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A NOVEL ONCO-FETAL GENE, GENE PRODUCT AND USES THEREFOR

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(57) Claim

1. A recombinant polypeptide comprising the amino acid sequence of a Pem protein encoded by the Pem gene.

5. A cDNA probe comprising the nucleotide sequence shown in Figures 1A and 1B.

6. A method for detecting neoplastic cells comprising incubation of a probe according to claim 5 with a target cell population under hybridizing conditions.

7. A method for treating neoplastic cells comprising insertion of an inactivated mutant Pem gene into neoplastic cells.

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(54) Title: A NOVEL ONCO-FETAL GENE, GENE PRODUCT AND USES THEREFOR

(57) Abstract

The present invention provides a novel cDNA sequence (Pem), a gene product protein, and uses for this novel cDNA sequence and the Pem gene product. The DNA sequence and recombinant DNA molecules of this invention are characterized in that each codes for a novel protein having the following characteristics: (1) is expressed by T-lymphoma cells, (2) is not expressed in normal thymus, activated spleen cells, gut associated lymphoid tissue, or bone marrow, and is not detectable in adult brain, liver, large intestine or ovary, (3) is expressed in immortalized or cancerous cell lines, and (4) is expressed in embryonic development.

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A NOVEL ONCO-FETAL GENE, GENE PRODUCT AND USES THEREFOR

Field of the Invention

This invention concerns a novel cDNA clone obtained from a T-lymphoma library. This novel cDNA clone, known as Pem, hybridizes to transcripts expressed in placenta and embryos in a stage-specific manner. The Pem cDNA sequence predicts an intracellular hydrophilic protein with no significant sequence similarity with other DNA or protein sequences. The DNA sequence and recombinant DNA molecules of this invention are characterized in that each codes for a novel protein having the following characteristics: (1) is expressed by T-lymphoma cells, (2) is not expressed in normal thymus, activated spleen cells, gut associated lymphoid tissue, or bone marrow, and is not detectable in adult brain, liver, large intestine or ovary, (3) is expressed in immortalized or cancerous cell lines, and (4) is expressed in embryonic development. As may be appreciated from the disclosure to follow, the DNA sequence, recombinant DNA molecules and process for producing the novel protein expressed in embryonic development and the novel Pem protein in substantially pure form may be useful in manipulating the regulation of embryonic development, altering the tumorigenic phenotype and may also be useful for localizing metastatic foci of tumors containing the Pem oncogene.

Background Art

Numerous genes have been identified in invertebrate organisms such as Drosophila melanogaster, Caenorhabditis elegans and Saccharomyces cerevisiae which participate in developmental events. In mammalian systems, only a few genes which play an integral role during development have been identified (Blau (1988) Cell 53:673). For example, the muscle-specific genes, MyoD1 and myogenin have served as important models of genetic control of murine differentiation. Somites and limb buds first express myogenin and MyoD1 on days 8 and 10 of gestation, respectively, (Sasson, et al. (1989) Nature 341:303-308) and are known to control the switch to the myoblast lineage in vitro (Davis, et al. (1987) Cell 51:987-1000). The Hox-5 gene complex exhibits an interesting pattern of expression in murine limb buds after the 9th day of gestation (Dolle, et al. (1989) Nature 342:161-111), although the function(s) of the encoded genes have yet to be defined. Few other genetic markers are known which tag cells in early and intermediate stages of murine development. For example, no gene has been associated uniquely with the initial process of segmentation, which occurs on day 8 of gestation in the mouse (for review, see Rossant and Joyner (1989) Trends in Genetics 5:277-283).

Immortalized and fully transformed cells frequently transcribe genes which are expressed in (and presumably influence) normal mammalian development (reviewed, Ruddon (1987) Gene Derepression in Cancer Cells, in Cancer Biology, Oxford University Press, New York, pages 431-436). In some cases, these "oncofetal" genes do not appear to contribute to the neoplastic phenotype. For example, a-fetoprotein is expressed by trophoblast cells and by many tumor cells. In other cases, developmentally regulated genes play a primary role in the conversion of cells to the transformed phenotype. For example, the proto-oncogenes c-myc, c-src, c-fos, and c-fms are expressed during embryonic development and have been shown to regulate developmental steps in vitro (for review, see Adamson (1987) Placenta 8:449-466).

In the development of the immune system, T-lymphocytes are derived from precursor stem cells which enter the thymus to undergo differentiation and maturation. Many genes are either activated or repressed as the T-cell passes through different stages of development within the thymus. For example, the cells acquire the IL-2 receptor, CD4, and/or CD8 on their surface during this time. These differentiation markers are important for T-cell development and/or function. Many gene products are increased in their levels of expression in developing T-lymphocytes. These include the T-cell receptor for antigen as well as the markers CD4 and CD8. Many other antigens have served as T-cell markers before their exact function in lymphocytes were known. Only recently has it been discovered that the T-cell antigen Pgpl aids thymocytes in their homing to the thymus whereas T200 (CD45) serves as a component for intracellular signalling. Another T-cell marker, Thy1, still has no known function associated with it.

The SL 12.4 cells exhibit a CD4/CD8 double negative phenotype and therefore resemble thymocytes at a relatively early stage of development. Furthermore, they do not express the T-cell receptor alpha subunit. SL 12.4 cells, however, can be induced to stably express CD4 and CD8 on their surface after co-cultivation upon thymic epithelial monolayers. TCR-alpha mRNA is also induced after these treatments. Thus, it appears that SL 12.4 cells have the capacity to undergo differentiation and maturation. This unique in vitro biological system mimics, to some extent, the thymic microenvironment.

A number of genes have been identified which are first expressed in developing thymocytes. Many of these genes encode proteins which must be expressed for T-cell precursors to become functional in the immune system, for example: (1) the TCR for antigen which is required for antigen recognition; (2) CD25 (the IL2 receptor) which must be expressed for the cells to respond to the cytokine IL2;

(3) gene products important for signal transduction during antigen recognition, such as CD3, CD4, CDS, CD45; (4) some of the gene products involved in thymocyte homing to target organs; and (5) gene products involved in T-cell activation (Fowlkes and Pardoll, Advances in Immunology 44:207-264 (1989); Hood, et al. (1985) Cell 40:225-229; Rothenberg and Lugo, Develop. Biol. 112:1-17 (1985); Adkins et al., Ann. Rev. Immunol. 5:325-365 (1987); Crabtree, Science 243:343-355 (1989); Kwon and Weissman, Proc. Natl. Acad. Sci.USA 86:1963-1967 (1989). There is remarkable heterogeneity in thymocyte subsets which express different combinations of expressed genes. Gene expression has been analyzed in detail in many, but not all, of the numerous classes of thymocytes; and it is likely that genes remain to be identified that encode products which function in T-cell development and homing, particularly those which are expressed in numerically infrequent, transient progenitor thymocytes.

Due to the extensive heterogeneity of thymocytes, it is not feasible to obtain fractionated progenitor thymocytes in sufficient numbers or purity to fully characterize the cascade of gene expression which occurs during development. For this reason, lymphoma and leukemia cell lines have been used extensively to study gene expression in lymphoid development (Greaves (1986) Science 234:697-704; Hanley-Hyde and Lynch (1986) Ann. Rev. Immunol. 4:621-649). A considerable body of literature indicates that numerically infrequent, transient progenitor cells are the target of transformation to malignancy, and further that some of the characteristics of the transformed target cells are preserved in the tumor cells. Unexpected gene expression in tumor cells was frequently dismissed as an aberration of transformation. However, careful analysis of "aberrant" gene expression in hematopoietic tumor cells has revealed rare subsets of normal progenitor cells which express such genes (Greaves (1986) Science 234:697-704;

According to one embodiment of the present invention there is provided a recombinant polypeptide comprising the amino acid sequence of a Pem protein encoded by the Pem gene.

According to a further embodiment of the present invention there is provided a cDNA probe comprising the nucleotide sequence shown in Figures 1A and 1B.

According to another embodiment of the present invention there is provided a method for treating neoplastic cells comprising insertion of an inactivated mutant Pem gene into neoplastic cells.

According to yet a further embodiment of the present invention there is provided an expression vehicle which comprises a DNA sequence coding for the Pem protein.

According to still another embodiment of the present invention there is provided a vector comprising a DNA sequence coding for Pem protein.

According to a further embodiment of the present invention there is provided a host transformed with a recombinant DNA molecule wherein said recombinant DNA molecule comprises a DNA sequence coding for the Pem protein.

According to another embodiment of the present invention there is provided a method of producing a Pem protein which comprises:

a) transforming a host with a DNA sequence coding for T-cell protein;

b) expressing said DNA sequence; and

c) recovering said Pem protein.

According to yet a further embodiment of the present invention there is provided a pharmaceutical composition useful for inducing the production of antibodies to Pem protein in an individual comprising an immunogenically effective amount of Pem protein.

In this

Hanley-Hyde and Lynch (1986) Ann. Rev. Immunol. 4:621-649; Pierce and Speers (1988) Cancer Res. 48:1996-2004).

The heterogeneity of murine and human lymphoma cell lines derived from a single individual can result from differences in the extent of maturation reached by individual cells. The heterogeneity of established T-lymphoma cell lines has been utilized to obtain closely related cell clones which differ in a limited number of characteristics. Hedrick, et al. (Hedrick, et al. (1984) Nature 308:149-153) using subtraction cloning techniques provided estimates that T- and B- cells differ in the expression of about 100 genes. It is likely that closely related T-lymphoma cells might differ in the expression of even fewer genes. Such cell clones provide an opportunity to work with pure populations of cells with defined and stable phenotypes which differ in a limited number of characteristics. The SL12 T-lymphoma model system was developed and utilized in the present application to provide such a closely related cell population. (Hays, et al. (1986) Int. J. Cancer 30:597-601; MacLeod, et al. (1984) Cancer Res. 44:1784-1790; MacLeod, et al. (1985) J. Nat. Cancer Inst. 74:875-882; MacLeod, et al. (1986) Proc. Natl. Acad. Sci. USA 83:6989-6993; Siegal, et al. (1987) J. Exp. Med. 166:1702-1715).

SL12.4 cells are similar to thymocytes at an intermediate stage of maturation (Fowlkes and Pardoll (1989) Advances in Immunol. 44:207-264). The two cell clones differ in their biological properties. SL12.4 cells generate extranodal tumors and are sensitive to glucocorticoid-induced lysis, whilst SL12.3 cells cause diffuse disseminated tumors resistant to lysis by glucocorticoids.

Brief Description of the Invention

The present invention provides a novel cDNA sequence, a gene product (Pem) protein, and uses for this novel cDNA sequence and the Pem gene product. ~~In this~~

invention, the sequence and expression characteristics of a novel gene represented by a cDNA clone (Pem) obtained from a T-lymphoma are described. Immortalized and transformed cell lines derived from several lineages express Pem transcripts. The DNA sequence and recombinant DNA molecules of this invention are characterized in that each codes for a novel protein having the following characteristics: (1) is expressed by T-lymphoma cells, (2) is not expressed in normal thymus, activated spleen cells, gut associated lymphoid tissue, or bone marrow, and is not detectable in adult brain, liver, large intestine or ovary, (3) is expressed in immortalized or cancerous cell lines, and (4) is expressed in embryonic development. As may be appreciated from the disclosure to follow, the DNA sequence, recombinant DNA molecules and process for producing the novel protein expressed in embryonic development and the novel Pem protein in substantially pure form may be useful in manipulating the regulation of embryonic development, altering the tumorigenic phenotype and may also be useful for localizing metastatic foci of tumors containing the Pem oncogene.

Although the Pem gene is not detectably expressed in adult tissue, it is sequentially expressed during murine fetal development, first in early embryos and subsequently in extraembryonic tissues. The expression of Pem during fetal development and its presence in immortalized and neoplastic cell lines are consistent with the properties expected of an "oncofetal" gene. This Pem cDNA sequence may be useful as a marker of neoplastic cells and of cells participating in early embryonic development.

Further objects, features and advantages of the present invention will become apparent from a review of the detailed description of the preferred embodiments which follows, in view of the drawings, a brief description of which follows.

Brief Description of the Drawings

Figure 1 shows the DNA and predicted protein sequence of Pem cDNA.

Figure 2 demonstrates the Pem mRNA expression in cell lines.

Figure 3 demonstrates Pem mRNA expression during fetal development.

Detailed Description of the Invention

In order that the invention herein described may be more fully understood, the following detailed description is set forth.

In the description the following terms are employed:

The term "host" as used herein is meant to include not only prokaryotes but also eukaryotes such as yeast and filamentous as well as plant and animal cells.

The term "prokaryote" is meant to include all bacteria which can be transformed with the DNA for the expression of the Pem or recombinant Pem proteins (rPemP) of the present invention.

The term "eukaryote" is meant to include all yeasts, fungi, animal and plant cells which can be transformed with the DNA for the expression of the Pem or recombinant Pem proteins of the present invention.

The DNA for the Pem proteins of the present invention can be derived from any mammalian species. All that is required is that the genetic sequence for the Pem protein (PemP) be expressed in the prokaryotic or eukaryotic organism. Preferred is the Pem DNA which expresses Pem protein(s) from mice. Especially preferred is the sequence of the Pem DNA which is immunologically cross reactive among multiple animal species (e.g., mice, rabbit, sea lion or human).

A recombinant DNA molecule coding for the Pem protein of the present invention can be used to transform a host using any of the techniques commonly known to those of ordinary skill in the art. Especially preferred is the use of a vector containing coding sequence for the Pem protein of the present invention for purposes of prokaryote transformation.

The T-cell recombinant protein (rPem) of the invention could have more or less amino acids at its flanking ends as compared to the amino acid sequence of native Pem protein.

The term "substantially pure" when applied to the Pem protein of the present invention means that the polypeptide is essentially free of other proteins normally associated with the Pem protein in its natural state and exhibiting constant and reproducible electrophoretic or chromatographic response, elution profiles, and antigen activity. The term "substantially pure" is not meant to exclude artificial or synthetic mixtures of the Pem protein with other compounds.

Methods for preparing fused, operably linked genes and expressing them in bacteria are known and are shown, for example, in U.S. Patent No. 4,366,246, herein incorporated by reference. The genetic constructs and methods described therein can be utilized for expression of Pem protein in prokaryotic or eukaryotic hosts.

Prokaryotic hosts may include Gram negative as well as Gram positive bacteria, such as E. coli, S. tymphimurium, Serratia marcescens, and Bacillus subtilis.

Eukaryotic hosts may include yeasts such as Pichia pastoris or mammalian cells.

In general, expression vectors (which may also be referred to as expression vehicles) containing promoter sequences which facilitate the efficient transcription of the inserted DNA fragment are used in connection with the host. The expression vector typically contains an origin of replication, promoter(s), terminator(s), as well as specific genes which are capable of providing phenotypic selection in transformed cells. The transformed hosts can be fermented and cultured according to means known in the art to achieve optimal cell growth.

Examples of promoters which can be used in the invention include, but are not limited to: rec A, trp, lac, tac, bacteriophage lambda pR or pL, MMTV, SV40. Examples of some of the plasmids or bacteriophage which can be used in the invention are listed in Maniatis, et al. Molecular Cloning, Cold Spring Harbor Laboratories, 1982; and others are known to those of skill in the art and can be easily ascertained.

The invention extends to any host modified according to the methods described, or modified by any other methods, commonly known to those of ordinary skill in the art, such as, for example, by transfer of genetic material using a lysogenic phage, and which yield a prokaryote or eukaryote expressing the gene for the Pem protein.

A gene is a DNA sequence which encodes through its template or messenger RNA a sequence of amino acids characteristic of a specific peptide. The term cDNA includes genes from which the intervening sequences have been removed. By the term rDNA is meant a molecule that has been recombined by splicing cDNA or genomic DNA sequences in vitro.

A cloning vehicle is a plasmid or phage DNA or other DNA sequence which is able to replicate in a host cell which is characterized by one or a small number of endonuclease recognition sites at which such DNA sequences may be cut in a determinable fashion without loss of an essential biological function of the DNA, and which contains a marker suitable for use in the identification of transformed cells. Markers, for example, are tetracycline resistance, neomycin resistance or ampicillin resistance. The word "vector" may be ~~is sometimes~~ used for cloning vehicle.

An expression vehicle is a vehicle similar to a cloning vehicle but which is capable of expressing a given structural gene in a host normally under control of certain control sequences.

Hosts transformed with the Pem genome for the Pem protein are particularly useful for the production of Pem polypeptide and protein.

The Pem protein may comprise the entire amino acid sequence of the Pem protein or may comprise only a specific determinant. An animal immunized with Pem recombinant protein will produce antibodies which will bind to epitopes present on the recombinant or naturally occurring polypeptides. Thus, the commercial production of Pem-containing recombinant proteins can be carried out.

The term "individual" is meant to include any animal, preferably a mammal, and most preferably a rodent, cat, dog, cow or human.

Detectable labels may be any molecule which may be detected. Commonly used detectable labels are radioactive labels including, but not limited to, ^{32}P, ^{14}C, ^{125}I, ^{3}H and ^{35}S. Biotin labeled nucleotides can be incorporated into DNA or RNA by nick translation, enzymatic, or chemical means. The biotinylated probes are detected after hybridization using avidin/streptavidin, fluorescent, enzymatic or colloidal gold conjugates. Nucleic acids may also be labeled with other fluorescent compounds, with immunodetectable fluorescent derivatives or with biotin analogues. Nucleic acids may also be labeled by means of attaching a protein. Nucleic acids cross-linked to radioactive or fluorescent histone H1, enzymes (alkaline phosphatase and peroxidases), or single-stranded binding (ssB) protein may also be used.

Two cell clones derived from the SL12 T-lymphoma cell line were chosen for the isolation of novel differentially expressed genes based on known differences in gene expression and on their different capacity to cause tumors in syngeneic host animals (Hays, et al. (1986) Int. J. Cancer 38:597-601; MacLeod, et al. (1984) Cancer Res. 44:1784-1790; MacLeod, et al. (1985) J. Nat. Cancer Inst. 74:875-882; MacLeod, et al. (1986) Proc. Natl. Acad. Sci.

USA 83:6989-6993; Siegal, et al. (1987) J. Exp. Med. 166:1702-1715; Weinroth, et al. (1985) Cancer Res. 45:4804-4809; Wilkinson, et al. (1988) EMBO J. 7:101-109 and Table 1 for a summary of phenotypes). The SL12.3 cell line expresses very few of the genes required for T-cell function, it is highly malignant in syngeneic animals and forms diffuse, aggressive tumors. In contrast, SL12.4 cells express mRNAs for all the components of the TCR/CD3 complex except TCR-alpha; and in several respects, the cells are similar to thymocytes at an intermediate stage in thymocyte development. SL12.4 cells are much less tumorigenic and induce prominent extranodal tumors.

To isolate cDNA clones representing genes expressed exclusively in SL12.4 cells and not in SL12.3 cells, a combination of subtraction hybridization-enriched probes and classical differential screening was used (similar to Filmus, et al. (1989) Mol. Cell. Biol. 8:4243-4249, as described in detail elsewhere (MacLeod, et al. (1990) Cell Growth Differ. 1:271-279).

By providing the DNA sequences and recombinant DNA molecules, the present invention also provides probes and methods to identify cells containing or lacking these sequences and means to administer these sequences to cells lacking these sequences.

Additionally, the present invention provides a means to inhibit the expression of the novel Pem sequence by providing to a cell containing the normal Pem DNA sequence an antisense RNA sequence or the DNA encoding said antisense RNA which can bind to and therefore block the synthesis of the RNA encoding the novel protein of the present invention. It will also be apparent to one of skill in the art from this disclosure that antibodies against any of the proteins of the present invention can be utilized to block the binding of ligands to the proteins and to target drugs or other agents (such as labels) to the cells expressing these proteins.

The present invention provides a novel cDNA sequence, a gene product (Pem) protein, and uses for this novel cDNA sequence and its associated Pem gene protein products. The cDNA sequence is 838 bp in length and contains a single long open reading frame that extends from bp 91 to 720 and predicts a protein containing 210 amino acids (Figure 1 and SEQ. ID. NO. 1). The numbers on the right of Figure 1 refer to the nucleotide sequence. The numbers on the left indicate the predicted amino acid position. The first methionine present in the sequence is underlined. The polyadenylation signal consensus sequence, AATAAA, is also underlined. The (X) at the 3' end of the DNA sequence refers to a string of 14 adenylate residues of the poly(A) tail. Potential phosphorylation sites for cAMP/cGMP-dependent kinase (AG), protein kinase C (C), and casein kinase II (CK) were identified using intelliGenetics programs which identify the appropriate consensus sequences surrounding Ser or Thr for each of the kinases. The consensus polyadenylation sequence, AATAAA, is located 50bp 5' of the poly(A) tract. The first methionine codon (91bp from the 5' end) is surrounded by the Kozak consensus sequence ($^G/_A$XXATGG) that provides an efficient translation start site (Kozak 1986). Pem sense transcripts prepared using T7 polymerase and translated in a reticulocyte lysate produce protein molecules of the predicted molecular weight (23 Kda, data not shown). Thus, the first methionine acts as a translation initiation site in in vitro transcription-translation experiments. The length of the Pem cDNA clone (838 bp, excluding the poly(A) tail) is similar to the size of Pem transcripts (0.9 kb) which have had their poly(A) tail removed (data not shown). Thus it is likely that the Pem cDNA clone is nearly full length.

The Pem protein is hydrophilic and contains no leader sequence, no N-linked glycosylation sites, nor any potential transmembrane spanning regions. Thus, Pem is not likely to be secreted or inserted into the cell membrane but has properties of an intracellular protein. The predicted Pem protein contains consensus sequences for phosphorylation of Ser and Thr by protein kinase C, casein kinase and cAMP/cGMP-dependent kinase as noted in Figure 1. Since DNA and predicted amino acid sequence searches of GenBank and Swiss Protein databases revealed no significant similarity to other known genes or gene products, Pem represents a new gene.

Having now generally described the invention, a more complete understanding can be obtained by reference to the following specific examples. These examples are provided for purposes of illustration only and are not intended to be limiting unless otherwise specified.

Example 1

Isolation, Characterization and Culture of Cells

A. Lymphoma Cell Lines. The isolation, characterization and culture requirements of the T-lymphoma cell lines SL12.1, SL12.3, SL12.4 and somatic cell hybrids formed among them have been described in detail in Hays, et al. (1986) Int. J. Cancer 38:597-601; MacLeod, et al. (1984) Cancer Res. 44:1784-1790; MacLeod, et al. (1985) J. Nat. Cancer Inst. 74:875-882; MacLeod, et al. (1986) Proc. Natl. Acad. Sci. USA 83:6989-6993; and Weinroth, et al. (1985) Cancer Res. 45:4804-4809; all of which are incorporated herein by reference.

The phenotypes of the SL12.3 and SL12.4 cell clones are summarized in Table 1. Transcript expression, surface protein expression, tumorigenicity and tumor type were determined by Northern analysis, flow cytometry and in vivo injection of cloned cells into syngeneic animals, respectively. TCR-ß 1.0 and 1.3 kb transcripts encode (D)-J-C and V-D-J-C sequences, respectively. The glucocorticoid response was determined by growth of the cells in 1 mM dexamethasone.

TABLE 1

Phenotypic Characteristics of SL12.4 and SL12.3 Cell Clones

		SL12.4	SL12.3
	Thy-1	++	+++
	TCR-alpha	-	+
	TCR-ß 1.0kb	+	-
mRNA	1.3kb	-	-
	TCR-gamma	-	-
	TCR-delta	-	-
	CD3-gamma	+	-
	CD3-delta	+	-
	CD3-epsilon	+	+/-
	CD3-zeta	+	+
	CD2	+	+
	CD4	-	-
	CD8	-	-
	Thy-1	++	++
	Pgp-1	-	+
Surface	ThB	+	-
Expression	TL	+	+
	T200	+	+
	$H-2K^k$	-	-
	IL2r	+	+
	J11d	+	+
	CD3-epsilon	-	-
	Mel-14	+	NT
Glucocorticoid Sensitivity		S	R
Tumorigenicity		Low	High
Tumor Type		Extra-Nodal (ovarian, muscle)	Diffuse

R = cells resistant to lysis, S = sensitive to lysis.

SAK8 cells (Gasson and Bourgeois J. Cell. Biol. 96:409-415 (1983) were obtained from Dr. Gasson. The lymphoma cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10 percent fetal calf serum, glutamine, penicillin and streptomycin. Two human ovarian carcinoma cell lines 2008 (Disaea, et al. Am. J. Obstet. Gynecol. 114:979-989 (1972)) and COLO 316 (Woods, et al. Cancer Res. 39:4449-4459 (1979) were cultured in RPMI medium 1640 supplemented with 5 percent bovine calf serum, glutamine and 1 percent Fungi-bact (Irvine Scientific, Santa Ana, California). When the cells were used to prepare RNA, they were harvested during exponential growth at a density near 5-8 x 10^5 cells per ml (Wilkinson and MacLeod EMBO J. 7:101-109 (1988). Splenocytes derived from BALB/c mice were seeded at 3 X 10^6 cells/ml and stimulated with 10 ug/ml ConA for two days before harvesting the RNA.

B. Co-cultivation of SL 12.4 cells and the thymic epithelial monolayers.

The co-cultivation conditions for SL 12.4 cells and the thymic epithelial monolayers. Briefly, SL 12.4 cells were seeded at a density such that their final concentration after the three day co-cultivation period was 1 x 10^6 cells/ml. TEL or TEPI were at confluency by the third day. The cells were grown in Dulbecco's Modified Eagle's Medium containing 10 percent fetal calf serum and supplemented with glutamine and penicillin/streptomycin at 37°C.

C. Cell lines for 20.2 Expression Studies.

Cell lines from the following sources were used in the 20.2 expression studies: Embryonal carcinomas F9 and PCC4 (Bernstine, et al. (1973) Proc. Natl. Acad. Sci. USA. 70:3899-3903, pituitary tumor ATt20 (Buonassisi, et al. (1962) Proc. Natl. Acad. Sci. USA. 48:1184-1192), thymic epithelial TEPI (Beardsley, et al. (1983) Proc. Natl. Acad. Sci. USA. 80:6005-6009), mammary epithelial MME (Evans (1988) Science 240:889-894)12.9), 3T3 (ATCC No. 92) and MEF were prepared according to Freshney (Freshney (1983) In Culture of Animal Cells. Alan R. Liss, Inc. pages 99-110), B-cell hybridoma PS.G8, B/T-lymphoma WEHI-21, macrophage P388D1, thymic epithelial TEL, basophil RBL-1, T-cell hybridoma 2H10^{v}, neuroblastoma N4TG1, myeloma S194/5, T-lymphomas: SL12.3, RS 4.2, SAK8, BW5147, AKR1, EL-4, somatic cell hybrid SL12.3 x SL12.4, and T-cell hybridoma BO-4H.H9.1. The cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10 percent fetal calf serum, glutamine, penicillin and streptomycin. Cells that were used to prepare RNA were harvested during exponential growth from cultures containing 5-8 x 10^5 cells per ml. Splenocytes obtained from BALB/c mice were seeded at 3 x 10^6 cells/ml in RPMI 1640 supplemented as above and stimulated with 10 ug/ml ConA for 6, 24, 48 or 72 hours before harvesting the RNA.

Example 2

Cloning and Screening Strategy

Poly(A)$^+$ mRNA from SL12.4 cells was used as a template to prepare double-stranded (ds) cDNA (Gubler and Hoffman (1983) Gene 25:263-269). EcoRI linkers were added to the ds DNA which was previously methylated. Dephosphorlyated lambda gt10 arms (Stratagene) were ligated to the cDNA and packaged into lambda phage using Stratagene packaging extract according to the manufacturer's instructions (Huynh, et al. in D. Glover (ed.) (1984) DNA Cloning Techniques: A Practical Approach. IRL Press, Oxford, U.K.).

Subtraction hybridization was performed essentially as originally described by Hedrick, et al. (1984) Nature 308:149-153 and Timberlake (1980) Dev. Biol. 78:497-503. Single stranded cDNA was prepared from 10 mg poly(A)$^+$ SL124 RNA using 250 mC of ^{32}P dCTP (Amersham) in the presence of 100 ug/ml of actinomycin D and hybridized to a Rot of 1260 (mol of nucleotide per liter x sec) with 25 mg poly(A)$^+$ RNA from SL12.3 cells in a volume of 8 ml at 68°C for 18 hours. After hybridization, the ss cDNA was collected by chromatography through a hydroxyapatite column. From 1 ug of starting SL12.4 cDNA, approximately 120 ng (12 percent of the input cDNA containing 3 x 10^7 cpm) was recovered and used to probe two 150 mm nitrocellulose filters containing 20,000 lambda gt10 plaques per filter. The first of two duplicate filter lifts from the SL12.4 lambda gt10 library was probed with total cDNA from SL12.3 mRNA, and the second filter lift was probed with the SL12.4 subtraction enriched cDNA prepared as described above. The strategy used was similar to that used by Filmus, et al. The plaque purified lambda phage clones were identified as SL12.4-specific by two screenings (using separately prepared subtracted probes). Subsequently Northern analysis was used to confirm that the clone hybridized only to mRNA from SL12.4 cells and not SL12.3 cells. The cDNA inserts were removed from lambda DNA by digestion with the restriction enzymes Hind III and Bgl II, isolated in low melting point agarose (Sea Kem) and subcloned into the plasmid vector pT7/T3 (Bethesda Research Laboratory) digested with Hind III and BamHI. The inserts could not be excised from the phage with EcoRI because the EcoRI sites were damaged in all of the isolates Kuziel, et al. (1987) Nucl. Acid Res. 15:3181.

Using the Pem cDNA, 15 additional clones were obtained from a lambda ZAPII SL12 cDNA library. These clones cover the full length of the mature mRNA transcript.

Example 3

Northern Blot Analysis

Total cellular RNA was isolated from cell lines and tissues by the guanidine isothiocyanate method (Maniatis, et al. (1983) In Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, modified as described (Wilkinson, et al. (1988) EMBO J. 7:101-109). Equal loading and transfer of RNA per lane was assessed by acridine orange staining (Maniatis, et al. (1983) In Molecular Cloning: A Laboratory

Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York) and by hybridization with actin, CHO-A and/or cyclophillin cDNA. For tissues, the method was modified such that the RNA pellet obtained after centrifugation in cesium chloride was resuspended in 10 mM Tris (pH 8), 0.5 percent SDS, 5 mM EDTA, followed by two extractions with phenol:chloroform:iso-amyl alcohol (24:24:1) and two extractions with chloroform:2-butanol:iso-amyl alcohol (20:5:1). For RNA blots, 10 mg of RNA was electrophoresed in 1 percent agarose-formaldehyde gels and transferred to Nytran membranes (Maniatis, et al. 1982). The Northern blots were hybridized with random oligomer primed ^{32}P-labeled cDNA inserts in the presence of 10 percent dextran sulphate and 50 percent formamide for 12-18 hours at 42°C (Meinkoth and Wahl 1984) To remove the labeled probe, RNA blots were washed with 0.1 x SSPE and 0.1 percent SDS at 90°C (Maniatis, et al. (1982) in "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), allowed to cool to room temperature, air-dried, and stored under vacuum until hybridized again. RNA sizes were determined by comparison with BRL RNA high and low molecular weight ladders.

Example 4

Southern Blot Analysis

Total cellular DNA was isolated from cells, T-lymphoma and murine-hamster somatic cell hybrids and tissues from other species was digested with the restriction enzyme Eco R.I. Twenty micro g of digested DNA was applied to each lane of a 0.7 percent agarose gel and electrophoresed and blotted onto Nytran supports essentially as described (Meinkoth and Wahl (1984) Anal. Biochem. 13:267-284), hybridized and washed as described for Northern blot analysis.

B. Southern blot analysis of 20.2.

Southern blot analysis of 20.2 was performed as above except as noted below.

Total cellular DNA was isolated from SL12.4 cells, murine and hamster liver and from somatic cell hybrids. DNA from chicken and human liver was obtained commercially from Clonetec, Palo Alto, California. The DNA was digested with the restriction enzymes noted in the examples according to the supplier's conditions. Ten ug of digested DNA was applied to each lane of a 0.8 percent agarose gel and electrophoresed in Tris acetate buffer for at least 48 hours and blotted onto Nytran supports, hybridized and washed as described for Northern blot analysis. The blots containing DNA from other species were washed at a lower stringency, the final wash was carried out at room temperature with 2 x SSPE.

Total cellular DNA was isolated as described (Maniatis, et al. 1982), digested with the restriction enzymes Eco R.I. Twenty micrograms of digested DNA was applied to each lane of a 0.7 percent agarose gel and electrophoresed in Tris acetate buffer, blotted onto a Nytran membrane essentially as described (Maniatis, et al. 1982), and washed as described for Northern blots.

Example 5

DNA Sequence Analysis

A restriction endonuclease map was determined from the Pem cDNA clone and fragments subcloned into pT7T3; the plasmid was purified over a cesium chloride gradient and directly sequenced by double-stranded dideoxy sequencing methods using Sequenase reagents (U.S. Biochemical Corp., Cleveland, Ohio). Part of the sequence was determined using primers to the host plasmid and other specific oligonucleotide primers (17mers) were prepared to the cDNA in the UCSD Cancer Center Core Molecular Biology Facility. Both DNA strands were sequenced in their entirety and all sequences were determined in at least two reactions performed in duplicate. Microgenia and IntelliGenetics computer programs were used to assemble the overlapping sequence information and perform the initial analysis of the DNA sequence. The properties of the predicted protein were assessed using the Prosite program from IntelliGenetics. The DNA and predicted protein sequence was used to search for similarities in the Swiss Protein and EMBL databases.

Example 6

cDNA and Predicted Protein Sequence of Pem.

In order to determine the cDNA and the protein sequence of the protein encoded by the Pem gene, novel cDNAs corresponding to genes involved in differentiation and/or neoplasia were sought. Toward that end, two closely related T-lymphoma cell clones SL12.4 and SL12.3 (MacLeod, et al. (1984) Cancer Res. 44:1784-1790) which differ in maturation level and tumorigenic properties were selected. On the basis of an analysis 10 cell surface antigens and 12 specific mRNAs, the cell clones represent distinct stages or lineages of T-cell development (MacLeod, et al. (1984) Cancer Res. 44:1784-1790; MacLeod, et al. (1985) J. Natl. Cancer Inst. 74:875-882; MacLeod, et al. (1986) Proc. Natl. Acad. Sci. USA 83:6989-6993; Hays, et al. (1986) Int. J. Cancer 38:597-601; MacLeod, et al. (1990) Cell Growth Differ. 1:271-279).

One cDNA clone, 20.2, identifies transcripts expressed in placenta and embryos and has been termed Pem.

The cDNA sequence is 838 bp in length and contains a single long open reading frame that extends from bp 91 to 720 and predicts a protein containing 210 amino acids (Figure 1). The numbers on the right of the figure refer to the nucleotide sequence. The numbers on the left indicate the predicted amino acid position. The first methionine present in the sequence is underlined. The polyadenylation signal consensus sequence, AATAAA, is also underlined. The (X) at the 3' end of the DNA sequence refers to a string of 14 adenylate residues of the poly(A) tail. Potential phosphorylation sites for cAMP/cGMP-dependent kinase (AG), protein kinase C (C), and casein kinase II (CK) were identified using IntelliGenetics programs which identify the appropriate consensus sequences surrounding Ser or Thr for each of the kinases. The consensus polyadenylation sequence, AATAAA, is located 50 bp 5' of the poly(A) tract. The first methionine codon (91 bp from the 5' end) is surrounded by the Kozak consensus sequence ($^{G}/_{A}$XXATGG) that provides an efficient translation start site (Kozak (1986) Cell 44:283-292). Pem sense transcripts prepared using T7 polymerase and translated in a reticulocyte lysate, produce protein molecules of the predicted molecular weight (23 Kda, data not shown). Thus, the first methionine acts as a translation initiation site in in vitro transcription-translation experiments. The length of the Pem cDNA clone (838 bp, excluding the poly(A) tail) is similar to the size of Pem transcripts (0.9 kb) which have had their poly(A) tail removed (data not shown). Thus it is likely that the Pem cDNA clone is nearly full length.

The Pem protein is hydrophilic and contains no leader sequence, no N-linked glycosylation sites, nor any potential transmembrane spanning regions. Thus, Pem is not likely to be secreted or inserted into the cell membrane, but has properties of an intracellular protein. The predicted Pem protein contains consensus sequences for phosphorylation of Ser and Thr by protein kinase C, casein kinase and cAMP/cGMP-dependent kinase as noted in Figure 1. Since DNA and predicted amino acid sequence searches of GenBank and Swiss Protein databases revealed no significant similarity to other known genes or gene products, Pem represents a new gene.

Example 7

Expression of Pem transcripts

In order to ascertain the expression of Pem transcripts in immortalized and transformed cell lines, northern analysis was used to identify the tissues and cell types which express Pem transcripts. Pem transcripts are abundant in a T-lymphoma cell line, but they are not detectable in lymphoid tissue from adult thymus, quiescent or activated splenocytes, gut associated lymphoid tissue or bone marrow, and are not detectable in adult brain, liver, large intestine, or ovary. Further, Pem transcripts were not found in pancreas, heart, lung, stomach, kidney or pituitary.

The microorganism PT7T3-20.2 relating to the present invention was deposited in the American Type Culture Collection on April 13, 1990 under the Budapest Treaty and was designated ATCC Accession No. 68304.

Since the Pem cDNA clone was isolated from a transformed cell line (SL12.4), Pem gene expression was assessed in other immortalized and transformed lines (Figure 2). To obtain the Pem mRNA expression in all lines, Northern blots were probed with ^{32}P labeled Pem inserts to assess the expression of Pem mRNA in the following murine cell lines: F9, PCC4 (embryonal carcinomas); ATt20 (pituitary); TEPI (thymic epithelial); MME (mammary epithelial); 3T3 (immortalized fibroblast); MEF (normal murine embryo fibroblasts); SL12.4 (T-lymphoma); PS.G8 (B cell hybridoma); WEHI-21 (B/T-lymphoma); P388D1 (macrophage); TEL (thymic epithelial); RBL-1 (basophil); 2H10^{V} (T-cell hybridoma); N4TG1 (neuroblastoma); S194/5 (myeloma); SL12.3, RS4.2, SAK8, BW5147, AKR1, EL-4 (T-lymphomas); SL12.3 x SL12.4 (somatic cell hybrid); and BO-4H.H.9.1 (T-cell hybridoma). Similar loading and transfer of RNA in all lanes was assessed by acridine orange staining of 18S and 28S rRNA, and by hybridization of the blot with a ^{32}P labeled cyclophillin (Cy) probe (Takahashi, et al. (1989) Nature 337:473-475) which recognizes transcripts present at similar levels in most murine cell lines.

Two embryonal carcinoma cell lines (F9 and PCC4), which have characteristics of pluripotent stem cells express the Pem gene but in strikingly different amounts. Immortalized 3T3 fibroblasts express Pem abundantly, whilst normal embryo fibroblast express at least 30-fold less Pem mRNA. Pem is expressed in cell lines of neuronal, pituitary, macrophage and mammary epithelial origin. In contrast, Pem transcripts are virtually undetectable in thymic epithelial cell lines and in most B- and T-cell tumor and hybridoma cell lines tested, although one B cell hybridoma (PS.G8) and two T-lymphoma cell lines (EL4 and SL12.4) express Pem transcripts (Fig. 2). Figure 2 shows that the 1.1-kb transcript is the most prominent transcript; however, some cells express an additional 3-kb mRNA. Both the 1.1 and 3-kb transcripts are enriched in poly(A)-selected RNA and are present in the cytoplasmic compartment in SL12.4 cells.

The lack of any obvious pattern of lineage specificity for Pem gene expression and the large variability in the amount of mRNA in the cell lines raises the possibility that the genes have been randomly lost, inactivated, translocated, or amplified either in vivo or after prolonged in vitro culture. Southern analysis has previously shown that Pem genes showed no detectable differences in band intensity or size between SL12.3 (Pem^-) and SL12.4 (Pem^+) cells, nor in another (Pem^-) T-lymphoma cell line, SAK8 (MacLeod, et al. (1990) Cell Growth Differ. 1:271-279). DNA has been subsequently tested from several of the cell lines expressing large amounts of Pem mRNA (SL12.4, ATt20, F9, MME, N4TG1, and EL4) and no differences in band intensity (copy number) or size were detected. Thus, it is unlikely that gene deletions, major genetic rearrangements, or gene amplification events are responsible for the differences in PEM mRNA accumulation in the cell lines.

Example 8

Pem gene expression in embryonic development.

The pattern of Pem gene expression is similar to oncofetal genes since Pem transcripts are present in transformed and immortalized cell lines, in embryonal carcinoma stem cells, but not in adult tissues (Ruddon (1987) Gene Derepression in Cancer Cells, In Cancer Biology, Oxford University Press, New York, pages 431-436). To further explore the possibility that Pem belongs to this class of genes, transcript levels were assessed in both embryos and in extraembryonic tissues at several stages of murine development. RNA was prepared from embryo, placenta (P), and yolk sac (Y) at the times of gestation indicated on Figure 3. Figure 3 shows that Pem transcripts are detectable as early as 6 days in embryonic development. Day 6 embryos include extra-embryonic tissue. Pem mRNA becomes abundant on day 7 or 8, but expression is sharply curtailed by day 9 and thereafter, although a faint signal is typically observed (Figure 3). In contrast, Pem transcripts are barely detectable in day 7 or 8 placenta and yolk sac, but increase to abundant levels on day 9, and all subsequent stages. Day 10 and 18 embryo, placenta, and yolk sac (not shown) possessed a similar pattern of Pem mRNA expression as days 12- 16. The blots were probed as described in Figure 2. The Pem gene expression patterns in embryonic and extraembryonic tissue lead to two significant conclusions: (1) Pem mRNA accumulates at high levels only briefly in embryos in toto (7 and 8 days) although it may persist in specific embryonic lineages at later stages; (2) The expression of Pem mRNA in embryos is reciprocal to that observed in extraembryonic tissue.

Example 9

Pem is an oncofetal gene.

The Pem gene is expressed in a stage-specific manner during embryogenesis, and it is expressed in a wide variety of immortalized and transformed cell lines. It is not detectably expressed in any adult tissues tested. Since these unusual expression characteristics are those expected of oncofetal genes (Ruddon (1987) Gene Derepression in Cancer Cells, in Cancer Biology, Oxford University Press, New York, pages 431-436), Pem may be a useful marker of immortalized cells and/or cells participating in early murine development. The observation that Pem transcripts decrease precipitously in day 9 embryos, the same point of gestation at which they become abundant in placenta and yolk sac, suggests the possibility that Pem^+ cells migrate from the embryonic to the extraembryonic compartment. Cells from the extraembryonic mesoderm (derived from the inner cell mass) give rise to placental and yolk sac cells, and thus have the characteristics expected of such a migrating cell. Although the Pem gene is specifically expressed during fetal development, it cannot be ruled out that it may also function in the adult. The Pem gene may be expressed by numerically infrequent or transient progenitor cells present in adult tissue. Since both adult and fetal progenitor cells are thought to be the targets of the transformation/immortalization process, (Pierce and Speers (1988) Cancer Res. 48:1996-2004), the observation that Pem mRNA is expressed by numerous immortalized and transformed cell lines is consistent with this concept. Deregulation resulting in overexpression or constitutive expression of Pem in immortalized cells might thereby contribute to their capacity for continuous cell proliferation.

In order to prevent or control the abnormal proliferation induced by the overexpression or constitutive expression of Pem, homologous recombinant gene therapy or targeted gene inactivation may be utilized to inhibit the function of the gene or the expression of a functional gene product. To this end, genomic clones of the Pem gene are utilized. The Pem gene is inactivated by inserting a selective marker within the gene in a position which inactivates the capacity of the gene to encode a functional marker or the capacity for the expression of a functional marker. The mutated gene is then transferred by electroporation or microinjection into an embryonic stem cell or an established cell line of same. Cells in which the selective marker is appropriately incorporated are selected and transferred into the blastocyst of an individual. Individuals may be selected which comprise either one or both alleles of the Pem gene so transformed.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The components, methods, procedures and techniques described herein are presently representative of the preferred embodiments, are intended to be exemplary, and are not intended as limitations on the scope of the present invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention and are defined by the scope of the appended claims.

(1) GENERAL INFORMATION:

(i) APPLICANT: Carol MacLeod

(ii) TITLE OF INVENTION: A Novel Onco-Fetal Gene, Gene Product and Uses Therefor

(iii) NUMBER OF SEQUENCES: 1

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy

(B) COMPUTER: IBM PC/AT

(C) OPERATING SYSTEM: MS-DOS

(D) SOFTWARE: WordPerfect

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(B) FILING DATE:

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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 838

(B) TYPE: nucleic acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA to mRNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mouse

(B) STRAIN: AKR1 Jackson

(C) INDIVIDUAL ISOLATE: SL12 cell line

(D) DEVELOPMENTAL STAGE: Bone marrow - adult

(E) HAPLOTYPE:

(F) TISSUE TYPE: Lymphoma

(G) CELL TYPE: T-cell

(H) CELL LINE: SL12.4 clone

(I) ORGANELLE:

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: ggt 10

(B) CLONE: 20.2

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Wilkinson, et al

(B) TITLE:

(C) JOURNAL: Developmental Biology

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(K) RELEVANT RESIDUES:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
GAA GAG CCA AAC AGC CAT CTC CCT GCA    27
Glu Glu Pro Asn Ser His Leu Pro Ala
                5

CAG TCC TTC AAG CTC ACC TCC TGC CTT    54
Gln Ser Phe Lys Leu Thr Ser Cys Leu
10                  15
```

CCG	TGG	ACA	AGA	GGA	AGC	ACA	AAG	AAT	81
Pro	Trp	Thr	Arg	Gly	Ser	Thr	Lys	Asn	
	20					25			
CAT	CCA	GGT	ATG	GAA	GCT	GAG	GGT	TCC	108
His	Pro	Gly	Met	Glu	Ala	Glu	Gly	Ser	
		30					35		
AGC	CGC	AAG	GTC	ACC	AGG	CTA	CTC	CGC	135
Ser	Arg	Lys	Val	Thr	Arg	Leu	Leu	Arg	
			40					45	
CTG	GGA	GTC	AAG	GAA	GAC	TCG	GAA	GAA	162
Leu	Gly	Val	Lys	Glu	Asp	Ser	Glu	Glu	
				50					
CAG	CAT	GAT	GTG	AAA	GCA	GAG	GCT	TTC	189
Gln	His	Asp	Val	Lys	Ala	Glu	Ala	Phe	
55					60				
TTC	CAG	GCT	GGA	GAG	GGG	AGA	GAT	GAG	216
Phe	Gln	Ala	Gly	Glu	Gly	Arg	Asp	Glu	
	65					70			
CAA	GGT	GCA	CAG	GGC	CAG	CCT	GGA	GTG	243
Gln	Gly	Ala	Gln	Gly	Gln	Pro	Gly	Val	
		75					80		
GGA	GCG	GTG	GGA	ACA	GAA	GGC	GAA	GGA	270
Gly	Ala	Val	Gly	Thr	Glu	Gly	Glu	Gly	
			85					90	
GAA	GAA	TTA	AAT	GGA	GGA	AAA	GGC	CAC	297
Glu	Glu	Leu	Asn	Gly	Gly	Lys	Gly	His	
				95					

TTT	GGT	CCT	GGT	GCT	CCT	GGT	CCT	ATG	324
Phe	Gly	Pro	Gly	Ala	Pro	Gly	Pro	Met	
100					105				
GGT	GAT	GGG	GAC	AAG	GAT	AGT	GGC	ACC	351
Gly	Asp	Gly	Asp	Lys	Asp	Ser	Gly	Thr	
	110					115			
AGG	GCT	GGT	GGT	GTG	GAG	CAG	GAA	CAA	378
Arg	Ala	Gly	Gly	Val	Glu	Gln	Glu	Gln	
		120					125		
AAT	GAG	CCA	GTT	GCT	GAG	GGC	ACT	GAG	405
Asn	Glu	Pro	Val	Ala	Glu	Gly	Thr	Glu	
			130					135	
AGC	CAG	GAG	AAT	GGA	AAT	CCT	GGG	GGT	432
Ser	Gln	Glu	Asn	Gly	Asn	Pro	Gly	Gly	
				140					
AGG	CAG	ATG	CCC	TCC	AGG	GCT	CTA	GGT	459
Arg	Gln	Met	Pro	Ser	Arg	Ala	Leu	Gly	
145					150				
TCG	CCC	AGC	TAT	CGA	CTG	AGG	GAA	CTG	486
Ser	Pro	Ser	Tyr	Arg	Leu	Arg	Glu	Leu	
	155					160			
GAG	TCC	ATT	TTG	CAG	CGC	ACT	AAT	TCC	513
Glu	Ser	Ile	Leu	Gln	Arg	Thr	Asn	Ser	
		165					170		
TTT	GAT	GTC	CCA	AGG	GAG	GAT	CTT	GAT	540
Phe	Asp	Val	Pro	Arg	Glu	Asp	Leu	Asp	
			175					180	

AGA	CTG	ATG	GAT	GCC	TGT	GTG	TCC	AGA	567
Arg	Leu	Met	Asp	Ala	Cys	Val	Ser	Arg	
				185					
GTG	CAG	AAT	TGG	TTT	AAG	ATC	AGG	AGG	594
Val	Gln	Asn	Trp	Phe	Lys	Ile	Arg	Arg	
190					195				
GCT	GCG	GCC	AGA	AGA	ACC	AGG	AGG	AGG	621
Ala	Ala	Ala	Arg	Arg	Thr	Arg	Arg	Arg	
	200					205			
GCA	ACA	CCA	GTC	CCT	GAA	CAT	TTT	AGA	648
Ala	Thr	Pro	Val	Pro	Glu	His	Phe	Arg	
		210					215		
GGA	ACA	TTC	GAG	TGT	CCT	GCT	TGT	CGT	675
Gly	Thr	Phe	Glu	Cys	Pro	Ala	Cys	Arg	
			220					225	
GGA	GTG	AGA	TGG	GGA	GAA	AGA	TGC	CCT	702
Gly	Val	Arg	Trp	Gly	Glu	Arg	Cys	Pro	
				230					
TTT	GCG	ACA	CCG	AGA	TTT				720
Phe	Ala	Thr	Pro	Arg	Phe				
235					240				

TGATTTGATC	ACATATGCCG	GCTATGACAG	CCCTTACTTT	760
TCAAGAATTC	AGCAATAAAG	AGGTGGATTC	CCAGTATGTT	800
TGTTCCATTA	CCTCTATGAT	TATTAAAATA	TTGATACA X	838

The claims defining the invention are as follows:

1. A recombinant polypeptide comprising the amino acid sequence of a Pem protein encoded by the Pem gene.

2. A polypeptide according to claim 1 in substantially pure form.

3. A polypeptide according to claim 1 or claim 2, wherein said Pem protein is encoded by DNA of a T-lymphoma cell.

4. A polypeptide according to claim 1, wherein said amino acid sequence is encoded by the nucleic acid sequence shown in Figures 1A and 1B.

5. A cDNA probe comprising the nucleotide sequence shown in Figures 1A and 1B.

6. A method for detecting neoplastic cells comprising incubation of a probe according to claim 5 with a target cell population under hybridizing conditions.

7. A method for treating neoplastic cells comprising insertion of an inactivated mutant Pem gene into neoplastic cells.

8. An expression vehicle which comprises a DNA sequence coding for the Pem protein.

9. An expression vehicle according to claim 8, wherein said expression vehicle is a plasmid capable of replication in a host which comprises, in operable linkage:

   a) an origin of replication;
   b) a promoter; and
   c) a DNA sequence coding for Pem protein.

10. An expression vehicle according to claim 9, wherein said expression vehicle is a phage or plasmid capable of replication in a prokaryotic host which comprises in operable linkage:

   a) a prokaryotic origin of replication;
   b) a prokaryotic promoter; and
   c) a DNA sequence coding for Pem protein.

11. An expression vehicle according to claim 9, wherein said expression vehicle is selected from the group consisting of pMAMneo, pNEO/Tfr-NC and

pMAM/neo/Tfr-NC.

12. A vector comprising a DNA sequence coding for Pem protein.

13. A vector according to claim 12 wherein said vector is isolated from the group consisting of a plasmid, a phage and a cosmid.

14. A vector according to claim 13, wherein said vector is pT7T3-20.2 having ATCC Accession number 68304.

15. A host transformed with a recombinant DNA molecule wherein said recombinant DNA molecule comprises a DNA sequence coding for the Pem protein.

16. A host according to claim 15 which is E. coli.

17. A method of producing Pem protein which comprises:

a) transforming a host with a DNA sequence coding for T-cell protein;

b) expressing said DNA sequence; and

c) recovering said Pem protein.

18. A pharmaceutical composition useful for inducing the production of antibodies to Pem protein in an individual comprising an immunogenically effective amount of Pem protein.

19. A pharmaceutical composition according to claim 18 wherein said Pem protein is produced by a method according to claim 17.

20. A recombinant polypeptide according to claim 1 substantially as hereinbefore described with reference to any one of the drawings and/or examples.

21. A cDNA probe according to claim 5 substantially as hereinbefore described with reference to any one of the drawings and/or examples.

22. A method according to any one of claims 6, 7 or 17 substantially as hereinbefore described with reference to any one of the drawings and/or examples.

23. An expression vehicle according to claim 8 substantially as hereinbefore described with reference to any one of the drawings and/or examples.

24. A vector according to claim 12 substantially as hereinbefore described with reference to any one of the drawings and/or examples.

25. A host according to claim 15 substantially as hereinbefore described with reference to any one of the drawings and/or examples.

26. A composition according to claim 18 substantially as hereinbefore described with reference to any one of the drawings and/or examples.

DATED this 7th day of September, 1995.

RESEARCH DEVELOPMENT FOUNDATION
By Their Patent Attorneys:
CALLINAN LAWRIE

89151/91

20.2 (PEM) cDNA and PREDICTED PROTEIN SEQUENCE

```
     GAA GAG CCA AAC AGC CAT CTC CCT GCA CAG TCC TTC AAG CTC   42
1    Glu Glu Pro Asn Ser His Leu Pro Ala Gln Ser Phe Lys Leu

     ACC TCC TGC CTT CCG TGG ACA AGA GGA AGC ACA AAG AAT CAT   84
15   Thr Ser Cys Leu Pro Trp Thr Arg Gly Ser Thr Lys Asn His
          |                             |
         (C)                           (C)
     CCA GGT ATG GAA GCT GAG GGT TCC AGC CGC AAG GTC ACC AGG  126
29   Pro Gly MET Glu Ala Glu Gly Ser Ser Arg Lys Val Thr Arg
                                  |   |               |
                                 (C   C)            (AG)
     CTA CTC CGC CTG GGA GTC AAG GAA GAC TCG GAA GAA CAG CAT  168
43   Leu Leu Arg Leu Gly Val Lys Glu Asp Ser Glu Glu Gln His

     GAT GTG AAA GCA GAG GCT TTC TTC CAG GCT GGA GAG GGG AGA  210
57   Asp Val Lys Ala Glu Ala Phe Phe Gln Ala Gly Glu Gly Arg

     GAT GAG CAA GGT GCA CAG GGC CAG CCT GGA GTG GGA GCG GTG  252
71   Asp Glu Gln Gly Ala Gln Gly Gln Pro Gly Val Gly Ala Val

     GGA ACA GAA GGC GAA GGA GAA GAA TTA AAT GGA GGA AAA GGC  294
85   Gly Thr Glu Gly Glu Gly Glu Glu Leu Asn Gly Gly Lys Gly
          |
         (CK)
     CAC TTT GGT CCT GGT GCT CCT GGT CCT ATG GGT GAT GGG GAC  336
99   His Phe Gly Pro Gly Ala Pro Gly Pro Met Gly Asp Gly Asp

     AAG GAT AGT GGC ACC AGG GCT GGT GGT GTG GAG CAG GAA CAA  378
113  Lys Asp Ser Gly Thr Arg Ala Gly Gly Val Glu Gln Glu Gln

     AAT GAG CCA GTT GCT GAG GGC ACT GAG AGC CAG GAG AAT GGA  420
127  Asn Glu Pro Val Ala Glu Gly Thr Glu Ser Gln Glu Asn Gly

     AAT CCT GGG GGT AGG CAG ATG CCC TCC AGG GCT CTA GGT TCG  462
141  Asn Pro Gly Gly Arg Gln Met Pro Ser Arg Ala Leu Gly Ser
```

FIG. 1A

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    CCC AGC TAT CGA CTG AGG GAA CTG GAG TCC ATT TTG CAG CGC  504
155 Pro Ser Tyr Arg Leu Arg Glu Leu Glu Ser Ile Leu Gln Arg
         |
        (C)
    ACT AAT TCC TTT GAT GTC CCA AGG GAG GAT CTT GAT AGA CTG  546
169 Thr Asn Ser Phe Asp Val Pro Arg Glu Asp Leu Asp Arg Leu

    ATG GAT GCC TGT GTG TCC AGA GTG CAG AAT TGG TTT AAG ATC  588
183 Met Asp Ala Cys Val Ser Arg Val Gln Asn Trp Phe Lys Ile
                                                     |
                                                    (C)
    AGG AGG GCT GCG GCC AGA AGA ACC AGG AGG AGG GCA ACA CCA  630
197 Arg Arg Ala Ala Ala Arg Arg Thr Arg Arg Arg Ala Thr Pro
                                 |                   |
                                (C)                 (AG)
    GTC CCT GAA CAT TTT AGA GGA ACA TTC GAG TGT CCT GCT TGT  672
211 Val Pro Glu His Phe Arg Gly Thr Phe Glu Cys Pro Ala Cys

    CGT GGA GTG AGA TGG GGA GAA AGA TGC CCT TTT GCG ACA CCG  714
225 Arg Gly Val Arg Trp Gly Glu Arg Cys Pro Phe Ala Thr Pro
                                                     |
                                                    (C)
    AGA TTT TGA TTT GAT CAC ATA TGC CGG CTA TGA CAG CCC TTA  756
239 Arg Phe

    CTT TTC AAG AAT TCA GCA ATA AAG AGG TGG ATT CCC AGT ATG  798
    TTT GTT CCA TTA CCT CTA TGA TTA TTA AAA TAT TGA TAC A(x) 840
```

C = Predicted glycoslyation site
AG & CK = Predicted phosphorlyation site
AATAAA = Predicted polyadenylation site

Number on left refers to amino acid sequence, on right to DNA sequence

FIG. 1B

FIG.2

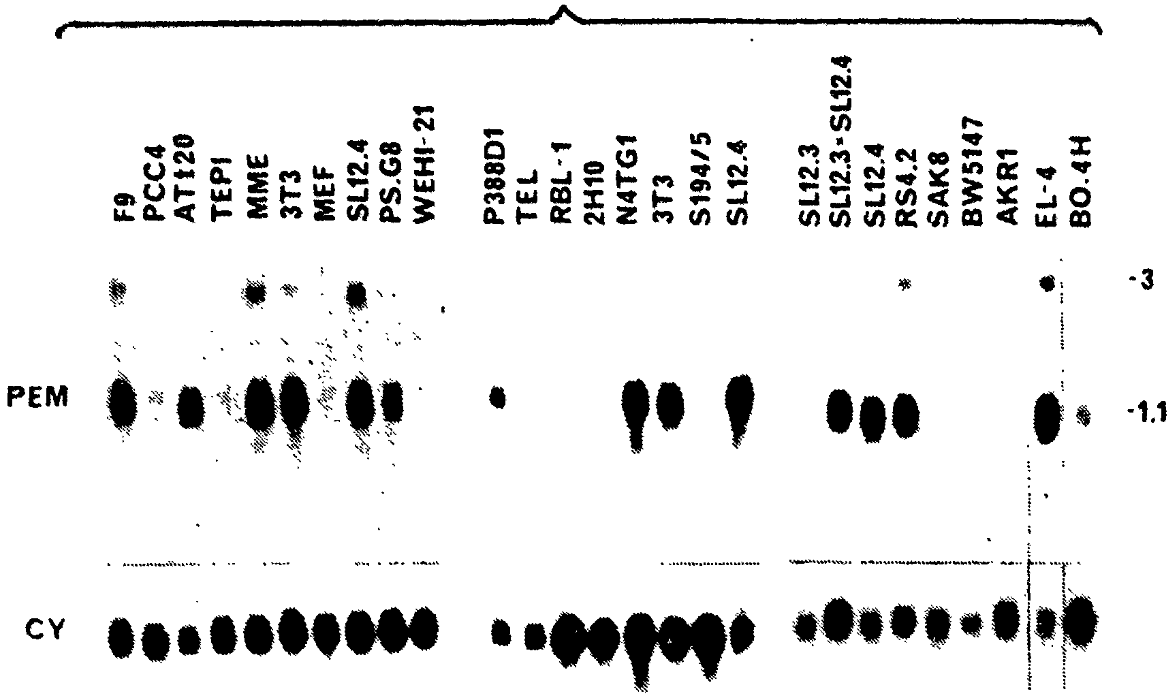
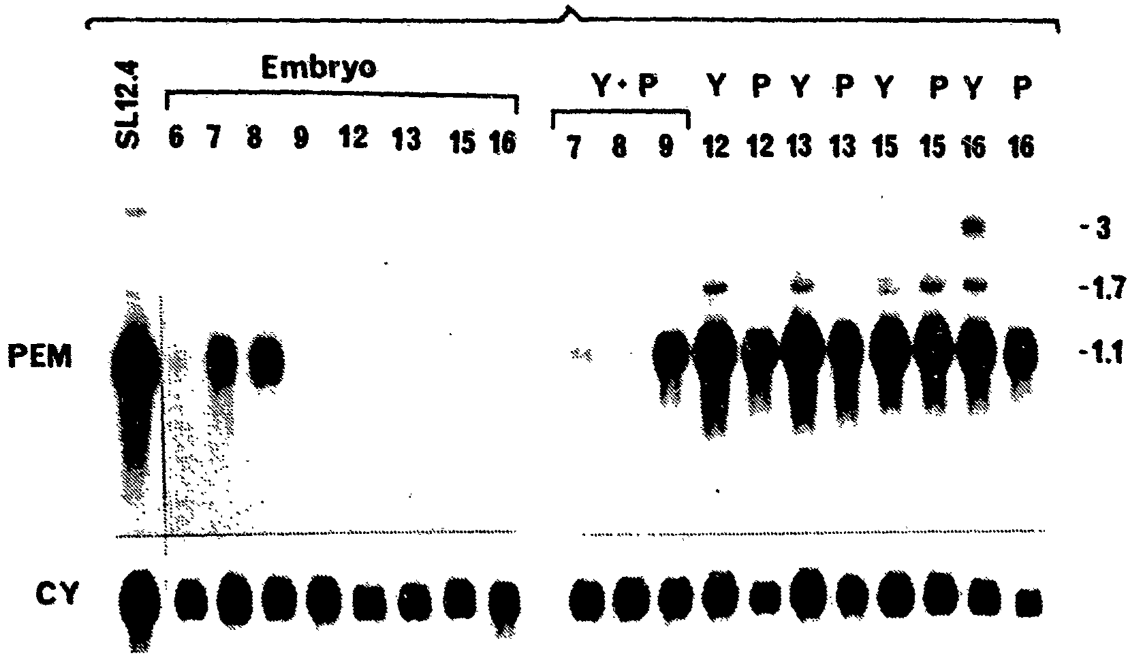

F9
PCC4
AT1.20
TEP1
MME
3T3
MEF
SL12.4
PS.G8
WEHI-21
P388D1
TEL
RBL-1
2H10
N4TG1
3T3
S194/5
SL12.4
SL12.3
SL12.3•SL12.4
SL12.4
RS4.2
SAK8
BW5147
AKR1
EL-4
BO.4H
PEM
CY
-3
-1.1

FIG.3


SL12.4
Embryo
6 7 8 9 12 13 15 16
Y•P
7 8 9
Y P Y P Y P Y P
12 12 13 13 15 15 16 16
PEM
CY
-3
-1.7
-1.1

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/07237

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

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

IPC(5): C12Q 1/68; C07 H 15/12
USCl: 536/27; 424/520; 514/44; 435/6

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
US CL	536/27; 435/6; 424/520; 514/44; 935/6, 78

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Nature. Vol. 308. issued 08 March 1984. Hedrick et al." Isolation of cDNA clones encoding T cell-specific proteins". pages 149-153. see entire document.	1-21
Y	Mc Michael et al "Leukocyte Typing III" published 1987 by Oxford University Press. pages 31-62. see entire document.	1-21
Y	International Journal of Cancer. Vol. 38. issued 1986. Hayes et al. "Tumorigenicity of T-lymphoma/T-lymphoma hybrids and T-Lymphoma/Normal Cell Hybrids". pages 597-601. see entire document.	1-21

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

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"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	Date of Mailing of this International Search Report
30 December 1991	28 JAN 1992
International Searching Authority	**Signature of Authorized Officer**
ISA/US	T. Michael Nisbet

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