



US 20050208626A1

(19) **United States**

(12) **Patent Application Publication**
Sheppard et al.

(10) **Pub. No.: US 2005/0208626 A1**

(43) **Pub. Date: Sep. 22, 2005**

(54) **SGIP PEPTIDES**

No. 09/608,810, filed on Jun. 30, 2000, now Pat. No. 6,420,521.

(75) Inventors: **Paul O. Sheppard**, Granite Falls, WA (US); **Stephen R. Jaspers**, Edmonds, WA (US); **Theresa A. Deisher**, Seattle, WA (US); **Paul D. Bishop**, Fall City, WA (US)

(60) Provisional application No. 60/141,592, filed on Jun. 30, 1999.

Publication Classification

Correspondence Address:
AMGEN INC.
LAW DEPARTMENT
1201 AMGEN COURT WEST
SEATTLE, WA 98119 (US)

(51) **Int. Cl.⁷** **C07H 21/04**; C07K 7/08
(52) **U.S. Cl.** **435/69.1**; 435/320.1; 435/325;
530/327; 536/23.1

(57) **ABSTRACT**

(73) Assignee: **ZymoGenetics, Inc.**

(21) Appl. No.: **11/133,912**

(22) Filed: **May 19, 2005**

Related U.S. Application Data

(63) Continuation of application No. 10/186,414, filed on Jul. 1, 2002, which is a continuation of application

The present invention is directed to polynucleotides, polypeptides, peptides, variants and uses thereof for novel peptide fragments which have homology to motilin. Tissue distribution of the mRNA for the novel polypeptide fragment is specific to the stomach, small intestine and pancreas. Binding of the peptide fragment has been shown in kidney and small intestine. The present invention further includes agonists, antagonists, variants, antibodies and host cells expressing the cDNA encoding the novel SGIP peptide.

SGIP PEPTIDES

[0001] This application is a continuation of application Ser. No. 10/186,414, filed Jul. 1, 2002, which is a continuation of application Ser. No. 09/608,810 filed Jun. 30, 2000, now U.S. Pat. No. 6,420,521, which claims benefit to Provisional Application No. 60/141,592 filed Jun. 30, 1999. The entire disclosures of these applications are relied upon and incorporated by reference herein.

BACKGROUND OF THE INVENTION

[0002] Many of the regulatory peptides that are important in maintaining nutritional homeostasis are found in the gastrointestinal environment. These peptides may be synthesized in the digestive system and act locally, but can also be identified in the brain as well. In addition, the reverse is also found, i.e., peptides are synthesized in the brain, but found to regulate cells in the gastrointestinal tract. This phenomena has been called the "brain-gut axis" and is important for signaling satiety, regulating body temperature and other physiological processes that require feedback between the brain and gut.

[0003] The gut peptide hormones include gastrin, cholecystokinin (CCK), secretin, gastric inhibitory peptide (GIP), vasoactive intestinal polypeptide (VIP), motilin, somatostatin, pancreatic peptide (PP), substance P and neuropeptide Y (NPY), and use several different mechanisms of action. For example, gastrin, motilin and CCK function as endocrine- and neurocrine-type hormones. Others, such as gastrin and GIP, are thought to act exclusively in an endocrine fashion. Other modes of action include a combination of endocrine, neurocrine and paracrine action (somatostatin); exclusively neurocrine action (NPY); and a combination of neurocrine and paracrine actions (VIP and Substance P). Most of the gut hormone actions are mediated by membrane-bound receptors and activate second messenger systems. For a review of gut peptides see, Mulvihill et al., in *Basic and Clinical Endocrinology*, pp. 551-570, 4th edition Greenspan F. S. and Baxter, J. D. editors., Appleton & Lange: Norwalk, Conn., 1994.

[0004] Many of these gut peptides are synthesized as inactive precursor molecules that require multiple peptide cleavages to be activated. The family known as the "glucagon-secretin" family which includes VIP, gastrin, secretin, motilin, glucagon and galanin exemplifies peptides regulated by multiple cleavages and post-translational modifications.

[0005] Motilin is a 22 amino acid peptide found in gut tissue of mammalian species (Domschke, W., *Digestive Diseases* 22(5):454-461, 1977). The DNA and amino acid sequences for porcine prepromotilin have been identified (U.S. Pat. No. 5,006,469). Motilin has been identified as a factor capable of increasing gastric motility, affecting the secretory function of the stomach by stimulating pepsin secretion (Brown et al., *Canadian J. of Physiol. Pharmacol.* 49:399405, 1971), and recent evidence suggests a role in myoelectric regulation of stomach and small intestine. Cyclic increases of motilin have been correlated with phase III of the interdigestive myoelectric complex and the hunger contraction of the duodenum (Chey et al., in *Gut Hormones*, (eds.) Bloom, S. R., pp. 355-358, Edinburgh, Churchill Livingstone, 1978; Lee et al, *Am. J. Digestive Diseases*, 23:789-795, 1978; and Itoh et al., *Am. J. Digestive Diseases*,

23:929-935, 1978). Motilin and analogues of motilin have been demonstrated to produce contraction of gastrointestinal smooth muscle, but not other types of smooth muscle cells (Strunz et al., *Gastroenterology* 68:1485-1491, 1975).

[0006] The present invention is directed to a novel peptide fragment, and the DNA segment encoding it, of a previously described secreted protein, zsig33 (Sheppard, P. O., WO98/42840: 1998). The present invention is also directed to a limited number of variants of said peptide fragment. The discovery of this novel peptide fragment is important for further elucidation of the how the body maintains its nutritional homeostasis and development of therapeutics to intervene in those processes, as well as other uses that will be apparent from the teachings herein.

SUMMARY OF THE INVENTION

[0007] Within one aspect, the present invention provides an isolated nucleic acid molecule encoding an isolated peptide molecule selected from the group consisting of (a) residue 1 (Gly) to residue 9 (His); (b) residue 2 (Ser) to residue 9 (His); (c) residue 3 (Ser) to residue 9 (His); and (d) residue 4 (Phe) to residue 9 (His); all of SEQ ID NO:2. Within one embodiment, the invention provides for the isolated peptide molecule encoded by said isolated nucleic acid molecule.

[0008] Within another aspect the invention provides an isolated polypeptide molecule comprising residues X through 9 of SEQ ID NO:6, wherein X is an integer from 1 to 4, inclusive, and wherein at least Y of said residues are as in the corresponding region of SEQ ID NO:2, wherein Y is 9 minus X. Within an embodiment the invention provides a method of modulating contractility and protein secretion in stomach, duodenum or jejunum tissue comprising applying said isolated polypeptide to said tissue. Within another embodiment is provided a method of modulating pancreatic secretion of hormones and digestive enzymes in a mammal comprising administering the isolated polypeptide of claim 1 to a mammal.

[0009] Within another aspect the present invention provides an isolated polypeptide molecule consisting of residues X through Y of SEQ ID NO:6, wherein Y is 10 or 11 and X is an integer from 1 to 4, inclusive, and at least (Y minus X) minus 3 residues are as in the corresponding region of SEQ ID NO:2.

[0010] Within another aspect is provided an isolated polypeptide molecule consisting of residues X through Y of SEQ ID NO:6, wherein Y is 10 or 11 and X is an integer from 1 to 4, inclusive, and at least (Y minus X) minus 2 residues are as in the corresponding region of SEQ ID NO:2. Within an embodiment the invention provides a method of modulating contractility in duodenum or jejunum tissue comprising applying the isolated polypeptide said tissue. Within another embodiment is provided a method of modulating pancreatic secretion of hormones and digestive enzymes comprising administering the isolated polypeptide to a mammal.

[0011] Within another aspect, the invention provides an isolated polypeptide molecule consisting of residues X through Y of SEQ ID NO:6, wherein Y is 10 or 11 and X is an integer from 1 to 4, inclusive, and at least (Y minus X) minus 1 residues are as in the corresponding region of SEQ

ID NO:2. Within an embodiment is provided a method of modulating contractility in duodenum or jejunum tissue comprising applying the isolated polypeptide to said tissue. Within another embodiment is provided a method of modulating pancreatic secretion of hormones and digestive enzymes comprising administering the isolated polypeptide to a mammal.

[0012] Within another aspect the invention provides an isolated polypeptide consisting of up to nine amino acids as shown in the amino acid sequence of SEQ ID NO:2. Within one embodiment the polypeptide has at its amino terminal residue 1 (Gly), residue 2 (Ser), residue 3 (Ser) or residue 4 (Phe) as shown in SEQ ID NO:2, and the polypeptide has at its carboxyl terminal residue 9 (His) as shown in SEQ ID NO:2. Within an embodiment, the polypeptide has up to one amino acid substitution. Within another embodiment, is provided a method of stimulating contractility in duodenum or jejunum tissue comprising administering the polypeptide, with or without amino acid substitutions to the tissue. Within another embodiment is provided a method of modulating pancreatic secretion of hormones and digestive enzymes comprising administering the polypeptide, with or without substitutions, to a mammal. Within another embodiment is provided a method of stimulating growth hormone secretion comprising administering the polypeptide, with or without substitutions, to a mammal.

[0013] Within another aspect the invention provides an isolated polypeptide wherein the polypeptide has at its amino terminal residue 2 (Ser), residue 3 (Ser) or residue 4 (Phe) as shown in SEQ ID NO:2, and the polypeptide has at its carboxyl terminal residue 10 (Gln) as shown in SEQ ID NO:2. Within an embodiment, the polypeptide has up to three amino acid substitutions. Within another embodiment, is provided a method of stimulating contractility in duodenum or jejunum tissue comprising administering the polypeptide, with or without amino acid substitutions to the tissue. Within another embodiment is provided a method of modulating pancreatic secretion of hormones and digestive enzymes comprising administering the polypeptide, with or without substitutions, to a mammal. Within another embodiment is provided a method of stimulating growth hormone secretion comprising administering the polypeptide, with or without substitutions, to a mammal.

[0014] Within another aspect the invention provides an isolated polypeptide wherein the polypeptide has at its amino terminal residue 3 (Ser) or residue 4 (Phe) as shown in SEQ ID NO:2, and the polypeptide has at its carboxyl terminal residue 10 (Gln) as shown in SEQ ID NO:2. Within an embodiment, the polypeptide has up to three amino acid substitution. Within another embodiment, is provided a method of stimulating contractility in duodenum or jejunum tissue comprising administering the polypeptide, with or without amino acid substitutions to the tissue. Within another embodiment is provided a method of modulating pancreatic secretion of hormones and digestive enzymes comprising administering the polypeptide, with or without substitutions, to a mammal. Within another embodiment is provided a method of stimulating growth hormone secretion comprising administering the polypeptide, with or without substitutions, to a mammal.

[0015] Within another aspect the invention provides an isolated polypeptide molecule, wherein the polypeptide mol-

ecule is selected from the group consisting of: a) a polypeptide molecule consisting of residues 1 to 9 as shown in SEQ ID NO:2; b) a polypeptide molecule consisting of residues 2 to 9 as shown in SEQ ID NO:2; c) a polypeptide molecule consisting of residues 3 to 9 as shown in SEQ ID NO:2; d) a polypeptide molecule consisting of residues 4 to 9 as shown in SEQ ID NO:2; e) a polypeptide molecule consisting of residues 2 to 10 as shown in SEQ ID NO:2; f) a polypeptide molecule consisting of residues 3 to 10 as shown in SEQ ID NO:2; g) a polypeptide molecule consisting of residues 4 to 10 as shown in SEQ ID NO:2; h) a polypeptide molecule consisting of residues 3 to 11 as shown in SEQ ID NO:2; and i) a polypeptide molecule consisting of residues 4 to 11 as shown in SEQ ID NO:2; wherein the polypeptide molecule has up to one amino acid substitution at residue X, wherein residue X is an integer from 1 to 11, inclusive, and wherein the amino acid is substituted with the corresponding amino acid at residue X as shown in SEQ ID NO:6. Within an embodiment, is provided a method of stimulating contractility in duodenum or jejunum tissue comprising administering the polypeptide, with or without amino acid substitutions to the tissue. Within another embodiment is provided a method of modulating pancreatic secretion of hormones and digestive enzymes comprising administering the polypeptide, with or without substitutions, to a mammal. Within another embodiment is provided a method of stimulating growth hormone secretion comprising administering the polypeptide, with or without substitutions, to a mammal. Within one embodiment, the isolated polypeptide molecule consists of residues 1 to 9 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 2 to 9 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 3 to 9 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 4 to 9 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 2 to 10 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 3 to 10 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 4 to 10 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 3 to 11 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 4 to 11 as shown in SEQ ID NO:2. Within another embodiment of the invention, residue 3 (Ser) is acylated. Within a further embodiment, residue 3 (Ser) has an n-octanoic acid acylation.

[0016] Within another aspect the invention provides an isolated polypeptide molecule, wherein the polypeptide molecule is selected from the group consisting of: a) a polypeptide molecule consisting of residues 1 to 9 as shown in SEQ ID NO:2; b) a polypeptide molecule consisting of residues 2 to 9 as shown in SEQ ID NO:2; c) a polypeptide molecule consisting of residues 3 to 9 as shown in SEQ ID NO:2; d) a polypeptide molecule consisting of residues 4 to 9 as shown in SEQ ID NO:2; e) a polypeptide molecule consisting of residues 2 to 10 as shown in SEQ ID NO:2; f) a polypeptide molecule consisting of residues 3 to 10 as shown in SEQ ID NO:2; g) a polypeptide molecule consisting of residues 4 to 10 as shown in SEQ ID NO:2; h) a polypeptide molecule consisting of residues 3 to 11 as shown in SEQ ID NO:2; and i) a polypeptide molecule

consisting of residues 4 to 1 as shown in SEQ ID NO:2. Within an embodiment, is provided a method of stimulating contractility in duodenum or jejunum tissue comprising administering the polypeptide, with or without amino acid substitutions to the tissue. Within another embodiment is provided a method of modulating pancreatic secretion of hormones and digestive enzymes comprising administering the polypeptide, with or without substitutions, to a mammal. Within another embodiment is provided a method of stimulating growth hormone secretion comprising administering the polypeptide, with or without substitutions, to a mammal. Within one embodiment, the isolated polypeptide molecule consists of residues 1 to 9 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 2 to 9 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 3 to 9 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 4 to 9 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 2 to 10 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 3 to 10 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 4 to 10 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 3 to 11 as shown in SEQ ID NO:2. Within another embodiment the polypeptide molecule consists of residues 4 to 11 as shown in SEQ ID NO:2. Within another embodiment of the invention, residue 3 (Ser) is acylated. Within a further embodiment, residue 3 (Ser) has an n-octanoic acid acylation.

[0017] Within another aspect, the invention provides an isolated polynucleotide molecule having a polynucleotide sequence selected from the group consisting of: a polynucleotide sequence as shown in SEQ ID NO:1; a polynucleotide molecule that is complementary to the polynucleotide sequence as shown in SEQ ID NO:1; and a polynucleotide sequence as shown in SEQ ID NO:7. Within an embodiment, is also provided a polynucleotide vector comprising said isolated polynucleotide molecule, a transcription promoter, and a transcription terminator, wherein the promoter is operably linked with the nucleic acid molecule, and wherein the nucleic acid molecule is operably linked with the transcription terminator.

DETAILED DESCRIPTION OF THE INVENTION

[0018] Prior to describing the present invention in detail, it may be helpful to define certain terms used herein:

[0019] The term “ortholog” denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

[0020] “Paralogs” are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, β -globin, γ -globin, and myoglobin are paralogs of each other.

[0021] The term “allelic variant” denotes any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymor-

phism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

[0022] The term “expression vector” denotes a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments may include promoter and terminator sequences, and may optionally include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, and the like. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

[0023] The term “isolated” when applied to a polynucleotide molecule, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems. Such isolated molecules are those that are separated from their natural environment and include cDNA and genomic clones. Isolated DNA molecules of the present invention are free of other genes with which they are ordinarily associated, but may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated regions will be evident to one of ordinary skill in the art (see for example, Dynan and Tijan, *Nature* 316:774-78, 1985). When applied to a protein, the term “isolated” indicates that the protein is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated protein is substantially free of other proteins, particularly other proteins of animal origin. It is preferred to provide the protein in a highly purified form, i.e., greater than 95% pure, more preferably greater than 99% pure.

[0024] The term “corresponding to”, when applied to positions of amino acid residues in sequences, means corresponding positions in a plurality of sequences when the sequences are optimally aligned.

[0025] The term “operably linked”, when referring to DNA segments, denotes that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in the promoter and proceeds through the coding segment to the terminator

[0026] The term “polynucleotide” denotes a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized in vitro, or prepared from a combination of natural and synthetic molecules.

[0027] The term “complements of polynucleotide molecules” denotes polynucleotide molecules having a complementary base sequence and reverse orientation as compared to a reference sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

[0028] The term “degenerate nucleotide sequence” denotes a sequence of nucleotides that includes one or more degenerate codons (as compared to a reference polynucleotide molecule that encodes a polypeptide). Degenerate

codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

[0029] The term “promoter” denotes a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

[0030] The term “secretory signal sequence” denotes a DNA sequence that encodes a polypeptide (a “secretory peptide”) that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger peptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

[0031] The term “receptor” denotes a cell-associated protein that binds to a bioactive molecule (i.e., a ligand) and mediates the effect of the ligand on the cell. Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. Binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell. This interaction in turn leads to an alteration in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. Most nuclear receptors also exhibit a multi-domain structure, including an amino-terminal, transactivating domain, a DNA binding domain and a ligand binding domain. In general, receptors can be membrane bound, cytosolic or nuclear; monomeric (e.g., thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (e.g., PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor).

[0032] The term “complement/anti-complement pair” denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of $<10^9 \text{ M}^{-1}$.

[0033] All references cited herein are incorporated by reference in their entirety.

[0034] The present invention is based in part upon the discovery of a novel peptide fragment, and the DNA segment encoding it, of a previously described secreted polypeptide, known as zsig33 (Sheppard, P. O., WO 98/42840). Zsig33 (shown in SEQ ID NOs: 3 and 4) has homology to motilin (shown in SEQ ID NO:5), and has been found to be transcribed in the gastrointestinal system. The novel peptide fragment of the present invention has homol-

ogy to the minimal basic unit of motilin which is required for binding of motilin to its receptor(s) (Miller, P. et al., *Peptides* 16(1):11-18). This novel peptide fragment and the cDNA encoding it has been designated SGIP and correlates to SEQ ID NO:s 1 and 2 for polynucleotides and polypeptides, respectively. Additionally, novel variants of this peptide fragment are described herein in SEQ ID NO:6.

[0035] Motilin is a member of a family of polypeptides that regulate the gastrointestinal physiology. The family of polypeptides important in gastrointestinal regulation to which motilin belongs includes glucagon, gastrin, galanin, and vasoactive intestinal peptide (VIP). These polypeptides are synthesized in a precursor form that requires multiple steps of processing to the active form. Particularly relevant to the polypeptide of the present invention are motilin, VIP and galanin, where processing involves removal of signal sequence, followed by cleavage of one or more accessory peptides to release the active peptide. The resulting active peptide may require further post-translational modifications, such as amidation, acylation, sulfation or pyrrolidone carboxylic acid modification of glutamic residues.

[0036] Analysis of the tissue distribution of the mRNA corresponding to said secreted protein, zsig33, showed that expression was highest in stomach, followed by apparent but decreased expression levels in small intestine and pancreas. The EST for the secreted protein, zsig33, was derived from a pancreatic library, and has been also been shown in lung cDNA libraries. Thus, the mRNA for the novel peptide fragment can also be localized to these tissues.

[0037] Studies involving the binding of porcine motilin to its receptor have shown that the minimal basic unit of motilin required for receptor binding is the sequence between residues 26 (Phe) and 32 (Tyr) of SEQ ID NO:5 and that the activity is mainly determined by these residues (Miller, P. et al., *Peptides* 16(1):11-18, 1995; and Peeters, T. L. et al., *Peptides* 13(6):1103-1107, 1992). It should be noted that serine (residue 29 of SEQ ID NO:5) of the receptor binding site of motilin has been shown to be an isoleucine by Schubert, H. et al., *Can. J. Biochem.* 52:7-8, 1974. A comparison of the polypeptide sequence of zsig33 (SEQ ID NO:4) with that of motilin (SEQ ID NO:5) predicts that residues 4 (Phe) to 9 (His) of SEQ ID NO:4 correspond to the receptor binding portion of motilin (residues 26 to 32 of SEQ ID NO:5). Thus, the active peptide, or minimal basic unit required for binding of zsig33 to its receptor can be as short as the sequence between residue 4 (Phe) and residue 9 (His) of SEQ ID NO:4. Substitutions of residues within this six residue peptide, can result in variants with altered affinity of the peptide for the receptor or altered activation of the receptor. Such alterations can result in agonistic as well as antagonistic activity. Furthermore, this comparison suggests that residues 4 (Phe), 5 (Leu), 6 (Ser) and 9 (His) of SEQ ID NO:2 (corresponding to residues 4, 5, 6, and 9 of SEQ ID NO:4) are particularly important residues for receptor binding and/or activity.

[0038] Additional substitutions of residues of SGIP polypeptides and peptides are further described herein. Substitutions of amino acids which can result in a SGIP variant which binds the receptor with high affinity, but causes low receptor activation are likely to be conservative substitutions at positions between residue 4 to residue 9 of SEQ ID NO:2,

preferably at residues 4 (Phe), 5 (Leu), 7 (Pro), and 9 (His). As such, substitutions at these positions are potential antagonists of SGIP.

[0039] Substitutions of residues of the SGIP peptides and polypeptides may also result in variants which are agonists. Such substitutions may be based on conservative amino acid substitutions as in Table 2, or based on predictions made by comparison to the active peptide of motilin as in Table A. These substitutions include positions 4 (Phe), 5 (Leu), and 9 (His). It is predicted, for example, that residue 4 (Phe) can be substituted with leucine, valine, isoleucine, tryptophan, or tyrosine; residue 5 (Leu) can be substituted with phenylalanine, valine, tyrosine or isoleucine; and that residue 9 (His) can be substituted with phenylalanine, arginine, tyrosine or lysine. Similarly, residue 7 (Pro) can be substituted with alanine, glycine, isoleucine, valine, or leucine.

[0040] Additionally, there are positions of SGIP at which mutations are not predicted to result in alteration of the binding affinity or activation of the receptor upon binding these mutants. These positions include, residue 6 (Ser) of SEQ ID NO:2, for example, at which position substitution with alanine, proline, threonine, methionine or glycine is not predicted to alter the binding of the mutant, or variants, as compared to wild-type SGIP.

[0041] Multiple cleavages by signal peptidase are expected in the present invention. Thus, the amino terminal of the SGIP active peptide may begin with glycine, residue 1 of SEQ ID NO:2, serine, residues 2 or 3 of SEQ ID NO:2 or phenylalanine, residue 4 of SEQ ID NO:2. These positions correlate to residues 1 through 4 SEQ ID NO:6.

[0042] Based on a comparison of the residues of SGIP to the residues of motilin which are known to be involved in binding the motilin receptor, there are variants of the fragment peptide of residues 1 to 11 of SEQ ID NO:2 which have increased receptor affinity or activation. These variants are also listed in SEQ ID NO:6. Thus, residues 1 through 11 of SEQ ID NOs:2 and 6 correspond to residues 1 through 11 of SEQ ID NO:4. Table A describes the possible substitutions for these variants. Variant peptides of SGIP may have more than one substitution. A variant peptide of having eleven or fewer amino acids has preferably three or fewer amino acid substitutions. A variant peptide of having eleven or fewer amino acids has more preferably two or fewer amino acid substitutions. Even more preferably, a variant peptide having eleven or fewer amino acids will have one amino acid substitution. Such substitutions are also supported by comparison of these variants with known analogs of motilin which are further described by Peeters, *ibid*.

[0043] A receptor for motilin has been identified in the gastrointestinal system (Feighner, S. D. et al., *Science* 284:2184-8, 1999). Two forms of the motilin receptor (GPR38-A, and GPR38-B) were shown resulting from alternative splicing events. Thus, the SGIP receptor is likely to be a member of this seven transmembrane G protein-coupled receptor homolog class. Receptors in this class can be used for screening variants of SGIP peptides for binding and activity. Members of this receptor class appear to activate the phospholipase C signal transduction pathway. Hence, variants of SGIP peptides can also be tested using an assay that measures phospholipase C transduction. An exemplary assay of this sort measures Ca^{2+} release with a aequorin, a bioluminescent Ca^{2+} -sensitive reporter protein. This assay is further described by Feighner, S. D. et al., *ibid*.

[0044] There are critical residues of the carboxyl terminal of motilin, which if the peptide is truncated at these residues, there is a sharp decrease in receptor binding (cloned human and native rabbit) and activity (Feighner, S. D. et al., *ibid*). These positions are residues 36 (Gln), and residue 37 (Arg) of motilin as shown in SEQ ID NO:5. Similarly, SGIP peptides which terminate in residues corresponding to residues 36 and 37 of motilin can result in a decrease in binding and activity of SGIP peptides. Specifically, these residues are residue 10 (Gln) and residue 11 (Arg). Potential amino acid substitutions of these residues are described in Table A.

[0045] Binding studies have suggested that motilin binds two heterogeneous receptors with varying affinities (Poitras, P., *Peptides* 17:701-707, 1996) suggesting that there are two forms of motilin binding these different receptors. One such receptor is located in the neural cells of the antrum, and the second receptor is located in the smooth muscle cells of the duodenum. Similarly, there may be more than one receptor which binds the SGIP ligand, or variants thereof, and the binding affinities may vary. Thus, the binding of SGIP polypeptides and peptides to its receptor(s) may result in different and varying biological events depending on the form of SGIP and the specific receptor-type to which it binds.

TABLE A

Peptide Substitutions			
Position: SEQ ID NO: 2	Position: SEQ ID NO: 4	Wild-Type Residue	Substitutions: SEQ ID NO: 6
1	1	Gly	Ser, Ala, Thr, Met
2	2	Ser	Gly, Ala, Thr, Met
3	3	Ser	Gly, Ala, Thr, Met
4	4	Phe	Trp, Tyr, Leu, Val, Ile
5	5	Leu	Ile, Val, Phe, Tyr
6	6	Ser	Gly, Ala, Thr, Met, Pro
7	7	Pro	Ala, Gly, Ile, Leu, Val
8	8	Glu	Asp
9	9	His	Arg, Lys, Phe, Tyr
10	10	Gln	Asn, Ser, Thr, His, Ala, Glu, Asp, Lys, Arg
11	11	Arg	Gln, Asn, Ser, Thr, His, Ala

[0046] Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules encoding SEQ ID NO:2, including all RNA sequences by substituting U for T. Thus, SGIP polypeptide-encoding polynucleotides and their RNA equivalents are contemplated by the present invention. Table 1 sets forth the one-letter codes used to denote degenerate nucleotide positions. "Resolutions" are the nucleotides denoted by a code letter. "Complement" indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, A being complementary to T, and G being complementary to C.

TABLE 1

Nucleotide	Resolution	Nucleotide	Complement
A	A	T	T
C	C	G	G
G	G	C	C

TABLE 1-continued

Nucleotide	Resolution	Nucleotide	Complement
T	T	A	A
R	A G	Y	C T
Y	C T	R	A G
M	A C	K	G T
K	G T	M	A C
S	C G	S	C G
W	A T	W	A T
H	A C T	D	A G T
B	C G T	V	A C G
V	A C G	B	C G T
D	A G T	H	A C T
N	A C G T	N	A C G T

[0047] The degenerate codons encompassing all possible codons for a given amino acid are set forth in Table 2.

TABLE 2

Amino Acid	One Letter Code	Codons	Degenerate Codon
Cys	C	TGC TGT	TGY
Ser	S	AGC AGT TCA TCC TCG TCT	WSN
Thr	T	ACA ACC ACG ACT	ACN
Pro	P	CCA CCC CCG CCT	CCN
Ala	A	GCA GCC GCG GCT	GCN
Gly	G	GGA GGC GGG GGT	GGN
Asn	N	AAC AAT	AAY
Asp	D	GAC GAT	GAY
Glu	E	GAA GAG	GAR
Gln	Q	CAA CAG	CAR
His	H	CAC CAT	CAY
Arg	R	AGA AGG CGA CGC CGG CGT	MGN
Lys	K	AAA AAG	AAR
Met	M	ATG	ATG
Ile	I	ATA ATC ATT	ATH
Leu	L	CTA CTC CTG CTT TTA TTG	YTN
Val	V	GTA GTC GTG GTT	GTN
Phe	F	TTC TTT	TTY
Tyr	Y	TAC TAT	TAY

TABLE 2-continued

Amino Acid	One Letter Code	Codons	Degenerate Codon
Trp	W	TGG	TGG
Ter	.	TAA TAG TGA	TRR
Asn Asp	B		RAY
Glu Gln	Z		SAR
Any	X		NNN

[0048] One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding each amino acid. For example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequence of SEQ ID NO:2. Variant sequences can be readily tested for functionality as described herein.

[0049] Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar sized regions of SEQ ID NO: 1, or a sequence complementary thereto, under stringent conditions. In general, stringent conditions are selected to be about 5° C. lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typical stringent conditions are those in which the salt concentration is at least about 0.02 M at pH 7 and the temperature is at least about 60° C.

[0050] As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for isolating DNA and RNA are well known in the art. It is generally preferred to isolate RNA from stomach, although DNA can also be prepared using RNA from other tissues or isolated as genomic DNA. Total RNA can be prepared using guanidine HCl extraction followed by isolation by centrifugation in a CsCl gradient (Chirgwin et al., *Biochemistry* 18:52-94, 1979). Poly (A)⁺ RNA is prepared from total RNA using the method of Aviv and Leder (*Proc. Natl. Acad. Sci. USA* 69:1408-1412, 1972). Complementary DNA (cDNA) is prepared from poly(A)⁺ RNA using known methods. Polynucleotides encoding SGIP polypeptides are then identified and isolated by, for example, hybridization or PCR.

[0051] The present invention further provides counterpart polypeptides and polynucleotides from other species (orthologs). Of particular interest are SGIP polypeptides from other mammalian species, including murine, rat, porcine, ovine, bovine, canine, feline, equine and other primate proteins. Orthologs of the human proteins can be cloned using information and compositions provided by the present

invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses the protein. Suitable sources of mRNA can be identified by probing Northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue of cell line. A SGIP ortholog-encoding cDNA can then be isolated by a variety of methods, such as

ventional methods. See, for example, Altschul et al., *Bull. Math. Bio.* 48: 603-616, 1986 and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915-10919, 1992. Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "blosum 62" scoring matrix of Henikoff and Henikoff (ibid.) as shown in Table 3 (amino acids are indicated by the standard one-letter codes).

TABLE 3

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	0	-1	-2	-1	4					
T	0	-1	0	-1	-1	-1	-2	-2	-1	-1	-1	-2	-1	1	5					
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Pat. No. 4,683,202), using primers designed from the sequences disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to SGIP. Similar techniques can also be applied to the isolation of genomic clones.

[0052] Those skilled in the art will recognize that the sequences disclosed in SEQ ID NO: 1, and polypeptide encoded thereby, represent a single allele of the human SGIP gene and polypeptide, and that allelic variation and alternative splicing are expected to occur. Allelic variants can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic variants of the DNA sequence shown in SEQ ID NO: 1, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are the product of allelic variation of SEQ ID NO: 2.

[0053] The present invention also provides isolated SGIP polypeptides that are substantially homologous to the polypeptides of SEQ ID NO: 2 and their orthologs. The term "substantially homologous" is used herein to denote polypeptides having 50%, preferably 60%, more preferably at least 80%, sequence identity to the sequences shown in SEQ ID NO: 2 or their orthologs. Such polypeptides will more preferably be at least 90% identical, and most preferably 95% or more identical to SEQ ID NO: 2 or its orthologs. Percent sequence identity is determined by con-

[0054] The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{\left[\begin{array}{l} \text{length of the longer sequence plus the number of} \\ \text{gaps introduced into the longer sequence in order} \\ \text{to align the two sequences} \end{array} \right]} \times 100$$

[0055] Sequence identity of polynucleotide molecules is determined by similar methods using a ratio as disclosed above.

[0056] Substantially homologous proteins and polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 4) and other substitutions that do not significantly affect the folding or activity of the protein or polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification (an affinity tag), such as a poly-histidine tract, protein A (Nilsson et al., *EMBO J.* 4:1075, 1985; Nilsson et al., *Methods Enzymol.* 198:3, 1991), glutathione S transferase (Smith and Johnson, *Gene* 67:31, 1988), maltose binding protein (Kellerman and Ferenci, *Methods Enzymol.* 90:459-463, 1982; Guan et al., *Gene* 67:21-30, 1987), thioredoxin, ubiquitin, cellulose binding protein, T7 polymerase, or other antigenic epitope or binding domain. See, in general Ford et al., *Protein Expression and Purification* 2: 95-107, 1991, which is

incorporated herein by reference. DNAs encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, N.J.; New England Biolabs, Beverly, Mass.).

TABLE 4

Conservative amino acid substitutions	
Basic:	arginine lysine histidine
Acidic:	glutamic acid aspartic acid
Polar:	glutamine asparagine
Hydrophobic:	leucine isoleucine valine
Aromatic:	phenylalanine tryptophan tyrosine
Small:	glycine alanine serine threonine methionine

[0057] In addition to the 20 standard amino acids, non-standard amino acids (such as 4-hydroxyproline, 6-N-methyl lysine, 2-aminoisobutyric acid, isovaline and γ -methyl serine) may be substituted for amino acid residues of SGIP. A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, and unnatural amino acids may be substituted for SGIP amino acid residues. "Unnatural amino acids" have been modified after protein synthesis, and/or have a chemical structure in their side chain(s) different from that of the standard amino acids. Unnatural amino acids can be chemically synthesized, or preferably, are commercially available, and include pipercolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, and 3,3-dimethylproline.

[0058] Essential amino acids in the SGIP polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244: 1081-1085, 1989). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (e.g., stimulation of gastrointestinal contractility, modulation of nutrient uptake, modulation of the secretion of digestive enzymes and hormones, modulation of secretion of enzymes and/or hormones in the pancreas, binding a SGIP receptor, or binding an antibody that specifically binds to residues 1 to 11 of SEQ ID NO:2) to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton et al., *J. Biol. Chem.* 271:4699-4708, 1996. Sites of ligand-receptor interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos et al., *Science* 255:306-312, 1992; Smith et al., *J. Mol. Biol.* 224:899-904, 1992; Wlodaver et al., *FEBS Lett.* 309:59-64, 1992. The identities of essential amino acids can also be inferred from

analysis of homologies with related members of the glucagon-secretin family of gut-brain peptide hormones.

[0059] Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (*Science* 241:53-57, 1988) or Bowie and Sauer (*Proc. Natl. Acad. Sci. USA* 86:2152-2156, 1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (e.g., Lowman et al., *Biochem.* 30:10832-10837, 1991; Ladner et al., U.S. Pat. No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis (Derbyshire et al., *Gene* 46:145, 1986; Ner et al., *DNA* 7:127, 1988).

[0060] Mutagenesis methods as disclosed above can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode active peptides or polypeptides (e.g., stimulation of gastrointestinal contractility, modulation of nutrient uptake, modulation of the secretion of digestive enzymes and hormones, modulation of secretion of enzymes and/or hormones in the pancreas, binding a SGIP receptor, or binding an antibody that specifically binds to residues 1 to 11 of SEQ ID NO:2) can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

[0061] Using the methods discussed above, one of ordinary skill in the art can identify and/or prepare a variety of peptides and polypeptides that are substantially homologous to residues 1 to 11 of SEQ ID NO: 2 or allelic variants thereof and retain properties of the wild-type protein. Such polypeptides may also include additional polypeptide segments as generally disclosed herein.

[0062] The polypeptides of the present invention, including full-length proteins and fragments thereof, can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, and Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY, 1987, which are incorporated herein by reference.

[0063] In general, a DNA sequence encoding a SGIP polypeptide of the present invention is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of

replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

[0064] To direct a SGIP polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be derived from a secreted protein (e.g., t-PA) or synthesized de novo. The secretory signal sequence is joined to the SGIP DNA sequence in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the propeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch et al., U.S. Pat. No. 5,037,743; Holland et al., U.S. Pat. No. 5,143,830).

[0065] Cultured mammalian cells are also preferred hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler et al., *Cell* 14:725, 1978; Corsaro and Pearson, *Somatic Cell Genetics* 7:603, 1981; Graham and Van der Eb, *Virology* 52:456, 1973), electroporation (Neumann et al., *EMBO J.* 1:841-845, 1982), DEAE-dextran mediated transfection (Ausubel et al., eds., *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY, 1987), liposome-mediated transfection (Hawley-Nelson et al., *Focus* 15:73, 1993; Ciccarone et al., *Focus* 15:80, 1993), and viral vectors (A. Miller and G. Rosman, *BioTechniques* 7:980-90, 1989; Q. Wang and M. Finer, *Nature Med.* 2:714-16, 1996), which are incorporated herein by reference. The production of recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson et al., U.S. Pat. No. 4,713,339; Hagen et al., U.S. Pat. No. 4,784,950; Palmiter et al., U.S. Pat. No. 4,579,821; and Ringold, U.S. Pat. No. 4,656,134, which are incorporated herein by reference. Preferred cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham et al., *J. Gen. Virol.* 36:59-72, 1977) and Chinese hamster ovary (e.g. CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Md. In general, strong transcription promoters are preferred, such as promoters from SV40 or cytomegalovirus. See, e.g., U.S. Pat. No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Pat. Nos. 4,579,821 and 4,601,978, which are incorporated herein by reference) and the adenovirus major late promoter.

[0066] Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the

presence of a neomycin-type drug, such as G-418 or the like. Selection systems may also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g., hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternative markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

[0067] Other higher eukaryotic cells can also be used as hosts, including plant cells, insect cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar et al., *J. Biosci. (Bangalore)* 11:47-58, 1987. Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino et al., U.S. Pat. No. 5,162,222 and WIPO publication WO 94/06463. Insect cells can be infected with recombinant baculovirus, commonly derived from *Autographa californica* nuclear polyhedrosis virus (AcNPV). DNA encoding the SGIP polypeptide is inserted into the baculoviral genome in place of the AcNPV polyhedrin gene coding sequence by one of two methods. The first is the traditional method of homologous DNA recombination between wild-type AcNPV and a transfer vector containing the SGIP flanked by AcNPV sequences. Suitable insect cells, e.g. SF9 cells, are infected with wild-type AcNPV and transfected with a transfer vector comprising a SGIP polynucleotide operably linked to an AcNPV polyhedrin gene promoter, terminator, and flanking sequences. See, King, L. A. and Possee, R. D., *The Baculovirus Expression System: A Laboratory Guide*, London, Chapman & Hall; O'Reilly, D. R. et al., *Baculovirus Expression Vectors: A Laboratory Manual*, New York, Oxford University Press., 1994; and, Richardson, C. D., Ed., *Baculovirus Expression Protocols. Methods in Molecular Biology*, Totowa, N.J., Humana Press, 1995. Natural recombination within an insect cell will result in a recombinant baculovirus which contains SGIP driven by the polyhedrin promoter. Recombinant viral stocks are made by methods commonly used in the art.

[0068] The second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow (Luckow, V. A., et al., *J. Virol* 67:4566-79, 1993). This system is sold in the Bac-to-Bac kit (Life Technologies, Rockville, Md.). This system utilizes a transfer vector, pFastBac1™ (Life Technologies) containing a Tn7 transposon to move the DNA encoding the SGIP polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." The pFastBac1™ transfer vector utilizes the AcNPV polyhedrin promoter to drive the expression of the gene of interest, in this case SGIP. However, pFastBac1™ can be modified to a considerable degree. The polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as Pcor, p6.9 or MP promoter) which is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins. See, Hill-Perkins, M.

S. and Possee, R. D., *J Gen Virol* 71:971-6, 1990; Bonning, B. C. et al., *J Gen Virol* 75:1551-6, 1994; and, Chazenbalk, G. D., and Rapoport, B., *J Biol Chem* 270:1543-9, 1995. In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen, Carlsbad, Calif.), or baculovirus gp67 (PharMingen, San Diego, Calif.) can be used. In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed SGIP polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer, T. et al., *Proc Natl Acad Sci* 82:7952-4, 1985). Using a technique known in the art, a transfer vector containing SGIP is transformed into *E. Coli*, and screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques, and used to transfect *Spodoptera frugiperda* cells, e.g. Sf9 cells. Recombinant virus that expresses SGIP is subsequently produced. Recombinant viral stocks are made by methods commonly used the art.

[0069] The recombinant virus is used to infect host cells, typically a cell line derived from the fall armyworm, *Spodoptera frugiperda*. See, in general, Glick and Pasternak, *Molecular Biotechnology: Principles and Applications of Recombinant DNA*, ASM Press, Washington, D.C., 1994. Another suitable cell line is the High FiveO™ cell line (Invitrogen) derived from *Trichoplusia ni* (U.S. Pat. No. 5,300,435). Commercially available serum-free media are used to grow and maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cello405™ (JRH Biosciences, Lenexa, Kans.) or Express FiveO™ (Life Technologies) for the *T. ni* cells. The cells are grown up from an inoculation density of approximately $2\text{-}5 \times 10^5$ cells to a density of $1\text{-}2 \times 10^6$ cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3. The recombinant virus-infected cells typically produce the recombinant SGIP polypeptide at 12-72 hours post-infection and secrete it with varying efficiency into the medium. The culture is usually harvested 48 hours post-infection. Centrifugation is used to separate the cells from the medium (supernatant). The supernatant containing the SGIP polypeptide is filtered through micropore filters, usually 0.45 μm pore size. Procedures used are generally described in available laboratory manuals (King, L. A. and Possee, R. D., *ibid.*; O'Reilly, D. R. et al., *ibid.*; Richardson, C. D., *ibid.*). Subsequent purification of the SGIP polypeptide from the supernatant can be achieved using methods described herein.

[0070] Fungal cells, including yeast cells, and particularly cells of the genera *Saccharomyces* and *Pichia*, can also be used within the present invention, such as for producing SGIP fragments or polypeptide fusions. Methods for transforming yeast cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Pat. No. 4,599,311; Kawasaki et al., U.S. Pat. No. 4,931,373; Brake, U.S. Pat. No. 4,870,008; Welch et al., U.S. Pat. No. 5,037,743; and Murray et al., U.S. Pat. No. 4,845,075, which are incorporated herein by reference. Transformed cells are selected by phenotype deter-

mined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in yeast is the POT1 vector system disclosed by Kawasaki et al. (U.S. Pat. No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Pat. No. 4,599,311; Kingsman et al., U.S. Pat. No. 4,615,974; and Bitter, U.S. Pat. No. 4,977,092, which are incorporated herein by reference) and alcohol dehydrogenase genes. See also U.S. Pat. Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454, which are incorporated herein by reference. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia guilliermondii*, *Pichia methanolica* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., *J. Gen. Microbiol.* 132:3459-3465, 1986 and Cregg, U.S. Pat. No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight et al., U.S. Pat. No. 4,935,349, which is incorporated herein by reference. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Pat. No. 5,162,228, which is incorporated herein by reference. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Pat. No. 4,486,533, which is incorporated herein by reference.

[0071] Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell. *P. methanolica* cells are cultured in a medium comprising adequate sources of carbon, nitrogen and trace nutrients at a temperature of about 25° C. to 35° C. Liquid cultures are provided with sufficient aeration by conventional means, such as shaking of small flasks or sparging of fermentors. A preferred culture medium for *P. methanolica* is YEPD (2% D-glucose, 2% Bacto™ Peptone (Difco Laboratories, Detroit, Mich.), 1% Bacto™ yeast extract (Difco Laboratories), 0.004% adenine and 0.006% L-leucine).

[0072] Expressed recombinant SGIP peptides or polypeptides can be purified using fractionation and/or conventional purification methods and media. Ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable anion exchange media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are preferred, with DEAE Fast-Flow Sepharose (Pharmacia, Piscataway, N.J.) being particularly preferred. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia),

Toyopearl butyl 650 (Toso Haas, Montgomeryville, Pa.), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties. Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Methods for binding receptor polypeptides to support media are well known in the art. Selection of a particular method is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, *Affinity Chromatography: Principles & Methods*, Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988.

[0073] The polypeptides, peptides and variants of the present invention can be isolated by exploitation of small size and low pI. For example, polypeptides, peptides and variants of the present invention can be bound to anionic exchanges at low pH values. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (*Methods in Enzymol.*, Vol. 182, "Guide to Protein Purification", M. Deutscher, (ed.), Acad. Press, San Diego, 1990, pp. 529-39). Alternatively, a fusion of the polypeptide, peptide or variant of interest and an affinity tag (e.g., polyhistidine, maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification.

[0074] Polypeptide fusions of the present invention will generally contain not more than about 1,500 amino acid residues, preferably not more than about 1,200 residues, more preferably not more than about 1,000 residues, and will in many cases be considerably smaller. For example, residues of SGIP polypeptide can be fused to *E. coli* β -galactosidase (1,021 residues; see Casadaban et al., *J. Bacteriol.* 143:971-980, 1980), a 10-residue spacer, and a 4-residue factor Xa cleavage site. In a second example, residues of SGIP polypeptide can be fused to maltose binding protein (approximately 370 residues), a 4-residue cleavage site, and a 6-residue polyhistidine tag.

[0075] Protein refolding (and optionally reoxidation) procedures may be advantageously used. It is preferred to purify the protein to >80% purity, more preferably to >90% purity, even more preferably >95%, and particularly preferred is a pharmaceutically pure state, that is greater than 99.9% pure with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. Preferably, a purified protein is substantially free of other proteins, particularly other proteins of animal origin.

[0076] SGIP polypeptides, peptides, variants and or fragments thereof may also be prepared through chemical synthesis. SGIP polypeptides may be monomers or multimers; glycosylated or non-glycosylated; pegylated or non-pegy-

lated; amidated or non-amidated; sulfated or non-sulfated; and may or may not include an initial methionine amino acid residue. For example, SGIP polypeptides can also be synthesized by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. The polypeptides are preferably prepared by solid phase peptide synthesis, for example as described by Merrifield, *J. Am. Chem. Soc.* 85:2149, 1963. The synthesis is carried out with amino acids that are protected at the alpha-amino terminus. Trifunctional amino acids with labile side-chains are also protected with suitable groups to prevent undesired chemical reactions from occurring during the assembly of the polypeptides. The alpha-amino protecting group is selectively removed to allow subsequent reaction to take place at the amino-terminus. The conditions for the removal of the alpha-amino protecting group do not remove the side-chain protecting groups.

[0077] The alpha-amino protecting groups are those known to be useful in the art of stepwise polypeptide synthesis. Included are acyl type protecting groups (e.g., formyl, trifluoroacetyl, acetyl), aryl type protecting groups (e.g., biotiny), aromatic urethane type protecting groups [e.g., benzyloxycarbonyl (Cbz), substituted benzyloxycarbonyl and 9-fluorenylmethyloxy-carbonyl (Fmoc)], aliphatic urethane protecting groups [e.g., t-butyloxycarbonyl (tBoc), isopropylloxycarbonyl, cyclohexylloxycarbonyl] and alkyl type protecting groups (e.g., benzyl, triphenylmethyl). The preferred protecting groups are tBoc and Fmoc.

[0078] The side-chain protecting groups selected must remain intact during coupling and not be removed during the deprotection of the amino-terminus protecting group or during coupling conditions. The side-chain protecting groups must also be removable upon the completion of synthesis using reaction conditions that will not alter the finished polypeptide. In tBoc chemistry, the side-chain protecting groups for trifunctional amino acids are mostly benzyl based. In Fmoc chemistry, they are mostly tert-butyl or trityl based.

[0079] In tBoc chemistry, the preferred side-chain protecting groups are tosyl for arginine, cyclohexyl for aspartic acid, 4-methylbenzyl (and acetamidomethyl) for cysteine, benzyl for glutamic acid, serine and threonine, benzyloxymethyl (and dinitrophenyl) for histidine, 2-Cl-benzyloxycarbonyl for lysine, formyl for tryptophan and 2-bromobenzyl for tyrosine. In Fmoc chemistry, the preferred side-chain protecting groups are 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc) or 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for arginine, trityl for asparagine, cysteine, glutamine and histidine, tert-butyl for aspartic acid, glutamic acid, serine, threonine and tyrosine, tBoc for lysine and tryptophan.

[0080] For the synthesis of phosphopeptides, either direct or post-assembly incorporation of the phosphate group is used. In the direct incorporation strategy, the phosphate group on serine, threonine or tyrosine may be protected by methyl, benzyl, or tert-butyl in Fmoc chemistry or by methyl, benzyl or phenyl in tBoc chemistry. Direct incorporation of phosphotyrosine without phosphate protection can also be used in Fmoc chemistry. In the post-assembly incorporation strategy, the unprotected hydroxyl groups of serine, threonine or tyrosine are derivatized on solid phase

with di-tert-butyl-, dibenzyl- or dimethyl-N,N'-diisopropylphosphoramidite and then oxidized by tert-butylhydroperoxide.

[0081] Solid phase synthesis is usually carried out from the carboxyl-terminus by coupling the alpha-amino protected (side-chain protected) amino acid to a suitable solid support. An ester linkage is formed when the attachment is made to a chloromethyl, chlorotriyl or hydroxymethyl resin, and the resulting polypeptide will have a free carboxyl group at the C-terminus. Alternatively, when an amide resin such as benzhydrylamine or p-methylbenzhydrylamine resin (for tBoc chemistry) and Rink amide or PAL resin (for Fmoc chemistry) are used, an amide bond is formed and the resulting polypeptide will have a carboxamide group at the C-terminus. These resins, whether polystyrene- or polyamide-based or polyethyleneglycol-grafted, with or without a handle or linker, with or without the first amino acid attached, are commercially available, and their preparations have been described by Stewart et al., "Solid Phase Peptide Synthesis" (2nd Edition), (Pierce Chemical Co., Rockford, Ill., 1984) and Bayer & Rapp Chem. Pept. Prot. 3:3 (1986); and Atherton et al., *Solid Phase Peptide Synthesis: A Practical Approach*, IRL Press, Oxford, 1989.

[0082] The C-terminal amino acid, protected at the side chain if necessary, and at the alpha-amino group, is attached to a hydroxymethyl resin using various activating agents including dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide (DIPCDI) and carbonyldiimidazole (CDI). It can be attached to chloromethyl or chlorotriyl resin directly in its cesium tetramethylammonium salt form or in the presence of triethylamine (TEA) or diisopropylethylamine (DIEA). First amino acid attachment to an amide resin is the same as amide bond formation during coupling reactions.

[0083] Following the attachment to the resin support, the alpha-amino protecting group is removed using various reagents depending on the protecting chemistry (e.g., tBoc, Fmoc). The extent of Fmoc removal can be monitored at 300-320 nm or by a conductivity cell. After removal of the alpha-amino protecting group, the remaining protected amino acids are coupled stepwise in the required order to obtain the desired sequence.

[0084] Various activating agents can be used for the coupling reactions including DCC, DIPCDI, 2-chloro-1,3-dimethylimidium hexafluorophosphate (CIP), benzotriazol-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) and its pyrrolidine analog (PyBOP), bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP), O-(benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HBTU) and its tetrafluoroborate analog (TBTU) or its pyrrolidine analog (HBPYU), O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HATU) and its tetrafluoroborate analog (TATU) or its pyrrolidine analog (HAPYU). The most common catalytic additives used in coupling reactions include 4-dimethylaminopyridine (DMAP), 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine (HODhbt), N-hydroxybenzotriazole (HOBT) and 1-hydroxy-7-azabenzotriazole (HOAt). Each protected amino acid is used in excess (>2.0 equivalents), and the couplings are usually carried out in N-methylpyrrolidone (NMP) or in DMF, CH₂Cl₂ or mixtures thereof. The extent of completion of the coupling

reaction can be monitored at each stage, e.g., by the ninhydrin reaction as described by Kaiser et al., *Anal. Biochem.* 34:595, 1970.

[0085] After the entire assembly of the desired peptide, the peptide-resin is cleaved with a reagent with proper scavengers. The Fmoc peptides are usually cleaved and deprotected by TFA with scavengers (e.g., H₂O, ethanedithiol, phenol and thioanisole). The tBoc peptides are usually cleaved and deprotected with liquid HF for 1-2 hours at -5 to 0° C., which cleaves the polypeptide from the resin and removes most of the side-chain protecting groups. Scavengers such as anisole, dimethylsulfide and p-thiocresol are usually used with the liquid HF to prevent cations formed during the cleavage from alkylating and acylating the amino acid residues present in the polypeptide. The formyl group of tryptophan and the dinitrophenyl group of histidine need to be removed, respectively by piperidine and thiophenyl in DMF prior to the HF cleavage. The acetamidomethyl group of cysteine can be removed by mercury(II)acetate and alternatively by iodine, thallium(III)trifluoroacetate or silver tetrafluoroborate which simultaneously oxidize cysteine to cystine. Other strong acids used for tBoc peptide cleavage and deprotection include trifluoromethanesulfonic acid (TFMSA) and trimethylsilyltrifluoroacetate (TMSOTf).

[0086] Amino acid modifications can be added to the synthetic peptides in a variety of ways. Such amino acid modifications can be beneficial, for example, to add glycosylation, acylation, or other modifications that would be added during host cell expression. These modifications may, for example, have an effect on the half-life of the peptide in the blood stream or may be necessary to transport the peptide in a lipid environment. In particular, acylation of the peptide can make it less polar and enhance its interaction with lipids, as well as to incorporate it into a liposome. The peptide may be orally available as an acylated variant due to an enhanced ability to cross the gut epithelium. The type of fatty acid chosen for this amino acid modification may affect the degree of lipid solubility as well as the activity of the SGIP peptide. One of ordinary skill in the art would be able to change the length, or the degree of saturation of the fatty acid side chain, and direct the peptides to different sites of action. For a small peptide of the gut hormone family, such sites of action may include, for example, a receptor in the gastrointestinal system versus a receptor in the brain or pituitary. Thus SGIP peptides of the present invention may be targeted to different tissues based on the modifications made to the peptides.

[0087] The activity of molecules of the present invention can be measured using a variety of assays that measure stimulation of gastrointestinal contractility, modulation of nutrient uptake, modulation of the secretion of digestive enzymes and hormones, modulation of secretion of enzymes and/or hormones in the pancreas, binding a SGIP receptor, or binding an antibody that specifically binds to residues 1 to 11 of SEQ ID NO:2. Of particular interest are changes in contractility of smooth muscle cells. For example, the contractile response of segments of mammalian duodenum or other gastrointestinal smooth muscles tissue (Depoortere et al., *J. Gastrointestinal Motility* 1:150-159, 1989, incorporated herein by reference). An exemplary in vivo assay uses an ultrasonic micrometer to measure the dimensional changes radially between commissures and longitudinally

to the plane of the valve base (Hansen et al., *Society of Thoracic Surgeons* 60:S384-390, 1995).

[0088] Gastric motility is generally measured in the clinical setting as the time required for gastric emptying and subsequent transit time through the gastrointestinal tract. Gastric emptying scans are well known to those skilled in the art, and briefly, comprise use of an oral contrast agent, such as barium, or a radiolabeled meal. Solids and liquids can be measured independently. A test food or liquid is radiolabeled with an isotope (e.g. ^{99m}Tc), and after ingestion or administration, transit time through the gastrointestinal tract and gastric emptying are measured by visualization using gamma cameras (Meyer et al., *Am. J. Dig. Dis.* 21:296, 1976; Collins et al., *Gut* 24:1117, 1983; Maughan et al., *Diabet. Med.* 13 9 *Supp.* 5:S6-10, 1996 and Horowitz et al., *Arch. Intern. Med.* 145:1467-1472, 1985). These studies may be performed before and after the administration of a promotility agent to quantify the efficacy of the drug.

[0089] Assays measuring SGIP polypeptides ability to affect cell proliferation or differentiation are well known in the art. For example, assays measuring proliferation include such assays as chemosensitivity to neutral red dye (Cavanaugh et al., *Investigational New Drugs* 8:347-354, 1990, incorporated herein by reference), incorporation of radiolabelled nucleotides (Cook et al., *Analytical Biochem.* 179:1-7, 1989, incorporated herein by reference), incorporation of 5-bromo-2'-deoxyuridine (BrdU) in the DNA of proliferating cells (Porstmann et al., *J. Immunol. Methods* 82:169-179, 1985, incorporated herein by reference), and use of tetrazolium salts (Mosmann, *J. Immunol. Methods* 65:55-63, 1983; Alley et al., *Cancer Res.* 48:589-601, 1988; Marshall et al., *Growth Reg.* 5:69-84, 1995; and Scudiero et al., *Cancer Res.* 48:4827-4833, 1988; all incorporated herein by reference). Assays measuring differentiation include, for example, measuring cell-surface markers associated with stage-specific expression of a tissue, enzymatic activity, functional activity or morphological changes (Watt, *FASEB*, 5:281-284, 1991; Francis, *Differentiation* 57:63-75, 1994; Raes, *Adv. Anim. Cell Biol. Technol. Bioprocesses*, 161-171, 1989; all incorporated herein by reference).

[0090] Assays can be used to measure other cellular responses, that include, chemotaxis, adhesion, changes in ion channel influx, regulation of second messenger levels and neurotransmitter release. Such assays are well known in the art. See, for example, in "Basic & Clinical Endocrinology Ser., Vol. Vol. 3," *Cytochemical Bioassays: Techniques & Applications*, Chayen; Chayen, Bitensky, eds., Dekker, N.Y., 1983.

[0091] In view of the tissue distribution observed for SGIP, agonists (including the natural ligand/substrate/cofactor/synthetic and naturally occurring peptides, and variants, etc.) and antagonists have enormous potential in both in vitro and in vivo applications. Compounds identified as SGIP agonists are useful for promoting stimulation of gastrointestinal contractility, modulation of nutrient uptake, modulation of the secretion of digestive enzymes and hormones, modulation of secretion of enzymes and/or hormones in the pancreas, binding a SGIP receptor, or binding an antibody that specifically binds to residues 1 to 11 of SEQ ID NO:2) in vivo and in vitro. For example, agonist compounds are useful as components of defined cell culture media and regulate the uptake of nutrients, and thus are

useful in specifically promoting the growth and/or development of gastrointestinal cells such as G cells, enterochromaffin cells and the epithelial mucosa of the stomach, duodenum, proximal jejunum, antrum and fundus.

[0092] The family of gut-brain peptides has been associated with neurological and CNS functions. For example, NPY, a peptide with receptors in both the brain and the gut has been shown to stimulate appetite when administered to the central nervous system (Gehlert, *Life Sciences* 55(6):551-562, 1994). Motilin immunoreactivity has been identified in different regions of the brain, particularly the cerebellum, and in the pituitary (Gasparini et al., *Hum. Genetics* 94(6):671-674, 1994). Motilin has been found to coexist with neurotransmitter γ -aminobutyric acid in cerebellum (Chan-Patay, *Proc. Sym. 50th Anniv. Meet. Br. Pharmacol. Soc.*: 1-24, 1982). Physiological studies have provided some evidence that motilin has an effect on feeding behavior (Rosenfield et al., *Phys. Behav.* 39(6):735-736, 1987), bladder control, pituitary growth hormone release. Other gut-brain peptides, such as CCK, enkephalin, VIP and secretin have been shown to be involved in control of blood pressure, heart rate, behavior, and pain modulation, in addition to be active in the digestive system. Therefore, SGIP including variants, or some portion thereof, could be expected to have some neurological association.

[0093] NPY has been implicated in cardiovascular effects such as increased sympathetic nerve activity in heart, which is associated with heart failure, as well as hypotension, and changes in blood pressure and vagal action (Feng, Q. et al *Acta. Physiol. Scand.* 166:285-291, 1999; McLean, K J. Et al. *Neuroscience* 92:1377-1387, 1999; Potter, E K et al; *Regul. Pept.* 25:167-177, 1989; Gardiner, S M *Brain Res. Brain Res. Review* 14:79-116, 1989).

[0094] Examples such as NPY and motilin emphasize the importance and broad activity of peptide hormones in the human body, and their impact on normal physiological function and disease. Peptide hormones are involved in regulatory aspects of cardiovascular regulation and homeostasis, digestion, brain, neuronal and other organ functions. Various peptide hormones have been shown to be involved in control of blood pressure, heart rate, arrhythmia, osmotic balance, influencing the release and action of cardiovascular transmitters, vasoconstriction and vasodilatation, vasoconstriction resulting in myocardial ischemia, vasomotor tone, contractility, food intake, respiration, behavior, and pain modulation, and the like. As a peptide hormone, SGIP peptides may similarly exert effects in heart, or other tissues in which it is expressed, or freely circulate through the body and exert effects elsewhere. Thus, SGIP peptides can regulate positively or negatively various physiological functions, or cause the release of other regulatory hormones from the heart, gut, CNS and other organs or tissues. Assays and models to test for such SGIP activity are well known in the art and described herein. For example, see amongst other methods known in the art: Feng, Q. et al supra. (pithed rat heart failure model to assess vascular sympathetic nerve activity); Horackova, et al., *Cell Tissue Res.* 297:409-421, 1999 (guinea pig atria model); McLean, K J. Et al. supra. (CNS response to hypotensive challenge to assess neuron response or activation within cardiovascular control); Potter, E K et al; supra. (Testing effects of polypeptides and peptide fragments on blood pressure and vagal action at the heart); Maturi, M F et al., *J. Clin. Invest* 83:1217-1224 (myocardial

ischemia and coronary constriction model in dogs); Haass, M. et al., *Naunyn-Schmiedeberg's Arch. Pharmacol.* 339:71-78, 1989 (pre-synaptic modulation in in situ perfused guinea pig heart); Hassall, C J, nad Burnstock, G. *Neurosci. Lett.* 52:111-115, 1984 (Cultured Guinea pig atria to study intrinsic innervation); Lundberg, J M. Et al., *Acta. Physiol. Scand.* 121:325-332, 1984 (effect of peptide on muscle tone, and autonomic transmission in Guinea pig atrium, vas deferens, urinary bladder, portal vein, and trachea); Mathias, C J J. *Neurosci. Methods* 34:193-200, 1990 (effect of food in take on cardiovascular control); Miyata, A. et al., *Ann. N.Y. Acad. Sci.* 865:73-81, 1998 (effect of peptides on rat aortic smooth muscle cell proliferation); Saita, M. et al., *Am. J. Physiol.* 274:R979-984, 1998 (Effects of centrally administered peptide on blood pressure, heart rate, renal sympathetic nerve activity in rats); Krowicki, Z K et al., *Am. J. Physiol.* 272:G1221-1229, 1997 (vagal mediated gastric motor excitation); Hall, M E et al., *Brain Res.* 497:280-290, 1989 (microinjection of peptides into the nucleus of the solitary tract (NTS) and effects on cardiovascular function).

[0095] Moreover, immunohistochemical and immunolabeling methods known in the art and described herein can be used to assess the influence of SGIP peptides on other cardiovascular effectors, such as, for example NPY and VIP (Wharton, J, and Gulbenkian S. *Experientia Suppl.* 56:292-316, 1989; and Forsgren, S. *Cell Tissue Res.* 256:125-135, 1989). As such, labeled SGIP peptides and antibodies can be used to assess cardiovascular function. In addition, such labeled SGIP peptides and antibodies can be used as diagnostics to assess human disease in comparison to normal controls, and described herein. Such histologic, immunohistochemical and immunolabeling methods and the like can be used in conjunction with the in vivo models described herein.

[0096] The cardiac activity of molecules of the present invention may be measured using a Langendorff assay. This preferred assay measures ex vivo cardiac function for an experimental animal, and is well known in the art. Experimental animals are, for example but not limited to, rats, rabbits and guinea pigs. Chronic effects on heart tissue can be measured after treating a test animal with SGIP peptides for 1 to 7 days, or longer. Control animals will have only received buffer. After treatment, the heart is removed and perfused retrograde through the aorta. During perfusion, several physiologic parameters are measured: coronary blood flow per time, left ventricular (LV) pressures, and heart rate. These parameters directly reflect cardiac function. Changes in these parameters, as measured by the Langendorff assay, following in vivo treatment with SGIP peptides relative to control animals indicates a chronic effect of the polypeptide on heart function. Moreover, the Langendorff assay can also be employed to measure the acute effects of SGIP peptides on heart. In such application, hearts from untreated animals are used and SGIP peptides are added to the perfusate in the assay. The parameters assessed above are measured and compared with the results from control hearts where SGIP peptides were omitted from the perfusate. Differences in heart rate, change in pressure per time, and/or coronary blood flow indicate an acute effect of the molecules of the present invention on heart function.

[0097] Additionally, other members of the gut-brain peptides, such as CCK, gastrin, and the like, have been shown

to modulate secretion of pancreatic enzymes and hormones. Thus, SGIP can be used to modulate secretion of pancreatic enzymes and hormones.

[0098] Similarly, other members of this family are known to modulate the secretion of endogenous proteins, such as the manner in which glucagon modulates the secretion of insulin. SGIP can be used to modulate the secretion of non-SGIP proteins such as, for example, GLP-1, growth hormone, somatostatin, and the like.

[0099] As a ligand, a SGIP peptide can bind a G protein coupled receptor, such as, for example, the growth hormone secretagogue receptor (GHS-R). Growth hormone secretagogues are a class of small peptides which stimulate the release of growth hormone from pituitary cells by a mechanism of action other than that of GHRH, i.e., by binding a different receptor (GHS-R) in the pituitary and hypothalamus. Thus, the binding of this receptor can play a role in regulating growth hormone secretion in extraneuroendocrine activities, such as, for example, sleep and food intake. Therefore, the secretion of growth hormone can be regulated by the formation of a peptide-receptor complex between SGIP peptides and GHS-R.

[0100] The release of growth hormone stimulates growth in many tissues and has effects on metabolic processes such as stimulating protein synthesis and free fatty acid mobilization as well as stimulating metabolism from a variety of energy sources from carbohydrates to fatty acids. Deficiency of growth hormone can result in medical disorders such as dwarfism.

[0101] One advantage of growth hormone secretagogues, in general, is their ability to amplify endogenous pulsatile growth hormone secretion while maintaining normal feedback mechanisms. Another important effect is the ability to restore serum insulin-like growth factor-I (IGF-I) levels in elderly adults to concentrations similar to those of young adults. See Hansen, B. S. et al., *Eur. J. Endocrinol.* 141:180-189, 1999. Thus, as a ligand for GHS-R, SGIP can be useful for modulating secretion of growth hormone and insulin-like growth factor I.

[0102] Using site-specific changes in the amino acid and DNA sequences of the present invention analogs can be made that are either antagonists, agonists or partial agonists (Macielay et al., *Peptides: Chem. Struct. Biol.* pp. 659, 1996). Antagonists are useful for clinical conditions associated with gastrointestinal hypermotility such as diarrhea and Crohn's disease. Antagonists are also useful as research reagents for characterizing sites of ligand-receptor interaction.

[0103] A SGIP polypeptide can also be used for purification of receptors. The polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting medium will generally be configured in the form of a column, and fluids containing ligand are passed through the column one or more times to allow ligand to bind to the receptor polypep-

tide. The ligand is then eluted using changes in salt concentration, chaotropic agents (guanidine HCl), or pH to disrupt ligand-receptor binding.

[0104] An assay system that uses a ligand-binding receptor (or an antibody, one member of a complement/anti-complement pair) or a binding fragment thereof, and a commercially available biosensor instrument (BIAcore™, Pharmacia Biosensor, Piscataway, N.J.) may be advantageously employed. Such receptor, antibody, member of a complement/anti-complement pair or fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, *J. Immunol. Methods* 145:229-40, 1991 and Cunningham and Wells, *J. Mol. Biol.* 234:554-63, 1993. A receptor, antibody, member or fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If a ligand, epitope, or opposite member of the complement/anti-complement pair is present in the sample, it will bind to the immobilized receptor, antibody or member, respectively, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding.

[0105] Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity (see Scatchard, *Ann. NY Acad. Sci.* 51: 660-72, 1949) and calorimetric assays (Cunningham et al., *Science* 253:545-48, 1991; Cunningham et al., *Science* 245:821-25, 1991).

[0106] Cells expressing functional GHS-R are used within screening assays. A variety of suitable assays are known in the art. These assays are based on the detection of a biological response in the target cell. One such assay is a cell proliferation assay. Cells are cultured in the presence or absence of a test compound, and cell proliferation is detected by, for example, measuring incorporation of tritiated thymidine or by colorimetric assay based on the metabolic breakdown of Alamar Blue™ (AccuMed, Chicago, Ill.) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Mosman, *J. Immunol. Meth.* 65: 55-63, 1983).

[0107] Another assay uses phospholipase C signal transduction to measure receptor binding. An exemplary assay of this sort measures release of Ca²⁺ with aequorin, a bioluminescent Ca²⁺-sensitive reporter protein. This assay is further described by Feighner, S. D. et al., supra. Hence, SGIP peptides can be tested using an assay that measures phospholipase C transduction. Yet another assay uses phospholipase C signal transduction to measure receptor binding. An exemplary assay of this sort measures release of Ca²⁺ with aequorin, a bioluminescent Ca²⁺-sensitive reporter protein. This assay is further described by Feighner, S. D. et al., supra. Hence, SGIP peptides can be tested using an assay that measures phospholipase C transduction. Alternative assays are also listed herein

[0108] SGIP polypeptides, peptides, and variants can also be used to prepare antibodies that specifically bind to SGIP epitopes, peptides or polypeptides. Methods for preparing polyclonal and monoclonal antibodies are well known in the art (see, for example, Sambrook et al., *Molecular Cloning:*

A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., 1989; and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, CRC Press, Inc., Boca Raton, Fla., 1982, which are incorporated herein by reference). As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from a variety of warm-blooded animals, such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats.

[0109] The immunogenicity of a SGIP polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of SGIP or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "haptene-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

[0110] As used herein, the term "antibodies" includes polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments, such as F(ab')₂ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting only non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking" them with a human-like surface by replacement of exposed residues, wherein the result is a "veneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced. Alternative techniques for generating or selecting antibodies useful herein include in vitro exposure of lymphocytes to SGIP protein or peptide, and selection of antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled SGIP protein or peptide).

[0111] Antibodies are defined to be specifically binding if they bind to a SGIP polypeptide with a binding affinity (K_a) of 10⁶ M⁻¹ or greater, preferably 10⁷ M⁻¹ or greater, more preferably 10⁸ M⁻¹ or greater, and most preferably 10⁹ M⁻¹ or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art (for example, by Scatchard analysis).

[0112] A variety of assays known to those skilled in the art can be utilized to detect antibodies which specifically bind to SGIP proteins or peptides. Exemplary assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: concurrent immunoelectrophoresis, radioimmunoassay, radioimmunoprecipitation, enzyme-linked immunosorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. In addition, antibodies can be screened for binding to wild-type versus mutant SGIP protein or peptide.

[0113] Antibodies to SGIP may be used for tagging cells that express SGIP for isolating SGIP by affinity purification; for diagnostic assays for determining circulating levels of SGIP polypeptides; for detecting or quantitating soluble SGIP as marker of underlying pathology or disease; in analytical methods employing FACS; for screening expression libraries; for generating anti-idiotypic antibodies; and as neutralizing antibodies or as antagonists to block SGIP activity in vitro and in vivo. Suitable direct tags or labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like; indirect tags or labels may feature use of biotin-avidin or other complement/anti-complement pairs as intermediates. Antibodies herein may also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for in vivo diagnostic or therapeutic applications.

[0114] Molecules of the present invention can be used to identify and isolate receptors that mediate the function of SGIP. For example, proteins and peptides of the present invention can be immobilized on a column and membrane preparations run over the column (*Immobilized Affinity Ligand Techniques*, Hermanson et al., eds., Academic Press, San Diego, Calif., 1992, pp. 195-202). Polypeptides and peptides which bind to the SGIP polypeptides, peptides, and variants of the present invention can then be eluted and characterized using methods known in the art. Proteins and peptides can also be radiolabeled (*Methods in Enzymol.*, vol. 182, "Guide to Protein Purification", M. Deutscher, ed., Acad. Press, San Diego, 1990, 721-737) or photoaffinity labeled (Brunner et al., *Ann. Rev. Biochem.* 62:483-514, 1993 and Fedan et al., *Biochem. Pharmacol.* 33:1167-1180, 1984) and specific cell-surface proteins can be identified in vivo and in vitro. Other detectable labels can also be used and include, for example, fluorescent labels (FITC, rhodamine, and fluorescent-biotinylated labels). Tissues which bind SGIP can be identified, for example, by binding assays as shown in Example 5, herein. Such tissues can be used as sources of cell extracts, membrane fractions, protein lysates, purified protein, and the like, and applied to a column on which the SGIP polypeptide or peptides have been immobilized and SGIP receptors can be isolated and characterized. Alternatively, such tissues can be harvested and tested in vitro or in vivo for binding to the SGIP ligand.

[0115] Another method to identify and purify the SGIP receptor measures the stimulation/inhibition of SGIP receptor-dependent cellular responses. For example, cell lines can be transfected with a reporter gene construct that is responsive to a receptor stimulated cellular pathway. Reporter gene constructs of this type are known in the art, and generally comprise a response element operably linked to a gene encoding an assay detectable protein, such as luciferase. DNA response elements can include, but are not limited to, cyclic AMP response elements (CRE), hormone response elements (HRE) insulin response element (IRE) (Nasrin et al., *Proc. Natl. Acad. Sci. USA* 87:5273-7, 1990) and serum response elements (SRE) (Shaw et al. *Cell* 56: 563-72, 1989). Cyclic AMP response elements are reviewed in Roestler et al., *J. Biol. Chem.* 263 (19):9063-6; 1988 and Habener, *Molec. Endocrinol.* 4 (8):1087-94; 1990. Hormone response elements are reviewed in Beato, *Cell* 56:335-44; 1989. The cell line can then be transfected with a cDNA library prepared from a tissue type which binds to SGIP polypeptides and peptides, for example, kidney, duodenum,

and jejunum, or others as identified by binding assays such as, for example, the assay in Example 5. Cell extracts, membrane fractions, protein lysates, purified protein, and the like, containing SGIP polypeptides, peptides, and variants stimulate the transfected cell lines by binding to cells expressing the cDNA of the receptor. The binding of SGIP, or a SGIP variant, to its receptor results in a change in the assayable protein or metabolite. Additionally, binding can be evidenced by the modulation of cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP). Measuring changes cAMP and cGMP is known to one skilled in the art, and kits are commercially available (Biotrak, Amersham Pharmacia Biotech, Piscataway, N.J.) for these determinations. In the alternative, cell extracts, membrane fractions, protein lysates, purified protein, and the like, of SGIP polypeptides, peptides and variants can be tested for direct binding to cells transfected with both the cDNA library (which contains the receptor) and the reporter gene using SGIP polypeptides, peptides or variants tagged with a detectable label (e.g., ^{125}I , biotin, horseradish peroxidase, FITC, and the like). Within assays of this type, the ability of a labeled test sample to bind to the receptor is indicative of ligand binding. Receptors used within binding assays may be cellular receptors or isolated, immobilized receptors.

[0116] As a ligand, the activity of SGIP polypeptide, peptide, or variant can be measured by a silicon-based biosensor microphysiometer which measures the extracellular acidification rate or proton excretion associated with receptor binding and subsequent physiologic cellular responses. An exemplary device is the Cytosensor™ Microphysiometer manufactured by Molecular Devices, Sunnyvale, Calif. A variety of cellular responses, such as cell proliferation, ion transport, energy production, inflammatory response, regulatory and receptor activation, and the like, can be measured by this method. See, for example, McConnell, H. M. et al., *Science* 257:1906-1912, 1992; Pitchford, S. et al., *Meth. Enzymol.* 228:84-108, 1997; Arimilli, S. et al., *J. Immunol. Meth.* 212:49-59, 1998; Van Liefde, I. et al., *Eur. J. Pharmacol.* 346:87-95, 1998. The microphysiometer can be used for assaying adherent or non-adherent eukaryotic or prokaryotic cells. By measuring extracellular acidification changes in cell media over time, the microphysiometer directly measures cellular responses to various stimuli, including SGIP polypeptide, peptide, variant, agonists, or antagonists. Preferably, the microphysiometer is used to measure responses of a SGIP-responsive eukaryotic cell, compared to a control eukaryotic cell that does not respond to SGIP polypeptide, peptide, or variant. SGIP-responsive eukaryotic cells comprise cells into which a receptor for SGIP has been transfected creating a cell that is responsive to SGIP polypeptide, peptide, or variant; or cells naturally responsive to SGIP such as, for example, cells derived from the kidney, small intestine or pituitary. Differences, measured by a change, for example, an increase or diminution in extracellular acidification, in the response of cells exposed to SGIP polypeptide, peptide, or variant relative to a control not exposed to SGIP polypeptide, peptide, or variant, are a direct measurement of SGIP-modulated cellular responses. Moreover, such SGIP-modulated responses can be assayed under a variety of stimuli. Using the microphysiometer, there is provided a method of identifying agonists of SGIP polypeptide, comprising providing cells responsive to a SGIP polypeptide, culturing a first

portion of the cells in the absence of a test compound, culturing a second portion of the cells in the presence of a test compound, and detecting a change, for example, an increase or diminution, in a cellular response of the second portion of the cells as compared to the first portion of the cells. The change in cellular response is shown as a measurable change in extracellular acidification rate. Moreover, culturing a third portion of the cells in the presence of SGIP polypeptide and the absence of a test compound can be used as a positive control for the SGIP-responsive cells, and as a control to compare the agonist activity of a test compound with that of the SGIP polypeptide. Moreover, using the microphysiometer, there is provided a method of identifying antagonists of SGIP polypeptide, comprising providing cells responsive to a SGIP polypeptide, culturing a first portion of the cells in the presence of SGIP and the absence of a test compound, culturing a second portion of the cells in the presence of SGIP and the presence of a test compound, and detecting a change, for example, an increase or a diminution in a cellular response of the second portion of the cells as compared to the first portion of the cells. The change in cellular response is shown as a measurable change in extracellular acidification rate. Antagonists and agonists, for SGIP polypeptide, can be rapidly identified using this method.

[0117] Moreover, polypeptides, peptides and variants of SGIP can be used to identify cells, tissues, or cell lines which respond to a SGIP-stimulated pathway. The microphysiometer, described above, can be used to rapidly identify ligand-responsive cells, such as cells responsive to SGIP polypeptides and variants of the present invention. Cells can be cultured in the presence or absence of SGIP polypeptides, peptides and variants. Those cells which elicit a measurable change in extracellular acidification in the presence of SGIP polypeptides, peptides and variants are responsive to SGIP. Such cell lines, can be used to identify antagonists and agonists of SGIP polypeptide as described above.

[0118] The chromosomal localization of zsig33, and thus of SGIP is 3p26.1 The present invention also provides reagents which will find use in diagnostic applications. For example, the SGIP gene, a probe comprising SGIP DNA or RNA or a subsequence thereof can be used to determine if the SGIP gene is present on chromosome 3p26.1 or if a mutation has occurred. Detectable chromosomal aberrations at the SGIP gene locus include, but are not limited to, aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, and other genetic linkage analysis techniques known in the art (Sambrook et al., *ibid.*; Ausubel et al., *ibid.*; Marian, *Chest* 108:255-65, 1995). Thus, molecules of the present invention can be used as diagnostics for specific diseases localized to the chromosome 3p26. 1.

[0119] Polynucleotides encoding SGIP polypeptides are useful within gene therapy applications where it is desired to increase or inhibit SGIP activity. If a mammal has a mutated or absent SGIP gene, the SGIP gene can be introduced into the cells of the mammal. In one embodiment, a gene

encoding a SGIP polypeptide is introduced in vivo in a viral vector. Such vectors include an attenuated or defective DNA virus, such as, but not limited to, herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), and the like. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. A defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Examples of particular vectors include, but are not limited to, a defective herpes simplex virus 1 (HSV1) vector (Kaplitt et al., *Molec. Cell. Neurosci.* 2:320-30, 1991); an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al., *J. Clin. Invest.* 90:626-30, 1992; and a defective adeno-associated virus vector (Samulski et al., *J. Virol.* 61:3096-101, 1987; Samulski et al., *J. Virol.* 63:3822-8, 1989).

[0120] In another embodiment, a SGIP gene can be introduced in a retroviral vector, e.g., as described in Anderson et al., U.S. Pat. No. 5,399,346; Mann et al. *Cell* 33:153, 1983; Temin et al., U.S. Pat. No. 4,650,764; Temin et al., U.S. Pat. No. 4,980,289; Markowitz et al., *J. Virol.* 62:1120, 1988; Temin et al., U.S. Pat. No. 5,124,263; International Patent Publication No. WO 95/07358, published Mar. 16, 1995 by Dougherty et al.; and Kuo et al., *Blood* 82:845, 1993. Alternatively, the vector can be introduced by lipofection in vivo using liposomes. Synthetic cationic lipids can be used to prepare liposomes for in vivo transfection of a gene encoding a marker (Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413-7, 1987; Mackey et al., *Proc. Natl. Acad. Sci. USA* 85:8027-31, 1988). The use of lipofection to introduce exogenous genes into specific organs in vivo has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. More particularly, directing transfection to particular cells represents one area of benefit. For instance, directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (e.g., hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

[0121] Similarly, the SGIP polynucleotides (SEQ ID NO:1 or SEQ ID NO:7), or the complementary proteins thereof, can be used to target specific tissues such as tissues of the bone marrow, peripheral blood lymphocytes, umbilical cord blood, prostate and in malignant and leukemic cell lines. It is possible to remove the target cells from the body; to introduce the vector as a naked DNA plasmid; and then to re-implant the transformed cells into the body. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun or use of a DNA vector transporter. See, e.g., Wu et al., *J. Biol. Chem.* 267:963-7, 1992; Wu et al., *J. Biol. Chem.* 263:14621-4, 1988.

[0122] Various techniques, including antisense and ribozyme methodologies, can be used to inhibit SGIP gene transcription and translation, such as to inhibit cell proliferation in vivo. Polynucleotides that are complementary to

a segment of a SGIP-encoding polynucleotide (e.g., a polynucleotide as set forth in SEQ ID NOs:1 or 3) are designed to bind to SGIP-encoding mRNA and to inhibit translation of such mRNA. Such antisense polynucleotides are used to inhibit expression of SGIP polypeptide-encoding genes in cell culture or in a subject.

[0123] The polypeptides, peptides, variants, nucleic acid and/or antibodies of the present invention may be used in treatment of disorders associated with gastrointestinal contractility, secretion of digestive enzymes, hormones, and acids, gastrointestinal motility, recruitment of digestive enzymes; inflammation, particularly as it affects the gastrointestinal system; reflux disease and regulation of nutrient absorption. Specific conditions that will benefit from treatment with molecules of the present invention include, but are not limited to, diabetic gastroparesis, post-surgical gastroparesis, vagotomy, chronic idiopathic intestinal pseudo-obstruction and gastroesophageal reflux disease. Additional uses include, gastric emptying for radiological studies, stimulating gallbladder contraction and antrectomy.

[0124] An association between gastrointestinal function and brain function has been observed for other hormones in this class. As an example, secretin infusion in autistic children resulted in amelioration of the gastrointestinal symptoms as well as a dramatic improvement in behavior (improved eye contact, alertness and expansion of expressive language). See Hovrath, K. et al., *J. Assoc. Acad. Minor Phys* 9(1):9-15, 1998. Similarly, a study of the upper gastrointestinal tract in autistic children with gastrointestinal symptoms showed that many had reflux esophagitis, chronic gastritis, and chronic duodenitis, as well as an elevated number of Paneth's cells in the duodenal crypts compared to non-autistic children. See Horvath, K. et al., *J. Pediatr.* 135(5):559-563, 1999. The administration of secretin to these autistic children resulted in increased pancreaticobiliary fluid output and higher fluid output. Gastrointestinal disorders, especially reflux esophagitis and disaccharide malabsorption may contribute to the behavioral problems of the non-verbal autistic patients. The observed increase in pancreaticobiliary secretion after secretin infusion suggests an upregulation of secretin receptors. As a member of the gut-hormone family of proteins, SGIP peptides by binding to a receptor, may have effects on neural development and/or utilization.

[0125] The motor and neurological affects of molecules of the present invention also make it useful for treatment of obesity and other metabolic disorders where neurological feedback modulates nutritional absorption. The molecules of the present invention are useful for regulating satiety, glucose absorption and metabolism, and neuropathy-associated gastrointestinal disorders.

[0126] Polypeptides of the present invention may be useful for evaluating functions of the hypothalamus-pituitary-adrenal axis by challenging the gastrointestinal system with SGIP, including variants, and measuring gastric motility and contractility, modulation of nutrient uptake, modulation of the secretion of digestive enzymes and hormones, or modulation of secretion of enzymes and/or hormones in the pancreas.

[0127] Potential uses of growth hormone are extensive and include treatment of diseases and conditions associated with bone formation (such as, for example, treatment of

osteoporosis, acceleration of bone formation and repair, stimulating osteoblasts, bone remodeling and cartilage growth, and skeletal dysplasia); immunity (such as, for example, stimulating the immune system, treating immunosuppressed patients); obesity, and metabolic disorders (such as, for example, preventing catabolic side effects of glucocorticoids, treatment of obesity and growth retardation related to obesity, attenuation of protein catabolic responses after surgery, reducing cachexia and protein loss due to chronic illness such as cancer or AIDS); dwarfism (such as, for example, treating growth retardation and physiological short stature including growth hormone deficiency and chronic illness, and intrauterine growth retardation); wound healing (such as, for example, accelerating wound repair, accelerating recovery of burn patients and treating patients with delayed wound healing); reproduction (such as, for example, as an adjuvant treatment for ovulation induction); as well as conditions associated with stress; conditions associated with kidney and lung dysfunction; conditions associated with aging and the elderly, including, muscle strength, bone fragility and skin thickness; and neuroendocrine activities such as sleep. Thus, as growth hormone secretagogues, SGIP peptides, would be useful to treat conditions associated with these disorders. Assays measuring the release of growth hormone are known in the art.

[0128] Additionally, molecules of SGIP may be used to detect or modulate the growth and/or differentiation of tumor cell, which are expressing a receptor which binds to SGIP. SGIP polypeptides can be labeled with radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like; indirect tags or labels may feature use of biotin-avidin or other complement/anti-complement pairs as intermediates. These labeled polypeptides can be applied in vitro or in vivo and are especially useful to identify SGIP or zsig33 receptors located on tumors in such tissues as, for example, stomach, brain, pancreas, kidney, duodenum, jejunum, and lung.

[0129] Molecules of the present invention are also useful as additives to anti-hypoglycemic preparations containing glucose and as adsorption enhancers for oral drugs which require fast nutrient action. Additionally, molecules of the present invention can be used to stimulate glucose-induced insulin release.

[0130] For pharmaceutical use, the proteins of the present invention are formulated for parenteral, particularly intravenous or subcutaneous, delivery according to conventional methods, as well as by nasal inhalation. Intravenous administration will be by bolus injection or infusion over a typical period of one to several hours. In general, pharmaceutical formulations will include a SGIP protein, polypeptides, peptides or variants in combination with a pharmaceutically acceptable vehicle, such as saline, buffered saline, 5% dextrose in water or the like. Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, etc. Methods of formulation are well known in the art and are disclosed, for example, in *Remington's Pharmaceutical Sciences*, Gennaro, ed., Mack Publishing Co., Easton Pa., 1990, which is incorporated herein by reference. Therapeutic doses will generally be in the range of 0.1 to 100 μ g/kg of patient weight per day, preferably 0.5-20 μ g/kg per day, with the exact dose determined by the clinician accord-

ing to accepted standards, taking into account the nature and severity of the condition to be treated, patient traits, etc. Determination of dose is within the level of ordinary skill in the art. The proteins may be administered for acute treatment, over one week or less, often over a period of one to three days or may be used in chronic treatment, over several months or years. For example, a therapeutically effective amount of SGIP is an amount sufficient to produce a clinically significant change in gastric motility and parameters used to measure changes in nutritional absorption. Specific tests for making such measurements are known to these ordinarily skilled in the art.

EXAMPLES

Example 1

Gastrointestinal Contractility

[0131] Two male Sprague-Dawley rats, approximately 12 weeks old (Harlan, Indianapolis, Ind.) are anesthetized with urethane and their stomachs are exposed through a small abdominal incision. Two 2.4 mm transducing crystals (Sonometrics, Ontario, Canada) are placed on the antral portion of the stomach such that circular contractions could be monitored as a change in the distance between the two crystals. The crystals are attached with VETBOND TISSUE ADHESIVE (3M, St. Paul, Minn.).

[0132] 10 μ l of 1 μ M acetylcholine, 10 μ l of norepinephrine (NE) at 1 μ M, or 10 μ l of phosphate buffer solution (PBS—a negative control) is applied topically between the crystals, and the distance between two crystals is measured.

[0133] SGIP peptides as described in Table A are dissolved separately in PBS and 10 μ l is applied topically for a final concentration of 1 μ g, 10 μ g or 100 μ g. Effects of the SGIP peptides are measured as above.

Example 2

Glucose Absorption

[0134] Eight female ob/ob mice, approximately 6 weeks old (Jackson Labs, Bar Harbor, Me.) are adapted to a 4 hour daily feeding schedule for two weeks. After two weeks on the feeding schedule, the mice are given 100 μ g of separate preparations of the SGIP peptides described in Table A in 100 μ l sterile 0.1% BSA by oral gavage, immediately after their eating period (post-prandially). Thirty minutes later, the mice are challenged orally with a 0.5 ml volume of 25% glucose. Retro-orbital bleeds are done to determine serum glucose levels. Blood is drawn prior to peptide dosing, prior to oral glucose challenge, and at 1, 2, 4, and 20 hours following the glucose challenge.

[0135] Post-prandial glucose absorption is measured when peptides are given orally at 100 μ g, 30 minutes prior to an oral glucose challenge.

Example 3

Peptide Synthesis

[0136] SGIP peptides as described in Table A are synthesized by solid phase peptide synthesis using a model 431A Peptide Synthesizer (Applied Biosystems/Perkin Elmer,

Foster City, Calif.). Fmoc-Glutamine resin (0.63 mmol/g; Advanced Chemtech, Louisville, Ky.) is used as the initial support resin. 1 mmol amino acid cartridges (Anaspec, Inc. San Jose, Calif.) are used for synthesis. A mixture of 2(1-Hbenzotriazol-y-yl 1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazol (HOBt), 2m N,N-Diisopropylethylamine, N-Methylpyrrolidone, Dichloromethane (all from Applied Biosystems/Perkin Elmer) and piperidine (Aldrich Chemical Co., St. Louis, Mo.), are used for synthesis reagents.

[0137] The Peptide Companion software (Peptides International, Louisville, Ky.) is used to predict the aggregation potential and difficulty level for synthesis for the these peptides. Synthesis is performed using single coupling programs, according to the manufacturer's specifications.

[0138] The peptide is cleaved from the solid phase following standard TFA cleavage procedure (according to Peptide Cleavage manual, Applied Biosystems/Perkin Elmer). Purification of the peptide is done by RP-HPLC using a C18, 10 μ m semi-preparative column (Vydac, Hesperia, Calif.). Eluted fractions from the column are collected and analyzed for correct mass and purity by electro-spray mass spectrometry.

Example 4

Gastric Emptying

[0139] The effect of topically applied SGIP peptides on the transit of phenol red through the stomachs of fasted male Sprague-Dawley rats (Harlan, Indianapolis, Ind.) is evaluated. The rats (6 animals, 8 weeks old) are fasted 24 hrs prior to being anesthetized with urethane (0.5 ml/100 grams of 25% solution). After anesthetizing, the animals are orally gaged with 1 ml of Phenol Red solution (50 mg/ml in 2% methylcellulose solution).

[0140] The stomach of each animal is exposed through a small abdominal incision and either 1 μ g of a SGIP peptide as described in Table A, or an amino acid control of a scrambled sequence peptide is applied topically to the stomach five minutes following the gavage. The amount of Phenol Red remaining in the stomach is determined by measuring optical density of the extracted stomach contents 30 minutes after the gavage.

Example 5

Body Weight and Glucose Clearance

[0141] Sixteen female ob/ob mice, 8 weeks old, (Jackson Labs, Bar Harbor, Me.) are adapted to a special 4 hour daily feeding schedule for two weeks. They are fed ad libitum from 7:30-11:30 am daily. After two weeks on the feeding schedule, the mice are divided into two groups of 8. One group is given 1.0 μ g/mouse of a preparation of a SGIP peptide as described in Table A. The other group is given a vehicle (i.e., a scrambled sequence peptide) in 100 μ l sterile 0.1% BSQA by oral gavage just prior to receiving food, and at the end of the 4 hour feeding period. The mice are injected twice daily for fourteen days, during which time food intake and body weight is measured daily. On day 14, immediately after the second oral gavage of the SGIP peptides, the mice are challenged orally with an 0.5 ml volume of 25% glucose. Retro-orbital bleeds are done to determine serum glucose

levels immediately prior to administration of the SGIP peptides or vehicle (t=30 min.), and also at 0, 1, 2, and 4 hours following the glucose challenge.

Example 6

In vivo Ligand Binding Assay

[0142] Ten week old Balb C male mice are anesthetized via intramuscular injection and tested for binding of SGIP in vivo.

[0143] SGIP peptides (as described in Table A) are tested. A single glycine is used as a negative control. Additionally, negative controls in which the residues of SGIP peptides have been rearranged, are also tested. The peptides and controls are coupled to fluorescein isothiocyanate (FITC, Molecular Probes, Eugene, Oreg.) in the following manner: The peptides, glycine control and FITC are dissolved in 0.1 M sodium bicarbonate at pH 9.0 to a concentration of 2.0 mg/ml for the peptides and glycine control and 5 mg/ml for FITC, avoiding exposure of the FITC to strong light. The FITC/sodium bicarbonate solution is added to the peptides at a ratio of 1 mg FITC to 1 mg peptide or glycine control, and allowed to react in the dark at ambient room temperature for 1 hour. The FITC-conjugated peptides and glycine control are dialyzed in a 1 K dialysis membrane and 0.1 M sodium bicarbonate buffer at 4° C. The buffer is changed daily and unbound FITC in the post-dialyzed buffer is measured by HPLC. After six days, the buffer is changed to phosphate buffered saline (PBS) and dialyzed for two days followed by another change in PBS and dialyzed for another 2 days. Peptide- or glycine-bound FITC is determined by measuring the absorbance of the dialyzed FITC-bound material at 498 nm and dividing by the extinction coefficient of fluorescein, 0.083 μ M. The molar ratio of fluorescein to peptide (mole FITC/mole peptide) is then determined.

[0144] The labeled peptides are administered via tail vein injections such that each mouse received 0.5 ml (0.5 mg) of labeled peptide which is allowed to circulate in the mice for 15 minutes following injection.

[0145] While under anesthesia the right atrium of each mouse is snipped to allow an exit path and 20 ml of PBS was injected into left ventricle and used to flush the circulatory system. The mice are then perfused with approximately 10 ml of formalin in neutral buffer (10% Neutral Buffered Formalin (NBF), Surgipath, Richmond, Ill.).

[0146] Tissues of interest are harvested by dissection, and fixed overnight in 10% NBF before processing for histological evaluation. Tissues are processed in the V.I.P. 2000 (Miles, Inc., Elkhart, Ind.) resulting in Paraffin® infiltration of the tissue. The tissue-Paraffin® blocks are sliced into 5 μ m sections in a Jung Biocut (Leica, Nussloch, Germany), placed on glass slides, and incubated at 60° C. for one hour to aid in adhering the tissue to the slide. The Paraffin® is removed by washing the slides three times in 100% xylene for 5 minutes. The slides are then rehydrated by 2 washes in 100% ethanol for 3 minutes; followed by one wash in 95% ethanol. The slides are allowed to dry and then mounted with 5 to 10 μ l of antifade medium [nine parts glycerol containing 2% DABCO (1,4-diazobicyclo-(2,2,2)-octane, Sigma, St. Louis, Mo.), dissolved at 55-70° C.; one part 0.2 M Tris/HCL, pH 7.5 DAPI (Sigma, St. Louis, Mo.) or propidium iodide (0.5 μ g/ml]. See also Kievits, T. et al., *Cytogenet Cell Genet* 53:134-136 (1990) for antifade medium. Slides are covered with cover slips and immediately examined by fluorescent microscopy at 495 nm.

[0147] Variants are measured for increased fluorescence in duodenum, jejunum, and convoluted tubules and collecting ducts of the kidney, as compared to the glycine.

Example 7

Acylated Peptide Synthesis

[0148] One or more serine residues of the peptides shown in Table A is be modified to include an n-octanoic acid side chain. The peptide is synthesized by Fmoc chemistry with all of the amino acids protected, except for the hydroxyl group of the serine in position 3 of SEQ ID NO:2. While still attached to the resin, this hydroxyl group of serine is acylated with n-octanoic acid (TCI America, Portland, Oreg.) by the action of 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (Pierce Chemical, Rockford, Ill.) in the presence of 4-(dimethylamino) pyridine (Fluka, Buchs, Switzerland). After acylation, the peptide is cleaved from the resin and protection groups removed. The peptide is then be purified by reverse phase HPLC.

[0149] From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 7

<210> SEQ ID NO 1

<211> LENGTH: 33

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

ggctccagct tcctgagccc tgaacaccag aga

33

<210> SEQ ID NO 2

-continued

```

<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2
Gly Ser Ser Phe Leu Ser Pro Glu His Gln Arg
  1           5           10

<210> SEQ ID NO 3
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)...(351)
<220> FEATURE:
<221> NAME/KEY: sig_peptide
<222> LOCATION: (1)...(69)
<220> FEATURE:
<221> NAME/KEY: mat_peptide
<222> LOCATION: (70)...(351)

<400> SEQUENCE: 3
atg ccc tcc cca ggg acc gtc tgc agc ctc ctg ctc ctc ggc atg ctc      48
Met Pro Ser Pro Gly Thr Val Cys Ser Leu Leu Leu Leu Gly Met Leu
          -20           -15           -10

tgg ctg gac ttg gcc atg gca ggc tcc agc ttc ctg agc cct gaa cac      96
Trp Leu Asp Leu Ala Met Ala Gly Ser Ser Phe Leu Ser Pro Glu His
          -5           1           5

cag aga gtc cag cag aga aag gag tcg aag aag cca cca gcc aag ctg      144
Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu
  10           15           20           25

cag ccc cga gct cta gca ggc tgg ctc cgc ccg gaa gat gga ggt caa      192
Gln Pro Arg Ala Leu Ala Gly Trp Leu Arg Pro Glu Asp Gly Gly Gln
          30           35           40

gca gaa ggg gca gag gat gaa ctg gaa gtc cgg ttc aac gcc ccc ttt      240
Ala Glu Gly Ala Glu Asp Glu Leu Glu Val Arg Phe Asn Ala Pro Phe
          45           50           55

gat gtt gga atc aag ctg tca ggg gtt cag tac cag cag cac agc cag      288
Asp Val Gly Ile Lys Leu Ser Gly Val Gln Tyr Gln Gln His Ser Gln
  60           65           70

gcc ctg ggg aag ttt ctt cag gac atc ctc tgg gaa gag gcc aaa gag      336
Ala Leu Gly Lys Phe Leu Gln Asp Ile Leu Trp Glu Glu Ala Lys Glu
  75           80           85

gcc cca gcc gac aag      351
Ala Pro Ala Asp Lys
  90

<210> SEQ ID NO 4
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: SIGNAL
<222> LOCATION: (1)...(23)

<400> SEQUENCE: 4
Met Pro Ser Pro Gly Thr Val Cys Ser Leu Leu Leu Leu Gly Met Leu
          -20           -15           -10

Trp Leu Asp Leu Ala Met Ala Gly Ser Ser Phe Leu Ser Pro Glu His
          -5           1           5

Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu

```


-continued

```

<222> LOCATION: (6)...(6)
<223> OTHER INFORMATION: Xaa is Gly, Ser, Ala, Thr, Pro, or Met
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (7)...(7)
<223> OTHER INFORMATION: Xaa is Pro, Ala, Gly, Ile, Leu, or Val
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (8)...(8)
<223> OTHER INFORMATION: Xaa is Glu or Asp
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (9)...(9)
<223> OTHER INFORMATION: Xaa is His, Arg, Lys, Phe, or Tyr
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: (10)...(10)
<223> OTHER INFORMATION: Xaa is Asn, Ser, Thr, His, Ala, Glu, Asp, Lys,
or Arg
<221> NAME/KEY: VARIANT
<222> LOCATION: (11)...(11)
<223> OTHER INFORMATION: Xaa is Gln, Asn, Ser, Thr, His, Ala, Glu, Asp,
or Lys
<220> FEATURE:
<223> OTHER INFORMATION: peptide

```

```

<400> SEQUENCE: 6

```

```

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1           5           10

```

```

<210> SEQ ID NO 7
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: degenerate sequence
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)...(33)
<223> OTHER INFORMATION: n = A,T,C or G

```

```

<400> SEQUENCE: 7

```

```

ggnwsnwsnt tyytnwsncc ngarcaycar mgn

```

33

1. An isolated polypeptide molecule consisting of an amino acid sequence selected from

- a. residues 3 to 9 of SEQ ID NO:2;
- b. residues 2 to 9 of SEQ ID NO:2;
- c. residues 1 to 9 of SEQ ID NO:2
- d. residues 3 to 10 of SEQ ID NO:2;
- e. residues 2 to 10 of SEQ ID NO:2; and
- f. residues 3 to 11 of SEQ ID NO:2,

wherein the polypeptide molecule has one amino acid substitution.

2. The isolated polypeptide molecule according to claim 1, wherein the amino acid substitution is selected from

- a. the amino acid at position 1 of SEQ ID NO:2 is substituted with serine, alanine, threonine, or methionine;
- b. the amino acid at position 2 of SEQ ID NO:2 is substituted with glycine, alanine, threonine, or methionine;

c. the amino acid at position 3 of SEQ ID NO:2 is substituted with glycine, alanine, threonine, or methionine;

d. the amino acid at position 4 of SEQ ID NO:2 is substituted with tryptophan, tyrosine, leucine, valine, or isoleucine;

e. the amino acid at position 5 of SEQ ID NO:2 is substituted with isoleucine, valine, phenylalanine, or tyrosine;

f. the amino acid at position 6 of SEQ ID NO:2 is substituted with glycine, alanine, threonine, methionine, or proline;

g. the amino acid at position 7 of SEQ ID NO:2 is substituted with alanine, glycine, isoleucine, leucine, or valine;

h. the amino acid at position 8 of SEQ ID NO:2 is substituted with aspartic acid;

i. the amino acid at position 9 of SEQ ID NO:2 is substituted with arginine, lysine, phenylalanine, or tyrosine;

- j. the amino acid at position 10 of SEQ ID NO:2 is substituted with asparagine, serine, threonine, histidine, alanine, glutamic acid, aspartic acid, lysine or arginine; or
 - k. the amino acid position 11 of SEQ ID NO:2 is substituted with glutamine, asparagine, serine, threonine, histidine, or alanine.
- 3.** An isolated polypeptide molecule consisting of an amino acid sequence selected from
- a. residues 4 to 9 of SEQ ID NO:2;
 - b. residues 4 to 10 of SEQ ID NO:2; and
 - c. residues 4 to 11 of SEQ ID NO:2;
- wherein the polypeptide molecule has one amino acid substitution.
- 4.** The isolated polypeptide molecule according to claim 3, wherein the amino acid substitution is selected from
- a. the amino acid at position 4 of SEQ ID NO:2 is substituted with tryptophan, tyrosine, leucine, valine, or isoleucine;
 - b. the amino acid at position 5 of SEQ ID NO:2 is substituted with isoleucine, valine, phenylalanine, or tyrosine;
 - c. the amino acid at position 6 of SEQ ID NO:2 is substituted with glycine, alanine, threonine, methionine, or proline;
 - d. the amino acid at position 7 of SEQ ID NO:2 is substituted with glycine, alanine, isoleucine, leucine or valine;
 - e. the amino acid at position 8 of SEQ ID NO:2 is substituted with aspartic acid;
 - f. the amino acid at position 9 of SEQ ID NO:2 is substituted with arginine, lysine, phenylalanine, or tyrosine;
 - g. the amino acid at position 10 of SEQ ID NO:2 is substituted with asparagine, serine, threonine, histidine, alanine, glutamic acid, aspartic acid, lysine, or arginine; or
 - h. the amino acid at position 11 of SEQ ID NO:2 is substituted with glutamine, asparagine, serine, threonine, histidine, or alanine.
- * * * * *