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(54) Title: PRAD1 CYCLIN AND ITS CDNA

(57) Abstract

A novel cyclin termed *prad1*, including human *prad1*; an antibody specific for *prad1*; a nucleic acid sequence which encodes *prad1* or a portion of *prad1*; and methods of using such antibody or nucleic acid to diagnose a neoplastic condition characterized by overexpression of *prad1*.

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PRAD1 CYCLIN AND ITS CDNA

Background of the Invention

Partial funding for the work described herein was
5 provided by the U.S. Government, which has certain rights
in the invention.

This invention relates to the field of cyclins.

The cyclins are a class of eukaryotic proteins
that were originally identified by their cyclic
10 accumulation and destruction at defined points in
embryonic cell cycles (Evans et al., Cell 33:389-396,
1983). They bind to and are essential for activation of
cdc2 protein kinase (reviewed in Murray et al., Science
246:614-621, 1989; Nurse, Nature 344:503-508, 1990;
15 Draetta et al., Cell 56:829-838, 1989). At present, the
cyclins can be divided into three families on the basis
of their kinetics of oscillation across the cell cycle,
their amino acid sequences, and, in some cases, genetic
experiments in yeast that determine when their functions
20 are needed (reviewed in Nurse, 1990; Nasmyth, Cell
63:1117-1120, 1990; Westendorf, J. Cell Biol. 108:1431-
1444, 1989). The B-type "mitotic" cyclins drive cells
into mitosis; their sequences are conserved from yeast to
human (Nurse, 1990; Westendorf et al., 1989; and Pines et
25 al., Cell 58:833-846, 1989). The A-type cyclins, which
are less well understood, may act earlier in the cell
cycle (Minshull et al., EMBO J. 9:2865-2875, 1990; Pines
et al., Nature 346:760-763, 1990; Swenson et al., Cell
47:861-870, 1986). The recently described CLNs (or "G1
30 cyclins") of budding yeast are thought to perform
analogous functions by interacting with *cdc2* homologues
at START, driving cells into S-phase (Nasmyth, 1990). A,
B, and CLN cyclins may act as stage-specific regulators
of progress across the cell cycle by conferring selective
35 substrate specificity upon *cdc2* kinase (Minshull et al.,

1990) or by selectively targeting *cdc2* to different intracellular compartments.

Summary of the Invention

The invention features a novel cyclin, *prad1*, and an isolated DNA (termed PRAD1) which encodes it. This DNA may be single-stranded or double-stranded, and may be genomic DNA, cDNA, or synthetic DNA. It may be identical to a naturally-occurring PRAD1 sequence (such as human PRAD1 cDNA, SEQ ID NO:1) or may differ from such sequence by the deletion, addition, or substitution of one or more nucleotides. By "isolated" is meant that the DNA is free of the coding sequences of genes that, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, immediately flank the gene encoding *prad1*. Included within the term *prad1* is human *prad1* and any homolog of human *prad1* (i.e., from another animal species, or a genetically altered version of a naturally-occurring *prad1* which exhibits a biological activity similar to that of the naturally-occurring protein) encoded by a DNA which is capable of hybridizing (1) under stringent hybridization conditions (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor, NY, 1989: herein incorporated by reference) to a single-stranded probe consisting of a segment of at least eight (preferably 18-40) nucleotides of human PRAD1 cDNA (SEQ ID NO:1) or human PRAD1 genomic DNA, or (2) under less stringent conditions (e.g., washing at 2xSSC, at 40°C) to a probe consisting of a segment of at least 40 (preferably 200-5000) nucleotides of human PRAD1 cDNA (SEQ ID NO:1) or human PRAD1 genomic DNA. Also within the invention are peptide fragments of a naturally-occurring *prad1*, which fragments are at least six amino acids in length and preferably 10-50 amino acids; and single-stranded DNA or RNA probes (preferably radioactively labelled) containing at least 8 nucleotides

of, but less than all of, human PRAD1-encoding RNA, human PRAD1 cDNA (SEQ ID NO:1) or human PRAD1 genomic DNA, and preferably between 10 and 5000 bases. Such DNA or RNA probes may be used in a diagnostic method which includes
5 the steps of obtaining a nucleic acid sample from an animal suspected of having a given neoplastic condition (or from a known tumor); contacting the nucleic acid sample with a single-stranded DNA or RNA probe capable of hybridizing to the PRAD1 homolog of the species to which
10 the animal belongs; and detecting the level of hybridization of the probe with the nucleic acid sample, such level being diagnostic for the neoplastic condition. Two examples of neoplastic conditions that may be diagnosed by this method include centrocytic lymphomas,
15 which appear to express abnormally high levels of PRAD1 mRNA, and those breast cancers which are characterized by a high degree of amplification of PRAD1 DNA.

The DNA sequence of the invention, which may be under the transcriptional control of a heterologous
20 promoter (defined as a promoter sequence other than the naturally-occurring promoter of the gene encoding *prad1*), may be incorporated into a vector (such as a phage) and thereby introduced into a cell. Included within the invention is a eukaryotic or prokaryotic cell (or an
25 essentially homogeneous population of such cells) containing (and preferably capable of expressing) a recombinant DNA molecule encoding *prad1*: that is, a cell into which (or into an ancestor of which) has been introduced, by means of genetic engineering, a DNA
30 molecule encoding *prad1*, resulting in that DNA molecule's being positioned adjacent to a DNA sequence to which it is not naturally adjacent (e.g., the *prad1*-encoding sequence is integrated into the genome of such cell). The *prad1* protein of the invention may be produced by
35 culturing such cells and recovering *prad1* from the cells,

or from their medium. Alternatively, DNA or mRNA encoding *prad1* may be combined with a standard *in vitro* expression system to produce *prad1*. *Prad1* so produced can be utilized in combination with a pharmacologically-acceptable carrier to promote wound healing in an animal, or can be used to promote proliferation of an animal cell by treating the cell with a proliferation-inducing amount of the protein of the invention (for example, by transfecting the cell with DNA encoding *prad1* so that the cell itself produces such a proliferation-inducing amount of *prad1*). Alternatively, the *prad1* (or an antigenic fragment thereof, determined by standard methodology) can be used to raise polyclonal or monoclonal antibodies capable of forming immune complexes with *prad1*, and thus useful as a diagnostic for certain neoplastic conditions characterized by abnormally high levels of *prad1* expression. The method of using such an antibody as a diagnostic would include the steps of obtaining a sample of a tissue of an animal suspected of having a such a neoplastic condition (e.g., certain lymphomas or breast cancers); contacting the sample with the antibody; and detecting the level of immune complexes formed by the antibody, such level being diagnostic for the neoplastic condition.

Also within the invention is a transgenic non-human vertebrate animal (preferably a mammal such as a rodent, e.g., a mouse) bearing a transgene (i.e., a piece of DNA which is artificially inserted into an embryonic cell, and becomes a part of the genome of the animal which develops from that cell) which includes a DNA sequence encoding *prad1*, and any cells or cell lines derived from such an animal. A transgenic animal is an animal having cells that contain a transgene, which transgene was introduced into the animal, or an ancestor of the animal, at an embryonic stage. If the embryonic

stage is a single-cell stage, then all nucleated cells of the animal will carry the transgene. The particular *prad1* encoded by the transgene may be endogenous to the species of the transgenic animal, or may be that of a 5 different species (e.g., human). By using a PRAD1 together with an appropriate promoter, a transgenic animal which readily develops neoplasias in a selected organ or tissue type will result, making such animal useful as a model for studying cancer in that organ or 10 tissue.

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

Detailed Description

15 The drawings are first described.

Drawings

Figure 1 is a Southern blot of Msp1-digested DNA probed with the 5' PTH gene probe (lanes 1, 2) and 3' PTH gene probe (lanes 3, 4).

20 Figure 2 is a diagrammatic representation of (a) the normal PTH gene, and (b) the two fragments resulting from the rearrangement in tumor M.

Figure 3 is diagrammatic representation of the D11S287 region, indicating known restriction sites and 25 the locations of the 500 bp fragment, the 1.6 kb XhoI fragment, and Probe B.

Figure 4 is a Northern analysis of D11S287 expression in various cell types.

Figure 5 is a diagrammatic representation of 30 PTH/D11S287 rearrangements in two parathyroid adenomas, and the relative locations of Probe B and a series of cloned cDNA segments.

Figure 6 is a representation of the nucleotide sequence and predicted amino acid sequence of human PRAD1 35 (SEQ ID NO:1) cDNA.

Figure 7 is an illustration of sequence homology between the "cyclin box" region of human *prad1* and the corresponding regions of some A-type, B-type, and G1 cyclins.

5 Figure 8 is a Northern blot analysis of D11S287 [human PRAD1 (SEQ ID NO:1)] expression in various cell types.

Figure 9 is (a) a Northern blot analysis of HeLa cell RNA probed with a human PRAD1 cDNA (SEQ ID NO:1)
10 probe, an H4 histone probe, and 28S rRNA; and (b) a graph depicting the results of the Northern blot.

Figure 10 is an analysis of the biological activity of recombinant human *prad1*.

Identification of Human PRAD1

15 Previous studies on DNA from cells of a benign parathyroid adenoma (reported in Arnold et al., J. Clin. Invest. 83:2034-2040, 1989) revealed evidence of a DNA rearrangement involving the parathyroid hormone (PTH) chromosomal locus (at chromosome 11, band p15) and a
20 segment of DNA (identified as Human Genome Database assignment D11S287) which normally maps to chromosome 11, band q13. It is now known that (a) although a number of previously-identified oncogenes (including INT-2 and HST-1), as well as the translocation breakpoint marker BCL-1
25 and possibly the gene for multiple endocrine neoplasia type I (MEN-I), map to the 11q13 region, the so-called D11S287 locus rearranged in at least some parathyroid adenomas is distinct from these previously-described markers; (b) D11S287 mRNA, while detectable in all
30 tissues analyzed, is significantly overexpressed in those parathyroid adenomas which have a 11q13/11p15 chromosomal rearrangement, and also in certain lymphomas (notably centrocytic lymphomas) characterized by rearrangement of the BCL-1 locus; and (c) the D11S287 locus is amplified
35 and expressed in many squamous cell and mammary

carcinomas. This evidence suggests that D11S287 (also referred to herein as human PRAD1, for parathyroid adenoma) is a newly-identified oncogene which figures in a variety of types of neoplasms.

5 Cloning Human PRAD1 cDNA (SEQ ID NO:1)

Human PRAD1 cDNA (SEQ ID NO: 1) has been cloned and sequenced by the methods described in detail below, yielding the sequence shown in Figure 6. The longest open reading frame, starting at the first ATG codon, 10 encodes a predicted protein of 295 amino acids (M_r 33,729). Screening the Genbank peptide database with this sequence reveals significant homology only to members of the cyclin family, with greatest similarity in the region conserved among cyclins, ranging from 19.1% to 15 33.6% identity, and 44.1% to 59.2% similarity. The human PRAD1 (SEQ ID NO:1) protein (*prad1*) has significant sequence similarities to all three types of cyclins (A, B, and CLN cyclins), but cannot readily be assigned to any one type. This suggests that *prad1* may represent a 20 new and different cyclin family member.

PRAD1 Expression

PRAD1 mRNA is expressed in many tissues and is highly conserved across species (Figure 7). As with other cyclin mRNAs expressed in human cells (Pines et 25 al., Cell 58:833-846, 1989; Pines et al., Nature 346:760-763, 1990), human PRAD1 mRNA levels vary across the cell cycle (Figure 9), consistent with but not proving a role in cell cycle regulation. The peak in PRAD1 mRNA levels occurs late in the cell cycle or in G1.

30 Biological Activity of Recombinant Human *Prad1* Protein

Bacterially expressed recombinant human *prad1*, produced as described in detail below, was used to further investigate the link between human PRAD1 and the cyclins. Cyclins are known to form complexes with $p34^{cdc2}$ 35 protein kinase, leading to its activation which can be

assayed using exogenous histone H1 as a substrate. In addition, cyclin/$p34^{cdc2}$ complexes can be purified by exploiting the ability of beads linked to $p13^{suc1}$, another cell cycle protein, to avidly bind $p34^{cdc2}$ and, in turn,
5 co-purify any proteins complexed with $p34^{cdc2}$ (Draetta et al., Cell 56:829-838, 1989). When recombinant human *prad1* was added to clam embryo interphase cell lysates (which lack endogenous cyclins and contain inactive $p34^{cdc2}$), both $p34^{cdc2}$ and *prad1* were bound by $p13^{suc1}$-
10 beads (Figure 10). As *prad1* does not bind to protein A-Sepharaose beads, its binding to $p13^{suc1}$-beads is most likely due to its interaction with $p34^{cdc2}$ or a closely related protein. Furthermore, kinase activity was induced by the addition of the human PRAD1 (SEQ ID NO:1)
15 *in vitro* translation product to interphase lysates (Figure 10). This kinase activity was lower than that seen with cyclin A. Cyclin B provided a negative control; for reasons not yet understood, our cyclin B translation product was not capable of activating $p34^{cdc2}$
20 in this type of assay. The difference between the activities induced by cyclin A and human *prad1* may be specific to this clam assay system, or may reflect a genuine difference between the functions of, or the substrate specificities conferred by, cyclin A vs. human
25 *prad1*.

<u>Use</u>

Both *prad1* and a nucleotide encoding *prad1* are useful for the preparation of diagnostic tools for the classification and/or prognosis of lymphomas, breast
30 cancers, and squamous cell cancers, as well as other cancers characterized by a high level of expression and/or amplification of the PRAD1 gene. For example, *prad1* or an antigenic peptide fragment of *prad1* could be used in accordance with standard methods (see, e.g.,
35 Harlow et al., *Antibodies: A Laboratory Manual*, Cold

Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988; Yanaihara et al., U.S. Patent No. 4,855,406; and Slamon et al., U.S. Patent No. 4,918,162; all of which are herein incorporated by reference) to raise polyclonal or
5 monoclonal antibodies capable of forming immune complexes with *prad1*, and useful for detecting abnormally high levels of *prad1* in a given tissue sample. Similarly, a hybridization probe prepared from a segment of at least 12 (and preferably greater than 250) nucleotides of human
10 PRAD1-encoding RNA, human PRAD1 cDNA (SEQ ID NO:1) or human PRAD1 genomic DNA may be employed as a means for determining the number of copies of PRAD1 present in the genomic DNA of a given sample, or the level of PRAD1 mRNA expressed in cells of such sample.

15 The nucleic acids of the invention may also be used therapeutically. Oligonucleotides which are antisense to human PRAD1 mRNA (or which express RNA that is antisense to human PRAD1 mRNA) may be synthesized to serve as an anticancer therapy in those cases diagnosed
20 as having a rearrangement or amplification of human PRAD1: such oligonucleotides would be introduced into tumor cells *in vivo* as a means to reduce production of *prad1* in such cells, and thereby to reduce neoplastic growth induced by an overabundance of *prad1*. (See, for
25 example, Weinberg et al., U.S. Patent No. 4,740,463, herein incorporated by reference.) By linking a PRAD1 sequence to a selected tissue-specific promoter or enhancer and introducing by standard methods (e.g., as described by Leder et al., U.S. Patent No.4,736,866,
30 herein incorporated by reference) the resultant hybrid gene into an animal embryo at an early developmental stage (e.g., the fertilized oocyte stage), a transgenic animal which expresses elevated levels of *prad1* in the selected tissue (e.g., breast, squamous cell, B-lymphoid
35 cell, parathyroid, and others) can be produced. The form

of PRAD1 utilized can be one which encodes a *prad1* similar to that of the animal species used, or it can encode the *prad1* homolog of a different species (e.g., human). Such an animal would be useful as an *in vivo* model for neoplastic disease in the selected tissue. In addition, cells derived from such a transgenic animal may be used to establish an immortal cell line that retains at least some of its differentiated characteristics while proliferating indefinitely *in vitro*. Alternatively, one could stably transfect primary cells (e.g., a type that has proven difficult to maintain in culture, such as pituitary cells) with a PRAD1 gene linked to an appropriate promoter (e.g., the metallothionin promoter) which ensures high levels of expression of the gene, and thereby establish an immortal cell line derived from such primary cells. PRAD1 sequences may be particularly useful in this regard because overexpression of PRAD1 (at least in parathyroid tissues) appears to trigger the proliferation of normally quiescent cells without causing them to completely lose their differentiated phenotype.

<u>Experimental Data</u>

The DNA abnormality in parathyroid tumor M was initially characterized by Southern analysis of MspI digests using probes specific for the 5' and 3' regions in the PTH gene (see below), which revealed a unique, tumor-specific band. Figure 1 illustrates these Southern blots of tumor M (T) and peripheral blood leukocyte (L) DNA pairs. MspI- digested DNA was probed with the 5' PTH gene probe (lanes 1, 2) and 3' PTH gene probe (lanes 3, 4). Squares indicate the normal gene (6.3 kb); arrows indicate the rearranged allele (1.5 kb in lane 1, 5.4 kb in lane 3). There is an MspI site within the DNA to which the 3' probe hybridizes (see Figure 2a); therefore, a smaller band (2.2 kb) representing the most 3' section of the normal PTH gene is present in lanes 3 and 4. The

intensities of the bands representing the abnormal allele were approximately equal to those representing the normal allele. Thus, in tumor M, as in tumor Y (Arnold et al., 1989), a clonal rearrangement of the PTH gene has occurred: in every tumor cell, one of the two alleles of the PTH gene remains normal but the other is disrupted. Figure 2(a) illustrates the normal PTH gene, with the positions of its three exons (Vasicek et al., Proc. Natl. Acad. Sci. USA 80:2127-2131, 1983), the 5' and 3' probes used in mapping and cloning, and the MspI sites indicated. In comparison, Figure 2(b) shows the two fragments resulting from the rearrangement in tumor M: one consists of the 5' PTH gene sequences plus juxtaposed non-PTH DNA (stippled area), while the other consists of 3' PTH gene sequences plus juxtaposed non-PTH DNA (cross-hatched area). In each fragment, the location of the breakpoint is shown by a diagonal line. The locations of several restriction enzyme sites, determined by Southern blot analysis of tumor DNA, are indicated: EcoRI (R), BamHI (B), HindIII (H), XhoI (X), SstI (S), MspI (M). The locations and sizes of the 1.5 kb and 5.4 kb rearranged MspI fragments, (shown in Figure 1) are indicated above each fragment. Below each fragment, lines ending in arrow tips depict the 1.5 kb and 16 kb cloned tumor DNA fragments. Analysis with multiple additional restriction enzymes indicated that the gene is separated into two parts, with the breakpoint located in the first intron (Figure 2b). Consequently, upstream regulatory elements and the first, non-coding exon in the 5' fragment are separated from the coding sequences in the 3' fragment. Each PTH gene fragment remains internally intact (within the limits of sensitivity of restriction mapping), but has become juxtaposed to non-PTH DNA.

To identify the rearranged non-PTH DNA (shaded and cross-hatched areas in Figure 2b), two DNA fragments containing PTH gene sequences plus breakpoint-adjacent DNA were cloned from tumor M DNA. One was a 16 kb BamHI fragment containing approximately 8 kb of non-PTH gene DNA adjacent to 8 kb of 3' PTH gene sequences (Figure 2b). Genomic Southern blots of normal DNA probed with subclones spanning most of the 8 kb of non-PTH DNA showed diffuse smears that did not yield to attempts at competition with excess human DNA (Sealy et al., 1985). This indicated that the non-PTH DNA in the 16 kb fragment contained sequences highly repeated in the human genome, and precluded its chromosomal localization.

We also cloned a 1.5 kb EcoRI fragment containing approximately 1 kb of the PTH gene's 5' region plus 500 bp of juxtaposed non-PTH DNA (Figure 2b). Probing normal human DNA blots with the subcloned 500 bp fragment demonstrated that it contained single-copy DNA; in situ hybridization and analysis of somatic cell hybrids revealed that the 500 bp fragment's normal chromosomal location is 11q13.

Hybridization of the 500 bp breakpoint-adjacent DNA fragment to an RNA blot of six parathyroid adenomas, including two with PTH gene rearrangements, was negative. To identify transcribed sequences near the breakpoint that could have been affected by the rearrangement, we walked along the chromosome by probing a normal human genomic library with the 500 bp subcloned fragment. We obtained a bacteriophage clone with a 14 kb insert, but Northern blot analyses revealed no hybridization of subclones spanning the entire insert. Mapping of the 14 kb insert showed that the 500 bp fragment was at one end, and demonstrated that the adjacent cloned DNA had a restriction map identical to that of the genomic DNA juxtaposed to tumor M's rearranged 5' PTH gene fragment.

(Compare Figures 2b and 3). At the other end of the 14 kb insert was a 1.6 kb XhoI fragment (Figure 3) identical in size to an XhoI fragment 1 kb from tumor Y's D11S287 breakpoint (Arnold et al., 1989). We subcloned these two independent 1.6 kb XhoI fragments (one from the above normal phage clone and one from a tumor Y-derived clone) and used them sequentially to probe blots of normal human genomic DNA digested with 7 restriction enzymes. With every enzyme, the two probes hybridized to precisely comigrating fragments. In addition, restriction maps of the two 1.6 kb fragments themselves were identical for all 6 enzymes used. Thus, the 1.6 kb XhoI fragment linked tumor M's breakpoint-adjacent DNA with that of tumor Y (D11S287), confirming that the 11q13 breakpoints in the two adenomas are both in the D11S287 region, separated by 15 kb. The composite restriction map of the unrearranged D11S287 region is shown in Figure 3, in which restriction sites for the enzymes HindIII (H), BamHI (B), EcoRI (E), SacI (S), MspI (M) and XhoI (X) are indicated. The locations of the 500 bp fragment, the 1.6 kb XhoI fragment, and probe B are shown. This map is derived from the maps of the phage clones described above and by Arnold et al. (1989), and Southern blots of DNA from tumors M and Y.

The proximity of the 11q13 breakpoints suggested that the rearrangements could have similar functional consequences. Because none of the DNA between the two tumors' breakpoints is transcribed in parathyroid cells, we looked for transcribed sequences distal to tumor Y's breakpoint. We used fragment B (Figure 3), a breakpoint-adjacent DNA fragment from tumor Y, to probe a blot containing total RNA from human placenta, several parathyroid adenomas lacking PTH gene rearrangements, and tumors M and Y. We also hybridized probe B to another blot containing total RNA from placenta and from another

parathyroid adenoma (tumor F) that was found recently to contain a clonal rearrangement of the PTH and D11S287 loci (Friedman et al., 1990); Southern blotting indicated that tumor F's rearrangement closely resembled tumor Y's.
5 Figure 4 presents the results of the Northern blots, in which 10 micrograms of total RNA was probed with Probe B (top panels), and with a 28S rRNA probe (bottom panels). Size determination was based on the migration of 28S rRNA. Lanes contain the following samples: lanes 1, 7:
10 placenta; lanes 2, 3, 4: parathyroid adenomas without PTH gene or D11S287 rearrangements; lanes 5, 6, 8: tumors Y, M, and F, respectively; lanes 7 and 8 are a separate Northern filter. The middle panel is a longer exposure of lanes 1-6 in the top panel. In lanes 5 and 8
15 (tumors Y and F) a faint band was visible, larger than the highly-overexpressed 4.5 kb band, which was not seen in lane 6 (tumor M) (data not shown). Exposure times: top row (probe B): lanes 1-6, 17h; lanes 7 and 8, 12h; Middle row (probe B): all lanes, 52h; Bottom row (28S
20 rRNA): all lanes, 1.5h. An approximately 4.5 kb transcript (slightly smaller than the 28S rRNA band) was seen in all lanes of Figure 4. However, the intensity of the 4.5 kb band in tumors M, Y and F was roughly 15-fold greater than that in any of the other specimens. We
25 demonstrated that the 4.5 kb band represents polyadenylated RNA by finding its intensity amplified in poly A+ RNA (data not shown).

Parathyroid adenoma M initially was identified as having an abnormal PTH gene during studies of the
30 monoclonality of parathyroid adenomas (tumor 1 in Arnold et al., N. Eng. J. Med. 318:658-662, 1988). All tumor specimens were frozen in liquid nitrogen shortly after surgical removal. Extraction of high molecular weight DNA, restriction enzyme digestion and Southern blotting
35 were performed as previously described (Arnold et al., N.

Eng. J. Med., 309:1593-1599, 1983). Total RNA was isolated by the guanidinium thiocyanate/cesium chloride method, electrophoresed on a denaturing formaldehyde-agarose gel, and transferred to nitrocellulose or nylon filters (Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 7.19-7.22, 7.37-7.39, 11.31-11.32, 1989). Hybridization conditions were similar to those used for Southern filters. Blots were washed at high stringency (0.1 x SSC, 65°).

PTH gene fragments used as hybridization probes were the 775 bp BglII fragment (5' PTH probe) and the 2.6 kb SstI-EcoRI fragment (3' PTH probe) from pPTHg108 (Igarashi et al., Mol. Cell. Biol. 6:1830-1833, 1986) (Figure 2a). The 500 bp fragment and probe B (Figure 3) were subcloned into pUC-18 from the breakpoint-adjacent DNA of the phage clones containing the rearranged PTH gene fragment plus juxtaposed DNA from tumor M (see above), and tumor Y (Arnold et al., 1989), respectively. The 1.6 kb XhoI fragment from the 14 kb insert cloned from the normal human genomic library was also sub-cloned into pUC-18. The 1.6 kb Xhol fragment from tumor Y was subcloned from a λphage 2001 clone containing the 17 kb HindIII fragment of tumor Y's unrearranged D11S287 allele (Arnold et al., 1989). All the above probes were random-primed and labelled with [^{32}P]dATP (Feinberg & Vogelstein, Anal. Biochem. 132:6-13, 1983). The 28S RNA oligonucleotide was end-labelled with [^{32}P]dATP (Sambrook et al., 1989) and used to probe the Northern filters to control for the amount of high molecular weight RNA present in each lane.

To clone the rearranged 5' PTH gene fragment (Figure 2b), an EcoRI library of tumor genomic DNA was constructed using the λZapII vector (Stratagene). This library was screened with the 5' PTH gene probe, and the

rearranged allele was distinguished from the normal allele by size, as DNA blots predicted that the rearranged EcoRI fragment would be 1.5 kb in size, and the normal fragment 3.5 kb. One clone containing the rearranged gene was identified in 1 x 10^6 plaques that were screened.

To clone the rearranged 3' PTH gene fragment (Figure 2b), a BamHI library of tumor genomic DNA was constructed in EMBL-3. Because restriction mapping indicated that both the normal and rearranged 3' PTH BamHI fragments were 16 kb in size, the library was screened with the 3' PTH probe (expected to hybridize to both the normal and rearranged PTH alleles) and then with the 5' PTH probe (expected to hybridize only to the normal allele). One clone containing the rearranged allele was identified in 6.5 x 10^3 plaques screened. As predicted, it contained 8 kb of 3' PTH gene sequences and 8 kb of newly-juxtaposed DNA. Most of this 8 kb was subcloned in roughly 2 kb units into pUC-18, and used to probe Southern filters of normal genomic DNA.

Prereassociation was performed by sonicating 1 mg of human placental genomic DNA and incubating it for 10-60 min with 50-100 ng of labelled repeat-containing subcloned DNA. This mix was then hybridized to a Southern filter containing normal human DNA using standard conditions.

The genomic library used to obtain the 14 kb insert was a partial Sau-3a digest of normal human DNA cloned into an EMBL-3 like vector (Clontech).

Chromosomal mapping using human-mouse somatic cell hybrids (Shows et al., Adv. Hum. Genet. 12:341-452, 1982; Shows et al., Somatic Cell Mol. Genet. 10:315-318, 1984); Southern blotting (Naylor et al., J. Exp. Med. 57:1020-1027, 1983); and in situ hybridization (Zabel et al., Cytogenet. Cell Genet. 39:200-205, 1985; Nakai et al.,

Cytogenet. Cell Genet. 43:215-217, 1986) was performed as previously described.

A λgt11 placental cDNA library (Clontech) was screened with radiolabeled Probe B. A clone denominated λP1-4 and another similar phage clone were isolated. Probe B and the insert of λP1-4 were sequenced. The region of genomic and cDNA overlap was followed in Probe B by a GT splice donor sequence in only one orientation, confirming hybridization data which had suggested transcription in the left to right orientation, as shown in Figure 5. The next probe was made by polymerase chain reaction amplification of the 3' region of the λP1-4 cDNA insert, and used to rescreen the same library. From $5x10^5$ pfu of this library, one of 16 positive clones, λP1-5, had an insert extending further downstream. The PstI/EcoRI fragment of λP1-5 was then used to rescreen the library, and 12 similar clones, the longest of which was λP2-3, were obtained. The sequence of the insert of λP2-3 revealed polyadenylation signals and a polyA stretch of 16 nucleotides in an appropriate position, consistent with the expected orientation. Standard methods for library screening and probe labeling were used (Davis et al., *Basic Methods in Molecular Biology* (Elsevier, New York, Amsterdam, London, 1986). These clones are illustrated in Figure 5, together with a schematic representation of PTH/D11S287 rearrangements in two parathyroid adenomas. The 5' PTH region (11p15, thick lines) was juxtaposed to the D11S287 region (11q13, thin lines) in each of these adenomas. The breakpoints in the D11S287 region are 15 kb apart. Genomic Probe B is shown as a darkened box, whose open area represents the first exon of PRAD1. Also shown are restriction maps of the inserts of representative overlapping PRAD1 cDNA clones, λP1-4, λP1-5, and λP2-3; and the deduced restriction map of the PRAD1 cDNA. The coding region is

shown as a crosshatched box. Scale of 1 kb is shown as arrows. Symbols used for restriction sites are: B, BamHI; E, EcoRI; H, HindIII; P, PstI.

The inserts of the clones λP1-4, λP1-5, and λP2-3
5 shown in Figure 5, and of other independent clones, were subcloned into pGEM7Zf(+) (Promega). Sequences were obtained using the double-stranded DNA sequencing technique (dideoxy method) with modified T7 DNa polymerase (Sequenase; U.S. Biochemical Corporation), as
10 described by the manufacturer. Several oligonucleotides were synthesized as internal primers to facilitate sequencing. The coding region was sequenced in both orientations and in at least two independent clones. Set forth in Figure 6 are the nucleotide sequence and
15 predicted amino acid sequence of human PRAD1 cDNA (SEQ ID NO:1). Nucleotide numbers are on the right. Nucleotide 3495, shown as W, indicates A or T because the sequences of two independent clones did not agree. Nucleotide 4017 is shown as R, meaning A or G, for the same reason.

20 Figure 7 illustrates sequence homology between the "cyclin box" region of the predicted PRAD1 protein (*prad1*) and that of A-type cyclins (human and clam cyclin A) (Swenson et al., Cell 47:861-870, 1986, and Wang et al., Nature 343:555-557, 1990); B-type cyclins (human
25 cyclin B and *S. pombe cdc13*) (Pines et al., Cell 58:833-846, 1989; and Booher et al., EMBO J. 7:2321-2327, 1988), and one *S. cerevisiae* G1 cyclin (*cln3*) (Nash et al., EMBO J. 7:4335-4346, 1988; Cross et al., Mol. Cell. Biol. 8:4675-4684, 1988) Clam cyclin A and *S. pombe cdc13*
30 homologies with *prad1* are representative of those found in their families; *cln3* alignes with *prad1* more closely than does *cln1* or *2*. Identical amino acids are shown as ¦. Conservative substitutions are shown as *. Alignment was made with the assistance of the BESTFIT program
35 (Devereux et al., Nucl. Acids Res. 12:387-395, 1984) and

conservative amino acids are grouped as follows: D, E, N, Q; H, K, R; A, G, P, S, T; I, L, M, V; F, W, Y. Amino acid numbers are on the right in this Figure.

RNAs were prepared for Northern blot analysis from the indicated tissues by standard procedures (Davis et al., 1986). 10 μg total RNA was loaded and separated on an agarose-formaldehyde gel, blotted onto nitrocellulose, and hybridized with Probe B or the 28S rRNA oligonucleotide. The filters were washed at high stringency (0.1x SSC, 60°C) and autoradiographed. Figure 8 illustrates a Northern blot analysis of total RNA from human thyroid (lane 1), human placenta (lane 2), bovine parathyroid (lane 3), bovine thyroid (lane 4), bovine lymph node (lane 5), bovine skeletal muscle (lane 6), murine heart (lane 7), and murine liver (lane 8). PRAD1 mRNA (shown in the upper panel) is approximately 4.5 kb in size, slightly smaller than the 28S rRNA; 28S rRNA hybridization is shown in the lower panel. Figure 9(a) shows a Northern blot analysis of total RNA from HeLa S3 cells after release from G1/S block. Hela S3 cells (American Type Culture Collection), maintained in Dulbecco Modified Eagle Medium (DMEM, GIBCO) with 7% fetal bovine serum (FBS), were synchronized at the G1/S boundary by sequential thymidine-aphidicolin treatment (Heintz, et al., Mol. Cell. Biol. 3:539-550, 1983) with a slight modification. Log-phase cells were incubated in complete medium (DMEM with 7% FBS, penicillin G, and streptomycin) with addition of 2mM thymidine (Sigma) for 12 h. After release from thymidine block by 3 washes with PBS, the cells were incubated for 10 h with 24 μM deoxycytidine (Sigma) and 24 μM thymidine, recovered by trypsinization, counted, and aliquoted equally (5.0×10^4 cells/cm^2). Incubation with 5 μg/ml aphidicolin (Sigma) for 14 h was followed by release from G1/S block with 4 DMEM washes and incubation in complete medium.

[^{3}H]Thymidine (NEN) was added to an aliquot 15 min before each indicated time point; a 30 min incubation and harvesting for trichloroacetic acid (TCA) precipitation followed. RNAs from parallel aliquots were extracted (Chomczynski et al., Anal. Biochem. 162:156-159, 1987) at the indicated times; time zero was just before release from aphidicolin. RNAs (5 μg per lane) were blotted onto nitrocellulose and sequentially hybridized with the PRAD1 λp1-4 cDNA insert, human H4 histone pF0108X (Pauli et al., Science 236:1308-1311, 1987), and a 28S rRNA oligonucleotide as described above. Human PRAD1 mRNA is shown in the upper panel of Figure 9(a); H4 histone mRNA in the middle panel shows the pattern expected in well-synchronized cells (Heintz et al., 1983); and 28S rRNA is shown in the lower panel as a control for RNA loading. In Figure 9(b) are compared the relative amounts of human PRAD1 mRNA (-•-), H4 histone mRNA (-∘-), and [^{3}H]thymidine incorporation (-□-) of HeLa S3 cells after release from G1/S block. The signals of the blot shown in Figure 9(a) were measured by densitometry and normalized to the 28S rRNA to produce the graph of Figure 9(b).

Clam embryo interphase cell lysates lacking endogenous cyclins were prepared by adding 100 μM emetine during first mitosis, as described previously (Luca et al., J. Cell Biol. 109:1895-1909, 1989), followed by homogenization and centrifugation at 150,000 x g. Aliquots of the supernatant were frozen in liquid nitrogen. [^{35}S]methionine-labeled *pradl* was produced in a reticulocyte lysate *in vitro* translation system (Promega) according to manufacturer's instructions, by using a plasmid (denominated pP1-8) containing the λP1-4 insert in pGEM7Zf(+) (Promega). To produce *pradl* in *E. coli*, pT4R-1 was constructed by insertion of the λP1-4 insert into the NcoI and BamHI sites of pET-3d (Studier et al.,

Meth. Enzym. 108:60-89, 1990). BL21(DE3) cells were transformed with pT4R-1, cultured, and treated with 0.4 mM isopropylthio-beta-galactosidase (IPTG) for 3 h to induce *prad1* expression. The induced product was
5 purified from cell culture as inclusion bodies (Gardella et al., J. Biol. Chem 265:15854-15859, 1990). On SDS-polyacrylamide gels, the apparent sizes of the *in vitro* translation product and the bacterially-expressed product were the same (M_r 35kD). Rabbit anti-*prad1* antisera were
10 raised against a synthetic peptide corresponding to amino acids 9-37 of *prad1*. Antisera were assayed by immunoprecipitation of the *in vitro* translation product. Antisera specificity was shown by comparison with normal rabbit serum and by successful competition with the (9-
15 37) peptide (data not shown).

Thawed clam embryo lysate (16.5 µl) and bacterially-expressed *prad1* (5.5 µl) were mixed and incubated at 18°C for 30 min before transfer to 4°C, dilution with 4 volumes of buffer A (50 mM Tris pH 7.4,
20 150 mM NaCl, 5mM EDTA, 5mM EGTA, 1 mM ammonium molybdate) and addition of p13^{suc1}- or protein A-Sepharose, followed by mixing for 1 h. Beads were then pelleted and washed in buffer A + 0.5% Tween-20; in buffer B (50 mM Tris pH 7.4, 1.0 M NaCl, 5 mM EDTA, 5 mM EGTA, 1 mM molybdate,
25 0.5% Tween-20); and finally in buffer A without Tween-20, all at 4°C. Washed beads were boiled in SDS sample buffer for 3 min and the supernatant split into three samples for electrophoresis. Gels were silver stained or blotted onto nitrocellulose filters and reacted with
30 rabbit antibodies generated against bacterially-expressed, full-length *S. pombe cdc2* protein or *prad1* peptide as above. Antibody binding was visualized by alkaline phosphatase-linked secondary antibodies, according to the manufacturer's directions (Promega).
35 Figure 10 demonstrates that *prad1* protein added to clam

embryo cell lysates binds to p13^{suc1}-Sepharose beads and activates histone H1 kinase activity. Bacterially expressed *prad1* was incubated with a clam embryo interphase lysate lacking endogenous cyclins A and B. The lysates were then mixed with p13^{suc1}- or protein A-Sepharose beads. The bound material was eluted, electrophoresed and either silver stained (a) or immunoblotted with anti-*prad1* antiserum (b) or anti-*cdc2* antiserum (c). Lane M shows molecular weight markers (from top to bottom) of 116, 94, 68, 56, 40, and 31 kD. Lane 1 shows whole clam embryo interphase lysate plus 18 ng *prad1* protein. Lanes 2, 3, 4, 5, and 6 represent clam embryo lysate to which 0, 18, 45, 225, or 18 ng of *prad1*, respectively, were added; these mixes were then assayed for material binding to p13^{suc1}-Sepharose (lanes 2-5) or protein A-Sepharose (lane 6) beads. Lane 7 shows bacterially-expressed *prad1*. Arrows indicate the positions of *prad1* and *cdc2* marker proteins.

Equal volumes of clam embryo interphase lysate and reticulocyte lysate containing [^{32}P]-labeled kinase products were then examined by SDS-PAGE, followed by autoradiography. Synthetic clam cyclins A and B (Westendorf et al., J. Cell Biol. 108:1431-1444; Swenson et al., Cell 47:861-870, 1986) and *prad1* mRNAs were transcribed and translated as described above. Translation product (3 μl) and clam embryo lysate (3 μl) were mixed. Samples were frozen immediately in liquid nitrogen. The remainder was incubated for 30 min at 18°C and then frozen. Samples were diluted with 1 volume of ice-cold buffer A, thawed on ice, and mixed with an equal volume of kinase mix (40 mM Hepes pH 7.3, 20 mM $MgCl_2$, 10 mM EGTA, 0.2 mg/ml histone H1, 10 μM cAMP-dependent kinase inhibitor (Sigma), 0.5 mCi/ml [γ-^{32}P]ATP and incubated at 23°C for 10 min. Double-strength SDS sample buffer was then added and the entire mix was analyzed by

SDS-PAGE followed by autoradiography, as shown in Figure 10(d).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Arnold, Andrew

(ii) TITLE OF INVENTION: PRAD1 CYCLIN AND ITS CDNA

(iii) NUMBER OF SEQUENCES: 1

(iv) CORRESPONDENCE ADDRESS:

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(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: U.S.A.
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb storage
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM: IBM P.C. DOS (Version 3.30)
(D) SOFTWARE: WordPerfect (Version 5.0)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 07/667,711
(B) FILING DATE: March 11, 1991

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Clark, Paul T.
(B) REGISTRATION NUMBER: 30,162
(C) REFERENCE/DOCKET NUMBER: 00786/070001

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(A) TELEPHONE: (617) 542-5070
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(C) TELEX: 200154

(2) **INFORMATION FOR SEQ ID NO.: 1:**

(i) **SEQUENCE CHARACTERISTICS:**

(A) **LENGTH:** 4244
(B) **TYPE:** nucleic acid
(C) **STRANDEDNESS:** single
(D) **TOPOLOGY:** linear

(ii) **SEQUENCE DESCRIPTION : 1:**

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GGCGCAGTAG CAGCGAGCAG CAGAGTCCGC ACGCTCCGGC GAGGGGCAGA AGAGCGCGAG      60

GGAGCGCGGG GCAGCAGAAG CGAGAGCCGA GCGCGGACCC AGCCAGGACC CACAGCCCTC     120

CCCAGCTGCC CAGGAAGAGC CCCAGCC ATG GAA CAC CAG CTC CTG TGC TGC GAA     174
                              Met Glu His Gln Leu Leu Cys Cys Glu
                               1               5

GTG GAA ACC ATC CGC CGC GCG TAC CCC GAT GCC AAC CTC CTC AAC GAC      222
Val Glu Thr Ile Arg Arg Ala Tyr Pro Asp Ala Asn Leu Leu Asn Asp
 10                  15                  20                  25

CGG GTG CTG CGG GCC ATG CTG AAG GCG GAG GAG ACC TGC GCG CCC TCG      270
Arg Val Leu Arg Ala Met Leu Lys Ala Glu Glu Thr Cys Ala Pro Ser
                 30                  35                  40

GTG TCC TAC TTC AAA TGT GTG CAG AAG GAG GTC CTG CCG TCC ATG CGG      318
Val Ser Tyr Phe Lys Cys Val Gln Lys Glu Val Leu Pro Ser Met Arg
             45                  50                  55

AAG ATC GTC GCC ACC TGG ATG CTG GAG GTC TGC GAG GAA CAG AAG TGC      366
Lys Ile Val Ala Thr Trp Met Leu Glu Val Cys Glu Glu Gln Lys Cys
         60                  65                  70

GAG GAG GAG GTC TTC CCG CTG GCC ATG AAC TAC CTG GAC CGC TTC CTG      414
Glu Glu Glu Val Phe Pro Leu Ala Met Asn Tyr Leu Asp Arg Phe Leu
     75                  80                  85

TCG CTG GAG CCC GTG AAA AAG AGC CGC CTG CAG CTG CTG GGG GCC ACT      462
Ser Leu Glu Pro Val Lys Lys Ser Arg Leu Gln Leu Leu Gly Ala Thr
 90                  95                 100                 105

TGC ATG TTC GTG GCC TCT AAG ATG AAG GAG ACC ATC CCC CTG ACG GCC      510
Cys Met Phe Val Ala Ser Lys Met Lys Glu Thr Ile Pro Leu Thr Ala
                110                 115                 120

GAG AAG CTG TGC ATC TAC ACC GAC AAC TCC ATC CGG CCC GAG GAG CTG      558
Glu Lys Leu Cys Ile Tyr Thr Asp Asn Ser Ile Arg Pro Glu Glu Leu
            125                 130                 135
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CTG CAA ATG GAG CTG CTC CTG GTG AAC AAG CTC AAG TGG AAC CTG GCC        606
Leu Gln Met Glu Leu Leu Leu Val Asn Lys Leu Lys Trp Asn Leu Ala
        140                 145                 150

GCA ATG ACC CCG CAC GAT TTC ATT GAA CAC TTC CTC TCC AAA ATG CCA        654
Ala Met Thr Pro His Asp Phe Ile Glu His Phe Leu Ser Lys Met Pro
    155                 160                 165

GAG GCG GAG GAG AAC AAA CAG ATC ATC CGC AAA CAC GCG CAG ACC TTC        702
Glu Ala Glu Glu Asn Lys Gln Ile Ile Arg Lys His Ala Gln Thr Phe
170                 175                 180                 185

GTT GCC CTC TGT GCC ACA GAT GTG AAG TTC ATT TCC AAT CCG CCC TCC        750
Val Ala Leu Cys Ala Thr Asp Val Lys Phe Ile Ser Asn Pro Pro Ser
                190                 195                 200

ATG GTG GCA GCG GGG AGC GTG GTG GCC GCA GTG CAA GGC CTG AAC CTG        798
Met Val Ala Ala Gly Ser Val Val Ala Ala Val Gln Gly Leu Asn Leu
            205                 210                 215

AGG AGC CCC AAC AAC TTC CTG TCC TAC TAC CGC CTC ACA CGC TTC CTC        846
Arg Ser Pro Asn Asn Phe Leu Ser Tyr Tyr Arg Leu Thr Arg Phe Leu
        220                 225                 230

TCC AGA GTG ATC AAG TGT GAC CCA GAC TGC CTC CGG GCC TGC CAG GAG        894
Ser Arg Val Ile Lys Cys Asp Pro Asp Cys Leu Arg Ala Cys Gln Glu
    235                 240                 245

CAG ATC GAA GCC CTG CTG GAG TCA AGC CTG CGC CAG GCC CAG CAG AAC        942
Gln Ile Glu Ala Leu Leu Glu Ser Ser Leu Arg Gln Ala Gln Gln Asn
250                 255                 260                 265

ATG GAC CCC AAG GCC GCC GAG GAG GAG GAA GAG GAG GAG GAG GAG GTG        990
Met Asp Pro Lys Ala Ala Glu Glu Glu Glu Glu Glu Glu Glu Glu Val
                270                 275                 280

GAC CTG GCT TGC ACA CCC ACC GAC GTG CGG GAC GTG GAC ATC TGA           1035
Asp Leu Ala Cys Thr Pro Thr Asp Val Arg Asp Val Asp Ile
            285                 290                 295

GGGCGCCAGG CAGGCGGGCG CCACCGCCAC CCGCAGCGAG GGCGGAGCCG GCCCCAGGTG    1095

CTCCACTGAC AGTCCCTCCT CTCCGGAGCA TTTTGATACC AGAAGGGAAA GCTTCATTCT    1155

CCTTGTTGTT GGTTGTTTTT TCCTTTGCTC TTTCCCCCTT CCATCTCTGA CTTAAGCAAA    1215

AGAAAAAGAT TACCCAAAAA CTGTCTTTAA AAGAGAGAGA GAGAAAAAAA AAATAGTATT    1275

TGCATAACCC TGAGCGGTGG GGGAGGAGGG TTGTGCTACA GATGATAGAG GATTTTATAC    1335

CCCAATAATC AACTCGTTTT TATATTAATG TACTTGTTTC TCTGTTGTAA GAATAGGCAT    1395

TAACACAAAG GAGGCGTCTC GGGAGAGGAT TAGGTTCCAT CCTTTACGTG TTTAAAAAAA    1455
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CCTCTTGGTT ACAGTAGCGT AGCGTGGCCG TGTGCATGTC CTTTGCGCCT GTGACCACCA 1755

CCCCAACAAA CCATCCAGTG ACAAACCATC CAGTGGAGGT TTGTCGGGCA CCAGCCAGCG 1815

TAGCAGGGTC GGGAAAGGCC ACCTGTCCCA CTCCTACGAT ACGCTACTAT AAAGAGAAGA 1875

CGAAATAGTG ACATAATATA TTCTATTTTT ATACTCTTCC TATTTTTGTA GTGACCTGTT 1935

TATGAGATGC TGGTTTTCTA CCCAACGGCC CTGCAGCCAG CTCACGTCCA GGTTCAACCC 1995

ACAGCTACTT GGTTTGTGTT CTTCTTCATA TTCTAAAACC ATTCCATTTC CAAGCACTTT 2055

CAGTCCAATA GGTGTAGGAA ATAGCGCTGT TTTTGTTGTG TGTGCAGGGA GGGCAGTTTT 2115

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GTGGGCCACT GTGGTGGCAG TGGAGGTGGG GTGTTTGGGA GGCTGCGTGC CAGTCAAGAA 2235

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AGTTGAAGTT TAGGAATCCT TTGGTGCCAA CTGGTGTTTG AAAGTAGGGA CCTCAGAGGT 2355

TTACCTAGAG AACAGGTGGT TTTTAAGGGT TATCTTAGAT GTTTCACACC GGAAGGTTTT 2415

TAAACACTAA AATATATAAT TTATAGTTAA GGCTAAAAAG TATATTTATT GCAGAGGATG 2475

TTCATAAGGC CAGTATGATT TATAAATGCA ATCTCCCCTT GATTTAAACA CACAGATACA 2535

CACACACACA CACACACACA CACAAACCTT CTGCCTTTGA TGTTACAGAT TTAATACAGT 2595

TTATTTTTAA AGATAGATCC TTTTATAGGT GAGAAAAAAA CAATCTGGAA GAAAAAAACC 2655

ACACAAAGAC ATTGATTCAG CCTGTTTGGC GTTTCCCAGA GTCATCTGAT TGGACAGGCA 2715

TGGGTGCAAG GAAAATTAGG GTACTCAACC TAAGTTCGGT TCCGATGAAT TCTTATCCCC 2775

TGCCCCTTCC TTTAAAAAAC TTAGTGACAA AATAGACAAT TTGCACATCT TGGCTATGTA 2835

ATTCTTGTAA TTTTTATTTA GGAAGTGTTG AAGGGAGGTG GCAAGAGTGT GGAGGCTGAC 2895

GTGTGAGGGA GGACAGGCGG GAGGAGGTGT GAGGAGGAGG CTCCCGAGGG GAAGGGGCGG 2955

TGCCCACACC GGGGACAGGC CGCAGCTCCA TTTTCTTATT GCGCTGCTAC CGTTGACTTC 3015

CAGGCACGGT TTGGAAATAT TCACATCGCT TCTGTGTATC TCTTTCACAT TGTTTGCTGC 3075

TATTGGAGGA TCAGTTTTTT GTTTTACAAT GTCATATACT GCCATGTACT AGTTTTAGTT 3135

TTCTCTTAGA ACATTGTATT ACAGATGCCT TTTTTGTAGT TTTTTTTTTT TTTATGTGAT 3195

CAATTTTGAC TTAATGTGAT TACTGCTCTA TTCCAAAAAG GTTGCTGTTT CACAATACCT 3255

CATGCTTCAC TTAGCCATGG TGGACCCAGC GGGCAGGTTC TGCCTGCTTT GGCGGGCAGA 3315

CACGCGGGCG CGATCCCACA CAGGCTGGCG GGGGCCGGCC CCGAGGCCGC GTGCGTGAGA 3375

ACCGCGCCGG TGTCCCCAGA GACCAGGCTG TGTCCCTCTT CTCTTCCCTG CGCCTGTGAT 3435

GCTGGGCACT TCATCTGATC GGGGGCGTAG CATCATAGTA GTTTTTACAG CTGTGTTATW 3495

CTTTGCGTGT AGCTATGGAA GTTGCATAAT TATTATTATT ATTATTATAA CAAGTGTGTC 3555

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CTGGAATTTG TTCAAGTTTT GGGTATGTTT AATCTGTTAT GTACTAGTGT TCTGTTTGTT 3675

ATTGTTTTGT TAATTACACC ATAATGCTAA TTTAAAGAGA CTCCAAATCT CAATGAAGCC 3735

AGCTCACAGT GCTGTGTGCC CCGGTCACCT AGCAAGCTGC CGAACCAAAA GAATTTGCAC 3795

CCCGCTGCGG GCCCACGTGG TTGGGGCCCT GCCCTGGCAG GGTCATCCTG TGCTCGGAGG 3855

CCATCTCGGG CACAGGCCCA CCCCGCCCCA CCCCTCCAGA ACACGGCTCA CGCTTACCTC 3915

AACCATCCTG GCTGCGGCGT CTGTCTGAAC CACGCGGGGG CCTTGAGGGA CGCTTTGTCT 3975

GTCGTGATGG GGCAAGGGCA CAAGTCCTGG ATGTTGTGTG TRTCGAGAGG CCAAAGGCTG 4035

GTGGCAAGTG CACGGGGCAC AGCGGAGTCT GTCCTGTGAC GCGCAAGTCT GAGGGTCTGG 4095

GCGGCGGGCG GCTGGGTCTG TGCATTTCTG GTTGCACCGC GGCGCTTCCC AGCACCAACA 4155

TGTAACCGGC ATGTTTCCAG CAGAAGACAA AAAGACAAAC ATGAAAGTCT AGAAATAAAA 4215

CTGGTAAAAC CCCAAAAAAA AAAAAAAAA 4244

Claims:

1 1. Isolated DNA comprising a sequence encoding
2 *prad1*.

1 2. A purified preparation of a vector, said
2 vector comprising a DNA sequence encoding *prad1*.

1 3. A cell comprising a recombinant DNA molecule
2 encoding *prad1*.

1 4. The cell of claim 3, wherein said recombinant
2 DNA molecule is integrated into the genome of said cell.

1 5. The cell of claim 4, wherein said cell is a
2 eukaryotic cell.

1 6. The cell of claim 3, wherein said cell is
2 capable of expressing *prad1* from said recombinant DNA
3 molecule.

1 7. The vector of claim 2, wherein said DNA
2 sequence encoding *prad1* is under the transcriptional
3 control of a heterologous promoter.

1 8. An essentially homogeneous population of
2 cells, each of which comprises a recombinant DNA molecule
3 encoding *prad1*.

1 9. The population of cells of claim 8, wherein
2 each of said cells is a eukaryotic cell.

1 10. The population of cells of claim 8, wherein
2 each of said cells is a prokaryotic cell.

1 11. *Prad1* produced by expression of a
2 recombinant DNA molecule encoding *prad1*.

1 12. *Prad1* synthesized by the cell of claim 3.

1 13. *Prad1* synthesized by the population of cells
2 of claim 8.

1 14. An essentially purified preparation of the
2 *prad1* of claim 11, 12, or 13.

1 15. A method for making *prad1*, which method
2 comprises
3 growing the cell of claim 3 in a medium to form a
4 population of cells which express said *prad1*; and
5 recovering said *prad1* from said population of
6 cells or said medium.

1 16. A method for making *prad1*, which method
2 comprises
3 combining the purified DNA of claim 1 with an
4 expression system to produce *prad1*; and
5 recovering said *prad1* from said expression
6 system.

1 17. A therapeutic composition comprising *prad1*
2 and a pharmacologically-acceptable carrier.

1 18. A method for promoting wound healing, which
2 method comprises
3 identifying an animal having a wound; and
4 administering a therapeutically-effective amount
5 of the therapeutic composition of claim 17 to said
6 animal.

1 19. A transgenic non-human vertebrate animal
2 bearing a transgene comprising a DNA sequence encoding
3 *prad1*.

1 20. The transgenic animal of claim 19, wherein
2 said animal is a mammal.

1 21. The transgenic animal of claim 20, wherein
2 said animal is a rodent.

1 22. The transgenic animal of claim 21, wherein
2 said animal is a mouse.

1 23. The transgenic animal of claim 19, wherein
2 said *prad1* is a *prad1* endogenous to the species of said
3 transgenic animal.

1 24. The transgenic animal of claim 19, wherein
2 said *prad1* is human *prad1*.

1 25. A cell derived from the transgenic animal of
2 claim 19.

1 26. A method for promoting proliferation of a
2 cell, which method comprises
3 providing an animal cell; and
4 treating said cell with a proliferation-inducing
5 amount of *prad1*.

1 27. A single-stranded DNA comprising a segment
2 of a PRAD1 at least 8 nucleotides in length.

1 28. The single-stranded DNA of claim 27, wherein
2 said segment of said PRAD1 is less than all of said
3 PRAD1.

1 29. The single-stranded DNA of claim 27, wherein
2 said segment of said PRAD1 is 10-3000 nucleotides in
3 length.

1 30. The single-stranded DNA of claim 27, wherein
2 said PRAD1 is a genomic PRAD1.

1 31. The single-stranded DNA of claim 27, wherein
2 said PRAD1 is a PRAD1 cDNA.

1 32. The single-stranded DNA of claim 27, wherein
2 said DNA is radioactively labelled.

1 33. The single-stranded DNA of claim 27, wherein
2 said DNA is antisense.

1 34. The single-stranded DNA of claim 27, wherein
2 said PRAD1 is human PRAD1.

1 35. The single-stranded DNA of claim 34, wherein
2 said PRAD1 is human PRAD1 cDNA (SEQ ID NO:1).

1 36. A diagnostic method comprising:
2 obtaining a nucleic acid sample from an animal
3 suspected of having a neoplastic condition;
4 contacting said nucleic acid sample with the
5 single-stranded DNA of claim 27, said PRAD1 being the
6 PRAD1 homolog of the species to which said animal
7 belongs; and
8 detecting the level of hybridization of said
9 single-stranded DNA with said nucleic acid sample, said
10 level being diagnostic for said neoplastic condition.

1 37. The method of claim 36, wherein said animal
2 is a mammal.

3 38. The method of claim 37, wherein said mammal
4 is a human.

5 39. An antibody capable of forming an immune
6 complex with *prad1*.

1 40. A purified preparation of the antibody of
2 claim 39.

1 41. The antibody of claim 39, wherein said *prad1*
2 is human *prad1*.

1 42. The purified preparation of claim 40,
2 wherein said *prad1* is human *prad1*.

1 43. A diagnostic method comprising:
2 obtaining a sample of a tissue of an animal
3 suspected of having a neoplastic condition;
4 contacting said sample with the antibody of
5 claim 39; and
6 detecting the level of immune complexes formed by
7 said antibody, said level being diagnostic for said
8 neoplastic condition.

1 44. The method of claim 43, wherein said animal
2 is a human.

1 45. A purified preparation of RNA comprising a
2 sequence encoding *prad1*.

1 46. An RNA molecule transcribed from the
2 isolated DNA of claim 1.

1 47. An RNA molecule comprising a sequence of at
2 least 8 nucleotides encoding a portion of a *prad1*.

1 48. The RNA molecule of claim 47, wherein said
2 sequence is 10-3000 nucleotides in length.

1 49. The RNA molecule of claim 48, wherein said
2 *prad1* is human *prad1*.

FIGURE 1

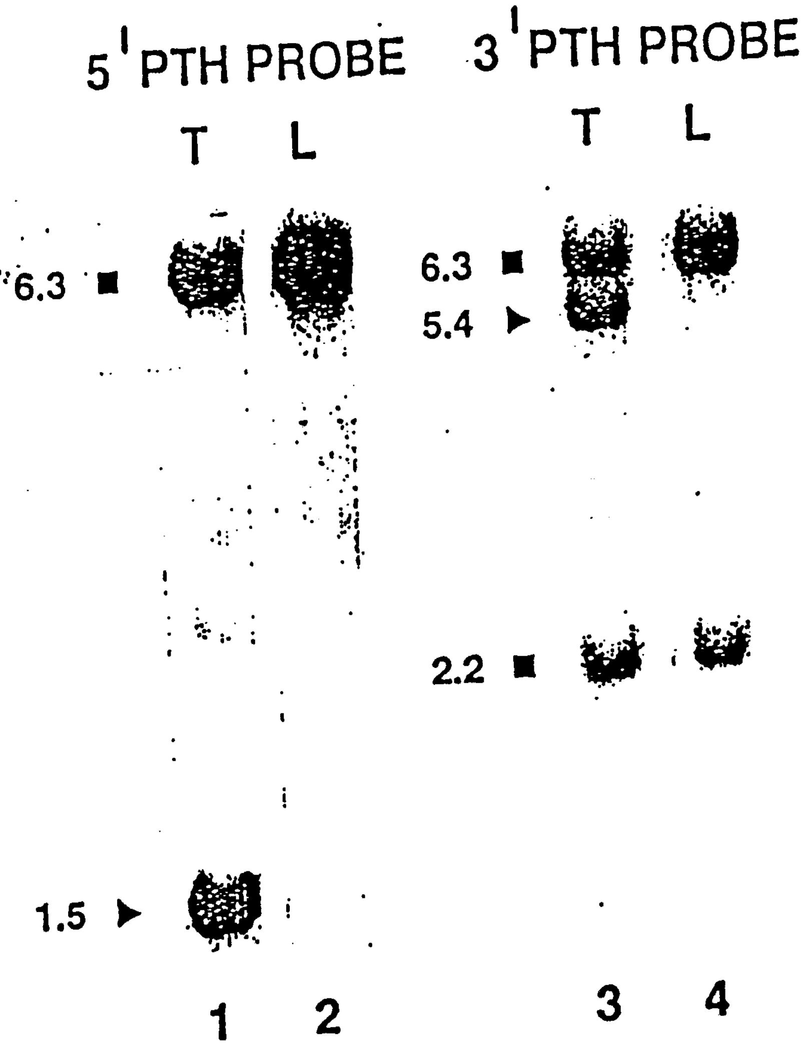
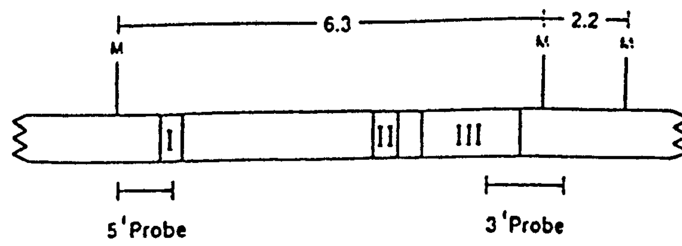


FIGURE 2

A. NORMAL PTH ALLELE



B. REARRANGED PTH ALLELE

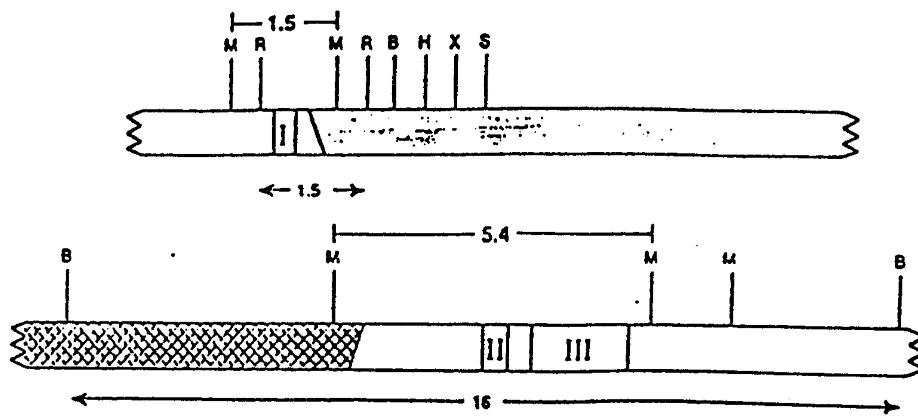


FIGURE 3

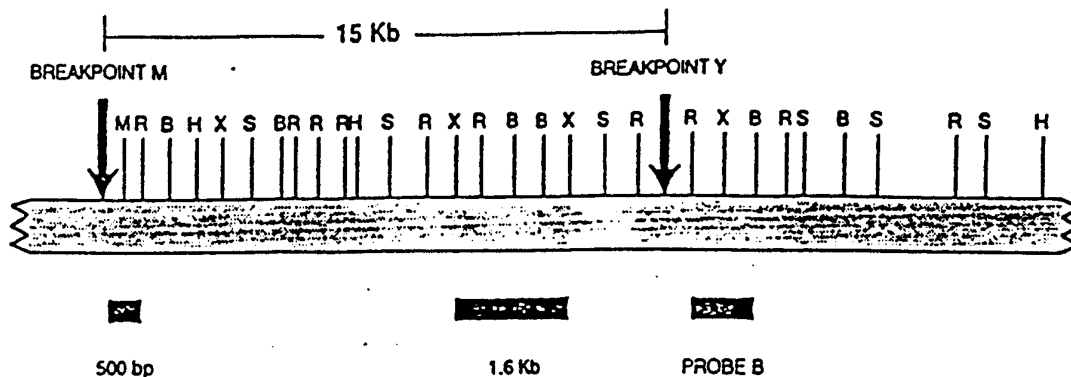


FIGURE 4 4/13

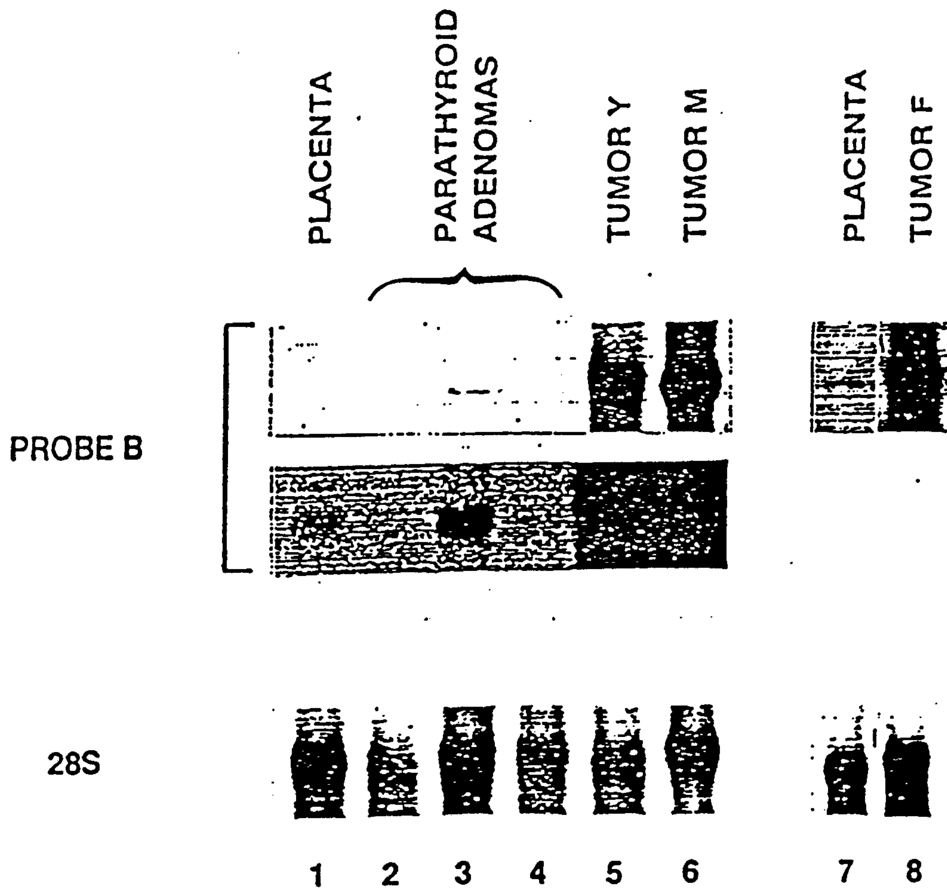


FIGURE 5

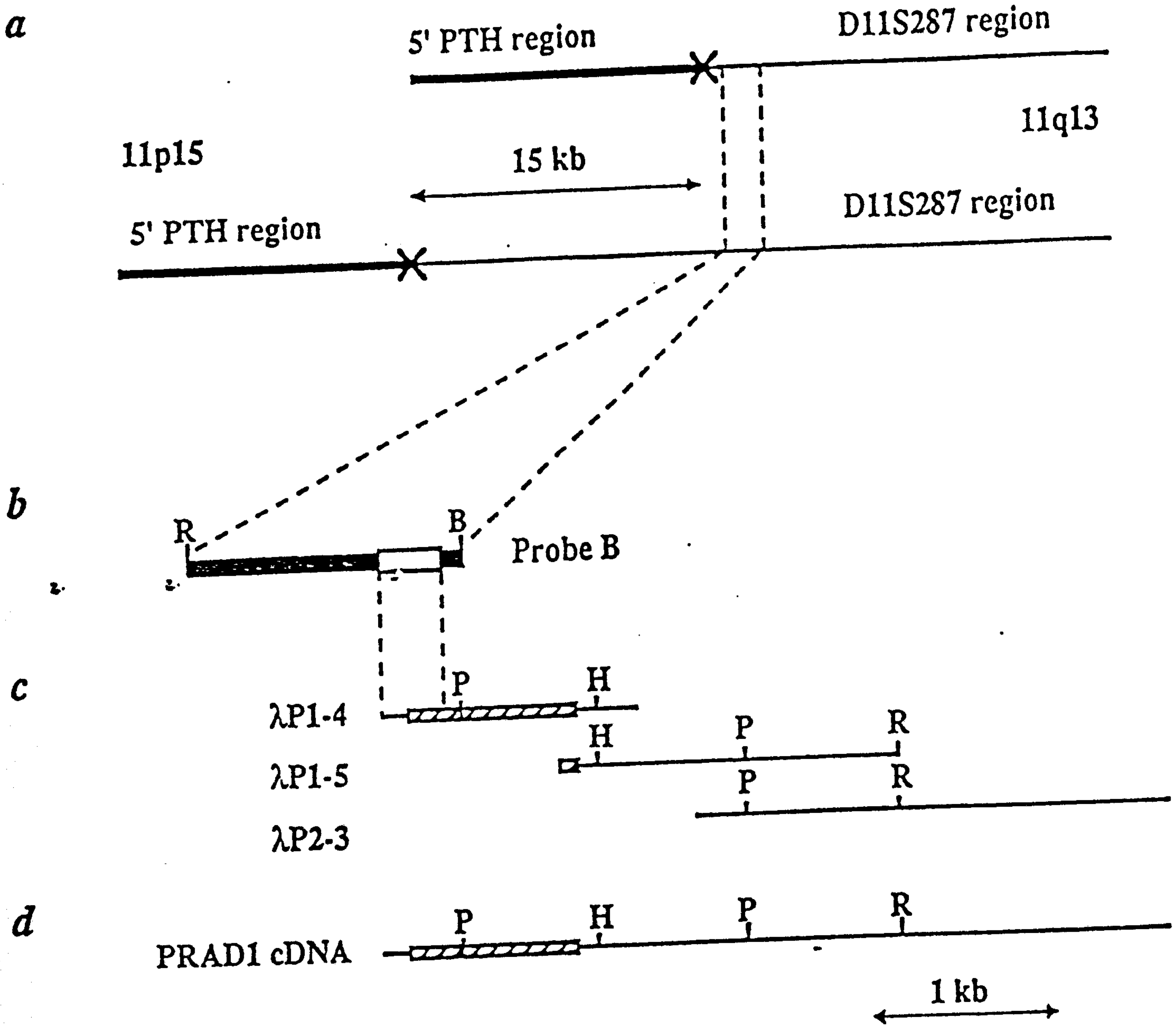


FIGURE 6

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GGCGCAGTAG CAGCGAGCAG CAGAGTCCGC ACGCTCCGGC GAGGGGCAGA AGAGCGCGAG    60

GGAGCGCGGG GCAGCAGAAG CGAGAGCCGA GCGCGGACCC AGCCAGGACC CACAGCCCTC   120

CCCAGCTGCC CAGGAAGAGC CCCAGCC ATG GAA CAC CAG CTC CTG TGC TGC GAA   174
                              Met Glu His Gln Leu Leu Cys Cys Glu
                               1               5

GTG GAA ACC ATC CGC CGC GCG TAC CCC GAT GCC AAC CTC CTC AAC GAC   222
Val Glu Thr Ile Arg Arg Ala Tyr Pro Asp Ala Asn Leu Leu Asn Asp
 10                 15                  20                  25

CGG GTG CTG CGG GCC ATG CTG AAG GCG GAG GAG ACC TGC GCG CCC TCG   270
Arg Val Leu Arg Ala Met Leu Lys Ala Glu Glu Thr Cys Ala Pro Ser
                30                  35                  40

GTG TCC TAC TTC AAA TGT GTG CAG AAG GAG GTC CTG CCG TCC ATG CGG   318
Val Ser Tyr Phe Lys Cys Val Gln Lys Glu Val Leu Pro Ser Met Arg
             45                  50                  55

AAG ATC GTC GCC ACC TGG ATG CTG GAG GTC TGC GAG GAA CAG AAG TGC   366
Lys Ile Val Ala Thr Trp Met Leu Glu Val Cys Glu Glu Gln Lys Cys
         60                  65                  70

GAG GAG GAG GTC TTC CCG CTG GCC ATG AAC TAC CTG GAC CGC TTC CTG   414
Glu Glu Glu Val Phe Pro Leu Ala Met Asn Tyr Leu Asp Arg Phe Leu
     75                  80                  85

TCG CTG GAG CCC GTG AAA AAG AGC CGC CTG CAG CTG CTG GGG GCC ACT   462
Ser Leu Glu Pro Val Lys Lys Ser Arg Leu Gln Leu Leu Gly Ala Thr
 90                  95                 100                 105

TGC ATG TTC GTG GCC TCT AAG ATG AAG GAG ACC ATC CCC CTG ACG GCC   510
Cys Met Phe Val Ala Ser Lys Met Lys Glu Thr Ile Pro Leu Thr Ala
                110                 115                 120

GAG AAG CTG TGC ATC TAC ACC GAC AAC TCC ATC CGG CCC GAG GAG CTG   558
Glu Lys Leu Cys Ile Tyr Thr Asp Asn Ser Ile Arg Pro Glu Glu Leu
            125                 130                 135

CTG CAA ATG GAG CTG CTC CTG GTG AAC AAG CTC AAG TGG AAC CTG GCC   606
Leu Gln Met Glu Leu Leu Leu Val Asn Lys Leu Lys Trp Asn Leu Ala
        140                 145                 150

GCA ATG ACC CCG CAC GAT TTC ATT GAA CAC TTC CTC TCC AAA ATG CCA   654
Ala Met Thr Pro His Asp Phe Ile Glu His Phe Leu Ser Lys Met Pro
    155                 160                 165

GAG GCG GAG GAG AAC AAA CAG ATC ATC CGC AAA CAC GCG CAG ACC TTC   702
Glu Ala Glu Glu Asn Lys Gln Ile Ile Arg Lys His Ala Gln Thr Phe
170                 175                 180                 185
```

FIGURE 6 (cont.)

```
GTT GCC CTC TGT GCC ACA GAT GTG AAG TTC ATT TCC AAT CCG CCC TCC     750
Val Ala Leu Cys Ala Thr Asp Val Lys Phe Ile Ser Asn Pro Pro Ser
                190                 195                 200

ATG GTG GCA GCG GGG AGC GTG GTG GCC GCA GTG CAA GGC CTG AAC CTG     798
Met Val Ala Ala Gly Ser Val Val Ala Ala Val Gln Gly Leu Asn Leu
            205                 210                 215

AGG AGC CCC AAC AAC TTC CTG TCC TAC TAC CGC CTC ACA CGC TTC CTC     846
Arg Ser Pro Asn Asn Phe Leu Ser Tyr Tyr Arg Leu Thr Arg Phe Leu
        220                 225                 230

TCC AGA GTG ATC AAG TGT GAC CCA GAC TGC CTC CGG GCC TGC CAG GAG     894
Ser Arg Val Ile Lys Cys Asp Pro Asp Cys Leu Arg Ala Cys Gln Glu
    235                 240                 245

CAG ATC GAA GCC CTG CTG GAG TCA AGC CTG CGC CAG GCC CAG CAG AAC     942
Gln Ile Glu Ala Leu Leu Glu Ser Ser Leu Arg Gln Ala Gln Gln Asn
250                 255                 260                 265

ATG GAC CCC AAG GCC GCC GAG GAG GAG GAA GAG GAG GAG GAG GAG GTG     990
Met Asp Pro Lys Ala Ala Glu Glu Glu Glu Glu Glu Glu Glu Glu Val
                270                 275                 280

GAC CTG GCT TGC ACA CCC ACC GAC GTG CGG GAC GTG GAC ATC TGA        1035
Asp Leu Ala Cys Thr Pro Thr Asp Val Arg Asp Val Asp Ile
            285                 290                 295

GGGCGCCAGG CAGGCGGGCG CCACCGCCAC CCGCAGCGAG GGCGGAGCCG GCCCCAGGTG  1095

CTCCACTGAC AGTCCCTCCT CTCCGGAGCA TTTTGATACC AGAAGGGAAA GCTTCATTCT  1155

CCTTGTTGTT GGTTGTTTTT TCCTTTGCTC TTTCCCCCTT CCATCTCTGA CTTAAGCAAA  1215

AGAAAAAGAT TACCCAAAAA CTGTCTTTAA AAGAGAGAGA GAGAAAAAAA AAATAGTATT  1275

TGCATAACCC TGAGCGGTGG GGGAGGAGGG TTGTGCTACA GATGATAGAG GATTTTATAC  1335

CCCAATAATC AACTCGTTTT TATATTAATG TACTTGTTTC TCTGTTGTAA GAATAGGCAT  1395

TAACACAAAG GAGGCGTCTC GGGAGAGGAT TAGGTTCCAT CCTTTACGTG TTTAAAAAAA  1455

AGCATAAAAA CATTTTAAAA ACATAGAAAA ATTCAGCAAA CCATTTTTAA AGTAGAAGAG  1515

GGTTTTAGGT AGAAAAACAT ATTCTTGTGC TTTTCCTGAT AAAGCACAGC TGTAGTGGGG  1575

TTCTAGGCAT CTCTGTACTT TGCTTGCTCA TATGCATGTA GTCACTTTAT AAGTCATTGT  1635

ATGTTATTAT ATTCCGTAGG TAGATGTGTA ACCTCTTCAC CTTATTCATG GCTGAAGTCA  1695

CCTCTTGGTT ACAGTAGCGT AGCGTGGCCG TGTGCATGTC CTTTGCGCCT GTGACCACCA  1755

CCCCAACAAA CCATCCAGTG ACAAACCATC CAGTGGAGGT TTGTCGGGCA CCAGCCAGCG  1815
```

FIGURE 6 (cont.)

```
TAGCAGGGTC GGGAAAGGCC ACCTGTCCCA CTCCTACGAT ACGCTACTAT AAAGAGAAGA 1875
CGAAATAGTG ACATAATATA TTCTATTTTT ATACTCTTCC TATTTTTGTA GTGACCTGTT 1935
TATGAGATGC TGGTTTTCTA CCCAACGGCC CTGCAGCCAG CTCACGTCCA GGTTCAACCC 1995
ACAGCTACTT GGTTTGTGTT CTTCTTCATA TTCTAAAACC ATTCCATTTC CAAGCACTTT 2055
CAGTCCAATA GGTGTAGGAA ATAGCGCTGT TTTTGTTGTG TGTGCAGGGA GGGCAGTTTT 2115
CTAATGGAAT GGTTTGGGAA TATCCATGTA CTTGTTTGCA AGCAGGACTT TGAGGCAAGT 2175
GTGGGCCACT GTGGTGGCAG TGGAGGTGGG GTGTTTGGGA GGCTGCGTGC CAGTCAAGAA 2235
GAAAAAGGTT TGCATTCTCA CATTGCCAGG ATGATAAGTT CCTTTCCTTT TCTTTAAAGA 2295
AGTTGAAGTT TAGGAATCCT TTGGTGCCAA CTGGTGTTTG AAAGTAGGGA CCTCAGAGGT 2355
TTACCTAGAG AACAGGTGGT TTTTAAGGGT TATCTTAGAT GTTTCACACC GGAAGGTTTT 2415
TAAACACTAA AATATATAAT TTATAGTTAA GGCTAAAAAG TATATTTATT GCAGAGGATG 2475
TTCATAAGGC CAGTATGATT TATAAATGCA ATCTCCCCTT GATTTAAACA CACAGATACA 2535
CACACACACA CACACACACA CACAAACCTT CTGCCTTTGA TGTTACAGAT TTAATACAGT 2595
TTATTTTTAA AGATAGATCC TTTTATAGGT GAGAAAAAAA CAATCTGGAA GAAAAAAACC 2655
ACACAAAGAC ATTGATTCAG CCTGTTTGGC GTTTCCCAGA GTCATCTGAT TGGACAGGCA 2715
TGGGTGCAAG GAAAATTAGG GTACTCAACC TAAGTTCGGT TCCGATGAAT TCTTATCCCC 2775
TGCCCCTTCC TTTAAAAAAC TTAGTGACAA AATAGACAAT TTGCACATCT TGGCTATGTA 2835
ATTCTTGTAA TTTTTATTTA GGAAGTGTTG AAGGGAGGTG GCAAGAGTGT GGAGGCTGAC 2895
GTGTGAGGGA GGACAGGCGG GAGGAGGTGT GAGGAGGAGG CTCCCGAGGG GAAGGGGCGG 2955
TGCCCACACC GGGGACAGGC CGCAGCTCCA TTTTCTTATT GCGCTGCTAC CGTTGACTTC 3015
CAGGCACGGT TTGGAAATAT TCACATCGCT TCTGTGTATC TCTTTCACAT TGTTTGCTGC 3075
TATTGGAGGA TCAGTTTTTT GTTTTACAAT GTCATATACT GCCATGTACT AGTTTTAGTT 3135
TTCTCTTAGA ACATTGTATT ACAGATGCCT TTTTTGTAGT TTTTTTTTTT TTTATGTGAT 3195
CAATTTTGAC TTAATGTGAT TACTGCTCTA TTCCAAAAAG GTTGCTGTTT CACAATACCT 3255
CATGCTTCAC TTAGCCATGG TGGACCCAGC GGGCAGGTTC TGCCTGCTTT GGCGGGCAGA 3315
CACGCGGGCG CGATCCCACA CAGGCTGGCG GGGGCCGGCC CCGAGGCCGC GTGCGTGAGA 3375
ACCGCGCCGG TGTCCCCAGA GACCAGGCTG TGTCCCTCTT CTCTTCCCTG CGCCTGTGAT 3435
```

FIGURE 6 (cont.)

```
GCTGGGCACT TCATCTGATC GGGGGCGTAG CATCATAGTA GTTTTTACAG CTGTGTTATW   3495
CTTTGCGTGT AGCTATGGAA GTTGCATAAT TATTATTATT ATTATTATAA CAAGTGTGTC   3555
TTACGTGCCA CCACGGCGTT GTACCTGTAG GACTCTCATT CGGGATGATT GGAATAGCTT   3615
CTGGAATTTG TTCAAGTTTT GGGTATGTTT AATCTGTTAT GTACTAGTGT TCTGTTTGTT   3675
ATTGTTTTGT TAATTACACC ATAATGCTAA TTTAAAGAGA CTCCAAATCT CAATGAAGCC   3735
AGCTCACAGT GCTGTGTGCC CCGGTCACCT AGCAAGCTGC CGAACCAAAA GAATTTGCAC   3795
CCCGCTGCGG GCCCACGTGG TTGGGGCCCT GCCCTGGCAG GGTCATCCTG TGCTCGGAGG   3855
CCATCTCGGG CACAGGCCCA CCCCGCCCCA CCCCTCCAGA ACACGGCTCA CGCTTACCTC   3915
AACCATCCTG GCTGCGGCGT CTGTCTGAAC CACGCGGGGG CCTTGAGGGA CGCTTTGTCT   3975
GTCGTGATGG GGCAAGGGCA CAAGTCCTGG ATGTTGTGTG TRTCGAGAGG CCAAAGGCTG   4035
GTGGCAAGTG CACGGGGCAC AGCGGAGTCT GTCCTGTGAC GCGCAAGTCT GAGGGTCTGG   4095
GCGGCGGGCG GCTGGGTCTG TGCATTTCTG GTTGCACCGC GGCGCTTCCC AGCACCAACA   4155
TGTAACCGGC ATGTTTCCAG CAGAAGACAA AAAGACAAAC ATGAAAGTCT AGAAATAAAA   4215
CTGGTAAAAC CCCAAAAAAA AAAAAAAAA                                     4244
```

APPL1534

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FIGURE 7

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human cyclin A:  MRAILVDWLVEVGEEYKLQNETLHLAVNYIDRFLSSMSVLRGKLQLVGTAAMLLASKFEEIYPPEVAEFVYITDDTYTK 288
                 || |*  |**|| || | **|   ||*||*|||||  *| ***|||*|** | *|||  |  |          ||**
pradl:           MRKIVATWMLEVCEEQKCEEEVFPLAMNYLDRFLSLEPVKKSRLQLLGATCMFVASKMKETIPLTAEKLCIYTDNSIRP 134
                 || |*  |**|| ||*|   |   |**||*|||||  *| ***|||*||* ||*|*|  |  |          ||**  *
clam cyclin A:   MRCILVDWLVEVSEEDKLHRETLFLGVNYIDRFLSKISVLRGKLQLVGAASMFLAAKYEEIYPPDVKEFAYITDDTYTS 273

human cyclin A:  KQVLRMEHLVLKVLTFDLAAPTVNQFLTQYFLHQQPANCKVESL...AMFLGELSLIDADPYLKYLPSVIAGAA 359
                  **| || |**  | **||| |  *|*  *  *  |*  * *  |     |   |   **   ||**|***
pradl:           EELLQMELLLVNKLKWNLAAMTPHDFIEHFLSKMPEAEENKQIIRKHAQTFVALCATDVK.FISNPPSMVAAGS 207
                 ***| || |**  | ***|  |*  | | || |  *|**   **  *     |   |*  **   ||* ||**
clam cyclin A:   QQVLRMEHLILKVLTFDVAVPTTNWFCEDFL.KSCDADDK...LKSLTMFLTELTLIDMDAYLKYLPSITAAAA 343

human cyclin B:  MRAILIDWLVQVQMKFRLLQETMYMTVSIIDRFMQNNCVPKKMLQLVGVTAMFIASKYEEMYPPEIGDFAFVTDNTYTK 279
                 || |*  |***|     *  *|   ***  *|||*  * | |  |||*| | ||*|||  |  |          |||*
pradl:           MRKIVATWMLEVCEEQKCEEEVFPLAMNYLDRFLSLEPVKKSRLQLLGATCMFVASKMKETIPLTAEKLCIYTDNSIRP 134
                 || |**  |**||    *   |   ||*| *||||||        *|||*| * *|*|||  | *  * *     *| *
cdc13:           MRGILTDWLIEVHSRFRLLPETLFLAVNIIDRFLSLRVCSLNKLQLVGIAALFIASKYEEVMCPSVQNFVYMADGGYDE 313

human cyclin B:  HQIRQMEMKILRALNFGLGRPLPLHFLRR.ASKIGEVDVEQHTL...AKYLMELTMLDYDMVHFPPSQIAAGA 348
                  ** |||* **   | * |*   |  |*   ||* | * *  *   |   * |   |   *  ||| *|||*
pradl:           EELLQMELLLVNKLKWNLAAMTPHDFIEHFLSKMPEAEENKQIIRKHAQTFVALCATDVKFISNPPSMVAAGS 207
                 ||*|| |   **  | *|||   | *    || *** |*       |   |   | *   | | ** |||   |**
cdc13:           EEILQAERYILRVLEFNLAYPNPMN....FLRRISKADFYDIQTRTVAKYLVEIGLLDHKLLPYPPSQQCAAA 382

pradl:           MRKIVATWMLEVCEEQKCEEEVFPLAMNYLDRFLSLEPVKKSRLQLLGATCMFVASKMKETIPLTA.....EKLCIYTD 129
                 || **  ***            |*    ||** |    *|    |||* | ****||  **      |       * ||   *
cln3:            MRFLIFDFIMYCHTRLNLSTSTLFLTFTILDKYSSRFIIKSYNYQLLSLTALWISSKFWDSKNRMATLKVLQNLC.CNQ 184

pradl:           NSIRPEELLQMELLLVNKLKWNLAAMTPHD.FIEHFLSKMPEAEENKQIIRKHAQTFVALCATDVKFISNPPSMVAAGS 207
                  ||*   *    ||*  |   | |  *    ** | *|*  ||    * *      **    * *  |       *  * |***      *
cln3:            YSIK..QFTTMEMHLFKSLDWSICQSATFDSYIDIFLFQSTSPLSPGVVL...SAPLEAFIQQKLALLNNAAGTAINKS 258
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FIGURE 8

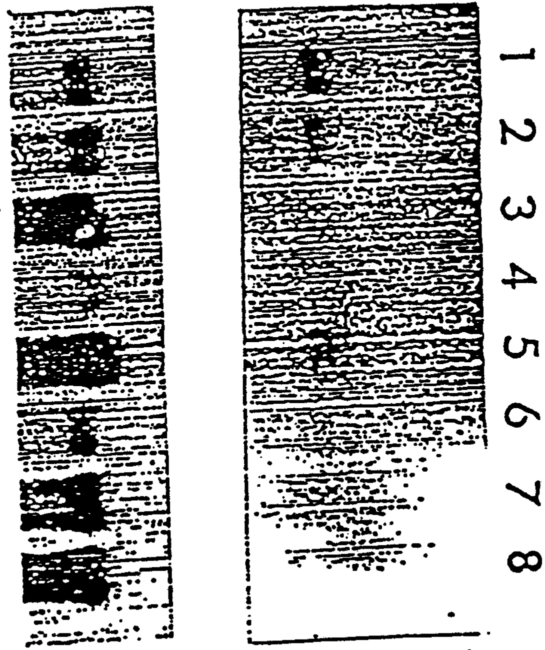
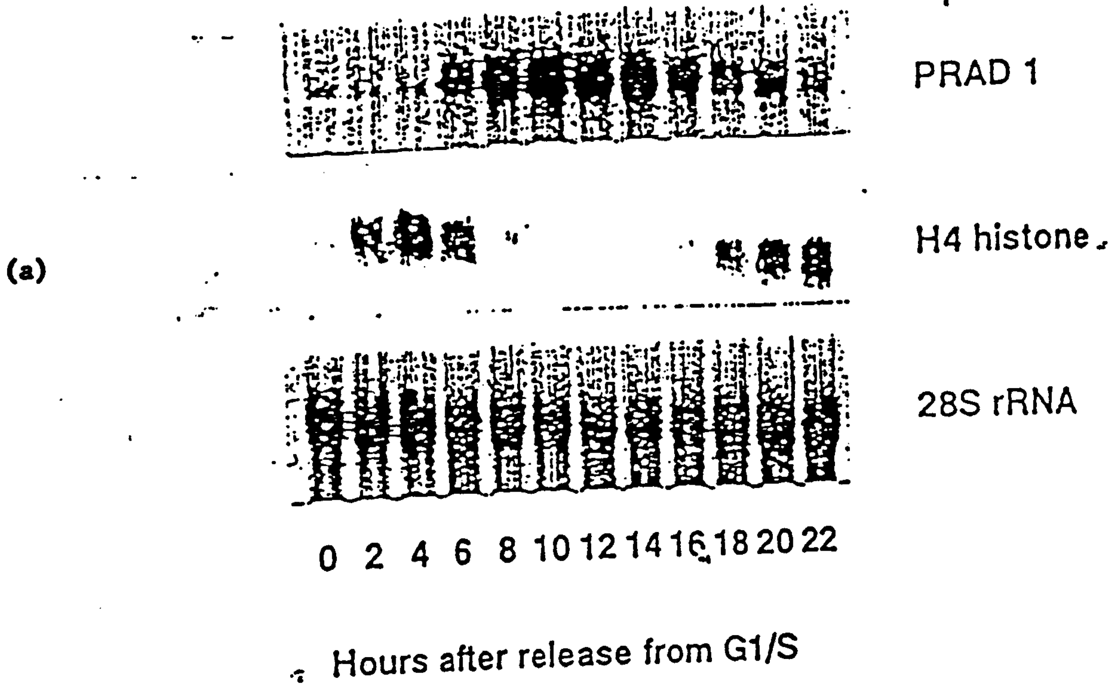


FIGURE 9

(a)



(b)

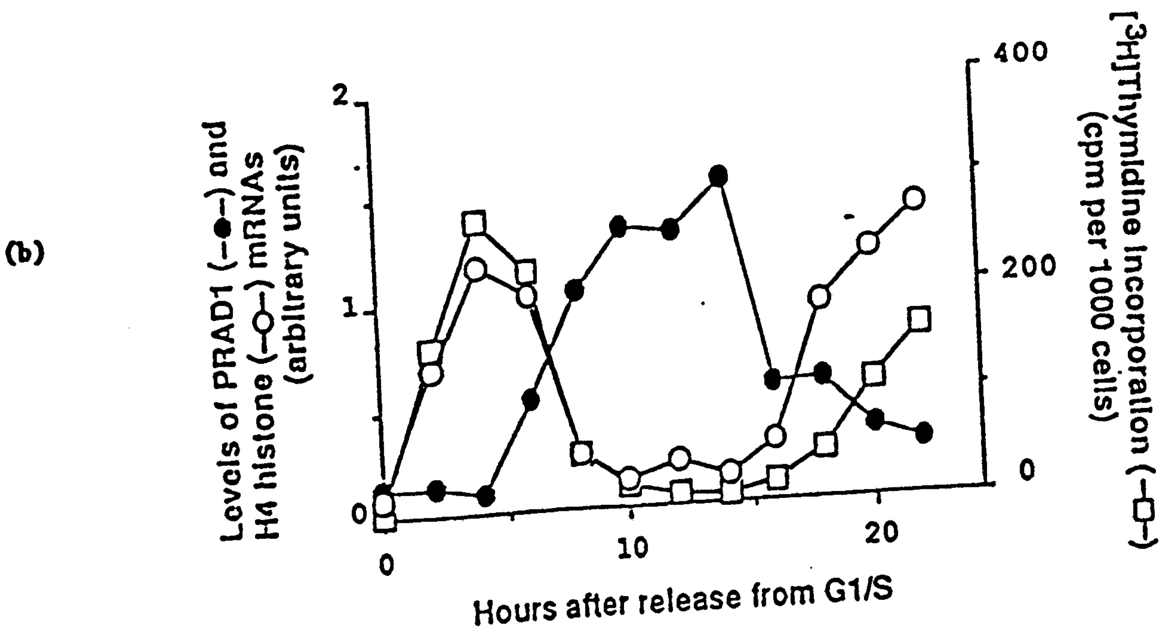
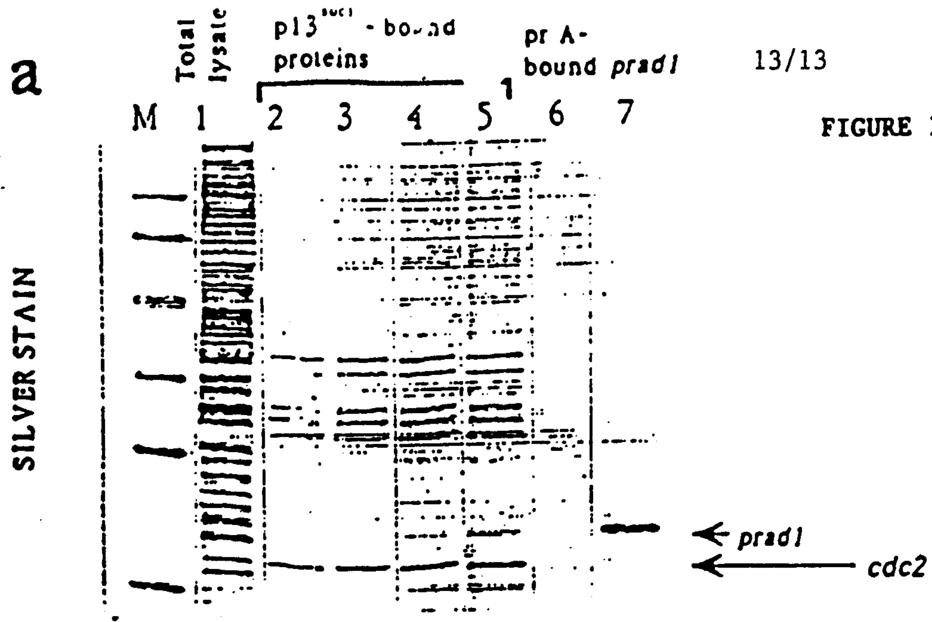
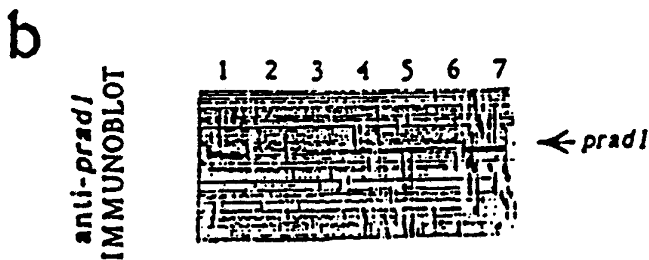
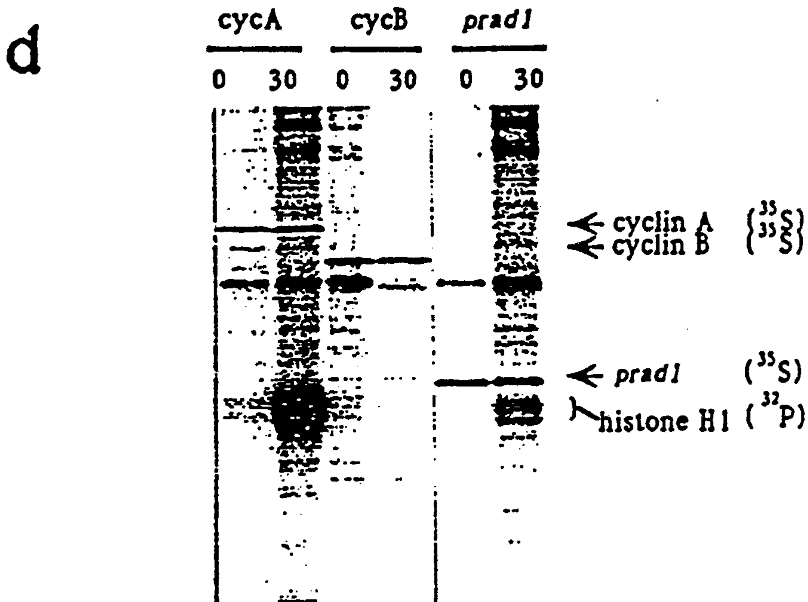


FIGURE 10







INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/01925

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C07H 21/02, 21/04; C12N 15/00, 15/09, 15/12, 15/27
US CL : 536/27; 435/69.1, 240.2, 252.3, 320.1

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	536/27; 435/69.1, 240.2, 252.3, 320.1

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

APS, DIALOG search terms: pradl, cyclin, centrocytic lymphoma, D11S287E, parathyroid, adenoma, 11q13, bcl1, oncogene

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No. [18]
X,P	NATURE, Volume 350, issued 11 April 1991, T. Motokura et al., "A Novel Cyclin encoded by a bcl1-linked candidate oncogene", pages 512-515, see entire document.	1-10,15-16,27-35, 45-49
A	NATURE, Volume 350, issued 11 April 1991, T. Hunt, "Cell Cycle Gets More Cyclins", pages 462-463, see entire document.	1-10,15-16, 27-35,45-49
X,P	CELL, Volume 65, issued 17 May 1991, H. Matsushime et al., "Colony-Stimulating Factor 1 Regulates Novel Cyclins during the G1 Phase of the Cell Cycle", pages 701-713, see entire document.	1-10,15-16, 27-35,45-49
X,P	CELL, Volume 66, issued 20 September 1991, D.J. Lew et al., "Isolation of Three Novel Human Cyclins by Rescue of G1 Cyclin (Cln) Function in Yeast", pages 1197-1206, see entire document.	1-10,15-16, 27-35,45-49
X,P	CELL, Volume 65, issued 17 May 1991, Y. Xiong et al., "Human D-Type Cyclin", pages 691-699, see entire document.	1-10,15-16, 27-35,45-49

* Special categories of cited documents:[15]
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
03 JUNE 1992	12 JUN 1992
International Searching Authority[1]	**Signature of Authorized Officer[20]**
ISA/US	MARIANNE PORTA ALLEN

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X,P	MOLECULAR AND CELLULAR BIOLOGY, Volume 11, No. 10, issued October 1991, D.A. Withers et al., "Characterization of a Candidate bcl-1 Gene", pages 4846-4853, see entire document.	1-10,15-16, 27-35,45-49
Y,P	BIOSIS Abstract, C.L. Rosenberg et al., "PRAD1 a Candidate BCL1 Oncogene Mapping and Expression in Centrocytic Lymphoma", pages 9638-9642, see abstract. Accession No. 93018797, for PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 88, No. 21, 1991.	1-10,15-16,27-35,45-49
Y,P	BIOSIS Abstract, C.L. Rosenberg et al., "Rearrangement and Overexpression of D11S287E a Candidate Oncogene on Chromosome 11Q13 in Benign Parathyroid Tumors", pages 449-454, see abstract. Accession No. 91131959, for ONCOGENE, Volume 6, No. 3, 1991.	1-10,15-16,27-35,45-49

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

1. ☐ Claim numbers _, because they relate to subject matter (1) not required to be searched by this Authority, namely:

2. ☐ Claim numbers _, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out (1), specifically:

3. ☐ Claim numbers _, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

Please See Attached Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

1-10, 15-16, 27-35, 45-49 (Telephone Practice)

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.

Remark on protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No.[18]
Y	JOURNAL OF BONE AND MINERAL RESEARCH, Volume 4, Supplement 1, issued 1989, A. Arnold et al., "DNA Rearranged Adjacent to the PTH Gene in a Parathyroid Adenoma Encodes an Abnormally Expressed Gene", page S262 (abstract), see entire document.	1-10,15-16,27-35,45-49
X/Y	NEW ENGLAND JOURNAL OF MEDICINE, Volume 318, No. 11, issued 17 March 1988, A. Arnold et al., "Monoclonality and Abnormal Parathyroid Hormone Genes in Parathyroid Adenomas", pages 658-662, see entire document.	1-10,27-34,45-49/15-16,35
X/Y	CLINICAL ENDOCRINOLOGY & METABOLISM, Volume 71, No. 2, issued August 1990, E. Friedman et al., "Genetic Abnormalities in Sporadic Parathyroid Adenomas", pages 293-297, see entire document.	1-10,27-34,45-49/15-16,35

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No.[18]
X/Y	JOURNAL OF CLINICAL INVESTIGATION, Vol.83, No.6, issued June 1989, Arnold et al, "Molecular Cloning and chromosomal Mapping...Adenoma" pages 2034-2040, see entire document.	1-10,27-34,45-49/ 15,16,35

FURTHER INFORMATION CONTINUED FROM PREVIOUS SHEETS

VI. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-10, 15-16, 27-35, and 45-49, drawn to nucleic acid sequences for prad1, vectors, transformed host cells, and a method of recombinant production of prad1, classified in at least, for example, Class 536, subclass 27, and Class 435, subclasses 69.1, 240.2, 252.3, and 320.1

II. Claims 11-14, and 17, drawn to protein sequences of prad1 and therapeutic composition, classified in at least, for example, Class 514, subclass 2.

III. Claim 18, drawn to a method for promoting wound healing, classified in Class 514, subclass 2.

IV. Claims 19-25, drawn to a transgenic animal and a cell derived therefrom, classified in Class 800, subclass 2.

V. Claim 26, drawn to a method for promoting proliferation of a cell, classified in Class 514, subclass 2.

VI. Claims 36-38, drawn to a diagnostic method using nucleic acids, classified in Class 435, subclass 6.

VII. Claims 39-42, drawn to an antibody to prad1, classified in Class 530, subclass 387.

VIII. Claims 43-44, drawn to a diagnostic method using antibodies, classified in Class 435, subclass 7.1.

The claims of these eight groups are drawn to distinct inventions which are not linked so as to form a single general inventive concept. PCT Rules 13.1 and 13.2 do not provide for multiple distinct methods and products within a single general inventive concept. 37 CFR 1.475 defines a single general inventive concept to be a first product and a first process of using the first product. As such, the claims of group I are deemed to be a single inventive concept. Group II is a second appearing product. Group III is a second appearing method of use. Group IV is a third appearing product. Group V is a third appearing method of use. Group VI is a fourth appearing method of use. Group VII is a fourth appearing product. Group VIII is a fifth appearing method of use.