



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/12, 15/11, C07K 14/475, 16/22, C12Q 1/68, G01N 33/53	A2	(11) International Publication Number: WO 00/06731 (43) International Publication Date: 10 February 2000 (10.02.00)
(21) International Application Number: PCT/US99/17277 (22) International Filing Date: 30 July 1999 (30.07.99) (30) Priority Data: 09/126,799 31 July 1998 (31.07.98) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 09/126,799 (CON) Filed on 31 July 1998 (31.07.98) (71) Applicants (for all designated States except US): WASHINGTON UNIVERSITY [US/US]; One Brookings Drive, St. Louis, MO 63130 (US). GENENTECH, INC. [US/US]; 1 DNA Way, South San Francisco, CA 94080 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): JOHNSON, Eugene, M., Jr. [US/US]; 13233 Amiot Drive, St. Louis, MO 63146 (US). MILBRANDT, Jeffrey, D. [US/US]; 75 Aberdeen Place, St. Louis, MO 63105 (US). KOTZBAUER, Paul, T. [US/US]; 100 Guernsey, Swarthmore, PA 19081 (US). LAMPE, Patricia, A. [US/US]; 10323 Grant Forest Lane, St. Louis, MO 63123 (US). KLEIN, Robert [US/US]; 1044		Webster Street, Palo Alto, CA 94301 (US). DE SAUVAGE, Fred [BE/US]; 166 Beach Park Boulevard, Foster City, CA 94404 (US). (74) Agents: HOLLAND, Donald, R. et al.; Howell & Haferkamp, L.C., 7733 Forsyth, Suite 1400, St. Louis, MO 63105 (US). (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: PERSEPHIN ARF, A PROTEIN ENCODED BY UNSPLICED PERSEPHIN mRNA		
(57) Abstract A novel protein, persephin ^{ARF} is disclosed and the human, mouse and rat amino acid sequences thereof are identified. Nucleotide sequences encoding these persephin ^{ARF} amino acid sequences are also disclosed.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

PERSEPHIN ARF, A PROTEIN ENCODED BY UNSPLICED PERSEPHIN mRNAReference to Government Grant

This invention was made with government support under Grant Numbers AG13729 and AG13730. The government has certain rights in this invention.

5 Background of the Invention

(1) Field of the Invention

This invention relates generally to novel expression products of the persephin gene and, more particularly to a novel protein encoded by an alternative reading frame in unspliced persephin mRNA.

10 (2) Description of the Related Art

Recently, a family of neurotrophic factors that are related to the TGF β superfamily of growth factors but structurally distinct from the neurotrophins has been identified. This family was identified on the basis of amino acid sequences that are conserved between glial cell line-derived neurotrophic factor (GDNF) and neurturin (NTN) which are not present in
15 other members of the TGF β superfamily. Kotzbauer et al., *Nature* 384:467-470, 1996. A PCR based strategy using degenerate primers corresponding to these conserved regions in what is now termed the GDNF family was used to discover an additional family member, persephin (PSP), as described in copending U.S. Serial No. 08/931,858 filed September 16, 1997; Milbrandt et al., *Neuron* 20:245-253, 1998.

GDNF, neurturin and persephin have similarities and differences in their biological activity profiles. While each family member exhibits neurotrophic activity on mesencephalic, dopaminergic and motor neurons and act as a kidney ramogen, persephin, unlike neurturin and GDNF, does not appear to act on peripheral sympathetic, sensory or enteric neurons.

5 Milbrandt et al., *supra*.

Sequence analysis of genomic and cDNA clones for GDNF, neurturin and persephin revealed that each family member is likely expressed within a cell as a pre-pro-protein, with the pre-region containing an amino terminal signal sequence followed by a pro domain which contains an RXXR proteolytic processing site immediately before the N-terminal amino acid
10 of the predicted mature protein. The genes encoding GDNF, neurturin and persephin are also similar in that each contains an intron that interrupts the coding region of the pro-domain. While the intron for neurturin was reported to be about 500 nt in length, the intron in the persephin gene was reported to be much shorter: 88 nt in mouse and rat genes and 85 nt in the human persephin gene. See U.S. Patent 5,739,307 and 08/931,858. While the latter
15 reference disclosed the sequence of the intron in the persephin rat and mouse genes and identified the location of the predicted splice site in human persephin protein, the intron sequence for the human persephin gene has not been disclosed.

Based on the observation that unspliced and spliced persephin mRNA have been reported to be present in equally low levels in rat heart, kidney, liver and brain, it has been
20 suggested that the regulation of persephin mRNA processing might be involved in regulating the production of persephin protein in tissues. Milbrandt et al., *supra*. This would be consistent with the reported use of alternative splicing of the GDNF intron to produce two GDNF mRNAs of differing lengths. The longer form, GDNF α , corresponds to the cDNA originally isolated by Lin et al., (GenBank citation) and the shorter form, GDNF β , contains a
25 78 bp deletion in the prepro region of the coding sequence probably resulting from use of an alternative donor splice site during GDNF mRNA processing. Trupp et al., *J. Cell Biol.* 130:137-148, 1995; Springer et al., *Exper. Neurol.* 131:47-52, 1995. However, this deletion does not include the secretion signal sequence or the consensus proteolytic cleavage sequence and does not alter the reading frame of the coding sequence downstream of the splice site.
30 Moreover, the deletion apparently does not interfere with the processing of the polypeptide encoded by GDNF β as identical mature GDNF proteins are believed to be produced from the GDNF α and GDNF β mRNA isoforms. Trupp et al., *supra*.

Alternative splicing resulting in mRNAs encoding entirely distinct proteins has been reported for other mammalian genes. For example, the human INK4a tumor suppressor gene
35 yields α and β transcripts of similar length which differ by the presence of alternative first

exons joined through the identical splice acceptor site to sequences in exon 2. Mao et al., *Cancer Res.* 55: 2995-2997, 1995, Stone et al., *Cancer Res.* 55:2988-2994, 1995. It has been determined that translation of the β transcript begins at a 5' AUG differing from that used in the α transcript and consequently the exon 2 nucleotide sequence is translated in an alternative reading frame from that used during translation of the α transcript. Quelle et al., *Cell* 83:993-1000, 1995. Although the polypeptides encoded by the α and β transcripts, named p16^{INK4a} and p19^{ARF}, respectively, have entirely different amino acid sequences, they have both been shown to be naturally expressed and surprisingly both are reported to induce growth arrest in mammalian fibroblasts. Quelle et al., *supra*.

Alternative splicing has also been shown to generate two transcripts of the murine periaxin gene in approximately equal amounts via a retained intron mechanism. Dytrych et al., *J. Biol. Chem.* 273:5794-5800, 1998. Because a stop codon is present in the retained intron, the larger mRNA containing the intron produces a truncated protein, S-periaxin, with an intron-encoded C terminus of 21 amino acids that is not present in the larger protein, L-periaxin, encoded by the shorter mRNA. Dytrych et al. reported that although both L-periaxin and S-periaxin are expressed in Schwann cells, they are targeted to different subcellular localizations, and suggested the two periaxin proteins may have distinct functions.

All references cited in this specification are hereby incorporated in their entirety by reference. The discussion of the references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinency of the cited references.

Summary of the Invention

Briefly, therefore, the present invention is directed to the identification of an alternative reading frame in the persephin genomic sequence that encodes a novel polypeptide. This polypeptide, referred to herein as persephin^{ARF}, is predicted to be the translation product of the unspliced transcript of the persephin gene. Because the unspliced transcript is present in various tissues in amounts comparable to or larger than quantities of the spliced transcript and because the unspliced transcript encodes a polypeptide with a secretion signal sequence, it is believed that persephin^{ARF} is synthesized *in vivo* and has biological activity by virtue of its secreted nature.

The invention thus provides isolated human, mouse, and rat persephin^{ARF} polypeptides which comprise the amino acid sequences set forth in SEQ ID NO:1, 2 and 3 respectively. In addition, the invention includes mature persephin^{ARF} polypeptides lacking the

secretion signal sequence and comprising SEQ ID NO:4 (human), SEQ ID NO:5 (mouse) or SEQ ID NO:6 (rat).

In another embodiment the present invention provides isolated polynucleotides encoding the human, mouse, and rat persephin^{ARF} polypeptides of SEQ ID NOS:1-6.

5 Preferred polynucleotides comprise human nucleotide sequences as set forth in SEQ ID NO:7 and 8; mouse nucleotide sequences as set forth in SEQ ID NOS:9 and 10; and rat nucleotide sequences as set forth in SEQ ID NO:11 and 12.

Expression vectors and stably transformed cells comprising polynucleotides encoding persephin^{ARF} are also within the scope of this invention. The transformed cells can be used in
10 a method for producing persephin^{ARF}.

In another embodiment, the invention provides isolated polynucleotides comprising nucleotide sequences complementary to SEQ ID NOS:7-12 as set forth in SEQ ID NOS:13-18 as well as isolated polynucleotides that specifically hybridize to the intronic portion of polynucleotides comprising any one of SEQ ID NOS:7, 8, 13 and 14. Such hybridizing
15 polynucleotides include but are not limited to SEQ ID NO:19 and its complement as well as fragments thereof. These complementary and hybridizing polynucleotides can be used in methods for detecting the persephin gene and unspliced transcription products thereof.

The present invention also provides antibodies which specifically react with persephin^{ARF} and methods for purifying persephin^{ARF} or detecting its expression using such
20 antibodies.

In still further embodiments, the present invention includes a composition comprising persephin^{ARF} and a pharmaceutically acceptable carrier as well as a method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of persephin^{ARF}.

25 Among the several advantages found to be achieved by the present invention, therefore, may be noted the provision of a novel protein, persephin^{ARF}, the provision of polynucleotides and methods for obtaining persephin^{ARF} by recombinant techniques; the provision of polynucleotides and antibodies useful in methods for detecting the persephin gene and expression products thereof; and the provision of methods for preventing or treating
30 medical conditions.

Brief Description of the Drawings

Figure 1 illustrates the alignment of the nucleotide sequences of the mouse, rat and human persephin genes (SEQ ID NOS:26, 27 and 7, respectively) with the arrows denoting
35 the beginning and ending of the intron that is not spliced out in the transcript encoding persephin^{ARF} and the stop codon for each species indicated by an asterisk (*);

Figure 2 illustrates the alignment of the amino acid sequences of human, mouse and rat persephin^{ARF} with the first amino acid of the predicted mature protein indicated by the asterisk (*) at amino acid position 24;

Figure 3 illustrates relative levels of unspliced and spliced persephin mRNA in various rat embryonic (E18) and adult tissues determined by semiquantitative RT/PCR using 32, 29 or 26 cycles, with unspliced mRNA indicated by the open arrowhead and spliced mRNA indicated by the closed arrowhead; and

Figure 4 illustrates an agarose gel showing products resulting from PCR analysis of human genomic DNA (lane 1) and reverse transcribed human fetal brain poly A⁺ RNA (lane 3, no RT control, and lane 4) using PCR primers corresponding to the start and stop sites of the coding sequence for human persephin, with size markers present in lane 2 and unspliced and spliced persephin mRNA species indicated by the filled and open arrowheads, respectively.

15 Description of the Preferred Embodiments

The present invention is based upon the identification of an alternative reading frame in unspliced persephin mRNA that encodes a novel protein, persephin^{ARF}. As used herein, "an alternative reading frame" in connection with a persephin gene or an unspliced transcript thereof is a reading frame which begins at the same initiation codon used in the synthesis of persephin and continues through the intron sequence. As shown in Figure 1, the alternative reading frame in the mouse and rat persephin genes stops at the end of the intron while in the human persephin gene the alternative reading frame continues for another 261 bases. Thus, the alternative reading frame in the human persephin gene encodes a 164 amino acid polypeptide that is twice as long as the 81 amino acid polypeptides encoded by the corresponding alternative reading frames in the mouse and rat persephin genes.

As shown in Fig. 2, alignment of the predicted amino sequences of the human, mouse and rat persephin^{ARF} polypeptides indicates that these three polypeptides share some homology in the region encoded by the intron. However, there is no homology between the persephin^{ARF} polypeptide and mature persephin. In addition, a search of the GenBank[®] database revealed no homologies between human persephin^{ARF} and other proteins.

As described more fully below, unspliced human, mouse and rat persephin transcripts are present in amounts comparable to or greater than the corresponding spliced transcripts. Thus, the inventors herein believe that the unspliced transcript is translated *in vivo* to produce persephin^{ARF} polypeptides whose predicted amino acid sequences are set forth in SEQ ID NOS:1-3. These persephin^{ARF} polypeptides are believed to be precursor forms in that they have a 23 amino acid long signal sequence identical to that present in precursor forms of

persephin. The presence of the signal sequence indicates that persephin^{ARF} is a secreted protein. The precursor form of persephin^{ARF} does not appear to contain an RXXR cleavage site; thus, cleavage of the predicted signal sequence is predicted to produce a 141 amino acid long mature human persephin^{ARF} consisting of SEQ ID NO:4 and 58 amino acid long mouse and rat persephin^{ARF} polypeptides consisting of SEQ ID NO:5 and SEQ ID NO:6, respectively.

As used herein, a "secreted protein" is one which, when expressed in a suitable host cell, is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence. Secreted proteins include without limitation proteins or polypeptides secreted wholly (e.g. soluble proteins) or partially (e.g. receptors) from the cell in which they are expressed. Secreted proteins also include without limitation proteins or polypeptides which are transported across the membrane of the endoplasmic reticulum.

Accordingly, one embodiment of the invention provides an isolated and purified polypeptide comprising a persephin^{ARF} amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6. As used herein, "isolated and purified" means that the polypeptide constitutes at least about 50 percent of a composition on a molar basis compared to total proteins or other macromolecular species present in the composition. Preferably, the polypeptide of the invention will constitute at least about 75 to about 80 mole percent of the total protein or other macromolecular species present. More preferably, an isolated and purified polypeptide will constitute about 85 to about 90 mole percent of a composition and still more preferably, at least about 95 mole percent or greater.

The invention also includes polypeptides comprising any naturally occurring allelic variants of the disclosed persephin^{ARF} amino acid sequences; that is, polypeptides encoded by the alternative reading frame in different alleles of the human, mouse or rat persephin genes.

Homologs of the disclosed mammalian persephin^{ARF} polypeptides are also embraced by the invention. As used herein, a persephin^{ARF} homolog is a polypeptide encoded by the alternative reading frame of a persephin gene of any other mammalian or nonmammalian species. A persephin gene in another species can be identified by making suitable probes or primers from the genomic sequences shown in Figure 1, screening a suitable library from the desired species and aligning the sequence of an identified clone against one or more of the genomic persephin sequences of Figure 1.

The scope of the present invention also includes conservatively substituted variants of the persephin^{ARF} amino acid sequences disclosed herein. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. Conservatively substituted amino acids can be grouped according to the chemical properties

of their side chains. For example, one grouping of amino acids includes those amino acids that have neutral and hydrophobic side chains (A, V, L, I, P, W, F, and M); another grouping is those amino acids having neutral and polar side chains (G, S, T, Y, C, N, and Q); another grouping is those amino acids having basic side chains (K, R, and H); another grouping is those amino acids having acidic side chains (D and E); another grouping is those amino acids having aliphatic side chains (G, A, V, L, and I); another grouping is those amino acids having aliphatic-hydroxyl side chains (S and T); another grouping is those amino acids having amine-containing side chains (N, Q, K, R, and H); another grouping is those amino acids having aromatic side chains (F, Y, and W); and another grouping is those amino acids having sulfur-containing side chains (C and M). Preferred conservative amino acid substitutions groups are: R-K; E-D, Y-F, L-M; V-I, and Q-H.

A persephin^{ARF} amino acid sequence as used herein can also include modified sequences in which one or more amino acids have been inserted, deleted, or replaced with a different amino acid or a modified amino acid or unusual amino acid, as well as modifications such as glycosylation or phosphorylation so long as the polypeptide containing the modified sequence retains the biological activity of persephin^{ARF}. By retaining the biological activity, it is meant that the modified polypeptide has a biological activity profile that is qualitatively similar to, but not necessarily quantitatively equivalent to, that of human, mouse or rat persephin^{ARF} identified herein.

Fragments of precursor or mature persephin^{ARF} are also encompassed by the present invention. Such fragments may be of any length but should retain the biological activity of precursor or mature persephin^{ARF} or should be antigenic. The minimum length of such biologically active or antigenic fragments can readily be determined by those skilled in the art using known techniques.

Antigenic fragments are capable of eliciting persephin^{ARF}-specific antibodies when administered to a host animal and includes those smaller fragments that require conjugation to a carrier molecule to be immunogenic. Typically, antigenic fragments will be at least 5 or 6 amino acids in length and may be any length up to the length of precursor persephin^{ARF}, preferably 10 to 12 amino acids in length and more preferably, an antigenic fragment will comprise at least 15 to 20 amino acids, or larger.

Because it is believed that unspliced persephin mRNA is translated, it is likely that a precursor or mature persephin^{ARF} polypeptide can be isolated from tissues or cells which naturally express persephin. Alternatively, polypeptides of the present invention can be made by recombinant DNA technology by expressing a nucleotide sequence encoding the desired persephin^{ARF} amino acid sequence in a suitable transformed host cell. Using methods well known in the art, a polynucleotide encoding persephin^{ARF} may be operably linked to an

expression vector, transformed into a host cell and culture conditions established that are suitable for expression of the polypeptide by the transformed cell.

Any suitable expression vector may be employed to produce recombinant persephin^{ARF} such as, for example, the mammalian expression vector pCB6 (Brewer, *Meth Cell Biol* 43:233-245, 1994) or the *E. coli* pET expression vectors, specifically, pET-30a (Studier et al., *Methods Enzymol* 185:60-89, 1990). Other suitable expression vectors for expression in mammalian and bacterial cells are known in the art as are expression vectors for use in yeast or insect cells. Baculovirus expression systems can also be employed.

A number of cell types may be suitable as host cells for expression of recombinant precursor or mature persephin^{ARF}. Mammalian host cells include, but are not limited to, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo 205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK and Jurkat cells. Yeast strains that may act as suitable host cells include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, and any other yeast strain capable of expressing heterologous proteins. Host bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium* and any other yeast strain capable of expressing heterologous proteins. If the polypeptide is made in yeast or bacteria, it may be necessary to modify the polypeptide, for example, by phosphorylation or glycosylation of the appropriate sites using known chemical or enzymatic methods, to obtain a biologically active polypeptide.

The polypeptide of the invention may also be expressed in transgenic animals, e.g., cows, goats, pigs, or sheep whose somatic or germ cells contain a nucleotide sequence encoding persephin^{ARF}.

The expressed persephin^{ARF} polypeptide can be purified using known purification procedures, such as gel filtration and ion exchange chromatography. Purification may also include affinity chromatography using an agent that will specifically bind the persephin^{ARF} polypeptide, such as a polyclonal or monoclonal antibody raised against persephin^{ARF} or fragment thereof. Other affinity resins typically used in protein purification may also be used such as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®. Purification of persephin^{ARF} can also include one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether.

It is also contemplated that a persephin^{ARF} polypeptide may be expressed as a fusion protein to facilitate purification. Such fusion proteins, for example, include a persephin^{ARF} amino acid sequence fused to a histidine tag such as when expressed in the pET bacterial expression system as well as the persephin^{ARF} amino acid sequence fused to the amino acid

sequence of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Similarly, the polypeptide of the invention can be tagged with a heterologous epitope and subsequently purified by immunoaffinity chromatography using an antibody that specifically binds such epitope. Kits for expression and purification of such fusion proteins
5 and tagged proteins are commercially available.

Precursor or mature persephin^{ARF} and fragments thereof may also be produced by chemical synthesis using methods known to those skilled in the art.

In another embodiment, the present invention provides an isolated and purified polynucleotide comprising a nucleotide sequence that encodes a persephin^{ARF} amino acid
10 sequence. As used herein, a polynucleotide includes DNA and/or RNA and thus the nucleotide sequences recited in the Sequence Listing as DNA sequences also include the identical RNA sequences with uracil substituted for thymine residues. Nucleotide sequences included in the invention are those encoding the human, mouse and rat persephin^{ARF} amino acid sequences set forth in SEQ ID NOS:1-6. It is understood by the skilled artisan that
15 degenerate nucleotide sequences can encode the persephin^{ARF} amino acid sequences described herein and these are also intended to be included within the present invention. Such nucleotide sequences include modifications of naturally-occurring sequences in which at least one codon is substituted with a corresponding redundant codon preferred by a given host cell, such as E. coli or insect cells, so as to improve expression of recombinant persephin^{ARF}
20 therein. A preferred polynucleotide of the invention encodes precursor human persephin^{ARF} and comprises SEQ ID NO:7. Another preferred polynucleotide encodes mature human persephin^{ARF} and comprises SEQ ID NO:8.

The present invention also encompasses vectors comprising an expression regulatory element operably linked to any of the persephin^{ARF}-encoding nucleotide sequences included
25 within the scope of the invention. This invention also includes host cells, of any variety, that have been transformed with such vectors.

In another embodiment, the present invention also provides a polynucleotide comprising a first nucleotide sequence which is complementary to a second nucleotide sequence encoding a persephin^{ARF} amino acid sequence. As used herein, complementary
30 nucleotide sequences have the same length. Complementary nucleotide sequences include SEQ ID NOS:13-18. Preferably the first nucleotide sequence is complementary to a human nucleotide sequence encoding precursor or mature persephin^{ARF} and comprises SEQ ID NO:13 or SEQ ID NO:14.

In yet another embodiment, a polynucleotide which specifically hybridizes to the
35 intronic portion of a human persephin^{ARF}-encoding polynucleotide or its complement is provided. Specific hybridization is defined herein as the formation of hybrids between a

polynucleotide, including oligonucleotides, and a specific reference polynucleotide (e.g., a polynucleotide comprising a nucleotide sequence complementary to a nucleotide sequence encoding persephin^{ARF}) wherein the polynucleotide preferentially hybridizes to the specific reference polynucleotide over other polynucleotides. Specific hybridization is preferably
5 done under high stringency conditions which, as well understood by those skilled in the art, can readily be determined by adjusting several factors during hybridization and during the washing procedure, including temperature, ionic strength, length of hybridization or washing times, and concentration of formamide (see for example, Sambrook et al., 1989, *supra*).

The present invention also provides methods for detecting expression of
10 persephin^{ARF} mRNA in a sample comprising detecting an unspliced transcript of the persephin gene. In one embodiment, the method comprises contacting the unspliced transcript with a polynucleotide probe which specifically hybridizes to the intron of the unspliced transcript and detecting a hybrid duplex between the probe and the unspliced transcript. The sample may comprise cultured cells of any type or may be a blood or tissue sample from a mammal.
15 The sample is treated using techniques known in the art to make its mRNA available for hybridization to the probe or, alternatively, the mRNA may be extracted from the sample and may then be subjected to gel electrophoresis or other size separation techniques.

The polynucleotide probe comprises a nucleotide sequence containing a minimum of at least about 6, preferably at least about 8, more preferably at least about 10 to 12, and even
20 more preferably at least about 15 to 20 contiguous nucleotides which are complementary to a sequence of the same length in the intron of unspliced persephin mRNA. Preferably, the polynucleotide probe is complementary to the intron sequence of human, rat or mouse persephin mRNA as set forth in SEQ ID NOS:19-21 or a fragment thereof. The polynucleotide probe may be composed of DNA and/or RNA, and/or a synthetic
25 oligonucleotide analog. As used herein, "synthetic oligonucleotide analog" includes oligonucleotides containing one or more modified bases, oligonucleotides with a modified phosphate backbone such as methylphosphonate, phosphorothioate and phosphoramidate, and oligonucleotides with a phosphate-free backbone such as polyamide (peptide nucleic acids). The polynucleotide probe may be prepared by any method known in the art such as, for
30 example, chemical synthesis, excision from a DNA fragment, transcription, or reverse transcription.

To enable detection of the hybrid duplex, the probe may be labeled with any detectable label known in the art such as, for example, radioactive or fluorescent labels or enzymatic markers. Labeling of the probe can be accomplished by any method known in the
35 art such as by PCR, random priming, end labeling, nick translation or the like. One skilled in the art will also recognize that other methods not employing a labeled probe can be used to

detect the hybrid duplex. Examples of methods that can be used for detecting hybridization include Southern blotting, fluorescence *in situ* hybridization, and single-strand conformation polymorphism with PCR amplification.

Hybridization conditions for the type of probe used may be readily determined by those skilled in the art. High stringency conditions are preferred in order to prevent false positives. The stringency of hybridization is determined by a number of factors in the hybridization and washing steps. Such factors are well known to those skilled in the art and outlined in, for example, Sambrook et al., 1989, *supra*.

In another embodiment, detection of the unspliced persephin transcript in the sample comprises performing a reverse transcription reaction on the unspliced transcript to produce a cDNA encoding persephin^{ARF}, amplifying a target sequence in the cDNA, and detecting the amplified target sequence. The target sequence can be of any length and can include the full length of the cDNA. In one embodiment, the target sequence comprises the intron sequence of unspliced persephin mRNA or a fragment thereof. Amplification of the target sequence can be by any amplification method known in the art, including polymerase chain reaction (PCR) and ligase chain reaction (LCR). Detection of the amplified target sequence can be accomplished using any known method such as separation of reaction products by gel electrophoresis and detecting a fragment of the expected size and/or incubation of the reaction products with a probe which specifically hybridizes to the target sequence.

Methods are also provided herein for producing persephin^{ARF}. Preparation can be by isolation from conditioned medium from a cell type that produces persephin^{ARF}. A second and preferred method involves utilization of recombinant methods by isolating a polynucleotide encoding precursor or mature persephin^{ARF}, cloning the polynucleotide along with appropriate regulatory elements into suitable vectors and cell types, and expressing the polynucleotide to produce precursor or mature persephin^{ARF}.

Another embodiment of the invention provides an isolated and purified antibody which specifically reacts with a persephin^{ARF} polypeptide or an epitope thereof. By epitope reference is made to an antigenic determinant of a polypeptide. An epitope could comprise 3 amino acids in a spatial conformation which is unique to the epitope. Methods of determining the spatial conformation of amino acid sequences are known in the art, and include, for example, x-ray crystallography and 2 dimensional nuclear magnetic resonance. Typically an epitope consists of at least 5 or 6 contiguous amino acids of a persephin^{ARF} amino acid sequence.

One approach for preparing antibodies to a protein is the selection and preparation of an amino acid sequence of all or part of the protein, chemically synthesizing the sequence and injecting it into an appropriate animal, usually a rabbit or a mouse. Peptide candidates for the

production of an antibody specific to a persephin^{ARF} polypeptide can be based upon amino acid sequences lying in hydrophilic regions which are thus likely to be exposed in the mature protein. Such peptides can be prepared by chemical synthesis using methods known in the art, e.g., the classical Merrifield method of solid phase peptide synthesis (Merrifield, *J Am Chem Soc* 85:2149, 1963 or the Fmoc strategy on a Rapid Automated Multiple Peptide Synthesis system (DuPont Company, Wilmington, DE) (Caprino and Han, *J Org Chem* 37:3404, 1972). In a preferred embodiment, the anti-persephin^{ARF} antibody is specific for human persephin^{ARF} and reacts with an epitope from SEQ ID NO:1 or SEQ ID NO:4.

The invention includes both polyclonal antibodies and monoclonal antibodies.

10 Polyclonal antibodies can be prepared by immunizing rabbits or other animals by injecting antigen followed by subsequent boosts at appropriate intervals. The animals are bled and sera assayed against purified persephin^{ARF}, usually by ELISA or by bioassay based upon the ability to block one or more of the biological activities of persephin^{ARF}. When using avian species, e.g. chicken, turkey and the like, the antibody can be isolated from the yolk of the egg. Monoclonal antibodies can be prepared, for example, by fusing antibody-producing cells 15 from immunized mice with continuously replicating tumor cells such as myeloma or lymphoma cells. (Kohler and Milstein, *Nature* 256:495-497, 1975; Galfre and Milstein, *Methods in Enzymology: Immunochemical Techniques* 73:1-46, Langone and Banatis eds., Academic Press, 1981). The hybridoma cells so formed are then cloned by limiting dilution 20 methods and supernates thereof are assayed for antibody production by ELISA, RIA or bioassay.

Polyclonal or monoclonal antibodies to persephin^{ARF} or an epitope thereof may be used in a method for detecting persephin^{ARF} in a sample. Any antibody-based method known in the art for detecting proteins may be used. Such methods include, but are not limited to 25 immunodiffusion, immunoelectrophoresis, immunochemical methods, binder-ligand immunoassays, immunohistochemical techniques, agglutination and complement assays. (For example, see *Basic and Clinical Immunology*, Sites and Terr, eds., Appleton & Lange, Norwalk, Conn. pp 217-262, 1991). Preferred are binder-ligand immunoassay methods which involve reacting antibodies with an epitope or epitopes of persephin^{ARF} or derivative thereof 30 to competitively displace a labeled persephin^{ARF} polypeptide.

Antibodies useful in detecting persephin^{ARF} are intended to include complete anti-persephin^{ARF} antibody molecules and persephin^{ARF}-binding fragments of such antibody molecules. The anti-persephin^{ARF} antibody may be unlabeled, for example as used in agglutination tests, or labeled for use in a wide variety of assay methods. Labels that can be 35 used include radionuclides, enzymes, fluorescers, chemiluminescers, enzyme substrates or co-factors, enzyme inhibitors, particles, dyes and the like for use in radioimmunoassay (RIA),

enzyme immunoassays, e.g., enzyme-linked immunosorbent assay (ELISA), fluorescent immunoassays and the like.

The present invention also includes therapeutic or pharmaceutical compositions comprising a persephin^{ARF} polypeptide and a method for treating a patient suffering from a medical condition associated with a lack of, or reduced amount of, at least one of the biological activities of persephin^{ARF}, the method comprising administering to the patient a therapeutically effective amount of a persephin^{ARF} polypeptide. Potential biological activities for persephin^{ARF} and medical conditions associated therewith are described below. As used herein, "therapeutically effective amount" means the amount of the persephin^{ARF} polypeptide sufficient to show a meaningful benefit to the patient, i.e., prevention, healing or amelioration of the relevant medical condition, or an increase in rate of healing or amelioration of the condition.

In certain circumstances, it may be desirable to modulate or decrease the effect of endogenously synthesized persephin^{ARF}. This may be achieved by blocking the activity of endogenously synthesized persephin^{ARF} or by decreasing expression of persephin^{ARF}. Thus, appropriate treatments, for example, may involve administration of anti-persephin^{ARF} antibodies or other compounds having persephin^{ARF} antagonist properties, or the use of antisense polynucleotides to modulate persephin^{ARF} expression.

Specific antibodies, either polyclonal or monoclonal, including persephin^{ARF}-binding fragments thereof, may be capable of preventing persephin^{ARF} from exerting one or more of its biological effects. Such antibodies can be produced by any suitable method known in the art. For example, murine or human monoclonal antibodies can be produced by hybridoma technology or by combinatorial antibody library technology, including panning a phage display library. The antibody may be engineered using recombinant techniques to produce an antibody with desirable characteristics such as being "humanized" to be better tolerated by the patient. Such antibody engineering techniques are known in the art. See for example, Hayden et al., *Curr. Opin. Immunol.* 9(2):201-212, 1997. Alternatively, a persephin^{ARF} polypeptide, or an immunologically active fragment thereof, or an anti-idiotypic antibody, or fragment thereof can be administered to an animal to elicit the production of antibodies capable of recognizing and binding to persephin^{ARF}. Such antibodies can be from any class of antibodies including, but not limited to IgG, IgA, IgM, IgD, and IgE or in the case of avian species, IgY and from any subclass of antibodies.

In another aspect of the present invention, persephin^{ARF} antisense oligonucleotides can be made and used in a method for diminishing the level of expression of persephin^{ARF} protein by a cell which comprises administering one or more persephin^{ARF} antisense oligonucleotides. By persephin^{ARF} antisense oligonucleotides reference is made to

oligonucleotides that have a nucleotide sequence that interacts through base pairing with a specific complementary nucleic acid sequence involved in the expression of persephin^{ARF} such that the expression of persephin^{ARF} is reduced. Preferably, the specific nucleic acid sequence involved in the expression of persephin^{ARF} is contained within a genomic DNA molecule or mRNA molecule that encodes persephin^{ARF}. The antisense oligonucleotide may be directed against a regulatory region of the persephin gene and/or coding sequences for precursor or mature persephin^{ARF}. By complementary to a nucleotide sequence in the context of antisense oligonucleotides and methods therefor is meant sufficiently complementary to such a sequence as to allow hybridization to that sequence in a cell, i.e., under physiological conditions. The persephin^{ARF} antisense oligonucleotides preferably comprise about 8 to about 100 nucleotides and more preferably the persephin^{ARF} antisense oligonucleotides comprise from about 15 to about 30 nucleotides.

The persephin^{ARF} antisense oligonucleotides can also include derivatives which contain a variety of modifications that confer resistance to nucleolytic degradation such as, for example, modified internucleoside linkages, modified nucleic acid bases and/or sugars and the like (Uhlmann and Peyman, *Chemical Reviews* 90:543-584, 1990; Schneider and Banner, *Tetrahedron Lett* 31:335, 1990; Milligan et al., *J Med Chem* 36:1923-1937, 1993; Tseng et al., *Cancer Gene Therap* 1:65-71, 1994; Miller et al., *Parasitology* 10:92-97, 1994). Such derivatives include but are not limited to backbone modifications such as phosphotriester, phosphorothioate, methylphosphonate, phosphoramidate, phosphorodithioate and formacetal as well as morpholino, peptide nucleic acid analogue and dithioate repeating units.

The therapeutic or pharmaceutical compositions of the present invention can be administered by any suitable route known in the art including for example intravenous, subcutaneous, intramuscular, transdermal, intrathecal or intracerebral or administration to cells in *ex vivo* treatment protocols. Administration can be either rapid as by injection or over a period of time as by slow infusion or administration of slow release formulation. For treating tissues in the central nervous system, administration can be by injection or infusion into the cerebrospinal fluid (CSF). When it is intended that a persephin^{ARF} polypeptide or an antibody thereto be administered to cells in the central nervous system, administration can be by intravenous injection with one or more agents capable of promoting penetration of persephin^{ARF} or the anti- persephin^{ARF} antibody across the blood-brain barrier such as an antibody to the transferrin receptor. Co-administration may comprise physically coupling any known blood-brain penetrating agent to the persephin^{ARF} polypeptide. (See for example, Friden et al., *Science* 259:373-377, 1993).

A persephin^{ARF} polypeptide or antibody thereto can also be linked or conjugated with agents that provide other desirable pharmaceutical or pharmacodynamic properties. For

example, a persephin^{ARF} polypeptide can be stably linked to a polymer such as polyethylene glycol to obtain desirable properties of solubility, stability, half-life and other pharmaceutically advantageous properties. (See, e.g., Davis et al. *Enzyme Eng* 4:169-73, 1978; Burnham, *Am J Hosp Pharm* 51:210-218, 1994).

5 The compositions are usually employed in the form of pharmaceutical preparations. Such preparations are made in a manner well known in the pharmaceutical art. One preferred preparation utilizes a vehicle of physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers such as physiological concentrations of other non-toxic salts, five percent aqueous glucose solution, sterile water or the like may also be used. It may
10 also be desirable that a suitable buffer be present in the composition. Such solutions can, if desired, be lyophilized and stored in a sterile ampoule ready for reconstitution by the addition of sterile water for ready injection. The primary solvent can be aqueous or alternatively non-aqueous. A persephin^{ARF} polypeptide can also be incorporated into a solid or semi-solid biologically compatible matrix which can be implanted into tissues requiring treatment.

15 The carrier can also contain other pharmaceutically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmaceutically-acceptable excipients for modifying or maintaining release or absorption or penetration across the blood-brain barrier. Such excipients are those substances usually and
20 customarily employed to formulate dosages for parenteral administration in either unit dosage or multi-dose form or for direct infusion into the cerebrospinal fluid by continuous or periodic infusion.

 It is also contemplated that certain formulations containing a persephin^{ARF} polypeptide are to be administered orally. Such formulations are preferably encapsulated and
25 formulated with suitable carriers in solid dosage forms. Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, gelatin, syrup, methyl cellulose, methyl- and propylhydroxybenzoates, talc, magnesium, stearate, water, mineral oil, and the like. The
30 formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions may be formulated so as to provide rapid, sustained, or delayed release of the active ingredients after administration to the patient by employing procedures well known in the art. The formulations can also contain substances that diminish proteolytic degradation
35 and promote absorption such as, for example, surface active agents.

The specific dose is calculated according to the approximate body weight or body surface area of the patient or the volume of body space to be occupied. The dose will also be calculated dependent upon the particular route of administration selected. Further refinement of the calculations necessary to determine the appropriate dosage for treatment is routinely made by those of ordinary skill in the art. Exact dosages are determined in conjunction with standard dose-response studies. It will be understood that the amount of the composition actually administered will be determined by a practitioner, in the light of the relevant circumstances including the condition or conditions to be treated, the choice of composition to be administered, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the chosen route of administration. Dose administration can be repeated depending upon the pharmacokinetic parameters of the dosage formulation and the route of administration used.

It is also contemplated that the expression of persephin^{ARF} could be increased in tissues defective in such expression by gene therapy. Patients may be implanted with vectors or cells capable of producing a biologically-active persephin^{ARF} polypeptide. Cells can be grown *ex vivo*, for example, for use in transplantation or engraftment into patients (Muench et al., *Leuk & Lymph* 16:1-11, 1994). In one approach, cells that secrete persephin^{ARF} may be encapsulated into semipermeable membranes for implantation into a patient. The cells can be those that normally express a persephin^{ARF} polypeptide or the cells can be transformed to express a persephin^{ARF} polypeptide. When the patient is human, it is preferred that the persephin^{ARF} be human persephin^{ARF}. However, the formulations and methods herein can be used for veterinary as well as human applications and the term "patient" as used herein is intended to include human and veterinary patients.

The isolated polynucleotides and polypeptides of the present invention are expected to exhibit one or more of the uses or biological activities identified below.

Research Uses and Utilities

The polynucleotides and polypeptides provided by the present invention can be commercialized as reagent grade research products or in kit format for use by the research community for various purposes.

For example, polynucleotides encoding persephin^{ARF} can be used to express recombinant persephin^{ARF} for characterizing its biological activity *in vitro* or *in vivo*. Polynucleotides complementary to persephin^{ARF}-encoding polynucleotides and fragments thereof can also be used to identify tissues that express persephin^{ARF} and in subtractive hybridization techniques that remove known polynucleotides to facilitate discovery of other novel polynucleotides expressed in those tissues. The polynucleotides of the invention can be used to analyze genomic DNA sequences in various populations to identify polymorphisms

that may be useful in DNA fingerprinting applications or may be linked to genetic disorders. In addition, persephin^{ARF}-encoding polynucleotides and fragments thereof can be administered to an animal to raise anti-persephin^{ARF} antibodies using DNA immunization techniques.

5 Similarly, persephin^{ARF} polypeptides provided by the invention can be used in various assays to characterize biological activity, including in a panel of multiple proteins for high-throughput screening to identify a candidate drug that interacts with a target receptor or to induce or inhibit a desired cellular response. In addition, persephin^{ARF} polypeptides can be used to raise antibodies for use in identifying cell types that synthesize and secrete
10 persephin^{ARF}. The polypeptides of the invention can also be used to identify proteins that bind to persephin^{ARF} including potential receptors or ligands.

Methods for performing the research activities described above are well known to those skilled in the art and are described in various references, including without limitation "Molecular Cloning: A Laboratory Manual", 2d ed. Cold Spring Harbor Laboratory Press,
15 Sambrook et al., eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

Cell Proliferation/Differentiation Activity

Because the persephin^{ARF} mRNA encodes a polypeptide with a secretion signal sequence and is expressed by a gene that also expresses persephin, a growth factor that
20 promotes the survival of certain types of neurons, persephin^{ARF} protein may also act as a cytokine, i.e., persephin^{ARF} may induce or inhibit cell proliferation or cell differentiation or may regulate the expression of other cytokines, including possibly persephin. A persephin^{ARF} polypeptide of the present invention can be tested for such activities on a number of known cytokine-dependent cell types grown as primary cultures or from established cell lines
25 including, without limitation, sympathetic neurons, sensory neurons, mesencephalic cells 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK, using well-known assays routinely performed in the art.

For example, persephin^{ARF} may be tested for neurotrophic activity using assays such
30 as, but not limited to, the superior cervical ganglion survival and nodose ganglion survival assays described in U.S. Patent No. 5,747,655, the dopaminergic neuron survival assay described in Lin et al., *Science* 260: 1130-1132, 1993, the chick embryo ciliary ganglia survival assay described in U.S. 5,011,914, and assays for nerve tissue generation as described in International Patent Publication No. WO95/05846. Demonstration of
35 neurotrophic activity for persephin^{ARF} would indicate its utility in treating one or more diseases and medical conditions associated with neuronal degeneration such as peripheral

neuropathy, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Shy-Drager syndrome, ischemic stroke, acute brain or spinal cord injury, nervous system tumors, multiple sclerosis, peripheral nerve trauma or injury, exposure to neurotoxins, metabolic diseases such as diabetes or renal dysfunction and damage caused
 5 by infectious agents.

Similarly, persephin^{ARF} may be tested for hematopoietic activity using assays for proliferation and differentiation of various cell types in the hematopoietic system, including without limitation (1) assays for stem cell survival and differentiation as described in
 10 Johansson et al., *Cellular Biology* 15:141-151, 1995; Keller et al., *Molecular and Cellular Biology* 13:473-486, 1993; McClanahan et al., *Blood* 81:2903-2915, 1993; Hirayama et al., *Proc. Natl. Acad. Sci. USA* 89:5907-5911, 1992; Neben et al., *Exper. Hematol.* 22:353-359; and *Culture of Hematopoietic Cells*, R. I. Freshney et al. eds., Wiley-Liss, Inc., New York, NY 1994, pp. 1-21, 23-39, 139-179, and 265-268; (2) assays for thymocyte or T-cell proliferation as described in *Current Protocols in Immunology*, J.E. Coligan et al. eds., John
 15 Wiley and Sons, 1998, Chapters 3 and 7; Takai et al., *J. Immunol.* 137:3494-3500, 1986; Bertagnoli et al., *J. Immunol.* 145:1706-1712, 1990; Bertagnoli et al., *Cellular Immunol.* 133:327-341, 1991; Bertagnoli et al., *J. Immunol.* 149:3778-3783, 1992; Bowman et al., *J. Immunol.* 152:1756-1761, 1994; (3) assays for proliferation and differentiation of other myeloid- and lymphoid-derived cells as described in *Current Protocols in Immunology*, *infra*
 20 at pp. 6.3.1-6.3.12, 6.6.1-6.6.5, 6.13.1 and 6.15.1; deVries et al., *J. Exp. Med.* 173:1205-1211, 1991; Moreau et al., *Nature* 336:690-692, 1988; Greenberger et al., *Proc. Natl. Acad. Sci. USA* 80:2935-2938, 1983; and Smith et al., *Proc. Natl. Acad. Sci. USA* 83:1857-1861, 1986; and (4) assays for stimulation or inhibition of cytokine production such as described in *Current Protocols in Immunology*, *infra* at pp. 3.12.1-3.12.14 and 6.8.1-6.8.8.

25 Demonstration that persephin^{ARF} has hematopoietic activity would indicate utility in treating one or more diseases or conditions associated with an insufficient number of blood cells, such as for example, leukopenias including eosinopenia and/or basopenia, lymphopenia, monocytopenia, neutropenia, anemias, thrombocytopenia or conditions associated with an insufficient number of stem cells including stem cell disorders such as aplastic anemia and
 30 paroxysmal nocturnal hemoglobinuria, and stem-cell deficient bone marrow following irradiation or chemotherapy.

Persephin^{ARF} may also promote or inhibit growth and differentiation of one or more of the following tissues: bone, cartilage, tendon, ligament, muscle (smooth, skeletal or elastic), vascular, and organs including without limitation endothelium, intestine, kidney, liver
 35 pancreas, skin, and pancreas. A growth promoting activity may promote the growth and/or differentiation of cells comprising such tissues and/or promote the generation or regeneration

of these tissues or alternatively, may inhibit fibrotic scarring to allow normal tissue to regenerate. Persephin^{ARF} may also exhibit wound healing activity.

A persephin^{ARF} polypeptide of the invention can be tested for tissue generation activity using assays known in the art including, without limitation, assays for promoting growth of bone, cartilage and tendon as described in International Patent Publication No. WO95/16035 and assays for promoting skin and endothelium growth as described in International Patent Publication No. WO/91/07491. The ability of persephin^{ARF} to promote wound healing can be tested using assays such as those described in *Epidermal Wound Healing*, Maibach, H.I. and Rovee, D.T., eds, Year Book Medical Publishers, Inc., Chicago, 1992, pp. 71-112, as modified by Eaglstein and Mertz, *J. Invest. Dermatol.* 71:382-84, 1978.

Demonstration that persephin^{ARF} promotes cartilage and/or bone growth would indicate its utility in healing bone fractures and cartilage damage, in improving fixation of artificial joints, in repair of congenital or trauma-induced craniofacial defects, and in cosmetic plastic surgery. A protein that promotes bone and cartilage growth may also be useful in treating osteoporosis, osteoarthritis or periodontal disease. The ability to induce tendon and/or ligament formation would make persephin^{ARF} a candidate agent for treating tears or deformities in tendon and/or ligament tissues, for improving fixation of tendon or ligament to bone or other tissues and for treating conditions such as tendinitis and carpal tunnel syndrome.

It is also expected that persephin^{ARF} may have activity in promoting better or faster healing of wounds. Such activity would make persephin^{ARF} useful, for example, in treating non-healing wounds such as pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

Immune Stimulating or Suppressing Activity

Persephin^{ARF} may also stimulate or suppress the immune system by, for example, regulating growth and proliferation of T, B, and NK cells, effecting or blocking the cytolytic activity of NK cells and other cytotoxic cell populations, inducing or suppressing T cell responses, regulating ratio of T_H1 and T_H2 cells, activating expression of class I and/or class II MHC molecules, and inducing or blocking the production of cytokines. Exhibition of an immune stimulating activity by persephin^{ARF} would indicate a utility in treating various immune deficiencies such as severe combined immunodeficiency (SCID) and AIDS or in treating infectious diseases caused by bacterial, viral and fungal infections, including infections by mycobacteria, *Leishmania* spp., malaria spp., *Salmonella typhi*, pathogenic strains of *E. coli*, HIV, hepatitis viruses, herpesviruses, and *candidiasis*. Alternatively, a determination that persephin^{ARF} has immune suppressing activity would indicate this protein would be useful in treating one or more autoimmune disorders and allergic reactions or

conditions including without limitation multiple sclerosis, systemic lupus erthematosus, rheumatoid arthritis, insulin dependent diabetes mellitis, connective tissue disease, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, Goodpasture's syndrome, myasthenia gravis, graft-versus-host disease and autoimmune
 5 inflammatory eye disease, asthma, hay fever, glomerulonephritis, and contact dermatitis.

Testing persephin^{ARF} for immune stimulating and/or suppressing activity may be done using assays known in the art, including without limitation the hematopoietic assays described above as well as the following methods:

Assays for modulation of T-cell dependent antibody responses and T_h1/T_h2 ratios
 10 including, e.g., those described in Maliszewski, *J. Immunol.* 144:3028-3033, 1990;

Assays for B cell function, as described, e.g., in *Current Protocols in Immunology*, *infra* at pp. 3.8.1-3.8.16;

Mixed lymphocyte reaction (MLR) assays, such as those described in *Current Protocols in Immunology*, *infra* at pp. 3.1-3.19 and Chapter 7, Takai et al., *J. Immunol.* 137,
 15 *supra*, Takai et al., *J. Immunol.* 140:508-512, 1988, and Bertagnoli et al., *J. Immunol.* 149, *supra*;

Assays for thymocyte or splenocyte cytotoxicity which are described, e.g., in *Current Protocols in Immunology*, *infra* at pp. 3.1-1.19 and Chapter 7, Hermann et al., *Proc. Natl. Acad. Sci USA* 78:2488-2492, 1981, Herrmann et al., *J. Immunol.* 128:1968-1974, 1982;
 20 Handa et al., *J. Immunol.* 135:1564-1572, 1985, Takai et al., *J. Immunol.* 137, *supra*, Bowman et al., *J. Virol.* 61:1992-1998, 1987, Takai et al., *J. Immunol.* 140, *supra*, Bertagnoli et al., *Cell. Immunol.* 133, *supra*, and Brown et al., *J. Immunol.* 153:3079-3092, 1994;

Assays for effect on T cell commitment and development include those described in Antica et al., *Blood* 84:111-117, 1994; Fine et al., *Cellular Immunol.* 155:111-122, 1994;
 25 Galy et al., *Blood* 85:2770-2778, 1995; Toki et al., *Proc. Nat. Acad. Sci. USA* 88:7548-7551, 1991; and

Assays for effect on lymphocyte survival and/or apoptosis such as those described in Darzynkiewicz et al., *Cytometry* 13:795-808, 1992; Gorczyca et al., *Leukemia* 7:659-670, 1993, Gorczyca et al., *Cancer Research* 53:1945-1951, 1993; Itoh et al., *Cell* 66:233-243,
 30 1991; Zacharchuk, *J. of Immunol.* 145:4037-4045; 1990; Zamai et al., *Cytometry* 14:891-897, 1993; Gorczyca et al., *Internat. J. Oncol.* 1:639-648, 1992.

Activin/Inhibin Activity

Persephin^{ARF} may also exhibit activity similar to that of activins and inhibins, polypeptide factors which induce or inhibit release of follicle stimulating hormone (FSH)
 35 from anterior pituitary cells, respectively. Thus, based on the ability of activins and inhibins to increase or decrease fertility in mammals, a similar activity for persephin^{ARF} would indicate

this protein, alone or in combination with another activin or inhibin, may be useful as a contraceptive or conversely, in treating infertility. Persephin^{ARF} may be tested for activin or inhibin activity using assays known in the art, including without limitation those described in Vale et al., *Endocrinol* 91:562-572, 1972; Ling et al., *Nature* 321:779-782, 1986; Vale et al.,
5 *Nature* 321:776-779, 1986; Mason et al., *Nature* 318:659-663, 1985; Forage et al., *Proc. Natl. Acad. Sci. USA* 83:3091-3095, 1986.

Chemotactic/Chemokinetic Activity

It is also expected that persephin^{ARF} may exhibit chemotactic or chemokinetic activity for one or more mammalian cells, including without limitation monocytes, fibroblasts,
10 neutrophils, T cells, mast cells, eosinophiles, epithelial and/or endothelial cells. A protein has chemotactic activity for a particular cell population if it can directly or indirectly stimulate directed orientation or movement of cells in the population. Due to an ability to attract cells involved in the immune response to a desired site of action, proteins having chemotactic or chemokinetic activity may be useful in treating wounds and other tissue damage, as well as in
15 treating localized infections and tumors.

Persephin^{ARF} may be tested for chemotactic activity using known assays that measure the ability of a protein to induce migration of cells across a membrane as well as the ability to induce adhesion of one cell population to another cell population. Such assays include, without limitation, those described in *Current Protocols in Immunology, infra* at Chapter 6.12;
20 Taub et al., *J. Clin. Invest.* 95:1370-1376, 1995; Lind et al., *APMIS* 103:140-146, 1995; Muller et al., *Eur. J. Immunol.* 25:1744-1748, 1995; Gruber et al., *J. Immunol.* 152:5860-5867, 1994; Johnston et al., *J. Immunol.* 153:1762-1768, 1994.

Anti-inflammatory Activity

A persephin^{ARF} polypeptide of the invention may have anti-inflammatory activity
25 which may include one or more of the following: inhibiting vascular permeability, inhibiting production or binding of cell adhesion molecules, inhibiting chemotaxis and release of chemical mediators by cells involved in the inflammatory response, and/or inhibiting activation or proliferation of cells involved in initiating or promoting the inflammatory response. A protein having anti-inflammatory activity may have utility in treating acute or
30 chronic inflammatory conditions, including without limitation conditions associated with infection such as septic shock, ischemia-reperfusion injury, arthritis, nephritis, inflammatory bowel disease, Crohn's disease, anaphylaxis, cytokine or chemokine-induced lung injury, complement-mediated hyperacute rejection, and overproduction of cytokines such as TNF or IL-1.

Hemostatic or Thrombolytic Activity

Persephin^{ARF} may exhibit hemostatic activity, which would indicate its utility in treating various coagulation disorders, such as hemophilias, or to enhance coagulation in treating wounds resulting from trauma, surgery and the like. Alternatively, persephin^{ARF} may have thrombolytic activity and would thus be a candidate therapeutic for inhibiting or dissolving formation of thrombosis and for preventing or treating conditions resulting therefrom. Persephin^{ARF} may be tested for hemostatic or thrombolytic activity using known assays such as Linet et al., *J. Clin. Pharmacol.* 26:131-140, 1986; Burdick et al., *Thrombosis Res.* 45:413-419, 1987; Humphrey et al., *Fibrinolysis* 5:71-79, 1991; Schaub, *Prostaglandins* 35:467-474, 1988.

Other Biological Activities

It is expected that persephin^{ARF} may have any one or more of other activities that would make persephin^{ARF} useful in treating medical conditions that would benefit from providing the activity. Such activities include without limitation: inhibiting tumor growth directly or indirectly; receptor activity for ligands such as growth factors and other cytokines; analgesic or other pain reducing activity; activities that inhibit or increase the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or components; activities that alter behavioral characteristics, including appetite, libido, stress, cognition, depression, addiction and violent behaviors; activities involved in regulating biorhythms; inhibiting or increasing fertility of male or female mammals; and activities that affect body characteristics such as height, weight, muscle mass, breast size, tissue pigmentation, hair growth or color.

Preferred embodiments of the invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims which follow the examples.

Example 1

This example illustrates the relative levels of unspliced and spliced transcripts of the persephin gene in various rat tissues as determined by semi-quantitative RT-PCR analysis.

Poly A⁺ RNA was isolated from heart, kidney, liver and brain tissues of embryonic and adult rat and semiquantitative RT-PCR was performed essentially as described by Freeman et al., *Neuron* 12:343-355, 1994. In brief, 1 µg of each RNA sample was used as template in separate reverse transcription (RT) reactions carried out at 37° C for 1 hr after

which the reaction mixtures were diluted (5:1) with H₂O and boiled for 5 min to kill the reverse transcriptase. Control reactions for each RNA sample were performed in the absence of reverse transcriptase to test for genomic DNA contamination.

Aliquots of the diluted RT reaction mixtures were used in PCR reactions with forward (5'-AGCTCAGCCACTGGTAGGGTCAGG-3') (SEQ ID NO:22) and reverse (5'-AGCTCAGCCACTGGTAGGGTCAGG-3') (SEQ ID NO:23) primers corresponding to the persephin cDNA sequence. The amount of each RT reaction used for PCR analysis was normalized by performing preliminary PCR reactions with primers to β -actin. The PCR parameters were 95°C for 30 s, 65°C for 30 s, then 72°C for 30 s for 26, 29 or 32 cycles.

Products of the PCR reactions were separated on a 2.8% agarose gel, blotted onto a nylon membrane and then detected by hybridization to a ³²P-labeled persephin probe. The results, as visualized by a PhosphorImager® (Molecular Dynamics, Sunnyvale, CA), are shown in Figure 3.

Two species of persephin mRNA were amplified by the RT/PCR reaction with the larger, more abundant species corresponding to the unspliced transcript of the persephin gene. The levels of both spliced and unspliced mRNAs were constant from embryonic day 10 through embryonic day 18 (E18) (data not shown). Slightly higher levels of both persephin mRNA species were observed in embryonic tissues other than brain.

Example 2

This example illustrates the detection of unspliced and spliced transcripts of the persephin gene in human fetal brain tissue.

Human fetal brain poly A⁺ RNA (1 μ g) (CLONTECH) was used as the template in a reverse transcriptase (RT) reaction. A control reaction was also performed in which no reverse transcriptase enzyme was added. These reactions were incubated at 37° C for 1 hr, diluted (5:1) with H₂O, and boiled for 5 min to kill the enzyme.

PCR reactions were then performed using forward and reverse primers, [5'-GTCACAATGGCCGTAGGGAAGTT-3'] (SEQ ID NO:24) and [5'-CACCTCAGCCACCACAGCCGCA-3'] (SEQ ID NO:25) corresponding to the start and stop sites in the human persephin coding sequence, respectively. The expected size of fragments amplified with these primers would be 566 nt and 481 nt corresponding to the unspliced and spliced species of persephin mRNA, respectively. Templates for the PCR reactions included human genomic DNA as a control for the size of the gene itself (including the intron); the human fetal brain RT reaction (5 μ l); and the mock human fetal brain RT reaction (5 μ l) in which the RT enzyme was omitted as a control for DNA contamination of the RNA sample. The PCR cycling parameters used were an initial denaturation at 98° C for

2 min, followed by 40 cycles of 68° C for 1.5 min and 98° C for 10 sec. The PCR products were electrophoresed on a 2.5 % agarose gel and detected by ethidium bromide staining.

5 As shown in Figure 4, fragments corresponding to the expected size for unspliced and spliced human persephin transcripts were amplified, with the larger unspliced transcript being present in a much larger amount than the smaller spliced form. These results indicate that human fetal brain tissue contains a significant amount of unspliced persephin mRNA, at least a portion of which should be available for translation into human precursor persephin^{ARF}.

In view of the above, it will be seen that the several advantages of the invention are achieved and other advantageous results attained.

10 As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

What is Claimed is:

1. An isolated and purified polypeptide comprising a persephin^{ARF} amino acid sequence.
2. The isolated and purified polypeptide of claim 1 wherein the persephin^{ARF} amino acid sequence is a human sequence comprising SEQ ID NO:1 or SEQ ID NO:4.
3. The isolated and purified polypeptide of claim 1 wherein the persephin^{ARF} amino acid sequence is a mouse sequence comprising SEQ ID NO:2 or SEQ ID NO:5.
4. The isolated and purified polypeptide of claim 1 wherein the persephin^{ARF} amino acid sequence is a rat sequence comprising SEQ ID NO:3 or SEQ ID NO:6.
5. A composition comprising the isolated and purified polypeptide of claim 2 and a pharmaceutically acceptable carrier.
6. An isolated and purified polynucleotide comprising a nucleotide sequence encoding a persephin^{ARF} amino acid sequence as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 or SEQ ID NO:6.
7. The isolated and purified polynucleotide of claim 6 wherein the nucleotide sequence comprises a human sequence as set forth in SEQ ID NO:7 or SEQ ID NO:8.
8. The isolated and purified polynucleotide of claim 6 wherein the nucleotide sequence comprises a mouse sequence as set forth in SEQ ID NO:9 or SEQ ID NO:10.
9. The isolated and purified polynucleotide of claim 6 wherein the nucleotide sequence comprises a rat sequence as set forth in SEQ ID NO:11 or SEQ ID NO:12.
10. A vector comprising a recombinant DNA molecule comprising expression regulatory elements operably linked to a nucleotide sequence encoding the human persephin^{ARF} amino acid sequence of claim 2.
11. A host cell transformed with the vector of claim 10.
12. A persephin^{ARF} polypeptide expressed by the host cell of claim 11.
13. An isolated and purified polynucleotide comprising a nucleotide sequence complementary to the nucleotide sequence of claim 6.
14. The isolated and purified polynucleotide of claim 12 wherein the complementary nucleotide sequence is SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17 or SEQ ID NO:18.
15. An isolated and purified polynucleotide which specifically hybridizes to a polynucleotide consisting of SEQ ID NO:19 or its complement.
16. The isolated and purified polynucleotide of claim 15 which is a persephin^{ARF} antisense oligonucleotide.

17. An isolated and purified antibody which specifically reacts with a persephin^{ARF} polypeptide or an epitope thereof, wherein the persephin^{ARF} polypeptide comprises SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

18. A method for detecting expression in a sample of a persephin^{ARF} polypeptide comprising reacting an antibody according to claim 17 with the persephin^{ARF} polypeptide and detecting binding of the antibody to the persephin^{ARF} polypeptide.

19. A method for detecting expression of persephin^{ARF} mRNA in a sample comprising detecting an unspliced transcript of the persephin gene in the sample.

20. The method of claim 19 wherein detecting the unspliced transcript comprises contacting the unspliced transcript with a polynucleotide probe which specifically hybridizes to the intron of the unspliced transcript and detecting a hybrid duplex between the probe and the unspliced transcript.

21. The method of claim 20 wherein detecting the unspliced persephin transcript comprises performing a reverse transcription reaction on the unspliced transcript to produce a cDNA encoding persephin^{ARF}, amplifying a target sequence in the cDNA, and detecting the amplified target sequence.

1	A T G G C T G C A G G A A G A C T T C G G A T C C T G T G T C T G C T G C T C C	mouse
1	A T G G C T G C A G G A A G A C T T C G G A T C T T G T T T C T G C T G C T C C	rat
1	A T G G C C G T A G G G A A G T T C C T G C T G G G C T C T C T G C T G C T C C	human
41	T G T C C T T G C A C C C G A G C C T C G G C T G G G T C C T T G A T C T T C A	mouse
41	T G T C C T T G C A C C T G G G C C T T G G C T G G G T C C T T G A T C T T C A	rat
41	T G T C C C T G C A G C T G G G A C A G G G C T G G G G C C C C G A T G C C C G	human
81	A G A G G C T T C T G T G G C A G A T A - - - A G C T C T C A T T T G G G A A G	mouse
81	A G A G G C T C C T G C G G C A G A T G - - - A G C T C T C A T C T G G G A A A	rat
81	T G G G G T T C C C G T G G C C G A T G G A G A G T T C T C G T C T G A A C A G	human
118	A T G G C A G A G A C T A G A G G G A C C T G G A C G C C C C A T C A G G G T A	mouse
118	A T G G C A G A G A C T G G A A G G A C C T G G A A G C C C C A T C A G G G T A	rat
121	G T G G C A A A G G C T G G A G G G A C C T G G - - - - - C T G G G T A	human
158	A G A A T T C C T G G G G G C C T C C C G - - - A C T C C C C A A T T C C T T C T	mouse
158	A G A A T T C T T G G G G G C C T C C T A - - - A C T C T A C A G T T C T T C C T	rat
152	A G G A C T T C C A G G G A C C C T C T G T G A C T T C C C A A C T T T C C C C	human
196	C T C A A A G C C C T C A C T T T G C C T T A C A A T C C T A C T C T A C C T T	mouse
196	C T C A A A G C C C T C A C T T T G C C T C A C A A T C C T A T T C T A C C T T	rat
192	A G C C C T G A C C C T G C T C A C T G T C A G C G C C C T T C C C T C C C A C	human
236	G C A C - T A G G T A A C A A C C A T G T C C G T C T T C C A A G A G C C T T G	mouse
236	G C A C - T A G G T A A C A A C A A T G T C C G C C T T C C A A G A G C C T T A	rat
232	A G G C A C C C A C C G C C C C C T T G C C C G C C T G C G C C G A G C C C T G	human
275	G C T G G T T C A T G C C G A C T G T G G A G C C T G A C C C T A C C A G T G G	mouse
275	C C T G G T T T G T G C C G G C T G T G G A G C C T G A C C C T A C C A G T G G	rat
272	T C T G G T C C A T G C C A G C T G T G G A G C C T G A C C C T G T C C G T G G	human
315	C T G A G C T G G G C C T G G G C T A T G C C T C G G A G G A G A A G G T C A T	mouse
315	C T G A G C T T G G C C T G G G C T A T G C C T C A G A G G A G A A G A T T A T	rat
312	C A G A G C T A G G C C T G G G C T A C G C C T C A G A G G A G A A G G T C A T	human
355	C T T C C G A T A C T G T G C T G G C A G C T G T C C C C A A G A G G C C C G T	mouse
355	C T T C C G A T A C T G T G C T G G C A G C T G T C C C C A A G A G G T C C G T	rat
352	C T T C C G C T A C T G C G C C G G C A G C T G C C C C C G T G G T G C C C G C	human
395	A C C C A G C A C A G T C T G G T A C T G G C C C G G C T T C G A G G G C G G G	mouse
395	A C C C A G C A C A G T C T G G T G C T G G C C C G T C T T C G A G G G C A G G	rat
392	A C C C A G C A T G G C C T G G C G C T G G C C C G G C T G C A G G G C C A G G	human
435	G T C G A G C C C A T G G C C G A C C C T G C T G C C A G C C C A C C A G C T A	mouse
435	G T C G A G C T C A T G G C A G A C C T T G C T G C C A G C C C A C C A G C T A	rat
432	G C C G A G C C C A C G G T G G G C C C T G C T G C C G G C C C A C T C G C T A	human
475	T G C T G A T G T G A C C T T C C T T G A T G A T C A G C A C C A T T G G C A G	mouse
475	T G C T G A T G T G A C C T T C C T T G A T G A C C A C C A C C A T T G G C A G	rat
472	C A C C G A C G T G G C C T T C C T C G A T G A C C G C C A C C G C T G G C A G	human
515	C A G C T G C C T C A G C T C T C A G C T G C A G C T T G T G G C T G T G G T G	mouse
515	C A G C T G C C T C A G C T C T C A G C C G C A G C T T G T G G C T G T G G T G	rat
512	C G G C T G C C C C A G C T C T C G G C G G C T G C C T G C G G C T G T G G T G	human
555	G C T G A A G G A G G C C A G T C T G G T G T C T C A G A A T C A C A A G C A T	mouse
555	G C T G A	rat
552	G C T G A	human

FIGURE 1

2/4

1	M A V G K F L L G S L L L L L S L Q L G Q G W G P D A R G V P V A D G E F S S E Q	HUMAN
1	M A A G R L R I L C L L L L S L H P S L G W V L D L Q E A S V A D - K L S F G K	MOUSE
1	M A A G R L R I L F L L L L S L H L G L G W V L D L Q E A P A D - E L S S G K	RAT
41	V A K A G G T W L - - G K D F Q G P S V T S Q L S - P A L T L L T V S A L P S	HUMAN
40	M A E T R G T W T P H Q K N S W G P P D S P I P S L K A L T L P Y N P T L P C	MOUSE
40	M A E T G R T W K P H Q G K N S W G P P N S T V L P L K A L T L P H N P I L P C	RAT
77	H R H P P P C P P A P S P V W S M P A V E P D P V R G R A R P G L R L R G E G	HUMAN MOUSE RAT
80	T R	
80	T R	
117	H L P L L R R Q L P P W C P H P A W P G A G P A A G P S P R W A L L P A H S	HUMAN
157	L H R R G L P R	HUMAN

FIGURE 2

3/4

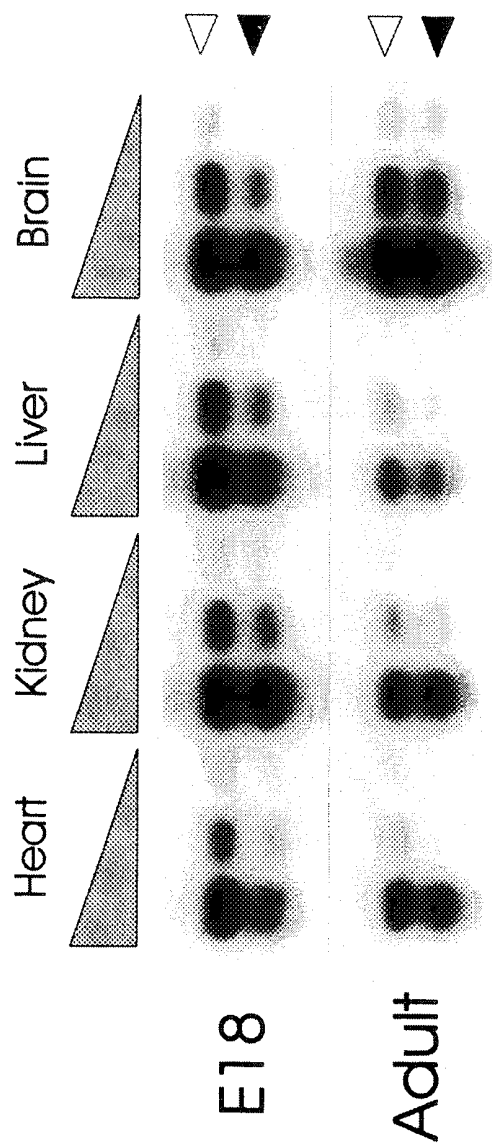


FIGURE 3

4/4

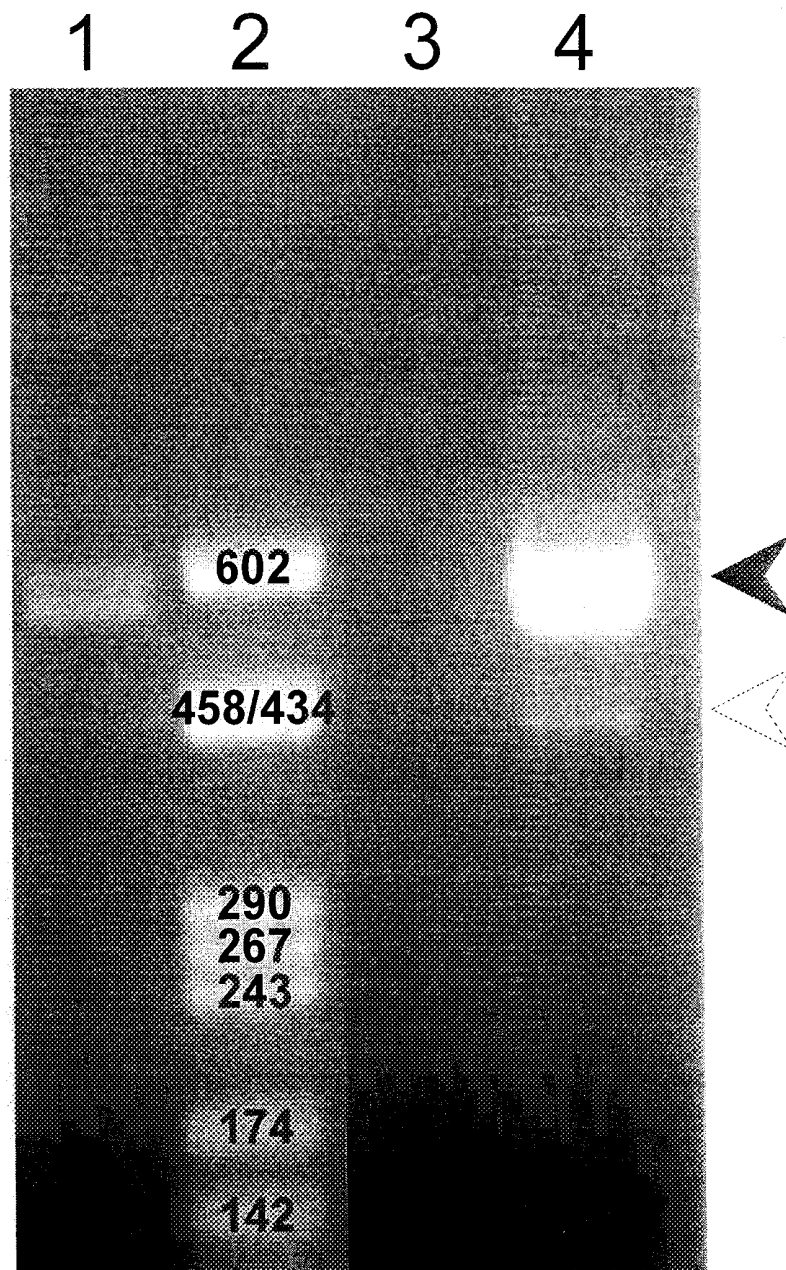


FIGURE 4

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: JOHNSON, EUGENE M
MILBRANDT, JEFFREY D
KOTZBAUER, PAUL T
LAMPE, PATRICIA A
KLEIN, ROBERT
DESAUVAGE, FRED
- (ii) TITLE OF INVENTION: Persephin and Related Growth Factors
- (iii) NUMBER OF SEQUENCES: 27
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Howell & Haferkamp, L.C.
 - (B) STREET: 7733 Forsyth Blvd., Suite 1400
 - (C) CITY: St. Louis
 - (D) STATE: MO
 - (E) COUNTRY: US
 - (F) ZIP: 63105
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Holland, Donald R
 - (B) REGISTRATION NUMBER: 35,197
 - (C) REFERENCE/DOCKET NUMBER: 6029-5274
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (314)727-5188
 - (B) TELEFAX: (314)727-6092

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 164 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

Met Ala Val Gly Lys Phe Leu Leu Gly Ser Leu Leu Leu Leu Ser Leu
1 5 10 15

Gln Leu Gly Gln Gly Trp Gly Pro Asp Ala Arg Gly Val Pro Val Ala
20 25 30

Asp Gly Glu Phe Ser Ser Glu Gln Val Ala Lys Ala Gly Gly Thr Trp
 35 40 45
 Leu Gly Lys Asp Phe Gln Gly Pro Ser Val Thr Ser Gln Leu Ser Pro
 50 55 60
 Ala Leu Thr Leu Leu Thr Val Ser Ala Leu Pro Ser His Arg His Pro
 65 70 75 80
 Pro Pro Pro Cys Pro Pro Ala Pro Ser Pro Val Trp Ser Met Pro Ala
 85 90 95
 Val Glu Pro Asp Pro Val Arg Gly Arg Ala Arg Pro Gly Leu Arg Leu
 100 105 110
 Arg Gly Glu Gly His Leu Pro Leu Leu Arg Arg Gln Leu Pro Pro Trp
 115 120 125
 Cys Pro His Pro Ala Trp Pro Gly Ala Gly Pro Ala Ala Gly Pro Gly
 130 135 140
 Pro Ser Pro Arg Trp Ala Leu Leu Pro Ala His Ser Leu His Arg Arg
 145 150 155 160
 Gly Leu Pro Arg

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 81 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Mouse

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Ala Gly Arg Leu Arg Ile Leu Cys Leu Leu Leu Ser Leu
 1 5 10 15
 His Pro Ser Leu Gly Trp Val Leu Asp Leu Gln Glu Ala Ser Val Ala
 20 25 30
 Asp Lys Leu Ser Phe Gly Lys Met Ala Glu Thr Arg Gly Thr Trp Thr
 35 40 45
 Pro His Gln Gly Lys Asn Ser Trp Gly Pro Pro Asp Ser Pro Ile Pro
 50 55 60
 Ser Leu Lys Ala Leu Thr Leu Pro Tyr Asn Pro Thr Leu Pro Cys Thr
 65 70 75 80
 Arg

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rat

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Met Ala Ala Gly Arg Leu Arg Ile Leu Phe Leu Leu Leu Leu Ser Leu
1           5           10           15

His Leu Gly Leu Gly Trp Val Leu Asp Leu Gln Glu Ala Pro Ala Ala
          20           25           30

Asp Glu Leu Ser Ser Gly Lys Met Ala Glu Thr Gly Arg Thr Trp Lys
          35           40           45

Pro His Gln Gly Lys Asn Ser Trp Gly Pro Pro Asn Ser Thr Val Leu
          50           55           60

Pro Leu Lys Ala Leu Thr Leu Pro His Asn Pro Ile Leu Pro Cys Thr
          65           70           75           80

Arg

```

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 141 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```

Pro Asp Ala Arg Gly Val Pro Val Ala Asp Gly Glu Phe Ser Ser Glu
1           5           10           15

Gln Val Ala Lys Ala Gly Gly Thr Trp Leu Gly Lys Asp Phe Gln Gly
          20           25           30

Pro Ser Val Thr Ser Gln Leu Ser Pro Ala Leu Thr Leu Leu Thr Val
          35           40           45

Ser Ala Leu Pro Ser His Arg His Pro Pro Pro Pro Cys Pro Pro Ala
          50           55           60

Pro Ser Pro Val Trp Ser Met Pro Ala Val Glu Pro Asp Pro Val Arg
          65           70           75           80

Gly Arg Ala Arg Pro Gly Leu Arg Leu Arg Gly Glu Gly His Leu Pro
          85           90           95

```

Leu Leu Arg Arg Gln Leu Pro Pro Trp Cys Pro His Pro Ala Trp Pro
 100 105 110

Gly Ala Gly Pro Ala Ala Gly Pro Gly Pro Ser Pro Arg Trp Ala Leu
 115 120 125

Leu Pro Ala His Ser Leu His Arg Arg Gly Leu Pro Arg
 130 135 140

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 58 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Mouse

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Leu Asp Leu Gln Glu Ala Ser Val Ala Asp Lys Leu Ser Phe Gly Lys
 1 5 10 15

Met Ala Glu Thr Arg Gly Thr Trp Thr Pro His Gln Gly Lys Asn Ser
 20 25 30

Trp Gly Pro Pro Asp Ser Pro Ile Pro Ser Leu Lys Ala Leu Thr Leu
 35 40 45

Pro Tyr Asn Pro Thr Leu Pro Cys Thr Arg
 50 55

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 58 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Rat

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu Asp Leu Gln Glu Ala Pro Ala Ala Asp Glu Leu Ser Ser Gly Lys
 1 5 10 15

Met Ala Glu Thr Gly Arg Thr Trp Lys Pro His Gln Gly Lys Asn Ser
 20 25 30

Trp Gly Pro Pro Asn Ser Thr Val Leu Pro Leu Lys Ala Leu Thr Leu
 35 40 45

Pro His Asn Pro Ile Leu Pro Cys Thr Arg
 50 55

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 556 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGGCCGTAG GGAAGTTCCT GCTGGGCTCT CTGCTGCTCC TGTCCCTGCA GCTGGGACAG	60
GGCTGGGGCC CCGATGCCCG TGGGGTTCCC GTGGCCGATG GAGAGTTCTC GTCTGAACAG	120
GTGGCAAAGG CTGGAGGGAC CTGGCTGGGT AAGGACTTCC AGGGACCCTC TGTGACTTCC	180
CAACTTTCCC CAGCCCTGAC CCTGCTCACT GTCAGCGCCC TTCCCTCCCA CAGGCACCCA	240
CCGCCCCCTT GCCCGCCTGC GCCGAGCCCT GTCTGGTCCA TGCCAGCTGT GGAGCCTGAC	300
CCTGTCCGTG GCAGAGCTAG GCCTGGGCTA CGCCTCAGAG GAGAAGGTCA TCTTCCGCTA	360
CTGCGCCGGC AGCTGCCCCC GTGGTGCCCG CACCCAGCAT GGCCTGGCGC TGGCCCCGGCT	420
GCAGGGCCAG GGCCGAGCCC ACGGTGGGCC CTGCTGCCGG CCCACTCGCT ACACCGACGT	480
GGCCTTCCTC GATGACCGCC ACCGCTGGCA GCGGCTGCCC CAGCTCTCGG CGGCTGCCTG	540
CGGCTGTGGT GGCTGA	556

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 487 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCCGATGCCC GTGGGGTTCC CGTGGCCGAT GGAGAGTTCT CGTCTGAACA GGTGGCAAAG	60
GCTGGAGGGA CCTGGCTGGG TAAGGACTTC CAGGGACCCT CTGTGACTTC CCAACTTTCC	120
CCAGCCCTGA CCCTGCTCAC TGTCAGCGCC CTTCCCTCCC ACAGGCACCC ACCGCCCCCT	180
TGCCCCGCTG CGCCGAGCCC TGTCTGGTCC ATGCCAGCTG TGGAGCCTGA CCCTGTCCGT	240
GGCAGAGCTA GGCCTGGGCT ACGCCTCAGA GGAGAAGGTC ATCTTCCGCT ACTGCGCCGG	300
CAGTGCCCCC CGTGGTGCCC GCACCCAGCA TGGCCTGGCG CTGGCCCCGC TGCAGGGCCA	360

GGGCCGAGCC CACGGTGGGC CCTGCTGCCG GCCCACTCGC TACACCGACG TGGCCTTCCT 420
CGATGACCGC CACCGCTGGC AGCGGCTGCC CCAGCTCTCG GCGGCTGCCT GCGGCTGTGG 480
TGGCTGA 487

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 242 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mouse

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGGCTGCAG GAAGACTTCG GATCCTGTGT CTGCTGCTCC TGTCTTGCA CCCGAGCCTC 60
GGCTGGGTCC TTGATCTTCA AGAGGCTTCT GTGGCAGATA AGCTCTCATT TGGGAAGATG 120
GCAGAGACTA GAGGGACCTG GACGCCCCAT CAGGGTAAGA ATTCCTGGGG GCCTCCCGAC 180
TCCCCAATTC CTTCTCTCAA AGCCCTCACT TTGCCTTACA ATCCTACTCT ACCTTGCACT 240
AG 242

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mouse

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTGATCTTCA AGAGGCTTCT GTGGCAGATA AGCTCTCATT TGGGAAGATG GCAGAGACTA 60
GAGGGACCTG GACGCCCCAT CAGGGTAAGA ATTCCTGGGG GCCTCCCGAC TCCCCAATTC 120
CTTCTCTCAA AGCCCTCACT TTGCCTTACA ATCCTACTCT ACCTTGCACT AG 172

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 242 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rat

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
ATGGCTGCAG GAAGACTTCG GATCTGTTT CTGCTGCTCC TGTCCTTGCA CCTGGGCCTT      60
GGCTGGGTCC TTGATCTTCA AGAGGCTCCT GCGGCAGATG AGCTCTCATC TGGGAAAATG      120
GCAGAGACTG GAAGGACCTG GAAGCCCCAT CAGGGTAAGA ATTCTTGGGG GCCTCCTAAC      180
TCTACAGTTC TTCCTCTCAA AGCCCTCACT TTGCCTCACA ATCCTATTCT ACCTTGCACT      240
AG                                                                 242
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rat

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
TTGATCTTCA AGAGGCTCCT GCGGCAGATG AGCTCTCATC TGGGAAAATG GCAGAGACTG      60
GAAGGACCTG GAAGCCCCAT CAGGGTAAGA ATTCTTGGGG GCCTCCTAAC TCTACAGTTC      120
TTCCTCTCAA AGCCCTCACT TTGCCTCACA ATCCTATTCT ACCTTGCACT AG              172
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 556 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
TCAGCCACCA CAGCCGCAGG CAGCCGCCGA GAGCTGGGGC AGCCGCTGCC AGCGGTGGCG      60
GTCATCGAGG AAGGCCACGT CGGTGTAGCG AGTGGGCCGG CAGCAGGGCC CACCGTGGGC      120
TCGGCCCTGG CCCTGCAGCC GGGCCAGCGC CAGGCCATGC TGGGTGCGGG CACCACGGGG      180
GCAGCTGCCG GCGCAGTAGC GGAAGATGAC CTTCTCTCTT GAGGCGTAGC CCAGGCCTAG      240
```

CTCTGCCACG GACAGGGTCA GGCTCCACAG CTGGCATGGA CCAGACAGGG CTCGGCGCAG	300
GCGGGCAAGG GGGCGGTGGG TGCCTGTGGG AGGGAAGGGC GCTGACAGTG AGCAGGGTCA	360
GGGCTGGGGA AAGTTGGGAA GTCACAGAGG GTCCCTGGAA GTCCTTACCC AGCCAGGTCC	420
CTCCAGCCTT TGCCACCTGT TCAGACGAGA ACTCTCCATC GGCCACGGGA ACCCCACGGG	480
CATCGGGGCC CCAGCCCTGT CCCAGCTGCA GGGACAGGAG CAGCAGAGAG CCCAGCAGGA	540
ACTTCCCTAC GGCCAT	556

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 487 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCAGCCACCA CAGCCGCAGG CAGCCGCCGA GAGCTGGGGC AGCCGCTGCC AGCGGTGGCG	60
GTCATCGAGG AAGGCCACGT CGGTGTAGCG AGTGGGCCGG CAGCAGGGCC CACCGTGGGC	120
TCGGCCCTGG CCCTGCAGCC GGGCCAGCGC CAGGCCATGC TGGGTGCGGG CACCACGGGG	180
GCAGCTGCCG GCGCAGTAGC GGAAGATGAC CTTCTCCTCT GAGGCGTAGC CCAGGCCTAG	240
CTCTGCCACG GACAGGGTCA GGCTCCACAG CTGGCATGGA CCAGACAGGG CTCGGCGCAG	300
GCGGGCAAGG GGGCGGTGGG TGCCTGTGGG AGGGAAGGGC GCTGACAGTG AGCAGGGTCA	360
GGGCTGGGGA AAGTTGGGAA GTCACAGAGG GTCCCTGGAA GTCCTTACCC AGCCAGGTCC	420
CTCCAGCCTT TGCCACCTGT TCAGACGAGA ACTCTCCATC GGCCACGGGA ACCCCACGGG	480
CATCGGG	487

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 242 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Mouse

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TACCGACGTC CTTCTGAAGC CTAGGACACA GACGACGAGG ACAGGAACGT GGGCTCGGAG	60
---	----

CCGACCCAGG AACTAGAAGT TCTCCGAAGA CACCGTCTAT TCGAGAGTAA ACCCTTCTAC 120
CGTCTCTGAT CTCCTGGAC CTGCGGGGTA GTCCCATCTT TAAGGACCCC CGGAGGGCTG 180
AGGGGTTAAG GAAGAGAGTT TCGGGAGTGA AACGGAATGT TAGGATGAGA TGGAACGTGA 240
TC 242

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Mouse

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AACTAGAAGT TCTCCGAAGA CACCGTCTAT TCGAGAGTAA ACCCTTCTAC CGTCTCTGAT 60
CTCCTGGAC CTGCGGGGTA GTCCCATCTT TAAGGACCCC CGGAGGGCTG AGGGGTTAAG 120
GAAGAGAGTT TCGGGAGTGA AACGGAATGT TAGGATGAGA TGGAACGTGA TC 172

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 242 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Rat

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TACCGACGTC CTTCTGAAGC CTAGAACAAA GACGACGAGG ACAGGAACGT GGACCCGGAA 60
CCGACCCAGG AACTAGAAGT TCTCCGAGGA CGCCGTCTAC TCGAGAGTAG ACCCTTTTAC 120
CGTCTCTGAC CTTCTGGAC CTTGCGGGTA GTCCCATCTT TAAGAACCCC CGGAGGATTG 180
AGATGTCAAG AAGGAGAGTT TCGGGAGTGA AACGGAGTGT TAGGATAAGA TGGAACGTGA 240
TC 242

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Rat

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AACTAGAAGT TCTCCGAGGA CGCCGTCTAC TCGAGAGTAG ACCCTTTTAC CGTCTCTGAC 60
CTTCCTGGAC CTTCCGGGGTA GTCCCATTCT TAAGAACCCC CGGAGGATTG AGATGTCAAG 120
AAGGAGAGTT TCGGGAGTGA AACGGAGTGT TAGGATAAGA TGGAACGTGA TC 172

- (2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTAAGGACTT CCAGGGACCC TCTGTGACTT CCCAACTTTC CCCAGCCCTG ACCCTGCTCA 60
CTGTCAGCGC CCTTCCCTCC CACAG 85

- (2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 88 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Rat

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTAAGAATTC CTGGGGGCCT CCCGACTCCC CAATTCCTTC TCTCAAAGCC CTCACCTTGC 60
CTTACAATCC TACTCTACCT TGCACTAG 88

- (2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 88 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rat

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GTAAGAATTC TTGGGGGCCT CCTAACTCTA CAGTTCTTCC TCTCAAAGCC CTCAC TTTGC 60
CTCACAATCC TATTCTACCT TGCAC TAG 88

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rat

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AGCTCAGCCA CTGGTAGGGT CAGG 24

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rat

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AGCTCAGCCA CTGGTAGGGT CAGG 24

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GTCACAATGG CCGTAGGGAA GTT

23

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CACCCCTCAGC CACCACAGCC GCA

23

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 594 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Mouse

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ATGGCTGCAG GAAGACTTCG GATCCTGTGT CTGCTGCTCC TGTCTTGCA CCCGAGCCTC	60
GGCTGGGTCC TTGATCTTCA AGAGGCTTCT GTGGCAGATA AGCTCTCATT TGGGAAGATG	120
GCAGAGACTA GAGGGACCTG GACGCCCCAT CAGGGTAAGA ATTCCTGGGG GCCTCCCGAC	180
TCCCCAATTC CTTCTCTCAA AGCCCTCACT TTGCCTTACA ATCCTACTCT ACCTTGCACT	240
AGGTAACAAC CATGTCCGTC TTCCAAGAGC CTTGGCTGGT TCATGCCGAC TGTGGAGCCT	300
GACCCTACCA GTGGCTGAGC TGGGCCTGGG CTATGCCTCG GAGGAGAAGG TCATCTTCCG	360
ATACTGTGCT GGCAGCTGTC CCCAAGAGGC CCGTACCCAG CACAGTCTGG TACTGGCCCCG	420
GCTTCGAGGG CGGGGTCGAG CCCATGGCCG ACCCTGCTGC CAGCCCACCA GCTATGCTGA	480
TGTGACCTTC CTTGATGATC AGCACCATTG GCAGCAGCTG CCTCAGCTCT CAGCTGCAGC	540
TTGTGGCTGT GGTGGCTGAA GGAGGCCAGT CTGGTGTCTC AGAATCACAA GCAT	594

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 559 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Rat

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATGGCTGCAG GAAGACTTCG GATCTTGTTT CTGCTGCTCC TGTCCCTGCA CCTGGGCCTT	60
GGCTGGGTCC TTGATCTTCA AGAGGCTCCT GCGGCAGATG AGCTCTCATC TGGGAAAATG	120
GCAGAGACTG GAAGGACCTG GAAGCCCCAT CAGGGTAAGA ATTCTTGGGG GCCTCCTAAC	180
TCTACAGTTC TTCCTCTCAA AGCCCTCACT TTGCCTCACA ATCCTATTCT ACCTTGCACT	240
AGGTAACAAC AATGTCCGCC TTCCAAGAGC CTTACCTGGT TTGTGCCGGC TGTGGAGCCT	300
GACCCACCA GTGGCTGAGC TTGGCCTGGG CTATGCCTCA GAGGAGAAGA TTATCTTCCG	360
ATACTGTGCT GGCAGCTGTC CCCAAGAGGT CCGTACCCAG CACAGTCTGG TGCTGGCCCG	420
TCTTCGAGGG CAGGGTCGAG CTCATGGCAG ACCTTGCTGC CAGCCCACCA GCTATGCTGA	480
TGTGACCTTC CTTGATGACC ACCACCATTG GCAGCAGCTG CCTCAGCTCT CAGCCGCAGC	540
TTGTGGCTGT GGTGGCTGA	559