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(21) International Application Number: PCT/US99/14201 (22) International Filing Date: 22 June 1999 (22.06.99) (30) Priority Data: 09/102,705 22 June 1998 (22.06.98) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 09/102,705 (CON) Filed on 22 June 1998 (22.06.98) (71) Applicant (for all designated States except US): MILLENIUM BIOTHERAPEUTICS, INC. [US/US]; 620 Memorial Drive, Cambridge, MA 02139 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): GOODEARL, Andrew, D., J. [GB/US]; 13 Indian Ridge Road, Natick, MA 01760 (US). (74) Agent: MEIKLEJOHN, Anita, L.; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: NOVEL MOLECULES OF THE T110-RELATED PROTEIN FAMILY AND USES THEREOF		
(57) Abstract Novel T110 polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated, full-length T110 proteins, the invention further provides isolated T110 fusion proteins, antigenic peptides and anti-T110 antibodies. The invention also provides T110 nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a T110 gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.		

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NOVEL MOLECULES OF THE T110-RELATED PROTEIN FAMILY
AND USES THEREOF

5 Cross Reference To Related Applications

This application is a continuation-in-part of application serial number 09/102,705, filed June 22, 1998.

Background of the Invention

10 The invention relates to a novel cell surface or secreted protein and the gene encoding it.

Many membrane-associated and secreted proteins, for example, cytokines, play a vital role in the regulation of cell growth, cell differentiation, and a variety of specific cellular responses. A number of medically useful proteins, including erythropoietin, granulocyte-macrophage colony stimulating factor, human growth hormone, and various interleukins, are secreted proteins. Thus, an important goal in the design and development of new therapies is the identification and characterization of membrane-associated and secreted proteins and the genes which encode them.

Many membrane-associated proteins are receptors which bind a ligand and transduce an intracellular signal, leading to a variety of cellular responses. The identification and characterization of such a receptor enables one to identify both the ligands which bind to the receptor and the intracellular molecules and signal transduction pathways associated with the receptor, permitting one to identify or design modulators of receptor activity, e.g., receptor agonists or antagonists and modulators of signal transduction.

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Summary of the Invention

The present invention is based, at least in part, on the discovery of a gene encoding T110. T110 protein is related to four-jointed (fj) protein of *Drosophila* Melanogaster. T110 is predicted to be a member of the type-II membrane protein superfamily. Such proteins usually employ a transmembrane domain as the internal signal sequence. The amino terminal end of such proteins is normally intracellular, and the carboxy terminal end is normally extracellular. However, some type II membrane proteins are secreted from the cell while others are initially expressed on the surface of the cell and are subsequently processed to release a soluble fragment.

The human T110 cDNA described below (SEQ ID NO:1) has a 1311 nucleotide open reading frame (nucleotides 131 to 1441 of SEQ ID NO:1; SEQ ID NO:3) which encodes a 437 amino acid protein (SEQ ID NO:2). Figure 8 depicts a potential alternative translation product (SEQ ID NO:4) for the above-described human T110 cDNA. It is possible that this alternative translation product is not full length. Those skilled in the art can isolate full-length clones having additional 5' coding sequence using the methods described below.

The mouse T110 cDNA described below (SEQ ID NO:5) has a 1350 nucleotide open reading frame (nucleotides 103 to 1452 of SEQ ID NO:5; SEQ ID NO:7) which encodes a 450 amino acid protein (SEQ ID NO:6). Figure 6 depicts a potential alternative translation product (SEQ ID NO:8) for the above-described murine T110 cDNA. It is possible that this alternative translation product is not full length. Those skilled in the art can isolate full-length clones having additional 5' coding sequence using the methods described below.

A partial rat T110 cDNA is also described below (SEQ ID NO:9). It has a 507 nucleotide open reading

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frame (nucleotides 1 to 507 of SEQ ID NO:9) which encodes a 169 amino acid peptide (SEQ ID NO:10). Those skilled in the art can isolate full-length clones having additional 5' sequence using the methods described below.

5 The T110 molecules of the present invention are useful as modulating agents in regulating a variety of cellular processes, e.g., cell proliferation and/or cell differentiation. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules
10 encoding T110 proteins or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of T110-encoding nucleic acids.

 The invention features a nucleic acid molecule
15 which includes a fragment of at least 400 (450, 500, 550, 600, 650, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, or 1420) nucleotides of the nucleotide sequence shown in SEQ ID NO:1 or a complement thereof; or a fragment of at least 200 (250, 300, 350, 400, 450, 500, 550, 600, 650,
20 700, 800, 900, 1000, 1110, 1200, 1300, 1400, or 1420) nucleotides of the nucleotide sequence shown in SEQ ID NO:3 or a complement thereof; or a fragment of at least 450 (500, 550, 600, 650, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, or 1450) nucleotides of the nucleotide
25 sequence shown in SEQ ID NO:5 or SEQ ID NO:7, or a complement thereof.

 In a preferred embodiment, a T110 nucleic acid molecule has the nucleotide sequence shown in SEQ ID NO:1, or SEQ ID NO:3, or SEQ ID NO:5, or SEQ ID NO:7.

30 Also within the invention is a nucleic acid molecule which encodes a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or SEQ ID NO:6 or SEQ ID NO:8, the fragment including at least 70 (80, 90, 100, 120, 140, 160, 180, 200, 250, 300,
35 350, 400, 450, or 480) contiguous amino acids of SEQ ID

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NO:2 or SEQ ID NO:4; or the fragment including at least 150 (160, 170, 180, 200, 250, 300, 350, 400, 450, or 480) contiguous amino acids of SEQ ID NO:6 or SEQ ID NO:8.

The invention includes a nucleic acid molecule
5 which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or SEQ ID NO:6 or SEQ ID NO:8, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1 or SEQ ID NO:3, or
10 SEQ ID NO:5 or SEQ ID NO:7 under stringent conditions.

Also within the invention is an isolated T110 protein which is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid
15 molecule having the nucleotide sequence of SEQ ID NO:3, SEQ ID NO:7, or SEQ ID NO:9.

Also within the invention is a polypeptide which is a naturally occurring allelic variant of a polypeptide that includes the amino acid sequence of SEQ ID NO:2 or
20 SEQ ID NO:4, or SEQ ID NO:6 or SEQ ID NO:8, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1 or SEQ ID NO:3, or SEQ ID NO:5 or SEQ ID NO:7 under stringent conditions;

25 Another embodiment of the invention features T110 nucleic acid molecules which specifically detect T110 nucleic acid molecules. For example, in one embodiment, a T110 nucleic acid molecule hybridizes under stringent conditions to a nucleic acid molecule comprising the
30 nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7, or a complement thereof. In another embodiment, the T110 nucleic acid molecule is at least 440 (450, 500, 550, 600, 650, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, or 1420) nucleotides in length
35 and hybridizes under stringent conditions to a nucleic

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acid molecule comprising the nucleotide sequence as shown in SEQ ID NO:1 or a complement thereof; or a fragment of at least 220 (250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, or
5 1420) nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence as shown in SEQ ID NO:3 or a complement thereof; or a fragment of at least 450 (500, 550, 600, 650, 700, 800, 900, 1000, 1100, 1200,
10 1300, 1400, or 1420) nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence as shown in SEQ ID NO:5 or SEQ ID NO:7, or a complement thereof. In another embodiment, the invention provides an isolated nucleic
15 acid molecule which is antisense to the coding strand of a T110 nucleic acid.

Another aspect of the invention provides a vector, e.g., a recombinant expression vector, comprising a T110 nucleic acid molecule of the invention. In another
20 embodiment the invention provides a host cell containing such a vector. The invention also provides a method for producing T110 protein by culturing, in a suitable medium, a host cell of the invention containing a recombinant expression vector such that a T110 protein is
25 produced.

Another aspect of this invention features isolated or recombinant T110 proteins and polypeptides. Preferred T110 proteins and polypeptides possess at least one biological activity possessed by naturally occurring
30 human T110, e.g., modulation of cellular proliferation. In one embodiment, an isolated T110 protein has an extracellular domain and lacks both a transmembrane and a cytoplasmic domain. In another embodiment, an isolated T110 protein is soluble under physiological conditions.

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The T110 proteins of the present invention, or biologically active portions thereof, can be operably linked to a non-T110 polypeptide (e.g., heterologous amino acid sequences) to form T110 fusion proteins. The invention further features antibodies that specifically bind T110 proteins, such as monoclonal or polyclonal antibodies. In addition, the T110 proteins or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of T110 activity or expression in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of T110 activity such that the presence of T110 activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating T110 activity comprising contacting a cell with an agent that modulates (inhibits or stimulates) T110 activity or expression such that T110 activity or expression in the cell is modulated. In one embodiment, the agent is an antibody that specifically binds to T110 protein. In another embodiment, the agent modulates expression of T110 by modulating transcription of a T110 gene, splicing of a T110 mRNA, or translation of a T110 mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of the T110 mRNA or the T110 gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant T110 protein activity or nucleic acid expression by administering an agent which is a T110 modulator to the subject. In one embodiment, the T110 modulator is a T110 protein. In another

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embodiment the T110 modulator is a T110 nucleic acid molecule. In other embodiments, the T110 modulator is a peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder characterized by
5 aberrant T110 protein or nucleic acid expression is neoplasia, inappropriate angiogenesis, or inappropriate tissue regeneration after injury.

The present invention also provides a diagnostic assay for identifying the presence or absence of a
10 genetic lesion or mutation characterized by at least one of: (i) aberrant modification or mutation (including partial or complete deletion or amplification) of a gene encoding a T110 protein; (ii) mis-regulation of a gene encoding a T110 protein; and (iii) aberrant post-
15 translational modification of a T110 protein, wherein a wild-type form of the gene encodes a protein with a T110 activity.

In another aspect, the invention provides a method for identifying a compound that binds to or
20 modulates the activity of a T110 protein. In general, such methods entail measuring a biological activity of a T110 protein in the presence and absence of a test compound and identifying those compounds which alter the activity of the T110 protein.

25 The invention also features methods for identifying a compound which modulates the expression of T110 by measuring the expression of T110 in the presence and absence of a compound.

A plasmid containing DNA encoding human T110 and a
30 plasmid containing DNA encoding murine T110 were deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia, 20110-2209, on June 22, 1998, and have been assigned ATCC Accession Nos. _____ and _____, respectively. The
35 deposits were made according to the terms of the Budapest

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Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. The plasmid containing human DNA was deposited in *E. coli* (strain designation Epfthb 110d), which contains a human
5 T110 DNA in the plasmid vector pZL1. The plasmid containing murine DNA was also deposited in *E. coli* (strain designation Epftmb 110g), which contains a murine T110 DNA in the plasmid vector pZL1. The deposits were made merely as a convenience for those of skill in the
10 art and are not an admission that deposits are required under 35 U.S.C. § 112.

The invention includes a nucleic acid molecule that contains the nucleotide sequence of the cDNA having ATCC Accession No. _____, the coding sequence of that
15 cDNA (i.e., the cDNA having ATCC Accession No. _____), or complements thereof. Similarly, the invention includes a nucleic acid molecule that contains the nucleotide sequence of the cDNA having ATCC Accession No. _____, the coding sequence of that cDNA (i.e., the cDNA
20 having ATCC Accession No. _____), or complements thereof.

The invention includes polypeptides encoded by the coding sequence of the nucleic acid molecules described above, i.e., sequence contained within the nucleic acid
25 molecules deposited with the ATCC and assigned ATCC Accession Nos. _____ and _____, and biologically active fragments thereof. Moreover, those of ordinary skill in the art will recognize that many, if not all, of the methods described herein can be practiced with the
30 nucleic acid molecules (or complements or fragments thereof) deposited with the ATCC, as described above, and/or the polypeptides (or fragments thereof) encoded by those molecules, just as they can be practiced as described herein by reference to a given SEQ ID No.

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Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

5 Figure 1 depicts the cDNA sequence (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2) of human T110. The open reading frame of SEQ ID NO:1 extends from nucleotide 131 to nucleotide 1441 (SEQ ID NO:3).

 Figure 2 is a hydropathy plot of human T110. The
10 location of the predicted transmembrane (TM), and extracellular (OUT) domains are indicated as are the position of cysteines (cys; vertical bars) and potential glycosylation sites (Ngly; vertical bars). Relative hydrophobicity is shown above the dotted line, and
15 relative hydrophilicity is shown below the dotted line.

 Figure 3 depicts the cDNA sequence (SEQ ID NO:5) and predicted amino acid sequence (SEQ ID NO:6) of mouse T110. The open reading frame of SEQ ID NO:5 extends from nucleotide 103 to nucleotide 1452 (SEQ ID NO:7).

20 Figure 4 is a hydropathy plot of mouse T110. The location of the predicted transmembrane (TM), and extracellular (OUT) domains are indicated as are the position of cysteines (cys; vertical bars) and potential glycosylation sites (Ngly; vertical bars). Relative
25 hydrophobicity is shown above the dotted line, and relative hydrophilicity is shown below the dotted line.

 Figure 5A depicts the partial cDNA sequence of rat T110 (SEQ ID NO:9).

 Figure 5B depicts the predicted amino acid
30 sequence (SEQ ID NO:10) of rat T110. The coding region of SEQ ID NO:10 extends from nucleotide 1 to nucleotide 507 of SEQ ID NO:9.

 Figure 6 depicts the cDNA sequence (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:4) of a

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potential alternative human T110 translation product. The open reading frame extends from nucleotide 2 to 1411 of SEQ ID NO:1 (SEQ ID NO:12).

Figure 7 is a hydropathy plot of a potential
5 alternative human T110 translation product. The location of the predicted transmembrane (TM), and extracellular (OUT) domains are indicated as are the position of cysteines (cys; vertical bars) and potential glycosylation sites (Ngly; vertical bars). Relative
10 hydrophobicity is shown above the dotted line, and relative hydrophilicity is shown below the dotted line.

Figure 8 depicts the cDNA sequence (SEQ ID NO:5) and predicted amino acid sequence (SEQ ID NO:8) of a potential alternative murine T110 translation product.
15 The open reading frame extends from nucleotide 1 to 1452 of SEQ ID NO:5 (SEQ ID NO:13).

Figure 9 is a hydropathy plot of a potential alternative murine T110 translation product. The location of the predicted transmembrane (TM), and
20 extracellular (OUT) domains are indicated as are the position of cysteines (cys; vertical bars) and potential glycosylation sites (Ngly; vertical bars). Relative hydrophobicity is shown above the dotted line, and relative hydrophilicity is shown below the dotted line.

25 Figure 10 depicts the sequence alignment of *D. melanogaster* four jointed protein (SEQ ID NO:11) with human T110 protein (SEQ ID NO:2).

Figure 11 is a plot showing predicted structural features of a potential alternative human T110 protein.

30 Detailed Description of the Invention

The present invention is based on the discovery of a cDNA molecule encoding human T110, a member of the type-II membrane protein superfamily. A nucleotide sequence encoding a human T110 protein is shown in Figure

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1 (SEQ ID NO:1; SEQ ID NO:3 includes the open reading
frame only). A predicted amino acid sequence of T110
protein is also shown in Figure 1 (SEQ ID NO: 2). This
protein includes a predicted signal peptide of about 28
5 amino acids (from amino acid 1 to about amino acid 28 of
SEQ ID NO:2). The predicted mature protein extends from
about amino acid 29 to amino acid 437 of SEQ ID NO:2 (SEQ
ID NO:14).

The human T110 cDNA of Figure 1 (SEQ ID NO:1),
10 which is approximately 2401 nucleotides long including
untranslated regions, encodes a protein amino acid having
a molecular weight of approximately 48 kDa (excluding
post-translational modifications).

Human T110 protein and *D. melanogaster* four-
15 jointed (fj) protein share many primary features. They
are proteins of similar size and both contain a single
predicted hydrophobic region near the N-terminus that may
be a transmembrane domain rather than a signal sequence.
Thus, the hydrophobic region from amino acids 1-28 (or 7-
20 30) might be a transmembrane domain that acts as an
internal signal sequence. Each protein contain two pairs
of conserved cysteine residues, one pair near the center
of the molecule (cys₁₆₁ and cys₁₇₈), the other pair near the
C-terminus of the molecule (cys₃₆₅ and cys₄₂₇). Regions of
25 highest identity between the two proteins surround the
two pairs of cysteines in the extracellular domains.
Each protein also contains putative N-glycosylation
sites, two of which are in approximately the same
position, i.e., between the two pairs of cysteines (amino
30 acid residuess 248 to 251 and amino acid residues 277 to
280). A sequence alignment of human T110 protein and
D. melanogaster fj protein is depicted in Figure 6. In
this alignment, human T110 protein and *D. melanogaster* fj
protein display about 30% identity and about 36%
35 similarity.

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An approximately 2.4 kb human T110 mRNA transcript is expressed at the highest level in brain, heart, placenta, and pancreas. Low levels of this transcript have been observed in liver, skeletal muscle, and kidney.

5 No detectable message is seen in lung. Embryonic expression is seen in week 8-9 fetus and week 20 liver and spleen mixed tissues. Embryonic expression is also observed in neuronal tissue.

Human T110 is one member of a family of molecules
10 (the "T110 family") having certain conserved structural and functional features. The present invention provides detailed description of various members of the "T110 family", e.g., human T110, mouse T110, and rat T110. The term "family" when referring to the protein and nucleic
15 acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain and having sufficient amino acid or nucleotide sequence identity as defined herein. Such family members can be naturally occurring and can be from
20 either the same or different species. For example, a family can contain a first protein of human origin and a homologue of that protein of murine origin, as well as a second, distinct protein of human origin and a murine homologue of that protein. Members of a family may also
25 have common functional characteristics.

Preferred T110 polypeptides of the present invention have an amino acid sequence sufficiently identical to the consensus amino acid sequence of human T110 protein. As used herein, the term "sufficiently
30 identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., an amino acid residue which has a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence
35 such that the first and second amino acid or nucleotide

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sequences have a common structural domain and/or common functional activity. For example, amino acid or nucleotide sequences which contain a common structural domain having about 65% identity, preferably 75%
5 identity, more preferably 85%, 95%, or 98% identity are defined herein as sufficiently identical.

As used interchangeably herein a "T110 activity", "biological activity of T110" or "functional activity of T110", refers to an activity exerted by a T110 protein,
10 polypeptide or nucleic acid molecule on a T110 responsive cell as determined *in vivo*, or *in vitro*, according to standard techniques. A T110 activity can be a direct activity, such as an association with or an enzymatic activity on a second protein or an indirect activity,
15 such as a cellular signaling activity mediated by interaction of the T110 protein with a second protein. In a preferred embodiment, a T110 activity includes at least one or more of the following activities: (i) the ability to interact with proteins in the T110 signalling
20 pathway (ii) the ability to interact with a T110 ligand or receptor (iii) the ability to interact with an intracellular target protein; and (iv) the ability to interact with proteins involved in cellular proliferation or differentiation.

25 Accordingly, another embodiment of the invention features isolated T110 proteins and polypeptides having a T110 activity.

Various aspects of the invention are described in further detail in the following subsections.

30 I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode T110 proteins or biologically active portions thereof, as well as nucleic acid molecules sufficient for use as hybridization probes

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to identify T110-encoding nucleic acids (e.g., T110 mRNA) and fragments for use as PCR primers for the amplification or mutation of T110 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

10 An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences (preferably protein encoding sequences) which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated T110 nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

30 A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or a complement of any of these nucleotide sequences, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid

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sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or
SEQ ID NO:7 as a hybridization probe, T110 nucleic acid
molecules can be isolated using standard hybridization
and cloning techniques (e.g., as described in Sambrook et
5 al., eds., *Molecular Cloning: A Laboratory Manual*. 2nd,
ed., Cold Spring Harbor Laboratory, Cold Spring Harbor
Laboratory Press, Cold Spring Harbor, NY, 1989).

A nucleic acid of the invention can be amplified
using cDNA, mRNA or genomic DNA as a template and
10 appropriate oligonucleotide primers according to standard
PCR amplification techniques. The nucleic acid so
amplified can be cloned into an appropriate vector and
characterized by DNA sequence analysis. Furthermore,
oligonucleotides corresponding to T110 nucleotide
15 sequences can be prepared by standard synthetic
techniques, e.g., using an automated DNA synthesizer.

In another preferred embodiment, an isolated
nucleic acid molecule of the invention comprises a
nucleic acid molecule which is a complement of the
20 nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3,
SEQ ID NO:5, SEQ ID NO:7, or a portion thereof. A
nucleic acid molecule which is complementary to a given
nucleotide sequence is one which is sufficiently
complementary to the given nucleotide sequence that it
25 can hybridize to the given nucleotide sequence thereby
forming a stable duplex.

Moreover, the nucleic acid molecule of the
invention can comprise only a portion of a nucleic acid
sequence encoding T110, for example, a fragment which can
30 be used as a probe or primer or a fragment encoding a
biologically active portion of T110. The nucleotide
sequence determined from the cloning of the human T110
gene allows for the generation of probes and primers
designed for use in identifying and/or cloning T110
35 homologues in other cell types, e.g., from other tissues,

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as well as T110 homologues from other mammals. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under
5 stringent conditions to at least about 12, preferably about 25, more preferably about 50, 75, 100, 125, 150, 175, 200, 250, 300, 350 or 400 consecutive nucleotides of the sense or anti-sense sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or of a naturally
10 occurring mutant of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7.

Probes based on the human T110 nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or identical proteins. The probe
15 comprises a label group attached thereto, e.g., a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissues which mis-express a T110 protein, such as by measuring a
20 level of a T110-encoding nucleic acid in a sample of cells from a subject, e.g., detecting T110 mRNA levels or determining whether a genomic T110 gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically
25 active portion of T110" can be prepared by isolating a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 which encodes a polypeptide having a T110 biological activity, expressing the encoded portion of T110 protein (e.g., by recombinant expression *in vitro*) and assessing
30 the activity of the encoded portion of T110.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7 due to degeneracy of the genetic code and thus encode the same
35 T110 protein as that encoded by the nucleotide sequence

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shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7.

In addition to the human T110 nucleotide sequence shown in SEQ ID NO:1 and SEQ ID NO:3, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of T110 may exist within a population (e.g., the human population). Such genetic polymorphism in the T110 gene may exist among individuals within a population due to natural allelic variation. An allele is one of a group of genes which occur alternatively at a given genetic locus. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a T110 protein, preferably a mammalian T110 protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the T110 gene. Alternative alleles can be identified by sequencing the gene of interest in a number of different individuals. This can be readily accomplished using hybridization probes recognizing T110 sequences to identify the same genetic locus in a variety of individuals. Any and all such nucleotide variations and resulting amino acid polymorphisms in T110 that are the result of natural allelic variation and that do not alter the functional activity of T110 are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding T110 proteins from other species (T110 homologues), which have a nucleotide sequence which differs from that of a human T110, are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the T110 cDNA of the invention can be isolated based on their identity to the human T110 nucleic acids disclosed herein using

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the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. For example, a soluble human T110 cDNA can be isolated based on its
5 identity to human membrane-bound T110. Likewise, a cDNA encoding a membrane-bound form of human T110 can be isolated based on its identity to soluble human T110.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 440
10 (450, 500, 550, 600, 650, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, or 1420) nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule having the nucleotide sequence as shown in SEQ ID NO:1 or a complement thereof; or the isolated nucleic
15 acid molecule is at least 220 (250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, or 1420) nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule having the nucleotide sequence as shown in SEQ ID NO:3 or
20 a complement thereof; or the isolated nucleic acid molecule is at least 450 (500, 550, 600, 650, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, or 1450) nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence
25 as shown in SEQ ID NO:5 or SEQ ID NO:7, or a complement thereof.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide
30 sequences at least 60% (65%, 70%, preferably 75%, 85%, or 95%) identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y.
35 (1989), 6.3.1-6.3.6. A preferred, non-limiting example

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of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule
5 of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7 corresponds to a naturally-occurring nucleic acid molecule. As used herein, a
10 "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

In addition to naturally-occurring allelic variants of the T110 sequence that may exist in the population, the skilled artisan will further appreciate
15 that changes can be introduced by mutation into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 or SEQ ID NO:7, thereby leading to changes in the amino acid sequence of the encoded T110 protein, without altering the functional ability of the T110 protein. For
20 example, one can make nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of T110 (e.g., the sequence of SEQ ID NO:2 or SEQ ID
25 NO:6) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the T110 proteins of various species are predicted to be particularly unamenable to
30 alteration.

For example, preferred T110 proteins of the present invention contain at least two pairs of conserved cysteines in the extracellular domain. Such conserved amino acids are less likely to be amenable to mutation.
35 Other amino acid residues, however, (e.g., those that are

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not conserved or only semi-conserved among T110 of various species) may not be essential for activity and thus are likely to be amenable to alteration.

In another example, preferred T110 proteins of the present invention contain residues that are identical between human T110 and mouse T110. Such conserved amino acids between human T110 and mouse T110 are likely to be structurally or functionally significant. Accordingly, it is preferable to preserve these conserved residues unless one wishes to decrease protein stability or activity.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding T110 proteins that contain changes in amino acid residues that are not essential for activity. Such T110 proteins differ in amino acid sequence from SEQ ID NO:2, 4, 6 or 8 yet retain biological activity. In one embodiment, the isolated nucleic acid molecule includes a nucleotide sequence encoding a protein that includes an amino acid sequence that is at least about 65% identical, 75%, 85%, 95%, or 98% identical to the amino acid sequence of SEQ ID NO:2, 4, 6, or 8.

An isolated nucleic acid molecule encoding a T110 protein having a sequence which differs from that of SEQ ID NO:2, 4, 6, or 8 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which

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the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids
5 with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine,
10 valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in T110
15 is preferably replaced with another amino acid residue from the same side chain family. Alternatively, mutations can be introduced randomly along all or part of a T110 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened
20 for T110 biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In a preferred embodiment, a mutant T110 protein
25 can be assayed for: (1) the ability to form protein:protein interactions with proteins in a T110 signalling pathway; (2) the ability to bind a T110 ligand or receptor (3) the ability to bind to an intracellular target protein; or (4) the ability to interact with a
30 protein involved in cellular proliferation or differentiation. In yet another preferred embodiment, a mutant T110 can be assayed for the ability to modulate cellular proliferation or cellular differentiation.

The present invention encompasses antisense
35 nucleic acid molecules, i.e., molecules which are

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complementary to a sense nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can
5 hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire T110 coding strand, or to only a portion thereof, e.g., all or part of the protein coding region (or open reading frame). An antisense nucleic acid molecule can be
10 antisense to a noncoding region of the coding strand of a nucleotide sequence encoding T110. The noncoding regions ("5' and 3' untranslated regions") are the 5' and 3' sequences which flank the coding region and are not translated into amino acids.

15 Given the coding strand sequences encoding T110 disclosed herein (e.g., SEQ ID NO:1 or SEQ ID NO:3), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary
20 to the entire coding region of T110 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of T110 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation
25 start site of T110 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using
30 procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to
35 increase the physical stability of the duplex formed

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

30 The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a T110 protein to thereby inhibit expression of the protein, 35 e.g., by inhibiting transcription and/or translation.

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

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

The invention also encompasses ribozymes. Ribozymes are catalytic RNA molecules with ribonuclease

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activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) Nature 334:585-591)) can be used to catalytically cleave T110 mRNA transcripts to thereby inhibit translation of T110 mRNA. A ribozyme having specificity for a T110-encoding nucleic acid can be designed based upon the nucleotide sequence of a T110 cDNA disclosed herein (e.g., SEQ ID NO:1, SEQ ID NO:3). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a T110-encoding mRNA. See, e.g., Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, T110 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel and Szostak (1993) *Science* 261:1411-1418.

20 The invention also encompasses nucleic acid molecules which form triple helical structures. For example, T110 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the T110 (e.g., the T110 promoter and/or enhancers) to form triple helical structures that prevent transcription of the T110 gene in target cells. See generally, Helene (1991) *Anticancer Drug Des.* 6(6):569-84; Helene (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14(12):807-15.

30 In preferred embodiments, the nucleic acid molecules of the invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate

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peptide nucleic acids (see Hyrup et al. (1996) *Bioorganic & Medicinal Chemistry* 4(1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose
5 phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA
10 oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup et al. (1996) *supra*; Perry-O'Keefe et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 14670-675.

PNAs of T110 can be used in therapeutic and
15 diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, e.g., inducing transcription or translation arrest or inhibiting replication. PNAs of T110 can also be used, e.g., in the
20 analysis of single base pair mutations in a gene by, e.g., PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, e.g., S1 nucleases (Hyrup (1996) *supra*; or as probes or primers for DNA sequence and hybridization
25 (Hyrup (1996) *supra*; Perry-O'Keefe et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 14670-675).

In another embodiment, PNAs of T110 can be modified, e.g., to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to
30 PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of T110 can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition
35 enzymes, e.g., RNase H and DNA polymerases, to interact

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with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds
5 between the nucleobases, and orientation (Hyrup (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996) *supra* and Finn et al. (1996) *Nucleic Acids Research* 24(17):3357-63. For example, a DNA chain can be synthesized on a solid
10 support using standard phosphoramidite coupling chemistry and modified nucleoside analogs. Compounds such as 5'-(4-meth-oxytrityl)amino-5'-deoxy-thymidine phosphoramidite can be used as a link between the PNA and the 5' end of DNA (Mag et al. (1989) *Nucleic Acid Res.*
15 17:5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn et al. (1996) *Nucleic Acids Research* 24(17):3357-63). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a
20 3' PNA segment (Peterser et al. (1975) *Bioorganic Med. Chem. Lett.* 5:1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents
25 facilitating transport across the cell membrane (see, e.g., Letsinger et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W0 88/09810) or the blood-brain barrier (see, e.g., PCT Publication No.
30 W0 89/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (see, e.g., Krol et al. (1988) *Bio/Techniques* 6:958-976) or intercalating agents (see, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be
35 conjugated to another molecule, e.g., a peptide,

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hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

II. Isolated T110 Proteins and Anti-T110 Antibodies

One aspect of the invention pertains to isolated
5 T110 proteins, and biologically active portions thereof,
as well as polypeptide fragments suitable for use as
immunogens to raise anti-T110 antibodies. In one
embodiment, native T110 proteins can be isolated from
cells or tissue sources by an appropriate purification
10 scheme using standard protein purification techniques.
In another embodiment, T110 proteins are produced by
recombinant DNA techniques. Alternative to recombinant
expression, a T110 protein or polypeptide can be
synthesized chemically using standard peptide synthesis
15 techniques.

An "isolated" or "purified" protein or
biologically active portion thereof is substantially free
of cellular material or other contaminating proteins from
the cell or tissue source from which the T110 protein is
20 derived, or substantially free of chemical precursors or
other chemicals when chemically synthesized. The
language "substantially free of cellular material"
includes preparations of T110 protein in which the
protein is separated from cellular components of the
25 cells from which it is isolated or recombinantly
produced. Thus, T110 protein that is substantially free
of cellular material includes preparations of T110
protein having less than about 30%, 20%, 10%, or 5% (by
dry weight) of non-T110 protein (also referred to herein
30 as a "contaminating protein"). When the T110 protein or
biologically active portion thereof is recombinantly
produced, it is also preferably substantially free of
culture medium, i.e., culture medium represents less than
about 20%, 10%, or 5% of the volume of the protein

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preparation. When T110 protein is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, i.e., it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly such preparations of T110 protein have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or non-T110 chemicals.

Biologically active portions of a T110 protein include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the T110 protein (e.g., the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8), which include fewer amino acids than the full length T110 proteins, and exhibit at least one activity of a T110 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the T110 protein. A biologically active portion of a T110 protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length. Preferred biologically active polypeptides include one or more identified T110 structural domains, e.g., the extracellular domain (SEQ ID NO:4 and SEQ ID NO:8).

Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native T110 protein. Preferred T110 protein has the amino acid sequence shown of SEQ ID NO:2 or SEQ ID NO:6. Other useful T110 proteins are substantially identical to SEQ ID NO:2 or SEQ ID NO:6 and retain the functional activity of the protein of SEQ ID NO:2 or SEQ ID NO:6 yet differ in amino acid sequence due to natural allelic variation or mutagenesis. Accordingly, a useful T110 protein is a

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protein which includes an amino acid sequence at least about 45%, preferably 55%, 65%, 75%, 85%, 95%, or 99% identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:6 and retains the functional activity of the
5 T110 proteins of SEQ ID NO:2. In other instances, the T110 protein is a protein having an amino acid sequence 55%, 65%, 75%, 85%, 95%, or 98% identical to the T110 extracellular domain (SEQ ID NO:4 or SEQ ID NO:8). In a preferred embodiment, the T110 protein retains a
10 functional activity of the T110 protein of SEQ ID NO:2 or SEQ ID NO:6.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can
15 be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position
20 in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical
25 positions shared by the sequences (i.e., % identity = # of identical positions/total # of overlapping positions x 100). Preferably, the two sequences are the same length.

The determination of percent homology between two sequences can be accomplished using a mathematical
30 algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Nat'l Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Nat'l Acad. Sci. USA*
35 90:5873-5877. Such an algorithm is incorporated into the

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NBLAST and XBLAST programs of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous
5 to T110 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to T110 protein molecules of the invention. To obtain gapped alignments for comparison
10 purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See
15 <http://www.ncbi.nlm.nih.gov>. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the
20 GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be
25 determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, only exact matches are counted.

The invention also provides T110 chimeric or fusion proteins. As used herein, a T110 "chimeric
30 protein" or "fusion protein" comprises a T110 polypeptide operably linked to a non-T110 polypeptide. A "T110 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to T110, whereas a "non-T110 polypeptide" refers to a polypeptide having an amino acid
35 sequence corresponding to a protein which is not

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substantially identical to the T110 protein, e.g., a protein which is different from the T110 protein and which is derived from the same or a different organism. Within a T110 fusion protein the T110 polypeptide can
5 correspond to all or a portion of a T110 protein, preferably at least one biologically active portion of a T110 protein. Within the fusion protein, the term "operably linked" is intended to indicate that the T110 polypeptide and the non-T110 polypeptide are fused in-
10 frame to each other. The non-T110 polypeptide can be fused to the N-terminus or C-terminus of the T110 polypeptide.

One useful fusion protein is a GST-T110 fusion protein in which the T110 sequences are fused to the
15 C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant T110.

In yet another embodiment, the fusion protein is an T110-immunoglobulin fusion protein in which all or part of T110 is fused to sequences derived from a member
20 of the immunoglobulin protein family. The T110-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a T110 ligand and a T110 protein on the surface
25 of a cell, to thereby suppress T110-mediated signal transduction *in vivo*. The T110-immunoglobulin fusion proteins can be used to affect the bioavailability of a T110 cognate ligand. Inhibition of the T110 ligand/T110 interaction may be useful therapeutically for both the
30 treatment of proliferative and differentiative disorders, as well as modulating (e.g. promoting or inhibiting) cell survival. Moreover, the T110-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-T110 antibodies in a subject, to purify T110
35 ligands and in screening assays to identify molecules

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which inhibit the interaction of T110 with a T110 ligand.

Preferably, a T110 chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the
5 different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as
10 appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene
15 fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, e.g., *Current Protocols in Molecular*
20 *Biology*, Ausubel et al. eds., John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). An T110-encoding nucleic acid can be cloned into such an expression vector such that the
25 fusion moiety is linked in-frame to the T110 protein.

The present invention also pertains to variants of the T110 proteins (i.e., proteins having a sequence which differs from that of a naturally occurring T110) which function as either T110 agonists (mimetics) or as T110
30 antagonists. Variants of the T110 protein can be generated by mutagenesis, e.g., discrete point mutation or truncation of the T110 protein. An agonist of the T110 protein can retain substantially the same, or a subset, of the biological activities of the naturally

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occurring form of the T110 protein. An antagonist of the T110 protein can inhibit one or more of the activities of the naturally occurring form of the T110 protein by, for example, competitively binding to a downstream or
5 upstream member of a cellular signaling cascade which includes the T110 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Treatment of a subject with a variant having a subset of the biological activities of the
10 naturally occurring form of the protein can have fewer side effects in a subject relative to treatment with the naturally occurring form of the T110 proteins.

Variants of the T110 protein which function as either T110 agonists (mimetics) or as T110 antagonists
15 can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of the T110 protein for T110 protein agonist or antagonist activity. In one embodiment, a variegated library of T110 variants is generated by combinatorial mutagenesis at the nucleic
20 acid level and is encoded by a variegated gene library. A variegated library of T110 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential T110 sequences is
25 expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of T110 sequences therein. There are a variety of methods which can be used to produce libraries of potential T110 variants from a
30 degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one
35 mixture, of all of the sequences encoding the desired set

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of potential T110 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang (1983) *Tetrahedron* 39:3; Itakura et al. (1984) *Annu. Rev. Biochem.* 53:323; Itakura et al. (1984) *Science* 198:1056; Ike et al. (1983) *Nucleic Acid Res.* 11:477).

In addition, libraries of fragments of the T110 protein coding sequence can be used to generate a variegated population of T110 fragments for screening and subsequent selection of variants of a T110 protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a T110 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes of the T110 protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of T110 proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions

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in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a technique which enhances the frequency of functional
5 mutants in the libraries, can be used in combination with the screening assays to identify T110 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave et al. (1993) *Protein Engineering* 6(3):327-331).

An isolated T110 protein, or a portion or fragment
10 thereof, can be used as an immunogen to generate antibodies that bind T110 using standard techniques for polyclonal and monoclonal antibody preparation. The full-length T110 protein can be used or, alternatively, the invention provides antigenic peptide fragments of
15 T110 for use as immunogens. The antigenic peptide of T110 comprises at least 8 (preferably 10, 15, 20, or 30) amino acid residues of the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:6 and encompasses an epitope of T110 such that an antibody raised against the peptide
20 forms a specific immune complex with T110.

Preferred epitopes encompassed by the antigenic peptide are regions of T110 that are located on the surface of the protein, e.g., hydrophilic regions. Other important criteria include a preference for a terminal
25 sequence, high antigenic index (e.g., as predicted by the Jameson-Wolf algorithm), ease of peptide synthesis (e.g., avoidance of prolines), and high surface probability (e.g., as predicted by the Emini algorithm).

A T110 immunogen typically is used to prepare
30 antibodies by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed T110 protein or a chemically synthesized T110 polypeptide. The preparation
35 can further include an adjuvant, such as Freund's

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complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic T110 preparation induces a polyclonal anti-T110 antibody response.

5 Accordingly, another aspect of the invention pertains to anti-T110 antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen
10 binding site which specifically binds an antigen, such as T110. A molecule which specifically binds to T110 is a molecule which binds T110, but does not substantially bind other molecules in a sample, e.g., a biological sample, which naturally contains T110. Examples of
15 immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind T110. The term "monoclonal
20 antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of T110. A monoclonal antibody composition thus typically
25 displays a single binding affinity for a particular T110 protein with which it immunoreacts.

Polyclonal anti-T110 antibodies can be prepared as described above by immunizing a suitable subject with a T110 immunogen. The anti-T110 antibody titer in the
30 immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized T110. If desired, the antibody molecules directed against T110 can be isolated from the mammal (e.g., from the blood) and further
35 purified by well-known techniques, such as protein A

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chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-T110 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to

5 prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497, the human B cell hybridoma technique (Kozbor et al. (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole et al.

10 (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing various antibodies and monoclonal antibody hybridomas is well known (see generally Current Protocols in Immunology (1994) Coligan

15 et al. (eds.) John Wiley & Sons, Inc., New York, NY). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a T110 immunogen as described above, and the culture supernatants of the resulting

20 hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds T110.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-T110

25 monoclonal antibody (see, e.g., Current Protocols in Immunology, *supra*; Galfre et al. (1977) *Nature* 266:55052; R.H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); and Lerner (1981) *Yale J. Biol.*

30 *Med.*, 54:387-402. Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For

35 example, murine hybridomas can be made by fusing

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lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line, e.g., a myeloma cell line that is sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind T110, e.g., using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-T110 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with T110 to thereby isolate immunoglobulin library members that bind T110. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene SurfZAP™ Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791; PCT Publication No. WO

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92/15679; PCT Publication No. WO 93/01288; PCT
Publication No. WO 92/01047; PCT Publication No. WO
92/09690; PCT Publication No. WO 90/02809; Fuchs et al.
(1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum.*
5 *Antibod. Hybridomas* 3:81-85; Huse et al. (1989) *Science*
246:1275-1281; Griffiths et al. (1993) *EMBO J* 12:725-734.

Additionally, recombinant anti-T110 antibodies,
such as chimeric and humanized monoclonal antibodies,
comprising both human and non-human portions, which can
10 be made using standard recombinant DNA techniques, are
within the scope of the invention. Such chimeric and
humanized monoclonal antibodies can be produced by
recombinant DNA techniques known in the art, for example
using methods described in PCT Publication No. WO
15 87/02671; European Patent Application 184,187; European
Patent Application 171,496; European Patent Application
173,494; PCT Publication No. WO 86/01533; U.S. Patent No.
4,816,567; European Patent Application 125,023; Better et
al. (1988) *Science* 240:1041-1043; Liu et al. (1987) *Proc.*
20 *Natl. Acad. Sci. USA* 84:3439-3443; Liu et al. (1987) *J.*
Immunol. 139:3521-3526; Sun et al. (1987) *Proc. Natl.*
Acad. Sci. USA 84:214-218; Nishimura et al. (1987) *Canc.*
Res. 47:999-1005; Wood et al. (1985) *Nature* 314:446-449;
and Shaw et al. (1988) *J. Natl. Cancer Inst.* 80:1553-
25 1559; Morrison, (1985) *Science* 229:1202-1207; Oi et al.
(1986) *Bio/Techniques* 4:214; U.S. Patent 5,225,539; Jones
et al. (1986) *Nature* 321:552-525; Verhoeyan et al. (1988)
Science 239:1534; and Beidler et al. (1988) *J. Immunol.*
141:4053-4060.

30 Completely human antibodies are particularly
desirable for therapeutic treatment of human patients.
Such antibodies can be produced using transgenic mice
which are incapable of expressing endogenous
immunoglobulin heavy and light chains genes, but which
35 can express human heavy and light chain genes. The

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transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of T110. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The

5 human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA and IgE

10 antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing

15 such antibodies, see, e.g., U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA), can be engaged to provide human antibodies directed against a

20 selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a

25 selected non-human monoclonal antibody, e.g., a murine antibody, is used to guide the selection of a completely human antibody recognizing the same epitope.

First, a non-human monoclonal antibody which binds a selected antigen (epitope), e.g., an antibody which

30 inhibits T110 activity, is identified. The heavy chain and the light chain of the non-human antibody are cloned and used to create phage display Fab fragments. For example, the heavy chain gene can be cloned into a plasmid vector so that the heavy chain can be secreted

35 from bacteria. The light chain gene can be cloned into a

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phage coat protein gene so that the light chain can be expressed on the surface of phage. A repertoire (random collection) of human light chains fused to phage is used to infect the bacteria which express the non-human heavy chain. The resulting progeny phage display hybrid antibodies (human light chain/non-human heavy chain). The selected antigen is used in a panning screen to select phage which bind the selected antigen. Several rounds of selection may be required to identify such phage. Next, human light chain genes are isolated from the selected phage which bind the selected antigen. These selected human light chain genes are then used to guide the selection of human heavy chain genes as follows. The selected human light chain genes are inserted into vectors for expression by bacteria. Bacteria expressing the selected human light chains are infected with a repertoire of human heavy chains fused to phage. The resulting progeny phage display human antibodies (human light chain/human heavy chain).

Next, the selected antigen is used in a panning screen to select phage which bind the selected antigen. The phage selected in this step display completely human antibody which recognize the same epitope recognized by the original selected, non-human monoclonal antibody. The genes encoding both the heavy and light chains are readily isolated and can be further manipulated for production of human antibody. This technology is described by Jespers et al. (1994, Bio/technology 12:899-903).

An anti-T110 antibody (e.g., monoclonal antibody) can be used to isolate T110 by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-T110 antibody can facilitate the purification of natural T110 from cells and of recombinantly produced T110 expressed in host cells. Moreover, an anti-T110

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antibody can be used to detect T110 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the T110 protein. Anti-T110 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

25 III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding T110 (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein

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additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other
5 vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain
10 vectors, expression vectors, are capable of directing the expression of genes to which they are operably linked. In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids (vectors). However, the invention is intended to include
15 such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the
20 invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for
25 expression, which is operably linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for
30 expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control
35 elements (e.g., polyadenylation signals). Such

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regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990).

Regulatory sequences include those which direct
5 constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design
10 of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including
15 fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., T110 proteins, mutant forms of T110, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of T110 in
20 prokaryotic or eukaryotic cells, e.g., bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185,
25 Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most
30 often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the
35 recombinant protein. Such fusion vectors typically serve

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three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity
5 purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion
10 protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5
15 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli*
20 expression vectors include pTrc (Amann et al., (1988) *Gene* 69:301-315) and pET 11d (Studier et al., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on
25 host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host
30 strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host
35 bacteria with an impaired capacity to proteolytically

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cleave the recombinant protein (Gottesman, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128).

Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada et al. (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be accomplished by standard DNA synthesis techniques.

In another embodiment, the T110 expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari et al. (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al. (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and picZ (Invitrogen Corp, San Diego, CA).

Alternatively, T110 can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith et al. (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable

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expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook et al. (*supra*).

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of
5 the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin
10 promoter (liver-specific; Pinkert et al. (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et
15 al. (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) *Science* 230:912-916), and
20 mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) *Science*
25 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an
30 antisense orientation. That is, the DNA molecule is operably linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to T110 mRNA. Regulatory sequences operably linked to a nucleic
35 acid cloned in the antisense orientation can be chosen

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which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific
5 or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the
10 activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub et al. (*Reviews - Trends in Genetics*, Vol. 1(1) 1986).

15 Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the
20 particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are
25 still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, T110 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or
30 mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or
35 transfection techniques. As used herein, the terms

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"transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-
5 precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (*supra*), and other laboratory manuals.

For stable transfection of mammalian cells, it is
10 known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., for resistance to
15 antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a
20 host cell on the same vector as that encoding T110 or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive,
25 while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) T110 protein. Accordingly, the invention further provides methods for
30 producing T110 protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding T110 has been introduced) in a suitable medium such that T110 protein
35 is produced. In another embodiment, the method further

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comprises isolating T110 from the medium or the host cell.

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in
5 one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which T110-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous T110 sequences have been
10 introduced into their genome or homologous recombinant animals in which endogenous T110 sequences have been altered. Such animals are useful for studying the function and/or activity of T110 and for identifying and/or evaluating modulators of T110 activity. As used
15 herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep,
20 dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene
25 product in one or more cell types or tissues of the transgenic animal. As used herein, an "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous T110 gene has been altered by homologous recombination
30 between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing T110-encoding nucleic acid into
35 the male pronuclei of a fertilized oocyte, e.g., by

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microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The T110 cDNA sequence e.g., that of (SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:7) can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of the human T110 gene, such as a mouse T110 gene can be used as a transgene. A nonhuman homologue of the human T110 can be isolated based on hybridization to the human T110 cDNA.

Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the T110 transgene to direct expression of T110 protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, U.S. Patent No. 4,873,191 and in Hogan, *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the T110 transgene in its genome and/or expression of T110 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding T110 can further be bred to other transgenic animals carrying other transgenes.

To create an homologous recombinant animal, a vector is prepared which contains at least a portion of a T110 gene (e.g., a human or a non-human homolog of the T110 gene, e.g., a murine T110 gene) into which a

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deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the T110 gene. In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous T110 gene is functionally disrupted (i.e., no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous T110 gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous T110 protein). In the homologous recombination vector, the altered portion of the T110 gene is flanked at its 5' and 3' ends by additional nucleic acid of the T110 gene to allow for homologous recombination to occur between the exogenous T110 gene carried by the vector and an endogenous T110 gene in an embryonic stem cell. The additional flanking T110 nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see, e.g., Thomas and Capecchi (1987) *Cell* 51:503 for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced T110 gene has homologously recombined with the endogenous T110 gene are selected (see, e.g., Li et al. (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see, e.g., Bradley in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the

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embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline
5 transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Current Opinion in Bio/Technology* 2:823-829 and in PCT Publication Nos. WO 90/11354, WO 91/01140, WO
10 92/0968, and WO 93/04169.

In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase
15 system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see, e.g., Lakso et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman et al.
20 (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of
25 "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals
30 described herein can also be produced according to the methods described in Wilmut et al. (1997) *Nature* 385:810-813 and PCT Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, e.g., a somatic cell, from the transgenic animal can be isolated and induced to exit the
35 growth cycle and enter G₀ phase. The quiescent cell can

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then be fused, e.g., through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it
5 develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, e.g., the somatic cell, is isolated.

10 IV. Pharmaceutical Compositions

The T110 nucleic acid molecules, T110 proteins, and anti-T110 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for
15 administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion
20 media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as
25 any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is
30 formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration.

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Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediamine-tetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injection include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF; Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by

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the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a T110 protein or anti-T110 antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or

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swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or
5 compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a
10 glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. For administration by inhalation, the compounds are delivered in the form of an aerosol spray
15 from a pressurized container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or
20 transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives.
25 Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

30 The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are
35 prepared with carriers that will protect the compound

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against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene
5 vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova
10 Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in
15 the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit
20 form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical
25 carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an
30 active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration
35 (U.S. Patent 5,328,470) or by stereotactic injection

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(see, e.g., Chen et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release
5 matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g. retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery
10 system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

15 The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) detection assays (e.g., chromosomal mapping, tissue typing, forensic biology), c) predictive medicine
20 (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenomics); and d) methods of treatment (e.g., therapeutic and prophylactic). T110 protein interacts with other cellular proteins and can thus be used for (i) regulation of cellular proliferation
25 and (ii) regulation of cellular differentiation. The isolated nucleic acid molecules of the invention can be used to express T110 protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect T110 mRNA (e.g., in a biological
30 sample) or a genetic lesion in a T110 gene, and to modulate T110 activity. In addition, the T110 proteins can be used to screen drugs or compounds which modulate T110 activity or expression as well as to treat disorders characterized by insufficient or excessive production of

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T110 protein or production of T110 protein forms which have decreased or aberrant activity compared to T110 wild type protein. In addition, the anti-T110 antibodies of the invention can be used to detect and isolate T110
5 proteins and modulate T110 activity.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

A. Screening Assays

10 The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) which bind to T110 proteins or have a
15 stimulatory or inhibitory effect on, for example, T110 expression or T110 activity.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of a
20 T110 protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially
25 addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library
30 approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des.* 12:145).

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Examples of methods for the synthesis of molecular libraries can be found in the art, for example in:

DeWitt et al. (1993) *Proc. Natl. Acad. Sci. U.S.A.*

90:6909; Erb et al. (1994) *Proc. Natl. Acad. Sci. USA*

5 91:11422; Zuckermann et al. (1994). *J. Med. Chem.*

37:2678; Cho et al. (1993) *Science* 261:1303; Carrell et

al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell et

al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and

Gallop et al. (1994) *J. Med. Chem.* 37:1233.

10 Libraries of compounds may be presented in
solution (e.g., Houghten (1992) *Bio/Techniques* 13:412-
421), or on beads (Lam (1991) *Nature* 354:82-84), chips
(Fodor (1993) *Nature* 364:555-556), bacteria (U.S. Patent
No. 5,223,409), spores (Patent Nos. 5,571,698; 5,403,484;
15 and 5,223,409), plasmids (Cull et al. (1992) *Proc. Natl.*
Acad. Sci. USA 89:1865-1869) or phage (Scott and Smith
(1990) *Science* 249:386-390; Devlin (1990) *Science*
249:404-406; Cwirla et al. (1990) *Proc. Natl. Acad. Sci.*
87:6378-6382; and Felici (1991) *J. Mol. Biol.* 222:301-
20 310).

In one embodiment, an assay is a cell-based assay
in which a cell which expresses a membrane-bound form of
T110 protein, or a biologically active portion thereof,
on the cell surface is contacted with a test compound and
25 the ability of the test compound to bind to a T110
protein determined. The cell, for example, can be a
yeast cell or a cell of mammalian origin. Determining
the ability of the test compound to bind to the T110
protein can be accomplished, for example, by coupling the
30 test compound with a radioisotope or enzymatic label such
that binding of the test compound to the T110 protein or
biologically active portion thereof can be determined by
detecting the labeled compound in a complex. For
example, test compounds can be labeled with ¹²⁵I, ³⁵S, ¹⁴C,
35 or ³H, either directly or indirectly, and the radioisotope

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detected by direct counting of radioemmission or by
scintillation counting. Alternatively, test compounds
can be enzymatically labeled with, for example,
horseradish peroxidase, alkaline phosphatase, or
5 luciferase, and the enzymatic label detected by
determination of conversion of an appropriate substrate
to product. In a preferred embodiment, the assay
comprises contacting a cell which expresses a membrane-
bound form of T110 protein, or a biologically active
10 portion thereof, on the cell surface with a known
compound which binds T110 to form an assay mixture,
contacting the assay mixture with a test compound, and
determining the ability of the test compound to interact
with a T110 protein, wherein determining the ability of
15 the test compound to interact with a T110 protein
comprises determining the ability of the test compound to
preferentially bind to T110 or a biologically active
portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based
20 assay comprising contacting a cell expressing a membrane-
bound form of T110 protein, or a biologically active
portion thereof, on the cell surface with a test compound
and determining the ability of the test compound to
modulate (e.g., stimulate or inhibit) the activity of the
25 T110 protein or biologically active portion thereof.
Determining the ability of the test compound to modulate
the activity of T110 or a biologically active portion
thereof can be accomplished, for example, by determining
the ability of the T110 protein to bind to or interact
30 with a T110 target molecule.

As used herein, a "target molecule" is a molecule
with which a T110 protein binds or interacts in nature,
for example, a molecule on the surface of a cell which
expresses a T110 protein, a molecule on the surface of a
35 second cell, a molecule in the extracellular milieu, a

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molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. A T110 target molecule can be a non-T110 molecule or a T110 protein or polypeptide of the present invention. In one embodiment, 5 a T110 target molecule is a component of a signal transduction pathway which facilitates transduction of an extracellular signal (e.g., a signal generated by binding of a compound to a membrane-bound T110 molecule or by binding of a soluble form of T110 to a cellular receptor) 10 through the cell membrane and into the cell. The target, for example, can be a second intercellular protein which has catalytic activity or a protein which facilitates the association of downstream signaling molecules with T110.

Determining the ability of the T110 protein to 15 bind to or interact with a T110 target molecule can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the T110 protein to bind to or interact with a T110 target molecule can be accomplished 20 by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (e.g., intracellular Ca^{2+} , diacylglycerol, IP3, etc.), detecting catalytic/enzymatic 25 activity of the target on an appropriate substrate, detecting the induction of a reporter gene (e.g., a T110-responsive regulatory element operably linked to a nucleic acid encoding a detectable marker, e.g. luciferase), or detecting a cellular response, for 30 example, cellular differentiation or cell proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting a T110 protein or biologically active portion thereof with a test compound and determining the ability of the test 35 compound to bind to the T110 protein or biologically

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active portion thereof (e.g., the extracellular domain of T110). Binding of the test compound to the T110 protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay
5 includes contacting the T110 protein or biologically active portion thereof with a known compound which binds T110 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a T110 protein,
10 wherein determining the ability of the test compound to interact with a T110 protein comprises determining the ability of the test compound to preferentially bind to T110 or biologically active portion thereof as compared to the known compound.

15 In another embodiment, an assay is a cell-free assay comprising contacting T110 protein or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the T110
20 protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of T110 can be accomplished, for example, by determining the ability of the T110 protein to bind to a T110 target molecule by one of the methods described
25 above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of T110 can be accomplished by determining the ability of the T110 protein to further modulate a T110 target molecule. For example, the
30 catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay comprises contacting the T110 protein or biologically
35 active portion thereof with a known compound which binds

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T110 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a T110 protein, wherein determining the ability of the test compound to
5 interact with a T110 protein comprises determining the ability of the T110 protein to preferentially bind to or modulate the activity of a T110 target molecule.

The cell-free assays of the present invention are amenable to use of both the soluble form or the membrane-
10 bound form of T110. In the case of cell-free assays comprising the membrane-bound form of T110, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of T110 is maintained in solution. Examples of such solubilizing agents include non-ionic
15 detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)_n, 3-[(3-cholamido-propyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-
20 [(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to
25 immobilize either T110 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. A test compound to T110, or interaction of T110 with a target molecule in the
30 presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a
35 domain that allows one or both of the proteins to be

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bound to a matrix. For example, glutathione-S-transferase/ T110 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical; St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or T110 protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components and complex formation is measured either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of T110 binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either T110 or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated T110 or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals; Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with T110 or target molecules but which do not interfere with binding of the T110 protein to its target molecule can be derivatized to the wells of the plate, and unbound target or T110 trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the T110 or target molecule, as well as enzyme-linked assays which

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rely on detecting an enzymatic activity associated with the T110 or target molecule.

In another embodiment, modulators of T110 expression are identified in a method in which a cell is
5 contacted with a candidate compound and the expression of T110 mRNA or protein in the cell is determined. The level of expression of T110 mRNA or protein in the presence of the candidate compound is compared to the level of expression of T110 mRNA or protein in the
10 absence of the candidate compound. The candidate compound can then be identified as a modulator of T110 expression based on this comparison. For example, when expression of T110 mRNA or protein is greater (statistically significantly greater) in the presence of
15 the candidate compound than in its absence, the candidate compound is identified as a stimulator of T110 mRNA or protein expression. Alternatively, when expression of T110 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in
20 its absence, the candidate compound is identified as an inhibitor of T110 mRNA or protein expression. The level of T110 mRNA or protein expression in the cells can be determined by methods described herein for detecting T110 mRNA or protein.

25 In yet another aspect of the invention, the T110 proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos et al. (1993) *Cell* 72:223-232; Madura et al. (1993) *J. Biol. Chem.* 268:12046-12054; Bartel et
30 al. (1993) *Bio/Techniques* 14:920-924; Iwabuchi et al. (1993) *Oncogene* 8:1693-1696; and PCT Publication No. WO 94/10300), to identify other proteins, which bind to or interact with T110 ("T110-binding proteins" or "T110-bp") and modulate T110 activity. Such T110-binding proteins
35 are also likely to be involved in the propagation of

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signals by the T110 proteins as, for example, upstream or downstream elements of the T110 pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of
5 separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for T110 is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other
10 construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact,
15 *in vivo*, forming an T110-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory
20 site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with T110.

25 This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences
30 identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to:
(i) map their respective genes on a chromosome and, thus, locate gene regions associated with genetic disease;

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(ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

5 1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome.

Accordingly, T110 nucleic acid molecules described herein
10 or fragments thereof, can be used to map the location of T110 genes on a chromosome. The mapping of the T110 sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

15 Briefly, T110 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the T110 sequences. Computer analysis of T110 sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus
20 complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the T110 sequences will yield an amplified fragment.

25 Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using
30 media in which mouse cells cannot grow (because they lack a particular enzyme), but human cells can, the one human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line

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in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio et al. 5 (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid 10 procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the T110 sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments 15 from specific chromosomes. Other mapping strategies which can similarly be used to map a T110 sequence to its chromosome include *in situ* hybridization (described in Fan et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and 20 pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one 25 step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical, e.g., colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands 30 develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location 35 with sufficient signal intensity for simple detection.

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Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma et al., (Human Chromosomes: A Manual of Basic Techniques
5 (Pergamon Press, New York, 1988)).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes.
10 Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

15 Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line
20 through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, e.g., Egeland et al. (1987) *Nature*,
25 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the T110 gene can be determined. If a mutation is observed in some or all of the affected
30 individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes such as
35 deletions or translocations that are visible from

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chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

The T110 sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the T110 sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from

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individuals and from tissue. The T110 sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater
5 degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be
10 compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 can comfortably provide positive individual identification
15 with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.
20 If a panel of reagents from T110 sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database,
25 positive identification of the individual, living or dead, can be made from extremely small tissue samples.

3. Use of Partial T110 Sequences in Forensic Biology

DNA-based identification techniques can also be
30 used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used

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to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a
5 standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can
10 enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for
15 identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions,
20 making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the T110 sequences or portions thereof, e.g., fragments derived from the noncoding regions of SEQ ID NO:1 or SEQ ID NO:5 having a length of at least 20 or 30 bases.

25 The T110 sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, e.g., brain tissue. This can be very useful in
30 cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such T110 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., T110
35 primers or probes can be used to screen tissue culture

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for contamination (i.e., screen for the presence of a mixture of different types of cells in a culture).

C. Predictive Medicine

The present invention also pertains to the field
5 of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trails are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates
10 to diagnostic assays for determining T110 protein and/or nucleic acid expression as well as T110 activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of
15 developing a disorder, associated with aberrant T110 expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with T110 protein, nucleic acid expression or
20 activity. For example, mutations in a T110 gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with T110
25 protein, nucleic acid expression or activity.

Another aspect of the invention provides methods for determining T110 protein, nucleic acid expression or T110 activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that
30 individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (e.g., drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (e.g., the genotype of the individual examined to

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determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs or other
5 compounds) on the expression or activity of T110 in clinical trials.

These and other agents are described in further detail in the following sections.

1. Diagnostic Assays

10 An exemplary method for detecting the presence or absence of T110 in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting T110 protein or nucleic acid (e.g., mRNA,
15 genomic DNA) that encodes T110 protein such that the presence of T110 is detected in the biological sample. A preferred agent for detecting T110 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to T110 mRNA or genomic DNA. The nucleic acid probe can be,
20 for example, a full-length T110 nucleic acid, such as the nucleic acid of SEQ ID NO: 1, 3, 5, or 7, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to
25 T110 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting T110 protein is an antibody capable of binding to T110 protein, preferably
30 an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the

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probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled.

5 Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended
10 to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect T110 mRNA, protein, or genomic DNA in a biological sample *in vitro*
15 as well as *in vivo*. For example, *in vitro* techniques for detection of T110 mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of T110 protein include enzyme linked immunosorbent assays (ELISAs), Western blots,
20 immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of T110 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of T110 protein include introducing into a subject a labeled anti-T110 antibody. For example, the
25 antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively,
30 the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

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In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting T110 protein, mRNA, or genomic DNA, such that the presence of T110 protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of T110 protein, mRNA or genomic DNA in the control sample with the presence of T110 protein, mRNA or genomic DNA in the test sample.

10 The invention also encompasses kits for detecting the presence of T110 in a biological sample (a test sample). Such kits can be used to determine if a subject is suffering from or is at increased risk of developing a disorder associated with aberrant expression of T110
15 (e.g., an immunological disorder). For example, the kit can comprise a labeled compound or agent capable of detecting T110 protein or mRNA in a biological sample and means for determining the amount of T110 in the sample (e.g., an anti-T110 antibody or an oligonucleotide probe
20 which binds to DNA encoding T110, e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7). Kits may also include instruction for observing that the tested subject is suffering from or is at risk of developing a disorder associated with aberrant expression of T110 if the amount
25 of T110 protein or mRNA is above or below a normal level.

For antibody-based kits, the kit may comprise, for example: (1) a first antibody (e.g., attached to a solid support) which binds to T110 protein; and, optionally, (2) a second, different antibody which binds to T110
30 protein or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit may comprise, for example: (1) an oligonucleotide, e.g., a detectably labelled oligonucleotide, which hybridizes to

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a T110 nucleic acid sequence or (2) a pair of primers useful for amplifying a T110 nucleic acid molecule;

The kit may also comprise, e.g., a buffering agent, a preservative, or a protein stabilizing agent.

5 The kit may also comprise components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit may also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of
10 the kit is usually enclosed within an individual container and all of the various containers are within a single package along with instructions for observing whether the tested subject is suffering from or is at risk of developing a disorder associated with aberrant
15 expression of T110.

2. Prognostic Assays

The methods described herein can furthermore be utilized as diagnostic or prognostic assays to identify subjects having or at risk of developing a disease or
20 disorder associated with aberrant T110 expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with T110
25 protein, nucleic acid expression or activity, e.g., a cell proliferation disorder. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing such a disease or disorder. Thus, the present invention provides a method
30 in which a test sample is obtained from a subject and T110 protein or nucleic acid (e.g., mRNA, genomic DNA) is detected, wherein the presence of T110 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant

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T110 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

5 Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or
10 disorder associated with aberrant T110 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with a specific agent or class of agents (e.g., agents of a type which decrease T110 activity). Thus, the present
15 invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant T110 expression or activity in which a test sample is obtained and T110 protein or nucleic acid is detected (e.g., wherein the
20 presence of T110 protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant T110 expression or activity).

 The methods of the invention can also be used to
25 detect genetic lesions or mutations in a T110 gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant T110 expression or activity, e.g., aberrant cell proliferation and/or differentiation. In preferred embodiments, the
30 methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion or mutation characterized by at least one of an alteration affecting the integrity of a gene encoding a T110-protein, or the mis-expression of the T110 gene. For
35 example, such genetic lesions can be detected by

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ascertaining the existence of at least one of: 1) a deletion of one or more nucleotides from a T110 gene; 2) an addition of one or more nucleotides to a T110 gene; 3) a substitution of one or more nucleotides of a T110 gene; 5 4) a chromosomal rearrangement of a T110 gene; 5) an alteration in the level of a messenger RNA transcript of a T110 gene; 6) aberrant modification of a T110 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a 10 messenger RNA transcript of a T110 gene, 8) a non-wild type level of a T110-protein, 9) allelic loss of a T110 gene, 10) inappropriate post-translational modification of a T110-protein, and 11) amplification of a T110 gene. As described herein, there are a large number of assay 15 techniques known in the art which can be used for detecting lesions in a T110 gene.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,195 and 20 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran et al. (1988) *Science* 241:1077-1080; and Nakazawa et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for 25 detecting point mutations in the T110-gene (see, e.g., Abravaya et al. (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, 30 contacting the nucleic acid sample with one or more primers which specifically hybridize to a T110 gene under conditions such that hybridization and amplification of the T110-gene (if present) occurs, and detecting the presence or absence of an amplification product, or 35 detecting the size of the amplification product and

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comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations

5 described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, et al. (1989) *Proc. Natl.*
10 *Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi et al. (1988) *Bio/Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection
15 schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a T110 gene from a sample cell can be identified by alterations
20 in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in
25 fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, e.g., U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme
30 cleavage site.

In other embodiments, genetic mutations in T110 can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides
35 probes (Cronin et al. (1996) *Human Mutation* 7:244-255;

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Kozal et al. (1996) *Nature Medicine* 2:753-759). For example, genetic mutations in T110 can be identified in two-dimensional arrays containing light-generated DNA probes as described in Cronin et al. supra. Briefly, a
5 first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This
10 step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to
15 the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the T110 gene and detect mutations by
20 comparing the sequence of the sample T110 with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a
25 variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Bio/Techniques* 19:448), including sequencing by mass spectrometry (see, e.g., PCT Publication No. WO 94/16101;
30 Cohen et al. (1996) *Adv. Chromatogr.* 36:127-162; and Griffin et al. (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the T110 gene include methods in which protection from cleavage
35 agents is used to detect mismatched bases in RNA/RNA or

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RNA/DNA heteroduplexes (Myers et al. (1985) *Science* 230:1242). In general, the technique of "mismatch cleavage" entails providing heteroduplexes formed by hybridizing (labeled) RNA or DNA containing the wild-type
5 T110 sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample
10 strands. RNA/DNA duplexes can be treated with RNase to digest mismatched regions, and DNA/DNA hybrids can be treated with S1 nuclease to digest mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and
15 with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, e.g., Cotton et al (1988) *Proc. Natl Acad Sci USA*
20 85:4397; Saleeba et al (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize
25 mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in T110 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the
30 thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu et al. (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a T110 sequence, e.g., a wild-type T110 sequence, is hybridized to a cDNA or other DNA product
35 from a test cell(s). The duplex is treated with a DNA

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mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, e.g., U.S. Patent No. 5,459,039.

In other embodiments, alterations in

5 electrophoretic mobility will be used to identify mutations in T110 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita et al. (1989) *Proc.*

10 *Natl. Acad. Sci USA* 86:2766; see also Cotton (1993) *Mutat. Res.* 285:125-144; Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control T110 nucleic acids will be denatured and allowed to renature. The secondary structure of

15 single-stranded nucleic acids varies according to sequence, and the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be

20 enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes

25 in electrophoretic mobility (Keen et al. (1991) *Trends Genet* 7:5).

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing

30 gradient gel electrophoresis (DGGE) (Myers et al. (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR.

35 In a further embodiment, a temperature gradient is used

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in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki et al. (1986) *Nature* 324:163); Saiki et al. (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs et al. (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition, it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini et al. (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at

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the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

5 The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients
10 exhibiting symptoms or family history of a disease or illness involving a T110 gene.

 Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which T110 is expressed may be utilized in the prognostic assays described
15 herein.

3. Pharmacogenomics

 Agents, or modulators which have a stimulatory or inhibitory effect on T110 activity (e.g., T110 gene
20 expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (e.g., a proliferative disorder) associated with aberrant T110 activity. In conjunction with such treatment, the
25 pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic
30 failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (e.g., drugs) for prophylactic or therapeutic treatments based on a
35 consideration of the individual's genotype. Such

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pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens.

Accordingly, the activity of T110 protein, expression of T110 nucleic acid, or mutation content of T110 genes in
5 an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to
10 altered drug disposition and abnormal action in affected persons. See, e.g., Linder (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body
15 are referred to as "altered drug action" conditions. Genetic conditions transmitted as single factors altering the way the body acts on drugs are referred to as "altered drug metabolism" conditions. These pharmacogenetic conditions can occur either as rare
20 defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics,
25 nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes
30 (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a
35 drug. These polymorphisms are expressed in two

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phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several
5 mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active
10 therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular
15 basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of T110 protein, expression of T110 nucleic acid, or mutation content of T110 genes in an individual can be determined to thereby select
20 appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug
25 responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a T110 modulator, such as a modulator identified by one of
30 the exemplary screening assays described herein.

4. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of T110 (e.g., the ability to modulate aberrant cell proliferation

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and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent, as determined by a screening assay as described herein to increase T110 gene expression, increase protein levels, or upregulate T110 activity, can be monitored in clinical trials of subjects exhibiting decreased T110 gene expression, decreased protein levels, or downregulated T110 activity. Alternatively, the effectiveness of an agent, as determined by a screening assay, to decrease T110 gene expression, decrease protein levels, or downregulate T110 activity, can be monitored in clinical trials of subjects exhibiting increased T110 gene expression, increased protein levels, or upregulated T110 activity. In such clinical trials, the expression or activity of T110 and, preferably, other genes that have been implicated in, for example, a cellular proliferation disorder can be used as a marker.

For example, and not by way of limitation, genes, including T110, that are modulated in cells by treatment with an agent (e.g., compound, drug or small molecule) which modulates T110 activity (e.g., as identified in a screening assay described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of T110 and other genes implicated in the disorder. The levels of gene expression (i.e., a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of T110 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the

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physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

5 In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified
10 by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a T110 protein, mRNA, or genomic DNA in the preadministration sample;
15 (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the T110 protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the T110 protein, mRNA, or
20 genomic DNA in the pre-administration sample with the T110 protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be
25 desirable to increase the expression or activity of T110 to higher levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of T110 to lower levels than
30 detected, i.e., to decrease the effectiveness of the agent.

C. Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a

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subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant T110 expression or activity. Such disorders include neoplasia, inappropriate angiogenesis, or inappropriate
5 tissue regeneration.

1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant T110 expression or activity,
10 by administering to the subject an agent which modulates T110 expression or at least one T110 activity. Subjects at risk for a disease which is caused or contributed to by aberrant T110 expression or activity can be identified by, for example, any or a combination of diagnostic or
15 prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the T110 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on
20 the type of T110 aberrancy, for example, a T110 agonist or T110 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

2. Therapeutic Methods

25 Another aspect of the invention pertains to methods of modulating T110 expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of T110 protein
30 activity associated with the cell. An agent that modulates T110 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of a T110 protein, a

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peptide, a T110 peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more of the biological activities of T110 protein. Examples of such stimulatory agents include active T110 protein and a
5 nucleic acid molecule encoding T110 that has been introduced into the cell. In another embodiment, the agent inhibits one or more of the biological activities of T110 protein. Examples of such inhibitory agents include antisense T110 nucleic acid molecules and anti-
10 T110 antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with
15 a disease or disorder characterized by aberrant expression or activity of a T110 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of
20 agents that modulates (e.g., upregulates or downregulates) T110 expression or activity. In another embodiment, the method involves administering a T110 protein or nucleic acid molecule as therapy to compensate for reduced or aberrant T110 expression or activity.

25 Stimulation of T110 activity is desirable in situations in which T110 is abnormally downregulated and/or in which increased T110 activity is likely to have a beneficial effect. Conversely, inhibition of T110 activity is desirable in situations in which T110 is
30 abnormally upregulated and/or in which decreased T110 activity is likely to have a beneficial effect.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and

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published patent applications cited throughout this application are hereby incorporated by reference.

EXAMPLES

Example 1: Isolation and Characterization of Human T110

5 CDNAs

A cDNA library was prepared from polyA mRNA isolated from ratPC12 cells (PC6-3 subline) that had been cultured in the absence of neurotrophic factors (NGF) for 12 hours. Random 5' sequencing yielded a single clone
10 with homology to the *D. melanogaster fj* gene. This partial rat clone was used to screen mouse and human fetal brain cDNA libraries. These screens have yielded clones containing mouse T110 and human T110.

Complete sequencing of the human T110 clone
15 revealed an approximately 2.4 kb cDNA insert with a 1311 base pair open reading frame predicted to encode a novel secreted protein, *i.e.*, human T110. Complete sequencing of the mouse T110 clone revealed an approximately 2.1 kb cDNA insert with a 1350 base pair open reading frame
20 predicted to encode a novel secreted protein, *i.e.*, mouse T110. The mouse and human protein sequences are about 85% identical. The major region of divergence is towards the N-terminus.

Figure 6 depicts the cDNA sequence (SEQ ID NO:1)
25 and predicted amino acid sequence (SEQ ID NO:4) of a potential alternative human T110 translation product. The open reading frame extends from nucleotide 2 to 1411 of SEQ ID NO:1).

Figure 8 depicts the cDNA sequence (SEQ ID NO:5)
30 and predicted amino acid sequence (SEQ ID NO:8) of a potential alternative murine T110 translation product. The open reading frame extends from nucleotide 1 to 1452 of SEQ ID NO:5.

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Example 2: Distribution of T110 mRNA in Human Tissues

The expression of T110 was analyzed using Northern blot hybridization. In rat, the Northern blot analysis of adult tissues showed highest expression in brain and
5 kidney. Expression was also observed in heart and lung. No mRNA was detected in spleen, liver, skeletal muscle or testis.

To examine the tissue distribution of human T110, the rat partial cDNA sequence was used as a probe for the
10 Northern blot analysis. The cDNA was radioactively labeled with ³²P-dCTP using the Prime-It kit (Stratagene; La Jolla, CA) according to the instructions of the supplier. Filters containing human mRNA (MTNI and MTNII: Clontech; Palo Alto, CA) were probed in ExpressHyb
15 hybridization solution (Clontech, Palo Alto, CA) and washed at high stringency according to manufacturer's recommendations.

These studies revealed that human T110 was expressed as an approximately 2.4 kilobase transcript at
20 highest level in brain, heart, placenta, and pancreas. Lower levels of transcript were seen in liver, skeletal muscle, and kidney. Transcript was not detected in lung. Embryonic expression was seen in week 8-9 fetus and week
20 liver and spleen mixed tissue.

25 *In situ* expression assays on mouse embryos revealed that T110 is expressed in the nervous system. In adult mice, *in situ* expression assays revealed that T110 is expressed in discrete regions of the brain, including the cerebellum and olfactory bulb, and in the
30 non-islet cells of the pancreas.

Example 3: Characterization of T110 Proteins

The human T110 cDNA (Figure 1; SEQ ID NO:1) isolated as described above encodes a 437 amino acid protein (Figure 1; SEQ ID NO:2). A hydropathy plot of

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T110 is presented in Figure 2. This plot shows the presence of a signal sequence (amino acids 1-28) and a hydrophobic region that may indicate a transmembrane domain (amino acid 7-30) that acts as an internal signal sequence.

Figure 7 is a plot showing predicted structural features of a potential alternative human T110 protein (SEQ ID NO:4). This figure shows predicted alpha helix regions (Garnier-Robson and Chou-Fasman), predicted beta sheet regions (Garnier-Robson and Chou-Fasman), predicted turn regions (Garnier-Robson and Chou-Fasman), predicted coil regions (Garnier-Robson), predicted hydrophilicity, predicted alpha amphipathic regions (Eisenberg) predicted beta amphipathic regions (Eisenberg), predicted flexible regions (Karplus-Schultz), predicted antigenic index (Jameson-Wolf), and surface probability (Emini).

A sequence alignment of human T110 protein and *D. melanogaster* fj protein, as shown in Figure 6, reveals that both proteins are of similar size, contain a single predicted hydrophobic region as the transmembrane and internal signal sequence, and include a large extracellular domain with two pairs of conserved cysteine residues. In this alignment, which includes gaps, the proteins are 20.7% identical and 35.9% similar.

Mature human T110 has a predicted MW of 48 kDa, not including post-translational modifications.

A secretion assay revealed that T110 is a secreted protein. It may be secreted using a signal peptide (amino acids 1-28) or a transmembrane region (amino acids 7-30) that acts as an internal signal sequence.

Example 4: Preparation of T110 Proteins

Recombinant T110 can be produced in a variety of expression systems. For example, the mature T110 peptide can be expressed as a recombinant glutathione-S-

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transferase (GST) fusion protein in *E. coli* and the fusion protein can be isolated and characterized. Specifically, as described above, T110 can be fused to GST and this fusion protein can be expressed in *E. coli* strain PEB199. Expression of the GST-T110 fusion protein in PEB199 can be induced with IPTG. The recombinant fusion protein can be purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads.

10 Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

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1. An isolated nucleic acid molecule selected from the group consisting of:

- a) a nucleic acid molecule comprising a fragment of at least 450 nucleotides of the nucleotide sequence of
5 SEQ ID NO:1 or a complement thereof;
- b) a nucleic acid molecule comprising a fragment of at least 220 nucleotides of the nucleotide sequence of SEQ ID NO:3 or a complement thereof;
- c) a nucleic acid molecule comprising a fragment
10 of at least 450 nucleotides of the nucleotide sequence of SEQ ID NO:5 or SEQ ID NO:7, or a complement thereof;
- d) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8;
- 15 e) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, wherein the fragment comprises at least 70 contiguous amino acids of SEQ ID NO:2 or SEQ ID NO:4;
- 20 f) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:8, wherein the fragment comprises at least 150 contiguous amino acids of SEQ ID NO:6 or SEQ ID NO:8; and
- 25 g) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule
30 comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7 under stringent conditions.

2. The isolated nucleic acid molecule of claim 1, which is selected from the group consisting of:

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a) a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7, or a complement thereof; and

b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.

3. The nucleic acid molecule of claim 1 further comprising vector nucleic acid sequences.

4. The nucleic acid molecule of claim 1 further comprising nucleic acid sequences encoding a heterologous polypeptide.

5. A host cell which contains the nucleic acid molecule of claim 1.

6. The host cell of claim 5 which is a mammalian host cell.

7. A non-human mammalian host cell containing the nucleic acid molecule of claim 1.

8. An isolated polypeptide selected from the group consisting of:

a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, wherein the fragment comprises at least 70 contiguous amino acids of SEQ ID NO:2 or SEQ ID NO:4;

b) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:8, wherein the fragment comprises at least 150 contiguous amino acids of SEQ ID NO:6 or SEQ ID NO:8; and

c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID

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NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7
5 under stringent conditions.

9. The isolated polypeptide of claim 8 comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 or SEQ ID NO:8.

10. The polypeptide of claim 8 further comprising
10 heterologous amino acid sequences.

11. An antibody which selectively binds to a polypeptide of claim 8.

12. A method for producing a polypeptide selected from the group consisting of:

15 a) a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8;

b) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4,
20 wherein the fragment comprises at least 70 contiguous amino acids of SEQ ID NO:2 or SEQ ID NO:4;

c) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:8, wherein the fragment comprises at least 150 contiguous
25 amino acids of SEQ ID NO:6 or SEQ ID NO:8; and

d) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8, wherein the polypeptide is encoded by a nucleic acid molecule
30 which hybridizes to a nucleic acid molecule comprising

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SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7
under stringent conditions;

comprising culturing the host cell of claim 5
under conditions in which the nucleic acid molecule is
5 expressed.

13. The isolated polypeptide of claim 8
comprising the amino acid sequence of SEQ ID NO:2, SEQ ID
NO:4, SEQ ID NO:6, or SEQ ID NO:8.

14. A method for detecting the presence of a
10 polypeptide of claim 8 in a sample, comprising:

- a) contacting the sample with a compound which
selectively binds to a polypeptide of claim 8; and
- b) determining whether the compound binds to the
polypeptide in the sample.

15 15. The method of claim 14, wherein the compound
which binds to the polypeptide is an antibody.

16. A kit comprising a compound which selectively
binds to a polypeptide of claim 8 and instructions for
use.

20 17. A method for detecting the presence of a
nucleic acid molecule of claim 1 in a sample, comprising
the steps of:

- a) contacting the sample with a nucleic acid
probe or primer which selectively hybridizes to the
25 nucleic acid molecule; and
- b) determining whether the nucleic acid probe or
primer binds to a nucleic acid molecule in the sample.

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18. The method of claim 17, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

19. A kit comprising a compound which selectively
5 hybridizes to a nucleic acid molecule of claim 1 and instructions for use.

20. A method for identifying a compound which binds to a polypeptide of claim 8 comprising the steps of:

- 10 a) contacting a polypeptide, or a cell expressing a polypeptide of claim 8 with a test compound; and
 b) determining whether the polypeptide binds to the test compound.

15 21. The method of claim 20, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

- a) detection of binding by direct detecting of test compound/polypeptide binding;
20 b) detection of binding using a competition binding assay;
 c) detection of binding using an assay for T110-mediated signal transduction.

22. A method for modulating the activity of a
25 polypeptide of claim 8 comprising contacting a polypeptide or a cell expressing a polypeptide of claim 8 with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

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23. A method for identifying a compound which modulates the activity of a polypeptide of claim 8, comprising:

- a) contacting a polypeptide of claim 8 with a
5 test compound; and
- b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

GCGGCCGCGATGGGGCCGAAGCGCCCGAAGCCCGGAGCCACAACTGCCGGGGCCGCTCGCCGCGGGACCCGGGT 79
 M G R R M R G 7
 GCCTGGGCTCGGCTTGAAGCGGCGGCGCACCGGCACAGCCGCGGGAGC ATG GGC AGG AGG ATG CGG GGC 151
 A A A T A G L W L L A L G S L L A L W G 27
 GCC GCC GCC ACC GCG GGG CTC TGG CTG CTG GCG CTG GGC TCG CTG CTG GCG CTG TGG GGA 211
 G L L P P R T E L P A S R P P E D R L P 47
 GGG CTC CTG CCG CCG CGG ACC GAG CTG CCC GCC TCC CGG CCG CCC GAA GAC CGA CTC CCA 271
 R R P A R S G G P A P A P R F P L P P P 67
 CGG CGC CCG GCC CGG AGC GGC GGC CCC GCG CCC GCG CCT CGC TTC CCT CTG CCC CCG CCC 331
 L A W D A R G G S L K T F R A L L T L A 87
 CTG GCG TGG GAC GCC CGC GGC GGC TCC CTG AAA ACT TTC CGG GCG CTG CTC ACC CTG GCG 391
 A G A D G P P R Q S R S E P R W H V S A 107
 GCC GGC GCG GAC GGC CCG CCC CGG CAG TCC CGG AGC GAG CCC AGG TGG CAC GTG TCA GCC 451
 R Q P R P E E S A A V H G G V F W S R G 127
 AGG CAG CCC CGG CCG GAG GAG AGC GCC GCG GTG CAC GGG GGC GTC TTC TGG AGC CGC GGC 511
 L E E Q V P P G F S E A Q A A A W L E A 147
 CTG GAG GAG CAG GTG CCC CCG GGC TTT TCG GAG GCC CAG GCG GCG GCG TGG CTG GAG GCG 571
 A R G A R M V A L E R G G C G R S S N R 167
 GCT CGC GGC GCC CGG ATG GTG GCC CTG GAG CGC GGG GGT TGC GGG CGC AGC TCC AAC CGA 631
 L A R F A D G T R A C V R Y G I N P E Q 187
 CTG GCC CGT TTT GCC GAC GGC ACC CGC GCC TGC GTG CGC TAC GGC ATC AAC CCG GAG CAG 691
 I Q G E A L S Y Y L A R L L G L Q R H V 207
 ATT CAG GGC GAG GCC CTG TCT TAC TAT CTG GCG CGC CTG CTG GGC CTC CAG CGC CAC GTG 751
 P P L A L A R V E A R G A Q W A Q V Q E 227
 CCG CCG CTG GCA CTG GCT CGG GTG GAG GCT CGG GGC GCG CAG TGG GCG CAG GTG CAG GAG 811
 E L R A A H W T E G S V V S L T R W L P 247
 GAG CTG CGC GCT GCG CAC TGG ACC GAG GGC AGC GTG GTG AGC CTG ACA CGC TGG CTG CCC 871
 N L T D V V V P A P W R S E D G R L R P 267
 AAC CTC ACG GAC GTG GTG GTG CCC GCG CCC TGG CGC TCG GAG GAC GGC CGT CTG CGC CCC 931
 L R D A G G E L A N L S Q A E L V D L V 287
 CTC CGG GAT GCC GGG GGT GAG CTG GCC AAC CTC AGC CAG GCG GAG CTG GTG GAC CTA GTA 991
 Q W T D L I L F D Y L T A N F D R L V S 307
 CAA TGG ACC GAC TTA ATC CTT TTC GAC TAC CTG ACG GCC AAC TTC GAC CGG CTC GTA AGC 1051
 N L F S L Q W D P R V M Q R A T S N L H 327
 AAC CTC TTC AGC CTG CAG TGG GAC CCG CGC GTC ATG CAG CGT GCC ACC AGC AAC CTG CAC 1111
 R G P G G A L V F L D N E A G L V H G Y 347
 CGC GGT CCG GGC GGG GCG CTG GTC TTT CTG GAC AAT GAG GCG GGC TTG GTG CAC GGC TAC 1171
 R V A G M W D K Y N E P L L Q S V C V F 367
 CGG GTA GCA GGC ATG TGG GAC AAG TAT AAC GAG CCG CTG TTG CAG TCA GTG TGC GTG TTC 1231

FIGURE 1 (1 of 2)

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R   E   R   T   A   R   R   V   L   E   L   H   R   G   Q   D   A   A   A   R   387
CGC GAG CGG ACC GCG CGG CGC GTC CTG GAG CTG CAC CGC GGA CAG GAC GCC GCG GCC CGG 1291

L   L   R   L   Y   R   R   H   E   P   R   F   P   E   L   A   A   L   A   D   407
CTG CTG CGC CTC TAC CGG CGC CAC GAG CCT CGC TTC CCC GAG CTG GCC GCC CTT GCA GAC 1351

P   H   A   Q   L   L   Q   R   R   L   D   F   L   A   K   H   I   L   H   C   427
CCC CAC GCT CAG CTG CTA CAG CGC CGC CTC GAC TTC CTC GCC AAG CAC ATT TTG CAC TGT 1411

K   A   K   Y   G   R   R   S   G   T   *   438
AAG GCC AAG TAC GGC CGC CGG TCT GGG ACT TAG 1444

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AGGGACCAGCCGCCAACGCCACCCGCAAAGGTGTCTAAAACTTCAGCTTTTCACCCACCTGCCCTTTCTTTCAAT 1602
CCCACGCTGGTTCTTTCAAAGTTCTGGGAGGACGAACCTCACCGAGGCGAGAAGTGTAAACATTCTCTCCACCCAGCTT 1681
ATAAAAGGATTCTTTACTGTGCCAGCACGGGGATTGGATCCGAAGAACTGGCTACTGGGGTTTGGCCCCCGAGTGGCC 1760
GTCCCTGTGGGAGATGCACCCCATTTCTTGGGCCCCCTCATTCCCTTTCCGAAAAGGAAAACCTTGCCTTTGAGCCGTT 1839
GAGCTAATTCTGCAATTTTCTACCAAACAGAGCGCTGGTGGCCCCGAGCAGGGCTGTGACATTGGCTGGTGGAGCCCC 1918
CTTCCTGTGTTCTCCCTTTGTTCAGCGCCGCGATGGTGAGATCACTGTTCCAAGCAGGGGGACGGCTCGCGATAGGAC 1997
AAAGAGAGCAGGACCTCCAGACTCTGGGGAGCCCTGCAGACCTTGACAATTTGCCTGACTCATTCTGACCTCTTGTC 2076
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CGACCAAAATTTTGACAACGATGATGTTCCACGAGAAGGAAAAAAATCAGTTTTATGCACTTTATTTTGTGTTTATTT 2234
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TGGGTCGAC 2401

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FIGURE 1 (2 of 2)

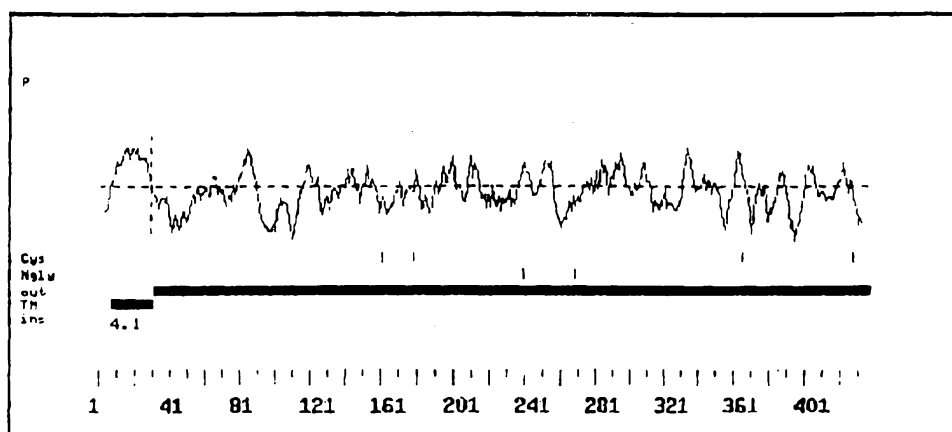


FIGURE 2

FIGURE 3 (1 of 2)

V L E L H R G Q D A A A R L L R L Y S R 394
GTC TTG GAG CTG CAC CGG GGT CAG GAC GCG GCG GCC CGG TTG CTG CGC CTC TAC AGT CGC 1284

H E P R F P E L A E L S E P H A Q L L Q 414
CAC GAA CCG CGT TTC CCA GAG CTG GCG GAG CTC TCA GAA CCC CAC GCT CAG CTG CTA CAG 1344

R R L D F L A K H I L H C K A K Y G R R 434
CGC CGC CTT GAC TTC CTC GCC AAA CAC ATT TTG CAC TGC AAG GCC AAG TAC GGC CGC CGG 1404

P G D L I T L R G R E G L G Y E * 451
CCC GGG GAC TTA ATA ACA CTC CGA GGA AGA GAG GGA CTG GGG TAT GAA TGA 1455

TGGGGGTTAGGGCTGTCTTCTCTGCCACGGGCAACGACCAACCAGCCAACGCCACCCAATGGTCTGATCGCCAAGCTG 1534

TCTAAAAAAAAAAATTGTGTGTACCCACTTGCCCCCTTCTTTTCATGCCAGGCTGTTTCCTTCCCAAGTTCTGGAAGG 1613

GCAAACTCACCGAGGCGAGAAGTGTAACATTCCCTCCACCTAGCTTATAAAAAGAGTTCTGTGCTTGTGTGGAGATTGG 1692

ATCCGAAGAAACTGGCTGCTGGGGTTTGGCCCCGGAATGGCAGTCTCTTTGGGAGATGCAGTCCATTTTTGTGCGCCCCC 1771

TCCACCCCTTCCCAAAAAGGAAAACCTCCGTTTGAGCTAATTCTCTATTTCTGAACTCCTATCATTTTTGGCCTGAAG 1850

GCTATGAATTCAGGACTAGTTATAAGCAGAGTCAATAATGAATTTCTTCTGCGTCTCCCAATTGACCAAAATTGACAA 1929

TGATGATGTTCAACCAGAGGGGGAAAAAATCAGTTTCATGCACCTTTACTTTTTTAATTTTAATTTTTTAGTAAGAAAA 2008

CTTTTTTATTTGACAGAATTTGCCTTCTGTGTATATATGTGCATAAATTGTGCTGTAAATAGACTAAACAACTTAT 2087

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FIGURE 3 (2 of 2)

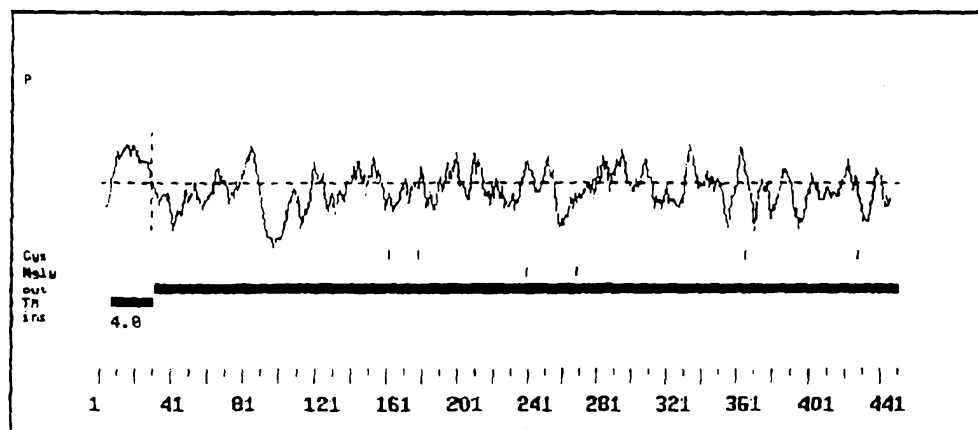


FIGURE 4

GAGCTGGTGGACCTGGTACAATGGACCGATCTGATCCTCTTCGA
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ACGCGTTATGCACCGCGCTACAAGCAACCTGCACCGAGGACCAGGAGGGGCGTTGGTCTT
TCTGGACAATGAGGCGGGCCTGGTGCACGGGTACCGGGTAGCCGGCATGTGGGACAAGTA
TAACGAACCGCTGCTACAGTCGGTGTGTGTATTCCGAGAGCGGACTGCTAGGCGCGTCTT
GGAGCTGCACCGGGGTCAGGACGCGGCAGCCCCGCTCCTGCGCCTCTACAGTCGCCACGA
ACGCGGTTTCCCAGAGCTGGCGGAGCTCGCAGACCCCCACGCTCAGCTGCTACAGCGCCG
CCTTGACTTCCTCGCCAAACACATTTTGCACTGTAAGGCCAAGTACGGCCGCCGACCCGG
GGACTTAATAACACTCCGAGGAAGAGAGGGGACTGGGTTATGAATGATGCGGGTTAGGGCT
GTCTTTTCTGCCACGGGCTATGACCAACCGGCCAACGCCCACCCAGTGGTCTGAATACCA
AGCTGTCTAAAACTTGTGTGTTACCCACTTGCCCCCTTCTTTTCATGCCAGGCTGTTTC
CTTTCCAAGTTCTGGAAGGGCAAACCTCACCGAGGCAAGAAGTGTAACATTCCTCCACCT
AGCTTATAAAAAGGAATCTGTGCTTGTGTGGAGATTGGATCCGAAGAACTGGCTGCTGG
GGTTTGGCCCCCTGAATGGCAGTCTCTTTGGGAGATGCAGTCCATTCTTGCCCCCCCCCTT
CCCAAAAAGGAAAACCTCCGTTTGAGCTAATTCCTCTATTTCTACTAAACACAGCGCC
GGTGGCCCCAGAGCAGGCTGTGACAAGGGCTGGTGGAGCCCCCTTCCTGTGTTCTCTCTT
TGTTCCAGCGCACGATGGTGAGATAACTGTTCCAAGCTGAGGGACAGCTCTGGATAGGAC
AAAGAGAGCAAGACTTCCCCGCCCCCTGGGAGCCCTACAGACCCTGACAGTTTGTCTGACC
CATTCTGAACCCCTGTCAATTTTAGCCTGAAGGCTATGAATTCGGAAC TAGTTATAAGCA
GAGTCAATAATGAATTTCTTCCCGCATCTCCCAATTGACCAAAATTGACAATGATGATGT
TCACCAGAAGAAAGAAAAAATCAGTTTCATGCACTTTACTTTTTTTAATTTTAATTTTT
TAGTAAGAAAACTTTTTTATTTGACAGAATTTGCCTTCTGTGTATATATGTGCATAAATC
GTGGTGTAATAGACTAAACAACTTATATTTCAATAAAAAGGGAGTTTAAAATTTAAAAA
AAAAAAAAAAGGGCGCCGC

Figure 5a

ELVDLVQWTD LILFDYLTAN FDRLVSNLFS LQWDPVMHR ATSNLHRGPG
GALVFLDNEA GLVHG YRVAG MWDKYNEPLL QSVCFREERT ARRVLELHRG
QDAAARLLRL YSRHEPRFPE LAELADPHAQ LLQRRLDFLA KHILHCKAKY
GRRPGDLITL RGREGLGYE

Figure 5b

3	CGG	CCG	CGA	TGG	GGC	CGA	AGC	GCC	CGA	AGC	CCC	CGA	GCC	CAC	AAA	CTG	43
	Arg	Pro	Arg	Trp	Gly	Arg	Ser	Ala	Arg	Ser	Pro	Gly	Ala	His	Lys	Leu	
	1				5				10					15			
	CCG	GGC	CCG	CCT	CGC	CGC	CGG	GAC	CCG	GGT	GCC	TGG	GCT	CGG	CTT	GAA	97
	Pro	Gly	Pro	Pro	Arg	Arg	Arg	Asp	Pro	Gly	Ala	Trp	Ala	Arg	Leu	Glu	
				20					25					30			
	GCG	GCG	GCG	GCG	CAC	CGG	CAC	AGC	CGC	GGG	AGC	ATG	GGC	AGG	AGG	ATG	145
	Ala	Ala	Ala	Ala	His	Arg	His	Ser	Arg	Gly	Ser	Met	Gly	Arg	Arg	Met	
				35				40					45				
	CGG	GGC	GCC	GCC	GCC	ACC	GCG	GGG	CTC	TGG	CTG	CTG	GCG	CTG	GGC	TCG	193
	Arg	Gly	Ala	Ala	Ala	Thr	Ala	Gly	Leu	Trp	Leu	Leu	Ala	Leu	Gly	Ser	
		50					55					60					
	CTG	CTG	GCG	CTG	TGG	GGA	GGG	CTC	CTG	CCG	CCG	CGG	ACC	GAG	CTG	CCC	241
	Leu	Leu	Ala	Leu	Trp	Gly	Gly	Leu	Leu	Pro	Pro	Arg	Thr	Glu	Leu	Pro	
	65					70				75						80	
	GCC	TCC	CGG	CCG	CCC	GAA	GAC	CGA	CTC	CCA	CGG	CGC	CCG	GCC	CGG	AGC	289
	Ala	Ser	Arg	Pro	Pro	Glu	Asp	Arg	Leu	Pro	Arg	Arg	Pro	Ala	Arg	Ser	
					85					90					95		
	GGC	GGC	CCC	GCG	CCC	GCG	CCT	CGC	TTC	CCT	CTG	CCC	CCG	CCC	CTG	GCG	337
	Gly	Gly	Pro	Ala	Pro	Ala	Pro	Arg	Phe	Pro	Leu	Pro	Pro	Pro	Leu	Ala	
				100					105					110			
	TGG	GAC	GCC	CGC	GGC	GGC	TCC	CTG	AAA	ACT	TTC	CGG	GCG	CTG	CTC	ACC	385
	Trp	Asp	Ala	Arg	Gly	Gly	Ser	Leu	Lys	Thr	Phe	Arg	Ala	Leu	Leu	Thr	
			115					120					125				
	CTG	GCG	GCC	GGC	GCG	GAC	GGC	CCG	CCC	CGG	CAG	TCC	CGG	AGC	GAG	CCC	433
	Leu	Ala	Ala	Gly	Ala	Asp	Gly	Pro	Pro	Arg	Gln	Ser	Arg	Ser	Glu	Pro	
		130					135					140					
	AGG	TGG	CAC	GTG	TCA	GCC	AGG	CAG	CCC	CGG	CCG	GAG	GAG	AGC	GCC	GCG	481
	Arg	Trp	His	Val	Ser	Ala	Arg	Gln	Pro	Arg	Pro	Glu	Glu	Ser	Ala	Ala	
	145					150				155						160	
	GTG	CAC	GGG	GGC	GTC	TTC	TGG	AGC	CGC	GGC	CTG	GAG	GAG	CAG	GTG	CCC	529
	Val	His	Gly	Gly	Val	Phe	Trp	Ser	Arg	Gly	Leu	Glu	Glu	Gln	Val	Pro	
					165					170					175		
	CCG	GGC	TTT	TCG	GAG	GCC	CAG	GCG	GCG	GCG	TGG	CTG	GAG	GCG	GCT	CGC	577
	Pro	Gly	Phe	Ser	Glu	Ala	Gln	Ala	Ala	Ala	Trp	Leu	Glu	Ala	Ala	Arg	
				180				185						190			
	GGC	GCC	CGG	ATG	GTG	GCC	CTG	GAG	CGC	GGG	GGT	TGC	GGG	CGC	AGC	TCC	625
	Gly	Ala	Arg	Met	Val	Ala	Leu	Glu	Arg	Gly	Gly	Cys	Gly	Arg	Ser	Ser	
			195					200					205				
	AAC	CGA	CTG	GCC	CGT	TTT	GCC	GAC	GGC	ACC	CGC	GCC	TGC	GTG	CGC	TAC	673
	Asn	Arg	Leu	Ala	Arg	Phe	Ala	Asp	Gly	Thr	Arg	Ala	Cys	Val	Arg	Tyr	
		210					215					220					
	GGC	ATC	AAC	CCG	GAG	CAG	ATT	CAG	GGC	GAG	GCC	CTG	TCT	TAC	TAT	CTG	721
	Gly	Ile	Asn	Pro	Glu	Gln	Ile	Gln	Gly	Glu	Ala	Leu	Ser	Tyr	Tyr	Leu	
	225					230				235						240	

FIGURE 6
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GCG	CGC	CTG	CTG	GGC	CTC	CAG	CGC	CAC	GTG	CCG	CCG	CTG	GCA	CTG	GCT	769
Ala	Arg	Leu	Leu	Gly	Leu	Gln	Arg	His	Val	Pro	Pro	Leu	Ala	Leu	Ala	
				245					250					255		
CGG	GTG	GAG	GCT	CGG	GGC	SCG	CAG	TGG	GCG	CAG	GTG	CAG	GAG	GAG	CTG	817
Arg	Val	Glu	Ala	Arg	Gly	Ala	Gln	Trp	Ala	Gln	Val	Gln	Glu	Glu	Leu	
			260					265					270			
CGC	GCT	GCG	CAC	TGG	ACC	GAG	GGC	AGC	GTG	GTG	AGC	CTG	ACA	CGC	TGG	965
Arg	Ala	Ala	His	Trp	Thr	Glu	Gly	Ser	Val	Val	Ser	Leu	Thr	Arg	Trp	
		275					280					285				
CTG	CCC	AAC	CTC	ACG	GAC	GTG	GTG	GTG	CCC	GCG	CCC	TGG	CGC	TCG	GAG	913
Leu	Pro	Asn	Leu	Thr	Asp	Val	Val	Val	Pro	Ala	Pro	Trp	Arg	Ser	Glu	
	290					295					300					
GAC	GGC	CGT	CTG	CGC	CCC	CTC	CGG	GAT	GCC	GGG	GGT	GAG	CTG	GCC	AAC	961
Asp	Gly	Arg	Leu	Arg	Pro	Leu	Arg	Asp	Ala	Gly	Gly	Glu	Leu	Ala	Asn	
305					310					315					320	
CTC	AGC	CAG	GCG	GAG	CTG	GTG	GAC	CTA	GTA	CAA	TGG	ACC	GAC	TTA	ATC	1009
Leu	Ser	Gln	Ala	Glu	Leu	Val	Asp	Leu	Val	Gln	Trp	Thr	Asp	Leu	Ile	
			325						330					335		
CTT	TTC	GAC	TAC	CTG	ACG	GCC	AAC	TTC	GAC	CGG	CTC	GTA	AGC	AAC	CTC	1057
Leu	Phe	Asp	Tyr	Leu	Thr	Ala	Asn	Phe	Asp	Arg	Leu	Val	Ser	Asn	Leu	
			340					345					350			
TTC	AGC	CTG	CAG	TGG	GAC	CCG	CGC	GTC	ATG	CAG	CGT	GCC	ACC	AGC	AAC	1105
Phe	Ser	Leu	Gln	Trp	Asp	Pro	Arg	Val	Met	Gln	Arg	Ala	Thr	Ser	Asn	
		355					360					365				
CTG	CAC	CGC	GGT	CCG	GGC	GGG	GCG	CTG	GTC	TTT	CTG	GAC	AAT	GAG	GCG	1153
Leu	His	Arg	Gly	Pro	Gly	Gly	Ala	Leu	Val	Phe	Leu	Asp	Asn	Glu	Ala	
	370					375					380					
GGC	TTG	GTG	CAC	GGC	TAC	CGG	GTA	GCA	GGC	ATG	TGG	GAC	AAG	TAT	AAC	1201
Gly	Leu	Val	His	Gly	Tyr	Arg	Val	Ala	Gly	Met	Trp	Asp	Lys	Tyr	Asn	
385					390					395					400	
GAG	CCG	CTG	TTG	CAG	TCA	GTG	TGC	GTG	TTC	CGC	GAG	CGG	ACC	GCG	CGG	1249
Glu	Pro	Leu	Leu	Gln	Ser	Val	Cys	Val	Phe	Arg	Glu	Arg	Thr	Ala	Arg	
				405					410					415		
CGC	GTC	CTG	GAG	CTG	CAC	CGC	GGA	CAG	GAC	GCC	GCG	GCC	CGG	CTG	CTG	1297
Arg	Val	Leu	Glu	Leu	His	Arg	Gly	Gln	Asp	Ala	Ala	Ala	Arg	Leu	Leu	
			420					425					430			
CGC	CTC	TAC	CGG	CGC	CAC	GAG	CCT	CGC	TTC	CCC	GAG	CTG	GCC	GCC	CTT	1345
Arg	Leu	Tyr	Arg	Arg	His	Glu	Pro	Arg	Phe	Pro	Glu	Leu	Ala	Ala	Leu	
		435					440					445				
GCA	GAC	CCC	CAC	GCT	CAG	CTG	CTA	CAG	CGC	CGC	CTC	GAC	TTC	CTC	GCC	1393
Ala	Asp	Pro	His	Ala	Gln	Leu	Leu	Gln	Arg	Arg	Leu	Asp	Phe	Leu	Ala	
	450					455					460					
AAG	CAC	ATT	TTG	CAC	TGT	AAG	GCC	AAG	TAC	GGC	CGC	CGG	TCT	GGG	ACT	1441
Lys	His	Ile	Leu	His	Cys	Lys	Ala	Lys	Tyr	Gly	Arg	Arg	Ser	Gly	Thr	
465					470					475					480	

FIGURE 6
SHEET 2 OF 3

TAGTGTCAACC	GGGAGGAAAA	GAGAGAGATC	TGGGGCTGGG	GTATGGATGA	TGGGGGGAAG
GGCGGTGCGCC	TCTGCCACTG	TCAGGGACCA	GCCGGGCAAC	GCCCACCCGC	AAAGGTGTCT
AAAAACTTCA	GCTTTTCACC	CACCTGCCCC	TTTCTTTCAA	TCCCACGCTG	TTCTTTTCA
AAGTTCTGGG	AGGACGAAC	TCACCGAGGC	GAGAAGTGTA	ACATTCTCTC	CACCCAGCTT
ATAAAAGGAT	TCTTTACTGT	GCCAGCACGG	GGATTGGATC	CGAAGAAACT	GGCTACTGGG
GTTTGGCCCC	CGAGTGGCCG	TCCCTGTGGG	AGATGCACCC	CATTCTTGGG	CCCCCCTCAT
TCCCTTTCCG	AAAAAGGAAA	ACTTGCGTTT	GAGCCGTTGA	GCTAATTCTG	CAATTTTCTA
CCAAACAGAG	CGCTGGTGGC	CCCGGAGCAG	GGCTGTGACA	TTGGCTGGTG	GAGCCCCCTT
CCTGTGTTCT	CCCTTTGTTC	CAGCGCCGCG	ATGGTGAGAT	CACTGTTCCA	AGCAGGGGGA
CGGCTCGCGA	TAGGACAAAG	AGAGCAGGAC	CTCCAGACTC	TGGGGAGCCC	TGCAGACCTT
GACAATTTGC	CTGACTCATT	CCTGACCTCT	TGTCATTTTG	GCCTGAAGGC	TACAAATTCA
GGGTCAGCTG	TATGCACTAA	GTCAAATAAT	GAATTTCTTC	CTCCCTCTCG	CAACCGACCA
AAATTTTGAC	AACGATGATG	TTCACCAGAA	GGAAAAAAA	ATCAGTTTTA	TGCACTTTAT
TTTGTTTTGA	TTTTCATTTT	TTATTAAGAA	AAAATTTTAT	TTTACAGAAT	TTACCTTCTC
TGTATATATG	TGCATAAAGT	GTGGTGTAAG	TATACTAAAC	AAACTTATAT	TTCAATAAAA
GGGAGTTTAA	AATTTAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAACGGACGC	GTGGGTGCGC

FIGURE 6
SHEET 3 OF 3

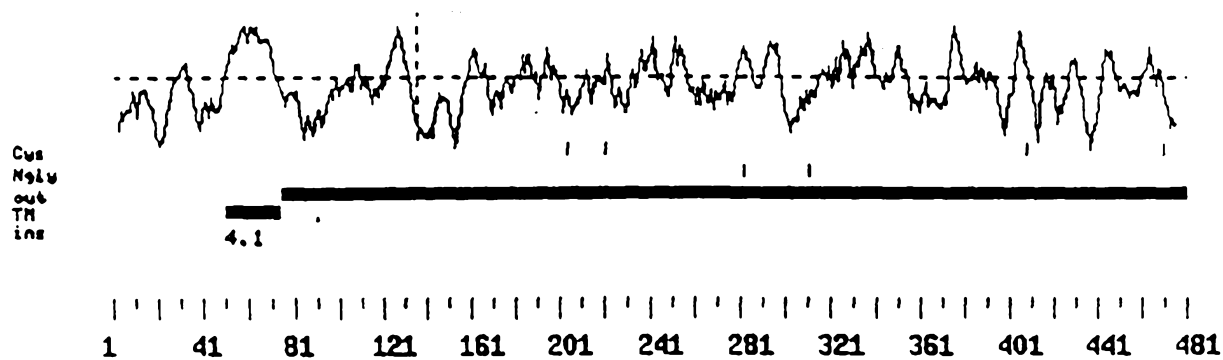


Figure 7

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GCC CCG GAG CCA GCT AGC AGC CGG GCC CGC CTC
Ala Pro Glu Pro Ala Ser Ser Arg Ala Arg Leu
1 5 10

GCC GCC CGC ACC CCG GCG CCC GGG CTT GGC TTG AAG CGG CGG CGG TGG
Ala Ala Arg Thr Arg Ala Pro Gly Leu Gly Leu Lys Arg Arg Arg Trp
15 20 25

CAC CGG CGC GCC GGC AGG AGC ATG GGG AGG AAG ATG CGG GGC GCC GCC
His Arg Arg Ala Gly Arg Ser Met Gly Arg Lys Met Arg Gly Ala Ala
30 35 40

GCC GCC GCG GGG CTC TGG CTG CTG GCT TTG AGC TCG CTG CTG ACG CTG
Ala Ala Ala Gly Leu Trp Leu Leu Ala Leu Ser Ser Leu Leu Thr Leu
45 50 55

TGG GGA GGA CTC CTG CCA CCG CGG ACC GAG CTG CCA GCC TCC CGG CCG
Trp Gly Gly Leu Leu Pro Pro Arg Thr Glu Leu Pro Ala Ser Arg Pro
60 65 70 75

CCC GAA GAT CGA CTC CCT CCG CAT CCG ATC CAG AGT GGC GGC CCC GCG
Pro Glu Asp Arg Leu Pro Pro His Pro Ile Gln Ser Gly Gly Pro Ala
80 85 90

CCC GAG CCG CGA TTC CCT CTG CCC CCG CCC CTA GTA TGG GAC GCC CGC
Pro Glu Pro Arg Phe Pro Leu Pro Pro Pro Leu Val Trp Asp Ala Arg
95 100 105

GGC GGC TCC CTG AAA ACT TTC CGG GCG CTG CTC ACC CTG GCG GCC GGC
Gly Gly Ser Leu Lys Thr Phe Arg Ala Leu Leu Thr Leu Ala Ala Gly
110 115 120

GCG GAT AAC CCG CCT AGG AGG CAC CAG GAC GAC CGC GGG CGG CAC GAG
Ala Asp Asn Pro Pro Arg Arg His Gln Asp Asp Arg Gly Arg His Glu
125 130 135

CCC TCC GGG CTG TCC TGG CCA GAG GAG CGC AGG GCG GTG CAC GGG GGC
Pro Ser Gly Leu Ser Trp Pro Glu Glu Arg Arg Ala Val His Gly Gly
140 145 150 155

GTC TTC TGG AGC CGC GGC CTG GAG GAG CAG GTT CCC CGG GGC TTT TCC
Val Phe Trp Ser Arg Gly Leu Glu Glu Gln Val Pro Arg Gly Phe Ser
160 165 170

GAA GCC CAA GCA GCA GCG TGG CTG GAG GTG GCA CGG GGT GCT CGG GTG
Glu Ala Gln Ala Ala Ala Trp Leu Glu Val Ala Arg Gly Ala Arg Val
175 180 185

GTG GCT CTG GAT CGC GGG GGC TGC GGA CGC AGT TCC AAC CGC CTA GCC
Val Ala Leu Asp Arg Gly Gly Cys Gly Arg Ser Ser Asn Arg Leu Ala
190 195 200

CGC TTT GCC GAC GGC ACC CGT GCC TGT GTA CGC TAC GGC ATC AAC CCA
Arg Phe Ala Asp Gly Thr Arg Ala Cys Val Arg Tyr Gly Ile Asn Pro
205 210 215

GAG CAG ATA CAG GGC GAG GCC CTG TCC TAC TAC CTT GCG CGC CTG CTG
Glu Gln Ile Gln Gly Glu Ala Leu Ser Tyr Tyr Leu Ala Arg Leu Leu
220 225 230 235

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FIGURE 8
SHEET 1 OF 3

GGC	CTC	CAG	CGC	CAC	GTG	CCG	CCG	CTG	GCA	CTG	GCT	CGG	GTG	GAG	GCT	240	245	250
Gly	Leu	Gln	Arg	His	Val	Pro	Pro	Leu	Ala	Leu	Ala	Arg	Val	Glu	Ala			
CGG	GGC	GCG	CAG	TGG	GTG	CAG	GTG	CAG	GAG	GAG	CTG	CGC	ACC	GCG	CAC	255	260	265
Arg	Gly	Ala	Gln	Trp	Val	Gln	Val	Gln	Glu	Glu	Leu	Arg	Thr	Ala	His			
TGG	ACC	GAG	GGC	AGC	GTG	GTG	AGC	CTG	ACG	CGC	TGG	CTG	CCT	AAC	CTC	270	275	280
Trp	Thr	Glu	Gly	Ser	Val	Val	Ser	Leu	Thr	Arg	Trp	Leu	Pro	Asn	Leu			
ACC	GAC	GTG	GTG	GTG	CCC	GAG	CCC	TGG	CGA	TCA	GAG	GAC	GGC	CGT	CTG	285	290	295
Thr	Asp	Val	Val	Val	Pro	Glu	Pro	Trp	Arg	Ser	Glu	Asp	Gly	Arg	Leu			
CGG	CCC	CTG	CGC	GAC	GCC	GGG	GGC	GAG	CTG	ACC	AAC	CTC	AGC	CAG	GCG	300	305	310
Arg	Pro	Leu	Arg	Asp	Ala	Gly	Gly	Glu	Leu	Thr	Asn	Leu	Ser	Gln	Ala			
GAG	CTG	GTG	GAC	TTG	GTA	CAA	TGG	ACC	GAT	CTG	ATC	CTC	TTC	GAT	TAC	320	325	330
Glu	Leu	Val	Asp	Leu	Val	Gln	Trp	Thr	Asp	Leu	Ile	Leu	Phe	Asp	Tyr			
CTG	ACG	GCC	AAC	TTT	GAC	CGG	CTT	GTG	AGC	AAC	CTC	TTC	AGC	TTA	CAG	335	340	345
Leu	Thr	Ala	Asn	Phe	Asp	Arg	Leu	Val	Ser	Asn	Leu	Phe	Ser	Leu	Gln			
TGG	GAC	CCA	CGC	GTT	ATG	CAC	CGC	GCT	ACG	AGC	AAC	CTG	CAC	CGA	GGA	350	355	360
Trp	Asp	Pro	Arg	Val	Met	His	Arg	Ala	Thr	Ser	Asn	Leu	His	Arg	Gly			
CCA	GGA	GGG	GCG	TTG	GTC	TTT	CTG	GAC	AAT	GAG	GCG	GGC	TTG	GTG	CAC	365	370	375
Pro	Gly	Gly	Ala	Leu	Val	Phe	Leu	Asp	Asn	Glu	Ala	Gly	Leu	Val	His			
GGC	TAC	CGG	GTA	GCC	GGC	ATG	TGG	GAC	AAG	TAT	AAC	GAA	CCG	CTG	CTA	380	385	390
Gly	Tyr	Arg	Val	Ala	Gly	Met	Trp	Asp	Lys	Tyr	Asn	Glu	Pro	Leu	Leu			
CAG	TCG	GTG	TGT	GTA	TTC	CGA	GAG	CGG	ACT	GCT	AGG	CGC	GTC	TTG	GAG	400	405	410
Gln	Ser	Val	Cys	Val	Phe	Arg	Glu	Arg	Thr	Ala	Arg	Arg	Val	Leu	Glu			
CTG	CAC	CGG	GGT	CAG	GAC	GCG	GCG	GCC	CGG	TTG	CTG	CGC	CTC	TAC	AGT	415	420	425
Leu	His	Arg	Gly	Gln	Asp	Ala	Ala	Ala	Arg	Leu	Leu	Arg	Leu	Tyr	Ser			
CGC	CAC	GAA	CCG	CGT	TTC	CCA	GAG	CTG	GCG	GAG	CTC	TCA	GAA	CCC	CAC	430	435	440
Arg	His	Glu	Pro	Arg	Phe	Pro	Glu	Leu	Ala	Glu	Leu	Ser	Glu	Pro	His			
GCT	CAG	CTG	CTA	CAG	CGC	CGC	CTT	GAC	TTC	CTC	GCC	AAA	CAC	ATT	TTG	445	450	455
Ala	Gln	Leu	Leu	Gln	Arg	Arg	Leu	Asp	Phe	Leu	Ala	Lys	His	Ile	Leu			
CAC	TGC	AAG	GCC	AAG	TAC	GGC	CGC	CGG	CCC	GGG	GAC	TTA	ATA	ACA	CTC	460	465	470
His	Cys	Lys	Ala	Lys	Tyr	Gly	Arg	Arg	Pro	Gly	Asp	Leu	Ile	Thr	Leu			

FIGURE 8
SHEET 2 OF 3

CGA GGA AGA GAG GGA CTG GGG TAT GAA TGATGGGGGT TAGGGCTGTC
Arg Gly Arg Glu Gly Leu Gly Tyr Glu
480

TTCTCTGCCA	CGGGCAACGA	CCAACCAGCC	AACGCCCACC	CAATGGTCTG	ATCGCCAAGC
TGTCTAAAAA	AAAAAATTGT	GTGTTACCCA	CTTGCCCCCTT	TCTTTTCATG	CCAGGCTGTT
TCCTTTCCAA	GTTCTGGAAG	GGCAAACCTCA	CCGAGGCGAG	AAGTGTAACA	TTCCCTCCAC
CTAGCTTATA	AAAAGAGTTC	TGTGCTTG TG	TGGAGATTGG	ATCCGAAGAA	ACTGGCTGCT
GGGGTTTGGC	CCGGGAATGG	CAGTCTCTTT	GGGAGATGCA	GTCCATTTTT	GTGCCCCCTT
CCACCCCTTC	CCAAAAAGGA	AAACTTCCGT	TTGAGCTAAT	TCCTCTATTT	CCTGAACTCC
TATCATTTTTG	GCCTGAAGGC	TATGAATTCA	GGACTAGTTA	TAAGCAGAGT	CAATAATGAA
TTTCTTCCTG	CGTCTCCCAA	TTGACCAAAA	TTGACAATGA	TGATGTTTAC	CAGAGGGGGA
AAAAAATCAG	TTTCATGCAC	TTTACTTTTT	TTAATTTTAA	TTTTTTAGTA	AGAAAACTTT
TTTATTTGAC	AGAATTTGCC	TTCTGTGTGT	ATATATGTGC	ATAAATTGTG	CTGTAAATAG
ACTAAACAAA	CTTATATTTT	AATAAAAGGG	AGTTTAAAAT	TTAAAAAAA	AAAAAAA
AAAAAAGGGC	GGCCGC				

FIGURE 8
SHEET 3 OF 3

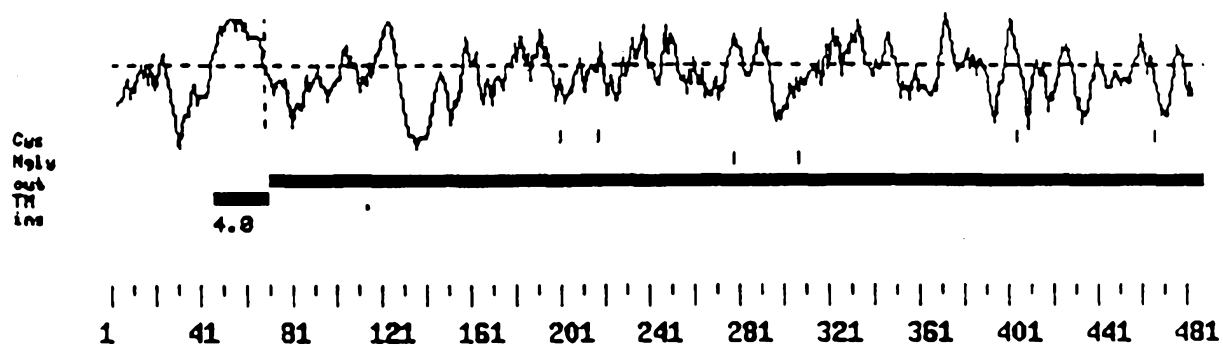


Figure 9

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DROS      LPEEQIQMVAVEPLSTYRV.EFIKETDELSAEQVFRNAFH
          : | | | | | | | | | | | | | | | |
T110      MGRMRGAAATAGLWLLALGSLALWGGLLPRTLPASR

LEQDKDAPDSMVVKLDTNDGSIKEFHVQRTASGRYRKGPERRMSKKMPE
          : | | | | | | | | | | | | | | | |
PPEDRLPRRPARSGGPAPAPRFPLPPPLAWDARGGSLKTFRALLTLAAGA

RVQPQETSRSP...TSPTNPTSEHQAGLIEEDVYWGPTVEQALPKGFAAK
          | : : | | | | | | | | | | | | | | | |
DGPFRQSRSEPRWHVSARQPRPEESAA.VHGGVFWSRGLEEQVPPGFSEA

DQVSWERFVGEQGRVVRLEQGCGRMQNRMVVFADGTRACAPYRQNTDQIQ
          . | | | | | | | | | | | | | | | | | | | |
QAAAWLEAARGARMVALERGGCGRSSNRLARFADGTRACVRYGINPEQIQ

GEIFSYTLGQLLNIS.NLAPSAATVVDSTPNWRAALGDITQAWKERRP
          | | | | | | | | | | | | | | | | | | | |
GEALSYTLARLLGLQRHVPPLALARVEARGAQWAQVQEEELRAAHWTESV

VVLTRWLSdlePAGIPQPFQPLERHLNKHdVWNLTRHMQSERQAQSQPHG
          | | | | | | | | | | | | | | | | | | | |
VSLTRWLPNLTdVVPAPWR.....SEDG

LLKRLGAASSPGSAHQSNAIETGTGTETANGALVQRLIELAQWSDLIVF
          | : | | | | | | | | | | | | | | | | | | |
RLRPLRDA.....GGELANLSQAE.LVDLVQWTDLILF

DYLIANLDRVNNLYNFQWNADIMAAFAHNLARQSASQLLVFLDNESGLL
          | | | | | | | | | | | | | | | | | | | |
DYLTANFDRLVSNLFSLQWDPRVMQRATSNLHR.GPGGALVFLDNEAGLV

HGYRLLKYEAYHSLLLDNLCVFRPTIDALRRL.RAAGAGRRLRDLFER
          | | | | | | | | | | | | | | | | | | | |
HGYRVAGMWDKYNEPLLQSVCFRERTARRVLELHRGQDAAARLLRLYRR

TTSAGVRDVLPSLPDKSVKILVERIDRVLGQVQKCGS.....
          | . | | | | | | | | | | | | | | | |
HEPRFPE..LAALADPHAQLLQRRLDLAKHILHCKAKYGRRSQT

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Figure 10

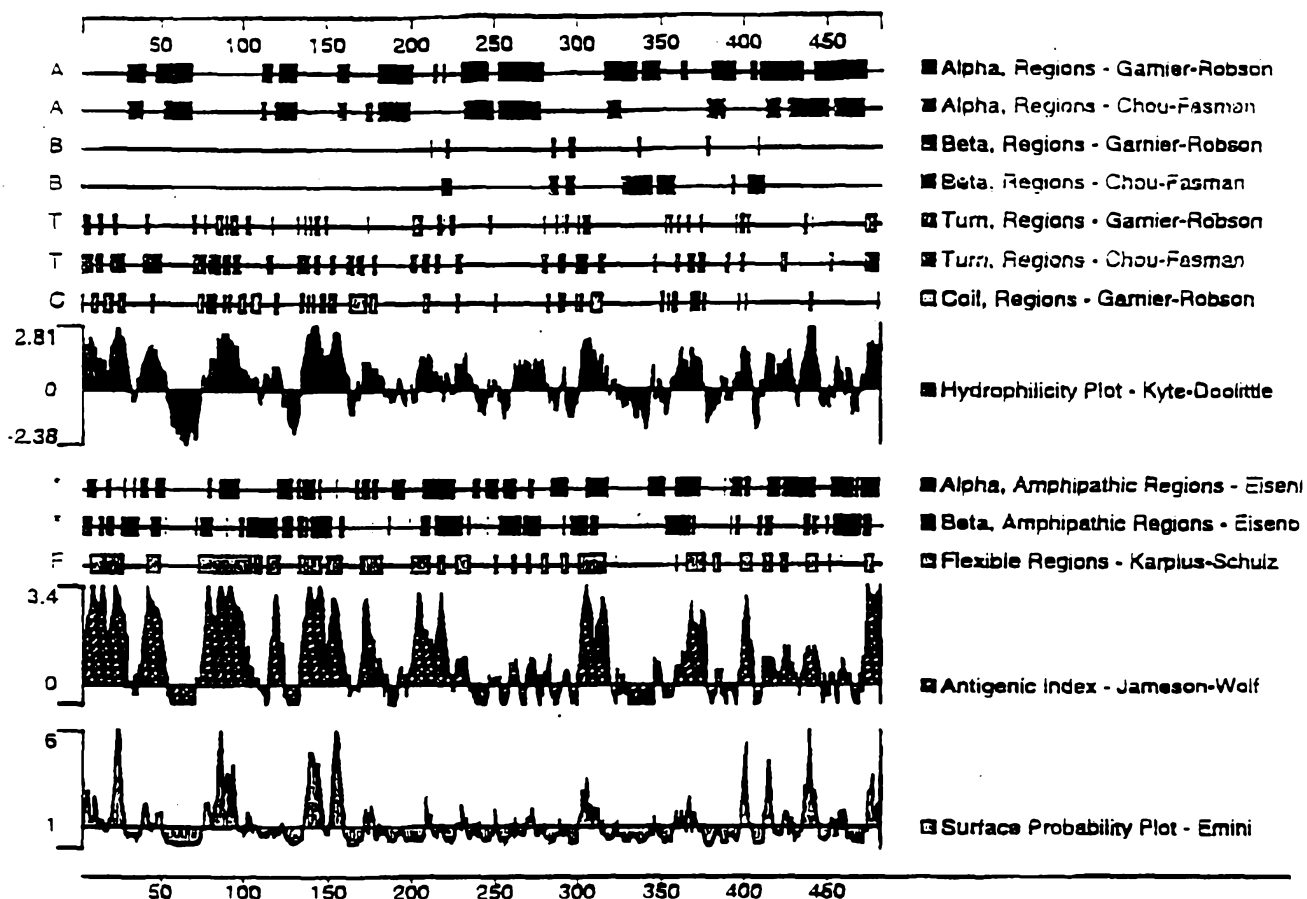


Figure 11