

【特許請求の範囲】

【請求項 1】

配列番号 2～3、10、12～13もしくは15、その翻訳された蛋白コード部分、その成熟タンパク質コード部分、その細胞外部分、またはその活性ドメインからなる群より選択されたヌクレオチド配列を含む、単離されたポリヌクレオチド。

【請求項 2】

生物活性を有するポリペプチドをコードする単離されたポリヌクレオチドであり、該ポリヌクレオチドはストリンジェントなハイブリダイゼーション条件下で請求項 1 に記載のポリヌクレオチドの相補体とハイブリダズする、ポリヌクレオチド。

【請求項 3】

生物活性を有するポリペプチドをコードする単離されたポリヌクレオチドであり、該ポリヌクレオチドは請求項 1 に記載のポリヌクレオチドと約 90% より大きい配列同一性を有する、ポリヌクレオチド。

【請求項 4】

請求項 1 に記載のポリヌクレオチドであって、DNA 配列である、ポリヌクレオチド。

【請求項 5】

請求項 1 に記載のポリヌクレオチドの相補体を含む、単離されたポリヌクレオチド。

【請求項 6】

請求項 1 に記載のポリヌクレオチドを含む、ベクター。

【請求項 7】

請求項 1 に記載のポリヌクレオチドを含む、発現ベクター。

【請求項 8】

請求項 1 に記載のポリヌクレオチドを発現するように、遺伝子操作された、宿主細胞。

【請求項 9】

請求項 8 に記載の宿主細胞であって、前記ポリヌクレオチドが、該宿主細胞のポリヌクレオチドの発現を制御する調節配列と作動可能に連結されている、宿主細胞。

【請求項 10】

配列番号 4～9、14もしくは16～18、その翻訳された蛋白コード部分、その成熟タンパク質コード部分、その細胞外部分、またはその活性ドメインからなる群より選択されたアミノ酸配列と少なくとも 80% 同一であるアミノ酸配列を含む、単離されたポリペプチド。

【請求項 11】

請求項 10 に記載のポリペプチドおよびキャリアを含む、組成物。

【請求項 12】

プロトンビナーゼ様活性を有するポリペプチドであって、配列番号 4～9、14もしくは16～18からなる群より選択されたポリペプチド配列の少なくとも 10 個連続するアミノ酸を含むポリペプチド。

【請求項 13】

請求項 12 に記載のポリペプチドであって、配列番号 4～9、14または16～18からなる群より選択されたポリペプチド配列の少なくとも 5 個連続するアミノ酸を含む、ポリペプチド。

【請求項 14】

請求項 12 に記載のポリペプチドをコードする、ポリヌクレオチド。

【請求項 15】

請求項 13 に記載のポリペプチドをコードする、ポリヌクレオチド。

【請求項 16】

請求項 10 に記載のポリペプチドをコードする、ポリヌクレオチド。

【請求項 17】

請求項 10 に記載のポリペプチドに特異的な、抗体。

【請求項 18】

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請求項 1 に記載のポリヌクレオチドをサンプル中で検出する方法であって、以下：

- a) 請求項 1 に記載のポリヌクレオチドと結合して、複合体を形成する化合物に、該複合体を形成するに必要な期間、該サンプルを接触させる工程、
 - b) 該複合体を検出し、複合体が検出されれば、請求項 1 に記載のポリヌクレオチドが検出されたものとする工程、
- を包含する、方法。

【請求項 19】

請求項 1 に記載のポリヌクレオチドをサンプル中で検出する方法であって、以下：

- a) 請求項 1 に記載のポリヌクレオチドとストリンジェントなハイブリダイゼーション条件の下でアニーリングする核酸プライマーを、そのようなハイブリダイゼーション条件下で該サンプルと接触させる工程； 10
 - b) 請求項 1 に記載のポリヌクレオチドの少なくとも一部を含む生成物を増幅する工程；および
 - c) 該生成物を検出し、それによって該サンプル中において請求項 1 に記載のポリヌクレオチドを検出する工程、
- を包含する、方法。

【請求項 20】

請求項 19 の方法であって、前記ポリヌクレオチドは、RNA 分子を含み、該方法はさらにアニーリングした RNA 分子を cDNA ポリヌクレオチドに逆転写する工程を含む、方法。 20

【請求項 21】

請求項 10 に記載のポリペプチドをサンプル中で検出する方法であって、以下：

- a) 該ポリペプチドと結合して、複合体を形成する化合物に対して、該複合体を形成するのに十分な条件下および期間、該サンプルを接触させる工程；
 - b) 該複合体の形成を検出し、複合体が検出されれば、請求項 10 に記載のポリヌクレオチドが検出されたものとする、工程、
- を包含する、方法。

【請求項 22】

請求項 10 に記載のポリペプチドと結合する化合物を同定する方法で、以下：

- a) 請求項 10 に記載のポリペプチドと、ポリペプチド／化合物の複合体を形成するのに十分な条件下および期間、該化合物を接触させる工程； 30
- b) 該複合体を検出し、該ポリペプチド／化合物の複合体が検出されれば、請求項 10 に記載のポリペプチドに結合する化合物が検出されたものとする工程、を包含する、方法。

【請求項 23】

請求項 10 に記載のポリペプチドと結合する化合物を同定する方法であって、以下：

- a) 請求項 10 に記載のポリペプチドと該化合物を、細胞の中で、ポリペプチド／化合物の複合体を形成するに十分な期間、接触させて、該複合体によって該細胞中にレポーター遺伝子配列を出現させる工程；および
 - b) レポーター遺伝子配列発現を検出することによって該複合体を検出し、該ポリペプチド／化合物の複合体が検出されれば、請求項 10 に記載のポリペプチドに結合する化合物が検出されたものとする工程、 40
- を包含する、方法。

【請求項 24】

プロントロンビナーゼ様のポリペプチドの生成方法であって、以下：

- a) 請求項 8 に記載の宿主細胞を該細胞中に該ポリペプチドを発現させるために十分な条件下で培養する工程
 - b) 細胞培養物または工程 (a) の細胞からポリペプチドを単離する工程、
- を包含する、方法。

【請求項 25】

請求項 10 に記載のポリペプチドを含む、キット。

【請求項 26】

請求項 1 に記載のポリヌクレオチド、または表面に付着した請求項 1 に記載のポリヌクレオチドの特有のセグメントを含む、核酸アレイ。

【請求項 27】

請求項 26 のアレイであって、請求項 1 に記載のポリヌクレオチドまたは該ポリヌクレオチドの特有のセグメントに対する完全適合（フルマッチ）を検出する、アレイ。

【請求項 28】

請求項 26 のアレイであって、請求項 1 に記載のポリヌクレオチドまたは該ポリヌクレオチドの特有のセグメントに対するミスマッチ（不適合）を検出するアレイ。

【請求項 29】

請求項 10 に記載のプロトロンビナーゼ様ポリペプチドの活性または発現の強化の必要が求められている被験体の治療方法であって、以下の項目よりなる群から選択される成分を該被験体に投与する工程を包含する、方法：

- a) 該ポリペプチドのアゴニストの治療量；
 - b) 該ポリペプチドの治療量；および
 - c) 該ポリペプチドが生成されるような形態と条件の下での、該ポリペプチドをコードするポリヌクレオチドの治療量、
- ならびに薬学的に受容可能なキャリア。

【請求項 30】

請求項 10 に記載のプロトロンビナーゼ様ポリペプチドの活性または発現を阻害する必要を有する被験体の治療方法であって、以下の項目よりなる群から選択される成分を該被験体に投与する工程を包含する、方法：

- a) 該ポリペプチドのアンタゴニストの治療量；
 - b) 該ペプチドをコードするヌクレオチド配列の発現を阻害するポリヌクレオチドの治療量；および
 - c) プロトロンビナーゼ様ポリペプチドとそのリガンドについて競合するポリペプチドの治療量、
- ならびに薬学的に受容可能なキャリア。

【請求項 31】

配列番号 2 もしくは 3 に記載のヌクレオチド配列、その成熟タンパク質コード部分、またはその活性ドメインを含む、ヒトのプロトロンビナーゼ活性を有する単離されたポリヌクレオチド。

【請求項 32】

生物活性を有するポリペプチドをコードする単離されたポリヌクレオチドであって、請求項 31 に記載のポリヌクレオチドの相補体にストリンジェントなハイブリダイゼーション条件下でハイブリダズするポリヌクレオチド。

【請求項 33】

生物活性を有するポリペプチドをコードする単離されたポリヌクレオチドであって、請求項 31 に記載のポリヌクレオチドと約 90% より大きい配列同一性を有するポリヌクレオチド。

【請求項 34】

請求項 31 に記載のポリヌクレオチドであって、DNA 配列であるポリヌクレオチド。

【請求項 35】

請求項 31 に記載のヌクレオチドの相補体を含む単離されたポリヌクレオチド。

【請求項 36】

請求項 31 に記載のポリヌクレオチドを含むベクター。

【請求項 37】

請求項 31 に記載のポリヌクレオチドを含む発現ベクター。

【請求項 38】

請求項 31 に記載のポリヌクレオチドを発現するように遺伝子操作された、宿主細胞。

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【請求項 3 9】

請求項 3 1 に記載のポリヌクレオチドを含むように遺伝子操作された宿主細胞であって、該ポリヌクレオチドは、該宿主細胞において該ポリヌクレオチドの発現を制御する調節配列と作動可能に連結されている、宿主細胞。

【請求項 4 0】

配列番号 4 ～ 9、その成熟タンパク質部分、および活性ドメインからなる群より選択されたアミノ酸を含む、単離されたポリペプチド。

【請求項 4 1】

請求項 4 0 に記載のポリペプチドおよびキャリアを含む、組成物。

【請求項 4 2】

請求項 4 0 に記載のポリペプチドに対する、抗体。

【請求項 4 3】

サンプルにおいて請求項 3 1 に記載のポリヌクレオチドを検出する方法であって、以下：
a) 請求項 3 1 に記載のポリヌクレオチドと結合して、複合体を形成する化合物に対して、該複合体を形成するのに十分な期間、該サンプルを接触させる工程；および
b) 該複合体を検出し、複合体が検出されれば、請求項 3 1 に記載のポリヌクレオチドが検出されたものとする工程、
を包含する、方法。

【請求項 4 4】

サンプルにおいて、請求項 3 1 に記載のポリヌクレオチドを検出する方法であって、以下 20
：

a) 請求項 3 1 に記載のポリヌクレオチドとストリンジェントなハイブリダイゼーション条件の下で、アニーリングする核酸プライマーをそのようなハイブリダイゼーション条件下で該サンプルと接触させる工程；
b) 請求項 3 1 に記載のポリヌクレオチドの少なくとも一部を含む生成物を増幅する工程；および
c) その生成物を検出し、それによって該サンプルで請求項 3 1 に記載のポリヌクレオチドを検出する工程、
を包含する、方法。

【請求項 4 5】

請求項 4 4 の方法であって、前記ポリヌクレオチドは請求項 4 0 に記載のポリペプチドをコードする RNA 分子であり、該方法はさらに、アニーリングした RNA 分子を cDNA ポリヌクレオチドに逆転写する工程を包含する、方法。

【請求項 4 6】

請求項 4 0 に記載のポリペプチドをサンプル中で検出する方法であって、以下：
a) 該ポリペプチドと結合して、複合体を形成する化合物に、該複合体を形成するのに十分な条件下および期間で該サンプルを接触させる工程；および
b) 該複合体の形成を検出し、複合体の形成が検出されれば、請求項 4 0 に記載のポリヌクレオチドが検出されたものとする工程、
を包含する、方法。 40

【請求項 4 7】

請求項 4 0 に記載のポリペプチドと結合する化合物を同定する方法であって、以下：
a) 請求項 4 0 に記載のポリペプチドと、ポリペプチド／化合物の複合体を形成するのに十分な条件下および期間で、該化合物を接触させる工程；
b) 該複合体を検出し、ポリペプチド／化合物の該複合体が検出されれば、請求項 4 0 に記載のポリペプチドに結合する化合物が検出されたものとする工程、を包含する、方法。

【請求項 4 8】

請求項 4 0 に記載のポリペプチドと結合する化合物を同定する方法であって、以下：
a) 請求項 4 0 に記載のポリペプチドと、該化合物を、細胞の中で、ポリペプチド／化合物の複合体を形成するに十分な期間、接触させて、該複合体によって該細胞中にレポータ 50

一遺伝子配列の発現を駆動させる工程；および

b) レポーター遺伝子配列発現を検出することによって該複合体を検出し、該ポリペプチド／化合物の複合体が検出されれば、請求項40に記載のポリペプチドに結合する化合物が検出されたものとする工程、
を包含する、方法。

【請求項49】

請求項40に記載のポリペプチドを作成する方法であって、以下：

a) 請求項38に記載の宿主細胞を、該細胞中に該ポリペプチドを発現させるために十分な期間、培養する工程；および

b) 該細胞培養物または工程(a)の細胞からポリペプチドを単離する工程、を包含する、方法。 10

【請求項50】

請求項40に記載のポリペプチドを含む、キット。

【請求項51】

請求項31に記載のポリヌクレオチド、または表面に付着した請求項31に記載のポリヌクレオチドに特有のセグメント、を含む核酸アレイ。

【請求項52】

請求項51のアレイであって、請求項31に記載のポリヌクレオチドまたは該ポリヌクレオチドの特有のセグメントに対する完全適合（フルマッチ）を検出するアレイ。

【請求項53】

請求項51のアレイであって、請求項31に記載のポリヌクレオチドまたは該ポリヌクレオチドの特有のセグメントに対する不適合（ミスマッチ）を検出するアレイ。 20

【請求項54】

請求項10に記載のポリペプチドおよび薬学的に受容可能なキャリアを含む組成物の治療量を、それを必要とする哺乳類被験体に、投与する工程を包含する、処置の方法。

【請求項55】

配列番号4～9からなる群から少なくとも10個連続するアミノ酸を含むプロトンビナーゼ活性を有するポリペプチド。

【請求項56】

配列番号4～9からなる群から少なくとも5個連続するアミノ酸を含む請求項55に記載のポリペプチド。 30

【請求項57】

請求項55に記載のポリペプチドをコードするポリヌクレオチド。

【請求項58】

請求項56に記載のポリペプチドをコードするポリヌクレオチド。

【請求項59】

請求項40に記載のポリペプチドをコードするポリヌクレオチド。

【請求項60】

配列番号9の少なくとも1個のアミノ酸配列を有するプロトンビナーゼ活性を有する単離されたポリペプチドであって、ただし、ここでA＝アラニン、C＝システイン、D＝アスパラギン酸、E＝グルタミン酸、F＝フェニルアラニン、G＝グリシン、H＝ヒスチジン、I＝イソロイシン、K＝リシン、L＝ロイシン、M＝メチオニン、N＝アスパラギン、P＝プロリン、Q＝グルタミン、R＝アルギニン、S＝セリン、T＝トレオニン、V＝バリン、W＝トリプトファン、Y＝チロシンである、単離されたポリペプチド。 40

【発明の詳細な説明】

【0001】

(1. 技術分野)

本発明は新規のポリヌクレオチドおよびそのようなポリヌクレオチドによってコードされたタンパク質を提供し、それと共にそれらのポリヌクレオチドおよびタンパク質の用途、例えば、治療法、診断法および研究法における用途を提供する。本発明は、特に新規のプ 50

ロトロンビナーゼ様ポリペプチドに関する。

【0002】

(2. 背景技術)

特定されているポリヌクレオチドおよびポリペプチド配列は、例えば、診断、法医学、遺伝子地図作成、遺伝子障害またはその他の特性の原因となる突然変異の特定、生物学的多様性を評価するため、およびDNAおよびアミノ酸配列に依存するその他の多くの型のデータおよび生成物の作成するため、数多くの用途を持っている。タンパク質は、たとえばリーダー配列クロニングにおけるその分泌性によるか、PCRベースの技術におけるその細胞または組織源によるか、または既知の生物学的活性の他の遺伝子との構造的な相同性により、生物学的な活性を持つことが知られている。本発明はポリペプチドおよびこのポリペプチドをコードするポリヌクレオチドに関する。本発明は特に新規の可溶性のプロトロンビナーゼ様ポリペプチドとポリヌクレオチドに関する。

【0003】

血管の損傷が生じた後の血流制御に関する人体の能力は生存の継続について、重要な要素である。損傷した組織が補修された後の血餅の発生と、その後の血餅の溶解の過程は止血と呼ばれる。止血は、1) 血管狭窄、2) 血小板活性化および凝集、3) 血栓形成、および4) 組織補修後の血餅の溶解から成る。血餅の発生過程を開始するために、不活性プロトロンビンがプロトロンビナーゼ複合体によってトロニンに変換され、後者は血小板リン脂質、Caイオン、プロトロンビンおよびVaとXaから構成される。プロトロンビナーゼは免疫凝結原の一種として認知され、性格づけられている(国際特許申請WO98/51335号)。プロトロンビンの切断によるトロニンの発生が中心的な調節プロセスである。トロニンは血小板を活性化して最初のカスケードを開始させ、そしてまた、主要なプラズマタンパク質であるフィブリノーゲンを分割する。フィブリンは繊維のネットワークを形成し、その結果、血餅が生じる。よって、プロトロンビナーゼは凝縮原であり、その活性の規制は生命維持上、不可欠である。フィブリノーゲン切断生成物はまた、血管収縮および走化活性を表することが示されており、そしていくつかの細胞種類に対して分裂誘発性である(ヘリックら、(1999) *Int. J. Biochem. Cell Biol.* 31, 741-746)。

【0004】

凝縮経路の調節不良は、血友病、心筋梗塞、糸状体疾患、糖尿病、腫瘍増殖、劇症型ウイルス肝炎、およびアテローム性動脈硬化症などの重篤な症状を起こし得る。これらの多くの症状は、フィブリン沈着および患部組織の壊死を伴う。血栓症または血液凝固はまた、従来ガンと関係づけられてきた(ドボルジャーク、H. F. Hum. Pathol. 18 (3), pp. 275-284 (1987))。プロトロンビナーゼはまたウイルス性肝炎とも関係づけられている(レビラ、Am. J. Pathol. 156 (4), pp. 1217-1225 (2000))。一部の研究者らは、プロトロンビナーゼの異常発現は、1型糖尿病の初期段階と関係することを示している(ファンデル・プランケンら、Clin. Appl. Thromb. Hemost. 6 (2), pp. 65-68 (2000))。プロトロンビナーゼまたはプロトロンビナーゼタンパク質は凝結を増進することで傷の治療に貢献し得るとされている(チアガラヤンら、Biochemistry 39 (21) pp. 6459-6465 (2000))。プロトロンビナーゼまたはプロトロンビナーゼタンパク質の抑制は、異常血栓の減少、病原性またはウイルス性肝炎の増加、肝臓の壊死の低減、または1型糖尿病の治療に役立つ。

【0005】

最近では、ウイルス感染またはサイトカイン治療のいずれかによって誘発される誘発可能なプロトロンビナーゼ遺伝子が存在することが明らかになってきつつある(レビラ、(2000) Am. J. Pathol. 150, pp. 1217-1225)。プロトロンビナーゼfg12タンパク質に対する抗体は、この疾患のマウスモデルにおける劇症型肝炎を排除し、肝臓の壊死を減少させることが証明された。同様に、妊娠中のマウスのサイトカイン治療においてfg12発現をアップレギュレートさせることは、自発的中絶を生じる

ことが示されている（クラークら、（1999）Am. J. Reproductive Immunol. 42, 37-43）。特定の病的状態の下においてのみ発現されるその他の誘発可能なプロトロンビナーゼ様タンパク質を識別することができれば有益である。

【0006】

（3．発明の要旨）

本発明は、新規のプロトロンビナーゼ様ポリペプチド、そのようなペプチドをコードする新規の単離されたポリヌクレオチド、それらに含まれるものとして、組み換えDNA分子、クローンされた遺伝子またはそれらの変性改変体、詳細には、天然に存在する改変体、例えば、対立遺伝子改変体、アンチセンスポリヌクレオチド分子、およびそれらのポリペプチド上に存在する1つあるいはそれ以上のエピトープを特に認識する抗体、さらにそれらの抗体を生み出すハイブリドーマの発見に基づいている。特に、本発明のポリヌクレオチドは、ヒトの卵巣から生成されたcDNAライブラリから単離されたプロトロンビナーゼ様ポリヌクレオチド（Hyseqクローン認識番号7735656（配列番号1）および7727065（配列番号11））に基づいている。

【0007】

本発明の組成物としては、さらに、本発明によるポリヌクレオチドを含む発現ベクターのようなベクター、そのようなポリヌクレオチドを含むような遺伝子工学的な処理をされた細胞、およびそのようなポリヌクレオチドを発現するような遺伝子工学的な処理をされた細胞が挙げられる。

【0008】

本発明の組成物は、以下のものを含むがそれらに限定されない、分離されたポリヌクレオチドを提供する：配列番号1～3、10～13、および15に指定されたヌクレオチドを含むポリヌクレオチド；または、配列番号1～3、10～13、および15のフラグメント；配列番号1～3、10～13、および15の完全長タンパク質コード配列を含むポリヌクレオチド（たとえば、配列番号4、9、14、18）；および任意の配列番号1～3、10～13、および15の成熟したタンパク質コード配列のヌクレオチド配列を含むポリヌクレオチド。本発明のポリヌクレオチドはまた、以下のものを含むが、それらに限定されないポリヌクレオチドを提供する：（a）配列番号1～3、10～13、および15に示される任意のヌクレオチド配列の補体；（b）配列番号4～9、14、16～18のいずれかをコードするヌクレオチド配列にストリンジェントなハイブリダイゼーション条件下でハイブリダイズするポリヌクレオチド；上記に列挙した任意のポリヌクレオチドの対立遺伝子改変体であり、このポリヌクレオチドに対して少なくとも70%のポリヌクレオチド配列同一性を持つ対立遺伝子改変体；上記に列挙した任意のペプチドの種ホモログ（例えばオーソログ）をコードするポリヌクレオチド；あるいは、配列番号4～9、14、16～18を含むポリペプチドの特定の領域または短縮物（truncation）を含むポリペプチドをコードするポリヌクレオチドである。

【0009】

本出願に使われる収集物は一つのポリヌクレオチドのみの収集物であっても良い。各配列の配列情報または独特な認識情報の収集物が、核酸アレイ上に提供されても良い。1つの実施形態において、配列情報のセグメントが、そのセグメントを含むポリヌクレオチドを検知するために核酸アレイ上に提供される。アレイは、そのセグメントを含むポリヌクレオチドに対する完全な一致または不一致を検知するように設計され得る。収集物は、コンピュータ読取可能な形式で提供されてもよい。

【0010】

本発明はさらに、上記に示すポリヌクレオチドの少なくともフラグメントを含むクローニングベクターまたは発現ベクターおよびそれらの発現ベクターによって形質転換された宿主細胞または生物を提供する。有益なベクターの中には、当該技術で周知のプラスミド、コスミド、ラムダファージ誘導體、ファージミドなどを含む。したがって、本発明はまた、本発明のポリヌクレオチドを含むベクターおよびこのポリヌクレオチドを含む宿主細胞を提供する。一般的に、ベクターは少なくとも1つの生物中での複製機能の起点を含み、

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便利な制限エンドヌクレアーゼ部位、および宿主細胞用の選択可能なマーカーを含む。本発明のベクターには、発現ベクター、複製ベクター、プローブ生成ベクター、および配列決定ベクターが含まれる。本発明の宿主細胞は原核細胞または真核細胞であり得、そして単細胞生物または多細胞生物であり得る。

【0011】

本発明の組成物は、配列番号4～9、14および16～18のアミノ酸配列を含む群から選択される単離されたポリペプチドを含むがそれに限定されないポリペプチド；または対応する完全長タンパク質もしくは成熟タンパク質を含む。本発明のポリペプチドはまた、
(a) 配列番号1～3、10～13、および15に示されたヌクレオチドを持つ任意のポリヌクレオチド；または (b) ストリンジェントなハイブリダイゼーション条件の下で (a) のポリヌクレオチドの相補体にハイブリダイズするポリヌクレオチドによってコードされる生物活性をもつポリペプチドをも含む。配列番号4～9、14、および16～18に挙げられている任意のタンパク質配列の生物学的または免疫学的活性の改変体、ならびに生物学的または免疫学的活性を保持するそれらの実質的等価物もまた意図される。本発明のポリペプチドは、その全部または一部を化学的に合成してもよいが、本発明の遺伝工学的に処理された細胞（たとえば宿主細胞）を利用して遺伝子組み換え手段で生成されることが望ましい。

【0012】

本発明はまた、本発明のポリペプチドを含む組成物を提供する。本発明の薬学的構成としては、本発明のポリペプチド、ならびに親水性の担体、例えば、薬学的受容可能な担体を含んでもよい。

【0013】

本発明はまた、望ましいポリペプチドの発現を許すような条件下で適切な培養液中で、本発明のポリペプチドをコードするポリヌクレオチドの少なくともフラグメントを含む発現ベクターを含む宿主細胞を培養し、培養物または宿主細胞からのタンパク質またはペプチドを精製することにより本発明のポリペプチドを生成する方法に関する。望ましい実施例には、そのようなプロセスで製造されたタンパク質が熟成した形態のタンパク質である場合を含む。

【0014】

本発明によるポリヌクレオチドは、分子生物学の当業者に公知の種々の技術における多数の適用を有する。それらの手法には、ハイブリダイゼーションプローブとしての用途、オリゴマーまたはプライマーとしての用途、PCRのために、アレイにおける用途、コンピューター読み取り可能な媒体での用途、染色体および遺伝子マッピングのための用途、タンパク質の組み換え産生における用途、およびアンチセンスDNAまたはRNAその化学アナログの生成における用途、などを含む。例えば、mRNAの発現が、特定の細胞または組織型に大きく限定される場合、本発明のポリヌクレオチドは、例えば、インサイチュハイブリダイゼーションを用いて、サンプル中の特定の細胞または組織mRNAの存在を検出するためのハイブリダイゼーションプローブとして用いられ得る。

【0015】

他の例示的な実施形態において、ポリヌクレオチドは、発現された遺伝子を同定するための発現された配列タグとして診断において（当該分野で周知であり、そしてVollrathら、Science 258:52-59 (1992) に例示されるように）または、ヒトゲノムの物理的マッピングのための発現された配列タグとして、用いられる。

【0016】

本発明のポリペプチドは、他のタンパク質に現在適用されている、種々の従来の手順および方法において用いられ得る。例えば、本発明のポリペプチドは、ポリペプチドに特異的に結合する抗体を生成するために用いられ得る。このような抗体、特にモノクローナル抗体は、組織におけるポリペプチドを検出または定量するために有用である。本発明のポリペプチドはまた、分子量マーカーとして、および食物補充物として用いられ得る。

【0017】

医学的状态を予防、処置、または緩和する方法がまた提供される。この方法は、本発明のペプチドおよび薬学的に受容可能なキャリアを含む組成物の治療上有効な量を、哺乳動物被験体に投与する工程を包含する。

【0018】

詳細には、本発明のポリペプチドおよびポリヌクレオチドは、たとえば、血友病、心筋梗塞、糸状体疾患、糖尿病、劇症ウイルス性肝炎およびアテローム性動脈硬化症などの凝結経路の調節不全に関連する疾病の予防および／または処置法の一部として利用することができる。このポリペプチドおよびポリヌクレオチドは、また、ウイルス性感染症の治療およびある種のガンの治療に有効であり得る。

【0019】

本発明の方法はまた、本明細書において挙げられるような障害の処置のための方法を提供する。この方法は、本明細書において挙げられる障害に関する症状または傾向を示す哺乳動物被験体への本発明のポリヌクレオチドまたはポリペプチドおよび薬学的に受容可能なキャリアを含む組成物の投与を包含し得る。さらに、本発明は、本明細書に挙げられるような疾患または障害を処置するための方法を包含する。この方法は、標的遺伝子産物の全体的活性を調節する組成物および他の物質ならびに薬学的に受容可能なキャリアを含む組成物を投与する工程を包含する。化合物および他の物質は、標的遺伝子／タンパク質発現または標的タンパク質活性のいずれかのレベルにこのような調整をもたらし得る。具体的には、本発明のポリペプチド、または本発明のポリヌクレオチドを含む組成物の治療の有効量、あるいは本発明のプロトロンビナーゼ様ポリペプチドの結合パートナー（例えば、
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プロトロンビナーゼ様ポリペプチドに特異的に反応性する抗体）を含む組成物の治療の有効量を、ヒトを含むがヒトに限らない哺乳動物に投与する工程を含む、ウイルス性疾患を含む病状の、予防、治療または回復のための方法を提供する。治療を必要とする個体にとって、本発明によるポリペプチド、またはヌクレオチド、またはポリペプチドの結合パートナー（またはインヒビター）のいずれかが有効であるかは、特定の状態または病理のメカニズムが決定する。

【0020】

本方法によると、本発明のポリペプチドは細胞機能のインビトロまたはインビボ抑制を発生させるために投与され得る。本発明のあるポリペプチドは、インビボで単独か、または他の治療法の補助剤（a d j u n c t）として、投与され得る。逆に、本発明のタンパク
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質または他の活性分は、特定の薬剤の処方に含ませて、そのような薬剤の副作用を最小化し得る。

【0021】

本発明はさらに、上記の方法において有用な医薬を製造するための方法を提供する。

【0022】

本発明はさらに、サンプル（例えば、組織またはサンプル）中における本発明のポリヌクレオチドまたはポリペプチドの存在を検出する方法に関する。このような方法は、例えば、本明細書中に列挙されたような障害の予後的または診断的な評価の一部として、および
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このような状態に対する素因を示す被験体の同定のために利用され得る。

【0023】

本発明は、本発明のポリペプチドが試料中に存在することを検知する方法として、複合体を形成するのに十分な条件下かつ期間、ポリペプチドと結合する化合物に試料を接触させ、そしてこの複合体の形成を検知し、それによって複合体が形成される場合、ポリペプチドが検知される方法を提供する。

【0024】

本発明はまた、本発明の方法を実施するための、ポリヌクレオチドプローブおよび／またはモノクローナル抗体、ならびに必要なに応じて定量基準を備えたキットを提供する。さらに、本発明は、上記に列挙されるような障害の処置のための臨床試験に関連した、薬物の効力を評価する方法および患者の経過をモニタリングする方法を提供する。

【0025】

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本発明はまた、本発明のポリヌクレオチドおよび／またはポリペプチドの発現または活性を調節（すなわち、増加または減少）する化合物を同定するための方法を提供する。このような方法は、例えば、本明細書中に列挙されたような障害の症状を改善し得る化合物の同定のために有用であり得る。このような方法としては、本発明のポリペプチドと相互作用する（例えば、結合する）化合物または他の物質を同定するためのアッセイが挙げられるが、これに限定されない。

【0026】

本発明は本発明のポリペプチドと結合する化合物を同定する方法として、複合体を形成するのに十分な条件下かつ時間で、化合物をポリペプチドに接触させて、ポリペプチド／化合物複合体を形成する工程、およびこの複合体を検知する工程、を包含し、その結果、ポリペプチド／化合物複合体が検知される場合、ポリペプチドと結合する化合物が同定される方法を提供する。

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【0027】

化合物を同定するための方法であって、ポリペプチド／化合物複合体が形成されるのに十分な時間、化合物を細胞の中でポリペプチドと接触させる工程であって、ここで、この複合体は、細胞中でのレポーター遺伝子の発現を誘導する、工程、およびこのレポーター遺伝子配列発現を検出することにより複合体を検出する工程を包含し、その結果、ポリペプチド／化合物複合体が検知される場合、ポリペプチドと結合する化合物が同定される方法もまた提供される。

【0028】

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（５．発明の詳細な説明）

配列番号４のプロトロンビナーゼ様ポリペプチドは、約３８８アミノ酸のタンパク質で、予測分子量が非グリコシル下で約４３kDaである。BLASTXアルゴリズムを使ったタンパク質データベース検索（アルトシュル、S. F. ら、J. Mol. Evol. 36: 290-300 (1993) およびアルトシュル、S. F. ら、J. Mol. Biol. 21: 403-10 (1990)、これらは本明細書中で参考として援用される）は、配列番号４はヒトのプロトロンビナーゼ Fg12 タンパク質に相同であることを示す。図１は（「プロトロンビナーゼ様」とも呼ばれる）配列番号３によってコードされたタンパク質（すなわち、配列番号４）とヒトのプロトロンビナーゼ Fg12 タンパク質（配列番号１９）との間のBLASTXアミノ酸配列アライメントを示し、これらは、２つの配列が、配列番号４の２９１のアミノ酸残基について５３％の相同性、配列番号４の２９１のアミノ酸残基について３７％の同一性があることを示している。

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【0029】

予測された約２０残基のシグナルペプチドは、配列番号４の残基約１から２０によってコードされる。成熟部分（配列番号９とも表示される）は、それ自身有用である。これは、哺乳類細胞における発現と切断生成物の配列決定によって確認されている。シグナルペプチドは、カイト／ドリトルのアルゴリズム（Kyte/Doolittle algorithm）（J. Molecular Biology, vol. 157, pp. 105-131 (1982)）によって予測された。当業者は、実際の切断部位はコンピュータプログラムで予測されたものと異なる可能性があることを理解するであろう。

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【0030】

プロトロンビナーゼ様はC末端領域でフィブリノーゲンβおよびγ鎖を有することがeM ATRIXソフトウェアパッケージ（スタンフォード大学、カリフォルニア州スタンフォード）を利用して予想される。具体的には、配列番号４の残基１８１から２１７、配列番号４の残基２２２から２３４、配列番号４の残基２７１から２８５、配列番号４の残基３２４から３５３にあることが予想される。配列番号４に対応するドメインは以下である：ここで、A＝アラニン、C＝システイン、D＝アスパラギン酸、E＝グルタミン酸、F＝フェニルアラニン、G＝グリシン、H＝ヒスチジン、I＝イソロイシン、K＝リシン、L＝ロイシン、M＝メチオニン、N＝アスパラギン、P＝プロリン、Q＝グルタミン、R＝アルギニン、S＝セリン、T＝トレオニン、V＝バリン、W＝トリプトファン、Y＝チロ

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シンである：

残基 181～217 VMCDMDYRGGGWTVIQKRIDGIIIDFQRLWCDYLDGFG（配列番号5と表示される）、

残基 324～353 CSHLHNKTGWWFNECGLANLNGIHHFSGKL（配列番号6と表示される）、

残基 271～285 FKMHLGRYSGNAGDA（配列番号7と表示される）、

残基 222～234 EFWLGLKKIFYIV（配列番号8と表示される）。

【0031】

配列番号4の予測される可溶性の分泌スプライス改変体は配列番号14である。ほぼ326のアミノ酸タンパク質であり、予測分子量は非グリコシル化されて約37kDaである。タンパク質データベースをBLASTXアルゴリズム（アルトシュール、S. F. ら、J. Mol. Evol. 36:290～300（1993）およびアルトシュール、S. F. ら、J. Mol. Biol. 21:403～10（1990）、これらは本明細書中で参考として援用される）での検索は、配列番号14はヒトのプロトロンビナーゼFg12タンパク質と相同であることを示す。図2と3は（「プロトロンビナーゼ様」として同定される）配列番号13でコードされたタンパク質（すなわち配列番号14）とヒトのプロトロンビナーゼFg12タンパク質（配列番号19）との間のBLASTPアミノ酸配列のアライメントを示す。

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【0032】

eMATRIXソフトウェアパッケージ（Stanford University, Stanford, CA）を用いて、プロトロンビナーゼ様が、C末端ドメインにおいてフィブリノーゲンβ鎖およびγ鎖を有することが予想される。特に、配列番号14の残基181～217、配列番号14の残基262～291にある。配列番号14に対応するドメインは以下のとおりである：ここで、A＝アラニン、C＝システイン、D＝アスパラギン酸、E＝グルタミン酸、F＝フェニルアラニン、G＝グリシン、H＝ヒスチジン、I＝イソロイシン、K＝リジン、L＝ロイシン、M＝メチオニン、N＝アスパラギン、P＝プロリン、Q＝グルタミン、R＝アルギニン、S＝セリン、T＝トレオニン、V＝バリン、W＝トリプトファン、Y＝チロシン：

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残基 181～217 VMCDMDYRGGGWTVIQKRIDGIIIDFQRLWCDYLDGFG（配列番号5と表示される）、

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残基 262～292 CSHLHNKTGWWFNECGLANLNGIHHFSGKL（配列番号6と表示される）。

【0033】

推定されるほぼ20残基のシグナルペプチドは配列番号14（配列番号16）の両方のほぼ残基1から残基20までコードされる。細胞外部分はそれ自体が有用である。これは哺乳動物の細胞における発現、および切断産物の配列決定によって確認することができる。シグナルペプチド領域は、Kyte-Doolittle疎水性予測アルゴリズム（J. Mol. Biol. 157:105～31頁（1982）（本明細書中に参考として援用される））によって予測された。当業者は、実際の切断部位が、コンピュータプログラムによって予測された切断部位と異なり得ることを認識する。

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【0034】

Molecular Simulations Inc. のGeneAtlasソフトウェア（Molecular Simulations Inc.、San Diego, CA）および配列プロファイルベース検索プロトコルPB90を使用し、PSI-BLASTにより定義されたドメイン上で最適化されたPSI-BLASTおよびSeqfoldベースの検索プロトコルを使用することによって、膜に結合された「プロトロンビナーゼ様」ポリペプチド配列番号14は、血液凝固に対する特性モチーフ（配列番号20）を持った残基82～321（配列番号17）に領域を持つことが決定された（図4参照）。配列番号14に対応するドメインは以下のとおりである：ここで、A＝アラニン、C＝システイン、D＝アスパラギン酸、E＝グルタミン酸、F＝フェニルアラニン、G＝グリ

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シン、H＝ヒスチジン、I＝イソロイシン、K＝リジン、L＝ロイシン、M＝メチオニン、N＝アスパラギン、P＝プロリン、Q＝グルタミン、R＝アルギニン、S＝セリン、T＝トレオニン、V＝バリン、W＝トリプトファン、Y＝チロシン：

血液凝固ドメイン（配列番号14のアミノ酸82～321と表示される）のP S I－B L A S T 値 1.3×10^{-66} 、タンパク質データベース同定番号入力＝l f z c _ B（R e s e a r c h C o l l a b o r a t o r y f o r S t r u c t u r a l B i o i n f o r m a t i c s . h t t p : / / w w . r c s b . o r g / p b d）。

【0035】

本発明のポリペプチドおよびポリヌクレオチドは、例えば、プロトロンビナーゼ様ポリペプチドの損失または過剰出現により媒介される障害の予防および／または処置のための方法の一部として利用することができる。そのような障害としては、とりわけ、血友病、心筋梗塞、糸状体疾患、糖尿病、劇症ウイルス性肝炎およびアテローム性動脈硬化症などの凝固経路の調節不全に関連する疾患が挙げられる。ポリヌクレオチドおよびポリペプチドは、ウイルス感染の処置において、およびいくつかの形態のガンの処置において有益であり得る。

【0036】

（5．1定義）

本明細書および添付の特許請求範囲において、単数形「a」、「an」および「the」は、特に文脈がそうでないことを示さない限り、複数形への言及をも含むことに留意しなければならない。

【0037】

用語「活性」は、任意の天然に存在するポリペプチドの生物学的活性および／または免疫原性活性を保持するポリペプチドの形態をいう。本発明に従って、用語「生物学的に活性」または「生物学的活性」は、天然に存在する分子の構造的機能、調節機能または生化学的機能を有するタンパク質またはペプチドをいう。用語「プロトロンビナーゼ様」とはヒトのプロトロンビナーゼの生物学的活性に類似する生物学的活性をいう。同様に、「生物学的に活性な」または「生物学的活性」は天然、組換えもしくは合成のプロトロンビナーゼ様ペプチド、またはそれらの任意のペプチドが、適切な動物または細胞において特異的な生物学的応答を誘導し、特異抗体と結合する能力をいう。

【0038】

用語「活性化された細胞」は、本出願中で使用される場合、細胞内または細胞外の膜輸送（正常なプロセスまたは罹患プロセスの一部としての分泌分子または酵素分子の外への輸送を含む）に参与する細胞である。

【0039】

用語「相補的な」または「相補性」とは、塩基対合によるポリヌクレオチドの天然の結合をいう。例えば、配列5'－A G T－3'は相補的な配列3'－T C A－5'と結合する。2つの一本鎖分子間の相補性は、「部分的」であってその結果、一部の核酸のみが結合し得るか、または「完全」であってその結果、全体的な相補性が一本鎖分子の間に存在し得る。核酸鎖間の相補性の程度は、核酸鎖間のハイブリダイゼーションの効率および強さに対して重大な効果を持つ。

【0040】

用語「胚性幹細胞（E S）」は、胚または成体へと多くの分化した細胞型（生殖細胞を含む）を生じさせ得る細胞をいう。用語「生殖系列幹細胞（G S C）」とは、配偶体の生産のために生殖細胞の定常的かつ連続した供給源を提供する、始原幹細胞に由来する幹細胞をいう。用語「始原生殖細胞（P G C）」とは、他の細胞系列、特に卵黄嚢、腸間膜、または胚形成中の生殖隆起を除いた、生殖細胞などの細胞に分化する可能性を有する、小集団の細胞セットをいう。P G CはG S CおよびE S細胞を誘導する供給源である。P G C、G S CおよびE S細胞は自己再生し得る。従って、これらの細胞は生殖系列を増殖させ、成人の特化器官を含めて複数の最終分化細胞を生じさせるだけでなく、自分自身をも再生し得る。

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【0041】

用語「発現調節フラグメント」EMFは、作動可能に連結されたORFまたは別のEMFの発現を調節する一連のヌクレオチドを意味する。

【0042】

本明細書中で使用される場合、配列は、その配列の発現がEMFの存在によって改変される場合に、「作動可能に連結された配列の発現を調節する」といわれる。EMFとしては、プロモーター、およびプロモーター調節配列（誘導性エレメント）が挙げられるが、これらに限定されない。1つのクラスのEMFは、特定の調節因子または生理学的事象に応答して、作動可能に連結されたORFの発現を誘導する核酸フラグメントである。

【0043】

用語「ヌクレオチド配列」または「核酸」または「ポリヌクレオチド」または「オリゴヌクレオチド」は、本明細書中では交換可能に使用され、そしてヌクレオチドのヘテロポリマーまたはこれらのヌクレオチドの配列をいう。これらの用語はまた、一本鎖もしくは二本鎖であり得て、センス鎖もしくはアンチセンス鎖を表し得る、ゲノムもしくは合成起源のDNAまたはRNA、ペプチド核酸（PNA）または任意のDNA様またはPNA様物質をいう。一般的に、本発明により提供される核酸セグメントは、ゲノムおよび短いオリゴヌクレオチドリンカーのフラグメントから、または一連のオリゴヌクレオチドから、または個々のヌクレオチドから組立てられて、微生物またはウイルスオペロン、または真核生物遺伝子由来の調節エレメントを含む組換え転写単位で発現され得る合成核酸を提供し得る。

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【0044】

用語「オリゴヌクレオチドフラグメント」もしくは「ポリヌクレオチドフラグメント」、「部分」または「セグメント」または「プローブ」または「プライマー」は、交換可能に使用され、そして少なくとも約5個のヌクレオチド、より好ましくは少なくとも約7個のヌクレオチド、より好ましくは少なくとも約9個のヌクレオチド、より好ましくは少なくとも約11個のヌクレオチド、そして最も好ましくは少なくとも約17個のヌクレオチドであるヌクレオチド残基の配列をいう。フラグメントは、好ましくは約500個未満のヌクレオチド、好ましくは約200個未満のヌクレオチド、より好ましくは約100個未満のヌクレオチド、より好ましくは約50個未満のヌクレオチド、そして最も好ましくは30個未満のヌクレオチドである。プローブは、好ましくは約6個のヌクレオチド～約200個のヌクレオチド、好ましくは約15個～約50個のヌクレオチド、より好ましくは約17個～30個のヌクレオチド、そして最も好ましくは、約20個～25個のヌクレオチドである。好ましくは、フラグメントは、ポリメラーゼ連鎖反応（PCR）、各種のハイブリダイゼーション手順またはマイクロアレイ手順において、mRNA分子またはDNA分子の同一または関連部分の同定または増幅に使われ得る。フラグメントまたはセグメントは本発明の各ポリヌクレオチド配列を独自に同定し得る。好ましくは、フラグメントは配列番号1～3、10～13および15の一部と実質的に類似する配列を含む。

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【0045】

プローブは例えば、細胞または組織の中に特定のmRNA分子が存在するかどうかを決定するため、またはWalshらによって記載された染色体DNAから類似の核酸配列を単離するために使われ得る（Walsh, P. S. ら、1992、PCR Methods Appl 1: 241～250）。これらは、ニックトランスレーション、クレノーフィリン反応、PCR、または当該分野で周知の他の方法によって標識され得る。本発明のプローブ、その調製および／または標識は、Sambrook J. ら、1989、Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY; またはAusubel, F. M. ら、1989、Current Protocols in Molecular Biology, John Wiley & Sons, New York, NYによって詳述されている（これらは両方とも、その全体が本明細書中に参考として援用される）。

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【0046】

本発明の核酸配列はまた、配列番号1～3、10～13および15の任意の核酸配列からの配列情報を含んでいる。配列情報は、配列番号1～3、10～13および15の配列情報を独自に同定するかまたは表す、配列番号1～3、10～13および15のセグメントであり得る。1つのそのようなセグメントは20マー核酸配列でもあり得る。なぜなら、その理由はヒトのゲノムにおいて20マーが完全に一致する確率は300分の1であるからである。ヒトのゲノムの場合、1組の染色体の中に30億個の塩基対がある。 4^{20} の20マーが存在し得るので、1組のヒトの染色体に存在する塩基対の数の300倍を超える20マーが存在することになる。同じ分析を用いて、17マーがヒトのゲノムにおいて完全にマッチする確率は約5分の1である。これらのセグメントを発現研究のためにアレイにおいて使用する場合は、15マーセグメントを使うことができる。同様に、15マーが発現された配列において完全にマッチする確率もまた、発現された配列がゲノム配列全体の約5%未満を構成するので、ほぼ5分の1である。

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【0047】

同様に、単一のミスマッチを検出するために配列情報を使う上で、1つのセグメントは25マーであり得る。ヒトのゲノムにおいて25マーがミスマッチ1個を伴って現れる確率は、完全マッチの確率($1 \div 4^{25}$)に各ヌクレオチド位置におけるミスマッチの増えた確率(3×25)を掛けることで計算される。1つのミスマッチを有する18マーが、発現研究のためのアレイにおいて検出され得る確率は約5分の1である。1つのミスマッチを有する20マーがヒトのゲノムの中で検出され得る確率は約5分の1である。

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【0048】

用語「オープンリーディングフレーム」ORFは、いかなる終結コドンをも含まない、アミノ酸をコードする一連のヌクレオチドトリプレットを意味し、タンパク質へと翻訳可能な配列である。

【0049】

用語「作動可能に連結された」または「作動可能に結合した」とは、機能的に関連した核酸配列をいう。例えば、あるプロモーターがあるコード配列の転写を制御するとき、そのプロモーターはそのコード配列と作動可能に結合または作動可能に連結されている。作動可能に連結された核酸配列は連続し得、同一のリーディングフレーム内にあり得、一方、ある遺伝的エレメント（例えば、リプレッサー遺伝子はコード配列に連続して連結されていないが、依然としてこのコード配列の転写／翻訳を制御する。

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【0050】

用語「多能性」は、細胞が、成体生物中に存在する多数の分化した細胞型へと分化する能力をいう。多能性細胞は、全能性細胞と比較して、その分化能力が制限されている。

【0051】

用語「ポリペプチド」または「ペプチド」または「アミノ酸配列」とは、オリゴペプチド、ペプチド、ポリペプチドもしくはタンパク質配列またはそれらのフラグメントをいい、天然に存在する分子または合成分子をいう。ポリペプチドの「フラグメント」、「部分」、または「セグメント」は、少なくとも5個のアミノ酸、好ましくは少なくとも約7個のアミノ酸、より好ましくは約9個のアミノ酸、そして最も好ましくは約17個以上のアミノ酸の一連のアミノ酸残基である。ペプチドは、好ましくは200個以下のアミノ酸、より好ましくは150個以下のアミノ酸、そして最も好ましくは100個以下のアミノ酸である。好ましくは、ペプチドは約5個から約200個のアミノ酸である。活性であるためには、任意のポリペプチドは、生物学的大約／または免疫学的活性を示すための十分な長さを持っていなければならない。

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【0052】

用語「天然に存在するポリペプチド」は、遺伝子操作されていない細胞によって産生されたポリペプチドをいい、そして特に、ポリペプチドの翻訳後改変（アセチル化、カルボキシル化、グリコシル化、リン酸化、脂質化（lipidation）、およびアシル化が挙げられるが、これらに限定されない）から生じる種々のポリペプチドを意図する。

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【0053】

用語「翻訳されたタンパク質コード部分」は、任意のリーダー配列またはプロセシング配列を含み得る、全長のタンパク質をコードする配列を意味する。

【0054】

用語「成熟タンパク質コード配列」は、何のリーダー／シグナル配列も伴わないペプチドまたはタンパク質をコードする配列をいう。ペプチドは、細胞中でのプロセシングの間に除去されるリーダー配列を有し得るか、またはこのタンパク質は、合成的に、もしくは成熟タンパク質コード配列のみをコードするポリヌクレオチドを使用して産生されてもよい。

【0055】

用語「誘導体」は、ヒトタンパク質において通常は生じない、ユビキチン結合、標識（例えば、放射性核種または種々の酵素による）、共有結合ポリマー付着（例えば、ペグ化（ポリエチレングリコールでの誘導体化））、およびオルニチンのようなアミノ酸の化学合成による挿入または置換のような技術によって化学的に改変されたポリペプチドをいう。

【0056】

用語「改変体」（または「アナログ」）は、例えば、組換えDNA技術を使用して作製された、アミノ酸の挿入、欠失および置換によって、天然に存在するポリペプチドとは異なる任意のポリペプチドをいう。どのアミノ酸残基が、目的の活性を損なうことなく置換、付加、または欠失され得るかを決定する際の指針は、特定のポリペプチドの配列を相同なペプチドの配列と比較すること、および相同性の高い領域（保存領域）においてなされるアミノ酸配列変化の数を最少にすることによってか、またはアミノ酸をコンセンサス配列で置換することによって、見出され得る。

【0057】

あるいは、これらの同一または類似のポリペプチドをコードする組換え改変体は、遺伝コードにおける「冗長性」を利用することにより、合成または選択することができる。各種のコドン置換（例えば、各種の制限部位を生じるサイレント変化）を導入して、プラスミドもしくはウイルスベクターへのクローニングまたは特定の原核生物系または真核生物系における発現を最適化することができる。ポリヌクレオチド配列における変異は、このポリペプチドまたはこのポリペプチドに付加される他のペプチドの領域に反映されて、このポリペプチドの任意の部分の特性を改変し得、リガンド結合親和性、鎖間親和性、または分解／代謝回転率のような特徴を変更し得る。

【0058】

好ましくは、アミノ酸「置換」は、1つのアミノ酸を、類似の構造的特性および／または化学的特性を有する別のアミノ酸で置換した結果（すなわち、保存的アミノ酸置換）である。「保存的」アミノ酸置換は、関与する残基の極性、電荷、可溶性、疎水性、親水性、および／または両親媒性の性質における類似性に基いてなされ得る。例えば、非極性（疎水性）アミノ酸としては、アラニン、ロイシン、イソロイシン、バリン、プロリン、フェニルアラニン、トリプトファン、およびメチオニンが挙げられ；極性の中性アミノ酸としては、グリシン、セリン、スレオニン、システイン、チロシン、アスパラギン、およびグルタミンが挙げられ；正に荷電した（塩基性）アミノ酸としては、アルギニン、リジン、およびヒスチジンが挙げられ；そして負に荷電した（酸性）アミノ酸としては、アスパラギン酸およびグルタミン酸が挙げられる。「挿入」または「欠失」は、好ましくは、約1～20、より好ましくは1～10アミノ酸の範囲である。許容されるバリエーションは、組換えDNA技術を使用して、ポリペプチド分子中にアミノ酸の挿入、欠失、または置換を体系的に作製すること、そして生じた組換え改変体を活性についてアッセイすることによって、実験的に決定され得る。

【0059】

あるいは、機能の改変が所望される場合には、挿入、欠失または非保存的改変を操作して、改変されたポリペプチドを生じさせ得る。このような改変は、例えば、本発明のポリペプチドの1以上の生物学的機能または生化学的特徴を改変し得る。例えば、このような改

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変は、リガンド結合親和性、鎖間親和性、または分解／代謝回転速度のようなポリペプチドの特徴を変化させ得る。さらに、このような改変は、発現のために選択された宿主細胞中における発現、スケールアップなどのためにより良く適したポリペプチドを生成するように選択され得る。例えば、システイン残基が、ジスルフィド架橋を排除するために、欠失され得るか、または別のアミノ酸残基で置換され得る。

【0060】

用語「精製された」または「実質的に精製された」は、本明細書中で使用される場合、示された核酸またはポリヌクレオチドは、他の生体高分子（例えば、ポリヌクレオチド、タンパク質など）が実質的に存在せずに存在することを示す。1つの実施形態では、ポリヌクレオチドまたはポリペプチドは、存在する示された生体高分子のうちの少なくとも95重量%、より好ましくは少なくとも99重量%を構成するように精製される（しかし、水、緩衝剤、および他の低分子（特に1000ダルトン未満の分子量を有する分子は存在し得る））。

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【0061】

用語「単離された」とは、本明細書中で使用される場合、その自然の供給源においてその核酸またはポリペプチドと共に存在する少なくとも1つの他の成分（例えば、核酸またはポリペプチド）から分離された核酸またはポリペプチドをいう。1つの実施形態では、核酸またはポリペプチドは、（もしあるとすれば）溶媒、緩衝剤、鉄、またはその溶液に通常存在する他の成分のみの存在下で見出される。用語「単離された」または「精製された」は、天然の供給源中に存在する核酸またはポリペプチドを包含しない。

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【0062】

用語「組換え」は、ポリペプチドまたはタンパク質をいうために本明細書中で使用される場合、そのポリペプチドまたはタンパク質が、組換え（例えば、微生物、昆虫または哺乳動物）発現系に由来することを意味する。「微生物（性）」は、細菌または真菌（例えば、酵母）の発現系において作製された組換えポリペプチドまたはタンパク質をいう。生成物として、「組換え微生物（性）」は、ネイティブな内因性物質を本質的に含まず、かつネイティブな結合グリコシル化を付随していないポリペプチドまたはタンパク質を規定する。大半の細菌培養物（例えば、E. coli）中で発現されるポリペプチドまたはタンパク質は、グリコシル化改変を含まず；酵母において発現されるポリペプチドまたはタンパク質は、哺乳動物細胞中において発現されるポリペプチドまたはタンパク質とは一般的に異なるグリコシル化パターンを有する。

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【0063】

用語「組換え発現ビヒクルまたはベクター」は、DNA（RNA）配列からポリペプチドを発現させるための、プラスミドまたはファージまたはウイルスまたはベクターをいう。発現ビヒクルは、以下のアセンブリを含む転写単位を含み得る：（1）遺伝子発現において調節的役割を有する遺伝的エレメント（1つまたは複数）（例えば、プロモーターまたはエンハンサー）；（2）mRNAへと転写され、そしてタンパク質へと翻訳される構造配列またはコード配列；ならびに（3）適切な転写開始配列および転写終結配列。酵母または真核生物の発現系において使用されることを意図された構造単位は、好ましくは、宿主細胞による翻訳タンパク質の細胞外分泌を可能にするリーダー配列を含む。あるいは、組換えタンパク質が、リーダー配列も輸送配列も伴わずに発現される場合、これは、アミノ末端メチオニン残基を含み得る。この残基は、最終産物を与えるために、発現された組換えタンパク質からその後切断されても、切断されなくともよい。

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【0064】

用語「組換え発現系」は、染色体DNA中に組換え転写単位が安定に組み込まれた宿主細胞、または染色体外で組換え転写単位を保有する宿主細胞を意味する。本明細書中で規定される組換え発現系は、発現されるべきDNAセグメントまたは合成遺伝子に連結された調節エレメントの誘導に際して、異種ポリペプチドまたは異種タンパク質を発現する。この用語はまた、遺伝子発現において調節的役割を有する組換え遺伝子エレメント（1つまたは複数）（例えば、プロモーターまたはエンハンサー）を安定的に組み込んだ宿主細胞

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を意味する。本明細書中で規定される組換え発現系は、発現されるべき内因性DNAセグメントまたは遺伝子に連結された調節エレメントの誘導に際して、細胞に対して内因性のポリペプチドまたはタンパク質を発現する。この細胞は、原核生物細胞であっても真核生物細胞であってもよい。

【0065】

用語「分泌」とは、膜を越えてかまたは膜を通して輸送されたタンパク質を包含し、適切な宿主細胞にそれが発現されたときに、そのアミノ酸配列中のシグナル配列の結果としての輸送を含む。「分泌」タンパク質としては、それが発現される細胞から完全に分泌されたタンパク質（例えば、可溶性タンパク質）かまたは部分的に分泌されたタンパク質（例えば、レセプター）が挙げられるが、これらに限定はしない。「分泌」タンパク質としてはまた、小胞体の膜を越えて輸送されるタンパク質が挙げられるが、限定はしない。「分泌」タンパク質はまた、非典型的シグナル配列を含むタンパク質（例えば、インターロイキン-1 β （Krasney, P. および Young, P. R. (1992) Cytokine 4 (2): 134~143) および損傷した細胞から放出される因子（例えば、インターロイキン-1レセプターアンタゴニスト）Arend, W. P. ら (1998) Annu. Rev. Immunol. 16: 27~55を参照))を包含することも意味する。

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【0066】

必要な場合は、発現ベクターは、ポリペプチドが細胞の膜を通ることを導く「シグナル配列またはリーダー配列」を含むように設計されていてもよい。そのような配列は、本発明のポリペプチド上に天然に存在していてもよいし、または組み換えDNA技術によって異種のタンパク質供給源から提供されてもよい。

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【0067】

「ストリンジェントな」という用語は、当該分野において通常ストリンジェントとして理解されている条件をいう。ストリンジェントな条件としては、高度にストリンジェントな条件（すなわち、フィルター固定したDNAを、0.5 M NaHPO₄、7%のドデシル硫酸ナトリウム（SDS）、1 mM EDTAに65℃でハイブリダイゼーションし、68℃の0.1X SSC/0.1% SDSで洗浄すること）、および中程度にストリンジェントな条件（すなわち、42℃の0.2X SSC/0.1% SDSで洗浄）が挙げられ得る。その他の例示的ハイブリダイゼーション条件は、本文中に実施例において説明する。

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【0068】

デオキシオリゴヌクレオチドのハイブリダイゼーションの場合、さらなるストリンジェントなハイブリダイゼーション条件としては、37℃（14塩基オリゴヌクレオチドに対して）、48℃（17塩基オリゴヌクレオチドに対して）、55℃（20塩基オリゴヌクレオチドに対して）および60℃（23塩基オリゴヌクレオチドに対して）の6X SSC/0.05%ピロリン酸ナトリウムで洗浄することが挙げられる。

【0069】

本明細書中で使用される場合、「実質的に等価」とは、ヌクレオチド配列とアミノ酸配列の両方をいい得、例えば、参照配列から1つ以上の置換、欠失または付加から変化する変異体配列があり、ここでその1つ以上の置換、欠失または付加の正味の効果は、参照配列と対象配列との間に機能上不利な差異を生じない。代表的には、そのような実質的に等価な配列は、本明細書中に列挙されたものの1つから35%以下異なる（すなわち、対応する参照配列との比較した場合に、実質的に等価な配列における個々の残基の置換、付加、および/または欠失の数を、その実質的に等価な配列の残基の総数で除した値が約0.35以下である）。そのような配列は列挙された配列に対して65%の配列同一性を有すると言われる。1つの実施形態において、本発明の実質的に等価な（例えば、変異体）配列は、列挙された配列から30%以下変化し（70%配列同一性）；本実施例のあるバリエーションでは、25%以下変化し（75%配列同一性）；本実施例のさらに別のバリエーションでは、20%以下変化し（80%配列同一性）；本実施例のさらに別のバリエーシ

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ョンでは、10%以下変化し（90%配列同一性）；本実施例のさらに別のバリエーションでは、5%以下変化する（95%配列同一性）。本発明による実質的に等価な（例えば、変異体）アミノ酸配列は、好ましくは、列挙されたアミノ酸配列と80%の同一性を有し、より好ましくは、90%の同一性を有する。本発明の実質的に等価なヌクレオチド配列は、遺伝子コードの重複性（redundancy）または縮重を考慮して、より低い配列同一性パーセントを有し得る。好ましくは、ヌクレオチド配列は、少なくとも約65%の同一性を有し、より好ましくは少なくとも約75%の同一性を有し、そして最も好ましくは少なくとも約95%の同一性を有する。本発明の目的のために、実質的に等価な生物学的活性特徴および実質的に等価な発現特徴を有する配列は、実質的に等価なものともみなされる。等価性の決定のためには、成熟配列の切断は（例えば、擬似ストップコドンを生成する変異を介して）無視されるべきである。配列同一性は、例えば、Jotun Hein法（Hein, J. (1990) Methods Enzymol. 183: 626~645）を使用して決定され得る。配列間の同一性はまた、当該分で公知の他の方法（例えば、ハイブリダイゼーション条件を変えること）によって、決定され得る。

【0070】

用語「全能」とは、成体生物の細胞型すべてへと細胞が分化する能力を指す。

【0071】

用語「形質転換」とは、適切な宿主細胞にDNAを導入して、そのDNAが染色体外エレメントとしてかまたは染色体組込みによってかのいずれかで複製可能であるようにすることを意味する。用語「トランスフェクション」とは、いくらかのコード配列が実際に発現されるかどうかにかかわらず、適切な宿主細胞による発現ベクターの取り込みをいう。用語「感染」とは、ウイルスまたはウイルスベクターの使用によって適切な宿主細胞に核酸を導入することを指す。

【0072】

本明細書中で使用される場合、「取り込み調節フラグメント」UMFとは、連結されたDNAフラグメントが細胞へ取り込まれるのを媒介する、一連のヌクレオチドを意味する。UMFは、既知のUMFを標的配列もしくは標的モチーフとして、以下に説明するようなコンピュータベースの系を使用して容易に同定され得る。UMFの存在および活性は、疑わしいUMFをマーカー配列に付着させることによって、確認され得る。その後、生じた核酸分子が、適切な条件下で、適切な宿主とともにインキュベートされ、そしてそのマーカー配列の取り込みを決定される。上述のように、UMFは、連結されたマーカー配列の取り込みの頻度を増加する。

【0073】

上述の各用語は、文脈上他の意味を示さないかぎり、各々について記載されたすべてを包含することが意味される。

【0074】

（5.2 本発明の核酸）

本発明は、新規な分泌プロトンビナーゼ様ポリペプチド、そのプロトンビナーゼ様ポリペプチドをコードするポリヌクレオチドの発見、そして神経学的状態および神経学的障害の診断、処置または予防のためのこれらの組成物の使用に基づく。

【0075】

本発明の単離されたポリヌクレオチドとしては、以下が挙げられるが、これらに限定されない：配列番号1~3、10~13および15のヌクレオチド配列のいずれかを含む、ポリヌクレオチド；配列番号1~3、10~13および15のフラグメント；配列番号1~3、10~13および15の全長タンパク質コード配列を含む、ポリヌクレオチド（例えば配列番号4、9、14、18）；ならびに配列番号1~3、10~13および15のいずれかのポリヌクレオチドの成熟タンパク質コード配列をコードするヌクレオチド配列を含む、ポリヌクレオチド。本発明のポリヌクレオチドとしてはまた、以下とストリンジェントな条件下でハイブリダイズするポリヌクレオチドが挙げられるが、これらに限定されない：（a）配列番号1~3、10~13または15のヌクレオチド配列のいずれかの相

補体；（b）配列番号4～9、14または16～18のポリペプチドのいずれか一つをコードするポリヌクレオチド；（c）上記の任意のポリヌクレオチドの対立遺伝子改変体であるポリヌクレオチド；（d）上記のタンパク質のいずれかの種ホモログをコードするポリヌクレオチド；あるいは（e）配列番号4～9、14または16～18のポリペプチドの特定のドメインもしくは短縮を含むポリペプチドをコードするポリヌクレオチド。目的のドメインは、コードされたポリペプチドの性質に依存し得、例えば、レセプター様ポリペプチド中のドメインとしては、リガンド結合ドメイン、細胞外ドメイン、膜貫通ドメイン、もしくは細胞質ドメイン、またはそれらの組み合わせが挙げられ；免疫グロブリン様タンパク質中のドメインとしては、免疫グロブリン様可変ドメインが挙げられ；酵素様ポリペプチド中のドメインとしては、触媒ドメインおよび基質結合ドメインが挙げられ；そしてリガンドポリペプチド中のドメインとしては、レセプター結合ドメインが挙げられる。

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【0076】

本発明のポリヌクレオチドとしては、天然に存在するDNAおよびRNA、または全合成DNAおよびRNAもしくは部分合成DNAおよびRNAが挙げられ、例えば、cDNAおよびゲノムDNAが挙げられ、例えば、mRNAが挙げられる。

【0077】

本発明はまた、本明細書中で開示されるcDNA配列に対応した遺伝子も提供する。これらの対応した遺伝子は、本明細書中で開示される配列情報を使って、既知の方法に従って単離され得る。そのような方法としては、適切なゲノムのライブラリー、または他のゲノム材料供給源における遺伝子の同定および／または増幅のための、開示された配列情報からプローブまたはプライマーの調製が挙げられる。さらなる5'配列および3'配列が、当該分野で公知の方法を使用して入手され得る。例えば、配列番号1～3、10～13および15のポリヌクレオチドのいずれかに対応する、全長cDNAもしくはゲノムDNAが、配列番号1～3、10～13および15のポリヌクレオチドのいずれかまたはその一部をプローブとして使用して、適切なハイブリダイゼーション条件下で適切なcDNAライブラリーもしくはゲノムDNAライブラリーをスクリーニングすることによって、入手され得る。あるいは、配列番号1～3、10～13および15のポリヌクレオチドは、適切なゲノムDNAライブラリーまたはcDNAライブラリーにおける遺伝子の同定および／または増幅を可能にする適切なプライマーについての基礎として使用され得る。

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【0078】

本発明の核酸配列は、一個以上の公開データベース（例えばdbEST、gbp riおよびUniGene）から取得されるESTおよび配列（cDNA配列およびゲノム配列を含む）から組み立てられ得る。このEST配列は、全長遺伝子に関する、配列情報、例示フラグメントもしくはセグメントの情報、または全長遺伝子についての新規なセグメント情報を提供し得る。

【0079】

本発明のポリヌクレオチドはまた、上記のポリヌクレオチドと実質的に等価のヌクレオチド配列を含むポリヌクレオチドを提供する。本発明によるポリヌクレオチドは、上記のポリヌクレオチドに対して、例えば、少なくとも約65%、少なくとも約70%、少なくとも約75%、少なくとも約80%、より典型的には少なくとも約90%、そしてさらにより典型的には少なくとも約95%の配列同一性を有し得る。

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【0080】

本発明における核酸配列の範囲に含まれるものは、配列番号1～3、10～13および15のヌクレオチドの配列のいずれかまたはその相補体にストリンジェントな条件下でハイブリダイズする核酸配列フラグメントであり、そのフラグメントは、約5ヌクレオチドより大きく、好ましくは7ヌクレオチドより大きく、より好ましくは9ヌクレオチドより大きく、そして最も好ましくは17ヌクレオチドより大きい。選択的な（すなわち、本発明のポリヌクレオチドのいずれか1つに特異的にハイブリダイズする）、例えば、15ヌクレオチド以上、17ヌクレオチド以上または20ヌクレオチド以上のフラグメントが、考

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慮される。ポリヌクレオチドに特異的にハイブリダイズすることができるプローブは、同じファミリーの遺伝子中の他のポリヌクレオチド配列から本発明のポリヌクレオチド配列を区別し得るか、あるいは他種の遺伝子からヒト遺伝子を区別し得、好ましくは、独自のヌクレオチド配列に基づく。

【0081】

本発明の範囲内にある配列は、これら特定の配列に限定されず、そしてまた、その対立遺伝子改変体および種改変体をも含む。対立遺伝子改変体と種改変体は、配列番号1～3、10～13および15にて提供される配列、その代表的なフラグメント、または配列番号1～3、10～13および15と少なくとも90%同一であり好ましくは95%同一のヌクレオチド配列を、同種の他の単離物からの配列と比較することによって、慣用的に決定され得る。さらに、コドンの可変性に順応するため、本発明は、本明細書中に開示された特定のORFと同様に、同じアミノ酸配列をコードする核酸分子を包含する。即ち、ORFのコード領域において、あるコドンをもつアミノ酸を、同じアミノ酸をコードする別のコドンで置換することが、明らかに意図される。

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【0082】

本発明の核酸（配列番号1～3、10～13および15を含む）の最も近接する近似物（the nearest neighbor）の結果は、アルゴリズムまたはプログラムを使ってデータベースを検索することにより取得され得る。好ましくは、BLAST（Basic Local alignment Search Toolの略語である）を使って、局所配列アラインメントが検索される（Altschul, S. F., J. Mol. Evol. 36: 290～300 (1993) および Altschul, S. F. et al., J. Mol. Biol. 21: 403～410 (1990)）。

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【0083】

開示されたポリヌクレオチドおよびタンパク質の種ホモログ（オルソログ）もた、本発明により提供される。種ホモログは、本明細書中に提供される配列から適切なプローブまたはプライマーを作製し、そして望ましい種から適切な核酸供給源をスクリーニングすることによって、単離され得る。そして同定され得る。

【0084】

本発明はまた、開示したポリヌクレオチドまたはタンパク質の対立遺伝子改変体も包含し；対立遺伝子改変体は即ち、単離されたポリヌクレオチドの天然に存在する代替形態であり、それはまたポリヌクレオチドによりコードされたものと同一であるか、相同であるか、または関連するタンパク質をコードする。

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【0085】

本発明の核酸配列はさらに、記載された核酸の改変体をコードする配列に関する。これらのアミノ酸配列改変体は、適切なヌクレオチド変化をネイティブポリヌクレオチドもしくは改変体ポリヌクレオチドに導入することによって、当該分野で公知の方法によって調製され得る。アミノ酸配列改変体の構築には、以下の2つの変数が存在する：変異の位置および変異の性質である。アミノ酸配列改変体をコードする核酸は、天然に存在しないアミノ酸配列をコードするようにそのポリヌクレオチドを変異させることによって、好ましくは構築される。これらの核酸変化は、異なる種からの核酸中で異なる部位（可変位置）または高度に保存された領域（定常領域）で作製され得る。そのような位置の部位は、代表的には、例えば、まず保存的選択枝で（例えば、疎水性アミノ酸を、異なる疎水性アミノ酸に）置換し、それからより遠い選択枝で（例えば、疎水性アミノ酸を、荷電アミノ酸に）置換することによって、連続して改変され、その後、欠失もしくは挿入が標的部位でなされ得る。アミノ酸配列の欠失は、一般的に、約1～30残基の範囲であり、好ましくは約1～10残基の範囲であり、代表的には連続している。アミノ酸の挿入としては、1～100残基以上の範囲のアミノ末端融合および／またはカルボキシル末端融合、ならびに単一アミノ酸残基または複数のアミノ酸残基の配列内挿入が挙げられる。配列内挿入は、一般には、約1～10アミノ酸残基の範囲であり得、好ましくは1～5残基の範囲であり得る。末端挿入の例としては、異なる宿主細胞中での分泌または細胞内ターゲティング

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に必要な、異種シグナル配列、ならびに発現されたタンパク質を精製するために有用な配列（例えば、FLAG配列またはポリヒスチジン配列）が挙げられる。

【0086】

好ましい方法において、新規なアミノ酸配列をコードするポリヌクレオチドが、部位特異変異誘発により変化される。この方法は、オリゴヌクレオチド配列を使って、ポリヌクレオチドが所望のアミノ酸改変体をコードするように変化させ、ならびに変化したアミノ酸の両側で隣接する十分な長さのヌクレオチドが、変化される部位のいずれの側でも安定な二重鎖を形成するように変化させる。一般に、部位特異的変異誘発の技術は、当業者に周知であり、そしてこの技術は、Edelmanら、DNA 2:183 (1983) のような刊行物によって例示される。ポリヌクレオチド配列において部位特異的变化を作製するための汎用的かつ効率的な方法は、ZollnerおよびSmith、Nucleic Acids Res. 10:6487~6500 (1982) により公開された。PCRもまた、新規な核酸のアミノ酸配列改変体を生成するために使用され得る。開始材料として少量のテンプレートDNAを使う場合、そのテンプレートDNA内の対応する領域とわずかに異なるプライマーが、望ましいアミノ酸改変体を生成し得る。PCR増幅は、そのプライマーにより特定の位置で、そのポリペプチドをコードするポリヌクレオチドテンプレートと異なる生成物DNAフラグメントの集団を生じる。この生成物DNAフラグメントは、プラスミド内の対応する領域を置換し、そしてこれは、望ましいアミノ酸改変体をコードするポリヌクレオチドを生じる。

【0087】

アミノ酸改変体を生成するさらなる技術は、カセット変異誘発技術（これはWellsら、Gene 34:315 (1985) に記載される）；ならびに当該分野で周知の他の変異誘発技術であり、これは例えば、前出のSambrookらおよびCurrent Protocols in Molecular Biology (Ausubelら) における技術である。遺伝コードの固有の縮重に起因して、実質的に同じアミノ酸配列または機能的に等価なアミノ酸配列をコードする他のDNA配列が、これらの新規な核酸のクローニングおよび発現のために本発明の実施において使用され得る。このようなDNA配列としては、ストリンジェントな条件の下、適切な新規な核酸配列にハイブリダイゼーション可能な配列が挙げられる。

【0088】

本発明の好ましいポリペプチド短縮物をコードするポリヌクレオチドは、本発明の1個以上のドメインと異種タンパク質配列とを含む、キメラタンパク質もしくは融合タンパク質をコードするポリヌクレオチドを生成するために使用され得る。

【0089】

本発明のポリヌクレオチドは、さらに、上記ポリヌクレオチドのいずれかの相補対を包含する。そのポリヌクレオチドは、DNA（ゲノムDNA、cDNA、増幅したDNA、または合成DNA）であってもRNAであっても良い。そのようなポリヌクレオチドを取得するための方法およびアルゴリズムは、当業者には周知であり、そしてそのような方法およびアルゴリズムとしては、例えば、所望の配列同一性のポリヌクレオチドを慣用的に単離し得るハイブリダイゼーション条件を決定する方法が挙げられ得る。

【0090】

本発明に従って、配列番号1~3、10~13および15、またはその機能的等価物のいずれか1つに対応する成熟タンパク質コード配列を含む、ポリヌクレオチドの配列が、適切な宿主細胞におけるその核酸の発現を指向する組換えDNA分子またはその機能的等価物を生成するために使用され得る。また、本明細書中で同定されたクローンのいずれかのDNAインサートも、包含される。

【0091】

本発明によるポリヌクレオチドは、十分に確立された組換えDNA技術により他の種々のヌクレオチド配列のいずれにも結合され得る（Sambrook Jら (1989) Molecular Cloning: A Laboratory Manual、Cold

Spring Harbor Laboratory、NYを参照のこと）。ポリヌクレオチドに結合するために有用なヌクレオチド配列としては、様々なベクター（例えば、当該分野で周知である、プラスミド、コスミド、ラムダファージ誘導体、ファージミドなど）が挙げられる。従って、本発明はまた、本発明のポリヌクレオチドを含むベクターおよびそのポリヌクレオチドを含む宿主細胞を提供する。一般に、そのベクターは、少なくとも1個の生物中で機能する複製起点、便利な制限エンドヌクレアーゼ部位、および宿主細胞についての選択マーカーを含む。本発明によるベクターとしては、発現ベクター、複製ベクター、プローブ生成ベクター、および配列決定ベクターが挙げられる。本発明による宿主細胞は、原核生物細胞であってもまたは真核生物細胞であっても良く、そして単細胞生物であってもまたは多細胞生物の一部であってもよい。

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【0092】

本発明はさらに、配列番号1～3、10～13および15のヌクレオチド配列またはそのフラグメント、あるいは本発明の他のポリヌクレオチドのいずれかを有する核酸を含む、組換え構築物を提供する。1つの実施形態では、本発明の組換え構築物は、ベクター（プラスミドベクターまたはウイルスベクター）を含み、そのベクターの中に、配列番号1～3、10～13および15のヌクレオチド配列もしくはそのフラグメントのいずれかを有する核酸が、順方向でかまたは逆方向に挿入されている。本発明のORFの1つを含むベクターの場合、そのベクターはさらに、調節配列を含み得、この調節配列としては、例えば、そのORFに作動可能に連結されたプロモーターが挙げられる。多数の適切なベクターおよびプロモーターが、当業者に公知であり、そして本発明の組換え構築物を作製するために商業的に入手可能である。下記のベクターは例として挙げる。細菌ベクター：pBs、phagescript、PsiX174、pBluescript SK、pBs KS、pNH8a、pNH16a、pNH18a、pNH46a（Stratagene）；pTrc99A、pKK223-3、pKK233-3、pDR540、pRIT5（Pharmacia）。真核生物ベクター：pWLneo、pSV2cat、pOG44、PXTI、pSG（Stratagene）、pSVK3、pBPV、pMSG、pSVL（Pharmacia）。

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【0093】

本発明の単離されたポリヌクレオチドは、そのタンパク質を組換え生成するために、Kauffmanら、Nucleic Acids Res. 19、4485～4490（1991）において開示された発現制御配列（pMT2発現ベクターまたはpED発現ベクター）に作動可能に連結され得る。多数の適切な発現制御配列が当該分野で公知である。組換えタンパク質を発現する一般的方法もまた公知であり、そしてR. Kaufman、Methods in Enzymology 185、537～566（1990）に例証されている。本明細書中にて定義される場合、「作動可能に連結される」とは、本発明の単離されたポリヌクレオチドおよび発現制御配列が、連結されたポリヌクレオチド／発現制御配列で形質転換（トランスフェクト）された宿主細胞によってそのタンパク質が発現されるように、ベクターまたは細胞の中に配列されることを意味する。

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【0094】

プロモーター領域は、CAT（クロラムフェニコールフェニコールトランスフェラーゼ）ベクター、または選択マーカーを含む他のベクターを使って、所望の任意の遺伝子から選択され得る。2個の適切なベクターは、pKK232-8およびpCM7である。特定の名前がついた細菌プロモーターとしてはlacI、lacZ、T3、T7、gpt、λPR、およびtrcが挙げられる。真核生物プロモーターとしては、以下が挙げられる：CMV極初期プロモーター、HSVチミジンキナーゼプロモーター、SV40初期プロモーターおよびSV40後期プロモーター、レトロウイルスからのLTRプロモーター、およびマウスメタロチオネイン-Iプロモーター。適切なベクターおよびプロモーターの選択は、十分に当業者のレベル内である。一般に、組換え発現ベクターは、複製起点、および宿主細胞の形質転換を可能にする選択マーカー（例えば、E. coliのアンピシリン耐性遺伝子およびS. cerevisiae TRP1遺伝子）、ならびに下流の構造配

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列の転写を指向するための、高度に発現される遺伝子由来のプロモーターを含む。このようなプロモーターは、とりわけ、解糖酵素（例えば、3-ホスホグリセリン酸キナーゼ（PGK））、 α -因子、酸ホスファターゼ、または熱ショックタンパク質をコードするオペロンに由来し得る。異種構造配列は、翻訳開始配列および翻訳終結配列と、そして好ましくは、翻訳されたタンパク質のペリプラズム空間もしくは細胞外媒質への分泌を指向し得るリーダー配列と、適切な位相でアセンブルされる。必要に応じて、異種配列が、所望の特徴（例えば、発現された組換え産物の安定化またはその精製の単純化）を付与するアミノ末端同定ペプチドを含む、融合タンパク質をコードし得る。細菌での使用に有用な発現ベクターは、機能的なプロモーターと作動可能な読み取り相で、適切な翻訳開始シグナルおよび翻訳終結シグナルとともに所望のタンパク質をコードする、構造DNA配列を挿入することによって構築される。このベクターは、宿主中でのそのベクターの維持を確実にし、所望する場合はそのベクターの増幅を確実にするために、1個以上の表現型選択マーカーおよび複製起点を含む。形質転換に適した原核生物宿主としては、*E. coli*、*Bacillus subtilis*、*Salmonella typhimurium*、および*Pseudomonas*属、*Streptomyces*属、および*Staphylococcus*属内の種々の種が挙げられるが、他のものもまた、選択肢として使用され得る。

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【0095】

代表的だがそれに限らない例として、細菌での使用に有用な発現ベクターは、選択マーカー、および周知のクローニングベクターpBR322（ATCC 37017）の遺伝子エレメントを含む市販のプラスミド由来の細菌複製起点を含み得る。そのような商業的ベクターとしては、例えば、pKK223-3（Pharmacia Fine Chemicals、Uppsala、Sweden）およびGEM 1（Promega Biotech、Madison、WI、USA）が挙げられる。これらのpBR322「骨格」部分は、適切なプロモーターおよび発現されるべき構造配列と結合される。適切な宿主菌株の形質転換ならびに適切な細胞密度までのその宿主株の増殖の後、選択されたプロモーターが、適切な手段（例えば、温度変更または化学的誘導）により誘導または抑制解除され、そして細胞はさらなる期間培養される。細胞は、代表的には、遠心分離により収集され、物理的手段または化学的手段により破壊され、そして生じた粗抽出物が、さらなる精製のために保持される。

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【0096】

本発明のポリヌクレオチドはまた、免疫応答を誘導するために使用され得る。例えば、Fandra、Nat. Biotech. 17: 870~872 (1999)（本明細書中に参考として援用される）に記述されるように、ポリペプチドをコードする核酸配列は、裸のプラスミドDNAの局所投与後またはそのDNAの注射（好ましくは、筋肉内注射）後に、コードされるポリペプチドに対する抗体を生成するために使用され得る。その核酸配列は、好ましくは、組換え発現ベクター中に挿入され、そして裸のDNAの形態であってもよい。

【0097】

（5.3 アンチセンス）

本発明の別の局面は、配列番号1~3、10~13および15のヌクレオチド配列、又はそのフラグメント、アナログ又は誘導体を含む核酸分子にハイブリダイズし得るか又は相補的な、単離されたアンチセンス核酸分子に関連する。「アンチセンス」核酸は、タンパク質をコードする「センス」核酸に相補的なヌクレオチド配列を含む：例えば、二本鎖のcDNA分子のコード鎖に相補的な、又はmRNAの配列に相補的である。特定の局面では、少なくとも約10、25、50、100、250もしくは500ヌクレオチドを、又はコード鎖全体、あるいはその一部のみに相補的な配列を含むアンチセンス核酸分子が提供される。配列番号4~9、14および16~18のいずれかのタンパク質のフラグメント、ホモログ、誘導体、およびアナログをコードする核酸分子、又は、配列番号1~3、10~13および15の核酸配列に相補的なアンチセンス核酸が更に提供される。

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【0098】

1つの実施形態では、アンチセンス核酸分子が、本発明のヌクレオチド配列のコード鎖の「コード領域」に対してアンチセンスである。「コード領域」という用語は、アミノ酸残基に翻訳されるコドンを含むヌクレオチド配列の領域をいう。別の実施形態では、アンチセンス核酸分子は、本発明のヌクレオチド配列のコード鎖の「非コード領域」に対してアンチセンスである。「非コード領域」という用語は、アミノ酸に翻訳されないコード領域（即ち、5'および3'非翻訳領域ともいう）に隣接した5'および3'の配列をいう。

【0099】

本明細書中に開示された核酸をコードする、コード鎖の配列（例えば、配列番号1～3、10～13および15）を考慮して、本発明のアンチセンス核酸は、ワトソンおよびクリック、又はフーグスティーンの塩基対形成の規則に従って設計され得る。アンチセンス核酸分子は、mRNAのコード領域全体に対して相補的であり得るが、より好ましくは、mRNAのコード領域又は非コード領域の一部分のみに対してアンチセンスであるオリゴヌクレオチドである。例えば、アンチセンスオリゴヌクレオチドはmRNAの翻訳開始部位周辺の領域に対して相補的であり得る。アンチセンスオリゴヌクレオチドは、例えば、約5、10、15、20、25、30、35、40、45又は50個のヌクレオチドの長さであり得る。本発明のアンチセンス核酸は、化学合成又は当該分野で公知の手順を使って酵素学的連結反応を使用して構築され得る。例えば、アンチセンス核酸（例えば、アンチセンスオリゴヌクレオチド）は、天然に存在するヌクレオチド、又は、分子の生物学的安定性を増加させるように、もしくは、アンチセンス核酸とセンス核酸との間に形成される二重鎖の物理的安定性を増加させるように設計された種々の改変ヌクレオチドを使って化学合成され得る。例えば、ホスホロチオエート誘導体およびアクリジン置換ヌクレオチドが使用され得る。

【0100】

アンチセンス核酸を生成する為に使用され得る改変ヌクレオチドの例には、下記のものが挙げられる：5-フルオロウラシル、5-ブロモウラシル、5-クロロウラシル、5-ヨードウラシル、ヒポキサンチン、キサンチン、4-アセチルシトシン、5-（カルボキシヒドロキシメチル）ウラシル、5-カルボキシメチルアミノメチル-2-チオウリジン、5-カルボキシメチルアミノメチルウラシル、ジヒドロウラシル、 β -D-ガラクトシルキューオシン（galactosylqueosine）、イノシン、N6-イソペンテニルアデニン、1-メチルグアニン、1-メチルイノシン、2,2-ジメチルグアニン、2-メチルアデニン、2-メチルグアニン、3-メチルシトシン、5-メチルシトシン、N6-アデニン、7-メチルグアニン、5-メチルアミノメチルウラシル、5-メトキシアミノメチル-2-チオウラシル、 β -D-マンノシルキューオシン、5'-メトキシカルボキシメチルウラシル、5-メトキシウラシル、2-メチルチオ-N6-イソペンテニルアデニン、ウラシル-5-オキシ酢酸（v）、ワイブトキソシン（wybutoxosine）、プソイドウラシル、キューオシン、2-チオシトシン、5-メチル-2-チオウラシル、2-チオウラシル、4-チオウラシル、5-メチルウラシル、ウラシル-5-オキシ酢酸メチルエステル、ウラシル-5-オキシ酢酸（v）、5-メチル-2-チオウラシル、3-（3-アミノ-3-N-2-カルボキシプロピル）ウラシル、（acp3）w、および2,6-ジアミノプリン。あるいは、アンチセンス核酸は、核酸がアンチセンス方向でサブクロニングされた発現ベクターを使って生物学的に生成され得る（即ち、挿入された核酸から転写されたRNAは目的の標的核酸に対してアンチセンス方向である。以下のサブセクションでさらに記載する）。

【0101】

本発明のアンチセンス核酸分子は、代表的に、被験体に投与されるか、またはそれらが本発明のタンパク質をコードする細胞のmRNAおよび／またはゲノムDNAとハイブリダイズ又は結合し、それによってタンパク質の発現を阻害する（例えば、転写および／または翻訳を阻害することにより）ようにインサイチュで生成される。そのハイブリダイゼーションは、安定した二重鎖を形成するための従来のヌクレオチドの相補性、又は、例えば

、DNA二重鎖に結合するアンチセンス核酸分子の場合、2重ヘリックスの大きな溝 (groove) 内での特異的相互作用により得る。本発明によるアンチセンス核酸分子の投与経路の一例は、組織部位へ直接注射することを含む。あるいは、アンチセンス核酸分子を、選択された細胞を標的化するように改変し得、次いで、全身的に投与され得る。例えば、全身的に投与する場合、アンチセンス分子を、選択された細胞の表面に発現されたレセプターまたは抗原に特異的に結合するように改変し得る (例えば、アンチセンス核酸分子を、細胞表面のレセプターまたは抗原に結合するペプチドまたは抗体に連結することによる)。アンチセンス核酸分子はまた、本明細書中に述べたベクターを使って細胞に送達される。アンチセンス分子の十分な細胞内濃度を達成する為に、アンチセンス核酸分子が強力な pol II 又は pol III プロモーターの制御下に置かれたベクター構築物が望ましい。

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【0102】

さらに別の実施形態では、本発明のアンチセンス核酸分子は、 α -アノマー核酸分子である。 α -アノマー核酸分子は、相補的なRNAと特異的な二本鎖ハイブリッドを形成し、ここでは、通常単位とは対照的に、鎖が互いに平行に走る (ゴーチエー (Gaultier) ほか (1987) *Nucleic Acids Res* 15: 6625~6641)。また、アンチセンス核酸分子は2'-O-メチルリボヌクレオチド (井上 (Inoue) ほか (1987) *Nucleic Acids Res* 15: 6131~6148)、又はキメラRNA-DNAアナログ (井上 (Inoue) ほか (1987) *FEBS Lett* 215: 327~330) を含む得る。

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(5.4 リボザイムおよびPNAの部分)

更に別の実施形態では、本発明のアンチセンス核酸がリボザイムである。リボザイムはリボヌクレアーゼ活性を有する触媒的なRNA分子であり、これは、それらが相補的領域を有するmRNAのような1本鎖の核酸を切断し得る。従って、リボザイム (例えば、ハマーヘッド型リボザイム; (ハーゼルホフおよびゲアラック (Hasek h o f f and Gerlach) 著 (1988) *Nature* 334: 585~591に記述) は、mRNAの転写物を触媒的に切断して、それによりmRNAの翻訳を阻害するために使用され得る。本発明の核酸に対する特異性を有するリボザイムは、本明細書中に開示したDNAのヌクレオチド配列に基づいて設計され得る (即ち、配列番号1~3、10~13および15)。例えば、テトラヒメナL-19 IVS RNAの誘導体を構築し得る。ここでは、活性部位のヌクレオチド配列が、本発明に従うmRNAのコード領域中の切断されるべきヌクレオチド配列に相補的である。例えば、チェック (Cech) ほか、米国特許第4, 987, 071号、及びチェック (Cech) ほか米国特許第5, 116, 742号を参照のこと。あるいは、mRNAを使って、RNA分子のプールから特定のリボヌクレアーゼ活性を有する触媒的なRNAを選択し得る。例えば、バーテル (Bartel) ほか (1993) *Science* 261: 1411~1418を参照のこと。

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【0103】

あるいは、調節領域 (例えば、プロモーターおよび/またはエンハンサー) に相補的なヌクレオチド配列を標的化して、標的細胞内の遺伝子の転写を防げる三重ヘリックス構造を形成することにより、遺伝子の発現が阻害され得る。一般的には、ヘレン (Helene) (1991) *Anticancer Drug Des.* 6: 569~84; ヘレン (Helene) ほか (1992) *Ann. N. Y. Acad. Sci.* 660: 27~36; 及びメーハー (Maher) (1992) *Bioassays* 14: 807~15を参照のこと。

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【0104】

種々の実施形態では、本発明の核酸は、例えば分子の安定性、ハイブリダイゼーション、又は可溶性を改善する為に、塩基部分、糖部分、又はリン酸骨格において改変され得る。例えば、核酸のデオキシリボースリン酸骨格は、ペプチド核酸を生成するように改変され得る (ハイラップ (Hyrup) ほか (1996) *Bioorg Med Chem* 4: 5~23)。本明細書中で使われる場合、「ペプチド核酸」又は「PNA」の用語は、

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核酸模擬体（例えば、DNA模擬体）をいう。ここでは、デオキシリボースリン酸骨格は、偽性ペプチド骨格に置換され、4個の天然の核酸塩基のみが保持される。PNAの中性骨格は、低イオン強度の条件下で、DNAとRNAとを特異的にハイブリダイズさせることが示された。PNAオリゴマーの合成は、標準的な固相ペプチド合成プロトコールを使って実施され得る。このプロトコールはハイラップ（Hyrup）ほか著の上記書（1996）；ペリーーオキーフ（Perry-O'Keefe）ほか著（1996） PNAS 93: 14670～675に説明されてある。

【0105】

本発明のPNAは、治療的適用および診断的適用に使用され得る。例えば、PNAは、配列特異的な遺伝子発現の調節のためのアンチセンスまたは抗遺伝子（antigene）薬剤として使用され得、これは例えば、転写もしくは翻訳停止を誘導することか、又は複製を阻害することによる。本発明のPNAはまた、例えば、PNA指向性PCRクランピング（clamping）による遺伝子における一塩基対変異の分析において；他の酵素（例えば、S1ヌクレアーゼ（ハイラップ ビー.（Hyrup B.）（1996）上記））と組合わせて使う場合の人工的制限酵素として；又はDNA配列およびハイブリダイゼーションのためのプローブもしくはプライマーとして（ハイラップ（Hyrup）ほか（1996）、前出；ペリーーオキーフ（Perry-O'Keefe）（1996）、前出）使用され得る。

【0106】

別の実施形態では、本発明のPNAを改変して、例えば、親油脂性基または他のヘルパー基をPNAに付加することによるか、PNA-DNAキメラの形成によるか、又はリボソームもしくは当該分野で公知の他の薬物送達技術を使用することによって、その安定性または細胞性取り込みを増強し得る。例えば、PNAおよびDNAの有利な特性を組み合わせ得るPNA-DNAキメラを生成し得る。その様なキメラは、DNA認識酵素、例えば、RNase HおよびDNAポリメラーゼをDNA部分と相互作用させ、同時にPNA部分は高度の結合親和性および特異性を提供する。PNA-DNAキメラを、塩基スタッキング、核酸塩基間の結合数、及び方向に関して選択された適切な長さのリンカーを使って連結し得る（ハイラップ（Hyrup）（1996）前出）。PNA-DNAキメラの合成は、ハイラップ（Hyrup）（1996）前出およびフィン（Finn）ほか（1996）Nucleic Acids Res 24: 3357～63に説明されたように実行され得る。例えば、DNA鎖は、標準的なホスホルアミダイト結合化学を使用して、固体支持体上で合成され得、そして改変されたヌクレオシドアナログ（例えば、5'-（4-メトキシトリチル）アミノ-5'-デオキシチミジンホスホルアミダイト）を、PNAと、DNAの5'末端との間で使用し得る（マグ（Mag）ほか（1989）Nucleic Acid Res 17: 5973～88）。次いで、PNAモノマーが段階様式で連結され、5' PNAセグメントおよび3' DNAセグメントを有するキメラ分子を生成する（フィン（Finn）ほか（1996）前出）。あるいは、キメラ分子は5' DNAセグメントおよび3' PNAセグメントで合成され得る。ピーターセン（Pettersen）ほか（1975）Bioorg Med Chem Lett 5: 1119～11124を参照のこと。

【0107】

他の実施形態では、オリゴヌクレオチドには、ペプチドのような他の付属基（例えば、インビボで宿主細胞レセプターを標的化するため）又は細胞膜（例えば、レツィンガー（Lettinger）ほか、1989、Proc. Natl. Acad. Sci. U. S. A. 86: 6553～6556；ルメートル（Lemaître）ほか、1987、Proc. Natl. Acad. Sci. 84: 648～652；PCT公開番号WO88/09810）、もしくは血液脳関門（例えば、PCT公開番号WO89/10134を参照のこと）を横切る輸送を容易にする薬剤を含み得る。更に、オリゴヌクレオチドは、ハイブリダイゼーション誘発切断薬剤（例えば、クロール（Kroll）ほか、1988、BioTechniques 6: 958～976を参照のこと）又はインターカレート剤

で（例えば、ゾン（Zon）、1988、Pharm. Res. 5: 539～549を参照のこと）改変され得る。この目的ために、オリゴヌクレオチドを別の分子、例えば、ペプチド、ハイブリダイゼーション誘発性架橋剤、輸送薬剤、ハイブリダイゼーション誘発性切断薬剤などに結合体化され得る。

【0108】

（5.5 宿主）

本発明は更に、本発明のポリヌクレオチドを含むように遺伝子操作された宿主細胞を提供する。例えば、そのような宿主細胞は、公知の形質転換、トランスフェクション又は感染方法を使ってその宿主細胞に導入された本発明の核酸を含み得る。本発明はなお更に、本発明のポリヌクレオチドを発現するように遺伝子操作された宿主細胞を提供し、その中では、このようなポリヌクレオチドは、細胞内でのポリヌクレオチドの発現を駆動する宿主細胞に対して異種の調節配列と作動可能に連結される。

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【0109】

プロトロンビナーゼ様DNA配列の知識は、プロトロンビナーゼ様ポリペプチドの発現を許容するか、又は増加させるように細胞を改変することを可能にする。細胞は、改変（例えば、相同組換えによって）されて、天然に存在するプロトロンビナーゼ様プロモーターの全部かまたは一部を、異種のプロモーターの全部かまたは一部で置換することにより、プロトロンビナーゼ様ポリペプチドの発現を増加を提供し得、その結果、細胞のプロトロンビナーゼ様ポリペプチドがより高いレベルで発現される。この異種プロモーターは、プロトロンビナーゼ様コード配列に作動的に連結される様な様式で挿入される。例えば、PCT国際公開番号WO94/12650、PCT国際公開番号WO92/20808、及びPCT国際公開番号WO91/09955を参照のこと。異種プロモーターDNAに加えて、増幅可能なマーカーDNA（例えば、ada、dhfr、及び多機能を有する、カルバミルリン酸シンターゼ、アスパルテートトランスカルバミラーゼ及びジヒドロオロターゼをコードするCAD遺伝子）および／またはイントロンDNAが異種プロモーターDNAと共に挿入され得ることもまた意図する。プロトロンビナーゼ様コード配列に連結される場合には、標準的な選択方法によるマーカーDNAの増幅が、細胞内でのプロトロンビナーゼ様コード配列の同時増幅を引き起こす。

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【0110】

宿主細胞は、哺乳動物細胞のような高等真核生物宿主細胞でも、酵母細胞のような下等真核生物宿主細胞でもよく、又は宿主細胞は、細菌細胞のような原核生物細胞でも良い。組換え構築物を宿主細胞へ導入することは、リン酸カルシウムトランスフェクション、DEAE、デキストランに媒介性トランスフェクション、又はエレクトロポレーションによってもたらされ得る。（デイビス、エル。（Davis, L.）ほか著、Basic Methods in Molecular Biology（1986））。本発明の1つのポリヌクレオチドを含む宿主細胞は、従来の方法で使用されて、単離されたフラグメントによってコードされる遺伝子産物を生成し得る（ORFの場合）か又はEMFの制御下で異種タンパク質を生成する為に使用され得る。

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【0111】

任意の宿主／ベクター系を使って、本発明の1以上のORFを発現させ得る。これらには、以下が挙げられるが、これらに限定されない：真核生物宿主、例えば：HeLa細胞、Cv-1細胞、COS細胞、293細胞、およびSf9細胞、ならびに原核生物宿主、例えば：E. coliおよびB. subtilis。最も好ましい細胞は、通常、特定のポリペプチドもしくはタンパク質を通常発現しない細胞、又はポリペプチドもしくはタンパク質を天然の低いレベルで発現する細胞である。成熟タンパク質は、適切なプロモーターの制御下において、哺乳動物細胞、酵母、細菌、又は他の細胞内で発現され得る。無細胞翻訳系もまた使用して、本発明のDNA構築物に由来するRNAを使ってそのようなタンパク質を生成し得る。原核生物および真核生物の宿主に使用するために適切なクローニングベクターおよび発現ベクターは、サムブルック（Sambrook）ほか著、Molecular Cloning: A Laboratory Manual、第2版、Co

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ld Spring Harbor, New York (1989) に記述されており、その開示は本明細書中で参考として援用される。

【0112】

種々の哺乳動物細胞の培養系もまた使用して、組換えタンパク質を発現し得る。哺乳動物の発現系の例は、グルズマン (Gluzman) 著、Cell、23:175 (1981) に記述された、サル腎臓線維芽細胞のCOS-7株を含む。適合可能なベクターを発現し得る他の細胞株は、例えば、C127、サルCOS細胞、チャイニーズハムスターの卵巣 (CHO) 細胞、ヒト腎臓293細胞、ヒト表皮A431細胞、ヒトColo205細胞、3T3細胞、CV-1細胞、他の形質転換された霊長類の細胞株、正常な二倍体細胞、一次組織のインビトロ培養物、一次外植片、HeLa細胞、マウスL細胞、BHK、H10
L-60、U937、HaK又はJurkat細胞である。哺乳動物の発現ベクターは、複製起点、適切なプロモーター及びまた任意の必要なリボソーム結合部位、ポリアデニル化部位、スプライスドナーおよびアクセプターの部位、転写終結配列、及び5'隣接非転写配列を含む。SV40のウイルスゲノム、例えば、SV40起点、初期プロモーター、エンハンサー、スプライス、及びポリアデニル化部位に由来するDNA配列を使って、必要な非転写遺伝子エレメントを提供し得る。細菌培養で生成された組換えポリペプチドおよびタンパク質は、通常、細胞ペレットからの最初の抽出、次に、1回以上の塩析、水性イオン交換クロマトグラフィー、又はサイズ排除クロマトグラフィー工程によって単離される。必要に応じて、成熟タンパク質の立体配置の完了においてタンパク質再折りたたみ工程が使用され得る。最後に、高速液体クロマトグラフィー (HPLC) を、最終精製20
手順のために使用し得る。タンパク質の発現に使用する微生物細胞は、凍結-融解サイクル、音波破砕、機械的破壊、又は細胞溶解剤の使用を含む任意の簡便な方法で破壊され得る。

【0113】

あるいは、タンパク質を酵母または昆虫のような下等真核生物中で、又は細菌のような原核生物中で生成することが可能であり得る。潜在的に適切な酵母株には、Saccharomyces cerevisiae、Schizosaccharomyces pombe、Kluyveromyces株、Candida、又は異種タンパク質を発現し得る任意の酵母株が挙げられる。潜在的に適切な細菌株には、Escherichia coli、Bacillus subtilis、Salmonella typhirium、又は異種タンパク質を発現し得る任意の細菌株を含む。タンパク質が酵母または細菌で作製される場合、機能的なタンパク質を得る為に、例えば、適切な部位のリン酸化、又はグリコシル化により、そこで生成されたタンパク質を改変する必要がある。そのような共有結合的付着は、公知の化学的又は酵素的な方法を使用して達成され得る。30

【0114】

本発明の別の実施形態では、細胞および組織は、誘導可能な調節エレメントの制御下で、本発明のポリヌクレオチドを含む内因性遺伝子を発現するように操作され得、その場合、内因性遺伝子の調節配列は相同組換えで置き換えられ得る。本明細書中で説明されたように、遺伝子標的化を使って、遺伝子の既存の調節領域を、異なる遺伝子から単離された調節配列、又は遺伝子工学方法で合成された新規な調節配列で置き換え得る。そのような調節配列は、プロモーター、エンハンサー、足場結合領域、負の調節エレメント、転写開始部位、調節タンパク質結合部位、又はそれらの配列の組合せで構成され得る。あるいは、生成されたRNA又はタンパク質の構造又は安定性に影響する配列は、置換され得るか、除去され得るか、付加され得るか、さもなくば標的化により改変され得る。これらの配列には、ポリアデニル化シグナル、mRNA安定性エレメント、スプライス部位、タンパク質の輸送又は分泌特性を増強または改変するためのリーダー配列、あるいは、タンパク質もしくはRNA分子の機能又は安定性を変更又は改善する他の配列を含む。40

【0115】

標的化事象は、単純に調節配列を挿入すること、遺伝子を新しい調節配列の制御下に置くこと、例えば、新しいプロモーターもしくはエンハンサー又は両方を遺伝子上流に挿入50

することであり得る。あるいは、標的化事象は、単純に調節エレメントを欠失させることで、例えば、組織特異的な負の調節エレメントを欠失させることであり得る。あるいは、標的化事象は、既存のエレメントを置き換え得る；例えば、組織特異的エンハンサーは、天然に存在するエレメントよりも広いが、又は天然に存在するエレメントとは異なった細胞型特異性を有するエンハンサーで置き換えられ得る。ここでは、天然に存在する配列は欠失され、そして新しい配列が付加される。いずれの場合でも、標的化事象の同定は、標的化DNAと連続した1個以上の選択可能なマーカー遺伝子を使って容易にされ得、外因性DNAが宿主細胞ゲノムに組み込まれた細胞の選択を可能にする。標的化事象の同定はまた、負の選択可能マーカーを外因性DNAに連結するように、陰性選択の特性を示す1以上のマーカー遺伝子を使用することによって容易にされ得るが、この負の選択可能マーカーが標的配列に隣接し、かつ宿主細胞ゲノム内における配列との正確な相同組換え事象が、この負の選択可能マーカーの安定な組み込みを引き起こさないように構成され得る。この目的のために有用なマーカーには、単純ヘルペスウイルスチミジンキナーゼ（TK）遺伝子、又は細菌性キサンチン-グアニンホスホリボシルトランスフェラーゼ（gpt）遺伝子が挙げられる。

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【0116】

本発明のこの局面に従って使用され得る遺伝子標的化、又は遺伝子活性化技術は、更に詳しく下記に説明されている：米国特許第5,272,071号、チャペル（Chappel）；米国特許第5,578,461号、シャーウィン（Sherwin）他；国際出願番号PCT/US92/09627（WO93/09222）セルデン（Selden）ほか；及び国際出願番号PCT/US90/06436（WO91/06667）スクールチ（Skoultschi）ほか。これらの各々の内容全体が本明細書中で参考として援用される。

【0117】

（5.6 本発明のポリペプチド）

本発明の単離されたポリペプチドとしては、下記を含むポリペプチドが挙げられるが、これらに限定されない：配列番号4～9、14もしくは16～18のいずれか1つとして示されるアミノ酸配列、又は配列番号1～3、10～13、もしくは15のヌクレオチド配列のいずれか1つによってコードされたアミノ酸配列、あるいは対応する全長タンパク質又は成熟タンパク質。本発明のポリペプチドはまた、好ましくは、以下によりコードされた生物学的活性又は免疫学的な活性のあるポリペプチドを含む：（a）配列番号1～3、10～13、もしくは15に示されるヌクレオチド配列のいずれか一つを有するポリヌクレオチド、又は（b）配列番号4～9、14又は16～18に示されるアミノ酸配列のいずれか1つをコードするポリヌクレオチド、又は（c）ストリンジェントなハイブリダイゼーション条件下で（a）もしくは（b）のポリヌクレオチドの相補体にハイブリダイズするポリヌクレオチド。本発明はまた、配列番号4～9、14もしくは16～18として示されるアミノ酸配列のいずれかの生物学的もしくは免疫学的に活性な改変体、又は対応する全長タンパク質もしくは成熟タンパク質；および生物学的活性を保持するそれらの「実質的に等価なもの」（例えば、少なくとも約65%、少なくとも約70%、少なくとも約75%、少なくとも約80%、少なくとも約85%、少なくとも約90%、典型的には少なくとも約95%、更に典型的には少なくとも約98%、又は最も典型的には少なくとも約99%のアミノ酸同一性を有する）を提供する。対立遺伝子改変体によりコードされたポリペプチドは、配列番号4～9、14又は16～18を含むポリペプチドと比較して、類似した活性か、より高い活性か、又はより低い活性を有し得る。

【0118】

生物学的活性を示し得る本発明のタンパク質のフラグメントもまた、本発明によって包含される。タンパク質のフラグメントは、直鎖形態であり得るか又は、公知の方法を使って環化され得る。例えば、これらの方法としては、エイチ・ユー・サラゴヴィ（H. U. Saragovi）ほか著、Bio/Technology 10、773-778（1992）およびアール・エス・マクドウェル（R. S. McDowell）ほか著、J. A

mer. Chem. Soc. 114、9245-9253 (1992)、に記述されており、この両方が本明細書中で参考として援用される。そのようなフラグメントは、タンパク質の結合部位の価数の増加を含む多くの目的のために免疫グロブリンのようなキャリア分子に融合され得る。

【0119】

本発明はまた、開示されたタンパク質の全長形態および成熟形態（例えば、シグナル配列も前駆配列も伴わない）の両方を提供する。このタンパク質コード配列は、開示されたヌクレオチド配列の翻訳によって、配列表において同定される。そのようなタンパク質の成熟形態は、適切な哺乳動物細胞、又は他の宿主細胞における全長のポリヌクレオチドの発現により獲得され得る。成熟形態のタンパク質の配列はまた、全長形態のアミノ酸配列から決定可能である。本発明のタンパク質が膜に結合している場合、可溶性形態のタンパク質もまた提供される。そのような形態では、タンパク質を膜に結合させる領域の一部又は全部を欠失させ、それによりタンパク質が、その発現される細胞から完全に分泌される。

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【0120】

本発明のタンパク質組成物は、受容可能なキャリア（例えば、親水性（例えば、薬学的に受容可能な）キャリア）をさらに含み得る。

【0121】

本発明はさらに、本発明の核酸フラグメントまたは本発明の核酸フラグメントの縮重改変体によってコードされる、単離されたポリペプチドを提供する。「縮重改変体」によって、本発明の核酸フラグメント（例えば、ORF）とヌクレオチドフラグメントで異なるが、遺伝コードの縮重性に起因して同一のポリペプチド配列をコードする、ヌクレオチドフラグメントが意図される。本発明の好ましい核酸フラグメントは、タンパク質をコードするORFである。

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【0122】

当該分野で公知の種々の方法を利用して、本発明の任意の単離されたポリペプチドまたはタンパク質を取得し得る。最も単純なレベルでは、このアミノ酸配列は、市販のペプチド合成機を使用して合成され得る。これらの合成的に構築したタンパク質配列は、一次、二次または三次構造上の特性および／あるいはコンフォメーション特性をタンパク質と共有するので、タンパク質と共通する生物学的特性（タンパク質活性を含む）を保持し得る。この技術は、大きいポリペプチドの小さいペプチドおよびフラグメントの生成に特に有用である。フラグメントは、例えば、ネイティブポリペプチドに対する抗体の生成において有用である。従って、これらは、治療化合物のスクリーニングおよび抗体開発のための免疫学的プロセスにおける、天然の精製されたタンパク質に対する生物学的に活性な代替物または免疫学的代替物として使用され得る。

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【0123】

あるいは、本発明のポリペプチドおよびタンパク質は、所望のポリペプチドまたはタンパク質を発現するように変更された細胞から精製され得る。本明細書中で使用される場合、細胞に、遺伝子操作を介して、その細胞が通常には産生しないかまたはその細胞が通常にはより低いレベルで産生するポリペプチドまたはタンパク質を産生させる場合に、この細胞は、所望のポリペプチドまたはタンパク質を発現するように変更されていると言われる。当業者は、真核生物細胞または原核生物細胞内へ組換え配列または合成配列のいずれかを導入および発現させるための手順を容易に適応し、本発明のポリペプチドまたはタンパク質のうちの1つを産生する細胞を産生し得る。

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【0124】

本発明はまた、ポリペプチドを産生するための方法に関し、この方法は、本発明の宿主細胞の培養物を適切な培養培地中で増殖させる工程、およびその細胞またはその細胞を増殖させる培養物からタンパク質を精製する工程を包含する。例えば、本発明の方法は、ポリペプチドを産生するためのプロセスを包含し、ここで、本発明のポリヌクレオチドを含む適切な発現ベクターを含む宿主細胞を、そのコードされるポリペプチドの発現を可能にする条件下で培養する。このポリペプチドは、培養物（都合よくは、培養培地）からか、ま

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たは宿主細胞から調製しそしてさらに精製した溶解物から、回収され得る。好ましい実施形態としては、このようなプロセスによって産生されたタンパク質が、そのタンパク質の全長形態または成熟形態である実施形態が挙げられる。

【0125】

代替的方法において、このポリペプチドまたはタンパク質は、このポリペプチドまたはタンパク質を天然で産生する細菌細胞から精製される。当業者は、ポリペプチドおよびタンパク質を単離するための公知の方法に容易に従って、本発明の単離されたポリペプチドまたはタンパク質を取得し得る。これらには、免疫クロマトグラフィー、HPLC、サイズ排除クロマトグラフィー、イオン交換クロマトグラフィーおよび免疫親和性クロマトグラフィーが挙げられるが、これらに限定されない。例えば、Scopes, Protein Purification: Principles and Practice, Springer-Verlag (1994); Sambrookら、Molecular Cloning: A Laboratory Manual; Ausubelら、Current Protocols in Molecular Biologyを参照のこと。生物学的／免疫学的活性を保持するポリペプチドフラグメントとしては、約100アミノ酸以上または約200アミノ酸以上を含むフラグメント、および特定のタンパク質ドメインをコードするフラグメントが挙げられる。

【0126】

精製されたポリペプチドを、当該分野で周知のインビトロ結合アッセイにおいて使用し、そのポリペプチドに結合する分子を同定し得る。これらの分子としては、例えば、小分子、コンビナトリアルライブラリー由来の分子、抗体または他のタンパク質が挙げられるが、これらに限定されない。次いで、この結合アッセイにおいて同定された分子を、当該分野で周知のインビボ組織培養物または動物モデルにおいて、アンタゴニスト活性またはアゴニスト活性について試験する。手短には、これらの分子を、複数の細胞培養物または動物において力価測定 (t i t r a t e) し、次いで、細胞／動物の死またはその細胞／動物の延長された生存性のいずれかについて試験する。

【0127】

さらに、本発明のペプチドまたはこれらのペプチドに結合し得る分子は、毒素（例えば、リシンまたはコレラ）と複合体化され得るかまたは細胞に対して毒性である他の化合物と複合体化され得る。次いで、この毒素－結合分子複合体を、配列番号4～9、14または16～18に対する結合分子の特異性によって、腫瘍または他の細胞に標的化する。

【0128】

本発明のタンパク質はまた、トランスジェニック動物の産物（例えば、トランスジェニックウシ、ヤギ、ブタまたはヒツジの乳汁の成分）として発現され得、これらのトランスジェニック動物は、このタンパク質をコードするヌクレオチド配列を含む体細胞または生殖細胞によって特徴付けられる。

【0129】

本明細書中に提供されるタンパク質にはまた、精製されたタンパク質のアミノ酸配列に類似するアミノ酸配列によって特徴付けられるが、その中に改変が天然において提供されるかまたは人為的に操作されているタンパク質が含まれる。例えば、改変（ペプチドまたはDNA配列における）は、公知の技術を使用して当業者に作製され得る。このタンパク質配列における目的の改変としては、そのコード配列の選択されたアミノ酸残基の変更、置換、交換 (r e p l a c e m e n t)、挿入または欠失が含まれ得る。例えば、1以上のシステイン残基が、欠失されるかまたは別のアミノ酸で置換され、この分子のコンフォメーションを変更し得る。変更、置換、交換、挿入または欠失についての技術は、当業者に周知である（例えば、米国特許第4,518,584号を参照のこと）。好ましくは、このような変更、置換、交換、挿入または欠失は、そのタンパク質の所望の活性を保持する。そのタンパク質機能に重要なタンパク質の領域は、当該分野で公知の種々の方法によって決定され得る。これらの方法としては、アラニンスキャニング方法が挙げられ、これは、アラニンでの単一のアミノ酸またはアミノ酸列の系統的置換を含み、その後、その得ら

れたアラニン含有改変体を生物学的活性について試験する。この型の分析は、生物学的活性におけるその置換されたアミノ酸の重要性を決定する。タンパク質機能に重要なタンパク質の領域は、eM A T R I Xプログラムによって決定され得る。

【0130】

全体または部分的にタンパク質活性を保持することが予測され、そしてスクリーニング方法または他の免疫学的方法に有用な、タンパク質配列の他のフラグメントおよび誘導体もまた、本明細書中の開示を考慮して当業者によって容易に作製され得る。このような改変は、本発明に含まれる。

【0131】

このタンパク質は、本発明の単離されたポリヌクレオチドを1以上の昆虫発現ベクター内の適切な制御配列に作動可能に連結し、そして昆虫発現系を使用することによって産生され得る。バキュロウイルス／昆虫細胞発現系についての材料および方法は、例えば、Invitrogen、San Diego、Calif.、U. S. A. (Max Bat^{T M} キット) からキット形態で市販されており、そしてこのような方法は、Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987) (本明細書中に参考として援用される) に記載されるように、当該分野で周知である。本明細書中で使用される場合、本発明のポリヌクレオチドを発現し得る昆虫細胞は、「形質転換」されている。

【0132】

本発明のタンパク質は、形質転換された宿主細胞を、組換えタンパク質を発現するのに適切な培養条件下で培養することによって調製され得る。次いで、その得られた発現タンパク質を、公知の精製プロセス (例えば、ゲル濾過およびイオン交換クロマトグラフィー) を使用して、このような培養物 (すなわち、培養培地または細胞抽出物) から精製し得る。このタンパク質の精製としては、このタンパク質に結合する薬剤を含むアフィニティークラム; コンカナバリンAアガロース、ヘパリントローヨパール^{T M} またはCibacromブルー3GA Sepharose^{T M} のようなアフィニティー樹脂上での1回以上のカラム工程; フェニルエーテル、ブチルエーテルまたはプロピルエーテルのような樹脂を使用する疎水性相互作用クロマトグラフィーを含む1回以上の工程; または免疫親和性クロマトグラフィーが挙げられる。

【0133】

あるいは、本発明のタンパク質は、精製を容易にする形態で発現され得る。例えば、本発明のタンパク質は、融合タンパク質 (例えば、マルトース結合タンパク質 (MBP)、グルタチオン-S-トランスフェラーゼ (GST) またはチオレドキシン (TRX) の融合タンパク質) としてかまたはHisタグとして発現され得る。このような融合タンパク質の発現および精製のためのキットは、それぞれ、New England BioLab (Beverly, Mass.)、Pharmacia (Piscataway, N. J.) およびInvitrogenから市販されている。このタンパク質はまた、エピトープでタグ化され、そしてその後、このようなエピトープに対する特異的抗体を使用して精製され得る。1つのこのようなエピトープ (「FLAG (登録商標)」) は、Kodak (New Haven, Conn.) から市販されている。

【0134】

最後に、1以上の逆相高速液体クロマトグラフィー (RP-HPLC) 工程 (疎水性RP-HPLC媒体 (例えば、ペンダントメチル基または他の脂肪族基を有するシルカゲル) を使用する) が、このタンパク質をさらに精製するために使用され得る。種々の組み合わせにおいて、前述の精製工程のいくつかまたは全てを使用して、実質的に均質な単離された組換えタンパク質を提供する。このように精製されたタンパク質は、実質的に他の哺乳動物タンパク質を含まず、そして本発明に従って「単離されたタンパク質」として定義される。

【0135】

本発明のポリペプチドは、アナログ (改変体) を含む。本発明のポリペプチドは、プロト

ロンビナーゼ様アナログを含む。これは、本発明のプロトロンビナーゼ様ポリペプチドのフラグメント、ならびに欠失、挿入または置換された1以上のアミノ酸を含むプロトロンビナーゼ様ポリペプチドを包含する。本発明のプロトロンビナーゼ様ポリペプチドのアナログは、プロトロンビナーゼ様ポリペプチドの融合体またはプロトロンビナーゼ様ポリペプチドの改変体を含み、ここで、このプロトロンビナーゼ様ポリペプチドまたはアナログは、別の部分（単数または複数）（例えば、標的化部分または別の治療的部分）に融合される。このようなアナログは、改善された特性（例えば、活性および／または安定性）を示し得る。プロトロンビナーゼ様ポリペプチドまたはアナログに融合され得る部分の例としては、例えば、ニューロンへのポリペプチドの送達を提供する標的化部分（例えば、中枢神経系に対する抗体、または神経細胞上に発現されるレセプターおよびリガンドに対する抗体）が挙げられる。プロトロンビナーゼ様に融合され得る他の部分としては、処置に使用される治療的薬剤（例えば、抗うつ剤または神経学的障害用の他の医薬）が挙げられる。また、プロトロンビナーゼ様ポリペプチドは、ニューロン増殖モジュレーター、および標的化された送達のための他のケモカインに融合され得る。

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【0136】

（5. 6. 1 ポリペプチドおよびポリヌクレオチドの同一性および類似性の決定）
好ましい同一性および／または類似性は、試験した配列間の最も大きい一致を与えるように設計される。同一性および類似性を決定するための方法は、以下を含むが、これらに限定されない、コンピュータープログラム中に体系化される：GCGプログラムパッケージ（GAPを含む）（Devereux, J., ら、Nucleic Acids Research 12 (1) : 387 (1984) ; Genetics Computer Group, University of Wisconsin, Madison, WI)、BLASTP、BLASTN、BLASTX、FASTA (Altschul, S. F. ら、J. Molec. Biol. 215 : 403-410 (1990))、PSI-BLAST (Altschul, S. F. ら、Nucleic Acids Res. vol. 25, pp. 3389-3402 (本明細書中に参考として援用される))、eMatrixソフトウェア (Wu ら、J. Comp. Biol., vol. 6, pp. 219-235 (1999) (本明細書中に参考として援用される))、eMotifソフトウェア (Nevill-Manning ら、ISMB-97, vol 4, pp. 202-209 (本明細書中に参考として援用される)) および Kyte-Doolittle 疎水性予測アルゴリズム (J. Mol Biol, 157, pp. 105-31 (1982) (本明細書中に参考として援用される))。BLASTプログラムは、National Center for Biotechnology Information (NCBI) および他の供給源 (BLAST Manual, Altschul, S. ら、NCB NLM NIH Bethesda, MD 20894 ; Altschul, S. ら、J. Mol. Biol. 215 : 403-410 (1990)) から公に入手可能である。

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【0137】

（5. 7 キメラタンパク質および融合タンパク質）
本発明はまた、キメラタンパク質または融合タンパク質を提供する。本明細書中で使用される場合、「キメラタンパク質」または「融合タンパク質」は、別のポリペプチドに作動可能に連結された、本発明のポリペプチドを含む。融合タンパク質内で、本発明のポリペプチドは、本発明のタンパク質の全てまたは一部に対応する。1つの実施形態において、融合タンパク質は、本発明のタンパク質の少なくとも1つの生物学的に活性な部分を含む。別の実施形態において、融合タンパク質は、本発明のタンパク質の少なくとも2つの生物学的に活性な部分を含む。融合タンパク質において、用語「作動可能に連結された」とは、本発明のポリペプチドおよび他のポリペプチドが互いにインフレームで融合されていることを示すことが意図される。このポリペプチドは、N末端またはC末端あるいは中間において融合され得る。

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【0138】

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例えば、1つの実施形態において、融合タンパク質は、第2のタンパク質の細胞外ドメインに作動可能に連結された、本発明のポリペプチドを含む。

【0139】

別の実施形態において、融合タンパク質は、GST融合タンパク質であり、ここで、本発明のポリペプチド配列は、GST（すなわち、グルタチオン S-トランスフェラーゼ）配列のC末端に融合される。

【0140】

別の実施形態において、融合タンパク質は、免疫グロブリン融合タンパク質であり、ここで、本発明のポリペプチド配列は、免疫グロブリンタンパク質ファミリーのメンバー由来の配列に融合される、1以上のドメインを含む。本発明の免疫グロブリン融合タンパク質は、薬学的組成物中に含まれ得、そして被験体に投与され、リガンドと細胞の表面上の本発明のタンパク質との間の相互作用を阻害し、これによって、インビボでのシグナル伝達を抑制し得る。免疫グロブリン融合タンパク質は、同族のリガンドのバイオアベイラビリティに影響を及ぼすために使用され得る。リガンド／タンパク質相互作用の阻害は、増殖障害および分化障害の処置（例えば、癌）ならびに細胞生存の調節の両方について治療的に有用であり得る。さらに、本発明の免疫グロブリン融合タンパク質は、レセプターに結合しそして二量体化し、これによって細胞内シグナルを伝達するために使用され得る。免疫グロブリン融合タンパク質はまた、被験体において抗体を産生するための免疫原として、リガンドを精製するために、そしてリガンドとの本発明のポリペプチドの相互作用を阻害する分子を同定するためのスクリーニングアッセイにおいて、使用され得る。

【0141】

本発明のキメラタンパク質または融合タンパク質は、標準的な組換えDNA技術によって産生され得る。例えば、異なるポリペプチド配列をコードするDNAフラグメントは、従来の技術に従って、例えば、連結のための平滑末端または突出末端、適切な末端を提供するための制限酵素消化、付着末端の適切な充填、望ましくない結合を回避するためのアルカリホスファターゼ処理、および酵素的連結を使用することによって、インフレイムで一緒に連結される。別の実施形態において、この融合遺伝子は、自動化DNA合成機を含む従来の技術によって合成され得る。あるいは、遺伝子フラグメントのPCR増幅を、2つの連続する遺伝子フラグメント間に相補的オーバーハングを生じるアンカープライマーを使用して行われ得、その後、これら2つのフラグメントは、アニールされそして再増幅され、キメラ遺伝子配列を生成し得る（例えば、Ausubelら（編）、Current Protocols in Molecular Biology, John Wiley & Sons, 1992を参照のこと）。さらに、融合部分（例えば、GSTポリペプチド）を既にコードする多くの発現ベクターが、市販されており。本発明のポリペプチドをコードする核酸は、その融合部分が、本発明のタンパク質にインフレイムで連結されるように、クローニングされ得る。

【0142】

（5.8 遺伝子治療）

本発明の遺伝子のポリヌクレオチドにおける変異は、そのコードされるタンパク質の正常な機能の損失を生じる。従って、本発明は、本発明のポリペプチドの正常な活性を回復するため；または本発明のポリペプチドに関連する疾患状態を処置するための、遺伝子治療を提供する。適切な細胞への本発明のポリペプチドをコードする機能的な遺伝子の送達は、ベクター、そしてより詳細にはウイルスベクター（例えば、アデノウイルス、アデノ随伴ウイルスまたはレトロウイルス）の使用によって、エキソビボ、インサイチュまたはインビボでもたらされるか、または物理的なDNA移入法（例えば、リポソームまたは化学的処理）の使用によってエキソビボでもたらされる。例えば、Anderson, Nature, vol. 392に対する補遺、no. 6679, pp. 25-20 (1998)；Verma, Scientific American: 68-84 (1990)；およびMiller, Nature, 357: 455-460 (1992)を参照のこと。本発明のヌクレオチドの任意の1つまたは本発明のポリペプチドをコードする遺伝子の導

入はまた、染色体外基質（一過的発現）または人工染色体（安定な発現）を用いて達成され得る。細胞はまた、このような細胞を増殖するため、あるいはこのような細胞に対する所望の効果またはこのような細胞における所望の活性を生じるために、本発明のタンパク質の存在下で、エキソピボで培養され得る。次いで、処理された細胞を、治療目的のためにインピボで導入し得る。あるいは、他のヒト疾患状態において、本発明のポリペプチドの発現を妨げることかまたは本発明のポリペプチドの活性を阻害することが、これらの疾患状態の処置において有用であることが意図される。アンチセンス治療または遺伝子治療を適用し、本発明のポリペプチドの発現をネガティブに調節し得ることが意図される。

【0143】

タンパク質の発現を阻害する他の方法としては、当該分野で公知の方法による、本発明の核酸、それらの相補体またはそれらの翻訳されるRNAに対するアンチセンス分子の導入が挙げられる。さらに、本発明のポリペプチドは、標的化欠失方法、または組織特異的なネガティブ調節エレメント（例えば、サイレンサー）の挿入によって、阻害され得る。

【0144】

本発明はなおさらに、本発明のポリヌクレオチドを発現するようにインピボで遺伝子操作された細胞を提供し、ここで、このようなポリヌクレオチドは、この宿主細胞においてこのポリヌクレオチドの発現を駆動するこの宿主細胞に対して異種の調節配列に、作動可能に連結されている。これらの方法は、本発明のポリヌクレオチドの発現を増加または減少するために使用され得る。

【0145】

本発明によって提供されるDNA配列の知識は、内因性ポリペプチドの発現を可能にするか、増加するかまたは減少するように細胞を改変することを可能にする。細胞は、天然に存在するプロモーターの全体または一部を異種プロモーターの全体または一部で置換することによって、ポリペプチド発現の増加を提供するように改変（例えば、相同組換えによって）改変され得、その結果、これらの細胞は、このタンパク質をより高いレベルで発現する。異種プロモーターは、それが、所望のタンパク質コード配列に作動可能に連結されている様式で挿入される。例えば、PCT国際公開番号WO94/12650、PCT国際公開番号WO92/20808、PCT国際公開番号WO91/09955を参照のこと。異種プロモーターDNAに加えて、増幅可能なマーカーDNA（例えば、ada、dhfr、および多機能CAD遺伝子（これは、カルバミルリン酸シンターゼ、アスパラギン酸トランスカルバミラーゼ、およびジヒドロオロターゼをコードする））および／またはイントロンDNAを、その異種プロモーターDNAと共に挿入され得ることもまた意図される。所望のタンパク質コード配列に連結される場合、標準的な選択方法によってマーカーDNAの増幅が、これらの細胞における所望のタンパク質コード配列の同時増幅を生じる。

【0146】

本発明の別の実施形態において、細胞および組織は、誘導性の調節エレメントの制御下で、本発明のポリヌクレオチドを含む内因性遺伝子を発現するように改変され得、この場合、この内因性遺伝子の調節配列は、相同組換えによって置換され得る。本明細書中に記載されるように、遺伝子標的化を使用して、遺伝子の既存の調節領域を、異なる遺伝子から単離された調節配列または遺伝子工学方法によって合成された新規調節配列と置換し得る。このような調節配列は、プロモーター、エンハンサー、足場付着領域、ネガティブ調節エレメント、転写開始部位、調節タンパク質結合部位または上記配列の組み合わせから構成され得る。あるいは、産生されるRNAまたはタンパク質の構造または安定性に影響する配列を、置換するか、除去するか、付加するか、または標的化によって改変し得る。これらの配列としては、ポリアデニル化シグナル、mRNA安定性エレメント、スプライシング部位、タンパク質の輸送または分泌特性を増強または改変するためのリーダー配列、あるいはタンパク質またはRNA分子の機能または安定性を変更または改善する他の配列が挙げられる。

【0147】

標的化事象は、調節配列の単純な挿入であり得、これは、その遺伝子を、新規調節配列の制御下に配置する（例えば、遺伝子上流への新規プロモーターまたはエンハンサーあるいは両方の挿入）。あるいは、標的化事象は、調節エレメント（例えば、組織特異的ネガティブ調節エレメントの欠失）の単純な欠失であり得る。あるいは、標的化事象は、既存のエレメントを置換し得；例えば、組織特異的エンハンサーが、天然に存在するエレメントよりも広いかまたは異なる細胞型特異性を有するエンハンサーによって置換され得る。ここで、この天然に存在する配列は、欠失され、そして新規配列が付加される。全ての場合において、標的化事象の同定は、その標的化DNAと連続する1以上の選択マーカー遺伝子の使用によって容易にされ得、これらは、その外因性DNAがその細胞ゲノムに組み込まれた細胞の選択を可能にする。標的化事象の同定はまた、ネガティブ選択の特性を示す1以上のマーカー遺伝子の使用（そのネガティブ選択マーカーが、外因性DNAに連結されているが、そのネガティブ選択マーカーが標的化配列に隣接するように構成され、その結果、宿主細胞ゲノムにおける配列との正確な相同組換え事象が、そのネガティブ選択マーカーの安定な組み込みを生じないように）によって容易にされ得る。この目的のための有用なマーカーは、単純ヘルペスウイルスチミジンキナーゼ（TK）遺伝子または細菌キサンチン・グアニンホスホリボシルトランスフェラーゼ（gpt）遺伝子が挙げられる。

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【0148】

本発明のこの局面に従って使用され得る遺伝子標的化技術または遺伝子活性化技術は、Chappelに対する米国特許第5,272,071号；Sherwinに対する米国特許第5,578,461号；Seldenらによる国際出願番号PCT/US92/09627（WO93/09222）；およびSkoultschikらによる国際出願番号PCT/US90/06436（WO91/06667）（これらの各々は、その全体が参考として本明細書中に援用される）に詳細に記載される。

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【0149】

（5.9 トランスジェニック動物）

本発明のポリペプチドのインビボでの生物学的機能を決める好ましい方法では、相同組換えを使っている動物の生殖細胞系で、本発明が提供する1個以上の遺伝子は過剰発現されているか、または不活性化されている〔カベッキ、Science 244:1288~1292（1989）〕。外因性または内因性プロモーターエレメントの調節制御の下で遺伝子が過剰発現されている動物は、トランスジェニック動物として公知である。相同組換えによって活性停止となっている内生の遺伝子をもつ動物は“ノックアウト”動物と称される。ノックアウト動物、好ましくは非ヒト哺乳動物、については本明細書中に参考として援用される米国特許No. 5,557,032に記載のとおり、調製できる。トランスジェニック動物は、生物学的なプロセスで、そして好ましくは疾患状態で、本発明のポリペプチドが演じる役割を決めるのに役立つ。トランスジェニック動物は、脂質の代謝を調節する化合物を識別するモデル系として有用である。トランスジェニック動物は、好ましくは非ヒト哺乳動物で、本明細書中に参考として援用される米国特許No. 5,489,743とPCT公開番号WO94/28122に記載された方法を使って生成される。

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【0150】

本発明のポリヌクレオチドのプロモーターの全部か一部が活性化か不活性化されて、本発明のポリヌクレオチドの発現のレベルが変更された、トランスジェニック動物が調製できる。不活性化は、上記の相同組換え方法を使って実行できる。活性化は、タンパク質発現を増加するために、同族性のプロモーターを補充するか、または取替えてでも達成できる。同族性のプロモーターは、特定の組織にプロモーター活性化を与えることが公知である1個以上の異種エンハンサーエレメントを挿入して補充できる。

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【0151】

本発明のポリヌクレオチドは又、例えば相同組換えまたはノックアウトストラテジーを使って、機能的プロトロンビナーゼ様ポリペプチドを発現しない、またはプロトロンビナーゼ様ポリペプチドの改変体を発現する動物を開発することを可能にする。そのような動物

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は、プロトロンビナーゼ様ポリペプチドのインビボの活性とプロトロンビナーゼ様ポリペプチドのモジュレーターを研究するモデルとして役に立つ。

【0152】

本発明のポリペプチドのインビボでの生物学的機能を決める好ましい方法では、相同組換えを使って動物の生殖細胞系で、本発明が提供する1個以上の遺伝子は超過発現されているか、または活性停止となっている〔カベッキ、Science 244:1288~1292(1989)〕。外生または内生プロモーターエレメントの規制管理の下で遺伝子が超過発現されている動物は、トランスジェニック動物として公知である。相同組換えによって活性停止となっている内生の遺伝子をもつ動物は”ノックアウト”動物と称される。ノックアウト動物、好ましくは非ヒト哺乳動物は、本明細書中に参考として援用される米国特許No. 5, 557, 032に記載のとおり、調製できる。トランスジェニック動物は、生物学的なプロセスで、そして望ましくは疾患の段階で、本発明のポリペプチドが演じる役割を決めるのに役立つ。トランスジェニック動物は、脂質の代謝を調節する化合物を識別するモデルシステムとして有用である。トランスジェニック動物は、望ましくはヒト以外の哺乳類で、ここに参考として含まれた米国特許No. 5, 489, 743とPCT公開番号WO94/28122に記載された方法を使って生成できる。

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【0153】

本発明のプロモーターのポリヌクレオチドの全部か一部が活性化か非活性化されて、本発明のポリペプチドの発現のレベルが変更されたトランスジェニック動物が、調製できる。不活性化は、上記の相同組換え方法を使って実行できる。活性化は、タンパク質発現を増加するために、同族性のプロモーターを補充するか、または取替えてでも達成できる。同族性のプロモーターは、特定の組織にプロモーター活性を与えるとされる1個以上の異族性のエンハンサーエレメントを挿入して補充できる。

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【0154】

(5.10, プロトロンビナーゼ様ポリペプチドの用途と生物学的活性)

本発明のポリヌクレオチドとタンパク質は、ここに特定された1個以上の用途または生物学的活性(ここに挙げたアッセイに関連するものを含む)を示すことが期待される。本発明のタンパク質について説明された用途または活性は、そのタンパク質またはそのタンパク質をコードするポリヌクレオチドの投与または使用によって提示され得る(例えば、遺伝子治療またはDNAの導入に適したベクター)。特殊な状態または病理学における機構が、本発明のポリペプチド、本発明のポリヌクレオチドまたはそのモジュレーター(アクティベーターまたはインヒビター)は治療を必要とする被験体に効果があるかどうかを決める。従って、“本発明の治療組成物”は、以下を含む組成物を含む:単離されたポリヌクレオチド(組換えDNA分子、クローンされた遺伝子とその変性改変体を含む)、または本発明のポリペプチド(全長タンパク質、成熟タンパク質および短縮物またはそのドメインを含む)、または化合物と他の物質で、標的遺伝子/タンパク質発現または標的タンパク質の活性のレベルでの、標的遺伝子産物の全体的活性を調整するもの。そのようなモジュレーターとしては、以下が挙げられる:フラグメントと融合タンパク質、抗体そして他の結合タンパク質を含む、ポリペプチド、アナログ、(改変体);本発明のポリペプチドを直接または間接に活性化または抑制する(本明細書中で述べられたように、薬物スクリーニングアッセイで同定された)化合物;三重螺旋の形成に適するアンチセンスポリヌクレオチドとポリヌクレオチド;そして特に、本発明のポリペプチドの1個以上のエピトープを特異的に認識する抗体または他の結合パートナー。

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【0155】

本発明のポリペプチドは、同様に細胞の活性化に関係し得るか、または本明細書中に述べられた、その他の生理学的な経路の1つに関連し得る。

【0156】

(5.10.1 調査用途と実用性)

本発明が提供するポリヌクレオチドは調査研究社会で色々な目的に使用できる。そのポリヌクレオチドは以下の様に使用できる:分析、特徴付けまたは治療の用途に組換えタンパ

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ク質を発現するために；対応するタンパク質が優先的に発現される組織のマーカーとして（構成的に、または組織分化、発達もしくは疾患状態の特定の段階で）；ゲル上の分子量マーカーとして；染色体識別、または関連した遺伝子位置のマッピングのための染色体のマーカー、またはのタグとして（標識される場合）；潜在的な遺伝子障害を特定するために、患者の内因性DNA配列と比較するために；ハイブリダイズのプローブとして、従って新規性のある、関連したDNA配列を発見するために；遺伝子のフィンガープリンティングのためにPCRプライマーを誘導する情報源として；他の新規のポリヌクレオチドを見つける過程で、既知の配列を“除外”するプローブとして；“遺伝子チップ”または発現パターンの検査などのその他の支持体に添付するためのオリゴマーを選択および作製するため；DNA免疫技術を使って、抗タンパク質抗体を惹起すること；そして、抗DNA抗体を惹起するか、または他の免疫反応を惹起する抗原として。ポリヌクレオチドが、他のタンパク質を結合するか、または潜在的に結合する、タンパク質をコードする場合（例えば、レセプターとリガンドの相互作用において）、ポリヌクレオチドはまた相互作用トラップアッセイにおいて（例えばギュリスほか、細胞 75：791-803（1993）で記述されたもの）、結合が起こる他のタンパク質をコードするポリヌクレオチドを同定するために、または結合の相互作用のインヒビターを同定するために使用する。

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【0157】

本発明が提供するポリペプチドは、同様に生物学的活性を決定するために、下記のようにアッセイで利用できる：高スループットスクリーニングのための複数のタンパク質のパネルで；抗体を惹起するため、または他の免疫反応を惹起するため；生物学的液体におけるタンパク質（またはそのレセプター）のレベルを定量的に決定するよう設計されたアッセイでの試薬（標識された試薬を含む）として；対応するポリペプチドが優先的に発現される組織のマーカーとして（構成的に、あるいは組織分化もしくは発達または疾患状態の特定の段階で）；そして勿論、関連したレセプターまたはリガンドを単離するため。これらの結合の相互作用に係わるタンパク質は、ペプチド、または結合相互作用の低分子インヒビターもしくはアゴニストのスクリーニングにも使用され得る。

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【0158】

本発明のポリペプチドはまた、プロトンビナーゼ様タンパク質と特異的に免疫反応する抗体物質を作るのに役立つ。本発明のポリペプチドに結合する抗体とその部分（例えば、Fabフラグメント）は、試料の中にその様なポリペプチドが存在することの確認に使用できる。その決定は、任意の適切な免疫アッセイ形式を使って行われ、この抗体に特異的に結合された任意の本発明のポリペプチドはポジティブコントロールとして使える。

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【0159】

これらの調査用途のいずれかまたは全てが、試薬の品質にまたはキット形態にして商品化のために調査用製品として開発可能である。

【0160】

上記に示した用途を実行する方法は当業者には周知である。その様な方法を開示した参考資料は、以下のものを制限なく含む：“分子のクローニング：研究所マニュアル”、第2版、Cold Spring Harbor Laboratory Press、サムブルック、J.、E. F. フリッチとT. マニアティス編、1989、および“酵素学方法論：分子クローニング技術への手引き”、Academic Press、バーガー、S. L. およびA. R. キンメル編、1987。

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【0161】

（5.10.2 栄養面での用途）

本発明のポリヌクレオチドとポリペプチドは、また栄養源または補充品として使用できる。そのような用途は、制限無く、タンパク質またはアミノ酸の補充品として、炭素、窒素、炭水化物のそれぞれの供給源として使用できる。その場合、本発明のポリペプチドまたはポリヌクレオチドは、特定の生物の飼料に添加できるか、あるいは粉末、錠剤、溶液、懸濁液、またはカプセルの形態のような、別個の固体または液体の調製物として投与できる。微生物の場合、本発明のポリペプチドまたはポリヌクレオチドは微生物が培養されて

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いる培地に添加できる。

【0162】

さらに、本発明のポリペプチドは、分子量マーカーとして、また食料補充品として使用できる。例えば、配列番号4からなるポリペプチドは、その未処理でグリコシル化されていない状態では、約43kDAの分子量を持つ。タンパク質の食料補充品は周知であり、本発明のポリペプチドを含む、適切な食料補充品の処方、食料調製の技術レベルで対応できる。

【0163】

(5.10.3 サイトカインと細胞増殖／分化の活性)

本発明のポリペプチドは、サイトカイン、細胞増殖（誘発または抑制のどちらか）、または細胞分化（誘発または抑制のどちらか）の活性を示し得るか、あるいは細胞集団における他のサイトカインの生成を誘発し得る。本発明のポリヌクレオチドはそのような性質を示すポリペプチドをコードすることができる。全ての公知のサイトカインを含む、これまで発見された多くのタンパク質因子は1回以上の因子依存の細胞増殖アッセイで活性を示した。従って、アッセイはサイトカイン活性の便利な確認方法として役立つ。本発明の治療的組成物の活性は、細胞株に対する多数の慣用的な因子に依存する細胞増殖のアッセイのいずれかによって立証され、これらの細胞株としては、制限無く、以下が挙げられる：32D、DA2、DA1G、T10、B9、B9/11、BaF3、MC9/G、M+（preB M+）、2E8、RB5、DA1、123、T1165、HT2、CTL2、TF-1、Mo7e、CMK、HUVEC、およびCaco。本発明の治療的組成物は 20
下記に使用できる：

T細胞または胸腺細胞増殖のアッセイは、下記に述べられたものを制限無く含む：免疫学における現在のプロトコル、編集 J. E. コリガン、A. M. クルイスピーク、D. H. マーグリース、E. M. シェバック、W. ストロバー、出版、Greene Publishing Associates and Wiley-Interscience（第3章、ネズミのリンパ球機能のインビトロアッセイ3.1～3.19；第7章、ヒトにおける免疫学の研究）；高井ほか、J. Immunol. 137:3494-3500、1986；バータニオリほか、J. Immunol. 145:1706-1712、1990；バータニオリほか、細胞免疫学133:327-341、1991；バータニオリほか、I. Immunol. 149:3778-3783、1992；ボウマンほか、I. Immunol. 152:1756-1761、1994。 30

サイトカイン生成および／または脾臓細胞、リンパ節細胞、もしくは胸腺細胞の増殖のアッセイは下記に述べられたものを制限なく含む：ポリクローナルT細胞の刺激、クルイスピーク、A. M. およびシェバック、E. M. 免疫学における現在のプロトコル、J. E. e. a. コリガン編集、第1巻pp. 3.12.1～3.12.14、John Wiley and Sons、Toronto、1994；および、ネズミとヒトのインターロイキン- γ の測定、シュライバー、R. D. 免疫学における現在のプロトコル、J. E. e. a. コリガン編集、Vol 1 pp. 6.8.1-6.8.8、John Wiley and Sons、Toronto、1994。

【0164】

造血およびリンパ球産生性細胞の増殖と分化のアッセイは、下記に述べられたものを制限無く含む：「ヒトとネズミ類のインターロイキン2およびインターロイキン4の測定」、ボトムリ、K.、デイビス、L. S. およびリプスキー P. E. ；「免疫学における現在のプロトコル」J. E. e. a. コリガン eds. Vol 1 pp. 6.3.1-6.3.12、John Wiley and Sons、Toronto、1991；デフリースほか、J. Exp. Med. 173:1205-1211、1991；モローほか、自然 336:690-692、1988；グリーンバーガーほか、Proc. Natl. Acad. Sci. U. S. A. 80:2931-2938、1983；ネズミとヒトのインターロイキン6の測定—ノーダン、R. 免疫学における現在のプロトコル、J. E. コリガン編、Vol 1 pp. 6.6.1-6.6.5、John Wile 40
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y and Sons, Toronto. 1991; スミスほか、Proc. Natl. Acad. Sci. U. S. A. 83: 1857-1861、1986; ヒトのインターロイキン11の測定—ベネット、F.、ジアノッティ、J.、クラーク、S. C. およびターナー、K. J. 免疫学における現在のプロトコル. J. E. コリガン編集. 第1巻 pp. 6. 15. 1 John Wiley and Sons, Toronto. 1991; ネズミとヒトのインターロイキン9の測定—シアレッタA.、ジアノッティ、J.、クラーク、S. C. およびターナー、K. J. 免疫学における現在のプロトコル. J. E. コリガン eds. 第1巻 pp-6. 13. 1、John Wiley and Sons, Toronto. 1991.

【0165】

T細胞クローンの抗体への応答のアッセイ（これは、とりわけ、APC-T細胞の相互作用と直接的なT細胞効果に影響するタンパク質を、増殖とサイトカイン生成を測定することによって識別する）は下記に述べられたものを制限無く含む：免疫学における現在のプロトコル、編集：J. E. コリガン、A. M. クルイスビーク、D. H. マーグリース、E. M. シェバック、W. ストロバー、出版社 Greene Publishing Associates and Wiley-Interscience（第3章、ネズミのリンパ球機能のインビトロアッセイ；第6章、サイトカインとそれらの細胞レセプター；第7章、ヒトにおける免疫学の研究）；ワインバーガーほか、Proc. Natl. Acad. Sci. USA 77: 6091-6095、1980；ワインバーガーほか、Eur. J. Immun. 11: 405-411、1981；高井ほか、J. Immunol. 137: 3494-3500、1986；高井ほか、J. Immunol. 140: 508-512、1988.

【0166】

（5. 10. 4 幹細胞増殖因子の活性）

本発明のポリペプチドは、幹細胞増殖因子活性を示し得、原始の生殖細胞、胚幹細胞、造血幹細胞および／または生殖株の幹細胞を含む多能性および全能な幹細胞の増殖、分化と生存に関与しているかもしれない。本発明のポリペプチドをインビボまたはエキソビボの幹細胞に投与すると、全能または多能性な状態で細胞集団を維持しそして拡大するかもしれない。それは、損傷した、または病気にかかった組織の再構築、移植、生物学的薬剤の製造、バイオセンサーの開発に役立つ。大量のヒト細胞を製造する能力は、ヒトタンパク質の生成に重要な実用用途を持ち、パーキンソン病、アルツハイマー病、その他の神経変性疾患を治療するために、現在それは非ヒト供給源、またはドナー、細胞移植用；移植用の組織、例えば骨髄、皮膚、軟骨、腱、骨、筋肉（心筋を含む）、血管、網膜、神経細胞、胃腸の細胞など；そして移植用の器官、例えば、腎臓、肝臓、膵臓（島細胞を含む）、心臓および肺から取得されねばならない。

【0167】

望ましい効果を達成するために、複数の異なった外因性の増殖因子および／またはサイトカインを本発明のポリペプチドと共に投与し得ることを考慮しており、これらは本明細書中に記載の増殖因子のいずれか、他の幹細胞の維持因子を含み、そして特に幹細胞因子（SCF）、白血病抑制因子（LIF）、Flt-3リガンド（Flt-3L）、インターロイキンのいずれか、IL-6に融合された、組換え可溶性のIL-6レセプター、マクロファージ炎症性タンパク質1- α （MIP-1- α ）、G-CSF、GM-CSF、トロポポイエチン（TPO）、血小板因子4（PF-4）、血小板由来の増殖因子（PDGF）、神経増殖因子、そして基本的な線維芽細胞増殖因子（bFGF）を含む。

【0168】

全能幹細胞は、実際的に任意の成熟細胞型を発生させるので、培養中のこれらの細胞の拡大は、大量の成熟細胞の生産を助ける。幹細胞の培養技術は当該分野において公知であり、本発明のポリペプチドの投与（必要に応じて他の増殖因子および／またはサイトカインと共に）は、幹細胞集団の生存と増殖を高めることが期待されている。これは、本発明のポリペプチドを培養培地に直接投与することによって達成できる。代替方法としては、本

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発明のポリペプチドに代わってコードするポリヌクレオチドでトランスフェクトされた支質細胞が培養物またはインビボの幹細胞集団のためのフィーダー層として使える。フィーダー層の支質支持細胞は、胚の骨髄線維芽細胞、骨髄支質細胞、胎児の肝細胞、または培養された胚の線維芽細胞を含み得る（参照：米国特許 No. 5, 690, 926）。

【0169】

幹細胞自体は、本発明のポリペプチドのオートクライン発現を誘発するために本発明のポリヌクレオチドでトランスフェクトされる。これによって、分化されていない全能／多能性な幹細胞株の生成が可能となり、それらはそのまま使用可能で、またはそれから望ましい成熟細胞型に分化できる。これらの安定した細胞株はまた分化されていない全能／多能性な mRNA の源となり、ポリメラーゼ連鎖反応実験用の cDNA のライブラリやテンプレートを作製できる。これらの研究によって、幹細胞の増殖および／または維持を規制する幹細胞集団において異なって発現された遺伝子の単離と同定が可能となる。

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【0170】

全能な幹細胞集団の拡大と維持は、多くの病理学的な症状の治療に役立つ。例えば、本発明のポリペプチドは、培養物中の幹細胞を操作するために使われ、病気、自己免疫疾患、事故による傷害、または遺伝障害によって損傷を受けた細胞を補充したり、取替えたりするために使用され得る神経上皮細胞を生じ得る。本発明のポリペプチドは、神経細胞の増殖を誘導したり、神経と脳の組織を再生したりする上で有用であり得、即ち、中枢および末梢神経の疾病やニューロパシー、ならびに神経細胞や神経組織の変性、死滅、または傷害を伴う機械的障害および外傷的障害の治療に使われる。さらに、拡大された幹細胞の集団は、遺伝子療法の目的のため、そして移植または注入の後、植え付けた組織を宿主が拒否する可能性を減らすため、遺伝学的に変更することもできる。

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【0171】

本発明のポリペプチドの発現と幹細胞に対するその影響も操作可能で、幹細胞の管理された分化を達成してさらに分化された細胞型にすることができる。分化されていない幹細胞集団から特定の分化された細胞型の純粋な集団を取得する広範的な方法は、選択マーカーを作動する、細胞型に特異的なプロモーターを使用することを含む。この選択マーカーは、望ましい型の細胞だけを生存させる。例えば、幹細胞は心筋細胞に（ウオバスほか、分化、48:173-182、(1991)；クルグほか、J. Clin. Invest.、98(1):216-224、(1998)）または骨格筋肉細胞に（ブラウダー、L. W. 組織工学理論 eds. ランザほか、Academic Press (1997)）分化するように誘発できる。代替方法としては、レチン酸のような分化因子と、内因性幹細胞因子活性の効果を抑制して分化を進めるように、本発明のポリペプチドのアンタゴニストとの存在下でその幹細胞を培養することによって、幹細胞の指向された分化を達成できる。

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【0172】

幹細胞のインビトロ培養物を使って、本発明のポリペプチドが幹細胞の増殖因子活性を示すかどうかを判定することができる。幹細胞は様々な細胞源（造血幹細胞と胚の幹細胞を含む）のいずれかから単離され、トンプソンほかによる Proc. Natl. Acad. Sci. U. S. A.、92:7844-7848 (1995) で述べられているように、本発明のポリペプチド単独で、または他の増殖因子またはサイトカインとの組合せの存在下で、フィーダー層で培養される。本発明のポリペプチドの、幹細胞の増殖を誘発する能力は、例えばバーンスタイン他の“血液”77:2316-2321 (1991) に述べられてあるように、半固体支持体上でのコロニー形成によって決定される。

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【0173】

(5.10.5 造血調節活性)

本発明のポリペプチドは造血調節に関連し得、従って、骨髄またはリンパ細胞障害の治療に関連し得る。コロニー形成細胞の支持、あるいは因子依存性細胞系の支持においてわずかでも生物学的活性があることから、造血調節への関与を示し、例えば、赤血球前駆細胞単独で、または他のサイトカインと共に成長や増殖を支持し、それによって、例えば、種

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々の貧血治療、または赤血球前駆体および／または赤血球の産生を刺激するための照射／化学療法と併用して；例えば、結果的に生じる骨髓抑制の予防または治療のための化学療法との併用において有用な顆粒球や単球／マクロファージ（すなわち、従来のCSF活性）などのような骨髓細胞の成長および増殖の支持において；巨核球の成長と増殖、したがって、血小板の成長および増殖を支持し、それによって、血小板減少症のような様々の血小板障害に、一般に血小板輸血の代わりに、または補助として使用してその予防または治療を可能にする際、および／あるいは、上記の造血細胞のいずれか、またはすべてに対して成熟を可能にする造血幹細胞の成長および増殖を支持する際に使用するなどの利用性が示され、したがって、種々の幹細胞障害（通常、再生不良性貧血および発作性夜間ヘモグロビン尿症をこれらに限らず含む移植によって通常治療される疾患など）、および、イン

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【0174】

本発明の治療的組成物は以下において使用できる。

【0175】

種々の造血細胞系の増殖および分化に適したアッセイは前記に引用した。

【0176】

胚性幹細胞分化（とりわけ、胚性分化造血に影響を及ぼすタンパク質を同定する）アッセイにはJohanssonらCellular Biology 15:141-151、1995；Kellerら、Molecular and Cellular Biology 13:473-486、1993；McClanahanら、Blood 81:2903-2915、1993に記載のアッセイがあり、またこれに限らない。

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【0177】

幹細胞の生存および分化のためのアッセイ（とりわけ、リンパー造血を調節するタンパク質を同定する）はMethylcellulose colony forming assays、Freshney、M. G. Culture of Hematopoietic Cells. R. 1. Freshney、ら編、Vol pp. 265-268、Wiley-Liss、Inc.、New York、N. Y. 1994；Hirayamaら、Proc. Natl. Acad. Sci. USA 89:5907-5911、1992；Primitive hematopoietic colony forming cells with high proliferative potential、McNiece、I. K. およびBriddell、R. A. Culture of Hematopoietic Cells. R. 1. Freshneyら編、Vol pp. 23-39、Wiley-Liss、Inc.、New York、N. Y. 1994；Nebenら、Experimental Hematology 22:353-359、1994；Cobblestone area forming cell assay、Ploemacher、R. E. Culture of Hematopoietic Cells. R. 1. Freshneyら編、Vol pp. 1-21、Wiley-Liss、Inc.、New York、N. Y. 1994；Long term bone marrow cultures in the presence of stromal cells、Sponcer、E.、Dexter、M. and Allen、T. Culture of Hematopoietic Cells. R. 1. Freshneyら編、Vol pp. 163-179、Wiley-Liss、Inc.、New York、N. Y. 1994；Long term culture initiating cell assay、Sutherland、H. J. Culture of Hematopoietic Cells. R. 1. Freshneyら編、Vol pp. 139-162、Wiley-Liss、Inc.、New York、N. Y. 1994に記載のアッセイを含み、またこれらに限らない

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【0178】

(5. 10. 6 組織成長活性)

本発明のポリペプチドはまた、骨、軟骨、腱、靱帯および／または神経組織の成長あるいは再生、ならびに創傷治癒および組織修復ならびに置換および火傷、切開および潰瘍の治癒にも関与し得る。

【0179】

骨が正常に形成されていない場合において、軟骨または骨あるいはその両者の成長を促す本発明のポリペプチドは、人および他の動物における骨折および軟骨損傷あるいは欠損症の治癒において使用できる。本発明のポリペプチド、抗体、結合パートナーまたは他のモジュレータの組成物は、開放骨折同様皮下骨折の軽減において予防的に使用でき、また人工関節の固定をも改善する場合がある。骨形成物質によって誘発される新規の骨形成は、先天性、外傷性、または腫瘍学的切除性頭蓋顔面欠損の修復に役立つし、また美容整形外科においても有用である。

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【0180】

本発明のポリペプチドはまた、骨形成細胞の誘引、骨形成細胞の成長刺激、骨形成細胞前駆体の分化の誘導に関与し得る。骨もしくは軟骨修復またはその両者の刺激によるか、あるいは、炎症または炎症過程により媒介される組織破壊過程（コラゲナーゼ活性化、破骨細胞活性化など）を阻止するによる、骨粗しょう症、変形性関節炎（骨関節炎）、骨変性疾患、または歯周病の治療もまた本発明の組成物の使用が可能な場合がある。

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【0181】

本発明のポリペプチドが関与し得る組織再生活性のもう一つのカテゴリーは、腱／靱帯様組織形成である。腱／靱帯様組織または他の組織形成の誘発は、これらの組織が正常に形成されていない場合において、人および他の動物における腱あるいは靱帯の損傷、奇形および他の腱または靱帯欠損の治癒に使用できる。このような腱／靱帯様組織誘発性タンパク質を使用した製剤は、腱または靱帯の骨あるいは他の組織への固定改善や、腱または靱帯組織の欠損修復にも使用できるだけでなく、腱または靱帯組織の破損を防ぐための予防にも使用できる場合がある。本発明の組成物によって誘発されたデノボ腱／靱帯様組織形成は、先天性、外傷性、または他の原因の腱あるいは靱帯欠損の修復に寄与し、また、腱または靱帯の取付け、修復のための美容整形手術にも使用できる。本発明の組成物は腱または靱帯形成細胞の誘発、腱または靱帯形成細胞の成長刺激、腱または靱帯形成細胞前駆体の分化の誘導、あるいは、腱／靱帯細胞または組織修復に作用するようインビボに戻すエキソビボ前駆体の成長の誘発を行う環境を提供し得る。本発明の組成物はまた、腱炎、手根管症候群および他の腱または靱帯欠損症の治療にも有用である。本組成物には、また適切な基質および／または当該分野で周知のキャリアとしての隔離作用物質も含まれていることがある。

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【0182】

本発明の組成物はまた、神経細胞の増殖と神経や脳組織の再生、すなわち、中枢および末梢神経系疾患や神経障害の治療に有用であるだけでなく、また神経細胞や神経組織の変性、死または外傷を含む機械的、外傷性障害にも有用である。さらに厳密には、末梢神経損傷、末梢神経障害および限局性神経障害などのような末梢神経系疾患、また、アルツハイマー病、パーキンソン病、ハンチントン舞蹈病、筋萎縮性側索硬化症およびシャイードレーガー症候群などの中枢神経系疾患の治療にも使用することができる。さらに、本発明によって治療され得る状態には、脊髄障害、脳外傷のような機械的、外傷性疾患および脳卒中のような脳血管疾患が含まれる。化学療法または他の医学療法から生じる末梢神経障害はまた、本発明の組成物を用いて処置され得る。

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【0183】

本発明の組成物はまた、プレッシャー潰瘍、血管性機能不全に関連する潰瘍、手術創傷および外傷性創傷などをこれらに限らず含む非治癒性創傷の閉合をより良く、より速く促進するのに役立つ場合がある。

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【0184】

本発明の組成物はまた、臓器（例えば、脾臓、肝臓、腸、腎臓、皮膚、内皮を含む）、筋肉（平滑筋、骨格筋肉、心筋）および血管（血管内皮を含む）組織などのような他の組織の産生または再生、あるいは、このような組織を構成する細胞の増殖の促進にも関与する。望ましい効果の一部は正常組織の再生を可能する線維性瘢痕の抑制または変調による。本発明のポリペプチドはまた、血管形成作用をも示す場合がある。

【0185】

本発明の組成物はまた、腸保護、または肺や肝臓線維症、種々の組織の再灌流損傷および全身性サイトカイン損傷から生じた状態の再生および処置にも有用である。

【0186】

本発明の組成物はまた、前駆体組織や前駆体細胞からの、前記した組織の分化を促進または阻害するために、または前記の組織の成長阻害にも有用であり得る。

【0187】

本発明の治療的組成物は以下において使用できる。

【0188】

組織生成アッセイには International Patent Publication No. WO95/16035（骨、軟骨、腱）；International Patent Publication No. WO95/05846（神経、ニューロン）；International Patent Publication No. WO91/07491（皮膚、内皮）に記載のアッセイがあり、またこれに限らない。

【0189】

創傷治癒活性アッセイには Winter, Epidermal Wound Healing, pp. 71-112 (Maibach, H. I. and Rovee, D. T., eds.), Year Book Medical Publishers, Inc., Chicago, Eaglstein and Mertz, J. Invest. Dermatol 71: 382-84 (1978) による改変に記載のアッセイがあり、またこれに限らない。

【0190】

（5.10.7 免疫刺激または抑制活性）

本発明のポリペプチドはまた、ここに記載のアッセイによる活性をこれに限らず含む免疫刺激活性または免疫抑制活性を示し得る。本発明のポリヌクレオチドはこのような活性を示すポリペプチドをコードできる。タンパク質は、種々の免疫不全や免疫疾患（重症複合型免疫不全症（SCID）など）の治療、例えば、Tおよび/またはBリンパ球の成長や増殖の調節（上方、または下方）と同様、NK細胞や他の細胞集団の細胞溶解活性への作用においても有用であり得る。これらの免疫不全は細菌あるいは真菌感染によるだけでなく、遺伝的またはウイルス（例えば、HIV）であり得るか、あるいは自己免疫疾患によって引き起こされる場合がある。さらに詳細には、HIV、肝炎ウイルス、ヘルペスウイルス、ミコプラズマ、Leishmania spp（リーシュマニア属）、マラリア種による感染症およびカンジダ症などのような種々の真菌感染症を含み、ウイルス、細菌、真菌または他の感染症によって生じる感染疾患は、本発明のタンパク質を用いて治療できる場合がある。もちろん、この点で、本発明のタンパク質は、免疫系へのブーストが一般に望ましい場合に、つまり、癌の治療においてもまた有用である。

【0191】

本発明のタンパク質を用いて処置される自己免疫疾患には、例えば、結合組織病、多発性硬化病、全身性エリテマトーデス、慢性関節リウマチ、自己免疫肺性炎症、ギラン・バレー症候群、自己免疫甲状腺炎、インシュリン依存性真性糖尿病、重症筋無力症、移植片対宿主疾患および、自己免疫炎症性眼疾患が挙げられる。このような本発明のタンパク質（または抗体を含むその拮抗物質）は、喘息（特にアレルギー性喘息）または他の呼吸器系障害のようなアレルギー反応および状態（例えば、アナフィラキシー、血清病、薬物反応、食物アレルギー、昆虫毒液アレルギー、肥満細胞症、アレルギー性鼻炎、過敏性肺炎

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、蕁麻疹、血管性浮腫、湿疹、アトピー性皮膚炎、アレルギー性接触皮膚炎、多型性紅疹、スティーヴンス・ジョンソン症候群、アレルギー性結膜炎、アトピー性角結膜炎、性病性角結膜炎、巨大乳頭状結膜炎、接触アレルギー）の処置にも有用である。免疫抑制が望まれる（例えば、臓器移植を含む）他の状態もまた、本発明のタンパク質（またはその拮抗物質）を用いて治療することができる場合がある。ポリペプチドまたはその拮抗物質のアレルギー反応に対する治療効果は、累積性接触増加テスト（Lastbom et al.、Toxicology 125：59－66、1998）、皮膚突き傷テスト（Hoffmann et al.、Allergy 54：446－54、1999）、モルモット皮膚感作テスト（Vohr et al.、Arch. Toxicol. 73：501－9）、およびマウス局所リンパ節試験 assay（Kimber et al.、J. Toxicol. Environ. Health 53：563－79）などのインビボ動物モデルによって評価できる。 10

【0192】

本発明のタンパク質を用いる場合、また、多くの方法で免疫反応を変調することも可能である。下方調節は、すでに進行中の免疫反応を抑制するか、または阻止するやり方で行われるか、あるいは、免疫反応の誘導の防御に關与する。活性T細胞の機能はT細胞反応の抑制によるか、またはT細胞における特異的トレランス（寛容）の誘導によって、あるいはその両方によって抑制される。T細胞反応の免疫抑制は一般に活性、非抗原特異的過程であり、これはT細胞の抑制物質への持続的暴露を必要とする。T細胞での非反応性または無反応の誘導に關連するトレランスは、それが一般に抗原特異的であり、寛容化物質への暴露が中止された後にも持続するという点で免疫抑制と区別される。操作的には、トレランスは、寛容化物質の無い状態における特定抗原への再暴露時にT細胞反応が起こらないことによって示される。 20

【0193】

下方調節または1つ以上の抗原機能（Bリンパ球抗原機能（例えば、B7のように）をこれに限らず含む）の防御、例えば、活性T細胞による高度レベルのリンホカイン合成の防御は、組織、皮膚および臓器移植の状態において、また、移植片対宿主疾患（GVHD）において有用である。例えば、T細胞機能を阻止することは、組織移植における組織破壊を減少する結果となるはずである。一般に、組織移植において移植の拒絶反応は、T細胞がそれを異物として認識することによって開始され、その後に移植片を破壊する免疫反応が続く。本発明の治療的組成物の投与は、T細胞のような免疫細胞によるサイトカインの合成を妨げ、したがって、免疫抑制剤として作用する。さらに、同時刺激がないことによって、T細胞が無反応化（アネルギー化）されることになり、これによって、被験者におけるトレランスが誘導される場合もある。Bリンパ球抗原阻止試薬による長期トレランスの誘導によって、これらの阻止剤の反復投与の必要性がなくなる。被験者において十分な免疫抑制またはトレランスを得るには、Bリンパ球抗原の組合せの機能を阻止する必要がある場合もある。 30

【0194】

臓器移植拒絶反応またはGVHDの予防における特定の治療的組成物の効力は、ヒトにおける有効性についての予測である動物モデルを用いて評価することができる。使用できる適切なシステムの例として、ラットにおける同種心臓移植片や、マウスにおける異種間膵島細胞移植片があり、これらのどちらもが、Lenschow et al.、Science 257：789－792（1992）およびTurka et al.、Proc. Natl. Acad. Sci. USA、89：11102－11105（1992）に述べられているようにインビボCTLA4Ig 融合タンパク質の免疫抑制効果を検査するために使われている。さらに、GVHD（移植片対宿主疾患）のマウスモデル（Paul et al.、Fundamental Immunology、Raven Press、New York、1989、pp. 846－847参照）はこの疾患の進展時の本発明の治療的組成物の効果を測定するために使うことができる。 40

【0195】

抗原機能の阻止はまた、自己免疫疾患の治療に対して治療的に有用である。多くの自己免疫疾患は、自己組織に反応的なT細胞の不適切な活性化の結果、および、その疾患の病態に関与するサイトカインや自己抗体の産生を促進した結果である。自己反応性T細胞の活性化の防御は病状を減少するか、または除去する。T細胞の刺激を阻止する試薬の投与は、T細胞活性化の抑制、およびこの疾患の過程に関与する自己抗体またはT細胞由来サイトカインの産出の防御に使うことができる。さらに、ブロック試薬の効力によって、この疾患からの長期的緩解につながる自己反応性T細胞の抗原特異的トレランスが誘導される場合がある。自己免疫疾患の予防または緩解におけるブロック試薬の効力は、多くのよく特徴化された、ヒト自己免疫疾患の動物モデルを用いて測定できる。この例には、マウス実験的自己免疫脳炎、MEL/lpr/lprマウスまたはNZB ハイブリッドマウスにおける全身性エリテマトーデス、マウス自己免疫性膠原関節炎、NODマウスおよびBBラットにおける真性糖尿病、マウス実験的重症筋無力症（Paul ed.、Fundamental Immunology、Raven Press、New York、1989、pp. 840-856参照）がある。

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【0196】

免疫反応を上げる手段としての抗原機能の上方調節（例、Bリンパ球抗原機能）は、また治療においても有用である。免疫反応の上方調節は既存の免疫反応を亢進させる形式か、または初期の免疫反応を誘発する形式で行われる。例えば、免疫反応の亢進は、インフルエンザ、感冒、および脳炎などのような全身性ウイルス疾患を含むウイルス感染の場合に有用な場合がある。

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【0197】

あるいは、感染した患者の抗ウイルス性免疫反応は、患者からT細胞を除去し、本発明のペプチドを発現するか、あるいは本発明の可溶性ペプチドの刺激性形態のものと共に、ウイルス抗原パルス化APCで、インビトロでT細胞を同時刺激し、そして、インビトロ活性T細胞を患者に再び導入することによって増強されることがある。抗ウイルス免疫反応を亢進させる他の方法は、患者から感染細胞を分離し、それらに、細胞がその表面にタンパク質のすべて、または一部を発現するように、ここに記載する本発明のタンパク質をコードする核酸で形質移入し、そして、形質移入された細胞を患者に再び導入することであろう。ここで、感染細胞は同時刺激シグナルを送ることができ、それによってインビボでT細胞を活性化できるであろう。

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【0198】

本発明のポリペプチドは形質移入された腫瘍細胞に対するT細胞媒介免疫反応を誘導するために必要な刺激シグナルを提供し得る。さらに、MHCクラスIまたはMHCクラスII分子を持たない、または、十分な量のMHCクラスIまたはMHCクラスII分子を再発現できない腫瘍細胞は、MHCクラスIアルファ鎖タンパク質と β_2 ミクログロブリンタンパク質、あるいは、MHCクラスIIアルファ鎖タンパク質とMHCクラスIIベータ鎖タンパク質のすべて、または一部（例えば、細胞質内ドメイン切断部分）のすべて、または一部をコードする核酸で形質移入して、それによって、細胞表面にMHCクラスIまたはMHCクラスIIタンパク質を発現させることができる。Bリンパ球抗原（例えば、B7-1、B7-2、B7-3）の活性を有するペプチドと共に、適切なクラスIまたはクラスII MHCを発現させることによって、形質移入された腫瘍細胞に対するT細胞媒介免疫反応が誘導される。随意に、インバリエント（不変異）鎖のようなMHCクラスII結合タンパク質の発現を阻止するアンチセンス構築物をコードする遺伝子は、Bリンパ球抗原の活性を有するペプチドをコードするDNAで同時形質移入して、腫瘍関連抗原の提示を促進し腫瘍特異的免疫を誘導することができる。このようにして、ヒト被験者におけるT細胞媒介免疫反応の誘導は、被験者における腫瘍特異的トレランスを十分克服できる。

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【0199】

本発明のタンパク質の活性は、他にも方法はあるが、次の方法によって測定され得る。

【0200】

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適切な胸腺または脾細胞の細胞毒性アッセイには、Current Protocols in Immunology、Ed by J. E. Coligan、A. M. Kruisbeek、D. H. Margulies、E. M. Shevach、W. Strober、Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3、In Vitro assays for Mouse Lymphocyte Function 3. 1～3. 19; Chapter 7、Immunologic studies in Humans); Herrmann et al.、Proc. Natl. Acad. Sci. USA 78: 2488-2492、1981; Herrmann et al.、J. Immunol. 128: 1968-1974、1982; Handa et al.、J. Immunol. 135: 1564-1572、1985; Takai et al.、J. Immunol. 137: 3494-3500、1986; Takai et al.、J. Immunol. 140: 508-512、1988; Bowman et al.、J. Virology 61: 1992-1998; Bertagnoli et al.、Cellular Immunology 133: 327-341、1991; Brown et al.、J. Immunol. 153: 3079-3092、1994に記載のアッセイがあり、またこれらに限らない。

【0201】

T細胞依存性免疫グロブリン反応およびアイソタイプスイッチング (T細胞依存性抗体反応を変調し、Th1/Th2プロフィールに影響するタンパク質を他から同定する) のアッセイにはMaliszewski、J. Immunol. 144: 3028-3033、1990;およびAssays for B cell function: In vitro antibody production、Mond、J. J. and Brunswick、M. In Current Protocols in Immunology. J. E. e. a. Coligan eds. Vol 1 pp. 3. 8. 1～3. 8. 16、John Wiley and Sons、Toronto. 1994に記載のアッセイがあり、またこれらに限らない。

【0202】

混合リンパ球反応 (MLR) アッセイ (主にTh1とCTL反応を生じるタンパク質を他から同定する) には、Current Protocols in Immunology、Ed by J. E. Coligan、A. M. Kruisbeek、D. H. Margulies、E. M. Shevach、W. Strober、Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3、In Vitro assays for Mouse Lymphocyte Function 3. 1～3. 19; Chapter 7、Immunologic studies in Humans); Takai et al.、J. Immunol. 137: 3494-3500、1986; Takai et al.、J. Immunol. 140: 508-512、1988; Bertagnoli et al.、J. Immunol. 149: 3778-3783、1992に記載のアッセイがあり、またこれらに限らない。

【0203】

樹状突起細胞依存性アッセイ (未処置のT細胞を活性化する樹状突起細胞によって発現されるタンパク質を他から同定する) にはGuery et al.、J. Immunol. 134: 536-544、1995; Inaba et al.、Journal of Experimental Medicine 173: 549-559、1991; Macatonia et al.、Journal of Immunology 154: 5071-5079、1995; Porgado et al.、Journal of Experimental Medicine 182: 255-260、1995; Nair et al.、Journal of Virology 67: 4062-4069、1993; Huang et al.、Science 264: 9

61-965、1994; Macatonia et al.、Journal of Experimental Medicine 169:1255-1264、1989; Bhardwaj et al.、Journal of Clinical Investigation 94:797-807、1994; および Inaba et al.、Journal of Experimental Medicine 172:631-640、1990に記載のアッセイがあり、またこれらに限らない。

【0204】

リンパ球生存／アポトーシスのアッセイ（スーパー抗原誘導後のアポトーシスを防ぐタンパク質およびリンパ球ホメオスタシスを調節するタンパク質を他から同定する）には、D
arzynkiewicz et al.、Cytometry 13:795-808 10
、1992; Gorczyca et al.、Leukemia 7:659-670
、1993; Gorczyca et al.、Cancer Research 53
:1945-1951、1993; Itoh et al.、Cell 66:233-
243、1991; Zacharchuk、Journal of Immunology 145:4037-4045、1990; Zamai et al.、Cytome
try 14:891-897、1993; Gorczyca et al.、Inte
rnational Journal of Oncology 1:639-648、
1992に記載のアッセイがあり、またこれらに限らない。

【0205】

T細胞コミットメントおよび発達の初期段階に影響するタンパク質のアッセイには： A
ntica et al.、Blood 84:111-117、1994; Fine
et al.、Cellular Immunology 155:111-122、1
994; Galy et al.、Blood 85:2770-2778、1995;
Toki et al.、Proc. Nat. Acad. Sci. USA 88:754
8-7551、1991に記載のアッセイがあり、またこれらに限らない。 20

【0206】

（5.10.8 アクチビン／インヒビン活性）

本発明のポリペプチドは、またアクチビンまたはインヒビン関連活性をも示す。本発明の
ポリヌクリオチドはこのような特性を示すポリペプチドをコードし得る。インヒビンは卵
胞刺激ホルモン（FSH）の遊離を抑制する能力によって特徴づけられており、一方、ア
クチビンは卵胞刺激ホルモン（FSH）の遊離を刺激する能力によって特徴づけられてい
る。このように、本発明のポリペプチドは、単独で、またはインヒビン系統群の一メンバ
ーとのヘテロダイマー（ヘテロ二量体）として、雌哺乳動物の受精能を減少させ、雄哺乳
動物の精子形成を減少させるインヒビンの機能に基づいた避妊薬として有用である。十分
量の他のインヒビンを投与することによって、これらの哺乳動物における不妊を誘発でき
る。あるいは、本発明のポリペプチドは、ホモダイマーとして、または他のタンパク質の
インヒビン群サブユニットを持つヘテロダイマーとして、脳下垂体前葉細胞からのFSH
（卵胞刺激ホルモン）遊離の刺激におけるアクチビン分子の機能に基づいた、受精能誘発
治療剤として有用である。例えば、米国特許番号、4,798,885を参照されたい。
本発明のポリペプチドは、また雌牛、羊および豚に限らず、このような家畜類の生涯生殖
能を増加するように、性的に未熟な哺乳動物の受精能の開始を前進させるためにも有用で
あり得る。 30 40

【0207】

本発明のポリペプチドの活性は、他にも方法はあるが、次の方法によって測定できる。

【0208】

アクチビン／インヒビン活性のアッセイには、Vale et al.、Endocri
nology 91:562-572、1972; Ling et al.、Natur
e 321:779-782、1986; Vale et al.、Nature 32
1:776-779、1986; Mason et al.、Nature 318:6
59-663、1985; Forage et al.、Proc. Natl. Acad 50

. Sci. USA 83:3091~3095、1986に記載のアッセイがあり、またこれらに限らない。

【0209】

(5.10.9 走化性／化学運動性活性)

本発明のポリペプチドは、例えば、単球、線維芽細胞、好中球、T細胞、肥満細胞、好酸球、上皮細胞または／および内皮細胞を含む哺乳動物細胞の走化性または化学運動性活性に参与し得る。本発明のポリヌクレオチドは、そのような属性を示すポリペプチドをコードすることができる。走化性および化学運動性レセプターの活性は、作用の望ましい部位へ望ましい細胞集団を動員するか、誘引するために使うことができる。走化性／化学運動性組成物（例えば、タンパク質、抗体、結合パートナー、または発明のモジュレータ）は、局所性感染症の治療においてだけでなく、創傷や他の組織の外傷の治療においても特定の利益を与える。例えば、リンパ球、単球または好中球の、腫瘍または感染部位への誘引によって、腫瘍または感染物質に対する免疫反応が改善される場合がある。

【0210】

タンパク質または、ペプチドは特定の細胞集団に対して、そのような細胞集団の方向または運動を直接または間接的に刺激できる場合、走化性活性を有する。好ましくは、タンパク質またはペプチドは細胞の有向運動を直接刺激する機能を有する。特定のタンパク質が細胞集団に対する走化性活性を有するかどうかは、そのタンパク質またはペプチドに公知の細胞走化性アッセイを行うことによって容易に判定できる。

【0211】

本発明の治療的組成物は以下において用いることができる。

【0212】

走化性活性のアッセイ（走化性を誘導する、または防ぐタンパク質を同定する）は、蛋白質が1つの細胞集団の他の細胞集団への付着を誘導する能力だけでなく、タンパク質が膜内で細胞の遊走を誘導する能力をも検定するアッセイからなる。移動および付着の適切なアッセイにはCurrent Protocols in Immunology、Ed by I. E. Coligan、A. M. Kruisbeek、D. H. Margules、E. M. Shevach、W. Strober、Pub. Greene Publishing Associates and Wiley-Interscience（Chapter 6.12、Measurement of alpha and beta Chemokines 6.12.1-6.12.28；Taub et al. J. Clin. Invest. 95:1370-1376、1995；Lind et al. APMIS 103:140-146、1995；Muller et al. Eur. J. Immunol. 25:1744-1748；Gruber et al. J. of Immunol. 152:5860-5867、1994；Johnston et al. J. of Immunol. 153:1762-1768、1994に記載のアッセイがあり、またこれらに限らない。

【0213】

(5.10.10 止血活性および血栓溶解活性)

本発明のポリペプチドはまた、止血、血栓溶解あるいは血栓症にも関与する場合がある。本発明のポリヌクレオチドは、そのような属性を示すポリペプチドをコードできる。組成物は種々の凝固障害（血友病のような遺伝的疾患を含む）の治療において、または外傷、手術あるいは他の原因から生じた創傷の治療において凝固や他の止血を促進させるために有用である。本発明の組成物はまた、血栓の溶解または形成抑制およびそれから生じる状態（例えば、心臓や中枢神経血管の梗塞（例えば、脳卒中）のような）の治療や予防にも有用である。

【0214】

本発明の治療的組成物は以下において使用できる。

【0215】

止血や血栓溶解活性のアッセイには、Lin et al.、J. Clin. Pha 50

rmacol. 26:131-140、1986; Burdick et al.、Thrombosis Res. 45:413-419、1987; Humphrey et al.、Fibrinolysis 5:71-79 (1991); Schaub、Prostaglandins 35:467-474、1988に記載のアッセイがあり、またこれに限らない。

【0216】

(5.10.11 癌の診断および治療)

本発明のポリペプチドは癌細胞の産生、増殖または転移に関与し得る。本発明のポリヌクレオチドまたはポリペプチドの存在または量の検出は、1種以上のタイプの癌の診断および／または予知に有用であり得る。例えば、本発明のポリヌクレオチド／ポリペプチドの存在または増加した発現は、癌の遺伝の危険性、前癌性状態または進行性悪性腫瘍を示し得る。逆に、遺伝子における欠損、またはこのポリペプチドの不在は、癌状態に関連し得る。また、癌または癌の素因に関連する単一ヌクレオチド多型の同定は、診断あるいは予測にも役立ち得る。

【0217】

癌の治療では、腫瘍細胞増殖の阻害、血管新生（腫瘍増殖の補助に必要な新血管の成長）の阻害および／または腫瘍細胞の運動性や侵襲性を減らすことによる転移の予防によって、腫瘍の退行を促進する。本発明の治療的組成物は成人および小児腫瘍において有効であり得、これには、固相腫瘍／悪性腫瘍、局所進行性腫瘍、ヒト軟部組織肉腫、転移性癌（リンパ性転移を含む）、血球悪性腫瘍（多発性骨髄腫、急性および慢性白血病、ならびにリンパ腫を含む）、頭部および頸部の癌（口癌、喉頭癌、甲状腺癌を含む）、肺癌（小細胞癌、および非小細胞癌を含む）、乳癌（小細胞癌および管性癌を含む）、消化管癌（食道癌、胃癌、大腸癌、結腸直腸および結腸直腸異常増殖に合併するポリープを含む）、膵臓癌、肝臓癌、泌尿器癌（膀胱癌および前立腺癌を含む）、女性生殖器官の悪性腫瘍（卵巣癌、子宮（子宮内膜を含む）癌、卵胞の固形腫瘍を含む）、腎臓癌（腎細胞癌を含む）、脳腫瘍（内因性脳腫瘍を含む）、神経芽細胞腫、アストロサイト（星状細胞）脳腫瘍、神経膠腫、中神経系における転移腫瘍細胞侵襲を含む脳腫瘍、骨癌（骨腫を含む）、皮膚癌（悪性黒色腫（メラノーマ）、ヒト皮膚ケラチノサイトの腫瘍進行、扁平上皮細胞癌、基底細胞癌、血管外皮細胞腫およびカポジ（Kaposi）肉腫を含む）が挙げられる。

【0218】

本発明のポリペプチド、ポリヌクレオチドまたはポリペプチドのモジュレータ（本発明のポリペプチドの生物活性のインヒビターおよび刺激物質を含む）は癌の治療のために投与され得る。治療的組成物は、単独に、または手術、化学療法、放射線療法、温熱療法およびレーザー療法のような癌の補助的療法と併せて治療的有效用量で投与することができ、有利な効果（例えば、腫瘍のサイズを減少させる効果、腫瘍の成長速度を低下させる効果、転移を阻害する効果、または他に、全体的な臨床状態を改善する効果）を、癌を必ずしも根絶させないで提供し得る。

【0219】

本組成物はまた、抗癌カクテルの一部として治療的有效量を投与することができる。抗癌カクテルは、本発明のポリペプチドまたはモジュレータと薬学的に受容可能な送達用キャリアに一種以上の抗癌剤を加えた配合剤である。癌の治療として抗癌カクテルを使用することは、日常的になっている。本発明のポリペプチドまたはモジュレータと組み合わせて治療として使用できる技術上周知の抗癌剤には、アクチノマイシンD、アミノグルテチミド、アスパラギナーゼ、ブレオマイシン、ブスルファン、カルボプラチン、カルムスチン、クロラムブチル、シスプラチン、（cis-DDP）、シクロホスファミド、塩酸シタラビン（シトシンアラビノシド）、ダカルバジン、ダクチノマイシン、塩酸ダウノルビシン、塩酸ドキソルビシン、エストラムスチン、リン酸ナトリウム、エトポシド（V16-213）、フロクスウリジン、5-フルオロウラシル（5-Fu）、フルタミデ、ヒドロキシウレア（ヒドロキシカルバミド）、イホスファミド、インターフェロンアルファ-2a

、インターフェロンアルファ-2b、酢酸リユープロライド（LHRH-遊離因子類似体）、ロムスチン、塩酸メクロレタミン（ナイトロジェンマスタード）、メルファラン、メルカプトプリン、メスナ、メトトレキセート（MTX）、マイトマイシン、塩酸ミトキサントロン、オクトレオチド、プリカマイシン、塩酸プロカルバジン、ストレプトゾシン、クエン酸タモキシフェン、チオグアニン、チオテパ、硫酸ビンブラスチン、硫酸ビンクリスチン、アムサクリン、アザシチジン、ヘキサメチルメラニン、インターロイキン-2、ミトグアゾン、ペントスタチン、セムスチン、テニポシデ、およびビンデシン硫酸がある。

【0220】

さらに、本発明の治療的組成物は、癌の予防的治療用に使うことができる。個体が癌になりやすい、技術上周知の遺伝的状態および／または環境状態（例えば、発癌物質への暴露）がある。これらの環境において、癌になる危険を少なくするために、治療的有効量の本発明のポリペプチドでこれらの個体を治療することは、有利な場合がある。

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【0221】

インビトロモデルを用いて、強力な癌の治療としての本発明のポリペプチドの有効量を測定することができる。これらのインビトロモデルには、培養腫瘍細胞の増殖アッセイ、軟寒天における培養腫瘍細胞の増殖（Freshney, (1987) Culture of Animal Cells: A manual of Basic Technique, Wiley-Liss, New York, NY Ch 18 and Ch 21を参照）、Giovanello et al., J. Natl. Can. Inst, 52: 921~30 (1974)に記載のヌードマウスにおける腫瘍システム、Pilkington et al., Anticancer Res., 17: 4107-9 (1997)に記載のBoyden Chamberアッセイにおける腫瘍細胞の移動性と侵襲性の可能性、ならびにRibatta et al., Intl. J. Dev. Biol., 40: 1189-97 (1999)およびLi et al., Clin. Exp. Metastasis, 17: 423-9 (1999)に各々記載の鶏絨毛尿膜の血管新生の誘導または血管内皮細胞遊走の誘導のような血管形成のアッセイがある。適切な腫瘍細胞系は、例えば、American Type Tissue Culture Collection カタログから入手できる。

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【0222】

（5.10.12 レセプター／リガンドの活性）

本発明のポリペプチドはまた、レセプター／リガンド相互作用の、レセプター、レセプターリガンドもしくはインヒビター、またはアゴニスト（作動薬）としての活性を示す。本発明のポリヌクレオチドはこのような特性を示すポリペプチドをコードし得る。このようなレセプターおよびリガンドの例には、サイトカインレセプターとそのリガンド、レセプターキナーゼとそのリガンド、レセプターホスファターゼとそのリガンド、細胞間相互作用に参与するレセプターとそのリガンド（セレクチン、インテグリンおよびそれらのリガンドのような）細胞接着分子をこれに限らず含む）、ならびに細胞性免疫応答および体液性免疫応答の抗原提示、抗原認識および進展に参与する、レセプター／リガンド対が挙げられるが、これらに限らない。レセプターとリガンドはまた、関連するレセプター／リガンド相互作用の可能性があるペプチドまたは小分子インヒビターのスクリーニングにも役立つ。本発明のタンパク質（レセプターおよびリガンドのフラグメントをこれらに限らず含む）は、それ自体レセプター／リガンド相互作用のインヒビターとして有用であり得る。

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【0223】

本発明のポリペプチドの活性は、他に方法もあるが、以下の方法によって測定できる。

【0224】

レセプターリガンド活性の適切なアッセイには、Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strob

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er、Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868、1987: Brierer et al., J. Exp. Med. 168:1145-1156、1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68、1994; Stitt et al., Cell 80:661-670、1995に記載のアッセイがあり、またアッセイはこれらに限らない。 10

【0225】

例として、本発明のポリペプチドは、1つまたは複数のリガンドのレセプターとして使われ、それによってそのリガンドの生物活性が伝達される。リガンドは結合アッセイ、アフィニティークロマトグラフィー、ダイハイブリッドスクリーニングアッセイ、BIAコアアッセイ、ゲルオーバーレイアッセイまたは技術上周知の他の方法によって同定できる。

【0226】

薬物またはタンパク質を、アゴニスト（作動薬）か、またはアンタゴニスト（拮抗薬）、あるいは部分的アゴニストか、または部分的アンタゴニストとして特徴付けする試験には、競合的リガンドとしての他のタンパク質を使う必要がある。本発明のポリペプチドまたはそのリガンドは、従来の方法によって放射性同位元素、比色定量分子または毒素分子に結合されて標識される（"Guide to Protein Purification" Murray P. Deutscher (ed) Methods in Enzymology Vol. 182 (1990) Academic Press, Inc. San Diego）。放射性同位元素の例としては、トリチウムや炭素-14があるが、これらに限定されない。比色定量分子の例には、フルオレサミンのような蛍光分子、またはローダミンや他の比色定量分子があり、またこれらに限定されない。毒素の例には、リシンがあり、またこれに限定されない。 20

【0227】

(5.10.13 薬物スクリーニング)

本発明は様々な薬物スクリーニング技法のいずれかにおいて、新しいポリペプチドまたはその結合フラグメントを用いて、化合物をスクリーニングするのに特に役立つ。このような試験に用いるポリペプチドまたはフラグメントは、溶液中に遊離しているか、固体支持体に付着しているか、細胞表面に保たれるか、または細胞内にあるかのいずれかであり得る。薬物スクリーニングの1つの方法では、真核または原核宿主細胞を利用し、これらはポリペプチドまたはそのフラグメントを発現する組み換え核酸で安定に形質転換される。薬物は、競合結合アッセイで、このような形質転換細胞に対してスクリーニングされる。生存能力のある、または固定化されたいずれかの型のこのような細胞は、標準結合アッセイに使うことができる。例えば、本発明のポリペプチドまたはフラグメントとテストされた薬剤との間の複合体形成を測定するか、または新規のポリペプチドと技術上周知の適切な細胞系との間の複合体形成における減少を調べることができる 30 40

結合能力、または本発明のポリペプチドの活性を変調する（すなわち、増加または減少させる）能力についてスクリーニングできる試験化合物の供給源として、（1）無機および有機化合物ライブラリー、（2）天然物ライブラリーおよび（3）ランダムまたは模倣ペプチド、オリゴヌクレオチド、または有機分子のいずれかを含むコンビナトリアルライブラリーがある。

【0228】

ケミカルライブラリーは多くの市販源から容易に合成または購入でき、また、これには既知の化合物の構造的類似体、または天然物スクリーニングを介して「ヒット」または「リード」と同定されている化合物が含まれ得る。

【0229】

天然物ライブラリーの供給源は、微生物（細菌や真菌を含む）、動物、植物もしくは他の植生、または海洋生物、およびスクリーニング用混合のライブラリーは、（１）土壌、植物または海洋微生物からのブrossの発酵および抽出、または（２）微生物自体からの抽出によって作製できる。天然物ライブラリーには、ポリケチド、非リボソームペプチドおよびその（非天然性発生）変異体がある。総説は、Science 282:63-68 (1998)を参照されたし。

【0230】

コンビナトリアルライブラリーは、多数のペプチド、オリゴヌクレオチドまたは有機化合物を含み、従来の自動合成法、PCR、クローニングまたは特許化された合成方法によって容易に作成することができる。ペプチドとオリゴヌクレオチドコンビナトリアル・ライブラリーが特に興味深い。なお、この他に興味深いライブラリーには、ペプチド、タンパク質、ペプチド模倣、多平行型合成収集、リコンビナトリアル、およびポリペプチドライブラリーがある。コンビナトリアルケミストリーとそれから作成されたライブラリーの総説は、Myers, Curr. Opin. Biotechnol. 8:701-707 (1997)を参照されたし。ペプチド模倣ライブラリーの総説および例は、Al-Obeidi et al., Mol. Biotechnol. 9(3):205-23 (1998); Hruby et al., Curr Opin Chem Biol. 1(1):114-19 (1997); Dörner et al., Bioorg Med Chem. 4(5):709-15 (1996)（アルキル化ジペプチド）を参照されたし。

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【0231】

本明細書中に記載する種々のライブラリーを使用してモジュレータを同定することによって、本発明のポリペプチドに結合する「ヒット」の能力を最適化するための「ヒット」（または「リード」）候補の修飾（変更）が可能になる。次に、結合アッセイで同定された分子を、インビボ組織培養または技術上周知の動物モデルにおけるアンタゴニストまたはアゴニスト活性について測定を行う。要するに、分子を多数の細胞培養または動物に滴定して、次に細胞／動物の死亡または細胞／動物の生存延長のいずれかについてテストする。

【0232】

このように、同定された結合分子は、毒素（例えば、リシンまたはコレラ）と複合体を作るか、または放射性同位元素のような細胞に対して毒性のある他の化合物と複合体を作る。次に、毒素結合分子複合体は、結合分子の本発明のポリペプチドに対する特異性によって腫瘍または他の細胞を標的にする。あるいは、結合分子は標的化および映像目的のためのイメージング（画像）剤と複合体を作る。

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【0233】

（5.10.14 レセプター活性のアッセイ）

本発明はまた、ポリペプチド（例えばリガンドまたはレセプター）の特異的結合を検出する方法をも提供する。この技術は、以前は不明であった、本発明のレセプターポリペプチドの結合パートナーの同定に、特に役立つ多くのアッセイを提供する。例えば、哺乳類または細菌の細胞、あるいはダイハイブリッドスクリーニングアッセイを用いた発現クローニングは、結合パートナーをコードするポリヌクレオチドの同定に使うことができる。他の例として、適切に固定化された本発明のポリペプチドを用いたアフィニティークロマトグラフィーは、本発明のポリペプチドを認識し、そして結合するポリペプチドを単離するために使用することができる。本発明のポリペプチドの生物活性を変調する（つまり、増加または減少する）化合物、特に小分子の同定に用いられる多くの異なったライブラリーがある。本発明のレセプターポリペプチド用リガンドは、本発明のレセプターの発現以外は遺伝学的に同じである２種の細胞集団に、外来性リガンドまたはリガンドのカクテルを加えることによって同定できる。１種の細胞集団は本発明のレセプターを発現するが、他の細胞集団は発現しない。次いで、加えられたリガンドに対する２種の細胞集団の応答を比較する。あるいは、発現ライブラリーは、細胞内で本発明のポリペプチドと同時発現さ

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せることができ、強力なリガンドを同定するためにオートクライン応答についてアッセイすることができる。さらに、他の例として、B I A コア 検 定、ゲルオーバーレイまたは技術的に周知の他の方法を用いて結合パートナーのポリペプチドを同定できるが、これには、(1) 有機および無機化学ライブラリー、(2) 天然物ライブラリーおよび(3) ランダムペプチド、オリゴヌクレオチドまたは有機分子のようなコンビナトリアルライブラリーが含まれる。

【0234】

下流細胞内情報伝達分子の、本発明のポリペプチドの情報伝達カスケードにおける役割は決定できる。例えば、本発明のポリペプチドの細胞質内ドメインが、リガンドが同定されているタンパク質の細胞外部分に融合したキメラタンパク質は、宿主細胞内で産生される。次に、この細胞をキメラタンパク質の細胞外部分に特異的なリガンドと一緒に培養し、それによってキメラレセプターを活性化する。次に、細胞内情報伝達に参与する既知の下流タンパク質は、予測される修飾、すなわち、リン酸化についてアッセイできる。また、技術上公知の他の方法も、また、レセプター活性に参与する情報伝達分子の同定に使用できる。

【0235】

(5.10.15 抗炎症活性)

本発明の組成物は、また抗炎症活性をも示す。抗炎症活性は、炎症反応に参与する細胞に刺激を与えるか、細胞間相互作用(例えば、細胞接着のような)を阻害または促進するか、炎症過程に参与する細胞の走化性を阻害または促進するか、細胞の血管外遊走を阻害または促進するか、あるいは、炎症応答をより直接的に阻害または促進する他の因子の産出を刺激または抑制することによって達成され得る。このような活性を持つ組成物は、感染(敗血症性ショック、敗血症または全身性炎症反応症候群(SIRS))、虚血再灌流傷害、菌体内毒素致死、関節炎、補体媒介超急性拒絶、腎炎、サイトカインまたはケモカイン誘導性肺傷害、炎症性腸疾患、クローン病、あるいはTNFやIL-1のようなサイトカインの産出過剰から生じる疾患に関連する、慢性または急性炎症状態の治療に、これらの適用に限らず用いることができる。本発明の組成物はまた、抗原性物質または抗原性材料に対するアナフィラキシーまたは過敏症の治療にも有用である。本発明の組成物は、敗血症、急性膵炎、エンドトキシンショック、サイトカイン性ショック、慢性関節リウマチ、慢性炎症性リウマチ、真性糖尿病タイプ1による膵臓細胞障害、移植片対宿主病、炎症性腸疾患、肺疾患に合併する炎症、他の自己免疫疾患もしくは炎症性疾患のような状態を予防または治療するために、急性もしくは慢性骨髄性白血病の抗増殖性剤として、または子宮内感染症の前段階における早期分娩の予防において使用することができ、また使用はこれらに限らない。

【0236】

(5.10.16 白血病)

白血病および関連障害は、本発明のポリヌクレオチドおよび/またはポリペプチドの機能を促進または阻害する治療剤を投与することによって治療または予防することができる場合がある。このような白血病および関連障害には、急性白血病、急性リンパ性白血病、急性骨髄球性白血病、骨髄芽球性、前骨髄球性、骨髄単球性、単球性、赤白血病、慢性白血病、慢性骨髄球(顆粒球)性白血病、および慢性リンパ性白血病(これらの疾患の総説については、Fishman et al.、1985、Medicine、2d Ed.、J. B. Lippincott Co.、Philadelphia参照)があり、またこれらに限らない。

【0237】

(5.10.17 神経系障害)

本発明のポリペプチドおよび/またはポリヌクレオチドの活性を変調する化合物の介入の効力をテストできる細胞タイプに関連し、またしたがって、治療的有用性の指標を見ながら治療できる神経系障害には、神経系損傷、およびアクソンの連絡切断、神経細胞の退縮もしくは変性、または脱髄のいずれかを生じる疾患または障害が、これらに限らず含まれ

る。本発明にしたがって患者（ヒトおよびヒト以外の哺乳動物を含む）において治療される神経系病変には、以下の、中枢（脊髄、脳を含む）または末梢神経系のどちらかの病変が含まれるが、これらに限らない：

（i）物理的傷害により生じるか、または手術（例えば、神経系の一部を切断する病変または圧迫損傷）に関連する病変を含む外傷性病変；

（i i）脳梗塞または脳虚血、あるいは脊髄の梗塞または虚血などの神経系の一部における酸素欠乏により、神経細胞損傷または死亡にいたる虚血性病変；

（i i i）神経系の一部が、例えば、膿瘍による感染の結果として破壊もしくは損傷を受けるか、あるいはヒト免疫不全症ウイルス、帯状疱疹、もしくは単純疱疹ウイルスによる感染、またはライム病、結核、梅毒に関連する、感染性病変；

（i v）パーキンソン病、アルツハイマー病、ハンチントン舞踏病または筋萎縮性側索硬化症に関連する変性に限らず、これらを含む変性過程の結果として、神経系の一部が破壊または損傷する変性（退縮）病変；

（v）栄養障害または代謝障害によって神経系の一部が破壊または損傷する、栄養疾患または栄養障害に関連する病変であって、ビタミンB12欠乏症、葉酸欠乏症、ウエルニッケ病、タバコ・アルコール性弱視、マルキャファーヴァ・ビニャミ病（脳梁の一次変性）およびアルコール性小脳変性が含まれるが、これらに限らない、病変；

（v i）全身性疾患に関連する神経性病変であって、糖尿病（糖尿病性神経障害、顔面麻痺）、全身性エリトマトーデス、癌または肉腫症が含まれるが、これらに限らない、神経性病変；

（v i i）アルコール、鉛または特定の神経毒を含む毒性物質によって生じた病変；

（v i i i）脱髄疾患によって神経系の一部が破壊または損傷する脱髄病変であって、多発性硬化症、ヒト免疫不全症ウイルス関連脊髄症、横断性脊髄症、種々の病因論、進行性多病巣性白質脳障害、および中央脳橋ミエリン溶解が含まれるが、これらに限らない、脱髄病変。

【0238】

本発明にしたがった有用な神経系疾患用治療剤は、神経細胞の生存または分化の促進についての生物活性を試験することによって選択できる。例えば、制限としてではなく、次の作用のいずれかを誘発する治療剤は本発明に従って、有用である：

（i）培養液において神経細胞の増加した生存時間；

（i i）培養液またはインビボにおいて、神経細胞の増加した出芽；

（i i i）培養液またはインビボにおいて、神経細胞関連分子（例えば、運動ニューロンに関連するコリンアセチルトランスフェラーゼまたはアセチルコリンエステラーゼ）の増加した産生；

（i v）インビボでの神経細胞機能障害の症状の減少。

【0239】

このような効果は技術上公知のいずれの方法によっても測定できる。好ましくは、実施例に限らず、神経細胞の生存率増加は、Arakawa et al.（1990、J. Neurosci. 10：3507-3515）に示される方法によって測定でき；神経細胞の出芽の増加は、Pestronk et al.（1980、Exp. Neurol. 70：65-82）またはBrown et al.（1981、Ann. Rev. Neurosci. 4：17-42）に示される方法によって検出でき；神経細胞関連分子の産生増加は、測定される分子に依存して、バイオアッセイ、酵素学的アッセイ、結合抗体、ノーザンブロットアッセイなどで測定でき；そして、運動ニューロン機能不全は、運動ニューロン障害（例えば、脱力感、運動ニューロン伝達速度または機能的な能力障害）の物理的症状の評価によって測定できる。

【0240】

特定の実施形態において、本発明に従って治療される運動ニューロン障害には、運動神経細胞および神経系の他の成分に影響を与え得る障害（梗塞、感染症、毒素への暴露、外傷、外科的損傷、変性疾患または悪性腫瘍）が挙げられるが、これに限定されず、そして筋

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萎縮性側索硬化症のような神経細胞に選択的に影響する障害には、進行性脊髄性筋萎縮症、進行性球麻痺、原発性側索硬化症、乳児性、若年性筋萎縮、幼児期の進行性球麻痺（ファチオ・ロンデ症候群）、灰白髄炎およびポリオ後症候群、ならびに遺伝性運動感覚ニューロパシー（シャルコー・マリー・トゥース病）が挙げられるが、これらに限定されない。

【0241】

（5.10.18 その他の活性）

本発明のポリペプチドはまた、さらに、次のような活性または作用のうちの1つ以上を示す：細菌、ウイルス、真菌および他の寄生虫をこれらに限らず含む、感染性物質の成長、感染もしくは機能を阻害または抑止すること；身長、体重、髪の色、眼の色、皮膚、脂肪の細い部分に対する割合もしくは他の組織色素沈着、または臓器もしくは身体部分のサイズもしくは形（例えば、乳房の増大もしくは縮小、または骨の形態もしくは形状における変化のような）をこれらに限らず含む身体的特徴に作用（抑制または亢進）すること；男性または女性の受精能に作用すること；食餌脂肪、脂質、タンパク質、炭水化物、ビタミン、ミネラル、補助因子、他の栄養的因子または成分の、代謝、異化、同化、処理、利用、保存、または排出に影響を与えること；食欲、性欲、ストレス、認知（認知障害を含む）、抑鬱（鬱病性障害を含む）および狂暴症をこれらに限らず含む行動性特徴に作用する、鎮痛効果または他の痛み減少作用を与える、造血系以外の系における胚幹細胞の分化および成長を促進すること；ホルモンまたは内分泌の活性化；酵素の場合、酵素の欠乏症を修正すること、および欠乏関連性疾患を治療すること；過剰増殖疾患（例えば、乾癬のような疾患）の治療；免疫グロブリン様活性（例えば、抗原または補体を結合する能力のような活性）；ならびにワクチン成分において抗原として作用して、そのようなタンパク質、または、そのようなタンパク質と交差反応する他の物質もしくは実体に対して免疫反応を促進すること。

【0242】

（5.10.19 多型の同定）

多型の実証は、ヒト被験体におけるこのような多型の同定ならびに診断および処置のためのこの情報の薬理学的使用を可能にする。このような多型は、例えば、種々の疾患状態（例えば、炎症または免疫応答に関連する障害）に対する差次的な素因または感受性あるいは薬物投与に対する差次的応答に関連し得、そしてこの遺伝情報は、予防的または治療的処置を適切に合わせるために使用され得る。例えば、炎症または自己免疫疾患に対する素因に関連する多型の存在は、その多型の存在を同定することによって、ヒトにおけるこの状態の診断を可能にする。

【0243】

多型は、当該分野で公知の種々の方法で同定され得、これら全ては、一般に、患者からサンプルを得る工程、そのサンプル由来のDNAを分析する工程（必要に応じて、DNAの単離または増幅を含む）、およびそのDNAにおける多型の存在を同定する工程を包含する。例えば、PCRを使用して、ゲノムDNAの適切なフラグメントを増幅し得、次いで、このゲノムDNAを配列決定し得る。あるいは、DNAを、対立遺伝子特異的オリゴヌクレオチドハイブリダイゼーション（ここでは、適切なオリゴヌクレオチドが、一塩基ミスマッチの検出を可能にする条件下で、そのDNAにハイブリダイズされる）または一ヌクレオチド伸長アッセイ（ここでは、多型の位置に直ぐ隣接してハイブリダイズするオリゴヌクレオチドが、1以上の標識されたヌクレオチドで伸長される）に供し得る。さらに、従来の制限フラグメント長多型分析（多型の存在または非存在に依存してゲノムDNAの差次的な消化を提供する制限酵素を使用する）を、行い得る。本発明のヌクレオチド配列を有するアレイを使用して、多型を検出し得る。このアレイは、本発明の改変されたヌクレオチド配列を検出するために、本発明のこれらのヌクレオチド配列を含み得る。代替的に、本発明のヌクレオチド配列のうちの任意の1つが、それらの配列由来の変化を検出するために、アレイ上に配置され得る。

【0244】

あるいは、アミノ酸配列における変化を生じる多型は、そのタンパク質のアミノ酸配列における対応する変化を検出することによって（例えば、改変体配列に特異的な抗体による）、検出され得る。

【0245】

（5.10.20 関節炎および炎症）

慢性関節リウマチに対する本発明の組成物の免疫抑制効果は、実験動物モデル系において決定される。この実験モデル系は、ラットにおけるアジュバント誘導性関節炎であり、そしてこのプロトコルは、J. Holoshitzら、1983, Science, 219: 56またはB. Waksmanら、1963, Int. Arch. Allergy Appl. Immunol., 23: 129によって記載される。この疾患の誘導は、完全フロイントアジュバント（CFA）中の死化Mycobacterium tuberculosisの懸濁液の単回注射（一般には、経皮的に）によって引き起こされ得る。注射の経路は、様々であり得るが、ラットは、その尾の基部にアジュバント混合物を注射され得る。ポリペプチドを、約1~5mg/kgの用量でリン酸緩衝化生理食塩水（PBS）中で投与する。コントロールは、PBSのみの投与からなる。

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【0246】

試験化合物の効果を試験するための手順は、CFA中の死化Mycobacterium tuberculosisの経皮注射、その直後の試験化合物の投与、およびその後の24日目までの1日おきの処置からなる。Mycobacterium CFAの注射後14日目、15日目、18日目、20日目、22日目および24日目に、全体的関節炎スコアを、上記のJ. Holoshitzに記載のように獲得し得る。これらのデータの分析は、その試験化合物が、この関節炎スコアの減少によって測定されるように、関節の隆起に対する劇的な影響を有することを示す。

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【0247】

（5.11 治療方法）

本発明の組成物（ポリペプチドフラグメント、アナログ、改変体および抗体、あるいは他の結合パートナーまたはモジュレーター（アンチセンスオリゴヌクレオチドを含む））は、種々の治療方法における多くの適用を有する。治療適用の例としては、本明細書中に例示される適用が挙げられるが、これらに限定されない。

【0248】

（5.11.1 実施例）

本発明の1つの実施形態は、本発明のプロトロンビナーゼ様ポリペプチドまたは他の組成物の有効量を、本発明の可溶性プロトロンビナーゼ様レセプターを調節することによって調節され得る疾患または障害に罹患する個体に対して投与することである。投与形態は、特に重要ではないが、非経口投与が、好ましい。例示的な投与形態は、静脈内ボラスを送達することである。本発明のプロトロンビナーゼ様ポリペプチドまたは他の組成物の投薬量は、通常、処方医によって決定される。この投薬量は、その個体患者の年齢、体重、状態および応答に従って変化することが予測される。代表的には、1用量あたりに投与されるポリペプチドの量は、約0.01μg/kg体重~100mg/kg体重の範囲であり、好ましく用量は、約0.1μg/kg患者体重~10mg/kg患者体重である。非経口投与のために、本発明のプロトロンビナーゼ様ポリペプチドは、薬学的に受容可能な非経口ビヒクルと合わせた注射形態に処方される。このようなビヒクルは、当該分野で周知であり、そしてこれらには、水、生理食塩水、リンゲル溶液、デキストロース溶液、および少量のヒト血清アルブミンからなる溶液が挙げられる。ビヒクルは、このポリペプチドまたは他の活性成分の等張性および安定性を維持する、わずかな量の添加剤を含み得る。このような溶液の調製物は、当業者の範囲内である。

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【0249】

（5.12 薬学的処方物および投与経路）

本発明のタンパク質または他の組成物（組換え供給源および非組換え供給源（これらに限定されない）を含む任意の供給源由来であり、抗体および本発明のポリペプチドの他の結

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合パートナーを含む)は、種々の障害を処置または改善するための用量で、それ自体でかまたは薬学的組成物(ここでは、それは、適切なキャリアまたは賦形剤と共に混合される)中で、必要とされる患者に投与され得る。このような組成物は、必要に応じて、(タンパク質または他の活性成分およびキャリアに加えて)希釈剤、充填剤、塩、緩衝液、安定剤、可溶化剤、および当該分野で周知の他の材料を含み得る。用語「薬学的に受容可能」とは、その活性成分の生物学的活性の効果を干渉しない、非毒性の材料を意味する。キャリアの特徴は、投与経路に依存する。本発明の薬学的組成物はまた、サイトカイン、リンホカインまたは他の造血因子(例えば、M-CSF、GM-CSF、TNF、IL-1、IL-2、IL-3、IL-4、IL-5、IL-6、IL-7、IL-8、IL-9、IL-10、IL-11、IL-12、IL-13、IL-14、IL-15、IFN、TNF0、TNF1、TNF2、G-CSF、Meg-CSF、トロンボポイエチン、幹細胞因子およびエリスロポイエチン)を含み得る。さらなる組成物において、本発明のタンパク質は、問題の疾患または障害の処置に有益な他の薬剤と合わせられ得る。これらの薬剤としては、種々の増殖因子(例えば、上皮増殖因子(EGF)、血小板由来増殖因子(PDGF)、トランスフォーミング増殖因子(TGF- α およびTGF- β)、インスリン様増殖因子(IGF))ならびに本明細書中に記載のサイトカインが挙げられる。

【0250】

薬学的組成物はさらに、タンパク質または他の活性成分の活性を増強するかあるいは処置におけるその活性または用途を補足する、他の薬剤を含み得る。このようなさらなる因子および/または薬剤が、薬学的組成物に含まれて、本発明のタンパク質または他の活性成分と相乗効果を生じ得るかまたは副作用を最小化し得る。逆に、本発明のタンパク質または他の活性成分は、特定の凝固因子、サイトカイン、リンホカイン、他の造血因子、血栓崩壊因子または抗血栓崩壊因子、あるいは抗炎症剤の処方物に含まれて、この凝固因子、サイトカイン、リンホカイン、他の造血因子、血栓崩壊因子または抗血栓崩壊因子、あるいは抗炎症剤(例えば、IL-1Ra、IL-1Hy1、IL-1Hy2、抗TNF、コルチコステロイド、免疫抑制剤)の副作用を最小化し得る。本発明のタンパク質は、マルチマー(例えば、ヘテロダイマーまたはホモダイマー)あるいはそれ自体または他のタンパク質との複合体において活性であり得る。結果として、本発明の薬学的組成物は、このようなマルチマーまたは複合体化形態における本発明のタンパク質を含み得る。

【0251】

第1のタンパク質を含む本発明の薬学的組成物に含まれる代替物として、第2のタンパク質または治療剤が、この第1のタンパク質と共に(例えば、同じ時点で、または異なる時点(これらの薬剤の組み合わせの治療濃度がその処置部位で達成される場合)で)投与され得る。即時適用の化合物の処方および投与の技術は、「Remington's Pharmaceutical Sciences」、Mack Publishing Co., Easton, PA(最新版)に見出され得る。治療有効用量はさらに、症状の改善(例えば、関連する医学状態の処置、治癒、予防または改善)あるいはこのような状態の処置、治癒、予防または改善の速度の増加を生じるに十分な化合物の量をいう。単独で投与される個々の活性成分に適用する場合、治療有効用量とは、その成分単独をいう。組み合わせに適用される場合、治療有効用量とは、連続的にかまたは同時にかのいずれで組み合わせ投与されるかに関わらず、治療効果を生じるそれらの活性成分の合わせた量をいう。

【0252】

本発明の処置または使用の方法の実施において、本発明のタンパク質または他の活性成分の治療有効量が、処置される状態を有する哺乳動物に投与される。本発明のタンパク質または活性成分は、本発明の方法に従って、単独でかまたは他の治療(例えば、サイトカイン、リンホカインまたは他の造血因子を使用する処置)との組み合わせで、投与され得る。1以上のサイトカイン、リンホカインまたは他の造血因子と共に共投与する場合、本発明のタンパク質または他の活性成分は、サイトカイン、リンホカイン、他の造血因子、血栓崩壊因子または抗血栓崩壊因子と同時にまたは連続的に投与され得る。連続的に投与

される場合、主治医が、本発明のタンパク質または他の活性成分を、サイトカイン、リンホカイン、他の造血因子、血栓崩壊因子または抗血栓崩壊因子と組み合わせて投与する適切な順序を決定する。

【0253】

(5.12.1 投与経路)

適切な投与経路には、例えば、以下が挙げられ得る：経口投与、直腸投与、経粘膜投与または腸投与；非経口送達（筋内注射、皮内注射、髄内注射、ならびに髄腔内注射、直接的な脳室内注射、静脈内注射、腹腔内注射、鼻内注射または眼内注射を含む）。薬学的組成物中においてあるいは本発明の方法を実施するために使用される本発明のタンパク質または他の活性成分の投与は、種々の従来の様式（例えば、経口摂取、吸入、局所適用、ある

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【0254】

あるいは、関節炎性の関節または線維症組織への化合物の直接的な注射を介して（しばしば、デポー処方物または持続放出处方物にて）、全身様式よりむしろ局所様式で化合物を投与し得る。緑内障手術の合併症として頻繁に起こる瘢痕プロセスを防止するために、この化合物は、例えば、点眼剤として局所的に投与され得る。さらに、例えば、関節炎または線維症組織を標的とする特定の抗体でコーティングしたリポソームの形態の、標的薬物送達システムの形態で、この薬物を投与し得る。このリポソームは、罹患組織に標的化され、そしてこの罹患組織によって選択的に取り込まれる。

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【0255】

本発明のポリペプチドは、作用の所望の部位に有効な投薬量を送達する任意の経路によって投与される。投与の適切な経路および特定の症状のために有効な投薬量の決定は、当業者のレベルの範囲に含まれる。好ましくは、創傷処置のために、治療化合物が部位に直接投与される。本発明のポリペプチドの適切な投薬量範囲は、これらの投薬量または適切な動物モデルにおける類似の研究から推定し得る。次いで、投薬量は、最大の治療効果を提供するように医師によって必要に応じて調整され得る。

【0256】

(5.12.2 組成物／処方物)

従って、本発明に従って使用するための薬学的組成物は、薬学的に利用される調製物への活性化化合物の加工を容易にする1つ以上の薬学的に受容可能なキャリア（賦形剤および補助剤を含む）を使用する従来の様式で処方され得る。これらの薬学的組成物は、それ自体が公知の様式（例えば、従来の混合、溶解、顆粒化、糖衣剤作製、微粒子化、乳化、カプセル化、封入または凍結乾燥プロセスによって）で製造され得る。適切な処方物は、選択された投与経路に依存する。治療的有效量の本発明のタンパク質または他の活性成分が経口投与される場合、本発明のタンパク質または他の活性成分は、錠剤、カプセル、散剤、溶液またはエリキシル剤の形態である。錠剤形態で投与される場合、本発明の薬学的組成物は、固体キャリア（例えば、ゼラチンまたはアジュバント）をさらに含み得る。錠剤、カプセル剤および散剤は、約5～95%の本発明のタンパク質または他の活性成分、および必要に応じて、約25～95%の本発明のタンパク質または他の活性成分を含む。液体

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【0257】

治療的有效量の本発明のタンパク質または他の活性成分が静脈内注射、皮膚注射または皮下注射によって投与される場合、本発明のタンパク質または活性成分は、発熱物質を含

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ない経口的に受容可能な水溶液の形態である。pH、等張性、および安定性に起因して、このような経口的に受容可能なタンパク質または他の活性成分の溶液の調製は当業者の範囲内である。静脈内注射、皮膚注射または皮下注射に好ましい薬学的組成物は、本発明のタンパク質または他の活性成分に加えて、等張性ビヒクル（例えば、塩化ナトリウム注射液、リンガー液、デキストロース注射液、デキストロース+塩化ナトリウム注射液、乳酸加リンガー液、または当該分野で公知の他のビヒクル）を含まなければならない。本発明の薬学的組成物はまた、安定剤、防腐剤、緩衝剤、酸化防止剤または他の当該分野で公知の添加剤を含み得る。注射について、本発明の薬剤は、水溶液の形態、好ましくは薬学的に適合性の緩衝液（例えば、ハンクス溶液、リンガー溶液、または生理食塩緩衝液）の形態で処方され得る。経粘膜投与について、浸透されるバリアに適切な浸透剤がこの処方物に使用される。このような浸透剤は一般的に当該分野で公知である。 10

【0258】

経口投与について、この化合物は、活性成分を当該分野で周知の薬学的に受容可能なキャリアと組み合わせることによって容易に処方され得る。このようなキャリアにより、本発明の化合物は、錠剤、丸剤、糖衣剤、カプセル剤、液体、ゲル、シロップ、スラリー、懸濁液などとして、処置される患者によって経口摂取のために処方され得る。経口用途のための薬学的調製物は、固体賦形剤で得られ、必要に応じて得られた混合物を顆粒化し、この下流の混合物を処理し、必要に応じてその後適切な賦形剤に添加し、錠剤または糖衣コアを得る。適切な賦形剤は、特に、フィラー（例えば、ラクトース、スクロース、マンニトールまたはソルビトールを含む糖）；セルロース調製物（例えば、トウモロコシデンプン、小麦デンプン、米デンプン、ジャガイモデンプン、ゼラチン、トラガカントゴム、メチルセルロース、ヒドロキシプロピルメチルセルロース、ナトリウムカルボキシメチルセルロース）、および／またはポリビニルピロリドン（PVP）である。所望ならば、崩壊剤（例えば、架橋ポリビニルピロリドン、寒天、またはアルギニン酸、またはアルギニン酸ナトリウムのようなそれらの塩）が添加され得る。糖衣コアは、適切なコーティングを用いて提供される。この目的のために、濃縮された糖溶液が使用され、これは必要に応じて、アラビアゴム、タルク、ポリビニルピロリドン、カルボポール（carbopol）ゲル、ポリエチレングリコール、および／または二酸化チタン、ラッカー溶液、および適切な有機溶媒および溶媒混合物を含み得る。染料または顔料は、同定のためまたは活性化化合物用量の異なる組み合わせの特徴付けのために錠剤または糖衣コーティングに添加され得る。 20 30

【0259】

経口で使用され得る薬学的調製物は、ゼラチンから作製されたプッシュフィットカプセルならびにゼラチンおよび可塑剤（例えば、グリセロールまたはソルビトール）から作製された軟質密閉カプセルを含む。このプッシュフィットカプセルは、フィラー（例えば、ラクトース）、結合剤（例えば、デンプン）、および／または潤滑剤（例えば、タルクまたはステアリン酸マグネシウム）、ならびに必要に応じて安定剤を混合された活性成分を含み得る。軟質カプセルにおいて、この活性化合物は、適切な液体（例えば、脂肪油、液体パラフィン、または液体ポリエチレングリコール）に溶解または懸濁され得る。さらに、安定剤が添加され得る。経口投与のための全ての処方物は、このような投与に適切な投薬量出なければならない。経頬粘膜投与について、この組成物は、従来の様式で処方される錠剤またはトロッチ剤の形態をとり得る。 40

【0260】

吸入による投与について、本発明に従って使用するための化合物は、適切な噴霧剤（例えば、ジクロロジフルオロメタン、トリクロロフルオロメタン、ジクロロテトラフルオロエタン、二酸化炭素または他の適切な気体）を使用して圧縮パックまたはネブライザーにより提供されるエアロゾルスプレーの形態で簡便に送達される。圧縮したエアロゾルの場合、投薬単位は、測定量を送達するバルブを提供することによって決定され得る。例えば、吸入器（inhalerまたはinsufflator）における使用のためのゼラチンのカプセルおよびカートリッジが、この化合物と適切な粉末基剤（例えば、ラクトースま 50

たはデンプン)の混合物を含んで処方され得る。これらの化合物は、注射(例えば、ボーラス入射または連続注入)による非経口投与のために処方され得る。注射のための処方物は、保存剤を添加した単位投薬形態(例えば、アンプル)または複数用量容器の形態で提供され得る。これらの組成物は、油性ビヒクルまたは水性ビヒクル中の懸濁液、溶液またはエマルジョンのような形態をとり得、そして処方剤(例えば、懸濁剤、安定剤および/または分散剤)を含み得る。

【0261】

非経口投与のための薬学的処方物は、水溶性形態の活性化合物の水溶液を含む。さらに、活性化合物の懸濁液は、適切な油性注射懸濁液として調製され得る。適切な親油性溶媒またはビヒクルとしては、脂肪油(例えば、胡麻油)、または合成脂肪酸エステル(例えば、オレイン酸エチルまたはトリグリセリド)、またはリポソームが挙げられる。水性注射懸濁液は、懸濁液の粘性を増加する物質(例えば、ナトリウムカルボキシメチルセルロース、ソルビトールまたはデキストラン)を含み得る。必要に応じて、この懸濁液はまた、この化合物の溶解度を増加し、高度に濃縮された溶液の調製を可能にする適切な安定剤または薬剤を含み得る。あるいは、この活性成分は、適切なビヒクル(例えば、滅菌した発熱物質を含まない水)で使用前に構築するための粉末形態であり得る。

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【0262】

この化合物はまた、ココアバターまたは他のグリセリドのような従来の坐剤基剤を含む直腸組成物(例えば、坐剤または保持浣腸)の形態で処方され得る。上で所望される処方物に加えて、この化合物はまた、蓄積調製物として処方され得る。このような長期作用処方物は、移植(例えば、皮下または筋肉内)によって、または筋肉内注射によって投与され得る。従って、例えば、これらの化合物は、適切なポリマー材料または疎水性材料(例えば、受容可能な油中のエマルジョンとして)、またはイオン交換樹脂と共に、不溶性の誘導体(例えば、不溶性の塩)として処方され得る。

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【0263】

本発明の疎水性化合物のための薬学的キャリアは、ベンジルアルコール、非極性界面活性剤、水混和性有機ポリマーおよび水相を含む共溶媒系である。この共溶媒系は、VPD共溶媒系であり得る。VPDは、3% w/vベンジルアルコール、8% w/vの非極性界面活性剤ポリソルベート80、および65% w/v ポリエチレングリコール300の溶液であり、無水エタノール中で作製される。VPD共溶媒系(VPD:5W)は、水溶液中5%デキストロースで1:1に希釈したVPDからなる。この共溶媒系は、疎水性化合物をよく溶解し、そしてそれ自体、全身投与の際に低い毒性を生じる。自然に、共溶媒系の比率は、その溶解度および毒性を壊すことなく、相当変化し得る。さらに、共溶媒成分の同一性は変化し得る:例えば、他の毒性比極性界面活性剤は、ポリソルベート80の代わりに使用され得;ポリエチレングリコールのフラクションサイズは変化し得;他の生体適合性ポリマーはポリエチレングリコール(例えば、ポリビニルピロリドンと交換し得;そして他の糖または多糖類は、デキストロースと交換し得る。あるいは、疎水性薬学的化合物のための他の送達系が用いられ得る。リポロームおよびエマルジョンは、疎水性薬物のための送達ビヒクルまたはの周知の例である。特定の有機溶媒(例えば、ジメチルスルホキシド)もまた用いられ得るが、通常、より大きな毒性という代償をはらう。さらに、これらの化合物は、持続性放出系(例えば、治療剤を含む固体疎水性ポリマーの半透性マトリクス)を使用して送達され得る。様々なタイプの持続性放出材料が確立されており、そして当業者に周知である。持続性放出カプセルは、それらの化学的性質に依存して、数週間から100日にわたって化合物を放出する。治療剤の化学的性質および生物学的活性に依存して、タンパク質または他の活性成分の安定化のためのさらなる戦略が用いられ得る。

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【0264】

薬学的組成物はまた、適切な固体またはゲル相キャリアまたは賦形剤を含み得る。このようなキャリアまたは賦形剤の例としては、炭酸カルシウム、リン酸カルシウム、様々な糖、デンプン、セルロース誘導体、ゼラチンおよびポリエチレングリコールのようなポリマ

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一が挙げられるが、これらに限定されない。本発明の活性成分の多くは、薬学的に受容可能な対イオンとの塩として提供され得る。このような薬学的に受容可能な塩基付加塩は、遊離酸の生物学的有効性および特性を保持する塩であり、無機塩基または有機塩基（例えば、水酸化ナトリウム、水酸化マグネシウム、アンモニア、トリアルキルアミン、ジアルキルアミン、モノアルキルアミン、二塩基性アミノ酸、酢酸ナトリウム、安息香酸カリウム、トリエタノールアミンなど）との反応によって得られる。

【0265】

本発明の薬学的組成物は、タンパク質またはペプチド抗原と共に、本発明のタンパク質または他の活性成分の複合体の形であり得る。タンパク質および／またはペプチド抗原は、Bリンパ球およびTリンパ球の両方に刺激シグナルを伝達する。Bリンパ球は、その表面にある免疫グロブリンレセプターを通して抗原に反応する。Tリンパ球は、MHCタンパク質による抗原の提示の後に、T細胞レセプター（TCR）を通して抗原に反応する。MHCおよび構造的に関連のあるタンパク質（クラスIおよびクラスII MHC遺伝子により宿主細胞上にコードされているものを含む）は、ペプチド抗原のTリンパ球への提示に貢献する。抗原成分もまた、精製されたMHCペプチド複合体単独で、またはT細胞に直接シグナル伝達し得る同時刺激分子と共に供給され得る。あるいは、表面免疫グロブリンおよびB細胞上の他の分子に結合し得る抗体、ならびにTCRおよびT細胞上の他の分子に結合し得る抗体は、本発明の薬学的組成物と組み合わせられ得る。

【0266】

本発明の薬学的組成物は、リポソームの形態であり得、ここで、本発明のタンパク質は、薬学的に受容可能な他のキャリアに加えて、脂質のような両親媒性の因子と組み合わせられる。脂質は、ミセル、不溶性の単一層、液晶、またはラメラ層のような凝集形態で水溶液中に存在する。リポソーム形成に適切な脂質としては、モノグリセリド、ジグリセリド、サルファチド、リゾレシチン、リン脂質、サポニン、胆汁酸などが挙げられるがこれらに限定されない。このようなリポソーム処方物の調製は、当業者の範囲内であり、例えば、米国特許第4,235,871号；同第4,501,728号；同第4,837,028号；および同第4,737,323号に開示され、これらは全て本明細書中に参考として援用される。

【0267】

本発明の薬学的組成物における、本発明のタンパク質または他の活性成分の量は、処置される状態の性質およびその重症度、ならびにこの患者が経験した以前の処置の性質に依存する。最終的には、本発明のタンパク質または他の活性成分の量は担当医が決定し、これによって各個々の患者が処置される。担当医は最初に、本発明のタンパク質または他の活性成分を少量投与し、患者の反応を観察する。この患者について最適な治療効果が得られるまで、より多くの用量の本発明のタンパク質または他の活性成分が投与され得、その点に達したらもはや用量は増加されない。本発明の方法を実施するために使用される種々の薬学的組成物は、本発明のタンパク質または他の活性成分を体重1kg当たり約0.01μg～約100mg（好ましくは約0.1μg～約10mg、より好ましくは約0.1μg～約1mg）含むべきであると意図される。骨、軟骨、腱、または靱帯の再生に有用な本発明の組成物について、この治療方法は、この組成物を、局所的、全身的、またはインプラントやデバイスとして局所的投与する工程を包含する。投与される場合、本発明における使用のための治療組成物は、当然ながら、発熱物質を含まない生理学的に受容可能な形態である。さらに、この組成物は、望ましくは、骨、軟骨、または組織の損傷部位へ送達するために、粘稠性形態でカプセル化または注入され得る。創傷治療および組織の修復のために、局所的投与が適切であり得る。本発明のタンパク質または他の活性成分以外の治療的に有用な因子（これらもまた、上記のような組成物中に必要に応じて含まれ得る）は、本発明の方法において、この組成物の代替として、またはこの組成物に加えて、同時または連続的に投与され得る。好ましくは、骨および／または軟骨の形成のために、この組成物は、タンパク質または他の活性成分を含む組成物を骨および／または軟骨の損傷部位に送達し得、骨および軟骨の発達のための構造を提供し、そして必要に応じて身体に再

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吸収され得るマトリックスを含む。このようなマトリックスは、現在他の移植医療用途で使われている材料で形成され得る。

【0268】

マトリックス材料の選択は、生体適合性、生物分解性、機械的性質、外見、および境界面の性質に基づく。この組成物の特定の適用は、適切な処方規定する。この組成物の可能性のあるマトリックスは、生物分解性かつ化学的に規定された硫酸カルシウム、リン酸三カルシウム、ヒドロキシアパタイト、ポリ乳酸、ポリグリコール酸、およびポリ無水物であり得る。他の可能性のある材料は、生物分解性でありかつ生物学的に十分に規定された、例えば骨や皮膚のコラーゲンである。さらなるマトリックスは、純粋なタンパク質または細胞外マトリックス成分で構成される。他の可能性のあるマトリックスは、非生物分解性でありかつ化学的に規定された、例えば焼結されたヒドロキシアパタイト、バイオガラス、アルミン酸塩、または他のセラミックである。マトリックスは、上記に言及した型の材料の任意の組合せ（例えば、ポリ乳酸およびヒドロキシアパタイトまたはコラーゲンおよびリン酸三カルシウム）からなり得る。生体セラミックは組成が変更され得る。例えば、カルシウム-アルミン酸塩-リン酸塩で、空孔サイズ、粒子サイズおよび粒子の形状、および生物分解性を変えるために処理され得る。150～800ミクロン範囲の直径を有する多孔質粒子の形態の、乳酸およびグリコール酸の50：50（モル重量）のコポリマーが現在好ましい。いくつかの適用において、タンパク質組成物のマトリックスからの解離を防ぐために、カルボキシメチルセルロースのような金属イオン封鎖剤または自家凝血を利用することが有用である。

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【0269】

金属イオン封鎖剤の好ましいファミリーは、アルキルセルロース（ヒドロキシアアルキルセルロースを含む）のようなセルロース材料であり、メチルセルロース、エチルセルロース、ヒドロキシエチルセルロース、ヒドロキシプロピルセルロース、ヒドロキシプロピルメチルセルロース、およびカルボキシメチルセルロースが挙げられ、カルボキシメチルセルロース（CMC）の陽イオン塩が最も好ましい。他の好ましい金属イオン封鎖剤としては、ヒアルロン酸、アルギン酸ナトリウム、ポリ（エチレングリコール）、ポリオキシエチレン酸化物、カルボキシビニルポリマー、およびポリ（ビニルアルコール）が挙げられる。本明細書中で有用な金属イオン封鎖剤の量は、全処方物重量に基づいて0.5～20重量%、好ましくは1～10重量%であり、これはタンパク質のポリマーマトリックスからの脱離を防ぎ、そしてこの組成物の適切な取り扱いを提供するために必要な量を表しており、しかも前駆細胞がマトリックスに侵入するのを妨げる程の多くはないので、これによって、タンパク質が前駆細胞の骨形成活性を助ける機会を提供する。さらなる組成物において、本発明のタンパク質または他の活性成分は、骨および／または軟骨の欠陥、創傷、または問題のある組織の処置に有益な他の因子と組み合わせられ得る。これらの因子としては、種々の増殖因子（例えば、上皮増殖因子（EGF）、血小板由来増殖因子（PDGF）、トランスホーミング増殖因子（TGF- α およびTGF- β ）、ならびにインスリン様増殖因子（IGF））が挙げられる。

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【0270】

この治療組成物はまた、現在獣医学用途についても価値が見出されている。ヒトに加えて、特定の家畜およびサラブレッドのウマは、本発明のタンパク質または他の活性成分を用いるこのような処置のための所望の患者である。組織再生において使用されるタンパク質含有薬学的組成物の投薬量レジメンは、このタンパク質の作用を改変する種々の要因（例えば、形成が所望される組織の重量、損傷部位、損傷を受けた組織の状態、創傷の大きさ、損傷を受けた組織の種類（例えば骨）、患者の年齢、性別、および食事、任意の感染の重症度、投与の時間、ならびに他の臨床的要因）を考慮して、担当医によって決定される。投薬量は、再生に使われるマトリックスの種類、または薬学的組成物中に含まれる他のタンパク質の有無によって変化し得る。例えば、最終組成物にIGF I（インスリン様増殖因子I）のような他の公知の増殖因子を加えることはまた、投与量に影響を及ぼし得る。進行は、組織および／または骨の成長および／または修復の定期的な診断によって（

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例えばX線、組織形態計測決定およびテトラサイクリン標識によって）モニターされ得る。

【0271】

本発明のポリヌクレオチドもまた、遺伝子治療に使用され得る。このようなポリヌクレオチドは、哺乳動物被験体における発現のために、インビボまたはエキソビボのいずれかで細胞に導入され得る。本発明のポリヌクレオチドはまた、核酸（ウイルスベクターまたは裸のDNAの形態が挙げられるがこれらに限定されない）を細胞または生物体に導入するための公知方法によって投与され得る。細胞はまた、増殖させるためまたはこのような細胞に所望の効果または活性を生じるために、本発明のタンパク質の存在下でエキソビボで培養され得る。次いで、処置された細胞は、治療目的のためインビボで導入され得る。

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【0272】

（5.12.3 有効投薬量）

本発明の使用に適した薬学的組成物は、意図される目的を達成するために活性成分を有効量含む組成物を含む。より具体的に述べると、治療的有效量とは、処置される被験体の現在の症状の進行を予防するかまたは緩和するのに有効な量を意味する。有効量の決定は、特に本明細書中で提供される詳細な開示を考慮すると、十分に当業者の範囲内である。本発明の方法において使用される任意の化合物について、治療的に有効な用量は、まず適切なインビトロアッセイから推定され得る。例えば用量は、動物モデルにおいて循環濃度範囲を達成するように処方され得、次にそれを使ってより正確にヒトにおける有用な用量が決定され得る。例えば、用量は、動物モデルにおいて循環濃度範囲を達成するように処方され得、が、その中に細胞培養物で決定される IC_{50} （すなわち、タンパク質の生物学的活性の最大値の半分の阻害を達成する試験化合物の濃度）が含まれる。このような情報は、ヒトにおいて有用な用量をより正確に決定するために使用され得る。

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【0273】

治療的に有効な用量とは、症状の回復または患者の生存期間の延長を生じる化合物の量という。このような化合物の毒性および治療効力は、細胞培養物または実験動物における標準的な薬学的手順により決定され得る（例えば、 LD_{50} （集団の50%が死ぬ用量）および ED_{50} （集団の50%に治療的に効果な用量）の決定のために）。毒性と治療効果との間の用量比は治療指数であり、そしてこれは LD_{50} と ED_{50} との間の比で表され得る。高い治療指数を示す化合物が好ましい。これらの細胞培養物アッセイおよび動物研究から得られるデータを使って、ヒトにおける使用のための投薬量の範囲を処方し得る。このような化合物の投薬量は、好ましくは ED_{50} を含む循環濃度の範囲内であり、毒性が全くあるいはほとんどない。この投薬量は、用いられる薬形および用いられる投与経路に基づいてその範囲内で変化し得る。正確な処方、投与経路、および投薬量は、患者の状態を見ながら、個々の医者によって選択され得る。例えば、Finglerら、1975、「The Pharmacological Basis of Therapeutics」、Ch. 1 p. 1を参照のこと。投薬量および間隔は、所望の効果を維持するのに十分な活性部分の血漿レベル、または最低有効濃度（MEC）を提供するように個々に調整され得る。MECは、各化合物について変化するが、インビトロデータから推測され得る。MECを達成するのに必要な投薬量は、個人の特性および投与経路に依存する。しかし、HPLCアッセイまたは生物検定は、血漿濃度を決定するために使用され得る。

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【0274】

投薬の間隔もまた、MEC値を使って決定され得る。化合物は、時間の10～90%、好ましくは30～90%の間、最も好ましくは50～90%の間、血漿レベルをMECより高く維持するレジメンを使って投与されるべきである。局所投与または選択的摂取の場合、この薬物の有効局所濃度は、血漿濃度とは無関係であり得る。

【0275】

本発明のポリペプチドまたは他の組成物についての例示的な投薬量レジメンは、毎日体重1kg当たり約0.01 μ g～100mgの範囲であり、好ましい用量は、毎日患者の体重1kg当たり約0.1 μ g～25mgの範囲であり、大人と子供では変化する。投薬は

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、1日1回であり得るか、または等量の用量がより長い間隔またはより短い間隔で送達され得る。

【0276】

当然ながら、投与される組成物の量は、処置される被験体、被験体の年齢および体重、苦痛の度合い、投与の様式、および処方医の判断に依存する。

【0277】

(5.12.4 包装)

この組成物は、所望の場合、活性成分を含む1つ以上の単位剤形を含み得るパックまたはディスペンサー装置において示され得る。このパックは、例えば金属またはブリスターパックのようなプラスチックホイルで構成され得る。このパックまたはディスペンサー装置は、投与のための指示書を添付し得る。適合性の薬学的キャリア中に処方される、本発明の化合物を含む組成物もまた調製され得、適切な容器に配置され、そして指示される状態の処置のためにラベルされ得る。

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【0278】

(5.13 抗体)

本発明の中には、本発明のタンパク質またはタンパク質のフラグメントに対する抗体も含まれる。本明細書中で使用される場合、用語「抗体」は、免疫グロブリン分子および免疫グロブリン(Ig)分子のうち免疫学的に活性な部分、すなわち抗原に特異的に結合する(免疫反応を生じる)抗原結合部位を含む分子をいう。このような抗体の中としては、ポリクローナル、モノクローナル、キメラ、単鎖、Fab、Fab'およびF(ab')₂フラグメント、ならびにFab発現ライブラリーが挙げられるがこれらに限定されない。一般に、ヒトから得られる抗体分子は、IgG、IgM、IgA、IgE、およびIgDのいずれかのクラスに関係しており、これらは、この分子中に存在する重鎖の性質により互いに異なる。特定のクラスは、さらにIgG₁、IgG₂などのようなサブクラスを有する。さらに、ヒトにおいて、軽鎖はκ鎖またはλ鎖であり得る。本明細書中での抗体への言及は、このような全てのクラス、サブクラス、そしてヒト抗体の型への言及を含む。

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【0279】

本発明の単離された関連タンパク質は、抗原またはその一部またはフラグメントとして役立つように意図され得、そしてさらに、ポリクローナル抗体およびモノクローナル抗体の調製についての標準的技術を使用して、この抗原を免疫特異的に結合する抗体を産生するための免疫原として使用され得る。全長タンパク質を使用して、あるいは本発明によって、免疫原として使用するための抗原の抗原性ペプチドフラグメントが提供され得る。抗原性ペプチドフラグメントは、配列番号4に示されるアミノ酸配列のような、全長タンパク質のアミノ酸配列のうち少なくとも6つのアミノ酸残基からなり、そしてこのペプチドに対して惹起された抗体が全長タンパク質またはこのエピトープを含む任意のフラグメントと特異的免疫複合体を形成するように、そのエピトープを含む。好ましくは、この抗原性ペプチドは、少なくとも10のアミノ酸残基、または少なくとも15のアミノ酸残基、または少なくとも20のアミノ酸残基、または少なくとも30のアミノ酸残基を含む。この抗原性ペプチドに含まれる好ましいエピトープは、タンパク質の表面に位置しているタンパク質の領域であり；通常これらは親水性領域である。

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【0280】

本発明の特定の実施形態において、抗原性ペプチドに含まれる少なくとも1つのエピトープは、タンパク質の表面に位置している関連タンパク質の領域(例えば、親水性領域)である。ヒトの関連タンパク質配列の疎水性分析は、関連タンパク質のどの領域が特に親水性であり、したがって標的抗体生産のために有用な表面性残基をコードしそうであることを示す。標的抗体生産の手段として、親水性および疎水性の領域を示すヒドロパシープロットは、フーリエ変換を用いてまたは用いずに、当該分野で周知の任意の方法(例えば、Kyte Doolittle法またはHopp Woods法を含む)によって作成され得る。HoppおよびWoods、1981、Proc. Nat. Acad. Sci. U S A 78:3824-3828; KyteおよびDoolittle 1982、J.

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Mol. Biol. 157:105-142 (いずれもその全体において本明細書中に参考として援用される)を参照のこと。抗原性タンパク質、あるいはその誘導体、フラグメント、アナログ、またはホモログ中の1つまたはそれ以上のドメインに特異的な抗体もまた、本明細書中で提供される。

【0281】

本発明のタンパク質、あるいは誘導体、フラグメント、アナログ、ホモログ、またはオルソログは、これらタンパク質構成成分と免疫特異的に結合する抗体の生産において、免疫抗原として利用され得る。

【0282】

当該分野で公知の種々の手順が、本発明のタンパク質、あるいはその誘導体、フラグメント、アナログ、ホモログ、またはオルソログに対するポリクローナル抗体またはモノクローナル抗体の生産のために用いられ得る(例えば、本明細書中で参考として援用される、Ank tibodies: A Laboratory Manual, Hallow E. および Lane D、1988、Cold Spring Harbor Laboratory Press、Cold Spring Harbor、NYを参照のこと)。これらの抗体のうちのいくつかは、以下で議論される。

【0283】

(5.13.1 ポリクローナル抗体)

ポリクローナル抗体の生産のために、種々の適切な宿主動物(例えばウサギ、ヤギ、マウスまたは他の哺乳動物)は、天然のタンパク質、その合成改変体、またはそれらの誘導体を1回またはそれ以上注射することによって免疫され得る。適切な免疫原性調製物は、例えば、天然に存在する免疫原性タンパク質、免疫原性タンパク質を表す化学的に合成されたポリペプチド、または組み換え発現された免疫原性タンパク質を含み得る。さらに、このタンパク質は、免疫される哺乳動物において免疫原性であることが公知の第2のタンパク質と結合体化され得る。このような免疫原性タンパク質の例としては、キーホールリンペットヘモシアニン、血清アルブミン、ウシサイログロブリン、ダイズのトリプシンインヒビターが挙げられるがこれらに限定されない。この調製物は、さらにアジュバントを含み得る。免疫反応を高めるために使用される種々のアジュバントとしては、フロイントアジュバント(完全および不完全)、鉍物ゲル(水酸化アルミニウム)、界面活性物質(例えばリゾレシチン、プルロニックポリオール、ポリアニオン、ペプチド、油乳濁液、ジニトロフェノールなど)、ヒトにおいて使用可能なアジュバント(例えば、カルメット-ゲラン杆菌およびCorynebacterium parvum)、または同様の免疫刺激因子が挙げられるがこれらに限定されない。用いられ得るアジュバントのさらなる例としては、MPL-TDMアジュバント(モノホスホリル脂質A、合成トレハロース・ジコリノミコール酸)が挙げられる。

【0284】

免疫原性タンパク質に対するポリクローナル抗体分子は、哺乳動物から(例えば、血液から)単離され得、そして周知の技術(例えば、プロテインAまたはプロテインGを使ったアフィニティークロマトグラフィー)によってさらに精製され得、主に免疫血清のIgG画分を提供する。引き続いて、または代替として、免疫グロブリン探求の標的である特定の抗原またはそのエピトープは、カラム上に固定され得、免疫親和性クロマトグラフィーによって免疫特異的抗体を精製する。免疫グロブリンの精製は、例えば、D. Wilkison (The Scientist, Inc., Philadelphia PA 発行のThe Scientist, Vol. 14, No. 8 (2000年4月17日), pp. 25-28)によって議論される。

【0285】

(5.13.2 モノクローナル抗体)

本明細書中で使用される場合、用語「モノクローナル抗体」(MAb)または「モノクローナル抗体組成物」は、独特の軽鎖遺伝子産物および独特の重鎖遺伝子産物からなる抗体分子の1つの分子種のみ含む抗体分子の集団をいう。特に、モノクローナル抗体の相補性

決定領域（CDR）は、この集団の全ての分子において同一である。従って、MAbは、抗原の特定のエピトープと免疫反応し得、これに対する独特の結合親和性によって特徴付けられる抗原結合部位を含む。

【0286】

モノクローナル抗体は、KohlerおよびMilstein、Nature、256：495（1975）に記述されているようなハイブリドーマ法を用いて調製され得る。ハイブリドーマ法において、マウス、ハムスター、または他の適切な宿主動物は、免疫剤に特異的に結合する抗体を産生するか、または産生し得るリンパ球を誘発するために、免疫剤で代表的に免疫される。あるいは、このリンパ球は、インビトロで免疫され得る。

【0287】

免疫剤としては、代表的に、タンパク質抗原、そのフラグメント、またはその融合タンパク質が挙げられる。一般に、ヒト起源の細胞が所望される場合には抹消血リンパ球が使用され、あるいは非ヒト哺乳動物源が所望される場合には脾臓細胞またはリンパ節細胞が使用されるかのいずれかである。次いで、このリンパ球は、ポリエチレングリコールのような適切な融合剤を使用して不死化細胞株と融合され、ハイブリドーマ細胞を形成する（Goding、Monoclonal Antibodies：Principles and Practice, Academic Press,（1986）, 59-103頁）。不死化細胞株は、通常、形質転換した哺乳動物細胞、特にげっ歯類、ウシ、およびヒト起源の骨髓腫細胞である。通常、ラットまたはマウスの骨髓腫細胞株が用いられる。ハイブリドーマ細胞は、好ましくは、非融合不死化細胞の増殖または生存を阻害する1つ以上の基質を含む適切な培養培地で培養され得る。例えば、親細胞に酵素ヒポキサンチン グアニンホスホリボシルトランスフェラーゼ（HGPRTまたはHPRRT）が欠如している場合、ハイブリドーマの培養培地は、通常、HGPRT欠乏細胞の増殖を阻害する基質であるヒポキサンチン、アミノプテリン、およびチミジン（HAT培地）を含む。

【0288】

好ましい不死化細胞株は、効率的に融合し、選択された抗体生産細胞による抗体発現を安定した高いレベルで支持するもの、およびHAT培地のような培地に感受性であるものである。より好ましい不死化細胞株は、例えばSalik Institute Cell Distribution Center, San Diego, California およびAmerican Type Culture Collection, Manassas, Virginiaから得られ得るマウスの骨髓腫細胞株である。ヒトの骨髓腫 およびマウス-ヒト異種骨髓腫細胞株もまた、ヒトモノクローナル抗体の生産に関して記載されている（Kozbor、J. Immunol.、133：3001（1984）；Brodeurら、Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York（1987）51-63頁）。

【0289】

次いで、このハイブリドーマが培養される培養培地を、この抗原に対するモノクローナル抗体の存在についてアッセイし得る。好ましくは、このハイブリドーマ細胞によって産生されるモノクローナル抗体の結合特異性は、免疫沈降反応により、あるいはラジオイムノアッセイ（RIA）または酵素結合イムノソルベント検定法（ELISA）のようなインビトロ結合アッセイによって決定される。このような技術およびアッセイは、当該分野で周知である。モノクローナル抗体の結合親和性は、例えば、MunsonおよびPollardのスキッチャード分析、Anal. Biochem., 107：220（1980）により決定される。好ましくは、標的抗原に対して高程度の特異性および高い結合親和性を有する抗体が単離される。

【0290】

所望のハイブリドーマ細胞が同定された後、クローンは、限界希釈法によりサブクローニングされ、そして標準的な方法によって成長させ得る。この目的に対して適切な培養培地として、例えば、ダルベッコのModified Eagle培地およびRPMI-16

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40 培地が挙げられる。あるいはハイブリドーマ細胞は、哺乳動物において腹水としてインビボで成長させ得る。

【0291】

サブクローンにより分泌されたモノクローナル抗体は、伝統的な免疫グロブリン精製手順（例えば、プロテインAセファロース、ヒドロキシルアパタイトクロマトグラフィー、ゲル電気泳動、透析、アフィニティークロマトグラフィー）により、培養培地または腹水流体から単離、または精製され得る。

【0292】

モノクローナル抗体は、米国特許第4,816,567号に記載されているような組み換えDNA法によって作製され得る。本発明のモノクローナル抗体をコードするDNAは、
10 伝統的な手順を用いて容易に単離され、そして配列決定でされ得る（例えば、ネズミ抗体の重鎖および軽鎖をコードする遺伝子に特異的に結合し得るオリゴヌクレオチドプローブを使用することによって）。本発明のハイブリドーマ細胞は、このようなDNAの好ましい供給源として役に立つ。一旦単離されたDNAは発現ベクターに置かれ得、次いで免疫グロブリンタンパク質を他で産生しない宿主細胞（例えば、猿COS細胞、チャイニーズハムスター卵巣（CHO）細胞、骨髓腫細胞）中にトランスフェクトされ、モノクローナル抗体の合成を得る。DNAはまた、例えば、相同性ネズミ配列の代わりにヒト重鎖および軽鎖定常ドメインに対するコード配列を用いることによって（米国特許第4,816,567号；Morrison、Nature 368、812-13（1994））、また
20 または非免疫グロブリンポリペプチドに対するコード配列の全てまたは一部を免疫グロブリンのコード配列に共有結合することによって改変され得る。このような非免疫グロブリンポリペプチドは、本発明の抗体の定常ドメインの代わりに用いられ得るか、または本発明の抗体の1つの抗原結合部位の可変ドメインの代わりに用いられ得、キメラ二価抗体を作製し得る。

【0293】

（5.13.2 ヒト化抗体）

本発明のタンパク質抗原に対する抗体は、ヒト化抗体またはヒト抗体をさらに含み得る。このような抗体は、投与された免疫グロブリンに対してヒトによる免疫応答を生じることがないので、ヒトへの投与に適している。抗体のヒト化された形態は、キメラ性の免疫グロブリン、免疫グロブリン鎖、またはそのフラグメント（例えばFv、Fab、Fab'、
30 F(ab')₂、または他の抗体の抗原結合サブ配列）であり、これらは主にヒト免疫グロブリンの配列で構成され、そして非ヒト免疫グロブリン由来の最小配列を含んでいる。ヒト化は、Winterおよび彼の同僚の方法を踏襲し（Jonesら、Nature、321：522-525（1986）；Riechmannら、Nature、332：323-327（1988）；Verhoeyenら、Science、239：1534-1536（1988））、ヒト抗体の対応する配列の代わりに、げっ歯類のCDRまたはCDR配列を用いることにより実行され得る（米国特許第5,225,539号もまた参照のこと）。いくつかの例において、ヒト免疫グロブリンのFv枠組み構造残基を、それに対応する非ヒト残基で置換される。

【0294】

ヒト化抗体は、レシピエント抗体にも、外来性CDRにも、枠組み構造配列にもいずれにも見出されない残基を含み得る。一般的に、ヒト化抗体は、少なくとも1つ、および代表的には2つの可変ドメインの全てを実質的に含む。その場合、CDR領域の全てまたは実質的に全てが非ヒト免疫グロブリンのCDR領域に対応しており、そして枠組み構造領域の全てまたは実質的に全てがヒト免疫グロブリンの共通配列の枠組み構造領域である。ヒト化抗体はまた、最適には、免疫グロブリンの定常領域（Fc）の少なくとも一部分、代表的にはヒト免疫グロブリンの定常領域（Fc）を含む（Jonesら 1986；Riechmannら、1988；およびPresta、Curr. Opin. Struct. Biol. 2：593-596（1992））。

【0295】

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(5. 13. 3 ヒト抗体)

完全なヒト抗体は、抗体分子に関連する。ここで軽鎖および重鎖の両方（CDRを含む）の全体の配列が、ヒト遺伝子から惹起される。このような抗体は、本明細書中では「ヒト抗体」または「完全なヒト抗体」という。ヒトモノクローナル抗体は、トリオマ（trio ma）技術（ヒトB細胞のハイブリドーマ技術（Kozborら、1983 Immunol Today 4:72を参照のこと）およびEBVハイブリドーマ技術（Coleら、1985: MONOCLONAL ANTIBODIES AND CANCER THERAPY、Alan R. Liss、Inc.、77-96頁を参照のこと））によって調整され得る。ヒトモノクローナル抗体は、本発明の実施において利用され得、そしてヒトハイブリドーマを使うことにより（Coteら、1983、Proc Natl Acad Sci USA 80:2026-2030を参照のこと）、またはインビトロでのエプスタインバーウイルスを用いてヒトB細胞を形質転換することにより（Coleら、1985: MONOCLONAL ANTIBODIES AND CANCER THERAPY、Alan R. Liss、Inc.、77-96頁を参照のこと）産生され得る。

【0296】

さらに、ヒト抗体はまた、さらなる技術（ファージ表示ライブラリー（HoogenboomおよびWinter、J. Mol. Biol. 227:381（1991）；Marksら、J. Mol. Biol. 222:581（1991））を含む）を使用して産生され得る。同様に、ヒト抗体は、ヒト免疫グロブリン遺伝子座をトランスジェニック動物（例えば、内因性の免疫グロブリン遺伝子が部分的にまたは完全に不活性化されたマウス）に導入することによって作製され得る。チャレンジに対し、ヒト抗体産生は観察され、全ての点（遺伝子の再配列、集合、および抗体のレパートリーを含む）においてヒトで見られる産生に厳密に似ている。このアプローチは、例えば、米国特許第5,545,807号；同第5,545,806号；同第5,569,825号；同第5,625,126号；同第5,633,425号；同第5,661,016号、およびMarksら、（Bio/Technology 10、779-783（1992）；Lonbergら（Nature 368、856-859（1994））；Morrison（Nature 368、812-13（1994））；Fishwildら、（Nature Biotechnology 14、845-51（1996））；Neuberger（Nature Biotechnology 14、826（1996））；ならびにLonbergおよびHuszar（Intern. Rev. Immunol. 13 65-93（1995））に記載される。

【0297】

さらに、ヒト抗体は、抗原によるチャレンジに対応する動物の内因性の抗体よりもむしろ完全なヒト抗体を産生するように改変されたトランスジェニック非ヒト動物を使って生成することも可能である（PCT公報WO94/02602を参照のこと）。非ヒト宿主において免疫グロブリンの重鎖および軽鎖をコードする内因性遺伝子は、不活性化し、そして免疫グロブリンの重鎖および軽鎖コードする活性遺伝子座が、宿主のゲノムの中に挿入される。ヒト遺伝子は、例えば、例えば、必要なヒトDNAセグメントを含む酵母の人工染色体を使って導入される。次いで所望の改変を全て提供する動物は、改変の完全な相補体をほとんど含まない中間トランスジェニック動物を交雑育種することにより後代として得られる。このような非ヒト動物の好ましい実施形態は、マウスであり、そしてPCT公報WO96/33735およびWO96/34086に開示されるように示されているように、XenomouseTMと呼ばれる。この動物は、完全なヒト免疫グロブリンを分泌するB細胞を産生する。抗体は、目的の免疫原で免疫された後の動物（例えば、ポリクローナル抗体の調整物）からあるいは動物由来のような不朽化B細胞（たとえば、モノクローナル抗体を産生するハイブリドーマ）から得られ得る。さらに、ヒト可変領域を持つ免疫グロブリンをコードする遺伝子が回収され得、そして発現させて、直接抗体を得、またはさらに改変され得、抗体アナログ（例えば、単鎖Fv分子）を得る。

【0298】

内因性の免疫グロブリン重鎖の発現を欠失している非ヒト宿主（例えばマウス）を生成する方法の例は、米国特許第5,939,598号に開示される。それは、胚性幹細胞における少なくとも1つの内因性の重鎖遺伝子座由来のJセグメント遺伝子を削除する工程を包含する方法によって得られ得、その遺伝子座の再配列を防ぎ、そして再配列された免疫グロブリン重鎖遺伝子座の転写の形成を防ぐ。削除する工程は、選択可能なマーカーをコードする遺伝子を含む標的ベクターによって影響され；そして胚性幹細胞から、選択可能なマーカーをコードする遺伝子を体細胞および生殖細胞が含むトランスジェニックマウスを生成する。

【0299】

目的の抗体（例えばヒト抗体）を生成する方法は、米国特許第5,916,771号に開示される。それは、重鎖をコードするヌクレオチド配列を含む発現ベクターを培養中の哺乳動物宿主細胞に導入する工程、軽鎖をコードするヌクレオチド配列を含む発現ベクターを別の哺乳動物宿主細胞に導入する工程、およびその2つの細胞を融合してハイブリッド細胞を作製する工程を包含する。ハイブリッド細胞は、重鎖および軽鎖を含む抗体を発現する。

【0300】

本手順におけるさらなる改善において、免疫原上の臨床的に関連するエピトープを同定する方法、およびその関連するエピトープに高い親和性で免疫特異的に結合する抗体を選択する相補的な方法は、PCT公報WO99/53049に開示される。

【0301】

(5.13.4 Fabフラグメントおよび単鎖抗体)

本発明に従って、技術が、本発明の抗原タンパク質に特異的な単鎖抗体を生成するために適用され得る（例えば米国特許第4,946,778号を参照のこと）。さらに、方法が、タンパク質、またはその誘導体、フラグメント、アナログ、相同体に対し所望の特異性を持つモノクローナルFabフラグメントの同定を迅速かつ効果的に得るため、Fab発現ライブラリーの構築について適用され得る（例えばHuseら、1989 Science 246:1275-1281を参照のこと）。タンパク質抗原に対するイディオタイプを含む抗体フラグメントは、当該分野で公知の技術を用いて生成され得る（(i) 抗体分子のペプシン消化により生成される $F(a_b)_2$ フラグメント；(ii) $F(a_b)_2$ のジスルフィド結合を減らすことにより生成される $F_a b$ フラグメント；(iii) パパインおよび還元剤で抗体分子を処理することにより生成される $F_a b$ フラグメント；および(iv) F_v フラグメントを含むが、これらに限定されない）。

【0302】

(5.13.5 生物特異的な抗体)

二重特異性(bispecific)抗体は、少なくとも2つの異なる抗原に対して結合特異性を有するモノクローナル（好ましくはヒトまたはヒト化）抗体である。本場合において、結合特異性の1つは、本発明の抗原タンパク質に対してである。第2の結合標的は、任意の他の抗原であり、そして有益な細胞表面のタンパク質またはレセプターまたはレセプターサブユニットである。

【0303】

二重特異性抗体を作製する方法は、当該分野で公知である。伝統的に、二重特異性抗体の遺伝子組み換え生成は、2つの免疫グロブリン重鎖／軽鎖対の共発現に基づく。この場合2つの重鎖は、異なる特異性を有している(MilsteinおよびCuello、Nature、305:537-539(1983))。免疫グロブリン重鎖および軽鎖のランダムな組み合わせのため、そのハイブリドーマ（クアドローマ）は、10個の異なる抗体分子の強力な混合物を産生する。その中で1つだけが、正しい二重特異性の構造を有する。その正しい分子の精製は、通常アフィニティークロマトグラフィー工程により達成される。同様の手順は、PCT公報WO93/08829(1993年5月13日公開)およびTraunckerら、1991 EMBO J.、10:3655-3659に

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開示される。

【0304】

所望の結合特異性（抗原抗体結合部位）を持つ抗体の可変ドメインは、免疫グロブリンの定常ドメイン配列に融合され得る。融合は好ましくは、ヒンジ、CH2およびCH3領域の少なくとも一部分を含む免疫グロブリン重鎖の定常ドメインとである。融合体の少なくとも1つに存在する軽鎖結合に必要な部位を含む第1重鎖定常領域（CH1）を有することが、好ましい。免疫グロブリン重鎖融合、そして所望の場合、免疫グロブリン軽鎖体をコードするDNAが、別個の発現ベクターに挿入され、そして適切な宿主生物体に共トランスフェクトされる。二重特異性抗体の生成のさらに詳細については、例えば、Sureshら、Methods in Enzymology、121:210（1986）を参照のこと。 10

【0305】

WO96/27011に記載される別のアプローチに従って、一对の抗体分子間の表面は、操作され、組み換え細胞培養物から回収されたヘテロダイマーの割合を最大にし得る。好ましい表面は、少なくとも一对の抗体定常ドメインのCH3領域を含む。本方法において、1つ以上の第一の抗体分子の表面由来の小さいアミノ酸側鎖は、より大きな側鎖（例えば、チロシンやトリプトファン）で置換される。大きな側鎖に対して同一のまたは同様のサイズの代償性「腔」は、大きいアミノ酸側鎖を小さいアミノ酸側鎖（例えば、アラニンまたはスレオニン）で置き換えることにより第2の抗体分子の表面上に作製される。この方法は、他の好ましくない最終産物（例えば、ホモダイマー）以上にヘテロダイマーの収量を増やす機構を提供する。 20

【0306】

二重特異性抗体は、完全長抗体、または抗体のフラグメント（例えばF(ab')₂二重特異性抗体）として調整され得る。抗体フラグメントから二重特異性抗体を生成する技術は、文献に記載されている。例えば、二重特異性抗体は化学結合を使用して調整され得る。Brennanら、Science 229:81（1985）は、インタクトな抗体がタンパク質分解的に切断され、F(ab')₂のフラグメントを生成する手順を記載する。これらのフラグメントは、ジチオール複合剤である亜ヒ酸ナトリウムの存在下で還元され、隣接するジチオールを安定化し、そして分子間ジスルフィド形成を妨げる。次いで、生成されたFab'フラグメントは、チオニトロ安息香酸（TNB）誘導体に変換される。次いでFab'-TNB誘導体の1つは、メルカプトエチルアミンで還元することでFab'-チオールに再変換され、そして等モル量の他のFab'-TNB誘導体と混合されて二重特異性抗体を形成する。生成された二重特異性抗体は、酵素の選択的固定化のための薬剤として使用され得る。 30

【0307】

さらに、Fab'フラグメントは、大腸菌から直接回収され、そして化学的に結合されて二重特異性抗体を形成し得る。Shalabyら、J. Exp. Med. 175:217-225（1992）は、完全なヒト化二重特異性抗体F(ab')₂分子の生成を記載する。各Fab'フラグメントは、大腸菌から別個に分泌され、インビトロで直接化学カップリングに供され、二重特異性抗体を形成する。従って、形成された二重特異性抗体は、ErbB2レセプターを過剰発現する細胞および正常なヒトT細胞に結合し得、そしてヒト乳房腫瘍標的に対して細胞傷害性リンパ球の溶解活性をトリガーし得る。 40

【0308】

組み換え細胞培養物から直接二重特異性抗体のフラグメントを作製し、そして単離する種々の技術もまた、記載されている。例えば、二重特異性抗体は、ロイシンジッパーを使用して生成されている。Kostelnýら、J. Immunol. 148（5）:1547-1553（1992）。FosおよびJunタンパク質由来のロイシンジッパーペプチドは、遺伝子融合により、2つの異なる抗体のFab'領域に連結された。抗体ホモダイマーは、ヒンジ領域で還元され、モノマーを形成し、そして次いで、再び酸化されて抗体ヘテロダイマーを形成した。本方法はまた、抗体ホモダイマーの生成について利用され 50

得る。Hollingerら、Proc. Natl. Acad. Sci. USA、90：6444-6448（1993）に記載される「diabody」技術は、二重特異性抗体のフラグメントを作製するための代替機構を提供している。そのフラグメントは、リンカーによって軽鎖可変ドメイン（V_L）に連結された重鎖可変ドメイン（V_H）を含む。リンカーは、短すぎて同じ鎖上の2つのドメイン間でペアリング不可能である。従って、1つのフラグメントのV_HドメインをV_Lドメインは、別のフラグメントのV_LドメインおよびV_Hドメインとペアリングするように強制され、その結果、2つの抗原結合部位を形成する。単鎖Fv（sFv）ダイマーの使用によって二重特異性抗体フラグメントを作製するための別の戦略もまた、報告されている。Gruberら、J. Immunol. 152：5368（1994）を参照のこと。

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【0309】

2価より多い抗体が、意図される。例えば、3重特異性抗体もまた、調整され得る。Tuttら、J. Immunol. 147：60（1991）。例示的な二重特異性抗体は、2つの異なるエピトープに結合し得る。そのうちの少なくとも1つは、本発明のタンパク質抗原に由来する。あるいは、免疫グロブリン分子の抗抗原性アームは、白血球上のトリガー分子（例えば、T細胞レセプター分子（例えば、CD2、CD3、CD28、B7））、またはIgG（FcγR）のFcレセプター（例えばFcγRI（CD64）、FcγRII（CD32）およびFcγRIII（CD16））に結合するアームと結合され得る。これは、細胞の防御機構を、特定の抗原を発現する細胞に集中させるためである。二重特異性抗体はまた、特定の抗原を発現する細胞に対する細胞傷害性薬剤を思考するために使用され得る。これらの抗体は、抗原結合アームおよび細胞傷害性薬剤または放射性核種キレート剤（例えば、EOTUBE、DPTA、DOTA、またはTETA）を結合するアームを有する。目的の別の二重特異性抗体は、明細書中に記載されるタンパク質抗原を結合し、そしてさらに組織因子（TF）を結合する。

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【0310】

（5.13.6 ヘテロ結合体抗体）

ヘテロ結合体抗体はまた、本発明の範囲内である。ヘテロ結合体抗体は、2つの共有結合をした抗体で構成される。このような抗体は、例えば、免疫系細胞を望ましくない細胞に対して標的にする（米国特許第4,676,980号）ため、および、HIV感染の処置（WO 91/00360；WO 92/200373；EP 03089）ため提供され得る。この抗体は、合成タンパク質化学において公知の方法（架橋剤を含む方法を包含する）を使用してインビトロで調整されることが意図される。例えば、免疫毒素は、ジスルフィド交換反応を使用して、またはチオエーテル結合を形成することにより構築され得る。本目的の適切な試薬の例は、イミノチオレートおよびメチルー4-メルカプトブチルイミデート、および例えば、米国特許第4,676,980号に開示載された試薬などである。

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【0311】

（5.13.7 エフェクター機能工学）

エフェクター機能に関する本発明の抗体を改変することが所望され得る。これは例えば、癌処置において抗体の効果を高めるためである。例えば、シスチン残基は、Fc部位に導入され得、それによりこの領域において鎖内ジスルフィド結合形成をし得る。従って生成されたホモダイマー抗体は、内面化能力を改良し、そして／または、相補体媒介細胞殺傷および抗体依存性細胞傷害性（ADCC）を増大させ得る。Caronら、J. Exp. Med.、176：1191-1195（1992）およびShopes、J. Immunol.、148：2918-2922（1992）を参照のこと。抗腫瘍活性を強められたホモダイマー抗体はまた、ヘテロ二機能性の架橋剤を使用して（Wolffら、Cancer Research、53：2560-2565（1993）に記載されるように）調整され得る。あるいは、抗体は、操作され得、二重Fc領域を持ち、そしてそれにより相補体溶解およびADCC能力を増進し得る。Stevensonら、Anti-Cancer Drug Design、3：219-230（1980）を参照のこと。

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【0312】

(5.13.8 免疫結合体)

本発明はまた、細胞傷害性薬剤（例えば、化学療法剤、毒素（例えば細菌、真菌、植物、または動物起源、またはそれらのフラグメントの酵素学的な活性毒素）または放射性アイソトープ（すなわち放射性結合体））に結合体化された抗体を含む免疫結合体に関係する。（、このような免疫結合体の生成において有用な化学療法剤は、上記されている。使用され得る酵素学的に活性な毒素およびそのフラグメントは、ジフテリアA鎖、ジフテリア毒素の非結合活性フラグメント、エキソトキシンA鎖（*Pseudomonas aeruginosa*由来）、リシンA鎖、アブリンA鎖、モデシンA鎖、 α サルシン、*Al eur ites ford i i*タンパク質、*d ianth in*タンパク質、*Phyt ol* 10
*aca americana*タンパク質（PAPI、PAPII、およびPAP-S）、*momordica charantia*インヒビター、クルシン、クロチン、*sap*
*aonaria officinalis*インヒビター、ゲロニン、ミトゲリン、レスト
リクトシン、フェノマイシン、エノマイシン、ならびにトリコテシンを含む。種々の放射
性核種は、放射性結合体化抗体の産物に対して入手可能である。例として、 ^{212}Bi 、 ^{131}I 、 ^{131}In 、 ^{90}Y 、 ^{186}Re が挙げられる。

【0313】

抗体および細胞傷害性薬剤の結合体は、種々の二機能性タンパク質カップリング剤（例えば、N-サクシンイミジル-3-(2-ピリジルジチオール)プロピオン酸塩（SPDP））、イミノチオレーン（IT）、イミドエステルの二機能性誘導体（ジメチル・アジピミ 20
デートHCLなど）、活性エステル（ジサクシンイミジル・スベリン酸塩など）、アルデ
ヒド（グルタルアルデヒドなど）、ビスアジド化合物（ビス（P-アジドベンゾイル）ヘ
キサンジアミンなど）、ビス-ジアゾニウム誘導体（ビス-（P-ジアゾニウムベンゾイ
ル）エチレンジアミンなど）、ジイソシアン酸塩（トリエン2, 6-ジイソシアン酸塩な
ど）、ビス活性フッ素化合物（1, 5-ジフルオロ-2, 4-ジニトロベンゼンなど）を
使用して作製される。例えば、リシン免疫毒素は、Vitettaら、Science、
238: 1098 (1987)に記載されるように調整され得る。炭素14標識化さ
れた1-イソチオシアナトベンジル-3-メチルジエチレントリアミンペンタ酢酸（MX- 30
DTPA）は、抗体に対する放射性核種の結合のための例示的なキレート剤である。W
O 94/11026を参照のこと。

【0314】

別の実施形態において、抗体は、腫瘍前標的における利用のため「レセプター」（例えば、ストレプトアビジン）に結合体化され得る。ここで、この抗体レセプター結合体は、患
者に投与され、引き続いて、結合されない結合体を洗浄剤および次いで細胞傷害性薬剤に
結合体化された「リガンド」（例えば、アビジン）の投与を使用して循環器系から除去さ
れる。

【0315】

(5.14 コンピューター読取り可能な配列)

本実施形態の1つの局面において、本発明のヌクレオチド配列は、コンピューター読取り
可能媒体上に記録され得る。本明細書中で使用する場合、「コンピューター読取り可能媒 40
体」は、コンピューターにより読取りおよびアクセスされ得る任意な媒体をいう。このよ
うな媒体として、磁気記憶媒体（例えば、フロッピー（登録商標）ディスク、ハードディ
スク記憶媒体、および磁気テープ）；光学記憶媒体（例えば、CD-ROM；電子記憶媒
体（RAMおよびROM）；これらのカテゴリーのハイブリッド（例えば、磁気／光学記
憶媒体）が挙げられるが、これらに限定されない。当業者は、任意の現在公知のコンピ
ューター読取り媒体が使用され、本発明のヌクレオチド配列をその上に記録しているコンピ
ューター読取り媒体を含む製品を作製し得ることを容易に理解し得る。した本明細書中で
使用される場合、「記録される」は、コンピューター読取り媒体に情報を保存するプロセ
スをいう。当業者は、本発明のヌクレオチド配列方法を含む製品を作成するためコンピ
ューター読取り媒体上に情報を記録する任意の現在公知の方法を容易に適用し得る。 50

【0316】

種々のデータ保存構造物は、本発明のヌクレオチド配列をその上に記録しているコンピューター読取り媒体を作成するため当業者にとって入手可能である。データ保存構造物の選択は、一般的に、保存された情報にアクセスするために選択された手段に基づく。さらに、種々のデータ処理プログラムおよびフォーマットは使用され、本発明のヌクレオチド配列情報をコンピューター読取り媒体上に記録し得る。配列情報は、市販されているソフトウェア（例えば、ワードパーフェクトやマイクロソフト・ワード）でフォーマットされたワードプロセッシングテキストファイルに表され得るか、または、データベースアプリケーションにおいて保存されるASCIIファイルの形式（例えば、DB2、Sybase、オラクル）で表され得る。当業者は、本発明のヌクレオチド配列情報をその上に記録されているコンピューター読取り媒体を得るために任意の数のデータ処理構造のフォーマット（例えば、テキストファイルかデータベース）を容易に適用し得る。 10

【0317】

ヌクレオチド配列番号 1～3、10～13もしくは15、またはその代表的なフラグメントのいずれか；又は配列番号 1～3、10～13または15のヌクレオチド配列のいずれかと少なくとも95%同一であるヌクレオチド配列を、コンピューター読取り可能な形で提供することにより、当業者は、種々の目的のために、その配列情報にいつでも日常的にアクセスし得る。コンピューター読取り可能な媒体で提供される配列情報にアクセスすることを可能にするコンピューターソフトウェアは、公に入手可能である。以下の例は、核酸配列内のオープン・リーディング・フレーム（ORF）を同定するために、どのよう 20
にしてSybaseシステム上でBLAST（Altschul等、J. Mol. Biol. 215: 403-410（1990））及びBLAZE（Brutlag等、Comp. Chem. 17: 203-207（1993））探索アルゴリズムを実行するソフトウェアが使用されるかを示している。このようなORFは、タンパク質をコードするフラグメントであるかも知れないし、発酵反応に使われる酵素や、商業的に有用な代謝産物の生産に使用される酵素のように、商業的に重要なタンパク質の産生に有用であり得る。

【0318】

本明細書中で使用される場合、「コンピューターベースシステム」は、本発明のヌクレオチド配列情報を分析するのに使われるハードウェア手段、ソフトウェア手段、及びデータ 30
記憶手段をいう。本発明のコンピューターベースシステムにとって最低限必要なハードウェア手段は、中央演算処理手段（CPU）、インプット手段、アウトプット装置、そしてデータ記憶手段を含む。当業者であれば、現在入手可能なコンピューターベースシステムのいずれかが、本発明の使用に適しているのがどれであるか容易に理解し得る。上述の如く、本発明のコンピューターベースシステムは、本発明のヌクレオチド配列をそこに記録したデータ記憶手段、及び探索手段の支援と実行に必要なハードウェア手段とソフトウェア手段からなる。

【0319】

本明細書中で使用される場合、「データ記憶装置」は、本発明のヌクレオチド配列情報を記録し得る記憶装置（メモリー）か、本発明のヌクレオチド配列情報をそこに記録している 40
製品にアクセスし得るメモリー・アクセス手段のいずれかをいう。

【0320】

本明細書中で使用される場合、「探索手段」は、標的配列又は標的構造モチーフとデータ記憶手段内に記録した配列情報とを比較するために、コンピューターベースシステム上で実行される1つ以上のプログラムをいう。探索手段は、特定の標的配列又は標的モチーフと一致するフラグメントや既知の配列の領域を同定するのに使用される。種々の公知のアルゴリズムが公に開示されており、そして、種々の市販されている検索手段を行うためのソフトウェアが、本発明のコンピューターベースシステムで使用されており、そして使用され得る。このようなソフトウェアの例としては、Smith-Waterman、MacPattern（EMBL）、BLASTNおよびBLASTA（NPOLYPEP 50

T I D E I A) 等があるがこれらに限定されない。当業者は、相同性探索を行うための利用可能なアルゴリズム又は実行ソフトウェアパッケージのいずれか1つが、本発明のコンピュータベースシステムでの使用のために適用され得ることを容易に理解する。本明細書中で使用される場合、「標的配列」は、任意の核酸または6つ以上のヌクレオチドのアミノ酸配列、あるいは2つ以上のアミノ酸を意味している。標的配列は長ければ長いほど、データベースにおけるランダムな出現として、標準配列は存在する可能性が低いことを当業者は容易に認識し得る。標的配列のうち最も好ましい配列の長さは、約10～100のアミノ酸、又は約30～300のヌクレオチド残基である。しかし既に良く知られていることであるが、遺伝子発現およびタンパク質プロセッシングに関連する配列フラグメントのような商業的に重要なフラグメントの探索は、より短い長さであり得る。

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【0321】

本文書に使用される「標的構造モチーフ」又は「標的モチーフ」という用語は、合理的に選択された配列又は配列の組み合わせのことであり、その配列は、標的モチーフのひだ上に形成される3次元構造に基づいて選ばれる。医学業界では種々の種類の標的モチーフが知られている。タンパク質の標的モチーフは、例えば、酵素の活性部位やシグナル配列などである。核酸の標的モチーフには、プロモーター（促進因子）配列、ヘアピン構造、誘発性発現要素（タンパク質結合配列）等が含まれる。

【0322】

このような方法における使用に適したポリヌクレオチドは、通常、長さが20～40塩基であり、転写に関係する遺伝子の領域（三重ヘリックス - Lee等、Nuc l . A c i d s R e s . 6 : 3 0 7 3 (1 9 7 9) ; Cooney等、Science、15241 : 456 (1988) ; およびDervan等、Science、251 : 1360 (1991)を参照のこと）、又はmRNA自身（アンチセンス - Olm no、J. Neurochem. 56 : 560 (1991) ; Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)）に相補的であるように設計されている。三重ヘリックス形成は、必要に応じてDNAからのRNA転写の遮断を生じ、アンチセンスRNAハイブリダイゼーションはmRNA分子のポリペプチドへの翻訳をブロックする。両技術とも、モデルシステムにおいて有効であることが実証されている。本発明の配列に含まれる情報は、アンチセン

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【0323】

（5.16 診断用アッセイおよびキット）

本発明は、核酸プローブ又は本発明の抗体（必要に応じて結合体化されるかまたは他に適切な標識と結合される）を使用することにより、本発明のORFの1つ又はそのホモログの存在又は発現を、試験サンプル中で同定する方法もさらに提供する。

【0324】

一般的に、本発明のポリヌクレオチドを検出するための方法は、ポリヌクレオチドに結合し複合体を形成する化合物をその複合体の形成に必要な時間サンプルと接触させる工程、およびその複合体を検出する工程を包含し得る。その結果、もし複合体が検出されれば、本発明のポリヌクレオチドもサンプル中で検出される。このような方法はまた、ストリンジェントなハイブリダイゼーション条件下で、このような条件下で本発明のポリヌクレオチドをアニールする核酸プライマーにサンプルと接触させる工程、そしてアニールされたポリヌクレオチドを増幅させる工程を包含し得る。その結果、もしポリヌクレオチドが増幅すれば、本発明のポリヌクレオチドがサンプル中に検出される。

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【0325】

一般的に、本発明のポリペプチドを検出する方法は、ポリペプチドに結合し複合体を形成する化合物をその複合体の形成に必要な時間サンプルと接触させる工程、そしてその複合体を検出する工程を包含する。その結果、もし複合体が検出されれば、本発明のポリペプチドもサンプルの中に検出される。

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【0326】

詳細には、このような方法は、試験サンプルを本発明の抗体の1つ以上、又は核酸プローブの1つ以上とインキュベートさせる工程、そして試験サンプル内の成分と核酸プローブまたは抗体の結合についてアッセイする工程を包含する。

【0327】

核酸プローブまたは抗体を試験サンプルと共にインキュベートする条件は変化する。インキュベーション条件は、アッセイに使われるフォーマット、使用される検出方法、アッセイに使用される核酸プローブまたは抗体の型及び性質に依存している。当業者は、一般に入手可能なハイブリダイゼーション、増幅、又は免疫学的アッセイフォーマットのうちのいずれか1つが、本発明の核酸プローブ又は抗体を使用するために容易に適合され得ることを認識する。このようなアッセイの例は、Chard, T., *An Introduction to Radioimmunoassay and Related Techniques*, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G. R., *Techniques in Immunocytochemistry*, Academic Press, Orlando, FL 第一巻 (1982)、第二巻 (1983)、第三巻 (1985); Tijssen, P., *Practice and Theory of Immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985) において見出され得る。本発明の試験サンプルとしては、細胞、タンパク質又は細胞の膜抽出物、生体液（痰、血液、血清、血漿、尿等）が挙げられる。上述の方法に使用される試験サンプルは、アッセイフォーマット、検出方法の性質、アッセイされるサンプルとして使われる組織、細胞、又は抽出物の性質等に依存して変化する。細胞のタンパク質抽出物又は膜抽出物の調製のための方法は当該分野で周知であり、利用されるシステムと適合性のサンプルを獲得するために容易に適合され得る。

【0328】

本発明の別の実施形態では、本発明のアッセイを実行するのに必要な試薬を含むキットが提供される。特に本発明は、1つ以上の容器を密接に閉じ込めて受容する区画キットを提供し、この容器は、以下を包含する：(a) 本発明のプローブ又は抗体のうちの1つを含む第一の容器；および(b) 以下の1つ以上を含む1つ以上の他の容器：洗浄試薬、結合プローブ又は抗体の存在を検出し得る試薬。

【0329】

詳細には、区画キットには、試薬を各々異なる容器に入れている任意のキットが含まれる。このような容器としては、小さいガラス容器、プラスチック容器、又はプラスチックや紙の切れ端が挙げられる。このような容器を使うと、試薬を1つの区画から別の区画に効果的に効率的に移すことが可能となり、その結果サンプルおよび試薬は混じり合うことがないし、各容器に入っている因子や溶液を定量的に、1つの区画キットから別の区画キットへ添加し得る。このような容器としては、試験サンプルを入れる容器、アッセイに使用される抗体を入れる容器、洗浄試薬（リン酸緩衝化生理食塩水、トリス緩衝液等）を入れる容器、および結合抗体やプローブを検出するのに使われる試薬を入れた容器等がある。検出試薬の型には、標識された核酸プローブ、標識された二次抗体、あるいは一次抗体が標識されている場合は、標識された抗体と反応可能な酵素又は抗体の結合試薬が含まれる。当業者は、本発明で開示されたプローブ又は抗体が、当該分野で周知の確立されたキットフォーマットのいずれに組み込まれているか容易に分かるはずである。

【0330】

(5.17 医療画像化)

本発明の新規のポリペプチド及び結合パートナーは、本発明の分子を発現する部位の医療画像化にとって有益である（例えば本発明のポリペプチドが免疫反応に関係している場所

、炎症又は感染部分の画像化等)。例えば、K u n k e l 等、米国特許第 5, 4 1 3, 7 7 8 号を参照のこと。このような方法には、標識薬又は画像剤の化学的付着、薬学的に受容可能なキャリア中で被験体に標識ポリペプチドを投与、標的部位でインビボでの標識ポリペプチドの画像化を含む。

【0331】

(5. 18 スクリーニングアッセイ)

本発明は、単離されたタンパク質及びポリヌクレオチドを使うことにより、配列番号 1 ～ 3、10 ～ 13 又は 15 に示されているヌクレオチド配列のいずれかに対応する O R F によりコードされているポリペプチドと結合した因子、又はその核酸によりコードされたポリペプチドの特異的ドメインと結合している因子を獲得および同定する方法をさらに提供

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する。上記方法の詳細は、以下の工程を包含する：
(a) 本発明の O R F によりコードされた単離されたタンパク質または本発明の核酸を因子に接触させる工程；および

(b) その因子が上記タンパク質又は上記核酸と結合するかどうか決定する工程。

従って一般的に、本発明のポリヌクレオチドに結合する化合物を同定するためのこのような方法は、本発明のポリヌクレオチドと化合物をポリヌクレオチド／化合物複合体を形成するのに必要な時間接触させる工程、およびその複合体を検出する工程を包含し、その結果、もしポリヌクレオチド／化合物複合体が検出されれば、本発明のポリヌクレオチドに結合する化合物が同定される。

【0332】

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従って同様に一般的に、本発明のポリペプチドに結合する化合物を同定するためのこのような方法は、本発明のポリペプチドと化合物をポリペプチド／化合物複合体を形成するのに必要な時間接触させる工程、およびその複合体を検出する工程を包含する。その結果、もしポリペプチド／化合物複合体が検出されれば、本発明のポリヌクレオチドに結合する化合物が同定される。

【0333】

本発明のポリペプチドに結合する化合物を同定する方法はまた、本発明のポリペプチドと化合物を細胞中で、ポリペプチド／化合物複合体を形成するのに必要な時間接触させる工程であって、ここで、その複合体は細胞中でレセプター遺伝子配列の発現を促す工程、レセプター遺伝子配列の発現を検出することによって、その複合体を検出する工程を包含する。その結果、もしポリペプチド／化合物複合体が検出されれば、本発明のポリペプチドに結合する化合物が同定される。

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【0334】

このような方法を介して同定される化合物には、本発明のポリペプチドの活性を調節する化合物（つまりその化合物の非存在下で観察される活性と比べポリペプチドの活性を増大させたり減少させたりする化合物）も含めることができる。あるいは、このような方法で同定される化合物の中には、本発明のポリヌクレオチドの発現を調節する化合物（つまりその化合物の非存在下で観察される発現レベルと比べ発現を増大させたり減少させたりする化合物）を含み得る。本発明の方法を使って同定されるような化合物は、当該分野で周知の標準アッセイ方法を用いて、活性／発現の調節能力について試験され得る。

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【0335】

上述のアッセイでスクリーニングされる因子には、ペプチド、炭水化物、ビタミン誘導体、他の薬学的な因子を含むがこれらに限定されない。これらの因子は、無作為に選別およびスクリーニングされ得るか、またはタンパク質モデリング技術を使って、合理的に選別もしくは設計され得る。

【0336】

無作為スクリーニングの場合、ペプチド、炭水化物、薬学的に受容可能な因子などは無作為に選択され、本発明の O R F によりコードされているタンパク質へ結合する能力についてアッセイされる。あるいは、因子は合理的に選別又は設計することも可能である。本明細書中では、ある因子が特定のタンパク質の構造に基づいて選択される時、その因子は「

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合理的に選別又は設計される」といわれる。例えば、当業者は、合理的に設計された抗ペプチドペプチド（例えばHurby等、Application of Synthetic Peptides: Antisense Peptides, 「In Synthetic Peptides, A User's Guide」, W. H. Freeman, NY (1992), 289-307、およびKaspiczakら、Biochemistry 28:9230-8 (1989)）、又は薬学的な因子などを作るために、現在利用可能な手順を容易に適用して、特定のペプチド配列に結合する能力を持つペプチドや薬学的因子などを生産する。

【0337】

前述の内容に加えて、広義に記載されるように、本発明のあるクラスの因子は、本発明のORF又はEMFの1つへの結合を通して、遺伝子発現をコントロールするために使用できる。上述のように、このような因子は無作為にスクリーニングされ得るか、または合理的に設計／選別され得る。当業者は、ORF又はEMFを標的にすることにより、単一のORF又は複数のORF（いずれも発現制御の面で同じEMFに依存）のいずれかの発現を調節し、配列特異的又は要素特異的の因子を設計することができる。あるクラスのDNA結合因子は、ハイブリダイズするか、あるいはDNA又はRNAに結合することにより三重ヘリックスを形成する、塩基の残基を含む因子である。このような因子は、古典的なリン酸ジエステル、リボ核酸骨格に基づいていても良いし、塩基付着能力を有する種々のスルフヒドリル誘導体又は重合誘導体でも良い。

【0338】

このような方法に使用される因子は、普通20～40の塩基を含んでおり、転写に関係する遺伝子の領域に相補的であるように（三重ヘリックス-Lee等、Nucleic Acids Res. 6:3073 (1979); Cooney等、Science, 241:456 (1988); 及びDervan等、Science 251:1360 (1991)を参照のこと）、あるいはmRNA自体に相補的であるように（アンチセンス-Okano、J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)）設計されている。三重ヘリックス形成は、必要に応じてDNAからRNAへの転写阻害をもたらす、アンチセンスRNAハイブリダイゼーションはmRNAをポリペプチドへの翻訳をブロックする。両技術とも、モデル・システムに於いて有効であることが証明されている。本発明の配列に含まれる情報は、アンチセンス、三重ヘリックスオリゴヌクレオチド、その他のDNA結合因子の設計にとって必要である。

【0339】

本発明のORFの1つによりコード化されているタンパク質に結合する因子は、診断剤として使用され得る。本発明のORFの1つによりコード化されているタンパク質に結合する因子は、薬学的組成物の生成のために既知の技術を使用して処方され得る。

【0340】

（5.19 プローブとしての核酸の使用）

本発明の別の局面は、天然に存在するヌクレオチド配列を使ってハイブリダイズし得るポリペプチド特有の核酸ハイブリダイゼーションプローブを提供することにある。本発明のハイブリダイゼーションプローブは、ヌクレオチド配列番号1～3、10～13または15のうちのいずれかに由来し得る。対応する遺伝子は限られた数の組織にしか発現されないもので、ヌクレオチド配列番号1～3、10～13および15のいずれかに由来するハイブリダイゼーションプローブは、サンプル中の組織のような細胞型のRNAの存在の指標として使用され得る。

【0341】

例えば、インサイチュハイブリダイゼーションのような任意の適切なハイブリダイゼーション手法が使用され得る。米国特許第4,683,195号と同4,965,188号に

記載されるPCRは、ヌクレオチド配列に基づくオリゴヌクレオチドのさらなる用途を提供する。PCRに用いられるこのようなプローブは組み換え起源であってもよいし、化学的に合成されてもよいし、またはその混合でもよい。プローブは、同一配列の検知のための別々のヌクレオチド配列、または密接に関係したゲノム配列の同定のための可能性のある配列の変性プールを含む。

【0342】

核酸のための特定のハイブリダイゼーションプローブを製造する他の手段には、mRNAプローブの製造のためのベクターに核酸配列をクローン化する手段が含まれる。このようなベクターは、当業者に公知であり、市販されており、T7またはSP6 RNAポリメラーゼのような適切なRNAポリメラーゼ、および適切に放射線標識したヌクレオチドを添加することによって、インビトロでRNAプローブを合成するために使用され得る。ヌクレオチド配列は、それぞれのゲノム配列をマップするためのハイブリダイゼーションプローブを構成するために使用され得る。本明細書中で提供されるヌクレオチド配列は、周知の遺伝子および／または染色体地図作成手法を使用して、ある染色体または染色体の特定領域に対してマップすることができる。これらの手法には、インサイチュハイブリダイゼーション、既知の染色体マーカーに対する連鎖分析、ライブラリーを使ったハイブリダイゼーションスクリーニングまたは既知の染色体特異的なフローソート (flow sorted) 染色体調製などがある。染色体分布の蛍光インサイチュハイブリダイゼーションの手法は、とりわけVermaら (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York NY. に記載されている。

【0343】

染色体調製物の蛍光インサイチュハイブリダイゼーションおよびその他の物理的染色体地図作成手法は、さらなる遺伝子地図データと相関し得る。遺伝子地図データの例は、1994 Genome Issue of Science (265:1981f) に見出され得る。物理的な染色体地図上の核酸の位置と特定の疾病（または特定の疾病に罹りやすい性質）の相関性は、その遺伝疾患と関連するDNAの領域を限定する上で役立つ。本発明のヌクレオチド配列は正常者、保菌者または罹患した個体の間の遺伝子配列の違いを検知するのに使用され得る。

【0344】

(5.20 支持体に結合されるオリゴヌクレオチドの調製)

オリゴヌクレオチド、すなわち、核酸の小セグメントは、例えば自動オリゴヌクレオチド合成装置を使って一般的に行われているように化学的方法によってオリゴヌクレオチドを直接合成することによって、容易に調製され得る。

【0345】

支持体結合されたオリゴヌクレオチドは、当業者には公知のガラス、ポリスチレンまたはテフロン（登録商標）のような任意の適当な支持体を使う方法のいずれかによって調製され得る。1つの戦略は、標準的な合成装置を使って合成されたオリゴヌクレオチドを正確にスポットする戦略である。固定は、受動的吸着を使用して (Inouye & Hondo, 1990 J. Clin Microbiol 28 (6) 1462-72) ; 紫外線を使用して (Nagataら、1985; Dahlenら、1987、MorrisseyおよびCollins、Mol. Cell Probes 1989 3 (2) 189-207) または改変されたDNAの共有結合 (Kellerら、1988; 1989) などによって達成され得、これらの参考文献は本明細書中に明確に援用される。

使用され得る別の戦略は、リンカーとして強力なバイオチン-ストレプトアビジン相互作用の使用である。例えば、Broudeら (1994) Proc. Natl. Acad. Sci USA 91 (8) 3072-6は、ビオチン化したプローブの使用について記載しており、それらはストレプトアビジン被覆された磁気ビーズ上に固定された二重鎖プローブである。ストレプトアビジンを被覆したビーズはDyna1, O

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s l oから購入され得る。もちろん、同じリンクのための化学はストレプトアビジンを用いる任意の表面を被覆するのに適用され得る。ビオチン化したプローブは、例えば、O p e r o n T e c h n o l o g i e s (A l a m e d a , C A) のような種々の供給源から入手され得る。

【0346】

N a n c L a b o r a t o r i e s (N a p e r v i l l e , I L) もまた、使用され得る適切な材料を販売している。N a n c L a b o r a t o r i e s は、DNA が、C o v a l i n k N H と呼ばれるマイクロウェル表面に共有結合し得る方法を開発した。C o v a l i n k N H は、更なる共有結合のための橋頭堡 (b r i d e - h e a d) として役立つ第2のアミノ基 (> H N) が移植されたポリスチレン面である。C o v a l i n k モジュールは、N a n c L a b o r a t o r i e s から購入され得る。DNA 分子はホスホラミダイト結合により C o v a l i n k の 5' 端に排他的に結合され得、1 p m o l 以上の DNA の固定を可能にする (R a s m a s s e n ら、(1991) A n a l B i o c h e m 198 (1) 138-42)。

【0347】

DNA 分子の 5' 端への共有結合のための C o v a l i n k N H ストリップの使用が記載されている (R a s m a s s e n ら、1991)。この技術においては、ホスホラミド結合が使用されている (C h u ら、1983 N u c l e i c A c i d s 11 (18) 6513-29)。これは、単一の共有結合のみを使用する固定が好ましいときに有利である。ホスホラミダイト結合が、DNA を C o v a l i n k N H 第2アミノ基に加える。これは、長さ 2 n m のスペーサーアームを介して、ポリスチレン表面に共有結合的に移植したスペーサーアームの端部に位置している。オリゴヌクレオチドを、ホスホラミダイト結合を介して C o v a l i n k N H にリンクするためには、オリゴヌクレオチド末端は 5' 端リン酸基を有さなければならない。おそらくビオチンを C o v a l i n k に共有結合し、ストレプトアビジンはプローブを結合するために使用され得る。

【0348】

より詳細には、この連結方法は DNA を水 (7.5 n g / u l) に溶解する工程、10 分間 95 °C で変性する工程、氷で 10 分間冷やす工程を包含する。次に、氷で冷やした 0.1 M 1-メチルイミダゾール、pH 7.0 (1-M e I m 7) を加えて、最終的な濃度を 10 mM 1-M e I m 7 とする。次いで、s s DNA 溶液を氷上の C o v a l i n k N H ストリップ (75 u l / w e l l) に分配する。

【0349】

10 mM 1-M e I m 7 に溶解したカルボジイミド 0.2 M 1-エチルー 3-(3-ジメチルアミノプロピル)-カルボジイミド (E D C) を新たに作り、ウェルあたり 25 u l 加えた。ストリップは 50 °C で 5 時間インキュベートした。インキュベーション後、ストリップを例えば N a n c - I m m u n o W a s h を使って洗浄した；最初に、ウェルを 3 回洗い、その後 5 分間洗浄液に浸漬し、最後に 3 回洗浄した（ここで、洗浄液は 50 °C に温めた 0.4 N N a O H、0.25 % S D S であった）。

【0350】

本発明の使用にさらに適した方法は、P C T 特許出願 W O 90 / 03382 (S o u t h e r n & M a s k o s) に記載される方法であることが意図され、これは、本明細書中に参考として援用される。支持体に結合されるオリゴヌクレオチドを調製する方法は、支持体によって保有される脂肪性ヒドロキシル基への共有結合リン酸ジエステル結合によって、リン酸基を介してヌクレオシド 3'-試薬を連結する工程を包含する。次いで、オリゴヌクレオチドは、支持されたヌクレオシドと合成オリゴヌクレオチド鎖から取り除かれた保護基に、支持体からオリゴヌクレオチドを切断しないような標準条件下で合成される。適切な試薬としては、ヌクレオシドホスホラミダイトおよびヌクレオシドリソホスファレート (p h o s p h o r a t e) が含まれる。

【0351】

DNA プローブアレイの調製のための DNA プローブの調製についてのオンチップのスト

ラテジーが用いられ得る。例えば、アドレス可能なレーザー操作光防護解除 (photo deprotection) は、ガラス表面上での直接的なオリゴヌクレオチドの化学合成に用いられ得る。このことに関しては、Fodor ((1991) Science 251 (4995) 767-73) らにより開示されており、本明細書中で参考として援用される。プローブはまた、ナイロン支持体の上に固定され得、このことに関しては、Van Nessel ((1991) Nucleic Acids Res. 19 (12) 3345-50) により開示される。また、プローブは、DuncanおよびCavallier ((1988) Anal Biochem 169 (1) 104-8) の方法を用いてテフロン (登録商標) に連結され得る；これらすべての参考文献は、本明細書中で参考として援用される。

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【0352】

オリゴヌクレオチドをナイロン支持体に連結するために、Van Nessel ((1991)) に記載されるように、ナイロン表面をアルキル化によって活性化および、シアヌル酸塩化物でオリゴヌクレオチドの 5'-アミンの選択的活性化が必要とされる。

【0353】

支持体に結合されたオリゴヌクレオチドを調製するための1つの特定の方法は、Pease ((1994) Proc. Natl. Acad. Sci. USA 91 (11) 5022-6) により記載された光合成法を用いることである。これらの著者は、固定されたオリゴヌクレオチドプローブのアレイ (DNAチップ) を作成するために近年の写真平版技術を用いた。光を使って高密度でのオリゴヌクレオチドプローブを合成に指向するこれらの方法、ミニチュア化されたアレイは、感光性 5'-保護N-アシルデオキシヌクレオシドホスホラミダイト、表面リンカー化学特性および多様な複合合成ストラテジーを用いる。この方法で、256個の空間的に定義されたオリゴヌクレオチドプローブのマトリクスが作成され得る。

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【0354】

(5.21 核酸フラグメントの調製)

核酸は、cDNA、ゲノムDNA、染色体DNA、マイクロ切断染色体バンド、コスミドまたはYACインサート、およびRNA (任意の増幅工程のないmRNAを含む) のような任意の適切な供給源から獲得され得る。例えば、Sambrook ((1989)) は、哺乳類細胞由来の高分子量DNAの単離のためのプロトコルについて記載している (9.14~9.23頁)。

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【0355】

DNAフラグメントは、M13、プラスミドまたはラムダベクターにクローンとして、および／もしくはPCRまたは他の増幅方法によってゲノムDNAまたはcDNAから直接調製され得る。サンプルは、マルチウェルプレート上で調製または分配され得る。約100-1000ngのDNAサンプルが、終容量2-500ml中に調製され得る。

【0356】

次いで核酸は、当業者に公知の任意の方法により (例えば、Sambrook ((1989)) の9.24~9.28に記載される制限酵素を用いることにより、超音波とNaOH処理で切断することにより)、断片化される。

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【0357】

低圧せん断法もまた、Schrieffer ((1990) Nucleic Acids Res. 18 (24) 7455-6) により記載されるように、適切である。この方法では、DNAサンプルは、低圧から中圧の種々の圧力の小型のフレンチプレッシャーセルを通過する。レバーデバイスは、細胞に対する低圧から中圧の圧力の制御された適用を可能にする。これらの研究の結果は、低圧切断が超音波および酵素によるDNAフラグメンテーション法の有用な代替であることが示す。

【0358】

DNAのフラグメンテーションのための1つの特定の適切な方法として、Fitzgerald ((1992) Nucleic Acids Res. 20 (14) 3753-50

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62) によって記載される2塩基認識エンドヌクラーゼ、CviJIを使用する方法が考慮される。これらの著者は、彼らがショットガンクローニングおよび配列決定に相当であると考え特定のサイズへのDNAの迅速なフラグメンテーションおよび分画のためのアプローチを記載した。

【0359】

制限エンドヌクラーゼCviJIは通常、GとCとの間の認識配列PuGCPyを切断して平滑末端を残す。この酵素(CviIL**)の特異性を変化させる特殊な反応条件は、低分子pUC19(2688個の塩基対)に形成される、DNAフラグメントの準無作為的な産生を可能にする。Fitzgeraldら(1992)は、高速ゲル過法によってサイズ分画されたpUC19のCviJI**消化および末端修復なしの、lacZマイナスM13クローニングベクターへの直接的な連結を用いて、このフラグメンテーションストラテジーの無作為度を定量的に評価した。76個のクローンの配列決定分析は、CviJI**が、pyGCPyとPuGCPu、それに加えてPuGCPy部位を制限すること、および新しい配列データがランダムフラグメンテーションと一致する割合で累積されることを示した。

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【0360】

文献で報告されているように、超音波破碎およびアガロースゲル分画と比較したこのアプローチの利点は：必要なDNAの量が少ないこと(2-5 μgの代わりに0.2-0.5 μg)；処理ステップが少ない(プレライゲーション、末端修復、化学的抽出、またはアガロースゲル電気泳動および溶出が不要)という点が挙げられる。

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【0361】

核酸フラグメントの獲得または調製の方法の如何にかかわらず、DNAを変性してハイブリダイゼーションに利用可能な一本鎖片を提供することが重要である。これは、DNA溶液を80-90℃で2-5分間インキュベートすることによって達成される。この溶液を次に2℃まで急冷し、DNAフラグメントがチップに接触する前に再び再生することを防ぐ。リン酸基はまた、当業者に公知の方法でゲノムDNAから除去されなければならない。

【0362】

(5.22 DNAアレイの調製)

アレイは、ナイロンメンブレンのような支持体の上にDNAサンプルをスポットすることによって調製され得る。スポットは、金属製ピンのアレイ(その位置はマイクロタイタープレートのウェルのアレイに対応する)を使い、約20nlのDNA溶液をナイロンメンブレンに移すことを繰り返すことで行われ得る。オフセットプリントにより、ウェルの密度より高いドット密度が得られる。1-25のドットは、使用されるラベルのタイプに依存して1mm²当たりで達成され得る。いくつかのあらかじめ選ばれた数の行と列にスポットすることを避けることにより、別のサブセット(サブアレイ)を形成し得る。1つのサブアレイ中のサンプルは、異なる個体のDNAの同じゲノムセグメント(または同じ遺伝子)であるか、または異なるオーバーラップするゲノムクローンであり得る。各サブアレイは同じサンプルのレプリカのスポットを表し得る。1つの例では、選択された遺伝子セグメントは、64人の患者から増幅されたものである。各患者に対して、増幅された遺伝子セグメントが、1枚の96ウェルプレート上にあり得る(全96ウェルが同じサンプルを持っている)。64人の患者1人1人に1枚のプレートが調製される。96ピンのデバイスを使うことにより、すべてのサンプルが1枚の8×12cmのメンブレンにスポットされ得る。サブアレイは、各患者から1つずつ、64サンプルを含み得る。96のサブアレイが同一であるとき、ドットスパンは1mm²で、サブアレイ間に1mmの間隔があり得る。

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【0363】

別のアプローチとしては、物理的なスペーサー(例えば、プラスチックグリッド(メンブレンに渡って成形されており、そのグリッドはマルチウェルプレート、または疎水性ストリップの底部に適用されるメンブレンの種類と類似である))によって分配され得るメン

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ブレンまたはプレート (N U N C, N a p e r v i l l e, I l l i n o i s) を使う方法である。固定された物理的スペーサーは、平面リン保存スクリーン (f l a t p h o s p h o r - s t o r a g e s c r e e n)、またはX線フィルムへの曝露によるイメージングには好ましくない。

【0364】

本発明を以下の例で説明する。この開示により、当業者には本発明の範囲の中で、多くの他の実施例や変更ができることは明らかである。したがって、本発明の広範囲の局面は、以下の実施例の開示によって制限されるべきではない。本発明は、本発明の1つの局面を示すことを意図した実施例によって範囲を制限されるべきではなく、そして、機能的に同等な組成物および方法は発明の範囲内にある。事実、ここに示す望ましい実施例を考察すれば当業者にとって本発明の実施において多くの修飾および改変が可能である。したがって、本発明の範囲における唯一の制限は、添付された請求項によるもののみである。

【0365】

本明細書中に列挙されるすべての参考文献は、その全体を参考として援用される。

【0366】

(6.0 実施例)

(実施例1)

(ヒト白血球のcDNAライブラリー由来の配列番号1の単離)

複数の新規の核酸を、標準のP C R、ハイブリダイゼーション配列シグネチャー分析による配列決定、S a n g e r 配列技術を用いて、ヒトの卵巣から調製されたcDNAライブラリーから得た (H y s e q クローン認識番号7735656 (配列番号1) および7727065 (配列番号11))。ライブラリーのインサートを、インサートに隣接するベクター配列に特異的なプライマーを使ってP C Rにより増幅した。このサンプルを、ナイロンのメンブレンにスポットし、そしてオリゴヌクレオチドプローブを使って検定し、配列シグネチャーを与えた。これらのクローンを、類似のまたは同一の配列ごとに分類し、単一の代表的なクローンを、ゲル配列決定のために各グループから選択した。次いで増幅されたインサートの5'配列を、典型的なS a n g e r 配列プロトコルにおける逆M13配列プライマーを使って推定した。P C R生成物を精製し、そして蛍光色素ターミネーターサイクル配列決定 (f l u o r e s c e n t d y e t e r m i n a t o r c y c l e s e q u e n c i n g) に供した。シングルパスゲル配列決定を、377 A p p l i e d B i o s y s t e m s (A B I) シーケンサーを使って行った。インサートを、このライブラリーからは従来得られなかったものであり、また、公開されているデータベースにはない新規な配列であると同定した。この配列は配列番号1および配列番号11と名付けられた。

【0367】

(実施例2)

(配列番号2および配列番号12の集合)

配列番号2および配列番号12と名付けられた本発明の核酸を、E S T 配列、配列番号1または配列番号11をそれぞれシードとして使って集合させた。次に因果循環 (r e c u r s i v e) アルゴリズムを用いて、異なるデータベース (すなわち、H y s e q の E S T 配列を含むデータベース、d b E S T バージョン114、g b p r i 114、およびU n i G e n e バージョン101) からこの集合体に属するさらなる配列を引き出すことによりシードを伸長し伸長集合体を得た。集合体を伸長する上述のデータベースからのさらなる配列がそれ以上なくなったとき、アルゴリズムを停止した。構成要素配列の集合体への組み入れは、伸長集合体に対するB L A S T N ヒットが300以上のB L A S T スコアであり、95%以上の同一性であることに基づいた。

【0368】

集合コンティグに対する最も近い結果を、F A S T X Y アルゴリズムを使い、G e n p e p t リリース114に対してF A S T A バージョン3のサーチを行うことにより得た。F A S T X Y は F A S T A アラインメントの改良版で、インコドンフレームシフトを可能に

する。最も近い結果は、Genpeptからの各集合体に対して最も近いホモログ（およびこの集合体がコードする翻訳されたアミノ酸配列を含む）を示した。最も近い結果を以下に示す。

【0369】

【表1】

配列番号	登録番号	詳細	Smith-Watermanスコア	同一性(%)
2	Z36531	ホモサピエンスフィブリノーゲン様タンパク質	500	38.462
12	U44830	<i>Rana sylvatica</i> 伸長フィブリノーゲン α 鎖	252	28.629

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シグナルペプチド配列およびその切断部位をコードする集合コンティグ内のヌクレオチド配列を、Neural Network SignalP V1.1プログラム（Center for Biological Sequence Analysis, The Technical University of Denmark）を使って決定した。原核生物および真核生物シグナルペプチドおよびそれらの切断部位を同定するためのプロセスはまた、Henrik Nielson, Jacob Engelbrecht, Soren BrunakおよびGunnar von Heijneの“Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites” Protein Engineering、第10巻、第1号、1-6頁（1997）によって開示されており、これは本明細書中で参考として援用される。Nielsonらの参考文献に記載される最大Sスコアおよび平均Sスコアは、それぞれの集合コンティグに対して獲得される。予測されるシグナル配列の最初のアミノ酸から始まり、45個のアミノ酸配列が記載される。配列中の45個のアミノ酸のすべてがシグナルペプチドを含むわけではない。

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【0370】

【表2】

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配列番号12について

アミノ酸セグメントに対応するの開始および終了ヌクレオチド位置	最大Sスコア	平均Sスコア	シグナルペプチドを含むアミノ酸セグメント (A=アラニン、C=システイン、D=アスパラギン酸、E=グルタミン酸、F=フェニルアラニン、G=グリシン、H=ヒスチジン、I=イソロイシン、K=リジン、L=ロイシン、M=メチオニン、N=アスパラギン、P=プロリン、Q=グルタミン、R=アルギニン、S=セリン、T=トレオニン、V=バリン、W=トリプトファン、Y=チロシン、X=未知、*=停止コドン、/=欠失の可能性あるヌクレオチド、\=挿入の可能性あるヌクレオチド)
54:189	0.922	0.765	MMSPSQASLLFLNVCIFICGEVVQGN CVHHSTDSSVYNIVEDGSN(配列番号22)

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配列番号2に対して予測されるアミノ酸配列は、翻訳された新規ポリヌクレオチドと既知のポリヌクレオチドとを比較することによって、ポリペプチドを選択するFASTY (<http://fasta.bioch.virginia.edu>から入手可能)と呼ばれるソフトウェアプログラムを使って得られた(W. R. Pearson, Methods in Enzymology, 183:63-98 (1990)、これは本明細書中

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【0371】

【表3】

配列番号2について

アミノ酸セグメントの最初のアミノ酸残基に対応する予測される開始ヌクレオチド位置	アミノ酸セグメントの最後のアミノ酸残基に対応する予測される終了ヌクレオチド位置	アミノ酸セグメント(A=アラニン、C=システイン、D=アスパラギン酸、E=グルタミン酸、F=フェニルアラニン、G=グリシン、H=ヒスチジン、I=イソロイシン、K=リジン、L=ロイシン、M=メチオニン、N=アスパラギン、P=プロリン、Q=グルタミン、R=アルギニン、S=セリン、T=トレオニン、V=バリン、W=トリプトファン、Y=チロシン、X=未知、*=停止コドン、/=欠失の可能性あるヌクレオチド、\=挿入の可能性のあるヌクレオチド)
3	717	DLKDTIGSVTKTPSGLYIHPGSSYPFEVMCDMDYR GGGWTVIQKRIDGIIDFQRLWCDYLDGFGDLLGEF WLGLKKIFYIVNQKNTSFMLYVALESEDDTLAYAS YDNFWLEDETRFFKMHLGRYSQGNAGDAFRGLKKE DNQNA MPFSTSDVDNDGCRPACLVNGQSVKSCSHL HNKGTGWWFNECGLANLNGIHHFSGKLLATGIQWG TWTKNNSPVKIKSVSMKIRRMYPYKF (配列番号21)

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(実施例3)

(配列番号3の集合)

配列番号3の新規のヌクレオチド配列の集合を、EST配列、配列番号1をシードとして使って得た。このシードを、BLASTのようなソフトウェアプログラムとHys eq (特許) ソフトウェアを使って、EST配列を含むHys eq (特許) データベースからさらなる配列を引き出すことにより、および3'末端を伸長するプライマーを用いるゲル配列決定(377 Applied Biosystems (ABI) シーケンサー)することにより伸長した。構成要素配列の集合体への組み入れは、BLASTスコアが1000以上、p値が $p=3$ (ホモロジーの長さ依存する) であることに基いた。5'末端は、Marathon-ReadyTM cDNAユーザーマニュアル (PT1156-1) (Clontech) (これは、本明細書中で参考として援用される) に開示されるようにRACEを使って伸長した。

【0372】

(実施例4)

(配列番号10の集合)

配列番号10の新規のヌクレオチド配列の集合を、EST配列、配列番号1をシードとして用いて達成した。このシードは、3'末端を伸長するためのプライマーを用いたゲル配列決定(377 Applied Biosystems (ABI) シーケンサー)により、さらに伸長された(プライマー伸長)。5'末端を、Marathon-ReadyTM cDNAユーザーマニュアル (PT1156-1) (Clontech) (これは、本明細書中で参考として援用される) に開示されるようにRACEを使って伸長した。

【0373】

(実施例5)

(配列番号 13 の集合)

PHRAP (Univ. of Washington) または CAP4 (Paracel) を使用して、全長遺伝子 cDNA 配列およびそれに対応するタンパク質配列を配列番号 12 の集合体から生成した。任意のフレームシフトおよび停止ゴドンの誤りは手動で訂正した。編集時に、配列を、FASTY および / または BLAST を使って Genbank (すなわち、Genepept リリース 115) に対してチェックした。編集過程で使用され得た他のコンピュータプログラムとしては、phred Phrap および Consed (Univ. of Washington) および ed-read, ed-ex および cgenz (Hyseq, Inc.) がある。この全長ヌクレオチド配列を配列番号 13 として示す。

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【0374】

ポリペプチド (配列番号 14) は、以下に示す配列番号 13 によってコードされると予測された。このポリペプチドを、翻訳された新規のポリヌクレオチドと既知のポリペプチドとの比較に基づいて、ポリペプチドを選択する BLASTX / FASTY と呼ばれるソフトウェアプログラムを使って予測した。最初のメチオニン ATG は、ヌクレオチド配列および推定ストップコドンの 54 位から始まり、TAA、1034 位のコード領域で終わる。

【0375】

(実施例 6)

(A. 細胞における配列番号 4 ~ 9、14 および 16 ~ 18 の発現)

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チャイニーズハムスター卵巣 (CHO) 細胞またはその他の適切な細胞型を、DMEM (ATCC) および 10% ウシ胎仔血清 (FBS) (Gibco) 中で、70% の集密度になるまで培養する。トランスフェクションの前に、培地を DMEM および 0.5% FCS に交換する。細胞を、FuGENE-6 トランスフェクション試薬 (Boehringer) によって、配列番号 2-3、10、12-13 または 15 の cDNA もしくは pBGal ベクターを用いてトランスフェクションする。要約すれば、4 μ l の FuGENE-6 を 100 μ l の DMEM に希釈し、5 分間インキュベートする。次いで、これに 1 μ g の DNA が加えられ、15 分間インキュベートしたのち、これを 35 mm ディッシュの CHO 細胞に加える。CHO 細胞を、37°C で 5% CO₂ 中でインキュベートする。24 時間後に、培地と細胞溶解産物を収集し、遠心分離し、そしてアッセイ緩衝液に対して透析する (15 mM Tris (pH 7.6)、134 mM NaCl、5 mM グルコース、3 mM CaCl₂ および MgCl₂)。

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【0376】

(B. 配列番号 2-3、10、12-13 または 15 を使った発現研究)

さまざまな組織における配列番号 2-3、10、12-13 または 15 の発現を、準定量的ポリメラーゼ連鎖反応をベースとする技術を使って分析した。ヒトの cDNA ライブラリーを、目的の組織 (成人の膀胱、成人の脳、成人の心臓、成人の腎臓、成人のリンパ節、成人の肝臓、成人の肺、成人の卵巣、成人の胎盤、成人の直腸、成人の脾臓、成人の精巣、骨髄、胸腺、甲状腺、胎児の腎臓、胎児の肝臓、胎児の肝臓-脾臓、胎児の皮膚、胎児の脳、胎児の白血球およびマクロファージ) から発現された遺伝子の供給源として用いる。遺伝子特異的プライマーを使って、サンプルから配列番号 2-3、10、12-13 または 15 の配列の部分を増幅する。増幅された生成物を、アガロースゲルで分離し、ナイロンフィルターに転移して化学的に連結させる。このフィルターは、次に配列番号 2-3、10、12-13 または 15 から生成された放射線標識 (³²P-dCTP) 二本鎖プローブと、Klenow ポリメラーゼを使い、ランダムプライム法でハイブリダイズさせる。そのフィルタを洗浄し (高ストリンジェンシー)、数時間にわたりホスホイメージングスクリーンに曝す。バンドは、特定のライブラリー内の配列番号 2-3、10、12-13 または 15 の配列を含む cDNA の存在、したがって対応する細胞タイプまたは組織中の mRNA の発現の存在を示す。

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【図面の簡単な説明】

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【図 1】

図 1 は、（「プロトロンビナーゼ様」として同定される）配列番号 3 でコードされたタンパク質（すなわち配列番号 4）とヒトのプロトロンビナーゼ F g 1 2 タンパク質（「プロトロンビナーゼ F g 1 2」）（配列番号 1 9）との間の B L A S T X アミノ酸配列のアライメントを示し、これは、この 2 つの配列が、配列番号 4 の 2 9 1 アミノ酸残基について 5 3 % の相同性、配列番号 4 の 2 9 1 のアミノ酸残基について 3 7 % の同一性を共有することを示す：ここで、A = アラニン、C = システイン、D = アスパラギン酸、E = グルタミン酸、F = フェニルアラニン、G = グリシン、H = ヒスチジン、I = イソロイシン、K = リシン、L = ロイシン、M = メチオニン、N = アスパラギン、P = プロリン、Q = グルタミン、R = アルギニン、S = セリン、T =トレオニン、V = バリン、W = トリプトファン、Y = チロシンである。ギャップを破線で示す。

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【図 2】

図 2 は、（「プロトロンビナーゼ様」として同定される）配列番号 1 3 でコードされたタンパク質（すなわち配列番号 1 4）とヒトのプロトロンビナーゼ F g 1 2 タンパク質（配列番号 1 9 のアミノ酸 1 - 2 6 9）との間の B L A S T P 配列のアライメントを示し、これは、2 つの配列が、配列番号 1 4 の 1 3 2 のアミノ酸残基について 5 4 % の相同性、配列番号 1 4 の 1 3 2 のアミノ酸残基について 3 7 % の同一性があることを示す：ここで、A = アラニン、C = システイン、D = アスパラギン酸、E = グルタミン酸、F = フェニルアラニン、G = グリシン、H = ヒスチジン、I = イソロイシン、K = リシン、L = ロイシン、M = メチオニン、N = アスパラギン、P = プロリン、Q = グルタミン、R = アルギニン、S = セリン、T =トレオニン、V = バリン、W = トリプトファン、Y = チロシンである。ギャップを破線で示す。

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【図 3】

図 3 は、（「プロトロンビナーゼ様」として同定される）配列番号 1 3 でコードされたタンパク質（すなわち配列番号 1 4）とヒトのプロトロンビナーゼ F g 1 2 タンパク質（配列番号 1 9 のアミノ酸 1 6 3 - 2 7 0）との間の B L A S T P アライメントを示し、これは、2 つの配列が、配列番号 1 4 の 1 1 5 のアミノ酸残基について 4 0 % の相同性、配列番号 1 4 の 1 1 5 のアミノ酸残基について 3 7 % の同一性があることを示す：ここで、A = アラニン、C = システイン、D = アスパラギン酸、E = グルタミン酸、F = フェニルアラニン、G = グリシン、H = ヒスチジン、I = イソロイシン、K = リシン、L = ロイシン、M = メチオニン、N = アスパラギン、P = プロリン、Q = グルタミン、R = アルギニン、S = セリン、T =トレオニン、V = バリン、W = トリプトファン、Y = チロシンである。ギャップを破線で示す。

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【図 4】

図 4 は、配列番号 1 4 と、凝血、p d b 番号 = I f z c _ B との M S I G e n e A t l a s （ジーンアトラス）アライメントを示す、これは、2 つの配列が、2 2 4 個のアミノ酸について 3 2 % 相同性、2 2 4 個のアミノ酸について 6 9 % の同一性が在ることを示す：ここで、A = アラニン、C = システイン、D = アスパラギン酸、E = グルタミン酸、F = フェニルアラニン、G = グリシン、H = ヒスチジン、I = イソロイシン、K = リシン、L = ロイシン、M = メチオニン、N = アスパラギン、P = プロリン、Q = グルタミン、R = アルギニン、S = セリン、T =トレオニン、V = バリン、W = トリプトファン、Y = チロシンである。スコア 8 6 . 2 2、推定 p 値は 7 . 2 e - 0 4 で、そして上限 p 値は 1 . 1 e - 1 1 である。M S I G e n e A t l a s レポートは、機能的予測に基づくタンパク質構造相同性（2 次および 3 次のタンパク質構造の両方とも）を可能にし、これはスレッディング（S E Q F o l d）、高スループットモデリング、および配列プロファイルに基づく検索（P B 9 0）手法を利用する。

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[Continued on next page]

(54) Title: METHODS AND MATERIALS RELATING TO PROTHROMBINASE-LIKE POLYPEPTIDES AND POLYNUCLEOTIDES

BLASTX ALIGNMENT OF SEQID NO: 4 (PROTHROMBINASE-LIKE) WITH HUMAN PROTHROMBINASE F₂ 12 PROTEIN (PROTHROMBINASE Fc12

The Genbank database indicates that the fibrinogen-like protein is homologous (37% identity)

```

>gi|5730075 ref|NP_006673.1|| fibrinogen-like 2 precursor; fibroleukin
>gi|1847273 pf49 protein - human >gi|137391 fibrinogen-like
protein expressed in T lymphocytes (pf49) - human >emb|CAD55296.1|
(236631) fibrinogen-like protein [Homo sapiens] >gi|AA010925.1|
(AV104015) fibrinogen-like protein 2 [Homo sapiens]
Length = 479

```

Score = 402 (141.5 bits), Expect = 5.7e-36, P = 5.7e-36
Identities = 109/291 (37%), Positives = 155/291 (53%)

PROTEINASE-LIKE: 98 LRRHDEQASGLDYLSNIVKLANVLLTYKVRKQLQFFSRFVQSGSL--CGTDND 155
+ N + D + A L + + V N L + + + Q + R F V Q N + D C + D

PROTHROMBINASE-LIKE: 156 TTGSVYTKTSGLETLIPESGSSYPFEVHCDMDYRGGGWTFIQHRIOSIIOFQRLNCDYLG 215
IG K S Y + P + REV DM+ GGQTV+Q N+DG + F R N DY G

PROTHROMBINASE-LIKE: 216 PGDLGGFWLSEKLEYIVNQHTSFHLYVALESLOOTLAYSTDNFKLSETRFFEDSL 275
 PQ+L GNKLC KI + K +L +LE + YA YD F++ +E ++R+

PROTEINOMINASE-3.1KB: 276 GRYGCGAGCAAFGLGLEDNQKAMPFSTGVYDNDGCRPACLYNQGVKGCSEHSEKQNF 335
G Y+G AGCA R K N + P+T D DND R+ G +C L+ +GRKT

PROTHROMBINASE-LIKE: 336 NECLGLNLG-IRHFGSKLLATGTCMQTW~TYNNQPVKIKSVMEKIRKWP-FTK 388
+ C AKNG +B + + GI NGTV P KS + + H P +TK

of that correspond to a novel secreted prothrombinase-like polypeptide. These pol

(57) Abstract: The invention provides novel polynucleotides and polypeptides encoded by such polynucleotides and mutants or variants thereof that correspond to a novel secreted prothrombinase-like polypeptide. These polynucleotides comprise nucleic acid sequences isolated from cDNA library from human ovary (Hyseq clone identification numbers 773565 (SEQ ID NO: 1) and 7727065 (SEQ ID NO: 11)). Other aspects of the invention include vectors containing processes for producing novel secreted prothrombinase-like polypeptides, and antibodies specific for such polypeptides.

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METHODS AND MATERIALS RELATING TO
PROTHROMBINASE-LIKE POLYPEPTIDES AND POLYNUCLEOTIDES

1. TECHNICAL FIELD

5 The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with uses for these polynucleotides and proteins, for example in therapeutic, diagnostic and research methods. In particular, the invention relates to a novel prothrombinase-like polypeptide.

10 2. BACKGROUND ART

Identified polynucleotide and polypeptide sequences have numerous applications in, for example, diagnostics, forensics, gene mapping; identification of mutations responsible for genetic disorders or other traits, to assess biodiversity, and to produce many other types of data and products dependent on DNA and amino acid sequences. Proteins are known to have biological activity, for example, by virtue of their secreted nature in the case of leader sequence cloning, by virtue of their cell or tissue source in the case of PCR-based techniques, or by virtue of structural similarity to other genes of known biological activity. It is to these polypeptides and the polynucleotides encoding them that the present invention is directed. In particular, this invention is directed to novel soluble prothrombinase-like polypeptides and polynucleotides.

The ability of the body to control the flow of blood following vascular injury is paramount to continued survival. The process of blood clotting and then the subsequent dissolution of the clot, following repair of the injured tissue, is termed hemostasis. Hemostasis consists of 1) vascular constriction, 2) platelet activation and clumping, 3) formation of thrombus, and 4) clot dissolution following tissue repair. In order to initiate the clotting process, inactive prothrombin is converted to thrombin by prothrombinase complex, which is composed of platelet phospholipids, Ca ions, prothrombin and factors Va and Xa. Prothrombinase has been identified and characterized as an immune procoagulant (International Patent Application No. WO98/51335). The generation of thrombin by cleavage of prothrombin is a central regulatory process. Thrombin activates platelets to start the initial cascade and also cleaves fibrinogen, a major plasma protein. Fibrin forms a network of fibers, which finally leads to formation of a clot. Thus, prothrombinase is a procoagulant and regulation of its activity is crucial for survival. Fibrinogen cleavage products are also shown

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to display vasoconstrictor and chemotactic activities and are mitogenic for several cell types (Herrick et al, (1999) *Int. J. Biochem. Cell Biol.* 31, 741-746).

Dysregulation of coagulation pathways can result in severe pathological conditions including hemophilia, myocardial infarction, glomerular diseases, diabetes, tumor growth, fulminant viral hepatitis, and atherosclerosis among others. Many of these pathologies involve fibrin deposition and necrosis of the affected tissue. Thrombosis or blood clotting has also been associated with cancer (Dvorak, H.F. *Hum. Pathol.* 18(3), pp. 275-284 (1987)). Prothrombinase is also associated with viral hepatitis. (Levy et al., *Am. J. Pathol.* 156(4), pp. 1217-1225 (2000)). Some researchers have shown that hyperexpression of prothrombinase is associated with early stages of Type 1 diabetes (van der Planken et al., *Clin. Appl. Thromb. Hemost.* 6(2), pp. 65-68 (2000)). Prothrombinase or prothrombinase-like protein may be beneficial in wound healing by increasing coagulation (Thiagarajan et al., *Biochemistry* 39(21) pp. 6459-6465 (2000)). Inhibition of prothrombinase or prothrombinase-like protein may reduce abnormal clotting, increase pathogenesis or viral hepatitis, decrease liver necrosis, or treat diabetes Type 1.

Recently, it is becoming apparent that there are inducible prothrombinase genes that are induced either upon viral infection or cytokine treatment (Levy et al (2000) *Am. J. Pathol.* 150, 1217-1225). Antibodies to the prothrombinase fgl2 protein were able to abrogate the fulminant hepatitis in murine model of the disease and reduce the liver necrosis. Similarly, it was shown that fgl2 expression is up regulated upon cytokine treatment of pregnant mice leading to spontaneous abortion (Clark et al (1999) *Am. J. Reproductive Immunol.* 42, 37-43). It would be beneficial to identify other inducible prothrombinase-like proteins that are expressed only under certain pathological conditions.

25

3. SUMMARY OF THE INVENTION

This invention is based on the discovery of novel prothrombinase-like polypeptides, novel isolated polynucleotides encoding such polypeptides, including recombinant DNA molecules, cloned genes or degenerate variants thereof, especially naturally occurring variants such as allelic variants, antisense polynucleotide molecules, and antibodies that specifically recognize one or more epitopes present on such polypeptides, as well as hybridomas producing such antibodies. Specifically, the polynucleotides of the present invention are based on a prothrombinase-like polynucleotide isolated from a cDNA library prepared from human ovary

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(Hyseq clone identification numbers 7735656 (SEQ ID NO: 1) and 7727065 (SEQ ID NO: 11)).

5 The compositions of the present invention additionally include vectors such as expression vectors containing the polynucleotides of the invention, cells genetically engineered to contain such polynucleotides and cells genetically engineered to express such polynucleotides.

10 The compositions of the invention provide isolated polynucleotides that include, but are not limited to, a polynucleotide comprising the nucleotide sequence set forth in the SEQ ID NO: 1-3, 10-13, and 15; or a fragment of SEQ ID NO: 1-3, 10-13 and 15; a polynucleotide comprising the full length protein coding sequence of the SEQ ID NO: 1-3, 10-13 and 15 (for example, SEQ ID NO: 4, 9, 14, 18); and a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of any of SEQ ID NO: 1-3, 10-13 and 15. The polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes under stringent hybridization conditions to (a) the complement of any of the nucleotide sequences set forth in SEQ ID NO: 1-3, 10-13 and 15; (b) a nucleotide sequence encoding any of SEQ ID NO: 4-9, 14, and 16-18; a polynucleotide which is an allelic variant of any polynucleotides recited above having at least 70% polynucleotide sequence identity to the polynucleotides; a polynucleotide which encodes a species homolog (e.g. orthologs) of any of the peptides recited above; or a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of the polypeptide comprising SEQ ID NO: 4-9, 14 and 16-18.

15 A collection as used in this application can be a collection of only one polynucleotide. The collection of sequence information or unique identifying information of each sequence can be provided on a nucleic acid array. In one embodiment, segments of sequence information are provided on a nucleic acid array to detect the polynucleotide that contains the segment. The array can be designed to detect full-match or mismatch to the polynucleotide that contains the segment. The collection can also be provided in a computer-readable format.

20 This invention further provides cloning or expression vectors comprising at least a fragment of the polynucleotides set forth above and host cells or organisms transformed with these expression vectors. Useful vectors include plasmids, cosmids, lambda phage derivatives, phagemids, and the like, that are well known in the art. Accordingly, the invention also provides a vector including a polynucleotide of the invention and a host cell containing the polynucleotide. In general, the vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and a selectable marker for the host cell. Vectors according to the invention include expression vectors, replication vectors, probe generation

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vectors, and sequencing vectors. A host cell according to the invention can be a prokaryotic or eukaryotic cell and can be a unicellular organism or part of a multicellular organism.

The compositions of the present invention include polypeptides comprising, but not limited to, an isolated polypeptide selected from the group comprising the amino acid sequence of SEQ ID NO: 4-9, 14, and 16-18; or the corresponding full length or mature protein. Polypeptides of the invention also include polypeptides with biological activity that are encoded by (a) any of the polynucleotides having a nucleotide sequence set forth in the SEQ ID NO: 1-3, 10-13 and 15; or (b) polynucleotides that hybridize to the complement of the polynucleotides of (a) under stringent hybridization conditions. Biologically or immunologically active variants of any of the protein sequences listed as SEQ ID NO: 4-9, 14 and 16-18 and substantial equivalents thereof that retain biological or immunological activity are also contemplated. The polypeptides of the invention may be wholly or partially chemically synthesized but are preferably produced by recombinant means using the genetically engineered cells (e.g. host cells) of the invention.

The invention also provides compositions comprising a polypeptide of the invention.

Pharmaceutical compositions of the invention may comprise a polypeptide of the invention and an acceptable carrier, such as a hydrophilic, e.g., pharmaceutically acceptable, carrier.

The invention also relates to methods for producing a polypeptide of the invention comprising culturing host cells comprising an expression vector containing at least a fragment of a polynucleotide encoding the polypeptide of the invention in a suitable culture medium under conditions permitting expression of the desired polypeptide, and purifying the protein or peptide from the culture or from the host cells. Preferred embodiments include those in which the protein produced by such a process is a mature form of the protein.

Polynucleotides according to the invention have numerous applications in a variety of techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use as oligomers, or primers, for PCR, use in an array, use in computer-readable media, use for chromosome and gene mapping, use in the recombinant production of protein, and use in generation of antisense DNA or RNA, their chemical analogs and the like. For example, when the expression of an mRNA is largely restricted to a particular cell or tissue type, polynucleotides of the invention can be used as hybridization probes to detect the presence of the particular cell or tissue mRNA in a sample using, e.g., *in situ* hybridization.

In other exemplary embodiments, the polynucleotides are used in diagnostics as expressed sequence tags for identifying expressed genes or, as well known in the art and

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exemplified by Vollrath et al., Science 258:52-59 (1992), as expressed sequence tags for physical mapping of the human genome.

The polypeptides according to the invention can be used in a variety of conventional procedures and methods that are currently applied to other proteins. For example, a polypeptide of the invention can be used to generate an antibody that specifically binds the polypeptide. Such antibodies, particularly monoclonal antibodies, are useful for detecting or quantitating the polypeptide in tissue. The polypeptides of the invention can also be used as molecular weight markers, and as a food supplement.

Methods are also provided for preventing, treating, or ameliorating a medical condition which comprises the step of administering to a mammalian subject a therapeutically effective amount of a composition comprising a peptide of the present invention and a pharmaceutically acceptable carrier.

In particular, the polypeptides and polynucleotides of the invention can be utilized, for example, as part of methods for the prevention and/or treatment of diseases associated with the dysregulation of coagulation pathways, such as hemophilia, myocardial infarction, glomerular disease, diabetes, fulminant viral hepatitis and atherosclerosis among others. The polynucleotides and polypeptides may also be beneficial in the treatment of viral infections and in the treatment of some forms of cancer.

The methods of the invention also provides methods for the treatment of disorders as recited herein which comprise the administration of a therapeutically effective amount of a composition comprising a polynucleotide or polypeptide of the invention and a pharmaceutically acceptable carrier to a mammalian subject exhibiting symptoms or tendencies related to disorders as recited herein. In addition, the invention encompasses methods for treating diseases or disorders as recited herein comprising the step of administering a composition comprising compounds and other substances that modulate the overall activity of the target gene products and a pharmaceutically acceptable carrier. Compounds and other substances can effect such modulation either on the level of target gene/protein expression or target protein activity. Specifically, methods are provided for preventing, treating or ameliorating a medical condition, including viral diseases, which comprises administering to a mammalian subject, including but not limited to humans, a therapeutically effective amount of a composition comprising a polypeptide of the invention, a polynucleotide of the invention or a therapeutically effective amount of a composition comprising a binding partner of (e.g., antibody specifically reactive for) prothrombinase-like polypeptides of the invention. The mechanics of the particular condition or pathology will dictate whether the polypeptides, or

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nucleotides of the invention or binding partners (or inhibitors) of the polypeptides would be beneficial to the individual in need of treatment.

According to this method, polypeptides of the invention can be administered to produce an *in vitro* or *in vivo* inhibition of cellular function. A polypeptide of the invention can be administered *in vivo* alone or as an adjunct to other therapies. Conversely, protein or other active ingredients of the present invention may be included in formulations of a particular agent to minimize side effects of such an agent.

The invention further provides methods for manufacturing medicaments useful in the above-described methods.

The present invention further relates to methods for detecting the presence of the polynucleotides or polypeptides of the invention in a sample (e.g., tissue or sample). Such methods can, for example, be utilized as part of prognostic and diagnostic evaluation of disorders as recited herein and for the identification of subjects exhibiting a predisposition to such conditions.

The invention provides a method for detecting a polypeptide of the invention in a sample comprising contacting the sample with a compound that binds to and forms a complex with the polypeptide under conditions and for a period sufficient to form the complex and detecting formation of the complex, so that if a complex is formed, the polypeptide is detected.

The invention also provides kits comprising polynucleotide probes and/or monoclonal antibodies, and optionally quantitative standards, for carrying out methods of the invention. Furthermore, the invention provides methods for evaluating the efficacy of drugs, and monitoring the progress of patients, involved in clinical trials for the treatment of disorders as recited above.

The invention also provides methods for the identification of compounds that modulate (i.e., increase or decrease) the expression or activity of the polynucleotides and/or polypeptides of the invention. Such methods can be utilized, for example, for the identification of compounds that can ameliorate symptoms of disorders as recited herein. Such methods can include, but are not limited to, assays for identifying compounds and other substances that interact with (e.g., bind to) the polypeptides of the invention.

The invention provides a method for identifying a compound that binds to the polypeptide of the present invention comprising contacting the compound with the polypeptide under conditions and for a time sufficient to form a polypeptide/compound complex and detecting the complex, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide is identified.

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Also provided is a method for identifying a compound that binds to the polypeptide comprising contacting the compound with the polypeptide in a cell for a time sufficient to form a polypeptide/compound complex wherein the complex drives expression of a reporter gene sequence in the cell and detecting the complex by detecting reporter gene sequence expression
 5 so that if the polypeptide/compound complex is detected a compound that binds to the polypeptide is identified.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the BLASTX amino acid sequence alignment between the protein
 10 encoded by SEQ ID NO:3 (i.e. SEQ ID NO:4) (also identified as "prothrombinase-like") and human prothrombinase Fg12 protein ("prothrombinase Fg12") (SEQ ID NO:19), indicating that the two sequence share 53% similarity over 291 amino acid residues of SEQ ID NO:4 and 37% identity over 291 amino acid residues of SEQ ID NO:4, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine,
 15 H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine. Gaps are presented as dashes.

Figure 2 shows the BLASTP sequence alignment between the protein encoded by SEQ ID :13 (i.e. SEQ ID NO:14) identified as "prothrombinase-like" and a portion of human
 20 prothrombinase Fg12 protein (amino acids 1-269 of SEQ ID NO:19), indicating that the two sequences share 54% similarity over 132 amino acid residues of SEQ ID NO:14 and 37% identity over the same 132 amino acid residues of SEQ ID NO:14, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Ptenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine,
 25 P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine. Gaps are presented as dashes.

Figure 3 shows the BLASTP alignment between the protein encoded by SEQ ID : 13 (i.e. SEQ ID NO: 14) identified as "prothrombinase-like" and human prothrombinase Fg12 protein (amino acids 163-270 of SEQ ID NO: 19), indicating that the two sequences share 34%
 30 similarity over that 115 amino acid residue portion of SEQ ID NO: 14 and 40% identity over the same 115 amino acid residue portion of SEQ ID NO: 14, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine,

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P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine,
W=Tryptophan, Y=Tyrosine. Gaps are presented as dashes.

Figure 4 shows the MSI GeneAtlas alignment of SEQ ID NO:14 with the blood coagulation, pdb number = 1fzc_B indicating that the two sequences share 32% identity over 224 amino acids and 69% over the same 224 amino acids, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine. The score 86.22, the estimated p-value is 7.2e-04 and the upper limit p value is 1.1e-11. The MSI GeneAtlas Report allows a functional prediction based protein structure homology (both secondary and tertiary protein structure), which utilizes threading (SEQFold), high throughput modeling and sequence profile-based searching (PB90) methodologies.

5. DETAILED DESCRIPTION OF THE INVENTION

The prothrombinase-like polypeptide of SEQ ID NO: 4 is an approximately 388-amino acid protein with a predicted molecular mass of approximately 43 kDa unglycosylated. Protein database searches with the BLASTX algorithm (Altschul S.F. et al., J. Mol. Evol. 36:290-300 (1993) and Altschul S.F. et al., J. Mol. Biol. 21:403-10 (1990), herein incorporated by reference) indicate that SEQ ID NO: 4 is homologous to human prothrombinase Fg12 protein. Figure 1 shows the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO:3 (i.e. SEQ ID NO: 4) (also identified as "prothrombinase-like") and the human prothrombinase Fg12 protein (SEQ ID NO: 19) indicating that the two sequences share 53% similarity over 291 amino acid residues of SEQ ID NO: 4 and 37% identity over the same 291 amino acid residues of SEQ ID NO: 4.

A predicted approximately twenty residue signal peptide is encoded from approximately residue 1 to 20 of SEQ ID NO:4. The mature portion (also represented as SEQ ID No: 9) is useful on its own. This can be confirmed by expression in mammalian cells and sequencing of the cleaved product. The signal peptide was predicted using the Kyte/Doolittle algorithm (J. Molecular Biology, vol. 157, pp. 105-131 (1982)). One of skill in the art will recognize that the actual cleavage site may be different than that predicted by the computer program.

Using eMATRIX software package (Stanford University, Stanford CA), prothrombinase-like is expected to have fibrinogen beta and gamma chains in the C-terminal domain. Specifically, at residues 181 through 217 of SEQ ID NO:4, at residues 222 through 234 of SEQ ID NO:4, at residues 271 through 285 of SEQ ID NO:4, at residues 324 through

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353 of SEQ ID NO:4. The domains corresponding to SEQ ID NO:4 are as follows, wherein
 A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine,
 G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine,
 N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine,

5 V=Valine, W=Tryptophan, Y=Tyrosine:

Residues 181-217 VMCDMDYRGGGWTVIQKRIDGIIDFQRLWCDYLDGFG (designated as
 SEQ ID NO:5),

Residues 324-353 CSHLHNKTGWWFNECGLANLNGIHHFSGKL (designated as SEQ ID
 NO:6),

10 Residues 271-285 FKMHLGRYSGNAGDA (designated as SEQ ID NO:7),

Residues 222-234 EFWLGLKKIFYIV (designated as SEQ ID NO:8).

A predicted soluble, secreted splice variant of SEQ ID NO: 4 is SEQ ID NO: 14. It is
 an approximately 326 amino acid protein with a predicted molecular mass of approximately 37
 kDa unglycosylated. Protein database searches with the BLASTX algorithm (Altschul S.F. et
 al., J. Mol. Evol. 36:290-300 (1993) and Altschul S.F. et al., J. Mol. Biol. 21:403-10 (1990),
 15 herein incorporated by reference) indicate that SEQ ID NO: 14 is homologous to human
 prothrombinase Fg12 protein. Figures 2 and 3 shows the BLASTP amino acid sequence
 alignment between the protein encoded by SEQ ID NO:13 (i.e. SEQ ID NO: 14) (also
 identified as "prothrombinase-like") and the human prothrombinase Fg12 protein (SEQ ID

20 NO: 19).

Using eMATRIX software package (Stanford University, Stanford CA),

prothrombinase-like is expected to have fibrinogen beta and gamma chains in the C-terminal
 domain. Specifically, at residues 181 through 217 of SEQ ID NO:14, at residues 262 through
 291 of SEQ ID NO:14. The domains corresponding to SEQ ID NO:14 are as follows, wherein

25 A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine,
 G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine,
 N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine,
 V=Valine, W=Tryptophan, Y=Tyrosine:

Residues 181-217 VMCDMDYRGGGWTVIQKRIDGIIDFQRLWCDYLDGFG (designated as
 30 SEQ ID NO:5),

Residues 262-292 CSHLHNKTGWWFNECGLANLNGIHHFSGKL (designated as SEQ ID
 NO:6).

A predicted approximately twenty residue signal peptide is encoded from approximately
 residue 1 to residue 20 of both SEQ ID NO: 14 (SEQ ID NO: 16). The extracellular portion is

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useful on its own. This can be confirmed by expression in mammalian cells and sequencing of the cleaved product. The signal peptide region was predicted using the Kyte-Doolittle hydrophobicity prediction algorithm (*J. Mol Biol*, 157, pp. 105-31 (1982), incorporated herein by reference). One of skill in the art will recognize that the actual cleavage site may be different than that predicted by the computer program.

Using Molecular Simulations Inc. GeneAtlas software (Molecular Simulations Inc., San Diego, CA) and the sequence profile-based searching protocol PB90 using optimized PSI-BLAST and the Seqfold based searching protocol on the PSI-BLAST defined domains, the membrane-bound prothrombinase-like polypeptide SEQ ID NO:14 was determined to have a region at residues 82 to 321 (SEQ ID NO:17) with characteristic motifs to blood coagulation (SEQ ID NO: 20) (See Figure 4). The domains corresponding to SEQ ID NO: 14 are as follows wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine:

Blood coagulation domain (designated as amino acids 82 - 321 of SEQ ID NO: 14) with PSI-BLAST e-value of 1.3e-66, protein database identification number entry = lfzc.B (Research Collaboratory for Structural Bioinformatics. <http://www.rcsb.org/pdb>).

The polypeptides and polynucleotides of the invention can be utilized, for example, as part of methods for the prevention and/or treatment of disorders mediated by loss or overexpression of prothrombinase-like polypeptide. Such disorders include diseases associated with the dysregulation of coagulation pathways, such as hemophilia, myocardial infarction, glomerular disease, diabetes, fulminant viral hepatitis and atherosclerosis among others. The polynucleotides and polypeptides may also be beneficial in the treatment of viral infections and in the treatment of some forms of cancer.

5.1 DEFINITIONS

It must be noted that as used herein and in the appended claims, the singular forms "a", "an" and "the" include plural references unless the context clearly dictates otherwise.

The term "active" refers to those forms of the polypeptide which retain the biologic and/or immunologic activities of any naturally occurring polypeptide. According to the invention, the terms "biologically active" or "biological activity" refer to a protein or peptide having structural, regulatory or biochemical functions of a naturally occurring molecule. The term "prothrombinase-like" refers to biological activity that is similar to the biological activity

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of a human prothrombinase. Likewise "biologically active" or "biological activity" refers to the capability of the natural, recombinant or synthetic prothrombinase-like peptide, or any peptide thereof, to induce a specific biological response in appropriate animals or cells and to bind with specific antibodies.

5 The term "activated cells" as used in this application are those cells which are engaged in extracellular or intracellular membrane trafficking, including the export of secretory or enzymatic molecules as part of a normal or disease process.

The terms "complementary" or "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence 5'-AGT-3' binds to the
10 complementary sequence 3'-TCA-5'. Complementarity between two single-stranded molecules may be "partial" such that only some of the nucleic acids bind or it may be "complete" such that total complementarity exists between the single stranded molecules. The degree of complementarity between the nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands.

15 The term "embryonic stem cells (ES)" refers to a cell that can give rise to many differentiated cell types in an embryo or an adult, including the germ cells. The term "germ line stem cells (GSCs)" refers to stem cells derived from primordial stem cells that provide a steady and continuous source of germ cells for the production of gametes. The term "primordial germ cells (PGCs)" refers to a small population of cells set aside from other cell
20 lineages particularly from the yolk sac, mesenteries, or gonadal ridges during embryogenesis that have the potential to differentiate into germ cells and other cells. PGCs are the source from which GSCs and ES cells are derived. The PGCs, the GSCs and the ES cells are capable of self-renewal. Thus these cells not only populate the germ line and give rise to a plurality of terminally differentiated cells that comprise the adult specialized organs, but are able to
25 regenerate themselves.

The term "expression modulating fragment," EMF, means a series of nucleotides which modulates the expression of an operably linked ORF or another EMF.

As used herein, a sequence is said to "modulate the expression of an operably linked sequence" when the expression of the sequence is altered by the presence of the EMF. EMFs
30 include, but are not limited to, promoters, and promoter modulating sequences (inducible elements). One class of EMFs is nucleic acid fragments which induce the expression of an operably linked ORF in response to a specific regulatory factor or physiological event.

The terms "nucleotide sequence" or "nucleic acid" or "polynucleotide" or "oligonucleotide" are used interchangeably and refer to a heteropolymer of nucleotides or the

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sequence of these nucleotides. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA) or to any DNA-like or RNA-like material. Generally, nucleic acid segments provided by this invention may be assembled from

5 fragments of the genome and short oligonucleotide linkers, or from a series of oligonucleotides, or from individual nucleotides, to provide a synthetic nucleic acid which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon, or a eukaryotic gene.

The terms "oligonucleotide fragment" or a "polynucleotide fragment", "portion," or

10 "segment" or "probe" or "primer" are used interchangeable and refer to a sequence of nucleotide residues which are at least about 5 nucleotides, more preferably at least about 7 nucleotides, more preferably at least about 9 nucleotides, more preferably at least about 11 nucleotides and most preferably at least about 17 nucleotides. The fragment is preferably less than about 500 nucleotides, preferably less than about 200 nucleotides, more preferably less

15 than about 100 nucleotides, more preferably less than about 50 nucleotides and most preferably less than 30 nucleotides. Preferably the probe is from about 6 nucleotides to about 200 nucleotides, preferably from about 15 to about 50 nucleotides, more preferably from about 17 to 30 nucleotides and most preferably from about 20 to 25 nucleotides. Preferably the fragments can be used in polymerase chain reaction (PCR), various hybridization procedures

20 or microarray procedures to identify or amplify identical or related parts of mRNA or DNA molecules. A fragment or segment may uniquely identify each polynucleotide sequence of the present invention. Preferably the fragment comprises a sequence substantially similar to a portion of SEQ ID NO: 1-3, 10-13 and 15.

Probes may, for example, be used to determine whether specific mRNA molecules are

25 present in a cell or tissue or to isolate similar nucleic acid sequences from chromosomal DNA as described by Walsh et al. (Walsh, P.S. et al., 1992, PCR Methods Appl 1:241-250). They may be labeled by nick translation, Klenow fill-in reaction, PCR, or other methods well known in the art. Probes of the present invention, their preparation and/or labeling are elaborated in Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor

30 Laboratory, NY; or Ausubel, F.M. et al., 1989, Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, both of which are incorporated herein by reference in their entirety.

The nucleic acid sequences of the present invention also include the sequence information from any of the nucleic acid sequences of SEQ ID NO: 1-3, 10-13 and 15. The

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sequence information can be a segment of SEQ ID NO: 1-3, 10-13 and 15 that uniquely identifies or represents the sequence information of SEQ ID NO: 1-3, 10-13 and 15. One such segment can be a twenty-mer nucleic acid sequence because the probability that a twenty-mer is fully matched in the human genome is 1 in 300. In the human genome, there are three billion base pairs in one set of chromosomes. Because 4^{20} possible twenty-mers exist, there are 300 times more twenty-mers than there are base pairs in a set of human chromosome. Using the same analysis, the probability for a seventeen-mer to be fully matched in the human genome is approximately 1 in 5. When these segments are used in arrays for expression studies, fifteen-mer segments can be used. The probability that the fifteen-mer is fully matched in the expressed sequences is also approximately one in five because expressed sequences comprise less than approximately 5% of the entire genome sequence.

Similarly, when using sequence information for detecting a single mismatch, a segment can be a twenty-five mer. The probability that the twenty-five mer would appear in a human genome with a single mismatch is calculated by multiplying the probability for a full match (1×4^{25}) times the increased probability for mismatch at each nucleotide position (3×25). The probability that an eighteen mer with a single mismatch can be detected in an array for expression studies is approximately one in five. The probability that a twenty-mer with a single mismatch can be detected in a human genome is approximately one in five.

The term "open reading frame," ORF, means a series of nucleotide triplets coding for amino acids without any termination codons and is a sequence translatable into protein.

The terms "operably linked" or "operably associated" refer to functionally related nucleic acid sequences. For example, a promoter is operably associated or operably linked with a coding sequence if the promoter controls the transcription of the coding sequence. While operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements e.g. repressor genes are not contiguously linked to the coding sequence but still control transcription/translation of the coding sequence.

The term "pluripotent" refers to the capability of a cell to differentiate into a number of differentiated cell types that are present in an adult organism. A pluripotent cell is restricted in its differentiation capability in comparison to a totipotent cell.

The terms "polypeptide" or "peptide" or "amino acid sequence" refer to an oligopeptide, peptide, polypeptide or protein sequence or fragment thereof and to naturally occurring or synthetic molecules. A polypeptide "fragment," "portion," or "segment" is a stretch of amino acid residues of at least about 5 amino acids, preferably at least about 7 amino

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acids, more preferably at least about 9 amino acids and most preferably at least about 17 or more amino acids. The peptide preferably is not greater than about 200 amino acids, more preferably less than 150 amino acids and most preferably less than 100 amino acids.

Preferably the peptide is from about 5 to about 200 amino acids. To be active, any

5 polypeptide must have sufficient length to display biological and/or immunological activity.

The term "naturally occurring polypeptide" refers to polypeptides produced by cells that have not been genetically engineered and specifically contemplates various polypeptides arising from post-translational modifications of the polypeptide including, but not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

10 The term "translated protein coding portion" means a sequence which encodes for the full length protein which may include any leader sequence or a processing sequence.

The term "mature protein coding sequence" refers to a sequence which encodes a peptide or protein without any leader/signal sequence. The peptide may have the leader sequences removed during processing in the cell or the protein may have been produced

15 synthetically or using a polynucleotide only encoding for the mature protein coding sequence.

The term "derivative" refers to polypeptides chemically modified by such techniques as ubiquitination, labeling (e.g., with radionuclides or various enzymes), covalent polymer attachment such as pegylation (derivatization with polyethylene glycol) and insertion or substitution by chemical synthesis of amino acids such as ornithine, which do not normally

20 occur in human proteins.

The term "variant"(or "analog") refers to any polypeptide differing from naturally occurring polypeptides by amino acid insertions, deletions, and substitutions, created using, *e.g.*, recombinant DNA techniques. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing activities of interest, may be found by comparing the sequence of the particular polypeptide with that of homologous peptides and minimizing the number of amino acid sequence changes made in regions of high homology (conserved regions) or by replacing amino acids with consensus sequence.

25 Alternatively, recombinant variants encoding these same or similar polypeptides may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations in the polynucleotide sequence may be reflected in the polypeptide or domains of other peptides added to the polypeptide to modify the

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properties of any part of the polypeptide, to change characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate.

Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, *i.e.*, conservative amino acid replacements. "Conservative" amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. "Insertions" or "deletions" are preferably in the range of about 1 to 20 amino acids, more preferably 1 to 10 amino acids. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in a polypeptide molecule using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

Alternatively, where alteration of function is desired, insertions, deletions or non-conservative alterations can be engineered to produce altered polypeptides. Such alterations can, for example, alter one or more of the biological functions or biochemical characteristics of the polypeptides of the invention. For example, such alterations may change polypeptide characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate. Further, such alterations can be selected so as to generate polypeptides that are better suited for expression, scale up and the like in the host cells chosen for expression. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges.

The terms "purified" or "substantially purified" as used herein denotes that the indicated nucleic acid or polypeptide is present in the substantial absence of other biological macromolecules, *e.g.*, polynucleotides, proteins, and the like. In one embodiment, the polynucleotide or polypeptide is purified such that it constitutes at least 95% by weight, more preferably at least 99% by weight, of the indicated biological macromolecules present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000 daltons, can be present).

The term "isolated" as used herein refers to a nucleic acid or polypeptide separated from at least one other component (*e.g.*, nucleic acid or polypeptide) present with the nucleic

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acid or polypeptide in its natural source. In one embodiment, the nucleic acid or polypeptide is found in the presence of (if anything) only a solvent, buffer, ion, or other components normally present in a solution of the same. The terms "isolated" and "purified" do not encompass nucleic acids or polypeptides present in their natural source.

5 The term "recombinant," when used herein to refer to a polypeptide or protein, means that a polypeptide or protein is derived from recombinant (*e.g.*, microbial, insect, or mammalian) expression systems. "Microbial" refers to recombinant polypeptides or proteins made in bacterial or fungal (*e.g.*, yeast) expression systems. As a product, "recombinant microbial" defines a polypeptide or protein essentially free of native endogenous substances
10 and unaccompanied by associated native glycosylation. Polypeptides or proteins expressed in most bacterial cultures, *e.g.*, *E. coli*, will be free of glycosylation modifications; polypeptides or proteins expressed in yeast will have a glycosylation pattern in general different from those expressed in mammalian cells.

 The term "recombinant expression vehicle or vector" refers to a plasmid or phage or
15 virus or vector, for expressing a polypeptide from a DNA (RNA) sequence. An expression vehicle can comprise a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences. Structural units intended
20 for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an amino terminal methionine residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final product.

25 The term "recombinant expression system" means host cells which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit extrachromosomally. Recombinant expression systems as defined herein will express heterologous polypeptides or proteins upon induction of the regulatory elements linked to the DNA segment or synthetic gene to be expressed. This term also means host cells
30 which have stably integrated a recombinant genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers. Recombinant expression systems as defined herein will express polypeptides or proteins endogenous to the cell upon induction of the regulatory elements linked to the endogenous DNA segment or gene to be expressed. The cells can be prokaryotic or eukaryotic.

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The term "secreted" includes a protein that is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence when it is expressed in a suitable host cell. "Secreted" proteins include without limitation proteins secreted wholly (*e.g.*, soluble proteins) or partially (*e.g.*, receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation proteins that are transported across the membrane of the endoplasmic reticulum. "Secreted" proteins are also intended to include proteins containing non-typical signal sequences (*e.g.* Interleukin-1 Beta, see Krasney, P.A. and Young, P.R. (1992) Cytokine 4(2):134 -143) and factors released from damaged cells (*e.g.* Interleukin-1 Receptor Antagonist, see Arend, W.P. et. al. (1998) Annu. Rev. Immunol. 16:27-55)

Where desired, an expression vector may be designed to contain a "signal or leader sequence" which will direct the polypeptide through the membrane of a cell. Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous protein sources by recombinant DNA techniques.

The term "stringent" is used to refer to conditions that are commonly understood in the art as stringent. Stringent conditions can include highly stringent conditions (*i.e.*, hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1X SSC/0.1% SDS at 68°C), and moderately stringent conditions (*i.e.*, washing in 0.2X SSC/0.1% SDS at 42°C). Other exemplary hybridization conditions are described herein in the examples.

In instances of hybridization of deoxyoligonucleotides, additional exemplary stringent hybridization conditions include washing in 6X SSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligonucleotides), 48°C (for 17-base oligonucleotides), 55°C (for 20-base oligonucleotides), and 60°C (for 23-base oligonucleotides).

As used herein, "substantially equivalent" can refer both to nucleotide and amino acid sequences, for example a mutant sequence, that varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between the reference and subject sequences. Typically, such a substantially equivalent sequence varies from one of those listed herein by no more than about 35% (*i.e.*, the number of individual residue substitutions, additions, and/or deletions in a substantially equivalent sequence, as compared to the corresponding reference sequence, divided by the total number of residues in the substantially equivalent sequence is about 0.35 or less). Such a sequence is said to have 65% sequence identity to the listed sequence. In one

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embodiment, a substantially equivalent, *e.g.*, mutant, sequence of the invention varies from a listed sequence by no more than 30% (70% sequence identity); in a variation of this embodiment, by no more than 25% (75% sequence identity); and in a further variation of this embodiment, by no more than 20% (80% sequence identity) and in a further variation of this embodiment, by no more than 10% (90% sequence identity) and in a further variation of this embodiment, by no more than 5% (95% sequence identity). Substantially equivalent, *e.g.*, mutant, amino acid sequences according to the invention preferably have at least 80% sequence identity with a listed amino acid sequence, more preferably at least 90% sequence identity. Substantially equivalent nucleotide sequence of the invention can have lower percent sequence identities, taking into account, for example, the redundancy or degeneracy of the genetic code. Preferably, nucleotide sequence has at least about 65% identity, more preferably at least about 75% identity, and most preferably at least about 95% identity. For the purposes of the present invention, sequences having substantially equivalent biological activity and substantially equivalent expression characteristics are considered substantially equivalent. For the purposes of determining equivalence, truncation of the mature sequence (*e.g.*, via a mutation which creates a spurious stop codon) should be disregarded. Sequence identity may be determined, *e.g.*, using the Jotun Hein method (Hein, J. (1990) *Methods Enzymol.* 183:626-645). Identity between sequences can also be determined by other methods known in the art, *e.g.* by varying hybridization conditions.

The term "totipotent" refers to the capability of a cell to differentiate into all of the cell types of an adult organism.

The term "transformation" means introducing DNA into a suitable host cell so that the DNA is replicable, either as an extrachromosomal element, or by chromosomal integration. The term "transfection" refers to the taking up of an expression vector by a suitable host cell, whether or not any coding sequences are in fact expressed. The term "infection" refers to the introduction of nucleic acids into a suitable host cell by use of a virus or viral vector.

As used herein, an "uptake modulating fragment," UMF, means a series of nucleotides which mediate the uptake of a linked DNA fragment into a cell. UMFs can be readily identified using known UMFs as a target sequence or target motif with the computer-based systems described below. The presence and activity of a UMF can be confirmed by attaching the suspected UMF to a marker sequence. The resulting nucleic acid molecule is then incubated with an appropriate host under appropriate conditions and the uptake of the marker sequence is determined. As described above, a UMF will increase the frequency of uptake of a linked marker sequence.

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Each of the above terms is meant to encompass all that is described for each, unless the context dictates otherwise.

5.2 NUCLEIC ACIDS OF THE INVENTION

5 The invention is based on the discovery of a novel secreted prothrombinase-like polypeptide, the polynucleotides encoding the prothrombinase-like polypeptide and the use of these compositions for the diagnosis, treatment or prevention of neurological conditions and disorders.

The isolated polynucleotides of the invention include, but are not limited to a
 10 polynucleotide comprising any of the nucleotide sequences of SEQ ID NO: 1-3, 10-13 and 15; a fragment of SEQ ID NO: 1-3, 10-13 and 15; a polynucleotide comprising the full length protein coding sequence of SEQ ID NO: 1-3, 10-13 and 15 (for example SEQ ID NO: 4, 9, 14, 18); and a polynucleotide comprising the nucleotide sequence encoding the mature protein coding sequence of the polynucleotides of any one of SEQ ID NO: 1-3, 10-13 and 15. The
 15 polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes under stringent conditions to (a) the complement of any of the nucleotides sequences of the SEQ ID NO: 1-3, 10-13 or 15; (b) a polynucleotide encoding any one of the polypeptides of SEQ ID NO: 4-9, 14 or 16-18; (c) a polynucleotide which is an allelic variant of any polynucleotides recited above; (d) a polynucleotide which encodes a species homolog of
 20 any of the proteins recited above; or (e) a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of the polypeptides of SEQ ID NO: 4-9, 14 or 16-18. Domains of interest may depend on the nature of the encoded polypeptide; e.g., domains in receptor-like polypeptides include ligand-binding, extracellular, transmembrane, or cytoplasmic domains, or combinations thereof; domains in immunoglobulin-like proteins
 25 include the variable immunoglobulin-like domains; domains in enzyme-like polypeptides include catalytic and substrate binding domains; and domains in ligand polypeptides include receptor-binding domains.

The polynucleotides of the invention include naturally occurring or wholly or partially synthetic DNA, e.g., cDNA and genomic DNA, and RNA, e.g., mRNA. The polynucleotides
 30 may include all of the coding region of the cDNA or may represent a portion of the coding region of the cDNA.

The present invention also provides genes corresponding to the cDNA sequences disclosed herein. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or

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primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. Further 5' and 3' sequence can be obtained using methods known in the art. For example, full length cDNA or genomic DNA that corresponds to any of the polynucleotides of the SEQ ID NO:1-3, 10-13 and 15 can be obtained by screening appropriate cDNA or genomic DNA libraries under suitable hybridization conditions using any of the polynucleotides of the SEQ ID NO:1-3, 10-13 and 15 or a portion thereof as a probe. Alternatively, the polynucleotides of the SEQ ID NO: 1-3, 10-13 and 15 may be used as the basis for suitable primer(s) that allow identification and/or amplification of genes in appropriate genomic DNA or cDNA libraries.

The nucleic acid sequences of the invention can be assembled from ESTs and sequences (including cDNA and genomic sequences) obtained from one or more public databases, such as dbEST, gbpro, and UniGene. The EST sequences can provide identifying sequence information, representative fragment or segment information, or novel segment information for the full-length gene.

The polynucleotides of the invention also provide polynucleotides including nucleotide sequences that are substantially equivalent to the polynucleotides recited above. Polynucleotides according to the invention can have, *e.g.*, at least about 65%, at least about 70%, at least about 75%, at least about 80%, more typically at least about 90%, and even more typically at least about 95%, sequence identity to a polynucleotide recited above.

Included within the scope of the nucleic acid sequences of the invention are nucleic acid sequence fragments that hybridize under stringent conditions to any of the nucleotide sequences of the SEQ ID NO: 1-3, 10-13 and 15, or complements thereof, which fragment is greater than about 5 nucleotides, preferably 7 nucleotides, more preferably greater than 9 nucleotides and most preferably greater than 17 nucleotides. Fragments of, *e.g.*, 15, 17, or 20 nucleotides or more that are selective for (*i.e.* specifically hybridize to any one of the polynucleotides of the invention) are contemplated. Probes capable of specifically hybridizing to a polynucleotide can differentiate polynucleotide sequences of the invention from other polynucleotide sequences in the same family of genes or can differentiate human genes from genes of other species, and are preferably based on unique nucleotide sequences.

The sequences falling within the scope of the present invention are not limited to these specific sequences, but also include allelic and species variations thereof. Allelic and species variations can be routinely determined by comparing the sequence provided in SEQ ID NO: 1-3, 10-13 and 15, a representative fragment thereof, or a nucleotide sequence at least 90% identical, preferably 95% identical, to SEQ ID NO: 1-3, 10-13 and 15 with a sequence from another isolate

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of the same species. Furthermore, to accommodate codon variability, the invention includes nucleic acid molecules coding for the same amino acid sequences as do the specific ORFs disclosed herein. In other words, in the coding region of an ORF, substitution of one codon for another codon that encodes the same amino acid is expressly contemplated.

5 The nearest neighbor result for the nucleic acids of the present invention, including SEQ ID NO: 1-3, 10-13 and 15, can be obtained by searching a database using an algorithm or a program. Preferably, a BLAST which stands for Basic Local alignment Search Tool is used to search for local sequence alignments (Altschul, S.F. J. Mol. Evol. 36 290-300 (1993) and Altschul S.F. et al. J. Mol. Biol. 21:403-410 (1990))

10 Species homologs (or orthologs) of the disclosed polynucleotides and proteins are also provided by the present invention. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

The invention also encompasses allelic variants of the disclosed polynucleotides or 15 proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous or related to that encoded by the polynucleotides.

The nucleic acid sequences of the invention are further directed to sequences which encode variants of the described nucleic acids. These amino acid sequence variants may be 20 prepared by methods known in the art by introducing appropriate nucleotide changes into a native or variant polynucleotide. There are two variables in the construction of amino acid sequence variants: the location of the mutation and the nature of the mutation. Nucleic acids encoding the amino acid sequence variants are preferably constructed by mutating the polynucleotide to encode an amino acid sequence that does not occur in nature. These nucleic acid alterations can be made at sites that differ in the nucleic acids from different species 25 (variable positions) or in highly conserved regions (constant regions). Sites at such locations will typically be modified in series, *e.g.*, by substituting first with conservative choices (*e.g.*, hydrophobic amino acid to a different hydrophobic amino acid) and then with more distant choices (*e.g.*, hydrophobic amino acid to a charged amino acid), and then deletions or 30 insertions may be made at the target site. Amino acid sequence deletions generally range from about 1 to 30 residues, preferably about 1 to 10 residues, and are typically contiguous. Amino acid insertions include amino- and/or carboxyl-terminal fusions ranging in length from one to one hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions may range generally from about 1 to 10 amino

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residues, preferably from 1 to 5 residues. Examples of terminal insertions include the heterologous signal sequences necessary for secretion or for intracellular targeting in different host cells and sequences such as FLAG or poly-histidine sequences useful for purifying the expressed protein.

5 In a preferred method, polynucleotides encoding the novel amino acid sequences are changed via site-directed mutagenesis. This method uses oligonucleotide sequences to alter a polynucleotide to encode the desired amino acid variant, as well as sufficient adjacent nucleotides on both sides of the changed amino acid to form a stable duplex on either side of the site being changed. In general, the techniques of site-directed mutagenesis are well known
10 to those of skill in the art and this technique is exemplified by publications such as, Edelman et al., *DNA* 2:183 (1983). A versatile and efficient method for producing site-specific changes in a polynucleotide sequence was published by Zoller and Smith, *Nucleic Acids Res.* 10:6487-6500 (1982). PCR may also be used to create amino acid sequence variants of the novel nucleic acids. When small amounts of template DNA are used as starting material, primer(s)
15 that differs slightly in sequence from the corresponding region in the template DNA can generate the desired amino acid variant. PCR amplification results in a population of product DNA fragments that differ from the polynucleotide template encoding the polypeptide at the position specified by the primer. The product DNA fragments replace the corresponding region in the plasmid and this gives a polynucleotide encoding the desired amino acid variant.

20 A further technique for generating amino acid variants is the cassette mutagenesis technique described in Wells et al., *Gene* 34:315 (1985); and other mutagenesis techniques well known in the art, such as, for example, the techniques in Sambrook et al., supra, and *Current Protocols in Molecular Biology*, Ausubel et al. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally
25 equivalent amino acid sequence may be used in the practice of the invention for the cloning and expression of these novel nucleic acids. Such DNA sequences include those which are capable of hybridizing to the appropriate novel nucleic acid sequence under stringent conditions.

Polynucleotides encoding preferred polypeptide truncations of the invention can be used to generate polynucleotides encoding chimeric or fusion proteins comprising one or more
30 domains of the invention and heterologous protein sequences.

The polynucleotides of the invention additionally include the complement of any of the polynucleotides recited above. The polynucleotide can be DNA (genomic, cDNA, amplified, or synthetic) or RNA. Methods and algorithms for obtaining such polynucleotides are well known to those of skill in the art and can include, for example, methods for determining

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hybridization conditions that can routinely isolate polynucleotides of the desired sequence identities.

In accordance with the invention, polynucleotide sequences comprising the mature protein coding sequences corresponding to any one of SEQ ID NO: 1-3, 10-13 and 15, or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of that nucleic acid, or a functional equivalent thereof, in appropriate host cells. Also included are the cDNA inserts of any of the clones identified herein.

A polynucleotide according to the invention can be joined to any of a variety of other nucleotide sequences by well-established recombinant DNA techniques (see Sambrook J et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY). Useful nucleotide sequences for joining to polynucleotides include an assortment of vectors, e.g., plasmids, cosmids, lambda phage derivatives, phagemids, and the like, that are well known in the art. Accordingly, the invention also provides a vector including a polynucleotide of the invention and a host cell containing the polynucleotide. In general, the vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and a selectable marker for the host cell. Vectors according to the invention include expression vectors, replication vectors, probe generation vectors, and sequencing vectors. A host cell according to the invention can be a prokaryotic or eukaryotic cell and can be a unicellular organism or part of a multicellular organism.

The present invention further provides recombinant constructs comprising a nucleic acid having any of the nucleotide sequences of the SEQ ID NO: 1-3, 10-13 and 15 or a fragment thereof or any other polynucleotides of the invention. In one embodiment, the recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a nucleic acid having any of the nucleotide sequences of the SEQ ID NO: 1-3, 10-13 and 15 or a fragment thereof is inserted, in a forward or reverse orientation. In the case of a vector comprising one of the ORFs of the present invention, the vector may further comprise regulatory sequences, including for example, a promoter, operably linked to the ORF. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLeo, pSV2cat, pOG44, PXTI, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia).

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The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., *Nucleic Acids Res.* 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of

5 expressing recombinant proteins are also known and are exemplified in R. Kaufman, *Methods in Enzymology* 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

10 Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda PR, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of

15 the appropriate vector and promoter is well within the level of ordinary skill in the art. Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from

20 operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), a-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode

25 a fusion protein including an amino terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product. Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or

30 more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

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As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3

5 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM 1 (Promega Biotech, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced or derepressed by appropriate means (*e.g.*, temperature shift or chemical induction) and cells are
10 cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Polynucleotides of the invention can also be used to induce immune responses. For example, as described in Fan et al., *Nat. Biotech.* 17:870-872 (1999), incorporated herein by reference, nucleic acid sequences encoding a polypeptide may be used to generate antibodies
15 against the encoded polypeptide following topical administration of naked plasmid DNA or following injection, and preferably intramuscular injection of the DNA. The nucleic acid sequences are preferably inserted in a recombinant expression vector and may be in the form of naked DNA.

5.3 ANTISENSE

20 Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1-3, 10-13 and 15, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded
25 cDNA molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of a protein of any of SEQ ID NO:4-9, 14 and 16-18 or antisense nucleic acids complementary to a
30 nucleic acid sequence of SEQ ID NO:1-3, 10-13 and 15 are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence of the invention. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to

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a "noncoding region" of the coding strand of a nucleotide sequence of the invention. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding a nucleic acid disclosed herein (*e.g.*, SEQ ID NO:1-3, 10-13 and 15), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of a mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of a mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of a mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

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The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a protein according to the invention to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res* 15: 6625-6641). The antisense nucleic acid molecule can also comprise a 2'-O-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett* 215: 327-330).

5.4 RIBOZYMES AND PNA MOIETIES

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as a mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gertach (1988) *Nature* 334:585-591)) can be used to catalytically cleave a mRNA transcripts to thereby inhibit translation of a mRNA. A ribozyme having specificity for a nucleic acid of the invention can be designed based upon the nucleotide sequence of a DNA disclosed herein (*i.e.*, SEQ ID NO:1-3, 10-13 and 15). For example, a derivative of a Tetrahymena L-19 IVS RNA can be

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constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an encoding mRNA according to the invention. See, *e.g.*, Cech *et al.* U.S. Pat. No. 4,987,071; and Cech *et al.* U.S. Pat. No. 5,116,742.

Alternatively, mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region (*e.g.*, promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells. See generally, Helene. (1991) *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14: 807-15.

In various embodiments, the nucleic acids of the invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* (1996) *PNAS* 93: 14670-675.

PNAs of the invention can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of the invention can also be used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S1 nucleases (Hyrup B. (1996) above); or as probes or primers for DNA sequence and hybridization (Hyrup *et al.* (1996), above; Perry-O'Keefe (1996), above).

In another embodiment, PNAs of the invention can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras can be generated that may

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combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, *e.g.*, RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996) above). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996) above and Finn *et al.* (1996) *Nucl Acids Res* 24: 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA (Mag *et al.* (1989) *Nucl Acid Res* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn *et al.* (1996) above). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, Petersen *et al.* (1975) *Bioorg Med Chem Lett* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, *Proc. Natl. Acad. Sci.* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (See, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon, 1988, *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, etc.

5.5 HOSTS

The present invention further provides host cells genetically engineered to contain the polynucleotides of the invention. For example, such host cells may contain nucleic acids of the invention introduced into the host cell using known transformation, transfection or infection methods. The present invention still further provides host cells genetically engineered to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell.

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Knowledge of prothrombinase-like DNA sequences allows for modification of cells to permit, or increase, expression of prothrombinase-like polypeptide. Cells can be modified (e.g., by homologous recombination) to provide increased prothrombinase-like polypeptide expression by replacing, in whole or in part, the naturally occurring prothrombinase-like promoter with all or part of a heterologous promoter so that the cells prothrombinase-like polypeptide is expressed at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to prothrombinase-like encoding sequences. See, for example, PCT International Publication No. WO94/12650, PCT International Publication No. WO92/20808, and PCT International Publication No. WO91/09955. It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (e.g., *ada*, *dhfr*, and the multifunctional CAD gene which encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the prothrombinase-like coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the prothrombinase-like coding sequences in the cells.

The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran-mediated transfection, or electroporation (Davis, L. et al., *Basic Methods in Molecular Biology* (1986)). The host cells containing one of the polynucleotides of the invention, can be used in conventional manners to produce the gene product encoded by the isolated fragment (in the case of an ORF) or can be used to produce a heterologous protein under the control of the EMF.

Any host/vector system can be used to express one or more of the ORFs of the present invention. These include, but are not limited to, eukaryotic hosts such as HeLa cells, Cv-1 cell, COS cells, 293 cells, and Sf9 cells, as well as prokaryotic host such as *E. coli* and *B. subtilis*. The most preferred cells are those which do not normally express the particular polypeptide or protein or which expresses the polypeptide or protein at low natural level. Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., in *Molecular Cloning: A Laboratory Manual*, Second Edition,

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Cold Spring Harbor, New York (1989), the disclosure of which is hereby incorporated by reference.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell 23:175 (1981). Other cell lines capable of expressing a compatible vector are, for example, the C127, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Recombinant polypeptides and proteins produced in bacterial culture are usually isolated by initial extraction from cell pellets, followed by one or more salting-out, aqueous ion exchange or size exclusion chromatography steps. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or insects or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous

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gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting. These sequence include polyadenylation signals, mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.*, inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the host cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (*gpt*) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No.

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PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

5.6 POLYPEPTIDES OF THE INVENTION

5 The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising: the amino acid sequence set forth as any one of SEQ ID NO: 4-9, 14 or 16-18 or an amino acid sequence encoded by any one of the nucleotide sequences SEQ ID NO: 1-3, 10-13, or 15 or the corresponding full length or mature protein. Polypeptides of the invention also include polypeptides preferably with biological or immunological activity that are encoded by:

10 (a) a polynucleotide having any one of the nucleotide sequences set forth in the SEQ ID NO: 1-3, 10-13, or 15 or (b) polynucleotides encoding any one of the amino acid sequences set forth as SEQ ID NO: 4-9, 14 or 16-18 or (c) polynucleotides that hybridize to the complement of the polynucleotides of either (a) or (b) under stringent hybridization conditions. The invention also provides biologically active or immunologically active variants of any of the amino acid

15 sequences set forth as SEQ ID NO: 4-9, 14 or 16-18 or the corresponding full length or mature protein; and "substantial equivalents" thereof (e.g., with at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, typically at least about 95%, more typically at least about 98%, or most typically at least about 99% amino acid identity) that retain biological activity. Polypeptides encoded by allelic variants

20 may have a similar, increased, or decreased activity compared to polypeptides comprising SEQ ID NO: 4-9, 14 or 16-18.

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as

25 described in H. U. Saragovi, et al., *Bio/Technology* 10, 773-778 (1992) and in R. S. McDowell, et al., *J. Amer. Chem. Soc.* 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites.

The present invention also provides both full-length and mature forms (for example,

30 without a signal sequence or precursor sequence) of the disclosed proteins. The protein coding sequence is identified in the sequence listing by translation of the disclosed nucleotide sequences. The mature form of such protein may be obtained by expression of a full-length polynucleotide in a suitable mammalian cell or other host cell. The sequence of the mature form of the protein is also determinable from the amino acid sequence of the full-length form.

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Where proteins of the present invention are membrane bound, soluble forms of the proteins are also provided. In such forms, part or all of the regions causing the proteins to be membrane bound are deleted so that the proteins are fully secreted from the cell in which it is expressed.

Protein compositions of the present invention may further comprise an acceptable carrier, such as a hydrophilic, *e.g.*, pharmaceutically acceptable, carrier.

The present invention further provides isolated polypeptides encoded by the nucleic acid fragments of the present invention or by degenerate variants of the nucleic acid fragments of the present invention. By "degenerate variant" is intended nucleotide fragments which differ from a nucleic acid fragment of the present invention (*e.g.*, an ORF) by nucleotide sequence but, due to the degeneracy of the genetic code, encode an identical polypeptide sequence. Preferred nucleic acid fragments of the present invention are the ORFs that encode proteins.

A variety of methodologies known in the art can be utilized to obtain any one of the isolated polypeptides or proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. This technique is particularly useful in producing small peptides and fragments of larger polypeptides. Fragments are useful, for example, in generating antibodies against the native polypeptide. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The polypeptides and proteins of the present invention can alternatively be purified from cells which have been altered to express the desired polypeptide or protein. As used herein, a cell is said to be altered to express a desired polypeptide or protein when the cell, through genetic manipulation, is made to produce a polypeptide or protein which it normally does not produce or which the cell normally produces at a lower level. One skilled in the art can readily adapt procedures for introducing and expressing either recombinant or synthetic sequences into eukaryotic or prokaryotic cells in order to generate a cell which produces one of the polypeptides or proteins of the present invention.

The invention also relates to methods for producing a polypeptide comprising growing a culture of host cells of the invention in a suitable culture medium, and purifying the protein from the cells or the culture in which the cells are grown. For example, the methods of the invention include a process for producing a polypeptide in which a host cell containing a suitable expression vector that includes a polynucleotide of the invention is cultured under

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conditions that allow expression of the encoded polypeptide. The polypeptide can be recovered from the culture, conveniently from the culture medium, or from a lysate prepared from the host cells and further purified. Preferred embodiments include those in which the protein produced by such process is a full length or mature form of the protein.

5 In an alternative method, the polypeptide or protein is purified from bacterial cells which naturally produce the polypeptide or protein. One skilled in the art can readily follow known methods for isolating polypeptides and proteins in order to obtain one of the isolated polypeptides or proteins of the present invention. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange
10 chromatography, and immuno-affinity chromatography. See, e.g., Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag (1994); Sambrook, et al., in *Molecular Cloning: A Laboratory Manual*; Ausubel et al., *Current Protocols in Molecular Biology*. Polypeptide fragments that retain biological/immunological activity include fragments comprising greater than about 100 amino acids, or greater than about 200 amino acids, and
15 fragments that encode specific protein domains.

The purified polypeptides can be used in *in vitro* binding assays which are well known in the art to identify molecules which bind to the polypeptides. These molecules include but are not limited to, for e.g., small molecules, molecules from combinatorial libraries, antibodies or other proteins. The molecules identified in the binding assay are then tested for antagonist
20 or agonist activity in *in vivo* tissue culture or animal models that are well known in the art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

In addition, the peptides of the invention or molecules capable of binding to the peptides may be complexed with toxins, e.g., ricin or cholera, or with other compounds that
25 are toxic to cells. The toxin-binding molecule complex is then targeted to a tumor or other cell by the specificity of the binding molecule for SEQ ID NO: 4-9, 14 or 16-18.

The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

30 The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications, in the peptide or DNA sequence, can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the alteration, substitution, replacement, insertion

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or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, *e.g.*, U.S. Pat. No.

5 4,518,584). Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein. Regions of the protein that are important for the protein function can be determined by various methods known in the art including the alanine-scanning method which involved systematic substitution of single or strings of amino acids with alanine, followed by testing the resulting alanine-containing variant for biological activity. This type of
10 analysis determines the importance of the substituted amino acid(s) in biological activity. Regions of the protein that are important for protein function can be determined by the eMATRIX program.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and are useful for screening or other
15 immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are encompassed by the present invention.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell
20 expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, Calif., U.S.A. (the MaxBat™ kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

25 The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (*i.e.*, from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will
30 bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl™ or Cibacrom blue 3GA Sepharose™; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

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Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX), or as a His tag. Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, Mass.), Pharmacia (Piscataway, N.J.) and Invitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("FLAG®") is commercially available from Kodak (New Haven, Conn.).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, *e.g.*, silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The polypeptides of the invention include analogs (variants). The polypeptides of the invention include prothrombinase-like analogs. This embraces fragments of prothrombinase-like polypeptide of the invention, as well prothrombinase-like polypeptides which comprise one or more amino acids deleted, inserted, or substituted. Also, analogs of the prothrombinase-like polypeptide of the invention embrace fusions of the prothrombinase-like polypeptides or modifications of the prothrombinase-like polypeptides, wherein the prothrombinase-like polypeptide or analog is fused to another moiety or moieties, *e.g.*, targeting moiety or another therapeutic agent. Such analogs may exhibit improved properties such as activity and/or stability. Examples of moieties which may be fused to the prothrombinase-like polypeptide or an analog include, for example, targeting moieties which provide for the delivery of polypeptide to neurons, *e.g.*, antibodies to central nervous system, or antibodies to receptor and ligands expressed on neuronal cells. Other moieties which may be fused to prothrombinase-like polypeptide include therapeutic agents which are used for treatment, for example anti-depressant drugs or other medications for neurological disorders. Also, prothrombinase-like polypeptides may be fused to neuron growth modulators, and other chemokines for targeted delivery.

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5.6.1 DETERMINING POLYPEPTIDE AND POLYNUCLEOTIDE IDENTITY AND SIMILARITY

Preferred identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in computer programs including, but are not limited to, the GCG program package, including GAP (Devereux, J., et al., Nucleic Acids Research 12(1):387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, BLASTX, FASTA (Altschul, S.F. et al., J. Molec. Biol. 215:403-410 (1990), PSI-BLAST (Altschul S.F. et al., Nucleic Acids Res. vol. 25, pp. 3389-3402, herein incorporated by reference), eMatrix software (Wu et al., J. Comp. Biol., vol. 6, pp. 219-235 (1999), herein incorporated by reference), eMotif software (Nevill-Manning et al, ISMB-97, vol 4, pp. 202-209, herein incorporated by reference) and the Kyte-Doolittle hydrophobicity prediction algorithm (J. Mol Biol, 157, pp. 105-31 (1982), incorporated herein by reference). The BLAST programs are publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul, S., et al. NCB NLM NIH Bethesda, MD 20894; Altschul, S., et al., J. Mol. Biol. 215:403-410 (1990).

5.7 CHIMERIC AND FUSION PROTEINS

The invention also provides chimeric or fusion proteins. As used herein, a "chimeric protein" or "fusion protein" comprises a polypeptide of the invention operatively linked to another polypeptide. Within a fusion protein the polypeptide according to the invention can correspond to all or a portion of a protein according to the invention. In one embodiment, a fusion protein comprises at least one biologically active portion of a protein according to the invention. In another embodiment, a fusion protein comprises at least two biologically active portions of a protein according to the invention. Within the fusion protein, the term "operatively linked" is intended to indicate that the polypeptide(s) according to the invention and the other polypeptide(s) are fused in-frame to each other. The polypeptide can be fused to the N-terminus or C-terminus or in the middle.

For example, in one embodiment a fusion protein comprises a polypeptide according to the invention operably linked to the extracellular domain of a second protein.

In another embodiment, the fusion protein is a GST-fusion protein in which the polypeptide sequences of the invention are fused to the C-terminus of the GST (i.e., glutathione S-transferase) sequences.

In another embodiment, the fusion protein is an immunoglobulin fusion protein in which the polypeptide sequences according to the invention comprise one or more domains

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fused to sequences derived from a member of the immunoglobulin protein family. The immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a ligand and a protein of the invention on the surface of a cell, to thereby suppress signal transduction *in vivo*.

- 5 The immunoglobulin fusion proteins can be used to affect the bioavailability of a cognate ligand. Inhibition of the ligand/protein interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, *e.g.*, cancer as well as modulating (*e.g.*, promoting or inhibiting) cell survival. Moreover, the immunoglobulin fusion proteins of the invention can be used to bind and to dimerize 2 receptors and thereby transduce an
- 10 intracellular signal. The immunoglobulin fusion proteins may also be used as immunogens to produce antibodies in a subject, to purify ligands, and in screening assays to identify molecules that inhibit the interaction of a polypeptide of the invention with a ligand.

- A chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide
- 15 sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA
- 20 synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel et al. (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially
- 25 available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A nucleic acid encoding a polypeptide of the invention can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the protein of the invention.

5.8 GENE THERAPY

- 30 Mutations in the polynucleotides of the invention gene may result in loss of normal function of the encoded protein. The invention thus provides gene therapy to restore normal activity of the polypeptides of the invention; or to treat disease states involving polypeptides of the invention. Delivery of a functional gene encoding polypeptides of the invention to appropriate cells is effected *ex vivo*, *in situ*, or *in vivo* by use of vectors, and more particularly

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viral vectors (e.g., adenovirus, adeno-associated virus, or a retrovirus), or *ex vivo* by use of physical DNA transfer methods (e.g., liposomes or chemical treatments). See, for example, Anderson, Nature, supplement to vol. 392, no. 6679, pp.25-20 (1998). For additional reviews of gene therapy technology see Friedmann, Science, 244: 1275-1281 (1989); Verma, Scientific American: 68-84 (1990); and Miller, Nature, 357: 455-460 (1992). Introduction of any one of the nucleotides of the present invention or a gene encoding the polypeptides of the present invention can also be accomplished with extrachromosomal substrates (transient expression) or artificial chromosomes (stable expression). Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes. Alternatively, it is contemplated that in other human disease states, preventing the expression of or inhibiting the activity of polypeptides of the invention will be useful in treating the disease states. It is contemplated that antisense therapy or gene therapy could be applied to negatively regulate the expression of polypeptides of the invention.

Other methods inhibiting expression of a protein include the introduction of antisense molecules to the nucleic acids of the present invention, their complements, or their translated RNA sequences, by methods known in the art. Further, the polypeptides of the present invention can be inhibited by using targeted deletion methods, or the insertion of a negative regulatory element such as a silencer, which is tissue specific.

The present invention still further provides cells genetically engineered *in vivo* to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell. These methods can be used to increase or decrease the expression of the polynucleotides of the present invention.

Knowledge of DNA sequences provided by the invention allows for modification of cells to permit, increase, or decrease, expression of endogenous polypeptide. Cells can be modified (e.g., by homologous recombination) to provide increased polypeptide expression by replacing, in whole or in part, the naturally occurring promoter with all or part of a heterologous promoter so that the cells express the protein at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to the desired protein encoding sequences. See, for example, PCT International Publication No. WO 94/12650, PCT International Publication No. WO 92/20808, and PCT International Publication No. WO 91/09955. It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (e.g., *ada*, *dhfr*, and the multifunctional CAD gene which encodes carbamyl phosphate synthase, aspartate

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transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the desired protein coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the desired protein coding sequences in the cells.

5 In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different
10 gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by
15 targeting. These sequences include polyadenylation signals, mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.*, inserting a new promoter or enhancer or
20 both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new
25 sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively
30 selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the

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Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (*gpt*) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

10 5.9 TRANSGENIC ANIMALS

In preferred methods to determine biological functions of the polypeptides of the invention *in vivo*, one or more genes provided by the invention are either over expressed or inactivated in the germ line of animals using homologous recombination [Capecchi, Science 244:1288-1292 (1989)]. Animals in which the gene is over expressed, under the regulatory control of exogenous or endogenous promoter elements, are known as transgenic animals. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals. Knockout animals, preferably non-human mammals, can be prepared as described in U.S. Patent No. 5,557,032, incorporated herein by reference. Transgenic animals are useful to determine the roles polypeptides of the invention play in biological processes, and preferably in disease states. Transgenic animals are useful as model systems to identify compounds that modulate lipid metabolism. Transgenic animals, preferably non-human mammals, are produced using methods as described in U.S. Patent No 5,489,743 and PCT Publication No. WO94/28122, incorporated herein by reference.

Transgenic animals can be prepared wherein all or part of a promoter of the polynucleotides of the invention is either activated or inactivated to alter the level of expression of the polypeptides of the invention. Inactivation can be carried out using homologous recombination methods described above. Activation can be achieved by supplementing or even replacing the homologous promoter to provide for increased protein expression. The homologous promoter can be supplemented by insertion of one or more heterologous enhancer elements known to confer promoter activation in a particular tissue.

The polynucleotides of the present invention also make possible the development, through, e.g., homologous recombination or knock out strategies, of animals that fail to express functional prothrombinase-like polypeptide or that express a variant of prothrombinase-like polypeptide. Such animals are useful as models for studying the *in vivo* activities of

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prothrombinase-like polypeptide as well as for studying modulators of the prothrombinase-like polypeptide.

In preferred methods to determine biological functions of the polypeptides of the invention *in vivo*, one or more genes provided by the invention are either over expressed or inactivated in the germ line of animals using homologous recombination [Capecchi, Science 244:1288-1292 (1989)]. Animals in which the gene is over expressed, under the regulatory control of exogenous or endogenous promoter elements, are known as transgenic animals. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals. Knockout animals, preferably non-human mammals, can be prepared as described in U.S. Patent No. 5,557,032, incorporated herein by reference. Transgenic animals are useful to determine the roles polypeptides of the invention play in biological processes, and preferably in disease states. Transgenic animals are useful as model systems to identify compounds that modulate lipid metabolism. Transgenic animals, preferably non-human mammals, are produced using methods as described in U.S. Patent No 5,489,743 and PCT Publication No. WO94/28122, incorporated herein by reference.

Transgenic animals can be prepared wherein all or part of the polynucleotides of the invention promoter is either activated or inactivated to alter the level of expression of the polypeptides of the invention. Inactivation can be carried out using homologous recombination methods described above. Activation can be achieved by supplementing or even replacing the homologous promoter to provide for increased protein expression. The homologous promoter can be supplemented by insertion of one or more heterologous enhancer elements known to confer promoter activation in a particular tissue.

5.10 USES AND BIOLOGICAL ACTIVITY OF PROTHROMBINASE-LIKE

25 POLYPEPTIDE

The polynucleotides and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified herein. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA). The mechanism underlying the particular condition or pathology will dictate whether the polypeptides of the invention, the polynucleotides of the invention or modulators (activators or inhibitors) thereof would be beneficial to the subject in need of treatment. Thus, "therapeutic compositions of the invention" include compositions comprising isolated polynucleotides

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(including recombinant DNA molecules, cloned genes and degenerate variants thereof) or polypeptides of the invention (including full length protein, mature protein and truncations or domains thereof), or compounds and other substances that modulate the overall activity of the target gene products, either at the level of target gene/protein expression or target protein activity. Such modulators include polypeptides, analogs, (variants), including fragments and fusion proteins, antibodies and other binding proteins; chemical compounds that directly or indirectly activate or inhibit the polypeptides of the invention (identified, e.g., via drug screening assays as described herein); antisense polynucleotides and polynucleotides suitable for triple helix formation; and in particular antibodies or other binding partners that specifically recognize one or more epitopes of the polypeptides of the invention.

The polypeptides of the present invention may likewise be involved in cellular activation or in one of the other physiological pathways described herein.

5.10.1 RESEARCH USES AND UTILITIES

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The polypeptides provided by the present invention can similarly be used in assays to determine biological activity, including in a panel of multiple proteins for high-throughput

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screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding polypeptide is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

The polypeptides of the invention are also useful for making antibody substances that are specifically immunoreactive with prothrombinase-like proteins. Antibodies and portions thereof (e.g., Fab fragments) which bind to the polypeptides of the invention can be used to identify the presence of such polypeptides in a sample. Such determinations are carried out using any suitable immunoassay format, and any polypeptide of the invention that is specifically bound by the antibody can be employed as a positive control.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E. F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S. L. and A. R. Kimmel eds., 1987.

5.10.2 NUTRITIONAL USES

Polynucleotides and polypeptides of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the polypeptide or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the polypeptide or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

Additionally, the polypeptides of the invention can be used as molecular weight markers, and as a food supplement. A polypeptide consisting of SEQ ID NO: 4, for example, has a molecular mass of approximately 43 kDa in its unprocessed and unglycosylated state. Protein

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food supplements are well known and the formulation of suitable food supplements including polypeptides of the invention is within the level of skill in the food preparation art.

5.10.3 CYTOKINE AND CELL PROLIFERATION/DIFFERENTIATION

5 ACTIVITY

A polypeptide of the present invention may exhibit activity relating to cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor-dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of therapeutic compositions of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+(preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e, CMK, HUVeC, and Caco. Therapeutic compositions of the invention can be used in the following:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnoli et al., J. Immunol. 145:1706-1712, 1990; Bertagnoli et al., Cellular Immunology 133:327-341, 1991; Bertagnoli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152:1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A. M. and Shevach, E. M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human interleukin- γ , Schreiber, R. D. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L. S. and Lipsky, P. E. In Current Protocols in

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- Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6--Nordan, R. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11--Bennett, F., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9-Ciarletta, A., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.
- Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

5.10.4 STEM CELL GROWTH FACTOR ACTIVITY

- A polypeptide of the present invention may exhibit stem cell growth factor activity and be involved in the proliferation, differentiation and survival of pluripotent and totipotent stem cells including primordial germ cells, embryonic stem cells, hematopoietic stem cells and/or germ line stem cells. Administration of the polypeptide of the invention to stem cells *in vivo* or *ex vivo* may maintain and expand cell populations in a totipotent or pluripotent state which would be useful for re-engineering damaged or diseased tissues, transplantation, manufacture of bio-pharmaceuticals and the development of bio-sensors. The ability to produce large quantities of human cells has important working applications for the production of human proteins which currently must be obtained from non-human sources or donors, implantation of cells to treat diseases such as Parkinson's, Alzheimer's and other neurodegenerative diseases; tissues for grafting such as bone marrow, skin, cartilage, tendons, bone, muscle (including cardiac muscle), blood vessels, cornea, neural cells, gastrointestinal

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cells and others; and organs for transplantation such as kidney, liver, pancreas (including islet cells), heart and lung.

It is contemplated that multiple different exogenous growth factors and/or cytokines may be administered in combination with the polypeptide of the invention to achieve the desired effect, including any of the growth factors listed herein, other stem cell maintenance factors, and specifically including stem cell factor (SCF), leukemia inhibitory factor (LIF), Flt-3 ligand (Flt-3L), any of the interleukins, recombinant soluble IL-6 receptor fused to IL-6, macrophage inflammatory protein 1-alpha (MIP-1-alpha), G-CSF, GM-CSF, thrombopoietin (TPO), platelet factor 4 (PF-4), platelet-derived growth factor (PDGF), neural growth factors and basic fibroblast growth factor (bFGF).

Since totipotent stem cells can give rise to virtually any mature cell type, expansion of these cells in culture will facilitate the production of large quantities of mature cells. Techniques for culturing stem cells are known in the art and administration of polypeptides of the invention, optionally with other growth factors and/or cytokines, is expected to enhance the survival and proliferation of the stem cell populations. This can be accomplished by direct administration of the polypeptide of the invention to the culture medium. Alternatively, stroma cells transfected with a polynucleotide that encodes for the polypeptide of the invention can be used as a feeder layer for the stem cell populations in culture or in vivo. Stromal support cells for feeder layers may include embryonic bone marrow fibroblasts, bone marrow stromal cells, fetal liver cells, or cultured embryonic fibroblasts (see U.S. Patent No. 5,690,926).

Stem cells themselves can be transfected with a polynucleotide of the invention to induce autocrine expression of the polypeptide of the invention. This will allow for generation of undifferentiated totipotent/pluripotent stem cell lines that are useful as is or that can then be differentiated into the desired mature cell types. These stable cell lines can also serve as a source of undifferentiated totipotent/pluripotent mRNA to create cDNA libraries and templates for polymerase chain reaction experiments. These studies would allow for the isolation and identification of differentially expressed genes in stem cell populations that regulate stem cell proliferation and/or maintenance.

Expansion and maintenance of totipotent stem cell populations will be useful in the treatment of many pathological conditions. For example, polypeptides of the present invention may be used to manipulate stem cells in culture to give rise to neuroepithelial cells that can be used to augment or replace cells damaged by illness, autoimmune disease, accidental damage or genetic disorders. The polypeptide of the invention may be useful for inducing the proliferation of neural cells and for the regeneration of nerve and brain tissue, i.e. for the

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treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders which involve degeneration, death or trauma to neural cells or nerve tissue. In addition, the expanded stem cell populations can also be genetically altered for gene therapy purposes and to decrease host rejection of replacement tissues after grafting or
5 implantation.

Expression of the polypeptide of the invention and its effect on stem cells can also be manipulated to achieve controlled differentiation of the stem cells into more differentiated cell types. A broadly applicable method of obtaining pure populations of a specific differentiated cell type from undifferentiated stem cell populations involves the use of a cell-type specific
10 promoter driving a selectable marker. The selectable marker allows only cells of the desired type to survive. For example, stem cells can be induced to differentiate into cardiomyocytes (Wobus et al., *Differentiation*, 48: 173-182, (1991); Klug et al., *J. Clin. Invest.*, 98(1): 216-224, (1998)) or skeletal muscle cells (Browder, L. W. In: *Principles of Tissue Engineering* eds. Lanza et al., Academic Press (1997)). Alternatively, directed differentiation of stem cells
15 can be accomplished by culturing the stem cells in the presence of a differentiation factor such as retinoic acid and an antagonist of the polypeptide of the invention which would inhibit the effects of endogenous stem cell factor activity and allow differentiation to proceed.

In vitro cultures of stem cells can be used to determine if the polypeptide of the invention exhibits stem cell growth factor activity. Stem cells are isolated from any one of
20 various cell sources (including hematopoietic stem cells and embryonic stem cells) and cultured on a feeder layer, as described by Thompson et al. *Proc. Natl. Acad. Sci. U.S.A.*, 92: 7844-7848 (1995), in the presence of the polypeptide of the invention alone or in combination with other growth factors or cytokines. The ability of the polypeptide of the invention to induce stem cells proliferation is determined by colony formation on semi-solid support e.g. as
25 described by Bernstein et al., *Blood*, 77: 2316-2321 (1991).

5.10.5 HEMATOPOIESIS REGULATING ACTIVITY

A polypeptide of the present invention may be involved in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell disorders. Even marginal
30 biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid

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cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

Therapeutic compositions of the invention can be used in the following:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M. G. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, N.Y. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I. K. and Briddell, R. A. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, N.Y. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R. E. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, N.Y. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, N.Y. 1994; Long term culture initiating cell assay, Sutherland, H. J. In

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Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, N.Y. 1994.

5.10.6 TISSUE GROWTH ACTIVITY

5 A polypeptide of the present invention also may be involved in bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as in wound healing and tissue repair and replacement, and in healing of burns, incisions and ulcers.

A polypeptide of the present invention which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Compositions of a polypeptide, antibody, binding partner, or other modulator of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

15 A polypeptide of this invention may also be involved in attracting bone-forming cells, stimulating growth of bone-forming cells, or inducing differentiation of progenitors of bone-forming cells. Treatment of osteoporosis, osteoarthritis, bone degenerative disorders, or periodontal disease, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes may also be possible using the composition of the invention.

Another category of tissue regeneration activity that may involve the polypeptide of the present invention is tendon/ligament formation. Induction of tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide environment to attract tendon- or ligament-

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forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or
5 ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The compositions of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, i.e. for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic
10 disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a composition may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which
15 may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a composition of the invention.

Compositions of the invention may also be useful to promote better or faster closure of
20 non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

Compositions of the present invention may also be involved in the generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including
25 vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring may allow normal tissue to regenerate. A polypeptide of the present invention may also exhibit angiogenic activity.

A composition of the present invention may also be useful for gut protection or
30 regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A composition of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

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Therapeutic compositions of the invention can be used in the following:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pp. 71-112 (Maibach, H. I. and Rovee, D. T., eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

5.10.7 IMMUNE STIMULATING OR SUPPRESSING ACTIVITY

A polypeptide of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A polynucleotide of the invention can encode a polypeptide exhibiting such activities.

A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, *Leishmania* spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, proteins of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein (or antagonists thereof, including antibodies) of the present invention may also be useful in the treatment of allergic reactions and conditions (e.g., anaphylaxis, serum sickness, drug reactions, food allergies, insect venom allergies, mastocytosis, allergic rhinitis, hypersensitivity pneumonitis, urticaria, angioedema, eczema, atopic dermatitis, allergic contact

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dermatitis, erythema multiforme, Stevens-Johnson syndrome, allergic conjunctivitis, atopic keratoconjunctivitis, venereal keratoconjunctivitis, giant papillary conjunctivitis and contact allergies), such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ
5 transplantation), may also be treatable using a protein (or antagonists thereof) of the present invention. The therapeutic effects of the polypeptides or antagonists thereof on allergic reactions can be evaluated by in vivo animals models such as the cumulative contact enhancement test (Lastbom et al., Toxicology 125: 59-66, 1998), skin prick test (Hoffmann et al., Allergy 54: 446-54, 1999), guinea pig skin sensitization test (Vohr et al., Arch. Toxicol.
10 73: 501-9), and murine local lymph node assay (Kimber et al., J. Toxicol. Environ. Health 53: 563-79).

Using the proteins of the invention it may also be possible to modulate immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune
15 response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-
20 specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7)), e.g., preventing high
25 level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a
30 therapeutic composition of the invention may prevent cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, a lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or

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tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular therapeutic compositions in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al., Science 257:789-792 (1992) and Turka et al., Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of therapeutic compositions of the invention on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block stimulation of T cells can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythematosus in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (e.g., a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response may be useful in cases of viral infection, including systemic viral diseases such as influenza, the common cold, and encephalitis.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed

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APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

A polypeptide of the present invention may provide the necessary stimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient mounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I alpha chain protein and β_2 microglobulin protein or an MHC class II alpha chain protein and an MHC class II beta chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J.

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Immunol. 140:508-512, 1988; Bowman et al., J. Virology 61:1992-1998; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production, Mond, J. J. and Brunswick, M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et

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al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad. Sci. USA 88:7548-7551, 1991.

5.10.8 ACTIVIN/INHIBIN ACTIVITY

5 A polypeptide of the present invention may also exhibit activin- or inhibin-related activities. A polynucleotide of the invention may encode a polypeptide exhibiting such characteristics. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a polypeptide of the present
10 invention, alone or in heterodimers with a member of the inhibin family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the polypeptide of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin
15 group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, U.S. Pat. No. 4,798,885. A polypeptide of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as, but not limited to, cows, sheep and
20 pigs.

The activity of a polypeptide of the invention may, among other means, be measured by the following methods.

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al.,
25 Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

5.10.9 CHEMOTACTIC/CHEMOKINETIC ACTIVITY

A polypeptide of the present invention may be involved in chemotactic or chemokinetic
30 activity for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Chemotactic and chemokinetic receptor activation can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic compositions (e.g. proteins, antibodies, binding

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partners, or modulators of the invention) provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

5 A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell

10 chemotaxis.

Therapeutic compositions of the invention can be used in the following:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell

15 population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: *Current Protocols in Immunology*, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margules, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. *J. Clin. Invest.* 95:1370-1376, 1995; Lind et al. *APMIS* 103:140-

20 146, 1995; Muller et al. *Eur. J. Immunol.* 25:1744-1748; Gruber et al. *J. of Immunol.* 152:5860-5867, 1994; Johnston et al. *J. of Immunol.* 153:1762-1768, 1994.

5.10.10 HEMOSTATIC AND THROMBOLYTIC ACTIVITY

A polypeptide of the invention may also be involved in hemostasis or thrombolysis or

25 thrombosis. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Compositions may be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A composition of the invention may also be useful for dissolving or inhibiting formation of

30 thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

Therapeutic compositions of the invention can be used in the following:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., *J. Clin. Pharmacol.* 26:131-140, 1986; Burdick et al., *Thrombosis*

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Rés. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

5.10.11 CANCER DIAGNOSIS AND THERAPY

5 Polypeptides of the invention may be involved in cancer cell generation, proliferation or metastasis. Detection of the presence or amount of polynucleotides or polypeptides of the invention may be useful for the diagnosis and/or prognosis of one or more types of cancer. For example, the presence or increased expression of a polynucleotide/polypeptide of the invention may indicate a hereditary risk of cancer, a precancerous condition, or an ongoing
10 malignancy. Conversely, a defect in the gene or absence of the polypeptide may be associated with a cancer condition. Identification of single nucleotide polymorphisms associated with cancer or a predisposition to cancer may also be useful for diagnosis or prognosis.

Cancer treatments promote tumor regression by inhibiting tumor cell proliferation, inhibiting angiogenesis (growth of new blood vessels that is necessary to support tumor
15 growth) and/or prohibiting metastasis by reducing tumor cell motility or invasiveness. Therapeutic compositions of the invention may be effective in adult and pediatric oncology including in solid phase tumors/malignancies, locally advanced tumors, human soft tissue sarcomas, metastatic cancer, including lymphatic metastases, blood cell malignancies including multiple myeloma, acute and chronic leukemias, and lymphomas, head and neck cancers
20 including mouth cancer, larynx cancer and thyroid cancer, lung cancers including small cell carcinoma and non-small cell cancers, breast cancers including small cell carcinoma and ductal carcinoma, gastrointestinal cancers including esophageal cancer, stomach cancer, colon cancer, colorectal cancer and polyps associated with colorectal neoplasia, pancreatic cancers, liver cancer, urologic cancers including bladder cancer and prostate cancer, malignancies of the
25 female genital tract including ovarian carcinoma, uterine (including endometrial) cancers, and solid tumor in the ovarian follicle, kidney cancers including renal cell carcinoma, brain cancers including intrinsic brain tumors, neuroblastoma, astrocytic brain tumors, gliomas, metastatic tumor cell invasion in the central nervous system, bone cancers including osteomas, skin cancers including malignant melanoma, tumor progression of human skin keratinocytes,
30 squamous cell carcinoma, basal cell carcinoma, hemangiopericytoma and Kaposi's sarcoma.

Polypeptides, polynucleotides, or modulators of polypeptides of the invention (including inhibitors and stimulators of the biological activity of the polypeptide of the invention) may be administered to treat cancer. Therapeutic compositions can be administered in therapeutically effective dosages alone or in combination with adjuvant cancer therapy such as surgery,

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chemotherapy, radiotherapy, thermotherapy, and laser therapy, and may provide a beneficial effect, e.g. reducing tumor size, slowing rate of tumor growth, inhibiting metastasis, or otherwise improving overall clinical condition, without necessarily eradicating the cancer.

- The composition can also be administered in therapeutically effective amounts as a portion of an anti-cancer cocktail. An anti-cancer cocktail is a mixture of the polypeptide or modulator of the invention with one or more anti-cancer drugs in addition to a pharmaceutically acceptable carrier for delivery. The use of anti-cancer cocktails as a cancer treatment is routine. Anti-cancer drugs that are well known in the art and can be used as a treatment in combination with the polypeptide or modulator of the invention include:
- 10 Actinomycin D, Aminoglutethimide, Asparaginase, Bleomycin, Busulfan, Carboplatin, Carmustine, Chlorambucil, Cisplatin (cis-DDP), Cyclophosphamide, Cytarabine HCl (Cytosine arabinoside), Dacarbazine, Dactinomycin, Daunorubicin HCl, Doxorubicin HCl, Estramustine phosphate sodium, Etoposide (V16-213), Floxuridine, 5-Fluorouracil (5-Fu), Flutamide, Hydroxyurea (hydroxycarbamide), Ifosfamide, Interferon Alpha-2a, Interferon
 - 15 Alpha-2b, Leuprolide acetate (LHRH-releasing factor analog), Lomustine, Mechlorethamine HCl (nitrogen mustard), Melphalan, Mercaptopurine, Mesna, Methotrexate (MTX), Mitomycin, Mitoxantrone HCl, Octreotide, Plicamycin, Procarbazine HCl, Streptozocin, Tamoxifen citrate, Thioguanine, Thiotepa, Vinblastine sulfate, Vincristine sulfate, Amsacrine, Azacitidine, Hexamethylmelamine, Interleukin-2, Mitoguanzone, Pentostatin, Semustine,
 - 20 Teniposide, and Vindesine sulfate.

- In addition, therapeutic compositions of the invention may be used for prophylactic treatment of cancer. There are hereditary conditions and/or environmental situations (e.g. exposure to carcinogens) known in the art that predispose an individual to developing cancers. Under these circumstances, it may be beneficial to treat these individuals with therapeutically effective doses of the polypeptide of the invention to reduce the risk of developing cancers.

- In vitro* models can be used to determine the effective doses of the polypeptide of the invention as a potential cancer treatment. These *in vitro* models include proliferation assays of cultured tumor cells, growth of cultured tumor cells in soft agar (see Freshney, (1987) Culture of Animal Cells: A Manual of Basic Technique, Wiley-Liss, New York, NY Ch 18 and Ch 21), tumor systems in nude mice as described in Giovanella et al., J. Natl. Can. Inst., 52: 921-30 (1974), mobility and invasive potential of tumor cells in Boyden Chamber assays as described in Pilkington et al., Anticancer Res., 17: 4107-9 (1997), and angiogenesis assays such as induction of vascularization of the chick chorioallantoic membrane or induction of vascular endothelial cell migration as described in Ribatta et al., Intl. J. Dev. Biol., 40: 1189-97 (1999)

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and Li et al., Clin. Exp. Metastasis, 17:423-9 (1999), respectively. Suitable tumor cells lines are available, e.g. from American Type Tissue Culture Collection catalogs.

5.10.12 RECEPTOR/LIGAND ACTIVITY

5 A polypeptide of the present invention may also demonstrate activity as receptor, receptor ligand or inhibitor or agonist of receptor/ligand interactions. A polynucleotide of the invention can encode a polypeptide exhibiting such characteristics. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell
10 interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses. Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention
15 (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a polypeptide of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in:

20 Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley- Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1- 7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenberg et al., J.
25 Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

By way of example, the polypeptides of the invention may be used as a receptor for a ligand(s) thereby transmitting the biological activity of that ligand(s). Ligands may be identified through binding assays, affinity chromatography, dihybrid screening assays, BIAcore assays, gel overlay assays, or other methods known in the art.

30 Studies characterizing drugs or proteins as agonist or antagonist or partial agonists or a partial antagonist require the use of other proteins as competing ligands. The polypeptides of the present invention or ligand(s) thereof may be labeled by being coupled to radioisotopes, colorimetric molecules or a toxin molecules by conventional methods. ("Guide to Protein Purification" Murray P. Deutscher (ed) Methods in Enzymology Vol. 182 (1990) Academic

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Press, Inc. San Diego). Examples of radioisotopes include, but are not limited to, tritium and carbon-14. Examples of colorimetric molecules include, but are not limited to, fluorescent molecules such as fluorescamine, or rhodamine or other colorimetric molecules. Examples of toxins include, but are not limited to, ricin.

5

5.10.13 DRUG SCREENING

This invention is particularly useful for screening chemical compounds by using the novel polypeptides or binding fragments thereof in any of a variety of drug screening techniques. The polypeptides or fragments employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or a fragment thereof. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between polypeptides of the invention or fragments and the agent being tested or examine the diminution in complex formation between the novel polypeptides and an appropriate cell line, which are well known in the art.

Sources for test compounds that may be screened for ability to bind to or modulate (i.e., increase or decrease) the activity of polypeptides of the invention include (1) inorganic and organic chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of either random or mimetic peptides, oligonucleotides or organic molecules.

Chemical libraries may be readily synthesized or purchased from a number of commercial sources, and may include structural analogs of known compounds or compounds that are identified as "hits" or "leads" via natural product screening.

The sources of natural product libraries are microorganisms (including bacteria and fungi), animals, plants or other vegetation, or marine organisms, and libraries of mixtures for screening may be created by: (1) fermentation and extraction of broths from soil, plant or marine microorganisms or (2) extraction of the organisms themselves. Natural product libraries include polyketides, non-ribosomal peptides, and (non-naturally occurring) variants thereof. For a review, see *Science* 282:63-68 (1998).

Combinatorial libraries are composed of large numbers of peptides, oligonucleotides or organic compounds and can be readily prepared by traditional automated synthesis methods, PCR, cloning or proprietary synthetic methods. Of particular interest are peptide and oligonucleotide combinatorial libraries. Still other libraries of interest include peptide, protein,

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peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see Myers, *Curr. Opin. Biotechnol.* 8:701-707 (1997). For reviews and examples of peptidomimetic libraries, see Al-Obeidi et al., *Mol. Biotechnol.*, 9(3):205-23 (1998); Hruby et al., *Curr Opin Chem Biol*, 1(1):114-19 (1997); Dorner et al., *Bioorg Med Chem*, 4(5):709-15 (1996) (alkylated dipeptides).

Identification of modulators through use of the various libraries described herein permits modification of the candidate "hit" (or "lead") to optimize the capacity of the "hit" to bind a polypeptide of the invention. The molecules identified in the binding assay are then tested for antagonist or agonist activity in *in vivo* tissue culture or animal models that are well known in the art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

The binding molecules thus identified may be complexed with toxins, e.g., ricin or cholera, or with other compounds that are toxic to cells such as radioisotopes. The toxin-binding molecule complex is then targeted to a tumor or other cell by the specificity of the binding molecule for a polypeptide of the invention. Alternatively, the binding molecules may be complexed with imaging agents for targeting and imaging purposes.

5.10.14 ASSAY FOR RECEPTOR ACTIVITY

The invention also provides methods to detect specific binding of a polypeptide e.g. a ligand or a receptor. The art provides numerous assays particularly useful for identifying previously unknown binding partners for receptor polypeptides of the invention. For example, expression cloning using mammalian or bacterial cells, or dihybrid screening assays can be used to identify polynucleotides encoding binding partners. As another example, affinity chromatography with the appropriate immobilized polypeptide of the invention can be used to isolate polypeptides that recognize and bind polypeptides of the invention. There are a number of different libraries used for the identification of compounds, and in particular small molecule, that modulate (*i.e.*, increase or decrease) biological activity of a polypeptide of the invention. Ligands for receptor polypeptides of the invention can also be identified by adding exogenous ligands, or cocktails of ligands to two cells populations that are genetically identical except for the expression of the receptor of the invention: one cell population expresses the receptor of the invention whereas the other does not. The response of the two cell populations to the addition of ligand(s) are then compared. Alternatively, an expression library can be co-expressed with the polypeptide of the invention in cells and assayed for an autocrine response

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to identify potential ligand(s). As still another example, BIAcore assays, gel overlay assays, or other methods known in the art can be used to identify binding partner polypeptides, including, (1) organic and inorganic chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of random peptides, oligonucleotides or organic molecules.

- 5 The role of downstream intracellular signaling molecules in the signaling cascade of the polypeptide of the invention can be determined. For example, a chimeric protein in which the cytoplasmic domain of the polypeptide of the invention is fused to the extracellular portion of a protein, whose ligand has been identified, is produced in a host cell. The cell is then incubated with the ligand specific for the extracellular portion of the chimeric protein, thereby activating the chimeric receptor. Known downstream proteins involved in intracellular signaling can then
10 be assayed for expected modifications i.e. phosphorylation. Other methods known to those in the art can also be used to identify signaling molecules involved in receptor activity.

5.10.15 ANTI-INFLAMMATORY ACTIVITY

- 15 Compositions of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production
20 of other factors which more directly inhibit or promote an inflammatory response. Compositions with such activities can be used to treat inflammatory conditions including chronic or acute conditions, including without limitation intimation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's
25 disease or resulting from over production of cytokines such as TNF or IL-1. Compositions of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material. Compositions of this invention may be utilized to prevent or treat conditions such as, but not limited to, sepsis, acute pancreatitis, endotoxin shock, cytokine
30 induced shock, rheumatoid arthritis, chronic inflammatory arthritis, pancreatic cell damage from diabetes mellitus type 1, graft versus host disease, inflammatory bowel disease, inflammation associated with pulmonary disease, other autoimmune disease or inflammatory disease, an antiproliferative agent such as for acute or chronic myelogenous leukemia or in the prevention of premature labor secondary to intrauterine infections.

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5.10.16 LEUKEMIAS

Leukemias and related disorders may be treated or prevented by administration of a therapeutic that promotes or inhibits function of the polynucleotides and/or polypeptides of the invention. Such leukemias and related disorders include but are not limited to acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia, chronic leukemia, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia (for a review of such disorders, see Fishman et al., 1985, Medicine, 2d Ed., J.B. Lippincott Co., Philadelphia).

5.10.17 NERVOUS SYSTEM DISORDERS

Nervous system disorders, involving cell types which can be tested for efficacy of intervention with compounds that modulate the activity of the polynucleotides and/or polypeptides of the invention, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the invention include but are not limited to the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems:

- (i) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;
- (ii) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;
- (iii) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis;
- (iv) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis;

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- (v) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration;
- (vi) neurological lesions associated with systemic diseases including but not limited to diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis;
- (vii) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and
- (viii) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including but not limited to multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.
- Therapeutics which are useful according to the invention for treatment of a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, therapeutics which elicit any of the following effects may be useful according to the invention:
- (i) increased survival time of neurons in culture;
 - (ii) increased sprouting of neurons in culture or *in vivo*;
 - (iii) increased production of a neuron-associated molecule in culture or *in vivo*, *e.g.*, choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or
 - (iv) decreased symptoms of neuron dysfunction *in vivo*.
- Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may be measured by the method set forth in Arakawa et al. (1990, J. Neurosci. 10:3507-3515); increased sprouting of neurons may be detected by methods set forth in Pestronk et al. (1980, Exp. Neurol. 70:65-82) or Brown et al. (1981, Ann. Rev. Neurosci. 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, *etc.*, depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, *e.g.*, weakness, motor neuron conduction velocity, or functional disability.
- In specific embodiments, motor neuron disorders that may be treated according to the invention include but are not limited to disorders such as infarction, infection, exposure to

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toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including but not limited to progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

5.10.18 OTHER ACTIVITIES

A polypeptide of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, co-factors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

5.10.19 IDENTIFICATION OF POLYMORPHISMS

The demonstration of polymorphisms makes possible the identification of such polymorphisms in human subjects and the pharmacogenetic use of this information for diagnosis and treatment. Such polymorphisms may be associated with, e.g., differential predisposition or susceptibility to various disease states (such as disorders involving

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inflammation or immune response) or a differential response to drug administration, and this genetic information can be used to tailor preventive or therapeutic treatment appropriately. For example, the existence of a polymorphism associated with a predisposition to inflammation or autoimmune disease makes possible the diagnosis of this condition in humans by identifying the presence of the polymorphism.

Polymorphisms can be identified in a variety of ways known in the art which all generally involve obtaining a sample from a patient, analyzing DNA from the sample, optionally involving isolation or amplification of the DNA, and identifying the presence of the polymorphism in the DNA. For example, PCR may be used to amplify an appropriate fragment of genomic DNA which may then be sequenced. Alternatively, the DNA may be subjected to allele-specific oligonucleotide hybridization (in which appropriate oligonucleotides are hybridized to the DNA under conditions permitting detection of a single base mismatch) or to a single nucleotide extension assay (in which an oligonucleotide that hybridizes immediately adjacent to the position of the polymorphism is extended with one or more labeled nucleotides). In addition, traditional restriction fragment length polymorphism analysis (using restriction enzymes that provide differential digestion of the genomic DNA depending on the presence or absence of the polymorphism) may be performed. Arrays with nucleotide sequences of the present invention can be used to detect polymorphisms. The array can comprise modified nucleotide sequences of the present invention in order to detect the nucleotide sequences of the present invention. In the alternative, any one of the nucleotide sequences of the present invention can be placed on the array to detect changes from those sequences.

Alternatively a polymorphism resulting in a change in the amino acid sequence could also be detected by detecting a corresponding change in amino acid sequence of the protein, e.g., by an antibody specific to the variant sequence.

5.10.20 ARTHRITIS AND INFLAMMATION

The immunosuppressive effects of the compositions of the invention against rheumatoid arthritis is determined in an experimental animal model system. The experimental model system is adjuvant induced arthritis in rats, and the protocol is described by J. Holoshitz, et al., 1983, Science, 219:56, or by B. Waksman et al., 1963, Int. Arch. Allergy Appl. Immunol., 23:129. Induction of the disease can be caused by a single injection, generally intradermally, of a suspension of killed Mycobacterium tuberculosis in complete Freund's adjuvant (CFA). The route of injection can vary, but rats may be injected at the base of the tail

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with an adjuvant mixture. The polypeptide is administered in phosphate buffered solution (PBS) at a dose of about 1-5 mg/kg. The control consists of administering PBS only.

The procedure for testing the effects of the test compound would consist of intradermally injecting killed *Mycobacterium tuberculosis* in CFA followed by immediately administering the test compound and subsequent treatment every other day until day 24. At 14, 15, 18, 20, 22, and 24 days after injection of *Mycobacterium* CFA, an overall arthritis score may be obtained as described by J. Holoskitz above. An analysis of the data would reveal that the test compound would have a dramatic affect on the swelling of the joints as measured by a decrease of the arthritis score.

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5.11 THERAPEUTIC METHODS

The compositions (including polypeptide fragments, analogs, variants and antibodies or other binding partners or modulators including antisense polynucleotides) of the invention have numerous applications in a variety of therapeutic methods. Examples of therapeutic applications include, but are not limited to, those exemplified herein.

5.11.1 EXAMPLE

One embodiment of the invention is the administration of an effective amount of the prothrombinase-like polypeptides or other composition of the invention to individuals affected by a disease or disorder that can be modulated by regulating the soluble prothrombinase-like receptor of the invention. While the mode of administration is not particularly important, parenteral administration is preferred. An exemplary mode of administration is to deliver an intravenous bolus. The dosage of prothrombinase-like polypeptides or other composition of the invention will normally be determined by the prescribing physician. It is to be expected that the dosage will vary according to the age, weight, condition and response of the individual patient. Typically, the amount of polypeptide administered per dose will be in the range of about 0.01 µg/kg to 100 mg/kg of body weight, with the preferred dose being about 0.1 µg/kg to 10 mg/kg of patient body weight. For parenteral administration, prothrombinase-like polypeptides of the invention will be formulated in an injectable form combined with a pharmaceutically acceptable parenteral vehicle. Such vehicles are well known in the art and examples include water, saline, Ringer's solution, dextrose solution, and solutions consisting of small amounts of the human serum albumin. The vehicle may contain minor amounts of

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additives that maintain the isotonicity and stability of the polypeptide or other active ingredient. The preparation of such solutions is within the skill of the art.

5 5.12 PHARMACEUTICAL FORMULATIONS AND ROUTES OF ADMINISTRATION

A protein or other composition of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources and including antibodies and other binding partners of the polypeptides of the invention) may be administered to a patient in need, by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s) at doses to treat or ameliorate a variety of disorders. Such a composition may optionally contain (in addition to protein or other active ingredient and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF α , TNF β , TNF γ , G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the disease or disorder in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factors (TGF- α and TGF- β), insulin-like growth factor (IGF), as well as cytokines described herein.

The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or other active ingredient or complement its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein or other active ingredient of the invention, or to minimize side effects. Conversely, protein or other active ingredient of the present invention may be included in formulations of the particular clotting factor, cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the clotting factor, cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent (such

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as IL-1Ra, IL-1 Hy1, IL-1 Hy2, anti-TNF, corticosteroids, immunosuppressive agents). A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or
 5 complexed form.

As an alternative to being included in a pharmaceutical composition of the invention including a first protein, a second protein or a therapeutic agent may be concurrently administered with the first protein (e.g., at the same time, or at differing times provided that therapeutic concentrations of the combination of agents is achieved at the treatment site).
 10 Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms, *e.g.*, treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing,
 15 prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein or other active ingredient of the present invention is administered to a mammal having a condition to be treated. Protein or other active ingredient of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or
 25 other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein or other active ingredient of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering
 30 protein or other active ingredient of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

5.12.1 ROUTES OF ADMINISTRATION

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Suitable routes of administration may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. Administration of protein or other active ingredient of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

Alternately, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a arthritic joints or in fibrotic tissue, often in a depot or sustained release formulation. In order to prevent the scarring process frequently occurring as complication of glaucoma surgery, the compounds may be administered topically, for example, as eye drops. Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with a specific antibody, targeting, for example, arthritic or fibrotic tissue. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

The polypeptides of the invention are administered by any route that delivers an effective dosage to the desired site of action. The determination of a suitable route of administration and an effective dosage for a particular indication is within the level of skill in the art. Preferably for wound treatment, one administers the therapeutic compound directly to the site. Suitable dosage ranges for the polypeptides of the invention can be extrapolated from these dosages or from similar studies in appropriate animal models. Dosages can then be adjusted as necessary by the clinician to provide maximal therapeutic benefit.

5.12.2 COMPOSITIONS/FORMULATIONS

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. These pharmaceutical compositions may be manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. Proper formulation is dependent upon the route of administration chosen. When a therapeutically effective amount of protein or other active ingredient of the present invention is administered orally, protein or other active ingredient of the present

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invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein or other active ingredient of the present invention, and preferably from about 25 to 90% protein or other active ingredient of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein or other active ingredient of the present invention, and preferably from about 1 to 50% protein or other active ingredient of the present invention.

When a therapeutically effective amount of protein or other active ingredient of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein or other active ingredient of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein or other active ingredient solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein or other active ingredient of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art. For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained solid excipient,

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optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, 5 gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, 10 talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of 15 gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In 20 addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration. For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from 25 pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound 30 and a suitable powder base such as lactose or starch. The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. The compositions may take such forms as

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suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active
5 compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers
10 or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other
15 glycerides. In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as
20 sparingly soluble derivatives, for example, as a sparingly soluble salt.

A pharmaceutical carrier for the hydrophobic compounds of the invention is a co-solvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The co-solvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate
25 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics.
30 Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, *e.g.* polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose. Alternatively, other delivery systems for hydrophobic pharmaceutical compounds

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may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid

5 hydrophobic polymers containing the therapeutic agent. Various types of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein or other active ingredient stabilization
10 may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols. Many of the active ingredients of the invention may be
15 provided as salts with pharmaceutically compatible counter ions. Such pharmaceutically acceptable base addition salts are those salts which retain the biological effectiveness and properties of the free acids and which are obtained by reaction with inorganic or organic bases such as sodium hydroxide, magnesium hydroxide, ammonia, trialkylamine, dialkylamine, monoalkylamine, dibasic amino acids, sodium acetate, potassium benzoate, triethanol amine
20 and the like.

The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) or other active ingredient of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes will respond to antigen through their surface immunoglobulin
25 receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T
30 cells. Alternatively antibodies able to bind surface immunoglobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other pharmaceutically

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acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithins, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323, all of which are incorporated herein by reference.

The amount of protein or other active ingredient of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein or other active ingredient of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein or other active ingredient of the present invention and observe the patient's response. Larger doses of protein or other active ingredient of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 μ g to about 100 mg (preferably about 0.1 μ g to about 10 mg, more preferably about 0.1 μ g to about 1 mg) of protein or other active ingredient of the present invention per kg body weight. For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systemically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein or other active ingredient of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing or other active ingredient-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into

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the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

- The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalcium phosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components.
- Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalcium phosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability. Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein compositions from disassociating from the matrix.
- A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt %, preferably 1-10 wt % based on total formulation weight, which represents the amount necessary to prevent desorption of the protein from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells. In further compositions, proteins or other active ingredient of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth

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factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF- α and TGF- β), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins or other active ingredient of the present invention. The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, *e.g.*, amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (*e.g.*, bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either *in vivo* or *ex vivo* into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA). Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

5.12.3 EFFECTIVE DOSAGE

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the subject being treated. Determination of the effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from appropriate *in vitro* assays. For example, a dose can be formulated in animal models to achieve

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a circulating concentration range that can be used to more accurately determine useful doses in humans. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of the protein's biological activity). Such information can be used to more accurately determine useful doses in humans.

A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD_{50} and ED_{50} . Compounds which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. See, *e.g.*, Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1. Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the desired effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

An exemplary dosage regimen for polypeptides or other compositions of the invention will be in the range of about 0.01 $\mu\text{g/kg}$ to 100 mg/kg of body weight daily, with the preferred dose being about 0.1 $\mu\text{g/kg}$ to 25 mg/kg of patient body weight daily, varying in adults and

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children. Dosing may be once daily, or equivalent doses may be delivered at longer or shorter intervals.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's age and weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

5.12.4 PACKAGING

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

5.13 ANTIBODIES

Also included in the invention are antibodies to proteins, or fragments of proteins of the invention. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab}, F_{ab'} and F_{(ab')2} fragments, and an F_{ab} expression library. In general, an antibody molecule obtained from humans relates to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG₁, IgG₂, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of human antibody species.

An isolated related protein of the invention may be intended to serve as an antigen, or a portion or fragment thereof, and additionally can be used as an immunogen to generate antibodies that immunospecifically bind the antigen, using standard techniques for polyclonal and monoclonal antibody preparation. The full-length protein can be used or, alternatively, the invention provides antigenic peptide fragments of the antigen for use as immunogens. An antigenic peptide fragment comprises at least 6 amino acid residues of the amino acid sequence of the full length protein, such as an amino acid sequence shown in SEQ ID NO: 4, and

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encompasses an epitope thereof such that an antibody raised against the peptide forms a specific immune complex with the full length protein or with any fragment that contains the epitope. Preferably, the antigenic peptide comprises at least 10 amino acid residues, or at least 15 amino acid residues, or at least 20 amino acid residues, or at least 30 amino acid residues.

- 5 Preferred epitopes encompassed by the antigenic peptide are regions of the protein that are located on its surface; commonly these are hydrophilic regions.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of -related protein that is located on the surface of the protein, *e.g.*, a hydrophilic region. A hydrophobicity analysis of the human related protein sequence will indicate which regions of a related protein are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle 1982, *J. Mol. Biol.* 157: 105-142, each of which is incorporated herein by reference in its entirety. Antibodies that are specific for one or more domains within an antigenic protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

- 20 A protein of the invention, or a derivative, fragment, analog, homolog or ortholog thereof, may be utilized as an immunogen in the generation of antibodies that immunospecifically bind these protein components.

Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies directed against a protein of the invention, or against derivatives, fragments, analogs homologs or orthologs thereof (see, for example, *Antibodies: A Laboratory Manual*, Harlow E, and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference). Some of these antibodies are discussed below.

5.13.1 Polyclonal Antibodies

- 30 For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by one or more injections with the native protein, a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, the naturally occurring immunogenic protein, a chemically synthesized polypeptide representing the immunogenic protein, or a

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recombinantly expressed immunogenic protein. Furthermore, the protein may be conjugated to a second protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (e.g., aluminum hydroxide), surface active substances (e.g., lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), adjuvants usable in humans such as Bacille Calmette-Guerin and *Corynebacterium parvum*, or similar immunostimulatory agents.

Additional examples of adjuvants which can be employed include MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

The polyclonal antibody molecules directed against the immunogenic protein can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as affinity chromatography using protein A or protein G, which provide primarily the IgG fraction of immune serum. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific antibody by immunoaffinity chromatography. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

5.13.2 Monoclonal Antibodies

The term "monoclonal antibody" (MAb) or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs thus contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

Monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized in vitro.

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The immunizing agent will typically include the protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63).

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980). Preferably, antibodies having a high degree of specificity and a high binding affinity for the target antigen are isolated.

After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods. Suitable culture media for this purpose

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include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown in vivo as ascites in a mammal.

The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel
5 electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by
10 using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to
15 obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, *Nature* 368, 812-13 (1994)) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a
20 non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

5.13.2 Humanized Antibodies

25 The antibodies directed against the protein antigens of the invention can further comprise humanized antibodies or human antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the human against the administered immunoglobulin. Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-
30 binding subsequences of antibodies) that are principally comprised of the sequence of a human immunoglobulin, and contain minimal sequence derived from a non-human immunoglobulin. Humanization can be performed following the method of Winter and co-workers (Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-327 (1988); Verhoeven et al., *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the

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corresponding sequences of a human antibody. (See also U.S. Patent No. 5,225,539.) In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences.

5 In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a

10 human immunoglobulin (Jones et al., 1986; Riechmann et al., 1988; and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)).

5.13.3 Human Antibodies

Fully human antibodies relate to antibody molecules in which essentially the entire sequences of both the light chain and the heavy chain, including the CDRs, arise from human genes. Such antibodies are termed "human antibodies", or "fully human antibodies" herein. Human monoclonal antibodies can be prepared by the trioma technique; the human B-cell hybridoma technique (see Kozbor, et al., 1983 *Immunol Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, et al., 1985 In: *MONOCLONAL*

20 *ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, et al., 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus in vitro (see Cole, et al., 1985 In: *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc., pp. 77-96).

25 In addition, human antibodies can also be produced using additional techniques, including phage display libraries (Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991)). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon

30 challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in Marks et al. (*Bio/Technology* 10, 779-783 (1992)); Lonberg et al. (*Nature* 368 856-859 (1994)); Morrison (*Nature* 368, 812-13 (1994)); Fishwild

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et al., (*Nature Biotechnology* 14, 845-51 (1996)); Neuberger (*Nature Biotechnology* 14, 826 (1996)); and Lonberg and Huszar (*Intern. Rev. Immunol.* 13 65-93 (1995)).

Human antibodies may additionally be produced using transgenic nonhuman animals which are modified so as to produce fully human antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. (See PCT publication WO94/02602). The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host have been incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's genome. The human genes are incorporated, for example, using yeast artificial chromosomes containing the requisite human DNA segments. An animal which provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals containing fewer than the full complement of the modifications. The preferred embodiment of such a nonhuman animal is a mouse, and is termed the Xenomouse™ as disclosed in PCT publications WO 96/33735 and WO 96/34096. This animal produces B cells which secrete fully human immunoglobulins. The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs of antibodies such as, for example, single chain Fv molecules.

An example of a method of producing a nonhuman host, exemplified as a mouse, lacking expression of an endogenous immunoglobulin heavy chain is disclosed in U.S. Patent No. 5,939,598. It can be obtained by a method including deleting the J segment genes from at least one endogenous heavy chain locus in an embryonic stem cell to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged immunoglobulin heavy chain locus, the deletion being effected by a targeting vector containing a gene encoding a selectable marker; and producing from the embryonic stem cell a transgenic mouse whose somatic and germ cells contain the gene encoding the selectable marker.

A method for producing an antibody of interest, such as a human antibody, is disclosed in U.S. Patent No. 5,916,771. It includes introducing an expression vector that contains a nucleotide sequence encoding a heavy chain into one mammalian host cell in culture, introducing an expression vector containing a nucleotide sequence encoding a light chain into

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another mammalian host cell, and fusing the two cells to form a hybrid cell. The hybrid cell expresses an antibody containing the heavy chain and the light chain.

In a further improvement on this procedure, a method for identifying a clinically relevant epitope on an immunogen, and a correlative method for selecting an antibody that binds immunospecifically to the relevant epitope with high affinity, are disclosed in PCT publication WO 99/53049.

5.13.4 F_{ab} Fragments and Single Chain Antibodies

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an antigenic protein of the invention (see e.g., U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F_{ab} expression libraries (see e.g., Huse, et al., 1989 Science 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for a protein or derivatives, fragments, analogs or homologs thereof. Antibody fragments that contain the idiotypes to a protein antigen may be produced by techniques known in the art including, but not limited to: (i) an F_{(ab)2} fragment produced by pepsin digestion of an antibody molecule; (ii) an F_{ab} fragment generated by reducing the disulfide bridges of an F_{(ab)2} fragment; (iii) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) F_v fragments.

5.13.5 Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for an antigenic protein of the invention. The second binding target is any other antigen, and advantageously is a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, 1991 *EMBO J.*, 10:3655-3659.

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Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')₂ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Additionally, Fab' fragments can be directly recovered from E. coli and chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med. 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')₂ molecule. Each Fab'

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fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

- 5 Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., *J. Immunol.* 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge
- 10 region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable
- 15 domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber et al., *J. Immunol.* 152:5368 (1994).
- 20 Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., *J. Immunol.* 147:60 (1991). Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the protein antigen of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule
- 25 on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG (Fc γ R), such as Fc γ RI (CD64), Fc γ RII (CD32) and Fc γ RIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic
- 30 agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the protein antigen described herein and further binds tissue factor (TF).

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5.13.6 Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention.

Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360; WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

5.13.7 Effector Function Engineering

It can be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, e.g., the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., *J. Exp. Med.*, 176: 1191-1195 (1992) and Shopes, *J. Immunol.*, 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity can also be prepared using heterobifunctional cross-linkers as described in Wolff et al. *Cancer Research*, 53: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., *Anti-Cancer Drug Design*, 3: 219-230 (1989).

5.13.8 Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and

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PAP-S), momordica charantia inhibitor, curcin, crocin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi , ^{131}I , ^{131}In , ^{90}Y , and ^{186}Re .

5 Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimide HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-
10 azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene
15 triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionuclide to the antibody. See WO94/11026.

In another embodiment, the antibody can be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) that is in turn
20 conjugated to a cytotoxic agent.

5.14 COMPUTER READABLE SEQUENCES

In one application of this embodiment, a nucleotide sequence of the present invention can be recorded on computer readable media. As used herein, "computer readable media"
25 refers to any medium which can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. A skilled artisan can readily appreciate how any of the presently known computer
30 readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a nucleotide sequence of the present invention. As used herein, "recorded" refers to a process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently known methods for recording information

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on computer readable medium to generate manufactures comprising the nucleotide sequence information of the present invention.

A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of data processor structuring formats (e.g. text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

By providing any of the nucleotide sequences SEQ ID NO: 1-3, 10-13 or 15 or a representative fragment thereof; or a nucleotide sequence at least 95% identical to any of the nucleotide sequences of the SEQ ID NO:1-3, 10-13 or 15 in computer readable form, a skilled artisan can routinely access the sequence information for a variety of purposes. Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium. The examples which follow demonstrate how software which implements the BLAST (Altschul et al., J. Mol. Biol. 215:403-410 (1990)) and BLAZE (Brutlag et al., Comp. Chem. 17:203-207 (1993)) search algorithms on a Sybase system is used to identify open reading frames (ORFs) within a nucleic acid sequence. Such ORFs may be protein encoding fragments and may be useful in producing commercially important proteins such as enzymes used in fermentation reactions and in the production of commercially useful metabolites.

As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based systems are suitable for use in the present invention. As stated above, the computer-based systems of the present invention comprise a data storage means having stored therein a nucleotide sequence of the present invention and the necessary hardware means

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and software means for supporting and implementing a search means. As used herein, "data storage means" refers to memory which can store nucleotide sequence information of the present invention, or a memory access means which can access manufactures having recorded thereon the nucleotide sequence information of the present invention.

5 As used herein, "search means" refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of a known sequence which match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a variety of commercially
10 available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software includes, but is not limited to, Smith-Waterman, MacPattern (EMBL), BLASTN and BLASTA (NPOLYPEPTIDEA). A skilled artisan can readily recognize that any one of the available algorithms or implementing software packages for conducting homology searches can be adapted for use in the present
15 computer-based systems. As used herein, a "target sequence" can be any nucleic acid or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. The most preferred sequence length of a target sequence is from about 10 to 100 amino acids, or from about 30 to 300 nucleotide
20 residues. However, it is well recognized that searches for commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a
25 three dimensional conformation which is formed upon the folding of the sequence.

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Polynucleotides suitable for use in these methods are usually 20 to 40 bases in length and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 15241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Olmno, J.

- 5 Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present
10 invention is necessary for the design of an antisense or triple helix oligonucleotide.

5.16 DIAGNOSTIC ASSAYS AND KITS

- The present invention further provides methods to identify the presence or expression of one of the ORFs of the present invention, or homolog thereof, in a test sample, using a
15 nucleic acid probe or antibodies of the present invention, optionally conjugated or otherwise associated with a suitable label.

- In general, methods for detecting a polynucleotide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polynucleotide for a period sufficient to form the complex, and detecting the complex, so that
20 if a complex is detected, a polynucleotide of the invention is detected in the sample. Such methods can also comprise contacting a sample under stringent hybridization conditions with nucleic acid primers that anneal to a polynucleotide of the invention under such conditions, and amplifying annealed polynucleotides, so that if a polynucleotide is amplified, a polynucleotide of the invention is detected in the sample.

- 25 In general, methods for detecting a polypeptide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polypeptide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, a polypeptide of the invention is detected in the sample.

- In detail, such methods comprise incubating a test sample with one or more of the
30 antibodies or one or more of the nucleic acid probes of the present invention and assaying for binding of the nucleic acid probes or antibodies to components within the test sample.

Conditions for incubating a nucleic acid probe or antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the nucleic acid probe or antibody used in the assay.

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One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the nucleic acid probes or antibodies of the present invention. Examples of such assays can be found in Chard, T., *An Introduction to Radioimmunoassay and Related Techniques*, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G.R. et al., *Techniques in Immunocytochemistry*, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., *Practice and Theory of immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985). The test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is compatible with the system utilized.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the assays of the present invention. Specifically, the invention provides a compartment kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the probes or antibodies of the present invention; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of a bound probe or antibody.

In detail, a compartment kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allows one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which contain the reagents used to detect the bound antibody or probe. Types of detection reagents include labeled nucleic acid probes, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. One skilled in the art will readily

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recognize that the disclosed probes and antibodies of the present invention can be readily incorporated into one of the established kit formats which are well known in the art.

5.17 MEDICAL IMAGING

5 The novel polypeptides and binding partners of the invention are useful in medical imaging of sites expressing the molecules of the invention (e.g., where the polypeptide of the invention is involved in the immune response, for imaging sites of inflammation or infection). See, e.g., Kunkel et al., U.S. Pat. NO. 5,413,778. Such methods involve chemical attachment of a labeling or imaging agent, administration of the labeled polypeptide to a subject in a
10 pharmaceutically acceptable carrier, and imaging the labeled polypeptide *in vivo* at the target site.

5.18 SCREENING ASSAYS

Using the isolated proteins and polynucleotides of the invention, the present invention
15 further provides methods of obtaining and identifying agents which bind to a polypeptide encoded by an ORF corresponding to any of the nucleotide sequences set forth in the SEQ ID NO: 1-3, 10-13 or 15, or bind to a specific domain of the polypeptide encoded by the nucleic acid. In detail, said method comprises the steps of:

- (a) contacting an agent with an isolated protein encoded by an ORF of the present
20 invention, or nucleic acid of the invention; and
- (b) determining whether the agent binds to said protein or said nucleic acid.

In general, therefore, such methods for identifying compounds that bind to a polynucleotide of the invention can comprise contacting a compound with a polynucleotide of the invention for a time sufficient to form a polynucleotide/compound complex, and detecting
25 the complex, so that if a polynucleotide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

Likewise, in general, therefore, such methods for identifying compounds that bind to a polypeptide of the invention can comprise contacting a compound with a polypeptide of the invention for a time sufficient to form a polypeptide/compound complex, and detecting the
30 complex, so that if a polypeptide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

Methods for identifying compounds that bind to a polypeptide of the invention can also comprise contacting a compound with a polypeptide of the invention in a cell for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of

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a receptor gene sequence in the cell, and detecting the complex by detecting reporter gene sequence expression, so that if a polypeptide/compound complex is detected, a compound that binds a polypeptide of the invention is identified.

Compounds identified via such methods can include compounds which modulate the activity of a polypeptide of the invention (that is, increase or decrease its activity, relative to activity observed in the absence of the compound). Alternatively, compounds identified via such methods can include compounds which modulate the expression of a polynucleotide of the invention (that is, increase or decrease expression relative to expression levels observed in the absence of the compound). Compounds, such as compounds identified via the methods of the invention, can be tested using standard assays well known to those of skill in the art for their ability to modulate activity/expression.

The agents screened in the above assay can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. The agents can be selected and screened at random or rationally selected or designed using protein modeling techniques.

For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to the protein encoded by the ORF of the present invention. Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the particular protein. For example, one skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like, capable of binding to a specific peptide sequence, in order to generate rationally designed antipeptide peptides, for example see Hurby et al., Application of Synthetic Peptides: Antisense Peptides, " In Synthetic Peptides, A User's Guide, W.H. Freeman, NY (1992), pp. 289-307, and Kaspczak et al., Biochemistry 28:9230-8 (1989), or pharmaceutical agents, or the like.

In addition to the foregoing, one class of agents of the present invention, as broadly described, can be used to control gene expression through binding to one of the ORFs or EMFs of the present invention. As described above, such agents can be randomly screened or rationally designed/selected. Targeting the ORF or EMF allows a skilled artisan to design sequence specific or element specific agents, modulating the expression of either a single ORF or multiple ORFs which rely on the same EMF for expression control. One class of DNA binding agents are agents which contain base residues which hybridize or form a triple helix formation by binding to DNA or RNA. Such agents can be based on the classic

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phosphodiester, ribonucleic acid backbone, or can be a variety of sulfhydryl or polymeric derivatives which have base attachment capacity.

Agents suitable for use in these methods usually contain 20 to 40 bases and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide and other DNA binding agents.

Agents which bind to a protein encoded by one of the ORFs of the present invention can be used as a diagnostic agent. Agents which bind to a protein encoded by one of the ORFs of the present invention can be formulated using known techniques to generate a pharmaceutical composition.

5.19 USE OF NUCLEIC ACIDS AS PROBES

Another aspect of the subject invention is to provide for polypeptide-specific nucleic acid hybridization probes capable of hybridizing with naturally occurring nucleotide sequences. The hybridization probes of the subject invention may be derived from any of the nucleotide sequences SEQ ID NO:1-3, 10-13 or 15. Because the corresponding gene is only expressed in a limited number of tissues, a hybridization probe derived from any of the nucleotide sequences SEQ ID NO: 1-3, 10-13 and 15 can be used as an indicator of the presence of RNA of cell type of such a tissue in a sample.

Any suitable hybridization technique can be employed, such as, for example, in situ hybridization. PCR as described in US Patents Nos. 4,683,195 and 4,965,188 provides additional uses for oligonucleotides based upon the nucleotide sequences. Such probes used in PCR may be of recombinant origin, may be chemically synthesized, or a mixture of both. The probe will comprise a discrete nucleotide sequence for the detection of identical sequences or a degenerate pool of possible sequences for identification of closely related genomic sequences.

Other means for producing specific hybridization probes for nucleic acids include the cloning of nucleic acid sequences into vectors for the production of mRNA probes. Such

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vectors are known in the art and are commercially available and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriate radioactively labeled nucleotides. The nucleotide sequences may be used to construct hybridization probes for mapping their respective genomic sequences. The nucleotide sequence provided herein may be mapped to a chromosome or specific regions of a chromosome using well known genetic and/or chromosomal mapping techniques. These techniques include *in situ* hybridization, linkage analysis against known chromosomal markers, hybridization screening with libraries or flow-sorted chromosomal preparations specific to known chromosomes, and the like. The technique of fluorescent *in situ* hybridization of chromosome spreads has been described, among other places, in Verma *et al.* (1988) *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York NY.

Fluorescent *in situ* hybridization of chromosomal preparations and other physical chromosome mapping techniques may be correlated with additional genetic map data. Examples of genetic map data can be found in the 1994 *Genome Issue of Science* (265:1981f). Correlation between the location of a nucleic acid on a physical chromosomal map and a specific disease (or predisposition to a specific disease) may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier or affected individuals.

5.20 PREPARATION OF SUPPORT BOUND OLIGONUCLEOTIDES

Oligonucleotides, i.e., small nucleic acid segments, may be readily prepared by, for example, directly synthesizing the oligonucleotide by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer.

Support bound oligonucleotides may be prepared by any of the methods known to those of skill in the art using any suitable support such as glass, polystyrene or Teflon. One strategy is to precisely spot oligonucleotides synthesized by standard synthesizers. Immobilization can be achieved using passive adsorption (Inouye & Hondo, 1990 *J. Clin Microbiol* 28(6) 1462-72); using UV light (Nagata *et al.*, 1985; Dahlen *et al.*, 1987; Morrissey & Collins, *Mol. Cell Probes* 1989 3(2) 189-207) or by covalent binding of base modified DNA (Keller *et al.*, 1988; 1989); all references being specifically incorporated herein.

Another strategy that may be employed is the use of the strong biotin-streptavidin interaction as a linker. For example, Broude *et al.* (1994) *Proc. Natl. Acad. Sci USA* 91(8) 3072-6 describe the use of biotinylated probes, although these are duplex probes, that are

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immobilized on streptavidin-coated magnetic beads. Streptavidin-coated beads may be purchased from Dynal, Oslo. Of course, this same linking chemistry is applicable to coating any surface with streptavidin. Biotinylated probes may be purchased from various sources, such as, e.g., Operon Technologies (Alameda, CA).

- 5 Nunc Laboratories (Naperville, IL) is also selling suitable material that could be used. Nunc Laboratories have developed a method by which DNA can be covalently bound to the microwell surface termed CovaLink NH. CovaLink NH is a polystyrene surface grafted with secondary amino groups ($>NH$) that serve as bridge-heads for further covalent coupling. CovaLink Modules may be purchased from Nunc Laboratories. DNA molecules may be bound
10 to CovaLink exclusively at the 5'-end by a phosphoramidate bond, allowing immobilization of more than 1 pmol of DNA (Rasmussen *et al.*, (1991) *Anal Biochem* 198(1) 138-42.

- The use of CovaLink NH strips for covalent binding of DNA molecules at the 5'-end has been described (Rasmussen *et al.*, 1991). In this technology, a phosphoramidate bond is employed (Chu *et al.*, 1983 *Nucleic Acids* 11(18) 6513-29). This is beneficial as immobilization
15 using only a single covalent bond is preferred. The phosphoramidate bond joins the DNA to the CovaLink NH secondary amino groups that are positioned at the end of spacer arms covalently grafted onto the polystyrene surface through a 2 nm long spacer arm. To link an oligonucleotide to CovaLink NH via an phosphoramidate bond, the oligonucleotide terminus must have a 5'-end phosphate group. It is, perhaps, even possible for biotin to be covalently bound to CovaLink and
20 then streptavidin used to bind the probes.

More specifically, the linkage method includes dissolving DNA in water (7.5 ng/ul) and denaturing for 10 min. at 95°C and cooling on ice for 10 min. Ice-cold 0.1 M 1-methylimidazole, pH 7.0 (1-MeIm), is then added to a final concentration of 10 mM 1-MeIm. A ss DNA solution is then dispensed into CovaLink NH strips (75 ul/well) standing on ice.

- 25 Carbodiimide 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide(EDC), dissolved in 10 mM 1-MeIm, is made fresh and 25 ul added per well. The strips are incubated for 5 hours at 50°C. After incubation the strips are washed using, e.g., Nunc-Immuno Wash; first the wells are washed 3 times, then they are soaked with washing solution for 5 min., and finally they are washed 3 times (where in the washing solution is 0.4 N NaOH, 0.25% SDS heated to 50°C).

- 30 It is contemplated that a further suitable method for use with the present invention is that described in PCT Patent Application WO 90/03382 (Southern & Maskos), incorporated herein by reference. This method of preparing an oligonucleotide bound to a support involves attaching a nucleoside 3'-reagent through the phosphate group by a covalent phosphodiester link to aliphatic hydroxyl groups carried by the support. The oligonucleotide is then synthesized on the supported

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nucleoside and protecting groups removed from the synthetic oligonucleotide chain under standard conditions that do not cleave the oligonucleotide from the support. Suitable reagents include nucleoside phosphoramidite and nucleoside hydrogen phosphorate.

An on-chip strategy for the preparation of DNA probe for the preparation of DNA probe arrays may be employed. For example, addressable laser-activated photodeprotection may be employed in the chemical synthesis of oligonucleotides directly on a glass surface, as described by Fodor *et al.* (1991) *Science* 251(4995) 767-73, incorporated herein by reference. Probes may also be immobilized on nylon supports as described by Van Ness *et al.* (1991) *Nucleic Acids Res.* 19(12) 3345-50; or linked to Teflon using the method of Duncan & Cavalier (1988) *Anal Biochem* 169(1) 104-8; all references being specifically incorporated herein.

To link an oligonucleotide to a nylon support, as described by Van Ness *et al.* (1991), requires activation of the nylon surface via alkylation and selective activation of the 5'-amine of oligonucleotides with cyanuric chloride.

One particular way to prepare support bound oligonucleotides is to utilize the light-generated synthesis described by Pease *et al.*, (1994) *Proc. Natl. Acad. Sci USA* 91(11) 5022-6. These authors used current photolithographic techniques to generate arrays of immobilized oligonucleotide probes (DNA chips). These methods, in which light is used to direct the synthesis of oligonucleotide probes in high-density, miniaturized arrays, utilize photolabile 5'-protected *N*-acyl-deoxynucleoside phosphoramidites, surface linker chemistry and versatile combinatorial synthesis strategies. A matrix of 256 spatially defined oligonucleotide probes may be generated in this manner.

5.21 PREPARATION OF NUCLEIC ACID FRAGMENTS

The nucleic acids may be obtained from any appropriate source, such as cDNAs, genomic DNA, chromosomal DNA, microdissected chromosome bands, cosmid or YAC inserts, and RNA, including mRNA without any amplification steps. For example, Sambrook *et al.* (1989) describes three protocols for the isolation of high molecular weight DNA from mammalian cells (p. 9.14-9.23).

DNA fragments may be prepared as clones in M13, plasmid or lambda vectors and/or prepared directly from genomic DNA or cDNA by PCR or other amplification methods. Samples may be prepared or dispensed in multiwell plates. About 100-1000 ng of DNA samples may be prepared in 2-500 µl of final volume.

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The nucleic acids would then be fragmented by any of the methods known to those of skill in the art including, for example, using restriction enzymes as described at 9.24-9.28 of Sambrook *et al.* (1989), shearing by ultrasound and NaOH treatment.

Low pressure shearing is also appropriate, as described by Schriefer *et al.* (1990) Nucleic Acids Res. 18(24) 7455-6. In this method, DNA samples are passed through a small French pressure cell at a variety of low to intermediate pressures. A lever device allows controlled application of low to intermediate pressures to the cell. The results of these studies indicate that low-pressure shearing is a useful alternative to sonic and enzymatic DNA fragmentation methods.

One particularly suitable way for fragmenting DNA is contemplated to be that using the two base recognition endonuclease, *Cvi*II, described by Fitzgerald *et al.* (1992) Nucleic Acids Res. 20(14) 3753-62. These authors described an approach for the rapid fragmentation and fractionation of DNA into particular sizes that they contemplated to be suitable for shotgun cloning and sequencing.

The restriction endonuclease *Cvi*II normally cleaves the recognition sequence PuGCPy between the G and C to leave blunt ends. Atypical reaction conditions, which alter the specificity of this enzyme (*Cvi*II**), yield a quasi-random distribution of DNA fragments from the small molecule pUC19 (2688 base pairs). Fitzgerald *et al.* (1992) quantitatively evaluated the randomness of this fragmentation strategy, using a *Cvi*II** digest of pUC19 that was size fractionated by a rapid gel filtration method and directly ligated, without end repair, to a lac Z minus M13 cloning vector. Sequence analysis of 76 clones showed that *Cvi*II** restricts pyGCPy and PuGCPy, in addition to PuGCPy sites, and that new sequence data is accumulated at a rate consistent with random fragmentation.

As reported in the literature, advantages of this approach compared to sonication and agarose gel fractionation include: smaller amounts of DNA are required (0.2-0.5 ug instead of 2-5 ug); and fewer steps are involved (no preligation, end repair, chemical extraction, or agarose gel electrophoresis and elution are needed).

Irrespective of the manner in which the nucleic acid fragments are obtained or prepared, it is important to denature the DNA to give single stranded pieces available for hybridization. This is achieved by incubating the DNA solution for 2-5 minutes at 80-90°C. The solution is then cooled quickly to 2°C to prevent renaturation of the DNA fragments before they are contacted with the chip. Phosphate groups must also be removed from genomic DNA by methods known in the art.

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5.22 PREPARATION OF DNA ARRAYS

Arrays may be prepared by spotting DNA samples on a support such as a nylon membrane. Spotting may be performed by using arrays of metal pins (the positions of which correspond to an array of wells in a microtiter plate) to repeated by transfer of about 20 nl of a DNA solution to a nylon membrane. By offset printing, a density of dots higher than the density of the wells is achieved. One to 25 dots may be accommodated in 1 mm², depending on the type of label used. By avoiding spotting in some preselected number of rows and columns, separate subsets (subarrays) may be formed. Samples in one subarray may be the same genomic segment of DNA (or the same gene) from different individuals, or may be different, overlapped genomic clones. Each of the subarrays may represent replica spotting of the same samples. In one example, a selected gene segment may be amplified from 64 patients. For each patient, the amplified gene segment may be in one 96-well plate (all 96 wells containing the same sample). A plate for each of the 64 patients is prepared. By using a 96-pin device, all samples may be spotted on one 8 x 12 cm membrane. Subarrays may contain 64 samples, one from each patient. Where the 96 subarrays are identical, the dot span may be 1 mm² and there may be a 1 mm space between subarrays.

Another approach is to use membranes or plates (available from NUNC, Naperville, Illinois) which may be partitioned by physical spacers e.g. a plastic grid molded over the membrane, the grid being similar to the sort of membrane applied to the bottom of multiwell plates, or hydrophobic strips. A fixed physical spacer is not preferred for imaging by exposure to flat phosphor-storage screens or x-ray films.

The present invention is illustrated in the following examples. Upon consideration of the present disclosure, one of skill in the art will appreciate that many other embodiments and variations may be made in the scope of the present invention. Accordingly, it is intended that the broader aspects of the present invention not be limited to the disclosure of the following examples. The present invention is not to be limited in scope by the exemplified embodiments which are intended as illustrations of single aspects of the invention, and compositions and methods which are functionally equivalent are within the scope of the invention. Indeed, numerous modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon consideration of the present preferred embodiments. Consequently, the only limitations which should be placed upon the scope of the invention are those which appear in the appended claims.

All references cited within the body of the instant specification are hereby incorporated by reference in their entirety.

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6.0 EXAMPLES

EXAMPLE 1

Isolation of SEQ ID NO: 1 from a cDNA Library of Human Leukocytes

5 A plurality of novel nucleic acids were obtained from a cDNA library prepared from human ovary (Hyseq clone identification numbers 7735656 (SEQ ID NO: 1) and 7727065 (SEQ ID NO: 11)) using standard PCR, sequencing by hybridization sequence signature analysis, and Sanger sequencing techniques. The inserts of the library were amplified with PCR using primers specific for vector sequences flanking the inserts. These samples were
10 spotted onto nylon membranes and interrogated with oligonucleotide probes to give sequence signatures. The clones were clustered into groups of similar or identical sequences, and single representative clones were selected from each group for gel sequencing. The 5' sequence of the amplified inserts was then deduced using the reverse M13 sequencing primer in a typical Sanger sequencing protocol. PCR products were purified and subjected to fluorescent dye
15 terminator cycle sequencing. Single-pass gel sequencing was done using a 377 Applied Biosystems (ABI) sequencer. The insert was identified as a novel sequence not previously obtained from this library and not previously reported in public databases. The sequences were designated as SEQ ID NO: 1 and SEQ ID NO:11.

20

EXAMPLE 2

ASSEMBLAGE OF SEQ ID NO: 2 AND SEQ ID NO: 12

The nucleic acids of the present invention, designated as SEQ ID NO: 2 and SEQ ID
25 NO:12 were assembled using an EST sequence, SEQ ID NO:1 or SEQ ID NO:11, respectively, as a seed. Then a recursive algorithm was used to extend the seed into an extended assemblage, by pulling additional sequences from different databases (i.e., Hyseq's database containing EST sequences, dbEST version 114, gb pri 114, and UniGene version 101) that belong to this assemblage. The algorithm terminated when there was no additional sequences from the above
30 databases that would extend the assemblage. Inclusion of component sequences into the assemblage was based on a BLASTN hit to the extending assemblage with BLAST score greater than 300 and percent identity greater than 95 %.

The nearest neighbor result for the assembled contigs were obtained by a FASTA version
3 search against Genpept release 114, using FASTXY algorithm. FASTXY is an improved
35 version of FASTA alignment which allows in-codon frame shifts. The nearest neighbor result

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showed the closest homologue for each assemblage from Genpept (and contains the translated amino acid sequences for which the assemblage encodes). The nearest neighbor results is set forth below:

5

SEQ ID NO:	Accession No.	Description	Smith-Waterman Score	% Identity
2	Z36531	Homo sapiens fibrinogen-like protein	500	38.462
12	U44830	Rana sylvatica extended fibrinogen alpha chain	252	28.629

The nucleotide sequence within the assembled contigs that codes for signal peptide sequences and their cleavage sites can be determined from using Neural Network SignalP V1.1 program (from Center for Biological Sequence Analysis, The Technical University of Denmark). The process for identifying prokaryotic and eukaryotic signal peptides and their cleavage sites are also disclosed by Henrik Nielson, Jacob Engelbrecht, Soren Brunak, and Gunnar von Heijne in the publication "Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites" Protein Engineering, vol. 10, no. 1, pp. 1-6 (1997), incorporated herein by reference. A maximum S score and a mean S score, as described in the Nielson et al reference, are obtained for each assembled contig. Starting from the first amino acid of the predicted signal sequence, a sequence of 45 amino acids is described. Not all forty-five amino acids in the sequence may comprise the signal peptide.

20 For SEQ ID NO: 12:

Beginning and end nucleotide location corresponding to amino acid segment	Maximum S score	mean S score	Amino acid segment containing signal peptide (A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q= Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine, X=Unknown, *=Stop Codon,

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			/=possible nucleotide deletion, \=possible nucleotide insertion)
54:189	0.922	0.765	MMSPSQASLLFLNVCIFCGEVVQGN CVHHSTDSVVNIVEDGSN (SEQ ID NO:22)

The predicted amino acid sequence for SEQ ID NO:2 was obtained by using a software program called FASTY (available from <http://fasta.bioch.virginia.edu>) which selects a polypeptide based on a comparison of translated novel polynucleotide to known polynucleotides (W.R. Pearson, Methods in Enzymology, 183:63-98 (1990), incorporated herein by reference).

For SEQ ID NO: 2:

Predicted beginning nucleotide location corresponding to first amino acid residue of amino acid segment	Predicted end nucleotide location corresponding to last amino acid residue of amino acid segment	AMINO ACID SEGMENT (A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine, X=Unknown, *=Stop Codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
3	717	DLKDTIGSVTKTPSGLYIHPEGSSYPFEVMCDMDYR GGGWTVIQKRIDGIDFQRLWCDYLDGFGDLIGEF WLGKKIFYIVNQKNTSFMLYVALESEDDTLAYAS YDNFWLEDETRFFKMHLGRYSNAGDAFRGLKKE DNQNAMPFSTSDVDNDGCRPACLVNGQSVKSCSHL HNKTGWWFNECGLANLNGIHFSGKLLATGIQWG TWTKNNSPVKIKSVSMKIRRMYPYKF (SEQ ID NO:21)

10

EXAMPLE 3

ASSEMBLAGE OF SEQ ID NO: 3

Assembly of novel nucleotide sequence of SEQ ID NO: 3 was accomplished by using an EST sequence SEQ ID NO: 1 as a seed. The seed was extended by using software programs such as BLAST and Hyseq proprietary software to pull additional sequences from Hyseq's proprietary database containing EST sequences and by gel sequencing (377 Applied Biosystems (ABI) sequencer) using primers to extend the 3' end. Inclusion of component sequences into the assemblage was based BLAST scores greater than 1000 and a p-value of p-3

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(depending on the length of homology). The 5' end was extended using RACE, as disclosed in Marathon-Ready™ cDNA User Manual (PT1156-1) (Clontech), herein incorporated by reference.

5

EXAMPLE 4

ASSEMBLAGE OF SEQ ID NO: 10

Assembly of novel nucleotide sequence of SEQ ID NO: 10 was accomplished by using an EST sequence SEQ ID NO: 1 as a seed. The seed was further extended by gel sequencing (377 Applied Biosystems (ABI) sequencer) using primers to extend the 3' end (primer extension). The 5' end was extended using RACE, as disclosed in Marathon-Ready™ cDNA User Manual (PT1156-1) (Clontech), herein incorporated by reference.

15

EXAMPLE 5

ASSEMBLAGE OF SEQ ID NO: 13

Using PHRAP (Univ. of Washington) or CAP4 (Paracel), a full length gene cDNA sequence and its corresponding protein sequence were generated from the assemblage of SEQ ID NO:12. Any frame shifts and incorrect stop codons were corrected by hand editing. During editing, the sequence was checked using FASTY and/or BLAST against Genbank (i.e. Genecept release 115). Other computer programs which may have been used in the editing process were phredPhrap and Consed (University of Washington) and ed-ready, ed-ext and cg-zip-2 (Hyseq, Inc.). The full-length nucleotide sequence is shown as SEQ ID NO: 13.

A polypeptide (SEQ ID NO: 14) was predicted to be encoded by SEQ ID NO: 13 as set forth below. The polypeptide was predicted using software programs called BLASTX/FASTY which selects a polypeptide based on a comparison of translated novel polynucleotide to known polypeptides. The initial methionine ATG starts at position 54 of the nucleotide sequence and the putative stop codon, TAA, ends the coding region at position 1034.

30

EXAMPLE 6

A. Expression of SEQ ID NO: 4-9, 14 and 16-18 in cells

Chinese Hamster Ovary (CHO) cells or other suitable cell types are grown in DMEM (ATCC) and 10% fetal bovine serum (FBS) (Gibco) to 70% confluence. Prior to transfection the media is changed to DMEM and 0.5% FCS. Cells are transfected with cDNAs for SEQ

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ID NO: 2-3, 10, 12-13 or 15 or with pBGal vector by the FuGENE-6 transfection reagent (Boehringer). In summary, 4 μ l of FuGENE-6 is diluted in 100 μ l of DMEM and incubated for 5 minutes. Then, this is added to 1 μ g of DNA and incubated for 15 minutes before adding it to a 35 mm dish of CHO cells. The CHO cells are incubated at 37°C with 5% CO₂. After 24 hours, media and cell lysates are collected, centrifuged and dialyzed against assay buffer (15 mM Tris pH 7.6, 134 mM NaCl, 5 mM glucose, 3 mM CaCl₂ and MgCl₂).

B. Expression Study Using SEQ ID NO: 2-3, 10, 12-13 or 15

The expression of SEQ ID NO: 2-3, 10, 12-13 or 15 in various tissues is analyzed using a semi-quantitative polymerase chain reaction-based technique. Human cDNA libraries are used as sources of expressed genes from tissues of interest (adult bladder, adult brain, adult heart, adult kidney, adult lymph node, adult liver, adult lung, adult ovary, adult placenta, adult rectum, adult spleen, adult testis, bone marrow, thymus, thyroid gland, fetal kidney, fetal liver, fetal liver-spleen, fetal skin, fetal brain, fetal leukocyte and macrophage). Gene-specific primers are used to amplify portions of the SEQ ID NO: 2-3, 10, 12-13 or 15 sequence from the samples. Amplified products are separated on an agarose gel, transferred and chemically linked to a nylon filter. The filter is then hybridized with a radioactively labeled (³²P-dCTP) double-stranded probe generated from SEQ ID NO: 2-3, 10, 12-13 or 15 using a Klenow polymerase, random-prime method. The filters are washed (high stringency) and used to expose a phosphorimaging screen for several hours. Bands indicate the presence of cDNA including SEQ ID NO: 2-3, 10, 12-13 or 15 sequences in a specific library, and thus mRNA expression in the corresponding cell type or tissue.

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CLAIMS

WE CLAIM:

1. An isolated polynucleotide comprising a nucleotide sequence selected from
5 the group consisting of SEQ ID NO: 2-3, 10, 12-13 or 15, the translated protein coding portion
thereof, the mature protein coding portion thereof, the extracellular portion thereof, or the active
domain thereof.
2. An isolated polynucleotide encoding a polypeptide with biological activity,
10 which polynucleotide hybridizes to the complement of a polynucleotide of claim 1 under stringent
hybridization conditions.
3. An isolated polynucleotide encoding a polypeptide with biological activity,
said polynucleotide having greater than about 90% sequence identity with the polynucleotide of
15 claim 1.
4. The polynucleotide of claim 1 which is a DNA sequence.
5. An isolated polynucleotide which comprises the complement of the
20 polynucleotide of claim 1.
6. A vector comprising the polynucleotide of claim 1.
7. An expression vector comprising the polynucleotide of claim 1.
25
8. A host cell genetically engineered to express the polynucleotide of claim
1.
9. The host cell of claim 8 wherein the polynucleotide is in operative
30 association with a regulatory sequence that controls expression of the polynucleotide in the host
cell.
10. An isolated polypeptide comprising an amino acid sequence which is at
least 80% identical to the amino acid sequence selected from the group consisting of SEQ ID NO:

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4-9, 14, or 16-18, the translated protein coding portion thereof, the mature protein coding portion thereof, the extracellular portion thereof, or the active domain thereof.

- 5
11. A composition comprising the polypeptide of claim 10 and a carrier.
12. A polypeptide, having prothrombinase-like activity, comprising at least ten consecutive amino acids from the polypeptide sequences selected from the group consisting of SEQ ID NO: 4-9, 14, or 16-18.
- 10
13. The polypeptide of claim 12, comprising at least five consecutive amino acids from the polypeptide sequences selected from the group consisting of SEQ ID NO. 4-9, 14, or 16-18.
- 15
14. A polynucleotide encoding a polypeptide according to claim 12.
15. A polynucleotide encoding a polypeptide according to claim 13.
16. A polynucleotide encoding a polypeptide according to claim 10.
- 20
17. An antibody specific for the polypeptide of claim 10.
18. A method for detecting the polynucleotide of claim 1 in a sample, comprising:
- 25
- a) contacting the sample with a compound that binds to and forms a complex with the polynucleotide of claim 1 for a period sufficient to form the complex; and
- b) detecting the complex, so that if a complex is detected, the polynucleotide of claim 1 is detected.
19. A method for detecting the polynucleotide of claim 1 in a sample, comprising:
- 30
- a) contacting the sample under stringent hybridization conditions with nucleic acid primers that anneal to the polynucleotide of claim 1 under such conditions;

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b) amplifying a product comprising at least a portion of the polynucleotide of claim 1; and
c) detecting said product and thereby the polynucleotide of claim 1 in the sample.

5

20. The method of claim 19, wherein the polynucleotide comprises an RNA molecule and the method further comprises reverse transcribing an annealed RNA molecule into a cDNA polynucleotide.

10

21. A method for detecting the polypeptide of claim 10 in a sample, comprising:

a) contacting the sample with a compound that binds to and forms a complex with the polypeptide under conditions and for a period sufficient to form the complex; and

15

b) detecting formation of the complex, so that if a complex formation is detected, the polypeptide of claim 10 is detected.

22. A method for identifying a compound that binds to the polypeptide of claim 10, comprising:

20

a) contacting the compound with the polypeptide of claim 10 under conditions and for a time sufficient to form a polypeptide/compound complex; and

b) detecting the complex, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide of claim 10 is identified.

25

23. A method for identifying a compound that binds to the polypeptide of claim 10, comprising:

a) contacting the compound with the polypeptide of claim 10, in a cell, for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a reporter gene sequence in the cell; and

30

b) detecting the complex by detecting reporter gene sequence expression, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide of claim 10 is identified.

24. A method of producing a prothrombinase-like polypeptide, comprising,

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- a) culturing the host cell of claim 8 under conditions sufficient to express the polypeptide in said cell; and
- b) isolating the polypeptide from the cell culture or cells of step (a).

5 25. A kit comprising the polypeptide of claim 10.

26. A nucleic acid array comprising the polynucleotide of claim 1 or a unique segment of the polynucleotide of claim 1 attached to a surface.

10 27. The array of claim 26, wherein the array detects full-matches to the polynucleotide or a unique segment of the polynucleotide of claim 1.

28. The array of claim 26, wherein the array detects mismatches to the polynucleotide or a unique segment of the polynucleotide of claim 1.

15

29. A method of treatment of a subject in need of enhanced activity or expression of prothrombinase-like polypeptide of claim 10 comprising administering to the subject a composition selected from the group consisting of:

- a) a therapeutic amount of an agonist of said polypeptide;
- 20 b) a therapeutic amount of the polypeptide; and
- c) a therapeutic amount of a polynucleotide encoding the polypeptide in a form and under conditions such that the polypeptide is produced, and a pharmaceutically acceptable carrier.

25 30. A method of treatment of a subject having need to inhibit activity or expression of prothrombinase-like polypeptide of claim 10 comprising administering to the subject a composition selected from the group consisting of:

- a) a therapeutic amount of an antagonist to said polypeptide;
- b) a therapeutic amount of a polynucleotide that inhibits the expression of
- 30 the nucleotide sequence encoding said polypeptide; and
- c) a therapeutic amount of a polypeptide that competes with the prothrombinase-like polypeptide for its ligand, and a pharmaceutically acceptable carrier.

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31. An isolated polynucleotide with human prothrombinase activity comprising a nucleotide sequence of SEQ ID NO: 2 or 3, the mature protein coding portion thereof, or the active domain thereof.
- 5 32. An isolated polynucleotide encoding a polypeptide with biological activity, said polynucleotide which hybridizes to the complement of a polynucleotide of claim 31 under stringent hybridization conditions.
- 10 33. An isolated polynucleotide encoding a polypeptide with biological activity, said polynucleotide having greater than about 90% sequence identity with the polynucleotide of claim 31.
34. The polynucleotide of claim 31 which is a DNA sequence.
- 15 35. An isolated polynucleotide which comprises the complement of the polynucleotide of claim 31.
36. A vector comprising the polynucleotide of claim 31.
- 20 37. An expression vector comprising the polynucleotide of claim 31.
38. A host cell genetically engineered to express the polynucleotide of claim 31.
- 25 39. A host cell genetically engineered to contain the polynucleotide of claim 31 in operative association with a regulatory sequence that controls expression of the polynucleotide in the host cell.
- 30 40. An isolated polypeptide comprising an amino acid selected from the group consisting of SEQ ID NO: 4-9, the mature protein portion thereof, and active domain thereof.
41. A composition comprising the polypeptide of claim 40 and a carrier.
42. An antibody directed against the polypeptide of claim 40.

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43. A method for detecting the polynucleotide of claim 31 in a sample, comprising:
- a) contacting the sample with a compound that binds to and forms a complex with the polynucleotide of claim 31 for a period sufficient to form the complex; and
 - b) detecting the complex, so that if a complex is detected, the polynucleotide of claim 31 is detected.
44. A method for detecting the polynucleotide of claim 31 in a sample, comprising:
- a) contacting the sample under stringent hybridization conditions with nucleic acid primers that anneal to the polynucleotide of claim 31 under such conditions;
 - b) amplifying a product comprising at least a portion of the polynucleotide of claim 31; and
 - c) detecting said product and thereby the polynucleotide of claim 31 in the sample.
45. The method of claim 44, wherein the polynucleotide is an RNA molecule that encodes a polypeptide of claim 40, and the method further comprises reverse transcribing an annealed RNA molecule into a cDNA polynucleotide.
46. A method for detecting the polypeptide of claim 40 in a sample, comprising:
- a) contacting the sample with a compound that binds to and forms a complex with the polypeptide under conditions and for a period sufficient to form the complex; and
 - b) detecting formation of the complex, so that if a complex formation is detected, the polypeptide of claim 40 is detected.
47. A method for identifying a compound that binds to the polypeptide of claim 40, comprising:
- a) contacting the compound with the polypeptide of claim 40 under conditions and for a time sufficient to form a polypeptide/compound complex; and

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b) detecting the complex, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide of claim 40 is identified.

48. A method for identifying a compound that binds to the polypeptide of claim 40, comprising:

a) contacting the compound with the polypeptide of claim 40, in a cell, for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a reporter gene sequence in the cell; and

b) detecting the complex by detecting reporter gene sequence expression, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide of claim 40 is identified.

49. A method of producing the polypeptide of claim 40, comprising,

a) culturing the host cell of claim 38 for a period of time sufficient to express the polypeptide in said cell; and

b) isolating the polypeptide from the cell culture or cells of step (a).

50. A kit comprising the polypeptide of claim 40.

51. A nucleic acid array comprising the polynucleotide of claim 31 or a unique segment of the polynucleotide of claim 31 attached to a surface.

52. The array of claim 51, wherein the array detects full-matches to the polynucleotide or a unique segment of the polynucleotide of claim 31.

53. The array of claim 51, wherein the array detects mismatches to the polynucleotide or a unique segment of the polynucleotide of claim 31.

54. A method of treatment comprising administering to a mammalian subject in need thereof a therapeutic amount of a composition comprising a polypeptide of claim 10 and a pharmaceutically acceptable carrier.

55. A polypeptide having prothrombinase activity comprising at least ten consecutive amino acids from the group consisting of SEQ ID NO: 4-9.

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56. The polypeptide of claim 55, comprising at least five consecutive amino acids from the group consisting of SEQ ID NO: 4-9.

5 57. A polynucleotide encoding a polypeptide according to claim 55.

58. A polynucleotide encoding a polypeptide according to claim 56.

59. A polynucleotide encoding a polypeptide according to claim 40.

10

60. An isolated polypeptide with prothrombinase activity having at least an amino acid sequence of SEQ ID NO: 9, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine,

15 S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine.

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BLASTX ALIGNMENT OF SEQID NO: 4 (PROTHROMBINASE-LIKE) WITH HUMAN PROTHROMBINASE Fg 12 PROTEIN (PROTHROMBINASE Fg12)

The Genbank database indicates that the fibrinogen-like protein is homologous (37% identity)...

>gi15730075 ref|NP_006573.1| fibrinogen-like 2 precursor: fibrinogen-like
 P01118(187273) P19103(187273) P19103(187273) P19103(187273) P19103(187273) P19103(187273)
 (236531) fibrinogen-like protein [Homo sapiens] >gb|AF010825.1|
 (AF010825) fibrinogen-like protein 2 [Homo sapiens]
 Length = 439

Score = 402 (141.5 bits), Expect = 5.7e-36, P = 5.7e-36
 Identities = 109/291 (37%), Positives = 155/291 (53%)

PROTHROMBINASE-LIKE: 96 LAMMDQKQASDYLSTNINRNLNRLVLLITVFVKQLDFFHVPVQSHL--DCTDIKD 155
 PROTHROMBINASE Fg12: 169 IENVDSKRNITV---VNSLQKCKCSQ--EQIQS--REVQ-HLITKDSVY- 217
 PROTHROMBINASE-LIKE: 156 TIGSVTPFSEILIHIPFESSYFVFKVIMQYAGSGWTVIQRIDGIDIFQRLAGDYLDG 215
 PROTHROMBINASE Fg12: 218 AIG---KSSSTYAVTPDFQNSSEVYCDMETKGGGWVQARLDSTNFKTWQDYKAG 274
 PROTHROMBINASE-LIKE: 216 FGLLGEFEGWLGKILFVYNQVTSFNLVALESDDTLAYSTNFKLEDFEFQAL 275
 PROTHROMBINASE Fg12: 275 FSNRREINQNDKILTKSE--MLKLIDDFNGVEDIALDFYVAREETKRLAV 332
 PROTHROMBINASE-LIKE: 276 GRYGQGDGAFGLAKENQWNPSTSDVNSGCRACLNGQSVYSGSHLNTQWPF 335
 PROTHROMBINASE Fg12: 333 GNYNGDGDALR-PKHYWDLKFEFTTPKNDRY-ES---G---NCG-LYSSSWWF 381
 PROTHROMBINASE-LIKE: 336 NSCGIATLNG-TNHFSGKLATGQWNT--TRNNSFVKSKSVKRLRYNP-YFK 388
 PROTHROMBINASE Fg12: 382 DCLSLNLRVYHQYRGVNGINGTTPGVSEAHFGYKSSKSKKAMLRERHEK 438

FIG. 1

BLASTP ALIGNMENT OF SEQ ID NO: 14 PROTHROMBINASE-LIKE POLYPEPTIDE (IDENTIFIED AS PROTHROMBINASE-LIKE) WITH HUMAN PROTHROMBINASE FGL2 PROTEIN SEQ ID NO: 19

Query: Prothrombinase-like polypeptide (SEQ ID 14)
Sbjct: Human prothrombinase Fgl2 protein (SEQ ID NO: 19)

sp|W88235|W88235 Human prothrombinase Fgl2 protein.
Length = 439

[illegible]

FIG. 2

BLASTP ALIGNMENT OF SEQ ID NO: 14 PROTHROMBINASE-LIKE POLYPEPTIDE (IDENTIFIED AS PROTHROMBINASE-LIKE) WITH HUMAN PROTHROMBINASE FGL2 PROTEIN SEQ ID NO: 19

Query: Prothrombinase-like polypeptide (SEQ ID NO: 14)
Sbjct: Human prothrombinase Fgl2 protein (SEQ ID NO: 19)
sp|W68235|W68235 Human prothrombinase Fgl2 protein.
Length = 435
Score = 129 (45.4 bits), Expect = 2.0e-18, Sum P(1) = 2.0e-18
Identities = 40/115 (34%), Positives = 50/115 (43%)
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Sbjct: 331 NVQNYGZGDLK-FKKNYHLLKFTTENDNY-FS---G---NQG-LYISGSH 379
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Sbjct: 867 NFEKGLAMG-LHHFSLKLLAGIQWSTV--TKNNSVYKSEVKKLRKANE-LPK 1011
Query: 380 WFLGLASLNGKTHQYTRVNGIFWQWVGVSEHGGYKSEKEMIDREK 438
Sbjct: 380 WFLGLASLNGKTHQYTRVNGIFWQWVGVSEHGGYKSEKEMIDREK 438

FIG. 3

$\frac{d}{dt} \left(\frac{\partial L}{\partial \dot{x}} \right) = \frac{\partial L}{\partial x}$

Query: Prothrombinase-like polypeptide (SEQ ID 14)
 Sbjct: Human blood coagulation protein 1fzc_B {pdb number} (SEQ ID NO: 20)

[illegible]

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SEQUENCE LISTING

<110> HVSFG, Inc.
Haley, Dana A
Boyle, Bryan J
Ho, Alice S
Zhou, Ping
Mize, Nancy K
Kuo, Chiauyun
Arterburn, Matthew C
Tang, Y. Tom
Liu, Chenghua
Drmanac, Radoje T

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 Phe Ile Cys Gly Glu Val Val Gln Gly Asn Cys Val His His Ser Thr
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 Ser His Gly Leu Asp Cys Thr Asp Ile Lys Asp Thr Ile Gly Ser Val
 145 150 155 160
 acc aaa aca cag agt ggt tta tac ata att cac cca gaa gga tct agc 528
 Thr Lys Thr Pro Ser Gly Leu Tyr Ile Ile His Pro Glu Gly Ser Ser
 165 170 175
 tac cca ttt gag gta atg tgt gac atg gat tac aga gga ggt gga tgg 576
 Tyr Pro Phe Glu Val Met Cys Asp Met Asp Tyr Arg Gly Gly Gly Trp
 180 185 190
 act gtg ata cag aaa aga att gat ggg ata att gat ttc cag ags ttg 624
 Thr Val Ile Gln Lys Arg Ile Asp Gly Ile Ile Asp Phe Gln Arg Leu
 195 200 205
 tgg tgt gat tat ctg gat gga ttt gga gat ctt cta gga gaa ttt tgg 672
 Trp Cys Asp Tyr Leu Asp Gly Phe Gly Asp Leu Leu Gly Glu Phe Trp
 210 215 220
 cta gga ctg aaa aag att ttt tat ata gta aat cag aaa aat acc agt 720
 Leu Gly Leu Lys Lys Ile Phe Tyr Ile Val Asn Gln Lys Asn Thr Ser
 225 230 235 240
 ttt atg ctg tat gtg gct ttg gaa tct gaa gat gac act ctt gct tat 768
 Phe Met Leu Tyr Val Ala Leu Glu Ser Glu Asp Asp Thr Leu Ala Tyr
 245 250 255
 gca tca tat gat aat ttt tgg cta gag gat gaa acg aga ttt ttt aaa 816
 Ala Ser Tyr Asp Asn Phe Trp Leu Glu Asp Glu Thr Arg Phe Phe Lys
 260 265 270
 atg cac tta gga cgg tat tca gga aat gct ggt gat gca ttc cgg ggt 864
 Met His Leu Gly Arg Tyr Ser Gly Asn Ala Gly Asp Ala Phe Arg Gly
 275 280 285
 ctc aaa aaa gaa gat aat caa aat gca atg cct ttt agc aca tca gat 912
 Leu Lys Lys Glu Asp Asn Gln Asn Ala Met Pro Phe Ser Thr Ser Asp
 290 295 300
 gtt gat aat gat ggg tgt cgc cct gca tgc ctg gtc aat ggt cag tct 960
 Val Asp Asn Asp Gly Cys Arg Pro Ala Cys Leu Val Asn Gly Gln Ser
 305 310 315 320
 gtg aag agc tgc agt cac ctc cat aac aag acc ggc tgg tgg ttt aac 1008
 Val Lys Ser Cys Ser His Leu His Asn Lys Thr Gly Trp Trp Phe Asn
 325 330 335
 gag tgt ggt cta gca aat cta aat ggc att cat cac ttc tct gga aaa 1056
 Glu Cys Gly Leu Ala Asn Leu Asn Gly Ile His His Phe Ser Gly Lys
 340 345 350
 ttg ctt gca act gga att caa tgg ggc acg tgg acc aaa aac aac tca 1104
 Leu Leu Ala Thr Gly Ile Gln Trp Gly Thr Trp Thr Lys Asn Asn Ser
 355 360 365

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cct gtc aag att aaa tct gtt tca atg aaa att aga aga atg tac aat 1152
Pro Val Lys ile Lys Ser Val Ser Met Lys ile Arg Arg Met Tyr Asn
370 375 380
cca tat ttt aaa taa tctcatttaa cattgtaag caagttctac aatgataa 1207
Pro Tyr Phe Lys
385
tattaagat ttttaaaagt ttatcttttc acttagtggt tcaaacatat taggcaaaa 1267
ttaactgtag atggcattta gatgttatga gtttaattag aaacttcaa tttgtagta 1327
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<210> 4
 <211> 388
 <212> PRT
 <213> Homo sapiens

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<400> 4
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1 5 10 15
Phe ile Cys Gly Glu Val Val Gln Gly Asn Cys Val His His Ser Thr
20 25 30
Asp Ser Ser Val Val Asn ile Val Glu Asp Gly Ser Asn Ala Lys Asp
35 40 45
Glu Ser Lys Ser Asn Asp Thr Val Cys Lys Glu Asp Cys Glu Glu Ser
50 55 60
Cys Asp Val Lys Thr Lys ile Thr Arg Glu Glu Lys His Phe Met Cys
65 70 75 80
Arg Asn Leu Gln Asn Ser ile Val Ser Tyr Thr Arg Ser Thr Lys Lys
85 90 95
Leu Leu Arg Asn Met Met Asp Glu Gln Gln Ala Ser Leu Asp Tyr Leu
100 105 110
Ser Asn Gln Val Asn Glu Leu Met Asn Arg Val Leu Leu Thr Thr
115 120 125
Glu Val Phe Arg Lys Gln Leu Asp Pro Phe Pro His Arg Pro Val Gln
130 135 140
Ser His Gly Leu Asp Cys Thr Asp ile Lys Asp Thr ile Gly Ser Val
145
4

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145          150          155          160
Thr Lys Thr Pro Ser Gly Leu Tyr Ile Ile His Pro Glu Gly Ser Ser
165          170          175
Tyr Pro Phe Glu Val Met Cys Asp Met Asp Tyr Arg Gly Gly Gly Trp
180          185          190
Thr Val Ile Gln Lys Arg Ile Asp Gly Ile Ile Asp Phe Gln Arg Leu
195          200          205
Trp Cys Asp Tyr Leu Asp Gly Phe Gly Asp Leu Leu Gly Glu Phe Trp
210          215          220
Leu Gly Leu Lys Lys Ile Phe Tyr Ile Val Asn Gln Lys Asn Thr Ser
225          230          235          240
Phe Met Leu Tyr Val Ala Leu Glu Ser Glu Asp Asp Thr Leu Ala Tyr
245          250          255
Ala Ser Tyr Asp Asn Phe Trp Leu Glu Asp Glu Thr Arg Phe Phe Lys
260          265          270
Met His Leu Gly Arg Tyr Ser Gly Asn Ala Gly Asp Ala Phe Arg Gly
275          280          285
Leu Lys Lys Glu Asp Asn Gln Asn Ala Met Pro Phe Ser Thr Ser Asp
290          295          300
Val Asp Asn Asp Gly Cys Arg Pro Ala Cys Leu Val Asn Gly Gln Ser
305          310          315          320
Val Lys Ser Cys Ser His Leu His Asn Lys Thr Gly Trp Trp Phe Asn
325          330          335
Glu Cys Gly Leu Ala Asn Leu Asn Gly Ile His His Phe Ser Gly Lys
340          345          350
Leu Leu Ala Thr Gly Ile Gln Trp Gly Thr Trp Thr Lys Asn Asn Ser
355          360          365
Pro Val Lys Ile Lys Ser Val Ser Met Lys Ile Arg Arg Met Tyr Asn
370          375          380
Pro Tyr Phe Lys
385

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<210> 5
<211> 37
<212> PRT
<213> Homo sapiens

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<400> 5
Val Met Cys Asp Met Asp Tyr Arg Gly Gly Gly Trp Thr Val Ile Gln
1          5          10          15
Lys Arg Ile Asp Gly Ile Ile Asp Phe Gln Arg Leu Trp Cys Asp Tyr
20          25          30
Leu Asp Gly Phe Gly
35

```

```

<210> 6
<211> 30
<212> PRT
<213> Homo sapiens

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<400> 6
Cys Ser His Leu His Asn Lys Thr Gly Trp Trp Phe Asn Glu Cys Gly
1          5          10          15
Leu Ala Asn Leu Asn Gly Ile His His Phe Ser Gly Lys Leu
20          25          30

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<210> 7
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 7
 Phe Lys Met His Leu Gly Arg Tyr Ser Gly Asn Ala Gly Asp Ala
 1 5 10 15

<210> 8
 <211> 13
 <212> PRT
 <213> Homo sapiens

<400> 8
 Glu Phe Trp Leu Gly Leu Lys Lys Ile Phe Tyr Ile Val
 1 5 10

<210> 9
 <211> 368
 <212> PRT
 <213> Homo sapiens

<400> 9
 Glu Val Val Gln Gly Asn Cys Val His His Ser Thr Asp Ser Ser Val
 1 5 10 15
 Val Asn Ile Val Glu Asp Gly Ser Asn Ala Lys Asp Glu Ser Lys Ser
 20 25 30
 Asn Asp Thr Val Cys Lys Glu Asp Cys Glu Glu Ser Cys Asp Val Lys
 35 40 45
 Thr Lys Ile Thr Arg Glu Glu Lys His Phe Met Cys Arg Asn Leu Gln
 50 55 60
 Asn Ser Ile Val Ser Tyr Thr Arg Ser Thr Lys Lys Leu Leu Arg Asn
 65 70 75 80
 Met Met Asp Glu Gln Gln Ala Ser Leu Asp Tyr Leu Ser Asn Gln Val
 85 90 95
 Asn Glu Leu Met Asn Arg Val Leu Leu Leu Thr Thr Glu Val Phe Arg
 100 105 110
 Lys Gln Leu Asp Pro Phe Pro His Arg Pro Val Gln Ser His Gly Leu
 115 120 125
 Asp Cys Thr Asp Ile Lys Asp Thr Ile Gly Ser Val Thr Lys Thr Pro
 130 135 140
 Ser Gly Leu Tyr Ile Ile His Pro Glu Gly Ser Ser Tyr Pro Phe Glu
 145 150 155 160
 Val Met Cys Asp Met Asp Tyr Arg Gly Gly Gly Thr Val Ile Gln
 165 170 175
 Lys Arg Ile Asp Gly Ile Ile Asp Phe Gln Arg Leu Trp Cys Asp Tyr
 180 185 190
 Leu Asp Gly Phe Gly Asp Leu Leu Gly Glu Phe Trp Leu Gly Leu Lys
 6

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195                200                205
Lys Ile Phe Tyr Ile Val Asn Gln Lys Asn Thr Ser Phe Met Leu Tyr
210                215                220
Val Ala Leu Glu Ser Glu Asp Asp Thr Leu Ala Tyr Ala Ser Tyr Asp
225                230                235                240
Asn Phe Trp Leu Glu Asp Glu Thr Arg Phe Phe Lys Met His Leu Gly
245                250                255
Arg Tyr Ser Gly Asn Ala Gly Asp Ala Phe Arg Gly Leu Lys Lys Glu
260                265                270
Asp Asn Gln Asn Ala Met Pro Phe Ser Thr Ser Asp Val Asp Asn Asp
275                280                285
Gly Cys Arg Pro Ala Cys Leu Val Asn Gly Gln Ser Val Lys Ser Cys
290                295                300
Ser His Leu His Asn Lys Thr Gly Trp Trp Phe Asn Glu Cys Gly Leu
305                310                315                320
Ala Asn Leu Asn Gly Ile His His Phe Ser Gly Lys Leu Leu Ala Thr
325                330                335
Gly Ile Gln Trp Gly Thr Trp Thr Lys Asn Asn Ser Pro Val Lys Ile
340                345                350
Lys Ser Val Ser Met Lys Ile Arg Arg Met Tyr Asn Pro Tyr Phe Lys
355                360                365

```

<210> 10
 <211> 2352
 <212> DNA
 <213> Homo sapiens

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<400> 10
ccagacgagc tacctaagaa cagctaaaag agcacacccg tctatgtagc aaaatagtg 60
gaagatttat aggtagaggc gacaaaccta ccgagccctg tgatagctgg ttgtccaa 120
tagaatctta gtccaacttt aaatttgccc acagaaacct ctaaatcccc ttgtaaaatt 180
aactgttagt ccaagagaga acagctcttt ggacactagg aaaaaacott gttagagaga 240
taaaaaattt aacacccata gtaggcctaa aagcagctac caaaaaaaa gcggcctgac 300
tgatatttga agaagtgttt tcatctatcc aagcagctac caaaaaaaa gcggcctgac 360
tcatctttat tcttaaatgt atgtattttt atttgtggag aagtgtgaca aggttaactgt 420
gtacatcatt ctacggactc ttcagtagtt aacattgtag agatggatc taatgcacaa 480
gatgaagta aaagtaatga tactgttgtt aaggaagact gtgaggatc atgtgttgtt 540
aaaaactaaa ttacacgaga agaaaaacat ttcattgtga gaaatttgc aaattctatt 600
gtttcttaca caagaagtac caaaaaacta ctaaggataa tgatggatga gcaacaagct 660
tccttggatt atttatctaa tcagggttaac gagctcatga atagagttct ccttttgact 720
acagaagttt ttgaaaaaca gctggatcc tttctctaca gacctgtca gtcacatggt 780
ttagattgca ctgatattaa ggataccatt ggctctgtca ccaaacacc gagtggttta 840
tacataattc aaccagaagg atctagctac ccatttgagg taatgtgtga catggattac 900
agaggaggtg gatggactgt gatacagaaa agaattgatg ggataattga ttccagagg 960
ttgtggtgtg attatctgga tggatttggg gatctcttag gagaattttg gctaggactg 1020
aaaaagattt ttatatagt aaatcagaaa aataccagtt taatgctgta tctggcttgg 1080
gaatctgaag atgacactct tgrttatgca tcatatgata attttggct agaggatgaa 1140
acgagatttt ttaaaatgca cttaggaagg tattcagaaa atgctgtgga tgcattccgg 1200
ggctcctaaa angaagataa tcaaaatgca atgctcttta gcacatcaga tgttgataat 1260

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gatgggtgtc gccctgcatg cctgggtcaat ggtcagtcgt tgaagagctg cagtcacctc 1320
cataacaaga cggctgtgtg gtttaacgag tgtgtgtctag caaatctaaa tggcattcat 1380
cacttctctg gaaaattgct tgcacactgga attcaatggg gcacgtggac caaaaacac 1440
tcacctgtca agattaaatc tgtttcaatg aaaattagaa gaatgtacaa tccatatttt 1500
aaataatctc atttaacatt gtaatgcaag ttctacaatg ataataact aaagattttt 1560
aaaagtttat cttttcaact agtgtttcaa acatcttagg caaatttaa ctgtagatgg 1620
catttagatg ttatgagttt aattagaaaa ctccaatttt gtagtattct ataaaagaaa 1680
acatggctta ttgtatgttt ttacttctga ctatattaac aatatacaat gaaatttgtt 1740
tcaagtgaac tacaacttgt cttcctaaaa tttatagtga ttttaaagga ttttgctttt 1800
tctttgaagc atttttaaac cataatatgt tgaaggaaa attgaaggga atattttact 1860
tatttttata ctttatatga ttatataatc tacagataat ttctaactga gaagttaca 1920
ataaataact ttatgcagat taatatataa gctacacatg atgtaaaaac cttactattt 1980
ctaggtgatg ccatccatt ttaaaagtag taagagtttg ctgcocaaat agtttttctt 2040
gttttcatat ctaatcatgg ttaactattt tgttattgtt tctaataaat atatgtactt 2100
tatatcttg aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaagggggg 2160
cccccttaac ttaagggggc cctttaaacc ccgggtaaac ccccgagggg ggcttttata 2220
gggccccccc ttggtgtgtg cccccccccc ggggtttttt ttgacggggg gggggccccc 2280
cccacatttt tttttttaa aaaaagagaa aattccccc tttgtggga aggggggttt 2340
tttttttgg gg 2352

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<210> 11
 <211> 453
 <212> DNA
 <213> Homo sapiens

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<400> 11
taatcgagag agcccgatatt nctgatattt gongnccgt tttcatctat ccaagaaaaa 60
tatgatgtct ccatccctag ccttactctt attcttaaat gtatgtattt ttgtttgtgg 120
agaagctgta caaggttaact gtgtacatca ttctacggac tcttcagtag ttaacattgt 180
agaagatgga tctaatgcaa aagatgaag aaaggaat gatactgttt gtaaggaaaga 240
ctgtgaggaa tcatgtgatg ttaaaactaa aattacaaga gaagaaaaac atttcatgtg 300
tgaattttg caaaattcta ttgtttccta cacaagaagt accaaaaaac tactaaggaa 360
tatgatggat gagcaacaag ctcccttgga ttatttatct aatcaggtaa tgcgtgacat 420
ggattacaga ggaggtggat ggactgtgat aca 453

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<210> 12
 <211> 1648
 <212> DNA
 <213> Homo sapiens

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<400> 12
ggtcgaccca cgcgtccgat ttgaagaagt gttttcatct atccaagaaa aatatgatgt 60
ctccatccca agcctcactc ttattcttaa atgtatgtat ttttatttgt ggagaagttg 120
tacaaggtaa ctgtgtacat cattctacgg actcctcagt agttaacatt gtagaagatg 180
gatctaagtc aaaagatgaa agtaaaagta atgatactgt ttgttaaggaa gactgtgagg 240
aatcatgtga tgttaaaact aaaaattcac gagaagaaaa acatttcaatg tgtagaattt 300
tgcataaattc tatgttttcc tacacaagaa gtacccaaaa actactaagg aatatgatgg 360
atgagcaaaa agcttccttg gattatttat ctaacaggt taacagatct atgaatagag 420
ttctcctttt gactacagaa gtttttagaa aacagctgga tctctttctt cacagacctg 480
ttcagtcaca tggtttagat tgcactgata ttaaggatc cattggctct gtcccaaaaa 540
caccgagtgg tttatacata attcaccag aaggatctag ctacccattt gaggtaatgt 600
gtgacatgga ttacagagga ggtggatgga ctgtgatata gaaaagaatt gatgggataa 660
ttgatttcca gaggttgggg tgtgattatc tggatggatt tggagatctt ctaggatgat 720
cattccgggg tctcaaaaaa gaagataatc aaaaatgcaat gccttttagc acatcagatg 780
ttgataatga tgggtgtcgc cctgcattgc tggtcnatgg tcagtctgtg aagagctgca 840
gtcacctcca taacaagacc ggctgggtgt ttaacagatg tggcttagca aatctaaatg 900
gcattcoatca cttctctgga aaattgcttg caactgggat tcaatggggg acgtggacca 960
aaaaacaatc accgtcctag attaaatctg ttctaatgaa aactgaaga atgtacatc 1020
catattttaa ataatctcat ttaacatttg aatgcaagtt ctacaatgat aatatattaa 1080
agatttttaa aagtttatct ttccacttag tgtttcaaac atattaggca aaatttaact 1140
gtagatgga tttagatgct atgagtttaa ttagnaaact tcaattttgt agtattatat 1200

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aaaagaaaac atggcttatt gtatgttttt acttctgact atattaacaa tatacaatga 1260
aatttgtttc aagtgaacta caactgtctt tcctaaaatt tatagtgttt ttaaaggatt 1320
ttgccttttc ttggaagcat ttttaaacca taatatgttg taaggaaaat tgaagggaat 1380
attttactta tttttatact ttatatgatt atataatcta cagataatbt ctactgaaga 1440
cagttacaat aastaacttt atgcagatta atataatagc tacacatgat gtaaaaacct 1500
tactatttct aggtgatgcc ataccatttt aaaagtagta agagtgttgc goccacaaatag 1560
tttttttgtt ttccatatct aatcatgggt aactattttg ttattgtttg taataaatat 1620
atgtactttt atatcctgaa aaaaaaaa

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<210> 13
 <211> 1651
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (54)..(1034)

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<400> 13
ggtcgaacca cgcgtccgat ttgaagaagt gttttcatct atccaagaaa aat atg 56
Met
1

atg tct cca tcc caa gcc tca ctc tta ttc tta aat gta tgt att ttt 104
Met Ser Pro Ser Gln Ala Ser Leu Leu Phe Leu Asn Val Cys Ile Phe
5 10 15

att tgt gga gaa gtt gta caa ggt aac tgt gta cat cat tct acg gac 152
Ile Cys Gly Glu Val Val Gln Gly Asn Cys Val His His Ser Thr Asp
20 25 30

tct tca gta gtt aac att gta gaa gat gga tct aat gca aaa gat gaa 200
Ser Ser Val Val Asn Ile Val Glu Asp Gly Ser Asn Ala Lys Asp Glu
35 40 45

agt aaa agt aat gat act gtt tgt aag gaa gac tgt gag gaa tca tgt 248
Ser Lys Ser Asn Asp Thr Val Cys Lys Glu Asp Cys Glu Glu Ser Cys
50 55 60

gat gtt aaa act aaa att aca cga gaa gaa aaa cat ttc atg tgt aga 296
Asp Val Lys Thr Lys Ile Thr Arg Glu Glu Lys His Phe Met Cys Arg
70 75 80

aat ttg caa aat tct att gtt tcc tac aca aga agt acc aaa aaa cta 344
Asn Leu Gln Asn Ser Ile Val Ser Tyr Thr Arg Ser Thr Lys Lys Leu
85 90 95

cta agg aat atg atg gat gag caa caa gct tcc ttg gat tat tta tct 392
Leu Arg Asn Met Met Asp Glu Gln Gln Ala Ser Leu Asp Tyr Leu Ser
100 105 110

aat cag gtt aac gag ctc atg aat aga gtt ctc ott ttg act aca gaa 440
Asn Gln Val Asn Glu Leu Met Asn Arg Val Leu Leu Leu Thr Thr Glu
115 120 125

gtt ttt aga aaa cag ctg gat cct ttt cct cac aga cct gtt cag tca 488
Val Phe Arg Lys Gln Leu Asp Pro Phe Pro His Arg Pro Val Gln Ser
130 135 140 145

cat ggt tta gat tgc act gat att aag gat acc att ggc tct gtc acc 536
His Gly Leu Asp Cys Thr Asp Ile Lys Asp Thr Ile Gly Ser Val Thr
150 155 160

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aaa aca ccg agt ggt tta tac ata att cac cca gaa gga tct agc tac 584
Lys Thr Pro Ser Gly Leu Tyr Ile Ile His Pro Glu Gly Ser Ser Tyr
      165              170              175

cca ttt gag gta atg tgt gac atg gat tac aga gga ggt gga tgg act 632
Pro Phe Glu Val Met Cys Asp Met Asp Tyr Arg Gly Gly Gly Trp Thr
      180              185              190

gtg ata cag aaa aga att gat ggg ata att gat ttc cag agg ttg tgg 680
Val Ile Gln Lys Arg Ile Asp Gly Ile Ile Asp Phe Gln Arg Leu Trp
      195              200              205

tgt gat tat ctg gat gga ttt gga gat ctt cta ggt gat gca ttc cgg 728
Cys Asp Tyr Leu Asp Gly Phe Gly Asp Leu Leu Gly Asp Ala Phe Arg
      210              215              220              225

ggt ctc aaa aaa gaa gat aat caa aat gca atg cct ttt agc aca tca 776
Gly Leu Lys Lys Glu Asp Asn Gln Asn Ala Met Pro Phe Ser Thr Ser
      230              235              240

gat gtt gat aat gat ggg tgt cgc cct gca tgc ctg gtc aat ggt cag 824
Asp Val Asp Asn Asp Gly Cys Arg Pro Ala Cys Leu Val Asn Gly Gln
      245              250              255

tct gtg aag agc tgc agt cac ctc cat aac aag acc ggc tgg tgg ttt 872
Ser Val Lys Ser Cys Ser His Leu His Asn Lys Thr Gly Trp Trp Phe
      260              265              270

aac gag tgt ggt cta gca aat cta aat ggc att cat cac ttc tct gga 920
Asn Glu Cys Gly Leu Ala Asn Leu Asn Gly Ile His His Phe Ser Gly
      275              280              285

aaa ttg ctt gca act gga att caa tgg ggc acg tgg acc aaa aac aac 968
Lys Leu Leu Ala Thr Gly Ile Gln Trp Gly Thr Trp Thr Lys Asn Asn
      290              295              300              305

tca cct gtc aag att aaa tct gtt tca atg aaa att aga aga atg tac 1016
Ser Pro Val Lys Ile Lys Ser Val Ser Met Lys Ile Arg Arg Met Tyr
      310              315              320

aat cca tat ttt aaa taa totcatttaa cattgtaatg caagtctac 1064
Asn Pro Tyr Phe Lys
      325

aatgataata tattaaagat ttttaaaagt ttatcttttc acttagtggt tcaaactat 1124
taggcataaat ttaactgtag atggcattta gatgttatga gtttaattag aaaacttcaa 1184
ttttgtagta ttctataaaa gaaaacatgg ottattgtat gtttttaact ctgaactatat 1244
taacaatata caatgaattt tgtttcaagt gaactacaac ttgtcttctt aaaatttata 1304
gtgatttttaa aggatttttg cttttctttg aagcattttt aaaccataat atgttgtaag 1364
gaaaattgaa gggaatattt taactatttt tatactttat atgattatat aatctacaga 1424
taattttctac tgaagacagt tacaataaat aactttatgc agattaatat ataagctaca 1484
catgatgtaa aaaccttact atttttaggt gatgccatgc catttttaaa gtagtaagag 1544
tttgctgccc aaatagtttt tcttggtttt atatctaato atggtttaact attttggtat 1604

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tggtttgtaat aaatatatgt actttttaaaa aaaacagaaa aaaaaa

1651

<210> 14
 <211> 326
 <212> PKT
 <213> Homo sapiens

<400> 14
 Met Met Ser Pro Ser Gln Ala Ser Leu Leu Phe Leu Asn Val Cys Ile
 1 5 10 15
 Phe Ile Cys Gly Glu Val Val Gln Gly Asn Cys Val His His Ser Thr
 20 25 30
 Asp Ser Ser Val Val Asn Ile Val Glu Asp Gly Ser Asn Ala Lys Asp
 35 40 45
 Glu Ser Lys Ser Asn Asp Thr Val Cys Lys Glu Asp Cys Glu Glu Ser
 50 55 60
 Cys Asp Val Lys Thr Lys Ile Thr Arg Glu Glu Lys His Phe Met Cys
 65 70 75 80
 Arg Asn Leu Gln Asn Ser Ile Val Ser Tyr Thr Arg Ser Thr Lys Lys
 85 90 95
 Leu Leu Arg Asn Met Met Asp Glu Gln Gln Ala Ser Leu Asp Tyr Leu
 100 105 110
 Ser Asn Gln Val Asn Glu Leu Met Asn Arg Val Leu Leu Thr Thr
 115 120 125
 Glu Val Phe Arg Lys Gln Leu Asp Pro Phe Pro His Arg Pro Val Gln
 130 135 140
 Ser His Gly Leu Asp Cys Thr Asp Ile Lys Asp Thr Ile Gly Ser Val
 145 150 155 160
 Thr Lys Thr Pro Ser Gly Leu Tyr Ile Ile His Pro Glu Gly Ser Ser
 165 170 175
 Tyr Pro Phe Glu Val Met Cys Asp Met Asp Tyr Arg Gly Gly Gly Trp
 180 185 190
 Thr Val Ile Gln Lys Arg Ile Asp Gly Ile Ile Asp Phe Gln Arg Leu
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 Phe Asn Glu Cys Gly Leu Ala Asn Leu Asn Gly Ile His His Phe Ser
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 50 55 60
 His Gly Leu Asp Cys Thr Asp Ile Lys Asp Thr Ile Gly Ser Val Thr
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 Lys Thr Pro Ser Gly Leu Tyr Ile Ile His Pro Glu Gly Ser Ser Tyr
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 Pro Phe Glu Val Met Cys Asp Met Asp Tyr Arg Gly Gly Gly Trp Thr
 100 105 110
 Val Ile Gln Lys Arg Ile Asp Gly Ile Ile Asp Phe Gln Arg Leu Trp
 115 120 125
 Cys Asp Tyr Leu Asp Gly Phe Gly Asp Leu Leu Gly Asp Ala Phe Arg
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Thr Lys Ile Thr Arg Glu Glu Lys His Phe Met Cys Arg Asn Leu Gln
50      55      60
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Lys Glu Asp Asn Gln Asn Ala Met Pro Phe Ser Thr Ser Asp Val Asp
210                215                220
Asn Asp Gly Cys Arg Pro Ala Cys Leu Val Asn Gly Gln Ser Val Lys
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Ser Cys Ser His Leu His Asn Lys Thr Gly Trp Trp Phe Asn Glu Cys
245                250                255
Gly Leu Ala Asn Leu Asn Gly Ile His His Phe Ser Gly Lys Leu Leu
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Ala Thr Gly Ile Gln Trp Gly Thr Trp Thr Lys Asn Asn Ser Pro Val
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Tyr Ala Ile Gly Lys Arg Ser Ser Glu Thr Tyr Arg Val Thr Pro Asp
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Leu Met Gly Glu Asn Arg Thr Met Thr Ile His Asn Gly Met Phe Phe
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Ser Thr Tyr Asp Arg Asp Asn Asp Gly Trp Leu Thr Ser Asp Pro Arg
225          230          235
Lys Gln Cys Ser Lys Glu Asp Gly Gly Gly Trp Trp Tyr Asn Arg Cys
245          250          255
His Ala Ala Asn Pro Asn Gly Arg Tyr Tyr Trp Gly Gly Gln Tyr Thr
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Trp Asp Met Ala Lys His Gly Thr Asp Asp Gly Val Val Trp Met Asn
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65     70     75     80
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Pro Ala Cys Leu Val Asn Gly Gln Ser Val Lys Ser Cys Ser His Leu
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1

1

【国際公開パンフレット（コレクトバージョン）】

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09/488,725 21 January 2000 (21.01.2000) US
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09/552,317 25 April 2000 (25.04.2000) US
09/596,196 17 June 2000 (17.06.2000) US
09/643,313 31 August 2000 (31.08.2000) US
- (63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:
US 09/488,725 (CIP)
Filed on 21 January 2000 (21.01.2000)
- (71) Applicant (for all designated States except US): HYSEQ, INC. [US/US]; 670 Almanor Avenue, Sunnyvale, CA 94086 (US).
- (72) Inventors; and
(75) Inventors/Applicants (for US only): HALEY, Dana, A. [US/US]; 1430 Glenwood Avenue, San Jose, CA 95125 (US); BOYLE, Bryan, J. [US/US]; 1947 10th Avenue, San Francisco, CA 94116 (US); SUK-YUE HO, Allee [US/US]; 30573 Meridian Circle, Union City, CA 94587 (US); ZHOU, Ping [CN/US]; 1461 Japaul Lane, San Jose, CA 95132 (US); MIZE, Nancy, K. [US/US]; 662 Mountain View Avenue, Mountain View, CA 94041 (US); KLO, Chiauynn [US/US]; 2121 Linwood Drive, San Jose, CA 95132 (US); ARTERBURN, Matthew, C. [US/US]; 5910 Via Del Cielo, Pleasanton, CA 94566 (US); TANG, Y., Tom [CN/US]; 4230 Ranwick Court, San Jose, CA

[Continued on next page]

(54) Title: METHODS AND MATERIALS RELATING TO PROTHROMBINASE-LIKE POLYPEPTIDES AND POLYNUCLEOTIDES

BLASTX ALIGNMENT OF SEQ ID NO: 4 (PROTHROMBINASE-LIKE WITH HUMAN PROTHROMBINASE FC 12 PROTEIN (PROTHROMBINASE Fg1))

The Genbank database indicates that the fibrinogen-like protein is homologous (37% identity)

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>gi |5730075 ref |NP_006673.1||fibrinogen-like 2 precursor; fibrinoleukin
>pir || 547273 pT49 protein-human >pir ||137391 fibrinogen-like
protein expressed in T lymphocytes (pT49) - human
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Length = 439

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Score = 402 (141.5 bits), Expect = 5.7e-36, P = 5.7e-36
 Identities = 109/291 (37%), Positives = 155/291 (53%)

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PROTHROMBINASE FC12:169 IENYVDSKVANLTFV---VNSLDGKCKSKCPSQ---EQIQS---RPVQ-HL IYKDCSDYY- 217

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(57) Abstract: The invention provides novel polynucleotides and polypeptides encoded by such polynucleotides and mutants or variants thereof that correspond to a novel secreted prothrombinase-like polypeptide. These polynucleotides comprise nucleic acid sequences isolated from cDNA library from human ovary (Hyseq clone identification numbers 773565 (SEQ ID NO: 1) and 7727065 (SEQ ID NO: 11)). Other aspects of the invention include vectors containing processes for producing novel secreted prothrombinase-like polypeptides, and antibodies specific for such polypeptides.

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(74) **Agent:** **ELRIFI, Ivor, R.**; Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P.C., One Financial Center, Boston, MA 02111 (US).

(81) **Designated States (national):** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) **Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
26 July 2001 (26.07.2001)

PCT

(10) International Publication Number
WO 01/053456 A3

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09/491,404 25 January 2000 (25.01.2000) US
09/552,317 25 April 2000 (25.04.2000) US
09/596,196 17 June 2000 (17.06.2000) US
09/643,313 31 August 2000 (31.08.2000) US
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Filed on 21 January 2000 (21.01.2000)
09/491,404 (CIP) US
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09/552,317 (CIP) US
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09/596,196 (CIP) US
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09/653,274 (CIP) US
Filed on 31 August 2000 (31.08.2000)
- (71) Applicant (for all designated States except US): HYSEQ, INC. [US/US]; 670 Almanor Avenue, Sunnyvale, CA 94086 (US).
- (72) Inventors; and
(75) Inventors/Applicants (for US only): HALEY, Dana, A. [US/US]; 1430 Glenwood Avenue, San Jose, CA 95125 (US); BOYLE, Bryan, J. [US/US]; 1947 10th Avenue, San Francisco, CA 94116 (US); SUK-YUE HO, Alice

[Continued on next page]

(54) Title: METHODS AND MATERIALS RELATING TO PROTHROMBINASE-LIKE POLYPEPTIDES AND POLYNUCLEOTIDES

BLAST ALIGNMENT OF SEQ ID NO. 4 (PROTHROMBINASE-LIKE WITH HUMAN PROTHROMBINASE FC 12 PROTEIN (PROTHROMBINASE FC 12))

The Genbank database indicates that the fibrinogen-like protein is homologous (37% identity).

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>gi|5730075 ref|NP_006872.1| fibrinogen-like 2 precursor, human  
      >gi|547273.9| g14 protein-human, >gi|537381| fibrinogen-like  
      protein expressed in lymphocytes (g14b) - human  
      >seq(CAB5838.1) (CAB5838.1) fibrinogen-like protein [ Homo sapiens ]  
      >gi|4010825.1| (AF104215) fibrinogen-like protein 2 [ Homo sapiens ]  
      Length = 429
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Score = 402 (141.5 bits), Expect = 5.7e-36, P = 5.7e-36  
Identities = 108/291 (37%), Positives = 155/291 (53%)
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PROTHROMBINASE-LIKE 1: M I R M A E D Q A L L V L S N G Y N E L M R V L L L T E V F R K G L D F P R P V S D H S --- D E T S I G S 105  
      * * * * *  
PROTHROMBINASE 102: M S I C H V V S S E V A L E T F Y --- V N G L D K C S C P S Q --- E G I G S --- R P V D R L Y V D C S D P T 207  
      * * * * *  
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      I G K S Y T * * * * * F E V C D M * C D M V A Q R A G D A T R W D Y I Z  
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      * * * * *  
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      F G L E F R L G K I * * * * * I L * * * * * Y A Y D F A A A E * * * * *  
PROTHROMBINASE 403: P A R A G --- M E E C E T P R P F P A S D F V C M E T A G A G M T L I A M L S E S T N T F T M D P A G 226  
      * * * * *  
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PROTHROMBINASE 403: A R L A G --- H * * * * * S I R C T W P R S D --- * * * * * P * F K  
      * * * * *  
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(57) Abstract: The invention provides novel polynucleotides and polypeptides encoded by such polynucleotides and mutants or variants thereof that correspond to a novel secreted prothrombinase-like polypeptide. These polynucleotides comprise nucleic acid sequences isolated from cDNA library from human ovary (Hyseq clone identification numbers 773565 (SEQ ID NO: 1) and 7727065 (SEQ ID NO: 11)). Other aspects of the invention include vectors containing processes for producing novel secreted prothrombinase-like polypeptides, and antibodies specific for such polypeptides.

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[US/US]; 30573 Meridian Circle, Union City, CA 94587 (US). **ZHOU, Ping** [CN/US]; 1461 Japaul Lane, San Jose, CA 95132 (US). **MIZE, Nancy, K.** [US/US]; 662 Mountain View Avenue, Mountain View, CA 94041 (US). **KUO, Chiauyun** [US/US]; 2121 Linewood Drive, San Jose, CA 95132 (US). **ARTERBURN, Matthew, C.** [US/US]; 5910 Via Del Cielo, Pleasanton, CA 94566 (US). **TANG, Y., Tom** [CN/US]; 4230 Ranwick Court, San Jose, CA 95118 (US). **LIU, Chenghua** [CN/US]; 1125 Ranchero Way #14, San Jose, CA 95117 (US). **DRMANAC, Radoje** [YU/US]; 850 East Greenwich Place, Palo Alto, CA 94303 (US).

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(81) **Designated States (national):** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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[Continued on next page]

(54) Title: METTIDS AND MATERIALS RELATING TO PROTIROMBINASE-LIKE POLYPEPTIDES AND POLYNUCLEOTIDES

FROM FIG. 1-1

PROTHROMBINASE-LIKE 156 TIGSVTKYPSGLYIHPGSSVPEVMQDMQVRSQMTVIQKRIQGIIFQRMWDYLDG 215
PROTHROMBINASE FIG. 218 AIG--KRSEETVRYVTPDKNSSEFVYCDMEIMGGMTVLQARLGSTNFTIRQDYKAG 274
PROTHROMBINASE-LIKE 218 FQDLLEFRLGLKIKFYVQKNTSMFYVALSEEDTLATAYONRILEDETRFFKKHL 275
FEHL EFWLG KI + K 4L + LE + YA YD F44 AE +++HL
PROTHROMBINASE FIG. 279 FQNLKRETNLQNDKHLTKSIE--MILRIIDLEPHNGVELYALYSQFYANFLKRYLHY 332
PROTHROMBINASE-LIKE 276 QKYSNAGADAFRLKKEEDONAMPFSTSDYNDGCRPAQLVNGDSKSCSLHKTQWRF 335
Q Y40 A22A S K N + FET D DNG P4 S 42 L4 +GDMF
PROTHROMBINASE FIG. 333 GKNYGTADALP-FNKHYNHDKFFFTFQKNDRY-P5----G----ACQ-LYSSQWRF 381
PROTHROMBINASE-LIKE 335 HECGLANLNG-IHMFSGKLLATG1QWGTN--TKNNSPVKIKSVSMKIRMYNPFYFK 388
+ C ANLNG +H + + G1 DGTW P KS + + M P +FK
PROTHROMBINASE FIG. 380 DAQLSAILNRYVYAGRTGYRNGIFQGTWPDVSEAHPCQTKSSFLEAAMIRPKHFK 438

BLAST ALIGNMENT OF SEQ. ID NO. 4 (PROTHROMBINASE-LIKE WITH HUMAN PROTHROMBINASE FIG. 12 PROTEIN (PROTHROMBINASE Fig. 1))

The Genbank database indicates that the fibrinogen-like protein is homologous (37% identity)

>gi |5733075 ref |NP_006873.1| fibrinogen-like 2 precursor; fibroleukin
>pir | 547273 p149 protelin-human >pir |127381 fibrinogen-like protein expressed in T lymphocytes (p149) = human
>emb |CA85298.1 | (Z36531) fibrinogen-like protein [Homo sapiens]
>gb |AF010825.1 | (AF0104015) fibrinogen-like protein 2 [Homo sapiens]
Length = 439

Score = 402 (141.5 bits), Expect = 5.7e-36, P = 5.7e-36
Identities = 109/291 (37%), Positives = 150/291 (53%)

PROTHROMBINASE-LIKE 381LSNANDEQDASLDYLSNDVNEIWRVLLTTIEVFRIKGLDPFPHRPVQSHGL--DCTD1KD 355
+ N 4D + A+L ++ VN L + ++ +G4 RPYQ H + DC10
PROTHROMBINASE FIG. 12181ENYVDSKVALTFV--VNSLDGCKSKCPG---ED1GS--RPVQ-HLTKDGSDFY- 217

TO FIG. 1-2

(57) Abstract: The invention provides novel polynucleotides and polypeptides encoded by such polynucleotides and mutants or variants thereof that correspond to a novel secreted prothrombinase-like polypeptide. These polynucleotides comprise nucleic acid sequences isolated from cDNA library from human ovary (Hyseq clone identification numbers 773565 (SEQ ID NO: 1) and 7727065 (SEQ ID NO: 11)). Other aspects of the invention include vectors containing processes for producing novel secreted prothrombinase-like polypeptides, and antibodies specific for such polypeptides.

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**METHODS AND MATERIALS RELATING TO
PROTHROMBINASE-LIKE POLYPEPTIDES AND POLYNUCLEOTIDES**

1. TECHNICAL FIELD

5 The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with uses for these polynucleotides and proteins, for example in therapeutic, diagnostic and research methods. In particular, the invention relates to a novel prothrombinase-like polypeptide.

10 2. BACKGROUND ART

Identified polynucleotide and polypeptide sequences have numerous applications in, for example, diagnostics, forensics, gene mapping; identification of mutations responsible for genetic disorders or other traits, to assess biodiversity, and to produce many other types of data and products dependent on DNA and amino acid sequences. Proteins are known to have
15 biological activity, for example, by virtue of their secreted nature in the case of leader sequence cloning, by virtue of their cell or tissue source in the case of PCR-based techniques, or by virtue of structural similarity to other genes of known biological activity. It is to these polypeptides and the polynucleotides encoding them that the present invention is directed. In particular, this invention is directed to novel soluble prothrombinase-like polypeptides and
20 polynucleotides.

The ability of the body to control the flow of blood following vascular injury is paramount to continued survival. The process of blood clotting and then the subsequent dissolution of the clot, following repair of the injured tissue, is termed hemostasis. Hemostasis consists of 1) vascular constriction, 2) platelet activation and clumping, 3) formation of
25 thrombus, and 4) clot dissolution following tissue repair. In order to initiate the clotting process, inactive prothrombin is converted to thrombin by prothrombinase complex, which is composed of platelet phospholipids, Ca ions, prothrombin and factors Va and Xa. Prothrombinase has been identified and characterized as an immune procoagulant (International Patent Application No. WO98/51335). The generation of thrombin by cleavage of
30 prothrombin is a central regulatory process. Thrombin activates platelets to start the initial cascade and also cleaves fibrinogen, a major plasma protein. Fibrin forms a network of fibers, which finally leads to formation of a clot. Thus, prothrombinase is a procoagulant and regulation of its activity is crucial for survival. Fibrinogen cleavage products are also shown

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to display vasoconstrictor and chemotactic activities and are mitogenic for several cell types (Herrick et al, (1999) *Int. J. Biochem. Cell Biol.* 31, 741-746).

Dysregulation of coagulation pathways can result in severe pathological conditions including hemophilia, myocardial infarction, glomerular diseases, diabetes, tumor growth, fulminant viral hepatitis, and atherosclerosis among others. Many of these pathologies involve fibrin deposition and necrosis of the affected tissue. Thrombosis or blood clotting has also been associated with cancer (Dvorak, H.F. *Hum. Pathol.* 18(3), pp. 275-284 (1987)). Prothrombinase is also associated with viral hepatitis. (Levy et al., *Am. J. Pathol.* 156(4), pp. 1217-1225 (2000)). Some researchers have shown that hyperexpression of prothrombinase is associated with early stages of Type 1 diabetes (van der Planken et al., *Clin. Appl. Thromb. Hemost.* 6(2), pp. 65-68 (2000)). Prothrombinase or prothrombinase-like protein may be beneficial in wound healing by increasing coagulation (Thiagarajan et al., *Biochemistry* 39(21) pp. 6459-6465 (2000)). Inhibition of prothrombinase or prothrombinase-like protein may reduce abnormal clotting, increase pathogenesis or viral hepatitis, decrease liver necrosis, or treat diabetes Type I.

Recently, it is becoming apparent that there are inducible prothrombinase genes that are induced either upon viral infection or cytokine treatment (Levy et al (2000) *Am. J. Pathol.* 150, 1217-1225). Antibodies to the prothrombinase fgl2 protein were able to abrogate the fulminant hepatitis in murine model of the disease and reduce the liver necrosis. Similarly, it was shown that fgl2 expression is up regulated upon cytokine treatment of pregnant mice leading to spontaneous abortion (Clark et al (1999) *Am. J. Reproductive Immunol.* 42, 37-43). It would be beneficial to identify other inducible prothrombinase-like proteins that are expressed only under certain pathological conditions.

25

3. SUMMARY OF THE INVENTION

This invention is based on the discovery of novel prothrombinase-like polypeptides, novel isolated polynucleotides encoding such polypeptides, including recombinant DNA molecules, cloned genes or degenerate variants thereof, especially naturally occurring variants such as allelic variants, antisense polynucleotide molecules, and antibodies that specifically recognize one or more epitopes present on such polypeptides, as well as hybridomas producing such antibodies. Specifically, the polynucleotides of the present invention are based on a prothrombinase-like polynucleotide isolated from a cDNA library prepared from human ovary

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(Hyseq clone identification numbers 7735656 (SEQ ID NO: 1) and 7727065 (SEQ ID NO: 11)).

5 The compositions of the present invention additionally include vectors such as expression vectors containing the polynucleotides of the invention, cells genetically engineered to contain such polynucleotides and cells genetically engineered to express such polynucleotides.

10 The compositions of the invention provide isolated polynucleotides that include, but are not limited to, a polynucleotide comprising the nucleotide sequence set forth in the SEQ ID NO: 1-3, 10-13, and 15; or a fragment of SEQ ID NO: 1-3, 10-13 and 15; a polynucleotide comprising the full length protein coding sequence of the SEQ ID NO: 1-3, 10-13 and 15 (for example, SEQ ID NO: 4, 9, 14, 18); and a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of any of SEQ ID NO: 1-3, 10-13 and 15. The polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes under stringent hybridization conditions to (a) the complement of any of the nucleotide sequences set forth in SEQ ID NO: 1-3, 10-13 and 15; (b) a nucleotide sequence encoding any of
15 SEQ ID NO: 4-9, 14, and 16-18; a polynucleotide which is an allelic variant of any polynucleotides recited above having at least 70% polynucleotide sequence identity to the polynucleotides; a polynucleotide which encodes a species homolog (e.g. orthologs) of any of the peptides recited above; or a polynucleotide that encodes a polypeptide comprising a specific
20 domain or truncation of the polypeptide comprising SEQ ID NO: 4-9, 14 and 16-18.

A collection as used in this application can be a collection of only one polynucleotide. The collection of sequence information or unique identifying information of each sequence can be provided on a nucleic acid array. In one embodiment, segments of sequence information are provided on a nucleic acid array to detect the polynucleotide that contains the segment. The array
25 can be designed to detect full-match or mismatch to the polynucleotide that contains the segment. The collection can also be provided in a computer-readable format.

This invention further provides cloning or expression vectors comprising at least a fragment of the polynucleotides set forth above and host cells or organisms transformed with these expression vectors. Useful vectors include plasmids, cosmids, lambda phage derivatives,
30 phagemids, and the like, that are well known in the art. Accordingly, the invention also provides a vector including a polynucleotide of the invention and a host cell containing the polynucleotide. In general, the vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and a selectable marker for the host cell. Vectors according to the invention include expression vectors, replication vectors, probe generation

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vectors, and sequencing vectors. A host cell according to the invention can be a prokaryotic or eukaryotic cell and can be a unicellular organism or part of a multicellular organism.

The compositions of the present invention include polypeptides comprising, but not limited to, an isolated polypeptide selected from the group comprising the amino acid sequence of SEQ ID NO: 4-9, 14, and 16-18; or the corresponding full length or mature protein. Polypeptides of the invention also include polypeptides with biological activity that are encoded by (a) any of the polynucleotides having a nucleotide sequence set forth in the SEQ ID NO: 1-3, 10-13 and 15; or (b) polynucleotides that hybridize to the complement of the polynucleotides of (a) under stringent hybridization conditions. Biologically or immunologically active variants of any of the protein sequences listed as SEQ ID NO: 4-9, 14 and 16-18 and substantial equivalents thereof that retain biological or immunological activity are also contemplated. The polypeptides of the invention may be wholly or partially chemically synthesized but are preferably produced by recombinant means using the genetically engineered cells (e.g. host cells) of the invention.

The invention also provides compositions comprising a polypeptide of the invention. Pharmaceutical compositions of the invention may comprise a polypeptide of the invention and an acceptable carrier, such as a hydrophilic, e.g., pharmaceutically acceptable, carrier.

The invention also relates to methods for producing a polypeptide of the invention comprising culturing host cells comprising an expression vector containing at least a fragment of a polynucleotide encoding the polypeptide of the invention in a suitable culture medium under conditions permitting expression of the desired polypeptide, and purifying the protein or peptide from the culture or from the host cells. Preferred embodiments include those in which the protein produced by such a process is a mature form of the protein.

Polynucleotides according to the invention have numerous applications in a variety of techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use as oligomers, or primers, for PCR, use in an array, use in computer-readable media, use for chromosome and gene mapping, use in the recombinant production of protein, and use in generation of antisense DNA or RNA, their chemical analogs and the like. For example, when the expression of an mRNA is largely restricted to a particular cell or tissue type, polynucleotides of the invention can be used as hybridization probes to detect the presence of the particular cell or tissue mRNA in a sample using, e.g., *in situ* hybridization.

In other exemplary embodiments, the polynucleotides are used in diagnostics as expressed sequence tags for identifying expressed genes or, as well known in the art and

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exemplified by Vollrath et al., Science 258:52-59 (1992), as expressed sequence tags for physical mapping of the human genome.

The polypeptides according to the invention can be used in a variety of conventional procedures and methods that are currently applied to other proteins. For example, a polypeptide of the invention can be used to generate an antibody that specifically binds the polypeptide. Such antibodies, particularly monoclonal antibodies, are useful for detecting or quantitating the polypeptide in tissue. The polypeptides of the invention can also be used as molecular weight markers, and as a food supplement.

Methods are also provided for preventing, treating, or ameliorating a medical condition which comprises the step of administering to a mammalian subject a therapeutically effective amount of a composition comprising a peptide of the present invention and a pharmaceutically acceptable carrier.

In particular, the polypeptides and polynucleotides of the invention can be utilized, for example, as part of methods for the prevention and/or treatment of diseases associated with the dysregulation of coagulation pathways, such as hemophilia, myocardial infarction, glomerular disease, diabetes, fulminant viral hepatitis and atherosclerosis among others. The polynucleotides and polypeptides may also be beneficial in the treatment of viral infections and in the treatment of some forms of cancer.

The methods of the invention also provides methods for the treatment of disorders as recited herein which comprise the administration of a therapeutically effective amount of a composition comprising a polynucleotide or polypeptide of the invention and a pharmaceutically acceptable carrier to a mammalian subject exhibiting symptoms or tendencies related to disorders as recited herein. In addition, the invention encompasses methods for treating diseases or disorders as recited herein comprising the step of administering a composition comprising compounds and other substances that modulate the overall activity of the target gene products and a pharmaceutically acceptable carrier. Compounds and other substances can effect such modulation either on the level of target gene/protein expression or target protein activity. Specifically, methods are provided for preventing, treating or ameliorating a medical condition, including viral diseases, which comprises administering to a mammalian subject, including but not limited to humans, a therapeutically effective amount of a composition comprising a polypeptide of the invention, a polynucleotide of the invention or a therapeutically effective amount of a composition comprising a binding partner of (e.g., antibody specifically reactive for) prothrombinase-like polypeptides of the invention. The mechanics of the particular condition or pathology will dictate whether the polypeptides, or

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nucleotides of the invention or binding partners (or inhibitors) of the polypeptides would be beneficial to the individual in need of treatment.

According to this method, polypeptides of the invention can be administered to produce an *in vitro* or *in vivo* inhibition of cellular function. A polypeptide of the invention can be administered *in vivo* alone or as an adjunct to other therapies. Conversely, protein or other active ingredients of the present invention may be included in formulations of a particular agent to minimize side effects of such an agent.

The invention further provides methods for manufacturing medicaments useful in the above-described methods.

10 The present invention further relates to methods for detecting the presence of the polynucleotides or polypeptides of the invention in a sample (e.g., tissue or sample). Such methods can, for example, be utilized as part of prognostic and diagnostic evaluation of disorders as recited herein and for the identification of subjects exhibiting a predisposition to such conditions.

15 The invention provides a method for detecting a polypeptide of the invention in a sample comprising contacting the sample with a compound that binds to and forms a complex with the polypeptide under conditions and for a period sufficient to form the complex and detecting formation of the complex, so that if a complex is formed, the polypeptide is detected.

The invention also provides kits comprising polynucleotide probes and/or monoclonal antibodies, and optionally quantitative standards, for carrying out methods of the invention. Furthermore, the invention provides methods for evaluating the efficacy of drugs, and monitoring the progress of patients, involved in clinical trials for the treatment of disorders as recited above.

25 The invention also provides methods for the identification of compounds that modulate (i.e., increase or decrease) the expression or activity of the polynucleotides and/or polypeptides of the invention. Such methods can be utilized, for example, for the identification of compounds that can ameliorate symptoms of disorders as recited herein. Such methods can include, but are not limited to, assays for identifying compounds and other substances that interact with (e.g., bind to) the polypeptides of the invention.

30 The invention provides a method for identifying a compound that binds to the polypeptide of the present invention comprising contacting the compound with the polypeptide under conditions and for a time sufficient to form a polypeptide/compound complex and detecting the complex, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide is identified.

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Also provided is a method for identifying a compound that binds to the polypeptide comprising contacting the compound with the polypeptide in a cell for a time sufficient to form a polypeptide/compound complex wherein the complex drives expression of a reporter gene sequence in the cell and detecting the complex by detecting reporter gene sequence expression so that if the polypeptide/compound complex is detected a compound that binds to the polypeptide is identified.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO:3 (i.e. SEQ ID NO:4) (also identified as "prothrombinase-like") and human prothrombinase Fg12 protein ("prothrombinase Fg12") (SEQ ID NO:19), indicating that the two sequence share 53% similarity over 291 amino acid residues of SEQ ID NO:4 and 37% identity over 291 amino acid residues of SEQ ID NO:4, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine. Gaps are presented as dashes.

Figure 2 shows the BLASTP sequence alignment between the protein encoded by SEQ ID :13 (i.e. SEQ ID NO:14) identified as "prothrombinase-like" and a portion of human prothrombinase Fg12 protein (amino acids 1-269 of SEQ ID NO:19), indicating that the two sequences share 54% similarity over 132 amino acid residues of SEQ ID NO:14 and 37% identity over the same 132 amino acid residues of SEQ ID NO:14, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine. Gaps are presented as dashes.

Figure 3 shows the BLASTP alignment between the protein encoded by SEQ ID : 13 (i.e. SEQ ID NO: 14) identified as "prothrombinase-like" and human prothrombinase Fg12 protein (amino acids 163-270 of SEQ ID NO: 19), indicating that the two sequences share 34% similarity over that 115 amino acid residue portion of SEQ ID NO: 14 and 40% identity over the same 115 amino acid residue portion of SEQ ID NO: 14, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine,

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P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine,
W=Tryptophan, Y=Tyrosine. Gaps are presented as dashes.

Figure 4 shows the MSI GeneAtlas alignment of SEQ ID NO:14 with the blood coagulation, pdb number = 1fzc_B indicating that the two sequences share 32% identity over 224 amino acids and 69% over the same 224 amino acids, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine. The score 86.22, the estimated p-value is 7.2e-04 and the upper limit p value is 1.1e-11. The MSI GeneAtlas Report allows a functional prediction based protein structure homology (both secondary and tertiary protein structure), which utilizes threading (SEQFold), high throughput modeling and sequence profile-based searching (PB90) methodologies.

5. DETAILED DESCRIPTION OF THE INVENTION

The prothrombinase-like polypeptide of SEQ ID NO: 4 is an approximately 388-amino acid protein with a predicted molecular mass of approximately 43 kDa unglycosylated. Protein database searches with the BLASTX algorithm (Altschul S.F. et al., J. Mol. Evol. 36:290-300 (1993) and Altschul S.F. et al., J. Mol. Biol. 21:403-10 (1990), herein incorporated by reference) indicate that SEQ ID NO: 4 is homologous to human prothrombinase Fg12 protein. Figure 1 shows the BLASTX amino acid sequence alignment between the protein encoded by SEQ ID NO:3 (i.e. SEQ ID NO: 4) (also identified as "prothrombinase-like") and the human prothrombinase Fg12 protein (SEQ ID NO: 19) indicating that the two sequences share 53% similarity over 291 amino acid residues of SEQ ID NO: 4 and 37% identity over the same 291 amino acid residues of SEQ ID NO: 4.

A predicted approximately twenty residue signal peptide is encoded from approximately residue 1 to 20 of SEQ ID NO:4. The mature portion (also represented as SEQ ID No: 9) is useful on its own. This can be confirmed by expression in mammalian cells and sequencing of the cleaved product. The signal peptide was predicted using the Kyte/Doolittle algorithm (J. Molecular Biology, vol. 157, pp. 105-131 (1982)). One of skill in the art will recognize that the actual cleavage site may be different than that predicted by the computer program.

Using eMATRIX software package (Stanford University, Stanford CA), prothrombinase-like is expected to have fibrinogen beta and gamma chains in the C-terminal domain. Specifically, at residues 181 through 217 of SEQ ID NO:4, at residues 222 through 234 of SEQ ID NO:4, at residues 271 through 285 of SEQ ID NO:4, at residues 324 through

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353 of SEQ ID NO:4. The domains corresponding to SEQ ID NO:4 are as follows, wherein
 A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine,
 G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine,
 N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine,

5 V=Valine, W=Tryptophan, Y=Tyrosine:

Residues 181-217 VMCDMDYRGGGWTVIQKRIDGIDFQRLWCDYLDGFG (designated as
 SEQ ID NO:5),

Residues 324-353 CSHLHNKTGWWFNECGLANLNGIHHSFGKL (designated as SEQ ID
 NO:6),

10 Residues 271-285 FKMHLGRYSGNAGDA (designated as SEQ ID NO:7),

Residues 222-234 EFWLGLKKIFYIV (designated as SEQ ID NO:8).

A predicted soluble, secreted splice variant of SEQ ID NO: 4 is SEQ ID NO: 14. It is
 an approximately 326 amino acid protein with a predicted molecular mass of approximately 37
 kDa unglycosylated. Protein database searches with the BLASTX algorithm (Altschul S.F. et
 al., J. Mol. Evol. 36:290-300 (1993) and Altschul S.F. et al., J. Mol. Biol. 21:403-10 (1990),
 15 herein incorporated by reference) indicate that SEQ ID NO: 14 is homologous to human
 prothrombinase Fg12 protein. Figures 2 and 3 shows the BLASTP amino acid sequence
 alignment between the protein encoded by SEQ ID NO:13 (i.e. SEQ ID NO: 14) (also
 identified as "prothrombinase-like") and the human prothrombinase Fg12 protein (SEQ ID

20 NO: 19).

Using eMATRIX software package (Stanford University, Stanford CA),
 prothrombinase-like is expected to have fibrinogen beta and gamma chains in the C-terminal
 domain. Specifically, at residues 181 through 217 of SEQ ID NO:14, at residues 262 through
 291 of SEQ ID NO:14. The domains corresponding to SEQ ID NO:14 are as follows, wherein
 25 A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine,
 G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine,
 N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine,
 V=Valine, W=Tryptophan, Y=Tyrosine:

Residues 181-217 VMCDMDYRGGGWTVIQKRIDGIDFQRLWCDYLDGFG (designated as
 30 SEQ ID NO:5),

Residues 262-292 CSHLHNKTGWWFNECGLANLNGIHHSFGKL (designated as SEQ ID
 NO:6).

A predicted approximately twenty residue signal peptide is encoded from approximately
 residue 1 to residue 20 of both SEQ ID NO: 14 (SEQ ID NO: 16). The extracellular portion is

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useful on its own. This can be confirmed by expression in mammalian cells and sequencing of the cleaved product. The signal peptide region was predicted using the Kyte-Doolittle hydrophobicity prediction algorithm (J. Mol Biol, 157, pp. 105-31 (1982), incorporated herein by reference). One of skill in the art will recognize that the actual cleavage site may be different than that predicted by the computer program.

Using Molecular Simulations Inc. GeneAtlas software (Molecular Simulations Inc., San Diego, CA) and the sequence profile-based searching protocol PB90 using optimized PSI-BLAST and the Seqfold based searching protocol on the PSI-BLAST defined domains, the membrane-bound prothrombinase-like polypeptide SEQ ID NO:14 was determined to have a region at residues 82 to 321 (SEQ ID NO:17) with characteristic motifs to blood coagulation (SEQ ID NO: 20) (See Figure 4). The domains corresponding to SEQ ID NO: 14 are as follows wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine:

Blood coagulation domain (designated as amino acids 82 - 321 of SEQ ID NO: 14) with PSI-BLAST e-value of 1.3e-66, protein database identification number entry = lfzc_B (Research Collaboratory for Structural Bioinformatics, <http://www.rcsb.org/pdb>).

The polypeptides and polynucleotides of the invention can be utilized, for example, as part of methods for the prevention and/or treatment of disorders mediated by loss or overexpression of prothrombinase-like polypeptide. Such disorders include diseases associated with the dysregulation of coagulation pathways, such as hemophilia, myocardial infarction, glomerular disease, diabetes, fulminant viral hepatitis and atherosclerosis among others. The polynucleotides and polypeptides may also be beneficial in the treatment of viral infections and in the treatment of some forms of cancer.

5.1 DEFINITIONS

It must be noted that as used herein and in the appended claims, the singular forms "a", "an" and "the" include plural references unless the context clearly dictates otherwise.

The term "active" refers to those forms of the polypeptide which retain the biologic and/or immunologic activities of any naturally occurring polypeptide. According to the invention, the terms "biologically active" or "biological activity" refer to a protein or peptide having structural, regulatory or biochemical functions of a naturally occurring molecule. The term "prothrombinase-like" refers to biological activity that is similar to the biological activity

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of a human prothrombinase. Likewise "biologically active" or "biological activity" refers to the capability of the natural, recombinant or synthetic prothrombinase-like peptide, or any peptide thereof, to induce a specific biological response in appropriate animals or cells and to bind with specific antibodies.

5 The term "activated cells" as used in this application are those cells which are engaged in extracellular or intracellular membrane trafficking, including the export of secretory or enzymatic molecules as part of a normal or disease process.

The terms "complementary" or "complementarity" refer to the natural binding of polynucleotides by base pairing. For example, the sequence 5'-AGT-3' binds to the
10 complementary sequence 3'-TCA-5'. Complementarity between two single-stranded molecules may be "partial" such that only some of the nucleic acids bind or it may be "complete" such that total complementarity exists between the single stranded molecules. The degree of complementarity between the nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands.

15 The term "embryonic stem cells (ES)" refers to a cell that can give rise to many differentiated cell types in an embryo or an adult, including the germ cells. The term "germ line stem cells (GSCs)" refers to stem cells derived from primordial stem cells that provide a steady and continuous source of germ cells for the production of gametes. The term
"primordial germ cells (PGCs)" refers to a small population of cells set aside from other cell
20 lineages particularly from the yolk sac, mesenteries, or gonadal ridges during embryogenesis that have the potential to differentiate into germ cells and other cells. PGCs are the source from which GSCs and ES cells are derived. The PGCs, the GSCs and the ES cells are capable of self-renewal. Thus these cells not only populate the germ line and give rise to a plurality of terminally differentiated cells that comprise the adult specialized organs, but are able to
25 regenerate themselves.

The term "expression modulating fragment," EMF, means a series of nucleotides which modulates the expression of an operably linked ORF or another EMF.

As used herein, a sequence is said to "modulate the expression of an operably linked sequence" when the expression of the sequence is altered by the presence of the EMF. EMFs
30 include, but are not limited to, promoters, and promoter modulating sequences (inducible elements). One class of EMFs is nucleic acid fragments which induce the expression of an operably linked ORF in response to a specific regulatory factor or physiological event.

The terms "nucleotide sequence" or "nucleic acid" or "polynucleotide" or "oligonucleotide" are used interchangeably and refer to a heteropolymer of nucleotides or the

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sequence of these nucleotides. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA) or to any DNA-like or RNA-like material. Generally, nucleic acid segments provided by this invention may be assembled from

5 fragments of the genome and short oligonucleotide linkers, or from a series of oligonucleotides, or from individual nucleotides, to provide a synthetic nucleic acid which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon, or a eukaryotic gene.

The terms "oligonucleotide fragment" or a "polynucleotide fragment", "portion," or

10 "segment" or "probe" or "primer" are used interchangeable and refer to a sequence of nucleotide residues which are at least about 5 nucleotides, more preferably at least about 7 nucleotides, more preferably at least about 9 nucleotides, more preferably at least about 11 nucleotides and most preferably at least about 17 nucleotides. The fragment is preferably less than about 500 nucleotides, preferably less than about 200 nucleotides, more preferably less

15 than about 100 nucleotides, more preferably less than about 50 nucleotides and most preferably less than 30 nucleotides. Preferably the probe is from about 6 nucleotides to about 200 nucleotides, preferably from about 15 to about 50 nucleotides, more preferably from about 17 to 30 nucleotides and most preferably from about 20 to 25 nucleotides. Preferably the

20 fragments can be used in polymerase chain reaction (PCR), various hybridization procedures or microarray procedures to identify or amplify identical or related parts of mRNA or DNA molecules. A fragment or segment may uniquely identify each polynucleotide sequence of the present invention. Preferably the fragment comprises a sequence substantially similar to a portion of SEQ ID NO: 1-3, 10-13 and 15.

Probes may, for example, be used to determine whether specific mRNA molecules are

25 present in a cell or tissue or to isolate similar nucleic acid sequences from chromosomal DNA as described by Walsh et al. (Walsh, P.S. et al., 1992, PCR Methods Appl 1:241-250). They may be labeled by nick translation, Klenow fill-in reaction, PCR, or other methods well known in the art. Probes of the present invention, their preparation and/or labeling are elaborated in Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor

30 Laboratory, NY; or Ausubel, F.M. et al., 1989, Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, both of which are incorporated herein by reference in their entirety.

The nucleic acid sequences of the present invention also include the sequence information from any of the nucleic acid sequences of SEQ ID NO: 1-3, 10-13 and 15. The

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sequence information can be a segment of SEQ ID NO: 1-3, 10-13 and 15 that uniquely identifies or represents the sequence information of SEQ ID NO: 1-3, 10-13 and 15. One such segment can be a twenty-mer nucleic acid sequence because the probability that a twenty-mer is fully matched in the human genome is 1 in 300. In the human genome, there are three billion base pairs in one set of chromosomes. Because 4^{20} possible twenty-mers exist, there are 300 times more twenty-mers than there are base pairs in a set of human chromosome. Using the same analysis, the probability for a seventeen-mer to be fully matched in the human genome is approximately 1 in 5. When these segments are used in arrays for expression studies, fifteen-mer segments can be used. The probability that the fifteen-mer is fully matched in the expressed sequences is also approximately one in five because expressed sequences comprise less than approximately 5% of the entire genome sequence.

Similarly, when using sequence information for detecting a single mismatch, a segment can be a twenty-five mer. The probability that the twenty-five mer would appear in a human genome with a single mismatch is calculated by multiplying the probability for a full match ($1+4^{25}$) times the increased probability for mismatch at each nucleotide position (3×25). The probability that an eighteen mer with a single mismatch can be detected in an array for expression studies is approximately one in five. The probability that a twenty-mer with a single mismatch can be detected in a human genome is approximately one in five.

The term "open reading frame," ORF, means a series of nucleotide triplets coding for amino acids without any termination codons and is a sequence translatable into protein.

The terms "operably linked" or "operably associated" refer to functionally related nucleic acid sequences. For example, a promoter is operably associated or operably linked with a coding sequence if the promoter controls the transcription of the coding sequence. While operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements e.g. repressor genes are not contiguously linked to the coding sequence but still control transcription/translation of the coding sequence.

The term "pluripotent" refers to the capability of a cell to differentiate into a number of differentiated cell types that are present in an adult organism. A pluripotent cell is restricted in its differentiation capability in comparison to a totipotent cell.

The terms "polypeptide" or "peptide" or "amino acid sequence" refer to an oligopeptide, peptide, polypeptide or protein sequence or fragment thereof and to naturally occurring or synthetic molecules. A polypeptide "fragment," "portion," or "segment" is a stretch of amino acid residues of at least about 5 amino acids, preferably at least about 7 amino

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acids, more preferably at least about 9 amino acids and most preferably at least about 17 or more amino acids. The peptide preferably is not greater than about 200 amino acids, more preferably less than 150 amino acids and most preferably less than 100 amino acids.

Preferably the peptide is from about 5 to about 200 amino acids. To be active, any

5 polypeptide must have sufficient length to display biological and/or immunological activity.

The term "naturally occurring polypeptide" refers to polypeptides produced by cells that have not been genetically engineered and specifically contemplates various polypeptides arising from post-translational modifications of the polypeptide including, but not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

10 The term "translated protein coding portion" means a sequence which encodes for the full length protein which may include any leader sequence or a processing sequence.

The term "mature protein coding sequence" refers to a sequence which encodes a peptide or protein without any leader/signal sequence. The peptide may have the leader sequences removed during processing in the cell or the protein may have been produced

15 synthetically or using a polynucleotide only encoding for the mature protein coding sequence.

The term "derivative" refers to polypeptides chemically modified by such techniques as ubiquitination, labeling (e.g., with radionuclides or various enzymes), covalent polymer attachment such as pegylation (derivatization with polyethylene glycol) and insertion or substitution by chemical synthesis of amino acids such as ornithine, which do not normally

20 occur in human proteins.

The term "variant" (or "analog") refers to any polypeptide differing from naturally occurring polypeptides by amino acid insertions, deletions, and substitutions, created using, *e.g.*, recombinant DNA techniques. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing activities of interest, may be found by comparing

25 the sequence of the particular polypeptide with that of homologous peptides and minimizing the number of amino acid sequence changes made in regions of high homology (conserved regions) or by replacing amino acids with consensus sequence.

Alternatively, recombinant variants encoding these same or similar polypeptides may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon

30 substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations in the polynucleotide sequence may be reflected in the polypeptide or domains of other peptides added to the polypeptide to modify the

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properties of any part of the polypeptide, to change characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate.

Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, *i.e.*, conservative amino acid replacements. "Conservative" amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. "Insertions" or "deletions" are preferably in the range of about 1 to 20 amino acids, more preferably 1 to 10 amino acids. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in a polypeptide molecule using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

Alternatively, where alteration of function is desired, insertions, deletions or non-conservative alterations can be engineered to produce altered polypeptides. Such alterations can, for example, alter one or more of the biological functions or biochemical characteristics of the polypeptides of the invention. For example, such alterations may change polypeptide characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate. Further, such alterations can be selected so as to generate polypeptides that are better suited for expression, scale up and the like in the host cells chosen for expression. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges.

The terms "purified" or "substantially purified" as used herein denotes that the indicated nucleic acid or polypeptide is present in the substantial absence of other biological macromolecules, *e.g.*, polynucleotides, proteins, and the like. In one embodiment, the polynucleotide or polypeptide is purified such that it constitutes at least 95% by weight, more preferably at least 99% by weight, of the indicated biological macromolecules present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000 daltons, can be present).

The term "isolated" as used herein refers to a nucleic acid or polypeptide separated from at least one other component (*e.g.*, nucleic acid or polypeptide) present with the nucleic

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acid or polypeptide in its natural source. In one embodiment, the nucleic acid or polypeptide is found in the presence of (if anything) only a solvent, buffer, ion, or other components normally present in a solution of the same. The terms "isolated" and "purified" do not encompass nucleic acids or polypeptides present in their natural source.

5 The term "recombinant," when used herein to refer to a polypeptide or protein, means that a polypeptide or protein is derived from recombinant (*e.g.*, microbial, insect, or mammalian) expression systems. "Microbial" refers to recombinant polypeptides or proteins made in bacterial or fungal (*e.g.*, yeast) expression systems. As a product, "recombinant microbial" defines a polypeptide or protein essentially free of native endogenous substances
10 and unaccompanied by associated native glycosylation. Polypeptides or proteins expressed in most bacterial cultures, *e.g.*, *E. coli*, will be free of glycosylation modifications; polypeptides or proteins expressed in yeast will have a glycosylation pattern in general different from those expressed in mammalian cells.

The term "recombinant expression vehicle or vector" refers to a plasmid or phage or
15 virus or vector, for expressing a polypeptide from a DNA (RNA) sequence. An expression vehicle can comprise a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences. Structural units intended
20 for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an amino terminal methionine residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final product.

25 The term "recombinant expression system" means host cells which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit extrachromosomally. Recombinant expression systems as defined herein will express heterologous polypeptides or proteins upon induction of the regulatory elements linked to the DNA segment or synthetic gene to be expressed. This term also means host cells
30 which have stably integrated a recombinant genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers. Recombinant expression systems as defined herein will express polypeptides or proteins endogenous to the cell upon induction of the regulatory elements linked to the endogenous DNA segment or gene to be expressed. The cells can be prokaryotic or eukaryotic.

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The term "secreted" includes a protein that is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence when it is expressed in a suitable host cell. "Secreted" proteins include without limitation proteins secreted wholly (e.g., soluble proteins) or partially (e.g., receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation proteins that are transported across the membrane of the endoplasmic reticulum. "Secreted" proteins are also intended to include proteins containing non-typical signal sequences (e.g. Interleukin-1 Beta, see Krasney, P.A. and Young, P.R. (1992) Cytokine 4(2):134 -143) and factors released from damaged cells (e.g. Interleukin-1 Receptor Antagonist, see Arend, W.P. et. al. (1998) Annu. Rev. Immunol. 16:27-55)

Where desired, an expression vector may be designed to contain a "signal or leader sequence" which will direct the polypeptide through the membrane of a cell. Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous protein sources by recombinant DNA techniques.

The term "stringent" is used to refer to conditions that are commonly understood in the art as stringent. Stringent conditions can include highly stringent conditions (i.e., hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1X SSC/0.1% SDS at 68°C), and moderately stringent conditions (i.e., washing in 0.2X SSC/0.1% SDS at 42°C). Other exemplary hybridization conditions are described herein in the examples.

In instances of hybridization of deoxyoligonucleotides, additional exemplary stringent hybridization conditions include washing in 6X SSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligonucleotides), 48°C (for 17-base oligonucleotides), 55°C (for 20-base oligonucleotides), and 60°C (for 23-base oligonucleotides).

As used herein, "substantially equivalent" can refer both to nucleotide and amino acid sequences, for example a mutant sequence, that varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between the reference and subject sequences. Typically, such a substantially equivalent sequence varies from one of those listed herein by no more than about 35% (i.e., the number of individual residue substitutions, additions, and/or deletions in a substantially equivalent sequence, as compared to the corresponding reference sequence, divided by the total number of residues in the substantially equivalent sequence is about 0.35 or less). Such a sequence is said to have 65% sequence identity to the listed sequence. In one

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embodiment, a substantially equivalent, *e.g.*, mutant, sequence of the invention varies from a listed sequence by no more than 30% (70% sequence identity); in a variation of this embodiment, by no more than 25% (75% sequence identity); and in a further variation of this embodiment, by no more than 20% (80% sequence identity) and in a further variation of this
 5 embodiment, by no more than 10% (90% sequence identity) and in a further variation of this embodiment, by no more than 5% (95% sequence identity). Substantially equivalent, *e.g.*, mutant, amino acid sequences according to the invention preferably have at least 80% sequence identity with a listed amino acid sequence, more preferably at least 90% sequence identity. Substantially equivalent nucleotide sequence of the invention can have lower percent sequence
 10 identities, taking into account, for example, the redundancy or degeneracy of the genetic code. Preferably, nucleotide sequence has at least about 65% identity, more preferably at least about 75% identity, and most preferably at least about 95% identity. For the purposes of the present invention, sequences having substantially equivalent biological activity and substantially equivalent expression characteristics are considered substantially equivalent. For the purposes
 15 of determining equivalence, truncation of the mature sequence (*e.g.*, via a mutation which creates a spurious stop codon) should be disregarded. Sequence identity may be determined, *e.g.*, using the Jotun Hein method (Hein, J. (1990) *Methods Enzymol.* 183:626-645). Identity between sequences can also be determined by other methods known in the art, *e.g.* by varying hybridization conditions.

20 The term "totipotent" refers to the capability of a cell to differentiate into all of the cell types of an adult organism.

The term "transformation" means introducing DNA into a suitable host cell so that the DNA is replicable, either as an extrachromosomal element, or by chromosomal integration. The term "transfection" refers to the taking up of an expression vector by a suitable host cell,
 25 whether or not any coding sequences are in fact expressed. The term "infection" refers to the introduction of nucleic acids into a suitable host cell by use of a virus or viral vector.

As used herein, an "uptake modulating fragment," UMF, means a series of nucleotides which mediate the uptake of a linked DNA fragment into a cell. UMFs can be readily identified using known UMFs as a target sequence or target motif with the computer-based
 30 systems described below. The presence and activity of a UMF can be confirmed by attaching the suspected UMF to a marker sequence. The resulting nucleic acid molecule is then incubated with an appropriate host under appropriate conditions and the uptake of the marker sequence is determined. As described above, a UMF will increase the frequency of uptake of a linked marker sequence.

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Each of the above terms is meant to encompass all that is described for each, unless the context dictates otherwise.

5.2 NUCLEIC ACIDS OF THE INVENTION

5 The invention is based on the discovery of a novel secreted prothrombinase-like polypeptide, the polynucleotides encoding the prothrombinase-like polypeptide and the use of these compositions for the diagnosis, treatment or prevention of neurological conditions and disorders.

The isolated polynucleotides of the invention include, but are not limited to a
 10 polynucleotide comprising any of the nucleotide sequences of SEQ ID NO: 1-3, 10-13 and 15; a fragment of SEQ ID NO: 1-3, 10-13 and 15; a polynucleotide comprising the full length protein coding sequence of SEQ ID NO: 1-3, 10-13 and 15 (for example SEQ ID NO: 4, 9, 14, 18); and a polynucleotide comprising the nucleotide sequence encoding the mature protein coding sequence of the polynucleotides of any one of SEQ ID NO: 1-3, 10-13 and 15. The
 15 polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes under stringent conditions to (a) the complement of any of the nucleotide sequences of the SEQ ID NO: 1-3, 10-13 or 15; (b) a polynucleotide encoding any one of the polypeptides of SEQ ID NO: 4-9, 14 or 16-18; (c) a polynucleotide which is an allelic variant of any polynucleotides recited above; (d) a polynucleotide which encodes a species homolog of
 20 any of the proteins recited above; or (e) a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of the polypeptides of SEQ ID NO: 4-9, 14 or 16-18. Domains of interest may depend on the nature of the encoded polypeptide; e.g., domains in receptor-like polypeptides include ligand-binding, extracellular, transmembrane, or cytoplasmic domains, or combinations thereof; domains in immunoglobulin-like proteins
 25 include the variable immunoglobulin-like domains; domains in enzyme-like polypeptides include catalytic and substrate binding domains; and domains in ligand polypeptides include receptor-binding domains.

The polynucleotides of the invention include naturally occurring or wholly or partially synthetic DNA, e.g., cDNA and genomic DNA, and RNA, e.g., mRNA. The polynucleotides
 30 may include all of the coding region of the cDNA or may represent a portion of the coding region of the cDNA.

The present invention also provides genes corresponding to the cDNA sequences disclosed herein. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or

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primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. Further 5' and 3' sequence can be obtained using methods known in the art. For example, full length cDNA or genomic DNA that corresponds to any of the polynucleotides of the SEQ ID NO: 1-3, 10-13 and 15 can be obtained by screening appropriate cDNA or genomic DNA libraries under suitable hybridization conditions using any of the polynucleotides of the SEQ ID NO: 1-3, 10-13 and 15 or a portion thereof as a probe. Alternatively, the polynucleotides of the SEQ ID NO: 1-3, 10-13 and 15 may be used as the basis for suitable primer(s) that allow identification and/or amplification of genes in appropriate genomic DNA or cDNA libraries.

The nucleic acid sequences of the invention can be assembled from ESTs and sequences (including cDNA and genomic sequences) obtained from one or more public databases, such as dbEST, gbpr, and UniGene. The EST sequences can provide identifying sequence information, representative fragment or segment information, or novel segment information for the full-length gene.

The polynucleotides of the invention also provide polynucleotides including nucleotide sequences that are substantially equivalent to the polynucleotides recited above. Polynucleotides according to the invention can have, *e.g.*, at least about 65%, at least about 70%, at least about 75%, at least about 80%, more typically at least about 90%, and even more typically at least about 95%, sequence identity to a polynucleotide recited above.

Included within the scope of the nucleic acid sequences of the invention are nucleic acid sequence fragments that hybridize under stringent conditions to any of the nucleotide sequences of the SEQ ID NO: 1-3, 10-13 and 15, or complements thereof, which fragment is greater than about 5 nucleotides, preferably 7 nucleotides, more preferably greater than 9 nucleotides and most preferably greater than 17 nucleotides. Fragments of, *e.g.* 15, 17, or 20 nucleotides or more that are selective for (*i.e.* specifically hybridize to any one of the polynucleotides of the invention) are contemplated. Probes capable of specifically hybridizing to a polynucleotide can differentiate polynucleotide sequences of the invention from other polynucleotide sequences in the same family of genes or can differentiate human genes from genes of other species, and are preferably based on unique nucleotide sequences.

The sequences falling within the scope of the present invention are not limited to these specific sequences, but also include allelic and species variations thereof. Allelic and species variations can be routinely determined by comparing the sequence provided in SEQ ID NO: 1-3, 10-13 and 15, a representative fragment thereof, or a nucleotide sequence at least 90% identical, preferably 95% identical, to SEQ ID NO: 1-3, 10-13 and 15 with a sequence from another isolate

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of the same species. Furthermore, to accommodate codon variability, the invention includes nucleic acid molecules coding for the same amino acid sequences as do the specific ORFs disclosed herein. In other words, in the coding region of an ORF, substitution of one codon for another codon that encodes the same amino acid is expressly contemplated.

5 The nearest neighbor result for the nucleic acids of the present invention, including SEQ ID NO: 1-3, 10-13 and 15, can be obtained by searching a database using an algorithm or a program. Preferably, a BLAST which stands for Basic Local alignment Search Tool is used to search for local sequence alignments (Altschul, S.F. J Mol. Evol. 36 290-300 (1993) and Altschul S.F. et al. J. Mol. Biol. 21:403-410 (1990))

10 Species homologs (or orthologs) of the disclosed polynucleotides and proteins are also provided by the present invention. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

15 The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous or related to that encoded by the polynucleotides.

The nucleic acid sequences of the invention are further directed to sequences which encode variants of the described nucleic acids. These amino acid sequence variants may be 20 prepared by methods known in the art by introducing appropriate nucleotide changes into a native or variant polynucleotide. There are two variables in the construction of amino acid sequence variants: the location of the mutation and the nature of the mutation. Nucleic acids encoding the amino acid sequence variants are preferably constructed by mutating the polynucleotide to encode an amino acid sequence that does not occur in nature. These nucleic 25 acid alterations can be made at sites that differ in the nucleic acids from different species (variable positions) or in highly conserved regions (constant regions). Sites at such locations will typically be modified in series, *e.g.*, by substituting first with conservative choices (*e.g.*, hydrophobic amino acid to a different hydrophobic amino acid) and then with more distant choices (*e.g.*, hydrophobic amino acid to a charged amino acid), and then deletions or 30 insertions may be made at the target site. Amino acid sequence deletions generally range from about 1 to 30 residues, preferably about 1 to 10 residues, and are typically contiguous. Amino acid insertions include amino- and/or carboxyl-terminal fusions ranging in length from one to one hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions may range generally from about 1 to 10 amino

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residues, preferably from 1 to 5 residues. Examples of terminal insertions include the heterologous signal sequences necessary for secretion or for intracellular targeting in different host cells and sequences such as FLAG or poly-histidine sequences useful for purifying the expressed protein.

5 In a preferred method, polynucleotides encoding the novel amino acid sequences are changed via site-directed mutagenesis. This method uses oligonucleotide sequences to alter a polynucleotide to encode the desired amino acid variant, as well as sufficient adjacent nucleotides on both sides of the changed amino acid to form a stable duplex on either side of the site being changed. In general, the techniques of site-directed mutagenesis are well known
10 to those of skill in the art and this technique is exemplified by publications such as, Edelman et al., *DNA* 2:183 (1983). A versatile and efficient method for producing site-specific changes in a polynucleotide sequence was published by Zoller and Smith, *Nucleic Acids Res.* 10:6487-6500 (1982). PCR may also be used to create amino acid sequence variants of the novel nucleic acids. When small amounts of template DNA are used as starting material, primer(s)
15 that differs slightly in sequence from the corresponding region in the template DNA can generate the desired amino acid variant. PCR amplification results in a population of product DNA fragments that differ from the polynucleotide template encoding the polypeptide at the position specified by the primer. The product DNA fragments replace the corresponding region in the plasmid and this gives a polynucleotide encoding the desired amino acid variant.

20 A further technique for generating amino acid variants is the cassette mutagenesis technique described in Wells et al., *Gene* 34:315 (1985); and other mutagenesis techniques well known in the art, such as, for example, the techniques in Sambrook et al., supra, and *Current Protocols in Molecular Biology*, Ausubel et al. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally
25 equivalent amino acid sequence may be used in the practice of the invention for the cloning and expression of these novel nucleic acids. Such DNA sequences include those which are capable of hybridizing to the appropriate novel nucleic acid sequence under stringent conditions.

Polynucleotides encoding preferred polypeptide truncations of the invention can be used to generate polynucleotides encoding chimeric or fusion proteins comprising one or more
30 domains of the invention and heterologous protein sequences.

The polynucleotides of the invention additionally include the complement of any of the polynucleotides recited above. The polynucleotide can be DNA (genomic, cDNA, amplified, or synthetic) or RNA. Methods and algorithms for obtaining such polynucleotides are well known to those of skill in the art and can include, for example, methods for determining

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hybridization conditions that can routinely isolate polynucleotides of the desired sequence identities.

In accordance with the invention, polynucleotide sequences comprising the mature protein coding sequences corresponding to any one of SEQ ID NO: 1-3, 10-13 and 15, or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of that nucleic acid, or a functional equivalent thereof, in appropriate host cells. Also included are the cDNA inserts of any of the clones identified herein.

A polynucleotide according to the invention can be joined to any of a variety of other nucleotide sequences by well-established recombinant DNA techniques (see Sambrook J et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY). Useful nucleotide sequences for joining to polynucleotides include an assortment of vectors, e.g., plasmids, cosmids, lambda phage derivatives, phagemids, and the like, that are well known in the art. Accordingly, the invention also provides a vector including a polynucleotide of the invention and a host cell containing the polynucleotide. In general, the vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and a selectable marker for the host cell. Vectors according to the invention include expression vectors, replication vectors, probe generation vectors, and sequencing vectors. A host cell according to the invention can be a prokaryotic or eukaryotic cell and can be a unicellular organism or part of a multicellular organism.

The present invention further provides recombinant constructs comprising a nucleic acid having any of the nucleotide sequences of the SEQ ID NO: 1-3, 10-13 and 15 or a fragment thereof or any other polynucleotides of the invention. In one embodiment, the recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a nucleic acid having any of the nucleotide sequences of the SEQ ID NO: 1-3, 10-13 and 15 or a fragment thereof is inserted, in a forward or reverse orientation. In the case of a vector comprising one of the ORFs of the present invention, the vector may further comprise regulatory sequences, including for example, a promoter, operably linked to the ORF. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrec99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLeo, pSV2cat, pOG44, PXTI, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia).

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The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., *Nucleic Acids Res.* 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, *Methods in Enzymology* 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda PR, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), a-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an amino terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product. Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

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As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3

5 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM 1 (Promega Biotech, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced or derepressed by appropriate means (*e.g.*, temperature shift or chemical induction) and cells are
10 cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Polynucleotides of the invention can also be used to induce immune responses. For example, as described in Fan et al., *Nat. Biotech.* 17:870-872 (1999), incorporated herein by reference, nucleic acid sequences encoding a polypeptide may be used to generate antibodies
15 against the encoded polypeptide following topical administration of naked plasmid DNA or following injection, and preferably intramuscular injection of the DNA. The nucleic acid sequences are preferably inserted in a recombinant expression vector and may be in the form of naked DNA.

5.3 ANTISENSE

20 Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1-3, 10-13 and 15, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded
25 cDNA molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of a protein of any of SEQ ID NO:4-9, 14 and 16-18 or antisense nucleic acids complementary to a
30 nucleic acid sequence of SEQ ID NO:1-3, 10-13 and 15 are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence of the invention. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to

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a "noncoding region" of the coding strand of a nucleotide sequence of the invention. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding a nucleic acid disclosed herein (*e.g.*, SEQ ID NO:1-3, 10-13 and 15), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of a mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of a mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of a mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (*v*), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (*v*), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

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The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a protein according to the invention to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gautier *et al.* (1987) *Nucleic Acids Res* 15: 6625-6641). The antisense nucleic acid molecule can also comprise a 2'-O-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett* 215: 327-330).

5.4 RIBOZYMES AND PNA MOIETIES

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as a mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave a mRNA transcripts to thereby inhibit translation of a mRNA. A ribozyme having specificity for a nucleic acid of the invention can be designed based upon the nucleotide sequence of a DNA disclosed herein (*i.e.*, SEQ ID NO:1-3, 10-13 and 15). For example, a derivative of a Tetrahymena L-19 IVS RNA can be

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constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a encoding a mRNA according to the invention. See, *e.g.*, Cech *et al.* U.S. Pat. No. 4,987,071; and Cech *et al.* U.S. Pat. No. 5,116,742.

Alternatively, mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region (*e.g.*, promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells. See generally, Helene. (1991) *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14: 807-15.

In various embodiments, the nucleic acids of the invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* (1996) *PNAS* 93: 14670-675.

PNAs of the invention can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of the invention can also be used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S1 nucleases (Hyrup B. (1996) above); or as probes or primers for DNA sequence and hybridization (Hyrup *et al.* (1996), above; Perry-O'Keefe (1996), above).

In another embodiment, PNAs of the invention can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras can be generated that may

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combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, *e.g.*, RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996) above). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996) above and Finn *et al.* (1996) *Nucl Acids Res* 24: 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA (Mag *et al.* (1989) *Nucl Acid Res* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn *et al.* (1996) above). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, Petersen *et al.* (1975) *Bioorg Med Chem Lett* 5: 1119-1124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, *Proc. Natl. Acad. Sci.* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (See, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon, 1988, *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, etc.

25 5.5 HOSTS

The present invention further provides host cells genetically engineered to contain the polynucleotides of the invention. For example, such host cells may contain nucleic acids of the invention introduced into the host cell using known transformation, transfection or infection methods. The present invention still further provides host cells genetically engineered to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell.

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Knowledge of prothrombinase-like DNA sequences allows for modification of cells to permit, or increase, expression of prothrombinase-like polypeptide. Cells can be modified (e.g., by homologous recombination) to provide increased prothrombinase-like polypeptide expression by replacing, in whole or in part, the naturally occurring prothrombinase-like promoter with all or part of a heterologous promoter so that the cells prothrombinase-like polypeptide is expressed at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to prothrombinase-like encoding sequences. See, for example, PCT International Publication No. WO94/12650, PCT International Publication No. WO92/20808, and PCT International Publication No. WO91/09955. It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (e.g., *ada*, *dhfr*, and the multifunctional CAD gene which encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the prothrombinase-like coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the prothrombinase-like coding sequences in the cells.

The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran-mediated transfection, or electroporation (Davis, L. et al., *Basic Methods in Molecular Biology* (1986)). The host cells containing one of the polynucleotides of the invention, can be used in conventional manners to produce the gene product encoded by the isolated fragment (in the case of an ORF) or can be used to produce a heterologous protein under the control of the EMF.

Any host/vector system can be used to express one or more of the ORFs of the present invention. These include, but are not limited to, eukaryotic hosts such as HeLa cells, Cv-1 cell, COS cells, 293 cells, and SF9 cells, as well as prokaryotic host such as *E. coli* and *B. subtilis*. The most preferred cells are those which do not normally express the particular polypeptide or protein or which expresses the polypeptide or protein at low natural level. Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., in *Molecular Cloning: A Laboratory Manual*, Second Edition,

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Cold Spring Harbor, New York (1989), the disclosure of which is hereby incorporated by reference.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell 23:175 (1981). Other cell lines capable of expressing a compatible vector are, for example, the C127, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HcLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Recombinant polypeptides and proteins produced in bacterial culture are usually isolated by initial extraction from cell pellets, followed by one or more salting-out, aqueous ion exchange or size exclusion chromatography steps. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or insects or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous

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gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting. These sequence include polyadenylation signals, mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.*, inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the host cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (*gpt*) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappell; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No.

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PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

5.6 POLYPEPTIDES OF THE INVENTION

5 The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising: the amino acid sequence set forth as any one of SEQ ID NO: 4-9, 14 or 16-18 or an amino acid sequence encoded by any one of the nucleotide sequences SEQ ID NO: 1-3, 10-13, or 15 or the corresponding full length or mature protein. Polypeptides of the invention also include polypeptides preferably with biological or immunological activity that are encoded by:

10 (a) a polynucleotide having any one of the nucleotide sequences set forth in the SEQ ID NO: 1-3, 10-13, or 15 or (b) polynucleotides encoding any one of the amino acid sequences set forth as SEQ ID NO: 4-9, 14 or 16-18 or (c) polynucleotides that hybridize to the complement of the polynucleotides of either (a) or (b) under stringent hybridization conditions. The invention also provides biologically active or immunologically active variants of any of the amino acid

15 sequences set forth as SEQ ID NO: 4-9, 14 or 16-18 or the corresponding full length or mature protein; and "substantial equivalents" thereof (e.g., with at least about 65 %, at least about 70 %, at least about 75 %, at least about 80 %, at least about 85 %, at least about 90 %, typically at least about 95 %, more typically at least about 98 %, or most typically at least about 99 % amino acid identity) that retain biological activity. Polypeptides encoded by allelic variants

20 may have a similar, increased, or decreased activity compared to polypeptides comprising SEQ ID NO: 4-9, 14 or 16-18.

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as

25 described in H. U. Saragovi, et al., *Bio/Technology* 10, 773-778 (1992) and in R. S. McDowell, et al., *J. Amer. Chem. Soc.* 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites.

The present invention also provides both full-length and mature forms (for example,

30 without a signal sequence or precursor sequence) of the disclosed proteins. The protein coding sequence is identified in the sequence listing by translation of the disclosed nucleotide sequences. The mature form of such protein may be obtained by expression of a full-length polynucleotide in a suitable mammalian cell or other host cell. The sequence of the mature form of the protein is also determinable from the amino acid sequence of the full-length form.

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Where proteins of the present invention are membrane bound, soluble forms of the proteins are also provided. In such forms, part or all of the regions causing the proteins to be membrane bound are deleted so that the proteins are fully secreted from the cell in which it is expressed.

Protein compositions of the present invention may further comprise an acceptable carrier, such as a hydrophilic, *e.g.*, pharmaceutically acceptable, carrier.

The present invention further provides isolated polypeptides encoded by the nucleic acid fragments of the present invention or by degenerate variants of the nucleic acid fragments of the present invention. By "degenerate variant" is intended nucleotide fragments which differ from a nucleic acid fragment of the present invention (*e.g.*, an ORF) by nucleotide sequence but, due to the degeneracy of the genetic code, encode an identical polypeptide sequence. Preferred nucleic acid fragments of the present invention are the ORFs that encode proteins.

A variety of methodologies known in the art can be utilized to obtain any one of the isolated polypeptides or proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. This technique is particularly useful in producing small peptides and fragments of larger polypeptides. Fragments are useful, for example, in generating antibodies against the native polypeptide. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The polypeptides and proteins of the present invention can alternatively be purified from cells which have been altered to express the desired polypeptide or protein. As used herein, a cell is said to be altered to express a desired polypeptide or protein when the cell, through genetic manipulation, is made to produce a polypeptide or protein which it normally does not produce or which the cell normally produces at a lower level. One skilled in the art can readily adapt procedures for introducing and expressing either recombinant or synthetic sequences into eukaryotic or prokaryotic cells in order to generate a cell which produces one of the polypeptides or proteins of the present invention.

The invention also relates to methods for producing a polypeptide comprising growing a culture of host cells of the invention in a suitable culture medium, and purifying the protein from the cells or the culture in which the cells are grown. For example, the methods of the invention include a process for producing a polypeptide in which a host cell containing a suitable expression vector that includes a polynucleotide of the invention is cultured under

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conditions that allow expression of the encoded polypeptide. The polypeptide can be recovered from the culture, conveniently from the culture medium, or from a lysate prepared from the host cells and further purified. Preferred embodiments include those in which the protein produced by such process is a full length or mature form of the protein.

5 In an alternative method, the polypeptide or protein is purified from bacterial cells which naturally produce the polypeptide or protein. One skilled in the art can readily follow known methods for isolating polypeptides and proteins in order to obtain one of the isolated polypeptides or proteins of the present invention. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange
10 chromatography, and immuno-affinity chromatography. See, e.g., Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag (1994); Sambrook, et al., in *Molecular Cloning: A Laboratory Manual*; Ausubel et al., *Current Protocols in Molecular Biology*. Polypeptide fragments that retain biological/immunological activity include fragments comprising greater than about 100 amino acids, or greater than about 200 amino acids, and
15 fragments that encode specific protein domains.

The purified polypeptides can be used in *in vitro* binding assays which are well known in the art to identify molecules which bind to the polypeptides. These molecules include but are not limited to, for e.g., small molecules, molecules from combinatorial libraries, antibodies, or other proteins. The molecules identified in the binding assay are then tested for antagonist
20 or agonist activity in *in vivo* tissue culture or animal models that are well known in the art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

In addition, the peptides of the invention or molecules capable of binding to the peptides may be complexed with toxins, e.g., ricin or cholera, or with other compounds that
25 are toxic to cells. The toxin-binding molecule complex is then targeted to a tumor or other cell by the specificity of the binding molecule for SEQ ID NO: 4-9, 14 or 16-18.

The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

30 The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications, in the peptide or DNA sequence, can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the alteration, substitution, replacement, insertion

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or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, *e.g.*, U.S. Pat. No. 4,518,584). Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein. Regions of the protein that are important for the protein function can be determined by various methods known in the art including the alanine-scanning method which involved systematic substitution of single or strings of amino acids with alanine, followed by testing the resulting alanine-containing variant for biological activity. This type of analysis determines the importance of the substituted amino acid(s) in biological activity. Regions of the protein that are important for protein function may be determined by the eMATRIX program.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and are useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are encompassed by the present invention.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, Calif., U.S.A. (the MaxBat™ kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (*i.e.*, from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl™ or Cibacrom blue 3GA Sepharose™; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

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Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX), or as a His tag. Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, Mass.), Pharmacia (Piscataway, N.J.) and Invitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("FLAG®") is commercially available from Kodak (New Haven, Conn.).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, *e.g.*, silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The polypeptides of the invention include analogs (variants). The polypeptides of the invention include prothrombinase-like analogs. This embraces fragments of prothrombinase-like polypeptide of the invention, as well prothrombinase-like polypeptides which comprise one or more amino acids deleted, inserted, or substituted. Also, analogs of the prothrombinase-like polypeptide of the invention embrace fusions of the prothrombinase-like polypeptides or modifications of the prothrombinase-like polypeptides, wherein the prothrombinase-like polypeptide or analog is fused to another moiety or moieties, *e.g.*, targeting moiety or another therapeutic agent. Such analogs may exhibit improved properties such as activity and/or stability. Examples of moieties which may be fused to the prothrombinase-like polypeptide or an analog include, for example, targeting moieties which provide for the delivery of polypeptide to neurons, *e.g.*, antibodies to central nervous system, or antibodies to receptor and ligands expressed on neuronal cells. Other moieties which may be fused to prothrombinase-like polypeptide include therapeutic agents which are used for treatment, for example anti-depressant drugs or other medications for neurological disorders. Also, prothrombinase-like polypeptides may be fused to neuron growth modulators, and other chemokines for targeted delivery.

5.6.1 DETERMINING POLYPEPTIDE AND POLYNUCLEOTIDE IDENTITY AND SIMILARITY

Preferred identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in computer programs including, but are not limited to, the GCG program package, including GAP (Devereux, J., et al., *Nucleic Acids Research* 12(1):387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, BLASTX, FASTA (Altschul, S.F. et al., *J. Molec. Biol.* 215:403-410 (1990), PSI-BLAST (Altschul S.F. et al., *Nucleic Acids Res.* vol. 25, pp. 3389-3402, herein incorporated by reference), eMatrix software (Wu et al., *J. Comp. Biol.*, vol. 6, pp. 219-235 (1999), herein incorporated by reference), eMotif software (Nevill-Manning et al., *ISMB-97*, vol 4, pp. 202-209, herein incorporated by reference) and the Kyte-Doolittle hydrophobicity prediction algorithm (*J. Mol Biol.*, 157, pp. 105-31 (1982), incorporated herein by reference). The BLAST programs are publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul, S., et al. NCB NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215:403-410 (1990).

5.7 CHIMERIC AND FUSION PROTEINS

The invention also provides chimeric or fusion proteins. As used herein, a "chimeric protein" or "fusion protein" comprises a polypeptide of the invention operatively linked to another polypeptide. Within a fusion protein the polypeptide according to the invention can correspond to all or a portion of a protein according to the invention. In one embodiment, a fusion protein comprises at least one biologically active portion of a protein according to the invention. In another embodiment, a fusion protein comprises at least two biologically active portions of a protein according to the invention. Within the fusion protein, the term "operatively linked" is intended to indicate that the polypeptide(s) according to the invention and the other polypeptide(s) are fused in-frame to each other. The polypeptide can be fused to the N-terminus or C-terminus or in the middle.

For example, in one embodiment a fusion protein comprises a polypeptide according to the invention operably linked to the extracellular domain of a second protein.

In another embodiment, the fusion protein is a GST-fusion protein in which the polypeptide sequences of the invention are fused to the C-terminus of the GST (i.e., glutathione S-transferase) sequences.

In another embodiment, the fusion protein is an immunoglobulin fusion protein in which the polypeptide sequences according to the invention comprise one or more domains

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fused to sequences derived from a member of the immunoglobulin protein family. The immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a ligand and a protein of the invention on the surface of a cell, to thereby suppress signal transduction *in vivo*.

- 5 The immunoglobulin fusion proteins can be used to affect the bioavailability of a cognate ligand. Inhibition of the ligand/protein interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, *e.g.*, cancer as well as modulating (*e.g.*, promoting or inhibiting) cell survival. Moreover, the immunoglobulin fusion proteins of the invention can be used to bind and to dimerize 2 receptors and thereby transduce an
- 10 intracellular signal. The immunoglobulin fusion proteins may also be used as immunogens to produce antibodies in a subject, to purify ligands, and in screening assays to identify molecules that inhibit the interaction of a polypeptide of the invention with a ligand.

- A chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide
- 15 sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA
- 20 synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel et al. (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially
- 25 available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A nucleic acid encoding a polypeptide of the invention can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the protein of the invention.

5.8 GENE THERAPY

- 30 Mutations in the polynucleotides of the invention gene may result in loss of normal function of the encoded protein. The invention thus provides gene therapy to restore normal activity of the polypeptides of the invention; or to treat disease states involving polypeptides of the invention. Delivery of a functional gene encoding polypeptides of the invention to appropriate cells is effected *ex vivo*, *in situ*, or *in vivo* by use of vectors, and more particularly

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viral vectors (e.g., adenovirus, adeno-associated virus, or a retrovirus), or *ex vivo* by use of physical DNA transfer methods (e.g., liposomes or chemical treatments). See, for example, Anderson, *Nature*, supplement to vol. 392, no. 6679, pp.25-20 (1998). For additional reviews of gene therapy technology see Friedmann, *Science*, 244: 1275-1281 (1989); Verma, *Scientific American*: 68-84 (1990); and Miller, *Nature*, 357: 455-460 (1992). Introduction of any one of the nucleotides of the present invention or a gene encoding the polypeptides of the present invention can also be accomplished with extrachromosomal substrates (transient expression) or artificial chromosomes (stable expression). Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes. Alternatively, it is contemplated that in other human disease states, preventing the expression of or inhibiting the activity of polypeptides of the invention will be useful in treating the disease states. It is contemplated that antisense therapy or gene therapy could be applied to negatively regulate the expression of polypeptides of the invention.

Other methods inhibiting expression of a protein include the introduction of antisense molecules to the nucleic acids of the present invention, their complements, or their translated RNA sequences, by methods known in the art. Further, the polypeptides of the present invention can be inhibited by using targeted deletion methods, or the insertion of a negative regulatory element such as a silencer, which is tissue specific.

The present invention still further provides cells genetically engineered *in vivo* to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell. These methods can be used to increase or decrease the expression of the polynucleotides of the present invention.

Knowledge of DNA sequences provided by the invention allows for modification of cells to permit, increase, or decrease, expression of endogenous polypeptide. Cells can be modified (e.g., by homologous recombination) to provide increased polypeptide expression by replacing, in whole or in part, the naturally occurring promoter with all or part of a heterologous promoter so that the cells express the protein at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to the desired protein encoding sequences. See, for example, PCT International Publication No. WO 94/12650, PCT International Publication No. WO 92/20808, and PCT International Publication No. WO 91/09955. It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (e.g., *ada*, *dhfr*, and the multifunctional CAD gene which encodes carbamyl phosphate synthase, aspartate

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transcarnamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the desired protein coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the desired protein coding sequences in the cells.

5 In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different
10 gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by
15 targeting. These sequences include polyadenylation signals, mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.*, inserting a new promoter or enhancer or
20 both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new
25 sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively
30 selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the

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Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (gpt) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

10 5.9 TRANSGENIC ANIMALS

In preferred methods to determine biological functions of the polypeptides of the invention *in vivo*, one or more genes provided by the invention are either over expressed or inactivated in the germ line of animals using homologous recombination [Capecchi, Science 244:1288-1292 (1989)]. Animals in which the gene is over expressed, under the regulatory control of exogenous or endogenous promoter elements, are known as transgenic animals. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals. Knockout animals, preferably non-human mammals, can be prepared as described in U.S. Patent No. 5,557,032, incorporated herein by reference. Transgenic animals are useful to determine the roles polypeptides of the invention play in biological processes, and preferably in disease states. Transgenic animals are useful as model systems to identify compounds that modulate lipid metabolism. Transgenic animals, preferably non-human mammals, are produced using methods as described in U.S. Patent No 5,489,743 and PCT Publication No. WO94/28122, incorporated herein by reference.

Transgenic animals can be prepared wherein all or part of a promoter of the polynucleotides of the invention is either activated or inactivated to alter the level of expression of the polypeptides of the invention. Inactivation can be carried out using homologous recombination methods described above. Activation can be achieved by supplementing or even replacing the homologous promoter to provide for increased protein expression. The homologous promoter can be supplemented by insertion of one or more heterologous enhancer elements known to confer promoter activation in a particular tissue.

The polynucleotides of the present invention also make possible the development, through, e.g., homologous recombination or knock out strategies, of animals that fail to express functional prothrombinase-like polypeptide or that express a variant of prothrombinase-like polypeptide. Such animals are useful as models for studying the *in vivo* activities of

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prothrombinase-like polypeptide as well as for studying modulators of the prothrombinase-like polypeptide.

In preferred methods to determine biological functions of the polypeptides of the invention *in vivo*, one or more genes provided by the invention are either over expressed or
5 inactivated in the germ line of animals using homologous recombination [Capecchi, Science 244:1288-1292 (1989)]. Animals in which the gene is over expressed, under the regulatory control of exogenous or endogenous promoter elements, are known as transgenic animals. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals. Knockout animals, preferably non-human mammals, can be
10 prepared as described in U.S. Patent No. 5,557,032, incorporated herein by reference. Transgenic animals are useful to determine the roles polypeptides of the invention play in biological processes, and preferably in disease states. Transgenic animals are useful as model systems to identify compounds that modulate lipid metabolism. Transgenic animals, preferably non-human mammals, are produced using methods as described in U.S. Patent No 5,489,743
15 and PCT Publication No. WO94/28122, incorporated herein by reference.

Transgenic animals can be prepared wherein all or part of the polynucleotides of the invention promoter is either activated or inactivated to alter the level of expression of the polypeptides of the invention. Inactivation can be carried out using homologous recombination methods described above. Activation can be achieved by supplementing or even replacing the
20 homologous promoter to provide for increased protein expression. The homologous promoter can be supplemented by insertion of one or more heterologous enhancer elements known to confer promoter activation in a particular tissue.

5.10 USES AND BIOLOGICAL ACTIVITY OF PROTHROMBINASE-LIKE 25 POLYPEPTIDE

The polynucleotides and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified herein. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or of polynucleotides encoding such
30 proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA). The mechanism underlying the particular condition or pathology will dictate whether the polypeptides of the invention, the polynucleotides of the invention or modulators (activators or inhibitors) thereof would be beneficial to the subject in need of treatment. Thus, "therapeutic compositions of the invention" include compositions comprising isolated polynucleotides

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(including recombinant DNA molecules, cloned genes and degenerate variants thereof) or polypeptides of the invention (including full length protein, mature protein and truncations or domains thereof), or compounds and other substances that modulate the overall activity of the target gene products, either at the level of target gene/protein expression or target protein activity. Such modulators include polypeptides, analogs, (variants), including fragments and fusion proteins, antibodies and other binding proteins; chemical compounds that directly or indirectly activate or inhibit the polypeptides of the invention (identified, e.g., via drug screening assays as described herein); antisense polynucleotides and polynucleotides suitable for triple helix formation; and in particular antibodies or other binding partners that specifically recognize one or more epitopes of the polypeptides of the invention.

The polypeptides of the present invention may likewise be involved in cellular activation or in one of the other physiological pathways described herein.

5.10.1 RESEARCH USES AND UTILITIES

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The polypeptides provided by the present invention can similarly be used in assays to determine biological activity, including in a panel of multiple proteins for high-throughput

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screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding polypeptide is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

The polypeptides of the invention are also useful for making antibody substances that are specifically immunoreactive with prothrombinase-like proteins. Antibodies and portions thereof (e.g., Fab fragments) which bind to the polypeptides of the invention can be used to identify the presence of such polypeptides in a sample. Such determinations are carried out using any suitable immunoassay format, and any polypeptide of the invention that is specifically bound by the antibody can be employed as a positive control.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E. F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S. L. and A. R. Kimmel eds., 1987.

5.10.2 NUTRITIONAL USES

Polynucleotides and polypeptides of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the polypeptide or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the polypeptide or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

Additionally, the polypeptides of the invention can be used as molecular weight markers, and as a food supplement. A polypeptide consisting of SEQ ID NO: 4, for example, has a molecular mass of approximately 43 kDa in its unprocessed and unglycosylated state. Protein

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food supplements are well known and the formulation of suitable food supplements including polypeptides of the invention is within the level of skill in the food preparation art.

5.10.3 CYTOKINE AND CELL PROLIFERATION/DIFFERENTIATION

5 ACTIVITY

A polypeptide of the present invention may exhibit activity relating to cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor-dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of therapeutic compositions of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+(preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e, CMK, HUVEC, and Caco. Therapeutic compositions of the invention can be used in the following:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., I. Immunol. 149:3778-3783, 1992; Bowman et al., I. Immunol. 152:1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A. M. and Shevach, E. M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human interleukin- γ , Schreiber, R. D. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L. S. and Lipsky, P. E. In Current Protocols in

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- Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6--Nordan, R. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11--Bennett, F., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9-Ciarletta, A., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.
- Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

5.10.4 STEM CELL GROWTH FACTOR ACTIVITY

- A polypeptide of the present invention may exhibit stem cell growth factor activity and be involved in the proliferation, differentiation and survival of pluripotent and totipotent stem cells including primordial germ cells, embryonic stem cells, hematopoietic stem cells and/or germ line stem cells. Administration of the polypeptide of the invention to stem cells *in vivo* or *ex vivo* may maintain and expand cell populations in a totipotent or pluripotent state which would be useful for re-engineering damaged or diseased tissues, transplantation, manufacture of bio-pharmaceuticals and the development of bio-sensors. The ability to produce large quantities of human cells has important working applications for the production of human proteins which currently must be obtained from non-human sources or donors, implantation of cells to treat diseases such as Parkinson's, Alzheimer's and other neurodegenerative diseases; tissues for grafting such as bone marrow, skin, cartilage, tendons, bone, muscle (including cardiac muscle), blood vessels, cornea, neural cells, gastrointestinal

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cells and others; and organs for transplantation such as kidney, liver, pancreas (including islet cells), heart and lung.

It is contemplated that multiple different exogenous growth factors and/or cytokines may be administered in combination with the polypeptide of the invention to achieve the desired effect, including any of the growth factors listed herein, other stem cell maintenance factors, and specifically including stem cell factor (SCF), leukemia inhibitory factor (LIF), Flt-3 ligand (Flt-3L), any of the interleukins, recombinant soluble IL-6 receptor fused to IL-6, macrophage inflammatory protein 1-alpha (MIP-1-alpha), G-CSF, GM-CSF, thrombopoietin (TPO), platelet factor 4 (PF-4), platelet-derived growth factor (PDGF), neural growth factors and basic fibroblast growth factor (bFGF).

Since totipotent stem cells can give rise to virtually any mature cell type, expansion of these cells in culture will facilitate the production of large quantities of mature cells. Techniques for culturing stem cells are known in the art and administration of polypeptides of the invention, optionally with other growth factors and/or cytokines, is expected to enhance the survival and proliferation of the stem cell populations. This can be accomplished by direct administration of the polypeptide of the invention to the culture medium. Alternatively, stroma cells transfected with a polynucleotide that encodes for the polypeptide of the invention can be used as a feeder layer for the stem cell populations in culture or in vivo. Stromal support cells for feeder layers may include embryonic bone marrow fibroblasts, bone marrow stromal cells, fetal liver cells, or cultured embryonic fibroblasts (see U.S. Patent No. 5,690,926).

Stem cells themselves can be transfected with a polynucleotide of the invention to induce autocrine expression of the polypeptide of the invention. This will allow for generation of undifferentiated totipotent/pluripotent stem cell lines that are useful as is or that can then be differentiated into the desired mature cell types. These stable cell lines can also serve as a source of undifferentiated totipotent/pluripotent mRNA to create cDNA libraries and templates for polymerase chain reaction experiments. These studies would allow for the isolation and identification of differentially expressed genes in stem cell populations that regulate stem cell proliferation and/or maintenance.

Expansion and maintenance of totipotent stem cell populations will be useful in the treatment of many pathological conditions. For example, polypeptides of the present invention may be used to manipulate stem cells in culture to give rise to neuroepithelial cells that can be used to augment or replace cells damaged by illness, autoimmune disease, accidental damage or genetic disorders. The polypeptide of the invention may be useful for inducing the proliferation of neural cells and for the regeneration of nerve and brain tissue, i.e. for the

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treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders which involve degeneration, death or trauma to neural cells or nerve tissue. In addition, the expanded stem cell populations can also be genetically altered for gene therapy purposes and to decrease host rejection of replacement tissues after grafting or
5 implantation.

Expression of the polypeptide of the invention and its effect on stem cells can also be manipulated to achieve controlled differentiation of the stem cells into more differentiated cell types. A broadly applicable method of obtaining pure populations of a specific differentiated cell type from undifferentiated stem cell populations involves the use of a cell-type specific
10 promoter driving a selectable marker. The selectable marker allows only cells of the desired type to survive. For example, stem cells can be induced to differentiate into cardiomyocytes (Wobus et al., *Differentiation*, 48: 173-182, (1991); Klug et al., *J. Clin. Invest.*, 98(1): 216-224, (1998)) or skeletal muscle cells (Browder, L. W. In: *Principles of Tissue Engineering eds.* Lanza et al., Academic Press (1997)). Alternatively, directed differentiation of stem cells
15 can be accomplished by culturing the stem cells in the presence of a differentiation factor such as retinoic acid and an antagonist of the polypeptide of the invention which would inhibit the effects of endogenous stem cell factor activity and allow differentiation to proceed.

In vitro cultures of stem cells can be used to determine if the polypeptide of the invention exhibits stem cell growth factor activity. Stem cells are isolated from any one of
20 various cell sources (including hematopoietic stem cells and embryonic stem cells) and cultured on a feeder layer, as described by Thompson et al. *Proc. Natl. Acad. Sci. U.S.A.*, 92: 7844-7848 (1995), in the presence of the polypeptide of the invention alone or in combination with other growth factors or cytokines. The ability of the polypeptide of the invention to induce stem cells proliferation is determined by colony formation on semi-solid support e.g. as
25 described by Bernstein et al., *Blood*, 77: 2316-2321 (1991).

5.10.5 HEMATOPOIESIS REGULATING ACTIVITY

A polypeptide of the present invention may be involved in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell disorders. Even marginal
30 biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid

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cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

Therapeutic compositions of the invention can be used in the following:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. *Cellular Biology* 15:141-151, 1995; Keller et al., *Molecular and Cellular Biology* 13:473-486, 1993; McClanahan et al., *Blood* 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methycellulose colony forming assays, Freshney, M. G. In *Culture of Hematopoietic Cells*. R. I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, N.Y. 1994; Hirayama et al., *Proc. Natl. Acad. Sci. USA* 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I. K. and Briddell, R. A. In *Culture of Hematopoietic Cells*. R. I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, N.Y. 1994; Neben et al., *Experimental Hematology* 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R. E. In *Culture of Hematopoietic Cells*. R. I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, N.Y. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooner, E., Dexter, M. and Allen, T. In *Culture of Hematopoietic Cells*. R. I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, N.Y. 1994; Long term culture initiating cell assay, Sutherland, H. J. In

Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, N.Y. 1994.

5.10.6 TISSUE GROWTH ACTIVITY

5 A polypeptide of the present invention also may be involved in bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as in wound healing and tissue repair and replacement, and in healing of burns, incisions and ulcers.

A polypeptide of the present invention which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Compositions of a polypeptide, antibody, binding partner, or other modulator of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

15 A polypeptide of this invention may also be involved in attracting bone-forming cells, stimulating growth of bone-forming cells, or inducing differentiation of progenitors of bone-forming cells. Treatment of osteoporosis, osteoarthritis, bone degenerative disorders, or periodontal disease, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes may also be possible using the composition of the invention.

Another category of tissue regeneration activity that may involve the polypeptide of the present invention is tendon/ligament formation. Induction of tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide environment to attract tendon- or ligament-

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forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or
5 ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The compositions of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, i.e. for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic
10 disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a composition may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which
15 may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a composition of the invention.

Compositions of the invention may also be useful to promote better or faster closure of
20 non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

Compositions of the present invention may also be involved in the generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including
25 vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring may allow normal tissue to regenerate. A polypeptide of the present invention may also exhibit angiogenic activity.

A composition of the present invention may also be useful for gut protection or
30 regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A composition of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

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Therapeutic compositions of the invention can be used in the following:

Assays for tissue generation activity include, without limitation, those described in:

International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in:

Winter, Epidermal Wound Healing, pp. 71-112 (Maibach, H. I. and Rovee, D. T., eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

5.10.7 IMMUNE STIMULATING OR SUPPRESSING ACTIVITY

A polypeptide of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A polynucleotide of the invention can encode a polypeptide exhibiting such activities.

A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, proteins of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein (or antagonists thereof, including antibodies) of the present invention may also be useful in the treatment of allergic reactions and conditions (e.g., anaphylaxis, serum sickness, drug reactions, food allergies, insect venom allergies, mastocytosis, allergic rhinitis, hypersensitivity pneumonitis, urticaria, angioedema, eczema, atopic dermatitis, allergic contact

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dermatitis, erythema multiforme, Stevens-Johnson syndrome, allergic conjunctivitis, atopic keratoconjunctivitis, venereal keratoconjunctivitis, giant papillary conjunctivitis and contact allergies), such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ

5 transplantation), may also be treatable using a protein (or antagonists thereof) of the present invention. The therapeutic effects of the polypeptides or antagonists thereof on allergic reactions can be evaluated by in vivo animals models such as the cumulative contact enhancement test (Lastbom et al., Toxicology 125: 59-66, 1998), skin prick test (Hoffmann et al., Allergy 54: 446-54, 1999), guinea pig skin sensitization test (Vohr et al., Arch. Toxicol. 10 73: 501-9), and murine local lymph node assay (Kimber et al., J. Toxicol. Environ. Health 53: 563-79).

Using the proteins of the invention it may also be possible to modulate immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses 15 or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance 20 can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7)), e.g., preventing high 25 level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a 30 therapeutic composition of the invention may prevent cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, a lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or

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tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular therapeutic compositions in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al., *Science* 257:789-792 (1992) and Turka et al., *Proc. Natl. Acad. Sci USA*, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., *Fundamental Immunology*, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of therapeutic compositions of the invention on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block stimulation of T cells can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythematosus in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., *Fundamental Immunology*, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (e.g., a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response may be useful in cases of viral infection, including systemic viral diseases such as influenza, the common cold, and encephalitis.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed

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APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

A polypeptide of the present invention may provide the necessary stimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I alpha chain protein and β_2 microglobulin protein or an MHC class II alpha chain protein and an MHC class II beta chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: *Current Protocols in Immunology*, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., *Proc. Natl. Acad. Sci. USA* 78:2488-2492, 1981; Herrmann et al., *J. Immunol.* 128:1968-1974, 1982; Handa et al., *J. Immunol.* 135:1564-1572, 1985; Takai et al., *J. Immunol.* 137:3494-3500, 1986; Takai et al., *J.*

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Immunol. 140:508-512, 1988; Bowman et al., J. Virology 61:1992-1998; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production, Mond, J. J. and Brunswick, M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et

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al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad. Sci. USA 88:7548-7551, 1991.

5.10.8 ACTIVIN/INHIBIN ACTIVITY

5 A polypeptide of the present invention may also exhibit activin- or inhibin-related activities. A polynucleotide of the invention may encode a polypeptide exhibiting such characteristics. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a polypeptide of the present
10 invention, alone or in heterodimers with a member of the inhibin family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the polypeptide of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin
15 group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, U.S. Pat. No. 4,798,885. A polypeptide of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as, but not limited to, cows, sheep and
20 pigs.

The activity of a polypeptide of the invention may, among other means, be measured by the following methods.

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al.,
25 Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

5.10.9 CHEMOTACTIC/CHEMOKINETIC ACTIVITY

A polypeptide of the present invention may be involved in chemotactic or chemokinetic
30 activity for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Chemotactic and chemokinetic receptor activation can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic compositions (e.g. proteins, antibodies, binding

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partners, or modulators of the invention) provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

5 A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell

10 chemotaxis.

Therapeutic compositions of the invention can be used in the following:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell

15 population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margules, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-

20 146, 1995; Muller et al Eur. J. Immunol. 25:1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153:1762-1768, 1994.

5.10.10 HEMOSTATIC AND THROMBOLYTIC ACTIVITY

A polypeptide of the invention may also be involved in hemostasis or thrombolysis or

25 thrombosis. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Compositions may be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A composition of the invention may also be useful for dissolving or inhibiting formation of

30 thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

Therapeutic compositions of the invention can be used in the following:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis

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Rés. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

5.10.11 CANCER DIAGNOSIS AND THERAPY

5 Polypeptides of the invention may be involved in cancer cell generation, proliferation or metastasis. Detection of the presence or amount of polynucleotides or polypeptides of the invention may be useful for the diagnosis and/or prognosis of one or more types of cancer. For example, the presence or increased expression of a polynucleotide/polypeptide of the invention may indicate a hereditary risk of cancer, a precancerous condition, or an ongoing malignancy. Conversely, a defect in the gene or absence of the polypeptide may be associated with a cancer condition. Identification of single nucleotide polymorphisms associated with cancer or a predisposition to cancer may also be useful for diagnosis or prognosis.

Cancer treatments promote tumor regression by inhibiting tumor cell proliferation, inhibiting angiogenesis (growth of new blood vessels that is necessary to support tumor growth) and/or prohibiting metastasis by reducing tumor cell motility or invasiveness. Therapeutic compositions of the invention may be effective in adult and pediatric oncology including in solid phase tumors/malignancies, locally advanced tumors, human soft tissue sarcomas, metastatic cancer, including lymphatic metastases, blood cell malignancies including multiple myeloma, acute and chronic leukemias, and lymphomas, head and neck cancers including mouth cancer, larynx cancer and thyroid cancer, lung cancers including small cell carcinoma and non-small cell cancers, breast cancers including small cell carcinoma and ductal carcinoma, gastrointestinal cancers including esophageal cancer, stomach cancer, colon cancer, colorectal cancer and polyps associated with colorectal neoplasia, pancreatic cancers, liver cancer, urologic cancers including bladder cancer and prostate cancer, malignancies of the female genital tract including ovarian carcinoma, uterine (including endometrial) cancers, and solid tumor in the ovarian follicle, kidney cancers including renal cell carcinoma, brain cancers including intrinsic brain tumors, neuroblastoma, astrocytic brain tumors, gliomas, metastatic tumor cell invasion in the central nervous system, bone cancers including osteomas, skin cancers including malignant melanoma, tumor progression of human skin keratinocytes, squamous cell carcinoma, basal cell carcinoma, hemangiopericytoma and Kaposi's sarcoma.

Polypeptides, polynucleotides, or modulators of polypeptides of the invention (including inhibitors and stimulators of the biological activity of the polypeptide of the invention) may be administered to treat cancer. Therapeutic compositions can be administered in therapeutically effective dosages alone or in combination with adjuvant cancer therapy such as surgery,

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chemotherapy, radiotherapy, thermotherapy, and laser therapy, and may provide a beneficial effect, e.g. reducing tumor size, slowing rate of tumor growth, inhibiting metastasis, or otherwise improving overall clinical condition, without necessarily eradicating the cancer.

The composition can also be administered in therapeutically effective amounts as a portion of an anti-cancer cocktail. An anti-cancer cocktail is a mixture of the polypeptide or modulator of the invention with one or more anti-cancer drugs in addition to a pharmaceutically acceptable carrier for delivery. The use of anti-cancer cocktails as a cancer treatment is routine. Anti-cancer drugs that are well known in the art and can be used as a treatment in combination with the polypeptide or modulator of the invention include:

Actinomycin D, Aminoglutethimide, Asparaginase, Bleomycin, Busulfan, Carboplatin, Carmustine, Chlorambucil, Cisplatin (cis-DDP), Cyclophosphamide, Cytarabine HCl (Cytosine arabinoside), Dacarbazine, Dactinomycin, Daunorubicin HCl, Doxorubicin HCl, Estramustine phosphate sodium, Etoposide (V16-213), Floxuridine, 5-Fluorouracil (5-Fu), Flutamide, Hydroxyurea (hydroxycarbamide), Ifosfamide, Interferon Alpha-2a, Interferon Alpha-2b, Leuprolide acetate (LHRH-releasing factor analog), Lomustine, Mechlorethamine HCl (nitrogen mustard), Melphalan, Mercaptopurine, Mesna, Methotrexate (MTX), Mitomycin, Mitoxantrone HCl, Octreotide, Plicamycin, Procarbazine HCl, Streptozocin, Tamoxifen citrate, Thioguanine, Thiotepa, Vinblastine sulfate, Vincristine sulfate, Amsacrine, Azacitidine, Hexamethylmelamine, Interleukin-2, Mitoguanzone, Pentostatin, Semustine, Teniposide, and Vindesine sulfate.

In addition, therapeutic compositions of the invention may be used for prophylactic treatment of cancer. There are hereditary conditions and/or environmental situations (e.g. exposure to carcinogens) known in the art that predispose an individual to developing cancers. Under these circumstances, it may be beneficial to treat these individuals with therapeutically effective doses of the polypeptide of the invention to reduce the risk of developing cancers.

In vitro models can be used to determine the effective doses of the polypeptide of the invention as a potential cancer treatment. These *in vitro* models include proliferation assays of cultured tumor cells, growth of cultured tumor cells in soft agar (see Freshney, (1987) Culture of Animal Cells: A Manual of Basic Technique, Wiley-Liss, New York, NY Ch 18 and Ch 21), tumor systems in nude mice as described in Giovanella et al., J. Natl. Can. Inst., 52: 921-30 (1974), mobility and invasive potential of tumor cells in Boyden Chamber assays as described in Pilkington et al., Anticancer Res., 17: 4107-9 (1997), and angiogenesis assays such as induction of vascularization of the chick chorioallantoic membrane or induction of vascular endothelial cell migration as described in Ribatta et al., Intl. J. Dev. Biol., 40: 1189-97 (1999).

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and Li et al., Clin. Exp. Metastasis, 17:423-9 (1999), respectively. Suitable tumor cells lines are available, e.g. from American Type Tissue Culture Collection catalogs.

5.10.12 RECEPTOR/LIGAND ACTIVITY

5 A polypeptide of the present invention may also demonstrate activity as receptor, receptor ligand or inhibitor or agonist of receptor/ligand interactions. A polynucleotide of the invention can encode a polypeptide exhibiting such characteristics. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell
10 interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses. Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention
15 (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a polypeptide of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in:

20 Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1- 7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenberg et al., J.
25 Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

By way of example, the polypeptides of the invention may be used as a receptor for a ligand(s) thereby transmitting the biological activity of that ligand(s). Ligands may be identified through binding assays, affinity chromatography, dihybrid screening assays, BIAcore assays, gel overlay assays, or other methods known in the art.

30 Studies characterizing drugs or proteins as agonist or antagonist or partial agonists or a partial antagonist require the use of other proteins as competing ligands. The polypeptides of the present invention or ligand(s) thereof may be labeled by being coupled to radioisotopes, colorimetric molecules or a toxin molecules by conventional methods. ("Guide to Protein Purification" Murray P. Deutscher (ed) Methods in Enzymology Vol. 182 (1990) Academic

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Press, Inc. San Diego). Examples of radioisotopes include, but are not limited to, tritium and carbon-14. Examples of colorimetric molecules include, but are not limited to, fluorescent molecules such as fluorescamine, or rhodamine or other colorimetric molecules. Examples of toxins include, but are not limited, to ricin.

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5.10.13 DRUG SCREENING

This invention is particularly useful for screening chemical compounds by using the novel polypeptides or binding fragments thereof in any of a variety of drug screening techniques. The polypeptides or fragments employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or a fragment thereof. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between polypeptides of the invention or fragments and the agent being tested or examine the diminution in complex formation between the novel polypeptides and an appropriate cell line, which are well known in the art.

Sources for test compounds that may be screened for ability to bind to or modulate (i.e., increase or decrease) the activity of polypeptides of the invention include (1) inorganic and organic chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of either random or mimetic peptides, oligonucleotides or organic molecules.

Chemical libraries may be readily synthesized or purchased from a number of commercial sources, and may include structural analogs of known compounds or compounds that are identified as "hits" or "leads" via natural product screening.

The sources of natural product libraries are microorganisms (including bacteria and fungi), animals, plants or other vegetation, or marine organisms, and libraries of mixtures for screening may be created by: (1) fermentation and extraction of broths from soil, plant or marine microorganisms or (2) extraction of the organisms themselves. Natural product libraries include polyketides, non-ribosomal peptides, and (non-naturally occurring) variants thereof. For a review, see *Science* 282:63-68 (1998).

Combinatorial libraries are composed of large numbers of peptides, oligonucleotides or organic compounds and can be readily prepared by traditional automated synthesis methods, PCR, cloning or proprietary synthetic methods. Of particular interest are peptide and oligonucleotide combinatorial libraries. Still other libraries of interest include peptide, protein,

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peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see Myers, *Curr. Opin. Biotechnol.* 8:701-707 (1997). For reviews and examples of peptidomimetic libraries, see Al-Obeidi et al., *Mol. Biotechnol.*, 9(3):205-23 (1998); Hruby et al., *Curr Opin Chem Biol*, 1(1):114-19 (1997); Dorner et al., *Bioorg Med Chem*, 4(5):709-15 (1996) (alkylated dipeptides).

Identification of modulators through use of the various libraries described herein permits modification of the candidate "hit" (or "lead") to optimize the capacity of the "hit" to bind a polypeptide of the invention. The molecules identified in the binding assay are then tested for antagonist or agonist activity in *in vivo* tissue culture or animal models that are well known in the art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

The binding molecules thus identified may be complexed with toxins, e.g., ricin or cholera, or with other compounds that are toxic to cells such as radioisotopes. The toxin-binding molecule complex is then targeted to a tumor or other cell by the specificity of the binding molecule for a polypeptide of the invention. Alternatively, the binding molecules may be complexed with imaging agents for targeting and imaging purposes.

5.10.14 ASSAY FOR RECEPTOR ACTIVITY

The invention also provides methods to detect specific binding of a polypeptide e.g. a ligand or a receptor. The art provides numerous assays particularly useful for identifying previously unknown binding partners for receptor polypeptides of the invention. For example, expression cloning using mammalian or bacterial cells, or dihybrid screening assays can be used to identify polynucleotides encoding binding partners. As another example, affinity chromatography with the appropriate immobilized polypeptide of the invention can be used to isolate polypeptides that recognize and bind polypeptides of the invention. There are a number of different libraries used for the identification of compounds, and in particular small molecule, that modulate (*i.e.*, increase or decrease) biological activity of a polypeptide of the invention. Ligands for receptor polypeptides of the invention can also be identified by adding exogenous ligands, or cocktails of ligands to two cells populations that are genetically identical except for the expression of the receptor of the invention: one cell population expresses the receptor of the invention whereas the other does not. The response of the two cell populations to the addition of ligands(s) are then compared. Alternatively, an expression library can be co-expressed with the polypeptide of the invention in cells and assayed for an autocrine response

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to identify potential ligand(s). As still another example, BIAcore assays, gel overlay assays, or other methods known in the art can be used to identify binding partner polypeptides, including, (1) organic and inorganic chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of random peptides, oligonucleotides or organic molecules.

- 5 The role of downstream intracellular signaling molecules in the signaling cascade of the polypeptide of the invention can be determined. For example, a chimeric protein in which the cytoplasmic domain of the polypeptide of the invention is fused to the extracellular portion of a protein, whose ligand has been identified, is produced in a host cell. The cell is then incubated with the ligand specific for the extracellular portion of the chimeric protein, thereby activating
10 the chimeric receptor. Known downstream proteins involved in intracellular signaling can then be assayed for expected modifications i.e. phosphorylation. Other methods known to those in the art can also be used to identify signaling molecules involved in receptor activity.

5.10.15 ANTI-INFLAMMATORY ACTIVITY

- 15 Compositions of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production
20 of other factors which more directly inhibit or promote an inflammatory response. Compositions with such activities can be used to treat inflammatory conditions including chronic or acute conditions, including without limitation intimation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's
25 disease or resulting from over production of cytokines such as TNF or IL-1. Compositions of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material. Compositions of this invention may be utilized to prevent or treat conditions such as, but not limited to, sepsis, acute pancreatitis, endotoxin shock, cytokine
30 induced shock, rheumatoid arthritis, chronic inflammatory arthritis, pancreatic cell damage from diabetes mellitus type 1, graft versus host disease, inflammatory bowel disease, inflammation associated with pulmonary disease, other autoimmune disease or inflammatory disease, an antiproliferative agent such as for acute or chronic myelogenous leukemia or in the prevention of premature labor secondary to intrauterine infections.

5.10.16 LEUKEMIAS

Leukemias and related disorders may be treated or prevented by administration of a therapeutic that promotes or inhibits function of the polynucleotides and/or polypeptides of the invention. Such leukemias and related disorders include but are not limited to acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia, chronic leukemia, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia (for a review of such disorders, see Fishman et al., 1985, Medicine, 2d Ed., J.B. Lippincott Co., Philadelphia).

5.10.17 NERVOUS SYSTEM DISORDERS

Nervous system disorders, involving cell types which can be tested for efficacy of intervention with compounds that modulate the activity of the polynucleotides and/or polypeptides of the invention, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the invention include but are not limited to the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems:

- (i) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;
- (ii) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;
- (iii) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis;
- (iv) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis;

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- (v) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration;
- (vi) neurological lesions associated with systemic diseases including but not limited to diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis;
- (vii) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and
- (viii) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including but not limited to multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.
- Therapeutics which are useful according to the invention for treatment of a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, therapeutics which elicit any of the following effects may be useful according to the invention:
- (i) increased survival time of neurons in culture;
 - (ii) increased sprouting of neurons in culture or *in vivo*;
 - (iii) increased production of a neuron-associated molecule in culture or *in vivo*, *e.g.*, choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or
 - (iv) decreased symptoms of neuron dysfunction *in vivo*.
- Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may be measured by the method set forth in Arakawa et al. (1990, J. Neurosci. 10:3507-3515); increased sprouting of neurons may be detected by methods set forth in Pestronk et al. (1980, Exp. Neurol. 70:65-82) or Brown et al. (1981, Ann. Rev. Neurosci. 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, *etc.*, depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, *e.g.*, weakness, motor neuron conduction velocity, or functional disability.
- In specific embodiments, motor neuron disorders that may be treated according to the invention include but are not limited to disorders such as infarction, infection, exposure to

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toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including but not limited to progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motor-sensory Neuropathy (Charcot-Marie-Tooth Disease).

5.10.18 OTHER ACTIVITIES

A polypeptide of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, co-factors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

5.10.19 IDENTIFICATION OF POLYMORPHISMS

The demonstration of polymorphisms makes possible the identification of such polymorphisms in human subjects and the pharmacogenetic use of this information for diagnosis and treatment. Such polymorphisms may be associated with, e.g., differential predisposition or susceptibility to various disease states (such as disorders involving

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inflammation or immune response) or a differential response to drug administration, and this genetic information can be used to tailor preventive or therapeutic treatment appropriately. For example, the existence of a polymorphism associated with a predisposition to inflammation or autoimmune disease makes possible the diagnosis of this condition in humans by identifying the presence of the polymorphism.

Polymorphisms can be identified in a variety of ways known in the art which all generally involve obtaining a sample from a patient, analyzing DNA from the sample, optionally involving isolation or amplification of the DNA, and identifying the presence of the polymorphism in the DNA. For example, PCR may be used to amplify an appropriate fragment of genomic DNA which may then be sequenced. Alternatively, the DNA may be subjected to allele-specific oligonucleotide hybridization (in which appropriate oligonucleotides are hybridized to the DNA under conditions permitting detection of a single base mismatch) or to a single nucleotide extension assay (in which an oligonucleotide that hybridizes immediately adjacent to the position of the polymorphism is extended with one or more labeled nucleotides). In addition, traditional restriction fragment length polymorphism analysis (using restriction enzymes that provide differential digestion of the genomic DNA depending on the presence or absence of the polymorphism) may be performed. Arrays with nucleotide sequences of the present invention can be used to detect polymorphisms. The array can comprise modified nucleotide sequences of the present invention in order to detect the nucleotide sequences of the present invention. In the alternative, any one of the nucleotide sequences of the present invention can be placed on the array to detect changes from those sequences.

Alternatively a polymorphism resulting in a change in the amino acid sequence could also be detected by detecting a corresponding change in amino acid sequence of the protein, e.g., by an antibody specific to the variant sequence.

5.10.20 ARTHRITIS AND INFLAMMATION

The immunosuppressive effects of the compositions of the invention against rheumatoid arthritis is determined in an experimental animal model system. The experimental model system is adjuvant induced arthritis in rats, and the protocol is described by J. Holoshitz, et al., 1983, Science, 219:56, or by B. Waksman et al., 1963, Int. Arch. Allergy Appl. Immunol., 23:129. Induction of the disease can be caused by a single injection, generally intradermally, of a suspension of killed Mycobacterium tuberculosis in complete Freund's adjuvant (CFA). The route of injection can vary, but rats may be injected at the base of the tail

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with an adjuvant mixture. The polypeptide is administered in phosphate buffered solution (PBS) at a dose of about 1-5 mg/kg. The control consists of administering PBS only.

The procedure for testing the effects of the test compound would consist of intradermally injecting killed *Mycobacterium tuberculosis* in CFA followed by immediately administering the test compound and subsequent treatment every other day until day 24. At 14, 15, 18, 20, 22, and 24 days after injection of *Mycobacterium* CFA, an overall arthritis score may be obtained as described by J. Holoskitz above. An analysis of the data would reveal that the test compound would have a dramatic affect on the swelling of the joints as measured by a decrease of the arthritis score.

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5.11 THERAPEUTIC METHODS

The compositions (including polypeptide fragments, analogs, variants and antibodies or other binding partners or modulators including antisense polynucleotides) of the invention have numerous applications in a variety of therapeutic methods. Examples of therapeutic applications include, but are not limited to, those exemplified herein.

5.11.1 EXAMPLE

One embodiment of the invention is the administration of an effective amount of the prothrombinase-like polypeptides or other composition of the invention to individuals affected by a disease or disorder that can be modulated by regulating the soluble prothrombinase-like receptor of the invention. While the mode of administration is not particularly important, parenteral administration is preferred. An exemplary mode of administration is to deliver an intravenous bolus. The dosage of prothrombinase-like polypeptides or other composition of the invention will normally be determined by the prescribing physician. It is to be expected that the dosage will vary according to the age, weight, condition and response of the individual patient. Typically, the amount of polypeptide administered per dose will be in the range of about 0.01µg/kg to 100 mg/kg of body weight, with the preferred dose being about 0.1µg/kg to 10 mg/kg of patient body weight. For parenteral administration, prothrombinase-like polypeptides of the invention will be formulated in an injectable form combined with a pharmaceutically acceptable parenteral vehicle. Such vehicles are well known in the art and examples include water, saline, Ringer's solution, dextrose solution, and solutions consisting of small amounts of the human serum albumin. The vehicle may contain minor amounts of

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additives that maintain the isotonicity and stability of the polypeptide or other active ingredient. The preparation of such solutions is within the skill of the art.

5 5.12 PHARMACEUTICAL FORMULATIONS AND ROUTES OF ADMINISTRATION

A protein or other composition of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources and including antibodies and other binding partners of the polypeptides of the invention) may be administered to a patient in need, by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s) at doses to treat or ameliorate a variety of disorders. Such a composition may optionally contain (in addition to protein or other active ingredient and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF α , TNF β , TNF γ , G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the disease or disorder in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factors (TGF- α and TGF- β), insulin-like growth factor (IGF), as well as cytokines described herein.

The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or other active ingredient or complement its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein or other active ingredient of the invention, or to minimize side effects. Conversely, protein or other active ingredient of the present invention may be included in formulations of the particular clotting factor, cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the clotting factor, cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent (such

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as IL-1Ra, IL-1 Hy1, IL-1 Hy2, anti-TNF, corticosteroids, immunosuppressive agents). A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or
 5 complexed form.

As an alternative to being included in a pharmaceutical composition of the invention including a first protein, a second protein or a therapeutic agent may be concurrently administered with the first protein (e.g., at the same time, or at differing times provided that therapeutic concentrations of the combination of agents is achieved at the treatment site).
 10 Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms, *e.g.*, treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing,
 15 prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein or other active ingredient of the present invention is administered to a mammal having a condition to be treated. Protein or other active ingredient of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or
 25 other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein or other active ingredient of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering
 30 protein or other active ingredient of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

5.12.1 ROUTES OF ADMINISTRATION

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Suitable routes of administration may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. Administration of protein or other active ingredient of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

Alternately, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a arthritic joints or in fibrotic tissue, often in a depot or sustained release formulation. In order to prevent the scarring process frequently occurring as complication of glaucoma surgery, the compounds may be administered topically, for example, as eye drops. Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with a specific antibody, targeting, for example, arthritic or fibrotic tissue. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

The polypeptides of the invention are administered by any route that delivers an effective dosage to the desired site of action. The determination of a suitable route of administration and an effective dosage for a particular indication is within the level of skill in the art. Preferably for wound treatment, one administers the therapeutic compound directly to the site. Suitable dosage ranges for the polypeptides of the invention can be extrapolated from these dosages or from similar studies in appropriate animal models. Dosages can then be adjusted as necessary by the clinician to provide maximal therapeutic benefit.

5.12.2 COMPOSITIONS/FORMULATIONS

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. These pharmaceutical compositions may be manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. Proper formulation is dependent upon the route of administration chosen. When a therapeutically effective amount of protein or other active ingredient of the present invention is administered orally, protein or other active ingredient of the present

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invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein or other active ingredient of the present invention, and preferably from about 25 to 90% protein or other active ingredient of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein or other active ingredient of the present invention, and preferably from about 1 to 50% protein or other active ingredient of the present invention.

When a therapeutically effective amount of protein or other active ingredient of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein or other active ingredient of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein or other active ingredient solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein or other active ingredient of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art. For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained solid excipient,

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optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration. For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. The compositions may take such forms as

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suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active
5 compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl
10 cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other
15 glycerides. In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as
20 sparingly soluble derivatives, for example, as a sparingly soluble salt.

A pharmaceutical carrier for the hydrophobic compounds of the invention is a co-solvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The co-solvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate
25 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics.
30 Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, *e.g.* polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose. Alternatively, other delivery systems for hydrophobic pharmaceutical compounds

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may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various types of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein or other active ingredient stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols. Many of the active ingredients of the invention may be provided as salts with pharmaceutically compatible counter ions. Such pharmaceutically acceptable base addition salts are those salts which retain the biological effectiveness and properties of the free acids and which are obtained by reaction with inorganic or organic bases such as sodium hydroxide, magnesium hydroxide, ammonia, trialkylamine, dialkylamine, monoalkylamine, dibasic amino acids, sodium acetate, potassium benzoate, triethanol amine and the like.

The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) or other active ingredient of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively antibodies able to bind surface immunoglobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other pharmaceutically

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acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithins, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323, all of which are incorporated herein by reference.

The amount of protein or other active ingredient of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein or other active ingredient of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein or other active ingredient of the present invention and observe the patient's response. Larger doses of protein or other active ingredient of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 μ g to about 100 mg (preferably about 0.1 μ g to about 10 mg, more preferably about 0.1 μ g to about 1 mg) of protein or other active ingredient of the present invention per kg body weight. For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systemically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein or other active ingredient of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing or other active ingredient-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into

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the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

- The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular
- 5 application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalcium phosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components.
- 10 Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalcium phosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle
- 15 size, particle shape, and biodegradability. Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein compositions from disassociating from the matrix.
- 20 A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate,
- 25 poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt %, preferably 1-10 wt % based on total formulation weight, which represents the amount necessary to prevent desorption of the protein from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby
- 30 providing the protein the opportunity to assist the osteogenic activity of the progenitor cells. In further compositions, proteins or other active ingredient of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth

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factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF- α and TGF- β), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications.

Particularly domestic animals and thoroughbred horses, in addition to humans, are desired

5 patients for such treatment with proteins or other active ingredient of the present invention.

The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, *e.g.*, amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue

10 (*e.g.*, bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic
15 assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such

polynucleotides can be introduced either in vivo or ex vivo into cells for expression in a

mammalian subject. Polynucleotides of the invention may also be administered by other known

20 methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA). Cells may also be cultured ex vivo in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced in vivo for therapeutic purposes.

25 5.12.3 EFFECTIVE DOSAGE

Pharmaceutical compositions suitable for use in the present invention include

compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount

effective to prevent development of or to alleviate the existing symptoms of the subject being

30 treated. Determination of the effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from appropriate in vitro assays. For example, a dose can be formulated in animal models to achieve

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a circulating concentration range that can be used to more accurately determine useful doses in humans. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of the protein's biological activity). Such information can be used to more accurately determine useful doses in humans.

5 A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD_{50} and ED_{50} . Compounds which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. See, *e.g.*, Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1. Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the desired effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

25 Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

30 An exemplary dosage regimen for polypeptides or other compositions of the invention will be in the range of about 0.01 $\mu\text{g/kg}$ to 100 mg/kg of body weight daily, with the preferred dose being about 0.1 $\mu\text{g/kg}$ to 25 mg/kg of patient body weight daily, varying in adults and

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children. Dosing may be once daily, or equivalent doses may be delivered at longer or shorter intervals.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's age and weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

5.12.4 PACKAGING

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

5.13 ANTIBODIES

Also included in the invention are antibodies to proteins, or fragments of proteins of the invention. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} , $F_{ab'}$ and $F_{(ab)2}$ fragments, and an F_{ab} expression library. In general, an antibody molecule obtained from humans relates to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG₁, IgG₂, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of human antibody species.

An isolated related protein of the invention may be intended to serve as an antigen, or a portion or fragment thereof, and additionally can be used as an immunogen to generate antibodies that immunospecifically bind the antigen, using standard techniques for polyclonal and monoclonal antibody preparation. The full-length protein can be used or, alternatively, the invention provides antigenic peptide fragments of the antigen for use as immunogens. An antigenic peptide fragment comprises at least 6 amino acid residues of the amino acid sequence of the full length protein, such as an amino acid sequence shown in SEQ ID NO: 4, and

encompasses an epitope thereof such that an antibody raised against the peptide forms a specific immune complex with the full length protein or with any fragment that contains the epitope. Preferably, the antigenic peptide comprises at least 10 amino acid residues, or at least 15 amino acid residues, or at least 20 amino acid residues, or at least 30 amino acid residues.

- 5 Preferred epitopes encompassed by the antigenic peptide are regions of the protein that are located on its surface; commonly these are hydrophilic regions.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of -related protein that is located on the surface of the protein, *e.g.*, a hydrophilic region. A hydrophobicity analysis of the human related protein sequence will indicate which regions of a related protein are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle 1982, *J. Mol. Biol.* 157: 105-142, each of which is incorporated herein by reference in its entirety. Antibodies that are specific for one or more domains within an antigenic protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

- 20 A protein of the invention, or a derivative, fragment, analog, homolog or ortholog thereof, may be utilized as an immunogen in the generation of antibodies that immunospecifically bind these protein components.

Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies directed against a protein of the invention, or against derivatives, fragments, analogs homologs or orthologs thereof (see, for example, *Antibodies: A Laboratory Manual*, Harlow E. and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference). Some of these antibodies are discussed below.

5.13.1 Polyclonal Antibodies

- 30 For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by one or more injections with the native protein, a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, the naturally occurring immunogenic protein, a chemically synthesized polypeptide representing the immunogenic protein, or a

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recombinantly expressed immunogenic protein. Furthermore, the protein may be conjugated to a second protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (e.g., aluminum hydroxide), surface active substances (e.g., lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), adjuvants usable in humans such as Bacille Calmette-Guerin and *Corynebacterium parvum*, or similar immunostimulatory agents.

Additional examples of adjuvants which can be employed include MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

The polyclonal antibody molecules directed against the immunogenic protein can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as affinity chromatography using protein A or protein G, which provide primarily the IgG fraction of immune serum. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific antibody by immunoaffinity chromatography. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

5.13.2 Monoclonal Antibodies

The term "monoclonal antibody" (MAb) or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs thus contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

Monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized in vitro.

The immunizing agent will typically include the protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63).

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980). Preferably, antibodies having a high degree of specificity and a high binding affinity for the target antigen are isolated.

After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods. Suitable culture media for this purpose

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include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown in vivo as ascites in a mammal.

The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel

electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, *Nature* 368, 812-13 (1994)) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

5.13.2 Humanized Antibodies

The antibodies directed against the protein antigens of the invention can further comprise humanized antibodies or human antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the human against the administered immunoglobulin. Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) that are principally comprised of the sequence of a human immunoglobulin, and contain minimal sequence derived from a non-human immunoglobulin. Humanization can be performed following the method of Winter and co-workers (Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-327 (1988); Verhoeven et al., *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the

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corresponding sequences of a human antibody. (See also U.S. Patent No. 5,225,539.) In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences.

5 In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a

10 human immunoglobulin (Jones et al., 1986; Riechmann et al., 1988; and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)).

5.13.3 Human Antibodies

Fully human antibodies relate to antibody molecules in which essentially the entire

15 sequences of both the light chain and the heavy chain, including the CDRs, arise from human genes. Such antibodies are termed "human antibodies", or "fully human antibodies" herein. Human monoclonal antibodies can be prepared by the trioma technique; the human B-cell hybridoma technique (see Kozbor, et al., 1983 Immunol Today 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, et al., 1985 In: MONOCLONAL

20 ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus in vitro (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

25 In addition, human antibodies can also be produced using additional techniques, including phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon

30 challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in Marks et al. (Bio/Technology 10, 779-783 (1992)); Lonberg et al. (Nature 368 856-859 (1994)); Morrison (Nature 368, 812-13 (1994)); Fishwild

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et al, (*Nature Biotechnology* 14, 845-51 (1996)); Neuberger (*Nature Biotechnology* 14, 826 (1996)); and Lonberg and Huszar (*Intern. Rev. Immunol.* 13 65-93 (1995)).

Human antibodies may additionally be produced using transgenic nonhuman animals which are modified so as to produce fully human antibodies rather than the animal's

- 5 endogenous antibodies in response to challenge by an antigen. (See PCT publication WO94/02602). The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host have been incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's genome. The human genes are incorporated, for example, using yeast artificial chromosomes containing the requisite human
- 10 DNA segments. An animal which provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals containing fewer than the full complement of the modifications. The preferred embodiment of such a nonhuman animal is a mouse, and is termed the Xenomouse™ as disclosed in PCT publications WO 96/33735 and WO 96/34096. This animal produces B cells which secrete fully human immunoglobulins.
- 15 The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly, or
- 20 can be further modified to obtain analogs of antibodies such as, for example, single chain Fv molecules.

An example of a method of producing a nonhuman host, exemplified as a mouse, lacking expression of an endogenous immunoglobulin heavy chain is disclosed in U.S. Patent No. 5,939,598. It can be obtained by a method including deleting the J segment genes from at

25 least one endogenous heavy chain locus in an embryonic stem cell to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged immunoglobulin heavy chain locus, the deletion being effected by a targeting vector containing a gene encoding a selectable marker; and producing from the embryonic stem cell a transgenic mouse whose somatic and germ cells contain the gene encoding the selectable marker.

- 30 A method for producing an antibody of interest, such as a human antibody, is disclosed in U.S. Patent No. 5,916,771. It includes introducing an expression vector that contains a nucleotide sequence encoding a heavy chain into one mammalian host cell in culture, introducing an expression vector containing a nucleotide sequence encoding a light chain into

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another mammalian host cell, and fusing the two cells to form a hybrid cell. The hybrid cell expresses an antibody containing the heavy chain and the light chain.

In a further improvement on this procedure, a method for identifying a clinically relevant epitope on an immunogen, and a correlative method for selecting an antibody that
 5 binds immunospecifically to the relevant epitope with high affinity, are disclosed in PCT publication WO 99/53049.

5.13.4 F_{ab} Fragments and Single Chain Antibodies

According to the invention, techniques can be adapted for the production of
 10 single-chain antibodies specific to an antigenic protein of the invention (see e.g., U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F_{ab} expression libraries (see e.g., Huse, et al., 1989 Science 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for a protein or derivatives, fragments, analogs or homologs thereof. Antibody fragments that contain the
 15 idiotypes to a protein antigen may be produced by techniques known in the art including, but not limited to: (i) an F_{(ab)₂} fragment produced by pepsin digestion of an antibody molecule; (ii) an F_{ab} fragment generated by reducing the disulfide bridges of an F_{(ab)₂} fragment; (iii) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent
 and (iv) F_v fragments.

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5.13.5 Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for an antigenic protein of the invention. The second binding target is
 25 any other antigen, and advantageously is a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, *Nature*, 305:537-539 (1983)). Because of the random
 30 assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, 1991 *EMBO J.*, 10:3655-3659.

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Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')₂ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., *Science* 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Additionally, Fab' fragments can be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.* 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')₂ molecule. Each Fab'

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fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

- 5 Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., *J. Immunol.* 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge
- 10 region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable
- 15 domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber et al., *J. Immunol.* 152:5368 (1994).
- 20 Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., *J. Immunol.* 147:60 (1991). Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the protein antigen of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule
- 25 on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG (Fc γ R), such as Fc γ RI (CD64), Fc γ RII (CD32) and Fc γ RIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic
- 30 agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the protein antigen described herein and further binds tissue factor (TF).

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5.13.6 Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention.

Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360; WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

5.13.7 Effector Function Engineering

It can be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, e.g., the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., *J. Exp Med.*, 176: 1191-1195 (1992) and Shopes, *J. Immunol.*, 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity can also be prepared using heterobifunctional cross-linkers as described in Wolff et al. *Cancer Research*, 53: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., *Anti-Cancer Drug Design*, 3: 219-230 (1989).

5.13.8 Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and

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PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi , ^{125}I , ^{131}I , ^{90}Y , and ^{186}Re .

5 Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives
10 (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene
15 triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionuclide to the antibody. See WO94/11026.

In another embodiment, the antibody can be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) that is in turn
20 conjugated to a cytotoxic agent.

5.14 COMPUTER READABLE SEQUENCES

In one application of this embodiment, a nucleotide sequence of the present invention can be recorded on computer readable media. As used herein, "computer readable media"
25 refers to any medium which can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. A skilled artisan can readily appreciate how any of the presently known computer
30 readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a nucleotide sequence of the present invention. As used herein, "recorded" refers to a process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently known methods for recording information

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on computer readable medium to generate manufactures comprising the nucleotide sequence information of the present invention.

A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of data processor structuring formats (*e.g.* text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

By providing any of the nucleotide sequences SEQ ID NO: 1-3, 10-13 or 15 or a representative fragment thereof, or a nucleotide sequence at least 95% identical to any of the nucleotide sequences of the SEQ ID NO:1-3, 10-13 or 15 in computer readable form, a skilled artisan can routinely access the sequence information for a variety of purposes. Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium. The examples which follow demonstrate how software which implements the BLAST (Altschul et al., J. Mol. Biol. 215:403-410 (1990)) and BLAZE (Brutlag et al., Comp. Chem. 17:203-207 (1993)) search algorithms on a Sybase system is used to identify open reading frames (ORFs) within a nucleic acid sequence. Such ORFs may be protein encoding fragments and may be useful in producing commercially important proteins such as enzymes used in fermentation reactions and in the production of commercially useful metabolites.

As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based systems are suitable for use in the present invention. As stated above, the computer-based systems of the present invention comprise a data storage means having stored therein a nucleotide sequence of the present invention and the necessary hardware means

and software means for supporting and implementing a search means. As used herein, "data storage means" refers to memory which can store nucleotide sequence information of the present invention, or a memory access means which can access manufactures having recorded thereon the nucleotide sequence information of the present invention.

5 As used herein, "search means" refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of a known sequence which match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software includes, but is not limited to, 10 Smith-Waterman, MacPattern (EMBL), BLASTN and BLASTA (NPOLYPEPTIDEIA). A skilled artisan can readily recognize that any one of the available algorithms or implementing software packages for conducting homology searches can be adapted for use in the present computer-based systems. As used herein, a "target sequence" can be any nucleic acid or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. The most preferred sequence length of a target sequence is from about 10 to 100 amino acids, or from about 30 to 300 nucleotide residues. However, it is well recognized that searches for commercially important fragments, 20 such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a three-dimensional configuration which is formed upon the folding of the target motif. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzyme active sites and signal sequences. Nucleic acid target motifs include, but are not limited to, promoter sequences, hairpin structures and inducible expression elements (protein binding sequences).

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5.15 TRIPLE HELIX FORMATION

In addition, the fragments of the present invention, as broadly described, can be used to control gene expression through triple helix formation or antisense DNA or RNA, both of which methods are based on the binding of a polynucleotide sequence to DNA or RNA.

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Polynucleotides suitable for use in these methods are usually 20 to 40 bases in length and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 15241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Olmno, J.

- 5 Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present
10 invention is necessary for the design of an antisense or triple helix oligonucleotide.

5.16 DIAGNOSTIC ASSAYS AND KITS

- The present invention further provides methods to identify the presence or expression of one of the ORFs of the present invention, or homolog thereof, in a test sample, using a
15 nucleic acid probe or antibodies of the present invention, optionally conjugated or otherwise associated with a suitable label.

- In general, methods for detecting a polynucleotide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polynucleotide for a period sufficient to form the complex, and detecting the complex, so that
20 if a complex is detected, a polynucleotide of the invention is detected in the sample. Such methods can also comprise contacting a sample under stringent hybridization conditions with nucleic acid primers that anneal to a polynucleotide of the invention under such conditions, and amplifying annealed polynucleotides, so that if a polynucleotide is amplified, a polynucleotide of the invention is detected in the sample.

- 25 In general, methods for detecting a polypeptide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polypeptide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, a polypeptide of the invention is detected in the sample.

- In detail, such methods comprise incubating a test sample with one or more of the
30 antibodies or one or more of the nucleic acid probes of the present invention and assaying for binding of the nucleic acid probes or antibodies to components within the test sample.

Conditions for incubating a nucleic acid probe or antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the nucleic acid probe or antibody used in the assay.

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One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the nucleic acid probes or antibodies of the present invention. Examples of such assays can be found in Chard, T., *An Introduction to Radioimmunoassay and Related Techniques*, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G.R. et al., *Techniques in Immunocytochemistry*, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., *Practice and Theory of immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985). The test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is compatible with the system utilized.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the assays of the present invention. Specifically, the invention provides a compartment kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the probes or antibodies of the present invention; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of a bound probe or antibody.

In detail, a compartment kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allows one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which contain the reagents used to detect the bound antibody or probe. Types of detection reagents include labeled nucleic acid probes, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. One skilled in the art will readily

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recognize that the disclosed probes and antibodies of the present invention can be readily incorporated into one of the established kit formats which are well known in the art.

5.17 MEDICAL IMAGING

5 The novel polypeptides and binding partners of the invention are useful in medical imaging of sites expressing the molecules of the invention (e.g., where the polypeptide of the invention is involved in the immune response, for imaging sites of inflammation or infection). See, e.g., Kunkel et al., U.S. Pat. NO. 5,413,778. Such methods involve chemical attachment of a labeling or imaging agent, administration of the labeled polypeptide to a subject in a
10 pharmaceutically acceptable carrier, and imaging the labeled polypeptide *in vivo* at the target site.

5.18 SCREENING ASSAYS

Using the isolated proteins and polynucleotides of the invention, the present invention
15 further provides methods of obtaining and identifying agents which bind to a polypeptide encoded by an ORF corresponding to any of the nucleotide sequences set forth in the SEQ ID NO: 1-3, 10-13 or 15, or bind to a specific domain of the polypeptide encoded by the nucleic acid. In detail, said method comprises the steps of:

- (a) contacting an agent with an isolated protein encoded by an ORF of the present
20 invention, or nucleic acid of the invention; and
- (b) determining whether the agent binds to said protein or said nucleic acid.

In general, therefore, such methods for identifying compounds that bind to a polynucleotide of the invention can comprise contacting a compound with a polynucleotide of the invention for a time sufficient to form a polynucleotide/compound complex, and detecting
25 the complex, so that if a polynucleotide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

Likewise, in general, therefore, such methods for identifying compounds that bind to a polypeptide of the invention can comprise contacting a compound with a polypeptide of the invention for a time sufficient to form a polypeptide/compound complex, and detecting the
30 complex, so that if a polypeptide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

Methods for identifying compounds that bind to a polypeptide of the invention can also comprise contacting a compound with a polypeptide of the invention in a cell for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of

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a receptor gene sequence in the cell, and detecting the complex by detecting reporter gene sequence expression, so that if a polypeptide/compound complex is detected, a compound that binds a polypeptide of the invention is identified.

Compounds identified via such methods can include compounds which modulate the activity of a polypeptide of the invention (that is, increase or decrease its activity, relative to activity observed in the absence of the compound). Alternatively, compounds identified via such methods can include compounds which modulate the expression of a polynucleotide of the invention (that is, increase or decrease expression relative to expression levels observed in the absence of the compound). Compounds, such as compounds identified via the methods of the invention, can be tested using standard assays well known to those of skill in the art for their ability to modulate activity/expression.

The agents screened in the above assay can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. The agents can be selected and screened at random or rationally selected or designed using protein modeling techniques.

For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to the protein encoded by the ORF of the present invention. Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the particular protein. For example, one skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like, capable of binding to a specific peptide sequence, in order to generate rationally designed antipeptide peptides, for example see Hurby et al., Application of Synthetic Peptides: Antisense Peptides, " In Synthetic Peptides, A User's Guide, W.H. Freeman, NY (1992), pp. 289-307, and Kasieczak et al., Biochemistry 28:9230-8 (1989), or pharmaceutical agents, or the like.

In addition to the foregoing, one class of agents of the present invention, as broadly described, can be used to control gene expression through binding to one of the ORFs or EMFs of the present invention. As described above, such agents can be randomly screened or rationally designed/selected. Targeting the ORF or EMF allows a skilled artisan to design sequence specific or element specific agents, modulating the expression of either a single ORF or multiple ORFs which rely on the same EMF for expression control. One class of DNA binding agents are agents which contain base residues which hybridize or form a triple helix formation by binding to DNA or RNA. Such agents can be based on the classic

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phosphodiester, ribonucleic acid backbone, or can be a variety of sulfhydryl or polymeric derivatives which have base attachment capacity.

Agents suitable for use in these methods usually contain 20 to 40 bases and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide and other DNA binding agents.

Agents which bind to a protein encoded by one of the ORFs of the present invention can be used as a diagnostic agent. Agents which bind to a protein encoded by one of the ORFs of the present invention can be formulated using known techniques to generate a pharmaceutical composition.

5.19 USE OF NUCLEIC ACIDS AS PROBES

Another aspect of the subject invention is to provide for polypeptide-specific nucleic acid hybridization probes capable of hybridizing with naturally occurring nucleotide sequences. The hybridization probes of the subject invention may be derived from any of the nucleotide sequences SEQ ID NO:1-3, 10-13 or 15. Because the corresponding gene is only expressed in a limited number of tissues, a hybridization probe derived from any of the nucleotide sequences SEQ ID NO: 1-3, 10-13 and 15 can be used as an indicator of the presence of RNA of cell type of such a tissue in a sample.

Any suitable hybridization technique can be employed, such as, for example, in situ hybridization. PCR as described in US Patents Nos. 4,683,195 and 4,965,188 provides additional uses for oligonucleotides based upon the nucleotide sequences. Such probes used in PCR may be of recombinant origin, may be chemically synthesized, or a mixture of both. The probe will comprise a discrete nucleotide sequence for the detection of identical sequences or a degenerate pool of possible sequences for identification of closely related genomic sequences.

Other means for producing specific hybridization probes for nucleic acids include the cloning of nucleic acid sequences into vectors for the production of mRNA probes. Such

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vectors are known in the art and are commercially available and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriate radioactively labeled nucleotides. The nucleotide sequences may be used to construct hybridization probes for mapping their respective genomic sequences. The nucleotide sequence provided herein may be mapped to a chromosome or specific regions of a chromosome using well known genetic and/or chromosomal mapping techniques. These techniques include *in situ* hybridization, linkage analysis against known chromosomal markers, hybridization screening with libraries or flow-sorted chromosomal preparations specific to known chromosomes, and the like. The technique of fluorescent *in situ* hybridization of chromosome spreads has been described, among other places, in Verma et al (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York NY.

Fluorescent *in situ* hybridization of chromosomal preparations and other physical chromosome mapping techniques may be correlated with additional genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of a nucleic acid on a physical chromosomal map and a specific disease (or predisposition to a specific disease) may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier or affected individuals.

5.20 PREPARATION OF SUPPORT BOUND OLIGONUCLEOTIDES

Oligonucleotides, i.e., small nucleic acid segments, may be readily prepared by, for example, directly synthesizing the oligonucleotide by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer.

Support bound oligonucleotides may be prepared by any of the methods known to those of skill in the art using any suitable support such as glass, polystyrene or Teflon. One strategy is to precisely spot oligonucleotides synthesized by standard synthesizers. Immobilization can be achieved using passive adsorption (Inouye & Hondo, 1990 J. Clin Microbiol 28(6) 1462-72); using UV light (Nagata *et al.*, 1985; Dahlen *et al.*, 1987; Morrissey & Collins, Mol. Cell Probes 1989 3(2) 189-207) or by covalent binding of base modified DNA (Keller *et al.*, 1988; 1989); all references being specifically incorporated herein.

Another strategy that may be employed is the use of the strong biotin-streptavidin interaction as a linker. For example, Broude *et al.* (1994) Proc. Natl. Acad. Sci USA 91(8) 3072-6 describe the use of biotinylated probes, although these are duplex probes, that are

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immobilized on streptavidin-coated magnetic beads. Streptavidin-coated beads may be purchased from Dynal, Oslo. Of course, this same linking chemistry is applicable to coating any surface with streptavidin. Biotinylated probes may be purchased from various sources, such as, e.g., Operon Technologies (Alameda, CA).

5 Nunc Laboratories (Naperville, IL) is also selling suitable material that could be used. Nunc Laboratories have developed a method by which DNA can be covalently bound to the microwell surface termed CovaLink NH. CovaLink NH is a polystyrene surface grafted with secondary amino groups ($>NH$) that serve as bridge-heads for further covalent coupling. CovaLink Modules may be purchased from Nunc Laboratories. DNA molecules may be bound
10 to CovaLink exclusively at the 5'-end by a phosphoramidate bond, allowing immobilization of more than 1 pmol of DNA (Rasmussen *et al.*, (1991) *Anal Biochem* 198(1) 138-42.

The use of CovaLink NH strips for covalent binding of DNA molecules at the 5'-end has been described (Rasmussen *et al.*, 1991). In this technology, a phosphoramidate bond is employed (Chu *et al.*, 1983 *Nucleic Acids* 11(18) 6513-29). This is beneficial as immobilization
15 using only a single covalent bond is preferred. The phosphoramidate bond joins the DNA to the CovaLink NH secondary amino groups that are positioned at the end of spacer arms covalently grafted onto the polystyrene surface through a 2 nm long spacer arm. To link an oligonucleotide to CovaLink NH via an phosphoramidate bond, the oligonucleotide terminus must have a 5'-end phosphate group. It is, perhaps, even possible for biotin to be covalently bound to CovaLink and
20 then streptavidin used to bind the probes.

More specifically, the linkage method includes dissolving DNA in water (7.5 ng/ul) and denaturing for 10 min. at 95°C and cooling on ice for 10 min. Ice-cold 0.1 M 1-methylimidazole, pH 7.0 (1-MeIm), is then added to a final concentration of 10 mM 1-MeIm. A ss DNA solution is then dispensed into CovaLink NH strips (75 ul/well) standing on ice.

25 Carbodiimide 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide(EDC), dissolved in 10 mM 1-MeIm, is made fresh and 25 ul added per well. The strips are incubated for 5 hours at 50°C. After incubation the strips are washed using, e.g., Nunc-Immuno Wash; first the wells are washed 3 times, then they are soaked with washing solution for 5 min., and finally they are washed 3 times (where in the washing solution is 0.4 N NaOH, 0.25% SDS heated to 50°C).

30 It is contemplated that a further suitable method for use with the present invention is that described in PCT Patent Application WO 90/03382 (Southern & Maskos), incorporated herein by reference. This method of preparing an oligonucleotide bound to a support involves attaching a nucleoside 3'-reagent through the phosphate group by a covalent phosphodiester link to aliphatic hydroxyl groups carried by the support. The oligonucleotide is then synthesized on the supported

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nucleoside and protecting groups removed from the synthetic oligonucleotide chain under standard conditions that do not cleave the oligonucleotide from the support. Suitable reagents include nucleoside phosphoramidite and nucleoside hydrogen phosphorate.

An on-chip strategy for the preparation of DNA probe for the preparation of DNA probe arrays may be employed. For example, addressable laser-activated photodeprotection may be employed in the chemical synthesis of oligonucleotides directly on a glass surface, as described by Fodor *et al.* (1991) *Science* 251(4995) 767-73, incorporated herein by reference. Probes may also be immobilized on nylon supports as described by Van Ness *et al.* (1991) *Nucleic Acids Res.* 19(12) 3345-50; or linked to Teflon using the method of Duncan & Cavalier (1988) *Anal Biochem* 169(1) 104-8; all references being specifically incorporated herein.

To link an oligonucleotide to a nylon support, as described by Van Ness *et al.* (1991), requires activation of the nylon surface via alkylation and selective activation of the 5'-amine of oligonucleotides with cyanuric chloride.

One particular way to prepare support bound oligonucleotides is to utilize the light-generated synthesis described by Pease *et al.*, (1994) *Proc. Natl. Acad. Sci USA* 91(11) 5022-6. These authors used current photolithographic techniques to generate arrays of immobilized oligonucleotide probes (DNA chips). These methods, in which light is used to direct the synthesis of oligonucleotide probes in high-density, miniaturized arrays, utilize photolabile 5'-protected-N-acyl-deoxynucleoside phosphoramidites, surface linker chemistry and versatile combinatorial synthesis strategies. A matrix of 256 spatially defined oligonucleotide probes may be generated in this manner.

5.21 PREPARATION OF NUCLEIC ACID FRAGMENTS

The nucleic acids may be obtained from any appropriate source, such as cDNAs, genomic DNA, chromosomal DNA, microdissected chromosome bands, cosmid or YAC inserts, and RNA, including mRNA without any amplification steps. For example, Sambrook *et al.* (1989) describes three protocols for the isolation of high molecular weight DNA from mammalian cells (p. 9.14-9.23).

DNA fragments may be prepared as clones in M13, plasmid or lambda vectors and/or prepared directly from genomic DNA or cDNA by PCR or other amplification methods. Samples may be prepared or dispensed in multiwell plates. About 100-1000 ng of DNA samples may be prepared in 2-500 µl of final volume.

The nucleic acids would then be fragmented by any of the methods known to those of skill in the art including, for example, using restriction enzymes as described at 9.24-9.28 of Sambrook *et al.* (1989), shearing by ultrasound and NaOH treatment.

Low pressure shearing is also appropriate, as described by Schriefer *et al.* (1990) Nucleic Acids Res. 18(24) 7455-6. In this method, DNA samples are passed through a small French pressure cell at a variety of low to intermediate pressures. A lever device allows controlled application of low to intermediate pressures to the cell. The results of these studies indicate that low-pressure shearing is a useful alternative to sonic and enzymatic DNA fragmentation methods.

One particularly suitable way for fragmenting DNA is contemplated to be that using the two base recognition endonuclease, CviJI, described by Fitzgerald *et al.* (1992) Nucleic Acids Res. 20(14) 3753-62. These authors described an approach for the rapid fragmentation and fractionation of DNA into particular sizes that they contemplated to be suitable for shotgun cloning and sequencing.

The restriction endonuclease CviJI normally cleaves the recognition sequence PuGCPy between the G and C to leave blunt ends. Atypical reaction conditions, which alter the specificity of this enzyme (CviJI**), yield a quasi-random distribution of DNA fragments from the small molecule pUC19 (2688 base pairs). Fitzgerald *et al.* (1992) quantitatively evaluated the randomness of this fragmentation strategy, using a CviJI** digest of pUC19 that was size fractionated by a rapid gel filtration method and directly ligated, without end repair, to a lac Z minus M13 cloning vector. Sequence analysis of 76 clones showed that CviJI** restricts pyGCPy and PuGCPy, in addition to PuGCPy sites, and that new sequence data is accumulated at a rate consistent with random fragmentation.

As reported in the literature, advantages of this approach compared to sonication and agarose gel fractionation include: smaller amounts of DNA are required (0.2-0.5 ug instead of 2-5 ug); and fewer steps are involved (no preligation, end repair, chemical extraction, or agarose gel electrophoresis and elution are needed).

Irrespective of the manner in which the nucleic acid fragments are obtained or prepared, it is important to denature the DNA to give single stranded pieces available for hybridization. This is achieved by incubating the DNA solution for 2-5 minutes at 80-90°C. The solution is then cooled quickly to 2°C to prevent renaturation of the DNA fragments before they are contacted with the chip. Phosphate groups must also be removed from genomic DNA by methods known in the art.

5.22 PREPARATION OF DNA ARRAYS

Arrays may be prepared by spotting DNA samples on a support such as a nylon membrane. Spotting may be performed by using arrays of metal pins (the positions of which correspond to an array of wells in a microtiter plate) to repeated by transfer of about 20 nl of a DNA solution to a nylon membrane. By offset printing, a density of dots higher than the density of the wells is achieved. One to 25 dots may be accommodated in 1 mm², depending on the type of label used. By avoiding spotting in some preselected number of rows and columns, separate subsets (subarrays) may be formed. Samples in one subarray may be the same genomic segment of DNA (or the same gene) from different individuals, or may be different, overlapped genomic clones. Each of the subarrays may represent replica spotting of the same samples. In one example, a selected gene segment may be amplified from 64 patients. For each patient, the amplified gene segment may be in one 96-well plate (all 96 wells containing the same sample). A plate for each of the 64 patients is prepared. By using a 96-pin device, all samples may be spotted on one 8 x 12 cm membrane. Subarrays may contain 64 samples, one from each patient. Where the 96 subarrays are identical, the dot span may be 1 mm² and there may be a 1 mm space between subarrays.

Another approach is to use membranes or plates (available from NUNC, Naperville, Illinois) which may be partitioned by physical spacers e.g. a plastic grid molded over the membrane, the grid being similar to the sort of membrane applied to the bottom of multiwell plates, or hydrophobic strips. A fixed physical spacer is not preferred for imaging by exposure to flat phosphor-storage screens or x-ray films.

The present invention is illustrated in the following examples. Upon consideration of the present disclosure, one of skill in the art will appreciate that many other embodiments and variations may be made in the scope of the present invention. Accordingly, it is intended that the broader aspects of the present invention not be limited to the disclosure of the following examples. The present invention is not to be limited in scope by the exemplified embodiments which are intended as illustrations of single aspects of the invention, and compositions and methods which are functionally equivalent are within the scope of the invention. Indeed, numerous modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon consideration of the present preferred embodiments. Consequently, the only limitations which should be placed upon the scope of the invention are those which appear in the appended claims.

All references cited within the body of the instant specification are hereby incorporated by reference in their entirety.

6.0 EXAMPLES**EXAMPLE 1**Isolation of SEQ ID NO: 1 from a cDNA Library of Human Leukocytes

5 A plurality of novel nucleic acids were obtained from a cDNA library prepared from human ovary (Hyseq clone identification numbers 7735656 (SEQ ID NO: 1) and 7727065 (SEQ ID NO: 11)) using standard PCR, sequencing by hybridization sequence signature analysis, and Sanger sequencing techniques. The inserts of the library were amplified with PCR using primers specific for vector sequences flanking the inserts. These samples were
10 spotted onto nylon membranes and interrogated with oligonucleotide probes to give sequence signatures. The clones were clustered into groups of similar or identical sequences, and single representative clones were selected from each group for gel sequencing. The 5' sequence of the amplified inserts was then deduced using the reverse M13 sequencing primer in a typical Sanger sequencing protocol. PCR products were purified and subjected to fluorescent dye
15 terminator cycle sequencing. Single-pass gel sequencing was done using a 377 Applied Biosystems (ABI) sequencer. The insert was identified as a novel sequence not previously obtained from this library and not previously reported in public databases. The sequences were designated as SEQ ID NO: 1 and SEQ ID NO: 11.

20

EXAMPLE 2ASSEMBLAGE OF SEQ ID NO: 2 AND SEQ ID NO: 12

The nucleic acids of the present invention, designated as SEQ ID NO: 2 and SEQ ID
25 NO:12 were assembled using an EST sequence, SEQ ID NO:1 or SEQ ID NO:11, respectively, as a seed. Then a recursive algorithm was used to extend the seed into an extended assemblage, by pulling additional sequences from different databases (i.e., Hyseq's database containing EST sequences, dbEST version 114, gb pri 114, and UniGene version 101) that belong to this assemblage. The algorithm terminated when there was no additional sequences from the above
30 databases that would extend the assemblage. Inclusion of component sequences into the assemblage was based on a BLASTN hit to the extending assemblage with BLAST score greater than 300 and percent identity greater than 95%.

The nearest neighbor result for the assembled contigs were obtained by a FASTA version
3 search against Genpept release 114, using FASTXY algorithm. FASTXY is an improved
35 version of FASTA alignment which allows in-codon frame shifts. The nearest neighbor result

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showed the closest homologue for each assemblage from Genpept (and contains the translated amino acid sequences for which the assemblage encodes). The nearest neighbor results is set forth below:

5

SEQ ID NO:	Accession No.	Description	Smith-Waterman Score	% Identity
2	Z36531	Homo sapiens fibrinogen-like protein	500	38.462
12	U44830	Rana sylvatica extended fibrinogen alpha chain	252	28.629

The nucleotide sequence within the assembled contigs that codes for signal peptide sequences and their cleavage sites can be determined from using Neural Network SignalP V1.1 program (from Center for Biological Sequence Analysis, The Technical University of Denmark). The process for identifying prokaryotic and eukaryotic signal peptides and their cleavage sites are also disclosed by Henrik Nielson, Jacob Engelbrecht, Soren Brunak, and Gunnar von Heijne in the publication "Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites" Protein Engineering, vol. 10, no. 1, pp. 1-6 (1997), incorporated herein by reference. A maximum S score and a mean S score, as described in the Nielson et al reference, are obtained for each assembled contig. Starting from the first amino acid of the predicted signal sequence, a sequence of 45 amino acids is described. Not all forty-five amino acids in the sequence may comprise the signal peptide.

20 For SEQ ID NO: 12:

Beginning and end nucleotide location corresponding to amino acid segment	Maximum S score	mean S score	Amino acid segment containing signal peptide (A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine, X=Unknown, *=Stop Codon,

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			/=possible nucleotide deletion, \=possible nucleotide insertion)
54:189	0.922	0.765	MMSPSQASLLFLNVCFICGEVVQGN CVHHSTDSSVVNIVEDGSN (SEQ ID NO:22)

The predicted amino acid sequence for SEQ ID NO:2 was obtained by using a software program called FASTY (available from <http://fasta.bioch.virginia.edu>) which selects a polypeptide based on a comparison of translated novel polynucleotide to known polynucleotides (W.R. Pearson, Methods in Enzymology, 183:63-98 (1990), incorporated herein by reference).

For SEQ ID NO: 2:

Predicted beginning nucleotide location corresponding to first amino acid residue of amino acid segment	Predicted end nucleotide location corresponding to last amino acid residue of amino acid segment	AMINO ACID SEGMENT (A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine, S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine, X=Unknown, *=Stop Codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
3	717	DLKDTIGSVTKTPSGLYIIHPEGSSYPFEVMCDMDYR GGGWTVIQKRIDGIIDFQRLWCDYLDGFGDLLGEF WLGKKIFYIVNQKNTSFMLYVALESEDDTLAYAS YDNFWLEDETRFFKMHLGRYSGNAGDAFRGLKKE DNQNAMPFSTSDVDNDGCRPACLVNGQSVKSCSHL HNKTGWWFNECGLANLNGIHHFSGKLLATGIQWVG TWTKNNSPVKIKSVSMKIRRMYPYKF (SEQ ID NO:21)

10

EXAMPLE 3

ASSEMBLAGE OF SEQ ID NO: 3

Assembly of novel nucleotide sequence of SEQ ID NO: 3 was accomplished by using an EST sequence SEQ ID NO: 1 as a seed. The seed was extended by using software programs such as BLAST and Hyseq proprietary software to pull additional sequences from Hyseq's proprietary database containing EST sequences and by gel sequencing (377 Applied Biosystems (ABI) sequencer) using primers to extend the 3' end. Inclusion of component sequences into the assemblage was based BLAST scores greater than 1000 and a p-value of p-3

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(depending on the length of homology). The 5' end was extended using RACE, as disclosed in Marathon-Ready™ cDNA User Manual (PT1156-1) (Clontech), herein incorporated by reference.

5

EXAMPLE 4

ASSEMBLAGE OF SEQ ID NO: 10

Assembly of novel nucleotide sequence of SEQ ID NO: 10 was accomplished by using an EST sequence SEQ ID NO: 1 as a seed. The seed was further extended by gel sequencing (377 Applied Biosystems (ABI) sequencer) using primers to extend the 3' end (primer extension). The 5' end was extended using RACE, as disclosed in Marathon-Ready™ cDNA User Manual (PT1156-1) (Clontech), herein incorporated by reference.

15

EXAMPLE 5

ASSEMBLAGE OF SEQ ID NO: 13

Using PHRAP (Univ. of Washington) or CAP4 (Paracel), a full length gene cDNA sequence and its corresponding protein sequence were generated from the assemblage of SEQ ID NO: 12. Any frame shifts and incorrect stop codons were corrected by hand editing. During editing, the sequence was checked using FASTY and/or BLAST against Genbank (i.e. Genecept release 115). Other computer programs which may have been used in the editing process were phredPhrap and Consed (University of Washington) and ed-ready, ed-ext and cg-zip-2 (Hyseq, Inc.). The full-length nucleotide sequence is shown as SEQ ID NO: 13.

A polypeptide (SEQ ID NO: 14) was predicted to be encoded by SEQ ID NO: 13 as set forth below. The polypeptide was predicted using software programs called BLASTX/FASTY which selects a polypeptide based on a comparison of translated novel polynucleotide to known polypeptides. The initial methionine ATG starts at position 54 of the nucleotide sequence and the putative stop codon, TAA, ends the coding region at position 1034.

30

EXAMPLE 6

A. Expression of SEQ ID NO: 4-9, 14 and 16-18 in cells

Chinese Hamster Ovary (CHO) cells or other suitable cell types are grown in DMEM (ATCC) and 10% fetal bovine serum (FBS) (Gibco) to 70% confluence. Prior to transfection the media is changed to DMEM and 0.5% FCS. Cells are transfected with cDNAs for SEQ

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ID NO: 2-3, 10, 12-13 or 15 or with pBGal vector by the FuGENE-6 transfection reagent (Boehringer). In summary, 4 μ l of FuGENE-6 is diluted in 100 μ l of DMEM and incubated for 5 minutes. Then, this is added to 1 μ g of DNA and incubated for 15 minutes before adding it to a 35 mm dish of CHO cells. The CHO cells are incubated at 37°C with 5% CO₂. After 5 24 hours, media and cell lysates are collected, centrifuged and dialyzed against assay buffer (15 mM Tris pH 7.6, 134 mM NaCl, 5 mM glucose, 3 mM CaCl₂ and MgCl₂).

B. Expression Study Using SEQ ID NO: 2-3, 10, 12-13 or 15

The expression of SEQ ID NO: 2-3, 10, 12-13 or 15 in various tissues is analyzed using a semi-quantitative polymerase chain reaction-based technique. Human cDNA libraries are used as sources of expressed genes from tissues of interest (adult bladder, adult brain, adult heart, adult kidney, adult lymph node, adult liver, adult lung, adult ovary, adult placenta, adult rectum, adult spleen, adult testis, bone marrow, thymus, thyroid gland, fetal kidney, fetal liver, fetal liver-spleen, fetal skin, fetal brain, fetal leukocyte and macrophage). Gene-specific 15 primers are used to amplify portions of the SEQ ID NO: 2-3, 10, 12-13 or 15 sequence from the samples. Amplified products are separated on an agarose gel, transferred and chemically linked to a nylon filter. The filter is then hybridized with a radioactively labeled (³²P-dCTP) double-stranded probe generated from SEQ ID NO: 2-3, 10, 12-13 or 15 using a Klenow polymerase, random-prime method. The filters are washed (high stringency) and used to 20 expose a phosphorimaging screen for several hours. Bands indicate the presence of cDNA including SEQ ID NO: 2-3, 10, 12-13 or 15 sequences in a specific library, and thus mRNA expression in the corresponding cell type or tissue.

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CLAIMS

WE CLAIM:

1. An isolated polynucleotide comprising a nucleotide sequence selected from
5 the group consisting of SEQ ID NO: 2-3, 10, 12-13 or 15, the translated protein coding portion
thereof, the mature protein coding portion thereof, the extracellular portion thereof, or the active
domain thereof.
2. An isolated polynucleotide encoding a polypeptide with biological activity,
10 which polynucleotide hybridizes to the complement of a polynucleotide of claim 1 under stringent
hybridization conditions.
3. An isolated polynucleotide encoding a polypeptide with biological activity,
said polynucleotide having greater than about 90% sequence identity with the polynucleotide of
15 claim 1.
4. The polynucleotide of claim 1 which is a DNA sequence.
5. An isolated polynucleotide which comprises the complement of the
20 polynucleotide of claim 1.
6. A vector comprising the polynucleotide of claim 1.
7. An expression vector comprising the polynucleotide of claim 1.
25
8. A host cell genetically engineered to express the polynucleotide of claim
1.
9. The host cell of claim 8 wherein the polynucleotide is in operative
30 association with a regulatory sequence that controls expression of the polynucleotide in the host
cell.
10. An isolated polypeptide comprising an amino acid sequence which is at
least 80% identical to the amino acid sequence selected from the group consisting of SEQ ID NO:

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4-9, 14, or 16-18, the translated protein coding portion thereof, the mature protein coding portion thereof, the extracellular portion thereof, or the active domain thereof.

- 5
11. A composition comprising the polypeptide of claim 10 and a carrier.
12. A polypeptide, having prothrombinase-like activity, comprising at least ten consecutive amino acids from the polypeptide sequences selected from the group consisting of SEQ ID NO: 4-9, 14, or 16-18.
- 10
13. The polypeptide of claim 12, comprising at least five consecutive amino acids from the polypeptide sequences selected from the group consisting of SEQ ID NO. 4-9, 14, or 16-18.
- 15
14. A polynucleotide encoding a polypeptide according to claim 12.
15. A polynucleotide encoding a polypeptide according to claim 13.
16. A polynucleotide encoding a polypeptide according to claim 10.
- 20
17. An antibody specific for the polypeptide of claim 10.
18. A method for detecting the polynucleotide of claim 1 in a sample, comprising:
- 25
- a) contacting the sample with a compound that binds to and forms a complex with the polynucleotide of claim 1 for a period sufficient to form the complex; and
- b) detecting the complex, so that if a complex is detected, the polynucleotide of claim 1 is detected.
19. A method for detecting the polynucleotide of claim 1 in a sample, comprising:
- 30
- a) contacting the sample under stringent hybridization conditions with nucleic acid primers that anneal to the polynucleotide of claim 1 under such conditions;

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b) amplifying a product comprising at least a portion of the polynucleotide of claim 1; and
c) detecting said product and thereby the polynucleotide of claim 1 in the sample.

5

20. The method of claim 19, wherein the polynucleotide comprises an RNA molecule and the method further comprises reverse transcribing an annealed RNA molecule into a cDNA polynucleotide.

10

21. A method for detecting the polypeptide of claim 10 in a sample, comprising:

a) contacting the sample with a compound that binds to and forms a complex with the polypeptide under conditions and for a period sufficient to form the complex; and

15

b) detecting formation of the complex, so that if a complex formation is detected, the polypeptide of claim 10 is detected.

22. A method for identifying a compound that binds to the polypeptide of claim 10, comprising:

20

a) contacting the compound with the polypeptide of claim 10 under conditions and for a time sufficient to form a polypeptide/compound complex; and

b) detecting the complex, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide of claim 10 is identified.

25

23. A method for identifying a compound that binds to the polypeptide of claim 10, comprising:

a) contacting the compound with the polypeptide of claim 10, in a cell, for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a reporter gene sequence in the cell; and

30

b) detecting the complex by detecting reporter gene sequence expression, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide of claim 10 is identified.

24. A method of producing a prothrombinase-like polypeptide, comprising,

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- a) culturing the host cell of claim 8 under conditions sufficient to express the polypeptide in said cell; and
- b) isolating the polypeptide from the cell culture or cells of step (a).

5 25. A kit comprising the polypeptide of claim 10.

 26. A nucleic acid array comprising the polynucleotide of claim 1 or a unique segment of the polynucleotide of claim 1 attached to a surface.

10 27. The array of claim 26, wherein the array detects full-matches to the polynucleotide or a unique segment of the polynucleotide of claim 1.

 28. The array of claim 26, wherein the array detects mismatches to the polynucleotide or a unique segment of the polynucleotide of claim 1.

15

 29. A method of treatment of a subject in need of enhanced activity or expression of prothrombinase-like polypeptide of claim 10 comprising administering to the subject a composition selected from the group consisting of:

- a) a therapeutic amount of an agonist of said polypeptide;
- 20 b) a therapeutic amount of the polypeptide; and
- c) a therapeutic amount of a polynucleotide encoding the polypeptide in a form and under conditions such that the polypeptide is produced, and a pharmaceutically acceptable carrier.

25 30. A method of treatment of a subject having need to inhibit activity or expression of prothrombinase-like polypeptide of claim 10 comprising administering to the subject a composition selected from the group consisting of:

- a) a therapeutic amount of an antagonist to said polypeptide;
- b) a therapeutic amount of a polynucleotide that inhibits the expression of
- 30 the nucleotide sequence encoding said polypeptide; and
- c) a therapeutic amount of a polypeptide that competes with the prothrombinase-like polypeptide for its ligand, and a pharmaceutically acceptable carrier.

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31. An isolated polynucleotide with human prothrombinase activity comprising a nucleotide sequence of SEQ ID NO: 2 or 3, the mature protein coding portion thereof, or the active domain thereof.
- 5 32. An isolated polynucleotide encoding a polypeptide with biological activity, said polynucleotide which hybridizes to the complement of a polynucleotide of claim 31 under stringent hybridization conditions.
- 10 33. An isolated polynucleotide encoding a polypeptide with biological activity, said polynucleotide having greater than about 90% sequence identity with the polynucleotide of claim 31.
34. The polynucleotide of claim 31 which is a DNA sequence.
- 15 35. An isolated polynucleotide which comprises the complement of the polynucleotide of claim 31.
36. A vector comprising the polynucleotide of claim 31.
- 20 37. An expression vector comprising the polynucleotide of claim 31.
38. A host cell genetically engineered to express the polynucleotide of claim 31.
- 25 39. A host cell genetically engineered to contain the polynucleotide of claim 31 in operative association with a regulatory sequence that controls expression of the polynucleotide in the host cell.
- 30 40. An isolated polypeptide comprising an amino acid selected from the group consisting of SEQ ID NO: 4-9, the mature protein portion thereof, and active domain thereof.
41. A composition comprising the polypeptide of claim 40 and a carrier.
42. An antibody directed against the polypeptide of claim 40.

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43. A method for detecting the polynucleotide of claim 31 in a sample, comprising:

- 5 a) contacting the sample with a compound that binds to and forms a complex with the polynucleotide of claim 31 for a period sufficient to form the complex; and
b) detecting the complex, so that if a complex is detected, the polynucleotide of claim 31 is detected.

44. A method for detecting the polynucleotide of claim 31 in a sample, comprising:

- 10 a) contacting the sample under stringent hybridization conditions with nucleic acid primers that anneal to the polynucleotide of claim 31 under such conditions;
b) amplifying a product comprising at least a portion of the polynucleotide of claim 31; and
15 c) detecting said product and thereby the polynucleotide of claim 31 in the sample.

45. The method of claim 44, wherein the polynucleotide is an RNA molecule that encodes a polypeptide of claim 40, and the method further comprises reverse transcribing an annealed RNA molecule into a cDNA polynucleotide.

46. A method for detecting the polypeptide of claim 40 in a sample, comprising:

- 25 a) contacting the sample with a compound that binds to and forms a complex with the polypeptide under conditions and for a period sufficient to form the complex; and
b) detecting formation of the complex, so that if a complex formation is detected, the polypeptide of claim 40 is detected.

47. A method for identifying a compound that binds to the polypeptide of claim 40, comprising:

- 30 a) contacting the compound with the polypeptide of claim 40 under conditions and for a time sufficient to form a polypeptide/compound complex; and

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b) detecting the complex, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide of claim 40 is identified.

48. A method for identifying a compound that binds to the polypeptide of claim 40, comprising:

- 5 a) contacting the compound with the polypeptide of claim 40, in a cell, for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a reporter gene sequence in the cell; and
- 10 b) detecting the complex by detecting reporter gene sequence expression, so that if the polypeptide/compound complex is detected, a compound that binds to the polypeptide of claim 40 is identified.

49. A method of producing the polypeptide of claim 40, comprising,

- 15 a) culturing the host cell of claim 38 for a period of time sufficient to express the polypeptide in said cell; and
- b) isolating the polypeptide from the cell culture or cells of step (a).

50. A kit comprising the polypeptide of claim 40.

20 51. A nucleic acid array comprising the polynucleotide of claim 31 or a unique segment of the polynucleotide of claim 31 attached to a surface.

52. The array of claim 51, wherein the array detects full-matches to the polynucleotide or a unique segment of the polynucleotide of claim 31.

25

53. The array of claim 51, wherein the array detects mismatches to the polynucleotide or a unique segment of the polynucleotide of claim 31.

30 54. A method of treatment comprising administering to a mammalian subject in need thereof a therapeutic amount of a composition comprising a polypeptide of claim 10 and a pharmaceutically acceptable carrier.

55. A polypeptide having prothrombinase activity comprising at least ten consecutive amino acids from the group consisting of SEQ ID NO: 4-9.

56. The polypeptide of claim 55, comprising at least five consecutive amino acids from the group consisting of SEQ ID NO: 4-9.
- 5 57. A polynucleotide encoding a polypeptide according to claim 55.
58. A polynucleotide encoding a polypeptide according to claim 56.
59. A polynucleotide encoding a polypeptide according to claim 40.
- 10 60. An isolated polypeptide with prothrombinase activity having at least an amino acid sequence of SEQ ID NO: 9, wherein A=Alanine, C=Cysteine, D=Aspartic Acid, E= Glutamic Acid, F=Phenylalanine, G=Glycine, H=Histidine, I=Isoleucine, K=Lysine, L=Leucine, M=Methionine, N=Asparagine, P=Proline, Q=Glutamine, R=Arginine,
- 15 S=Serine, T=Threonine, V=Valine, W=Tryptophan, Y=Tyrosine.

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FIG.1-1

BLASTX ALIGNMENT OF SEQ ID NO: 4 (PROTHROMBINASE-LIKE WITH HUMAN PROTHROMBINASE FG 12 PROTEIN (PROTHROMBINASE Fg1)

The Genbank database indicates that the fibrinogen-like protein is homologous (37% identity)

>gi | 5730075 ref | NP_006673.1 || fibrinogen-like 2 precursor; fibroblastin
>pir || S47273 pT49 protein-human >pir || |37391 fibrinogen-like
protein expressed in T lymphocytes (pT49) - human
>emb | CAA85298.1 | (Z36531) fibrinogen-like protein [Homo sapiens]
>gb | AAD10825.1 | (AF104015) fibrinogen-like protein 2 [Homo sapiens]
Length = 439

Score = 402 (141.5 bits), Expect = 5.7e-36, P = 5.7e-36
Identities = 109/291 (37%), Positives = 155/291 (53%)

PROTHROMBINASE-LIKE: 98 L RNMDEQASLDYL SNQVNELMNRVLLTTEVFRKQLDPFHRPVQSHGL---DCTDIDK 155
+ N +D + A+L ++ VN L + ++ +Q+ RPVQ H + DC+D
PROTHROMBINASE Fg12: 169 I ENYVDSKVANLTFV---YNSLDGKCKSCPSQ---EQ IQS---RPVQ-HL IYKDCSDYY- 217

TO FIG.1-2

FIG. 1-2

FROM FIG. 1-1

PROTHROMBINASE-LIKE: 156 TIGSVTKTPSGLYI IHPEGSSYPFEVWCDMDYRGGWTVIQKRIDGIDFQRLWCDYLDG 215
 IG K S Y + P+ + FEV CDM+ GGGWTV+Q R+DG +F R W DY G
 PROTHROMBINASE FG12:218 AIG---KRSSETYRVTDPKNSSEFVYCDMETMGGGWTVLQARLDGSTNFTRTWQDYKAG 274

 PROTHROMBINASE-LIKE: 216 FGDLLGEFWLGLKIFYIYNQKNTSFMLYVALESEDDTLAYASYDNFWLEDETRFFKMHL 275
 FG+L EFWLG KI + K +L + LE + YA YD F++ +E +++H+
 PROTHROMBINASE FG12:275 FGNLRREFWLGNDKIHLTKSKE---MILRIDLEDFNGVELYALYDQFYVANEFLKYRLHV 332

 PROTHROMBINASE-LIKE: 276 GRYSGNAGDAFRGLKKEDNQNPFFSTDVDNDGCRPACLVNGQSVKSCSHLHNKTGWWF 335
 G Y+G AGDA R K N + F+T D DND P+ G +C L+ +GWWF
 PROTHROMBINASE FG12:333 GNYNGTAGDALP-FNKHYNHDLKFFTTPDKNDRY-PS---G----NCG-LYYSSGWWF 381

 PROTHROMBINASE-LIKE: 336 NECGLANLNG- IHHFSGKLLATG IQWGTW---TKNNSPVKIKSVSMKIRRMYPN-YFK 388
 + C ANLNG +H + + GI WGTW P KS + + M P +FK
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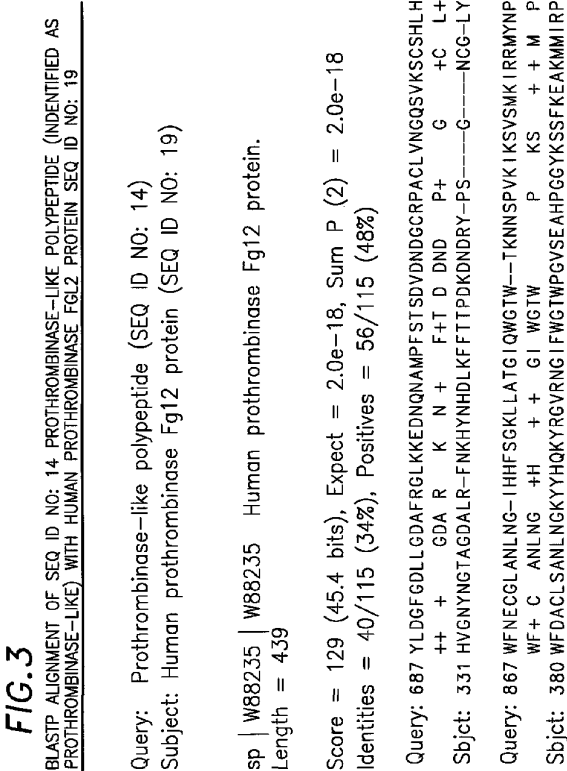
PCT/US00/35061

FIG. 2

BLASTP ALIGNMENT OF SEQ ID NO: 14 PROTHROMBINASE-LIKE POLYPEPTIDE (IDENTIFIED AS PROTHROMBINASE-LIKE) WITH HUMAN PROTHROMBINASE Fg12 PROTEIN SEQ ID NO: 19

Query: Prothrombinase-like polypeptide (SEQ ID NO: 14)
Subject: Human prothrombinase Fg12 protein (SEQ ID NO: 19)
sp | W88235 | W88235 Human prothrombinase Fg12 protein.
Length = 439
Score = 175 (61.6 bits), Expect = 4.0e-18, Sum P (2) = 4.0e-18
Identities = 49/132 (37%), Positives = 72/132 (54%)

Query: 345 LRNMDEQQASLDYLSNQVNLNMRVLLLTTEVFRKQLDPFPHRPVQSHGL---DCTDIKD 518
+ N +D + A+L ++ VN L + ++ +Q+ RPVQ H + DC+D
Sbjct: 169 IENYVDSKVANLTFV---VNSLDGKCKSKPSQ---EQIQS---RPVQ-HLIYKDCSDYY- 217
Query: 519 TIGSVTKTPSGLYIHPGSSYPFEVCMMDYRGSGWTVIOKRIDGIIIDFQRLWCDYLDG 698
IG K S Y + P+ + FEV CDM+ GGGWTV+Q R+DG +F R W DY G
Sbjct: 218 AIG---KRSSETYRVTDPDKNSSFEVYCDMETMGGGWTVLQARLDGSTNFTRTWQDYKAG 274
Query: 699 FGDLLGDAFRGLKK 740
FG+L + + G K
Sbjct: 275 FGNLRRREFWLGNDK 288



MSI GENEATLAS ALIGNMENT OF SEQ ID NO: 14 PROTHROMBINASE-LIKE POLYPEPTIDE (IDENTIFIED AS "PROTHROMBINASE-LIKE") WITH HUMAN BLOOD COAGULATION PROTEIN SEQ ID NO: 20

Query: Prothrombinase-like polypeptide (SEQ ID NO: 14)
 Subject: Human blood coagulation protein 1fzc_B (pdb number) (SEQ ID NO: 20)
 Score = 86.22 (bits), estimated P Value = 7.2e-04, upper limit P Value = 1.1e-11
 Identities = 72/224 (32%), Similarities = 155/224 (69%) Bits per residue = 2.0 (gapless)

[illegible]

Query:

PVQSHGLDCTDKDTIGSVTKTPSGLYIHPEGSSYPFEVMCDMDYRGGWTVIQRIDG
||| : : : : : ||| : : : : : ||| : : : : : ||| : : : : : |||

Subject:

PVWS.GKECEEIRKGETISE...MYLIQPDSSVKPYRYCYDDMTENGWTVIQNRQDG
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TO FIG. 4-2

FROM FIG. 4-1

[illegible]

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SEQUENCE LISTING

<110> HYSEQ, Inc.
 Haley, Dana A
 Boyle, Bryan J
 Ho, Alice S
 Zhou, Ping
 Mize, Nancy K
 Kuo, Chia-yun
 Arterburn, Matthew C
 Tang, Y. Tom
 Liu, Chengkua
 Drmanac, Radoje T

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 Polypeptides and Polynucleotides

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gggggggggc ccccccact ttttttttt taaaaaaaag agaaaattcc ccccttttgt 1987
gggaaggggg gttttttttt ttggggg 2013

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<210> 4
 <211> 388
 <212> PRT
 <213> Homo sapiens

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<400> 4
Met Met Ser Pro Ser Gln Ala Ser Leu Leu Phe Leu Asn Val Cys Ile
1 5 10 15
Phe Ile Cys Gly Glu Val Val Gln Gly Asn Cys Val His His Ser Thr
20 25 30
Asp Ser Ser Val Val Asn Ile Val Glu Asp Gly Ser Asn Ala Lys Asp
35 40 45
Glu Ser Lys Ser Asn Asp Thr Val Cys Lys Glu Asp Cys Glu Glu Ser
50 55 60
Cys Asp Val Lys Thr Lys Ile Thr Arg Glu Glu Lys His Phe Met Cys
65 70 75 80
Arg Asn Leu Gln Asn Ser Ile Val Ser Tyr Thr Arg Ser Thr Lys Lys
85 90 95
Leu Leu Arg Asn Met Met Asp Glu Gln Gln Ala Ser Leu Asp Tyr Leu
100 105 110
Ser Asn Gln Val Asn Glu Leu Met Asn Arg Val Leu Leu Leu Thr Thr
115 120 125
Glu Val Phe Arg Lys Gln Leu Asp Pro Phe Pro His Arg Pro Val Gln
130 135 140
Ser His Gly Leu Asp Cys Thr Asp Ile Lys Asp Thr Ile Gly Ser Val

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145          150          155          160
Thr Lys Thr Pro Ser Gly Leu Tyr Ile Ile His Pro Glu Gly Ser Ser
Tyr Pro Phe Glu Val Met Cys Asp Met Asp Tyr Arg Gly Gly Gly Trp
165          170          175
Thr Val Ile Gln Lys Arg Ile Asp Gly Ile Ile Asp Phe Gln Arg Leu
180          185          190
Trp Cys Asp Tyr Leu Asp Gly Phe Gly Asp Leu Leu Gly Glu Phe Trp
195          200          205
Leu Gly Leu Lys Lys Ile Phe Tyr Ile Val Asn Gln Lys Asn Thr Ser
210          215          220
Phe Met Leu Tyr Val Ala Leu Glu Ser Glu Asp Asp Thr Leu Ala Tyr
225          230          235
Ala Ser Tyr Asp Asn Phe Trp Leu Glu Asp Glu Thr Arg Phe Phe Lys
240          245          250
Met His Leu Gly Arg Tyr Ser Gly Asn Ala Gly Asp Ala Phe Arg Gly
255          260          265
Leu Lys Lys Glu Asp Asn Gln Asn Ala Met Pro Phe Ser Thr Ser Asp
270          275          280
Val Asp Asn Asp Gly Cys Arg Pro Ala Cys Leu Val Asn Gly Gln Ser
285          290          300
Val Lys Ser Cys Ser His Leu His Asn Lys Thr Gly Trp Trp Phe Asn
305          310          315
Glu Cys Gly Leu Ala Asn Leu Asn Gly Ile His His Phe Ser Gly Lys
320          325          330
Leu Leu Ala Thr Gly Ile Gln Trp Gly Thr Trp Thr Lys Asn Asn Ser
335          340          345
Pro Val Lys Ile Lys Ser Val Ser Met Lys Ile Arg Arg Met Tyr Asn
350          355          360
Pro Tyr Phe Lys
365          370          375          380          385

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<210> 5
<211> 37
<212> PRT
<213> Homo sapiens

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<400> 5
Val Met Cys Asp Met Asp Tyr Arg Gly Gly Gly Trp Thr Val Ile Gln
1          5          10          15
Lys Arg Ile Asp Gly Ile Ile Asp Phe Gln Arg Leu Trp Cys Asp Tyr
20          25          30
Leu Asp Gly Phe Gly
35

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<210> 6
<211> 30
<212> PRT
<213> Homo sapiens

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<400> 6
Cys Ser His Leu His Asn Lys Thr Gly Trp Trp Phe Asn Glu Cys Gly
1          5          10          15
Leu Ala Asn Leu Asn Gly Ile His His Phe Ser Gly Lys Leu
20          25          30

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<210> 7
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 7
 Phe Lys Met His Leu Gly Arg Tyr Ser Gly Asn Ala Gly Asp Ala
 1 5 10 15

<210> 8
 <211> 13
 <212> PRT
 <213> Homo sapiens

<400> 8
 Glu Phe Trp Leu Gly Leu Lys Lys Ile Phe Tyr Ile Val
 1 5 10

<210> 9
 <211> 368
 <212> PRT
 <213> Homo sapiens

<400> 9
 Glu Val Val Gln Gly Asn Cys Val His His Ser Thr Asp Ser Ser Val
 1 5 10 15
 Val Asn Ile Val Glu Asp Gly Ser Asn Ala Lys Asp Glu Ser Lys Ser
 20 25 30
 Asn Asp Thr Val Cys Lys Glu Asp Cys Glu Glu Ser Cys Asp Val Lys
 35 40 45
 Thr Lys Ile Thr Arg Glu Glu Lys His Phe Met Cys Arg Asn Leu Gln
 50 55 60
 Asn Ser Ile Val Ser Tyr Thr Arg Ser Thr Lys Lys Leu Leu Arg Asn
 65 70 75 80
 Met Met Asp Glu Gln Gln Ala Ser Leu Asp Tyr Leu Ser Asn Gln Val
 85 90 95
 Asn Glu Leu Met Asn Arg Val Leu Leu Leu Thr Thr Glu Val Phe Arg
 100 105 110
 Lys Gln Leu Asp Pro Phe Pro His Arg Pro Val Gln Ser His Gly Leu
 115 120 125
 Asp Cys Thr Asp Ile Lys Asp Thr Ile Gly Ser Val Thr Lys Thr Pro
 130 135 140
 Ser Gly Leu Tyr Ile Ile His Pro Glu Gly Ser Ser Tyr Pro Phe Glu
 145 150 155 160
 Val Met Cys Asp Met Asp Tyr Arg Gly Gly Gly Trp Thr Val Ile Gln
 165 170 175
 Lys Arg Ile Asp Gly Ile Ile Asp Phe Gln Arg Leu Trp Cys Asp Tyr
 180 185 190
 Leu Asp Gly Phe Gly Asp Leu Leu Gly Glu Phe Trp Leu Gly Leu Lys
 6

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195 200 205
 Lys Ile Phe Tyr Ile Val Asn Gln Lys Asn Thr Ser Phe Met Leu Tyr
 210 215 220
 Val Ala Leu Glu Ser Glu Asp Asp Thr Leu Ala Tyr Ala Ser Tyr Asp
 225 230 235 240
 Asn Phe Trp Leu Glu Asp Glu Thr Arg Phe Phe Lys Met His Leu Gly
 245 250 255
 Arg Tyr Ser Gly Asn Ala Gly Asp Ala Phe Arg Gly Leu Lys Lys Glu
 260 265 270
 Asp Asn Gln Asn Ala Met Pro Phe Ser Thr Ser Asp Val Asp Asn Asp
 275 280 285
 Gly Cys Arg Pro Ala Cys Leu Val Asn Gly Gln Ser Val Lys Ser Cys
 290 295 300
 Ser His Leu His Asn Lys Thr Gly Trp Trp Phe Asn Glu Cys Gly Leu
 305 310 315 320
 Ala Asn Leu Asn Gly Ile His His Phe Ser Gly Lys Leu Leu Ala Thr
 325 330 335
 Gly Ile Gln Trp Gly Thr Trp Thr Lys Asn Asn Ser Pro Val Lys Ile
 340 345 350
 Lys Ser Val Ser Met Lys Ile Arg Arg Met Tyr Asn Pro Tyr Phe Lys
 355 360 365

<210> 10
 <211> 2352
 <212> DNA
 <213> Homo sapiens

<400> 10
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 gaagatttat aggttagaggc gacaaacctt ccgggctctg tcatagctgg ttgtccaaaga 120
 tagaatutta gtccaacttt aaatttgccc acggaacctt ctaaatcccc ttgttaaatit 180
 aactgttagt ccaagcagga acagctcttt ggacactagg aaaaaaactt gttagagagag 240
 taaaaaattt anccccata gtaggcctaa aagcagccac caaaaaaaa gccggcctgac 300
 tcatatttga agaagtgttt tcatctatcc aagaaaaata tcatgtctcc atccccagcc 360
 tcatctttat tottaaatgt atgtattttt atttgggag aagttgtaca aggttaactgt 420
 gtacatcatt ctacggactc ttcagtagtt aacattgtag aagatggato taatgcanaa 480
 gatgaagta aaagtaatga tactgtttgt aaggaagact gtgaggatc atgtgatgtt 540
 aaactaana ttacacgaga agnaaaacat ttcattgtga gaattttgca aaattctatt 600
 gtttcttaca caagaagtac caaaaaacta ctaaggaata tcatggatga gcaacagct 660
 tcttgggatt atttatctaa tcaaggtaac gactcactga atagatttct ccttttgact 720
 acagaagtgt ttgaaaaaca gctggatcct tttctcaca gacctgttca gtacatgggt 780
 tttagattga ctgatattaa ggtaccattt ggctctgcca ccaaaacacc gactgggtta 840
 taataatc acccgaagg atctagctac caatttgagg taatgtgtga catggattac 900
 agaggagggt gatggactgt gatcacagaa gaattgatg ggataattga tttccagagg 960
 ttgtgggtgt attatcttga tggatttggg gatcttctag gagaatttgg gctaggacgt 1020
 aaaaagattt ttatatagt aaatcagaaa aatccagttt ttatgctgta tgtggctttg 1080
 gaatcagaag atgacactct tgcattatga tcatatgata atttttggct agaggatgaa 1140
 acgagatttt ttaaaatgca cttaggcggg tattcaggaa atgctgttga tgcattccgg 1200
 ggtctcaaaa aagaagataa tcaaaatgca atgcctttta gcaatcaga tgttgataat 1260

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gatgggtgtc gccctgcacg cctggccaat ggtcagctcg tgaagagctg cagtcacctc 1320
catacaaga cggctgggtg gtttaacagag tctggcttag caaatctaaa tggcattccat 1380
ccctctcttg gaaatttgtt tgnactgga attcaatggg gncctggnc caaaacacac 1440
tcacctgtca agattaaatc tgtttcaatg aaatttagaa gaatgtacaa tccatatttt 1500
aaataatctc atttaacatt gtaatgcaag ttctacaatg alaatatatt aaagattttt 1560
asaagtattat cttttccatt agtggttoan acatatingg caaatrttaa ctgtagatcg 1620
catttagatg ttatgagttt aattagaaaa cttaattttt gtatatttct ataaagaaa 1680
acatggotta ttgtatgttt ttactctbga ctatattaac aatatacaat gaaattttgt 1740
toagtgaaac tacaacttgt ctctctaaaa ttatagtgaa ttttaagga ttttgccttt 1800
tccttggaac atttttaaac cataatatgt tgaaggnaa atgaaggga atattttact 1860
tatttttata ctttatatga ttatataatc tacagataat ttctaactga gacagttaac 1920
ataataaact ttatgcagat taatataaa gctacacatg atgtaaaaac cttaatttt 1980
ctaggtgatg ccataccatt ttaaaagtag taagagtttg ctgccccaat agtttttctt 2040
gttttcabat ctaatcatgg ttaactattt tgttattgtt tgaataaat atagttaact 2100
ttatatcttg aaaaaaanaa aaaaaaanaa aaaaaaanaa aaaaaaanaa aaaaagggcg 2160
ccctttcaat taaaaagggc cttttaaacc cgggttaaac cccggagggg ggtttttata 2220
gggccccccc ttggtgtgtg cccccccccc ggggtttttt ttgaoggggg gggggggccc 2280
ccactttttt tttttttaa aaaaagagaa aattccccc ttttgggga aggggggttt 2340
ttttttttgg gg

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<210> 11
 <211> 453
 <212> DNA
 <213> Homo sapiens

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<400> 11
taatcgang agcccgatt nctgatattt gcngncccg ttctatctat ccaagaaaaa 60
tagatgtct ccacccctag ccttaactctt attcttaaat gtagtatttt ttgtttgtgg 120
agaagctgta caaggttaact gtgtacatca ttctaaaggac tcttaagtag ttaacattgt 180
agaagctgga tctaatgcaa agatgaag tanaagtant gatactgttt gtaagaaaga 240
ctgtgaggaat tcatgtgatg ttaaaactaa aattacacga gaagaaaaac atttca:gtg 300
tagaattttg caaatctcta ttgttctcta cacaagaagt accaaaaaac tactaaggaa 360
tagatggat gagcaacaag cttccttggg ttatttatct aatcaaggtaa tgtgtgcat 420
ggattacaga ggaggtggat ggaactgtgat aca 453

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<210> 12
 <211> 1648
 <212> DNA
 <213> Homo sapiens

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<400> 12
ggcgaccca cgggtccgat ttgaagaagt gttttcatct atccaagaaa aatatgatgt 60
ctccatccca agcctcactc ttatctctaa atgtatgtat ttttatitgt ggaagaagtg 120
tacaaggtaa ctgtgtacat catctctacg actcttcaag agttaacatt gtagaagatg 180
gatctaagtc anaagatgaa agtaaaagta atgatactgt ttgaaggaa gactgtgagg 240
aatcatgtga tgtttaaact aaaaatacac gagaagaaaa acatttcatg tgaagaattt 300
tgcaaaaattc tattgttttc tacacaagaa gtaccaaana actactaagg aatatgatgg 360
atgagcaaca agcttctctg gattatttat ctaatcaggt taaagagctc atgaatagag 420
ttctctctttt gactacagaa gtttttagaa aacagctgga tctctttctc caccagacctg 480
caccagctgg ttataacata altcaccacg aaggatctag ctacccattt gaggtaatgt 600
gtgacatgga ttacagagga ggtggatgga ctgtgataca gaaaagaatt gatgggataa 660
ttgattttca gaggttgtgg tgtgattatc tggatggatt tgaagatctt ctagggtgatg 720
catttcgggg totcaaaaaa gaagataatc aaaaatgcaat gcccttttagc acatcagatg 780
ltgataatga tgggtgtcgc cctgcaatgc ttgtcaatgg tgaactgtgg aagagcgaga 840
gtacactcca taacaagacc ggtgtgtgtt ttacacagtg tggctcagtg aatctaaatg 900
gcattcaaca ctctctctgga aaatgtgttg caactgggaat tcaatggggc acgtggacca 960
aaaaacaactc acctgtcaag attaaactgt ttctaatgaa aattagaaga atgtacatc 1020
catatttttaa ataatctcat ttaacattgt aatgcaagtt ctacaatgat aatattattaa 1080
agattttttaa aagtttatct tttcacttag tgtttcaaac atattaggca aaattttaact 1140
gtagatggca tttagatgtt atgagtttaa ttgaaaaact tcaattttgt agtattctat 1200

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aaaagaaaac atggcttatt gtatgttttt acttctgact atattaacaa tatacaatga 1260
aatttgtttc aagtgaacta caacttgtct toctaaaatt tatagtgaat ttaagggaat 1320
ttgctttttc ttgaagat tttaaaacca taatagttg taaggaaaat tgaagggaat 1380
attttactta tttttatact ttataagatt atataatga cagataatt ctaatgaaga 1440
cagttacaat aataacttt atgcagatta atataaaga taacatgat gtaaaaacct 1500
tactatttct aggtgatgcc ataccatttt aaaagtagta agagtttgct gcccaaatag 1560
tttttcttgt ttctatatct aatcatggtt aactattttg ttattgtttg taataaatat 1620
atgtactttt atactctgaa aaaaaaaa 1648

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<210> 13
<211> 1651
<212> DNA
<213> Homo sapiens

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<220>
<221> CDS
<222> (54)..(1034)

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<400> 13
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Met
1
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Met Ser Pro Ser Gln Ala Ser Leu Leu Phe Leu Asn Val Cys Ile Phe
5 10 15
att tgt gga gaa gtt gta caa ggt aac tgt gta cat cat tct acg gac 152
Ile Cys Gly Glu Val Val Gln Gly Asn Cys Val His His Ser Thr Asp
20 25 30
tct tca gta gtt aac att gta gaa gat gga tct aat gca aaa gat gaa 200
Ser Ser Val Val Asn Ile Val Glu Asp Gly Ser Asn Ala Lys Asp Glu
35 40 45
agt aaa agt aat gat act gtt tgt aag gaa gac tgt gag gaa tca tgt 248
Ser Lys Ser Asn Asp Thr Val Cys Lys Glu Asp Cys Glu Glu Ser Cys
50 55 60 65
gat gtt aaa act aaa att aca cga gaa gaa aaa cat ttc atg tgt aga 296
Asp Val Lys Thr Lys Ile Thr Arg Glu Glu Lys His Phe Met Cys Arg
70 75 80
aat ttg caa aat tct att gtt tcc tac tca aga agt acc aaa aaa cta 344
Asn Leu Gln Asn Ser Ile Val Ser Tyr Thr Arg Ser Thr Lys Lys Leu
85 90 95
cta agg aat atg atg gat gag caa caa gct tcc ttg gat tat tta tct 392
Leu Arg Asn Met Met Asp Glu Gln Gln Ala Ser Leu Asp Tyr Leu Ser
100 105 110
aat cag gtt aac gag ctc atg aat aga gtt ctc ctt ttg act aca gaa 440
Asn Gln Val Asn Glu Leu Met Asn Arg Val Leu Leu Leu Thr Thr Glu
115 120 125
gtt ttt aga aaa cag ctg gat cct ttt cct cac aga cct gtt cag tca 488
Val Phe Arg Lys Gln Leu Asp Pro Phe Pro His Arg Pro Val Gln Ser
130 135 140 145
cat ggt tta gat tgc act gat att aag gat acc att ggc tot gtc acc 536
His Gly Leu Asp Cys Thr Asp Ile Lys Asp Thr Ile Gly Ser Val Thr
150 155 160

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aaa aca cag agt ggt tta tac ata att cac cca gaa gga tct agc tac 584
Lys Thr Pro Ser Gly Leu Tyr Ile Ile His Pro Glu Gly Ser Ser Tyr
165 170 175

cca ttt gag gta atg tgt gac atg gat tac aga gga ggt gga tgg act 632
Pro Phe Glu Val Met Cys Asp Met Asp Tyr Arg Gly Gly Gly Trp Thr
180 185 190

gtg ata cag aaa aga att gat ggg ata att gat ttc cag agg ttg tgg 680
Val Ile Gln Lys Arg Ile Asp Gly Ile Ile Asp Phe Gln Arg Leu Trp
195 200 205

tgt gat tat ctg gat gga ttt gga gat ctt cta ggt gat gca ttc cgg 728
Cys Asp Tyr Leu Asp Gly Phe Gly Asp Leu Leu Gly Asp Ala Phe Arg
210 215 220 225

ggt ctc aaa aaa gaa gat aat caa aat gca atg cct ttt agc aca tca 776
Gly Leu Lys Lys Glu Asp Asn Gln Asn Ala Met Pro Phe Ser Thr Ser
230 235 240

gat gtt gat aat gat ggg tgt cgc cct gca tgc ctg gtc aat ggt cag 824
Asp Val Asp Asn Asp Gly Cys Arg Pro Ala Cys Leu Val Asn Gly Gln
245 250 255

tct gtg aag agc tgc agt cac ctc cat aac aag acc ggc tgg tgg ttt 872
Ser Val Lys Ser Cys Ser His Leu His Asn Lys Thr Gly Trp Trp Phe
260 265 270

aac gag tgt ggt cta gca aat cta aat ggc att cat cac ttc tct gga 920
Asn Glu Cys Gly Leu Ala Asn Leu Asn Gly Ile His His Phe Ser Gly
275 280 285

aaa ttg ctt gca act gga att caa tgg ggc acg tgg acc aaa aac aac 968
Lys Leu Leu Ala Thr Gly Ile Gln Trp Gly Thr Trp Thr Lys Asn Asn
290 295 300 305

tca cct gtc aag att aaa tct gtt tca atg aaa att aga aga atg tac 1016
Ser Pro Val Lys Ile Lys Ser Val Ser Met Lys Ile Arg Arg Met Tyr
310 315 320

aat cca tat ttt aaa taa tctcatttaa cattgtaatg caagttctac 1064
Asn Pro Tyr Phe Lys
325

aatgataata tattaagat ttttaaaagt ttatcttttc acttagtgtt tcaaacatat 1124

taggcataaat ttaactgtag atggcattta gatgttatga gtttaattag aaaacttcaa 1184

ttttgtagta ttctataaaa gaaaacatgg cttattgtat gtttttaact ctgactatat 1244

taacaatata caatgaaatt tgtttcaagt gaactacaac ttgtcttact aaaatttata 1304

gtgattttta aggattttgc cttttctttg aagcaatttt aaaccataat atgttgtaag 1364

gaaaattgan gggaaatatt tacttatttt tatactttat atgattatat aactacacaga 1424

taatttttac tgaagacagt tacaataaat aactttatgc agattaatat ataagctaca 1484

catgatgtaa aaaccttact atttctaggt gatgccatad ctttttaaaa gtagtaagag 1544

tttgtctgcc aaatagtttt tcttgttttc atatctaac atggttaact atttggttat 1604

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tggttgtaaat aaatatatgt actttttaaaa aaacacagaaa aaaaaaa

1651

<210> 14

<211> 326

<212> PRT

<213> Homo sapiens

<400> 14

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Met Met Ser Pro Ser Gln Ala Ser Leu Leu Phe Leu Asn Val Cys Ile
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Phe Ile Cys Gly Glu Val Val Gln Gly Asn Cys Val His His Ser Thr
 20           25           30
Asp Ser Ser Val Val Asn Ile Val Glu Asp Gly Ser Asn Ala Lys Asp
 35           40           45
Glu Ser Lys Ser Asn Asp Thr Val Cys Lys Glu Asp Cys Glu Glu Ser
 50           55           60
Cys Asp Val Lys Thr Lys Ile Thr Arg Glu Glu Lys His Phe Met Cys
 65           70           75
Arg Asn Leu Gln Asn Ser Ile Val Ser Tyr Thr Arg Ser Thr Lys Lys
 80           85           90
Leu Leu Arg Asn Met Met Asp Glu Gln Gln Ala Ser Leu Asp Tyr Leu
100          105          110
Ser Asn Gln Val Asn Glu Leu Met Asn Arg Val Leu Leu Leu Thr Thr
115          120          125
Glu Val Phe Arg Lys Gln Leu Asp Pro Phe Pro His Arg Pro Val Gln
130          135          140
Ser His Gly Leu Asp Cys Thr Asp Ile Lys Asp Thr Ile Gly Ser Val
145          150          155
Thr Lys Thr Pro Ser Gly Leu Tyr Ile Ile His Pro Glu Gly Ser Ser
160          165          170
Tyr Pro Phe Glu Val Met Cys Asp Met Asp Tyr Arg Gly Gly Gly Trp
175          180          185
Thr Val Ile Gln Lys Arg Ile Asp Gly Ile Ile Asp Phe Gln Arg Leu
190          195          200
Trp Cys Asp Tyr Leu Asp Gly Phe Gly Asp Leu Leu Gly Asp Ala Phe
205          210          215
Arg Gly Leu Lys Lys Glu Asp Asn Gln Asn Ala Met Pro Phe Ser Thr
220          225          230
Ser Asp Val Asp Asn Asp Gly Cys Arg Pro Ala Cys Leu Val Asn Gly
235          240          245
Gln Ser Val Lys Ser Cys Ser His Leu His Asn Lys Thr Gly Trp Trp
250          255          260
Phe Asn Glu Cys Gly Leu Ala Asn Leu Asn Gly Ile His His Phe Ser
265          270          275
Gly Lys Leu Leu Ala Thr Gly Ile Gln Trp Gly Thr Trp Thr Lys Asn
280          285          290
Asn Ser Pro Val Lys Ile Lys Ser Val Ser Met Lys Ile Arg Arg Met
295          300          305
Tyr Asn Pro Tyr Phe Lys
310          315          320
325

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<210> 15

<211> 981

<212> DNA

<213> Homo sapiens

<400> 15

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atgatgtctc catcccaagc ctccactctta ttcttaaatg tatgtatttt tatttgagg 60
gaagttgtac aaggttaactg tgtacatcat tctacggact cttcagtagt taacattgta 120
gaagatggat ctaatgcaaa agatgaaagt aaaagtaatg atactgtttg taaggaagac 180

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tgtggggaat catgtgatgt taaaactaaa attacacgag aagaaaaaca ttctatgtgt 240
agaaatttgc aaatttctat tgtttcctac acaagaagta ccaaaaaact actaagggaat 300
atgatggatg agcaacaagc ttctttggat tatttatcta atcaggtraa cgagctcag 360
aatagagttc tctttttgac tacagaagtt tttagaaaac agctggatcc ttctctcacc 420
agacctgttc agtcacatgg tttagattgc actgatatta aggataccat tggctctgtc 480
acaaaaacac cgatgggttt atacataatt caccagaag gatctagcta cccatttgag 540
gtaatgtgtg acatggatta cagaggaggt ggatggactg tgatacagaa aagaattgat 600
gggataattg atttccagag gttgtggtgt gattatctgg atggatttgg agatcttcta 660
gggtgatcat tccgggtctc caaaaaagaa gataatcaaa atgcaatgcc ttttagcaca 720
tcagatgttg ataagtgatg gtgtgcctct gcctgcctgg tcaatgggtc gtctgtgaag 780
agctgcagtc aactccataa caagaccggc tgggtggtta acgagtgtgg cttagcaaat 840
ctaaatggca ttcatcattt ctctgaaaaa tgccttgcaa ctggaattca atggggcacc 900
tggaccaaaa acaactcacc tgtcaagatt aaatctgttt caatgaaat tagaagaatg 960
tacaatccat attttaata a

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<210> 16
 <211> 20
 <212> PRT
 <213> Homo sapiens

<400> 16
 Met Met Ser Pro Ser Gln Ala Ser Leu Leu Phe Leu Asn Val Cys Ile
 1 5 10 15

Phe Ile Cys Gly
 20

<210> 17
 <211> 240
 <212> PRT
 <213> Homo sapiens

<400> 17
 Asn Leu Gln Asn Ser Ile Val Ser Tyr Thr Arg Ser Thr Lys Lys Leu
 1 5 10 15

Leu Arg Asn Met Met Asp Glu Gln Gln Ala Ser Leu Asp Tyr Leu Ser
 20 25 30

Asn Gln Val Asn Glu Leu Met Asn Arg Val Leu Leu Leu Thr Thr Glu
 35 40 45

Val Phe Arg Lys Gln Leu Asp Pro Phe Pro His Arg Pro Val Gln Ser
 50 55 60

His Gly Leu Asp Cys Thr Asp Ile Lys Asp Thr Ile Gly Ser Val Thr
 65 70 75 80

Lys Thr Pro Ser Gly Leu Tyr Ile Ile His Pro Glu Gly Ser Ser Tyr
 85 90 95

Pro Phe Glu Val Met Cys Asp Met Asp Tyr Arg Gly Gly Gly Trp Thr
 100 105 110

Val Ile Gln Lys Arg Ile Asp Gly Ile Ile Asp Phe Gln Arg Leu Trp
 115 120 125

Cys Asp Tyr Leu Asp Gly Phe Gly Asp Leu Leu Gly Asp Ala Phe Arg
 130 135 140

Gly Leu Lys Lys Glu Asp Asn Gln Asn Ala Met Pro Phe Ser Thr Ser
 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240

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145 150 155 160
 Asp Val Asp Asn Asp Gly Cys Arg Pro Ala Cys Leu Val Asn Gly Gln
 165 170 175
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【国際調査報告】

INTERNATIONAL SEARCH REPORT		International application No. PCT/US00/55081
A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : Please See Extra Sheet. US CL : 435/69.1, 69.2, 183, 252.9, 320.1, 325, ; 580/550; 586/23.1 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/69.1, 69.2, 183, 252.9, 320.1, 325, ; 580/550; 586/23.1 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN (Bioscience), East (all databases), sequence search for SEQ ID NO.2, search terms: angiopoietin, prothrombinase, fg12.		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Database GenEmbl on GenCore, Compugen Ltd. Acc. No. AC015500, BIRREN et al. Homo Sapiens, clone RP11-21G19, Direct Submission, March 2000.	1, 2, 4, 31-34
Y,P		6-9, 24, 26, 36-39, 49
A,P	LEVY et al. Molecular and Functional Analysis of the Human Prothrombinase Gene (HFGL2) and its Role in Viral Hepatitis. Am. J. Path. April 2000. Vol. 156, No. 4, pp. 1217-1225.	1-4, 6-16, 24-26, 31-34, 36-41, 49-50, 55-60
A	GALE et al. Growth factors acting via endothelial cell-specific receptor tyrosine kinases: VEGFs, Angiopoietins, and ephrins in vascular development. Genes & Dev. 1999, Vol. 13, pp. 1055-1066.	1-4, 6-16, 24-26, 31-34, 36-41, 49-50, 55-60
☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents "A" document defining the general state of the art which is not considered to be of particular relevance "B" earlier document published on or after the international filing date "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "Z" document number of the same patent family		
Date of the actual completion of the international search 05 JULY 2001		Date of mailing of the international search report 07 AUG 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 905-8930		Authorized officer HOLLY SCHMIDT Telephone No. (703) 508-6196

INTERNATIONAL SEARCH REPORT		International application No. PCT/US00/55061
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database GenEmbl on GenCore, Compugen Ltd. Acc. No. AX079973, BALLINGER et al. Novel angiopoietin materials and methods, WO 0105825, January 25, 2001, see especially title.	1-4, 6-16, 24-26, 31-34, 36-41, 49-50, 53-60

INTERNATIONAL SEARCH REPORT		International application No. PCT/US00/55061
Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)		
This international report has not been established in respect of certain claims under Article 17(a)(a) for the following reasons:		
1.	☐	Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2.	☐	Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.	☒	Claims Nos.: 27, 28, 46, 52, AND 55 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)		
This International Searching Authority found multiple inventions in this international application, as follows:		
Please See Extra Sheet.		
1.	☐	As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.	☐	As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.	☐	As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.	☒	No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-4, 6-16, 24-25, 31-34, 36-41, 49, 50, 55-60 as they relate to SEQ ID NO:2
Remark on Protest		☐ The additional search fees were accompanied by the applicant's protest.
		☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/35061

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C07H 21/02, 21/04; C07K 14/00; C12N 1/21, 2/00, 15/00, 15/65, 15/85, 15/86

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-4, 6-16, 24-26, 31-34, 36-41, 49-50, and 55-60, drawn to a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 8-5, 10, 12-15, and 15, a recombinant vector, a host cell, a method of producing the encoded polypeptide, and a polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence selected from the group consisting of SEQ ID NO: 4-9, 14, and 16-18. Applicant must elect one nucleic acid sequence and one protein sequence (see species election below).

Group II, claim(s) 17 and 42, drawn to an antibody that selectively binds the claimed polypeptides.

Group III, claim(s) 18 and 43, drawn to a method of detecting a polynucleotide by hybridization assay.

Group IV, claims 19-20 and 44 drawn to a method of detecting a polynucleotide by PCR.

Group V, claims 21-22 and 45-47, drawn to a protein binding assay.

Group VI, claims 23 and 48, drawn to a method of identifying a compound that binds the claimed polypeptide by reporter gene expression.

Group VII, claims 1-6, 26, 31, 34-36, and 51, drawn to a nucleic acid array comprising the claimed polynucleotides.

Group VIII, claims 29a and c, drawn to a method of treatment by administering an agonist of the claimed polypeptides.

Group IX, claims 29b and 54, drawn to a method of treatment by administering the claimed polypeptide.

Group X, claim 29c, drawn to a method of gene therapy using the claimed polynucleotides.

Group XI, claim 30a, drawn to a method of treatment using an antagonist.

Group XII, claim 30b, drawn to a method of treatment using antisense therapy.

The inventions listed as Groups I-XII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Pursuant to 37 C.F.R. 1.475(d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first recited product; the polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 2-5, 10, 12-15, and 15, a recombinant vector, a host cell, a method of producing the encoded polypeptide, and a polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence selected from the group consisting of SEQ ID NO: 4-9, 14, and 16-18. Further pursuant to 37 C.F.R. 1.475(d), the ISA/US considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

- I. The polynucleotide of SEQ ID NO: 2,
- II. The polynucleotide of SEQ ID NO: 3 and the polypeptide it encodes, SEQ ID NOs 4 and 5-9.
- III. The polynucleotide of SEQ ID NO: 10,
- IV. The polynucleotide of SEQ ID NO: 12,
- V. The polynucleotide of SEQ ID NO: 15 and the polypeptide it encodes, SEQ ID NO: 14, 5-6, and 16-18,
- VI. The polynucleotide of SEQ ID NO: 15.

All of the claims are deemed to correspond to the species listed above.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/55061

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

The polynucleotides with the nucleotide sequences set forth in SEQ ID NOs: 2-3, 10, 12-15, and 15 and the polypeptides they encode have different structural and functional features.

Therefore, Group I will be searched with respect to species I (the polynucleotide of SEQ ID NO:2). The appropriate fees must be paid for searches of additional groups and species.

In the absence of any response from the applicant, this Authority will establish the International Preliminary Examination Report based on the main invention. The claims drawn to the main invention are as follows:

Claims 1-4, 6-16, 24-25, 31-34, 36-41, 49-50, and 55-60 as they relate to SEQ ID NO: 2.

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(51)Int.Cl. ⁷	F I	テーマコード (参考)
A 6 1 K 48/00	A 6 1 P 3/10	4 B 0 6 5
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(33)優先権主張国 米国(US)

(31)優先権主張番号 09/643,313

(32)優先日 平成12年8月31日(2000.8.31)

(33)優先権主張国 米国(US)

(81)指定国 AP(GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE,TR),OA(BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG),AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CR,CU,CZ,DE,DK,DM,DZ,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,MZ,NO,NZ,PL,PT,RO,RU,SD,SE,S G,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,US,UZ,VN,YU,ZA,ZW

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アメリカ合衆国 カリフォルニア 95132, サン ノゼ, ジャポール レーン 1461
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