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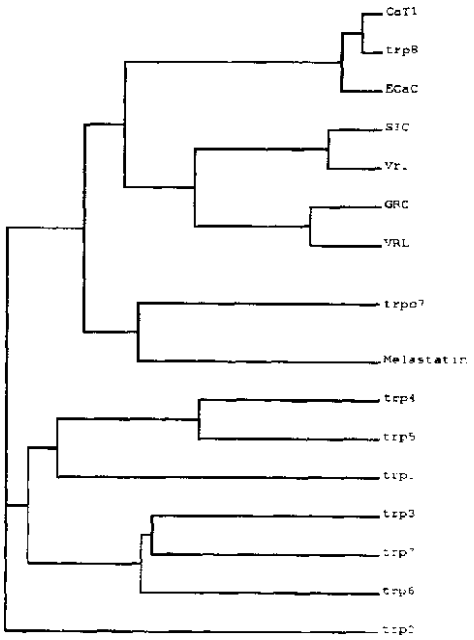
(54)【発明の名称】Ｔｒｐ８、Ｔｒｐ９およびＴｒｐ１０、癌の新規マーカー

(57)【要約】
本発明は、正常細胞および悪性腫瘍の細胞における遺伝子発現に関し、特に癌に関連する新規マーカーＴｒｐ８、Ｔｒｐ９およびＴｒｐ１０、ならびにＴｒｐ８、Ｔｒｐ９、Ｔｒｐ１０をコードする遺伝子に関する。ベクター、宿主細胞、抗体、およびこれらのヒトタンパク質を産生するための組換え方法も提供される。本発明はさらに、腫瘍を診断および治療するのに有用な診断方法および治療方法に関する。



【特許請求の範囲】
【請求項１】
ヒト前立腺癌に関連するタンパク質Ｔｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ９、Ｔｒｐ１０ａもしくはＴｒｐ１０ｂまたはＴｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ９、Ｔｒｐ１０ａもしくはＴｒｐ１０ｂの生物学的特性を示すタンパク質をコードし、かつ
（ａ） 図７、８Ａ、９、１０または１１に示されるアミノ酸配列を含むタンパク質をコードする核酸分子、
（ｂ） 図７、８Ａ、９、１０または１１に示されるヌクレオチド配列を含む核酸分子、
（ｃ） ＤＳＭＺ寄託番号ＤＳＭ １３５７９、ＤＳＭ １３５８０、ＤＳＭ １３５８４、ＤＳＭ １３５８１ またはＤＳＭ．．．．に含まれる核酸分子、
（ｄ） （ａ）～（ｃ）に規定された核酸分子にハイブリダイズする核酸分子、
（ｅ） （ａ）～（ｄ）に規定された核酸配列から遺伝子コードの縮重により変化した核酸配列の核酸分子、ならびに
（ｆ） （ａ）～（ｅ）に規定された核酸配列の断片、誘導体または対立遺伝子バリエーションを示す核酸分子、
からなる群より選ばれるものである、単離された核酸分子。
【請求項２】
請求項１記載の核酸分子を含んでなる組換えベクター。
【請求項３】
核酸分子が調節エレメントに操作可能に連結され、原核生物宿主細胞および／または真核生物宿主細胞において翻訳可能なＲＮＡ の翻訳および合成が可能である請求項２記載の組換えベクター。
【請求項４】
請求項３記載の組換えベクターを含んでなる組換え宿主細胞。
【請求項５】
哺乳動物細胞、細菌細胞、昆虫細胞または酵母細胞である、請求項４記載の組換え宿主細胞。
【請求項６】
請求項１記載の核酸分子によりコードされるヒト前立腺癌に関連するタンパク質Ｔｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂの生物学的特性を示す単離されたタンパク質。
【請求項７】
請求項６記載の単離されたタンパク質を発現する組換え宿主細胞。
【請求項８】
ヒト前立腺癌に関連するタンパク質Ｔｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂの生物学的特性を示す単離されたタンパク質を作製する方法であって、
（ａ） 該タンパク質が発現される条件下で請求項６記載の組換え宿主細胞を培養すること、ならびに
（ｂ） 該タンパク質を回収すること
を含む、方法。
【請求項９】
請求項８記載の方法により作製されたタンパク質。
【請求項１０】
請求項１記載の核酸分子から転写されたｍＲＮＡまたはその一部に相補的であり、該ｍＲＮＡまたはその一部に選択的に結合しうることを特徴とし、該核酸分子によりコードされるタンパク質の合成を阻害しうるアンチセンスＲＮＡ 配列。
【請求項１１】
請求項１記載の核酸分子から転写されたｍＲＮＡまたはその一部に相補的であり、該ｍＲ

ＮＡまたはその一部に選択的に結合し、かつ切断しうることを特徴とし、したがって該核酸分子によりコードされるタンパク質の合成を阻害するリボザイム。
【請求項１２】
請求項６記載のタンパク質の活性を抑制しうることを特徴とするインヒビター。
【請求項１３】
タンパク質Ｔｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂ、あるいはＴｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂをコードするｍＲＮＡを含むことが推測される標的試料と、Ｔｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂ、あるいはＴｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂをコードするｍＲＮＡと反応する試薬とを接触させること、ならびにＴｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂ、あるいはＴｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂをコードするｍＲＮＡを検出することを含む、前立腺癌の診断方法。
【請求項１４】
試薬が核酸である請求項１３記載の方法。
【請求項１５】
試薬が抗体である請求項１３記載の方法。
【請求項１６】
試薬が検出可能に標識されている請求項１３記載の方法。
【請求項１７】
標識が、放射性同位体、生物発光化合物、化学発光化合物、蛍光化合物、金属キレート、または酵素からなる群より選択される、請求項１６記載の方法。
【請求項１８】
タンパク質Ｔｒｐ８ａ および／またはＴｒｐ８ｂ、あるいはＴｒｐ８ａ および／またはＴｒｐ８ｂ をコードするｍＲＮＡを含むことが推測される標的試料と、Ｔｒｐ８ａ および／またはＴｒｐ８ｂ、あるいはＴｒｐ８ａ および／またはＴｒｐ８ａ および／またはＴｒｐ８ｂ をコードするｍＲＮＡと反応する試薬とを接触させること、ならびにＴｒｐ８ａ および／またはＴｒｐ８ｂ、あるいはＴｒｐ８ａ および／またはＴｒｐ８ｂをコードするｍＲＮＡを検出することを含む、子宮内膜癌（子宮癌）の診断方法。
【請求項１９】
試薬が核酸である請求項１８記載の方法。
【請求項２０】
試薬が抗体である請求項１８記載の方法。
【請求項２１】
試薬が検出可能に標識されている請求項１８記載の方法。
【請求項２２】
標識が、放射性同位体、生物発光化合物、化学発光化合物、蛍光化合物、金属キレート、または酵素からなる群より選択される、請求項２１記載の方法。
【請求項２３】
試料と、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂアンチセンスＲＮＡ、あるいはＴｒｐ１０ａおよび／またはＴｒｐ１０ｂに関連するアンチセンスＲＮＡ を検出する試薬とを接触させることを含む、被験体の組織における黒色腫、絨毛膜癌、肺癌および前立腺癌の診断方法。
【請求項２４】
Ｔｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂの発現、および／またはＴｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂの活性を減少または阻害する試薬の治療有効量を哺乳動物被験体に投与することを含む、前立腺腫瘍、子宮内膜癌（子宮癌）腫瘍、絨毛膜癌、肺癌または黒色腫の予防、治療、または改善方法。
【請求項２５】

試薬が、アンチセンスＲＮＡ を含むヌクレオチド配列である、請求項２４記載の方法。
【請求項２６】
試薬が、リボザイムを含むヌクレオチド配列である、請求項２４記載の方法。
【請求項２７】
試薬が、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂのインヒビターである、請求項２４記載の方法。
【請求項２８】
試薬が、抗Ｔｒｐ８ａ 抗体、抗Ｔｒｐ８ｂ 抗体、抗Ｔｒｐ１０ａ抗体および／または抗Ｔｒｐ１０ｂ抗体、またはそれらの断片である、請求項２７記載の方法。
【請求項２９】
試料中のＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂ、あるいはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂをコードするｍＲＮＡまたはＴｒｐ１０ａおよび／またはＴｒｐ１０ｂアンチセンス転写物の検出に有用であり、ここで、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ９、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂ、あるいはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ９、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂをコードするｍＲＮＡまたはＴｒｐ１０ａおよび／またはＴｒｐ１０ｂアンチセンス転写物の増大した濃度の存在が、前立腺腫瘍、子宮内膜癌（子宮癌）腫瘍、絨毛膜癌、肺癌または黒色腫を示すものであり、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂ、あるいはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ９、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂをコードするｍＲＮＡまたはＴｒｐ１０ａおよび／またはＴｒｐ１０ｂアンチセンス転写物の検出のためのプローブを含むキット。
【請求項３０】
検出される標的成分がＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ９、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂであり、プローブが抗体である、請求項２９記載のキット。
【請求項３１】
試験化合物とイオンチャンネルＴｒｐ８、Ｔｒｐ９および／またはＴｒｐ１０ｂとを接触させること、ならびに該試験化合物がカルシウム取り込みに影響を与えるかどうかを決定することを含む、イオンチャンネルＴｒｐ８、Ｔｒｐ９および／またはＴｒｐ１０ｂのアゴニストまたはアンタゴニストとして作用する化合物の同定方法。
【発明の詳細な説明】
【０００１】
技術分野
本発明は、正常細胞および悪性腫瘍細胞における遺伝子発現に関し、特に癌に関連する新規マーカー（Ｔｒｐ８、Ｔｒｐ９およびＴｒｐ１０ ）ならびにＴｒｐ８、Ｔｒｐ９およびＴｒｐ１０ をコードする遺伝子に関する。
【０００２】
発明の背景
前立腺癌は、世界中の高齢男性の最も一般的な疾患の１つである。前立腺癌の診断およびモニタリングは、疾患の不均質性に起因して、困難である。診断について、悪性疾患の種々の格付けは、Ｇｌｅａｓｏｎ－Ｓｃｏｒｅ Ｄｉａｇｎｏｓｉｓ によって区別され得る。この診断のために、前立腺組織サンプルが、患者から生検によって採取され、組織の形態が調査される。しかし、このアプローチは、病理学者の経験に依存する主観的な結果を得るのみである。これらの結果の確認のため、かつ早期診断を得るため、さらなる診断方法が適用され得る。この診断方法は、前立腺特異的抗原（ＰＳＡ ）の検出に基づく。ＰＳＡ は、血清サンプル、血液サンプルなどにおいて、抗ＰＳＡ 抗体を使用してアッセイされる。しかし、原則としてＰＳＡ はまた、正常な前立腺組織においても発現されるので、正常前立腺組織と悪性前立腺組織との間の区別を可能にするため、閾値の規定（例えば、約４ｎｇ／ｍｌ ＰＳＡ）を必要とする。あいにく、この診断方法は、全く感度が低く、しばしば偽陽性結果を得る。さらに、この診断方法を使用することによっては、悪性疾患の格付け、腫瘍の進行およびその転移の可能性に関する判定が全く得られ得ない。従

って、分子マーカーの使用は、特に非常に悪い予後を有し、前立腺癌が転移している患者に対する、良性組織と悪性組織の区別ならびに前立腺癌の格付けおよび病気分類に有用である。

【０００３】

前立腺腫瘍、特に転移腫瘍、の存在に相互に関係ある有意義な特異的マーカーを提供するための先行技術の上で考察した限定および欠点が、この疾患の進行に沿って診断的に、予後的にかつ治療的に使用され得るマーカーの必要性を引き起こしている。本発明は、Ｔｒｐ８、Ｔｒｐ９およびＴｒｐ１０ ならびにＴｒｐ８、Ｔｒｐ９およびＴｒｐ１０ をコードする遺伝子の供給によってこのような必要性を満たす：Ｔｒｐ８およびＴｒｐ１０ をコードする遺伝子は、前立腺癌および前立腺転移において発現されるが、正常な前立腺、良性肥厚（ＢＨＰ ）および前立腺上皮内腫瘍（ＰＩＮ ）には発現されない。さらに、Ｔｒｐ１０ 転写物の発現は、癌において検出可能であるが、肺、前立腺、胎盤の健常な組織および黒色腫においては検出不可能である。

【０００４】

発明の要旨

本発明は、癌に関連する新規マーカー（Ｔｒｐ８、Ｔｒｐ９およびＴｒｐ１０ ）をコードする遺伝子の単離に基づく。新規カルシウムチャンネルタンパク質Ｔｒｐ８、Ｔｒｐ９およびＴｒｐ１０ は、ｔｒｐ （過渡レセプターポテンシャル（ｔｒａｎｓｉｅｎｔ ｒｅｃｅｐｔｏｒ ｐｏｔｅｎｔｉａｌ））ファミリーのメンバーであり、ヒト胎盤（Ｔｒｐ８ａ およびＴｒｐ８ｂ ）ならびにヒト前立腺（Ｔｒｐ９、Ｔｒｐ１０ａおよびＴｒｐ１０ｂ）から単離される。Ｔｒｐ タンパク質は、Ca^{2+} 選択イオンチャンネルおよび非選択イオンチャンネルの着実成長（ｓｔｅａｄｉｌｙ ｇｒｏｗｉｎｇ）ファミリーに属する。近年、７つのＴｒｐ タンパク質（ｔｒｐ１～ｔｒｐ７）が、同定されており、陽イオンエントリー、レセプター作動カルシウムエントリーおよびフェロモン感覚シグナル伝達に関連することが示唆されている。構造的に関連するｔｒｐ タンパク質は、心臓によって引き起こされる侵害（ｎｏｃｉｏｃｅｐｔｉｏｎ）に共に関連するバニロイド（ｖａｎｉｌｌｏｉｄ ）レセプター（ＶＲ１ ）およびバニロイド用レセプター（ＶＲＬ－１ ）である。さらに、２つのカルシウム透過性チャンネルが、ラット小腸（ＣａＴ１）およびウサギ腎臓（ＥＣａＣ）において同定された。これらの遠縁に関連するチャンネルは、小腸（ＣａＴ１）の管腔からのカルシウムイオンの取り込みまたは腎臓（ＥＣａＣ）の遠位細管におけるカルシウムイオンの再取り込みに関連すると示唆される。一般的特性またはＴｒｐ および関連チャンネルは、いくつかの保存アミノ酸モチーフを含む６つの膜貫通ドメインを構成すると提案された構造である。本発明において、ヒト胎盤由来のＣａＴ１様カルシウムチャンネル（Ｔｒｐ８）ならびにＴｒｐ９およびＴｒｐ１０ （２つのバリアント、Ｔｒｐ１０ａおよびＴｒｐ１０ｂ）のクローニングおよび発現が記載される。Ｔｒｐ８ ｃＤＮＡ の２つの多型改変体は、胎盤から単離された（Ｔｒｐ８ａ およびＴｒｐ８ｂ ）。ＨＥＫ （ヒト胎児腎臓）細胞におけるＴｒｐ８ｂ ｃＤＮＡの一過的発現は、Ｔｒｐ８チャンネルが発現系において構造的開放であることを意味する細胞質ゾルのカルシウム過負荷を生じる。Ｔｒｐ８は、ＨＥＫ 細胞において高度なカルシウム選択的内部流動を誘導する。Ｔｒｐ８タンパク質のＣ 末端は、カルシウム依存様式でカルモジュリンを結合する。Ｔｒｐ９チャンネルは、胎盤の栄養芽細胞および合胞体栄養細胞層においてならびに膵臓腺房細胞において発現される。さらに、Ｔｒｐ８チャンネルは、前立腺癌および前立腺転移において発現されるが、前立腺の正常組織においては発現されない。Ｔｒｐ８転写物の発現は、良性前立腺肥厚（ＢＰＨ ）または前立腺上皮内腫瘍（ＰＩＮ ）においては検出不可能である。従って、Ｔｒｐ８チャンネルは、もっぱら悪性前立腺組織において発現され、前立腺癌に対する分子マーカーとして利用される。実験結果から、Ｔｒｐ８および／またはＴｒｐ１０ のモジュレーション（例えば、発現または活性の阻害）が治療目的（例えば、腫瘍進行の予防に対する）であることもまた明らかである。

【０００５】

従って、本発明は、Ｔｒｐ８、Ｔｒｐ９およびＴｒｐ１０ タンパク質をそれぞれ、ならびにこのタンパク質をコードする核酸分子、そしてさらにＴｒｐ８、Ｔｒｐ９および／またはＴｒｐ１０ の発現あるいは活性を阻害し得るアンチセンスＲＮＡ 、リボザイムならびにインヒビターを提供する。

【０００６】

１つの実施形態において、本発明は、被験体の組織中のＴｒｐ８またはＴｒｐ１０ に関連する前立腺癌または子宮内膜癌（子宮の癌）を検出するための診断方法を提供し、この方法は、Ｔｒｐ８および／またはＴｒｐ１０ をコードするｍＲＮＡを含むサンプルをＴｒｐ８および／またはＴｒｐ１０ あるいは対応するｍＲＮＡを検出する試薬と接触させることを含む。

【０００７】

さらなる実施形態において、本発明は、被験体の組織において黒色腫、絨毛膜癌、肺の癌および前立腺の癌を検出するための診断方法を提供し、この方法は、サンプルをＴｒｐ１０ａアンチセンス転写物および／またはＴｒｐ１０ｂアンチセンス転写物あるいはＴｒｐ１０ａ関連アンチセンス転写物および／またはＴｒｐ１０ｂ関連アンチセンス転写物を検出する試薬と接触させることを含む。

【０００８】

別の実施形態において、本発明は、Ｔｒｐ８および／またはＴｒｐ１０ に関連する前立腺腫瘍、肺の癌、胎盤の癌（絨毛膜癌）または黒色腫の処置の方法を提供し、この方法は、このような障害に罹患している被験体に治療的有効量の試薬（Ｔｒｐ８および／またはＴｒｐ１０ の発現あるいはこのタンパク質の活性を調節（例えば、阻害）する）（例えば、上記化合物）を投与することを含む。

【０００９】

最後に、本発明は、遺伝子治療の方法を提供し、この方法は、被験体の細胞へ上記のアンチセンスＲＮＡ またはリボザイムをコードするヌクレオチド配列（プロモーターに作動可能に連結している）を含む発現ベクターを導入することを含む。

【００１０】

発明の詳細な説明

本発明は、ヒト前立腺癌関連タンパク質Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂあるいはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂの生物学的特性を示すタンパク質をコードし、そして以下からなる群より選択される単離された核酸分子に関する：

（ａ） 図７ 、８Ａ、９ 、１０、または１１に示されるアミノ酸配列を含むタンパク質をコードする核酸分子；

（ｂ） 図７ 、８Ａ、９ 、１０、または１１に示されるヌクレオチド配列を含む核酸分子；

（ｃ）ＤＳＭＺ 受託番号ＤＳＭ１３５７９（寄託日：２０００年６ 月２８日）、ＤＳＭ１３５８０（寄託日：２０００年６ 月２８日）、ＤＳＭ１３５８４（寄託日：２０００年７ 月５ 日）、ＤＳＭ１３５８１（寄託日：２０００年６ 月２８日）またはＤＳＭ．．．．（寄託日：．．．．）に含まれる核酸分子；

（ｄ）（ａ）～（ｃ） に規定された核酸分子にハイブリダイズする核酸分子；

（ｅ） （ａ） ～（ｄ） に規定された核酸配列から遺伝子コードの縮重により変化した核酸配列の核酸分子；および

（ｆ） （ａ） ～（ｅ） に規定された核酸配列のフラグメント、誘導体または対立遺伝子バリエーションを示す核酸分子。

【００１１】

本明細書中で使用される場合、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂの生物学的特性を示すタンパク質は、以下の実施例に例示するような少なくとも１つの活性を有するタンパク質であることが理解される。

【００１２】

本明細書中で使用される場合、用語「単離された核酸分子」としては、天然に付随する他の核酸、タンパク質、脂質、炭水化物または他の物質を実質的に有さない核酸分子が挙げられる。

【００１３】

第１の実施形態において、本発明は、図７、８Ａ、９、１０、または１１に示されるアミノ酸配列を含むヒト前立腺癌関連タンパク質Ｔｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ９、Ｔｒｐ１０ａまたは Ｔｒｐ１０ｂをコードする単離された核酸分子を提供する。本発明はまた、図７、８Ａ、９、１０、または１１に示されるヌクレオチド配列を含む核酸分子を提供する。

【００１４】

本発明は、図７、８Ａ、９、１０、または１１においてそれぞれ同定された生成ヌクレオチド配列だけではなく、それぞれの予測翻訳アミノ酸配列、ならびにＤＳＭＺにＤＳＭ１３５７９で寄託されたＴｒｐ８ａ ｃＤＮＡ、ＤＳＭＺにＤＳＭ１３５８０で寄託されたＴｒｐ８ｂ ｃＤＮＡ、ＤＳＭＺにＤＳＭ１３５８４で寄託されたＴｒｐ９ ｃＤＮＡ、ＤＳＭＺにＤＳＭ１３５８１で寄託されたＴｒｐ１０ａ ｃＤＮＡ、およびＤＳＭＺにＤＳＭ．．．．で寄託されたＴｒｐ１０ｂ ｃＤＮＡ をそれぞれ含むプラスミドＤＮＡを提供する。各寄託Ｔｒｐ クローンのヌクレオチド配列は、公知の方法に従って寄託クローンを配列決定することによってたやすく決定され得る。次いで、予測アミノ酸配列が、このような寄託物から実証され得る。さらに、各寄託クローンによってコードされるタンパク質のアミノ酸配列はまた、ペプチド配列決定によってか、または寄託Ｔｒｐ コードＤＮＡ を含む適切な宿主細胞においてタンパク質を発現し、このタンパク質を回収し、そしてその配列を決定することによって直接決定され得る。

【００１５】

本発明の核酸分子は、ＤＮＡ 分子およびＲＮＡ 分子の両方であり得る。例えば、適切なＤＮＡ 分子は、ゲノム分子またはｃＤＮＡ分子である。Ｔｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂの全てあるいは一部をコードする全ての核酸分子がまた、生物学的活性を有するポリペプチドをコードする限り、含まれることが理解される。本発明の核酸分子は、天然供給源から単離され得るか、または公知の方法に従って合成され得る。

【００１６】

本発明はまた、上記核酸分子にハイブリダイズする核酸分子を提供する。本明細書中で使用される場合、用語「ハイブリダイズする」とは、従来のハイブリダーゼーション条件下、好ましくは例えばＳａｍｂｒｏｏｋら、Ｍｏｌｅｃｕｌａｒ Ｃｌｏｎｉｎｇ，Ａ Ｌａｂｏｒａｔｏｒｙ Ｍａｎｕａｌ第2版（１９８９）Ｃｏｌｄ Ｓｐｒｉｎｇ Ｈａｒｂｏｒ Ｌａｂｏｒａｔｏｒｙ Ｐｒｅｓｓ，Ｃｏｌｄ Ｓｐｒｉｎｇ Ｈａｒｂｏｒ，ＮＹ に記載されるようなストリンジェントな条件下でのハイブリダイゼーションの意味を有する。意図されるものはまた、より低いストリンジェンシーハイブリダイゼーション条件でＴｒｐ 核酸分子にハイブリダイズする核酸分子である。ハイブリダイゼーションおよびシグナル検出のストリンジェンシーにおける変化は、ホルムアミド濃度（より低いパーセンテージのホルムアミドはより低いストリンジェンシーを生じる）、塩条件または温度の操作を介して主に達成される。例えば、より低いストリンジェンシー条件としては、６×ＳＳＰＥ（２０×ＳＳＰＥ＝３Ｍ ＮａＣｌ；９．２Ｍ NaH_2PO_4；０．０２Ｍ ＥＤＴＡ，ｐＨ７．４）、０．５％ ＳＤＳ、３０％ ホルムアミド、ＤＮＡ をブロックする１００ μｇ／ｍｌのサケ精子を含有する溶液中での３７℃一晩インキュベーション、次いで５０℃での１ ×ＳＳＰＥ、０．１％ ＳＤＳを用いた洗浄が挙げられる。加えて、さらにより低いストリンジェンシーを達成するために、ストリンジェントハイブリダーゼーション後に実施する洗浄は、より高い塩濃度（例えば、５ ×ＳＳＣ ）でなされ得る。上記条件における変動は、ハイブリダイゼーション実験におけるバックグランドを抑制するために使用される代替のブロック試薬の含有および／または置換を介して達成され得る。特異的なブロック試薬の含有は、互換性の問題に起因して、上記のハイブリダイゼ

ーション条件の改変を必要とし得る。

【００１７】

本発明の分子にハイブリダイズする核酸分子は、例えば、ヒト細胞株または組織から生成されるゲノムライブラリーまたはｃＤＮＡライブラリーから単離され得る。このような核酸分子を同定して単離するために、本発明の分子またはこれらの分子の一部またはこれらの分子の逆（ｒｅｖｅｒｓｅ）相補体が、例えば、従来の方法に従うハイブリダイゼーション（例えば、Ｓａｍｂｒｏｏｋら、前出）によって使用され得る。ハイブリダイゼーションプローブとして、例えば、図７、８Ａ、９、１０、または１１にそれぞれ示されるヌクレオチド配列あるいはこれらの配列の一部を正確にまたは基本的に有する核酸分子が、使用され得る。ハイブリダイゼーションプローブとして使用されるフラグメントは、従来の合成方法によって生成された合成フラグメントであり得、その配列は本発明の核酸分子の配列に基本的に対応する。

【００１８】

本発明の核酸分子としてはまた、遺伝子コードの結果として縮重した配列を有する分子が挙げられる。

【００１９】

さらなる実施形態において、本発明は、本発明のタンパク質をコードする上記の核酸分子のフラグメント、誘導体および対立遺伝子バリアントを含む核酸分子を提供する。「フラグメント」は、記載のタンパク質の１つをコードするのに十分な長さである核酸分子の一部であることが理解される。これらのフラグメントは、本発明の核酸分子の転写物に特異的にハイブリダイズする核酸分子を含む。これらの核酸分子は、例えば、プローブまたはプライマーとして、下記の診断アッセイおよび／またはキットにおいて使用され得、好ましくは、少なくとも１０ヌクレオチドの長さ、特に少なくとも１５ヌクレオチドの長さ、特に好ましくは少なくとも５０ヌクレオチドの長さを有するオリゴヌクレオチドである。本発明の核酸分子およびオリゴヌクレオチドはまた、例えば、ＰＣＲ 反応のためのプライマーとして使用され得る。特に有用なプローブ（プライマー）の例は、表１および２に示される。

【００２０】

【表１】

表1

インサイチュハイブリダイゼーションに使用したTrp8プローブ：

プローブ（アンチセンス）

1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'

2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'

3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

対照（センス）

1.) 5' GGCAAGATCTCAACCGGCAGCGGA 3'

2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'

3.) 5' TTCCACACCCGGGGCTCTGAGGAT 3'

【００２１】

【表２】

表2

図14に示したインサイチュハイブリダイゼーションに使用したTrp10プローブ：
プローブ（アンチセンス）

1.) 5' GCTTCCACCCCAAGCTTCACAGGAATAGA 3' (図 14 A, 14B)
2.) 5' GGCGATGAAATGCTGGTCTGTGGC 3' (図 14C, 14D, 14N, 14S, 14O)
3.) 5' ATCTTCCAGTTCTTGGTGTCTCGG 3' (図 14E, 14K)
4.) 5' GCTGCAGTACTCCTGCACCAGGAA 3' (図 14L, 14M)

プローブ（センス）

1.) 5' TCTATTCCTGTGAAGCTTGGGGTGGAAGC 3' (図 14F, 14G)
2.) 5' GCCACAGACCAGCATTTCATCGCC 3' (図 14H, 14I, 14T)
3.) 5' CCGAGACACCAAGAACTGGAAGAT 3' (図 14J, 14P)
4.) 5' TTCCTGGTGCAGGAGTACTGCAGC 3' (図 14Q, 14R)

【００２２】
本文脈における用語「誘導体」とは、これらの分子の配列が１つの位置または数個の位置で上記の核酸分子の配列と異なっているが、これらの配列と高いレベルの相同性を有していることを意味する。この結果、相同性とは、少なくとも４０％ の配列同一性、特に少なくとも６０％ の同一性、好ましくは８０％ より高い同一性、特に好ましくは９０％ より高い同一性を意味する。核酸分子によってコードされるこれらのタンパク質は、図７、８Ａ、９、１０、または１１にそれぞれ示されるアミノ酸配列に少なくとも８０％、好ましくは８５％、そして特に好ましくは９０％ より高い（９７％ および９９％）配列同一性を有する。上記核酸分子に対するずれは、欠失、置換、挿入または組換えによって生成され得る。誘導体の定義としてはまた、スプライスバリアント（例えば、図８Ｂ～８Ｅおよび９Ｂに示されるスプライスバリアント）が挙げられる。
【００２３】
上記分子に相同であり、かつこれらの分子の誘導体になる核酸分子は、通常、同じ生物学的機能を有する改変を表すこれらの分子のバリエーションである。これらは、天然に存在するバリエーション（例えば、他の生物由来の配列）または天然に存在し得るか、もしくは特定の変異誘発によって誘導され得るかのいずれかの変異体である。さらに、バリエーションは、合成的に生成された配列であり得る。この対立遺伝子バリアントは、天然に存在するバリアント、または合成的に生成されたバリアントまたは組換えＤＮＡ プロセスによって生成されたバリアントのいずれかであり得る。
【００２４】
一般的に、従来の分子生物学的プロセスによって、異なる変異を本発明の核酸分子へ導入することは可能である（例えば、Ｓａｍｂｒｏｏｋら、前出、参照のこと）。結果として、おそらく変更された生物学的特性を有するＴｒｐ タンパク質またはＴｒｐ 関連タンパク質が合成される。１つの可能性は、核酸分子がコードＤＮＡ 配列の５’末端または３’末端から連続的な欠失によって生成され、それに応じて短縮されたタンパク質の合成を導く欠失変異体の生成である。別の可能性は、アミノ酸配列の修飾が例えば、イオンチャンネル特性またはｔｒｐ イオンチャンネルの調節に影響を及ぼす位置での単一の点変異の導入である。この方法により、例えば、改変されたイオン伝導孔、改変されたK_m 値を有するか、または通常細胞内に存在する調節機構（例えば、アロステリック調節もしくは共有結合修飾に関して）をもはや受けないムテインが生成され得る。このようなムテインはまた、それぞれ、Ｔｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂの治療的に有用なアンタゴニストとして貴重であり得る。
【００２５】
遺伝子工学による原核生物細胞における操作のため、本発明の核酸分子またはこれらの分

子の一部が、プラスミドへ導入され得、ＤＮＡ 配列の組換えによる配列の変異誘発または改変を可能にする。従来の方法（Ｓａｍｂｒｏｏｋら、前出、参照）によって、塩基が交換され得、天然または合成配列が付加され得る。ＤＮＡ フラグメントを互いに連結するため、アダプターまたはリンカーがフラグメントに付加され得る。さらに、適切な切断部位を提供するか、または余分なＤＮＡ もしくは切断部位を除去する操作が、実施され得る。挿入、欠失または置換が可能な場合、インビトロ変異誘発、プライマー修復、拘束またはライゲーションが実施され得る。解析方法として、通常、配列解析、拘束解析および他の生化学的または分子生物学的方法が使用される。

【００２６】

本発明の核酸分子の種々のバリアントによってコードされるタンパク質は、特定の一般的な特徴（例えば、イオンチャンネル活性、分子量、免疫学的反応性または立体配座または物理的特性（例えば、電気泳動移動度、クロマトグラフィー性質（ｂｅｈａｖｉｏｒ）、沈降係数、可溶性、スペクトル特性、安定性、ｐＨ最適条件、温度最適条件など））を示す。

【００２７】

本発明はさらに、本発明の核酸分子を含むベクターに関する。好ましくは、それらは、遺伝子工学の分野で通常使用されるプラスミド、コスミド、ウイルス、バクテリオファージおよび他のベクターである。本発明の使用に適切なベクターとしては、哺乳動物細胞における発現用のＴ７ベース発現ベクターおよび昆虫細胞における発現用のバキュロウイルス由来ベクターが挙げられるが、それらに限定されない。好ましくは、本発明の核酸分子は、翻訳され得る原核生物細胞および／または真核生物細胞におけるＲＮＡ の転写および合成を請け負う本発明の組換えベクター中の調節エレメントに作動可能に連結される。転写されるべきヌクレオチド配列は、Ｔ７、メタロチオネシンＩ またはポリヘドリン（ｐｏｌｙｈｅｄｒｉｎ）プロモーターなどのプロモーターに作動可能に連結され得る。

【００２８】

さらなる実施形態において、本発明は、一過的にまたは安定に本発明の核酸分子またはベクターを含む組換え宿主細胞に関する。宿主細胞は、インビトロで組換えＤＮＡ を組み込み、場合により本発明の核酸分子によってコードされるタンパク質を合成し得る生物と理解される。好ましくは、これらの細胞は、原核生物細胞または真核生物細胞（例えば、哺乳動物細胞、細菌細胞、昆虫細胞または酵母細胞）である。本発明の宿主細胞は、好ましくは、本発明の導入核酸分子が、形質転換細胞に関して異種（すなわちこれらの細胞中に天然には存在しない）であるか、または対応する天然に存在する配列のゲノム中の場所とは異なる場所に局在化することにより特徴付けられる。

【００２９】

本発明のさらなる実施形態は、ヒト前立腺癌関連タンパク質Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂの生物学的特性を示し、かつ本発明の核酸分子によってコードされる単離されたタンパク質、ならびにそれらの産生方法に関する。それにより、例えば、本発明の宿主細胞は、タンパク質の合成を可能にする条件下で培養され、そしてこのタンパク質が続いて培養細胞および／または培養培地から単離される。組換え的に産生されたタンパク質の単離および精製は、従来の手段（予備クロマトグラフィーならびに抗Ｔｒｐ８ａ 抗体、抗Ｔｒｐ８ｂ 抗体、抗Ｔｒｐ９抗体、抗Ｔｒｐ１０ａ抗体または抗Ｔｒｐ１０ｂ抗体それぞれとの親和性に関連する親和性分離および免疫学的分離が挙げられる）によって実施され得る。

【００３０】

本明細書中で使用される場合、用語「単離されたタンパク質」としては、天然に付随する他のタンパク質、核酸、脂質、炭水化物または他の物質を実質的に有さないタンパク質が挙げられる。しかし、このようなタンパク質は、組換え的に産生されたタンパク質を含むだけではなく、単離された天然に存在するタンパク質、合成的に生成されたタンパク質、またはこれらの方法の組み合わせによって生成されたタンパク質も含む。このようなタンパク質を調製するための手段は、当該分野で十分に理解される。Ｔｒｐ タンパク質は、

好ましくは実質的に精製された形態である。ヒト前立腺癌関連タンパク質Ｔｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂタンパク質（分泌タンパク質を含む）の組換え的に生成されたバージョンは、Ｓｍｉｔｈ およびＪｏｈｎｓｏｎ，Ｇｅｎｅ ６７；３１－４０（１９８８）に記載される１工程方法によって実質的に精製され得る。

【００３１】

さらに好ましい実施形態において、本発明は、アンチセンスＲＮＡ 配列およびリボザイムに関する。このアンチセンスＲＮＡ 配列は、本発明の核酸分子から転写されたｍＲＮＡまたはその一部に相補的であり、かつこのｍＲＮＡに選択的に結合し得ることに特徴を有し、この配列は、この核酸分子によってコードされるタンパク質の合成を阻害し得る。このリボザイムは、本発明の核酸分子から転写されたｍＲＮＡまたはその一部に相補的であり、かつこのｍＲＮＡに選択的に結合そして切断し得ることに特徴を有し、従って、この核酸分子によってコードされるタンパク質の合成を阻害する。単一のＲＮＡ 鎖から構成されるリボザイムは、標的ＲＮＡ（例えば、Ｔｒｐ 遺伝子のうちの１つから転写されたｍＲＮＡ）を分子間切断し得るＲＮＡ 酵素、すなわち、触媒ＲＮＡ である。現在、論文（例えば、Ｔａｎｎｅｒら、Ａｎｔｉｓｅｎｓｅ Ｒｅｓｅａｒｃｈ ａｎｄ Ａｐｐｌｉｃａｔｉｏｎｓ，ＣＲＣ Ｐｒｅｓｓ Ｉｎｃ．（１９９３），４１５－４２６ を参照のこと）に記載されるストラテジーに従って標的ＲＮＡ を特異的部位で切断し得るリボザイムを構築することは可能である。このようなリボザイムに対する２つの主な必要条件は、切断のために必須の、標的ＲＮＡ に相補的であり、かつその基質に結合可能な触媒ドメインおよび領域である。この相補的配列、すなわちアンチセンスＲＮＡ またはリボザイムは、Ｔｒｐ８ａ 発現、Ｔｒｐ８ｂ 発現、Ｔｒｐ９発現、Ｔｒｐ１０ａ発現およびＴｒｐ１０ｂ発現のそれぞれの抑制、すなわち、前立腺癌または子宮内膜癌（子宮の癌）の処置の場合に有用である。好ましくは、本発明のアンチセンスＲＮＡ およびリボザイムは、コード領域に相補的である。本発明の核酸分子の配列を提供された当業者は、上記アンチセンスＲＮＡ またはリボザイムを産生および利用する立場にいる。本発明の核酸分子から転写されたｍＲＮＡに相補性を示すアンチセンスＲＮＡ およびリボザイムのそれぞれの領域は、好ましくは、少なくとも１０ヌクレオチド長、特に少なくとも１５ヌクレオチド長および特に好ましくは少なくとも５０ヌクレオチド長を有する。

【００３２】

さらなる実施形態において、本発明は、上記のアンチセンスＲＮＡ またはリボザイムと類似の目的（すなわち、生物学的に活性なＴｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂ分子の減少または除去）を満たすＴｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ９、Ｔｒｐ１０ａおよびＴｒｐ１０ｂのそれぞれのインヒビターに関する。このようなインヒビターは、例えば、アンタゴニストとして作用する対応するタンパク質の構造アナログであり得る。さらに、このようなインヒビターは、組換え的に生成されたタンパク質の使用によって（例えば、適切な条件下でタンパク質に結合する強力なインヒビターの能力を利用することによって）同定された（例えば、この組換え的に生成されたタンパク質を使用して、インヒビターをスクリーニングかつ同定し得る）分子を含む。このインヒビターは、例えば、試験混合物を調製することによって同定され得、ここで、このインヒビター候補は、Ｔｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂがネイティブ構造であり得る適切な条件下でＴｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂそれぞれと共にインキュベートされる。このようなインビトロ試験系は、当該分野で周知の方法に従って確立され得る。インヒビターは、例えば、組換え的に生成されたＴｒｐ タンパク質に結合する合成または天然に存在する分子のいずれかに対する第一スクリーニング、次いで第二工程において、以下の実施例に記載されるような生物学的活性のうち少なくとも１つの阻害によって影響を及ぼされるようなＴｒｐ タンパク質の阻害に対する細胞アッセイにおいてこれらの選択された分子を試験することによって同定され得る。Ｔｒｐ８ａ、Ｔｒｐ８ｂ、Ｔｒｐ９、Ｔｒｐ１０ａまたはＴｒｐ１０ｂを結合する分子のこのようなスクリーニングは、ラージスケールで、例えば

、合成および／または天然分子のライブラリーから候補分子をスクリーニングすることによって容易に実施し得た。このようなインヒビターは、例えば、合成有機化学物質、天然発酵産物、微生物、植物もしくは動物から抽出された基質、またはペプチドである。インヒビターのさらなる例は、特異的抗原、好ましくはモノクローナル抗体である。さらに、本発明のヌクレオチド配列およびコードされるタンパク質を使用して、腫瘍発達および進行に関するさらなる因子を同定し得る。本文脈においては、ｔｒｐ ファミリーのメンバーのカルシウムチャンネルの改変はＴ リンパ球の増殖を導くＴ リンパ球の免疫応答の刺激を生じ得ることが強調されるべきである。本発明のタンパク質は、例えば、タンパク質／タンパク質相互作用に基づくスクリーニング方法（例えば、ツーハイブリッドシステム Ｆｉｅｌｄｓ，Ｓ．およびＳｏｎｇ，Ｏ．（１９８９）Ｎａｔｕｒｅ（３４０）：２４５－２４６）を使用して腫瘍に関連するさらなる（非関連）タンパク質を同定するために使用され得る。

【００３３】

本発明はまた、タンパク質Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂあるいはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂコードｍＲＮＡを含むと考えられる標的サンプルを、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂあるいはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂコードｍＲＮＡと反応する試薬と接触させて、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂあるいはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂコードｍＲＮＡを検出することを含む前立腺癌の診断方法を提供する。

【００３４】

胎盤の癌細胞（絨毛膜癌）、肺の癌細胞および前立腺の癌細胞はＴｒｐ１０ 転写物ならびにＴｒｐ１０ アンチセンス転写物およびＴｒｐ１０ アンチセンス転写物に部分的に相補的な転写物を発現することがわかっている。従って、本発明はまた、被験体の組織中の黒色腫、絨毛膜癌、肺の癌および前立腺の癌を診断する方法を提供する。この方法は、サンプルをＴｒｐ１０ａおよび／またはＴｒｐ１０ｂアンチセンスＲＮＡ を検出する試薬と接触させることを含む。

【００３５】

標的がｍＲＮＡ（またはアンチセンスＲＮＡ ）である場合、試薬は、典型的に核酸プローブまたはＰＣＲ に対するプライマーである。当業者は、図７ 、８ａ、１０および１１にそれぞれに、または上記の表１および２に示されるようなＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａまたはＴｒｐ１０ｂのヌクレオチド配列に関する情報に基づいて、適切な核酸プローブを設計する立場にある。標的がタンパク質である場合、試薬は、典型的に、抗体プローブである。用語「抗体」とは、好ましくは、異なるエピトープ特異性を有するプールモノクローナル抗体から本質的になる抗体および独特なモノクローナル抗体調製物をいう。モノクローナル抗体は、当業者に周知の方法によって本発明のタンパク質のフラグメントを含む抗原から作製される（例えば、Ｋｏｅｈｌｅｒ ら、Ｎａｔｕｒｅ ２５６（１９７５），４９５を参照のこと）。本明細書中で使用される場合、用語「抗体」（Ａｂ）または「モノクローナル抗体」（Ｍａｂ ）は、タンパク質に特異的に結合し得るインタクトな分子および抗体フラグメント（例えば、Ｆａｂ およびＦ（ａｂ’）２ フラグメントなど）を含むことが意味される。Ｆａｂ およびＦ（ａｂ’）２ フラグメントは、インタクトな抗体のＦｃフラグメントを欠き、循環からより速やかになくなり、そしてインタクトな抗体よりも低い非特異的組織結合を有し得る（Ｗａｈｌら、Ｊ．Ｎｕｃｌ．Ｍｅｄ．２４：３１６－３２５（１９８３））。従って、これらのフラグメントおよびＦＡＢ または他の免疫グロブリン発現ライブラリーの産物が好ましい。さらに、本発明の抗体としては、キメラ抗体、一本鎖抗体、およびヒト化抗体が挙げられる。例えば、生物学的流体または組織における標的細胞成分、すなわち、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂ、あるいはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂコードｍＲＮＡあるいはＴｒｐ１０ａ／ｂアンチセン

ス転写物は、例えば、インサイチュハイブリダーゼーション（例えば、以下の実施例に従って）によって直接インサイチュで検出され得るか、またはプローブと接触させる前に当業者に公知の一般的方法によって他の細胞成分から単離され得る。検出方法としては、ノザンブロット分析、ＲＮａｓｅ 保護、インサイチュ方法（例えば、インサイチュハイブリダイゼーション）、インビトロ増幅方法（ＰＣＲ 、ＬＣＲ 、ＱＲＮＡレプリカーゼまたはＲＮＡ 転写／増幅（ＴＡＳ 、３ＳＲ ）、逆ドットブロット（ＥＰ－Ｂ１ Ｏ ２３７ ３６２ に開示される））、免疫アッセイ、ウエスタンブロットおよび当業者に公知の他の検出アッセイが挙げられる。

【００３６】

インビトロ増幅によって得られた産物は、確立された方法に従って（例えば、産物をアガロースゲル上で分離し、続いてエチジウムブロマイドで染色することによって）検出され得る。あるいは、増幅産物は、増幅または標識ｄＮＴＰに対する標識プライマーを使用することによって検出され得る。

【００３７】

このプローブは、例えば、放射性同位体、生物発光化合物、化学発光化合物、蛍光化合物、金属キレート剤または酵素を用いて検出可能に標識され得る。

【００３８】

組織におけるＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよびＴｒｐ１０ｂそれぞれの発現は、古典的免疫組織学方法を用いて研究され得る（Ｊａｌｋａｎｅｎら、Ｊ．Ｃｅｌｌ．Ｂｉｏｌ．１０１（１９８５），９７６－９８５；Ｊａｌｋａｎｅｎら、Ｊ．Ｃｅｌｌ．Ｂｉｏｌ．１０５（１９８７），３０８７－３０９６；Ｓｏｂｏｌら、Ｃｌｉｎ．Ｉｍｍｕｎｐａｔｈｏｌ．２４（１９８２），１３９－１４４；Ｓｏｂｏｌら、Ｃａｎｃｅｒ ６５（１９８５），２００５－２０１０）。タンパク質遺伝子発現を検出するために有用な他の抗体ベースの方法としては、酵素結合イムノソルベント検定法（ＥＬＩＳＡ）および放射免疫測定法（ＲＩＡ）などの免疫アッセイが挙げられる。適切な抗体アッセイ標識は、当該分野で公知であり、グルコースオキシダーゼなどの酵素標識、および放射性同位体（例えば、ヨウ素（^{125}Ｉ、^{121}Ｉ）、炭素（^{14}Ｃ）、硫黄（^{35}Ｓ）、トリチウム（^{3}Ｈ）、インジウム（^{112}Ｉｎ）およびテクネチウムローダミン）、ならびにビオチンが挙げられる。生物学的サンプルにおけるＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａまたはＴｒｐ１０ｂレベルをアッセイすることに加えて、タンパク質はまた、画像化によってインビボで検出され得る。タンパク質のインビボ画像化のための抗体標識またはマーカーとしては、Ｘ－線撮影法、ＮＭＲ またはＥＳＲによって検出可能なものが挙げられる。Ｘ－線撮影法について、適切な標識としては、検出可能な放射線を放射するが被験体に対して明白に有害ではないバリウムまたはセシウムなどの放射性同位体が挙げられる。ＮＭＲ およびＥＳＲ に対する適切なマーカーとしては、直接的なハイブリドーマに対する栄養素を標識することによって抗体に組み込まれ得る重水素などの検出可能な特徴的なスピンを有するものが挙げられる。適切な検出可能な画像化部分（例えば、放射性同位体（例えば、^{131}Ｉ、^{112}Ｉｎ、^{99}ｍＴｃ）、放射性不透明（ｒａｄｉｏ－ｏｐａｑｕｅ）基質、または核磁気共鳴で検出可能な物質）で標識されているタンパク質特異的抗体または抗体フラグメントは、哺乳動物へ導入される（例えば、非経口、皮下、または腹腔内）。被験体のサイズおよび使用される画像化システムが診断画像を生成するのに必要な画像化部分の量を決定することが当該分野で理解される。放射性同位体部分の場合、ヒト被験体に対して、注入される放射性同位体の量は、通常約５～２０ミリキュールの^{99}ｍＴｃ の範囲である。標識された抗体または抗体フラグメントは、次いで、特異的タンパク質を含む細胞の位置で好ましくは蓄積する。インビボ腫瘍画像化は、Ｓ．Ｗ．Ｂｕｒｃｈｉｅｌら、「Ｉｍｍｕｎｏｐｈａｒｍａｃｏｋｉｎｅｔｉｃｓ ｏｆ Ｒａｄｉｏｌａｂｅｌｅｄ Ａｎｔｉｂｏｄｉｅｓ ａｎｄ Ｔｈｅｉｒ Ｆｒａｇｍｅｎｔ」（Ｔｕｍｏｒ Ｉｍａｇｉｎｇ 第１３章：Ｔｈｅ Ｒａｄｉｏｃｈｅｍｉｃａｌ Ｄｅｔｅｃｔｉｏｎ ｏｆ Ｃａｎｃｅｒ，Ｓ．Ｗ．ＢｕｒｃｈｉｅｌおよびＢ．Ａ．Ｒｈｏｄｅｓ 編、Ｍａｓｓｏｎ Ｐｕｂｌｉｓｈｉｎｇ Ｉｎｃ．（１

９８２））に記載される。

【００３９】

マーカーＴｒｐ８ａ およびＴｒｐ８ｂ はまた、例えば、インサイチュハイブリダイゼーションを使用することによって腫瘍の進行および前立腺腫瘍の悪性の程度の診断評価（格付けおよび病気分類）をモニターするため、予後に有用である。原発性癌においては、Ｔｒｐ８は約２ ～１０％ の癌細胞中において発現され、レジディブ（ｒｅｚｉｄｉｖｅ）癌においては約１０～６０％ の細胞において発現され、および転移においては約６０～９０％ の細胞において発現される。

【００４０】

本発明はまた、タンパク質Ｔｒｐ８ａ および／もしくはＴｒｐ８ｂ 、またはＴｒｐ８ａ および／もしくはＴｒｐ８ｂ コードｍＲＮＡを含むと考えられる標的サンプルを、Ｔｒｐ８ａ および／もしくはＴｒｐ８ｂ またはそのコードｍＲＮＡと反応する試薬と接触させて、Ｔｒｐ８ａ および／またはＴｒｐ８ｂ コードｍＲＮＡを検出することを含む子宮内膜癌（子宮の癌）の診断方法に関する。本方法の特定の実施形態に関して、参考は、上記の前立腺癌の診断方法の特定の実施形態に対してなされる。

【００４１】

Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａもしくはＴｒｐ１０ｂの濃度またはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａもしくはＴｒｐ１０ｂコードｍＲＮＡの濃度が正常であるか、あるいは増加しているか、従って、悪性腫瘍の存在についての指標を評価するため、測定濃度は、好ましくは数量化のためにＴｒｐ８ａ ：Ｔｒｐ９、Ｔｒｐ８ｂ ：Ｔｒｐ９またはＴｒｐ１０ （ａ もしくはｂ ）／Ｔｒｐ９ の比を使用することによって正常組織における濃度と比較される。

【００４２】

前立腺癌は侵襲的に増加している場合、それ自身の基底膜を形成するので、Ｔｒｐ８およびＴｒｐ１０ を発現する細胞のみが、この現象に関連すると結論付けられ得る。従って、これらのタンパク質の発現および／または活性を阻害することによって、ＰＣＡ のような癌の有効治療が提供されると結論付けられ得る。

【００４３】

従って、本発明はまた、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／もしくはＴｒｐ１０ｂ発現またはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／もしくはＴｒｐ１０ｂの活性を減少あるいは阻害する試薬を含む医薬組成物、ならびに哺乳動物被験体に治療的に有効な量の試薬（Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／もしくはＴｒｐ１０ｂ発現またはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／もしくはＴｒｐ１０ｂの活性を減少あるいは阻害する）を投与することを含む前立腺腫瘍、子宮内膜癌（子宮癌）腫瘍、絨毛膜癌、肺の癌または黒色腫を予防、処置、または改善する方法に関する。このような試薬の例は、上記のアンチセンスＲＮＡ 、リボザイムまたはインヒビター（例えば、特異的抗体）である。さらに、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ９、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂの生物学的機能を阻害あるいは調節するペプチドは、治療試薬として有用であり得る。例えば、これらのペプチドは、Ｒｏｔｔｇｅｎ， Ｐ．およびＣｏｌｌｉｎｓ， Ｊ．（Ｇｅｎｅ （１９９５）１６４（２）：２４３－２５０）に記載されるように、組み合わせファージディスプレイライブラリー（Ｃｏｓｍｉｘ，Ｂｒａｕｎｓｃｈｗｅｉｇ， Ｇｅｒｍａｎｙ ）のスクリーニングによって得られ得る。さらに、Ｔｒｐ８およびＴｒｐ１０ タンパク質の抗原性エピトープは、Ｅ．ｃｏｌｉにおける組換えＴｒｐ８およびＴｒｐ１０ エピトープライブラリーの発現（Ｍａｒｑｕａｒｔ， Ａ ＆ Ｆｌｏｃｋｅｒｚｉ， Ｖ．， ＦＥＢＳ Ｌｅｔｔ． ４０７（１９９７），１３７－１４０； Ｔｒｏｓｔ， Ｃ．ら、ＦＥＢＳ Ｌｅｔｔ． ４５１（１９９９），２５７－２６３）および前立腺の癌または子宮内膜の癌に罹患する患者の血清を用いたこれらのライブラリーの連続スクリーニングによって同定され得る。次いで、免疫原性であり、かつ患者の血清中の抗体の形成を導くこれらのＴｒｐ８およびＴｒｐ１０ エピトープは、Ｔｒｐ８またはＴｒｐ１０ を発現する癌細胞に対する免疫発見のためのＴｒｐ８

またはＴｒｐ１０ 誘導ペプチドワクチンとして使用され得る。あるいは、Ｅ．ｃｏｌｉ発現系に対して、Ｔｒｐ８もしくはＴｒｐ１０ またはＴｒｐ８およびＴｒｐ１０ のエピトープは、ヒト胎児腎臓（Ｈｅｋ２９３）細胞（Ａｍｅｒｉｃａｎ Ｔｙｐｅ Ｃｕｌｔｕｒｅ Ｃｏｌｌｅｃｔｉｏｎ，ＡＴＣＣ ＣＲＬ １５７３ ）などの哺乳動物細胞株において発現され得る。

【００４４】

最後に、上記疾患の治療に有用な化合物は、イオンチャンネルＴｒｐ８、Ｔｒｐ９およびＴｒｐ１０ 上でアンタゴニストまたはアゴニストとして作用する化合物を含む。Ｔｒｐ８は、一価イオン（すなわちナトリウム）および二価イオン（すなわちカルシウム）の存在下でカルシウムイオンに対してのみ透過性である高いカルシウム選択的イオンチャンネルであることが示され得た（以下の実施例４ 、および図３Ａ、Ｃ 、Ｅ を参照のこと）。生理学的条件下で、Ｔｒｐ８は、大きな内部電流を示すカルシウム選択チャンネルである。Ｔｒｐ８チャンネル（ならびにＴｒｐ９およびＴｒｐ１０ａ／ｂチャンネル）のこの非常に大きな伝導性は、Ｔｒｐ チャンネルと相互作用する薬理学化合物をスクリーニングするための系（ハイスループットスクリーニング系を含む）を確立するのに有用である。有用なハイスループットスクリーニング系は、当業者に周知であり、例えば、生物学的系におけるカルシウムシグナル伝達を検出するためのアッセイにおいてＴｒｐ８、Ｔｒｐ９およびＴｒｐ１０ チャンネルをコードするＤＮＡ 配列で安定にまたは一過的にトランスフェクトされた細胞株を使用することが挙げられる。このような系としては、Ｃａ感受性色素（例えば、エクオリン、アポエクオリン、Ｆｕｒａ－２、Ｆｌｕｏ－３およびＩｎｄｏ－１など）に基づくアッセイが挙げられる。

【００４５】

したがって、本発明はまた、イオンチャンネルＴｒｐ８、Ｔｒｐ９および／またはＴｒｐ１０ におけるアゴニストまたはアンタゴニストとして作用する化合物を同定する方法に関し、該方法は、好ましくは、Ｔｒｐ８、Ｔｒｐ９および／またはＴｒｐ１０ をコードするＤＮＡ 配列と安定にまたは一過的にトランスフェクトした細胞に基づくシステムを用いることにより、試験化合物とイオンチャンネルＴｒｐ８、Ｔｒｐ９および／またはＴｒｐ１０ とを接触させ、該試験化合物がカルシウム取り込みに影響を与えるかどうかを決定することを含む。

【００４６】

投与のために、上記試薬は好ましくは適切な医薬キャリアと組み合わされる。適切な医薬キャリアの例は、当該分野において周知であり、リン酸緩衝化生理食塩水、水、オイル／水エマルジョンなどのエマルジョン、種々の型の湿潤剤、滅菌溶液などが挙げられる。かかるキャリアは、通常法により配合され得、適切な用量で被験体に投与されうる。適切な組成物の投与は、静脈内、腹腔内、皮下、筋内、局所または皮内投与などの異なる経路によりもたらされうる。投与経路は、もちろん腫瘍の性質および医薬組成物に含まれる化合物の種類に依存する。用量養生法は、担当医師および他の臨床因子により決定されうる。医学分野で周知のように、任意の一患者の用量は、患者のサイズ、体表面積、年齢、性別、投与される特定の化合物、投与時間および投与経路、腫瘍の種類および段階、全身の健康および同時に投与される他の薬物を含む多くの因子に依存する。

【００４７】

本発明のアンチセンスＲＮＡ またはリボザイムの送達は、直接適用により、または好ましくは、これらの化合物を含むキメラウイルスなどの組換え発現ベクターまたはコロイド分散系を用いることにより、達成されうる。所望の標的にこれらの核酸を送達することにより、例えば、ＰＣＡ の転移形成に関して、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂの細胞内発現、およびしたがって、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂのレベルが減少されえ、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂの陰性効果の阻害を生じうる。

【００４８】

例えば、コロイド分散系として衝撃送達により、または動脈における部位へカテーテルに

より、標的部位への直接適用を行ないうる。上記核酸の送達に使用しうるコロイド分散系は、高分子複合体、ナノカプセル、ミクロスフェア、ビーズおよび水中油エマルジョンを含む液体基準系（混合）、ミセル、リポソームおよびリポプレックス（ｌｉｐｏｐｌｅｘ）が挙げられる。好ましいコロイド系は、リポソームである。リポソームの組成は、通常リン脂質およびステロイド、特にコレステロールの組み合わせである。当業者は、所望の核酸分子の送達に適切なかかるリポソームを選択する位置にある。組織特異的または細胞特異的リポソームを、所望の腫瘍へのみの送達を達成するために使用しうる。リポソームの標的化は、一般に公知の方法を適用することにより、当業者により行われうる。この標的化は、受動的標的化（洞様毛細血管を含む器官においてＲＥＳ の細胞に分配するリポソームの自然の傾向を利用する）および能動的標的化（例えば、既知の方法により、例えば、抗体、レセプター、糖、糖脂質、タンパク質などの特異的リガンドにリポソームを結合させることにより）を含む。本発明では、特異的細胞表面リガンドを介して特異的腫瘍へのリポソームを標的化させるために、好ましくはモノクローナル抗体が使用される。

【００４９】

遺伝子治療に有用な好ましい組換えベクターは、ウイルスベクター、例えば、アデノウイルス、ヘルペスウイルス、ワクシニア、またはより好ましくはレトロウイルスなどのＲＮＡ ウイルスである。よりいっそう好ましくは、レトロウイルスベクターは、マウスまたはトリレトロウイルスの誘導体である。本発明で使用されうるかかるレトロウイルスベクターの例は、Ｍｏｌｏｎｅｙ マウス白血病ウイルス（ＭｏＭｕＬＶ）、Ｈａｒｖｅｙマウス肉腫ウイルス（ＨａＭｕＳＶ）、マウス乳房腫瘍ウイルス（ＭｕＭＴＶ） およびＲｏｕｓ肉腫ウイルス（ＲＳＶ） である。最も好ましくは、マウスベクターと比較して、より広い宿主範囲を提供するテナガザル白血病ウイルス（ＧａＬＶ）などの非ヒト霊長類レトロウイルスベクターが使用される。組換えレトロウイルスは不安定であるので、伝染性粒子を作製するために援助が必要である。かかる援助は、例えば、ＬＴＲ 内での調節配列の制御下でレトロウイルスの構造遺伝子の全てをコードするプラスミドを含むヘルパー細胞株を用いることにより、提供されうる。適切なヘルパー細胞株が、当業者に周知である。かかるベクターは、選択可能なマーカーをコードする遺伝子をさらに含みうるので、形質導入細胞が同定されうる。さらに、レトロウイルスベクターは、標的特異的になるように改変されうる。これは、例えば、糖、糖脂質、またはタンパク質、好ましくは抗体をコードするポリヌクレオチドを挿入することにより、達成されうる。当業者は、標的特異的ベクターのさらなる作製方法を知っている。インビトロ遺伝子療法またはインビボ遺伝子療法に関するさらに適切なベクターおよび方法が、文献に記載されており、当業者に公知である。例えば、ＷＯ９４／２９４６９またはＷＯ９７／００９５７参照。

【００５０】

標的器官、すなわち処置対象の腫瘍においてのみ発現を達成するために、例えば、アンチセンスＲＮＡ またはリボザイムをコードする核酸がまた、作動可能に組織特異的プロモーターに連結され、遺伝子療法に使用されうる。かかるプロモーターは、当業者に周知である（Ｚｉｍｍｅｒｍａｎｎ ら、（１９９４） Ｎｅｕｒｏｎ １２、１１－２４； Ｖｉｄａｌら；（１９９０） ＥＭＢＯ Ｊ． ９、８３３－８４０； Ｍａｙｆｏｒｄら、（１９９５）、Ｃｅｌｌ ８１ 、８９１－９０４； Ｐｉｎｋｅｒｔら、（１９８７） Ｇｅｎｅｓ ＆ Ｄｅｖ． １ 、２６８－７６参照）。

【００５１】

前記で論じた診断的研究における使用に対して、キットがまた本発明により提供される。かかるキットは、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂ、または代替的にはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂをコードするｍＲＮＡまたはＴｒｐ１０ａ／ｂアンチセンス転写物である標的細胞成分の検出に有用であり、ここで、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂ、または代替的にはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂをコードするｍＲＮＡまたはＴｒｐ１０ａ／ｂアンチセンス転写物の存在または増大した濃度は、前立腺腫瘍、子宮内膜癌、黒色腫、絨毛膜癌または肺癌を示し、

該キットは、Ｔｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ９、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂ、または代替的にはＴｒｐ８ａ 、Ｔｒｐ８ｂ 、Ｔｒｐ９、Ｔｒｐ１０ａおよび／またはＴｒｐ１０ｂをコードするｍＲＮＡまたはＴｒｐ１０ａ／ｂアンチセンス転写物の検出のためのプローブを含む。プローブは、検出可能に標識されうる。かかるプローブは、特異的抗体または特異的オリゴヌクレオチドでありうる。好ましい態様において、前記キットは、抗Ｔｒｐ８ａ 抗体、抗Ｔｒｐ８ｂ 抗体、抗Ｔｒｐ９抗体、抗Ｔｒｐ１０ａ抗体および／または抗Ｔｒｐ１０ｂ抗体を含み、例えばＥＬＩＳＡ により前記診断を可能にし、当該分野で公知の技術を用いて、固体支持体、例えば、ポリスチレンマイクロタイター皿またはニトロセルロース紙に結合した抗体を含む。代替的には、前記キットは、ＲＩＡ に基づき、放射性同位体で標識された前記抗体を含む。本発明のキットの好ましい態様において、抗体は、酵素、蛍光化合物、発光化合物、強磁性プローブまたは放射性化合物で標識される。本発明のキットは、例えば、本発明の１つ以上のプローブが充填された１つ以上の容器を含みうる。医薬品または生物学的産物の製造、使用または販売を調整する政府機関により規定された形態における通知がキットの容器に付随し、通知は、ヒト投与のための製造、使用または販売による機関による承認を反映する。

【００５２】

実施例

以下の実施例は、説明を意図し、本発明を限定しない。かかる実施例は、使用されうる代表的なものである一方、当業者に公知の他の方法が代替的に利用されうる。

【００５３】

実施例１：材料および方法

（Ａ）ｃＤＮＡクローンの単離およびノーザンブロット解析

全ＲＮＡ を標準技術を用いてヒト胎盤および前立腺から単離した。製造業者の説明書にしたがって、ポリ（Ａ）$^{+}$ ＲＮＡ－スピンカラム（Ｎｅｗ Ｅｎｇｌａｎｄ Ｂｉｏｌａｂｓ， Ｂｅｖｅｒｌｙ， ＵＳＡ） を用いてｍＲＮＡの単離を行なった。ｃＤＮＡ選択システム（Ｇｉｂｃｏ－ＢＲＬ， Ｒｏｃｋｖｉｌｌｅ， ＵＳＡ） を用いてポリ（ａ）$^{+}$ ＲＮＡ を逆転写し、λ－Ｚａｐファージ（Ｓｔｒａｔａｇｅｎｅ， Ｌａ Ｊｏｌｌａ， ＵＳＡ） にサブクローン化した。ヒト発現配列タグ（ＧｅｎＢａｎｋアクセッション番号１４０４０４２）を用いて、オリゴｄ（Ｔ）プライム化（ｐｒｉｍｅｄ）ヒト胎盤ｃＤＮＡライブラリーをスクリーニングした。いくつかのｃＤＮＡクローンを同定し、単離した。プライマー５’－ｇｃａ ｔａｇ ｇａａ ｇｇｇ ａｃａ ｇｇｔ ｇｇ－３’および５’－ｇａｇ ａｇｔ ｃｇａ ｇｇｔ ｃａｇ ｔｇｇ ｔｃｃ－３’ を用いて、さらなるｃＤＮＡクローンを２つの特異的なプライム化ｃＤＮＡライブラリーから単離した。

【００５４】

ｃＤＮＡクローンをサーモサイクラー（ＰＥ Ａｐｐｌｉｅｄ Ｂｉｏｓｙｓｔｅｍｓ， ＵＳＡ）およびＴｈｅｒｍｏ Ｓｅｑｕｅｎａｓｅ（Ａｍｅｒｓｈａｍ Ｐｈａｒｍａｃｉａ Ｂｉｏｔｅｃｈ Ｅｕｒｏｐｅ， Ｆｒｅｉｂｕｒｇ， Ｇｅｒｍａｎｙ）を用いて配列決定した。ＤＮＡ 配列を、自動シークエンサー（Ｌｉｃｏｒ， Ｌｉｎｃｃｏｌｎ， ＵＳＡ）を用いて解析した。

【００５５】

ノーザンブロット解析のために、０．８ ％アガロースゲル上の電気泳動により、ヒト胎盤または前立腺由来の５ μｇ のヒトポリ（Ａ）$^{+}$ ＲＮＡ を分離した。ポリ（Ａ）$^{+}$ ＲＮＡ をＨｙｂｏｎｄ Ｎナイロン膜（Ａｍｅｒｓｈａｍ Ｐｈａｒｍａｃｉａ Ｂｉｏｔｅｃｈ Ｅｕｒｏｐｅ， Ｆｒｅｉｂｕｒｇ， Ｇｅｒｍａｎｙ）に移した。膜を４２℃で一晩５０％ホルムアミドの存在下でハイブリダイズした。［α^{32}Ｐ］ｄＣＴＰおよび「ｒｅａｄｙ ｐｒｉｍｅ 」標識キット（Ａｍｅｒｓｈａｍ Ｐｈａｒｍａｃｉａ Ｂｉｏｔｅｃｈ Ｅｕｒｏｐｅ， Ｆｒｅｉｂｕｒｇ， Ｇｅｒｍａｎｙ）を用いて、ＤＮＡ プローブを標識した。市販のノーザンブロットを配給業者の説明書にしたがってハイブリダイズさせた（Ｃｌｏｎｔｅｃｈ， Ｐａｏｌｏ Ａｌｔｏ， ＵＳＡ）。

【００５６】

（Ｂ）発現プラスミドの構築およびＨＥＫ２９３細胞のトランスフェクション
Ｔｒｐ８ｂ のｃＤＮＡを含む組換えジシストロン（ｄｉｃｉｓｔｒｏｎｉｃ ）真核生物発現プラスミドｐｄｉＴＲＰ８ を用いて、トリβアクチンプロモーター、次いで内部リボソームエントリーサイド（ＩＲＥＳ）およびグリーン蛍光タンパク質（ＧＦＰ） のｃＤＮＡの制御下で、リポフェクションを行なった。ＴＲＰ８ｂ およびＧＦＰ の全タンパク質コード領域を保有するｐｄｉＴＲＰ８ を得るために（Ｐｒａｓｈｅｒ，Ｄ．Ｃ．ら（１９９２）、Ｇｅｎｅ １１１、２２９－２３３）、ＴＲＰ８ｂ ｃＤＮＡの５’および３’－ 非翻訳配列を除去し、脊椎動物における翻訳の開始に対するコンセンサス配列（Ｋｏｚａｋ，Ｍ．（１９８７）Ｎｕｃｌｅｉｃ Ａｃｉｄｓ Ｒｅｓｅａｒｃｈ１５、８１２５－８１４８）を翻訳開始コドンのすぐ５’に導入し、得られたｃＤＮＡを、トリβアクチンプロモーターの下流のｐＣＡＧＧＳベクター（Ｎｉｗａ，Ｈ．、Ｙａｍａｍｕｒａ，Ｋ．およびＭｉｙａｚａｋｉ，Ｊ．（１９９１）、Ｇｅｎｅ ８、１９３－１９９）にサブクローン化した。脳心筋炎ウイルスに由来するＩＲＥＳ（Ｋｉｍ，Ｄ．Ｇ．、Ｋａｎｇ，Ｈ．Ｍ．、Ｊａｎｇ，Ｓ．Ｋ．およびＳｈｉｎ Ｈ．Ｓ．（１９９２）Ｍｏｌ．Ｃｅｌｌ．Ｂｉｏｌ．１２，３６３６－３６４３）、それに続いてＳｅｒ６５Ｔｈｒ変異を含むＧＦＰ ｃＤＮＡ（Ｈｅｉｍ，Ｒ、Ｃｕｂｉｔｔ，Ａ．Ｂ．、Ｔｓｉｅｎ，Ｒ．Ｙ．（１９９５）Ｎａｔｕｒｅ ３７３、６６３－６６４）を、ＴＲＰ８ｂ ｃＤＮＡに３’をクローン化した。ＩＲＥＳ配列は１つの転写物からＴＲＰ８ｂ およびＧＦＰ の同時翻訳を可能にする。したがって、グリーン蛍光の発現により、トランスフェクト細胞を明白に検出しうる。

【００５７】
細胞内Ca^{2+}濃度のモニタリングのために、リポフェクタミン（Ｑｕｉａｇｅｎ，Ｈｉｌｄｒｅｎ，Ｇｅｒｍａｎｙ） の存在下に４：１ のモル比でｐｃＤＮＡ３－ＴＲＰ８ｂベクターおよびｐｃＤＮＡ３－ＧＦＰベクターを用いて、ヒト胎児腎臓（ＨＥＫ ２９３） 細胞を同時トランスフェクトした。ｐｃＤＮＡ３－ＴＲＰ８ｂを得るために、脊椎動物における翻訳の開始に対するコンセンサス配列（Ｋｏｚａｋ，Ｍ．（１９８７）Ｎｕｃｌｅｉｃ Ａｃｉｄｓ Ｒｅｓｅａｒｃｈ１５，８１２５－８１４８） を含むＴＲＰ８ｂ の全タンパク質コード領域をｐｃＤＮＡ３ベクター（Ｉｎｖｉｔｒｏｇｅｎ，Ｇｒｏｎｉｎｇｅｎ，Ｎｅｔｈｅｒｌａｎｄｓ）にサブクローン化した。カルシウムモニタリングおよびパッチクランプ試験を、それぞれ２日およびトランスフェクション後１日行なった。

【００５８】
（Ｃ）Ｔｒｐ８遺伝子の染色体局在化
ＮＩＧＭＳ 体ハイブリッドマッピングパネルＮｏ．２（Ｃｏｒｉｅｌｌ Ｉｎｓｔｉｔｕｔｅ，Ｃａｍｄｅｎ，ＮＪ，ＵＳＡ）を用いて、以前記載されたようにヒトＴＲＰ８遺伝子の染色体局在化を行なった（Ｄｒｗｉｎｇａ，Ｈ．Ｌ．、Ｔｏｊｉ，Ｌ．Ｈ．、Ｋｉｍ，Ｃ．Ｈ．、Ｇｒｅｅｎｅ，Ａ．Ｅ．、Ｍｕｌｉｖｏｒ，Ｒ．Ａ．（１９９３）Ｇｅｎｏｍｉｃｓ １６、３１１－３１４；Ｄｕｂｏｉｓ，Ｂ．Ｌ．およびＮａｙｌｏｒ，Ｓ．Ｌ．（１９９３）Ｇｅｎｏｍｉｃｓ １６、３１５－３１９）。

【００５９】
（Ｄ）インビトロ翻訳、グルタチオン－セファロースおよびカルモジュリンアガロース結合アッセイ
Ｎ－およびＣ－末端Ｔｒｐ８－ フラグメントをｐＧＥＸ－４Ｔ２ベクター（Ａｍｅｒｓｈａｍ Ｐｈａｒｍａｃｉａ Ｅｕｒｏｐｅ，Ｆｒｅｉｂｕｒｇ，Ｇｅｒｍａｎｙ）にサブクローン化し、グルタチオン－Ｓ－ トランスフェラーゼ（ＧＳＴ）－Ｔｒｐ８融合構築物を生じた（図４）。大腸菌ＢＬ ２１ 細胞においてＧＳＴ－ＴＲＰ８－ 融合タンパク質を発現させ、グルタチオン－セファロースビーズ（Ａｍｅｒｓｈａｍ Ｐｈａｒｍａｃｉａ Ｂｉｏｔｅｃｈ Ｅｕｒｏｐｅ，Ｆｒｅｉｂｕｒｇ，Ｇｅｒｍａｎｙ）を用いて精製した。

【００６０】

ＴＮＴ 結合転写／翻訳キット（Ｐｒｏｍｅｇａ，Ｍａｄｉｓｏｎ，ＵＳＡ）を用いて、^{35}Ｓ－メチオニンの存在下で、ヒトＴｒｐ８ ｃＤＮＡ およびアフリカツメガエルカルモジュリン（Ｘｅｎｏｐｕｓ ｌａｅｖｉｓ）ｃＤＮＡ（Ｄａｖｉｓ，Ｔ．Ｎ．およびＴｈｏｒｎｅｒ，Ｊ．Ｐｒｏｃ．Ｎａｔｌ．Ａｃａｄ．Ｓｃｉ．ＵＳＡ８６，７９０９－７９１３）のインビトロ翻訳を行なった。翻訳産物をゲル濾過（Ｓｅｐｈａｄｅｘ Ｇ５０、Ａｍｅｒｓｈａｍ Ｐｈａｒｍａｃｉａ Ｂｉｏｔｅｃｈ Ｅｕｒｏｐｅ，Ｆｒｅｉｂｕｒｇ，Ｇｅｒｍａｎｙ）により精製し、１ｍＭ Ca^{2+} または２ｍＭ ＥＧＴＡの存在下で５０ｍＭ Ｔｒｉｓ－ＨＣｌ、ｐＨ７．４、０．１％Ｔｒｉｔｏｎ Ｘ－１００、１５０ｍＭ ＮａＣｌ中にて、ＧＳＴ－Ｔｒｐ８またはカルモジュリン－アガロースに結合したグルタチオンビーズ（Ｃａｌｂｉｏｃｈｅｍ）と共に等量の^{35}Ｓ標識プローブを２時間インキュベートした。３回洗浄した後、結合タンパク質をＳＤＳサンプルバッファーを用いて溶出し、ＳＤＳ－ＰＡＧＥにより分画し、^{35}Ｓ 標識タンパク質をＰｈｏｓｐｈｏｒ Ｉｍａｇｅｒ（Ｆｕｊｉｆｉｌｍ，Ｔｏｋｙｏ，Ｊａｐａｎ）を用いて検出した。

【００６１】

（Ｅ）カルシウム測定

細胞内 Ca^{2+} 濃度（$[Ca^{2+}]_i$）をデジタル画像化システム（Ｔ．Ｉ．Ｌ．Ｌ．Ｐｈｏｔｏｎｉｃｓ，Ｐｌａｎｅｇｇ，Ｇｅｒｍａｎｙ）を用いて二重波長ｆｕｒａ－２蛍光比測定（Ｔｓｉｅｎ，Ｒ．Ｙ．（１９８８）Ｔｒｅｎｄｓ Ｎｅｕｒｏｓｃｉ．１１、４１９－４２４）により決定した。ＨＥＫ 細胞を１０％ウシ胎仔血清の存在下で最少必須培地において増殖させ、前記（Ｂ）に記載のようにｐｃＤＮＡ３－ＴＲＰ８ｂベクターおよびｐＣＤＮＡ３－ＧＦＰベクターを用いて同時トランスフェクトした。トランスフェクト細胞をグリーン蛍光の発現により検出した。４ μＭ ｆｕｒａ－２／ＡＭ（Ｍｏｌｅｃｕｌａｒ Ｐｒｏｂｅ，Ｏｒｅｇｏｎ，ＵＳＡ）を細胞に１時間負荷した。負荷後、細胞をバッファーＢ１（１０ｍＭ Ｈｅｐｅｓ、１１５ｍＭ ＮａＣｌ、２ｍＭ $MgCl_2$、５ｍＭ ＫＣｌ、ｐＨ７．４）で３回リンスし、（Ｇａｒｃｉａ，Ｄ．Ｅ．、Ｃａｖａｌｉｅ，Ａ．およびＬｕｘ，Ｈ．Ｄ．（１９９４）Ｊ．Ｎｅｕｒｏｓｃｉ １４、５４５－５５３）に記載のように３４０ｎｍ および３８０ｎｍ の励起波長で得られる蛍光比から $[Ca^{2+}]_i$ を計算した。

【００６２】

（Ｆ）電気生理学的記録

ＨＥＫ 細胞を（Ｂ）に記載の真核生物発現プラスミドｐｄｉＴＲＰ８ を用いてトランスフェクトし、トランスフェクション後１日電気力学的記録を行なった。（Ｈａｍｉｌｌ，Ｏ．Ｐ．、Ｍａｒｔｙ，Ａ．、Ｎｅｈｅｒ，Ｅ．、Ｓａｋｍａｎｎ，Ｂ．およびＳｉｇｗｏｒｔｈ，Ｆ．Ｊ．（１９８１）Ｐｆｌｕｅｇｅｒｓ Ａｒｃｈ．３９１、８５－１００；Ｐｈｉｌｉｐｐ，Ｓ．、Ｃａｖａｌｉｅ，Ａ．、Ｆｒｅｉｃｈｅｌ，Ｍ．、Ｗｉｓｓｅｎｂａｃｈ，Ｕ．、Ｚｉｍｍｅｒ，Ｓ．、Ｔｒｏｓｔ，Ｃ．、Ｍａｒｑｕａｒｔ，Ａ．、Ｍｕｒａｋａｍｉ，Ｍ．およびＦｌｏｃｋｅｒｚｉ，Ｖ．（１９９６）ＥＭＢＯ Ｊ．６１６６－６１７１）に記載のように、パッチクランプ技術のホールセルモードで、単細胞を電圧固定した。ピペット溶液は、（ｍＭ）で含まれる：１４０ アスパラギン酸、１０ ＥＧＴＡ、１０ ＮａＣｌ、１ $MgCl_2$、１０ Ｈｅｐｅｓ（ＣｓＯＨ を有してｐＨ７．２）または１２５ ＣｓＣｌ、１０ ＥＧＴＡ、４ $CaCl_2$、１０ Ｈｅｐｅｓ（ＣｓＯＨ を有してｐＨ７．２）を含んだ。容器の溶液は、（ｍＭ）で含まれる：１００ＮａＣｌ、１０ＣｓＣｌ、２ $MgCl_2$、５０マンニトール、１０グルコース、２０Ｈｅｐｅｓ（ＣｓＯＨを有してｐＨ７．４）および２ $CaCl_2$ を含むか、または $CaCl_2$ を添加しなかった（－Ca^{2+} 溶液）。二価を含まない容器の溶液は、（ｍＭ）：１１０Ｎ－メチル－Ｄ－ グルカミン（ＮＭＧＤ）を含んだ。変化する保持電位（ｈｏｌｄｉｎｇ ｐｏｔｅｎｔｉａｌｓ）で－１００～＋１００ｍＶの１００ｍｓｅｃ 電圧ランプの間ホールセル電流を記録した。

【００６３】

（Ｇ）インサイチュハイブリダイゼーション
６～８μＭの厚さのホルマリン固定組織スライスを用いて、インサイチュハイブリダイゼーションを行なった。スライスを水和し、１０μｇ／ｍｌのプロテイナーゼＫ（Ｒｏｃｈｅ Ｄｉａｇｎｏｓｔｉｃｓ，Ｍａｎｎｈｅｉｍ，Ｇｅｒｍａｎｙ）を含むＰＢＳバッファーの存在下で０．５時間インキュベートした。ビオチン標識デオキシ－オリゴヌクレオチド（０．５ｐｍｏｌ／μｌ）を用いて３３％ホルムアミドの存在下にスライスを３７℃で１２時間ハイブリダイズさせた。さらに、スライスを２×ＳＳＣで数回リンスし、アビジン／ビオチン標識西洋ワサビペルオキシダーゼ複合体（ＡＢＣ，ＤＡＫＯ，Ｓａｎｔａ Ｂａｒｂａｒａ，ＵＳＡ）を用いて２５℃で０．５時間インキュベートした。ＰＢＳバッファーを用いて数回洗浄した後、スライスをビオチン標識チラミド（ｔｙｒａｍｉｄ）および過酸化物（０．１５％ｗ／ｖ）の存在下で１０分間インキュベートし、ＰＢＳバッファーでリンスし、さらにＡＢＣ複合体と共に０．５時間インキュベートした。スライスをＰＢＳバッファーを用いて洗浄し、ＤＡＢ溶液（Ｎ，Ｎ－ジメチル－ホルムアミド中にジアミノベンジジン（５０μｇ／ｍｌ）、５０ｍＭＴｒｉｓ／ＥＤＴＡバッファーｐＨ８．４、０．１５％ H_2O_2；Ｍｅｒｃｋ，Ｄａｒｍｓｔａｄｔ，Ｇｅｒｍａｎｙ）の存在下でインキュベートした。４分後、水中でスライドをインキュベートすることにより検出を停止した。ＮＨＳ－ＬＣビオチン（スルホスクシニミジル－６－（ビオチニミド）－ヘキサノエート）、２．５ｍｇ／ｍｌ；Ｐｉｅｒｃｅ，Ｒｏｃｋｆｏｒｄ，ＵＳＡ）およびチラミン－ＨＣｌ（０．７５ｍｇ／ｍｌ，Ｓｉｇｍａ）を２５ｍＭホウ酸バッファー、ｐＨ８．５中で１２時間インキュベートすることにより、チラミドをビオチン標識した。チラミド溶液をＰＢＳバッファー中で１～５：１０００に希釈した。

【００６４】
（Ｈ）ＧｅｎＢａｎｋ アクセッション番号：ＴＲＰ８ａ、Ａｊ２４３５００；ＴＲＰ８ｂ Ａｊ２４３５０１

【００６５】
実施例２：ＴＲＰ８転写物の発現
イオンチャンネルのＴＲＰファミリーに遠く関連するタンパク質の検索において、ヒト発現配列タグ（ＥＳＴ，ＧｅｎＢａｎｋ アクセッション番号１４０４０４２）をＶＲ１遺伝子に少し相同であるＢＬＡＳＴプログラム（Ｎａｔｉｏｎａｌ Ｃｅｎｔｅｒ ｆｏｒ Ｂｉｏｔｅｃｈｎｏｌｏｇｙ Ｉｎｆｏｒｍａｔｉｏｎ（ＮＣＢＩ）にて；Ａｌｔｓｃｈｕｌ，Ｓ．Ｆ．、Ｇｉｓｈ，Ｗ．、Ｍｉｌｌｅｒ，Ｗ．、Ｍｙｅｒｓ，Ｅ．Ｗ．およびＬｉｐｍａｎ，Ｄ．Ｊ．Ｊ．（１９９０）Ｍｏｌ．Ｂｉｏｌ．５、４０３－４１０）を用いてＧｅｎＢａｎｋ データベースにおいて同定した。数個のヒト胎盤ｃＤＮＡライブラリーを構築し、プローブとしてこのＥＳＴ ＤＮＡを用いてスクリーニングした。数個の全長ｃＤＮＡクローンを同定し、単離した。全長ｃＤＮＡクローンは、３個のアミノ酸において異なる２つの推定上のタンパク質をコードし、Ｔｒｐ８ａおよびＴｒｐ８ｂと呼んだ（図１ｃ、２ａ、７および８Ａ）。２個体の胎盤から構築された２つのｃＤＮＡライブラリーからｃＤＮＡクローンを単離することにより、この知見を再現した。誘導されたタンパク質配列は、６個の膜貫通ドメイン、ｔｒｐチャンネルに特有の全般的な特徴および関連するタンパク質を含む（図１ｂ）。配列は、一方で公開された、ラット腸から単離されたカルシウム取り込み輸送タンパク質１（ＣａＴ１）（Ｐｅｎｇ，Ｊ．Ｂ．、Ｃｈｅｎ，Ｘ．Ｚ．、Ｂｅｒｇｅｒ，Ｕ．Ｖ．、Ｖａｓｓｉｌｅｖ，Ｐ．Ｍ．、Ｔｓｕｋａｇｕｃｈｉ，Ｈ．、Ｂｒｏｗｎ，Ｅ．Ｍ．およびＨｅｄｉｇｅｒ Ｍ．Ａ．（１９９９）Ｊ．Ｂｉｏｌ Ｃｈｅｍ．６；２７４、２２７３９－２２７４６）およびウサギ腎臓から単離された上皮カルシウム取り込みチャンネル（ＥＣａＣ）（Ｈｏｅｎｄｅｒｏｐ，Ｊ．Ｇ．、ｖａｎ ｄｅｒ Ｋｅｍｐ，Ａ．Ｗ．、Ｈａｒｔｏｇ，Ａ．、ｖａｎ ｄｅ Ｇｒａａｆ，Ｓ．Ｆ．、ｖａｎ Ｏｓ，Ｃ．Ｈ．、Ｗｉｌｌｅｍｓ，Ｐ．Ｈ．およびＢｉｎｄｅｌｓ，Ｒ．Ｊ．（１９９９）Ｊ．Ｂｉｏｌ Ｃｈｅｍ．２６；２７４、８３７５－８３７８）に密接に関連した。Ｔｒｐ８ａ／ｂ転写物の発現は、ヒト

胎盤、膵臓および前立腺において検出可能であり（図５）、ノーザンシグナルのサイズ（３．０ｋｂ）は、単離された全長ｃＤＮＡのサイズに対応する。さらに、１．８ｋｂのより短い転写物、おそらくスプライスバリアントは、ヒト精巣において検出可能である。Ｔｒｐ８ ｍＲＮＡは小腸または結腸では発現されないこと（図５）は、Ｔｒｐ８がラットＣａＴ１またはウサギＥＣａＣタンパク質のヒトオルソログではないことを意味する。他の関連配列があるかどうかを調査するために、Ｔｒｐ８ａ／ｂ 由来プライマー（ＵＷ２４１、５’－ＴＡＴ ＧＡＧ ＧＧＴ ＴＣＡ ＧＡＣ ＴＧＣ－３’およびＵＷ２４２、５’－ＣＡＡ ＡＧＴ ＡＧＡ ＴＧＡ ＧＧＴ ＴＧＣ－３’）を使用して、Ｔｒｐ８配列にヌクレオチドレベルで９５％同一である１０５ｂｐ フラグメントをヒトゲノムＤＮＡから増幅した（データは示さず）。これは、ヒトにおける少なくともゲノムレベルでの数個の類似配列の存在を示す。

【００６６】

実施例３：Ｔｒｐ８タンパク質の２つのバリアント（Ｔｒｐ８ａおよびＴｒｐ８ｂ）は多型を生じる

Ｔｒｐ８ ｃＤＮＡの２つのバリアントをヒト胎盤から単離し（図２Ａ、７および８Ａ）、それは３つのアミノ酸において異なる２つのタンパク質をコードし、Ｔｒｐ８ａおよびＴｒｐ８ｂと呼んだ。Ｔｒｐ８ａ／ｂ 特異的プライマーを設計し、ヒトＴ－リンパ球から単離したゲノムＤＮＡ 由来のＴｒｐ８遺伝子の４５８ｂｐ のＤＮＡ フラグメントを増幅した（プライマー対：ＵＷ２４３、５’－ＣＡＣ ＣＡＴ ＧＴＧ ＣＴＧ ＣＡＴ ＣＴＡ ＣＣ－３’およびＵＷ２４４、５’－ＣＡＡ ＴＧＡ ＣＡＧ ＴＣＡ ＣＣＡ ＧＣＴ ＣＣ－３’）。増幅産物は、Ｔｒｐ８ａ の由来するタンパク質の配列がアミノ酸のバリンを含み、Ｔｒｐ８ｂ 配列がメチオニンを含む１部の配列ならびにサイレント塩基対置換（ｇ対ａ）および３０３ｂｐ のイントロンを含む（図２Ａ、Ｂ）。Ｔｒｐ８遺伝子の両方のバリアント（ａ、ｂ）が等量でゲノムＤＮＡ から増幅された。このことは、ヒトゲノムにおける両方のバリアントの存在を示し、それゆえにＲＮＡ 編集の結果でない（図２Ｂ）。Ｔｒｐ８ａ 遺伝子は、制限酵素Ｂｓｐ１２８６Ｉを用いる４５８ｂｐのゲノムフラグメントを切り出すことにより、Ｔｒｐ８ｂ 遺伝子と区別されうる（図２Ｂ）。鋳型として１２人のヒト被験体の血液から単離したヒトゲノムＤＮＡ を用いて、４５８ｂｐ フラグメントを増幅し、ＢＳＰ１２８６Ｉを用いて拘束した。試験した被験体１１人において、Ｔｒｐ８ｂ 遺伝子のみが検出可能であり、一方１人の被験体（７）がＴｒｐ８ａ およびＴｒｐ８ｂ 遺伝子を含む（図２Ｄ）。これらは、２ つのＴｒｐ８バリアントが多型により生じ、個体の遺伝子を表さないことを示す。Ｔｒｐ８特異的プライマーおよび染色体ＤＮＡ を鋳型として用いると、Ｔｒｐ８遺伝子座は第７ 染色体で検出可能である（図２Ｃ）。

【００６７】

実施例４：Ｔｒｐ８ｂ はカルシウム透過チャンネルである

Ｔｒｐ８ｂ ｃＤＮＡのタンパク質コード配列を、サイトメガロウイルスプロモーター（ＣＭＶ）の制御下でｐｃＤＮＡ３ベクター（Ｉｎｖｉｔｒｏｇｅｎ，Ｇｒｏｎｉｎｇｅｎ，Ｎｅｔｈｅｒｌａｎｄｓ）にサブクローン化した。Ｔｒｐ８ｂ ｐｃＤＮＡ３構築物（ｐｃＤＮＡ３－Ｔｒｐ８ｂ ベクター）およびグリーン蛍光タンパク質（ＧＦＰ）をコードするｐｃＤＮＡ３－ＧＦＰベクターを４：１ の比で用いてヒト胎児腎臓（ＨＥＫ２９３）細胞を同時トランスフェクトした。Ｔｒｐ８ｂ ｃＤＮＡおよびレポーターのｃＤＮＡ、ＧＦＰ をヒト胎児腎臓（ＨＥＫ ２９３）細胞において、一過性に発現させた。レポーター遺伝子ＧＦＰ のグリーン蛍光により同定した同時トランスフェクト細胞において、二重波長ｆｕｒａ－２蛍光比測定により、細胞内Ca^{2+}濃度（$[Ca^{2+}]_i$）および$[Ca^{2+}]_i$ の変化を決定した（図３Ｆ）。

【００６８】

二重波長ｆｕｒａ－２蛍光比測定は、蛍光Ca^{2+}感受性染料であり、ＥＧＴＡの構造に基づいてＲ．Ｙ．Ｔｓｉｅｎ により設計された（例えば、Ｔｒｅｎｄｓ Ｎｅｕｒｏｓｃｉ．１１、４１９－４２４（１９８８））ｆｕｒａ－２を用いる標準手順である（例え

ば、An introduction of Molecular Neurobiology（Hall, Z.W. 編）Sinauer Associates、Sunderland、USA（１９９２））。その蛍光放出スペクトルは、生理学的濃度範囲におけるCa^{2+}への結合により変化する。Ca^{2+}の不在下で、fura－2は、３８５nm の励起波長で最も強く蛍光を発し、fura－2がCa^{2+}に結合する場合、最も有効な励起波長は、３４５nm にシフトする。この特性を使用して細胞内の局部的なCa^{2+}濃度を測定する。細胞膜を横切って拡散し、サイトゾルエステラーゼにより活性化fura－2に加水分解されるfura－2エステル（例えば、fura－2AM）と共に細胞に負荷をかけうる。

【００６９】

１mM Ca^{2+}の存在下で、Trp8発現細胞は３００nM より多くのサイトゾルCa^{2+}を典型的に含み、一方非トランスフェクト対照は１００nM 未満のCa^{2+}イオンを含んだ（図３F）。細胞外Ca^{2+}なしでTrp8b トランスフェクト細胞をインキュベートしたとき、細胞内Ca^{2+}濃度（$[Ca^{2+}]_i$）は、非トランスフェクト細胞に匹敵するレベルに減少した。容器への１mM Ca^{2+}の再添加は、Trp8b トランスフェクト細胞においてサイトゾル$[Ca^{2+}]$の有意な増加を生じたが、対照では生じなかった（図３F）。Ca^{2+}イオンの容器溶液への再添加の後、サイトゾルCa^{2+}濃度は、Trp8b トランスフェクト細胞において高い定常状態レベルのままである。

【００７０】

実施例５：Trp8発現細胞はカルシウム選択内部電流を示す

TRP8の電気生理学的特性を詳細に特徴付けるために、ジシストロン発現ベクターpdiTRP8 を用いて、HEK ２９３ 細胞においてTRP8およびGFP を同時発現させ、パッチクランプ技術を用いてホールセルモードで電流を測定した（Hamill, O.P.、Marty, A.、Neher, E.、Sakmann, B. およびSigworth, F.J.（１９８１）Pflugers Arch.、３９１、８５－１００）。

【００７１】

真核生物発現プラスミドpdiTRP8 は、トリβアクチンプロモーター、次に内部リボソームエントリーサイド（IRES）およびグリーン蛍光タンパク質（GFP）のcDNAの制御下でTrp8b のcDNAを含む。TRP8b およびGFP の全タンパク質コード領域を保有するpdiTRP8 を得るために（Prasher, D.C.ら（１９９２）、Gene １１１、２２９－２３３）、TRP8b cDNAの５’および３’－ 非翻訳配列を除去し、脊椎動物における翻訳の開始に対するコンセンサス配列（Kozak, M.（１９８７）Nucleic Acids Research１５, ８１２５－８１４８）を翻訳開始コドンの５’直前に導入し、得られたcDNAを、トリβアクチンプロモーターの下流のpCAGGSベクター（Niwa, H.、Yamamura, K. およびMiyazaki, J.（１９９１）、Gene ８、１９３－１９９）にサブクローン化した。次いで、脳心筋炎ウイルスに由来するIRES（Kim, D.G.、Kang, H.M.、Jang, S.K. およびShin H.S.（１９９２）Mol. Cell. Biol. １２、３６３６－３６４３）、それに続いてSer６５Thr変異を含むGFP cDNA（Heim, R, Cubitt, A.B.、Tsien, R.Y.（１９９５）Nature ３７３、６６３－６６４）を、TRP8b cDNAに３’をクローン化した。IRES配列は１つの転写物からTRP8b およびGFP の同時翻訳を可能にする。したがって、グリーン蛍光の顕色により、トランスフェクト細胞を明白に検出しうる。

【００７２】

２mM の外部カルシウムの存在下で、Trp8b トランスフェクトHEK 細胞は内部整流電流を示し、その大きさは細胞内カルシウムのレベルおよび電気化学的推進力に依存する。静止膜電位を、－４０mV で保持するか、またはパルス間でカルシウムの流入に

対する推進力を下げるために＋７０ｍＶ のいずれかで保持した。電流の跡を－１００から＋１００ｍＶまで電圧スロープに応答して記録し、毎秒ごとに適用した。内部および外部電流を経時的にモニターするために、本発明者らはスロープの－８０ｍＶ および＋８０ｍＶ で電流の大きさを解析した。図３Ａは、経時的な－８０ｍＶ での電流の代表的な跡を示す。－４０ｍＶ または＋７０ｍＶ の両方の保持電位で、電流は、ＧＦＰ 含有ベクターのみを有するトランスフェクトされた細胞においてよりも有意に大きい（図３Ｅ）。驚くべきことに、正の保持電位への変更後、Ｔｒｐ８トランスフェクト細胞における電流の大きさはゆっくり増加し、約７０秒後に定常状態に届く（図３Ａ）。誘導電流の選択性を決定するために、次に、本発明者らは、ナトリウムを含まずCa^{2+}を添加しない溶液（図３Ａ、Ｃ ）、またはナトリウムを含むが二価のイオンを含まない容器溶液のいずれかを用いて細胞を灌流した。溶液変化のみの効果についての対照のために、本発明者らはまた、標準容器を用いて灌流した（図３Ａのパフを参照）。外部Ca^{2+}の除去は、ｔｒｐ８誘導電流を完全に廃止する－残りの電流は大きさおよび形において対照と同一である一方（図３Ａ、Ｃ 、Ｅ ）、外部ナトリウムの除去は効果がない（図３Ｅ）。カルシウム選択チャンネル（例えば、Ｖｅｎｎｅｋｅｎｓ，Ｒ．、Ｈｏｅｎｄｅｒｏｐ，Ｇ．Ｊ．、Ｐｒｅｎｅｎ，Ｊ．、Ｓｔｕｉｏｖｅｒ，Ｍ．、Ｗｉｌｌｅｍｓ 、ＰＨＧＭ、Ｄｒｏｏｇｍａｎｓ，Ｇ．、Ｎｉｌｉｕｓ，Ｂ．およびＢｉｎｄｅｌｓ，Ｒ．Ｊ．Ｍ（１９９９）Ｊ．Ｂｉｏｌ．Ｃｈｅｍ．２７５、３９６３－３９６９ ）の重要な性質は、全ての外部二価イオン、すなわちCa^{2+}およびマグネシウムを除去した場合にのみナトリウムを処理するための能力である。ｔｒｐ８チャンネルがこの現象と一致するかどうかを調べるために、標準の容器溶液をナトリウムと１ｍＭ のＥＧＴＡのみを含む溶液に切り替えた。図３ＢおよびＤ に見られうるように、Ｔｒｐ８トランスフェクト細胞は、このとき非常に大きいナトリウム電流を伝導しうる。驚くべきことに、溶液変更直後、電流は迅速に増加する前にまずより小さくなり、孔は最初はまだカルシウムにより遮断されたまま（変則モル画分挙動と通常呼ばれる現象（Ｗａｒｎａｔ，Ｊ，、Ｐｈｉｌｉｐｐ，Ｓ．、Ｚｉｍｍｅｒ，Ｓ．、Ｆｌｏｃｋｅｒｚｉ，Ｖ．、およびＣａｖａｌｉｅ Ａ．（１９９９）Ｊ．Ｐｈｙｓｉｏｌ．（Ｌｏｎｄ） ５１８、６３１－６３８ ））であり得ることを示す。標準の容器溶液におけるＴｒｐ８トランスフェクト細胞の測定外部電流は、非トランスフェクト対照細胞またはレポーター遺伝子ＧＦＰ を発現するのみである細胞と有意に異ならない。外部Ca^{2+}の除去がＴｒｐ８特異的電流を廃止する場合、残りの電流を溶液変化の前の電流から引き算し、非混入Ｔｒｐ８コンダクタンスを得た（図３Ｃにおける挿入図を参照）。所定のイオン条件（内部の高いＥＧＴＡ、外部の２ｍＭ Ca^{2+}）から予期されるように、電流－電圧の関係は、ここで外部電流がほとんどないか全くない顕著な内部整流を示す。

【００７３】

Ｔｒｐ８電流の発生の時間経過と電流の大きさの両方が、刺激の頻度（データ示さず）、内部Ca^{2+}濃度および外部Ca^{2+}濃度ならびに静止膜電位に依存し、このことは、Ｔｒｐ８カルシウムコンダクタンスがCa^{2+}媒介フィードバック機構により複雑に（ｉｎｔｒｉｃａｌｌｙ）調節されることを示唆する。

【００７４】

実施例６：Ca^{2+}／カルモジュリンはＴｒｐ８タンパク質のＣ 末端に結合する

カルシウム調節フィードバックの主な媒介物質であるカルモジュリンが関与するかどうかを試験するために、まずＴｒｐ８タンパク質がカルモジュリンに結合しるうるかどうかを生化学的に調査した。インビトロで^{35}Ｓ－メチオニンの存在下にてＴｒｐ８ ｃＤＮＡを翻訳し、産物をカルモジュリンアガロースビーズと共にインキュベートした。Ca^{2+}の存在下または不在下のいずれかで数回洗浄した後、ビーズをＬａｅｍｍｌｉ バッファー中でインキュベートし、ＳＤＳ－ポリアクリルアミドゲル電気泳動に供した。Ca^{2+}の不在下ではなく、Ca^{2+}（１ｍＭ ）の存在下で、Ｔｒｐ８タンパク質はカルモジュリンに結合する（図４Ｂ）。

【００７５】

結合部位を厳密にするために、２つのアプローチを行った。第１に、Ｔｒｐ８の様々な細胞内ドメインのＧＳＴ－ＴＲＰ８融合タンパク質を構築し、大腸菌において発現させ、グルタチオンセファロースビーズに結合させた。次にこれらのビーズをインビトロで翻訳された^{35}Ｓ－標識カルモジュリンとインキュベートし、洗浄し、ゲル電気泳動に供した。第２に、インビトロで翻訳されたＴｒｐ８タンパク質の切断型を、上記カルモジュリン－アガロースへの結合に使用した。図４ＡおよびＣ で示されるように、Ｔｒｐ８のＮ 末端領域（Ｎ１、Ｎ２）の融合タンパク質はカルモジュリンに結合しなかった一方、Ｃ 末端フラグメント（Ｃ１、Ｃ２、Ｃ３、Ｃ４）は、カルシウムの存在下でカルモジュリン結合を示した（全体のＴｒｐ８タンパク質内のフラグメントの局在下について、図４Ｃ参照）。したがって、インビトロで翻訳されたＴｒｐ８の切断型は、Ｃ 末端の３２個のアミノ酸残基を欠如し、カルモジュリン－アガロースに結合しなかった（４Ｂ）。本発明者らは、カルモジュリン結合部位をＴｒｐ８タンパク質のアミノ酸残基６９１ ～７１１ に制限している。このカルモジュリン結合部位は、典型的に保存されたＩＱ（通常のミオシンのモチーフ）に類似しないが、数個の荷電アミノ酸残基が保存されたショウジョウバエメラノガスター（Ｄｒｏｓｏｐｈｉｌａ ｍｅｌａｎｏｇａｓｔｅｒ） の一過性レセプター電位様（ｔｒｐｌ ）タンパク質（ＷａｒｒおよびＫｅｌｌｙ 、１９９６）のカルシウム依存性カルモジュリン結合部位１に対する制限された配列相同性を有する。Ｔｒｐ８タンパク質のカルモジュリン結合部位の配列は、Ｅｒｉｃｋｓｏｎ－ＶｉｔａｎｅｎおよびＤｅ Ｇｒａｄｏにより提案されたモデル（１９８７， Ｍｅｔｈｏｄｓ Ｅｎｚｙｍｏｌ． １３９、４５５－４７８ ）による、帯電し、かつ疎水的部位を有する推定の両親媒性のαヘリックスホイール構造に似ている。

【００７６】

実施例７：ヒト胎盤および膵臓におけるＴｒｐ８転写物の発現

インサイチュでのハイブリダイゼーション実験のために、１０週齢の発育不全のヒト胎盤由来の数個のスライドを使用した。インサイチュでのハイブリダイゼーション実験は、ヒト胎盤でのＴｒｐ８転写物の発現を示した（図５Ｂ）。発現は、胎盤の栄養膜および合胞体層で検出可能であったが、ランゲルハンス細胞では検出不可能であった。

【００７７】

Ｔｒｐ８転写物は、ヒト膵臓において検出可能である（図５Ａ）。したがって、Ｔｒｐ８プローブを、ヒト膵臓の組織切片にハイブリダイズさせた。膵臓癌を罹患する患者から膵臓組織を取り出した。Ｔｒｐ８発現は、膵臓腺房細胞において検出可能であるが、ランゲルハンス島においては検出不可能である（図５Ｃ）。膵臓癌の領域には、Ｔｒｐ８発現を見出さなかった（データ示さず）。

【００７８】

さらに、インサイチュでのハイブリダイゼーションならびにノーザン解析により、ヒト結腸においてもヒト腎臓においてもＴｒｐ８ ｃＤＮＡ は、検出不可能である（図５Ａ、Ｄ）。インサイチュ発現データとあわせたノーザン結果は、Ｔｒｐ８タンパク質が、ラット腸から（Ｐｅｎｇ，Ｊ．Ｂ． 、Ｃｈｅｎ，Ｘ．Ｚ． 、Ｂｅｒｇｅｒ，Ｕ．Ｖ． 、Ｖａｓｓｉｌｅｖ，Ｐ．Ｍ． 、Ｔｓｕｋａｇｕｃｈｉ，Ｈ．、Ｂｒｏｗｎ，Ｅ．Ｍ．およびＨｅｄｉｇｅｒ，Ｍ．Ａ．（１９９９）Ｊ．Ｂｉｏｌ．Ｃｈｅｍ．６；２７４，２２７３９－２２７４６ ）ならびにウサギ腎臓から（Ｈｏｅｎｄｅｒｏｐ，Ｊ．Ｇ．、ｖａｎ ｄｅｒ Ｋｅｍｐ，Ａ．Ｗ． 、Ｈａｒｔｏｇ，Ａ． 、ｖａｎ ｄｅ Ｇｒａａｆ，Ｓ．Ｆ．、ｖａｎ Ｏｓ，Ｃ．Ｈ． 、Ｗｉｌｌｅｍｓ，Ｐ．Ｈ．およびＢｉｎｄｅｌｓ，Ｒ．Ｊ．（１９９９）Ｊ．Ｂｉｏｌ．Ｃｈｅｍ．２６；２７４、８３７５－８３７８ ）それぞれクローン化されたＣａＴ１およびＥＣａＣチャンネルのヒトオルソログではないことを示している。Ｔｒｐ８は、その発現がＣａＴ１が豊富に発現される小腸および結腸組織において検出不可能であるので、ＣａＴ１のヒト型を表さないようである。しかしながら、Ｔｒｐ８がラットＣａＴ１のヒト型である場合、第２の遺伝子産物は、ラット小腸および結腸におけるＣａＴ１に帰するヒト小腸および結腸におけるCa^{2+}取り込みに必要とされるようである。

【００７９】
実施例８：前立腺の良性組織および悪性組織におけるＴｒｐ８転写物の異なる発現
市販のノーザンブロット（Ｃｌｏｎｔｅｃｈ，Ｐａｌｏ Ａｌｔｏ，ＵＳＡ）に対するＴｒｐ８プローブのハイブリダイゼーションにより示されるように、Ｔｒｐ８転写物をヒト前立腺において発現させる（図５Ａ）。Ｔｒｐ８転写物は、良性前立腺過形成（ＢＰＨ）を罹患する患者のプールされたｍＲＮＡを用いるノーザンブロット解析により、検出不可能であった（図５Ａ、前立腺＊）。細胞レベルでのＴｒｐ８発現を試験するために、Ｔｒｐ８特異的ｃＤＮＡプローブを用いて前立腺組織の切片をハイブリダイズさせた（表３）。Ｔｒｐ８転写物の発現は、正常前立腺（ｎ＝３）、良性過形成（ＢＰＨ、ｎ＝１５）または前立腺上皮内腫瘍（ＰＩＮ、ｎ＝９）において検出不可能である（図６Ａ、Ｃ、Ｅ）。Ｔｒｐ８転写物は、発現レベルは異なるが、前立腺癌（ＰＣＡ）においてのみ検出可能であった。低い発現レベルが、原発性癌において見出された（癌細胞の２～１０％、ｎ＝８）（図７Ｂ）。非常に強い発現がレジディブ（ｒｅｚｉｄｉｖｅ）癌（１０～６０％）（図７Ｄ、ｎ＝６）および前立腺の転移（６０～９０％、ｎ＝４）（図７Ｆ）において検出可能であった。したがって、図５Ａで使用された市販のノーザンブロットは、配給業者により示されるような正常前立腺ｍＲＮＡを含むのみではないことが結論づけられるべきである。配給業者の説明書により、このノーザンブロットに使用した前立腺ｍＲＮＡは１４～６０歳の範囲の１５人のヒト被験体から回収した。この前立腺組織を病理学的手段により実験しなかった。Ｔｒｐ８発現は、正常または良性前立腺において検出不可能であるので、この知見は、このノーザンブロットに使用したｍＲＮＡが、前立腺癌組織からいくぶん抽出されたことを示す。要約すると、Ｔｒｐ８発現は、悪性前立腺においてのみ検出可能であり、したがって、Ｔｒｐ８ ｃＤＮＡ は、前立腺癌に対するマーカーである。結果を表４にまとめる。
【００８０】
【表３】

表３

インサイチュハイブリダイゼーションに使用したTrp8プローブ：

プローブ（アンチセンス）

1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'

2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'

3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

対照（センス）

1.) 5' GGCAAGATCTCAACCGGCAGCGGA 3'

2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'

3.) 5' TTCCACACCCGGGGCTCTGAGGAT 3'

【００８１】
【表４】

表４

前立腺	合計	陰性	陽性
正常	3	3	0
BPH	15	15	0
PIN	9	9	0
癌	18	1	17

【００８２】
（Ｂ）子宮の良性組織および悪性組織におけるＴｒｐ８転写物の特異的発現
さらに、Ｔｒｐ８が、子宮内膜癌（子宮癌とも呼ばれ、子宮肉腫または子宮頸の癌とは区別される）において発現される一方、正常な子宮組織では発現が観察されなかったことが示されうる。したがって、Ｔｒｐ８もまた、前記癌の診断に対する特異的なマーカーである（図１２）。
【００８３】
実施例９：Ｔｒｐ９の特徴付け
Ｔｒｐ９の完全なタンパク質コード配列を決定した（図９ ）。Ｔｒｐ９転写物は、ヒト前立腺およびヒト結腸において優勢に発現する。ノーザンブロット解析により示されうるように、良性前立腺過形成（ＢＰＨ 、図１３、上のパネル左） または前立腺癌（図１３、上のパネル右） においてＴＲＰ９の発現の差異はない。しかしながら、Ｔｒｐ９は、前立腺癌に対する参照マーカーとして有用である、すなわち、Ｔｒｐ８の発現レベルの定量に使用されうる。患者および健常個体におけるＴｒｐ８：Ｔｒｐ９の発現の比は、定量アッセイの開発に有用である。
【００８４】
実施例１０：Ｔｒｐ１０ の特徴付け
ＴＲＰ１０ （ａ およびｂ ）の完全なタンパク質コード配列をバイオコンピューティングにより決定した（図１０および１１）。ノーザンブロット解析におけるプローブとしてＴｒｐ１０ ｃＤＮＡの２３５ｂｐ フラグメントを用いると、ＴＲＰ１０ 転写物は、前立腺癌を罹患する個体から単離されたｍＲＮＡにおいてのみ検出されうるが（図１３、下のパネル） 、前立腺の良性組織（前立腺ＢＰＨ ）から単離されたｍＲＮＡにおいても、心臓、脳、胎盤、肺、肝臓、骨格筋、腎臓および膵臓から単離されたｍＲＮＡにおいても検出され得ない。Ｔｒｐ１０ ｃＤＮＡの２３５ｂｐ ｃＤＮＡフラグメントを、プライマー対ＵＷ２４８ 、５’－ＡＣＡ ＧＣＴ ＧＣＴ ＧＧＴ ＣＴＡ ＴＴＣ Ｃ－３’およびＵＷ２４９ ５’－ＴＡＴ ＧＴＧ ＣＣＴ ＴＧＧ ＴＴＴ ＧＴＡ ＣＣ －３’および前立腺ｃＤＮＡを鋳型として用いて増幅した。要約すると、ＴＲＰ８に類似するＴｒｐ１０ａおよびＴｒｐ１０ｂはまた、悪性前立腺組織において発現される。ここまでは、その発現は、試験した他のいずれの組織においても観察され得なかった（上記参照のこと）。したがって、Ｔｒｐ１０ａおよびＴｒｐ１０ｂはまた、悪性前立腺組織に特異的である有用なマーカーである。
【００８５】
さらに、生物学的情報のための国立センター（ＮＣＢＩ）の公開データベースにおけるデータベース検索は、Ｔｒｐ１０ 配列と部分的に同一である数個の発現配列タグ（ＥＳＴクローン）の存在を明らかにした。これらのＥＳＴ クローンを肺、胎盤、前立腺の癌組織から、および黒色腫から最初から単離した。これらのクローンは、次のアクセッション番号：ＢＥ２７４４４８、ＢＥ４０８８８０、ＢＥ２０７０８３、ＢＥ７９１１７３、ＡＩ６７１８５３、ＢＥ３９０６２７を有するクローンを含む。結果は、これらの組織の癌細胞がＴｒｐ１０ 関連転写物を発現する一方、対応する健常組織におけるＴｒｐ１０ 転写物の発現が検出不可能であることを示す（図１３）。さらに、黒色腫および前立腺癌の癌細胞において、Ｔｒｐ１０ 転写物は、４個のアンチセンスプローブを用いるインサイチュハイブリダイゼーションにより示されるように発現されることが示され得た（図１４Ａ－Ｅ および１３Ｋ－Ｏ および表２、前記）。さらに、Ｔｒｐ１０ 転写物を発現するこれらの組織の癌細胞はまた、４個のセンスプローブを用いるインサイチュハイブリダイゼーションにより、図１４Ｆ－Ｊ 、図１４Ｐ－Ｒ および図１４Ｔ に示されるようにＴｒｐ１０－アンチセンス転写物を発現することが明らかに示され得た（表２、前記）。インサイチュハイブリダイゼーション実験は、肺、胎盤、前立腺の癌および黒色腫に由来する癌細胞のサブセットの検出が、それぞれＴｒｐ１０ 転写物に相補的であるかまたはＴｒｐ１０－アンチセンス転写物に相補的であるアンチセンスならびにセンスプローブを用いて可能であることを証明する。

【００８６】
前記は説明を意図し、本発明の範囲の限定ではない。前記教示に基づいて、過度の実験なしに、当業者は、さらなる態様を容易に構想し、製造することができる。
【図面の簡単な説明】
【図１】
図１Ａは、ｔｒｐ および関連タンパク質の系統発生的関係である。図１Ｂは、ＫｙｔｅおよびＤｏｏｌｉｔｔｌｅ に従うＴｒｐ８タンパク質配列のヒドロパシープロットである。図１Ｃは、上皮カルシウムチャンネルＥＣａＣ（ウサギ由来）およびＶｒ１ （ラット由来）に対するＴｒｐ８ａ／ｂ のアラインメントである。推定膜貫通ドメインに下線を施している。
【図２】
図２Ａは、Ｔｒｐ８遺伝子の多型である。多型改変体Ｔｒｐ８ａ およびＴｒｐ８ｂ は、５つの塩基対で異なり、誘導タンパク質配列において３つのアミノ酸変換を生じる。特異的プライマーは、矢印で示されるようにＴｒｐ８遺伝子由来であった。図２Ｂは、Ｔｒｐ８ａ およびＴｒｐ８ｂ 遺伝子が単一の制限部位で区別できることを示す。Ｔｒｐ８遺伝子のゲノムフラグメントは、特異的プライマーを使用して増幅され得る（図２Ａに示される）。Ｔｒｐ８ｂ 遺伝子のゲノムフラグメントは、制限酵素ＢＳＰ１２８６Ｉのさらなる部位を含む（ 図２Ｂ） 。図２Ｃは、Ｔｒｐ８遺伝子が第７ 染色体上に位置されることを示す。図２Ｄは、７人のヒト被験体の遺伝子型である。Ｔｒｐ８遺伝子の４５８ｂｐ のゲノムフラグメントは、特異的プライマーを用いて増幅され（図２Ａに示される）、ＢＳＰ１２８６Ｉで拘束された。生じたフラグメントは、ＰＡＧＥ電気泳動によって分析された。
【図３】
図３は、Ｔｒｐ８ｂ タンパク質がカルシウム選択イオンチャンネルであることを示す。Ａは、ｐｄｉＴｒｐ８ｂトランスフェクトＨＥＫ２９３細胞の代表的なトレースである。Ｔｒｐ８ｂ 媒介電流は、－４０ｍＶ または＋７０ｍＶ での保持電位で１００ｍｓｅｃの電圧スロープ（－１００ｍＶ～＋１００ｍＶ）によって活性化され；１、Ｔｒｐ８電流は、２ｍｍ［Ca^{2+}］$_0$で存在し；２、溶液スイッチの効果は３のみ、名目上０ カルシウム溶液へスイッチする。Ｂは、０ 個の二価陽イオンの存在下でのＴｒｐ８電流である。Ｃは、Ａに示される電流の電流電圧関係（流入、電流を減ずるリーク）である。Ｄは、Ｂに示される電流の電流電圧関係である。Ｅは、代表的な実験の統計である。黒：Ｔｒｐ８トランフェクト細胞、灰色：対照細胞。左から右への列：－４０ｍＶ でのＴｒｐ８電流（ｎ＝８ ）および＋７０ｍＶ の保持電位でのＴｒｐ８電流（ｎ＝１２）。標準的な電解槽溶液（ナトリウムを含まない１２０ｍＭ ＮＭＤＧ（ｎ＝７ ）および名目上０ のカルシウムイオンを含む１２０ｍＭ ＮＭＤＧ（ｎ＝８ ）を含む、または０ 個の二価陽イオンを含む１ｍＭ ＥＧＴＡの存在する（ｎ＝６ ））におけるＴｒｐ８電流。Ｆは、１ｍＭ［Ca^{2+}］$_0$の存在または非存在下でのＴｒｐ８ｂ トランスフェクトＨＥＫ 細胞（灰色）および対照（黒）中の［Ca^{2+}］$_i$ における代表的な変化である。対照細胞と比較した、１ｍＭ［Ca^{2+}］$_0$の再添加前および後のＴｒｐ８ｂ トランスフェクトＨＥＫ 細胞の細胞質ゾルカルシウム濃度の流入、相対的増加。
【図４】
図４は、Ｔｒｐ８タンパク質のＣ 末端領域がカルモジュリンと結合することを示す。Ａは、カルモジュリン結合研究に使用されるＴｒｐ８タンパク質のＮ 末端フラグメントおよびＣ 末端フラグメントである。Ｂは、Ｔｒｐ８タンパク質およびｃＤＮＡのＭｕｎＩ切断後にインビトロで翻訳された切断Ｔｒｐ８タンパク質である。このタンパク質は、Ｃ 末端の３２個のアミノ酸残基が欠失する。これらのタンパク質は、^{35}S メチオニンの存在下でインビトロで翻訳され、１ｍＭ のＣａ$^{2+}$ または２ｍＭ のＥＧＴＡの存在下でカルモジュリン結合アガロースビーズと共にインキュベートされた。Ｃは、Ｃａ$^{2+}$（１ｍＭ ）またはＥＧＴＡ（２ｍＭ ）の存在下でのＴｒｐ８タンパク質のＮ 末端フラグメントおよびＣ 末端フラグメントへのカルモジュリン結合である。

【図５】
図５は、Ｔｒｐ８ ｃＤＮＡ の発現パターンである。Ａは、ノザンブロット（左パネル、Ｃｌｏｎｔｅｃｈ Ｐｏｌｏ Ａｌｔｏ）がＴｒｐ９ ｃＤＮＡ の３４８ｂｐ のＮｃｏＩ／ＢａｍＨＴフラグメントを用いてハイブリダイズされたことを示す。このプローブは、市販用のブロットから単離されたｍＲＮＡ種へハイブリダイズするが、良性前立腺肥厚から単離されたｍＲＮＡ種（右パネル、良性前立腺肥厚に罹患している２０人のヒト被験体から単離したｍＲＮＡ）へはハイブリダイズしない。Ｂ、Ｃは、ヒト組織のスライド上のビオチニル化Ｔｒｐ８特異的オリゴヌクレオチドを用いたインサイチュハイブリダイゼーションである。左列はアンチセンスプローブ、右列はセンスプローブ、Ｄはアンチセンスプローブである。

【図６】
図６は、ヒト前立腺におけるＴｒｐ８ ｃＤＮＡ の特異的発現である。Ａ～Ｆは、前立線組織を用いたインサイチュハイブリダイゼーションである。Ａは、正常前立腺。Ｂは、原発性癌、Ｃは、良性肥厚、Ｄは、レジディブ癌、Ｅは、前立腺上皮内新形成、Ｆは、前立腺のリンパ節転移である。

【図７】
図７は、Ｔｒｐ８ａ ｃＤＮＡ配列および誘導アミノ酸配列である。

【図８】
図８Ａは、Ｔｒｐ８ｂ ｃＤＮＡ配列および誘導アミノ酸配列である。図８Ｂは、スプライスバリアント１のｃＤＮＡ配列である（１２Ｂ１）。図８Ｃは、スプライスバリアント２のｃＤＮＡ配列である（１７－３）。図８Ｄは、スプライスバリアント３のｃＤＮＡ配列である（２３Ａ３）。図８Ｅは、スプライスバリアント４のｃＤＮＡ配列である（２３Ｃ３）。

【図９】
図９Ａは、Ｔｒｐ９ ｃＤＮＡ 配列および誘導アミノ酸配列である。図９Ｂは、スプライスバリアント１５のｃＤＮＡ配列および誘導アミノ酸配列である。

【図１０】
図１０Ａは、Ｔｒｐ１０ａのｃＤＮＡ配列および誘導アミノ酸配列である。図１０Ｂは、Ｔｒｐ１０ａのｃＤＮＡフラグメントおよび誘導アミノ酸配列である。

【図１１】
図１１は、Ｔｒｐ１０ｂのｃＤＮＡ配列および誘導アミノ酸配列である。

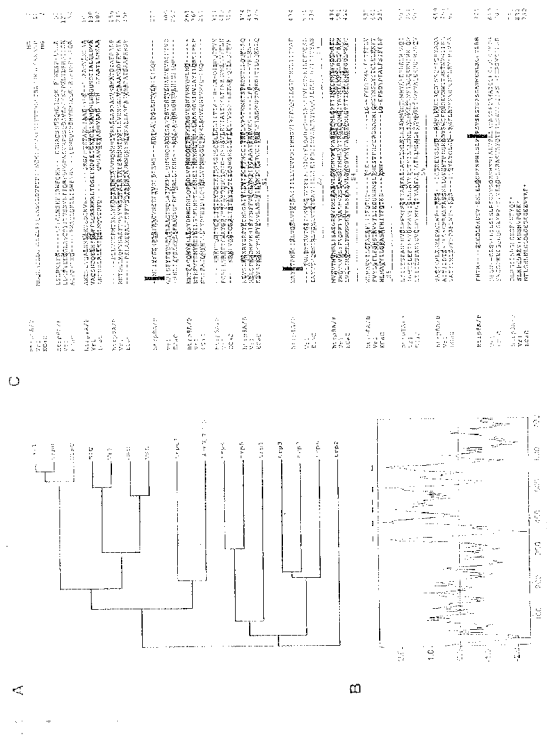
【図１２】
図１２は、ヒト子宮内膜癌または子宮の癌におけるＴｒｐ８ ｍＲＮＡ の発現である。Ａ～Ｄは、Ｔｒｐ８アンチセンスとハイブリダイズされた子宮内膜癌のスライドを用いたインサイチュハイブリダイゼーション（左列）または対照としてのセンスプローブ（右列）である。Ｅ～Ｆは、正常な子宮内膜のスライドにハイブリダイズされたＴｒｐ８アンチセンスプローブである。正常な子宮内膜組織ではハイブリダイゼーションは起こらないことが明らかに見え得る。

【図１３】
図１３は、ヒトＴｒｐ９およびＴｒｐ１０ 遺伝子の発現である。ノザンブロットは、Ｔｒｐ９（上パネル）またはＴｒｐ１０ （下パネル）特異的プローブを使用してハイブリダイズされた。Ｔｒｐ９ ｃＤＮＡ の発現は、ヒト前立腺および結腸を含む多くの組織ならびに良性前立腺肥厚において検出可能である。Ｔｒｐ１０ ｃＤＮＡの発現は、市販のノザンブロット（Ｃｌｏｎｔｅｃｈ、右側）のヒト前立腺において検出可能である。このノザンブロットは、１４～６０歳の範囲の１５人のヒト被験体から収集した前立腺組織を含む。Ｔｒｐ１０ ｃＤＮＡの発現は、良性前立腺肥厚においては検出不可能であった（左側）。

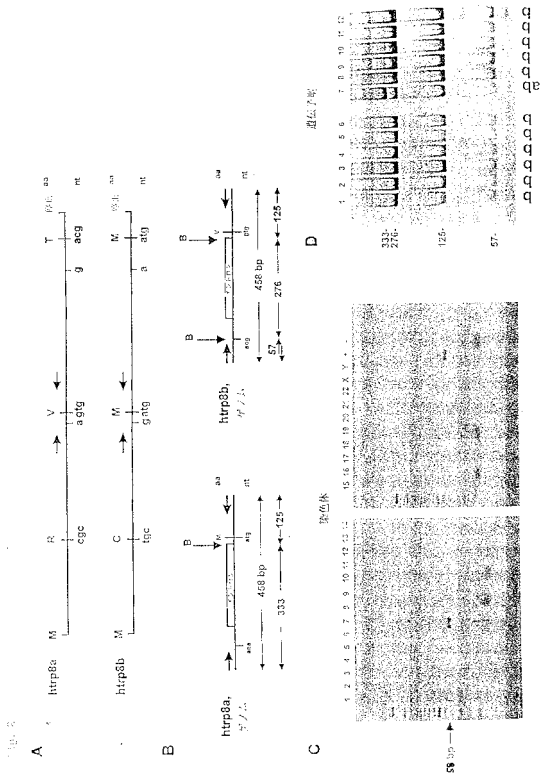
【図１４】
図１４は、ヒト前立腺癌および黒色腫の転移におけるＴｒｐ１０ 転写物およびＴｒｐ１０ アンチセンス転写物の発現である。Ｔｒｐ１０ アンチセンス（Ａ～Ｅ、Ｋ～Ｎ）およ

びＴｒｐ１０ 関連センスプローブ（Ｆ～Ｊ、Ｐ～Ｒ）でハイブリダイズされたスライドのインサイチュハイブリダーゼーション。両プローブは、これらの癌細胞がＴｒｐ１０ 転写物およびＴｒｐ１０ アンチセンス転写物を発現することを示す同じ癌細胞を検出することが明らかにわかり得る。Ｓは、Ｔｒｐ１０ 発現が前立腺の良性肥厚（ＢＰＨ）において検出不可能であることを示す。ＯおよびＴは、ヒト肺における黒色腫の転移におけるＴｒｐ１０ 転写物（Ｏ）およびＴｒｐ１０ アンチセンス転写物（Ｔ）の発現を示す。黒色腫癌細胞は、Ｔｒｐ１０ 転写物およびＴｒｐ１０ アンチセンス転写物の両方を発現する。

【図１】

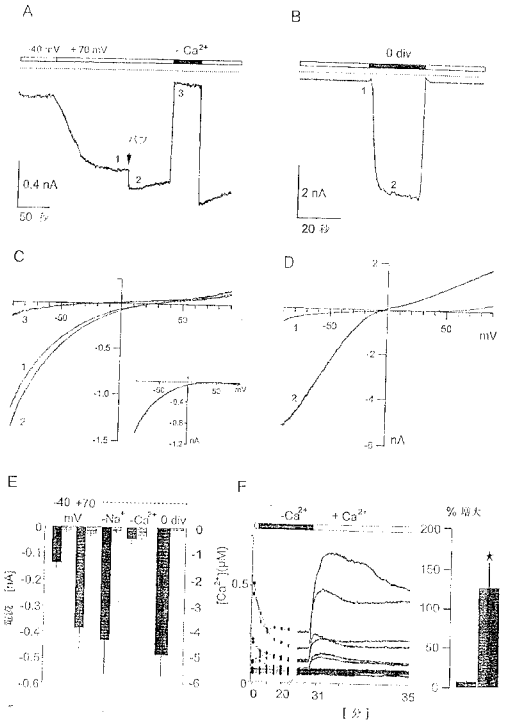


【図２】



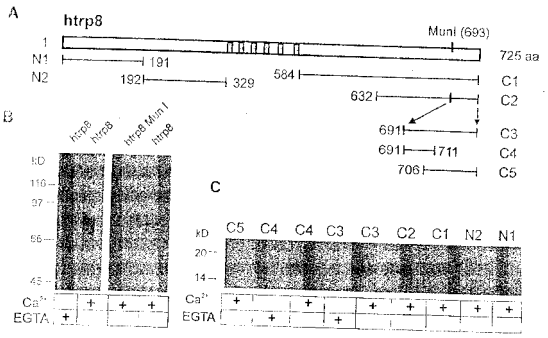
【図３】

Fig. 3



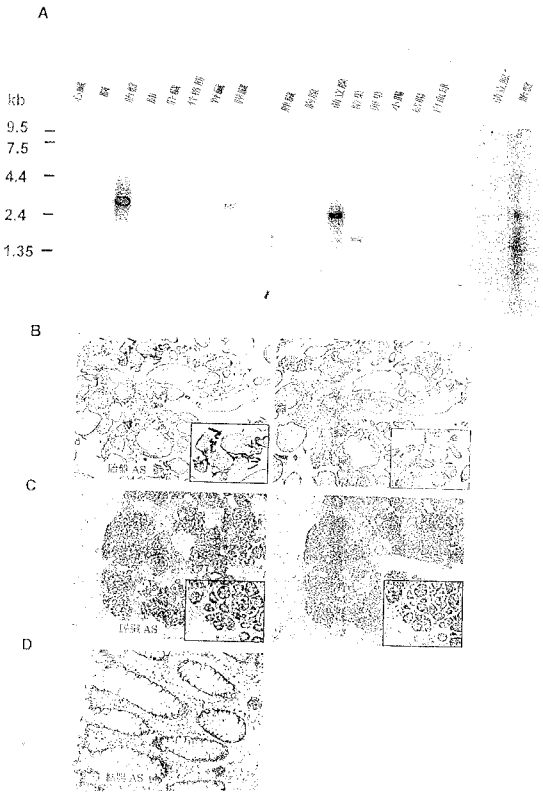
【図４】

Fig. 4



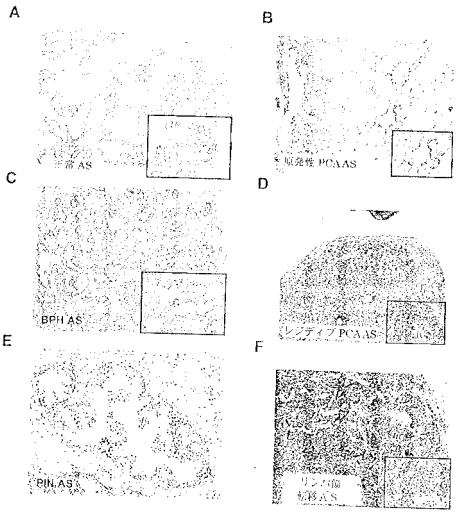
【図５】

Fig. 5



【図６】

Fig. 6



【図７】

Fig. 7

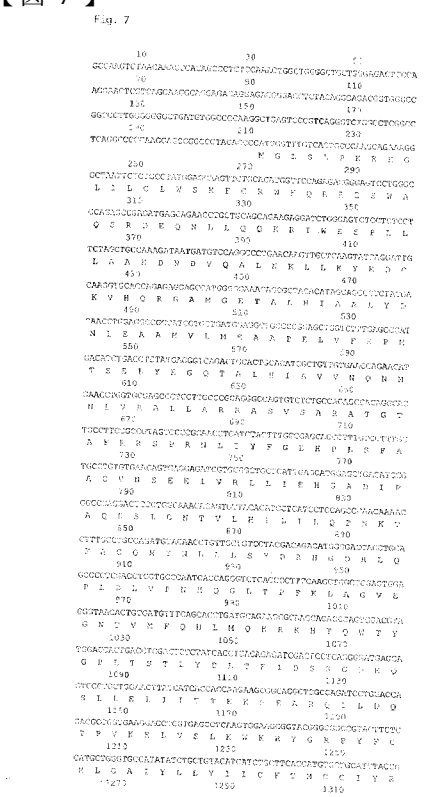


Fig. 7 / 続き1

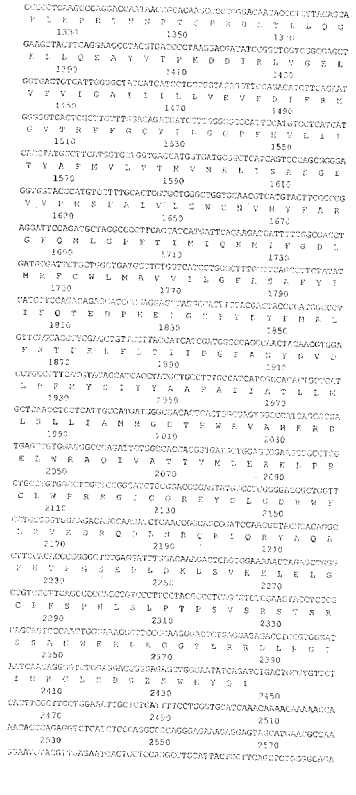
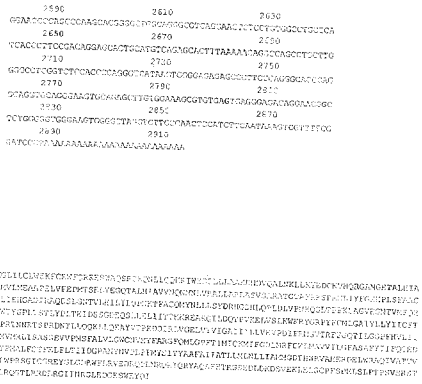


Fig. 7 / 続き2



【図８】

Figure 8:

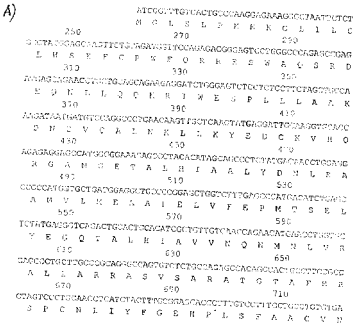


Fig. 8 / 続き

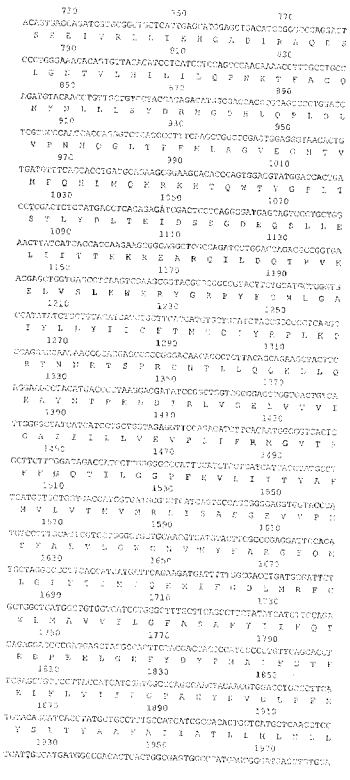


Fig. 8 / 続き2

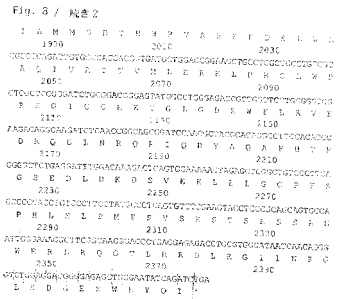
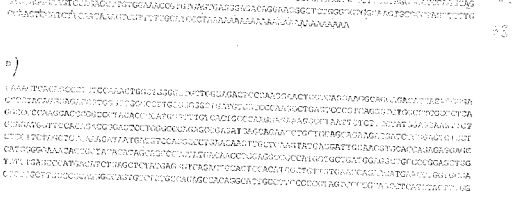


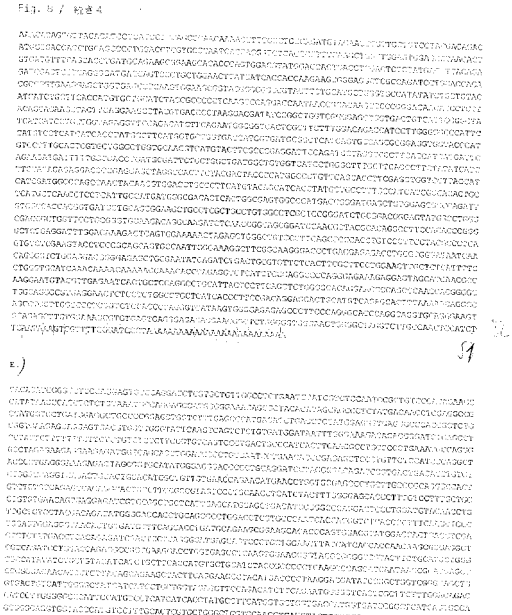
Fig. 8 / 続き3

c)



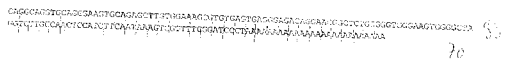
d)

Fig. 8 / 続き4



e)

Fig. 8 / 続き8



【図９】

Figure 9.

A.

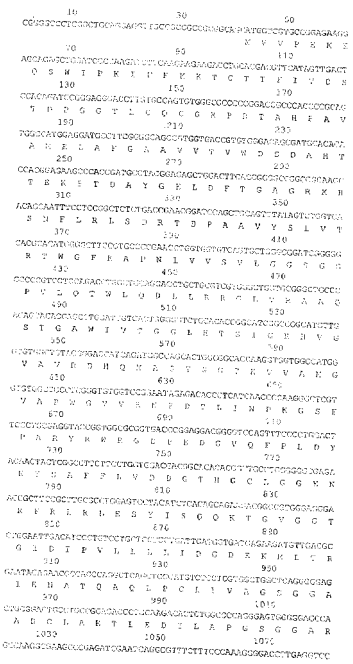


Fig. 9 / 続き1

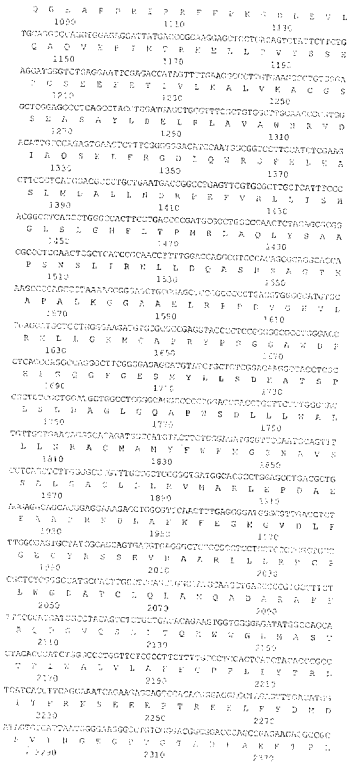


Fig. 9 / 続き2

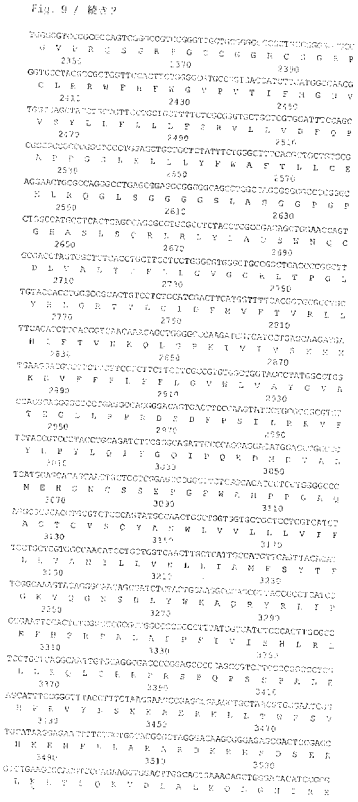


Fig. 9 / 続き３

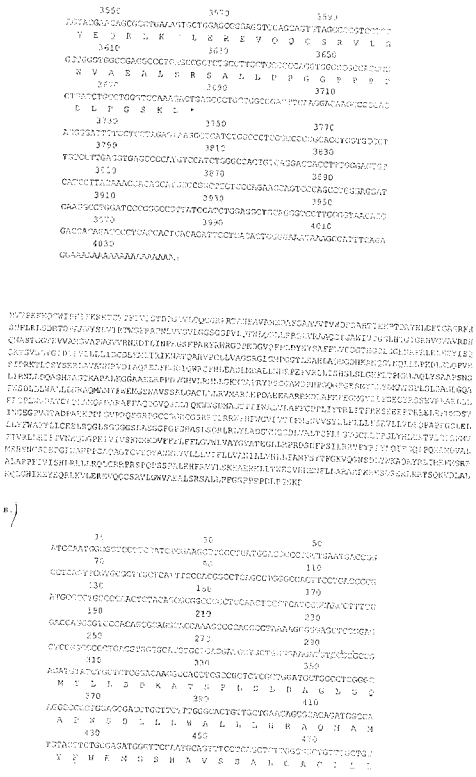


Fig. 9 / 続き４

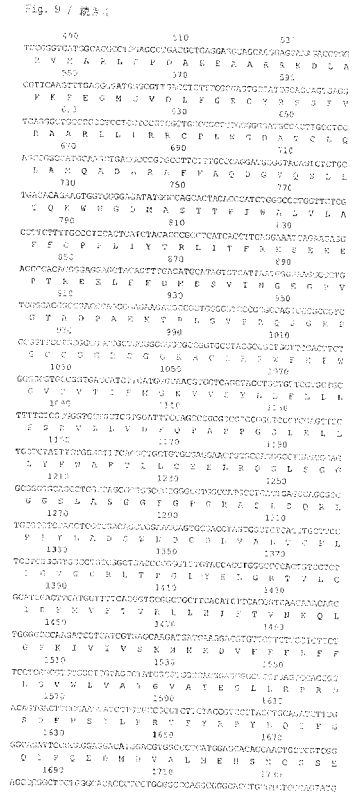


Fig. 9 / 続き５

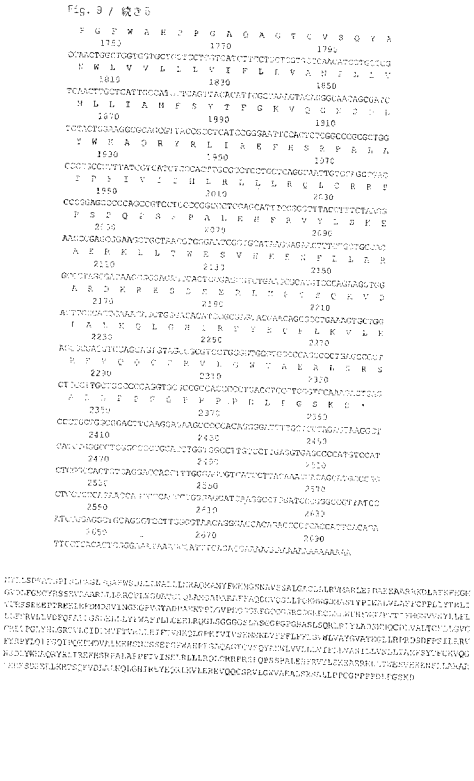
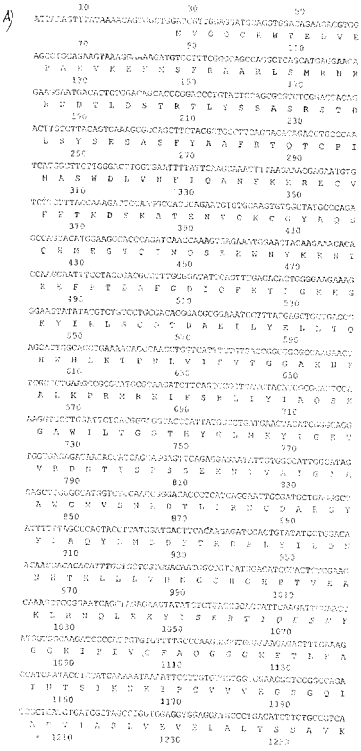
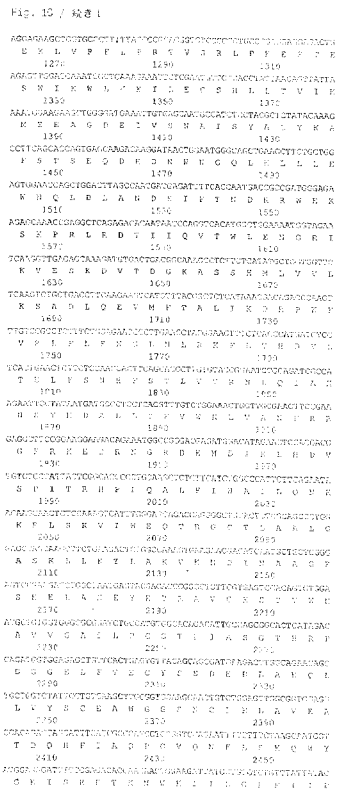


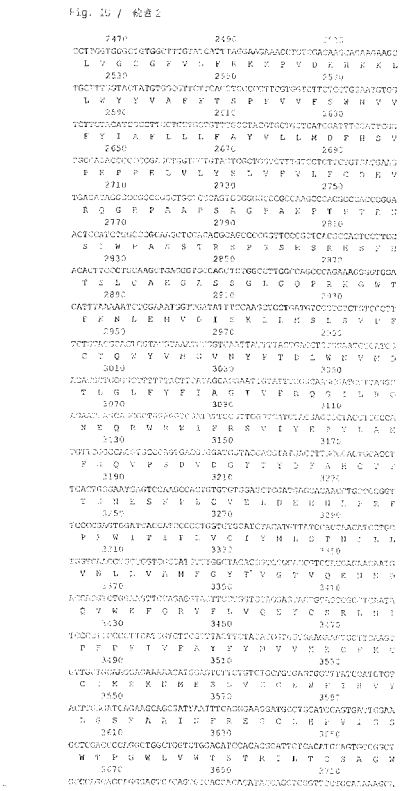
Fig. 9 / 続き６

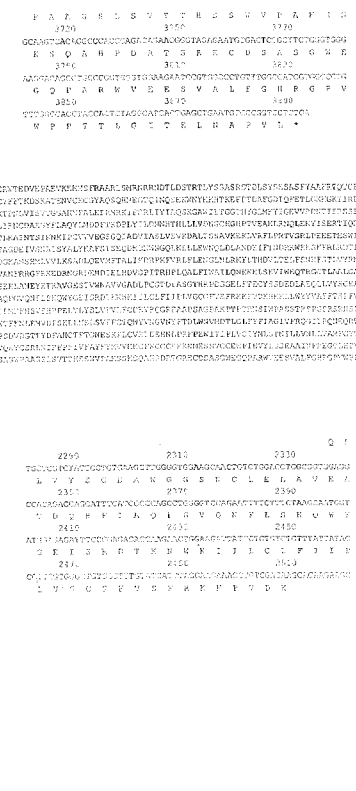
【図１０】

Fig. 10

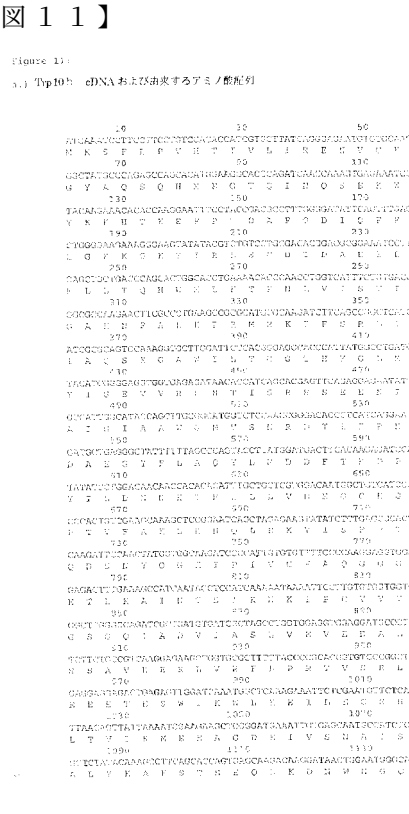


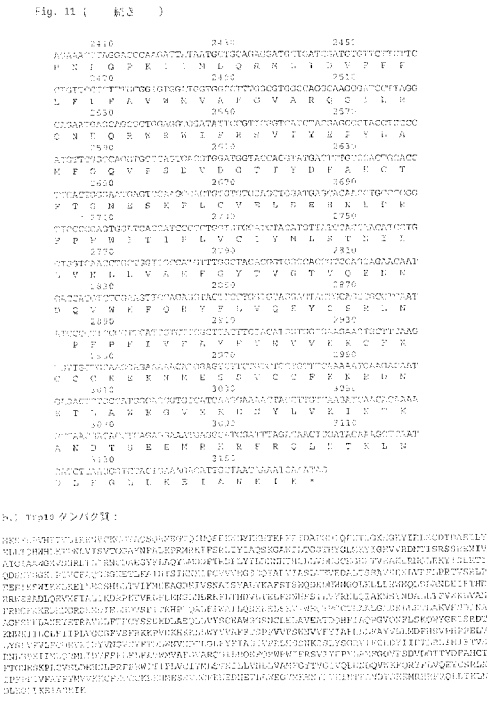




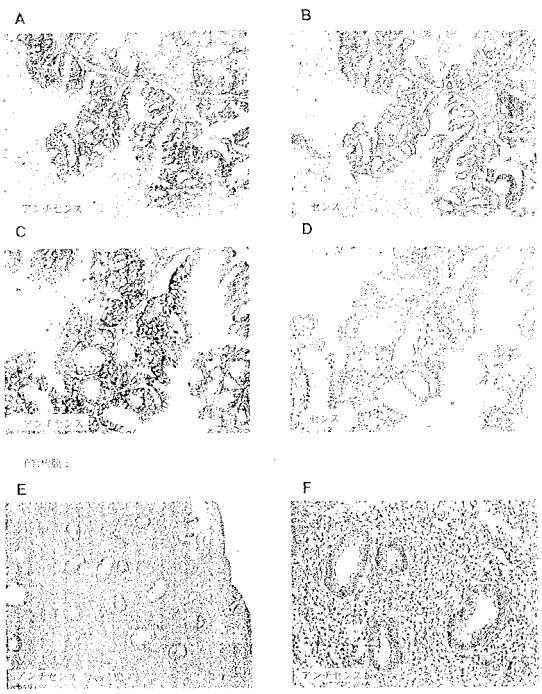


【図１１】





【図１２】



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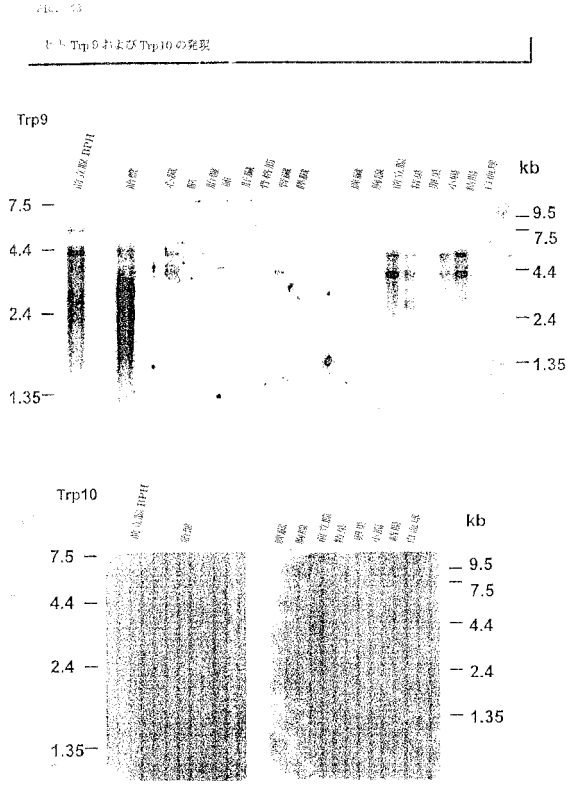

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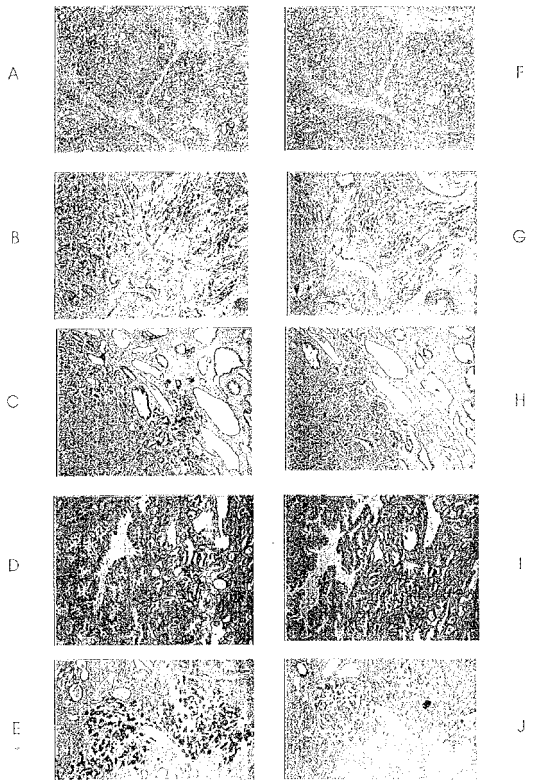

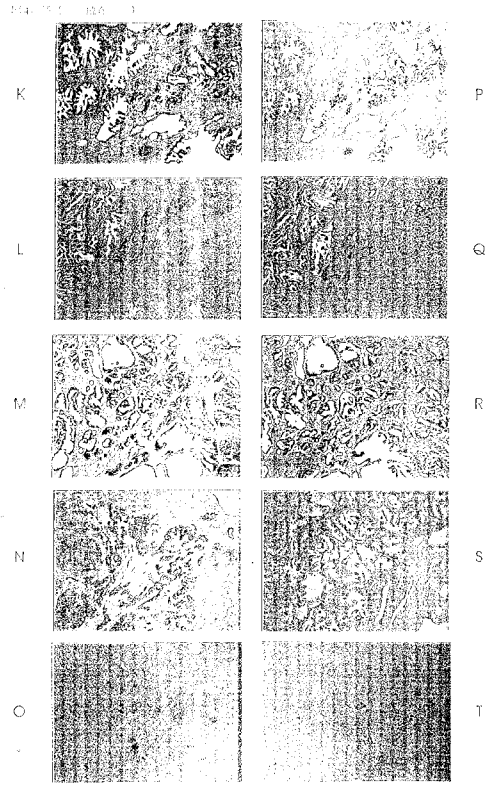
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(54) **Title:** TRP8, TRP9 AND TRP10, NOVEL MARKERS FOR CANCER

(57) **Abstract:** The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10. Also provided are vectors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating a tumor.

Trp8, Trp9 and Trp10, novel markers for cancer

FIELD OF THE INVENTION

The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10

BACKROUND OF THE TECHNOLOGY

Prostate cancer is one of the most common diseases of older men world wide. Diagnosis and monitoring of prostate cancer is difficult because of the heterogeneity of the disease. For diagnosis different grades of malignancy can be distinguished according to the Gleason-Score Diagnosis. For this diagnosis a prostate tissue sample is taken from the patient by biopsy and the morphology of the tissue is investigated. However, this approach only yields subjective results depending on the experience of the pathologist. For confirmation of these results and for obtaining an early diagnosis an additional diagnostic method can be applied which is based on the detection of a prostate specific antigen (PSA). PSA is assayed in serum samples, blood samples etc. using an anti-PSA-antibody. However, since in principle PSA is also expressed in normal prostate tissue there is a requirement for the definition of a threshold value (about 4 ng/ml PSA) in order to be able to distinguish between normal and malign prostate tissue. Unfortunately, this diagnostic method is quite insensitive and often yields false-positive results. Moreover, by using this diagnostic method any conclusions as regards the grade of malignancy, the progression of the tumor and its potential for metastasizing cannot be drawn. Thus, the use of molecular markers would be helpful to distinguish benign from malign tissue and for grading and staging prostate carcinoma, particularly for patients with metastasizing prostate cancer having a very bad prognosis.

The above discussed limitations and failings of the prior art to provide meaningful specific markers which correlate with the presence of prostate tumors, in particular metastasizing tumors, has created a need for markers which can be used diagnostically, prognostically and therapeutically over the course of this disease. The present invention fulfils such a need by the provision of Tpr8, Trp9 and Trp10 and the genes encoding Trp8, Trp9 and Trp10: The genes encoding Trp8 and Trp10 are expressed in prostate carcinoma and prostatic metastasis, but

not in normal prostate, benign hyperplasia (BHP) and intraepithelial prostatic neoplasia (PIN). Furthermore, expression of Trp10 transcripts is detectable in carcinoma but not in healthy tissue of the lung, the prostate, the placenta and in melanoma.

SUMMARY OF THE INVENTION

The present invention is based on the isolation of genes encoding novel markers associated witha cancer, Trp8, Trp9 and Trp10. The new calcium channel proteins Trp8, Trp9 and Trp10 are members of the trp (transient receptor potential) - family, isolated from human placenta (Trp8a and Trp8b) and humane prostate (Trp9, Trp10a and Trp10b). Trp proteins belong to a steadily growing family of Ca^{2+} selective and non selective ion channels. In the recent years seven Trp proteins (trp1 - trp7) have been identified and suggested to be involved in cation entry, receptor operated calcium entry and pheromone sensory signaling. Structurally related to the trp proteins are the vanilloid receptor (VR1) and the vanilloid like receptor (VRL-1) both involved in nociception triggered by heat. Furthermore, two calcium permeable channels were identified in rat small intestine (CaT1) and rabbit kidney (ECaC). These distantly related channels are suggested to be involved in the uptake of calcium ions from the lumen of the small intestine (CaT1) or in the reuptake of calcium ions in the distal tubule of the kidney (ECaC). Common features or the Trp and related channels are a proposed structure comprising six transmembrane domains including several conserved amino acid motifs. In the present invention the cloning and expression of a CaT1 like calcium channel (Trp8) from human placenta as well as Trp9 and Trp10 (two variants, Trp10a and Trp10b) is described. Two polymorphic variants of the Trp8 cDNA were isolated from placenta (Trp8a and Trp8b). Transient expression of the Trp8b cDNA in HEK (human embryonic kidney) cells results in cytosolic calcium overload implicating that the Trp8 channel is constitutive open in the expression system. Trp8 induces highly calcium selective inward currents in HEK cells. The C -terminus of the Trp8 protein binds calmodulin in a calcium dependent manner. The Trp9 channel is expressed in trophoblasts and syncytiotrophoblasts of placenta and in pancreatic acinar cells. Furthermore, the Trp8 channel is expressed in prostatic carcinoma and prostatic metastases, but not in normal tissue of the prostate. No expression of Trp8 transcripts is detectable in benign prostatic hyperplasia (BPH) or prostatic intraepithelial neoplasia (PIN). Therefore, the Trp8 channel is exclusively expressed in malign prostatic tissues and serves as molecular marker for prostate cancer. From the experimental results it is also apparent that the

modulation of Trp8 and/or Trp10, e.g. the inhibition of expression or activity , is of therapeutic interest, e.g. for the prevention of tumor progression.

The present invention, thus, provides a Trp8, Trp9 and Trp10 protein, respectively, as well as nucleic acid molecule encoding the protein and, moreover, an antisense RNA, a ribozyme and an inhibitor, which allow to inhibit the expression or the activity of Trp8, Trp9 and/or Trp10.

In one embodiment, the present invention provides a diagnostic method for detecting a prostate cancer or endometrial cancer (cancer of the uterus) associated with Trp8 or Trp10 in a tissue of a subject, comprising contacting a sample containing Trp8 and/or Trp10 encoding mRNA with a reagent which detects Trp8 and/or Trp10 or the corresponding mRNA.

In a further embodiment, the present invention provides a diagnostic method for detecting a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense transcripts or Trp10a and/or Trp10b related antisense transcripts.

In another embodiment, the present invention provides a method of treating a prostate tumor, carcinoma of the lung, carcinoma of the placenta (chorion carcinoma) or melanoma associated with Trp8 and/or Trp10, comprising administering to a subject with such an disorder a therapeutically effect amount of a reagent which modulates, e.g. inhibits, expression of Trp8 and/or Trp10 or the activity of the protein, e.g. the above described compounds.

Finally, the present invention provides a method of gene therapy comprising introducing into cells of a subject an expression vector comprising a nucleotide sequence encoding the above mentioned antisense RNA or ribozyme, in operable linkage with a promoter.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: A, phylogenetic relationship of trp and related proteins. B, hydropathy plot of the Trp8 protein sequence according to Kyte and Doolittle. C, alignment of Trp8a/b to the epithelial calcium channels ECaC (from rabbit) and Vr1 (from rat). Putative transmembrane domains are underlined.

Figure 2: A, polymorphism of the Trp8 gene. The polymorphic variants Trp8a and Trp8b differ in five base pairs resulting in three amino acid exchanges in the derived protein sequences. Specific primers were derived from the Trp8 gene as indicated by arrows. B, the Trp8a and Trp8b genes are distinguishable by a single restriction site. Genomic fragments of the Trp8 gene can be amplified using specific primers (shown in A). The genomic fragment of the Trp8b gene contains an additional site of the restriction enzyme BSP1286I (B). C, the Trp8 gene is located on chromosome 7. D, genotyping of eleven human subjects. A 458 bp genomic fragment of the Trp8 gene was amplified using specific primers (shown in A) and restricted with BSP1286I. The resulting fragments were analyzed by PAGE electrophoresis.

Figure 3: The Trp8b protein is a calcium selective ion channel. A, representative trace of a pdiTrp8b transfected HEK 293 cell. Trp8b mediated currents are activated by voltage ramps (-100 mV - +100 mV) of 100 msec at -40 mV or +70 mV holding potential. 1, Trp8b currents in the presence at 2mm $[Ca^{2+}]_o$;2, effect of solution switch alone 3, switch to nominal zero calcium solution. B, Trp8b currents in the presence of zero divalent cations. C, current voltage relationship of the currents shown in A. Inset, leak subtracted current. D, current voltage relationship of the current shown in B. E, statistics of representative experiments. Black: Trp8 transfected cells, gray: control cells. Columns from left to right: Trp8 currents at - 40 mV (n =12) and + 70 mV holding potential (n = 12). Trp8 currents in standard bath solution including 120 mM NMDG without sodium (n = 7) and with nominal zero calcium ions (n = 8) or in the presence of 1mM EGTA with zero divalent cations (n = 6). F, representative changes in $[Ca^{2+}]_i$ in Trp8b transfected HEK cells (gray) and controls (black) in the presence or absence of 1mM $[Ca^{2+}]_o$. Inset, relative increase of cytosolic calcium concentration of Trp8b transfected HEK cells, before and after readdition of 1 mM $[Ca^{2+}]_o$ in comparison to control cells.

Figure 4: The C-terminal region of the Trp8 protein binds calmodulin. A, N- and C-terminal fragments of the Trp8 protein used for calmodulin binding studies. B, the Trp8 protein and a truncated Trp8 protein which was in vitro translated after MunI cut of the cDNA, which lacks the C-terminal 32 amino acid residues, were in vitro translated in the presence of ^{35}S-methionine and incubated with calmodulin coupled agarose beads in the presence of 1 mM Ca^{2+} or 2 mM EGTA. C, calmodulin binding to N- and C-terminal fragments of the Trp8protein in the presence of Ca^{2+} (1 mM) or EGTA (2 mM)

Figure 5: Expression pattern of the Trp8 cDNA. A, Northern blots (left panels, Clontech, Palo Alto) were hybridized using a 348 bp NcoI/BamHT fragment of the Trp9 cDNA. The probe hybridizes to mRNA species isolated from the commercial blot, but not to mRNA species isolated from benign prostate hyperplasia (right panel, mRNA isolated from 20 human subjects with benign prostate hyperplasia). B,C, in situ hybridization with biotinylated Trp8 specific oligonucleotides on slides of human tissues. Left column antisense probes, right column sense probes. D, antinsense probes.

Figure 6: Differential expression of Trp8 cDNA in human prostate. A - F, in situ hybridization with prostatic tissues. A, normal prostate, B, primary carcinoma, C, benign hyperplasia, D, rezidive carcinoma, E, prostatic intraepithelial neoplasia, F, lymphnode metastasis of the prostata.

Figure 7: Trp8a cDNA sequence and derived amino acid sequence

Figure 8: A, Trp8b cDNA sequence and derived amino acid sequence
B, cDNA sequence of splice variant 1 (12B1)
C, cDNA sequence of splice variant 2 (17-3)
D, cDNA sequence of splice variant 3 (23A3)
E, cDNA sequence of splice variant 4 (23C3)

Figure 9: A, Trp9 cDNA sequence and derived amino acid sequence B, cDNA sequence of splice variant 15 and derived amino acid sequence.

Figure 10: A, cDNA sequence of Trp10a and derived amino acid sequence, B, cDNA fragment of Trp10a and derived amino acid sequence.

Figure 11: cDNA sequence of Trp10b and derived amino acid sequence.
Figure 12: Expression of Trp8 mRNA in human endometrial cancer or cancer of the uterus. A - D, in situ hybridization with slides of endometrial cancer hybridized with Trp8 antisense (left column) or sense probes as controls (right column). E - F, Trp8 antisense probes hybridized to slides of normal endometrium. It can be clearly seen no hybridization occurs with normal endometrial tissue.

Figure 13: Expression of human Trp9 and Trp10 genes
Northern blots were hybridized using Trp9 (upper panel) or Trp10 (lower panel) specific probes. Expression of the Trp9 cDNA is detectable in many tissues including human prostate and colon as well as in benign prostatic hyperplasia. Expression of Trp10 cDNA is detectable in human prostate of a commercial northern blot (Clontech, right side). This Northern blot contains prostatic tissue collected from 15 human subjects in the range of 14 - 60 years of age. No expression of Trp10 cDNA was detectable in benign prostatic hyperplasia (left side).

Figure 14: Expression of Trp10 transcripts and Trp10-antisense transcripts in human prostate cancer and metastasis of a melanoma. In situ hybridizations of slides hybridized with Trp10-antisense (A-E, K-N) and Trp10 related sense probes (F-J, P-R). It can clearly be seen that both probes detect the same cancer cells indicating that these cancer cells express Trp10 transcripts as well as Trp10-antisense transcripts. S, no Trp10 expression is detectable in benign hyperplasia of the prostate (BPH). O and T, show expression of Trp10 transcripts (O) and Trp10-antisense transcripts (T) in a metastasis of a melanoma in human lung. Melanoma cancer cells express both Trp10 transcripts and Trp10-antisense transcripts.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b or a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being selected from the group consisting of

(a) a nucleic acid molecule encoding a protein that comprises the amino acid sequence depicted in Figure 7, 8A, 9,10 or 11;
(b) a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9,10, or 11;
(c) a nucleic acid molecule included in DSMZ Deposit no. DSM 13579 (deposit date: 28 June 2000), DSM 13580 (deposit date: 28 June 2000), DSM 13584 (deposit date: 5 July 2000), DSM 13581 (deposit date: 28 June 2000) or DSM ....(deposit date:.....);
(d) a nucleic acid molecule with hybridizes to a nucleic acid molecule specified in (a) to (c)

(e) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) to (d) due to the degeneration of the genetic code; and

(f) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a) to (e).

As used herein, a protein exhibiting biological properties of Trp8a, Trp8b, Trp9,Trp10a or Trp10b is understood to be a protein having at least one of the activities as illustrated in the Examples, below.

As used herein, the term „isolated nucleic acid molecule„ includes nucleic acid molecules substantially free of other nucleic acids, proteins, lipids, carbohydrates or other materials with which it is naturally associated.

In a first embodiment, the invention provides an isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9,Trp10a or Trp10b comprising the amino acid sequence depicted in Figure 7, 8A, 9,10 or 11. The present invention also provides a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9,10 or 11.

The present invention provides not only the generated nucleotide sequence identified in Figure 7, 8A, 9,10 or 11, respectively and the predicted translated amino acid sequence, respectively, but also plasmid DNA containing a Trp8a cDNA deposited with the DSMZ, under DSM 13579, a Trp8b cDNA deposited with the DSMZ, under DSM 13580, a Trp9 cDNA deposited with the DSMZ, under DSM 13584, a Trp10a cDNA deposited with the DSMZ, under DSM 13581, and a Trp10b cDNA deposited with the DSMZ, under DSM...., respectively. The nucleotide sequence of each deposited Trp-clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by each deposited clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited Trp-encoding DNA, collecting the protein, and determining its sequence.

The nucleic acid molecules of the invention can be both DNA and RNA molecules. Suitable DNA molecules are, for example, genomic or cDNA molecules. It is understood that all

nucleic acid molecules encoding all or a portion of Trp8a, Trp8b, Trp9,Trp10a or Trp10b are also included, as long as they encode a polypeptide with biological activity. The nucleic acid molecules of the invention an be isolated from natural sources or can be synthesized according to know methods.

The present invention also provides nucleic acid molecules which hybridize to the above nucleic acid molecules. As used herein, the term „hybridize„ has the meaning of hybridization under conventional hybridization conditions, preferably under stringent conditions as described, for example, in Sambrook et al., Molecular Cloning, A Laboratory Manual 2nd edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Also contemplated are nucleic acid molecules that hybridize to the Trp nucleic acid molecules at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency), salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°Cin a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 9.2M NaH_2PO_4; 0.02M EDTA, pH7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA, following by washes at 50°C with 1 X SSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC). Variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Nucleic acid molecules that hybridize to the molecules of the invention can be isolated, e.g., from genomic or cDNA libraries that were produced from human cell lines or tissues. In order to identify and isolate such nucleic acid molecules the molecules of the invention or parts of these molecules or the reverse complements of these molecules can be used, for example by means of hybridization according to conventional methods (see, e.g., Sambrook et al., supra). As a hybridization probe nucleic acid molecules can be used, for example, that have exactly or basically the nucleotide sequence depicted in Figure 7, 8A, 9,10 or 11, respectively, or parts of these sequences. The fragments used as hybridization probe can be synthetic

fragments that were produced by means of conventional synthetic methods and the sequence of which basically corresponds to the sequence of a nucleic acid molecule of the invention.

The nucleic acid molecules of the present invention also include molecules with sequences that are degenerate as a result of the genetic code.

In a further embodiment, the present invention provides nucleic acid molecules which comprise fragments, derivatives and allelic variants of the nucleic acid molecules described above encoding a protein of the invention. „Fragments„ are understood to be parts of the nucleic acid molecules that are long enough to encode one of the described proteins. These fragments comprise nucleic acid molecules specifically hybridizing to transcripts of the nucleic acid molecules of the invention. These nucleic acid molecules can be used, for example, as probes or primers in the diagnostic assay and/or kit described below and, preferably, are oligonucleotides having a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides. The nucleic acid molecules and oligonucleotides of the invention can also be used, for example, as primers for a PCR reaction. Examples of particular useful probes (primers) are shown in Tables 1 and 2.

Table 1

Trp8 probes used for in situ hybridization:

Probes (antisense)

1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'

2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'

3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

Controls (sense)

1.) 5' GGCAAGATCTCAACCGGCAGCGGA 3'

2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'

3.) 5' TTCCACACCCGGGGCTCTGAGGAT 3'

Tabelle 2

Trp10 probes used for the in situ hybridizations shown in Figure 14:

Probes (antisense)

1.) 5' GCTTCCACCCCAAGCTTCACAGGAATAGA 3' (Figure 14 A, 14B)

2.) 5' GGCGATGAAATGCTGGTCTGTGGC 3' (Figure 14C, 14D, 14N, 14S, 14O)

3.) 5' ATCTTCCAGTTCTTGGTGTCTCGG 3' (Figure 14E, 14K)

4.) 5' GCTGCAGTACTCCTGCACCAGGAA 3' (Figure 14L, 14M)

Probes (sense)

1.) 5' TCTATTCCTGTGAAGCTTGGGGTGGAAGC 3' (Figure 14F, 14G)

2.) 5' GCCACAGACCAGCATTTCATCGCC 3' (Figure 14H, 14I, 14T)

3.) 5' CCGAGACACCAAGAACTGGAAGAT 3' (Figure 14J, 14P)

4.) 5' TTCCTGGTGCAGGAGTACTGCAGC 3' (Figure 14Q, 14R)

The term „derivative„ in this context means that the sequences of these molecules differ from the sequences of the nucleic acid molecules described above at one or several positions but have a high level of homology to these sequences. Homology hereby means a sequence identity of at least 40%, in particular an identity of at least 60%, preferably of more than 80% and particularly preferred of more than 90%. These proteins encoded by the nucleic acid molecules have a sequence identity to the amino acid sequence depicted in Figure 7, 8A, 9, 10 and 11, respectively, of at least 80%, preferably of 85% and particularly preferred of more than 90%, 97% and 99%. The deviations to the above-described nucleic acid molecules may have been produced by deletion, substitution, insertion or recombination. The definition of the derivatives also includes splice variants, e.g. the splice variants shown in Figures 8B to 8E and 9B.

The nucleic acid molecules that are homologous to the above-described molecules and that represent derivatives of these molecules usually are variations of these molecules that represent modifications having the same biological function. They can be naturally occurring variations, for example sequences from other organisms, or mutations that can either occur naturally or that have been introduced by specific mutagenesis. Furthermore the variations can be synthetically produced sequences. The allelic variants can be either naturally occurring variants or synthetically produced variants or variants produced by recombinant DNA processes.

Generally, by means of conventional molecular biological processes it is possible (see, e.g., Sambrook et al., supra) to introduce different mutations into the nucleic acid molecules of the invention. As a result Trp proteins or Trp related proteins with possibly modified biological properties are synthesized. One possibility is the production of deletion mutants in which nucleic acid molecules are produced by continuous deletions from the 5'- or 3'-terminal of the coding DNA sequence and that lead to the synthesis of proteins that are shortened accordingly. Another possibility is the introduction of single-point mutation at positions where a modification of the amino aid sequence influences, e.g., the ion channel properties or the regulations of the trp-ion channel. By this method muteins can be produced, for example, that possess a modified ion conducting pore, a modified K_m-value or that are no longer subject to the regulation mechanisms that normally exist in the cell, e.g. with regard to allosteric regulation or covalent modification. Such muteins might also be valuable as therapeutically useful antagonists of Trp8a, Trp8b, Trp9,Trp10a or Trp10b, respectively.

For the manipulation in prokaryotic cells by means of genetic engineering the nucleic acid molecules of the invention or parts of these molecules can be introduced into plasmids allowing a mutagenesis or a modification of a sequence by recombination of DNA sequences. By means of conventional methods (cf. Sambrook et al., supra) bases can be exchanged and natural or synthetic sequences can be added. In order to link the DNA fragments with each other adapters or linkers can be added to the fragments. Furthermore, manipulations can be performed that provide suitable cleavage sites or that remove superfluous DNA or cleavage sites. If insertions, deletions or substitutions are possible, in vitro mutagenesis, primer repair, restriction or ligation can be performed. As analysis method usually sequence analysis, restriction analysis and other biochemical or molecular biological methods are used.

The proteins encoded by the various variants of the nucleic acid molecules of the invention show certain common characteristics, such as ion channel activity, molecular weight, immunological reactivity or conformation or physical properties like the electrophoretical mobilty, chromatographic behavior, sedimentation coefficients, solubility, spectroscopic properties, stability; pH optimum, temperature optimum.

The invention furthermore relates to vectors containing the nucleic acid molecules of the invention. Preferably, they are plasmids, cosmids, viruses, bacteriophages and other vectors

usually used in the field of genetic engineering. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in mammalian cells and baculovirus-derived vectors for expression in insect cells. Preferably, the nucleic acid molecule of the invention is operatively linked to the regulatory elements in the recombinant vector of the invention that guarantee the transcription and synthesis of an RNA in prokryotic and/or eukaryotic cells that can be translated. The nucleotide sequence to be transcribed can be operably linked to a promoter like a T7, metallothionein I or polyhedrin promoter.

In a further embodiment, the present invention relates to recombinant host cells transiently or stable containing the nucleic acid molecules or vectors or the invention. A host cell is understood to be an organism that is capable to take up *in vitro* recombinant DNA and, if the case may be, to synthesize the proteins encoded by the nucleic acid molecules of the invention. Preferably, these cells are prokaryotic or eukaryotic cells, for example mammalian cells, bacterial cells, insect cells or yeast cells. The host cells of the invention are preferably characterized by the fact that the introduced nucleic acid molecule of the invention either is heterologous with regard to the transformed cell, i.e. that it does not naturally occur in these cells, or is localized at a place in the genome different from that of the corresponding naturally occurring sequence.

A further embodiment of the invention relates to isolated proteins exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9,Trp10a or Trp10b and being encoded by the nucleic acid molecules of the invention, as well as to methods for their production, whereby, e.g., a host cell of the invention is cultivated under conditions allowing the synthesis of the protein and the protein is subsequently isolated from the cultivated cells and/or the culture medium. Isolation and purification of the recombinantly produced proteins may be carried out by conventional means including preparative chromatography and affinity and immunological separations involving affinity with an anti-Trp8a-, anti-Trp8b-, anti-Trp9-,anti-Trp10a- or anti-Trp10b-antibody, respectively.

As used herein, the term „isolated protein„ includes proteins substantially free of other proteins, nucleic acids, lipids, carbohydrates or other materials with which it is naturally associated. Such proteins however not only comprise recombinantly produced proteins but include isolated naturally occurring proteins, synthetically produced proteins, or proteins

produced by a combination of these methods. Means for preparing such proteins are well understood in the art. The Trp proteins are preferably in a substantially purified form. A recombinantly produced version of a human prostate carcinoma associated protein Trp8a, Trp8b, Trp9,Trp10a or Trp10b protein, including the secreted protein, can be substantially purified by the one-step method described in Smith and Johnson, Gene 67; 31-40 (1988).

In a further preferred embodiment, the present invention relates to an antisense RNA sequence characterised that it is complementary to an mRNA transcribed from a nucleic acid molecule of the present invention or a part thereof and can selectively bind to said mRNA, said sequence being capable of inhibiting the synthesis of the protein encoded by said nucleic acid molecules, and a ribozyme characterised in that it is complementary to an mRNA transcribed from a nucleic acid molecule of the present invention or a part thereof and can selectively bind to and cleave said mRNA, thus inhibiting the synthesis of the proteins encoded by said nucleic acid molecules. Riboyzmes which are composed of a single RNA chain are RNA enzymes, i.e. catalytic RNAs, which can intermolecularly cleave a target RNA, for example the mRNA transcribed from one of the Trp genes. It is now possible to construct ribozymes which are able to cleave the target RNA at a specific site by following the strategies described in the literature. (see, e.g., Tanner et al., in: Antisense Research and Applications, CRC Press Inc. (1993), 415-426). The two main requirements for such ribozymes are the catalytic domain and regions which are complementary to the target RNA and which allow them to bind to its substrate, which is a prerequisite for cleavage. Said complementary sequences, i.e., the antisense RNA or ribozyme, are useful for repression of Trp8a-, Trp8b, Trp9-,Trp10a- and Trp10b-expression, respectively, i.e. in the case of the treatment of a prostate cancer or endometrial cancer (carcinoma of the uterus). Preferably, the antisense RNA and ribozyme of the invention are complementary to the coding region. The person skilled in the art provided with the sequences of the nucleic acid molecules of the present invention will be in a position to produce and utilise the above described antisense RNAs or ribozymes. The region of the antisense RNA and ribozyme, respectively, which shows complementarity to the mRNA transcribed from the nucleic acid molecules of the present invention preferably has a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides.

In still a further embodiment, the present invention relates to inhibitors of Trp8a, Trp8b, Trp9, Trp10a and Trp10b, respectively, which fulfill a similar purpose as the antisense RNAs or

ribozymes mentioned above, i.e. reduction or elimination of biologically active Trp8a, Trp8b, Trp9, Trp10a or Trp10b molecules. Such inhibitors can be, for instance, structural analogues of the corresponding protein that act as antagonists. In addition, such inhibitors comprise molecules identified by the use of the recombinantly produced proteins, e.g. the recombinantly produces protein can be used to screen for and identify inhibitors, for example, by exploiting the capability of potential inhibitors to bind to the protein under appropriate conditions. The inhibitors can, for example, be identified by preparing a test mixture wherein the inhibitor candidate is incubated with Trp8a, Trp8b, Trp9, Trp10a or Trp10b, respectively, under appropriate conditions that allow Trp8a, Trp8b, Trp9, Trp10a or Trp10b to be in a native conformation. Such an in vitro test system can be established according to methods well known in the art. Inhibitors can be identified, for example, by first screening for either synthetic or naturally occurring molecules that bind to the recombinantly produced Trp protein and then, in a second step, by testing those selected molecules in cellular assays for inhibition of the Trp protein, as reflected by inhibition of at least one of the biological activities as described in the examples, below. Such screening for molecules that bind Trp8a, Trp8b, Trp9, Trp10a or Trp10b could easily performed on a large scale, e.g. by screening candidate molecules from libraries of synthetic and/or natural molecules. Such an inhibitor is, e.g., a synthetic organic chemical, a natural fermentation product, a substance extracted from a microorganism, plant or animal, or a peptide. Additional examples of inhibitors are specific antibodies, preferably monoclonal antibodies. Moreover, the nucleic sequences of the invention and the encoded proteins can be used to identify further factors involved in tumor development and progression. In this context it should be emphasized that the modulation of the calcium channel of a member of the trp family can result in the stimulation of the immune response of T lymphocytes leading to proliferation of the T lymphocytes. The proteins of the invention can, e.g., be used to identify further (unrelated) proteins which are associated with the tumor using screening methods based on protein/protein interactions, e.g. the two-hybrid-system Fields, S. and Song, O. (1989) Nature (340): 245-246.

The present invention also provides a method for diagnosing a prostate carcinoma which comprises contacting a target sample suspected to contain the protein Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA with a reagent which reacts with Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA and detecting Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp 10b encoding mRNA.

It has been found that carcinoma cells of placenta (chorion carcinoma), lung and prostate express Trp10 transcripts as well as Trp10 antisense transcripts and transcripts being in part complementary to Trp10 antisense transcripts. Accordingly, the present invention also provides a method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense RNA.

When the target is mRNA (or antisense RNA), the reagent is typically a nucleic acid probe or a primer for PCR. The person skilled in the art is in a position to design suitable nucleic acids probes based on the information as regards the nucleotide sequence of Trp8a, Trp8b, Trp10a or Trp10b as depicted in figure 7, 8a, 10 and 11, respectively, or tables 1 and 2, above. When the target is the protein, the reagent is typically an antibody probe. The term „antibody", preferably, relates to antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specifities, as well as distinct monoclonal antibody preparations. Monoclonal antibodies are made from an antigen containing fragments of the proteins of the invention by methods well known to those skilled in the art (see, e.g., Köhler et al., Nature 256 (1975), 495). As used herein, the term „antibody" (Ab) or „monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab') 2 fragments) which are capable of specifically binding to protein. Fab and f(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. (Wahl et al., J. Nucl. Med. 24: 316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimerical, single chain, and humanized antibodies. The target cellular component, i.e. Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts, e.g., in biological fluids or tissues, may be detected directly in situ, e.g. by in situ hybridization (e.g., according to the examples, below) or it may be isolated from other cell components by common methods known to those skilled in the art before contacting with a probe. Detection methods include Northern blot analysis, RNase protection, in situ methods, e.g. in situ hybridization, in vitro amplification methods (PCR, LCR, QRNA replicase or RNA-transcription/amplification (TAS, 3SR), reverse dot blot disclosed in EP-B1 O 237 362)), immunoassays, Western blot and other detection assays that are known to those skilled in the art.

Products obtained by in vitro amplification can be detected according to established methods, e.g. by separating the products on agarose gels and by subsequent staining with ethidium bromide. Alternatively, the amplified products can be detected by using labeled primers for amplification or labeled dNTPs.

The probes can be detectable labeled, for example, with a radioisotope, a bioluminescent, compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

Expression of Trp8a, Trp8b, Trp10a and Trp10b, respectively, in tissues can be studied with classical immunohistological methods (Jalkanen et al., J. Cell. Biol. 101 (1985), 976-985; Jalkanen et al., J. Cell. Biol. 105 (1987), 3087-3096; Sobol et al. Clin. Immunpathol. 24 (1982), 139-144; Sobol et al., Cancer 65 (1985), 2005-2010). Other antibody based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium rhodamine, and biotin. In addition to assaying Trp8a, Trp8b, Trp 10a or Trp10b levels in a biological sample, the protein can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99}mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99}mTc. The labeled antibody or antibody fragment will then preferentially accumulate at he location of cells which contain the specific protein. In

vivo tumor imaging is described in S.W. Burchiel et al., „Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments". (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B.A. Rhodes, eds., Masson Publishing Inc. (1982)).

The marker Trp8a and Trp8b is also useful for prognosis, for monitoring the progression of the tumor and the diagnostic evaluation of the degree of malignancy of a prostate tumor (grading and staging), e.g. by using in situ hybridization: In a primary carcinoma Trp8 is expressed in about 2 to 10% of carcinoma cells, in a rezidive carcinoma in about 10 to 60% of cells and in metastases in about 60 to 90% of cells.

The present invention also relates to a method for diagnosing endometrial cancer (cancer of the uterus) which comprises contacting a target sample suspected to contain the protein Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA with a reagent which reacts with Trp8a and/or Trp8b or the encoding mRNA and detecting Trp8a and/or Trp8b encoding mRNA. As regards particular embodiments of this method reference is made to the particular embodiments of the method of diagnosing a prostate cancer outlined above.

For evaluating whether the concentration of Trp8a, Trp8b, Trp10a or Trp10b or the concentration of Trp8a, Trp8b, Trp10a or Trp10b encoding mRNA is normal or increased, thus indicative for the presence of a malignant tumor, the measured concentration is compared with the concentration in a normal tissue, preferably by using the ratio of Trp8a:Trp9, Trp8b:Trp9 or Trp10(a or b)/Trp9 for quantification.

Since the prostate carcinoma forms its own basement membrane when growing invasively, it can be concluded that only cells expressing Trp8 and Trp10 are involved in this phenomenon. Thus, it can be concluded that by inhibiting the expression and/or activity of these proteins an effective therapy of cancers like PCA is provided.

Thus, the present invention also relates to a pharmaceutical composition containing a reagent which decreases or inhibits Trp8a, Trp8b, Trp10a and/or Trp10b expression or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b, and a method for preventing, treating, or ameliorating a prostate tumor, endometrial cancer (uterine carcinoma) tumor, a chorion carcinoma, cancer of the lung or melanoma,which comprises administering to a mammalian subject a

therapeutically effective amount of a reagent which decreases or inhibits Trp8a, Trp8b, Trp10a and/or Trp10b expression or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b. Examples of such reagents are the above described antisense RNAs, ribozymes or inhibitors, e.g. specific antibodies. Furthermore, peptides, which inhibit or modulate the biological function of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b may be useful as therapeutical reagents. For example, these peptides can be obtained by screening combina torial phage display libraries (Cosmix, Braunschweig, Germany) as described by Rottgen, P. and Collins, J. (Gene (1995) 164 (2): 243-250). Furthermore, antigenic epitopes of the Trp8 and Trp10 proteins can be identified by the expression of recombinant Trp8 and Trp10 epitope libraries in E. coli (Marquart, A. & Flockerzi, V., FEBS Lett. 407 (1997), 137-140; Trost, C., et al., FEBS Lett. 451 (1999) 257-263 and the consecutive screening of these libraries with serum of patients with cancer of the prostate or of the endometrium. Those Trp8 and Trp10 epitopes which are immunogenic and which lead to the formation of antibodies in the serum of the patients can be then be used as Trp8 or Trp10 derived peptide vaccines for immune inventions against cancer cells which express Trp8 or Trp10. Alternatively to the E. coli expression system, Trp8 or Trp10 or epitopes of Trp8 and Trp10 can be expressed in mammalian cell lines such as human embryonic kidney (Hek 293) cells (American Type Culture Collection, ATCC CRL 1573).

Finally, compounds useful for therapy of the above described diseases comprise compounds which act as antagonists or agonists on the ion channels Trp8, Trp9 and Trp10. It could be shown that Trp8 is a highly calcium selective ion channel which in the presence of monovalent (namely sodium) and divalent ions (namely calcium) is only permeable for calcium ions (see Example 4, below, and Figures 3A, C, E). Under physiological conditions, Trp8 is a calcium selective channel exhibiting large inward currents. This very large conductance of Trp8 channels (as wells as Trp9 and Trp10a/b channels) is useful to establish systems for screening pharmacological compounds interacting with Trp-channels including high throughput screening systems. Useful high throughput screening systems are well known to the person skilled in the art and include, e.g., the use of cell lines stably or transiently transfected with DNA sequences encoding Trp8, Trp9 and Trp10 channels in assays to detect calcium signaling in biological systems. Such systems include assays based on Ca-sensitive dyes such as aequorin, apoaequorin, Fura-2, Fluo-3 and Indo-1.

Accordingly, the present invention also relates to a method for identifying compounds which act as agonists or antagonists on the ion channels Trp8, Trp9 and/or Trp10, said method comprising contacting a test compound with the ion channel Trp8, Trp9 and/or Trp10, preferably by using a system based on cells stably or transiently transfected with DNA sequences encoding Trp8, Trp9 and/or Trp10, and determining whether said test compound affects the calcium uptake.

For administration the above described reagents are preferably combined with suitable pharmaceutical carriers. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Such carriers can be formulated by conventional methods and can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g. by intravenous, intraperetoneal, subcutaneous, intramuscular, topical or intradermal administration. The route of administration, of course, depends on the nature of the tumor and the kind of compound contained in the pharmaceutical composition. The dosage regimen will be determined by the attending physician and other clinical factors. As is well known in the medical arts, dosages for any one patient depends on many factors, including the patient's size, body surface area, age, sex, the particular compound to be administered, time and route of administration, the kind and stage of the tumor, general health and other drugs being administered concurrently.

The delivery of the antisense RNAs or ribozymes of the invention can be achieved by direct application or, preferably, by using a recombinant expression vector such as a chimeric virus containing these compounds or a colloidal dispersion system. By delivering these nucleic acids to the desired target, the intracellular expression of Trp8a, Trp8b, Trp10a and/or Trp10b and, thus, the level of Trp8a, Trp8b, Trp10a and/or Trp10b can be decreased resulting in the inhibition of the negative effects of Trp8a, Trp8b, Trp10a and/or Trp10b, e.g. as regards the metastasis formation of PCA.

Direct application to the target site can be performed, e.g., by ballistic delivery, as a colloidal dispersion system or by catheter to a site in artery. The colloidal dispersion systems which can be used for delivery of the above nucleic, acids include macromolecule complexes, nanocapsules, microspheres, beads and lipid-based systems including oil-in-water emulsions

(mixed), micelles, liposomes and lipoplexes, The preferred colloidal system is a liposome. The composition of the liposome is usually a combination of phospholipids and steroids, especially cholesterol. The skilled person is in a position to select such liposomes which are suitable for the delivery of the desired nucleic acid molecule. Organ-specific or cell-specific liposomes can be used in order to achieve delivery only to the desired tumor. The targeting of liposomes can be carried out by the person skilled in the art by applying commonly known methods. This targeting includes passive targeting (utilizing the natural tendency of the liposomes to distribute to cells of the RES in organs which contain sinusoidal capillaries) or active targeting (for example by coupling the liposome to a specific ligand, e.g., an antibody, a receptor, sugar, glycolipid, protein etc., by well known methods). In the present invention monoclonal antibodies are preferably used to target liposomes to specific tumors via specific cell-surface ligands.

Preferred recombinant vectors useful for gene therapy are viral vectors, e.g. adenovirus, herpes virus, vaccinia, or, more preferably, an RNA virus such as a Retrovirus. Even more preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of such retroviral vectors which can be used in the present invention are: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV) and Rous sarcoma virus (RSV). Most preferably, a non-human primate retroviral vector is employed, such as the gibbon ape leukemia virus (GaLV), providing a broader host range compared to murine vectors. Since recombinant retroviruses are defective, assistance is required in order to produce infectious particles. Such assistance can be provided, e.g., by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. Suitable helper cell lines are well known to those skilled in the art. Said vectors can additionally contain a gene encoding a selectable marker so that the transduced cells can be identified. Moreover, the retroviral vectors can be modified in such a way that they become target specific. This can be achieved, e.g., by inserting a polynucleotide encoding a sugar, a glycolipid, or a protein, preferably an antibody. Those skilled in the art know additional methods for generating target specific vectors. Further suitable vectors and methods for in vitro- or in vivo-gene therapy are described in the literature and are known to the persons skilled in the art; see, e.g., WO 94/29469 or WO 97/00957.

In order to achieve expression only in the target organ, i.e. tumor to be treated, the nucleic acids encoding, e.g. an antisense RNA or ribozyme can also be operably linked to a tissue specific promoter and used for gene therapy. Such promoters are well known to those skilled in the art (see e.g. Zimmermann et al., (1994) Neuron 12, 11-24; Vidal et al.; (1990) EMBO J. 9, 833-840; Mayford et al., (1995), Cell 81, 891-904; Pinkert et al., (1987) Genes & Dev. 1, 268-76).

For use in the diagnostic research discussed above, kits are also provided by the present invention. Such kits are useful for the detection of a target cellular component, which is Trp8a, Trp8b, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts, wherein the presence or an increased concentration of Trp8a, Trp8b, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts is indicative for a prostate tumor, endometrial cancer, melanoma, chorion carcinoma or cancer of the lung, said kit comprising a probe for detection of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts. The probe can be detectably labeled. Such probe may be a specific antibody or specific oligonucleotide. In a preferred embodiment, said kit contains an anti-Trp8a-, anti-Trp8b-, anti-Trp9-, anti-Trp10a-and/or anti-Trp10b-antibody and allows said diagnosis, e.g., by ELISA and contains the antibody bound to a solid support, for example, a polystyrene microtiter dish or nitrocellulose paper, using techniques known in the art. Alternatively, said kits are based on a RIA and contain said antibody marked with a radioactive isotope. In a preferred embodiment of the kit of the invention the antibody is labeled with enzymes, fluorescent compounds, luminescent compounds, ferromagnetic probes or radioactive compounds. The kit of the invention may comprise one or more containers filled with, for example, one or more probes of the invention. Associated with container (s) of the kit can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, us or sale for human administration.

EXAMPLES

The following Examples are intended to illustrate, but not to limit the invention. While such Examples are typical of those that might be used, other methods known to those skilled in the art may alternatively be utilized.

Example 1: ***Materials and Methods***

(A) Isolation of cDNA clones and Northern blot analysis

Total RNA was isolated from human placenta an prostate using standard techniques. Isolation of mRNA was performed with poly (A)$^+$RNA - spin columns (New England Biolabs, Beverly, USA) according to the instructions of the manufacturer. Poly (a) $^+$RNA was reverse transcribed using the cDNA choice system (Gibco-BRL, Rockville, USA) and subcloned in λ-Zap phages (Stratagene, La Jolla, USA). An human expressed sequence tag (GenBank accession number 1404042) was used to screen an oligo d(T) primed human placenta cDNA library. Several cDNA clones were identified and isolated. Additional cDNA clones were isolated from two specifically primed cDNA libraries using primers 5'-gca tag gaa ggg aca ggt gg-3' and 5'-gag agt cga ggt cag tgg tcc-3'.

cDNA clones were sequenced using a thermocycler (PE Applied Biosystems, USA) and Thermo Sequenase (Amersham Pharmacia Biotech Europe, Freiburg, Germany). DNA sequences were analyzed with an automated sequencer (Licor, Linccoln, USA).

For Northern blot analysis 5 µg human poly (A)$^+$ RNA from human placenta or prostate were separated by electrophoresis on 0.8 % agarose gels. Poly (A)$^+$ RNA was transferred to Hybond N nylon membranes (Amersham Pharmacia Biotech Europe, Freiburg, Germany). The membranes were hyridized in the presence of 50 % formamide at 42^{0}C over night. DNA probes were labelled using [α^{32}P]dCTP and the „ready prime„ labelling kit (Amersham Pharmacia Biotech Europe, Freiburg, Germany). Commercial Northern blots were hybridized according to the distributors instructions (Clontech, Paolo Alto, USA).

(B) Construction of expression plasmids and transfection of HEK 293 cells

Lipofections were carried out with the recombinant dicistronic eucaryotic expression plasmid pdiTRP8 containing the cDNA of Trp8b under the control of the chicken ß-actin promotor followed by an internal ribosome entry side (IRES) and the cDNA of the green fluorescent protein (GFP). To obtain pdiTRP8 carrying the entire protein coding regions of TRP8b and

the GFP (Prasher, D.C. et al. (1992), Gene 111, 229-233), the 5`and 3´-untranslated sequences of the TRP8b cDNA were removed, the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) Nucleic Acids Research15, 8125-8148) was introduced immediately 5`of the translation initiation codon and the resulting cDNA was subcloned into the pCAGGS vector (Niwa, H., Yamamura, K. and Miyazaki, J (1991), Gene 8, 193-199) downstream of the chicken β-actin promotor. The IRES derived from encephalmyocarditis virus (Kim, D.G., Kang, H.M., Jang, S.K. and Shin H.S. (1992) Mol.Cell.Biol. 12, 3636-3643) followed by the GFP cDNA containing a Ser65Thr mutation (Heim, R., Cubitt, A.B., Tsien, R.Y. (1995) Nature 373, 663-664) was then cloned 3` to the TRP8b cDNA. The IRES sequence allows the simultaneous translation of TRP8b and GFP from one transcript. Thus, transfected cells can be detected unequivocally by the development of green fluorescence.

For monitoring of the intracellular Ca^{2+} concentration human embryonic kidney (HEK 293) cells were cotransfected with the pcDNA3-TRP8b vector and the pcDNA3-GFPvector in a molar ratio of 4 : 1 in the presence of lipofectamine (Quiagen, Hilden, Germany). To obtain pcDNA3-TRP8b the entire protein coding region of TRP8b including the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) Nucleic Acids Research15, 8125-8148) was subcloned into the pcDNA3 vector (Invitrogen, Groningen, Netherlands). Calcium monitoring and patch clamp experiments were carried out two days and one day after transfection, respectively.

(C) Chromosomal localization of the Trp8 gene

The chromosomal localization of the human TRP8 gene was performed using NIGMS somatic hybrid mapping panel No.2 (Coriell Institute, Camden, NJ, USA) previously described (Drwinga, H.L., Toji, L.H., Kim, C.H., Greene, A.E., Mulivor, R.A. (1993) Genomics 16, 311-314; Dubois, B.L. and Naylor, S.L. (1993) Genomics 16, 315-319).

(D) **In Vitro Translation, glutathione - sepharose and calmodulin agarose binding assay**

N– and C-terminal Trp8-fragments were subcloned into the pGEX-4T2 vector (Amersham Pharmacia Europe, Freiburg, Germany) resulting in glutathione-S-transferase (GST)-Trp8 fusion constructs (Fig. 4). The GST-TRP8-fusion proteins were expressed in E. coli BL 21 cells and purified using glutathione - sepharose beads (Amersham Pharmacia Biotech Europe, Freiburg, Germany).

In vitro translation of human Trp8 cDNA and Xenopus laevis calmodulin cDNA (Davis, T.N. and Thorner, J. Proc.Natl.Acad.Sci. USA 86, 7909-7913.) was performed in the presence of ^{35}S-methionine using the TNT coupled transcription/translation kit (Promega, Madison, USA). Translation products were purified by gel fliltration (Sephadex G50, Amersham Pharmacia Biotech Europe, Freiburg, Germany) and equal amounts of ^{35}S labeled probes were incubated for 2 h with glutathione beads bound to GST - Trp8 or calmodulin - agarose (Calbiochem) in 50 mM Tris-HCl, pH 7.4, 0.1 % Triton X-100, 150 mM NaCl in the presence of 1 mM Ca^{2+} or 2 mM EGTA. After three washes, bound proteins were eluted with SDS sample buffer, fractionated by SDS-PAGE and ^{35}S labeled proteins were detected using a Phosphor Imager (Fujifilm, Tokyo, Japan).

(E) Calcium measurements

The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined by dual wavelength fura-2 fluorescence ratio measurements (Tsien, R.Y. (1988) Trends Neurosci. 11, 419-424) using a digital imaging system (T.I.L.L. Photonics, Planegg, Germany). HEK cells were grown in minimal essential medium in the presence of 10 % fetal calf serum and cotransfected with the pcDNA3-TRP8b vector and the pCDNA3-GFPvector as described above (B). Transfected cells were detected by development of green fluorescence. The cells were loaded with 4μM fura-2/AM (Molecular Probes, Oregon, USA) for one hour. After loading the cells were rinsed 3 times with buffer B1 (10 mM Hepes, 115 mM NaCl, 2 mM $MgCl_2$, 5mM KCl, pH 7.4) and the $[Ca^{2+}]_i$ was calculated from the fluorescence ratios obtained at 340 and 380 nm excitation wavelengths as described (Garcia, D.E., Cavalié, A. and Lux, H.D. (1994) J. Neurosci 14, 545-553).

(F) Electrophysiological recordings

HEK cells were transfected with the eucaryotic expression plasmid pdiTRP8 described in (B) and electrophysiolocigal recordings were carried out one day after transfection. Single cells were voltage clamped in the whole cell mode of the patch clamp technique as described (Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85-100; Philipp, S., Cavalié, A., Freichel, M., Wissenbach, U., Zimmer, S., Trost, C., Marquart, A., Murakami, M. and Flockerzi, V. (1996) EMBO J. 6166-6171). The pipette solution contained contained (mM): 140 aspartic acid, 10 EGTA, 10 NaCl, 1 MgCl2, 10 Hepes (pH 7.2 with CsOH) or 125 CsCl, 10 EGTA, 4 $CaCl_2$ 10 Hepes (pH 7,2 with CsOH). The bath solution contained (mM): 100 NaCl, 10 CsCl, 2 $MgCl_2$, 50 mannitol, 10 glucose, 20

Hepes (pH 7,4 with CsOH) and 2 $CaCl_2$, or no added $CaCl_2$ (-Ca^{2+} solution). Divalent free bath solution contained (mM): 110 N-methyl-D-glucamine (NMDG). Whole cell currents were recorded during 100 msec voltage ramps from −100 to +100 mV at varying holding potentials.

(G) In Situ Hybridization

In situ hybridizations were carried out using formalin fixed tissue slices of 6 - 8 µM thickness. The slices were hydrated and incubated in the presence of PBS buffer including 10 µg / ml proteinase K (Roche Diagnostics, Mannheim, Germany) for 0.5 h. The slices were hybridized at 37°C using biotinylated deoxy-oligonucleotides (0.5 pmol / µl) in the presence of 33 % formamide for 12 h. Furthermore the slices were several times rinsed with 2 x SSC and incubated at 25°C for 0.5 h with avidin / biotinylated horse raddish peroxidase complex (ABC, DAKO, Santa Barbara, USA). After several washes with PBS buffer the slices were incubated in the presence of biotinylated tyramid and peroxide (0.15 % w/v) for 10 min, rinsed with PBS buffer and additionally incubated with ABC complex for 0.5 h. The slices were washed with PBS buffer and incubated in the presence of DAB solution (diaminobenzidine (50µg / ml), 50 mM Tris/EDTA buffer pH 8.4, 0.15 % H_2O_2 in N,N - dimethyl-formamide; Merck, Darmstadt, Germany), The detection was stopped after 4 minutes by incubating the slides in water. Tyramid was biotinylated by incubating NHS-LC Biotin (sulfosuccinimidyl-6-(biotinimid)-hexanoat), 2.5 mg / ml; Pierce, Rockford, USA) and tyramin-HCl (0.75 mg / ml, Sigma) in 25 mM borate buffer pH 8.5 for 12 h. The tyramid solution was diluted 1 - 5 : 1000 in PBS buffer.

(H) GenBank accession numbers: TRP8a, Aj243500; TRP8b Aj243501

Example 2: Expression of TRP8 transcripts

In search of proteins distantly related to the TRP family of ion channels, an human expressed sequence tag (EST, GenBank accession number 1404042) was identified in the GenBank database using BLAST programms (at the National Center for Biotechnology Information (NCBI); Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J.J. (1990) Mol. Biol. 5, 403-410) being slightly homologous to the VR1 gene. Several human placenta cDNA libraries were constructed and screeened with this EST DNA as probe. Several full length

cDNA clones were identified and isolated. The full length cDNA clones encoded two putative proteins differing in three amino acids and were termed Trp8a and Trp8b (Fig. 1c, 2a, 7 and 8A). This finding was reproduced by isolating cDNA clones from two cDNA libraries constructed from two individual placentas. The derived protein sequence(s) comprises six transmembrane domains, a characteristic overall feature of trp channels and related proteins (Fig.: 1b). The sequence is closely related to the meanwhile published calcium uptake transport protein 1 (CaT1), isolated from rat intestine (Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M. and Hediger M.A.(1999) J Biol Chem. 6;274, 22739-22746) and to the epithelial calcium uptake channel (ECaC) isolated from rabbit kidney (Hoenderop, J.G., van der Kemp, A.W., Hartog, A., van de Graaf, S.F., van Os, C.H., Willems, P.H. and Bindels, R.J. (1999) J Biol Chem. 26;274, 8375-8378). Expression of Trp8a/b transcripts are detectable in human placenta, pancreas and prostate (Fig.: 5) and the size of the Northern signal (3.0 kb) corresponds with the size of the isolated full length cDNAs. In addition, a shorter transcript of 1.8 kb, probably a splice variant, is detectable in human testis. The Trp8 mRNA is not expressed in small intestine or colon (Fig.: 5) implicating that Trp8 is not the human ortholog of the rat CaT1 or rabbit ECaC proteins. To investigate whether there are other related sequences Trp8a/b derived primers (UW241, 5'-TAT GAG GGT TCA GAC TGC-3' and UW242, 5'-CAA AGT AGA TGA GGT TGC-3') were used to amplify a 105 bp fragment from human genomic DNA being 95% identical on the nucleotide level to the Trp8 sequence (data not shown). This indicates the existence of several similar sequences in humans at least at the genomic level.

Example 3: Two variants of the Trp8 protein (Trp8a and Trp8b) arise by polymorphism

Two variants of the Trp8 cDNA were isolated from human placenta (Fig.: 2A, 7 and 8A) which encoded two proteins which differ in three amino acids and were termed Trp8a and Trp8b. Trp8a/b specific primers were designed to amplify a DNA fragment of 458 bp of the Trp8 gene from genomic DNA isolated from human T-lymphocytes (primer pair: UW243, 5'-CAC CAT GTG CTG CAT CTA CC-3' and UW244, 5'-CAA TGA CAG TCA CCA GCT CC-3'). The amplification product contains a part of the sequence where the derived protein sequence of Trp8a comprises the amino acid valine and the Trp8b sequence methionine as well as a silent base pair exchange (g versus a) and an intron of 303bp (Fig.: 2.A, B). Both variants of the Trp8 genes (a,b) were amplified from genomic DNA in equal amounts indicating the existence of both variants in the human genome and therefore being not the

result of RNA editing (Fig.: 2B). The Trp8a gene can be distinguished from the Trp8b gene by cutting the genomic fragment of 458bp with the restriction enzyme Bsp1286I (Fig. 2B). Using human genomic DNA isolated from blood of twelve human subjects as template, the 458bp fragment was amplified and restricted with BSP1286I. In eleven of the tested subjects only the Trp8b gene is detectable, while one subject (7) contains Trp8a and Trp8b genes (Fig.: 2D). These implicates that the two Trp8 variants arise by polymorphism and do not represent individual genes. Using Trp8 specific primers and chromosomal DNA as template, the Trp8 locus is detectable on chromosome 7 (Fig.: 2C).

Example 4: Trp8b is a calcium permeable channel

The protein coding sequence of the Trp8b cDNA was subcloned into pcDNA3 vector (Invitrogen, Groningen, Netherlands) under the control of the cytomegalovirus promotor (CMV). Human embryonic kidney (HEK 293) cells were cotransfected with the Trp8b pcDNA3 construct (pcDNA3-Trp8b vector) and the pcDNA3-GFPvector encoding the green fluorescent protein (GFP) in 4:1 ratio. The Trp8b cDNA and the cDNA of the reporter, GFP, was transiently expressed in human embryonic kidney (HEK 293) cells. The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and changes of $[Ca^{2+}]_i$ were determined by dual wavelength fura-2 fluorescence ratio measurements (Fig.: 3F) in cotransfected cells which were identified by the green fluorescence of the reporter gene GFP.

Dual wavelength fura-2 fluorescence ratio measurement is a standard procedure (e.g. in: An introduction of Molecular Neurobiology (ed. Hall, Z.W.)Sinauer Associates, Sunderland, USA (1992)) using fura-2, which is a fluorescent Ca^{2+} sensitive dye and which was designed by R.Y.Tsien (e.g. Trends Neurosci. 11, 419-424 (1988) based upon the structure of EGTA. Its fluorescence emission spectrum is altered by binding to Ca^{2+} in the physiological concentration range. In the absence of Ca^{2+}, fura-2 fluoresces most strongly at an excitation wavelength of 385 nm; when it binds Ca^{2+}, the most effective excitation wavelength shifts to 345 nm. This property is used to measure local Ca^{2+} concentrations within cells. Cells can be loaded with fura-2 esters (e.g. fura-2AM) that diffuse across cell membranes and are hydrolyzed to active fura-2 by cytosolic esterases.

In the presence of 1mM Ca^{2+}, Trp8 expressing cells typically contained more than 300 nM cytosolic Ca^{2+}, while non transfected controls contained less than 100 nM Ca^{2+} ions (Fig. 3F).

When Trp8b transfected cells were incubated without extracellular Ca^{2+}, the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) decreased to levels comparable to non transfected cells. Readdition of 1mM Ca^{2+} to the bath resulted in significant increase of the cytosolic $[Ca^{2+}]$ in Trp8b transfected cells, but not in controls (Fig.: 3F). After readdition of Ca^{2+} ions to the bath solution, the cytosolic Ca^{2+} concentration remains on a high steady state level in the Trp8b transfected cells.

Example 5: Trp8 expressing cells show calcium selective inward currents

To characterize in detail the electrophysiological properties of TRP8, TRP8 and GFP were coexpressed in HEK293 cells using the dicistronic expression vector pdiTRP8 and measured currents using the patch clamp technique in the whole cell mode (Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflugers Arch., 391, 85-100).

The eucaryotic expression plasmid pdiTRP8 contains the cDNA of Trp8b under the control of the chicken ß-actin promotor followed by an internal ribosome entry side (IRES) and the cDNA of the green fluorescent protein (GFP). To obtain pdiTRP8 carrying the entire protein coding regions of TRP8b and the GFP (Prasher, D.C. et al. (1992), Gene 111, 229-233), the 5`and 3´-untranslated sequences of the TRP8b cDNA were removed, the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) Nucleic Acids Research15, 8125-8148) was introduced immediately 5`of the translation initiation codon and the resulting cDNA was subcloned into the pCAGGS vector (Niwa, H., Yamamura, K. and Miyazaki, J (1991), Gene 8, 193-199) downstream of the chicken β-actin promotor. The IRES derived from encephalmyocarditis virus (Kim, D.G., Kang, H.M., Jang, S.K. and Shin H.S. (1992) Mol.Cell.Biol. 12, 3636-3643) followed by the GFP cDNA containing a Ser65Thr mutation (Heim, R., Cubitt, A.B., Tsien, R.Y. (1995) Nature 373, 663-664) was then cloned 3` to the TRP8b cDNA. The IRES sequence allows the simultaneous translation of TRP8b and GFP from one transcript. Thus, transfected cells can be detected unequivocally by the development of green fluorescence.

In the presence of 2 mM external calcium, Trp8b transfected HEK cells show inwardly rectifying currents, the size of which depends on the level of intracellular calcium and the electrochemical driving force. The resting membrane potential was held either at –40 mV, or, to lower the driving force for calcium influx in between pulses, at + 70 mV. Current traces

were recorded in response to voltage ramps from –100 to +100 mV, that were applied every second. To monitor inward and outward currents over time, we analyzed the current size at – 80 and + 80 mV of the ramps. Figure 3A shows a representative trace of the current at – 80 mV over time. Both at a holding potential of –40 mV or at +70 mV, the currents are significantly larger than in cells transfected with only the GFP containing vector (Fig.: 3E). Interestingly, after changing to a positive holding potential, current size in Trp8 transfected cells slowly increases and reaches steady state after approximately 70 seconds (Fig.: 3A). To determine the selectivity of the induced currents, we then perfused the cells with solutions that either contain no sodium, no added Ca^{2+} (Fig. 3A, C) or a sodium containing, but divalent ion free bath solution. To control for the effect of the solution change alone, we also perfused with normal bath (see puff in Fig. 3A). While removal of external Ca^{2+} completely abolishes the trp 8 induced currents - the remaining current being identical in size and shape to the control (Fig.: 3A, C, E), removal of external sodium has no effect (Fig.: 3E). An important hallmark of calcium selective channels (e.g. Vennekens, R., Hoenderop, G.J., Prenen, J., Stuiover, M., Willems, PHGM, Droogmans, G., Nilius, B.and Bindels, R.J.M (1999) J. Biol. Chem. 275, 3963-3969), is their ability to conduct sodium only if all external divalent ions, namely Ca^{2+} and magnesium are removed. To test whether the trp 8 channel conforms with this phenomenon normal bath solution was switched to a solution containing only sodium and 1 mM EGTA. As can be seen in Figure 3B and D, Trp8 transfected cells can now conduct very large sodium currents. Interestingly, immediately after the solution change, the currents first become smaller before increasing rapidly, indicating that the pore may initially still be blocked by calcium a phenomenon usually called anomalous mole fraction behaviour (Warnat, J., Philipp, S., Zimmer, S.,.Flockerzi, V., and Cavalié A.(1999) J.Physiol. (Lond) 518, 631-638). The measured outward currents of Trp8 transfected cells in normal bath solution are not significantly different from non-transfected control cells or cells which only express the reporter gene GFP. As the removal of external Ca^{2+} abolishes the Trp8 specific current, the remaining current was subtracted from the current before the solution change to obtain the uncontaminated Trp8 conductance (see inset in Fig.: 3C). As expected from the given ionic conditions (high EGTA inside, 2 mM Ca^{2+} outside), the current-voltage relationship now shows prominent inward rectification with little to no outward current.

Both the time course of the development of Trp8 currents and the size of the currents depend on the frequency of stimulation (data not shown), the internal and external Ca^{2+} concentration

and the resting membrane potential, suggesting that Trp8 calcium conductance is intrically regulated by a Ca^{2+} mediated feedback mechanisms.

Example 6: Ca^{2+} / calmodulin binds to the C-terminus of the Trp8 protein

To test whether calmodulin, a prime mediator of calcium regulated feedback, is involved, first it was investigated biochemically whether Trp8 protein can bind calmodulin. Trp8 cDNA was in vitro translated in the presence of ^{35}S-methionine and the product incubated with calmodulin-agarose beads. After several washes either in the presence or abscence of Ca^{2+}, the beads were incubated in Laemmli buffer and subjected to SDS-polyacrylamide gel electrophoresis. In the presence of Ca^{2+} (1mM), but not in the absence of Ca^{2+}, Trp8 protein binds to calmodulin (Fig.: 4B).

To narrow down the binding site, two approaches were undertaken: Firstly, GST-TRP8 fusion proteins of various intracellular domains of Trp8 were constructed, expressed in E. coli and bound to gluthathione sepharose beads. These beads were then incubated with in vitro translated ^{35}S- labeled calmodulin, washed and subjected to gel electrophoresis. Secondly, truncated versions of in vitro translated Trp8 protein were used in the above described binding to calmodulin-agarose. As shown in Figure 4A, and C, fusion proteins of the N-terminal region (N1, N2) of Trp8 did not bind calmodulin, while C-terminal fragments (C1, C2, C3, C4) showed calmodulin binding in the presence of calcium (for localization of fragments within the entire Trp8 protein see Fig. 4C). Accordingly, a truncated version of in vitro translated Trp8, which lacks the C-terminal 32 amino acid residues did not bind to calmodulin-agarose (4B). We have restricted the calmodulin binding site to amino acid residues 691 to 711 of the Trp8 protein. This calmodulin binding site does not resemble the typical conserved IQ - motif of conventional myosins, but has limited sequence homology to the calcium dependent calmodulin binding site 1 of the transient receptor potential like (trpl) protein of Drosophila melanogaster (Warr and Kelly, 1996) with several charged amino acid residues conserved. The sequence of the calmodulin binding site of the Trp8 protein resembles a putative amphipathic α-helical wheel structure with a charged and a hydrophobic site according to a model proposed by Erickson-Vitanen and De Grado (1987, Methods Enzymol. 139, 455-478.).

Example 7: Expression of Trp8 transcripts in human placenta and pancreas

Several slides from a human placenta of a ten week old abort were used for in situ hybridization experiments. The in situ hybridization experiments revealed expression of Trp8 transcripts in human placenta (Fig.: 5B). Expression was detectable in trophoblasts and syncytiotrophoblasts of the placenta, but not in Langhans cells.

Trp8 transcripts are detectable in human pancreas (Fig.: 5A). Therefore Trp8 probes were hybridized to tissue sections of human pancreas. The pancreatic tissues were removed from patients with pancreas cancer. Trp8 expression is detectable in pancreatic acinar cells, but not in Langerhans islets (Fig.: 5C). No Trp8 expression was found in regions of pancreatic carcinomas (data not shown).

Furthermore, the Trp8 cDNA is not detectable in human colon nor in human kidney by in situ hybridization as well as by Northern analysis (Fig.: 5A, D). The Northern results taken together with the in situ expression data indicate that the Trp8 protein is not the human ortholog of the CaT1 and ECaC channels cloned from rat intestine (Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M. and Hediger M.A.(1999) J Biol Chem. 6;274, 22739-22746) and from rabbit kidney (Hoenderop, J.G., van der Kemp, A.W., Hartog, A., van de Graaf, S.F., van Os, C.H., Willems, P.H. and Bindels, R.J. (1999) J Biol Chem. 26;274, 8375-8378), respectively. Trp8 is unlikely to represent the human version of CaT1 as its expression is undetectable in the small intestine and colon tissues where CaT1 is abundantly expressed. If, however, Trp8 is the human version of rat CaT1, a second gene product appears to be required for Ca^{2+} uptake in human small intestine and colon attributed to CaT1 in rat small intestine and colon.

Example 8: Differential expression of Trp8 transcripts in benign and malign tissue of the prostate

The Trp8 transcripts are expressed in human prostate as shown by hybridization of a Trp8 probe to a commercial Northern blot (Clontech, Palo Alto, USA) (Fig.: 5A). Trp8 transcripts were not detectable by Northern blot analysis using pooled mRNA of patients with benign prostatic hyperplasia (BPH) (Fig.: 5A, prostate*). To examine Trp8 expression on the cellular

level, sections of prostate tissues were hybridized using Trp8 specific cDNA probes (Table 3). Expression of Trp8 transcripts is not detectable in normal prostate (n = 3), benign hyperplasia (BPH, n = 15) or prostatic intraepithelial neoplasia (PIN, n = 9) (Fig.: 6A, C, E). Trp8 transcripts were only detectable in prostate carcinoma (PCA), although with different expression levels. Low expression levels were found in primary carcinomas (2 - 10 % of the carcinoma cells, n = 8) (Fig.: 7B) . Much stronger expression was detectable in rezidive carcinoma (10 - 60 %) (Fig.: 7D, n = 6) and metastases of the prostate (60 - 90 %, n = 4) (Fig.: 7F). Thus it has to be concluded that the commercial Northern blot used in Fig.: 5A contains not only normal prostate mRNA as indicated by the distributor. According to the distributors instructions the prostate mRNA used for this Northern blot was collected from 15 human subjects in the range of 14 to 60 years of age. This prostate tissue was not examined by pathologic means. Since Trp8 expression is not detectable in normal or benign prostate, this finding implicates that the mRNA used for this Northern blot was extracted in part from prostatic carcinoma tissue. To summarize, Trp8 expression is only detectable in malign prostate and, thus, the Trp8 cDNA is a marker for prostate carcinoma. The results are summarized in Table 4.

Table 3

Trp8 probes used for in situ hybridization:

Probes (antisense)

1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'

2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'

3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

Controls (sense)

1.) 5' GGCAAGATCTCAACCGGCAGCGGA 3'

2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'

3.) 5' TTCCACACCCGGGGCTCTGAGGAT 3'

Table 4

Prostate	total	negative	positive
normal	3	3	0
BPH	15	15	0
PIN	9	9	0

carcinoma	18	1	17

(B) Differential expression of Trp8 transcripts in benign and malign tissue of the uterus

Moreover it could be shown that Trp8 is expresed in endometrial cancer (also called cancer of the uterus, to be distinguished from uterine sarcoma or cancer of the cervix) whereas no expression was observed in normal uterus tissue. Thus, Trp8 also is a specific marker for the diagnosis of the above cancer (Fig. 12).

Example 9: Characterization of Trp9

The complete protein coding sequence of Trp9 was determined (Fig. 9). Trp 9 transcripts are predominantly expressed in the human prostate and in human colon. As it could be shown by Northern blot analysis, there is no difference of the expression of TRP9 in benigne prostata hyperplasia (BPH, Fig. 13, upper panel left) or prostate carcinoma (Fig. 13, upper panel right). However, Trp9 is useful as a reference marker for prostata carcinoma, i.e. can be used for quantifying the expression level of Trp8. The ratio of the expression of Trp8:Trp9 in patients and healthy individuals is useful for the development of a quantitative assay.

Example 10: Characterization of Trp10

The complete protein coding sequence of TRP10 (a and b) was determined by biocomputing (Fig. 10 and 11). Using a 235 bp fragment of the Trp10 cDNA as probe in Northern blot analysis TRP10 transcripts could only be detected in mRNA isolated from individuals with prostate cancer (Fig. 13, bottom panel) but not in mRNA isolated from benign tissue of the prostate (prostate BPH) nor in mRNA isolated from heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. The 235 bp cDNA fragment of the Trp10 cDNA was amplified using the primer pair UW248 5'-ACA GCT GCT GGT CTA TTC C-3' and UW249 5'-TAT

GTG CCT TGG TTT GTA CC-3' and prostate cDNA as template. In summary, Trp10a and Trp10b , like TRP8 are also expressed in malignant prostate tissue. So far, its expression could not be observed in any other tissue examined (see above). Thus, Trp 10a and Trp10b are also useful markers which are specific for malignant prostate tissue.

Furthermore, database searches in public databases of the national center for biological information (NCBI) revealed the existence of several expressed sequence tags (EST clones) being in part identical to the Trp10 sequence. These EST clones were originally isolated from cancer tissues of lung, placenta, prostate and from melanoma. These clones include the clones with the following accession numbers: BE274448, BE408880, BE207083, BE791173, AI671853, BE390627. The results demonstrate that cancer cells of these tissues express Trp10 related transcripts whereas no expression of Trp10 transcripts in the corresponding healthy tissues are detectable (Figure 13). Furthermore, it could be shown that in cancer cells of melanoma and prostate cancer Trp10 transcripts are expressed as shown by in situ hybridizations using 4 antisense probes (Figure 14A – E and 13K-O and Table 2, above). Furthermore, it could clearly be shown that cancer cells of these tissues expressing Trp10 transcripts also express Trp10-antisense transcripts as shown in Figure 14F-J, Figure 14P-R and Figure14T by in situ hybridizations using 4 sense probes (Table 2, above). The in situ hybridization experiments demonstrate that detection of a subset of cancer cells derived from carcinoma of lung, placenta, prostate and melanoma is feasible using antisense as well as sense probes complementary to Trp10 transcripts or complementary to Trp10-antisense transcripts, respectively.

The foregoing is meant to illustrate but not to limit the scope of the invention. The person skilled in the art can readily envision and produce further embodiment, based on the above teachings, without undue experimentation.

What Is claimed Is:

1. An isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b or a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being selected from the group consisting of
(a) a nucleic acid molecule encoding a protein that comprises the amino acid sequence depicted in Figure 7, 8A, 9, 10 or 11;
(b) a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9, 10 or 11;
(c) a nucleic acid molecule included in DSMZ Deposit No. DSM 13579, DSM 13580, DSM 13584 , DSM 13581 or DSM....;
(d) a nucleic acid molecule which hybridizes to a nucleic acid molecule specified in (a) to (c);
(e) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) to (d) due to the degeneration of the genetic code; and
(f) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a) to (e).

2. A recombinant vector containing the nucleic acid molecule of claim 1

3. The recombinant vector of claim 2 wherein the nucleic acid molecule is operatively linked to regulatory elements allowing transcription and synthesis of a translatable RNA in prokaryotic and/or eukaryotic host cells.

4. A recombinant host cell which contains the recombinant vector of claim 3.

5. The recombinant host cell of claim 4, which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.

6. An isolated protein exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b which is encoded by a nucleic acid molecule of claim 1.

7. A recombinant host cell that expresses the isolated protein of claim 6.

8. A method of making an isolated protein exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b comprising:
(a) culturing the recombinant host cell of claim 6 under conditions such that said protein is expressed; and
(b) recovering said protein.

9. The protein produced by the method of claim 8.

10. An antisense RNA sequence characterized in that it is complementary to an mRNA transcribed from a nucleic acid molecule of claim 1 or a part thereof and can selectively bind to said mRNA or part thereof, said sequence being capable of inhibiting the synthesis of the protein encoded by said nucleic acid molecule.

11. A ribozyme characterized in that it is complementary to an mRNA transcribed from a nucleic acid molecule of claim 1 or a part thereof and can selectively bind to and cleave said mRNA or part thereof, thus inhibiting the synthesis of the protein encoded by said nucleic acid molecule.

12. An inhibitor characterized in that it can suppress the activity of the protein of claim 6.

13. A method for diagnosing a prostate carcinoma which comprises contacting a target sample suspected to contain the protein Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA with a reagent which reacts with Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA and detecting Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA.

14. The method of claim 13, wherein the reagent is a nucleic acid.

15. The method of claim 13, wherein the reagent is an antibody.

16. The method of claim 13, wherein the reagent is detectably labeled.

17. The method of claim 16, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

18. A method for diagnosing an endometrial cancer (carcinoma of the uterus) which comprises contacting a target sample suspected to contain the protein Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA with a reagent which reacts with Trp8a and/or Trp8b or the Trp8a and/or Trp8a and/or trp8b encoding mRNA and detecting Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA.

19. The method of claim 18, wherein the reagent is a nucleic acid.

20. The method of claim 18, wherein the reagent is an antibody.

21. The method of claim 18, wherein the reagent is detectably labeled.

22. The method of claim 21, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

23. A method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense RNA or Trp10a and/or Trp10b related antisense RNA.

24. A method for preventing, treating, or ameliorating a prostate tumor, endometrial cancer (carcinoma of the uterus) tumor, a chorion carcinoma, cancer of the lung or melanoma, which comprises administering to a mammalian subject a therapeutically effective amount of a reagent which decreases or inhibits expression of Trp8a, Trp8b, Trp10a and/or Trp10b and/or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b.

25. The method of claim 24, wherein the reagent is a nucleotide sequence comprising an antisense RNA.

26. The method of claim 24, wherein the reagent is a nucleotide sequence comprising a ribozyme.

27. The method of claim 24, wherein the reagent is an inhibitor of Trp8a, Trp8b, Trp10a and/or Trp10b.

28. The method of claim 27, wherein the reagent is an anti-Trp8a-, anti Trp8b-, anti-Trp10a- and/or anti-Trp10b antibody or a fragment thereof.

29. A diagnostic kit useful for the detection of Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts in a sample, wherein the presence of an increased concentration of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts is indicative for a prostate tumor, endometrial cancer (cancer of the uterus) tumor, a chorion carcinoma, cancer of the lung or melanoma, said kit comprising a probe for detection of Trp8a, Trp8b, Trp9, Trp10a or Trp10b or Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts.

30. The kit of claim 29, wherein the target component to be detected is Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b and the probe is an antibody.

31. A method for identifying a compound which acts as an agonist or antagonist on the ion channels Trp8, Trp9 and/or Trp10, said method comprising contacting a test compound with the ion channel Trp8, Trp9 and/or Trp10,and determining whether said test compound affects the calcium uptake.

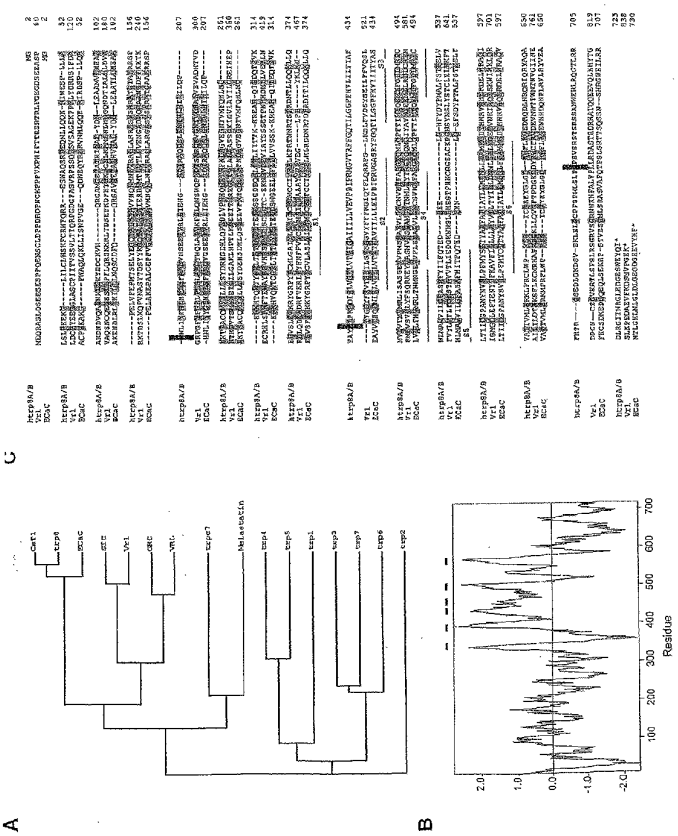
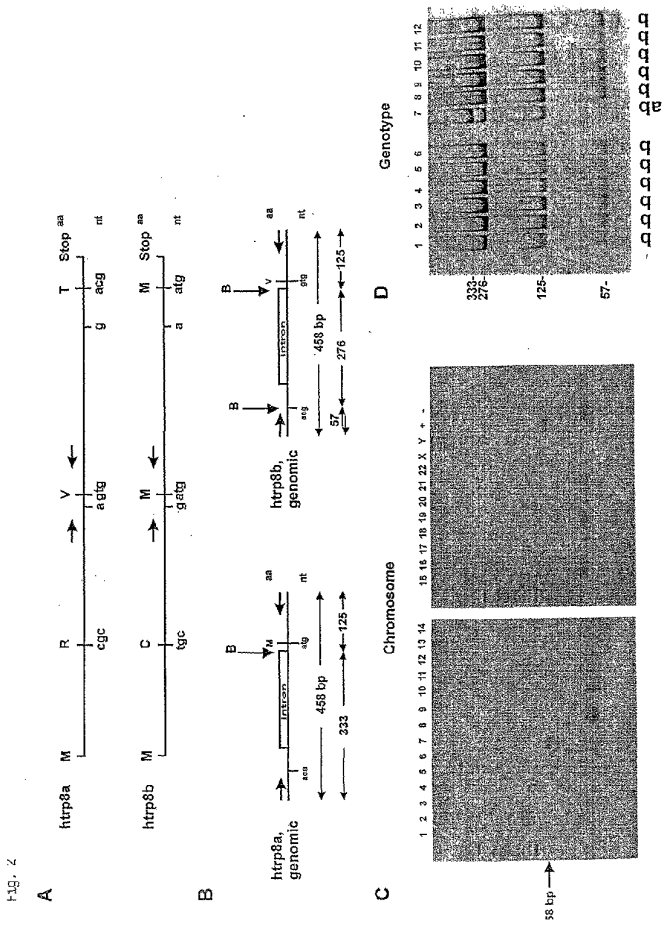


Fig. 1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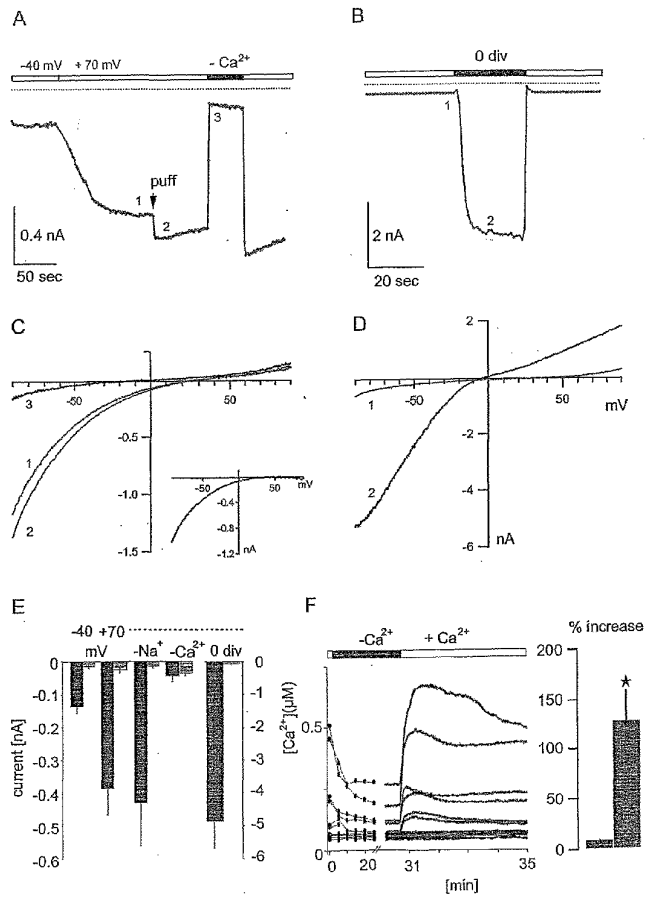
htrp8b
B
htrp8a, genomic
htrp8b, genomic
intron
458 bp
333
125
276
57
C
Chromosome
58 bp
D
Genotype
333-
276-
125-
57-

Fig. 2

Fig. 3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+ 70 mV
- Ca2+
puff
1
2
3
0.4 nA
50 sec
B
0 div
1
2
2 nA
20 sec
C
3
-50
50
-0.5
-1.0
-1.5
1
2
-50
50
mV
-0.4
-0.8
-1.2
nA
D
2
1
-50
50
mV
-2
-4
-6
nA
E
-40 +70
mV
-Na+
-Ca2+
0 div
current [nA]
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
0
-1
-2
-3
-4
-5
-6
F
-Ca2+
+ Ca2+
% increase
[Ca2+](µM)
0.5
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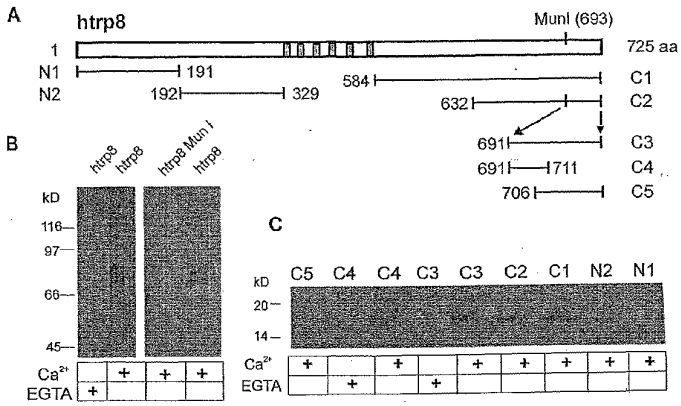
0
20
31
35
[min]
200
150
100
50
0

Fig. 4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htrp8
MunI (693)
1
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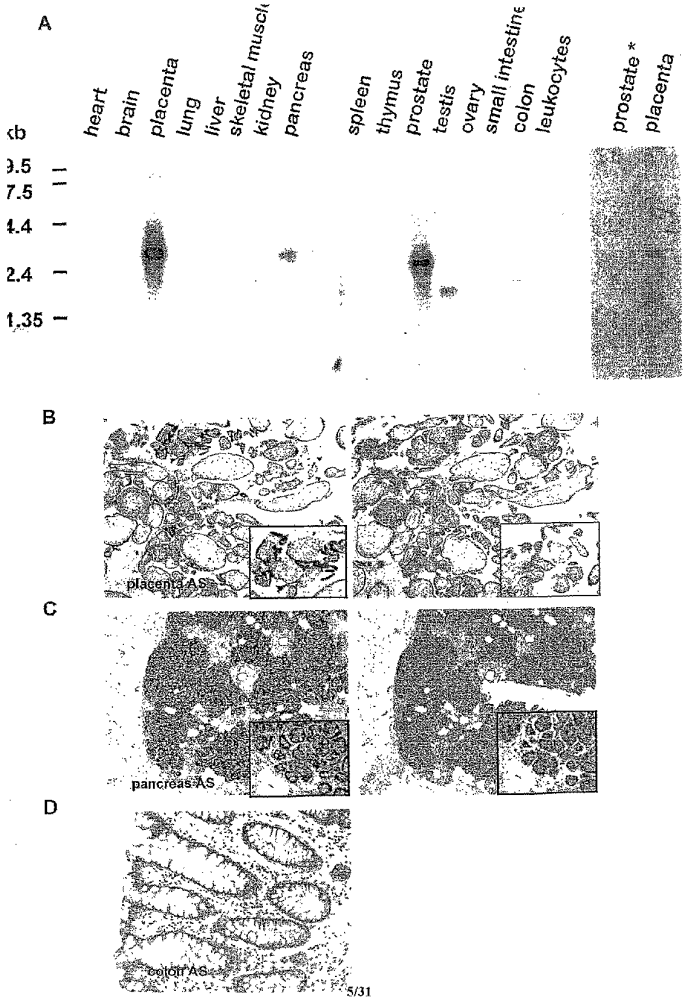
N1
191
N2
192
329
584
C1
632
C2
691
C3
691
711
C4
706
C5
B
htrp8
htrp8
htrp8 MunI
htrp8
kD
116
97
66
45
Ca2+
EGTA
C
kD
C5 C4 C4 C3 C3 C2 C1 N2 N1
20
14
Ca2+
EGTA

Fig. 5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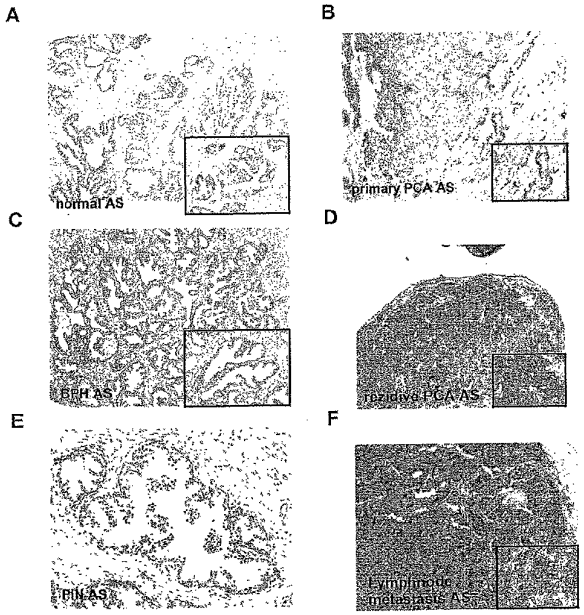
placenta
lung
liver
skeletal muscl
kidney
pancreas
spleen
thymus
prostate
testis
ovary
small intestine
colon
leukocytes
prostate *
placenta
kb
9.5
7.5
4.4
2.4
1.35
B
placenta AS
C
pancreas AS
D
colon AS

Fig. 6


A
normal AS
B
primary PCA AS
C
BPH AS
D
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E
PIN AS
F
Lymphnode
metastasis AS

Fig. 7

```
       10                  30                  50
GCCAAGTGTAACAAACTCACAGCCCTCTCCAAACTGGCTGGGGCTGCTGGGAGACTCCCA
       70                  90                 110
AGGAACTCGTCAGGAAGGCAGGAGACAGGAGACGGGACCTCTACAGGGAGACGGTGGGCC
      130                 150                 170
GGCCCTTGGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGTCTGGCCTCGGCC
      190                 210                 230
TCAGGCCCCCAAGGAGCCGGCCCTACACCCCATGGGTTTGTCACTGCCCAAGGAGAAAGG
                              M  G  L  S  L  P  K  E  K  G
      250                 270                 290
GCTAATTCTCTGCCTATGGAGCAAGTTCTGCAGATGGTTCCAGAGACGGGAGTCCTGGGC
 L  I  L  C  L  W  S  K  F  C  R  W  F  Q  R  R  E  S  W  A
      310                 330                 350
CCAGAGCCGAGATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCCTCTCCT
 Q  S  R  D  E  Q  N  L  L  Q  Q  K  R  I  W  E  S  P  L  L
      370                 390                 410
TCTAGCTGCCAAAGATAATGATGTCCAGCCCTGAACAAGTTGCTCAAGTATGAGGATTG
 L  A  A  K  D  N  D  V  Q  A  L  N  K  L  L  K  Y  E  D  C
      430                 450                 470
CAAGGTGCACCAGAGAGGAGCCATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGA
 E  V  H  Q  R  G  A  M  G  E  T  A  L  H  I  A  A  L  Y  D
      490                 510                 530
CAACCTGGAGGCCGCCATGGTGCTGATGGAGGCTGCCCCGGAGCTGGTCTTTGAGCCCAT
 N  L  E  A  A  M  V  L  M  E  A  A  P  E  L  V  F  E  P  M
      550                 570                 590
GACATCTGAGCTCTATGAGGGTCAGACTGCACTGCACATCGCTGTTGTGAACCAGAACAT
 T  S  E  L  Y  E  G  Q  T  A  L  H  I  A  V  V  N  Q  N  M
      610                 630                 650
GAACCTGGTGCGAGCCCTGCTTGCCCGCAGGGCCAGTGTCTCTGCCAGAGCCACAGGCAC
 N  L  V  R  A  L  L  A  R  R  A  S  V  S  A  R  A  T  G  T
      670                 690                 710
TGCCTTCCGCCGTAGTCCCCGCAACCTCATCTACTTTGGGGAGCACCCTTTGTCCTTTGC
 A  F  R  R  S  P  R  N  L  I  Y  F  G  E  H  P  L  S  F  A
      730                 750                 770
TGCCTGTGTGAACAGTGAGGAGATCGTGCGGCTGCTCATTGAGCATGGAGCTGACATCCG
 A  C  V  N  S  E  E  I  V  R  L  L  I  E  H  G  A  D  I  R
      790                 810                 830
GGCCCAGGACTCCCTGGGAAACACAGTGTTACACATCCTCATCCTCCAGCCCAACAAAAC
 A  Q  D  S  L  G  N  T  V  L  H  I  L  I  L  Q  P  N  X  T
      850                 870                 890
CTTTGCCTGCCAGATGTACAACCTGTTGCTGTCCTACGACAGACATGGGGACCACCTGCA
 F  A  C  Q  M  Y  N  L  L  L  S  Y  D  R  H  G  D  H  L  Q
      910                 930                 950
GCCCCTGGACCTCGTGCCCAATCACCAGGGTCTCACCCCTTTCAAGCTGGCTGGAGTGGA
 P  L  D  L  V  P  N  H  Q  G  L  T  P  F  K  L  A  G  V  E
      970                 990                1010
GGGTAACACTGTGATGTTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGACGTA
 G  N  T  V  M  F  Q  H  L  M  Q  K  R  K  H  T  Q  W  T  Y
     1030                1050                1070
TGGACCACTGACCTCGACTCTCTATGACCTCACAGAGATCGACTCCTCAGGGGATGAGCA
 G  P  L  T  S  T  L  Y  D  L  T  E  I  D  S  S  G  D  E  Q
     1090                1110                1130
GTCCCTGCTGGAACTTATCATCACCACCAAGAAGCGGGAGGCTCGCCAGATCCTGGACCA
 S  L  L  E  L  I  I  T  T  K  K  R  E  A  R  Q  I  L  D  Q
     1150                1170                1190
GACGCCGGTGAAGGAGCTGGTGAGCCTCAAGTGGAAGCGGTACGGGGGGCCGTACTTCTG
 T  P  V  K  E  L  V  S  L  K  W  K  R  Y  G  R  P  Y  F  C
     1210                1230                1250
CATGCTGGGTGCCATATATCTGCTGTACATCATCTGCTTCACCATGTGCTGCATCTACCG
 M  L  G  A  I  Y  L  L  Y  I  I  C  F  T  M  C  C  I  Y  R
     1270                1290                1310
```

Fig. 7 / continuation 1

```
CCCCCTCAAGCCCAGGACCAATAACCGCACAAGCCCCCGGGACAACACCCTCTTACAGCA
 P  L  K  P  R  T  N  N  R  T  S  P  R  D  N  T  L  L  Q  Q
    1330                1350                1370
GAAGCTACTTCAGGAAGCCTACGTGACCCCTAAGGACGATATCCGGCTGGTCGGGGAGCT
 K  L  L  Q  E  A  Y  V  T  P  K  D  D  I  R  L  V  G  E  L
    1390                1410                1430
GGTGACTGTCATTGGGGCTATCATCATCCTGCTGGTAGAGGTTCCAGACATCTTCAGAAT
 V  T  V  I  G  A  I  I  I  L  L  V  E  V  P  D  I  F  R  M
    1450                1470                1490
GGGGGTCACTCGCTTCTTTGGACAGACCATCCTTGGGGGCCCATTCCATGTCCTCATCAT
 G  V  T  R  F  F  G  Q  T  I  L  G  G  P  F  H  V  L  I  I
    1510                1530                1550
CACCTATGCCTTCATGGTGCTGGTGACCATGGTGATGCGGCTCATCAGTGCCAGCGGGGA
 T  Y  A  F  M  V  L  V  T  M  V  M  R  L  I  S  A  S  G  E
    1570                1590                1610
GGTGGTACCCATGTCCTTTGCACTCGTGCTGGGCTGGTGCAACGTCATGTACTTCGCCCG
 V  V  P  M  S  F  A  L  V  L  G  W  C  N  V  M  Y  F  A  R
    1630                1650                1670
AGGATTCCAGATGCTAGGCCCCTTCACCATCATGATTCAGAAGATGATTTTTGGCGACCT
 G  F  Q  M  L  G  P  F  T  I  M  I  Q  K  M  I  F  G  D  L
    1690                1710                1730
GATGCGATTCTGCTGGCTGATGGCTGTGGTCATCCTGGGCTTTGCTTCAGCCTTCTATAT
 M  R  F  C  W  L  M  A  V  V  I  L  G  F  A  S  A  F  Y  I
    1750                1770                1790
CATCTTCCAGACAGAGGACCCCGAGGAGCTAGGCCACTTCTACGACTACCCCATGGCCCT
 I  F  Q  T  E  D  P  E  E  L  G  H  F  Y  D  Y  P  M  A  L
    1810                1830                1850
GTTCAGCACCTTCGAGCTGTTCCTTACCATCATCGATGGCCCAGCCAACTACAACGTGGA
 F  S  T  F  E  L  F  L  T  I  I  D  G  P  A  N  Y  N  V  D
    1870                1890                1910
CCTGCCCTTCATGTACAGCATCACCTATGCTGCCTTTGCCATCATCGCCACACTGCTCAT
 L  P  F  M  Y  S  I  T  Y  A  A  F  A  I  I  A  T  L  L  M
    1930                1950                1970
GCTCAACCTCCTCATTGCCATGATGGGCGACACTCACTGGCGAGTGGCCCATGAGCGGGA
 L  N  L  L  I  A  M  M  G  D  T  H  W  R  V  A  H  E  R  D
    1990                2010                2030
TGAGCTGTGGAGGGCCCAGATTGTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCCTCG
 E  L  W  R  A  Q  I  V  A  T  T  V  M  L  E  R  K  L  P  R
    2050                2070                2090
CTGCCTGTGGCCTCGCTCCGGGATCTGCGGACGGGAGTATGGCCTGGGGGACCGCTGGTT
 C  L  W  P  R  S  G  I  C  G  R  E  Y  G  L  G  D  R  W  F
    2110                2130                2150
CCTGCGGGTGGAAGACAGGCAAGATCTCAACCGGCAGCGGATCCAACGCTACGCACAGGC
 L  R  V  E  D  R  Q  D  L  N  R  Q  R  I  Q  R  Y  A  Q  A
    2170                2190                2210
CTTCCACACCCGGGGCTCTGAGGATTTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGG
 F  H  T  R  G  S  E  D  L  D  K  D  S  V  E  K  L  E  L  G
    2230                2250                2270
CTGTCCCTTCAGCCCCCACCTGTCCCTTCCTACGCCCTCAGTGTCTCGAAGTACCTCCCG
 C  P  F  S  P  H  L  S  L  F  T  P  S  V  S  R  S  T  S  R
    2290                2310                2330
CAGCAGTGCCAATTGGGAAAGGCTTCGGCAAGGSACCCTGAGGAGAGACCTGCGTGGGAT
 S  S  A  N  W  E  R  L  R  Q  G  T  L  R  R  D  L  R  G  I
    2350                2370                2390
AATCARCAGGGGTCTGGAGGACGGGGAGAGCTGGGRATATCAGATCTGACTGCGTGTTCT
 I  N  R  G  L  E  D  G  E  S  W  E  Y  Q  I
    2410                2430                2450
CACTTCGCTTCCTGGAACTTGCTCTCATTTTCCYGGGTGCATCAAACAAAACAAAAACCA
    2470                2490                2510
AACACCCAGAGGTCTCATCTCCCAGGCCCCAGGGAGAAAGAGGAGTAGCATGAACGCCAA
    2530                2550                2570
GGAATGTACGTTGAGAATCACTGCTCCAGGCCTGCATTACTCCTTCAGCTCTGGGGCAGA
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Fig. 7 / continuation 2

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      2590                2610                2630
GGAAGCCCAGCCCAAGCACGGGGCTGGCAGGGCGTGAGGAACTCTCCTGTGGCCTGCTCA
      2650                2670                2690
TCACCCTTCCGACAGGAGCACTGCATGTCAGAGCACTTTAAAAACAGGCCAGCCTGCTTG
      2710                2730                2750
GGCCCTCGGTCTCCACCCCAGGGTCATAAGTGGGGAGAGAGCCCTTCCCAGCGCACCCAG
      2770                2790                2810
GCAGGTGCAGGGAAGTGCAGAGCTTGTGGAAAGCGTGTGAGTGAGGGAGACAGGAACGGC
      2830                2850                2870
TCTGGGGGTGGGAAGTGGGGCTAGGTCTTGCCAACTCCATCTTCAATAAAGTCGTTTTCG
      2890                2910
GATCCCTAAAAAAAAAAAAAAAAAAAAAAAAAAA
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SRSSAMWERLRQGILRRDLRGIINRGLEDGESWEYQI
```

Figure 8:

A)

```
                    ATGGGTTTGTCACTGCCCAAGGAGAAAGGGCTAATTCTCT
                    M  G  L  S  L  P  K  E  K  G  L  I  L  C
          250                 270                 290
GCCTATGGAGCAAGTTCTGCAGATGGTTCCAGAGACGGGAGTCCTGGGCCCAGAGCCGAG
  L  W  S  K  F  C  R  W  F  Q  R  R  E  S  W  A  Q  S  R  D
          310                 330                 350
ATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCCTCTCCTTCTAGCTGCCA
  E  Q  N  L  L  Q  Q  K  R  I  W  E  S  P  L  L  L  A  A  K
          370                 390                 410
AAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTGCAAGGTGCACC
  D  N  D  V  Q  A  L  N  K  L  L  K  Y  E  D  C  K  V  H  Q
          430                 450                 470
AGAGAGGAGCCATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACCTGGAGG
  R  G  A  M  G  E  T  A  L  H  I  A  A  L  Y  D  N  L  E  A
          490                 510                 530
CCGCCATGGTGCTGATGGAGGCTGCCCCGGAGCTGGTCTTTGAGCCCATGACATCTGAGC
  A  M  V  L  M  E  A  A  P  E  L  V  F  E  P  M  T  S  E  L
          550                 570                 590
TCTATGAGGGTCAGACTGCACTGCACATCGCTGTTGTGAACCAGAACATGAACCTGGTGC
  Y  E  G  Q  T  A  L  H  I  A  V  V  N  Q  N  M  N  L  V  R
          610                 630                 650
GAGCCCTGCTTGCCCGCAGGGCCAGTGTCTCTGCCAGAGCCACAGGCACTGCCTTCCGCC
  A  L  L  A  R  R  A  S  V  S  A  R  A  T  G  T  A  F  R  R
          670                 690                 710
GTAGTCCCTGCAACCTCATCTACTTTGGGGAGCACCCTTTGTCCTTTGCTGCCTGTGTGA
  S  P  C  N  L  I  Y  F  G  E  H  P  L  S  F  A  A  C  V  N
```

Fig. 8 / contin ı 1

```
      730                 750                 770
ACAGTGAGGAGATCGTGCGGCTGCTCATTGAGCATGGAGCTGACATCCGGGCCCAGGACT
  S  E  E  I  V  R  L  L  I  E  H  G  A  D  I  R  A  Q  D  S
      790                 810                 830
CCCTGGGAAACACAGTGTTACACATCCTCATCCTCCAGCCCAACAAAACCTTTGCCTGCC
  L  G  N  T  V  L  H  I  L  I  L  Q  P  N  K  T  F  A  C  Q
      850                 870                 890
AGATGTACAACCTGTTCCTGTCCTACGACAGACATGGGGACCACCTGCAGCCCCTGGACC
  M  Y  N  L  L  S  Y  D  R  H  G  D  H  L  Q  P  L  D  L
      910                 930                 950
TCGTGCCCAATCACCAGGGTCTCACCCCTTTCAAGCTGGCTGGAGTGGAGGGTAACACTG
  V  P  N  H  Q  G  L  T  P  F  K  L  A  G  V  E  G  N  T  V
      970                 990                1010
TGATGTTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGACGTATGGACCACTGA
  M  F  Q  H  L  M  Q  K  R  K  H  T  Q  W  T  Y  G  P  L  T
     1030                1050                1070
CCTCGACTCTCTATGACCTCACAGAGATCGACTCCTCAGGGGATGAGCAGTCCCTGCTGG
  S  T  L  Y  D  L  T  E  I  D  S  S  G  D  E  Q  S  L  L  E
     1090                1110                1130
AACTTATCATCACCACCAAGAAGCGGGAGGCTCGCCAGATCCTGGACCAGACGCCGGTGA
  L  I  I  T  T  K  K  R  E  A  R  Q  I  L  D  Q  T  P  V  K
     1150                1170                1190
AGGAGCTGGTGAGCCTCAAGTGGAAGCGGTACGGGCGGCCGTACTTCTGCATGCTGGGTG
  E  L  V  S  L  K  W  K  R  Y  G  R  P  Y  F  C  M  L  G  A
     1210                1230                1250
CCATATATCTGCTGTACATCATCTGCTTCACCATGTGCTGCATCTACGGCCCCCTCAAGC
  I  Y  L  L  Y  I  I  C  F  T  M  C  C  I  Y  R  P  L  K  P
     1270                1290                1310
CCAGGACCAATAACCGCACGAGCCCCCGGGACAACACCCTCTTACAGCAGAAGCTACTTC
  R  T  N  N  R  T  S  P  R  D  N  T  L  L  Q  Q  K  L  L  Q
     1330                1350                1370
AGGAAGCCTACATGACCCCTAAGGACGATATCCGGCTGGTCGGGGAGCTGGTGACTGTCA
  E  A  Y  M  T  P  K  D  D  I  R  L  V  G  E  L  V  T  V  I
     1390                1410                1430
TTGGGGCTATCATCATCCTGCTGGTAGAGGTTCCAGACATCTTCAGAATGGGGGTCACTC
  G  A  I  I  I  L  L  V  E  V  P  D  I  F  R  M  G  V  T  R
     1450                1470                1490
GCTTCTTTGGACAGACCATCCTTGGGGGCCCATTCCATGTCCTCATCATCACCTATGCCT
  F  F  G  Q  T  I  L  G  G  P  F  H  V  L  I  I  T  Y  A  F
     1510                1530                1550
TCATGGTGCTGGTGACCATGGTGATGCGGCTCATCAGTGCCAGCGGGGAGGTGGTACCCA
  M  V  L  V  T  M  V  M  R  L  I  S  A  S  G  E  V  V  P  M
     1570                1590                1610
TGTCCTTGCACTCGTGCTGGGCTGGTGCAACGTCATGTACTTCGCCCGAGGATTCCAGA
  S  F  A  L  V  L  G  W  C  N  V  M  Y  F  A  R  G  F  Q  M
     1630                1650                1670
TGCTAGGCCCCTTCACCATCATGATTCAGAAGATGATTTTTGGCGACCTGATGCGATTCT
  L  G  P  F  T  I  M  I  Q  K  M  I  F  G  D  L  M  R  F  C
     1690                1710                1730
GCTGGCTGATGGCTGTGGTCATCCTGGGCTTTGCTTCAGCCTTCTATATCATCTTCCAGA
  W  L  M  A  V  V  I  L  G  F  A  S  A  F  Y  I  I  F  Q  T
     1750                1770                1790
CAGAGGACCCCGAGGAGCTAGGCCACTTCTACGACTACCCCATGGCCCTGTTCAGCACCT
  E  D  P  E  E  L  G  H  F  Y  D  Y  P  M  A  L  F  S  T  F
     1810                1830                1850
TCGAGCTGTTCCTTACCATCATCGATGGCCCAGCCAACTACAACGTGGACCTGCCCTTCA
  E  L  F  L  T  I  I  D  G  P  A  N  Y  N  V  D  L  P  F  M
     1870                1890                1910
TGTACAGCATCACCTATGCTGCCTTTGCCATCATCGCCACACTGCTCATGCTCAACCTCC
  Y  S  I  T  Y  A  A  F  A  I  I  A  T  L  L  M  L  N  L  L
     1930                1950                1970
TCATTGCCATGATGGGCGACACTCACTGGCGAGTGGCCCATCAGCGGGATGAGCTGTGGA
```

Fig. 8 / continuation 2

```
  I  A  M  M  G  D  T  H  W  R  V  A  H  E  R  D  E  L  W  R
     1990              2010              2030
GGGCCCAGATTGTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCCTCGCTGCCTGTGGC
  A  Q  I  V  A  T  T  V  M  L  E  R  K  L  P  R  C  L  W  P
     2050              2070              2090
CTCGCTCCGGGATCTGCGGACGGGAGTATGGCCTGGGAGACCGCTGGTTCCTGCGGGTGG
  R  S  G  I  C  G  R  E  Y  G  L  G  D  R  W  F  L  R  V  E
     2110              2130              2150
AAGACAGGCAAGATCTCAACCGGCAGCGGATCCAACGCTACGCACAGGCCTTCCACACCC
  D  R  Q  D  L  N  R  Q  R  I  Q  R  Y  A  Q  A  F  H  T  R
     2170              2190              2210
GGGGCTCTGAGGATTTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGGCTGTCCCTTCA
  G  S  E  D  L  D  K  D  S  V  E  K  L  E  L  G  C  P  F  S
     2230              2250              2270
GCCCCCACCTGTCCCTTCTATGCCCTCAGTGTCTCGAAGTACCTCCCGCAGCAGTGCCA
  P  H  L  S  L  P  M  P  S  V  S  R  S  T  S  R  S  S  A  N
     2290              2310              2330
ATTGGGAAAGGCTTCGGCAAGGGACCCTGAGGAGAGACCTGCGTGGGATAATCAACAGGG
  W  E  R  L  R  Q  G  T  L  R  R  D  L  R  G  I  I  N  R  G
     2350              2370              2390
GTCTGGAGGACGGGGAGAGCTGGGAATATCAGATCTGA
  L  E  D  G  E  S  W  E  Y  Q  I  *
```

```
MGLSLPKENGLILCLWSKFCRNFQRESWAQSRDEQNLLQQKRIWESPLLLAAKDNDVQALNKLLKYEDCKVHQRGAMGETALHIA
ALYDNLEAANVLMEAAPELVFEPMTSELYEGQTALHIAVVNQMMNLVRALLARRASVSARATGTAFRRSPCNLIYFGEHPLSFAAC
VNSEEIVRLLIEHGADIRAQDSLGNTVLHILILQPNKTFACQMYNLLLSYDRHGDHLQPLDLVPNHQGLTPFKLAGVEGNTVMFQH
LMQKRKHTQWTYGPLTSTLYDLTEIDSSGDEQSLLELIITTKKREARQILDQTPVKELVSLKWKRYGRPYFCMLGAIYLLYIICFT
MCCIYRFLKPRTNNRTSPRDNTLLQQKLLQEAYHTEKDDIRLVGELVTVIGAIILLVEVPDIFRMGVTRFFGQTILGGPFHVLII
TYAFMVLVTMVMRLISASGEVVPMSFALVLGWCNVMYFARGFQMLGPFTIMIQKMIFGDLMRFCWLMAVVILGFASAFYIIFQTED
PEELGHFYDYPMALFSTFELFLTIIDGPANYNVDLPFMYSITYAAFAIIATLLMLNLLIAMMGDTHWRVAHERDELWRAQIVATTV
MLERKLPRCLWPRSGICGREYGLGDRWFLRVEDRQDLNRQRIQRYAQAFHTRGSEDLDKDSVEKLELGCFFSPHLSLPMPSVSRST
SRSSANWERLRQGTLRRDLRGIINRGLEDGESWEYQI
```

B)

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CAAACTCACAGCCCTCTCCAAACTGGCTGGGGCTGCTGGGAGACTCCCAAGGAACTCGTCAGGAAGGCAGGAGACAGGAGACGGGA
CCTCTACAGGGAGACGGTGGGCCGGCCCTTGGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGTCTGGCCTCGGCCTCA
GGCCCCCAAGGAGCCGGCCCTACACCCCATGGGTTTGTCACTGCCCAAGGAGAAAGGCTAATTCTCTGCCTATGGAGCAAGTTCT
GCAGATGGTTCCAGACACGGGAGTCCTGGGCCCAGACCCGAGATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCCT
CTCCTTCTAGCTGCCAAAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTGCAAGGTGCACCAGAGAGGAGC
CATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACCTGGAGGCCGCCATGGTGCTGATGGAGGCTGCCCCGGAGCTGG
TCTTTGAGCCCATGACATCTGAGCTCTATGAGGGTCAGACTGCACTGCACATCGCTGTTGTGAACCAGAACATGAACCTGGTGCGA
GCCCTGCTTGCCCGCAGGGCCAGTGTCTCTGCCGAGCCACAGGCACTGCCTTCCGCCGTAGTCCCGCAACCTCATCTACTTTGG
GGAGCACCCTTTGTCCTTTGCTGCCTGTGTGAACAGTGAGGAGATCGTGCGGCTGCTCATTGAGCATGGAGCTGACATCCGGGCCC
AGGACTCCCTGGCCCAACAAAACCTTTGCCTGCCAGATGTACAACCTGTTGCTGTCCTACGACAGACATGGGGACCACCTGCAGCC
CCTGGACCTCGTGCCCAATCACCAGGGTCTCACCCCTTTCAAGCTGGCTGGAGTGGAGGGTAACACTGTGATGTTTCAGCACCTGA
TGCAGAAGCGGAAGCACACCCAGTGGACGTATGGACCACTGACCTCGACTCTCTATGACCTCACAGAGATCGACTCCTCAGGGGAT
GAGCAGTCCCTGCTGGAACTTATCATCACCACCAAGAAGCGGGAGGCTCGCCAGATCCTGGACCAGACGCCGGTGAAGGAGCTGGT
GAGCCTCAAGTGGAAGCGGTACGGGCGGCCGTACTTCTGCATGCTGGGTGCCATATATCTGCTGTACATCATCTGCTTCACCATGT
GCTGCATCTACCGCCCCCCTCAAGCCCAGGACCAATAACCGCACAAGCCCCCGGGACAACACCCTCTTACAGCACGAAGCTACTTCAG
GAAGCCTACGTGACCCCTAAGGACGATATCCGGCTGGTCGGCGAGCTGGTGACTGTCATTGGGGCTATCATCATCCTGCTGGTAGA
GGTTCCAGACATCTTCAGAATGGGGGTCACTCGCTTCTTTGGACAGACCATCCTTGGGGGCCCATTCCATGTCCTCATCATCACCT
ATGCCTTCATGGTGCTGGTGACCATGGTGATGCGGCTCATATGATTTTTGGCGACCTGATGCGATTCTGCTGGCTGATGGCTGTGG
TCATCCTGGGCTTTGCTTCAGCCTTCTATATCATCTTCCAGACAGAGGACCCCGAGGAGCTAGGCCACTTCTACGACTACCCCATG
GCCCTGTTCAGCACCTTCGAGCTGGTCCTTACCATCATCGATGGCCCAGCCAACTACAACGTGGACCTGCCCTTCATGTACAGCAT
CACCATATGCTGCCTTTGCCATCATCGCCACACTGCTCATGCTCAACCTCCTCATTGCCATGATGGGCGACACTCACTGGCGAGTGG
CCCATGAGCGGGATGAGCTGTGGAGGCCCAGATTGTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCCTCGCTGCCTGTGGCCT
CGCTCCCGGATCTGCGGACGGGAGTATGGCCTGGGGGACCGCTGGTTCCTGCGGGTGGAAGACAGGCAAGATCTCAACCGGCAGCG
```

Fig. 8 / continuation 3

GATCCAACGCTACGCACAGGCCTTCCACACCCGGGGCTCTGAGGATTTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGGCTGTC
CCTTCAGCCCCCACCTGTCCCTTCCTACGCCCTCAGTGTCTCGAAGTACCTCCCGCAGCAGTGCCAATTGGGAAAGGCTTCGGCAA
GGGACCCTGAGGAGAGACCTGCGTGGGATAATCAACAGGGGTCTGGAGGACGGGCAGAGCTGGGAATATCAGATCTGACTGCGTGT
TCTCACTTCGCTTCCTGGAACTTGCTCTCATTTTCCTGGGTGCATCAAACAAAACAAAAACCAAACACCCAGAGGTCTCATCTCCC
AGGCCCCAGGGAGAAAGAGGAGTAGCATGAACGCCAAGGAATGTACGTTGAGAATCACTGCTCCAGGCCTGCATTACTCCTTCAGC
TCTGGGGCAGAGGAAGCCCAGCCCAAGCACGGGGCTGGCAGGGCGTGAGGAACTCTCCTGTGGCCTGCTCATCACCCTTCCGACAG
GAGCACTGCATGTCAGAGCACTTTAAAAACAGGCCAGCCTGCTTGGGCCCTCGGTCTCCACCCCAGGGTCATAAGTGGGGAGAGAG
CCCTTCCCAGGGCACCCAGGCAGGTGCAGGGAAGTGCAGAGCTTGTGGAAAGCGTGTGAGTGAGGGAGACAGGAACGGCTCTGGGG
GTGGGAAGTGGGGCTAGGTCTTGCCAACTCCATCTTCAATAAAGTCGTTTCGGATCCCTAAAAAAAAAAAAAAAAAAAAAAAAAAA

c.)

CAAACTCACAGCCCTCTCCAAACTGGCTGGGGCTGCTGGGAGACTCCCAAGGAACTCGTCAGGAAGGCAGGAGACAGGAGACGGGA
CCTCTACAGGGAGACGGTGGGCCGGCCCTTGGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGTCTGGCCTCGGCCTCA
GGCCCCCAAGGAGCCGGCCCTACACCCCATGGGTTTGTCACTGCCCAAGGAGAAGGGCTAATTCTCTGCCTATGGAGCAAGTTCT
GCAGATGGTTCCAGAGACGGGAGTCCTGGGCCCAGAGCCGAGATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCCT
CTCCTTCTAGCTGCCAAACATAATGATGTCCAGCCCTGAACAAGTTGCTCAAGTATGAGGATTGCAAGGTGCACCAGAGAGGAGC
CATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACCTGGAGGCCGCCATGGTGCTGATGGAGGCTGCCCCGGAGCTGG
TCTTTGAGCCCATGACATCTGAGCTCTATGAGGTCCTGACTGCCCATCACTTGAACGCCTGCCCCCTGAAATGCCAGGGCCTAGAG
AAGAGCAAGAGATGGGCAGCAGCTGGATCCCCTGGGAATCCTGAACACCCGAGAGCTCCCTGTTCTCCATCCCAGGCTACCCCTGA
GGGAAAGAGACTGGGGTGCATATGGGAGGGACCCCCTGCAGGATCCTGGGGACAGACCCGTGACTGACAGCTGTCTCTGGGCCAGG
TCAGACTGCACTGCACATCGCTGTTGTGAACCAGAACATGAACCTGGTGCGAGCCCTGCTTGCCCGCAGGGCCAGTGTCTCTGCCA
GAGCCACAGGCACTGCCTTCCGCCGTAGTCCCTGCAACCTCATCTACTTTGGGGAGCACCCTTTGTCCTTTGCTGCCTGTGTGAAC
AGTGAGGAGATCGTGCGCTGCTCATTGAGCATGGAGCTGACATCCGGGCCCAGGACTCCCTGGCCCAACAAAACCTTTGCCTGCC
AGATGTACAACCTGTTGCTGTCCTACGACAGACATGGGGACCACCTGCAGCCCCTGGACCTCGTGCCCAATCACCAGGGTCTCACC
CCTTTCAAGCTGGCTGGAGTGGAGGGTAACACTGTGATGTTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGACGTATGG
ACCACTGACCTCGACTCTCTATGACCTCACAGAGATCGACTCCTCAGGGGATGAGCAGTCCCTGCTGGAACTTATCATCACCACCA
AGAAGCGGGAGGCTCGCCAGATCCTGGACCAGACGCCGGTGAAGGAGGTGGTGAGCCTCAAGTGGAAGCGGTACGGGCGGCCGTAC
TTCTGCATGCTGGGTGCCATATATCTGCTGTACATCATCTGCTTCACCATGTGCTGCATCTACCGCCCCCTCAAGCCCAGGACCAA
TAACCGCACGAGCCCCCGGGACAACACCCTCTTACAGCRGAAGCTACTTCAGGAAGCCTACATGACCCCTAAGGACGATATCGGC
TGGTCGGGGAGCTGGTGACTGTCATTGGGGCTATCATCATCCTGCTGGTAGAGGTTCCAGACATCTTCAGAATGGGGGTCACTGGC
TTCTTTGGACAGACCATCCTTGGGGGCCCATTCCATGTCCTCATCATCACCTATGCCTTCATGGTGCTGGTGACCATGGTGATGCG
GCTCATCAGTGCCAGCGGGAGGIGGTACCCATGTCCTTTGCACTCGTGCTGGGCTGGTGCAACGTCATGTACTTCGCCCGAGGAT
TCCAGATGCTAGGCCCCTTCACCATCATGATTCAGAAGATGATTTTGGCGACCTGATGCGATTCTGCTGGCTGATGGCTGTGGTC
ATCCTGGGCTTTGCTTAGACAGAGGACCCCGAGGAGCIAGGCCACTTCTACGACTACCCCATGGCCCTGTTCAGCACCTTCGAGCT
GGTCCTTACCATCATCGATGGCCCAGCCAACTACAACGTGGACCTGCCCTTCATGTACAGCATCACCTATGCTGCCTTTGCCATCA
TCGCCACACTGCTCATGCTCAACCTCCTCATTGCCATGATGGGCGACACTCACTGGCGAGTGGCCCATGAGCGGGATGAGCTGTGG
AGGGCCCAGATTGTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCCTCGCTGCCTGTGGCCTCGCTCCGGGATCTGCGGACGGGA
GTATGGCCTGGGACACCGCTGGTTCCTGCGGGTGGAAGACAGGCAAGATCTCAACCGGCAGCGGATCCAACGCTACGCACAGGCCT
TCCACACCCGGGGCTCTGAGGATTTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGGCTGTCCCTTCAGCCCCCACCTGTCCCTT
CCTATGCCCTCAGTGTCTCGAAGTACCTCCCGCAGCAGTGCCAATTGGGAAAGGCTTCGGCAAGGGACCCTGAGGAGAGACCTGCG
TGGGATAATCAACAGGGGTCTGGAGGACGGGGAGAGCTGGGAATATCAGATCTGACTGCGTGTTCTCACTTCGCTTCCTGGAACTT
GCTCTCATTTTCCTGGGTGCATCAAACAAAACAAAAACCAAACACCCAGAGGTCTCATCTCCCAGGCCCCAGGGAGAAAGAGGAGT
AGCATGAACGCCAAGGAATGTACGTTGAGAATCACTGCTCCAGGCCTGCATTACTCCTTCAGCTCTGGGGCAGAGGAAGCCCAGCC
CAAGCACGGGGCTGGCAGGGCGTGAGGAACTCTCCTGTGGCCTGCTCATCACCCTTCCGACAGGAGCACTGCATGTCAGAGCACTT
TAAAAACAGGCCAGCCTGCTTGGGCCCTCGGTCTCCACCCCAGGGTCATAAGTGGGGAGAGAGCCCTTCCCAGGGCACCCAGGCAG
GTGCAGGGAAGTGCAGAGCTTGTGGAAAGCGTGTGAGTGAGGGAGACAGGAACGGCTCTGGGGGTGGGAAGTGGGGCTAGGTCTTG
CCAACTCCATCTTCAATAAAGTCGTTTCGGATCCCTAAAAAAAAAAAAAAAAAAAAAAAAAAA

D.)

CAAACTCACAGCCCTCTCCAAACTGGCTGGGGCTGCTGGGAGACTCCCAAGGAACTCGTCAGGAAGGCAGGAGACAGGAGACGGGA
CCTCTACAGGGAGACGGTGGGCCGGCCCTTGGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGTCTGGCCTCGGCCTCA
GGCCCCCAAGGAGCCGGCCCTACACCCCATGGGTTTGTCACTGCCCAAGGAGAAGGGCTAATTCTCTGCCTATGGAGCAAGTTCT
GCAGATGGTTCCAGAGACGGGAGTCCTGGGCCCAGAGCCGAGATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCCT
CTCCTTCTAGCTGCCAAAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTGCAAGGTGCACCAGAGAGGAGC
CATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACCTGGAGGCCGCCATGGTGCTGATGGAGGCTGCCCCGGAGCTGG
TCTTTGAGCCCATGACATCTGAGCTCTATGAGGGTCAGACTGCACTGCACATCGCTGTTGTGAACCAGAACATGAACCTGGTGCCA
GCCCTGCTTGCCCGCAGGGCCAGTGTCTCTGCCAGAGCCACAGGCACTGCCTTCCGCCGTAGTCCCCGCAACCTCATCTACTTTGG

Fig. 8 / continuation

AAACACAGTGTTACACATCCTCATCCTCCAGCCCAACAAAACCTTTGCCTGCCAGATGTACAACCTGTTGCTGTCCTACGACAGAC
ATGGGGACCACCTGCAGCCCCTGGACCTCGTGCCCAATCACCAGGGTCTCACCCCTTTCAAGCTGGCTGGAGTGGAGGGTAACACT
GTGATGTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGACGTATGGACCACTGACCTCGACTCTCTATGACCTCACAGA
GATCGACTCCTCAGGGGATGAGCAGTCCCTGCTGGAACTTATCATCACCACCAGAAGCGGGAGGCTCGCCAGATCCTGGACCAGA
CGCCGGTGAAGGAGCTGGTGAGCCTCAAGTGGAAGCGGTACGGGCGGCCGTACTTCTGCATGCTGGGTGCCATATATCTGCTGTAC
ATCATCTGCTTCACCATGTGCTGCATCTACCGCCCCCTCAAGCCCAGGACCAATAACCGCACAAGCCCCCGGGACAACACCCTCTT
ACAGCAGAAGCTACTTCAGGAAGCCTACGTGACCCCTAAGGACGATATCCGGCTGGTCGGGGAGCTGGTGACTGTCATTGGGGCTA
TCATCATCCTGCTGGTAGAGGTTCCAGACATCTTCAGAATGGGGGTCACTCGCTTCTTTGACAGACCATCCTTGGCGGCCCATTC
CATGTCCTCATCATCACCTATGCCTTCATGGTGCTGGTGACCATGGTGATGCGCCTCATCAGTGCCAGCGGGGAGGTGGTACCCAT
GTCCTTTGCACTCGTGCTGGGCTGGTGCAACGTCATGTACTTCGCCCGAGGATTCCAGATGCTAGGCCCCTTCACCATCATGATTC
AGAAGATGATTTTGGCGACCTGATGCGATTCTGCTGGCTGATGGCTGTGGTCATCCTGGGCTTTGCTTCAGCCTTCTATATCATC
TTCCAGACAGAGGACCCCGAGGAGCTAGGCCACTTCTACGACTACCCCATGGCCCTGTTCAGCACCTTGAGCTGGTCCTTACCAT
CATCGATGGCCCAGCCAACTACAACGTGGACCTGCCCTTCATGTACAGCATCACCTATGCTGCCTTTGCCATCATCGCCACACTGC
TCATGCTCAACCTCCTCATTGCCATGATGGGCGACACTCACTGGCGAGTGGCCCATGAGCGGGATGAGCTGTGGAGGCCCAGATT
GTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCCTCGCTGCCTGTGGCCTCGCTCCGGGATCTGCGGACGGGAGTATGGCCTGGG
GGACCGCTGGTTCCTGCGGGTGGAAGACAGGCAAGATCTCAACCGGCAGCGGATCCAACGCTACGCACAGGCCTTCCACACCCGGG
GCTCTGAGGATTTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGGCTGTCCCTTCAGCCCCCACCTGTCCCTTCCTACGCCCTCA
GTGTCTCGAAGTACCTCCCGCAGCAGTGCCAATTGGGAAAGGCTTCGGCAAGGGACCCTGAGGAGAGACCTGCGTGGGATAATCAA
CAGGGGTCTGGAGGACGGGGAGAGCTGGGAATATCAGATCTGACTGCGTGTTCTCACTTCGCTTCCTGGAACTTGCTCTCATTTTC
CTGGGTGCATCAAACAAAACAAAAACCAAACACCCAGAGGTCTCATCTCCCAGGCCCCAGGGAGAAAGAGGAGTAGCATGAACGCC
AAGGAATGTACGTTGAGAATCACTGCTCCAGGCCTGCATTACTCCTTCAGCTCTGGGGCAGAGGAAGCCCAGCCCAAGCACGGGGC
TGGCAGGGCGTGAGGAACTCTCCTGTGGCCTGCTCATCACCCTTCCGACAGGAGCACTGCATGTCAGAGCACTTTAAAAACAGGCC
AGCCTGGTTGGGCCCTCGGTCTCCACCCCAGGGTCATAAGTGGGGAGAGAGCCCTTCCCAGGGCACCCAGGCAGGTGCAGGGAAGT
GCAGAGCTTGTGGAAAGCGTGTGAGTGAGGGAGACAGGAACGGCTCTGGGGGTGGGAAGTGGGGCTAGGTCTTGCCAACTCCATCT
TCAATAAAGTCGTTTTCGGATCCCTAAAAAAAAAAAAAAAAAAAAAAAAAAAA

E.)

CACACATGGGGCCTCCCAGGAGTGCCCAGGACCTCGTGCTGTTGGCCTCTGAATCTATCGTCTCCAATCCGCTGTCCCACAGAAGC
CATATAACCCACCTCTCTGTAAATGCCAGGAGCCATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACCTGGAGGCCG
CCATGGTGCTGATGGAGGCTGCCCCGGAGCTGGTCTTTGAGCCCATGACATCTGAGCTCTATGGAGGGTGAGGGCCCACGGGTCTG
GGGTGAAGAGCAGGAGTGACGTGGTTGGGTATTCAAGTCAGTCTCTGTGATGGATAATTTGGGAAAGACACAGGGGATCTGAGCCT
CCTACTCTTTTTTCTTCTCTGTCTCCCTTCCGTGTCAGTCCCTGACTGCCCATCACTTGAACGCCTGCCCCCTGAAATGCCAGGG
GCCTAGAGAAGAGGAAGAGATGGCAGCAGCTGGATCCCCTGGGAATCCTGAACACCCGAGAGCTCCCTGTTCTCCATCCCAGGCT
ACCCCTGAGGGAAAGAGACTAGGGGTGCATATGGGAGGGACCCCCTGCAGGATCCTAGGGGACAGACCCGTGACTGACAGCTGTCT
CTGGGCCAGGTCAGACTGCACTGCACATCGCTGTTGTGAACCAGAACATGAACCTGGTGCGAGCCCTGCTTGCCCGCAGGGCCAGT
GTCTCTGCCAGAGCCACAGCACTGCCTTCCGCCGTAGTCCCCTGCAACCTCATCTACTTTGGGGAGCACCCTTTGTCCTTTGCTGC
CTGTGTGAACAGTGAGGAGATCGTGCGGCTGCTCATTGAGCATGGAGCTGACATCCGGGCCCAGGACTCCCTGGATGTACAACCTG
TTGCTGTCCTACGACAGACATGGGGACCACCTGCAGCCCCTGGACCTCGTGCCCAATCACCAGGGTCTCACCCCTTCAAGCTGGC
TGGAGTGGAGGGTAACACTGTGATGTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGACGTATGGACCACTGACCTCGA
CTCTCTATGACCTCACAGAGATCGACTCCTCAGGGGATGAGCAGTCCCTGCTGGAACTTATCATCACCACCAAGAAGCGGGAGGCT
CGCCAGATCCTGGACCAGACGCCGGTGAAGGAGCTGGTGAGCCTCAAGTGGAAGCGGTACGGGCGGCCGTACTTCTGCATGCTGGG
TGCCATATATCTGCTGTACATCATCTGCTTCACCATGTGCTGCATCTACCGCCCCCTCAAGCCCAGGACCAATAACCGCACGAGCC
CCCGGGACAACACCCTCTTACAGCAGAAGCTACTTCAGGAAGCCTACATGACCCCTAAGGACGATATCCGGCTGGTCGGGGAGCTG
GTGACTGTCATTGGGGCTATCATCATCCTGCTGGTAGAGGTTCCAGACATCTTCAGAATGGGGGTCACTCGCTTCTTTGGACAGAC
CATCCTTGGGGGCCCATTCCATGTCCTCATCATCACCTATGCCTTCATGGTGCTGGTGACCATGGTGATCCGCCTCATCAGTGCCA
GCGGGGAGGTGGTACCCATGTCCTTTGCACTCGTGCTGGGCTGGTGCAACGTCATGTACTTCGCCCGAGGATTCCAGATGCTAGGC
CCCTTCACCATCATGATTCAGAAGATGATTTTGGCGACCTGATGCGATTCTGCTGGCTGATGGCTGTGGTCATCCTGGGCTTTGC
TTCAGCCTTCTATATCATCTTCCAGACAGAGGACCCCGAGGAGCTAGGCCACTTCTACGACTACCCCATGGCCCTGTTCAGCACCT
TCGAGCTGGTCCTTACCATCATCGATGGCCCAGCCAACTACAACGTGGACCTGCCCTTCATGTACAGCATCACCTATGCTGCCTTT
GCCATCATCGCCACACTGCTCATGCTCAACCTCCTCATTGCCATGATGGGCGACACTCACTGGCGAGTGGCCCATGAGCGGGATGA
GCTGTGGAGGCCCAGATTGTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCCTCGCTGCCTGTGGCCTCGCTCCGGGATCTGCG
GACGGGAGTATGGCCTGGGAGACCGCTGGTTCCTGCGGGTGGAAGACAGGCAAGATCTCAACCGGCAGCGGATCCAACGCTACGCA
CAGGCCTTCCACACCCGGGGCTCTGAGGATTTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGGCTGTCCCTTCAGCCCCCACCT
GTCCCTTCCTATGCCCTCAGTGTCTCGAAGTACCTCCCGCAGCAGTGCCAATTGGGAAAGGCTTCGGCAAGGGACCCTGAGGAGAG
ACCTGCGTGGGATAATCAACAGGGGTCTGGAGGACGGGGAGAGCTGGGAATATCAGATCTGACTGCGTGTTCTCACTTCGCTTCCT
GGAACTTGCTCTCATTTTCCTGGGTGCATCAAACAAAACAAAAACCAAACACCCAGAGGTCTCATCTCCCAGGCCCCAGGGAGAAA
GAGGAGTAGCATGAACGCCAAGGAATGTACGTTGAGAATCACTGCTCCAGCCTGCATTACTCCTTCAGCTCTGGGGCAGAGGAAG
CCCAGCCCAAGCACGGGGCTGGCAGGGCGTGAGGAACTCTCCTGTGGCCTGCTCATCACCCTTCCGACAGGAGCACTGCATGTCAG
AGCACTTTAAAAACAGGCCAGCCTGCTTGGGCCCTCGGTCTCCACCCCAGGGTCATAAGTGGGGAGAGAGCCCTTCCCAGGGCACC

Fig. 8 / continuation 5

CAGGCAGGTGCAGGGAAGTGCAGAGCTTGTGGAAAGCGTGTGAGTGAGGGAGACAGGAACGGCTCTGGGGGTGGGAAGTGGGGCTA
GGTCTTGCCAACTCCATCTTCAATAAAGTCGTTTTCGGATCCCTAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Figure 9:

A.

```
        10                  30                  50
CGGGGCCCTGGGCTGCAGGAGGTTGCGGCCGGCCGCCGCAGCATGGTGGTGCCGGAGAAGG
                                                M  V  V  P  E  K  E
        70                  90                  110
AGCAGAGCTGGATCCCCAAGATCTTCAAGAAGAAGACCTGCACGACGTTCATAGTTGACT
  Q  S  W  I  P  K  I  F  K  K  K  T  C  T  T  F  I  V  D  S
       130                 150                 170
CCACAGATCCGGGAGGGACCTTGTGCCAGTGTGGGCGCCCCCGGACCGCCCACCCCGCAG
  T  D  P  G  G  T  L  C  Q  C  G  R  P  R  T  A  H  P  A  V
       190                 210                 230
TGGCCATGGAGGATGCCTTCGGGGCAGCCGTGGTGACCGTGTGGGACAGCGATGCACACA
  A  M  E  D  A  F  G  A  A  V  V  T  V  W  D  S  D  A  H  T
       250                 270                 290
CCACGGAGAAGCCCACCGATGCCTACGGAGAGCTGGACTTCACGGGGGCCGGCCGCAAGC
  T  E  K  P  T  D  A  Y  G  E  L  D  F  T  G  A  G  R  K  H
       310                 330                 350
ACAGCAATTTCCTCCGGCTCTCTGACCGAACGGATCCAGCTGCAGTTTATAGTCTGGTCA
  S  N  F  L  R  L  S  D  R  T  D  P  A  A  V  Y  S  L  V  T
       370                 390                 410
CAGGCACATGGGGCTTCCGTGCCCCGAACCTGGTGGTGTCAGTGCTGGGGGGATCGGGGG
  R  T  W  G  F  R  A  P  N  L  V  V  S  V  L  G  G  S  G  G
       430                 450                 470
GCCCCGTCCTCCAGACCTGGCTGCAGGACCTGCTGCGTCGTGGGCTGGTGCGGGCTGCCC
  P  V  L  Q  T  W  L  Q  D  L  L  R  R  G  L  V  R  A  A  Q
       490                 510                 530
AGAGCACAGGAGCCTGGATTGTCACTGGGGGTCTGCACACGGGCATCGGCCGGCATGTTG
  S  T  G  A  W  I  V  T  G  G  L  H  T  G  I  G  R  H  V  G
       550                 570                 590
GTGTGGCTGTACGGGACCATCAGATGGCCAGCACTGGGGGCACCAAGGTGGTGGCCATGG
  V  A  V  R  D  H  Q  M  A  S  T  G  G  T  K  V  V  A  M  G
       610                 630                 650
GTGTGGCCCCCTGGGGTGTGGTCCGGAATAGAGGACACCCTCATCAACCCCAAGGGCTCGT
  V  A  P  W  G  V  V  R  N  R  D  T  L  I  N  P  K  G  S  F
       670                 690                 710
TCCCTGCGAGGTACCGGTGGCGCGGTGACCCGGAGGACGGGGTCCAGTTTCCCCTGGACT
  P  A  R  Y  R  W  R  G  D  P  E  D  G  V  Q  F  P  L  D  Y
       730                 750                 770
ACAACTACTCGGCCTTCTTCCTGGTGGACGACGGCACACACGGCTGCCTGGGGGGCGAGA
  N  Y  S  A  F  F  L  V  D  D  G  T  H  G  C  L  G  G  E  N
       790                 810                 830
ACCGCTTCCGCTTGCGCCTGGAGTCCTACATCTCACAGCAGAAGACCGGCGTGGGAGGGA
  R  F  R  L  R  L  E  S  Y  I  S  Q  Q  K  T  G  V  G  G  T
       850                 870                 890
CTGGAATTGACATCCCTGTCCTGCTCCTCCTGATTGATGGTGATGAGAAGATGTTGACGC
  G  I  D  I  P  V  L  L  L  L  I  D  G  D  E  K  M  L  T  R
       910                 930                 950
GAATAGAGAACGCCACCCAGGCTCAGCTCCCATGTCTCCTCGTGGCTGGCTCAGGGGGAG
  I  E  N  A  T  Q  A  Q  L  P  C  L  L  V  A  G  S  G  G  A
       970                 990                 1010
CTGCGGACTGCCTGGCGGAGACCCTGGAAGACACTCTGGCCCCAGGGAGTGGGGGAGCCA
  A  D  C  L  A  E  T  L  E  D  T  L  A  P  G  S  G  G  A  R
      1030                1050                1070
GGCAAGGCGAAGCCCGAGATCGAATCAGGCGTTTCTTTCCCAAAGGGGACCTTGAGGTCC
```

Fig. 9 / continuation 1

```
  Q  G  E  A  R  D  R  I  R  R  F  F  P  K  G  D  L  E  V  L
     1090              1110              1130
TGCAGGCCCAGGTGGAGAGGATTATGACCGGAAGGAGCTCCTGACAGTCTATTCTTCTG
  Q  A  Q  V  E  R  I  M  T  R  K  E  L  L  T  V  Y  S  S  E
     1150              1170              1190
AGGATGGCTCTGAGGAATTCGAGACCATAGTTTTGAAGGCCCTTGTGAAGGCCTGTGCGA
  D  G  S  E  E  F  E  T  I  V  L  K  A  L  V  K  A  C  G  S
     1210              1230              1250
GCTCGGAGGCCTCAGCCTACCTGGATGAGCTGCGTTTGGCTGTGGCTTGGAACCGCGTGG
  S  E  A  S  A  Y  L  D  E  L  R  L  A  V  A  W  N  R  V  D
     1270              1290              1310
ACATTGCCCAGAGTGAACTCTTTCGGGGGGACATCCAATGGCGGTCCTTCCATCTCGAAG
  I  A  Q  S  E  L  F  R  G  D  I  Q  W  R  S  F  H  L  E  A
     1330              1350              1370
CTTCCCCCATGGACGCCCTGCTGAATGACCGCCCTGAGTTCTGCGCTTGCTCATTTCCC
  S  L  N  D  A  L  L  N  D  R  P  E  F  V  R  L  L  I  S  H
     1390              1410              1430
ACGGCCTCAGCCTGGGCCACTTCCTGACCCCGATGCGCCTGGCCCAACTCTACAGCGCGG
  G  L  S  L  G  H  F  L  T  P  N  R  L  A  Q  L  Y  S  A  A
     1450              1470              1490
CGCCCTCCAACTCGCTCATCCGCAACCTTTTGGACCAGGCGTCCCACAGCGCAGGCACCA
  P  S  N  S  L  I  R  N  L  L  D  Q  A  S  H  S  A  G  T  K
     1510              1530              1550
AAGCCCCAGCCCTAAAAGGGGAGCTGCGGAGCTCCGGCCCCCTGACGTGGGGCATCTCC
  A  P  A  L  K  G  G  A  A  E  L  R  P  P  D  V  G  H  V  L
     1570              1590              1610
TGAGGATGCTGCTGGGGAAGATGTGCGCGCCGAGGTACCCCTCCGGGGGCGCCTGGGACC
  R  M  L  L  G  K  M  C  A  P  R  Y  P  S  G  G  A  W  D  P
     1630              1650              1670
CTCACCCAGGCCAGGGCTTCGGGGAGAGCATGTATCTGCTCTCGGACAAGGCCACCTCGC
  H  P  G  Q  G  F  G  E  S  M  Y  L  L  S  D  K  A  T  S  P
     1690              1710              1730
CGCTCTCGCTGGATGCTGGCCTCGGGCAGGCCCCCTGGAGCGACCTGCTTCTTTGGGCAC
  L  S  L  D  A  G  L  G  Q  A  P  W  S  D  L  L  L  W  A  L
     1750              1770              1790
TGTTGCTGAACAGGGCACAGATGGCCATGTACTTCTGGGAGATGGGTTCCAATGCAGTTT
  L  L  N  R  A  Q  M  A  M  Y  F  W  E  M  G  S  N  A  V  S
     1810              1830              1850
CCTCAGCTCTTGGGGCCTGTTTGCTGCTCCGGCTGATGGCACGCCTGGAGCCTGACGCTG
  S  A  L  G  A  C  L  L  L  R  V  M  A  R  L  E  P  D  A  E
     1870              1890              1910
AGGAGGCAGCACGGAGGAAACACCTGGCGTTCAAGTTTGAGGGGATGGGCGTTGACCTCT
  E  A  A  R  R  K  D  L  A  F  K  F  E  G  M  G  V  D  L  F
     1930              1950              1970
TTGGCGAGTGCTATCGCAGCAGTGAGGTGAGGGCTGCCCGCCTCCTCCTCCGTCGCTGCC
  G  E  C  Y  R  S  S  E  V  R  A  A  R  L  L  L  R  R  C  P
     1990              2010              2030
CGCTCTGGGGGGATGCCACTTGCCTCCAGCTGGCCATGCAAGCTGACGCCCGTGCCTTCT
  L  W  G  D  A  T  C  L  Q  L  A  M  Q  A  D  A  R  A  F  F
     2050              2070              2090
TTGCCCAGGATGGGGTACAGTCTCTGCTGACACAGAAGTGGTGGGGAGATATGGCCAGCA
  A  Q  D  G  V  Q  S  L  L  T  Q  K  W  W  G  D  M  A  S  T
     2110              2130              2150
CTACACCCATCTGGGCCCTGGTTCTCGCCTTCTTTTGCCCTCCACTCATCTACACCCGCC
  T  P  I  W  A  L  V  L  A  F  F  C  F  P  L  I  Y  T  R  L
     2170              2190              2210
TCATCACCTTCAGGAAATCAGAAGAGGAGCCCACACGGGAGGAGCTAGAGTTTGACATGG
  I  T  F  R  K  S  E  E  E  P  T  R  E  E  L  E  F  D  M  D
     2230              2250              2270
ATAGTGTCATTAATGGGGAAGGGCCTGTCGGGACGGCGGACCCAGCCGAGAAGACGCCGC
  S  V  I  N  G  E  G  P  V  G  T  A  D  P  A  E  K  T  P  L
     2290              2310              2330
```

Fig. 9 / continuation 2

```
TGGGGGTCCCGCCCAGTCGGCCGTCCGGGTTGCTGCGGGGGCCGCTGCGGGGGGCGCC
  G  V  P  R  Q  S  G  R  P  G  C  C  G  G  R  C  G  G  R  R
     2350                 2370                 2390
GGTGCCTACGCCGCTGGTTCCACTTCTGGGGCGTGCCGGTGACCATCTTCATGGCAACG
  C  L  R  R  W  F  H  F  W  G  V  P  V  T  I  F  M  G  N  V
     2410                 2430                 2450
TGGTCAGCTACCTGCTGTTCCTGCTGCTTTCTCGCGGGTGCTGCTCGTGGATTTCCAGC
  V  S  Y  L  L  F  L  L  F  S  R  V  L  L  V  D  F  Q  P
     2470                 2490                 2510
CGGCGCCGCCCGGCTCCCTGGAGCTGCTGCTCTATTTCTGGGCTTTCACGCTGCTGTGCG
  A  P  P  G  S  L  E  L  L  L  Y  F  W  A  F  T  L  L  C  E
     2530                 2550                 2570
AGGAACTGCGCCAGGGCCTGAGCGGAGGCGGGGGCAGCCTCGCCAGCGGGGGCCCCGGGC
  E  L  R  Q  G  L  S  G  G  G  G  S  L  A  S  G  G  P  G  P
     2590                 2610                 2630
CTGGCCATGCCTCACTGACCCAGCCCCTCCGCCTCTACCTCGCCGACAGCTGGAACCAGT
  G  H  A  S  L  S  Q  R  L  R  L  Y  L  A  D  S  W  N  Q  C
     2650                 2670                 2690
GCGACCTAGTGGCTCTCACCTGCTTCCTCCTGGGCGTGGGCTGCCGGCTGACCCCGGGTT
  D  L  V  A  L  T  C  F  L  L  G  V  G  C  R  L  T  P  G  L
     2710                 2730                 2750
TGTACCACCTGGGCCGCACTGTCCTCTGCATCGACTTCATGGTTTCACGCTGCGGCTGC
  Y  H  L  G  R  T  V  L  C  I  D  F  M  V  F  T  V  R  L  L
     2770                 2790                 2810
TTCACATCTTCACGGTCAACAAACAGCTGGGGCCCAAGATCGTCATCGTGAGCAAGATGA
  F  I  F  T  V  N  K  Q  L  G  P  K  I  V  I  V  S  K  M  M
     2830                 2850                 2870
TGAAGGACGTGTTCTTCTTCCTCTTCCTCGGCGTGTGCTGGTAGCCTATGCCGTGG
  K  D  V  F  F  F  L  F  F  L  G  V  W  L  V  A  Y  G  V  A
     2890                 2910                 2930
CCACGGAGGGGCTCCTGAGGCCACGGGACAGTGACTTCCCAAGTATCCTGCGCCGCGTCT
  T  E  G  L  L  R  P  R  D  S  D  F  P  S  I  L  R  R  V  F
     2950                 2970                 2990
TCTACCGTCCCTACCTGCAGATCTTCGGGCAGATTCCCCAGGAGGACATGGACGTGGCCC
  Y  R  P  Y  L  Q  I  F  G  Q  I  P  Q  E  D  M  D  V  A  L
     3010                 3030                 3050
TCATGGAGCACAGCAACTGCTCGTCGGAGCCCGGCTTCTGGGCACACCCTCCTGGGCCCC
  M  E  H  S  N  C  S  S  E  P  G  F  W  A  H  P  P  G  A  Q
     3070                 3090                 3110
AGGCGGGCACCTGCCTCTCCCAGTATGCCAACTGGCTGCTGGTGCTGCTCCTCGTCATCT
  A  G  T  C  V  S  Q  Y  A  N  W  L  V  V  L  L  L  V  I  F
     3130                 3150                 3170
TCCTGCTCGTGGCCAACATCCTGCTGGTCAACTTGCTCATTGCCATGTTCAGTTACACAT
  L  L  V  A  N  I  L  L  V  N  L  L  I  A  M  F  S  Y  T  F
     3190                 3210                 3230
TCGGCAAAGTACAGGGCAACAGCGATCTCTACTGGAAGGCGCAGCGTTACCGCCTCATCC
  G  K  V  Q  G  N  S  D  L  Y  W  K  A  Q  R  Y  R  L  I  R
     3250                 3270                 3290
GGGAATTCCACTCTCGGCCCGCGCTGGCCCCGCCCTTTATCGTCATCTCCCACTTGCGCC
  E  F  H  S  R  P  A  L  A  P  P  F  I  V  I  S  H  L  R  L
     3310                 3330                 3350
TCCTGCTCAGGCAATTGTGCAGGCGACCCCGGAGCCCCCAGCCGTCCTCCCCGGCCCTCG
  L  L  R  Q  L  C  R  R  P  R  S  P  Q  P  S  S  P  A  L  E
     3370                 3390                 3410
AGCATTTCCGGGTTTACCTTCTAAGGAAGCCGAGCGGAAGCTGCTAACGTGGGAATCGG
  H  F  R  V  Y  L  S  K  E  A  E  R  K  L  L  T  W  E  S  V
     3430                 3450                 3470
TGCATAAGGAGAACTTTCTGCTGGCACGCGCTAGGGACAAGCGGGAGAGCGACTCCGAGC
  H  K  E  N  F  L  L  A  R  A  R  D  K  R  E  S  D  S  E  R
     3490                 3510                 3530
GTCTGAAGCGCAGGTCCCAGAAGGTGGACTTGGCACTGAAACAGCTGGGACACATCCGCG
  L  K  R  T  S  Q  K  V  D  L  A  L  K  Q  L  G  H  I  R  E
```

Fig. 9 / continuation 3

```
      3550                3570                3590
AGTACGAACAGCGCCTGAAAGTGCTGGAGCGGGAGGTCCAGCAGTGTAGCCGCGTCCTGG
  Y  E  Q  R  L  K  V  L  E  R  E  V  Q  Q  C  S  R  V  L  G
      3610                3630                3650
GGTGGGTGGCCGAGGCCCTGAGCCGCTCTGCCTTGCTGCCCCCAGGTGGGCCGCCACCCC
  W  V  A  E  A  L  S  R  S  A  L  L  P  P  G  G  P  P  P  P
      3670                3690                3710
CTGACCTGCCTGGGTCCAAAGACTGAGCCCTGCTGGCGGACTTCAAGGAGAAGCCCCCAC
  D  L  P  G  S  K  D  *
      3730                3750                3770
AGGGGATTTTGCTCCTAGAGTAAGGCTCATCTGGGCCTCGGCCCCCGCACCTGGTGGCCT
      3790                3810                3830
TGTCCTTGAGGTGAGCCCCATGTCCATCTGGGCCACTGTCAGGACCACCTTTGGGAGTGT
      3850                3870                3890
CATCCTTACAAACCACAGCATGCCCGGCTCCTCCCAGAACCAGTCCCAGCCTGGGAGGAT
      3910                3930                3950
CAAGGCCTGGATCCCCGGGCCGTTATCCATCTGGAGGCTGCAGGGTCCTTGGGGTAACAGG
      3970                3990                4010
GACCACAGACCCCTCACCACTCACAGATTCCTCACACTGGGGAAATAAAGCCATTTCAGA
      4030
GGAAAAAAAAAAAAAAAAAAAA
```

```
MVTPEKDQSNIDKIFKKKTCTTFIVDSTDPGGTLCQCGRPRTARPAVAMEDAFGAAVVTVMDSDAHTTEKPTDAYELDFTGAG
SNFLRLSDRTDPAAVVSLVTRTWGFRAPNLVVSVLGGSGGPVLQTWLQDLLRRGLVRAAQSTGAWIVTGCLHTGIGRHVGVAV
QMASTGGTKVVAMGVAPRGVVRNPDTLINPKGSFFARYEWEGDPEDGVQFPLDYNYSAFFLVDDGTHGCLGGENRFELRLESY
QKTGVGGTGIDIFVLLLLIDSDERMLTRIENATQAHVPCLLVAGSRGLGMPGGTLEAHLAQDGDHKANQSTMQLLLPEDLSLQ
SIDRKTLQSYSERLAVANNEVDIAQSELFRGDIGWRSFHLEASLMDALLNDRPEFVRLLISHGLSLGHFLTPMRLAQLYSAAI
LIRNLLDQASHSAGTKAPALKGGAELRPPDVGHVLRMLLGKMCAPRYPSGGAWDPHPGQGPGESMYLLSDKATSPLSLDAGI
PWSDLLLWALLLNRAQMAMYPWEMGSNAVSSALGRCLLLRVNARLSPDAEEAARRKDLAAFKFGNGVDLPGRCYRSSEVRAAI
RRCPLWGDATCLQLAMQADARAFFAQDGVQSLLTQKWNGDMASTTPIWAIVLAFFCPPLIYTRLITFRKSEEEPTRELEFDK
INGEGPVGTADPAEKTFLGVPRQSGRPGCCSGRCGGRRCLRRWFHEWGVEVTIFMGNVVSYLLPLLLFSRVLLVDFQPAPPGS
LLYFWAFTLLCEELRQGLSGGGGSLASGGPGPGHASLSQRLRLYLADSWNQCDLVALTCFLLGVGCRLTPGLVHLGRTVLCIC
FTVRLLHIFTVNKQLGPKIVIVSKMMKDVFFFLFFLGVWLVATGVATEGLLRPRDSDFPSILRAVFYRFYLQIFGQIPQEDMC
MEHSNCSSEPGFWAHPPGAQRGTCVSQYANPLVVLLLVIFLLVANILLVNLLIAMFSYTFGKVQGNSDLYWKAQRYRLIREFH
ALAPPFIVISHLRLLLRQLCRRPRSPQPSSPALEHFRVYLSKEASRKLLTWESVHKENFLLARARDKRESDSERLKRTSQKVT
KQLGHIREYEQRLKVLEREVQQCSRVLGWVARALSRSALLPPGGPPPPDLPGSKD
```

B.)

```
       10                  30                  50
ATCCAATGGCGGTCCTTCCATCTCGAAGCTTCCCTCATGGACGCCCTGCTGAATGACCGG
       70                  90                 110
CCTGAGTTCGGTGCGCTTGCTCATTTCCCACGGCCTCAGCCTGGGCCACTTCCTGACCCCG
      130                 150                 170
ATGCGCCTGGCCCCAACTCTACAGCGCGGCGCCCTCCAACTCGCTCATCCGCAACCTTTTG
      190                 210                 230
GACCAGGCGTCCCACAGCGCAGGCACCAAAGCCCCAGCCCTAAAAGGGGGAGCTGCGGAG
      250                 270                 290
CTCCGGCCCCCTGACGTGGGGCATGTGCTGAGGATGCTGCTGGGGAAGATGTGCGCGCCG
      310                 330                 350
AGATGTATCTGCTCTCGGACAAGGCCACCTCGCCGCTCTCGCTGGATGCTGGCCTCGGGC
  M   Y   L   L   S   D   K   A   T   S   P   L   S   L   D   A   G   L   G   Q
      370                 390                 410
AGGCCCCCTGGAGCGACCTGCTCTTTGGGCACTGTTGCTGAACAGGGCACAGATGGCCA
  A   P   W   S   D   L   L   L   W   A   L   L   L   N   R   A   Q   M   A   M
      430                 450                 470
TGTACTTCTGGGAGATGGGTTCCAATGCAGTTTCCTCAGCTCTTGGGGCCTGTTTGCTGC
  Y   F   W   E   M   G   S   N   A   V   S   S   A   L   G   A   C   L   L   L
```

Fig. 9 / continuation 4

```
      490                 510                 530
TCCGGGTGATGGCACGCCTGGAGCCTGACGCTGAGGAGGCAGCACGGAGGAAAGACCTGG
  R  V  M  A  R  L  E  P  D  A  E  E  A  A  R  R  K  D  L  A
      550                 570                 590
CGTTCAAGTTTGAGGGGATGGGCGTTGACCTCTTTGGCGAGTGCTATCCCAGCAGTGAGG
  F  K  F  E  G  M  G  V  D  L  F  G  E  C  Y  R  S  S  E  V
      610                 630                 650
TGAGGGCTGCCCGCCTCCTCCTCCGTCGCTGCCCGCTCTGGGGGGATGCCACTTGCCTCC
  R  A  A  R  L  L  L  R  R  C  F  L  W  G  D  A  T  C  L  Q
      670                 690                 710
AGCTGGCCATGCAAGCTGACGCCCGTGCCTTCTTTGCCCAGGATGGGGTACAGTCTCTGC
  L  A  M  Q  A  D  A  R  A  F  F  A  Q  D  G  V  Q  S  L  L
      730                 750                 770
TGACACAGAAGTGGTGGGGAGATATGGCCAGCACTACACCCATCTGGGCCCTGGTTCTCG
  T  Q  K  W  W  G  D  M  A  S  T  T  P  I  W  A  L  V  L  A
      790                 810                 830
CCTTCTTTTGCCCTCCACTCATCTACACCCGCCTCATCACCTTCAGGAAATCAGAAGAGG
  F  F  C  P  P  L  I  Y  T  R  L  I  T  F  R  K  S  E  E  E
      850                 870                 890
AGCCCACACGGGAGGAGCTAGAGTTTGACATGGATAGTGTCATTAATGGGGAAGGGCCTG
  P  T  R  E  E  L  E  F  D  M  D  S  V  I  N  G  E  G  P  V
      910                 930                 950
TCGGGACGGCGGACCCAGCCGAGAAGACGCCGCTGGGGGTCCCGCGCCAGTCGGGCCGTC
  G  T  A  D  P  A  E  K  T  P  L  G  V  P  R  Q  S  G  R  P
      970                 990                1010
CGGGTTGCTGCGGGGGCCGCTGCGGGGGCGCCGGTGCCTACGCCGCTGGTTCCACTTCT
  G  C  C  G  G  R  C  G  G  R  R  C  L  R  R  W  F  H  F  W
     1030                1050                1070
GGGGCGTGCCGGTGACCATCTTCATGGGCAACGTGGTCAGCTACCTGCTGTTCCTGCTGC
  G  V  P  V  T  I  F  M  G  N  V  V  S  Y  L  L  F  L  L  L
     1090                1110                1130
TTTTCTCGCGGGTGCTGCTCGTGGATTTCCAGCCGGCCCCGCCCGGCTCCCTGGAGCTGC
  F  S  R  V  L  L  V  D  F  Q  P  A  P  P  G  S  L  E  L  L
     1150                1170                1190
TGCTCTATTTCTGGGCTTTCACGCTGCTGTGCGAGGAACTGCGCCCAGGGCCTGAGCGGAG
  L  Y  F  W  A  F  T  L  L  C  E  E  L  R  Q  G  L  S  G  G
     1210                1230                1250
GCGGGGGCAGCCTCGCCAGCGGGGGCCCCGGGCCTGGCCATGCCTCACTGAGCCAGCGCC
  G  G  S  L  A  S  G  G  P  G  P  G  H  A  S  L  S  Q  R  L
     1270                1290                1310
TGCGCCTCTACCTCGCCGACAGCTGGAACCAGTGCGACCTAGTGGCTCTCACCTGCTTCC
  R  L  Y  L  A  D  S  W  N  Q  C  D  L  V  A  L  T  C  F  L
     1330                1350                1370
TCCTGGGCGTGGGCTGCCGGCTGACCCCGGGTTTGTACCACCTGGGCCGCACTGTCCTCT
  L  G  V  G  C  R  L  T  P  G  L  Y  H  L  G  R  T  V  L  C
     1390                1410                1430
GCATCGACTTCATGGTTTTCACGGTGCGGCTGCTTCACATCTTCACGGTCAACAAACAGC
  I  D  F  M  V  F  T  V  R  L  L  H  I  F  T  V  N  E  Q  L
     1450                1470                1490
TGGGGCCCAAGATCGTCATCGTGAGCAAGATGATGAAGGACGTGTTCTTCTTCTTCTT
  G  P  K  I  V  I  V  S  K  M  M  K  D  V  F  F  F  L  F  F
     1510                1530                1550
TCCTCGGCGTGTGGCTGGTAGCCTATGGCGTGGCCACGGAGGGGCTCCTGAGGCCACGGG
  L  G  V  W  L  V  A  Y  G  V  A  T  E  G  L  L  R  P  R  D
     1570                1590                1610
ACAGTGACTTCCCAAGTATCCTGCGCCGCGTCTTCTACCGTCCCTACCTGCAGATCTTCG
  S  D  F  P  S  I  L  R  R  V  F  Y  R  P  Y  L  Q  I  F  G
     1630                1650                1670
GGCAGATTCCCCAGGAGGACATGGACGTGGCCCTCATGGAGCACAGCAACTGCTCGTCGG
  Q  I  P  Q  E  D  M  D  V  A  L  M  E  H  S  N  C  S  S  E
     1690                1710                1730
AGCCCGGCTTCTGGGCACACCCTCCTGGGGCCCAGGCGGGCACCTGCGTCTCCCAGTATG
```

Fig. 9 / continuation 5

```
  P  G  F  W  A  H  P  P  G  A  Q  A  G  T  C  V  S  Q  Y  A
     1750              1770              1790
CCAACTGGCTGGTGGTGCTGCTCCTCGTCATCTTCCTGCTCGTGGCCAACATCCTGCTGG
  N  W  L  V  V  L  L  L  V  I  F  L  L  V  A  N  I  L  L  V
     1810              1830              1850
TCAACTTGCTCATTGCCATGTTCAGTTACACATTCGGCAAAGTACAGGGCAACAGCGATC
  N  L  L  I  A  M  F  S  Y  T  F  G  K  V  Q  G  N  S  D  L
     1870              1890              1910
TCTACTGGAAGGCGCAGCGTTACCGCCTCATCCGGGAATTCCACTCTCGCCCCGCGCTGG
  Y  W  K  A  Q  R  Y  R  L  I  R  E  F  H  S  R  P  A  L  A
     1930              1950              1970
CCCCGCCCTTTATCGTCATCTCCCACTTGCGCCTCCTGCTCAGGCAATTGTGCAGGCGAC
  P  P  F  I  V  I  S  H  L  R  L  L  L  R  Q  L  C  R  R  P
     1990              2010              2030
CCCGGAGCCCCCAGCCGTCCTCCCCGGCCCTCGAGCATTTCCGGGTTTACCTTCTAAGG
  R  S  P  Q  P  S  S  P  A  L  E  H  F  R  V  Y  L  S  K  E
     2050              2070              2090
AAGCCGAGCGGAAGCTGCTAAAGTGGCAATCGGTGCATAAGGAGAACTTTCTGCTGGCAC
  A  E  R  K  L  L  T  W  E  S  V  H  K  E  N  F  L  L  A  R
     2110              2130              2150
GCGCTAGGGACAAGCGGGAGAGCGAGTCCGAGCGTCTGAAGCGCACGTCCCAGAAGGTGG
  A  R  D  K  R  E  S  D  S  E  R  L  K  R  T  S  Q  K  V  D
     2170              2190              2210
ACTTGGCACTGAAACAGCTGGGACACATCCGCGAGTACGAACAGCGCCTGAAAGTGCTGG
  L  A  L  K  Q  L  G  H  I  R  E  Y  E  Q  R  L  K  V  L  E
     2230              2250              2270
AGCGGGAGGTCCAGCAGTGTAGCCGCGTCCTGGGGTGGGTGGCCGAGGCCCTGAGCCGCT
  R  E  V  Q  Q  C  S  R  V  L  G  W  V  A  E  A  L  S  R  S
     2290              2310              2330
CTGCCTTGCTGCCCCCAGGTGGGCCGCCACCCCCTGACCTGCCTGGGTCCAAAGACTGAG
  A  L  L  P  P  G  G  P  P  P  P  D  L  P  G  S  K  D  *
     2350              2370              2390
CCCTGCTGGCGGACTTCAAGGAGAAGCCCCCACAGGGGATTTTGCTCCTAGAGTAAGGCT
     2410              2430              2450
CATCTGGGCCTCGGCCCCCGCACCTGGTGGCCTTGTCCTTGAGGTGAGCCCCATGTCCAT
     2470              2490              2510
CTGGCCACTGTCAGGACCACCTTTGGGAGTGTCATCCTTACAAACCACAGCATGCCCGG
     2530              2550              2570
CTCCTCCCAGAACCAGTCCCAGCCTGGGAGGATCAAGGCCTGGATCCCGGGCCGTTATCC
     2590              2610              2630
ATCTGGAGGCTGCAGGGTCCTTGGGGTAACAGGGACCACAGACCCCTCACCACTCACAGA
     2650              2670              2690
TTCCTCACACTGGGGAAATAAAGCCATTTCAGAGGAAAAAAAAAAAAAAAAAAAA
```

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MYLLSDKATSPLSLDAGLGQAPWSDLLLWALLLNRAQMANYFWEMGSNAVSSALGACLLLRVMARLEPDAEEAARRKDLAFKFEGM
GVDLFGECYRSSEVRAARLLLRRCPLWGDATCLQLAMQADARAFFAQDGVQSLLTQKWWGDMASTTPIWALVLAEFCPPLIYTRLI
TFRKSEEEPTREELEFDMDSVINGEGPVGTADPAEKTPLGVPRQSGRPGCCGGRCGGRRCLRRWFSFWGVFVTIFNGNVVSYLLFL
LLFSRVLLVDEQPAPGSLELLLYFWAFTLLCELRQGLSGGGGSLASGGPGPGHASLSQRLRLYLADSWNQCDLVALTCFLLGVG
CRLTPGLYHLGRTVLCIDFMVFTVRLLHIFTVNKQLGPKIVIVSKMMKDVFFFLFFLGVWLVAYGVATEGLLRPRDSDFPSILRRV
FYRPYLQIFGQIPQEDMDVALMEHSNCSSEPGFWAHPPGAQAGTCVSQYANWLVVLLLVIFLLVANILLVNLLIAMFSYTFGKVQG
NSDLYWKAQRYRLIREFHSRPALAPPFIVISHLRLLLRQLCRRPRSPQPSSPALEHFRVYLSKEAERKLLTWESVHKENFLLARAR
DKRESDSERLKRTSQKVDLALKQLGHIREYEQRLKVLEREVQQCSRVLGWVAEALSRSALLPPGGPPPPDLPGSKD
```

Fig. 10

A)

```
        10                  30                  50
ATTAAAGTTTATAAAACAGTGGCTGGATGGTTGGAGGATGCAGGTGGACAGAAGACGTGG
                           M  V  G  G  C  R  W  T  E  D  V  E
        70                  90                  110
AGCCTGCAGAAGTAAAGGAAAAGATGTCCTTTCGGGCAGCCAGGCTCAGCATGAGGAACA
  P  A  E  V  K  E  K  M  S  F  R  A  A  R  L  S  M  R  N  R
       130                 150                 170
GAAGGAATGACACTCTGGACAGCACCCGGACCCTGTACTCCAGCGCGTCTCGGAGCACAG
  R  N  D  T  L  D  S  T  R  T  L  Y  S  S  A  S  R  S  T  D
       190                 210                 230
ACTTGTCTTACAGTGAAAGCGCCAGCTTCTACGCTGCCTTCAGGACACAGACGTGCCCAA
  L  S  Y  S  E  S  A  S  F  Y  A  A  F  R  T  Q  T  C  P  I
       250                 270                 290
TCATGGCTTCTTGGGACTTGGTGAATTTTATTCAAGCAAATTTAAGAAACGAGAATGTG
  M  A  S  W  D  L  V  N  F  I  Q  A  N  F  K  K  R  E  C  V
       310                 330                 350
TCTTCTTTACCAAAGATTCCAAGGCCACGGAGAATGTGTGCAAGTGTGGCTATGCCCAGA
  F  F  T  K  D  S  K  A  T  E  N  V  C  K  C  G  Y  A  Q  S
       370                 390                 410
GCCAGCACATGGAAGGCACCCAGATCAACCAAAGTGAGAAATGGAACTACAAGAAACACA
  Q  H  M  E  G  T  Q  I  N  Q  S  E  K  W  N  Y  K  K  H  T
       430                 450                 470
CCAAGGAATTTCCTACCGACGCCTTTGGGGATATTCAGTTTGAGACACTGGGGAAGAAAG
  K  E  F  P  T  D  A  F  G  D  I  Q  F  E  T  L  G  K  K  G
       490                 510                 530
GGAAGTATATACGTCTGTCCTGCGACACGGACGCGGAAATCCTTTACGAGCTGCTGACCC
  K  Y  I  R  L  S  C  D  T  D  A  E  I  L  Y  E  L  L  T  Q
       550                 570                 590
AGCACTGGCACCTGAAAACACCCAACCTGGTCATTTCTGTGACCGGGGGGCGCCAAGAACT
  H  W  H  L  K  T  P  N  L  V  I  S  V  T  G  G  A  K  N  F
       610                 630                 650
TCGCCCTGAAGCCGCGCATGCGCAAGATCTTCAGCCGGCTCATCTACATCGCGCAGTCCA
  A  L  K  P  R  M  R  K  I  F  S  R  L  I  Y  I  A  Q  S  K
       670                 690                 710
AAGGTGCTTGGATTCTCACGGGAGGCACCCATTATGGCCTGATGAAGTACATCGGGGAGG
  G  A  W  I  L  T  G  G  T  H  Y  G  L  M  K  Y  I  G  E  V
       730                 750                 770
TGGTGAGAGATAACACCATCAGCAGGAGTTCAGAGGAGAATATTGTGGCCATTGGCATAG
  V  R  D  N  T  I  S  R  S  S  E  E  N  I  V  A  I  G  I  A
       790                 810                 830
CAGCTTGGGGCATGGTCTCCAACCGGGACACCCTCATCAGGAATTGCGATGCTGAGGGCT
  A  W  G  M  V  S  N  R  D  T  L  I  R  N  C  D  A  E  G  Y
       850                 870                 890
ATTTTTTAGCCCAGTACCTTATGGATGACTTCACAAGAGATCCACTGTATATCCTGGACA
  F  L  A  Q  Y  L  M  D  D  F  T  R  D  P  L  Y  I  L  D  N
       910                 930                 950
ACAACCACACACATTTGCTGCTCGTGGACAATGGCTGTCATGGACATCCCACTGTCGAAG
  N  H  T  H  L  L  L  V  D  N  G  C  H  G  H  P  T  V  E  A
       970                 990                 1010
CAAAGCTCCGGAATCAGCTAGAGAAGTATATCTCTGAGCGCACTATTCAAGATTCCAACT
  K  L  R  N  Q  L  E  K  Y  I  S  E  R  T  I  Q  D  S  N  Y
      1030                1050                1070
ATGGTGGCAAGATCCCCATTGTGTGTTTGCCCAAGGAGGTGGAAAAGAGACTTTGAAAG
  G  G  K  I  P  I  V  C  F  A  Q  G  G  G  K  E  T  L  K  A
      1090                1110                1130
CCATCAATACCTCCATCAAAAATAAAATTCCTTGTGTGGTGGTGGAAGGCTCGGGCCAGA
  I  N  T  S  I  K  N  K  I  P  C  V  V  V  E  G  S  G  Q  I
      1150                1170                1190
TCGCTGATGTGATCGCTAGCCTGGTGGAGGTGGAGGATGCCCTGACATCTTCTGCCGTCA
  A  D  V  I  A  S  L  V  E  V  E  D  A  L  T  S  S  A  V  K
      1210                1230                1250
```

Fig. 10 / continuation 1

```
AGGAGAAGCTGGTGCGCTTTTTACCCCGCACGGTGTCCCGGCTGCCTGAGGAGGAGACTG
  E K L V R F L P R T V S R L P E E E T E
     1270                1290                1310
AGAGTTCGATCAAATGGCTCAAAGAAATTCTCGAATGTTCTCACCTATTAACAGTTATTA
  S W I K W L K E I L E C S H L L T V I K
     1330                1350                1370
AAATGGAAGAAGCTGGGGATGAAATTGTGAGCAATGCCATCTCCTACGCTCTATACAAAG
  M E E A G D E I V S N A I S Y A L Y K A
     1390                1410                1430
CCTTCAGCACCAGTGAGCAAGACAAGGATAACTGGAATGGGCAGCTGAAGCTTCTGCTGG
  F S T S E Q D K D N W N G Q L K L L L E
     1450                1470                1490
AGTGGAACCAGCTGGACTTAGCCAATGATGAGATTTTCACCAATGACCGCCGATGGGAGA
  W N Q L D L A N D E I F T N D R R W E K
     1510                1530                1550
AGAGCAAACCGAGGCTCAGAGACACAATAATCCAGGTCACATGGCTGGAAAATGGTAGAA
  S K P R L R D T I I Q V T W L E N G R I
     1570                1590                1610
TCAAGGTTGAGAGCAAAGATGTGACTGACGGCAAAGCCTCTTCTCATATGCTGGTGGTTC
  K V E S K D V T D G K A S S H M L V V L
     1630                1650                1670
TCAAGTCTGCTGACCTTCAAGAAGTCATGTTTACGGCTCTCATAAAGGACAGACCCAAGT
  K S A D L Q E V M F T A L I K D R P K F
     1690                1710                1730
TTGTCCGCCTCTTTCTGGAGAATGGCTTGAACCTACGGAAGTTTCTCACCCATGATGTCC
  V R L F L E N G L N L R K F L T H D V L
     1750                1770                1790
TCACTGAACTCTTCTCCAACCACTTCAGCACGCTTGTGTACCGGAATCTGCAGATCGCCA
  T E L F S N H F S T L V Y R N L Q I A K
     1810                1830                1850
AGAATTCCTATAATGATGCCCTCCTCACGTTTGTCTGGAAACTGGTTGCGACTTCCGAA
  N S Y N D A L L T F V W K L V A N F R R
     1870                1890                1910
GAGGCTTCCGGAAGGAAGACAGAATGGCCGGGACGAGATGGACATAGAACTCCACGACG
  G F R K E D R N G R D E M D I E L H D V
     1930                1950                1970
TGTCTCCTATTACTCGGCACCCCCTGCAAGCTCTCTTCATCTGGGCCATTCTTCAGAATA
  S F I T R H P L Q A L F I W A I L Q N K
     1990                2010                2030
AGAAGGAACTCTCCAAAGTCATTTGGGAGCAGACCAGGGGCTGCACTCTGGCAGCCCTGG
  K E L S K V I W E Q T R G C T L A A L G
     2050                2070                2090
GAGCCAGCAAGCTTCTGAAGACTCTGGCCAAAGTGAAGAACGACATCAATGCTGCTGGGG
  A S K L L K T L A K V K N D I N A A G E
     2110                2130                2150
AGTCCGAGGAGCTGGCTAATGAGTACGAGACCCGGGCTGTTGGTGAGTCCACAGTGTGGA
  S E K L A N E Y E T R A V G E S T V W N
     2170                2190                2210
ATGCTGTGGTGGGCGCGGATCTGCCATGTGGCACAGACATTGCCAGCGGCACTCATAGAC
  A V V G A D L P C G T D I A S G T H R P
     2230                2250                2270
CAGATGGTGGAGAGCTGTTCACTGAGTGTTACAGCAGCGATGAAGACTTGGCAGAACAGC
  D G G E L F T E C Y S S D E D L A E Q L
     2290                2310                2330
TGCTGGTCTATTCCTGTGAAGCTTGGGGTGGAAGCAACTGTCTGGAGCTGGCGGTGGAGG
  L V Y S C E A W G G S N C L E L A V E A
     2350                2370                2390
CCACAGACCAGCATTTCATCGCCCAGCCTGGGGTCCAGAATTTTCTTTCTAAGCAATGGT
  T D Q H F I A Q P G V Q N F L S K Q W Y
     2410                2430                2450
ATGGAGAGATTTCCCGAGACACCAAGAACTGGAAGATTATCCTGTGTCTGTTTATTATAC
  G E I S R D T K N W K I I L C L F I I P
```

Fig. 10 / continuation 2

```
      2470                2490                2510
CCTTGGTGGGCTGTGGCTTTGTATCATTTAGGAAGAAACCTGTCGACAAGCACAAGAAGC
  L  V  G  C  G  F  V  S  F  R  K  K  P  V  D  K  H  K  K  L
      2530                2550                2570
TGCTTTGGTACTATGTGGCGTTCTTCACCTCCCCCTTCGTGGTCTTCTCCTGGAATGTGG
  L  W  Y  Y  V  A  F  F  T  S  P  F  V  V  F  S  W  N  V  V
      2590                2610                2630
TCTTCTACATCGCCTTCCTCCTGCTGTTTGCCTACGTGCTGCTCATGGATTTCCATTCGG
  F  Y  I  A  F  L  L  L  F  A  Y  V  L  L  M  D  F  H  S  V
      2650                2670                2690
TGCCACACCCCCCCGAGCTGGTCCTGTACTCGCTGGTCTTTGTCCTCTTCTGTGATGAAG
  P  H  P  P  E  L  V  L  Y  S  L  V  F  V  L  F  C  D  E  V
      2710                2730                2750
TGAGACAGGGCCGGCCGGCTGCTCCCAGTGCGGGGCCCGCCAAGCCCACGCCCACCCGGA
  R  Q  G  R  P  A  A  P  S  A  G  P  A  K  P  T  P  T  R  N
      2770                2790                2810
ACTCCATCTGGCCCGCAAGCTCCACACGCAGCCCCGGTTCCCGCTCACGCCACTCCTTCC
  S  I  W  P  A  S  S  T  R  S  P  G  S  R  S  R  H  S  F  H
      2830                2850                2870
ACACTTCCCTGCAAGCTGAGGGTGCCAGCTCTGGCCTTGGCCAGCCCAGAAAGGGGTGGA
  T  S  L  Q  A  E  G  A  S  S  G  L  G  Q  P  R  K  G  W  T
      2890                2910                2930
CATTTAAAAATCTGGAAATGGTTGATATTTCCAAGCTGCTGATGTCCCTCTCTGTCCCTT
  F  K  N  L  K  M  V  D  I  S  K  L  L  M  S  L  S  V  P  F
      2950                2970                2990
TCTGTACGCAGTGGTACGTAAATGGGGTGAATTATTTTACTGACCTGTGGAATGTGATGG
  C  T  Q  W  Y  V  N  G  V  N  Y  F  T  D  L  W  N  V  M  D
      3010                3030                3050
ACACGCTGGGGCTTTTTTACTTCATAGCAGGAATTGTATTTCGGCAAGGGATCCTTAGGC
  T  L  G  L  F  Y  F  I  A  G  I  V  F  R  Q  G  I  L  R  Q
      3070                3090                3110
AGAATGAGCAGCGCTGGAGGTGGATATTCCGTTCGGTCATCTACGAGCCCTACCTGGCCA
  N  E  Q  R  W  R  W  I  F  R  S  V  I  Y  E  P  Y  L  A  M
      3130                3150                3170
TGTTCGGCCAGGTGCCCAGTGACGTGGATGGTACCACGTATGACTTTGCCCACTGCACCT
  F  G  Q  V  P  S  D  V  D  G  T  T  Y  D  F  A  H  C  T  F
      3190                3210                3230
TCACTGGGAATGAGTCCAAGCCACTGTGTGTGGAGCTGGATGAGCACAACCTGCCCCGGT
  T  G  N  E  S  K  P  L  C  V  E  L  D  E  H  N  L  P  R  F
      3250                3270                3290
TCCCCGAGTGGATCACCATCCCCCTGGTGTGCATCTACATGTTATCCACCAACATCCTGC
  P  E  W  I  T  I  P  L  V  C  I  Y  M  L  S  T  N  I  L  L
      3310                3330                3350
TGGTCAACCTGCTGGTCGCCATGTTTGGCTACACGGTGGGCACCGTCCAGGAGAACAATG
  V  N  L  L  V  A  M  F  G  Y  T  V  G  T  V  Q  E  N  N  D
      3370                3390                3410
ACCAGGTCTGGAAGTTCCAGAGGTACTTCCTGGTGCAGGAGTACTGCAGCCGCCTCAATA
  Q  V  W  K  F  Q  R  Y  F  L  V  Q  E  Y  C  S  R  L  N  I
      3430                3450                3470
TCCCCTTCCCCTTCATCGTCTTCGCTTACTTCTACATGGTGGTGAAGAAGTGCTTCAAGT
  P  F  P  F  I  V  F  A  Y  F  Y  M  V  V  K  K  C  F  K  C
      3490                3510                3530
GTTGCTGCAAGGAGAAAAACATGGAGTCTTCTGTCTGCTGTGAGTGGTTTATCCATGTGT
  C  C  K  E  K  N  M  E  S  S  V  C  C  E  W  F  I  H  V  Y
      3550                3570                3590
ACTTGGGATCAGAAGCAGCGATTAATTTCAGGGAAGGATGCCTGCATCCAGTGATTGGAA
  L  G  S  E  A  A  I  N  F  R  E  G  C  L  H  P  V  I  G  S
      3610                3630                3650
GCTGGACCCCAGGCTGGCTGGTCTGGACATCCACACGCATTCTCACATGCAGTGCCGGCT
  W  T  P  G  N  L  V  W  T  S  T  R  I  L  T  C  S  A  G  W
      3670                3690                3710
GGCCAGCAGCAGGGAGTCTCAGTGTCACCACACATAGCAGCTGGGTTCCTGCAAAAAGCA
```

Fig. 10 / continuation 3

```
  P  A  A  G  S  L  S  V  T  T  H  S  S  W  V  P  A  K  S  S
     3730                3750                3770
GCAAGTCACAGGCCCACCCAGACAGAACGGGTAGAGAATGTGACTCTGCTTCTGGGTGGG
  K  S  Q  A  H  P  D  R  T  G  R  E  C  D  S  A  S  G  W  E
     3790                3810                3830
AAGGACAGCCTGCCCGGTGGGTGGAAGAATCCGTGGCCCTGTTTGGCCATCGTGCCCTG
  G  Q  P  A  R  W  V  E  E  S  V  A  L  F  G  H  R  G  P  V
     3850                3870                3890
TTTGGCCACCTACCACTCTAGGCATCACTGAGCTGAATGCGCCGGTCCTCTGA
  W  P  P  T  T  L  G  I  T  E  L  N  A  P  V  L  *
```

```
MVGGCRWIEDVEPAEVKEKMSFRAARLSMRHRRNDTLDSTRTLYSSASRSTDLSYSESASFYAAFRTQTCPIMASWDLVNFIQANF
KKRECVFFTKDSKATENVCKCGYAQSQMMEGTQINGSEKWNYKKHTKEFFTDAFGDIQFETLGKKGKYIRLSCDTDAELLYELLTQ
HWHLKTPNLVISVTGGAKNFALKPRMRKIFSRLIYIAQSKGAWILTGGTHYGLMKYIGEVVRDNTISRSSEENIVAIGIAAWGMVS
NRDTLIRNCDAEGYFLAQYLMDDFTRDPLYILDNNHTHLLLVDNGCHGHPTVEAKLRNQLEKYISERTIQDSNYGGKIPIVCFAQG
GGKETLKAINTSIKNKIPCVVVEGSGQIADVIASLVEVEDALTSSAVKEKLVRFLPATVSRLPEEETESWIKWLKELECSHLLTV
IKMEEAGEIVSNAISYALYEAFSTSEQDKDNWNGQLKLLLEWNQLDLANDEIFTNDRWEESKPRLRDTIIQVTWLENGRIKVES
KDVTDGKASSHMLVVLKSADLQEVMFTALIKDRPKFVRLFLENGINLRKFLTHDVLTELFSNHFSTLVYRNLQLAKNSYNDALLTF
VWKLVANFRRGFAKEDRNGRDEMDIELHDVSPITRHPLQALFTWAILQNKKELSKVIWEQTRGCTLAALGASKLLKTLAKVKNDIN
AAGESEELANEYETRAVGESTVWNAVVGADLPCGTDIASGTHRPDGGELFTECYSSDEDLAEQLLVYSCEAWGGSNCLELAVEAFD
QHFIAQPGVQNFLSKQWYGEISRDTKNWKIILCLFIIPLVGCGFVSFRKKFVDKHKKLLWYYVAFFTSPFVVFSWNVVFYIAFLLL
FAYVLIMDFHSVPHPPELVLYSLVFVLFCDEVRQGRPAAPSAGPAKPTPTRNSIWPASSTRSPGSRSRHSFHTSLQAEGASSGLGQ
PRKGWTFKNLEMVDISKLLMSLSVPFCTQWYVNGVNYFTDLANVMDTLGLEYFIAGIVFRQGILRQNEQRWAWIFRSVIYEPYLAM
FGQVPSDVDGTTYDFAHCTFTGNESKPLCVELDSHNLPAFDEWITIPLVCIYMLSTNILLVNLLVAMFGYTVGTVQENNDQVWKFQ
RYFLVQEYCSRLNIPFPFIVFAYFYMVVKKCFKCCCKEKNMESSVCCEWFIHVYLGSEAAIMFREGCLEPVIGSWTPGWLVWTSTR
ILTCSAGWEAAGSLSVTTHSSWVPAKSSKSQAHPDRTGRECDSASGWEGQPARWVEESVALFGHRGPVWPPTTLGITELNAPVL
```

a.

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                                                  Q  L
      2290                2310                2330
TGCTGGTCTATTCCTGTGAAGCTTGGGGTGGAAGCAACTGTCTGGAGCTGGCGGTGGAGG
  L  V  Y  S  C  E  A  W  G  G  S  N  C  L  E  L  A  V  E  A
      2350                2370                2390
CCACGACCAGCATTTCATCGCCCAGCCTGGGGTCCAGAATTTTCTTTCTAAGCAATGGT
  T  D  Q  H  F  I  A  Q  P  G  V  Q  N  F  L  S  K  Q  W  Y
      2410                2430                2450
ATGGAGAGATTTCCCGAGACACCAAGAACTGGAAGATTTATCCTGTGTCTGTTTATTATAC
  G  E  I  S  R  D  T  K  N  W  K  I  I  L  C  L  F  I  I  P
      2470                2490                2510
CCTTGGTGGGCTGTGGCTTTGTATCATTTAGGAAGAAACCTGTCGACAAGCACAAGAAGC
  L  V  G  C  G  F  V  S  F  R  K  K  P  V  D  K
```

Figure 11:

a.) Trp10b cDNA and derived amino acid sequence

```
        10                  30                  50
ATGAAATCCTTCCTTCCTGTCCACACCATCGTGCTTATCAGGGAGAATGTGTGCAAGTGT
M  K  S  F  L  P  V  H  T  I  V  L  I  R  E  N  V  C  K  C
        70                  90                  110
GGCTATGCCCAGAGCCAGCACATGGAAGGCACCCAGATCAACCAAAGTGAGAAATGGAAC
G  Y  A  Q  S  Q  H  M  E  G  T  Q  I  N  Q  S  E  K  W  N
       130                 150                 170
TACAAGAAACACACCAAGGAATTTCCTACCGACGCCTTTGGGGATATTCAGTTTGAGACA
Y  K  K  H  T  K  E  F  P  T  D  A  F  G  D  I  Q  F  E  T
       190                 210                 230
CTGGGGAAGAAAGGAAGTATATACGTCTGTCCTGCGACACGGACGCGGAAATCCTTTAC
L  G  K  K  G  K  Y  I  R  L  S  C  D  T  D  A  E  I  L  Y
       250                 270                 290
GAGCTGCTGACCCAGCACTGGCACCTGAAAACACCCAACCTGGTCATTTCTGTGACCGGG
E  L  L  T  Q  H  W  H  L  K  T  P  N  L  V  I  S  V  T  G
       310                 330                 350
GGCGCCAAGAACTTCGCCCTGAAGCCGCGCATGCGCAAGATCTTCAGCCTGGCTCATCTAC
G  A  K  N  F  A  L  K  P  R  M  R  K  I  F  S  R  L  I  Y
       370                 390                 410
ATCGCGCAGTCCAAAGGTGCTTGGATTCTCACGGGAGGCACCCATTATGGCCTGATGAAG
I  A  Q  S  K  G  A  W  I  L  T  G  G  T  H  Y  G  L  M  K
       430                 450                 470
TACATCGGGGAGGTGGTGAGAGATAACACCATCAGCAGGAGTTCAGAGGAGAAATATTGTG
Y  I  G  E  V  V  R  D  N  T  I  S  R  S  S  E  E  N  I  V
       490                 510                 530
GCCATTGGCATAGCAGCTTGGGGCATGGTCTCCAACCGGGACACCCTCATCAGGAATTGC
A  I  G  I  A  A  W  G  M  V  S  N  R  D  T  L  I  R  N  C
       550                 570                 590
GATGCTGAGGGCTATTTTTTAGCCCAGTACCTTATGGATGACTTCACAAGAGATCCACTG
D  A  E  G  Y  F  L  A  Q  Y  L  M  D  D  F  T  R  D  P  L
       610                 630                 650
TATATCCTGGACAACACCACACACATTTGCTGCTCGTGGACAATGGCTGTCATGGACAT
Y  I  L  D  N  N  E  T  H  L  L  L  V  D  N  G  C  H  G  H
       670                 690                 710
CCCACTGTCGAAGCAAAGCTCCGGAATCAGCTAGAGAAGTATATCTCTGAGCGCACTATT
P  T  V  E  A  K  L  R  N  Q  L  E  K  Y  I  S  E  R  T  I
       730                 750                 770
CAAGATTCCAACTATGGTGGCAAGATCCCCATTGTGTGTTTTGCCCAAGGAGGTGGAAAA
Q  D  S  N  Y  G  G  K  I  P  I  V  C  F  A  Q  G  G  G  K
       790                 810                 830
GAGACTTTGAAAGCCATCAATACCTCCATCAAAAATAAAATTCCTTGTGTGGTGGTGGAA
E  T  L  K  A  I  N  T  S  I  K  N  K  I  P  C  V  V  V  E
       850                 870                 890
GGCTCGGGCCAGATCGCTGATGTGATCGCTAGCCTGGTGGAGGTGGAGGATGCCCTGACA
G  S  G  Q  I  A  D  V  I  A  S  L  V  E  V  E  D  A  L  T
       910                 930                 950
TCTTCTGCCGTCAAGGAGAAGCTGGTGCGCTTTTTACCCCGCACGGTGTCCCGGCTGCCT
S  S  A  V  K  E  K  L  V  R  F  L  P  R  T  V  S  R  L  P
       970                 990                 1010
GAGGAGGAGACTGAGAGTTGGATCAAATGGCTCAAAGAAATTCTCGAATGTTCTCACCTA
E  E  E  T  E  S  W  I  K  W  L  K  E  I  L  E  C  S  H  L
      1030                1050                1070
TTAACAGTTATTAAAATGGAAGAAGCTGGGGATGAAATTGTGAGCAATGCCATCTCCTAC
L  T  V  I  K  M  E  E  A  G  D  E  I  V  S  N  A  I  S  Y
      1090                1110                1130
GCTCTATACAAAGCCTTCAGCACCAGTGAGCAAGACAAGGATAACTGGAATGGGCAGCTG
A  L  Y  K  A  F  S  T  S  E  Q  D  K  D  N  W  N  G  Q  L
```

Fig. 11 (Continuation)

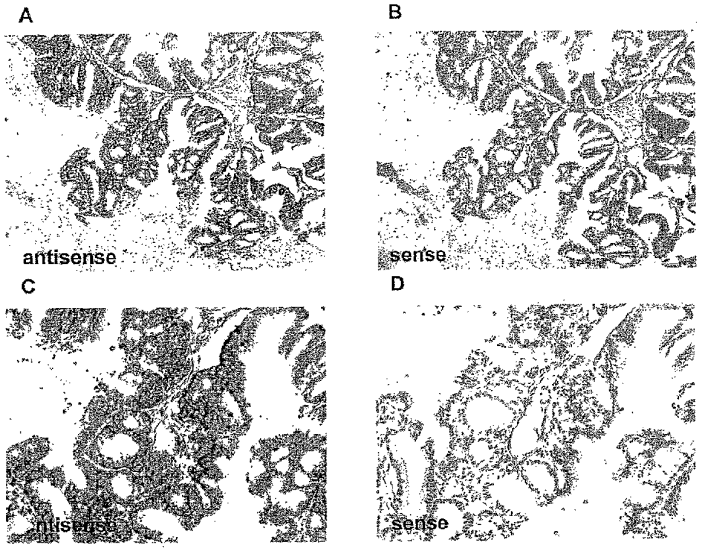
```
      2410                2430                2450
AGAAACTTAGGACCCAAGATTATAATGCTGCAGAGGATGCTGATCGATGTGTTCTTCTTC
R  N  L  G  P  K  I  I  M  L  Q  R  M  L  I  D  V  F  F  F
      2470                2490                2510
CTGTTCCTCTTTGCGGTGTGGATGGTGGCCTTTGGCGTGGCCAGGCAAGGGATCCTTAGG
L  F  L  F  A  V  W  M  V  A  F  G  V  A  R  Q  G  I  L  R
      2530                2550                2570
CAGAATGAGCAGCGCTGGAGGTGGATATTCCGTTCGGTCATCTACGAGCCCTACCTGGCC
Q  N  E  Q  R  W  R  W  I  F  R  S  V  I  Y  E  P  Y  L  A
      2590                2610                2630
ATGTTCGGCCAGGTGCCCAGTGACGTGGATGGTACCACGTATGACTTTGCCCACTGCACC
M  F  G  Q  V  P  S  D  V  D  G  T  T  Y  D  F  A  H  C  T
      2650                2670                2690
TTCACTGGGAATGAGTCCAAGCCACTGTGTGTGGAGCTGGATGAGCACAACCTGCCCCGG
F  T  G  N  E  S  K  P  L  C  V  E  L  D  E  H  N  L  P  R
      2710                2730                2750
TTCCCCGAGTGGATCACCATCCCCCTGGTGTGCATCTACATGTTATCCACCAACATCCTG
F  P  E  W  I  T  I  P  L  V  C  I  Y  M  L  S  T  N  I  L
      2770                2790                2810
CTGGTCAACCTGCTGGTCGCCATGTTTGGCTACACGGTGGGCACCGTCCAGGAGAACAAT
L  V  N  L  L  V  A  M  F  G  Y  T  V  G  T  V  Q  E  N  N
      2830                2850                2870
GACCAGGTCTGGAAGTTCCAGAGGTACTTCCTGGTGCAGGAGTACTGCAGCCGCCTCAAT
D  Q  V  W  K  F  Q  R  Y  F  L  V  Q  E  Y  C  S  R  L  N
      2890                2910                2930
ATCCCCTTCCCCTTCATCGTCTTCGCTTACTTCTACATGGTGGTGAAGAAGTGCTTCAAG
I  P  F  P  F  I  V  F  A  Y  F  Y  M  V  V  K  K  C  F  K
      2950                2970                2990
TGTTGCTGCAAGGAGAAAAACATGGAGTCTTCTGTCTGCTGTTTCAAAAATGAAGACAAT
C  C  C  K  E  K  N  M  E  S  G  V  C  C  F  K  N  E  D  N
      3010                3030                3050
GAGACTCTGGCATGGGAGGGTGTCATGAAGGAAAACTACCTTGTCAAGATCAACACAAAA
E  T  L  A  W  E  G  V  M  K  E  N  Y  L  V  K  I  N  T  K
      3070                3090                3110
GCCAACGACACCTCAGAGGAAATGAGGCATCGATTTAGACAACTGGATACAAAGCTTAAT
A  N  D  T  S  E  E  M  R  H  R  F  R  Q  L  D  T  K  L  N
      3130                3150
GATCTCAAGGGTCTACTGAAAGAGATTGCTAATAAAATCAAATAG
D  L  K  G  L  L  K  E  I  A  N  K  I  K  *
```

b.) Trp10 protein:

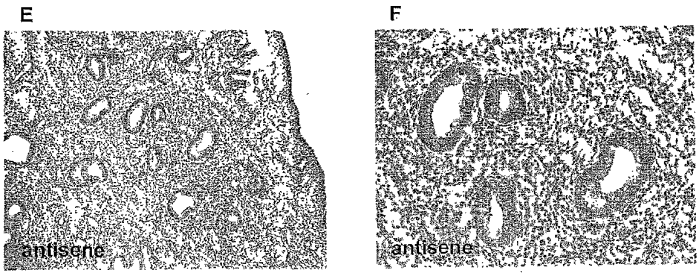
```
MKSFLPVHTIVLIRENVCKCGYAQSQHMEGTQINQSEKWNYKKHTKEPPTDAFGDIQFETLGKKGKYIRLSCDTDAEILY
ELLTQHWHLKTPNLVISVTGGAKNFALKPRMRKIFSRLIYIAQSKGAWILTGGTHYGLMKYIGEVVRDNTISRSSEENIV
AIGIAAWGMVSNRDTLIRNCDAEGYFLAQYLMDDFTRDPLYILDNNHTHLLLVDNGCHGHPTVEAKLRNQLEKYISERTI
QDSNYGGKIPIVCFAQGGGKETLKAINTSIKWKIPCVVVEGSGQIADVIASLVEVEDALTSSAVKEKLVRFLPRTVSRLP
EEETESWIKWLKELLECSHLLTVIKMEEAGDEIVSNAISYALYKAFSTSRQDKDWWNGQLKLLLEWNQLDLANDEIFTND
RRWESADLQEVMFTALIKDRPKFVRLFLENGLNLRKFLTHDVLTSLPSNHFSTLVYRNLQIAKNSYNDALLTFVWKLVAN
FRRGFRKEDRNGRDEMDIELHDVSPITRHPLQALFIWAILQNKKELSKVIWEQTRGCTLAALGASKLLKTLAKVKNDINA
AGESEELANEYETRAVELFTECYSSDEDLAEQLLVYSCEAWGGSNCLELAVEATDQHFIAQFGVQNPLSKQMYGEISRDT
KNWKIILCLFIIFLVGCGFVSFRKKPVDKHKKLLWYYVAFFTSPFVVFSWNVVFYIAFLLLFAYVLLMDFHSVPHPPELV
LYSLVFVLFCDEVRQWYVNGVNYFTDLWNVMDTLGLFYFIAGIVFRLHSSNKSSLYSGRVIFCLDYIIFTLRLIHIFTVS
RNLGPKIIMLQRMLIDVFFFLFLFAVWMVAFGVARQGILRQNEQRWRWIFRSVIYEPYLAMFGQVPSDVDGTTYDFAHCT
FTGNESKPLCVELDEENLPRFPEWITIPLVCIYMLSTNILLVNLLVAMFGYTVGTVQENNDQVWKFQRYFLVQEYCSRLN
IPFPFIVFAYFYMVVKKCPKCCCKEKNMESSVCCFKNEDNSTLAWEGVMKENYLVKINTKANDTSEEMRHRFRQLDTKLN
DLKGLLKEIANKIK
```

The Trp8 Gene is expre d in endometrial or uteri
in normal endometrium

Endometrial cancer:



Endometrium:



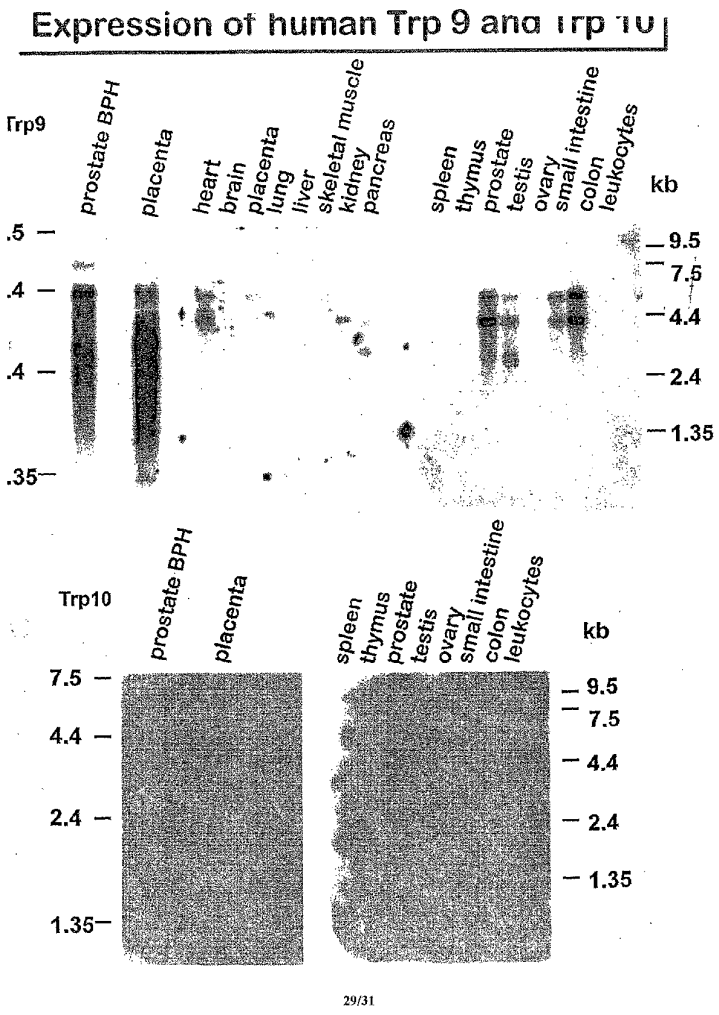
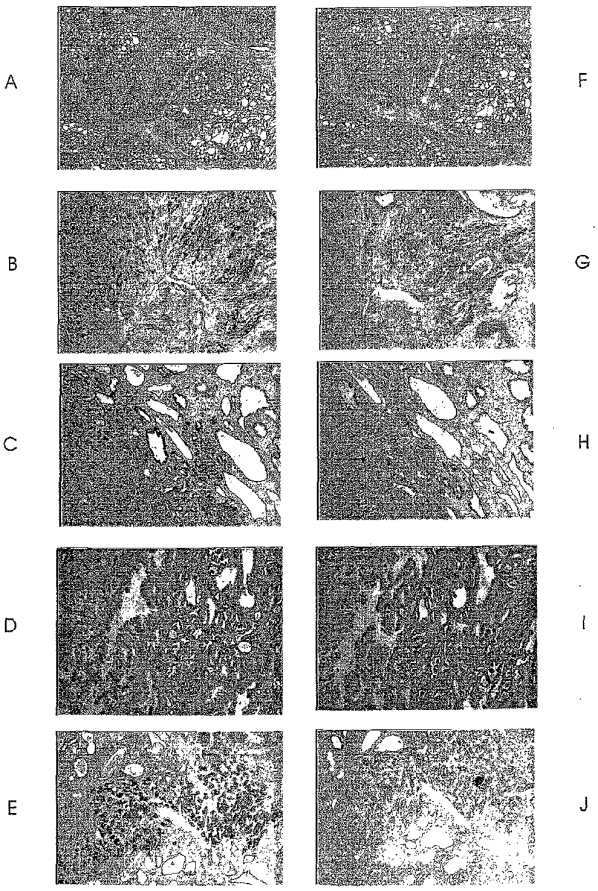
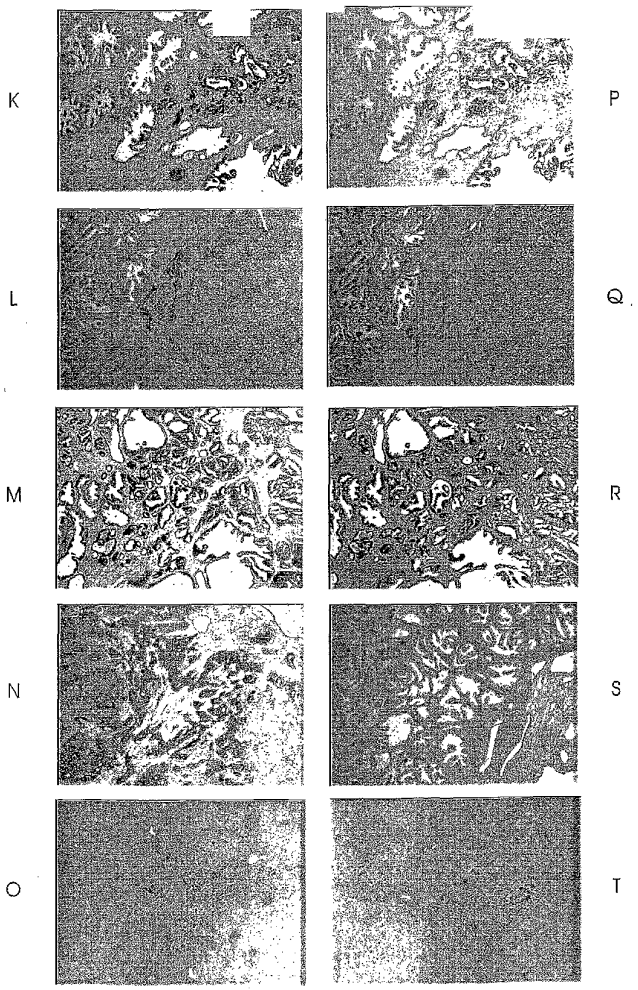

Expression of human Trp 9 and Trp 10
Trp9
prostate BPH
placenta
heart
brain
placenta
lung
liver
skeletal muscle
kidney
pancreas
spleen
thymus
prostate
testis
ovary
small intestine
colon
leukocytes
kb
.5
.4
.4
.35
9.5
7.5
4.4
2.4
1.35
Trp10
prostate BPH
placenta
spleen
thymus
prostate
testis
ovary
small intestine
colon
leukocytes
kb
7.5
4.4
2.4
1.35
9.5
7.5
4.4
2.4
1.35

Fig. 14

Expression of Trp10 transcripts and Trp10-antisense transcripts in human prostate cancer and in malignant melanoma

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(54) Title: TRP8, TRP9 AND TRP10, NOVEL MARKERS FOR CANCER

(57) Abstract: The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10. Also provided are vectors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating a tumor.

WO 02/010382 PCT/EP01/08309

Trp8, Trp9 and Trp10, novel markers for cancer

FIELD OF THE INVENTION

The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10

BACKROUND OF THE TECHNOLOGY

Prostate cancer is one of the most common diseases of older men world wide. Diagnosis and monitoring of prostate cancer is difficult because of the heterogeneity of the disease. For diagnosis different grades of malignancy can be distinguished according to the Gleason-Score Diagnosis. For this diagnosis a prostate tissue sample is taken from the patient by biopsy and the morphology of the tissue is investigated. However, this approach only yields subjective results depending on the experience of the pathologist. For confirmation of these results and for obtaining an early diagnosis an additional diagnostic method can be applied which is based on the detection of a prostate specific antigen (PSA). PSA is assayed in serum samples, blood samples etc. using an anti-PSA-antibody. However, since in principle PSA is also expressed in normal prostate tissue there is a requirement for the definition of a threshold value (about 4 ng/ml PSA) in order to be able to distinguish between normal and malign prostate tissue. Unfortunately, this diagnostic method is quite insensitive and often yields false-positive results. Moreover, by using this diagnostic method any conclusions as regards the grade of malignancy, the progression of the tumor and its potential for metastasizing cannot be drawn. Thus, the use of molecular markers would be helpful to distinguish benign from malign tissue and for grading and staging prostate carcinoma, particularly for patients with metastasizing prostate cancer having a very bad prognosis.

The above discussed limitations and failings of the prior art to provide meaningful specific markers which correlate with the presence of prostate tumors, in particular metastasizing tumors, has created a need for markers which can be used diagnostically, prognostically and therapeutically over the course of this disease. The present invention fulfils such a need by the provision of Tpr8, Trp9 and Trp10 and the genes encoding Trp8, Trp9 and Trp10: The genes encoding Trp8 and Trp10 are expressed in prostate carcinoma and prostatic metastasis, but

not in normal prostate, benign hyperplasia (BHP) and intraepithelial prostatic neoplasia (PIN). Furthermore, expression of Trp10 transcripts is detectable in carcinoma but not in healthy tissue of the lung, the prostate, the placenta and in melanoma.

SUMMARY OF THE INVENTION

The present invention is based on the isolation of genes encoding novel markers associated witha cancer, Trp8, Trp9 and Trp10. The new calcium channel proteins Trp8, Trp9 and Trp10 are members of the trp (transient receptor potential) - family, isolated from human placenta (Trp8a and Trp8b) and humane prostate (Trp9, Trp10a and Trp10b). Trp proteins belong to a steadily growing family of Ca^{2+} selective and non selective ion channels. In the recent years seven Trp proteins (trp1 - trp7) have been identified and suggested to be involved in cation entry, receptor operated calcium entry and pheromone sensory signaling. Structurally related to the trp proteins are the vanilloid receptor (VR1) and the vanilloid like receptor (VRL-1) both involved in nociception triggered by heat. Furthermore, two calcium permeable channels were identified in rat small intestine (CaT1) and rabbit kidney (ECaC). These distantly related channels are suggested to be involved in the uptake of calcium ions from the lumen of the small intestine (CaT1) or in the reuptake of calcium ions in the distal tubule of the kidney (ECaC). Common features or the Trp and related channels are a proposed structure comprising six transmembrane domains including several conserved amino acid motifs. In the present invention the cloning and expression of a CaT1 like calcium channel (Trp8) from human placenta as well as Trp9 and Trp10 (two variants, Trp10a and Trp10b) is described. Two polymorphic variants of the Trp8 cDNA were isolated from placenta (Trp8a and Trp8b). Transient expression of the Trp8b cDNA in HEK (human embryonic kidney) cells results in cytosolic calcium overload implicating that the Trp8 channel is constitutive open in the expression system. Trp8 induces highly calcium selective inward currents in HEK cells. The C -terminus of the Trp8 protein binds calmodulin in a calcium dependent manner. The Trp9 channel is expressed in trophoblasts and syncytiotrophoblasts of placenta and in pancreatic acinar cells. Furthermore, the Trp8 channel is expressed in prostatic carcinoma and prostatic metastases, but not in normal tissue of the prostate. No expression of Trp8 transcripts is detectable in benign prostatic hyperplasia (BPH) or prostatic intraepithelial neoplasia (PIN). Therefore, the Trp8 channel is exclusively expressed in malign prostatic tissues and serves as molecular marker for prostate cancer. From the experimental results it is also apparent that the

modulation of Trp8 and/or Trp10, e.g. the inhibition of expression or activity , is of therapeutic interest, e.g. for the prevention of tumor progression.

The present invention, thus, provides a Trp8, Trp9 and Trp10 protein, respectively, as well as nucleic acid molecule encoding the protein and, moreover, an antisense RNA, a ribozyme and an inhibitor, which allow to inhibit the expression or the activity of Trp8, Trp9 and/or Trp10.

In one embodiment, the present invention provides a diagnostic method for detecting a prostate cancer or endometrial cancer (cancer of the uterus) associated with Trp8 or Trp10 in a tissue of a subject, comprising contacting a sample containing Trp8 and/or Trp10 encoding mRNA with a reagent which detects Trp8 and/or Trp10 or the corresponding mRNA.

In a further embodiment, the present invention provides a diagnostic method for detecting a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense transcripts or Trp10a and/or Trp10b related antisense transcripts.

In another embodiment, the present invention provides a method of treating a prostate tumor, carcinoma of the lung, carcinoma of the placenta (chorion carcinoma) or melanoma associated with Trp8 and/or Trp10, comprising administering to a subject with such an disorder a therapeutically effect amount of a reagent which modulates, e.g. inhibits, expression of Trp8 and/or Trp10 or the activity of the protein, e.g. the above described compounds.

Finally, the present invention provides a method of gene therapy comprising introducing into cells of a subject an expression vector comprising a nucleotide sequence encoding the above mentioned antisense RNA or ribozyme, in operable linkage with a promoter.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: A, phylogenetic relationship of trp and related proteins. B, hydropathy plot of the Trp8 protein sequence according to Kyte and Doolittle. C, alignment of Trp8a/b to the epithelial calcium channels ECaC (from rabbit) and Vr1 (from rat). Putative transmembrane domains are underlined.

Figure 2: A, polymorphism of the Trp8 gene. The polymorphic variants Trp8a and Trp8b differ in five base pairs resulting in three amino acid exchanges in the derived protein sequences. Specific primers were derived from the Trp8 gene as indicated by arrows. B, the Trp8a and Trp8b genes are distinguishable by a single restriction site. Genomic fragments of the Trp8 gene can be amplified using specific primers (shown in A). The genomic fragment of the Trp8b gene contains an additional site of the restriction enzyme BSP1286I (B). C, the Trp8 gene is located on chromosome 7. D, genotyping of eleven human subjects. A 458 bp genomic fragment of the Trp8 gene was amplified using specific primers (shown in A) and restricted with BSP1286I. The resulting fragments were analyzed by PAGE electrophoresis.

Figure 3: The Trp8b protein is a calcium selective ion channel. A, representative trace of a pdiTrp8b transfected HEK 293 cell. Trp8b mediated currents are activated by voltage ramps (-100 mV - +100 mV) of 100 msec at -40 mV or +70 mV holding potential. 1, Trp8b currents in the presence at 2mm $[Ca^{2+}]_o$;2, effect of solution switch alone 3, switch to nominal zero calcium solution. B, Trp8b currents in the presence of zero divalent cations. C, current voltage relationship of the currents shown in A. Inset, leak subtracted current. D, current voltage relationship of the current shown in B. E, statistics of representative experiments. Black: Trp8 transfected cells, gray: control cells. Columns from left to right: Trp8 currents at - 40 mV (n =12) and + 70 mV holding potential (n = 12). Trp8 currents in standard bath solution including 120 mM NMDG without sodium (n = 7) and with nominal zero calcium ions (n = 8) or in the presence of 1mM EGTA with zero divalent cations (n = 6). F, representative changes in $[Ca^{2+}]_i$ in Trp8b transfected HEK cells (gray) and controls (black) in the presence or absence of 1mM $[Ca^{2+}]_o$. Inset, relative increase of cytosolic calcium concentration of Trp8b transfected HEK cells, before and after readdition of 1 mM $[Ca^{2+}]_o$ in comparison to control cells.

Figure 4: The C-terminal region of the Trp8 protein binds calmodulin. A, N- and C-terminal fragments of the Trp8 protein used for calmodulin binding studies. B, the Trp8 protein and a truncated Trp8 protein which was in vitro translated after MunI cut of the cDNA, which lacks the C-terminal 32 amino acid residues, were in vitro translated in the presence of ^{35}S-methionine and incubated with calmodulin coupled agarose beads in the presence of 1 mM Ca^{2+} or 2 mM EGTA. C, calmodulin binding to N- and C-terminal fragments of the Trp8protein in the presence of Ca^{2+} (1 mM) or EGTA (2 mM)

Figure 5: Expression pattern of the Trp8 cDNA. A, Northern blots (left panels, Clontech, Palo Alto) were hybridized using a 348 bp NcoI/BamHT fragment of the Trp9 cDNA. The probe hybridizes to mRNA species isolated from the commercial blot, but not to mRNA species isolated from benign prostate hyperplasia (right panel, mRNA isolated from 20 human subjects with benign prostate hyperplasia). B,C, in situ hybridization with biotinylated Trp8 specific oligonucleotides on slides of human tissues. Left column antisense probes, right column sense probes. D, antinsense probes.

Figure 6: Differential expression of Trp8 cDNA in human prostate. A - F, in situ hybridization with prostatic tissues. A, normal prostate, B, primary carcinoma, C, benign hyperplasia, D, rezidive carcinoma, E, prostatic intraepithelial neoplasia, F, lymphnode metastasis of the prostata.

Figure 7: Trp8a cDNA sequence and derived amino acid sequence

Figure 8: A, Trp8b cDNA sequence and derived amino acid sequence
B, cDNA sequence of splice variant 1 (12B1)
C, cDNA sequence of splice variant 2 (17-3)
D, cDNA sequence of splice variant 3 (23A3)
E, cDNA sequence of splice variant 4 (23C3)

Figure 9: A, Trp9 cDNA sequence and derived amino acid sequence B, cDNA sequence of splice variant 15 and derived amino acid sequence.

Figure 10: A, cDNA sequence of Trp10a and derived amino acid sequence, B, cDNA fragment of Trp10a and derived amino acid sequence.

Figure 11: cDNA sequence of Trp10b and derived amino acid sequence.

Figure 12: Expression of Trp8 mRNA in human endometrial cancer or cancer of the uterus. A - D, in situ hybridization with slides of endometrial cancer hybridized with Trp8 antisense (left column) or sense probes as controls (right column). E - F, Trp8 antisense probes hybridized to slides of normal endometrium. It can be clearly seen no hybridization occurs with normal endometrial tissue.

Figure 13: Expression of human Trp9 and Trp10 genes
Northern blots were hybridized using Trp9 (upper panel) or Trp10 (lower panel) specific probes. Expression of the Trp9 cDNA is detectable in many tissues including human prostate and colon as well as in benign prostatic hyperplasia. Expression of Trp10 cDNA is detectable in human prostate of a commercial northern blot (Clontech, right side). This Northern blot contains prostatic tissue collected from 15 human subjects in the range of 14 - 60 years of age. No expression of Trp10 cDNA was detectable in benign prostatic hyperplasia (left side).

Figure 14: Expression of Trp10 transcripts and Trp10-antisense transcripts in human prostate cancer and metastasis of a melanoma. In situ hybridizations of slides hybridized with Trp10-antisense (A-E, K-N) and Trp10 related sense probes (F-J, P-R). It can clearly be seen that both probes detect the same cancer cells indicating that these cancer cells express Trp10 transcripts as well as Trp10-antisense transcripts. S, no Trp10 expression is detectable in benign hyperplasia of the prostate (BPH). O and T, show expression of Trp10 transcripts (O) and Trp10-antisense transcripts (T) in a metastasis of a melanoma in human lung. Melanoma cancer cells express both Trp10 transcripts and Trp10-antisense transcripts.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b or a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being selected from the group consisting of

(a) a nucleic acid molecule encoding a protein that comprises the amino acid sequence depicted in Figure 7, 8A, 9,10 or 11;

(b) a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9,10, or 11;

(c) a nucleic acid molecule included in DSMZ Deposit no. DSM 13579 (deposit date: 28 June 2000), DSM 13580 (deposit date: 28 June 2000), DSM 13584 (deposit date: 5 July 2000), DSM 13581 (deposit date: 28 June 2000) or DSM ....(deposit date:....);

(d) a nucleic acid molecule with hybridizes to a nucleic acid molecule specified in (a) to (c)

(e) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) to (d) due to the degeneration of the genetic code; and

(f) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a) to (e).

As used herein, a protein exhibiting biological properties of Trp8a, Trp8b, Trp9,Trp10a or Trp10b is understood to be a protein having at least one of the activities as illustrated in the Examples, below.

As used herein, the term „isolated nucleic acid molecule„ includes nucleic acid molecules substantially free of other nucleic acids, proteins, lipids, carbohydrates or other materials with which it is naturally associated.

In a first embodiment, the invention provides an isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9,Trp10a or Trp10b comprising the amino acid sequence depicted in Figure 7, 8A, 9,10 or 11. The present invention also provides a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9,10 or 11.

The present invention provides not only the generated nucleotide sequence identified in Figure 7, 8A, 9,10 or 11, respectively and the predicted translated amino acid sequence, respectively, but also plasmid DNA containing a Trp8a cDNA deposited with the DSMZ, under DSM 13579, a Trp8b cDNA deposited with the DSMZ, under DSM 13580, a Trp9 cDNA deposited with the DSMZ, under DSM 13584, a Trp10a cDNA deposited with the DSMZ, under DSM 13581, and a Trp10b cDNA deposited with the DSMZ, under DSM...., respectively. The nucleotide sequence of each deposited Trp-clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by each deposited clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited Trp-encoding DNA, collecting the protein, and determining its sequence.

The nucleic acid molecules of the invention can be both DNA and RNA molecules. Suitable DNA molecules are, for example, genomic or cDNA molecules. It is understood that all

nucleic acid molecules encoding all or a portion of Trp8a, Trp8b, Trp9,Trp10a or Trp10b are also included, as long as they encode a polypeptide with biological activity. The nucleic acid molecules of the invention an be isolated from natural sources or can be synthesized according to know methods.

The present invention also provides nucleic acid molecules which hybridize to the above nucleic acid molecules. As used herein, the term „hybridize„ has the meaning of hybridization under conventional hybridization conditions, preferably under stringent conditions as described, for example, in Sambrook et al., Molecular Cloning, A Laboratory Manual 2nd edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Also contemplated are nucleic acid molecules that hybridize to the Trp nucleic acid molecules at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency), salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°Cin a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 9.2M NaH_2PO_4; 0.02M EDTA, pH7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA, following by washes at 50°C with 1 X SSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC). Variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Nucleic acid molecules that hybridize to the molecules of the invention can be isolated, e.g., from genomic or cDNA libraries that were produced from human cell lines or tissues. In order to identify and isolate such nucleic acid molecules the molecules of the invention or parts of these molecules or the reverse complements of these molecules can be used, for example by means of hybridization according to conventional methods (see, e.g., Sambrook et al., supra). As a hybridization probe nucleic acid molecules can be used, for example, that have exactly or basically the nucleotide sequence depicted in Figure 7, 8A, 9,10 or 11, respectively, or parts of these sequences. The fragments used as hybridization probe can be synthetic

fragments that were produced by means of conventional synthetic methods and the sequence of which basically corresponds to the sequence of a nucleic acid molecule of the invention.

The nucleic acid molecules of the present invention also include molecules with sequences that are degenerate as a result of the genetic code.

In a further embodiment, the present invention provides nucleic acid molecules which comprise fragments, derivatives and allelic variants of the nucleic acid molecules described above encoding a protein of the invention. „Fragments„ are understood to be parts of the nucleic acid molecules that are long enough to encode one of the described proteins. These fragments comprise nucleic acid molecules specifically hybridizing to transcripts of the nucleic acid molecules of the invention. These nucleic acid molecules can be used, for example, as probes or primers in the diagnostic assay and/or kit described below and, preferably, are oligonucleotides having a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides. The nucleic acid molecules and oligonucleotides of the invention can also be used, for example, as primers for a PCR reaction. Examples of particular useful probes (primers) are shown in Tables 1 and 2.

Table 1

Trp8 probes used for in situ hybridization:

Probes (antisense)

1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'

2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'

3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

Controls (sense)

1.) 5' GGCAAGATCTCAACCGGCAGCGGA 3'

2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'

3.) 5' TTCCACACCCGGGGCTCTGAGGAT 3'

Tabelle 2

Trp10 probes used for the in situ hybridizations shown in Figure 14:

Probes (antisense)

1.) 5' GCTTCCACCCCAAGCTTCACAGGAATAGA 3' (Figure 14 A, 14B)
2.) 5' GGCGATGAAATGCTGGTCTGTGGC 3' (Figure 14C, 14D, 14N, 14S, 14O)
3.) 5' ATCTTCCAGTTCTTGGTGTCTCGG 3' (Figure 14E, 14K)
4.) 5' GCTGCAGTACTCCTGCACCAGGAA 3' (Figure 14L, 14M)

Probes (sense)

1.) 5' TCTATTCCTGTGAAGCTTGGGGTGGAAGC 3' (Figure 14F, 14G)
2.) 5' GCCACAGACCAGCATTTCATCGCC 3' (Figure 14H, 14I, 14T)
3.) 5' CCGAGACACCAAGAACTGGAAGAT 3' (Figure 14J, 14P)
4.) 5' TTCCTGGTGCAGGAGTACTGCAGC 3' (Figure 14Q, 14R)

The term „derivative„ in this context means that the sequences of these molecules differ from the sequences of the nucleic acid molecules described above at one or several positions but have a high level of homology to these sequences. Homology hereby means a sequence identity of at least 40%, in particular an identity of at least 60%, preferably of more than 80% and particularly preferred of more than 90%. These proteins encoded by the nucleic acid molecules have a sequence identity to the amino acid sequence depicted in Figure 7, 8A, 9, 10 and 11, respectively, of at least 80%, preferably of 85% and particularly preferred of more than 90%, 97% and 99%. The deviations to the above-described nucleic acid molecules may have been produced by deletion, substitution, insertion or recombination. The definition of the derivatives also includes splice variants, e.g. the splice variants shown in Figures 8B to 8E and 9B.

The nucleic acid molecules that are homologous to the above-described molecules and that represent derivatives of these molecules usually are variations of these molecules that represent modifications having the same biological function. They can be naturally occurring variations, for example sequences from other organisms, or mutations that can either occur naturally or that have been introduced by specific mutagenesis. Furthermore the variations can be synthetically produced sequences. The allelic variants can be either naturally occurring variants or synthetically produced variants or variants produced by recombinant DNA processes.

Generally, by means of conventional molecular biological processes it is possible (see, e.g., Sambrook et al., supra) to introduce different mutations into the nucleic acid molecules of the invention. As a result Trp proteins or Trp related proteins with possibly modified biological properties are synthesized. One possibility is the production of deletion mutants in which nucleic acid molecules are produced by continuous deletions from the 5'- or 3'-terminal of the coding DNA sequence and that lead to the synthesis of proteins that are shortened accordingly. Another possibility is the introduction of single-point mutation at positions where a modification of the amino aid sequence influences, e.g., the ion channel properties or the regulations of the trp-ion channel. By this method muteins can be produced, for example, that possess a modified ion conducting pore, a modified K_m-value or that are no longer subject to the regulation mechanisms that normally exist in the cell, e.g. with regard to allosteric regulation or covalent modification. Such muteins might also be valuable as therapeutically useful antagonists of Trp8a, Trp8b, Trp9, Trp10a or Trp10b, respectively.

For the manipulation in prokaryotic cells by means of genetic engineering the nucleic acid molecules of the invention or parts of these molecules can be introduced into plasmids allowing a mutagenesis or a modification of a sequence by recombination of DNA sequences. By means of conventional methods (cf. Sambrook et al., supra) bases can be exchanged and natural or synthetic sequences can be added. In order to link the DNA fragments with each other adapters or linkers can be added to the fragments. Furthermore, manipulations can be performed that provide suitable cleavage sites or that remove superfluous DNA or cleavage sites. If insertions, deletions or substitutions are possible, in vitro mutagenesis, primer repair, restriction or ligation can be performed. As analysis method usually sequence analysis, restriction analysis and other biochemical or molecular biological methods are used.

The proteins encoded by the various variants of the nucleic acid molecules of the invention show certain common characteristics, such as ion channel activity, molecular weight, immunological reactivity or conformation or physical properties like the electrophoretical mobilty, chromatographic behavior, sedimentation coefficients, solubility, spectroscopic properties, stability; pH optimum, temperature optimum.

The invention furthermore relates to vectors containing the nucleic acid molecules of the invention. Preferably, they are plasmids, cosmids, viruses, bacteriophages and other vectors

usually used in the field of genetic engineering. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in mammalian cells and baculovirus-derived vectors for expression in insect cells. Preferably, the nucleic acid molecule of the invention is operatively linked to the regulatory elements in the recombinant vector of the invention that guarantee the transcription and synthesis of an RNA in prokryotic and/or eukaryotic cells that can be translated. The nucleotide sequence to be transcribed can be operably linked to a promoter like a T7, metallothionein I or polyhedrin promoter.

In a further embodiment, the present invention relates to recombinant host cells transiently or stable containing the nucleic acid molecules or vectors or the invention. A host cell is understood to be an organism that is capable to take up *in vitro* recombinant DNA and, if the case may be, to synthesize the proteins encoded by the nucleic acid molecules of the invention. Preferably, these cells are prokaryotic or eukaryotic cells, for example mammalian cells, bacterial cells, insect cells or yeast cells. The host cells of the invention are preferably characterized by the fact that the introduced nucleic acid molecule of the invention either is heterologous with regard to the transformed cell, i.e. that it does not naturally occur in these cells, or is localized at a place in the genome different from that of the corresponding naturally occurring sequence.

A further embodiment of the invention relates to isolated proteins exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9,Trp10a or Trp10b and being encoded by the nucleic acid molecules of the invention, as well as to methods for their production, whereby, e.g., a host cell of the invention is cultivated under conditions allowing the synthesis of the protein and the protein is subsequently isolated from the cultivated cells and/or the culture medium. Isolation and purification of the recombinantly produced proteins may be carried out by conventional means including preparative chromatography and affinity and immunological separations involving affinity with an anti-Trp8a-, anti-Trp8b-, anti-Trp9-,anti-Trp10a- or anti-Trp10b-antibody, respectively.

As used herein, the term „isolated protein„ includes proteins substantially free of other proteins, nucleic acids, lipids, carbohydrates or other materials with which it is naturally associated. Such proteins however not only comprise recombinantly produced proteins but include isolated naturally occurring proteins, synthetically produced proteins, or proteins

produced by a combination of these methods. Means for preparing such proteins are well understood in the art. The Trp proteins are preferably in a substantially purified form. A recombinantly produced version of a human prostate carcinoma associated protein Trp8a, Trp8b, Trp9,Trp10a or Trp10b protein, including the secreted protein, can be substantially purified by the one-step method described in Smith and Johnson, Gene 67; 31-40 (1988).

In a further preferred embodiment, the present invention relates to an antisense RNA sequence characterised that it is complementary to an mRNA transcribed from a nucleic acid molecule of the present invention or a part thereof and can selectively bind to said mRNA, said sequence being capable of inhibiting the synthesis of the protein encoded by said nucleic acid molecules, and a ribozyme characterised in that it is complementary to an mRNA transcribed from a nucleic acid molecule of the present invention or a part thereof and can selectively bind to and cleave said mRNA, thus inhibiting the synthesis of the proteins encoded by said nucleic acid molecules. Riboyzmes which are composed of a single RNA chain are RNA enzymes, i.e. catalytic RNAs, which can intermolecularly cleave a target RNA, for example the mRNA transcribed from one of the Trp genes. It is now possible to construct ribozymes which are able to cleave the target RNA at a specific site by following the strategies described in the literature. (see, e.g., Tanner et al., in: Antisense Research and Applications, CRC Press Inc. (1993), 415-426). The two main requirements for such ribozymes are the catalytic domain and regions which are complementary to the target RNA and which allow them to bind to its substrate, which is a prerequisite for cleavage. Said complementary sequences, i.e., the antisense RNA or ribozyme, are useful for repression of Trp8a-, Trp8b, Trp9-,Trp10a- and Trp10b-expression, respectively, i.e. in the case of the treatment of a prostate cancer or endometrial cancer (carcinoma of the uterus). Preferably, the antisense RNA and ribozyme of the invention are complementary to the coding region. The person skilled in the art provided with the sequences of the nucleic acid molecules of the present invention will be in a position to produce and utilise the above described antisense RNAs or ribozymes. The region of the antisense RNA and ribozyme, respectively, which shows complementarity to the mRNA transcribed from the nucleic acid molecules of the present invention preferably has a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides.

In still a further embodiment, the present invention relates to inhibitors of Trp8a, Trp8b, Trp9, Trp10a and Trp10b, respectively, which fulfill a similar purpose as the antisense RNAs or

ribozymes mentioned above, i.e. reduction or elimination of biologically active Trp8a, Trp8b, Trp9, Trp10a or Trp10b molecules. Such inhibitors can be, for instance, structural analogues of the corresponding protein that act as antagonists. In addition, such inhibitors comprise molecules identified by the use of the recombinantly produced proteins, e.g. the recombinantly produces protein can be used to screen for and identify inhibitors, for example, by exploiting the capability of potential inhibitors to bind to the protein under appropriate conditions. The inhibitors can, for example, be identified by preparing a test mixture wherein the inhibitor candidate is incubated with Trp8a, Trp8b, Trp9, Trp10a or Trp10b, respectively, under appropriate conditions that allow Trp8a, Trp8b, Trp9, Trp10a or Trp10b to be in a native conformation. Such an in vitro test system can be established according to methods well known in the art. Inhibitors can be identified, for example, by first screening for either synthetic or naturally occurring molecules that bind to the recombinantly produced Trp protein and then, in a second step, by testing those selected molecules in cellular assays for inhibition of the Trp protein, as reflected by inhibition of at least one of the biological activities as described in the examples, below. Such screening for molecules that bind Trp8a, Trp8b, Trp9, Trp10a or Trp10b could easily performed on a large scale, e.g. by screening candidate molecules from libraries of synthetic and/or natural molecules. Such an inhibitor is, e.g., a synthetic organic chemical, a natural fermentation product, a substance extracted from a microorganism, plant or animal, or a peptide. Additional examples of inhibitors are specific antibodies, preferably monoclonal antibodies. Moreover, the nucleic sequences of the invention and the encoded proteins can be used to identify further factors involved in tumor development and progression. In this context it should be emphasized that the modulation of the calcium channel of a member of the trp family can result in the stimulation of the immune response of T lymphocytes leading to proliferation of the T lymphocytes. The proteins of the invention can, e.g., be used to identify further (unrelated) proteins which are associated with the tumor using screening methods based on protein/protein interactions, e.g. the two-hybrid-system Fields, S. and Song, O. (1989) Nature (340): 245-246.

The present invention also provides a method for diagnosing a prostate carcinoma which comprises contacting a target sample suspected to contain the protein Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA with a reagent which reacts with Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA and detecting Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp 10b encoding mRNA.

It has been found that carcinoma cells of placenta (chorion carcinoma), lung and prostate express Trp10 transcripts as well as Trp10 antisense transcripts and transcripts being in part complementary to Trp10 antisense transcripts. Accordingly, the present invention also provides a method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense RNA.

When the target is mRNA (or antisense RNA), the reagent is typically a nucleic acid probe or a primer for PCR. The person skilled in the art is in a position to design suitable nucleic acids probes based on the information as regards the nucleotide sequence of Trp8a, Trp8b, Trp10a or Trp10b as depicted in figure 7, 8a, 10 and 11, respectively, or tables 1 and 2, above. When the target is the protein, the reagent is typically an antibody probe. The term „antibody", preferably, relates to antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specifities, as well as distinct monoclonal antibody preparations. Monoclonal antibodies are made from an antigen containing fragments of the proteins of the invention by methods well known to those skilled in the art (see, e.g., Köhler et al., Nature 256 (1975), 495). As used herein, the term „antibody" (Ab) or „monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab') 2 fragments) which are capable of specifically binding to protein. Fab and f(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. (Wahl et al., J. Nucl. Med. 24: 316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimerical, single chain, and humanized antibodies. The target cellular component, i.e. Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts, e.g., in biological fluids or tissues, may be detected directly in situ, e.g. by in situ hybridization (e.g., according to the examples, below) or it may be isolated from other cell components by common methods known to those skilled in the art before contacting with a probe. Detection methods include Northern blot analysis, RNase protection, in situ methods, e.g. in situ hybridization, in vitro amplification methods (PCR, LCR, QRNA replicase or RNA-transcription/amplification (TAS, 3SR), reverse dot blot disclosed in EP-B1 O 237 362)), immunoassays, Western blot and other detection assays that are known to those skilled in the art.

Products obtained by in vitro amplification can be detected according to established methods, e.g. by separating the products on agarose gels and by subsequent staining with ethidium bromide. Alternatively, the amplified products can be detected by using labeled primers for amplification or labeled dNTPs.

The probes can be detectable labeled, for example, with a radioisotope, a bioluminescent, compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

Expression of Trp8a, Trp8b, Trp10a and Trp10b, respectively, in tissues can be studied with classical immunohistological methods (Jalkanen et al., J. Cell. Biol. 101 (1985), 976-985; Jalkanen et al., J. Cell. Biol. 105 (1987), 3087-3096; Sobol et al. Clin. Immunpathol. 24 (1982), 139-144; Sobol et al., Cancer 65 (1985), 2005-2010). Other antibody based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium rhodamine, and biotin. In addition to assaying Trp8a, Trp8b, Trp 10a or Trp10b levels in a biological sample, the protein can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99}mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99}mTc. The labeled antibody or antibody fragment will then preferentially accumulate at he location of cells which contain the specific protein. In

vivo tumor imaging is described in S.W. Burchiel et al., „Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments". (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B.A. Rhodes, eds., Masson Publishing Inc. (1982)).

The marker Trp8a and Trp8b is also useful for prognosis, for monitoring the progression of the tumor and the diagnostic evaluation of the degree of malignancy of a prostate tumor (grading and staging), e.g. by using in situ hybridization: In a primary carcinoma Trp8 is expressed in about 2 to 10% of carcinoma cells, in a rezidive carcinoma in about 10 to 60% of cells and in metastases in about 60 to 90% of cells.

The present invention also relates to a method for diagnosing endometrial cancer (cancer of the uterus) which comprises contacting a target sample suspected to contain the protein Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA with a reagent which reacts with Trp8a and/or Trp8b or the encoding mRNA and detecting Trp8a and/or Trp8b encoding mRNA. As regards particular embodiments of this method reference is made to the particular embodiments of the method of diagnosing a prostate cancer outlined above.

For evaluating whether the concentration of Trp8a, Trp8b, Trp10a or Trp10b or the concentration of Trp8a, Trp8b, Trp10a or Trp10b encoding mRNA is normal or increased, thus indicative for the presence of a malignant tumor, the measured concentration is compared with the concentration in a normal tissue, preferably by using the ratio of Trp8a:Trp9, Trp8b:Trp9 or Trp10(a or b)/Trp9 for quantification.

Since the prostate carcinoma forms its own basement membrane when growing invasively, it can be concluded that only cells expressing Trp8 and Trp10 are involved in this phenomenon. Thus, it can be concluded that by inhibiting the expression and/or activity of these proteins an effective therapy of cancers like PCA is provided.

Thus, the present invention also relates to a pharmaceutical composition containing a reagent which decreases or inhibits Trp8a, Trp8b, Trp10a and/or Trp10b expression or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b, and a method for preventing, treating, or ameliorating a prostate tumor, endometrial cancer (uterine carcinoma) tumor, a chorion carcinoma, cancer of the lung or melanoma,which comprises administering to a mammalian subject a

therapeutically effective amount of a reagent which decreases or inhibits Trp8a, Trp8b, Trp10a and/or Trp10b expression or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b. Examples of such reagents are the above described antisense RNAs, ribozymes or inhibitors, e.g. specific antibodies. Furthermore, peptides, which inhibit or modulate the biological function of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b may be useful as therapeutical reagents. For example, these peptides can be obtained by screening combina torial phage display libraries (Cosmix, Braunschweig, Germany) as described by Rottgen, P. and Collins, J. (Gene (1995) 164 (2): 243-250). Furthermore, antigenic epitopes of the Trp8 and Trp10 proteins can be identified by the expression of recombinant Trp8 and Trp10 epitope libraries in E. coli (Marquart, A. & Flockerzi, V., FEBS Lett. 407 (1997), 137-140; Trost, C., et al., FEBS Lett. 451 (1999) 257-263 and the consecutive screening of these libraries with serum of patients with cancer of the prostate or of the endometrium. Those Trp8 and Trp10 epitopes which are immunogenic and which lead to the formation of antibodies in the serum of the patients can be then be used as Trp8 or Trp10 derived peptide vaccines for immune inventions against cancer cells which express Trp8 or Trp10. Alternatively to the E. coli expression system, Trp8 or Trp10 or epitopes of Trp8 and Trp10 can be expressed in mammalian cell lines such as human embryonic kidney (Hek 293) cells (American Type Culture Collection, ATCC CRL 1573).

Finally, compounds useful for therapy of the above described diseases comprise compounds which act as antagonists or agonists on the ion channels Trp8, Trp9 and Trp10. It could be shown that Trp8 is a highly calcium selective ion channel which in the presence of monovalent (namely sodium) and divalent ions (namely calcium) is only permeable for calcium ions (see Example 4, below, and Figures 3A, C, E). Under physiological conditions, Trp8 is a calcium selective channel exhibiting large inward currents. This very large conductance of Trp8 channels (as wells as Trp9 and Trp10a/b channels) is useful to establish systems for screening pharmacological compounds interacting with Trp-channels including high throughput screening systems. Useful high throughput screening systems are well known to the person skilled in the art and include, e.g., the use of cell lines stably or transiently transfected with DNA sequences encoding Trp8, Trp9 and Trp10 channels in assays to detect calcium signaling in biological systems. Such systems include assays based on Ca-sensitive dyes such as aequorin, apoaequorin, Fura-2, Fluo-3 and Indo-1.

Accordingly, the present invention also relates to a method for identifying compounds which act as agonists or antagonists on the ion channels Trp8, Trp9 and/or Trp10, said method comprising contacting a test compound with the ion channel Trp8, Trp9 and/or Trp10, preferably by using a system based on cells stably or transiently transfected with DNA sequences encoding Trp8, Trp9 and/or Trp10, and determining whether said test compound affects the calcium uptake.

For administration the above described reagents are preferably combined with suitable pharmaceutical carriers. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Such carriers can be formulated by conventional methods and can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g. by intravenous, intraperetoneal, subcutaneous, intramuscular, topical or intradermal administration. The route of administration, of course, depends on the nature of the tumor and the kind of compound contained in the pharmaceutical composition. The dosage regimen will be determined by the attending physician and other clinical factors. As is well known in the medical arts, dosages for any one patient depends on many factors, including the patient's size, body surface area, age, sex, the particular compound to be administered, time and route of administration, the kind and stage of the tumor, general health and other drugs being administered concurrently.

The delivery of the antisense RNAs or ribozymes of the invention can be achieved by direct application or, preferably, by using a recombinant expression vector such as a chimeric virus containing these compounds or a colloidal dispersion system. By delivering these nucleic acids to the desired target, the intracellular expression of Trp8a, Trp8b, Trp10a and/or Trp10b and, thus, the level of Trp8a, Trp8b, Trp10a and/or Trp10b can be decreased resulting in the inhibition of the negative effects of Trp8a, Trp8b, Trp10a and/or Trp10b, e.g. as regards the metastasis formation of PCA.

Direct application to the target site can be performed, e.g., by ballistic delivery, as a colloidal dispersion system or by catheter to a site in artery. The colloidal dispersion systems which can be used for delivery of the above nucleic, acids include macromolecule complexes, nanocapsules, microspheres, beads and lipid-based systems including oil-in-water emulsions

(mixed), micelles, liposomes and lipoplexes, The preferred colloidal system is a liposome. The composition of the liposome is usually a combination of phospholipids and steroids, especially cholesterol. The skilled person is in a position to select such liposomes which are suitable for the delivery of the desired nucleic acid molecule. Organ-specific or cell-specific liposomes can be used in order to achieve delivery only to the desired tumor. The targeting of liposomes can be carried out by the person skilled in the art by applying commonly known methods. This targeting includes passive targeting (utilizing the natural tendency of the liposomes to distribute to cells of the RES in organs which contain sinusoidal capillaries) or active targeting (for example by coupling the liposome to a specific ligand, e.g., an antibody, a receptor, sugar, glycolipid, protein etc., by well known methods). In the present invention monoclonal antibodies are preferably used to target liposomes to specific tumors via specific cell-surface ligands.

Preferred recombinant vectors useful for gene therapy are viral vectors, e.g. adenovirus, herpes virus, vaccinia, or, more preferably, an RNA virus such as a Retrovirus. Even more preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of such retroviral vectors which can be used in the present invention are: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV) and Rous sarcoma virus (RSV). Most preferably, a non-human primate retroviral vector is employed, such as the gibbon ape leukemia virus (GaLV), providing a broader host range compared to murine vectors. Since recombinant retroviruses are defective, assistance is required in order to produce infectious particles. Such assistance can be provided, e.g., by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. Suitable helper cell lines are well known to those skilled in the art. Said vectors can additionally contain a gene encoding a selectable marker so that the transduced cells can be identified. Moreover, the retroviral vectors can be modified in such a way that they become target specific. This can be achieved, e.g., by inserting a polynucleotide encoding a sugar, a glycolipid, or a protein, preferably an antibody. Those skilled in the art know additional methods for generating target specific vectors. Further suitable vectors and methods for in vitro- or in vivo-gene therapy are described in the literature and are known to the persons skilled in the art; see, e.g., WO 94/29469 or WO 97/00957.

In order to achieve expression only in the target organ, i.e. tumor to be treated, the nucleic acids encoding, e.g. an antisense RNA or ribozyme can also be operably linked to a tissue specific promoter and used for gene therapy. Such promoters are well known to those skilled in the art (see e.g. Zimmermann et al., (1994) Neuron 12, 11-24; Vidal et al.; (1990) EMBO J. 9, 833-840; Mayford et al., (1995), Cell 81, 891-904; Pinkert et al., (1987) Genes & Dev. 1, 268-76).

For use in the diagnostic research discussed above, kits are also provided by the present invention. Such kits are useful for the detection of a target cellular component, which is Trp8a, Trp8b, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts, wherein the presence or an increased concentration of Trp8a, Trp8b, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts is indicative for a prostate tumor, endometrial cancer, melanoma, chorion carcinoma or cancer of the lung, said kit comprising a probe for detection of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b or, alternatively, Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a/b antisense transcripts. The probe can be detectably labeled. Such probe may be a specific antibody or specific oligonucleotide. In a preferred embodiment, said kit contains an anti-Trp8a-, anti-Trp8b-, anti-Trp9-, anti-Trp10a-and/or anti-Trp10b-antibody and allows said diagnosis, e.g., by ELISA and contains the antibody bound to a solid support, for example, a polystyrene microtiter dish or nitrocellulose paper, using techniques known in the art. Alternatively, said kits are based on a RIA and contain said antibody marked with a radioactive isotope. In a preferred embodiment of the kit of the invention the antibody is labeled with enzymes, fluorescent compounds, luminescent compounds, ferromagnetic probes or radioactive compounds. The kit of the invention may comprise one or more containers filled with, for example, one or more probes of the invention. Associated with container (s) of the kit can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, us or sale for human administration.

EXAMPLES

The following Examples are intended to illustrate, but not to limit the invention. While such Examples are typical of those that might be used, other methods known to those skilled in the art may alternatively be utilized.

Example 1: Materials and Methods

(A) Isolation of cDNA clones and Northern blot analysis

Total RNA was isolated from human placenta an prostate using standard techniques. Isolation of mRNA was performed with poly $(A)^+$RNA - spin columns (New England Biolabs, Beverly, USA) according to the instructions of the manufacturer. Poly (a) $^+$RNA was reverse transcribed using the cDNA choice system (Gibco-BRL, Rockville, USA) and subcloned in λ-Zap phages (Stratagene, La Jolla, USA). An human expressed sequence tag (GenBank accession number 1404042) was used to screen an oligo d(T) primed human placenta cDNA library. Several cDNA clones were identified and isolated. Additional cDNA clones were isolated from two specifically primed cDNA libraries using primers 5'-gca tag gaa ggg aca ggt gg-3' and 5'-gag agt cga ggt cag tgg tcc-3'.

cDNA clones were sequenced using a thermocycler (PE Applied Biosystems, USA) and Thermo Sequenase (Amersham Pharmacia Biotech Europe, Freiburg, Germany). DNA sequences were analyzed with an automated sequencer (Licor, Linccoln, USA).

For Northern blot analysis 5 µg human poly $(A)^+$ RNA from human placenta or prostate were separated by electrophoresis on 0.8 % agarose gels. Poly $(A)^+$ RNA was transferred to Hybond N nylon membranes (Amersham Pharmacia Biotech Europe, Freiburg, Germany). The membranes were hyridized in the presence of 50 % formamide at 42^0C over night. DNA probes were labelled using [α^{32}P]dCTP and the „ready prime„ labelling kit (Amersham Pharmacia Biotech Europe, Freiburg, Germany). Commercial Northern blots were hybridized according to the distributors instructions (Clontech, Paolo Alto, USA).

(B) Construction of expression plasmids and transfection of HEK 293 cells

Lipofections were carried out with the recombinant dicistronic eucaryotic expression plasmid pdiTRP8 containing the cDNA of Trp8b under the control of the chicken ß-actin promotor followed by an internal ribosome entry side (IRES) and the cDNA of the green fluorescent protein (GFP). To obtain pdiTRP8 carrying the entire protein coding regions of TRP8b and

the GFP (Prasher, D.C. et al. (1992), Gene 111, 229-233), the 5`and 3´-untranslated sequences of the TRP8b cDNA were removed, the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) Nucleic Acids Research15, 8125-8148) was introduced immediately 5`of the translation initiation codon and the resulting cDNA was subcloned into the pCAGGS vector (Niwa, H., Yamamura, K. and Miyazaki, J (1991), Gene 8, 193-199) downstream of the chicken β-actin promotor. The IRES derived from encephalmyocarditis virus (Kim, D.G., Kang, H.M., Jang, S.K. and Shin H.S. (1992) Mol.Cell.Biol. 12, 3636-3643) followed by the GFP cDNA containing a Ser65Thr mutation (Heim, R., Cubitt, A.B., Tsien, R.Y. (1995) Nature 373, 663-664) was then cloned 3` to the TRP8b cDNA. The IRES sequence allows the simultaneous translation of TRP8b and GFP from one transcript. Thus, transfected cells can be detected unequivocally by the development of green fluorescence.

For monitoring of the intracellular Ca^{2+} concentration human embryonic kidney (HEK 293) cells were cotransfected with the pcDNA3-TRP8b vector and the pcDNA3-GFPvector in a molar ratio of 4 : 1 in the presence of lipofectamine (Quiagen, Hilden, Germany). To obtain pcDNA3-TRP8b the entire protein coding region of TRP8b including the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) Nucleic Acids Research15, 8125-8148) was subcloned into the pcDNA3 vector (Invitrogen, Groningen, Netherlands). Calcium monitoring and patch clamp experiments were carried out two days and one day after transfection, respectively.

(C) Chromosomal localization of the Trp8 gene

The chromosomal localization of the human TRP8 gene was performed using NIGMS somatic hybrid mapping panel No.2 (Coriell Institute, Camden, NJ, USA) previously described (Drwinga, H.L., Toji, L.H., Kim, C.H., Greene, A.E., Mulivor, R.A. (1993) Genomics 16, 311-314; Dubois, B.L. and Naylor, S.L. (1993) Genomics 16, 315-319).

(D) In Vitro Translation, glutathione - sepharose and calmodulin agarose binding assay

N– and C-terminal Trp8-fragments were subcloned into the pGEX-4T2 vector (Amersham Pharmacia Europe, Freiburg, Germany) resulting in glutathione-S-transferase (GST)-Trp8 fusion constructs (Fig. 4). The GST-TRP8-fusion proteins were expressed in E. coli BL 21 cells and purified using glutathione - sepharose beads (Amersham Pharmacia Biotech Europe, Freiburg, Germany).

In vitro translation of human Trp8 cDNA and Xenopus laevis calmodulin cDNA (Davis, T.N. and Thorner, J. Proc.Natl.Acad.Sci. USA 86, 7909-7913.) was performed in the presence of ^{35}S-methionine using the TNT coupled transcription/translation kit (Promega, Madison, USA). Translation products were purified by gel fliltration (Sephadex G50, Amersham Pharmacia Biotech Europe, Freiburg, Germany) and equal amounts of ^{35}S labeled probes were incubated for 2 h with glutathione beads bound to GST - Trp8 or calmodulin - agarose (Calbiochem) in 50 mM Tris-HCl, pH 7.4, 0.1 % Triton X-100, 150 mM NaCl in the presence of 1 mM Ca^{2+} or 2 mM EGTA. After three washes, bound proteins were eluted with SDS sample buffer, fractionated by SDS-PAGE and ^{35}S labeled proteins were detected using a Phosphor Imager (Fujifilm, Tokyo, Japan).

(E) Calcium measurements

The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined by dual wavelength fura-2 fluorescence ratio measurements (Tsien, R.Y. (1988) Trends Neurosci. 11, 419-424) using a digital imaging system (T.I.L.L. Photonics, Planegg, Germany). HEK cells were grown in minimal essential medium in the presence of 10 % fetal calf serum and cotransfected with the pcDNA3-TRP8b vector and the pCDNA3-GFPvector as described above (B). Transfected cells were detected by development of green fluorescence. The cells were loaded with 4μM fura-2/AM (Molecular Probes, Oregon, USA) for one hour. After loading the cells were rinsed 3 times with buffer B1 (10 mM Hepes, 115 mM NaCl, 2 mM $MgCl_2$, 5mM KCl, pH 7.4) and the $[Ca^{2+}]_i$ was calculated from the fluorescence ratios obtained at 340 and 380 nm excitation wavelengths as described (Garcia, D.E., Cavalié, A. and Lux, H.D. (1994) J. Neurosci 14, 545-553).

(F) Electrophysiological recordings

HEK cells were transfected with the eucaryotic expression plasmid pdiTRP8 described in (B) and electrophysiolocigal recordings were carried out one day after transfection. Single cells were voltage clamped in the whole cell mode of the patch clamp technique as described (Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85-100; Philipp, S., Cavalié, A., Freichel, M., Wissenbach, U., Zimmer, S., Trost, C., Marquart, A., Murakami, M. and Flockerzi, V. (1996) EMBO J. 6166-6171). The pipette solution contained contained (mM): 140 aspartic acid, 10 EGTA, 10 NaCl, 1 MgCl2, 10 Hepes (pH 7.2 with CsOH) or 125 CsCl, 10 EGTA, 4 $CaCl_2$ 10 Hepes (pH 7,2 with CsOH). The bath solution contained (mM): 100 NaCl, 10 CsCl, 2 $MgCl_2$, 50 mannitol, 10 glucose, 20

Hepes (pH 7,4 with CsOH) and 2 $CaCl_2$, or no added $CaCl_2$ (-Ca^{2+} solution). Divalent free bath solution contained (mM): 110 N-methyl-D-glucamine (NMDG). Whole cell currents were recorded during 100 msec voltage ramps from -100 to +100 mV at varying holding potentials.

(G) In Situ Hybridization

In situ hybridizations were carried out using formalin fixed tissue slices of 6 - 8 µM thickness. The slices were hydrated and incubated in the presence of PBS buffer including 10 µg / ml proteinase K (Roche Diagnostics, Mannheim, Germany) for 0.5 h. The slices were hybridized at 37°C using biotinylated deoxy-oligonucleotides (0.5 pmol / µl) in the presence of 33 % formamide for 12 h. Furthermore the slices were several times rinsed with 2 x SSC and incubated at 25°C for 0.5 h with avidin / biotinylated horse raddish peroxidase complex (ABC, DAKO, Santa Barbara, USA). After several washes with PBS buffer the slices were incubated in the presence of biotinylated tyramid and peroxide (0.15 % w/v) for 10 min, rinsed with PBS buffer and additionally incubated with ABC complex for 0.5 h. The slices were washed with PBS buffer and incubated in the presence of DAB solution (diaminobenzidine (50µg / ml), 50 mM Tris/EDTA buffer pH 8.4, 0.15 % H_2O_2 in N,N - dimethyl-formamide; Merck, Darmstadt, Germany), The detection was stopped after 4 minutes by incubating the slides in water. Tyramid was biotinylated by incubating NHS-LC Biotin (sulfosuccinimidyl-6-(biotinimid)-hexanoat), 2.5 mg / ml; Pierce, Rockford, USA) and tyramin-HCl (0.75 mg / ml, Sigma) in 25 mM borate buffer pH 8.5 for 12 h. The tyramid solution was diluted 1 - 5 : 1000 in PBS buffer.

(H) GenBank accession numbers: TRP8a, Aj243500; TRP8b Aj243501

Example 2: Expression of TRP8 transcripts

In search of proteins distantly related to the TRP family of ion channels, an human expressed sequence tag (EST, GenBank accession number 1404042) was identified in the GenBank database using BLAST programms (at the National Center for Biotechnology Information (NCBI); Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J.J. (1990) Mol. Biol. 5, 403-410) being slightly homologous to the VR1 gene. Several human placenta cDNA libraries were constructed and screeened with this EST DNA as probe. Several full length

cDNA clones were identified and isolated. The full length cDNA clones encoded two putative proteins differing in three amino acids and were termed Trp8a and Trp8b (Fig. 1c, 2a, 7 and 8A). This finding was reproduced by isolating cDNA clones from two cDNA libraries constructed from two individual placentas. The derived protein sequence(s) comprises six transmembrane domains, a characteristic overall feature of trp channels and related proteins (Fig.: 1b). The sequence is closely related to the meanwhile published calcium uptake transport protein 1 (CaT1), isolated from rat intestine (Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M. and Hediger M.A.(1999) J Biol Chem. 6;274, 22739-22746) and to the epithelial calcium uptake channel (ECaC) isolated from rabbit kidney (Hoenderop, J.G., van der Kemp, A.W., Hartog, A., van de Graaf, S.F., van Os, C.H., Willems, P.H. and Bindels, R.J. (1999) J Biol Chem. 26;274, 8375-8378). Expression of Trp8a/b transcripts are detectable in human placenta, pancreas and prostate (Fig.: 5) and the size of the Northern signal (3.0 kb) corresponds with the size of the isolated full length cDNAs. In addition, a shorter transcript of 1.8 kb, probably a splice variant, is detectable in human testis. The Trp8 mRNA is not expressed in small intestine or colon (Fig.: 5) implicating that Trp8 is not the human ortholog of the rat CaT1 or rabbit ECaC proteins. To investigate whether there are other related sequences Trp8a/b derived primers (UW241, 5'-TAT GAG GGT TCA GAC TGC-3' and UW242, 5'-CAA AGT AGA TGA GGT TGC-3') were used to amplify a 105 bp fragment from human genomic DNA being 95% identical on the nucleotide level to the Trp8 sequence (data not shown). This indicates the existence of several similar sequences in humans at least at the genomic level.

Example 3: Two variants of the Trp8 protein (Trp8a and Trp8b) arise by polymorphism

Two variants of the Trp8 cDNA were isolated from human placenta (Fig.: 2A, 7 and 8A) which encoded two proteins which differ in three amino acids and were termed Trp8a and Trp8b. Trp8a/b specific primers were designed to amplify a DNA fragment of 458 bp of the Trp8 gene from genomic DNA isolated from human T-lymphocytes (primer pair: UW243, 5'-CAC CAT GTG CTG CAT CTA CC-3' and UW244, 5'-CAA TGA CAG TCA CCA GCT CC-3'). The amplification product contains a part of the sequence where the derived protein sequence of Trp8a comprises the amino acid valine and the Trp8b sequence methionine as well as a silent base pair exchange (g versus a) and an intron of 303bp (Fig.: 2.A, B). Both variants of the Trp8 genes (a,b) were amplified from genomic DNA in equal amounts indicating the existence of both variants in the human genome and therefore being not the

result of RNA editing (Fig.: 2B). The Trp8a gene can be distinguished from the Trp8b gene by cutting the genomic fragment of 458bp with the restriction enzyme Bsp1286I (Fig. 2B). Using human genomic DNA isolated from blood of twelve human subjects as template, the 458bp fragment was amplified and restricted with BSP1286I. In eleven of the tested subjects only the Trp8b gene is detectable, while one subject (7) contains Trp8a and Trp8b genes (Fig.: 2D). These implicates that the two Trp8 variants arise by polymorphism and do not represent individual genes. Using Trp8 specific primers and chromosomal DNA as template, the Trp8 locus is detectable on chromosome 7 (Fig.: 2C).

Example 4: Trp8b is a calcium permeable channel

The protein coding sequence of the Trp8b cDNA was subcloned into pcDNA3 vector (Invitrogen, Groningen, Netherlands) under the control of the cytomegalovirus promotor (CMV). Human embryonic kidney (HEK 293) cells were cotransfected with the Trp8b pcDNA3 construct (pcDNA3-Trp8b vector) and the pcDNA3-GFPvector encoding the green fluorescent protein (GFP) in 4:1 ratio. The Trp8b cDNA and the cDNA of the reporter, GFP, was transiently expressed in human embryonic kidney (HEK 293) cells. The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and changes of $[Ca^{2+}]_i$ were determined by dual wavelength fura-2 fluorescence ratio measurements (Fig.: 3F) in cotransfected cells which were identified by the green fluorescence of the reporter gene GFP.

Dual wavelength fura-2 fluorescence ratio measurement is a standard procedure (e.g. in: An introduction of Molecular Neurobiology (ed. Hall, Z.W.)Sinauer Associates, Sunderland, USA (1992)) using fura-2, which is a fluorescent Ca^{2+} sensitive dye and which was designed by R.Y.Tsien (e.g. Trends Neurosci. 11, 419-424 (1988) based upon the structure of EGTA. Its fluorescence emission spectrum is altered by binding to Ca^{2+} in the physiological concentration range. In the absence of Ca^{2+}, fura-2 fluoresces most strongly at an excitation wavelength of 385 nm; when it binds Ca^{2+}, the most effective excitation wavelength shifts to 345 nm. This property is used to measure local Ca^{2+} concentrations within cells. Cells can be loaded with fura-2 esters (e.g. fura-2AM) that diffuse across cell membranes and are hydrolyzed to active fura-2 by cytosolic esterases.

In the presence of 1mM Ca^{2+}, Trp8 expressing cells typically contained more than 300 nM cytosolic Ca^{2+}, while non transfected controls contained less than 100 nM Ca^{2+} ions (Fig. 3F).

When Trp8b transfected cells were incubated without extracellular Ca^{2+}, the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) decreased to levels comparable to non transfected cells. Readdition of 1mM Ca^{2+} to the bath resulted in significant increase of the cytosolic $[Ca^{2+}]$ in Trp8b transfected cells, but not in controls (Fig.: 3F). After readdition of Ca^{2+} ions to the bath solution, the cytosolic Ca^{2+} concentration remains on a high steady state level in the Trp8b transfected cells.

Example 5: Trp8 expressing cells show calcium selective inward currents

To characterize in detail the electrophysiological properties of TRP8, TRP8 and GFP were coexpressed in HEK293 cells using the dicistronic expression vector pdiTRP8 and measured currents using the patch clamp technique in the whole cell mode (Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflugers Arch., 391, 85-100).

The eucaryotic expression plasmid pdiTRP8 contains the cDNA of Trp8b under the control of the chicken ß-actin promotor followed by an internal ribosome entry side (IRES) and the cDNA of the green fluorescent protein (GFP). To obtain pdiTRP8 carrying the entire protein coding regions of TRP8b and the GFP (Prasher, D.C. et al. (1992), Gene 111, 229-233), the 5'and 3'-untranslated sequences of the TRP8b cDNA were removed, the consensus sequence for initiation of translation in vertebrates (Kozak, M. (1987) Nucleic Acids Research15, 8125-8148) was introduced immediately 5'of the translation initiation codon and the resulting cDNA was subcloned into the pCAGGS vector (Niwa, H., Yamamura, K. and Miyazaki, J (1991), Gene 8, 193-199) downstream of the chicken β-actin promotor. The IRES derived from encephalmyocarditis virus (Kim, D.G., Kang, H.M., Jang, S.K. and Shin H.S. (1992) Mol.Cell.Biol. 12, 3636-3643) followed by the GFP cDNA containing a Ser65Thr mutation (Heim, R., Cubitt, A.B., Tsien, R.Y. (1995) Nature 373, 663-664) was then cloned 3' to the TRP8b cDNA. The IRES sequence allows the simultaneous translation of TRP8b and GFP from one transcript. Thus, transfected cells can be detected unequivocally by the development of green fluorescence.

In the presence of 2 mM external calcium, Trp8b transfected HEK cells show inwardly rectifying currents, the size of which depends on the level of intracellular calcium and the electrochemical driving force. The resting membrane potential was held either at –40 mV, or, to lower the driving force for calcium influx in between pulses, at + 70 mV. Current traces

were recorded in response to voltage ramps from −100 to +100 mV, that were applied every second. To monitor inward and outward currents over time, we analyzed the current size at − 80 and + 80 mV of the ramps. Figure 3A shows a representative trace of the current at − 80 mV over time. Both at a holding potential of −40 mV or at +70 mV, the currents are significantly larger than in cells transfected with only the GFP containing vector (Fig.: 3E). Interestingly, after changing to a positive holding potential, current size in Trp8 transfected cells slowly increases and reaches steady state after approximately 70 seconds (Fig.: 3A). To determine the selectivity of the induced currents, we then perfused the cells with solutions that either contain no sodium, no added Ca^{2+} (Fig. 3A, C) or a sodium containing, but divalent ion free bath solution. To control for the effect of the solution change alone, we also perfused with normal bath (see puff in Fig. 3A). While removal of external Ca^{2+} completely abolishes the trp 8 induced currents - the remaining current being identical in size and shape to the control (Fig.: 3A, C, E), removal of external sodium has no effect (Fig.: 3E). An important hallmark of calcium selective channels (e.g. Vennekens, R., Hoenderop, G.J., Prenen, J., Stuiover, M., Willems, PHGM, Droogmans, G., Nilius, B.and Bindels, R.J.M (1999) J. Biol. Chem. 275, 3963-3969), is their ability to conduct sodium only if all external divalent ions, namely Ca^{2+} and magnesium are removed. To test whether the trp 8 channel conforms with this phenomenon normal bath solution was switched to a solution containing only sodium and 1 mM EGTA. As can be seen in Figure 3B and D, Trp8 transfected cells can now conduct very large sodium currents. Interestingly, immediately after the solution change, the currents first become smaller before increasing rapidly, indicating that the pore may initially still be blocked by calcium a phenomenon usually called anomalous mole fraction behaviour (Warnat, J., Philipp, S., Zimmer, S.,.Flockerzi, V., and Cavalié A.(1999) J.Physiol. (Lond) 518, 631-638). The measured outward currents of Trp8 transfected cells in normal bath solution are not significantly different from non-transfected control cells or cells which only express the reporter gene GFP. As the removal of external Ca^{2+} abolishes the Trp8 specific current, the remaining current was subtracted from the current before the solution change to obtain the uncontaminated Trp8 conductance (see inset in Fig.: 3C). As expected from the given ionic conditions (high EGTA inside, 2 mM Ca^{2+} outside), the current-voltage relationship now shows prominent inward rectification with little to no outward current.

Both the time course of the development of Trp8 currents and the size of the currents depend on the frequency of stimulation (data not shown), the internal and external Ca^{2+} concentration

and the resting membrane potential, suggesting that Trp8 calcium conductance is intrically regulated by a Ca^{2+} mediated feedback mechanisms.

Example 6: Ca^{2+} / calmodulin binds to the C-terminus of the Trp8 protein

To test whether calmodulin, a prime mediator of calcium regulated feedback, is involved, first it was investigated biochemically whether Trp8 protein can bind calmodulin. Trp8 cDNA was in vitro translated in the presence of ^{35}S-methionine and the product incubated with calmodulin-agarose beads. After several washes either in the presence or abscence of Ca^{2+}, the beads were incubated in Laemmli buffer and subjected to SDS-polyacrylamide gel electrophoresis. In the presence of Ca^{2+} (1mM), but not in the absence of Ca^{2+}, Trp8 protein binds to calmodulin (Fig.: 4B).

To narrow down the binding site, two approaches were undertaken: Firstly, GST-TRP8 fusion proteins of various intracellular domains of Trp8 were constructed, expressed in E. coli and bound to gluthathione sepharose beads. These beads were then incubated with in vitro translated ^{35}S- labeled calmodulin, washed and subjected to gel electrophoresis. Secondly, truncated versions of in vitro translated Trp8 protein were used in the above described binding to calmodulin-agarose. As shown in Figure 4A, and C, fusion proteins of the N-terminal region (N1, N2) of Trp8 did not bind calmodulin, while C-terminal fragments (C1, C2, C3, C4) showed calmodulin binding in the presence of calcium (for localization of fragments within the entire Trp8 protein see Fig. 4C). Accordingly, a truncated version of in vitro translated Trp8, which lacks the C-terminal 32 amino acid residues did not bind to calmodulin-agarose (4B). We have restricted the calmodulin binding site to amino acid residues 691 to 711 of the Trp8 protein. This calmodulin binding site does not resemble the typical conserved IQ - motif of conventional myosins, but has limited sequence homology to the calcium dependent calmodulin binding site 1 of the transient receptor potential like (trpl) protein of Drosophila melanogaster (Warr and Kelly, 1996) with several charged amino acid residues conserved. The sequence of the calmodulin binding site of the Trp8 protein resembles a putative amphipathic α-helical wheel structure with a charged and a hydrophobic site according to a model proposed by Erickson-Vitanen and De Grado (1987, Methods Enzymol. 139, 455-478.).

Example 7: Expression of Trp8 transcripts in human placenta and pancreas

Several slides from a human placenta of a ten week old abort were used for in situ hybridization experiments. The in situ hybridization experiments revealed expression of Trp8 transcripts in human placenta (Fig.: 5B). Expression was detectable in trophoblasts and syncytiotrophoblasts of the placenta, but not in Langhans cells.

Trp8 transcripts are detectable in human pancreas (Fig.: 5A). Therefore Trp8 probes were hybridized to tissue sections of human pancreas. The pancreatic tissues were removed from patients with pancreas cancer. Trp8 expression is detectable in pancreatic acinar cells, but not in Langerhans islets (Fig.: 5C). No Trp8 expression was found in regions of pancreatic carcinomas (data not shown).

Furthermore, the Trp8 cDNA is not detectable in human colon nor in human kidney by in situ hybridization as well as by Northern analysis (Fig.: 5A, D). The Northern results taken together with the in situ expression data indicate that the Trp8 protein is not the human ortholog of the CaT1 and ECaC channels cloned from rat intestine (Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M. and Hediger M.A.(1999) J Biol Chem. 6;274, 22739-22746) and from rabbit kidney (Hoenderop, J.G., van der Kemp, A.W., Hartog, A., van de Graaf, S.F., van Os, C.H., Willems, P.H. and Bindels, R.J. (1999) J Biol Chem. 26;274, 8375-8378), respectively. Trp8 is unlikely to represent the human version of CaT1 as its expression is undetectable in the small intestine and colon tissues where CaT1 is abundantly expressed. If, however, Trp8 is the human version of rat CaT1, a second gene product appears to be required for Ca^{2+} uptake in human small intestine and colon attributed to CaT1 in rat small intestine and colon.

Example 8: Differential expression of Trp8 transcripts in benign and malign tissue of the prostate

The Trp8 transcripts are expressed in human prostate as shown by hybridization of a Trp8 probe to a commercial Northern blot (Clontech, Palo Alto, USA) (Fig.: 5A). Trp8 transcripts were not detectable by Northern blot analysis using pooled mRNA of patients with benign prostatic hyperplasia (BPH) (Fig.: 5A, prostate*). To examine Trp8 expression on the cellular

level, sections of prostate tissues were hybridized using Trp8 specific cDNA probes (Table 3). Expression of Trp8 transcripts is not detectable in normal prostate (n = 3), benign hyperplasia (BPH, n = 15) or prostatic intraepithelial neoplasia (PIN, n = 9) (Fig.: 6A, C, E). Trp8 transcripts were only detectable in prostate carcinoma (PCA), although with different expression levels. Low expression levels were found in primary carcinomas (2 - 10 % of the carcinoma cells, n = 8) (Fig.: 7B) . Much stronger expression was detectable in rezidive carcinoma (10 - 60 %) (Fig.: 7D, n = 6) and metastases of the prostate (60 - 90 %, n = 4) (Fig.: 7F). Thus it has to be concluded that the commercial Northern blot used in Fig.: 5A contains not only normal prostate mRNA as indicated by the distributor. According to the distributors instructions the prostate mRNA used for this Northern blot was collected from 15 human subjects in the range of 14 to 60 years of age. This prostate tissue was not examined by pathologic means. Since Trp8 expression is not detectable in normal or benign prostate, this finding implicates that the mRNA used for this Northern blot was extracted in part from prostatic carcinoma tissue. To summarize, Trp8 expression is only detectable in malign prostate and, thus, the Trp8 cDNA is a marker for prostate carcinoma. The results are summarized in Table 4.

Table 3

Trp8 probes used for in situ hybridization:

Probes (antisense)

1.) 5' TCCGCTGCCGGTTGAGATCTTGCC 3'

2.) 5' CTTGCTCCATAGGCAGAGAATTAG 3'

3.) 5' ATCCTCAGAGCCCCGGGTGTGGAA3'

Controls (sense)

1.) 5' GGCAAGATCTCAACCGGCAGCGGA 3'

2.) 5' CTAATTCTCTGCCTATGGAGCAAG 3'

3.) 5' TTCCACACCCGGGGCTCTGAGGAT 3'

Table 4

Prostate	total	negative	positive
normal	3	3	0
BPH	15	15	0
PIN	9	9	0

carcinoma	18	1	17

(B) Differential expression of Trp8 transcripts in benign and malign tissue of the uterus

Moreover it could be shown that Trp8 is expresed in endometrial cancer (also called cancer of the uterus, to be distinguished from uterine sarcoma or cancer of the cervix) whereas no expression was observed in normal uterus tissue. Thus, Trp8 also is a specific marker for the diagnosis of the above cancer (Fig. 12).

Example 9: Characterization of Trp9

The complete protein coding sequence of Trp9 was determined (Fig. 9). Trp 9 transcripts are predominantly expressed in the human prostate and in human colon. As it could be shown by Northern blot analysis, there is no difference of the expression of TRP9 in benigne prostata hyperplasia (BPH, Fig. 13, upper panel left) or prostate carcinoma (Fig. 13, upper panel right). However, Trp9 is useful as a reference marker for prostata carcinoma, i.e. can be used for quantifying the expression level of Trp8. The ratio of the expression of Trp8:Trp9 in patients and healthy individuals is useful for the development of a quantitative assay.

Example 10: Characterization of Trp10

The complete protein coding sequence of TRP10 (a and b) was determined by biocomputing (Fig. 10 and 11). Using a 235 bp fragment of the Trp10 cDNA as probe in Northern blot analysis TRP10 transcripts could only be detected in mRNA isolated from individuals with prostate cancer (Fig. 13, bottom panel) but not in mRNA isolated from benign tissue of the prostate (prostate BPH) nor in mRNA isolated from heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. The 235 bp cDNA fragment of the Trp10 cDNA was amplified using the primer pair UW248 5'-ACA GCT GCT GGT CTA TTC C-3' and UW249 5'-TAT

GTG CCT TGG TTT GTA CC-3' and prostate cDNA as template. In summary, Trp10a and Trp10b , like TRP8 are also expressed in malignant prostate tissue. So far, its expression could not be observed in any other tissue examined (see above). Thus, Trp 10a and Trp10b are also useful markers which are specific for malignant prostate tissue.

Furthermore, database searches in public databases of the national center for biological information (NCBI) revealed the existence of several expressed sequence tags (EST clones) being in part identical to the Trp10 sequence. These EST clones were originally isolated from cancer tissues of lung, placenta, prostate and from melanoma. These clones include the clones with the following accession numbers: BE274448, BE408880, BE207083, BE791173, AI671853, BE390627. The results demonstrate that cancer cells of these tissues express Trp10 related transcripts whereas no expression of Trp10 transcripts in the corresponding healthy tissues are detectable (Figure 13). Furthermore, it could be shown that in cancer cells of melanoma and prostate cancer Trp10 transcripts are expressed as shown by in situ hybridizations using 4 antisense probes (Figure 14A – E and 13K-O and Table 2, above). Furthermore, it could clearly be shown that cancer cells of these tissues expressing Trp10 transcripts also express Trp10-antisense transcripts as shown in Figure 14F-J, Figure 14P-R and Figure14T by in situ hybridizations using 4 sense probes (Table 2, above). The in situ hybridization experiments demonstrate that detection of a subset of cancer cells derived from carcinoma of lung, placenta, prostate and melanoma is feasible using antisense as well as sense probes complementary to Trp10 transcripts or complementary to Trp10-antisense transcripts, respectively.

The foregoing is meant to illustrate but not to limit the scope of the invention. The person skilled in the art can readily envision and produce further embodiment, based on the above teachings, without undue experimentation.

What Is claimed Is:

1. An isolated nucleic acid molecule encoding the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b or a protein exhibiting biological properties of Trp8a, Trp8b, Trp9, Trp10a or Trp10b and being selected from the group consisting of

(a) a nucleic acid molecule encoding a protein that comprises the amino acid sequence depicted in Figure 7, 8A, 9, 10 or 11;

(b) a nucleic acid molecule comprising the nucleotide sequence depicted in Figure 7, 8A, 9, 10 or 11;

(c) a nucleic acid molecule included in DSMZ Deposit No. DSM 13579, DSM 13580, DSM 13584 , DSM 13581 or DSM....;

(d) a nucleic acid molecule which hybridizes to a nucleic acid molecule specified in (a) to (c);

(e) a nucleic acid molecule the nucleic acid sequence of which deviates from the nucleic sequences specified in (a) to (d) due to the degeneration of the genetic code; and

(f) a nucleic acid molecule, which represents a fragment, derivative or allelic variation of a nucleic acid sequence specified in (a) to (e).

2. A recombinant vector containing the nucleic acid molecule of claim 1

3. The recombinant vector of claim 2 wherein the nucleic acid molecule is operatively linked to regulatory elements allowing transcription and synthesis of a translatable RNA in prokaryotic and/or eukaryotic host cells.

4. A recombinant host cell which contains the recombinant vector of claim 3.

5. The recombinant host cell of claim 4, which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.

6. An isolated protein exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b which is encoded by a nucleic acid molecule of claim 1.

7. A recombinant host cell that expresses the isolated protein of claim 6.

8. A method of making an isolated protein exhibiting biological properties of the human prostate carcinoma associated protein Trp8a, Trp8b, Trp9, Trp10a or Trp10b comprising:
(a) culturing the recombinant host cell of claim 6 under conditions such that said protein is expressed; and
(b) recovering said protein.

9. The protein produced by the method of claim 8.

10. An antisense RNA sequence characterized in that it is complementary to an mRNA transcribed from a nucleic acid molecule of claim 1 or a part thereof and can selectively bind to said mRNA or part thereof, said sequence being capable of inhibiting the synthesis of the protein encoded by said nucleic acid molecule.

11. A ribozyme characterized in that it is complementary to an mRNA transcribed from a nucleic acid molecule of claim 1 or a part thereof and can selectively bind to and cleave said mRNA or part thereof, thus inhibiting the synthesis of the protein encoded by said nucleic acid molecule.

12. An inhibitor characterized in that it can suppress the activity of the protein of claim 6.

13. A method for diagnosing a prostate carcinoma which comprises contacting a target sample suspected to contain the protein Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA with a reagent which reacts with Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA and detecting Trp8a, Trp8b, Trp10a and/or Trp10b or the Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA.

14. The method of claim 13, wherein the reagent is a nucleic acid.

15. The method of claim 13, wherein the reagent is an antibody.

16. The method of claim 13, wherein the reagent is detectably labeled.

17. The method of claim 16, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

18. A method for diagnosing an endometrial cancer (carcinoma of the uterus) which comprises contacting a target sample suspected to contain the protein Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA with a reagent which reacts with Trp8a and/or Trp8b or the Trp8a and/or Trp8a and/or trp8b encoding mRNA and detecting Trp8a and/or Trp8b or the Trp8a and/or Trp8b encoding mRNA.

19. The method of claim 18, wherein the reagent is a nucleic acid.

20. The method of claim 18, wherein the reagent is an antibody.

21. The method of claim 18, wherein the reagent is detectably labeled.

22. The method of claim 21, wherein the label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

23. A method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and of the prostate in a tissue of a subject, comprising contacting a sample with a reagent which detects Trp10a and/or Trp10b antisense RNA or Trp10a and/or Trp10b related antisense RNA.

24. A method for preventing, treating, or ameliorating a prostate tumor, endometrial cancer (carcinoma of the uterus) tumor, a chorion carcinoma, cancer of the lung or melanoma, which comprises administering to a mammalian subject a therapeutically effective amount of a reagent which decreases or inhibits expression of Trp8a, Trp8b, Trp10a and/or Trp10b and/or the activity of Trp8a, Trp8b, Trp10a and/or Trp10b.

25. The method of claim 24, wherein the reagent is a nucleotide sequence comprising an antisense RNA.

26. The method of claim 24, wherein the reagent is a nucleotide sequence comprising a ribozyme.

27. The method of claim 24, wherein the reagent is an inhibitor of Trp8a, Trp8b, Trp10a and/or Trp10b.

28. The method of claim 27, wherein the reagent is an anti-Trp8a-, anti Trp8b-, anti-Trp10a- and/or anti-Trp10b antibody or a fragment thereof.

29. A diagnostic kit useful for the detection of Trp8a, Trp8b, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts in a sample, wherein the presence of an increased concentration of Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b or Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts is indicative for a prostate tumor, endometrial cancer (cancer of the uterus) tumor, a chorion carcinoma, cancer of the lung or melanoma, said kit comprising a probe for detection of Trp8a, Trp8b, Trp9, Trp10a or Trp10b or Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b encoding mRNA or Trp10a and/or Trp10b antisense transcripts.

30. The kit of claim 29, wherein the target component to be detected is Trp8a, Trp8b, Trp9, Trp10a and/or Trp10b and the probe is an antibody.

31. A method for identifying a compound which acts as an agonist or antagonist on the ion channels Trp8, Trp9 and/or Trp10, said method comprising contacting a test compound with the ion channel Trp8, Trp9 and/or Trp10,and determining whether said test compound affects the calcium uptake.

Figs. 1A and 1B

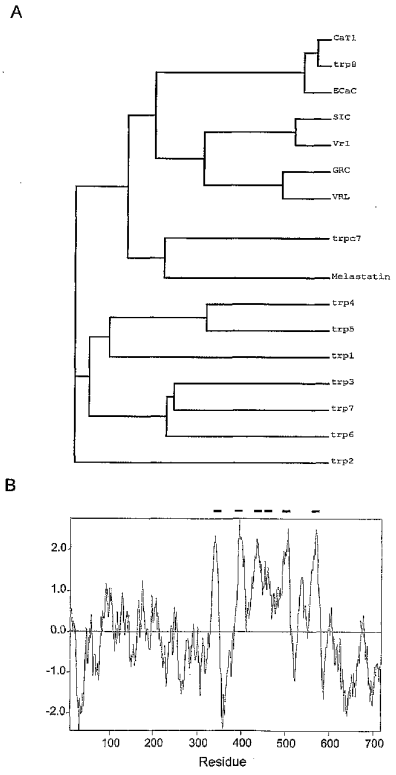
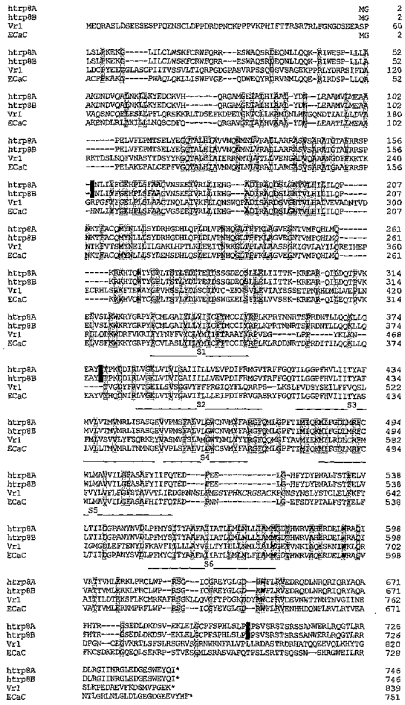
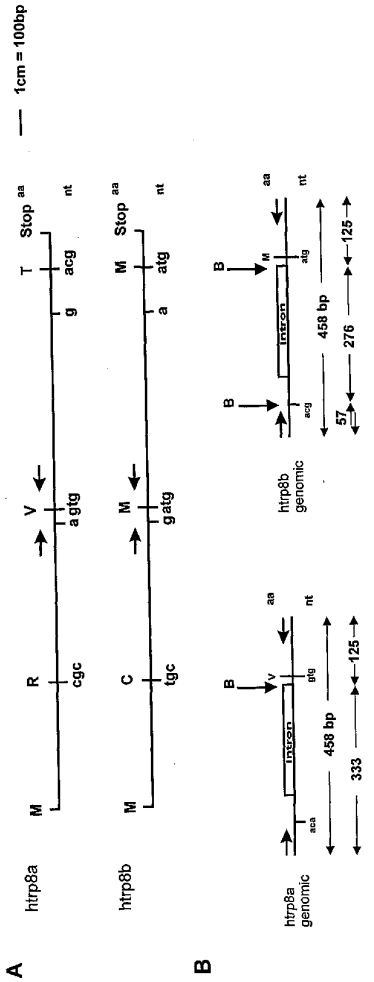


Fig. 1C

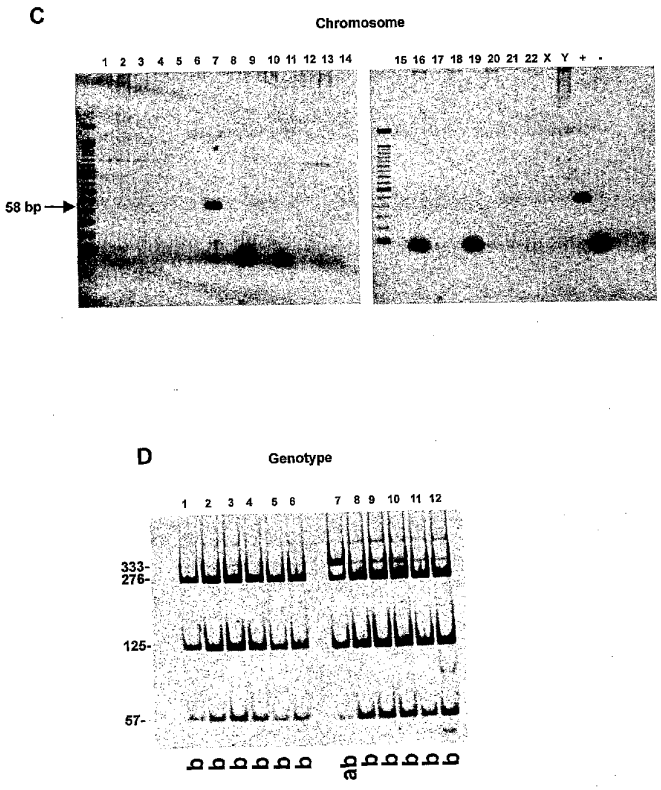
C



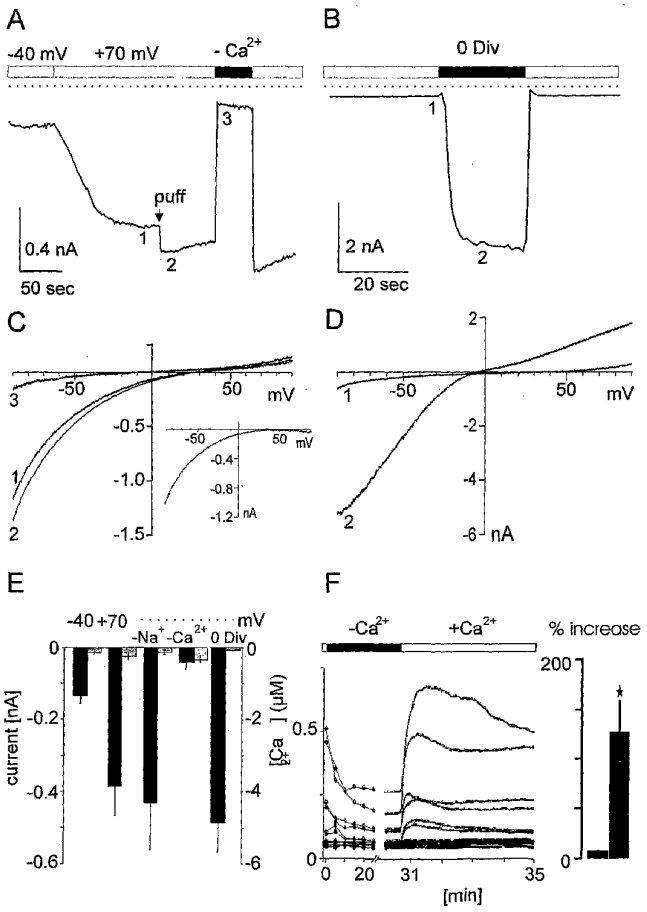


Figs. 2A and 2B

Figs. 2C and 2D



Figs. 3A – 3F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+70 mV
- Ca2+
puff
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B
0 Div
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20 sec
C
mV
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nA
E
-40 +70
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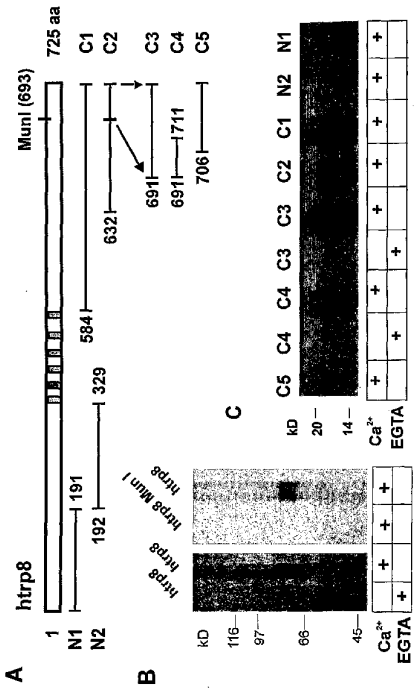
F
-Ca2+
+Ca2+
% increase
[min]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MunI (693)
N1
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C1
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C2
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C3
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691
711
C5
706
B
htrp8 MunI
htrp8
htrp8
kD
116
97
66
45
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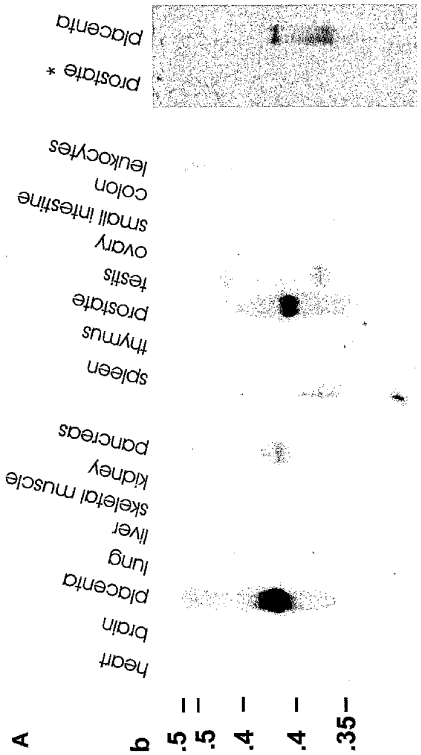
C
kD
20
14
C5 C4 C4 C3 C3 C2 C1 N2 N1
Ca2+
EGTA

Figs. 4A – 4C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prostate *
leukocytes
colon
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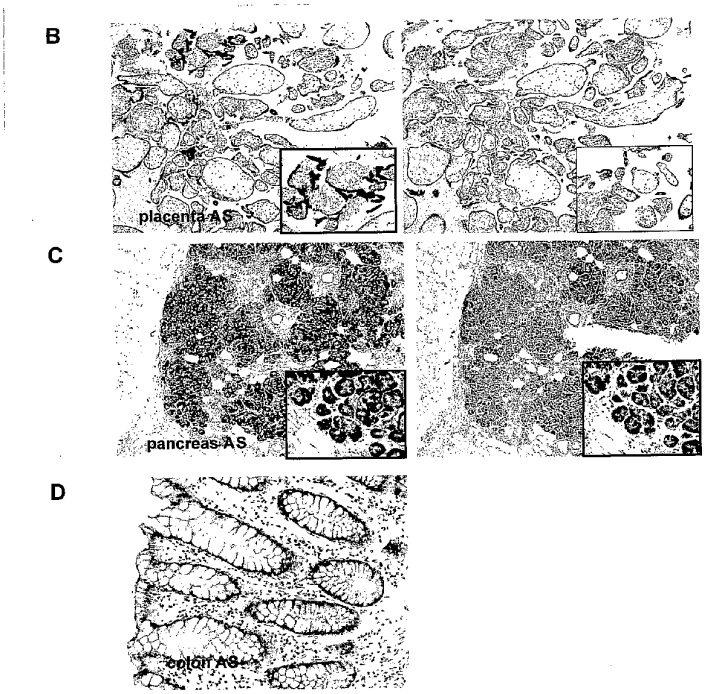
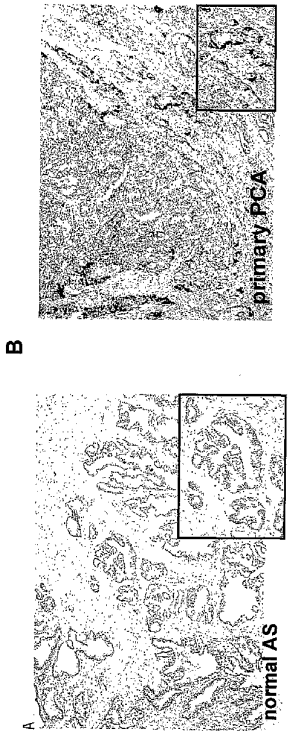
spleen
pancreas
kidney
skeletal muscle
liver
lung
placenta
brain
heart
A
b
.5
.5
.4
.4
.35

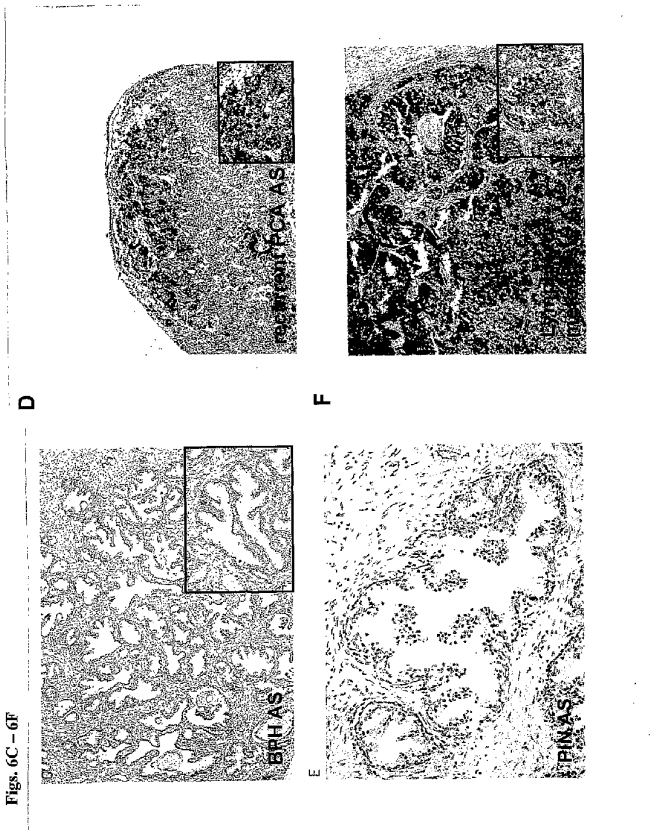
Fig. 5A

Figs. 5B – 5D





Figs. 6A and 6B



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Figs. 6C – 6F

Fig. 7

```
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       130                 150                 170
GGCCCTTGGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGTCTGGCCTCGGCC
       190                 210                 230
TCAGGCCCCCAAGGAGCCGGCCCTACACCCATGGGTTTGTCACTGCCCAAGGAGAAAGG
                                  M  G  L  S  L  P  K  E  K  G
       250                 270                 290
GCTAATTCTCTGCCTATGGAGCAAGTTCTGCAGATGGTTCCAGAGACGGGAGTCCTGGGC
 L  I  L  C  L  W  S  K  F  C  R  W  F  Q  R  R  E  S  W  A
       310                 330                 350
CCAGAGCCGAGATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCCTCTCCT
 Q  S  R  D  E  Q  N  L  L  Q  Q  K  R  I  W  E  S  P  L  L
       370                 390                 410
TCTAGCTGCCAAAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTG
 L  A  A  K  D  N  D  V  Q  A  L  N  K  L  L  K  Y  E  D  C
       430                 450                 470
CAAGGTGCACCAGAGAGGAGCCATGGGGGAAACAGCGCTACACATAGCAGCCCCTCTATGA
 K  V  H  Q  R  G  A  M  G  E  T  A  L  H  I  A  A  L  Y  D
       490                 510                 530
CAACCTGGAGGCCGCCATGGTGCTGATGGAGGCTGCCCCGGGAGCTGGTCTTTGAGCCCAT
 N  L  E  A  A  M  V  L  M  E  A  A  P  E  L  V  F  E  P  M
       550                 570                 590
GACATCTGAGCTCTATGAGGGTCAGACTGCACTGCACATCGCTGTTGTGAACCAGAACAT
 T  S  E  L  Y  E  G  Q  T  A  L  H  I  A  V  V  N  Q  N  M
       610                 630                 650
GAACCTGGTGCGAGCCCTGCTTGCCCGCAGGGCCAGTGTCTCTGCCAGAGCCACAGGCAC
 N  L  V  R  A  L  L  A  R  R  A  S  V  S  A  R  A  T  G  T
       670                 690                 710
TGCCTTCCGCCGTAGTCCCCGCAACCTCATCTACTTTGGGGAGCACCCTTTGTCCTTTGC
 A  F  R  R  S  P  R  N  L  I  Y  F  G  E  H  P  L  S  F  A
       730                 750                 770
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 A  C  V  N  S  E  E  I  V  R  L  L  I  E  H  G  A  D  I  R
       790                 810                 830
GGCCCAGGACTCCCTGGGAAACACAGTGTTACACATCCTCATCCTCCAGCCCAACAAAAC
 A  Q  D  S  L  G  N  T  V  L  H  I  L  I  L  Q  P  N  K  T
       850                 870                 890
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 F  A  C  Q  M  Y  N  L  L  L  S  Y  D  R  H  G  D  H  L  Q
       910                 930                 950
GCCCCTGGACCTCGTGCCCAATCACCAGGGTCTCACCCCTTTCAAGCTGGCTGGAGTGGA
 P  L  D  L  V  P  N  H  Q  G  L  T  P  F  K  L  A  G  V  E
       970                 990                1010
GGGTAACACTGTGATGTTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGACCTA
 G  N  T  V  M  F  Q  H  L  M  Q  K  R  K  H  T  Q  W  T  Y
      1030                1050                1070
TGGACCACTGACCTCGACTCTCTATGACCTCACAGAGATCGACTCCTCAGGGGATGAGCA
 G  P  L  T  S  T  L  Y  D  L  T  E  I  D  S  S  G  D  E  Q
      1090                1110                1130
GTCCCTGCTGGAACTTATCATCACCACCAAGAAGCGGGAGGCTCGCCAGATCCTGGACCA
 S  L  L  E  L  I  I  T  T  K  K  R  E  A  R  Q  I  L  D  Q
      1150                1170                1190
GACGCCGGTGAAGGAGCTGGTGAGCCTCAAGTGGAAGCGGTACGGGCGGCCGTACTTCTG
 T  P  V  K  E  L  V  S  L  K  W  K  R  Y  G  R  P  Y  F  C
      1210                1230                1250
CATGCTGGGTGCCATATATCTGCTGTACATCATCTGCTTCACCATGTGCTGCATCTACCG
 M  L  G  A  I  Y  L  L  Y  I  I  C  F  T  M  C  C  I  Y  R
      1270                1290                1310
```

CCCCCTCAAGCCCAGGACCAATAACCGCACAAGCCCCCGGGACAACACCCTCTTACAGCA
P L K P R T N N R T S P R D N T L L Q Q
1330 1350 1370
GAAGCTACTTCAGGAAGCCTACGTGACCCTAAGGACGATATCCGGCTGGTCGGGGAGCT
K L L Q E A Y V T P K D D I R L V G E L
1390 1410 1430
GGTGACTGTCATTGGGGCTATCATCATCCTGCTGGTAGAGGTTCCAGACATCTTCAGAAT
V T V I G A I I I L L V E V P D I F R M
1450 1470 1490
GGGGGTCACTCGCTTCTTTGGACAGACCATCCTTGGGGGCCCATTCCATGTCCTCATCAT
G V T R F F G Q T I L G G P F H V L I I
1510 1530 1550
CACCTATGCCTTCATGGTGCTGGTGACCATGGTGATGCGGCTCATCAGTGCCAGCGGGGA
T Y A F M V L V T M V M R L I S A S G E
1570 1590 1610
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V V P M S F A L V L G W C N V M Y F A R
1630 1650 1670
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G F Q M L G P F T I M I Q K M I F G D L
1690 1710 1730
GATGCGATTCTGCTGGCTGATGGCTGTGGTCATCCTGGGCTTTGCTTCAGCCTTCTATAT
M R F C W L M A V V I L G F A S A F Y I
1750 1770 1790
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I F Q T E D P E E L G H F Y D Y P M A L
1810 1830 1850
GTTCAGCACCTTCGAGCTGTTCCTTACCATCATCGATGGCCCAGCCAACTACAACGTGGA
F S T F E L F L T I I D G P A N Y N V D
1870 1890 1910
CCTGCCCTTCATGTACAGCATCACCTATGCTGCCTTGCCATCATCGCCACACTGCTCAT
L P F M Y S I T Y A A F A I I A T L L M
1930 1950 1970
GCTCAACCTCCTCATTGCCATGATGGGCGACACTCACTGGCGAGTGGCCCATGAGCGGGA
L N L L I A M M G D T H W R V A H E R D
1990 2010 2030
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E L W R A Q I V A T T V M L E R K L P R
2050 2070 2090
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C L W P R S G I C G R E Y G L G D R W F
2110 2130 2150
CCTGCGGGTGGAAGACAGGCAAGATCTCAACCGGCAGCGGATCCAACGCTACGCACAGGC
L R V E D R Q D L N R Q R I Q R Y A Q A
2170 2190 2210
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F H T R G S E D L D K D S V E K L E L G
2230 2250 2270
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C P F S P H L S L P T P S V S R S T S R
2290 2310 2330
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S S A N W E R L R Q G T L R R D L R G I
2350 2370 2390
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I N R G L E D G E S W E Y Q I
2410 2430 2450
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2470 2490 2510
AACACCCAGAGGTCTCATCTCCCAGGCCCCAGGGAGAAAGAGGAGTAGCATGAACGCCAA
2530 2550 2570
GGAATGTACGTTGAGAATCACTGCTCCAGGCCTGCATTACTCCTTCAGCTCTGGGGCAGA

Fig. 7 / continuation 2

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      2590                2610                2630
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      2650                2670                2690
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      2710                2730                2750
GGCCCTCGGTCTCCACCCCAGGGTCATAAGTGGGGAGAGAGCCCTTCCCAGGGCACCCAG
      2770                2790                2810
GCAGGTGCAGGGAAGTGCAGAGCTTGTGGAAAGCGTGTGAGTGAGGGAGACAGGAACGGC
      2830                2850                2870
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      2890                2910
GATCCCTAAAAAAAAAAAAAAAAAAAAAAAAAA
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```

Figure 8:

A)

```
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  L  W  S  K  F  C  R  W  F  Q  R  R  E  S  W  A  Q  S  R  D
       310                  330                  350
ATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCCTCTCCTTCTAGCTGCCA
  E  Q  N  L  L  Q  Q  K  R  I  W  E  S  P  L  L  L  A  A  K
       370                  390                  410
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  D  N  D  V  Q  A  L  N  K  L  L  K  Y  E  D  C  K  V  H  Q
       430                  450                  470
AGAGAGGAGCCATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACCTGGAGG
  R  G  A  M  G  E  T  A  L  H  I  A  A  L  Y  D  N  L  E  A
       490                  510                  530
CCGCCATGGTGCTGATGGAGGCTGCCCCGGAGCTGGTCTTTGAGCCCATGACATCTGAGC
  A  M  V  L  M  E  A  A  P  E  L  V  F  E  P  M  T  S  E  L
       550                  570                  590
TCTATGAGGGTCAGACTGCACTGCACATCGCTGTTGTGAACCAGAACATGAACCTGGTGC
  Y  E  G  Q  T  A  L  H  I  A  V  V  N  Q  N  M  N  L  V  R
       610                  630                  650
GAGCCCTGCTTGCCCGCAGGGCCAGTGTCTCTGCCAGAGCCACAGGCACTGCCTTCCGCC
  A  L  L  A  R  R  A  S  V  S  A  R  A  T  G  T  A  F  R  R
       670                  690                  710
GTAGTCCCTGCAACCTCATCTACTTTGGGGAGCACCCTTTGTCCTTTGCTGCCTGTGTGA
  S  P  C  N  L  I  Y  F  G  E  H  P  L  S  F  A  A  C  V  N
```

Fig. 8 / continuation 1

```
      730                 750                 770
ACAGTGAGGAGATCGTGCGGCTGCTCATTGAGCATGGAGCTGACATCCGGGCCCAGGACT
  S  E  E  I  V  R  L  L  I  E  H  G  A  D  I  R  A  Q  D  S
      790                 810                 830
CCCTGGGAAACACAGTGTTACACATCCTCATCCTCCAGCCCAACAAAACCTTTGCCTGCC
  L  G  N  T  V  L  H  I  L  I  L  Q  P  N  K  T  F  A  C  Q
      850                 870                 890
AGATGTACAACCTGTTGCTGTCCTACGACAGACATGGGGACCACCTGCAGCCCCTGGACC
  M  Y  N  L  L  L  S  Y  D  R  H  G  D  H  L  Q  P  L  D  L
      910                 930                 950
TCGTGCCCAATCACCAGGGTCTCACCCCTTTCAAGCTGGCTGGAGTGGAGGGTAACACTG
  V  P  N  H  Q  G  L  T  P  F  K  L  A  G  V  E  G  N  T  V
      970                 990                1010
TGATGTTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGACGTATGGACCACTGA
  M  F  Q  H  L  M  Q  K  R  K  H  T  Q  W  T  Y  G  P  L  T
     1030                1050                1070
CCTCGACTCTCTATGACCTCACAGAGATCGACTCCTCAGGGGATGAGCAGTCCCTGCTGG
  S  T  L  Y  D  L  T  E  I  D  S  S  G  D  E  Q  S  L  L  E
     1090                1110                1130
AACTTATCATCACCACCAAGAAGCGGGAGGCTGCCCAGATCCTGGACCAGACGCCGGTGA
  L  I  I  T  T  K  K  R  E  A  R  Q  I  L  D  Q  T  P  V  K
     1150                1170                1190
AGGAGCTGGTGAGCCTCAAGTGGAAGCGGTACGGGCGGCCGTACTTCTGCATGCTGGGTG
  E  L  V  S  L  K  W  K  R  Y  G  R  P  Y  F  C  M  L  G  A
     1210                1230                1250
CCATATATCTGCTGTACATCATCTGCTTCACCATGTGCTGCATCTACCGCCCCCTCAAGC
  I  Y  L  L  Y  I  I  C  F  T  M  C  C  I  Y  R  P  L  K  P
     1270                1290                1310
CCAGGACCAATAACCGCACGAGCCCCCGGGACAACACCCTCTTACAGCAGAAGCTACTTC
  R  T  N  N  R  T  S  P  R  D  N  T  L  L  Q  Q  K  L  L  Q
     1330                1350                1370
AGGAAGCCTACATGACCCCTAAGGACGATATCCGGCTGGTCGGGGAGCTGGTGACTGTCA
  E  A  Y  M  T  P  K  D  D  I  R  L  V  G  E  L  V  T  V  I
     1390                1410                1430
TTGGGGCTATCATCATCCTGCTGGTAGAGGTTCCAGACATCTTCAGAATGGGGGTCACTC
  G  A  I  I  I  L  L  V  E  V  P  D  I  F  R  M  G  V  T  R
     1450                1470                1490
GCTTCTTTGGACAGACCATCCTTGGGGGCCCATTCCATGTCCTCATCATCACCTATGCCT
  F  F  G  Q  T  I  L  G  P  F  H  V  L  I  I  T  Y  A  F
     1510                1530                1550
TCATGGTGCTGGTGACCATGGTGATGCGGCTCATCAGTGCCAGCGGGGAGGTGGTACCCA
  M  V  L  V  T  M  V  M  R  L  I  S  A  S  G  E  V  V  P  M
     1570                1590                1610
TGTCCTTTGCACTCGTGCTGGGCTGGTGCAACGTCATGTACTTCGCCCGAGGATTCCAGA
  S  F  A  L  V  L  G  W  C  N  V  M  Y  F  A  R  G  F  Q  M
     1630                1650                1670
TGCTAGGCCCCTTCACCATCATGATTCAGAAGATGATTTTGGCGACCTGATGCGATTCT
  L  G  P  F  T  I  M  I  Q  K  M  I  F  G  D  L  M  R  F  C
     1690                1710                1730
GCTGGCTGATGGCTGTGGTCATCCTGGGCTTTGCTTCAGCCTTCTATATCATCTTCCAGA
  W  L  M  A  V  V  I  L  G  F  A  S  A  F  Y  I  I  F  Q  T
     1750                1770                1790
CAGAGGACCCCGAGGAGCTAGGCCACTTCTACGACTACCCCATGGCCCTGTTCAGCACCT
  E  D  P  E  E  L  G  H  F  Y  D  Y  P  M  A  L  F  S  T  F
     1810                1830                1850
TCGAGCTGTTCCTTACCATCATCGATGGCCCAGCCAACTACAACGTGGACCTGCCCTTCA
  E  L  F  L  T  I  I  D  G  P  A  N  Y  N  V  D  L  P  F  M
     1870                1890                1910
TGTACAGCATCACCTATGCTGCCTTTGCCATCATCGCCACACTGCTCATGCTCAACCTCC
  Y  S  I  T  Y  A  A  F  A  I  I  A  T  L  L  M  L  N  L  L
     1930                1950                1970
TCATTGCCATGATGGGCGACACTCACTGGCGAGTGGCCCATGAGCGGGATGAGCTGTGGA
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Fig. 8 / continu on 2

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 I  A  M  M  G  D  T  H  W  R  V  A  H  E  R  D  E  L  W  R
    1990              2010              2030
GGGCCCAGATTGTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCCTCGCTGCCTGTGGC
 A  Q  I  V  A  T  T  V  M  L  E  R  K  L  P  R  C  L  W  P
    2050              2070              2090
CTCGCTCCGGGATGTGCGGACGGGAGTATGGCCTGGGAGACCGCTGGTTCCTGCGGGTGG
 R  S  G  I  C  G  R  E  Y  G  L  G  D  R  W  F  L  R  V  E
    2110              2130              2150
AAGACAGGCAAGATCTCAACGGCAGCGGATCCAACGCTACGCACAGGCCTTCCACACCC
 D  R  Q  D  L  N  R  Q  R  I  Q  R  Y  A  Q  A  F  H  T  R
    2170              2190              2210
GGGCCTCTGAGGATTTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGGCTGTCCCTTCA
 G  S  E  D  L  D  K  D  S  V  E  K  L  E  L  G  C  P  F  S
    2230              2250              2270
GCCCCCACCTGTCCCTTCCTATGCCCTCAGTGTCTCGAAGTACCTCCCGCAGCAGTGCCA
 P  H  L  S  L  P  M  P  S  V  S  R  S  T  S  R  S  S  A  N
    2290              2310              2330
ATTGGGAAAGGCTTCGGCAAGGGACCCTGAGGAGAGACCTGCGTGGGATAATCAACAGGG
 W  E  R  L  R  Q  G  T  L  R  R  D  L  R  G  I  I  N  R  G
    2350              2370              2390
GTCTGGAGGACGGGGAGAGCTGGGAATATCAGATCTGA
 L  E  D  G  E  S  W  E  Y  Q  I  *
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MGLSLPKEKGLILCLWSKFCRWFQRRESWAQSRDEQNLLQQKRIWESPLLLAAKDNDVQALNKLLKYEDCKVHQRGAMGETALHIA
ALYDNLEAANVLMEAAPELVFEPMTSELYEGQTALHIAVVNQNMNLVRALLARRASVSARATGTAFRRSPCNLIYFGEHPLSFAAC
VNSEEIVRLLIEHGADIRAQDSLGNTVLHILILQPNKTFACQMYNLLLSYDRHGDHLQPLDLVPNHQGLTPFKLAGVEGNTVMFQH
LMQKRKHTQWTYGPLTSTLYDLTEIDSSGDEQSLLELIITTKKRRARQILDQTPVKELVSLKWKRYGRPYFCMLGAIYLLYIICFT
MCCIYRELKPRTNNRTSPRDNTLLQQKLLQEAYMTPKDPIRLVGELVTVIGAIIILVEVPDIFRMGVTRFFGQTILGGPFHVLII
TYAFMVLVTMVMRLISASGEVVPMSFALVLGWCNVHYFARGFQMLGPFTINIQKMIFGDLMRFCWLMAVVILGFASAFYIIFQTED
PSELGHFYDYPMALFSTFELFLTIIDGPANYNVDLPFMYSITYAAFAIIATLLMLNLLIAMMGDTHWRVAHERDELWRAQIVATTV
MLERKLPRCLWPRSGICGREYGLGDRWFLRVEDRQDLNRQRIQRYAQAFHTRGSEDLDKDSVEKLELGCPFSPHLSLPMPSVSRST
SRSSANWERLRQGTLRRDLRGIINRGLEDGESWEYQI
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b)

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CAAACTCACAGCCCTCTCCAAACTGGCTGGGGCTGCTGGGAGACTCCCAAGGAACTGGTCAGGAAGGCAGGAGACAGGAGACGGGA
CCTCTACAGGGAGACGGTGGGCCGGCCCTTGGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGTCTGGCCTCGGCCTCA
GGCCCCCAAGGAGCCGGCCCTACACCCATGGGTTTGTCACTGCCCAAGGAGAAAGGCTAATTCTCTGCCTATGGAGCAAGTTCT
GCAGATGGTTCCAGAGACGGGAGTCCTGGGCCCAGAGCCCAGATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCCT
CTCCTTCTAGCTGCCAAAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTGCAAGGTGCACCAGAGAGGAGC
CATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACCTGGAGCCCGCCATGGTGCTGATGGAGGCTGCCCCGGAGCTGG
TCTTTGAGCCCATGACATCTGAGCTCTATGAGGGTCAGACTGCACTGCACATCGCTGTTGTGAACCAGAACATGAACCTGGTGCCA
GCCCTGCTTGCCCCGCAGGGCCAGTCTCTCTGCCAGAGCCACAGGCACTGCCTTCCGCGTAGTCCCCGCAACCTCATCTACTTTGG
GGAGCACCCTTTGTCCTTTGCTGCCTGTGTGAACAGTGAGGAGATCGTGCGGCTGCTCATTGAGCATGGAGCTGACATCCGGCCCC
AGGACTCCCTGGCCCAACAAAACCTTTGCCTGCCAGATGTACAACCTGTTGCTGTCCTACGACAGACATGGGGACCACCTGCAGCC
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GAGCCTCAAGTGGAAGCGGTACGCGGCGGCCGTACTTCTGCATGCTGGGTGCCATATATCTGCTGTACATCATCTGCTTCACCATGT
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GAAGCCTACGTGACCCCTAAGGACGATATCCGGCTGGTCGGGGAGCTGGTGACTGTCATTGGGGCTATCATCATCCTGCTGGTAGA
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ATGCCTTCATGGTGCTGGTGACCATGGTGATGCGGCTCATATGATTTTGGCGACCTGATGCGATTCTGCTGGCTGATGGCTGTGG
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GCCCTGTTCAGCACCTTCGAGCTGGTCTTACCATCATCGATGGCCCAGCCAACTACAACGTGGACCTGCCCTCATGTACAGCAT
CACCTATGCTGCCTTTGCCATCATCGCCACACTGCTCATGCTCAACCTCCTCATTGCCATGATGGGCGACACTCACTGGCGAGTGG
CCCATGAGCGGGATGAGCTGTGGAGGGCCCAGATTGTGGCCACCACGGTGATGCTGGAGCGGAAGCTGCCTCGCTGCCTGTGGCCT
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GATCCAACGCTACGCACAGGCCTTCCACACCCGGGGCTCTGAGGATTTGGACAAAGACTCAGTGGAAAAACTAGAGCTGGGCTGTC
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GGGACCCTGAGGAGAGACCTGCGTGGGATAATCAACAGGGGTCTGGAGGACGGGGAGAGCTGGGAATATCAGATCTGACTGCGTGT
TCTCACTTCGCTTCCTGGAACTTGCTCTCATTTTCCTGGGTGCATCAAACAAAACAAAAACCAAACACCCAGAGGTCTCATCTCCC
AGGCCCCAGGGAGAAAGAGGAGTAGCATGAACGCCAAGGAATGTACGTTGAGAATCACTGCTCCAGGCCTGCATTACTCCTTCAGC
TCTGGGGCAGAGGAAGCCCAGCCCAAGCACGGGGCTGGCAGGCGTGAGGAACTCTCCTGTGGCCTGCTCATCACCCTCCGACAG
GAGCACTGCATGTCAGACCACTTTAAAAACAGGCCAGCCTGCTTGGCCCTCGGTCTCCACCCCAGGGTCATAAGTGCGCAGAGG
CCCTTCCCAGGGCACCCAGGCAGGTGCAGGGAAGTGCAGAGCTTGTGGAAGCGTGTGACTGAGGGAGACAGGAACGGCTCTGGGG
GTGGGAAGTGGGGCTAGGTCTTGCCAACTCCATCTTCAATAAAGTCGTTTTCGGATCCCTAAAAAAAAAAAAAAAAAAAAAAAAA

c.)

CAAACTCACAGCCCTCTCCAAACTGGCTGGGGCTGCTGGGAGACTCCCAAGGAACTCGTCAGGAAGGCAGGAGACAGGAGACGGGA
CCTCTACAGGGAGAGGTGGGCCGGCCTTGGCGGGGCCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGTCTGGCCTCGGCCTCA
GGCCCCCAAGGAGCCGGCCCTACACCCCATGGGTTTGTCACTGCCCAAGGAGAAAGGGCTAATTCTCTGCCTATGGAGCAAGTTCT
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CTCCTTCTAGCTGCCAAAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTCCAAGGTGCACCAGAGAGGAGC
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CCTTTCAAGCTGGCTGGAGTGGAGGGTAACACTGTGATGTTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGACGTATGG
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GTGCAGGGAAGTGCAGAGCTTGTGGAAAGCGTGTGAGTGACGGAGACAGGAACGGCTCTGGGGGTGGGAAGTGGGGCTAGGTCTTG
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D.)

CAAACTCACAGCCCTCTCCAAACTGGCTGGGGCTGCTGGGAGACTCCCAAGGAACTCGTCAGGAAGGCAGGAGACAGGAGACGGGA
CCTCTACAGGGAGAGCGGTGGGCCGGCCTTGGGGGGGCTGATGTGGCCCCAAGGCTGAGTCCCGTCAGGGTCTGGCCTCGGCCTCA
GGCCCCCAAGGAGCCGGCCCTACACCCCATGGGTTTGTCACTGCCCAAGGAGAAAGGGCTAATTCTCTGCCTATGGAGCAAGTTCT
GCAGATGGTTCCAGAGACGGGAGTCCTGGGCCCAGAGCCGAGATGAGCAGAACCTGCTGCAGCAGAAGAGGATCTGGGAGTCTCCT
CTCCTTCTAGCTGCCAAAGATAATGATGTCCAGGCCCTGAACAAGTTGCTCAAGTATGAGGATTGCAAGGTGCACCAGAGAGGAGC
CATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACCTGGAGGCCGCCATGGTGCTGATGGAGGCTGCCCCGGAGCTGG
TCTTTGAGCCCATGACATCTGAGCTCTATGAGGGTCAGACTGCACTGCACATGGCTGTTGTGAACCAGAACATGAACCTGGTGCGA
GCCCTGCTTGCCCGCAGGGCCAGTGTCTCTGCCAGAGCCACAGGCACTGCCTTCCGCCGTAGTCCCGCAACCTCATGTACTTTGC

AAACACAGTGTTACACATCCTCATCCTCCAGCCCAACAAAACCTTTGCCTGCCAGATGTACAACCTGTTGCTGTCCTACGACAGAC
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GTGATGTTTCAGCACCTGATGCAGAAGCGGAAGCACACCCAGTGGACGTATGGACCACTGACCTCGACTCTCTATGACCTCACAGA
GATCGACTCCTCAGGGGATGAGCAGTCCCTGCTGGAACTTATCATCACCACCAAGAAGCGGGAGGCTCGCCAGATCCTGGACCAGA
CGCCGGTGAAGGAGCTGGTGAGCCTCAAGTGGAAGCGGTACGGGCGGCCGTACTTCTGCATGCTGGGTGCCATATAPCTGCTGTAC
ATCATCTGCTTCACCATGTGCTGCATCTACCGCCCCCTCAAGCCCAGCACCAATAACCGCACAAGCCCCCGGGACAACACCCTCTT
ACAGCAGAAGCTACTTCAGGAAGCCTACGTGACCCCTAAGGACGATATCCGGCTGGTCCGGGAGCTGGTGACTGTCATTGGGGCTA
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AGAAGATGATTTTTGGCGACCTGATGCGATTCTGCTGGCTGATGGCTGTGGTCATCCTGGGCTTTGCTTCAGCCTTCTATATCATC
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B.)

CACACATGGGGGCTGCCAGGAGTGCCCAGGACCTCGTGCTGTTGGCCTCTGAATCTATCGTCTCCAATCCGCTGTCCCACAGAAGC
CATATAACCCACCTCTCGTAAATGCCAGGAGCCATGGGGGAAACAGCGCTACACATAGCAGCCCTCTATGACAACCTGGAGGCCG
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CCTACTCTTTTTSTCTTCTCTGTCTCCCTTCCGTGTCAGTCCCTGACTGCCCATCACTTGAACGCCTGCCCCCTGAAATGCCAGGG
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CCCAGCCCAAGCACGGGGCTGGCAGGGCGTGAGGAACTCTCCTGTGGCCTGCTCATCACCCTTCCGACAGGAGCACTGCATGTCAG
AGCACTTTAAAAACAGGCCAGCCTGCTTGGGCCCTCGGTCTCCACCCCAGGGTCATAAGTGGGGAGAGAGCCCTTCCCAGGGCACC

Fig. 8 / continuation 5

CAGGCAGGTGCAGGGAAGTGCAGAGCTTGTGGAAAGCGTGTGAGTGAGGGAGACAGGAACGGCTCTGGGGGTGGGAAGTGGGGCTA
GGTCTTGCCAACTCCATCTTCAATAAAGTCGTTTTCGGATCCCTAAAAAAAAAAAAAAAAAAAAAAAAA

Figure 9:

A.

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        10                  30                  50
CGGGGCCCTGGGCTGCAGGAGGTTGCGGCGGCCGCGGCAGCATGGTGGTGCCGGAGAAGG
                                          M  V  V  P  E  K  E
        70                  90                 110
AGCAGAGCTGGATCCCCAAGATCTTCAAGAAGAAGACCTGCACGACGTTCATAGTTGACT
  Q  S  W  I  P  K  I  F  K  K  K  T  C  T  T  F  I  V  D  S
       130                 150                 170
CCACAGATCCGGCAGGGACCTTGTGCCAGTGTGGGCGCCCCCGGACCGCCCACCCCGCAG
  T  D  P  G  G  T  L  C  Q  C  G  R  P  R  T  A  H  P  A  V
       190                 210                 230
TGGCCATGGAGGATGCCTTCGGGGCAGCCGTGGTGACCGTGTGGGACAGCGATGCACACA
  A  M  E  D  A  F  G  A  A  V  V  T  V  W  D  S  D  A  H  T
       250                 270                 290
CCACGGAGAAGCCCACCGATGCCTACGGAGAGCTGGACTTCACGGGGGCCGGCCGCAAGC
  T  E  K  P  T  D  A  Y  G  E  L  D  F  T  G  A  G  R  K  H
       310                 330                 350
ACAGCAATTTCCTCCGGCTCTCTGACCGAACGGATCCAGCTGCAGTTTATAGTCTGGTCA
  S  N  F  L  R  L  S  D  R  T  D  P  A  A  V  Y  S  L  V  T
       370                 390                 410
CACGCACATGGGGCTTCCGTGCCCCGAACCTGGTGGTGTCAGTGCTGGGGGGATCGGGGG
  R  T  W  G  F  R  A  P  N  L  V  V  S  V  L  G  G  S  G  G
       430                 450                 470
GCCCCGTCCTCCAGACCTGGCTGCAGGACCTGCTGCGTCGTGGGCTGGTGCGGGCTGCCC
  P  V  L  Q  T  W  L  Q  D  L  L  R  R  G  L  V  R  A  A  Q
       490                 510                 530
AGAGCACAGGAGCCTGGATTGTCACTGGGGGTCTGCACACGGCCATCGGCCGGCATGTTG
  S  T  G  A  W  I  V  T  G  G  L  H  T  G  I  G  R  H  V  G
       550                 570                 590
GTGTGGCTGTACGGGACCATCAGATGGCCAGCACTGGGGGCACCAAGGTGGTGGCCATGG
  V  A  V  R  D  H  Q  M  A  S  T  G  G  T  K  V  V  A  M  G
       610                 630                 650
GTGTGGCCCCCTGGGGTGTGGTCCGGAATAGAGACACCCTCATCAACCCCAAGGGCTCGT
  V  A  P  W  G  V  V  R  N  R  D  T  L  I  N  P  K  G  S  F
       670                 690                 710
TCCCTGCGAGGTACCGGTGGCGCGGTGACCCGGAGGACGGGGTCCAGTTTCCCCTGGACT
  P  A  R  Y  R  W  R  G  D  P  E  D  G  V  Q  F  P  L  D  Y
       730                 750                 770
ACAACTACTCGGCCTTCTTCCTGGTGGACGACGGCACACACGGCTGCCTGGGGGGCGAGA
  N  Y  S  A  F  F  L  V  D  D  G  T  H  G  C  L  G  G  E  N
       790                 810                 830
ACCGCTTCCGCTTGCGCCTGGAGTCCTACATCTCACAGCAGAAGACGGGCGTGGGAGGGA
  R  F  R  L  R  L  E  S  Y  I  S  Q  Q  K  T  G  V  G  G  T
       850                 870                 890
CTGGAATTGACATCCCTGTCCTGCTCCTCCTGATTGATGGTGATGAGAAGATGTTGACGC
  G  I  D  I  P  V  L  L  L  L  I  D  G  D  E  K  M  L  T  R
       910                 930                 950
GAATAGAGAACGCCACCCAGGCTCAGCTCCCATGTCTCCTCGTGGCTGGCTCAGGGGGAG
  I  E  N  A  T  Q  A  Q  L  P  C  L  L  V  A  G  S  G  G  A
       970                 990                1010
CTGCGGACTGCCTGGCGGAGACCCTGGAAGACACTCTGGCCCCAGGGAGTGGGGGAGCCA
  A  D  C  L  A  E  T  L  E  D  T  L  A  P  G  S  G  G  A  R
      1030                1050                1070
GGCAAGGCGAAGCCCGAGATCGAATCAGGCGTTTCTTTCCCAAAGGGGACCTTGAGGTCC
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Fig. 9 / continu: n 1

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  Q  G  E  A  R  D  R  I  R  R  F  F  P  K  G  D  L  E  V  L
     1090              1110              1130
TGCAGGCCCAGGTGGAGAGGATTATGACCCGGAAGGAGCTCCTGACAGTCTATTCTTCTG
  Q  A  Q  V  E  R  I  M  T  R  K  E  L  L  T  V  Y  S  S  E
     1150              1170              1190
AGGATGGGTCTGAGGAATTCGAGACCATAGTTTGAAGGCCCTTGTGAAGGCCTGTGGGA
  D  G  S  E  E  F  E  T  I  V  L  K  A  L  V  K  A  C  G  S
     1210              1230              1250
GCTCGGAGGCCTCAGCCTACCTGGATCAGCTGCGTTTGGCTGTGGCTTGGAACCGCGTGG
  S  E  A  S  A  Y  L  D  E  L  R  L  A  V  A  W  N  R  V  D
     1270              1290              1310
ACATTGCCCAGAGTGAACTCTTTCGGGGGGACATCCAATGGCGGTCCTTCCATCTCGAAG
  I  A  Q  S  E  L  F  R  G  D  I  Q  W  R  S  F  H  L  E  A
     1330              1350              1370
CTTCCCTCATGGACGCCCTGCTGAATGACCGGCCTGAGTTCGTGCGCTTGCTCATTTCCC
  S  L  M  D  A  L  L  N  D  R  P  E  F  V  R  L  L  I  S  H
     1390              1410              1430
ACGGCCTCAGCCTGGGCCACTTCCTGACCCCGATGCGCCTGGCCCAACTCTACAGCGCGG
  G  L  S  L  G  H  F  L  T  P  M  R  L  A  Q  L  Y  S  A  A
     1450              1470              1490
CGCCCTCCAACTCGCTCATCCGCAACCTTTTGGACCAGGCGTCCCACAGCGCAGGCACCA
  P  S  N  S  L  I  R  N  L  L  D  Q  A  S  H  S  A  G  T  K
     1510              1530              1550
AAGCCCCAGCCCTAAAAGGGGAGCTGCGGAGCTCCGGCCCCCTGACGTGGGGCATGTGC
  A  P  A  L  K  G  G  A  A  E  L  R  P  P  D  V  G  H  V  L
     1570              1590              1610
TGAGGATGCTGCTGGGGAAGATGTGCGCGCCGAGGTACCCCTCCGGGGGCGCCTGGGACC
  R  M  L  L  G  K  M  C  A  P  R  Y  P  S  G  G  A  W  D  P
     1630              1650              1670
CTCACCCAGGCCAGGGCTTCGGGGACAGCATGTATCTGCTCTCGGACAAGGCCACCTCGC
  H  P  G  Q  G  F  G  E  S  M  Y  L  L  S  D  K  A  T  S  P
     1690              1710              1730
CGCTCTCGCTGGATGCTGGCCTCGGGCAGGCCCCCTGGAGCGACCTGCTTCTTTGGGCAC
  L  S  L  D  A  G  L  G  Q  A  P  W  S  D  L  L  L  W  A  L
     1750              1770              1790
TGTTGCTGAACAGGGCACAGATGGCCATGTACTTCTGGGAGATGGGTTCCAATGCAGTTT
  L  L  N  R  A  Q  M  A  M  Y  F  W  E  M  G  S  N  A  V  S
     1810              1830              1850
CCTCAGCTCTTGGCGCCTGTTTGCTGCTCCGGGTGATGCCACGCCTGGAGCCTGACGCTG
  S  A  L  G  A  C  L  L  L  R  V  M  A  R  L  E  F  D  A  E
     1870              1890              1910
AGGAGGCAGCACGGAGGAAAGACCTGGCGTTCAAGTTTGAGGGGATGGGCGTTGACCTCT
  E  A  A  R  R  K  D  L  A  F  K  F  E  G  M  G  V  D  L  F
     1930              1950              1970
TTGGCGAGTGCTATCGCAGCAGTGAGGTGAGGGCTGCCCGCCTCCTCCTCCGTCGCTGCC
  G  E  C  Y  R  S  S  E  V  R  A  A  R  L  L  L  R  R  C  P
     1990              2010              2030
CGCTCTGGGGGGATGCCACTTGCCTCCAGCTGGCCATGCAAGCTGACGCCCGTGCCTTCT
  L  W  G  D  A  T  C  L  Q  L  A  M  Q  A  D  A  R  A  F  F
     2050              2070              2090
TTGCCCAGGATGGGGTACAGTCTCTGCTGACACAGAAGTGGTGGGGAGATATGGCCAGCA
  A  Q  D  G  V  Q  S  L  L  T  Q  K  W  W  G  D  M  A  S  T
     2110              2130              2150
CTACACCCATCTGGGCCCTGGTTCTCGCCTTCTTTTGCCCTCCACTCATCTACACCCGCC
  T  P  I  W  A  L  V  L  A  F  F  C  P  P  L  I  Y  T  R  L
     2170              2190              2210
TCATCACCTTCAGGAAATCAGAAGAGGAGCCCACACGGGAGGAGCTAGAGTTTCACATGG
  I  T  F  R  K  S  E  E  E  P  T  R  E  E  L  E  F  D  M  D
     2230              2250              2270
ATAGTGTCATTAATGGGGAAGGGCCTGTCGGGACGGCGGACCCAGCCGAGAAGACGCCGC
  S  V  I  N  G  E  G  F  V  G  T  A  D  P  A  E  K  T  P  L
     2290              2310              2330
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TGGGGGTCCCGCGCCAGTGGGCCGTCCGGGTTGCTGCGGGGGCCGCTGCGGGGGGCGCC
  G  V  P  R  Q  S  G  R  P  G  C  C  G  G  R  C  G  G  R  R
     2350                 2370                 2390
GGTGCCTACGCCGCTGGTTCCACTTCTGGGGCGTGCCGGTGACCATCTTCATGGGCAACG
  C  L  R  R  W  F  H  F  W  G  V  P  V  T  I  F  M  G  N  V
     2410                 2430                 2450
TGGTCAGCTACCTGCTGTTCCTGCTGCTTTCTCGCGGGTGCTGCTCGTGGATTTCCAGC
  V  S  Y  L  L  F  L  L  F  S  R  V  L  L  V  D  F  Q  P
     2470                 2490                 2510
CGGCGCCGCCCGGCTCCCTGGAGCTGCTGCTCTATTTCTGGGCTTTCACGCTGCTGTGCG
  A  P  P  G  S  L  E  L  L  L  Y  F  W  A  F  T  L  L  C  E
     2530                 2550                 2570
AGGAACTGCGCCAGGGCCTGAGCGGAGGCGGGGGCAGCCTCGCCAGCGGGGGCCCCGGGC
  E  L  R  Q  G  L  S  G  G  G  G  S  L  A  S  G  G  P  G  P
     2590                 2610                 2630
CTGGCCATGCCTCACTGAGCCAGCGCCTGCGCCTCTACCTCGCCGACAGCTGGAACCAGT
  G  H  A  S  L  S  Q  R  L  R  L  Y  L  A  D  S  W  N  Q  C
     2650                 2670                 2690
GCGACCTAGTGGCTCTCACCTGCTTCCTCCTGGGCGTGGCTGCCGGCTGACCCCGGGTT
  D  L  V  A  L  T  C  F  L  L  G  V  G  C  R  L  T  P  G  L
     2710                 2730                 2750
TGTACCACCTGGGCCGCACTGTCCTCTGCATCGACTTCATGGTTTTCACGGTGCGGCTGC
  Y  H  L  G  R  T  V  L  C  I  D  F  M  V  F  T  V  R  L  L
     2770                 2790                 2810
TTCACATCTTCACGGTCAACAAACAGCTGGGGCCCAAGATCGTCATCGTGAGCAAGATGA
  H  I  F  T  V  N  K  Q  L  G  P  K  I  V  I  V  S  K  M  M
     2830                 2850                 2870
TGAAGGACGTGTTCTTCTTCCTCTTCTTCCTCGGCGTGTGGCTGGTAGCCTATGGCGTGG
  K  D  V  F  F  F  L  F  F  L  G  V  W  L  V  A  Y  G  V  A
     2890                 2910                 2930
CCACGGAGGGGCTCCTGAGGCCACGGGACAGTGACTTCCCAAGTATCCTGCGCCGCGTCT
  T  E  G  L  L  R  P  R  D  S  D  F  P  S  I  L  R  R  V  F
     2950                 2970                 2990
TCTACCGTCCCTACCTGCAGATCTTCGGGCAGATTCCCCAGGAGGACATGGACGTGGCCC
  Y  R  P  Y  L  Q  I  F  G  Q  I  P  Q  E  D  M  D  V  A  L
     3010                 3030                 3050
TCATGGAGCACAGCAACTGCTCGTGGGAGCCCGGCTTCTGGGCACACCCTCCTGGGGCCC
  M  E  H  S  N  C  S  S  E  P  G  F  W  A  H  P  P  G  A  Q
     3070                 3090                 3110
AGGCGGGCACCTGCGTCTCCCAGTATGCCAACTGGCTGGTGCTGCTCCTCGTCATCT
  A  G  T  C  V  S  Q  Y  A  N  W  L  V  V  L  L  L  V  I  F
     3130                 3150                 3170
TCCTGCTCGTGGCCAACATCCTGCTGGTCAACTTGCTCATTGCCATGTTCAGTTACACAT
  L  L  V  A  N  I  L  L  V  N  L  L  I  A  M  F  S  Y  T  F
     3190                 3210                 3230
TCNGCAAAGTACAGGGCAACAGCGATCTCTACTGGAAGGCGCAGCGTTACCGCCTCATCC
  G  K  V  Q  G  N  S  D  L  Y  W  K  A  Q  R  Y  R  L  I  R
     3250                 3270                 3290
GGGAATTCCACTCTCGGCCCGCGCTGGCCCCGCCCTTTATCGTCATCTCCCACTTGCGCC
  E  F  H  S  R  P  A  L  A  P  P  F  I  V  I  S  H  L  R  L
     3310                 3330                 3350
TCCTGCTCAGGCAATTGTGCAGGCGACCCCGGAGCCCCCAGCCGTCCTCCCCGGCCCTCG
  L  L  R  Q  L  C  R  R  P  R  S  P  Q  P  S  S  P  A  L  E
     3370                 3390                 3410
AGCATTTCCGGGTTTACCTTCTAAGGAAGCCGAGCGGAAGCTGCTAACGTGGGATCGG
  H  F  R  V  Y  L  S  K  E  A  E  R  K  L  L  T  W  E  S  V
     3430                 3450                 3470
TGCATAAGGAGAACTTTCTGCTGGCACGCGCTAGGGACAAGCGGGAGAGCGACTCCGAGC
  H  K  E  N  F  L  L  A  R  A  R  D  K  R  E  S  D  S  E  R
     3490                 3510                 3530
GTCTGAAGCGCACGTCCCAGAAGGTGGACTTGGCACTGAAACAGCTGGGACACATCCGCG
  L  K  R  T  S  Q  K  V  D  L  A  L  K  Q  L  G  H  I  R  E
```

Fig. 9 / continua ↑ 3

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      3550                3570                3590
AGTACGAACAGCGCCTGAAAGTGCTCGGAGCGGGAGGTCCAGCAGTGTAGCCGCGTCCTGG
  Y  E  Q  R  L  K  V  L  E  R  E  V  Q  Q  C  S  R  V  L  G
      3610                3630                3650
GGTGGGTGGCCGAGGCCCTGAGCCGCTCTGCCTTGCTGCCCCCAGGTGGGCCGCCACCCC
  W  V  A  E  A  L  S  R  S  A  L  L  P  P  G  G  P  P  P  P
      3670                3690                3710
CTGACCTGCCTGGGTCCAAAGACTGAGCCCTGCTGGCGGACTTCAAGGAGAAGCCCCCAC
  D  L  P  G  S  K  D  *
      3730                3750                3770
AGGGGATTTTGCTCCTAGAGTAAGCCTCATCTGGGCCTCGGCCCCCCSCACCTGGTGGCCT
      3790                3810                3830
TGTCCTTCAGGTGAGCCCCATGTCCATCTGGGCCACTGTCAGGACCACCTTTGGGAGTGT
      3850                3870                3890
CATCCTTACAAACCACAGCATGCCCGGCTCCTCCCAGAACCAGTCCCAGCCTGGGAGGAT
      3910                3930                3950
CAAGCCCTGGATCCCGGGCCGTTATCCATCTGGAGGCTGCAGGGTCCTTGGGGTAACAGG
      3970                3990                4010
GACCACAGACCCCTCACCACTCACAGATTCCTCACACTGGGGAAATAAAGCCATTTCAGA
      4030
CGMAAAAAAAAAAAAAAAAAA
```

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MVVPEKEQSWIPKLFKKKTCTTFIVDGTDPGGTLCQCGRPHTAHPAVAMEDAFGAAVVTVWDSDAHTTEKPTDAYELDFTGAGRKH
SNFLRLSDRTDPAAVYSLVTRTWGFRAFNLVVSVLGGSGGPVLQTWLQDLLRRGLVRAAQSTGAWIVTGGLHTGIGRHVGVAVRDH
QMASTGGTKVVAMGVAPWGVVRNRDTLINPKGSFPARYRWRGDPEDGVQFPLDYNYSAFFLVDDGTHGCLGGENRFRLRLESYISQ
QKTGVGGTGIDIPVLLLLTDGDEKMLTRIENATQAHVFCLLVAGSRGLGMPGGTLRAHLAQDGDHKANQSTNQLLLPKDLSLQEVE
SIDRKTLQSYSERLAVAWNRVDIAQSELFRGDIQWRSFHLEASLMDALLNDRPEFVRLLISHGLSLGHFLTPMRLAQLYSAAPSNS
LIRNLLDQASHSAGTKAPALKGGAAELRPPDVGHVLRMLLGKMCAPRYPSGGAWDPHPGQGFGESMYLLSDKATSPLSLRAGLGQA
PWSDLLLWALLLNRAQMAMYFWEMGSNAVSSALGACLLLRVMARLEPDAREAARRKDLAFKFEGMGVDLFGECYRSSEVRAARLLL
RRCFLWGDATCLQLAMQADARAFEAQDGVQSLLTQKWWGDMASTTPIWALVLAFFCPPLIYTRLITFRKSEEEPTREELEFDMDSV
INGEGPVGTADPAEKTPLGVPRQSGRPGCCGGRCGGRRCLRRWFHFWGVPVTIFMGNVVSYLLFLLLFSRVLLVDFQPAPPGSLEL
LLYFWAFTLLCEELRQCLSGGGGSLASGGPGPGHASLSQRLRLYLADSWNQCDLVALTCFLLGVGCRLTPGLYHLGRTVLCIDFMV
FTVRLLHIFTVNKQLGPKIVISFMMKDVFFFLFFLGVWLVAYGVATEGLLRPRDSDFPSILRRVFYRPYLQIFGQIPQEDMDVAL
MEHSNCSSEPGFWAHFPGAQAGTCVSQYANWLVVLLVIPLLVANILLVNLLIAMFSYTFGKVQGNSDLYWKAQRYRLIREFHERP
ALAPPFIVISHLRLLRQLCRRPRSPQPSSPALEHFRVYLSKEAERKLLTWESVHKENFLLARARDKRESDSERLKRTSQKVDLAL
KQLGHIREYEQRLKVLEREVQQCSRVLGWVAEALSRSALLPPGGPPPPDLPGSKD
```

B.)

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        10                  30                  50
ATCCAATGGCGGTCCTTCCATCTCGAAGCTTCCCTCATGGACGCCCTGCTGAATGACCGG
        70                  90                 110
CCTGAGTTCGTGCGCTTGCTCATTTCCCACGGCCTCAGCCTGGGCCACTTCCTGACCCCG
       130                 150                 170
ATGCGCCTGGCCCAACTCTACAGCCGCGGCGCCCTCCAACTCGCTCATCCGCAACCTTTTG
       190                 210                 230
GACCAGGCGTCCCACAGCGCAGGCACCAAAGCCCCAGCCCTAAAAGGGGAGCTGCGGAG
       250                 270                 290
CTCCGGCCCCCTGACGTGGGGCATGTGCTGAGGATGCTGCTGGGGAAGATGTGCGCGCCG
       310                 330                 350
AGATGTATCTGCTCTCGGACAAGGCCACCTCGCCGCTCTGGCTGGATGCTGGCCTCGGGC
  M  Y  L  L  S  D  K  A  T  S  P  L  S  L  D  A  G  L  G  Q
       370                 390                 410
AGGCCCCCTGCAGCGACCTGCTTCTTTGGGCACTGTTGCTGAACAGGGCACAGATGGCCA
  A  P  W  S  D  L  L  L  W  A  L  L  L  N  R  A  Q  M  A  M
       430                 450                 470
TGTACTTCTGGGAGATGGGTTCCAATGCAGTTTCCTCAGCTCTTGGGGCCTGTTTGCTGC
  Y  F  W  E  M  G  S  N  A  V  S  S  A  L  G  A  C  L  L  L
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Fig. 9 / continua 14

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      490                 510                 530
TCCGGGTGATGGCACGCCTGGAGCCTGACGCTGAGGAGGCAGCACGGAGGAAAGACCTGG
  R  V  M  A  R  L  E  P  D  A  E  E  A  A  R  R  K  D  L  A
      550                 570                 590
CGTTCAAGTTTGAGGGGATGGGCGTTGACCTCTTTGGCGAGTGCTATCGCAGCAGTGAGG
  F  K  F  E  G  M  G  V  D  L  F  G  E  C  Y  R  S  S  E  V
      610                 630                 650
TGAGGGCTGCCCGGCCTCCTCCTCCGTCGCTGCCCGCTCTGGGGGGATGCCACTTGCCTCC
  R  A  A  R  L  L  L  R  R  C  P  L  W  G  D  A  T  C  L  Q
      670                 690                 710
AGCTGGCCATGCAAGCTGACGCCCGTGCCTTCTTTGCCCAGGATGGGGTACAGTCTCTGC
  L  A  M  Q  A  D  A  R  A  F  F  A  Q  D  G  V  Q  S  L  L
      730                 750                 770
TGACACAGAAGTGGTGGGGAGATATGGCCAGCACTACACCCATCTGGGCCCTGGTTCTCG
  T  Q  K  W  W  G  D  M  A  S  T  T  P  I  W  A  L  V  L  A
      790                 810                 830
CCTTCTTTTGCCCTCCACTCATCTACACCCGCCTCATCACCTTCAGGAAACTCAGAAGAGG
  F  F  C  P  P  L  I  Y  T  R  L  I  T  F  R  K  S  E  E  E
      850                 870                 890
AGCCCACACGGGAGGAGCTAGAGTTTGACATGGATAGTGTCATTAATGGGGAAGGGCCTG
  P  T  R  E  E  L  E  F  D  M  D  S  V  I  N  G  E  G  P  V
      910                 930                 950
TCGGGACGGCGGACCCAGCCGAGAAGACCCCGCTGGGGGTCCCGCTGCCAGTCGGGCCGTC
  G  T  A  D  P  A  E  K  T  P  L  G  V  P  R  Q  S  G  R  P
      970                 990                1010
CGGGTTGCTGCGGGGGCCGCTGCGGGGGGCGCCGGTGCCTACGCCGCTGGTTCCACTTCT
  G  C  C  G  G  R  C  G  G  R  R  C  L  R  R  W  F  H  F  W
     1030                1050                1070
GGGGCGTGCCGGTGACCATCTTCATGGGCAACGTGGTCAGCTACCTGCTGTTCCTGCTGC
  G  V  P  V  T  I  F  M  G  N  V  V  S  Y  L  L  F  L  L  L
     1090                1110                1130
TTTTCTCGCGGGTGCTGCTCGTGGATTTCCAGCCGGCGCCGCCGGCTCCCTGGAGCTGC
  F  S  R  V  L  L  V  D  F  Q  P  A  P  P  G  S  L  E  L  L
     1150                1170                1190
TGCTCTATTTCTGGGCTTTCACGCTGCTGTGCGAGGAACTGCGCCAGGGCCTGAGCGGAG
  L  Y  F  W  A  F  T  L  L  C  E  E  L  R  Q  G  L  S  G  G
     1210                1230                1250
GCGGGGGCAGCCTCGCCAGCGGGGGCCCCGGGCCTGGCCATGCCTCACTGAGCCAGCGCC
  G  G  S  L  A  S  G  G  P  G  P  G  H  A  S  L  S  Q  R  L
     1270                1290                1310
TGCGCCTCTACCTCGCCGACAGCTGGAACCAGTGCGACCTAGTGGCTCTCACCTGCTTCC
  R  L  Y  L  A  D  S  W  N  Q  C  D  L  V  A  L  T  C  F  L
     1330                1350                1370
TCCTGGGCGTGGGCTGCCGGCTGACCCCGGGTTTGTACCACCTGGGCCGCACTGTCCTCT
  L  G  V  G  C  R  L  T  P  G  L  Y  H  L  G  R  T  V  L  C
     1390                1410                1430
GCATCGACTTCATGGTTTTCACGGTGCGGCTGCTTCACATCTTCACGGTCAACAAACAGC
  I  D  F  M  V  F  T  V  R  L  L  H  I  F  T  V  N  K  Q  L
     1450                1470                1490
TGGGGCCCAAGATCGTCATCGTGAGCAAGATGATGAAGGACGTGTTCTTCTTCCTCTTCT
  G  P  K  I  V  I  V  S  K  M  M  K  D  V  F  F  F  L  F  F
     1510                1530                1550
TCCTCGGCGTGTGGCTCGTAGCCTATGGCGTGGCCACGGAGGGGCTCCTGAGGCCACGGG
  L  G  V  W  L  V  A  Y  G  V  A  T  E  G  L  L  R  P  R  D
     1570                1590                1610
ACAGTGACTTCCCAAGTATCCTGCGCCGCGTCTTCTACCGTCCCTACCTGCAGATCTTCG
  S  D  F  P  S  I  L  R  R  V  F  Y  R  P  Y  L  Q  I  F  G
     1630                1650                1670
GGCAGATTCCCCAGGAGGACATGGACGTGGCCCTCATGGAGCACAGCAACTGCTCGTCGG
  Q  I  P  Q  E  D  M  D  V  A  L  M  E  H  S  N  C  S  S  E
     1690                1710                1730
AGCCCGGCTTCTGGGCACACCCTCCTGGGGCCCAGGCGGGCACCTGCGTCTCCCAGTATG
```

Fig. 9 / continuation 5

```
  P  G  F  W  A  H  P  P  G  A  Q  A  G  T  C  V  S  Q  Y  A
     1750              1770              1790
CCAACTGGCTGGTGGTGCTGCTCCTCGTCATCTTCCTGCTCGTGGCCAACATCCTGCTGG
  N  W  L  V  V  L  L  L  V  I  F  L  L  V  A  N  I  L  L  V
     1810              1830              1850
TCAACTTGCTCATTGCCATGTTCAGTTACACATTCGGCAAAGTACAGGGCAACAGCGATC
  N  L  L  I  A  M  F  S  Y  T  F  G  K  V  Q  G  N  S  D  L
     1870              1890              1910
TCTACTGGAAGGCGCAGCGTTACCGCCTCATCCGGGAATTCCACTCTCGGCCCGCGCTGG
  Y  W  K  A  Q  R  Y  R  L  I  R  E  F  H  S  R  P  A  L  A
     1930              1950              1970
CCCCGCCCTTTATCGTCATCTCCCACTTGCGCCTCCTGCTCAGGCAATTGTGCAGGCGAC
  P  P  F  I  V  I  S  H  L  R  L  L  L  R  Q  L  C  R  R  P
     1990              2010              2030
CCCGGAGCCCCCAGCCGTCCTCCCCGGCCCTCGAGCATTTCCGGGTTTACCTTTCTAAGG
  R  S  P  Q  P  S  S  P  A  L  E  H  F  R  V  Y  L  S  K  E
     2050              2070              2090
AAGCCGAGCGGAAGCTGCTAACGTGGGAATCGGTGCATAAGGAGAACTTTCTGCTGGCAC
  A  E  R  K  L  L  T  W  E  S  V  H  K  E  N  F  L  L  A  R
     2110              2130              2150
GCGCTAGGGACAAGCGGGAGAGCGACTCCGAGCGTCTGAAGCGCACGTCCCAGAAGGTGG
  A  R  D  K  R  E  S  D  S  E  R  L  K  R  T  S  Q  K  V  D
     2170              2190              2210
ACTTGGCACTGAAACAGCTGGGACACATCCGCGAGTACGAACAGCGCCTGAAAGTGCTGG
  L  A  L  K  Q  L  G  H  I  R  E  Y  E  Q  R  L  K  V  L  E
     2230              2250              2270
AGCGGGAGGTCCAGCAGTGTAGCCGCGTCCTGGGGTGGGTGGCCGAGGCCCTGAGCCGCT
  R  E  V  Q  Q  C  S  R  V  L  G  W  V  A  E  A  L  S  R  S
     2290              2310              2330
CTGCCTTGCTGCCCCCAGGTGGGCCGCCACCCCCTGACCTGCCTGGGTCCAAAGACTGAG
  A  L  L  P  P  G  G  P  P  P  P  D  L  P  G  S  K  D  *
     2350              2370              2390
CCCTGCTGGCGGACTTCAAGGAGAAGCCCCCACAGGGGATTTTGCTCCTAGAGTAAGGCT
     2410              2430              2450
CATCTGGGCCTCGGCCCCCGCACCTGGTGGCCTTGTCCTTGAGGTGAGCCCCATGTCCAT
     2470              2490              2510
CTGGGCCACTGTCAGGACCACCTTTGGGAGTGTCATCCTTACAAACCACAGCATGCCCGG
     2530              2550              2570
CTCCTCCCAGAACCAGTCCCAGCCTGGGAGGATCAAGGCCTGGATCCCGGGCCGTTATCC
     2590              2610              2630
ATCTGGAGGCTGCAGGGTCCTTGGGGTAACAGGGACCACAGACCCCTCACCACTCACAGA
     2650              2670              2690
TTCCTCACACTGGGGAAATAAAGCCATTTCAGAGGAAAAAAAAAAAAAAAAAAAAA
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MYLLSDKATSPLSLDAGLGQAPWSDLLLWALLLNRAQMAMYFWEMGSNAVSSALGACLLLRVMARLEPDAEEAARRKDLAFKFEGM
GVDLFGECYRSSEVRAARLLLRRCPLWGDATCLQLAMQADARAFFAQDGVQSLLTQKWWGDMASTTPIWALVLAFFCPPLIYTRLI
TFRKSEEEPTRREELEFDMDSVINGEGPVGTADPAEKTPLGVERQSGRPGCCGGRCGGRRCLRRWFHFWGVPVTIFMGNVVSYLLFL
LLFSRVLLVDFQPAPPGSLELLLYFWAFTLLCEELRQGLSGGGGSLASGGPGPGHASLSQRLRLYLADSWNQCDLVALTCFLLGVG
CRLTPGLYHLGRTVLCIDFMVFTVRLLHIFTVNKQLGPKIVIVSKMMKDVFFFLFFLGVWLVAYGVATEGLLRPRDSDFPSILRRV
FYRPYLQIFGQIPQEDMDVALMEHSNCSSEPGFWAHPPGAQAGTCVSQYANWLVVLLLVIFLLVANILLVNLLIAMFSYTFGKVQG
NSDLYWKAQRYRLIREFHSRPALAPPFIVISHLRLLLRQLCRRPRSPQPSSPALEHFRVYLSKEAERKLLTWESVHKENFLLARAR
DKRESDSERLKRTSQKVDLALKQLGHIREYEQRLKVLEREVQQCSRVLGWVAEALSRSALLPPGGPPPPDLPGSKD
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A)

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        10                  30                  50
ATTAAAGTTTATAAAACAGTGGCTGGATGGTTGGAGGATGCAGGTGGACAGAAGACGTGG
                           M  V  G  G  C  R  W  T  E  D  V  E
        70                  90                 110
AGCCTGCAGAAGTAAAGGAAAAGATGTCCTTTCGGGCAGCCAGGCTCAGCATGAGGAACA
  P  A  E  V  K  E  K  M  S  F  R  A  A  R  L  S  M  R  N  R
       130                 150                 170
GAAGGAATGACACTCTGGACAGCACCCGGACCCTGTACTCCAGCGCGTCTCGGAGCACAG
  R  N  D  T  L  D  S  T  R  T  L  Y  S  S  A  S  R  S  T  D
       190                 210                 230
ACTTGTCTTACAGTGAAAGCGCCAGCTTCTACGCTGCCTTCAGGACACAGACGTGCCCAA
  L  S  Y  S  E  S  A  S  F  Y  A  A  F  R  T  Q  T  C  P  I
       250                 270                 290
TCATGGCTTCTTGGGACTTGGTGAATTTTATTCAAGCAAATTTAAGAAACGAGAATGTG
  M  A  S  W  D  L  V  N  F  I  Q  A  N  F  K  K  R  E  C  V
       310                 330                 350
TCTTCTTTACCAAAGATTCCAAGGCCACGGAGAATGTGTGCAAGTGTGGCTATGCCCAGA
  F  F  T  K  D  S  K  A  T  E  N  V  C  K  C  G  Y  A  Q  S
       370                 390                 410
GCCAGCACATGGAAGGCACCCAGATCAACCAAAGTGAGAAATGGAACTACAAGAAACACA
  Q  H  M  E  G  T  Q  I  N  Q  S  E  K  W  N  Y  K  K  H  T
       430                 450                 470
CCAAGGAATTTCCTACCGACGCCTTTGGGGATATTCAGTTTGAGACACTGGGGAAGAAAG
  K  E  F  P  T  D  A  F  G  D  I  Q  F  E  T  L  G  K  K  G
       490                 510                 530
GGAAGTATATACGTCTGTCCTGCGACACGGACGCGGAAATCCTTTACGAGCTGCTGACCC
  K  Y  I  R  L  S  C  D  T  D  A  E  I  L  Y  E  L  L  T  Q
       550                 570                 590
AGCACTGGCACCTGAAAACACCCAACCTGGTCATTTCTGTGACCGGGGGCGCCAAGAACT
  H  W  H  L  K  T  P  N  L  V  I  S  V  T  G  G  A  K  N  F
       610                 630                 650
TCGCCCTGAAGCCGCGCATGCGCAAGATCTTCAGCCGGCTCATCTACATCGCGCAGTCCA
  A  L  K  P  R  M  R  K  I  F  S  R  L  I  Y  I  A  Q  S  K
       670                 690                 710
AAGGTGCTTGGATTCTCACGGGAGGCACCCATTATGGCCTGATGAAGTACATCGGGGAGG
  G  A  W  I  L  T  G  G  T  H  Y  G  L  M  K  Y  I  G  E  V
       730                 750                 770
TGGTGAGAGATAACACCATCAGCAGGAGTTCAGAGGAGAATATTGTGGCCATTGGCATAG
  V  R  D  N  T  I  S  R  S  S  E  E  N  I  V  A  I  G  I  A
       790                 810                 830
CAGCTTGGGGCATGGTCTCCAACCGGGACACCCTCATCAGGAATTGCGATGCTGAGGGCT
  A  W  G  M  V  S  N  R  D  T  L  I  R  N  C  D  A  E  G  Y
       850                 870                 890
ATTTTTTAGCCCAGTACCTTATGGATGACTTCACAAGAGATCCACTGTATATCCTGGACA
  F  L  A  Q  Y  L  M  D  D  F  T  R  D  P  L  Y  I  L  D  N
       910                 930                 950
ACAACCACACACATTTGCTGCTCGTGGACAATGGCTGTCATGGACATCCCACTGTCGAAG
  N  H  T  H  L  L  L  V  D  N  G  C  H  G  H  P  T  V  E  A
       970                 990                1010
CAAAGCTCCGGAATCAGCTAGAGAAGTATATCTCTGAGDGCACTATTCAAGATTCCAACT
  K  L  R  N  Q  L  E  K  Y  I  S  E  R  T  I  Q  D  S  N  Y
      1030                1050                1070
ATGGTGGCAAGATCCCCATTGTGTTTTGCCCAAGGAGGTGGAAAAGAGACTTTGAAAG
  G  G  K  I  P  I  V  C  F  A  Q  G  G  G  K  E  T  L  K  A
      1090                1110                1130
CCATCAATACCTCCATCAAAATAAAATTCCTTGTGTGGTGGTGGAAGGCTCGGGCCAGA
  I  N  T  S  I  K  N  K  I  P  C  V  V  V  E  G  S  G  Q  I
      1150                1170                1190
TCGCTGATGTGATCGCTAGCCTGGTGGAGGTGGAGGATGCCCTGACATCTTCTGCCGTCA
  A  D  V  I  A  S  L  V  E  V  E  D  A  L  T  S  S  A  V  K
      1210                1230                1250
```

Fig. 10 / continuation 1

```
AGGAGAAGCTGGTGCGCTTTTACCCCGCACGGTGTCCCGGCTGCCTGAGGAGGAGACTG
  E K L V R F L P R T V S R L P E E E T E
    1270                1290                1310
AGAGTTGGATCAAATGGCTCAAAGAAATTCTCGAATGTTCTCACCTATTAACAGTTATTA
  S W I K W L K E I L E C S H L L T V I K
    1330                1350                1370
AAATGGAAGAAGCTGGGGATGAAATTGTGAGCAATGCCATCTCCTACGCTCTATACAAAG
  M E E A G D E I V S N A I S Y A L Y K A
    1390                1410                1430
CCTTCAGCACCAGTGAGCAAGACAAGGATAACTGGAATGGGCAGCTGAAGCTTCTGCTGG
  F S T S E Q D K D N W N G Q L K L L L E
    1450                1470                1490
AGTGGAACCAGCTGGACTTAGCCAATGATGAGATTTTCACCAATGACCGCCGATGGGAGA
  W N Q L D L A N D E I F T N D R R W E K
    1510                1530                1550
AGAGCAAACCGAGGCTCAGAGACACAATAATCCAGGTCACATGGCTGGAAAATGGTAGAA
  S K P R L R D T I I Q V T W L E N G R I
    1570                1590                1610
TCAAGGTTGAGAGCAAAGATGTGACTGACGGCAAAGCCTCTTCTCATATGCTGGTGGTTC
  K V E S K D V T D G K A S S H M L V V L
    1630                1650                1670
TCAAGTCTGCTGACCTTCAAGAAGTCATGTTTACCGCTCTCATAAAGGACAGACCCAAGT
  K S A D L Q E V M F T A L I K D R P K F
    1690                1710                1730
TTGTCCGCCTCTTTCTGGAGAATGGCTTGAACCTACGGAAGTTTCTCACCCATGATGTCC
  V R L F L E N G L N L R K F L T H D V L
    1750                1770                1790
TCACTGAACTCTTCTCCAACCACTTCAGCACGCTTGTGTACCGGATCTGCAGATCGCCA
  T E L F S N H F S T L V Y R N L Q I A K
    1810                1830                1850
AGAATTCCTATAATGATGCCCTCCTCACGTTTGTCTGGAAACTGGTTGCGAACTTCCGAA
  N S Y N D A L L T F V W K L V A N F R R
    1870                1890                1910
GAGGCTTCCGGAAGGAAGACAGAAATGGCCGGGACGAGATGGACATAGAACTCCACGACG
  G F R K E D R N G R D E M D I E L H D V
    1930                1950                1970
TGTCTCCTATTACTCGGCACCCCCTGCAAGCTCTCTTCATCTGGGCCATTCTTCAGAATA
  S P I T R H P L Q A L F I W A I L Q N K
    1990                2010                2030
AGAAGGAACTCTCCAAAGTCATTTGGGAGCAGACCAGGGCTGCACTCTGGCAGCCCTGG
  K E L S K V I W E Q T R G C T L A A L G
    2050                2070                2090
GAGCCAGCAAGCTTCTGAAGACTCTGGCCAAAGTGAAGAACGACATCAATGCTGCTGGGG
  A S K L L K T L A K V K N D I N A A G E
    2110                2130                2150
AGTCCGAGGAGCTGGCTAATGAGTACGAGACCCGGGCTGTTGGTGAGTCCACAGTGTGGA
  S E E L A N E Y E T R A V G E S T V W N
    2170                2190                2210
ATGCTGTGGTGGGCGCGGATCTGCCATGTGGCACAGACATTGCCAGCGGCACTCATAGAC
  A V V G A D L P C G T D I A S G T H R P
    2230                2250                2270
CAGATGGTGGAGAGCTGTTCACTGAGTGTTACAGCAGCGATGAAGACTTGGCAGAACAGC
  D G G E L F T E C Y S S D E D L A E Q L
    2290                2310                2330
TGCTGGTCTATTCCTGTGAAGCTTGGGGTGGAAGCAACTGTCTGGAGCTGGCGGTGGAGG
  L V Y S C E A W G G S N C L E L A V E A
    2350                2370                2390
CCACAGACCAGCATTCATGGCCCAGCCTGGGGTCCAGAATTTTCTTTCTAAGCAATGGT
  T D Q H F I A Q P G V Q N F L S K Q W Y
    2410                2430                2450
ATGGAGAGATTTCCCGAGACACCAAGAACTGGAAGATTATCCTGTGTCTGTTTATTATAC
  G E I S R D T K N W K I I L C L F I I P
```

Fig. 10 / continuation 2

```
     2470                2490                2510
CCTTGGIGGGCTGTGGCTTTGTATCATTTAGGAAGAAACCTGTCGACAAGCACAAGAAGC
  L  V  G  C  G  F  V  S  F  R  K  K  P  V  D  K  H  K  K  L
     2530                2550                2570
TGCTTTGGTACTATGTGGCGTTCTTCACCTCCCCCTTCGTGGTCTTCTCCTGGATGTGG
  L  W  Y  Y  V  A  F  F  T  S  P  F  V  V  F  S  W  N  V  V
     2590                2610                2630
TCTTCTACATCGCCTTCCTCCTGCTGTTTGCCTACGTGCTGCTCATGGATTTCCATTCGG
  F  Y  I  A  F  L  L  L  F  A  Y  V  L  L  M  D  F  H  S  V
     2650                2670                2690
TGCCACACCCCCCCGAGCTGGTCCTGTACTCGCTGGTCTTTGTCCTCTTCTGTGATGAAG
  P  H  P  P  E  L  V  L  Y  S  L  V  F  V  L  F  C  D  E  V
     2710                2730                2750
TGAGACAGGGCCGSCCGGCTGCTCCCACTGCGGGGCCCGCCAAGCCCACGCCCACCCGGA
  R  Q  G  R  P  A  A  P  S  A  G  P  A  K  P  T  P  T  R  N
     2770                2790                2810
ACTCCATCTGGCCCGCAAGCTCCACACGCAGCCCCGGTTCCGCTCACGCCACTCCTTCC
  S  I  W  P  A  S  S  T  R  S  P  G  S  R  S  R  H  S  F  H
     2830                2850                2870
ACACTTCCCTGCAAGCTGAGGGTGCCAGCTCTGGCCTTGGCCACCCCAGAAAGGGGTGGA
  T  S  L  Q  A  E  G  A  S  S  G  L  G  Q  P  R  K  G  W  T
     2890                2910                2930
CATTTAAAAATCTGGAAATGGTTGATATTTCCAAGCTGCTGATGTCCCTCTCTGTCCCTT
  F  K  N  L  E  M  V  D  I  S  K  L  L  M  S  L  S  V  P  F
     2950                2970                2990
TCTGTACGCAGTGGTACGTAAATGGGGTGAATTATTTACTGACCTGTGGAATGTGATGG
  C  T  Q  W  Y  V  N  G  V  N  Y  F  T  D  L  W  N  V  M  D
     3010                3030                3050
ACACGCTGGGGCTTTTTACTTCATAGCAGGAATTGTATTTCGGCAAGGGATCCTTAGGC
  T  L  G  L  F  Y  F  I  A  G  I  V  F  R  Q  G  I  L  R  Q
     3070                3090                3110
AGAATGAGCAGCGCTGGAGGTGGATATTCCGTTCGGTCATCTACGAGCCCTACCTGGCCA
  N  E  Q  R  W  R  W  I  F  R  S  V  I  Y  E  P  Y  L  A  M
     3130                3150                3170
TGTTCGGCCAGGTGCCCAGTGACGTGGATCGTACCACGTATGACTTTGCCCACTGCACCT
  F  G  Q  V  P  S  D  V  D  G  T  T  Y  D  F  A  H  C  T  F
     3190                3210                3230
TCACTGGGAATGAGTCCAAGCCACTGTGTGTGGAGCTGGATGAGCACAACCTGCCCCGGT
  T  G  N  E  S  K  P  L  C  V  E  L  D  E  H  N  L  P  R  F
     3250                3270                3290
TCCCCGAGTCGATCACCATCCCCCTGGTGTGCATCTACATGTTATCCACCAACATCCTGC
  P  E  W  I  T  I  P  L  V  C  I  Y  M  L  S  T  N  I  L  L
     3310                3330                3350
TGGTCAACCTGCTGGTCGCCATGTTTGGCTACACGGTGGGCACCGTCCAGGAGAACAATG
  V  N  L  L  V  A  M  F  G  Y  T  V  G  T  V  Q  E  N  N  D
     3370                3390                3410
ACCAGGTCTGGAAGTTCCAGAGGTACTTCCTGGTGCAGGAGTACTGCAGCCGCCTCAATA
  Q  V  W  K  F  Q  R  Y  F  L  V  Q  E  Y  C  S  R  L  N  I
     3430                3450                3470
TCCCGTTCCCCTTCATCGTCTTCGCTTACTTCTACATGGTGGTGAAGAAGTGCTTCAAGT
  P  F  P  F  I  V  F  A  Y  F  Y  M  V  V  K  K  C  F  K  C
     3490                3510                3530
GTTGCTGCAAGGAGAAAAACATGGAGTCTTCTGTCTGCTGTGAGTGGTTTATCCATGTGT
  C  C  K  E  K  N  M  E  S  S  V  C  C  E  W  F  I  H  V  Y
     3550                3570                3590
ACTTGGGATCAGAAGCAGCGATTAATTTCAGGGAAGCATGCCTGCATCCAGTGATTGGAA
  L  G  S  E  A  A  I  N  F  R  E  G  C  L  H  P  V  I  G  S
     3610                3630                3650
GCTGGACCCCAGGCTGGCTGGTCTGGACATCCACACGCATTCTCACATGCAGTGCCGGCT
  W  T  P  G  W  L  V  W  T  S  T  R  I  L  T  C  S  A  G  W
     3670                3690                3710
GGCCAGCAGCAGGGAGTCTCAGTGTCACCACACATAGCAGCTGGGTTCCTGCAAAAAGCA
```

Fig. 10 / continuation 3

```
  P  A  A  G  S  L  S  V  T  T  H  S  S  W  V  P  A  K  S  S
     3730                 3750                 3770
GCAAGTCACAGGCCCACCCAGACAGAACGGGTAGAGAATGTGACTCTGCTTCTGGGTGGG
  K  S  Q  A  H  P  D  R  T  G  R  E  C  D  S  A  S  G  W  E
     3790                 3810                 3830
AAGGACAGCCTGCCCGGTGGGTGGAAGAATCCGTGGCCCTGTTTGGCCATCGTGGCCCTG
  G  Q  P  A  R  W  V  E  E  S  V  A  L  F  G  H  R  G  P  V
     3850                 3870                 3890
TTTGGCCACCTACCACTCTAGGCATCACTGAGCTGAATGCGCCGGTCCTCTGA
  W  P  P  T  T  L  G  I  T  E  L  N  A  P  V  L  *
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```
MVGGCRWTEDVEPAEVKEKMSFRAARLSMRNRRNDTLDSTRTLYSSASRSTDLSYSESASFYAAFRTQTCPIMASWDLVNFIQANF
KKRECVFFTKDSKATENVCKCGYAQSQHMEGTQINQSEKWNYKKHTKEFPTDAFGDIQFETLGKKGKYIRLSCDTDAEILYELLTQ
HWHLKTPNLVISVTGGAKNFALKPRMRKIFSRLIYIAQSKGAWILTGGTHYGLMKYIGEVVRDNTISRSSEENIVAIGIAAWGMVS
NRDTLIRNCDAEGYFLAQYLMDDFTRDPLYILDNNHTHLLLVDNGCHGHPTVEAKLRNQLEKYISERTIQDSNYGGKIPIVCFAQG
GGKETLKAINTSIKNKIPCVVVEGSGQIADVIASLVEVEDALTSSAVKEKLVRFLPRTVSRLPEEETESWIKWLKEILECSHLLTV
IKMEEAGDEIVSNAISYALYKAFSTSEQDKDNWNCQLKLLLEWNQLDLANDEIFTNDRRWEKSKPRLRDTIIQVTWLENGRIKVES
KDVTDGKASSHMLVVLKSADLQEVMFTALIKDRPKEVRLFLENGLNLRKFLTHDVLTELFSNHFSTLVYRNLQIAKNSYNDALLTF
VWKLVANFRRGFRKEDRNGRDEMDIELHDVSPITRHPLQALFIWAILQNKKELSKVIWEQTRGCTLAALGASKLLKTLAKVKNDIN
AAGESEELANEYETRAVGESTVWNAVVGADLPCGTDIASGTHRPDGGELFTECYSSDEDLAEQLLVYSCEAWGGSNCLELAVEATD
QHFIAQPGVQNFLSKQWYGEISRDTKNWKIILCLFIIPLVGCGFVSFRKKPVDKHKKLLWYYVAFFTSPFVVFSWNVVFYIAFLLL
FAYVLLMDFHSVPHPPELVLYSLVFVLFCDEVRQGRPAAPSAGPAKPTFTRNSIWPASSTRSPGSRSRHSFHTSLQAEGASSGLGQ
PRKGWTFKNLEMVDISKLLMSLSVPFCTQWYVNGVNYFTDLWNVMDTLGLFYFIAGIVFRQGILRQNEQRWRWIFRSVIYEPYLAM
FGQVPSDVDGTTYDFAHCTFTGNESKFLCVELDEHNLPRFPEWITIPLVCIYMLSTNILLVNLLVAMFGYTVGTVQENNDQVWKFQ
RYFLVQEYCSRLNIPFPFIVFAYFYMVVKKCFKCCCKEKNMESSVCCEWFIHVYLGSEAAINFREGCLHPVIGSWTPGWLVWTSTR
ILTCSAGWPAAGSLSVTTHSSWVPAKSSKSQAHPDRTGRECDSASGWEGQPARWVEESVALFGHRGPVWPPTTLGITELNAPVL
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B.

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                                                    Q  L
      2290                 2310                 2330
TGCTGGTCTATTCCTGTGAAGCTTGGGGTGGAAGCAACTGTCTGGAGCTGGCGGTGGAGG
  L  V  Y  S  C  E  A  W  G  G  S  N  C  L  E  L  A  V  E  A
      2350                 2370                 2390
CCACAGACCAGCATTTCATCGCCCAGCCTGGGGTCCAGAATTTTCTTTCTAAGCAATGGT
  T  D  Q  H  F  I  A  Q  P  G  V  Q  N  F  L  S  K  Q  W  Y
      2410                 2430                 2450
ATGGAGAGATTTCCCGAGACACCAAGAACTGGAAGATTATCCTGTGTCTGTTTATTATAC
  G  E  I  S  R  D  T  K  N  W  K  I  I  L  C  L  F  I  I  P
      2470                 2490                 2510
CCTTGGTGGGCTGTGGCTTTGTATCATTTAGGAAGAAACCTGTCGACAAGCACAAGAAGC
  L  V  G  C  G  F  V  S  F  R  K  K  P  V  D  K
```

Figure 11:

a.) Trp10b cDNA and derived amino acid sequence

```
        10                  30                  50
ATGAAATCCTTCCTTCCTGTCCACACCATCGTGCTTATCAGGGAGAATGTGTGCAAGTGT
M  K  S  F  L  P  V  H  T  I  V  L  I  R  E  N  V  C  K  C
        70                  90                 110
GGCTATGCCCAGAGCCAGCACATGGAAGGCACCCAGATCAACCAAAGTGAGAAATGGAAC
G  Y  A  Q  S  Q  H  M  E  G  T  Q  I  N  Q  S  E  K  W  N
       130                 150                 170
TACAAGAAACACACCAAGGAATTTCCTACCGACGCCTTTGGGGATATTCAGTTTGAGACA
Y  K  K  H  T  K  E  F  P  T  D  A  F  G  D  I  Q  F  E  T
       190                 210                 230
CTGGGGAAGAAAGGGAAGTATATACGTCTGTCCTGCGACACGGACGCCGAAATCCTTTAC
L  G  K  K  G  K  Y  I  R  L  S  C  D  T  D  A  E  I  L  Y
       250                 270                 290
GAGCTGCTGACCCAGCACTGGCACCTGAAAACACCCAACCTGGTCATTTCTGTGACCGGG
E  L  L  T  Q  H  W  H  L  K  T  P  N  L  V  I  S  V  T  G
       310                 330                 350
GGCGCCAAGAACTTCGCCCTGAAGCCGCGCATGCGCAAGATCTTCAGCCGGCTCATCTAC
G  A  K  N  F  A  L  K  P  R  M  R  K  I  F  S  R  L  I  Y
       370                 390                 410
ATCGCGCAGTCCAAAGGTGCTTGGATTCTCACGGGAGGCACCCATTATGGCCTGATGAAG
I  A  Q  S  K  G  A  W  I  L  T  G  G  T  H  Y  G  L  M  K
       430                 450                 470
TACATCGGGGAGGTGGTGAGAGATAACACCATCAGCAGGAGTTCAGAGGAGAATATTGTG
Y  I  G  E  V  V  R  D  N  T  I  S  R  S  S  E  E  N  I  V
       490                 510                 530
GCCATTGGCATAGCAGCTTGGGGCATGGTCTCCAACCGGGACACCCTCATCAGGAATTGC
A  I  G  I  A  A  W  G  M  V  S  N  R  D  T  L  I  R  N  C
       550                 570                 590
GATGCTGAGGGCTATTTTTTAGCCCAGTACCTTATGGATGACTTCACAAGAGATCCACTG
D  A  E  G  Y  F  L  A  Q  Y  L  M  D  D  F  T  R  D  P  L
       610                 630                 650
TATATCCTGGACAACAACCACACACATTTGCTGCTCGTGGACAATGGCTGTCATGGACAT
Y  I  L  D  N  N  H  T  H  L  L  L  V  D  N  G  C  H  G  H
       670                 690                 710
CCCACTGTCGAAGCAAAGCTCCGGAATCAGCTAGAGAAGTATATCTCTGAGCGCACTATT
P  T  V  E  A  K  L  R  N  Q  L  E  K  Y  I  S  E  R  T  I
       730                 750                 770
CAAGATTCCAACTATGGTGGCAAGATCCCCATTGTGTGTTTTGCCCAAGGAGGTGGAAAA
Q  D  S  N  Y  G  G  K  I  P  I  V  C  F  A  Q  G  G  G  K
       790                 810                 830
GAGACTTTGAAAGCCATCAATACCTCCATCAAAAATAAAATTCCTTGTGTGGTGGTGGAA
E  T  L  K  A  I  N  T  S  I  K  N  K  I  P  C  V  V  V  E
       850                 870                 890
GGCTCGGGCCAGATCGCTGATGTGATCGCTAGCCTGGTGGAGGTGGAGGATGCCCTGACA
G  S  G  Q  I  A  D  V  I  A  S  L  V  E  V  E  D  A  L  T
       910                 930                 950
TCTTCTGCCGTCAAGGAGAAGCTGGTGCGCTTTTACCCCGCACGGTGTCCCGGCTGCCT
S  S  A  V  K  E  K  L  V  R  F  L  P  R  T  V  S  R  L  P
       970                1010
GAGGAGGAGACTGAGAGTTGGATCAAATGGCTCAAAGAAATTCTCGAATGTTCTCACCTA
E  E  E  T  E  S  W  I  K  W  L  K  E  I  L  E  C  S  H  L
      1030                1050                1070
TTAACAGTTATTAAAATGGAAGAAGCTGGGGATGAAATTGTGAGCAATGCCATCTCCTAC
L  T  V  I  K  M  E  E  A  G  D  E  I  V  S  N  A  I  S  Y
      1090                1110                1130
GCTCTATACAAAGCCTTCAGCACCAGTGAGCAAGACAAGGATAACTGGAATGGGCAGCTG
A  L  Y  K  A  F  S  T  S  E  Q  D  K  D  N  W  N  G  Q  L
```

Fig. 11 (Continuation)

```
      2410                2430                2450
AGAAACTTAGGACCCAAGATTATAATGCTGCAGAGGATGCTGATCGATGTGTTCTTCTTC
R  N  L  G  P  K  I  I  M  L  Q  R  M  L  I  D  V  F  F  F
      2470                2490                2510
CTGTTCCTCTTTGCGGTGTGGATGGTGGCCTTTGGCGTGGCCAGGCAAGGGATCCTTAGG
L  F  L  F  A  V  W  M  V  A  F  G  V  A  R  Q  G  I  L  R
      2530                2550                2570
CAGAATGAGCAGCGCTGGAGGTGGATATTCCGTTCGGTCATCTACGAGCCCTACCTGGCC
Q  N  E  Q  R  W  R  W  I  F  R  S  V  I  Y  E  P  Y  L  A
      2590                2610                2630
ATGTTCGGCCAGGTGCCCAGTGACGTGGATGGTACCACGTATGACTTTGCCCACTGCACC
M  F  G  Q  V  P  S  D  V  D  G  T  T  Y  D  F  A  H  C  T
      2650                2670                2690
TTCACTGGGAATGAGTCCAAGCCACTGTGTGTGGAGCTGGATGAGCACAACCTGCCCCGG
F  T  G  N  E  S  K  P  L  C  V  E  L  D  E  H  N  L  P  R
      2710                2730                2750
TTCCCCGAGTGGATCACCATCCCCCTGGTGTGCATCTACATGTTATCCACCAACATCCTG
F  P  E  W  I  T  I  P  L  V  C  I  Y  M  L  S  T  N  I  L
      2770                2790                2810
CTGGTCAACCTGCTGGTCGCCATGTTTGGCTACACGGTGGGCACCGTCCAGGAGAACAAT
L  V  N  L  L  V  A  M  F  G  Y  T  V  G  T  V  Q  E  N  N
      2830                2850                2870
GACCAGGTCTGGAAGTTCCAGAGGTACTTCCTGGTGCAGGAGTACTGCAGCCGCCTCAAT
D  Q  V  W  K  F  Q  R  Y  F  L  V  Q  E  Y  C  S  R  L  N
      2890                2910                2930
ATCCCCTTCCCCTTCATCGTCTTCGCTTACTTCTACATGGTGGTGAAGAAGTGCTTCAAG
I  P  F  P  F  I  V  F  A  Y  F  Y  M  V  V  K  K  C  F  K
      2950                2970                2990
TGTTGCTGCAAGGAGAAAAACATGGAGTCTTCTGTCTGCTGTTTCAAAAATGAAGACAAT
C  C  C  K  E  K  N  M  E  S  S  V  C  C  F  K  N  E  D  N
      3010                3030                3050
GAGACTCTGGCATGGGAGGGTGTCATGAAGGAAAACTACCTTGTCAAGATCAACACAAAA
E  T  L  A  W  E  G  V  M  K  E  N  Y  L  V  K  I  N  T  K
      3070                3090                3110
GCCAACGACACCTCAGAGGAAATGAGGCATCGATTTAGACAACTGGATACAAAGCTTAAT
A  N  D  T  S  E  E  M  R  H  R  F  R  Q  L  D  T  K  L  N
      3130                3150
GATCTCAAGGGTCTACTGAAAGAGATTGCTAATAAAATCAAATAG
D  L  K  G  L  L  K  E  I  A  N  K  I  K  *
```

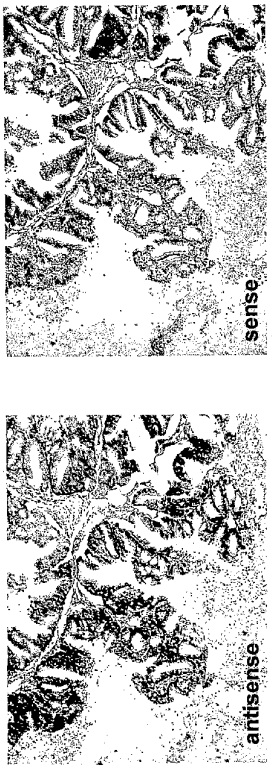
b.) Trp10 protein:

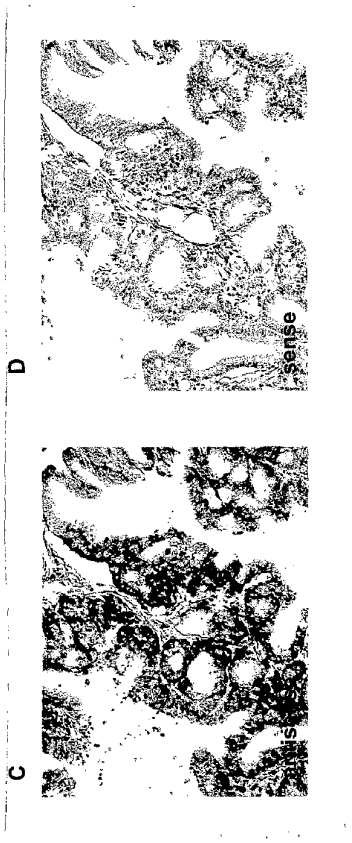
```
MKSFLPVHTIVLIRENVCKCGYAQSQHMEGTQINQSEKWNYKKHTKEFPTDAFGDIQFETLGKKGKYIRLSCDTDAEILY
ELLTQHWHLKTPNLVISVTGGAKNFALKPRMRKIFSRLIYIAQSKGAWILTGGTHYGLMKYIGEVVRDNTISRSSEENIV
AIGIAAWGMVSNRDTLIRNCDAEGYFLAQYLMDDFTRDPLYILDNNHTHLLLVDNGCHGHPTVEAKLRNQLEKYISERTI
QDSNYGGKIPIVCFAQGGGKETLKAINTSIKNKIPCVVVEGSGQIADVIASLVEVEDALTSSAVKEKLVRFLPRTVSRLP
EEETESWIKWLKEILECSHLLTVIKMEEAGDEIVSNAISYALYKAFSTSEQDKDNWNGQLKLLLEWNQLDLANDEIFTND
RRWESADLQEVMFTALIKDRPKFVRLFLENGLNLRKFLTHDVLTELFSNHFSTLVYRNLQIAKNSYNDALLTFVWKLVAN
FRRGFRKEDRNGRDEMDIELHDVSPITRHPLQALFIWAILQNKKELSKVIWEQTRGCTLAALGASKLLKTLAKVKNDINA
AGESEELANEYETRAVELFTECVSSDEDLAEQLLVYSCEAWGGSNCLELAVEATDQHFIAQPGVQNFLSKQWYGEISRDT
KNWKIILCLFIIPLVGCGFVSFRKKPVDKHKKLLWYYVAFFTSPFVVFSWNVVFYIAFLLLFAYVLLMDFHSVPHPPELV
LYSLVFVLFCDEVRQWYVNGVNYFTDLWNVMDTLGLFYFIAGIVFRLHSSNKSSLYSGRVIFCLDYIIFTLRLIHIFTVS
RNLGPKIIMLQRMLIDVPFFLFLFAVWMVAFGVARQGILRQNEQRWRWIFRSVIYEPYLAMFGQVPSDVDGTTYDFAHCT
FTGNESKPLCVELDEHNLPRFPEWITIPLVCIYMLSTNILLVNLLVAMFGYTVGTVQENNDQVWKFQRYFLVQEYCSRLN
IPFPFIVFAYFYMVVKKCFKCCCKEKNMESSVCCFKNEDNETLAWEGVMKENYLVKINTKANDTSEEMRHRFRQLDTKLN
DLKGLLKEIANKIK
```

Figs. 12A and 12B

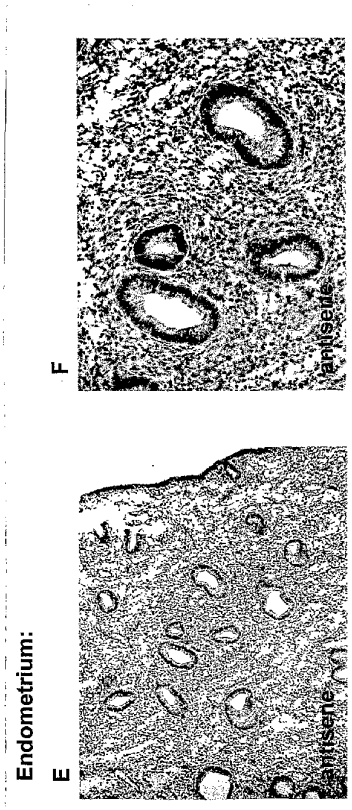
The Trp8 gene is expressed in endometrial or uterine cancer, but not in normal endometrium

Endometrial cancer:





Figs. 12C and 12D



Figs. 12E and 12F

Expression of human Trp 9 and Trp 10

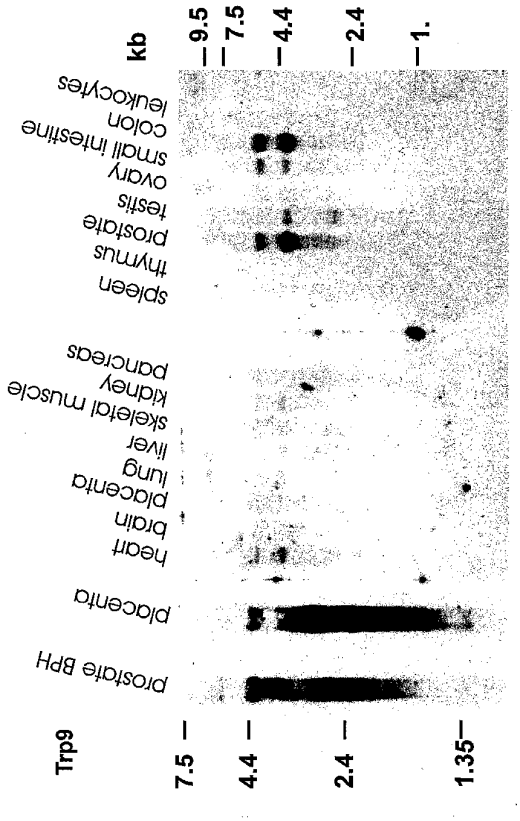


Fig. 13

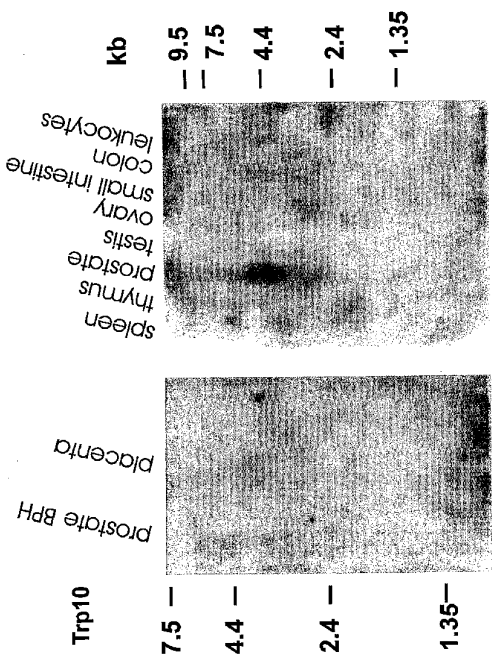
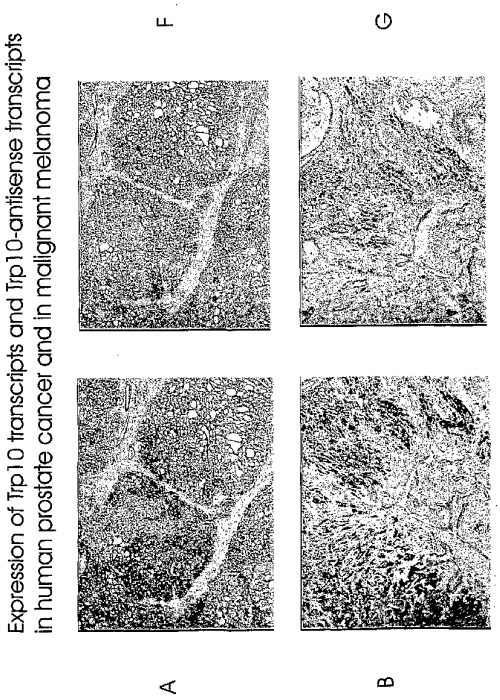
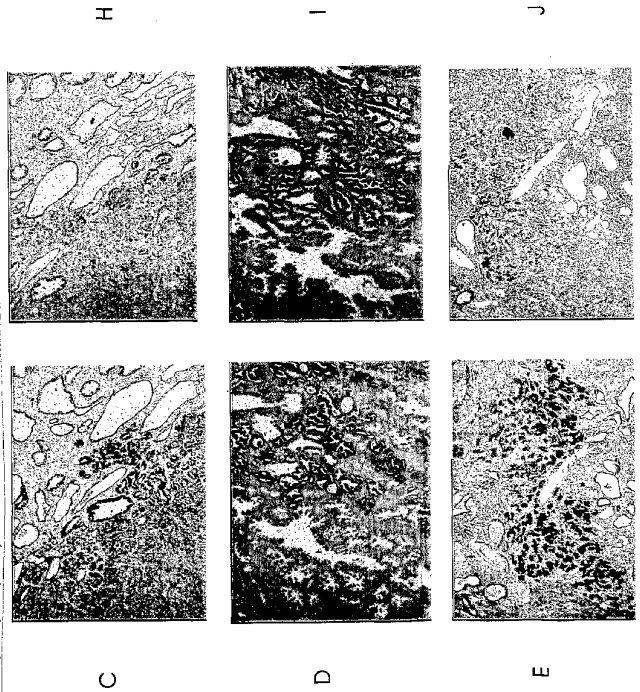


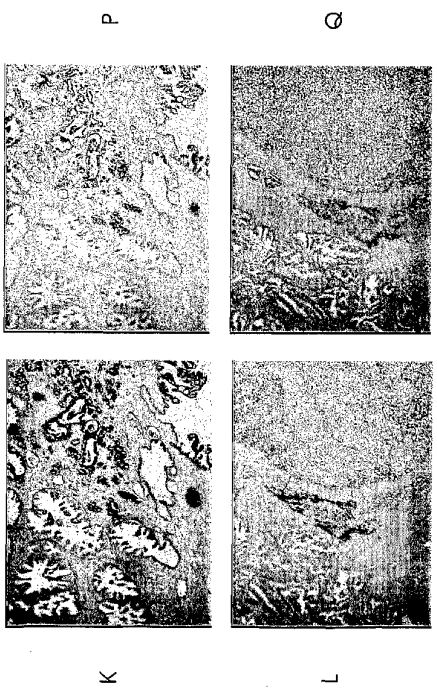
Fig. 13 / Continuation 1

Figs. 14A, 14B, 14F and 14G





Figs. 14C, 14D, 14E, 14H, 14I and 14J



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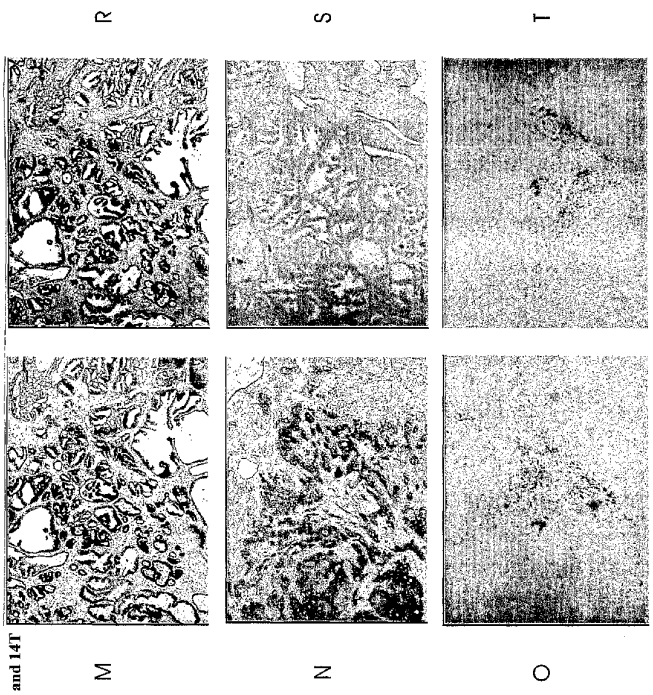
Figs. 14K, 14L, 14P and 14Q



Figs. 14M, 14N, 14O, 14R, 14S and 14T

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, 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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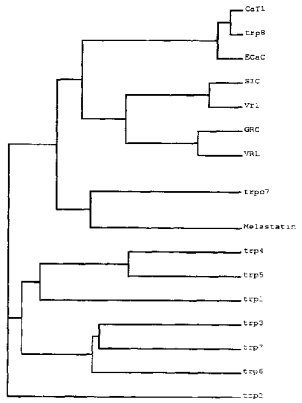
Published:
— *with international search report*

[Continued on next page]

(54) **Title:** TRP8, TRP9 AND TRP10, MARKERS FOR CANCER



(57) **Abstract:** The present invention relates to gene expression in normal cells and cells of malignant tumors and particularly to novel markers associated with cancer, Trp8, Trp9 and Trp10, and the genes encoding Trp8, Trp9 and Trp10. Also provided are vectors, host cells, antibodies, and recombinant methods for producing these human proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating a tumor.

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【国際調査報告】

INTERNATIONAL SEARCH REPORT

rnational Application No
PCT/EP 01/08309

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/11 C12N9/00 C07K14/47 C12Q1/68
G01N33/577 A61K31/713 C07K14/705

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)
IPC 7 C07K

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 practical, search terms used)
BIOSIS, EPO-Internal, SEQUENCE SEARCH, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 09166 A (SHAPERO MICHAEL H ;DENDREON CORP (US); LAUS REINER (US); TSAVALER) 25 February 1999 (1999-02-25) see SEQID14 + 15, pages 2,3, 28,29, Example 4 table 3 ---	1-10, 12-17, 23,29-31
X	WO 00 40614 A (BETH ISRAEL HOSPITAL ;SCHARENBERG ANDREW M (US)) 13 July 2000 (2000-07-13) see seqid31 + 32, page 11, first paragraph, page 44, lines 13-15 --- -/--	1-10,12, 31

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

° Special categories of cited documents :
"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"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
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"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.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 March 2003	13. 03. 2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Holtorf, S

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INTERNATIONAL SEARCH REPORT

National Application No
PCT/EP 01/08309

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MULLER D ET AL: "Molecular cloning, tissue distribution, and chromosomal mapping of the human epithelial Ca2+ channel (ECAC1)." GENOMICS, vol. 67, no. 1, 1 July 2000 (2000-07-01), pages 48-53, XP002222953 ISSN: 0888-7543 the whole document	1
X	WO 98 15657 A (ABBOTT LAB) 16 April 1998 (1998-04-16) the whole document	1-12, 29-31
X	WO 98 37093 A (CORIXA CORP) 27 August 1998 (1998-08-27) the whole document	1-12, 29-31
A	TSAVALER LARISA ET AL: "TRP-P8, a novel prostate-specific gene, is upregulated in prostate cancer and other malignancies and shares high homology with TRP calcium channel proteins." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, no. 41, March 2000 (2000-03), page 694 XP008011242 91st Annual Meeting of the American Association for Cancer Research.;San Francisco, California, USA; April 01-05, 2000, March, 2000 ISSN: 0197-016X the whole document	
A	HARTENECK C ET AL: "FROM WORM TO MAN: THREE SUBFAMILIES OF TRP CHANNELS" TRENDS IN NEUROSCIENCE, ELSEVIER, AMSTERDAM, NL, vol. 23, no. 4, April 2000 (2000-04), pages 159-166, XP001012870 ISSN: 0166-2236	
P,X	WO 01 14423 A (SMITHKLINE BEECHAM PLC) 1 March 2001 (2001-03-01) see SEQid1 + 2; see example 1	1-9,31

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/08309

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WISSENBACH ULRICH ET AL: "Expression of CaT-like, a novel calcium-selective channel, correlates with the malignancy of prostate cancer." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 22, 1 June 2001 (2001-06-01), pages 19461-19468, XP002222954 ISSN: 0021-9258 the whole document	1-9,13, 14, 16-19, 21-23,29
P,X	WO 01 04303 A (HEDIGER MATTHIAS A) 18 January 2001 (2001-01-18) see SEQID1 + 2 the whole document	1-5
P,X	WO 01 42467 A (MILLENNIUM PREDICTIVE MEDICINE) 14 June 2001 (2001-06-14) see SEQID 4615	1
E	WO 01 51633 A (FANGER GARY RICHARD ;HARLOCKER SUSAN L (US); MEAGHER MADELEINE JOY) 19 July 2001 (2001-07-19) see SEQID764, example 3, claims	1
E	WO 02 14361 A (AGENSYS INC) 21 February 2002 (2002-02-21) see SEQID1479, examples 1-4 the whole document	1-10, 13-23
E	WO 02 00722 A (SILOS SANTIAGO INMACULADA ;CURTIS RORY A J (US); MILLENNIUM PHARM) 3 January 2002 (2002-01-03) see SEQID4	1-5
E	WO 01 68857 A (CURTIS RORY A J ;COOK WILLIAM JAMES (US); MILLENNIUM PHARM INC (US) 20 September 2001 (2001-09-20) see SEQID1, examples	1-5
E	WO 01 53348 A (SQUIBB BRISTOL MYERS CO ;GAUGHAN GLEN T (US); RAMANATHAN CHANDRA S) 26 July 2001 (2001-07-26) see SEQID5 the whole document	1
E	WO 01 62794 A (LORA JOSE M ;CURTIS RORY A J (US); GLUCKSMANN MARIA ALEXANDRA (US)) 30 August 2001 (2001-08-30) the whole document	1-9
E	WO 02 30268 A (EOS BIOTECHNOLOGY INC) 18 April 2002 (2002-04-18) see SEQID53	1,6

-/--

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/08309

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	BOEDDING MATTHIAS ET AL: "The recombinant human TRPV6 channel functions as Ca2+ sensor in human embryonic kidney and rat basophilic leukemia cells." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 39, 27 September 2002 (2002-09-27), pages 36656-36664, XP002222955 September 27, 2002 ISSN: 0021-9258 the whole document -----	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 01/08309

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 24-28 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 12 partially
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:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
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:

see additional sheet

1. [X] 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.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

International Application No. PCT/EP 01/08309

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-12, 29-31 partially, 13-28 completely

Isolated nucleic acid molecules encoding human prostate carcinom associated proteins as characterized by SEQIDs5,45,11,3 and SEQIDs 6,46,12,4, respectively; the recombinant expression of the same in host cells; the isolated proteins as characterized by SEQIDs 6,46,12,4; antisense RNA sequence and ribozyme complementary to said nucleic acid molecules; inhibitor that can suppress the activity of said prostate carcinom associated proteins; method for diagnosing a prostate carcinoma by contacting a sample with a nucleic acid, an antibody or other reagent that reacts with the mRNA of SEQIDs5,45,11,3; method for diagnosing endomertial cancer by contacting a target sample with a nucleic acid, an antibody or other reagent that reacts with the mRNA of SEQIDs5,45,11,3; method for diagnosing a melanoma, chorion carcinoma, cancer of the lung and ot the prostate comprising contacting a target sample with a reagent which detects antisense RNA of SEQIDs 11 and 3; method for preventing prostate tumour, endometrial cancer, choroin carcinoma or cancer of the lung comprising administering an inhibiting reagent of human prostate carcinom associated proteins; diagnostic kit containing an antibody; method for identifyng an agonist or an antagonist of human prostate carcinom associated proteins.

2. Claims: 1-12, 29-31 partially

Isolated nucleic acid molecule encoding human prostate carcinom associated protein as characterized by SEQIDs 7 and SEQIDs 8, respectively; the recombinant expression of the same in host cells; the isolated protein as characterized by SEQIDs 8; antisense RNA sequence and ribozyme complementary to said nucleic acid molecule; inhibitor that can suppress the activity of said prostate carcinom associated protein; diagnostic kit containing an antibody; method for identifyng an agonist or an antagonist of human prostate carcinom associated proteins.

International Application No. PCT/EP 01/08309

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 12 partially

Present claim 12 relates to an inhibitor wich is defined by reference to a desirable characteristic or property, namely suppressing the activity of the protein of claim 6.

The claims cover all inhibitors having this characteristic or property, whereas the application provides only support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for a limited number of such inhibitors.

In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the inhibitors by reference to a result to be achieved.
Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those parts of the claim 12 which appear to be clear, supported and disclosed, namely those parts relating to the Trp8/10 corresponding antibody , Trp8/10 corresponding antisense construct, a Trp8/10 corresponding ribozyme.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 01/08309

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
WO 9909166	A	25-02-1999	US	6194152	B1	27-02-2001
			AU	744875	B2	07-03-2002
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