(86) Date de dépot PCT/PCT Filing Date: 2002/03/29
(87) Date publication PCT/PCT Publication Date: 2002/10/10
(45) Date de délivrance/issue Date: 2013/11/19
(85) Entrée phase nationale/National Entry: 2003/09/29
(86) N° demande PCT/PCT Application No.: US 2002/009885
(87) N° publication PCT/PCT Publication No.: 2002/078640
(30) Priorité/Priority: 2001/03/29 (US09/821,726)

(54) Titre : REGULATION DE LA CROISSANCE ET DE LA REPARATION DE TISSUS GASTRO-INTESTINAUX PAR DES GASTROKINES ET DES INHIBITEURS
(54) Title: CONTROL OF GROWTH AND REPAIR OF GASTRO-INTESTINAL TISSUES BY GASTROKINES AND INHIBITORS

A

HUMAN 1

MKPTTVPAAGLQTVTLPALAAINPIMWNIDNNNSAGSGGSQVSVSGHSNVHNN 50

PIG 1

MKPTTVPAAGLQTVTLPALAAINPIMWNIDNNNSAGSGGSQVSVSGHSNVHNN 50

51 VEDRNQMDWSQSTMDTQGFRPTLQXKCTCDEMTRPFMPISTQQDDAL 100

51 VEDRNQMDWSQSTMDTQGFRPTLQXKCTCDEMTRPFMPISTQQDDAL 100

101 VRXRLQGKGPGGPRPGPQGLSTSHQVHGQIYKGOGTATHMRGKPFMTMA 150

101 VRXRLQGKGPGGPRPGPQGLSTSHQVHGQIYKGOGTATHMRGKPFMTMA 150

151 HRKQBAELFPSGNCITTSSLWTVSVGQLTVVEN* 186

*termination

151 HRKQBAELFPSGNCITTSSLWTVSVGQLTVVEN* 186

B

Predicted Structure for the praAMP-18 ORF

Human

HNQ signal peptide   N-dom  C-dom  amphipathic helix

amphipathic helix

amphipathic helix

GP turn

C

helix

amphipathic helix

DNA

(57) Abrégé/Abstract:
A novel group of gastrokines called Gastric Antrum Mucosal Protein is characterized. A member of the group is designated AMP-18. AMP-18 genomic DNA, cDNA and the AMP-18 protein are sequenced for human, mouse and pig. The AMP-18 protein and
active peptides derived from it are cellular growth factors. Surprisingly, peptides capable of inhibiting the effects of the complete protein, are also derived from the AMP-18 protein. Cytoprotection and control of mammalian gastro-intestinal tissue growth and repair (restitution) is facilitated by the use of the proteins, making the protein candidates for therapies in inflammatory bowel disease and gastric ulcers.
CONTROL OF GROWTH AND REPAIR OF GASTRO-INTESTINAL TISSUES BY GASTROKINES AND INHIBITORS

A novel group of gastrokines called Gastric Antrum Mucosal Protein is characterized. A member of the group is designated AMP-18. AMP-18 genomic DNA, cDNA and the AMP-18 protein are sequenced for human, mouse and pig. The AMP-18 protein and active peptides derived from it are cellular growth factors. Surprisingly, peptides capable of inhibiting the effects of the complete protein, are also derived from the AMP-18 protein. Cytoprotection and control of mammalian gastro-intestinal tissue growth and repair (restitution) is facilitated by the use of the proteins, making the protein candidates for therapies in inflammatory bowel disease and gastric ulcers.
Control of Growth and Repair of Gastro-Intestinal Tissues by Gastrokines and Inhibitors

Inventors: F. Gary Toback, Terence E. Martin, and Margaret M. Walsh-Reitz

BACKGROUND

A novel group of Gastric Antrum Mucosal Proteins that are gastrokines, is characterized. A member of the gastrokine group is designated AMP-18. AMP-18 genomic DNA, and cDNA molecules are sequenced for human and mouse, and the protein sequences are predicted from the nucleotide sequences. The cDNA molecule for pig AMP-18 is sequenced and confirmed by partial sequencing of the natural protein. The AMP-18 protein and active peptides derived from its sequence are cellular growth factors. Surprisingly, peptides capable of inhibiting the effects of the complete protein, are also derived from the AMP-18 protein sequence. Control of mammalian gastro-intestinal tissues growth and repair is facilitated by the use of the protein or peptides, making the protein and the derived peptides candidates for therapies.

Searches for factors affecting the mammalian gastro-intestinal (GI) tract are motivated by need for diagnostic and therapeutic agents. A protein may remain part of the mucin layer, providing mechanical (e.g., lubricant or gel stabilizer) and chemical (e.g. against stomach acid, perhaps helping to maintain the mucus pH gradient and/or hydrophobic barrier) protection for the underlying tissues. The trefoil peptide family has been suggested to have such general cytoprotectant roles (see Sands and Podolsky, 1996). Alternatively, a cytokine-like activity could help restore damaged epithelia. A suggestion that the trefoil peptides may act in concert with other factors to maintain and repair the epithelium, further underlines the complexity of interactions that take place in the gastrointestinal tract (Podolsky, 1997). The maintenance of the integrity of the GI epithelium is essential to the continued well-being of a mammal, and wound closing after damage normally occurs very rapidly (Lacy, 1988), followed by proliferation and differentiation soon thereafter to reestablish epithelial integrity (Nursat et al., 1992). Thus protection and restitution are two critical features of the healthy gastrointestinal tract, and may be important in
the relatively harsh extracellular environment of the stomach.

Searches for GI proteins have met with some success. Complementary DNA (cDNA) sequences to messenger RNAs (mRNA) isolated from human and porcine stomach cells were described in the University of Chicago Ph.D. thesis "Characterization of a novel messenger RNA and immunochemical detection of its protein from porcine gastric mucosa," December 1987, by one of the present inventors working with the other inventors. However, there were several cDNA sequencing errors that led to significant amino acid changes from the AMP-18 protein disclosed herein. The protein itself was isolated and purified only as an aspect of the present invention, and functional analyses were performed to determine utility.

Nucleic acid sequences were sought.

SUMMARY OF THE INVENTION

A novel gene product designated Antrum Mucosal Protein 18 ("AMP-18") is a gastrokine. The protein was discovered in cells of the stomach antrum mucosa by analysis of cDNA clones obtained from humans, pigs, and mice. The protein is a member of a group of cellular growth factors or cytokines, more specifically gastrokines. The AMP-18 cDNA sequences predict a protein 185 amino acids in length for both pig and man. The nucleotide sequences also predict a 20-amino acid N-terminal signal sequence for secreted proteins. The cleavage of this N-terminal peptide from the precursor (preAMP-18) was confirmed for the pig protein; this cleavage yields a secreted protein 165 amino acids in length and ca.18,000 Daltons (18kD) in size. Human and mouse genomic DNA sequences were also obtained and sequenced. A human genomic DNA was isolated in 4 overlapping fragments of sizes 1.6kb, 3kb, 3.3 kb and 1.1 kb respectively. The mouse genomic DNA sequence was isolated in a single BAC clone.

The gastrokine designated AMP-18 protein is expressed at high levels in cells of the gastric antrum. The protein is barely detectable in the rest of the stomach or duodenum, and was not found, or was found in low levels, in other body tissues tested. AMP-18 is synthesized in luminal surface mucosal cells, and is secreted together with mucin granules.

Studies in humans confirm the location and expression of the AMP-18 peptide
in human gastric mucosa.

Compositions of AMP-18 isolated from mouse and pig antrum tissue stimulate growth of confluent stomach, intestinal, and kidney epithelial cells in culture; human, monkey, dog and rat cells are also shown to respond. This mitogenic (growth stimulating) effect is inhibited by specific antisera (antibodies) to AMP-18, supporting the conclusion that AMP-18, or its products, e.g. peptides derived from the protein by isolation of segments of the protein or synthesis, is a growth factor. Indeed, certain synthetic peptides whose amino acid sequences represent a central region of the AMP-18 protein also have growth-factor activity. The peptides also speed wound repair in tissue culture assays, indicating a stimulatory effect on cell migration, the process which mediates restitution of stomach mucosal injury. Thus, the protein and its active peptides are motogens. Unexpectedly, peptides derived from sub-domains of the parent molecule can inhibit the mitogenic effect of bioactive synthetic peptides and of the intact, natural protein present in stomach extracts.

There are 3 activities of the gastrokine proteins and peptides of the present invention. The proteins are motogens because they stimulate cells to migrate. They are mitogens because they stimulate cell division. They function as cytoprotective agents because they maintain the integrity of the epithelium (as shown by the protection conferred on electrically resistant epithelial cell layers in tissue culture treated with damaging agents such as oxidants or non-steroidal anti-inflammatory drugs NSAIDs).

The synthesis of AMP-18 is confined to luminal mucosal lining epithelial cells of the gastric antrum of humans and other mammals. Inside cells the protein is co-localized with mucins in secretion granules, and appears to be secreted into the mucus overlying the apical plasma membrane. Recombinant human AMP-18 in E. coli exerts its mitogenic effect at a concentration an order of magnitude lower than growth-promoting peptides derived from the center of the mature protein. Peptide 77-97, the most potent mitogenic peptide, is amino acid sequence-specific AMP peptides appears to be cell-type specific as it does not stimulate growth of fibroblasts or HeLa cells. Mitogenesis by specific AMP peptides appears to be mediated by a cell surface receptor because certain peptides that are not active mitogens can competitively
inhibit, in a concentration-dependent manner, the growth stimulating effects of peptide 58-99 and antrum cell extracts. AMP-18 and its derived peptides exhibit diverse effects on stomach and intestinal epithelial cells which suggest they could play a critical role in repair after gastric mucosal injury. These include cytoprotection, mitogenesis, restitution, and maturation of barrier function after oxidant-and/or indomethacin-mediated injury. Possible mechanisms by which AMP-18 or its peptide derivatives mediate their pleiotropic effects include stimulation of protein tyrosine kinase activity, prolongation of heat shock protein expression after cell stress, and enhanced accumulation of the tight junction-associated protein ZO-1 and occludin. Certain of these physiological effects can occur at concentrations that are relatively low for rhAMP-18 (<50 nM) compared to the concentrations of other gastric peptide mediators such as trefoil peptides or the α-defensin, cryptdin 3 (>100 μM). Immunoreactive AMP-18 is apparently released by cells of the mouse antrum after indomethacin gavage, and by canine antrum cells in primary culture exposed to forskolin, suggest that the protein is subject to regulation. These results imply that AMP-18 could play a role in physiological and pathological processes such as wound healing in the gastric mucosal epithelium in vivo.

The invention relates a group of isolated homologous cellular growth stimulating proteins designated gastrokines, that are produced by gastric epithelial cells and include the consensus amino acid sequences VKE(K/Q)KXXGKGPGGXPPK (SEQ ID NO: 10) wherein XX can be LQ or absent (which results in SEQ ID NOS 25 and 26, respectively). An isolated protein of the group has an amino acid sequence as shown in FIG 8. The protein present in pig gastric epithelia in a processed form lacking the 20 amino acids which constitute a signal peptide sequence, has 165 amino acids and an estimated molecular weight of approximately 18kD as measured by polyacrylamide gel electrophoresis. Signal peptides are cleaved after passage through endoplasmic reticulum (ER). The protein is capable of being secreted. The amino acid sequence shown in FIG. 3 was deduced from a human cDNA sequence. An embodiment of the protein is shown with an amino acid sequence as in FIG. 6, a sequence predicted from mouse RNA and DNA.

A growth stimulating (bioactive) peptide may be derived from a protein of the
gastrokine group. Bioactive peptides rather than proteins are preferred for use because they are smaller, consequently the cost of synthesizing them is lower than for an entire protein.

In addition, a modified peptide may be produced by the following method:

(a) eliminating major protease sites in an unmodified peptide amino acid sequence by amino acid substitution or deletion; and/or
(b) introducing into the modified amino acid analogs of amino acids in the unmodified peptide.

An aspect of the invention is a synthetic growth stimulating peptide, having a sequence of amino acids from positions 78 to 119 as shown in FIG. 3.

Another peptide has a sequence of amino acids from position 97 to position 117 as shown in FIG. 3.

Another peptide has a sequence of amino acids from position 97 to position 121 as shown in FIG. 3.

Another peptide has a sequence of amino acids from position 104 to position 117 as shown in FIG. 3.

An embodiment of an isolated bioactive peptide has one of the following sequences: KKLQGKPGPGPPPK (SEQ ID NO:11), LDALVKEKKLQGKPGGPPPK (SEQ ID NO: 12), or LDALVKEKKLQGKPGGPPPKGLMY (SEQ ID NO: 13). An embodiment of an inhibitor of a protein of the gastrokine group has the amino acid sequence KKTCIVHKKK (SEQ ID NO: 14) or KKEVMSIQSLDALVKEKK. (SEQ ID NO: 15) (see also Table 1)

The invention also relates a pharmaceutical composition including at least a growth stimulating peptide.

A pharmaceutical composition for the treatment of diseases associated with overgrowth of gastric epithelia, includes an inhibitor of a protein of the group of gastrokines or of a growth stimulating peptide derived from the gastrokine proteins.

A pharmaceutical composition for the treatment of diseases of the colon and small intestine includes at least a growth stimulating peptide of the present invention. Examples of such diseases include ulcerative colitis and Crohn’s Disease.
Antibodies to the protein product AMP-18 encoded by the human cDNA expressed in bacteria were produced in rabbits; these antibodies reacted with 18kD antrum antigens of all mammalian species tested (human, pig, goat, sheep, rat and mouse), providing a useful method to detect gastrokines. An antibody to a protein of the group recognizes an epitope within a peptide of the protein that includes an amino acid sequence from position 78 to position 119 as in FIG. 3.

The invention is also directed to an isolated genomic DNA molecule with the nucleotide sequence of a human as shown in FIG. 1 and an isolated cDNA molecule encoding a human protein, that the nucleotide sequence as shown in FIG 2.

Another aspect of the invention is an isolated DNA molecule having the genomic sequence found in DNA derived from a mouse, as shown in FIG. 4.

Genomic DNA has value because it includes regulatory elements for gastric expression of genes, consequently, the regulatory elements can be isolated and used to express other gene sequences than gastrokines in gastric tissue.

An aspect of the invention is a mouse with a targeted deletion in a nucleotide sequence in the mouse genome that, when expressed without the deletion, encodes a protein of the group of gastrokines of the present invention.

An aspect of the invention is a method of making a gastrokine protein or a peptide derived from a gastrokine protein. The method includes:

a) obtaining an isolated cDNA molecule with a sequence such as that shown in FIG. 2;

b) placing the molecule in a recombinant DNA expression vector;

c) transfecting a host cell with the recombinant DNA expression vector;

d) providing environmental conditions allowing the transfected host cell to produce a protein encoded by the cDNA molecule; and

e) purifying the protein from the host cell.

Host cells in which expression has been successful include baculovirus, which allows large amounts of gastrokines to be provided for commercial and research uses. For example, human AMP-18 protein without the signal peptide was produced.

A recombinant human protein AMP-18 expressed in E. coli has the sequence in FIG. 14, left panel.
An aspect of the invention is a method to stimulate growth of epithelial cells in the gastrointestinal tract of mammals. The method includes the steps of:

(a) contacting the epithelial cells with a composition comprising a gastrokine protein or a peptide derived from a protein of the group; and

(b) providing environmental conditions for stimulating growth of the epithelial cells.

A method to inhibit cellular growth stimulating activity of a protein of the group includes the steps of:

(a) contacting the protein with an inhibitor; and

(b) providing environmental conditions suitable for cellular growth stimulating activity of the protein.

The inhibitor may be an antibody directed toward at least one epitope of the protein, e.g. an epitope with an amino acid sequence from position 78 to position 119 of the deduced amino acid sequence in FIG. 3 or an inhibitor peptide such as those in Table 1.

A method of testing the effects of different levels of expression of a protein on mammalian gastrointestinal tract epithelia, includes the steps of:

(a) obtaining a mouse with an inactive or absent gastrokine protein;

(b) determining the effects of a lack of the protein in the mouse;

(c) administering increasing levels of the protein to the mouse; and

(d) correlating changes in the gastrointestinal tract epithelia with the levels of the protein in the epithelia.

Kits are contemplated that will use antibodies to gastrokines to measure their levels by quantitative immunology. Levels may be correlated with disease states and treatment effects.

A method to stimulate migration of epithelial cells after injury to the gastrointestinal tract of mammals, includes the steps of:

(a) contacting the epithelial cells with a composition comprising a peptide derived from the protein; and

(b) providing environmental conditions allowing migration of the epithelial cells.
A method for cytoprotection of damaged epithelial cells in the gastrointestinal tract of mammals, includes the following steps:

(a) contacting the damaged epithelial cells with a composition including a protein of the gastrokine group or a peptide derived from the protein; and

(b) providing environmental conditions allowing repair of the epithelial cells. The damaged cells may form an ulcer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a human genomic nucleotide sequence (SEQ ID NO: 1) of a pre-gastrokine; sequence features were determined from cDNA and PCR of human genomic DNA amph-ge8.seq Length: 7995 predicted promoter: 1405; exon 1: 1436-1490; exon 2: 4292-4345; exon 3: 4434-4571; exon 4: 5668-5778; exon 5: 6709-6856; exon 6: 7525-7770; polyA site: 7751.

FIG. 2 is a human cDNA sequence (SEQ ID NO: 2); the DNA clone was obtained by differential expression cloning from human gastric cDNA libraries.

FIG. 3 is a human preAMP-18 protein sequence (SEQ ID NO: 3) predicted from a cDNA clone based on Powell (1987) and revised by the present inventors; N-21 is the expected N-terminus of the mature protein.

FIG. 4 is a mouse preAMP-18 sequence (SEQ ID NO: 4) determined from RT-PCR of mRNA and PCR of BAC-clones of mouse genomic DNA sequences:


FIG. 5 is a mouse cDNA sequence (SEQ ID NO: 5) for preAMP-18.

FIG. 6 is mouse preAMP-18 amino acid sequence (SEQ ID NO: 6); RT-PCR performed on RNA isolated from mouse stomach antrum: Y-21 is the predicted N-terminus of the mature protein; the spaces indicated by .. mean there are no amino acids there to align with other sequences in FIG. 10.

FIG. 7 is a cDNA expressing porcine AMP-18 (SEQ ID NO: 7).

FIG. 8 is pig pre-gastrokine (pre-AMP-18) protein sequence. (SEQ ID NO: 8)

predicted from a cDNA clone based on Powell (1987) D-21 is the N-terminus of the mature protein - confirmed by sequencing of the protein isolated from pig stomach.
FIG. 9 is a comparison between the amino acid sequences of human (SEQ ID NO: 3) versus pig (SEQ ID NO: 8) pre-gastrokine.

FIG. 10 shows a computer-generated alignment comparison of human (SEQ ID NO: 3), pig (SEQ ID NO: 8) and mouse (SEQ ID NO: 6) predicted protein sequences determined from sequencing of cDNA clones for human and pig AMP-18, and by polymerase chain reaction of mouse RNA and DNA using preAMP-18 specific oligonucleotide primers; in each case the first 20 amino acids constitute the signal peptide, cleaved after passage through the endoplasmic reticulum membrane.

FIG. 11 shows the effect of porcine gastric antrum mucosal extract, human AMP peptide 77-97, of the mature protein (same as peptide 97-117 of human precursor protein; Table 1) and EGF on growth of gastric epithelial cells; AGS cells were grown in DMEM containing fetal bovine serum (5%) in 60-mm dishes; different amounts of pig antrum extract, HPLC purified peptide 77-97, and/or EGF were added; four days later the cells were dispersed and counted with a hemocytometer; antrum extract and peptides each stimulated cell growth in a concentration-dependent manner; the bar graph shows that at saturating doses, peptide 77-97 (8μg/ml) or EGF (50ng/ml) was mitogenic; together they were additive suggesting that the two mitogens act using different receptors and/or signaling pathways; anti-AMP antibodies inhibited the antrum extract but did not inhibit peptide 77-97.

FIG. 12 shows the structure of the human and mouse preAMP-18 genes; the number of base pairs in introns are shown above the bars; exons are indicated E1-E6 and introns II-I5; there are minor differences in intron length.

FIG. 13 shows Left panel. Amino acid sequence of recombinant human AMP-18 (residues 21-185 of SEQ ID NO: 3) expressed in E. coli. Note the His6-tag (SEQ ID NO: 16) within a 12 amino acid domain (SEQ ID NO: 9) at the N-terminus that has replaced the putative hydrophobic signal peptide. Right panel. Effect of rhAMP-18 and AMP peptide 77-97 on growth of confluent cultures of IEC-18 cells. Although maximal growth stimulation is similar, the half-maximal concentration (K_{1/2}) for rhAMP-18 (~30 nM) is about an order of magnitude lower than for the peptide (~300 nM).

FIG. 14 shows Left Panel. Alignment of the open reading frames (ORF)
derived from the cDNA clones for AMP-18 for the precursor proteins of human (SEQ ID NO: 3) and pig (SEQ ID NO: 8) antrum. Similarity was 78.50% and identity was 75.27%. Computer analysis was carried out using the GAP and PEPTIDESTRUCTRE programs of the Wisconsin Package (GCG). *Right Panel.*

Model of the predicted secondary structure for the human preAMP ORF. Attention is drawn to the asparagine rich N-terminal domain, the short tryptophan (W)-rich and glycine-proline (GP) regions, and the conserved positions of the four cysteine (C) residues. Possible amphipathic helices are indicated.

FIG. 15 shows the effect of porcine antrum cell extract, peptide 77-97, and EGF on growth of intestinal epithelial cells. IEC-6 cells were grown in 60-mm dishes. Antrum cell extract (*left panel*) and peptide 77-97 (*center panel*) each stimulated growth in a concentration-dependent manner. Peptide 77-97 (1μg/ml) appeared more potent than EGF (50 ng/ml) (*right panel*). Values are means ± SE for 3 cultures.

FIG. 16 shows the effect of AMP peptide 77-99 and EGF on growth and wound restitution by human antrum epithelial cells. To measure *growth* (*left panel*), HAE cells were plated in 60-mm dishes. Peptide 77-97 (8 μg/ml), or EGF (50 ng/ml), or both were added to the medium and the number of cells counted 4 days later. Peptide 77-97 and EGF each stimulated proliferation, and appeared to be additive. Values are means ± SE for 3 cultures. To measure migration (*right panel*), cells were grown in 60-mm dishes to prepare a confluent monolayer. The medium was aspirated and replaced with fresh medium containing 0.01% calf serum (CS). The monolayer was mechanically wounded by scraping with a razor blade. Detached cells were removed by aspirating medium, and rinsing the remaining cells twice with fresh medium containing 0.01% CS. Fresh medium (5 ml) containing CS (0.01%) and insulin (100 U/L) was added to wounded cultures. Either peptide 77-97 (8μg/ml), EGF (50ng/ml), or both were added to duplicate cultures. Migration was assessed at 24, 48 and 72 hr after wounding by measuring the distance (in mm) that cells had migrated from the wound edge using a microscope eyepiece reticle (10-mm long; 0.1-mm markings). Migrating cells at 12 randomly chosen sites along a 0.25-mm stretch of the wound edge were measured at 40-fold magnification. Migration at 2 different
sites was measured for each of 2 separate wounds made in each culture. Values are the mean distance cells moved into the denuded area from the edge of 4 different wounds in 2 cultures ± SE. Cells exposed to peptide 77-97 migrated further from the wound edge than those exposed to vehicle at 72 hr. EGF also stimulated cell movement, and the two agents acting together markedly enhanced migration.

FIG. 17 shows the effect of AMP peptide 67-85 on growth of intestinal epithelial cells stimulated by peptide 58-99. Confluent cultures of IEC-18 cells were prepared. One day later, medium was aspirated and replaced with 5 ml of DMEM containing CS (0.5%) and insulin, without (control) or with mitogenic peptide 58-99 (8 µg/ml). Sister plates receiving 1 ml medium and different amounts of peptide 67-85 were incubated at 1 hr at 38°C on a CO₂ incubator, and then an additional 4 ml of medium was added to each dish. Peptide 58-99 was added to 2 of the 4-sister plates at each concentration of peptide 67-85, and the number of cells was counted. In the absence of peptide 67-85, cell number increased by 290%, whereas cells exposed to peptide 58-99 increased in number by 407%, and EGF-treated (50 ng/ml) cells increased by 402% (not shown) during the next 3 days. Stimulation of cell growth by mitogenic peptide 58-99 was completely abolished by preincubation of cells with 0.25 µg/ml of peptide 67-85. When added alone, peptide 67-85 (0.25 to 8 µg/ml) was not a mitogen. Values for the number of cells per culture are shown relative to multiplication of cells exposed to the vehicle during the same period.

FIG. 18 shows the effect of rabbit antiserum to AMP-18 on mitogenic effect of rhAMP-18 on confluent IEC-18 cells. When rhAMP-18 (50 nanomolar) was preincubated for 30 min with antiserum (1:100 dilution) +Ab), growth stimulation was reduced by ~95%; preimmune serum had no effect on cell growth. The half-maximal concentration (K₁₀) for growth stimulation of this recently purified rhAMP-18 is about 5 nanomolar.

FIG. 19 shows the effect of AMP peptide 77-97 on wound restitution in human antrum (HAE) and rat intestinal (IEC-18) epithelial cells. Confluent monolayer cultures were mechanically wounded by scraping with a razor blade, and the distance that cells migrated from the wound edge was measured using a microscope eyepiece reticle. Cells migrated further in the presence of AMP peptide
at each time point studied (P<0.005).

FIG. 20 shows the effect of AMP peptide 77-97 on maturation of TER. Monolayer cultures of MDCK cells were grown on permeable polycarbonate filters (0.4-μm pore size) (Transell) in DMEM containing FBS (2%) without (control) or with peptide 77-97 (8 μg/ml) for 8 days. TER was measured 24 hr after the cells were plated, and at specified times thereafter using an epithelial volt-ohm meter (EVOM, Millipore). Following each measurement, medium containing FBS without or with peptide was changed (0, 48, and 144 hr), and additional peptide 77-97 (8 μg/ml) was added at 30 and 72 hr. At 72 hr, TER in cultures that received peptide 77-97 was twice as high as in control cultures. Values are means for 3 cultures; variance is <10% of the mean. TER was measured from 3 different areas on filter.

FIG. 21 shows the effect of AMP peptide 77-97 on TER in monolayers injured with the oxidant monochloramine or indomethacin. Panel A: When a stable TER was reached (330 Ω·cm²) in MDCK cell monolayers the medium was changed to DMEM containing FBS (0.2%), and either peptide 77-97 (8μg/ml) or EGF (50 ng/ml). After 18 hr, peptide 77-97 or EGF were added to the specified wells. One hour later monochloramine (0.1 mM), like the other agents, was added to the apical and basal compartments of the Transwell. Monochloramine-injured cultures treated with vehicle or EGF sustained ~35-40% loss of TER 90 min after oxidant exposure, whereas the TER of oxidant-injured cells treated with peptide 77-97 was similar to control cultures not exposed to the oxidant. Panels B, C: Caco2/bbe (C2) subclone monolayers were grown on collagen-coated polycarbonate filters until a stable TER was reached (225 Ω·cm²). Spent medium was replaced with fresh medium containing FBS (0.1%) alone or with peptide 77-97 (8 μg/ml). After 18 hr, monochloramine (0.3 mM, B) or indomethacin (0.1 mM, C) was added to both compartments of the Transwell. At time 0, cultures received either vehicle (control), vehicle plus oxidant or indomethacin, or peptide 77-97 and oxidant or indomethacin. TER of injured cultures treated with vehicle decreased by ~35% at 90 min, whereas peptide-treated cultures declined ~10%. The peptide did not alter TER of non-injured cells (not shown).

FIG. 22 shows the effect of AMP peptide 77-97 on TER following injury by
DSS. C2 cell monolayers were grown in DMEM containing FBS (5%) and transferrin (10 μg/ml) on collagen-coated polycarbonate filters until a stable TER was reached (225 Ω cm²). At time 0, cells were exposed to no DSS (control), or DSS (4%) in the upper compartment of the Transwell. AMP peptide 77-97 (8 μg/ml) was added to the upper and lower compartments of the Transwell 1 day prior to the addition of DSS at time 0. TER of DSS-injured cultures treated with vehicle decreased by ~70% at 45 min, whereas peptide-treated cultures declined ~10% at that time. The peptide did not alter TER of non-injured cells. Values are means for ≥ 6 cultures.

FIG. 23 shows the effect of AMP peptide 77-97 on ZO-1 and occludin after oxidant injury of C2 cells. This immunoblot shows that protein levels in the insoluble fraction are ~ two-fold greater after exposure of cells to AMP peptide than to the vehicle.

FIG. 24 shows the effect of AMP peptide on C2 cells. Cultures were exposed to the peptide for different periods of time and the insoluble fraction was obtained. Proteins were separated, immunoblots were probed with specific antisera, and the amount of each protein was quantified using laser densitometry.

FIG. 25 shows the effect of rhAMP-18 on TER of monolayers subjected to oxidant injury. Confluent C2 cell monolayers were prepared on Transwells until a stable TER was established. Medium was replaced with fresh medium containing FBS (0.1%) alone (control), or with either rhAMP-18 (100 nanomolar) or peptide 77-97 (3.7 micromolar). After 18 hr, monochloramine (0.3 mM) was added to both compartments of the Transwell, and cultures received either vehicle (control), vehicle plus oxidant, rhAMP-18 and oxidant, or peptide 77-97 and oxidant, after which TER was measured.

FIG. 26 shows the effect of rhAMP-19 on levels of ZO-1 and occludin in C2 cells. Monolayer cultures were treated with rhAMP-19 (100 nanomolar) or the vehicle for 8 hr. Following cell lysis, an insoluble (particulate) fraction representing cell membranes and cytoskeleton-associated TJ protein was prepared and then subjected to immunoblotting. The amount of immunoreactive ZO-1 and occludin is about two-fold greater in rhRMP-18-treated (+) cells than vehicle-treated (1) cells as
estimated by laser densitometry of the same immunoblot. Equal protein loading in each lane was documented by re-probing the blot with an antibody to heat shock protein 73 which is constitutively expressed by these cells.

FIG. 27 shows Left Panel. Mice (n=10) were given 3% DSS, and stools were assayed daily. Fewer animals given AMP peptide (3 mg/kg body weight/day, s.c.) than vehicle and homocult-positive stool. Right panel. After animals received DSS for 4 days, they were switched to water (day 0 on graph). Mice given AMP peptide daily lost less weight than those given vehicle by day 5 (P<0.04). Weight of peptide-treated animals on days 4 and 5 appeared to increase; it declined in those given vehicle.

DETAILED DESCRIPTION OF THE INVENTION

1. General

A novel gene product, a member of a group of gastrokines, was detected in mammalian gastric antrum mucosal by a differential screen of cDNA libraries obtained from different regions of the pig stomach. The cDNA sequence predicted a protein of 185 amino acids including a signal peptide leader sequence. A cDNA was also isolated from a human library. The predicted amino acid sequence identity between pig and human in 76.3%. The sequences predicted a 20 amino acid signal peptide characteristic for secreted proteins. The cleavage of this N-terminal signal peptide was confirmed for the pig protein. Antibodies to the product of the human cDNA expressed in bacteria were raised in rabbits; these antibodies reacted with 18-20kD antrum antigens of all mammalian species tested (pig, goat, sheep, rat and mouse). In agreement with mRNA levels, the AMP-18 protein is expressed at high levels only in the gastric antrum; it is barely detectable in the rest of the stomach or duodenum, and was not detected in a variety of other tissues tested. AMP-18 is synthesized in the luminal surface mucosal cells; immuno-electron microscopy locates AMP-18 in the secretion granules of these cells. Partially purified AMP-18 preparations from mouse and pig antrum tissue are mitogenic to confluent stomach and kidney epithelial cells in culture; this effect is inhibited by the specific antisera, implying that AMP-18, or its products, is a growth factor.

AMP-18 is likely secreted with the mucus and functions, perhaps as peptide
derivatives, within the mucus gel to maintain epithelial integrity directly, and possibly to act against pathogens. In view of the growth factor activity observed on epithelial cell lines in culture, it is likely that AMP-18 or its peptide derivative(s) serves as an autocrine (and possible paracrine) factor for the gastric epithelium. The function of AMP-18 may not be simply as a mitogen, but in addition it may act as differentiation factor providing the signals for replenishment of the mature lumenal surface cells. The AMP-18 protein or its derivatives are likely important to the normal maintenance of the highly dynamic gastric mucosa, as well as playing a critical role in the restitution of the antrum epithelium following damage. This protein has not been characterized in any publication, however, related nucleic acid sequences have been reported as ESTs and as a similar full length gene. Limitations of EST data cannot yield information on starting sequences, signal peptides, or sequences in the protein responsible for bioactivity, as disclosed in the present invention. A number of these ESTs have been reported for mammalian stomach cDNAs, but related ESTs have also been reported or pancreas and also pregnant uterus libraries. Although expression of AMP-18 RNA in these other tissues appears to be low (as indicated for pancreas by PCR analysis), these results suggest that this growth factor may have broader developmental and physiological roles than that implied by the specific high levels of expression found for the stomach.

The AMP-18 protein appears to be expressed at the surface of the cellular layers of the gastrointestinal (GI) tract. The expressing cells may be releasing stored growth factor where needed - in the crypts and crevices of the GI tract where cellular repair is needed due to surface damage.

AMP-18 may act on the mucosal, apical surfaces of the epithelial cells, collaborating with prostaglandins and other growth factors that operate via basolateral cell surface receptors on the serosal side. The protein or its derivatives are likely important for the normal maintenance of the highly dynamic gastric mucosa, in face of the mechanical stress and high acidity of the stomach. AMP-18 may play a critical role in the repair of the stomach epithelium following damage by agents such as alcohol, nonsteroidal anti-inflammatory drugs (NSAIDs), or pathogens, in particular Helicobacter pylori, which predominantly infects the antrum and is a causative agent.
of gastric ulcers and possibly cancers.

2. **Bioactivity**

A synthetic peptide (42 amino acids, a "42-mer") representing a central region of the AMP-18 amino acid sequence also has growth factor activity, which is inhibited by specific antisera; some related shorter peptides also have stimulatory activity, while others can inhibit the activity of the 42-mer. This result suggests that a saturatable epithelial receptor exists for AMP-18, and opens direct avenues to analyzing the bioactive regions of the protein and identifying the putative receptor(s). Because AMP-18 does not resemble in structure any known cytokine or cytoprotectant protein (such as the trefoil peptides), the analysis of the interactions of the protein, and its active and inhibitory related peptides, with cells offers the opportunity to reveal novel molecular interactions involved in cell growth control.

BSC-1 cell growth was stimulated by gel-fractionated porcine antrum extract; porcine extract protein (250µg) was loaded into each of 2 lanes and subjected to electrophoresis in a polyacrylamide gel (12.5%); the 5 thin slices (2-3 mm) from each area between M, 14 kDa and 21.5 kDa were cut from the experimental lanes. Each pair of slices was placed in a silanized microfuge tube with 200µl sterile PBS, 3% acetonitrile and 1% BSA, and macerated; proteins were eluted from the gel for 18 hr at 22°C with vigorous shaking; the samples were then microcentrifuged and a sample of a supernatant was added to a confluent culture of BSC-1 cells; the number of cells was counted 4 days later; maximal growth stimulation was observed in cultures receiving extracts eluted from gel slices corresponding to a M, of ~18 kDa; antisera to recombinant human AMP-18 added to the culture medium completely inhibited growth stimulation by the 18 kDa fraction (+Ab); values are means of 2 cultures; SE is less than 10% of the mean.

The biological activity (mitogenic for epithelial cells in the gastro-intestinal tract) of the AMP-18 is located in the C-terminal half of the protein. The epitopic sequence(s) appear(s) to be immediately N-terminal to the mitogenic sequence.

The biological activity that is a growth factor, is exhibited by a peptide comprising at least 42 amino acids from positions 78 to 119 of the full-length protein sequence. An antibody to this region blocked mitogenic activity. Although a peptide
having an amino acid sequence of 104 to 117 had mitogenic activity, an antibody to this region did not block (inhibit) the activity. A peptide with an amino acid sequence from positions 97 - 117 has the same mitogenic activity as a peptide with the 42 amino acid sequence, but is less expensive to produce as a synthetic peptide.

3. **Inhibition of Bioactivity**

Epithelial cell growth that was stimulated by murine or porcine antrum cell extract was blocked by rabbit antiserum to a complete, recombinant human AMP-18 precursor protein; confluent cultures of BSC-1 cells were prepared; murine or porcine antrum cell extract was prepared and its protein concentration was measured; cell extracts alone and with different dilutions of the antiserum, or antiserum alone (1:100 dilution was added to the culture medium, and the number of cells was counted 4 days later). Growth stimulation by murine antrum gastrokines was maximally inhibited by the antiserum (93%) at a dilution of 1:400, whereas stimulation by the porcine antrum protein extract was totally inhibited at a dilution of 1:100. Scored values were means for 3 cultures; standard error of the mean (SE) was less than 10% of the mean.

Antibodies to the AMP-18 protein have diagnostic uses to determine different levels of the protein in the gastro-intestinal tract in vivo. Ulcers are likely to develop if less than normal levels of AMP-18 protein are present. Normal values are determined by technologies known to those of skill in the art, that is, obtaining representative samples of persons to be tested (age, sex, clinical condition categories) and applying standard techniques of protein quantitation. The effects of aspirin and indamethacin on AMP-18 levels are also useful to monitor deleterious levels of the drugs including the non-steroidal anti-inflammatory drugs (NSAIDs). Stomach cancer cell lines do not express the AMP-18 proteins at least by detection methods disclosed herein.

4. **Genomic DNA**

Genomic AMP-18 DNA sequences have been cloned for human and mouse as a prelude to the analysis of the gene regulatory elements, which presumably determine the great differences in the levels of expression of the gene in tissues where the gene may be active. Upstream and downstream flanking sequences have been
isolated from mouse genomic DNA preparatory to a gene knockout. The flanking genomic sequences likely determine the very different levels of expression of the gene in the stomach and few other tissues where it may be expressed. With the involvement of different regulatory elements, gastrokine genes could be expressed as a growth factor in other tissues.

5. **Uses of Gastrokines of the Present Invention**

Because the AMP-18 protein and certain peptides derived from it can stimulate growth and wound repair by stomach and intestinal epithelial cells (as well as kidney) these gastrokine molecules are candidates for therapeutic agents to speed recovery of the injured GI tract following pharmacological interventions, radiotherapy, or surgery. In addition, the antibodies developed to gastrokines may be used in kits to measure the levels of AMP-18 protein or peptide in tissue of blood in diverse pathological states. These novel molecules have great therapeutic potential in the treatment of gastric ulcers, and inflammatory bowel disease, whereas new agents that inhibit its function could prove useful in the treatment of cancers of the GI tract.

The stomach is not a congenial location for many bacteria, and those that can survive the acidity do not establish themselves there (Rotimi et al., 1990). It is of interest therefore that the antrum region is the favored site for the attachment, penetration and cytolytic effects of *Helicobacter pylori*, an agent which infects a major proportion of the human population (>60% by the seventh decade) and has been associated with gastritis, gastric and duodenal ulcers (Goodwin et al., 1986; Blaser, 1987) and gastric adenocarcinomas (Nomura et al., 1991; Parsonnet et al., 1991). Thus as an epithelial cell growth factor, AMP-18 may act to ameliorate the damage caused by bacterial infiltration and cytosis. Given the conjunction of the specific antrum expression of AMP-18 and the preferred site of binding of *H. pylori*, it is possible that the bacteria use AMP-18 as a tropic factor. *H. pylori* attaches to cells of the antrum having fucose-containing mucin granules (Falk et al., 1993; Baczko et al., 1995). These granules also may contain AMP-18. Anti-microbial peptides have been found in the stomach of the amphibian *Xenopus laevis* (Moore et al., 1991). Some domains of the AMP-18 structure resemble that of the magainins, and possibly AMP-18 interacts with enteric bacteria.
6. **Isolation of Pig AMP-18**

Antisera against human AMP-18 protein were used to assist in the purification of the protein from extracts of pig antrum mucosa. Immunoaffinity methods applied to total tissue extracts have not proven very effective, but by using immunobLOTS to monitor cell-fractionation, gradient centrifugation and gel electrophoresis sufficient amounts of the pig 18 kDa polypeptide was purified to confirm by sequencing that the native N-terminus is the one predicted by cleavage of 20 amino acids from the N-terminus of the ORF precisely at the alanine-aspartate site anticipated for signal peptide removal. Despite the abundance of asparagine residues in the mature protein, none fit the consensus context characteristic of glycosylation. Fairly extensive regions of the protein may possess amphipathic helix forming propensity. The latter may represent units within the protein yielding bioactive peptides after processing. Using circular dichroism the synthetic peptide representing amino acids 126-143 in the human preAMP sequence (FIG. 3) is readily induced to become helical in moderate concentrations of trifluoroethanol conditions used to assess helix propensity for some bioactive peptides, including anti-microbial peptides of the magainin type (see, for example, Park et al., 1997).

7. **Preparation of active recombinant human AMP-18 in E. coli**

A cDNA encoding human AMP-18 was designed in which the 20-amino acid hydrophobic signal peptide sequence was replaced with an N-terminal 12-amino acid peptide that included a starch of 6 histidine residues (FIG. 13, left panel). Expression of this modified cDNA sequence was predicted to yield a 177-amino acid protein product (Mr 19, 653) that could be readily purified using Ni-NTA resin to bind the His6-tag (SEQ ID NO: 16). The cDNA sequence lacking the region coding for the N-terminal signal peptide (see FIG. 14) was amplified by PCR using oligonucleotides that provided suitable linkers for inserting the product into the BamHI site of a QE30 expression vector (QLAGEN); the sequence of the recombinant vector was confirmed. The recombinant human (rh) AMP-18 engineered with the His6-tag (SEQ ID NO: 16) was subsequently expressed in E. coli cells. To harvest it, the bacteria were lysed and aliquots of the soluble and insoluble fractions were subjected to SDS-PAGE followed by immunoblotting using the specific rabbit antiserum to the rhAMP-18 precursor.
Very little of the expressed protein was detected in the soluble fraction of the lysate. Urea (6 M) was employed to release proteins from the insoluble fraction solubilize rhAMP-18 containing the His6-tag (SEQ ID NO: 16), and make it available to bind to the \( \text{Ni}^{2+} \) -charged resin from which it was subsequently eluted with a gradient of imidazole (0 to 200 mM). The amount of eluted rhAMP-18 was measured using the BCA assay, and the appearance of a single band at the predicted size of 19-20 kD was confirmed by SDS-PAGE followed by immunoblotting. To determine if eluted rhAMP-18 renatured to assume a structure that was mitogenic, aliquots of the eluate (following removal of urea and imidazole by dialysis) were added to cultures of IEC-18 cells and the number of cells was counted 4 days later. FIG. 13 (right panel) indicates that the recombinant protein stimulates cell proliferation to the same maximal extent as does mitogenic AMP peptide 77-97 (or soluble antrum tissue extracts from pig shown in FIG. 11), but that it does so at a half-maximal concentration an order of magnitude lower than for peptide 77-97. AMP peptide 77-97 refers to the mature protein; same as peptide 97-117 of human precursor protein: Table 1. These observations indicate that biologically active recombinant human AMP-18 that can be utilized in diverse clinical situations is available. The mitogenic potency of rhAMP-18 is in the nanomolar range which would be expected for a native gastric cell growth factor that participates in the maintenance and repair of the stomach \textit{in vivo}.

8. \textbf{Stimulation of growth and restitution of stomach and intestinal epithelial cells by AMP-18 and derived peptides}

To characterize the capacity of gastric and intestinal cells to respond to AMP-18, AGS gastric adenocarcinoma cells, HAE human gastric antrum mucosa primary cultures transformed with SV40 large T antigen, rat diploid small intestinal epithelial cells of the IEC-6 (FIG. 15) and IEC-18 lines, NCI N-87 gastric carcinoma cells, and SK-GT5 gastroesophageal adenocarcinoma cells were studied; human WI-38 fibroblasts and HeLa cells served as non-GI control cell lines. Mitogenesis was assayed by performing cell counts 3 to 4 days after exposing cells to the agent of interest, trypsinizing the culture to prepare single cells, and confirming this while counting them in a hemocytometer.
Antrum extracts containing AMP-18, peptide 77-97, or EGF each stimulated growth of AGS cells, and as expected, the rabbit antiserum to recombinant human AMP-18 precursor protein inhibited the activity of the antrum extract but not of peptide 77-97 which lacks the epitope (FIG. 11). Growth stimulation by peptide 77-97 was additive with that of EGF. Growth of AGS cells is not stimulated by scrambled peptide 77-97 or by peptide 67-85, and peptide 67-85 completely inhibits growth stimulation by peptide 58-99. HAE cells were used to test whether AMP-18 can exert an effect on epithelial cells that exist in the local environment of its synthesis. These cells, provided by Dr. Duane Smoot, Howard University College of Medicine, are not completely immortalized and therefore have limited passage number. Growth stimulation of HAE cells by peptide 77-97 was apparently additive with that of EGF (Fig. 16, left panel). Not only does the AMP peptide stimulate growth but it also acted as a motogen, resulting in more rapid migration (restitution) of cells into scrape wounds made in confluent cultures. This enhancement of wound restitution also showed high additivity with EGF (FIG. 16 right panel). Whether there is a synergism or not, the observed additivity supports that AMP-18 may play an important role in maintaining an intact stomach mucosal epithelium, and in facilitating its repair after injury. The growth of rat diploid IEC-6 cells was also stimulated by the antrum extract, peptide 77-97, and EGF, although the peptide appeared a more potent mitogen than EGF (FIG. 15). Near-maximal growth stimulation was detected at an AMP peptide concentration of 0.5 µg/ml (0.23 µM) (FIG. 15, center panel), a much lower value than the concentration needed for trefoil peptides (1 µg/µl (~150 µM) or the α-defensin, cryptdin 3 (660 µm/ml (~140 µM) to exert their effects in culture. The maximal mitogenic effect of rhAMP-18 on IEC-18 cells has been observed at 5 nanomolar (FIG. 18). The mitogenic effect of peptide 77-97 was corroborated by measuring [³H]thymidine incorporation into DNA in IEC-6 cells which was stimulated by 68% (P<0.001) from 16,668 ± 616 to 28,036 ± 882 by the peptide. Stimulation of wound restitution was comparable to EGF, and apparently additive with it. Scrambled peptide 77-97 did NOT stimulate growth of IEC-18 cells or BSC-1 cells at concentrations up to 8 µg/ml. Growth of gastric NCI N-87 cells and gastric SK-GT5 cells was also stimulated by peptide 77-97, antrum extract, of EGF in a
concentration-dependent manner. AMP-18 antiserum blocked the mitogenic effect of antrum extract, or EGF in a concentration-dependent manner. AMP-18 antiserum blocked the mitogenic effect of antrum extract on these two gastric epithelial cell lines, but not the proliferative effects of peptide 77-97 or EGF. Preimmune serum had no effect on growth. These results suggest that AMP-18 and its peptide derivatives could function in vivo to stimulate growth and restitution during repair after injury. Growth of human fibroblastic (WI-38) or epidermoid (HeLa) cells at concentrations up to 8 μg/ml, suggesting that its mitogenic effect is relatively epithelial-cell specific. AMP peptide 77-97 does not stimulate growth.

9. **Competitive inhibition of IEC-18 cell growth by AMP derived peptides**

To gain additional information about the interaction between AMP peptides and their binding site(s) on the cell surface, non-transformed rat IEC-18 cells were studied. Progressively increasing the concentration of non-mitogenic peptide 67-85 blocks growth-stimulation by peptide 58-99 if this mitogenic 42-mer exerts its effect by a receptor-mediated mechanism. Peptide 58-99 stimulated an increase in cell number of 407% compared to 290% by the vehicle in a 3-day assay. As the concentration of peptide 67-85 was raised progressively to ~0.1 μg/ml, the growth-stimulatory effect of peptide 58-99 was nearly abolished (FIG. 17). This result suggests that the two peptides compete for the same surface “receptor” site.

10. **Antiserum to AMP-18 neutralizes the mitogenic effect of rhAMP-18**

Ongoing studies reveal that rabbit antiserum to AMP-18 precursor recognizes rhAMP-18 on immunoblots. The antiserum also blocks the mitogenic effect of porcine antral tissue extracts (FIG. 11) and AMP peptide 58-99, and immunolocalizes AMP-18 in cells of human and murine gastric antral tissue. FIG. 18 shows that the antiserum neutralizes the mitogenic effect of rhAMP-18 in confluent cultures of IEC-18 cells, thereby extending its utility to study the recombinant as well as native protein.

Using GI epithelial cells, results suggest that the cytoprotective effect of rhAMP-18 may be mediated by its capacity to facilitate accumulation of tight junction
(TJ) proteins, and that it is a potent mitogen. The results also imply that AMP peptide 77-97 is an appropriate surrogate for rhAMP-18, although the peptide requires a relatively higher concentration to exert its physiological effects (FIG. 13).

To improve the yield of rhAMP-18, an EDTA-free protease-inhibitor cocktail is used, lysozyme is added to digest *E. coli* cell debris, and recombinant protein is eluted from Ni^{2+} beads with 1M imidazole.

11. **AMP peptide stimulates restitution of gastric and intestinal epithelial cells after scrape-wounding.**

Data presented in FIG. 19 were obtained after 24 to 48 hr exposure to AMP peptide, times before a mitogenic effect can be detected by an increase in cell number. The results indicate that AMP peptide stimulates restitution in scrape-wounded human gastric adenocarcinoma-derived cells of the HAE line, and in nontransformed rat intestinal cells of the IEC-18 line. Thus AMP peptide rapidly stimulates restitution of gastric and intestinal epithelial cells in culture, and presumably could speed resurfacing of the injured gastric mucosa *in vivo*.

12. **AMP peptide induces tyrosine kinase activity suggesting that its functional effects are mediated by a cell surface receptor**

To obtain evidence that the physiological effects of AMP-18 are specific and receptor-mediated, AMP peptide was tested to see if it induces tyrosine phosphorylation in GI epithelial cells.

IEC-18 cells were treated with AMP peptide 77-97 at a concentration previously shown to be in excess of that required for maximal growth stimulation for different periods of time up to 60 min. The cells were then lysed, the protein extracted and separated on SDS-polyacrylamide gels, blotted, and the blot probed with 4G10 anti-phosphotyrosine monoclonal antibody. The blot showed that exposure of cells to AMP peptide (8 µg/ml) results in tyrosine phosphorylation of several proteins after two min, including those having molecular masses of 42- and 55-kDa, suggesting a role for activated ERK1 and ERK2 in AMP-18 signaling. There was a decline in the extent of tyrosine phosphorylation of several of the proteins after 5 min, and persistence of others for up to 60 min.

AMP peptide and presumably AMP-18 may signal their mitogenic,
motogenic, and cytoprotective effects via a cell surface receptor, and possibly stimulate tyrosine phosphorylation of specific cell proteins.

13. **AMP peptide 77-97 enhances development of barrier function of epithelial cells and is cytoprotective**

Maintenance of barrier function is essential for preventing entry of foreign antigens and bacteria from the gastric lumen, and for other functions such as vectorial transport of electrolytes, water and nutrients. Acting alone or in concert with other agents, AMP-18 might mediate the rapid return of barrier function following mucosal injury. To determine whether AMP peptide 77-97 could facilitate development of barrier function, and could also serve as a cytoprotective agent to prevent loss of function when reactive oxygen metabolites, indomethacin, or dextran sulfate sodium (DSS), increases mucosal permeability and compromises cell integrity needed to maintain epithelial tight junctions. Cell lines known to develop relatively high values for TER as a marker of epithelial tight junctions were used. Initially, peptide 77-97 modulates maturation of TER in monolayer cultures of well-characterized, nontransformed MDCK cells. FIG. 20 shows that exposure to the peptide increases TER in the monolayer by 24 hr, and to a greater extent thereafter. This observation suggests that AMP-18 or AMP peptide could speed recovery of the GI epithelium after injury, and enhance development of barrier function.

To determine whether AMP peptide protects barrier function in a tissue culture model of mucosal oxidant injury, cell monolayers were subjected to reactive oxygen metabolite injury using monochloramine. The results in FIG. 21 (panel A) indicate that after 60 min of exposure to monochloramine, MDCK cells treated with vehicle or EGF show a substantial loss of TER, whereas the TER of cultures treated with peptide 77-97 is similar to non-injured monolayers. These results are of considerable interest because they suggest that AMP peptide but not EGF is cytoprotective under this set of conditions, whereas these two molecules were previously found to be equivalent and additive mitogens and motogens for gastric and intestinal epithelial cells. The cytoprotective effect of peptide 77-97 was also apparent in Caco2/bbe (C2) cells derived from a human colonic adenocarcinoma line in the setting of exodontant (FIG. 21, panel B) or indomethacin-mediated (panel C)
injury.

14. **Cytoprotective effect of AMP peptide following DSS injury**

To evaluate the potential capacity of AMP peptide to exert a cytoprotective effect in colitis *in vivo*, a solution of dextran sulfate sodium (DSS) was added to the culture medium of C2 cell monolayers used to model the colonic epithelium. DSS-mediated injury of barrier function was quantified by measuring TER in these monolayer cultures. FIG. 22 indicates that DSS (4%) reduced the TER to ~30% of the control value after 45 min, and that AMP peptide was cytoprotective. This observation provides a strong physiological rationale for evaluating AMP peptide as a therapeutic agent in the murine model of DSS-mediated colitis.

To determine whether AMP peptide could speed recovery of TER after DSS-induced colonic cell injury, a highly sought-after functional characteristic of an agent designed to treat IBD, C2 cell monolayers were exposed to DSS (5%) for 10 min which reduced TER to 33 ± 6% of the control value. DSS was removed by aspirating the medium and replacing it with fresh medium. AMP peptide 77-97 (8 μg/ml) or vehicle was added to the culture medium, and TER was measured 18 hr later. In the presence of the vehicle, TER increased from 33% to 66 ± 7% of the control value, whereas cells exposed to AMP peptide reached a value 112 ± 4% of control. The salutary results in a tissue culture model of DSS-mediated colitis suggest that AMP peptide can speed recovery of barrier function in the injured colonic epithelium *in vivo*.

15. **The cytoprotective effect of AMP peptide in colonic epithelial cells may be mediated by increased accumulation of tight junction proteins**

FIG. 21B shows that AMP peptide 77-97 blunts the fall in transepithelial electrical resistance (TER) in Caco2/bbe (C2) cells after oxidant injury. To find out how the peptide exerts its cytoprotective effect, C2 cell monolayers were treated with AMP peptide, and oxidant injury was induced with monochloramine 18 hours later. Changes in the levels of specific tight junction (TJ) proteins were checked. Cells were lysed, and proteins of the insoluble/particulate fraction were studied by immunoblotting. FIG. 23 shows that there is more immunoreactive ZO-1 and occludin...
in AMP peptide-treated than in vehicle-treated cells at time 0, and for 60 minutes following oxidant-induced injury, suggesting that the greater abundance of these TJ proteins thereby blunts loss of TER in the monolayer and preserves barrier function. These observations implied that AMP peptide enhanced TJ protein accumulation during the 18 hours before cells were subjected to oxidant injury. Non-injured cells were studied and showed that AMP peptide (or rhAMP-18) rapidly increased the amount of immunoreactive ZO-1 and occludin compared to untreated cells (FIG. 24). These changes appear relatively specific for ZO-1 and occludin as they were not observed for several other TJ proteins (ZO-2, claudin-1, claudin-2, claudin-5), or heat shock protein (HSC) 73.

16. **Cytoprotective effect of rhAMP-18**

A sufficient amount of purified rhAMP-18 was prepared to test whether rhAMP-18 was cytoprotective compared to AMP peptide 77-87 which blunted the fall in transepithelial electrical resistance (TER) in Caco2/bbe (C2) cells following monochloramine-mediated oxidant injury (FIG. 21B). FIG. 25 shows that exposure to monochloramine reduces TER by ~35% at 45 min, whereas cells pre-treated with either rhAMP-18 or peptide 77-97 exhibited only a ~10% decline in TER. FIG. 26 indicates that treatment of C2 cells with rhAMP-18 for 8 hr increases the amount of immunoreactive ZO-1 and occludin compared to vehicle-treated cells. These results suggest that AMP-18 could mediate its cytoprotective effect by enhancing accumulation of specific TJ proteins and thereby preserve barrier function along the GI tract following mucosal injury.

17. **Administration of AMP peptide delays appearance of blood in the stool and reduces weight loss in mice with DSS induced colitis**

To evaluate the therapeutic efficacy of AMP peptide, DSS colitis was induced in C57/BL6 male mice by giving animals (12-15 gm each) 3% DSS (M, 36-44 kDa) in the drinking water. Evidence of colitis (blood in the stool) was found as early as day 1 (FIG. 27, left panel), and in all animals by day 4. AMP peptide, administered daily by subcutaneous (s.c.) injection, delayed the appearance of hemoccult-positive stool, and also reduced the extent of weight loss (FIG. 27, right panel). These positive findings strongly support AMP peptide as a useful therapeutic agent in colitis.
and other diseases that injure the mucosal surface of the GI tract.

The synthesis of AMP-18 is confined to luminal mucosal lining epithelial cells of the gastric antrum and other mammals. Inside cells the protein is co-localized with mucins in secretion granules, and appears to be secreted into the mucus overlying the apical plasma membrane. **Recombinant human AMP-18 prepared in E. coli exerts its mitogenic effect at a concentration an order of magnitude lower than growth-promoting peptides derived from the center of the mature protein.** Peptide 77-97, the most potent mitogenic peptide, is amino acid sequence-specific, and appears to be cell-type specific as it does not stimulate growth of fibroblasts or HeLa cells. Mitogenesis by specific AMP peptides appears to be mediated by a cell surface receptor because certain peptides that are not active mitogens can competitively inhibit, in a concentration-dependent manner, the growth-stimulating effects of peptide 58-99 and antrum cell extracts. AMP-18 and its derived peptides exhibit diverse effects on stomach and intestinal epithelial cells which suggest they could play a critical role in repair after gastric mucosal injury. These include cytoprotection, mitogenesis, restitution, and maturation of barrier function after oxidant, DSS, and/or indomethacin-mediated injury. **Possible mechanisms by which AMP-18 or its peptide derivatives mediate their pleiotropic effects include stimulation of protein tyrosine kinase activity, prolongation of heat shock protein expression after cell stress, and/or enhanced accumulation of the tight junction-associated proteins ZO-1 and occludin.** Certain of these physiological effects can occur at concentrations that are relatively low for rhAMP-18 (<50 nM) compared to the concentrations of other gastric peptide mediators such as trefoil peptides or the α-defensin, cryptdin 3 (> 100 μM). **Immunoreactive AMP-18 is apparently released by cells of the mouse antrum after indomethacin gavage, and by canine antrum cells in primary culture exposed to forskolin, suggesting that the protein is subject to regulation.** AMP-18 likely plays a role in physiological and pathological processes such as wound healing in the gastric mucosal epithelium in vivo. The capacity of AMP peptide to delay the onset of bloody stool in the DSS model of mouse colitis, and reduce the extent of weight loss suggests therapeutic efficacy in diverse diseases that injure the mucosa of the GI tract.
Attorney Docket No. 21459/93376

-28-

(inflammatory bowel diseases, gastric ulcer, and the like)

MATERIALS AND METHODS

1. **Isolation of Antrum-Specific cDNA Clones**

   cDNA clones for the gastrointestinal (GI) peptide gastrin, which regulates gastric acid secretion as well as mucosal and pancreatic cell growth (Yoo et al., 1982) were isolated. From these screens several other mRNAs expressed relatively specifically in the antrum of the stomach were found. The open reading frame (ORF) in one of these RNAs was highly conserved between pig and man, and predicted a novel conserved protein of no immediately apparent function. Using specific antibodies, it was shown that similar protein species are present in the stomach antrum mucosa of all mammals tested. There is tissue specificity of expression of these sequences and they are apparently ubiquitously present in the antrum mucosa of mammalian species.

2. **RNA Expression**

   The isolation of the cDNA clones was predicted on a preferential expression in the mucosa of the stomach antrum and this has been confirmed initially by Northern blot hybridization of RNAs from various tissues probed with the cDNA sequences and subsequently by protein analysis. The Northern blots showed the specificity of mRNA expression within the gastrointestinal tract of the pig. Highest mRNA expression was in the antrum mucosa, variable amounts in the adjacent corpus mucosa and undetectable levels in fundus, esophagus and duodenum. The non-mucosal tissue of the antrum and corpus contained little RNA reacting with the cDNA probe.

3. **Antibodies to Expressed Protein**

   The open reading frames (ORFs) of the human and pig cDNA clones predict very similar relatively low molecular weight (MW) proteins, which have no close homologs to known proteins in the computer databases and therefore give little indication of possible function. As an approach to study the biological role of the presumptive proteins, the full cDNA sequences were expressed in *E. coli*, using a vector that also encoded an N-terminal His6-tag (SEQ ID NO: 16). Unfortunately, as
expressed in bacteria the polypeptide products are insoluble and not readily amenable to biochemical studies. However, the bacterial product of the human cDNA was separated on sodium dodecyl sulfate (SDS) gels used as an immunogen in rabbits to elicit antisera. The sera were screened against protein extracts of antral tissue from a number of mammalian species. This procedure has successfully produced several high-titer, low background antisera capable of recognizing both the immunogen and proteins of about 18 kDa expressed in the antrum of the mammals tested. The bacterially-expressed protein migrates more slowly because it contains the signal peptide sequence was well as a His6-tag (SEQ ID NO: 16). The preimmune sera showed no significant 18 kDa reactivity. The cross-reactivity of the antisera raised against the protein expressed from the human cDNA clone with proteins of very similar MW in antrum extracts from a variety of mammals (pig, goat, sheep, rat and mouse; the last consistently migrates slightly more rapidly in SDS gels) supports the level of conservation of amino acid sequence predicted by comparison of the ORFs of the human and pig cDNAs (See FIG. 10). In subsequent experiments, human AMP-18 with a signal peptide was produced in bacteria.

The preimmune sera give insignificant reactions on Western blots of all tissue extracts, while the two immune sera (at up to 1:50000 dilution) both give major bands of 18-20 kDa only, and those only in stomach antrum extracts, and to a lesser degree in the adjacent corpus extracts. The sera were raised against bacterially-expressed protein so there is no possibility of other exogenous immunogens of animal origin.

As determined by immunoblots, the specificity of expression to the antrum is even greater than the Northern blots would suggest, and the strength of the signal from antrum extracts implies a relatively high abundance of the protein, although quantitative estimates were not made. Significant antigen was not detected in non-stomach tissues tested.

The immunohistochemistry showed insignificant staining of antral tissue by both preimmune sera, while both immune sera stained the surface mucosal cells very strongly at considerable dilutions. The preimmune sera did not lead to immunogold staining in the immunoelectron microscope study. The growth factor activity of antrum extracts is inhibited by both immune, but not preimmune sera. Finally, the
results with a synthetic peptide, which has growth factor activity, is inhibited by the immune but not the preimmune sera, and carries epitopes recognized by the immune but not the preimmune sera, further validate the specificity of these reagents.

4. **Northern Blot Hybridization of RNAs From Pig Gut Mucosal Tissues**

Total RNA was electrophoresed, transferred to a membrane and hybridized with a labeled pig AMP-18 cDNA probe. The source of the RNA sample for each lane was: 1. Distal duodenum; 2. Proximal duodenum; 3. Antrum; 4. Adjacent corpus; 5. Fundus; 6. Esophagus. Equal amounts of RNA were loaded. The signal from RNA of the antrum adjacent corpus was variable. Size markers (nucleotides) were run on the same gel for comparison.

5. **Immunoblots Using A Rabbit Antiserum Raised Against the Bacterial-Expressed Protein Directed By the Human Antrum-Specific cDNA Clone**

Whole tissue proteins were dissolved in SDS buffer, electrophoresed, and transferred to membranes that were reacted with immune serum (1:50000). Bound antibody molecules were detected using peroxidase-labeled anti-rabbit antibody. Preimmune serum gave no specific staining of parallel blots at 1:200 dilution. Lanes: 1,6,13,17 contained markers. 2 HeLa cells. 3 mouse TLT cells. 4 expressed human protein + HEla cells. 7 mouse corpus. 8 mouse antrum. 9 mouse duodenum. 10 mouse intestine. 11 mouse liver. 12 expressed human protein + TLT cells. 14 mouse antrum. 15 mouse brain. 16 mouse Kidney. 18 pig antrum. 19 mouse antrum.

Immunoblots of high percentage acrylamide gels showed that the antisera recognized epitopes on the synthetic peptide 78-119. The reaction of peptide 78-119 with the antibodies was not unexpected because this region of the sequence was predicted to be exposed on the surface of the protein and to be antigenic. Not only does this further substantiate a belief that AMP-18 or its immediate precursor, is a growth factor, for epithelial cells, but also provides a basis for analysis of the bioactive (and antigenic) regions of AMP-18, and a tool for the assessment of cell receptor number and identity. Chemical synthesis of peptides also makes available a
convenient and rapid source of considerable quantities of pure "wild-type" and "mutant" reagents for further cell studies. The synthetic peptide 78-119 apparently acts by the same mechanism as the antrum protein, because their maximal effects are not additive.

6. **Sequence and Predicted Structure of the Pre-AMP Open Reading Frame**

The predicted amino acid sequences for human and pig are 76% identical. The predicted signal peptides are not bold; the N-terminus of native pig AMP has been shown to be aspartate (FIG. 10).

7. **Structure of the Native Protein**

The ORF's of the human and pig cDNAs predicted polypeptides of similar general structure (FIG. 10). The predicted molecular weights for the otherwise unmodified human and pig proteins was 18.3 and 18.0 respectively; these values are in good agreement with electrophoretic mobility in SDS the of antrum proteins reacting with the antisera of the present invention.

The antisera was used to assist in the purification of the protein from extracts of pig antrum mucosa. Immunoaffinity methods applied to total tissue extracts have not proven very effective, but by using immunoblots to monitor cell-fractionation, gradient centrifugation and gel electrophoresis sufficient amounts of the pig 18 kDa polypeptide was purified to confirm by sequencing that the native N-terminus is one predicted by cleavage of about 20 amino acids from the N-terminus of the ORF precisely at the alanine-aspartate site anticipated for signal peptide removal. Despite the abundance of asparagine residues, none fit the consensus context for glycosylation. Fairly extensive regions which may possess amphipathic helix forming propensity. The latter may represent units within the protein or as peptides after processing. Using circular dichroism the synthetic peptide representing amino acids 126-143 in the human preAMP sequence (FIG.3) is readily induced to become helical in moderate concentrations of trifluoroethanol conditions used to assess helix propensity for some bioactive peptides, including anti-microbial peptides of the magainin type (see for example Park et al., 1997).
8. **Localization of AMP-18**

The antisera to AMP-18 have proven to be excellent histochemical probes, reacting strongly with sections of the mouse antrum region but not with the fundus, duodenum or intestine, confirming the results of the immunoblots. The preimmune sera give negligible reactions even at much higher concentration. The AMP-18 protein appears to be concentrated in mucosal epithelial cells lining the stomach lumen, although lesser signals in cells deeper in the tissue and along the upper crypt regions suggest that cells may begin to express the protein as they migrate toward the lumenal layer. Higher magnification of the histochemical preparations indicates only a general cytoplasmic staining at this level of resolution; there are some patches of intense staining that may be the light microscope equivalent of granule-packed regions of some lumenal surface cells seen by electron microscopy (EM). The localization of AMP-18 in the antrum mucosa is therefore very different from those cells synthesizing gastrin which are deep in the mucosal layer.

9. **Immunoelectron microscope localization of the AMP-18 antigens in the mouse stomach antrum mucosal cells**

The tissue pieces were fixed in 4% formaldehyde and processed for embedding in Unicryl. Thin sections were reacted with rabbit anti-human AMP-18 antisera (1:200); bound antibodies detected by Protein-A conjugated to 10nm colloidal gold. The reacted sections were stained with lead citrate before viewing (20,000x). The gold particles are visible over the semi-translucent secretion granules, which appear much more translucent here than in the standard glutaraldehyde-osmium-epoxy procedure (11,400x) because of the requirements for immunoreactivity. Negligible background was seen on other cytoplasmic structures.

The general structure of the protein implies a possible secretory role so a precise intracellular localization would be valuable. This requires EM immunocytochemical procedures. Standard embedding and staining methods reveal that, as previously reported by many others, the antrum region (e.g. Johnson and McMinn, 1970) contains mucosal epithelial cells which are very rich in secretory granules. Preliminary immuno-EM data show the immune sera used at 1:200-1:800 dilution react specifically with the secretion granules. The latter appear somewhat swollen.
and less electron opaque than in standard fixation conditions and the differences in density are harder to discern, but overall the cell structure is quite well-preserved for stomach tissue fixed and embedded under the less stringent conditions required to preserve immuno-reactivity. At 1:100 dilution, the preimmune sera exhibited negligible backgrounds with no preference for the secretion granules.

10. **Growth Factor Activity on Epithelial Cell Cultures.**

A function for AMP-18 is that it is a growth factor at least partly responsible for the maintenance of a functional mucosal epithelium in the pyloric antrum and possibly elsewhere in the stomach. Initially, stomach epithelial cell lines were not immediately available, but kidney epithelial cell systems (Kartha et al., 1992; Aithal et al., 1994; Lieske et al., 1994) were used. A fractionated antrum mucosal cell extract was used for these experiments. Using immunoblotting as a probe to follow fractionation, on lysis of the mucosal cells scraped from either pig or mouse antrum, the AMP-18 antigen was recovered in the 35S fraction on sucrose density gradients. Such high speed supernatant fractions served as the starting material for studies on cell growth. Unexpectedly, these extracts stimulated a 50% increase in confluent renal epithelial cells of monkey (BSC-1 cells), but had no effect on HeLa or WI-38 fibroblast cells. The stimulation of BSC-1 cells was at least as effective as that observed with diverse polypeptide mitogens, including EGF, IGF-I, aFGF, bFGF and vasopressin, assayed at their optimal concentrations. Comparable growth stimulation by the antrum extracts was observed when DNA synthesis was assessed by measuring [³H]thymidine incorporation into acid-insoluble material. The biological activity of the antrum extracts survived heating for 5 minutes at 65°C, and dialysis using a membrane with Mₜ cutoff of 10 kDa, which would eliminate most oligopeptides; this treatment removes 60-70% of polypeptide material, but spared AMP-18 as assayed by immunoblots. More importantly, mitogenic stimulation of BSC-1 cells by the mouse or pig antrum extract was inhibited when either of two different antisera to the human recombinant preAMP-18 (expressed in bacteria) was added to the culture medium. Preimmune sera (1:100 to 1:800) had no effect on cell growth, nor did they alter the mitogenic effect of the antrum extracts. These observations suggest that gastric mucosal cell AMP-18 functions as a potent mitogen for kidney epithelial cells, which
do not normally express this protein.

To gain further evidence that the growth-promoting activity in the partially fractionated antrum extracts was mediated by the AMP-18 protein, an aliquot of the mouse extract was subjected to SDS-polyacrylamide gel electrophoresis; the method used previously to determine the N-terminal sequence of the natural protein. The gel was cut into 2-mm slices and each slice was extracted with 3% acetonitrile in phosphate-buffered saline containing 1% BSA. The extract supernatants were assayed for mitogenic activity. The results indicated that one slice containing protein in the 16-19 kDa range possessed growth-promoting activity. Significantly, this growth response was blocked by the immune but not the pre-immune sera. Taken together with the relatively low sedimentation rate of the protein, these findings provide additional evidence to support the conclusion that AMP-18 is an epithelial cell mitogen and that it functions as a monomer or possibly a homotypic dimer. It also implies that the structure of the protein is such that it can readily reacquire a native conformation after the denaturing conditions of SDS-gel electrophoresis.

To assess the interaction of the antrum growth factor activity with other cytokines, its activity was tested to determine if it was additive with EGF in epithelial cell cultures. EGF (50 ng/ml) added with untreated mouse antrum extract (10 μg/ml), or heated, dialyzed pig extract (10 μg/ml) exhibited additive stimulation of mitogenesis; up to 74% increase in cell number above the quiescent level; the greatest stimulation observed so far for any factor using the BSC-1 cell assay. An example of this additivity is shown for an AMP-peptide and EGF on AGS cells in FIG. 11. This observation suggests that AMP-18 and EGF initiate proliferation by acting on different cell surface receptors. It also implies that AMP-18 growth factor activity might normally collaborate with other autocrine and paracrine factors in the maintenance or restitution of the epithelium. In view of the results with EGF, it is likely that AMP-18 is secreted at and acts upon the apical face (i.e., stomach lumenal face) of the epithelial cell layer while other factors (for which EGF may serve as an example) act from the basal surface.

11. **Bioactivity of Gastrokin (AMP-18) Related Peptides.**

The activities of synthetic peptides of the present invention are unexpected.
Peptides based on the ORF of the human cDNA clone peptides were synthesized in
the University of Chicago Cancer Center Peptide Core Facility, which checks the
sequence and mass spectra of the products. The peptides were further purified by
HPLC. Five relatively large oligopeptides (of about 40 amino acids each)
approximately spanning the length of the protein without including the signal peptide,
were analyzed. One peptide 42 amino acids long spanning amino acids lys-78 to leu-
119 of the pre-AMP sequence (peptide 58-99 of the matured form of the protein; see
Table 1), including a predicted helix and glycine-proline (GP) turns, gave good
mitogenic activity. This response was blocked by the specific antiserum, but not by
the preimmune sera.

**TABLE 1: BIOACTIVITY OF SYNTHETIC PEPTIDES BASED ON THE
SEQUENCE OF PRE-GASTROKINE (PRE-AMP-18)**

<table>
<thead>
<tr>
<th>Name of Peptide Sequence in Human</th>
<th>#AA</th>
<th>AMINO ACID SEQUENCE</th>
<th>K_{1/2} \mu M</th>
</tr>
</thead>
<tbody>
<tr>
<td>78-119</td>
<td>42</td>
<td>KKTcvHMKKEvMPSIQSDLvKEKKeKLQGKGPGPpPKGL (SEQ ID NO: 17)</td>
<td>0.3</td>
</tr>
<tr>
<td>78-88</td>
<td>11</td>
<td>KKTcvHMKK (SEQ ID NO:14)</td>
<td>Inactive</td>
</tr>
<tr>
<td>87-105</td>
<td>19</td>
<td>KKEvMPSIQSDLvKEKK (SEQ ID NO:15)</td>
<td>Inactive</td>
</tr>
<tr>
<td>104-117</td>
<td>14</td>
<td>KKLQGKGPGPpPK (SEQ ID NO: 11)</td>
<td>0.8</td>
</tr>
<tr>
<td>104-111</td>
<td>18</td>
<td>KKLQGKGPGPpPKGLMY (SEQ ID NO: 18)</td>
<td>1.0</td>
</tr>
<tr>
<td>97-117</td>
<td>21</td>
<td>LDALvKEKKLQGKGPGPpPK (SEQ ID NO: 12)</td>
<td>0.3</td>
</tr>
<tr>
<td>97-117**</td>
<td>21</td>
<td>GKLQPGKPvKLDGKEPLAK (SEQ ID NO: 19)</td>
<td>Inactive</td>
</tr>
<tr>
<td>97-121</td>
<td>25</td>
<td>LDALvKEKKLQGKGPGPpPKGLMY (SEQ ID NO: 13)</td>
<td>0.2</td>
</tr>
<tr>
<td>109-117</td>
<td>9</td>
<td>KGPGPpPK (SEQ ID NO: 20)</td>
<td>2.5</td>
</tr>
<tr>
<td>104-109</td>
<td>6</td>
<td>KKLQGK (SEQ ID NO: 21)</td>
<td>7.4</td>
</tr>
<tr>
<td>110-113</td>
<td>4</td>
<td>GPGG (SEQ ID NO: 22)</td>
<td>Inactive</td>
</tr>
<tr>
<td><strong>mouse</strong></td>
<td></td>
<td><strong>LDTMvKEQK..GKGPGPpKDLMY (SEQ ID NO: 23)</strong></td>
<td>0.2</td>
</tr>
</tbody>
</table>

**Table 1: Analysis of mitogenic peptides derived from the human and
mouse pre-gastrokine (pre-AMP-18) sequence.** A 14 amino acid mitogenic domain
is in bold type. *Peptides are identified by their position in the amino acid sequence of the pre-gastrokine (preAMP-18). #AA; number of amino acids in a peptide. K_{1/2}; concentration for half-maximal growth stimulation.

Overlapping inactive peptides can inhibit the activity of the mitogenic peptides: that is, human peptides 78-88 and 87-105 block the activity of peptide 78-119, and while peptide 87-105 blocks the activity of peptide 104-117, the peptide 78-88 does not. Peptides 78-88 and 87-105 block the activity of the protein in stomach extracts.

**scrambled

12. **The Growth Stimulatory Domain of Gastrokine (AMP-18).**

Finding that a 42-amino acid peptide representing a central region of the novel antrum mucosal cell protein AMP-18 had mitogenic activity similar in character to that of the intact protein in pig and mouse antrum extracts (Table 1), has facilitated the characterization of the bio-active region of the molecule. A peptide including amino acids at positions 78-119, gave similar maximal stimulation of growth of the BSC-1 epithelial cell line to that given by the tissue extracts and was similarly inhibited by several different antisera raised in rabbits to the bacterially-expressed complete antrum protein. The mitogenic activity of a number of synthetic "deletion" peptides related to peptide "78-119" are summarized in Table 1. Growth activity determinations have so far been accomplished with the kidney epithelial cell line as well as several gastric and intestinal lines.

The original 42 amino acid sequence of peptide 78-119 was broken into three segments bounded by lysine (K) residues; N-terminal to C-terminal these are peptides with amino acids at positions 78-88, 87-105 and 104-117. Of these only peptide 104-117 possessed mitogenic activity giving a similar plateau of growth stimulation but requiring a higher molar concentration than the original peptide "78-119"; this is reflected in the higher K_{1/2} value, which suggests that 14-amino acid peptide has 30-40% of the activity of the 42-amino acid peptide. A conclusion from this is that the smaller peptide has less binding affinity for a cell receptor, perhaps due to a lessened ability to form the correct conformation, or alternatively because of the loss of ancillary binding regions. The latter notion is supported by the observations that
peptides "78-88" and 87-105" can antagonize the activity of intact 42-mer-peptide 78-119; these peptides also antagonize the activity of antrum extracts further supporting the validity of synthetic peptides as a means to analyze the biological function of the novel protein. An additional aspect of the invention is that peptide 87-105, but NOT 68-88, antagonizes the activity of peptide 104-117; note that peptide 87-105 overlaps the adjacent 104-117 sequence by two residues.

Taken together these results suggest a relatively simple linear model for the growth-stimulatory region of AMP-18; viz there is an N-terminal extended binding domain (predicted to be largely helix, the relative rigidity of which may explain the linear organization of the relevant sequences as determined in the cell growth studies), followed by a region high in glycine and proline with no predicted structure beyond the likelihood of turns. It is this latter region which contains the trigger for growth stimulation. The specificity of antagonism by peptides 78-88 and 87-105 may be based on whether they overlap or not the agonist peptides 78-119 and 104-117; for example 78-88 overlaps and inhibits 78-119, but does not overlap or inhibit 104-117. The specificity of competition by these peptides taken with the inactivity of the 78-119 scrambled peptide, strengthens a conclusion that AMP-18 interacts with specific cellular components. Further evidence that the receptor binding region extends N-terminally from peptide 104-117 is provided by the enhanced activity of peptide 97-117 which contains a seven amino acid N-terminal extension of 104-117. A peptide with a four amino acid extension in the C-terminal direction (peptide 104-121) appears to have slightly less activity to the parent 104-117, but does include a natural tyrosine, which makes possible labeling with radioactive iodine, which allows determination of the binding of AMP-related peptides to cells, initially by assessment of number of binding sites and subsequently detection of the receptor protein(s).

The peptide 97-117 was used for most test because of its activity (equal to the 42-mer) and its relative economy (21 amino acids in length). However, a C-terminal extension to the tyr-121 gives the most active peptide thus far, perhaps because it stabilizes secondary structure. Even though this peptide does not match the nanomolar activity of EGF, for example, it is much more potent than reported for trefoil peptides (Podolsky, 1997). An estimate for the activity the intact AMP protein
13. **Expression of Recombinant Protein**

(a) **E. coli.** Recombinant constructs are generally engineered by polymerase-chain-reactions using synthetic oligonucleotides complementary to the appropriate regions of the full-length cDNA sequences within the PT/CEBP vector and extended by convenient restriction enzyme sites to enable ready insertion into standard vector polylinkers. The initial experiments with expression of the AMP ORF in bacterial systems employed an expression vector PT/CEBP, which included an N-terminal His6-tag (SEQ ID NO: 16) (Jeon *et al.*, 1994), intended to facilitate the purification of the expressed protein on Ni-NTA resin (Qiagen). Expression of the full-length human cDNA within this vector in the host BL21(DE3)pLysS gave good yields of insoluble protein, which after electrophoresis under denaturing conditions was suitable for use as an immunogen in rabbits to obtain specific high-titer antibodies, but which has not been useful for analysis of the protein’s native structure and function. This insolubility is most probably due to the presence of an unnatural N-terminus, having a His6-tag (SEQ ID NO: 16) upstream of hydrophobic signal peptide, in the expressed protein. Engineering vectors which will express the ORF without the hydrophobic signal peptide sequence are also useful. These are constructed using bacterial expression vectors with and without N- or C-terminal His-tags. The human AMP-18 sequence lacking the 20 amino acid signal peptide and containing a His6-tag (SEQ ID NO: 16) was also expressed in bacteria.

(b) **Pichia pastoris.** Among the simple eukaryotes, the budding yeast *P. pastoris* is gaining wide popularity as an expression system of choice for production and secretion of functional recombinant proteins (Romanos *et al.*, 1992; Cregg *et al.*, 1993). In this system, secretion of the foreign protein may utilize either its own signal peptide or the highly compatible yeast mating-type alpha signal. This organism will correctly process and secrete and at least partially modify the AMP-18 protein. Vectors for constitutive and regulated expression of foreign genes are developed in *Pichia* (Sears *et al.*, 1998). In addition to a poly-linker cloning site, these vectors contain either the high expression constitutive glyceraldehyde-3-phosphate dehydrogenase (GAP) or the methanol-regulated alcohol oxidase promoter.
(AOX1). The latter is an extremely stringent promoter yielding insignificant product in normal culture conditions while giving the highest expression of the vectors tested in the presence of methanol, amounting to as much as 30% of the cell protein. The advantage that the yeast *Pichia* has over the mammalian and insect alternatives is that it is continuously grown in protein-free media, thus simplifying the purification of the expressed protein and eliminating extraneous bioactivities originating in the serum or the host animal cells. A pIB4 construct (inducible by methanol-containing medium) contains the complete human preAMP-18 cDNA sequence.

(c) **Baculovirus/Insect cells.** An alternative, frequently successful, non-mammalian eukaryotic expression system is that using recombinant Baculovirus, such as *Autographa californica*, in an insect cell culture system. As with *Pichia*, a large repertoire of convenient vectors are available in this system, containing both glutathione S-transferase (GST)-and His6-tags (SEQ ID NO: 16) (Pharmingen). Transfections are carried out into *Spodoptera frugiperda* (*Sf*) cells; these cells can be slowly adapted to protein-free medium to favor the purification of secreted proteins. If an endogenous signal peptide does not function in these cells, secretion of foreign proteins can also be forced using vectors containing the viral gp67 secretion signal upstream of the cloning site. Recombinant proteins can be expressed at levels ranging from 0.1-50% total cell protein. Some protein modifications may be more favored in this insect cell system relative to yeast, but still may not duplicate the mammalian system. It appears that the insect expression system would be somewhat more onerous than *Pichia*, and not entirely substitute for expression in mammalian cells. The human AMP-18 sequence lacking the 20 amino acid signal peptide and containing a His6-tag (SEQ ID NO: 16) was expressed in Baculovirus.

(d) **Mammalian cells.** Modifications not detectable by immunoblot analysis may take place in mammalian cells that are not duplicated in cells of other eukaryotes. Although not as convenient as prokaryotic and simple eukaryotic systems, mammalian cells are now frequently used for both transient and continuous expression of foreign proteins. Several growth factors have been expressed and secreted in significant amounts using these systems.
The plasmid pcDNA3/human kidney 293 system: pcDNA3 contains a polylinker cloning site flanked by the strong constitutive cytomegalovirus (CMV) promoter and a SV40 polyA signal (Invitrogen). Laboratory experience is that 60-90% transient transfection levels can be achieved. To this end, PCR amplification of the human preAMP cDNA clone is performed with oligonucleotides that contain the initiation codon and native ribosome binding site (Kozak sequence) as well as suitable restriction enzyme linkers for correct orientation into pcDNA3. Favorable constructs were identified in the transient assay using the potent antibiotic blasticidin S and a vector containing the resistance gene, stable mammalian transfectant cell lines can be established “in less than one week” (Invitrogen). The available vectors also include the constitutive CMV promoter, a polylinker cloning site, an elective V5-epitope/His6-tag (SEQ ID NO: 16) and the SV40 poly(A) signal (PcDNA6/V5-His).

14. **Expression and Analysis of Altered (Modified) Forms of AMP-18**

Given an efficient expression system for the production of “wild-type” AMP-18, a series of mutant proteins, containing either deletions or substitutions may be created, which will permit analysis of the functional domains. The amphipathic helices, the conserved cystine (C) residues and the basic amino acids doublets, which may be cleavage sites, are attractive targets. Although not as simple as an enzyme assay, the mitogenesis assay is routine and replicable, and would enable “mutants” to be characterized as fast as they are constructed. Dominant negative (or positive) “mutants” will be as significant as mutations exhibiting simple loss of function, because these will imply interactions with other factors including possible cell receptors.

15. **Biochemical and Immunoaffinity Fractionation of Expressed and Native Gastrokine Proteins**

In the case of some of the expressed forms of gastrokine AMP-18, the recombinant protein will contain peptide tags that will permit the rapid purification of soluble protein. The presence of these tags, if they do not severely interfere with the protein’s normal functions, will also permit analysis of interactions with other relevant macromolecules. His6-tags (SEQ ID NO: 16) permit purification by binding the recombinant proteins to Ni-NTA resin beads (Janknecht et al., 1991; Ni-NTA
resin from Qiagen). The tagged protein is bound with greater affinity than most antigen-antibody complexes and can be washed rigorously before the Ni$_2^+$-histidine chelation complex is disrupted by excess imidazole to release the purified protein.

GST-tagged recombinant proteins are purified on glutathione-agarose, washed and then eluted with reduced glutathione (Smith and Johnson, 1988). As with all the proposed expression systems, each protein preparation may be tested at the earliest possible stage for its growth factor activity.

Conventional fractionation procedures are used to achieve the desired purity, particularly in the case of the isolation of the natural protein from tissue. Pig antrum mucosa is a preferred starting point for the latter, using initial centrifugation and heat-treatment protocol, followed by a size-exclusion column: BioGel™ P60 is suitable, given the evidence that the 18 kDa protein exists, most probably as a monomer in the extracts. The eluant is loaded on an immunoaffinity matrix created by crosslinking anti-AMP antibodies purified on HiTrap™ Protein A to CNBr-activated Sepharose™ 4B (Pharmacia). Further modification of the immunoaffinity matrix may be helpful, either by extension of the linker to the matrix, which has proven useful in the past (Aithal et al, 1994), or by crosslinking the antibody to immobilized protein-A. Because active protein can be recovered by SDS-gel elution, active protein may also be recovered from the antigen-antibody complexes. Further fractionation could be achieved by C8 reversed-phase high-performance liquid chromatography (HPLC) column. A final step is the use of the SDS-gel elution technique with confirmation of identity by N-terminal sequencing. In all of these steps the immunodetectable AMP-18 and the growth factor activity should fractionate together.

16. **AMP-18 Related Synthetic Peptides**

AMP-18 maybe precursor to one or several bioactive peptides. Synthetic peptides provide a convenient avenue to explore the function of a protein; peptides may mimic aspects of the function or antagonize them. If a peptide either duplicates or inhibits the protein's activity, then it suggests the identity of functional domains of the intact protein, and also provides the possibility of synthesizing specifically tagged probes to explore protein-cell interactions.
Finding that a synthetic 42 amino acid peptide, representing a middle region of the human protein, is capable of mimicking the growth factor activity of the partially fractionated antrum mucosal extracts has provided a short-cut to the analysis of AMP-18 function. This peptide (designated peptide 58-99; amino acids are at positions 58-99 of the mature protein after removal of the signal peptide) in addition to several possible protein processing sites at lysine pairs, contains one of the regions capable of extended helix formation as well as a glycine-proline loop. An added advantage of this peptide is that it contains epitopes recognized by both of the antisera disclosed herein. Some smaller peptides derived from this sequence were synthesized to focus on the bioactive regions. Initially sequences bounded by the lysine residues were studied because they may indicate distinct domains within the protein structure, by virtue of being exposed on the surface of the protein, as witnessed by the antigenicity of this region, and may be sites of cleavage in vivo to bioactive peptides. The glycine-proline region is important (see Table 1 illustrating the bioactive domains of AMP-18). Glycine-proline sequences are known to be involved in SH3 (src homology domain type 3) ligands (see Cohen et al., 1995; Nguyen et al., 1998), because SH domains are involved in protein-protein interactions that GP region of AMP-18 may be involved in the interaction of the protein with a cell surface receptor. The exact GPGGPPP (SEQ ID NO: 24) sequence found in AMP-18 has not been reported for the intracellular-acting SH3 domains, so the intriguing possibility exists that it represents a novel protein interaction domain for extracellular ligands. A 21-mer derived from amino acids at positions 97-117 of the mature sequence has activity similar to the 42-mer. This shorter peptide is useful for growth assays on various epithelial cell lines. This peptide does not express the epitope recognized by the antisera disclosed herein.

All of the AMP-18 derived peptides were synthesized by the Cancer Center Peptide Core Facility of the University of Chicago, which also confirmed the molecular mass and amino acid sequence of the purified peptides that are isolated by HPLC. The biological activity of peptide 78-119 not only provides the basis for seeking smaller peptides with mitogenic activity, but permits amino acid substitutions that have positive or negative effects to be found rapidly. Inactive peptides were
tested for their ability to block the function of active peptides or intact AMP-18. The possible inclusion of D-amino acids in the peptides (in normal or reverse order) may stabilize them to degradation while permitting retention of biological function. Further the ability to synthesize active peptides enables tags that facilitate studies of the nature, tissue distribution and number of cellular receptors. Such tags include His-6 biotin or iodinated tyrosine residues appended to the peptide sequence (several of the bioactive peptides have a naturally occurring tyrosine at the C-terminus).

Synthetic peptides also permit assessment of the role of potential secondary structure on function. The finding that a 4 amino acid C-terminal extension of the active peptide 97-117, predicted to promote a helix similar to that for the intact AMP-18 sequence, led to a more active peptide 97-121, is interesting. The helix-propensity of these active peptides e.g. peptide 126-143, which resembles an antimicrobial magainin peptide, provides useful information. With respect to antimicrobial peptides, the function of the magain in class is related to their ability to form amphipathic helices (Boman, 1995). Synthetic peptides that can be locked in the helical form by lactam bridges (Houston et al., 1996) enhanced biological activity; at least one pair of appropriate acidic and basic amino acid residues for lactam formation already exist in potential helix regions of AMP-18.

Another equally significant aspect of the peptide studies is the potential availability of specific anti-AMP-18 peptides that antagonize its biological functions. Tissue culture studies show that sub-peptides of the growth-promoting peptide 78-119 can antagonize the activity of the intact peptide (see Table 1). Peptides that can occupy cellular binding sites but lack some essential residues for activity may block the action of AMP-18 and its active peptides. This makes available another set of reagents for the analysis of cellular receptors and for assessing receptor-ligand affinity constants. Availability of defined peptide antagonists is useful in whole animal studies, and may eventually serve to regulate the activity of the natural protein in humans.

17. **Interactions of AMP-18 and Related Peptides with Cells:**

   **Assessment of Cell Growth**

   Non-transformed monkey kidney epithelial cell line BSC-1 and other
epithelial cell lines were used to assess effects on growth. In general, conditions were chosen for each line such that cells are grown to confluence in plastic dishes in supplemented growth medium with minimal calf (or fetal) serum for growth (Lieske et al., 1997); BSC-1 cells become confluent at 10^5/60mm dish with 1% calf serum. At the start of the growth assay the medium on the confluent culture was aspirated and replaced with fresh medium with minimal serum to maintain viability (0.01% for BSC-1 cells). AMP-18 preparations were added to the culture medium and 4 days later the cell monolayer was rinsed, detached with trypsin, and the cells were counted using a hemocytometer. Determination of the capacity of AMP-18 to initiate DNA synthesis was measured by the incorporation of [3H]thymidine (Toback, 1980); to confirm the DNA synthesis assay, autoradiograms of leveled cells were counted (Karth and Toback, 1985).

The protein AMP-18 is expressed in the antrum mucosa and to a lesser extent in the adjacent corpus mucosa. However, both antrum extracts and the active synthetic peptides stimulate proliferation of most simple epithelial cell lines. The major criterion used, apart from cells which might be natural targets for AMP-18 or its peptides, was that of growth control, particularly cell-density restriction. Many transformed stomach lines derived from human cancer patients are available from various sources, but most of these do not exhibit growth control. For example, a gastric AGS adenocarcinoma cell subline from Dr. Duane Smoot (Howard University College of Medicine) showed a greater degree of contact inhibition, and responded well to AMP-18 and its derived peptides. These cells do not naturally synthesize AMP-18. Similar responses were observed with the non-transformed rat IEC intestinal epithelial cells (provided by Dr. Mark Musch, Dept. Medicine, University of Chicago); the latter show excellent epithelial cell characteristics in culture (Quaroni et al., 1979; Digass et al., 1998).

18. Receptors for AMP-18 on the Surface of Epithelial Cells

Characterization of the target cell receptors of AMP-18 is intriguing because of the apparent existence of receptors on cells which are not expected ever to contact this protein. Initial growth response assays were performed on kidney-derived epithelial cell lines, which responded well to the stomach factor. Gastric cell lines, as
well as the non-transformed rat intestinal epithelial IEC-6 cells, were used to address the receptors in cells that are likely the true physiological targets for the antrum factor. The specificity for the action of this protein in vivo likely arises from the extremely tissue specific nature of its expression, rather than that of its receptor. It is possible that AMP-18 may interact with receptors shared with other growth factors. However, the additive growth stimulus of EGF and the antrum extracts suggest that AMP-18 may have novel receptors.

Protein molecules in cell membranes that interact with AMP-18 may be sought in several different ways. Pure AMP-18 or related peptides labeled, e.g. with biotin or radioactive iodine, are used to estimate the number of saturatable sites on the cell surface. Scatchard analysis of the binding values as used to determine the number and affinity of receptors. For quantitative studies, binding is measured at increasing AMP ligand concentrations, and non-specific components are identified by measuring binding in the presence of excess unlabeled factor. Iodinated growth factors have been cross-linked to cellular receptors enabling their identification (Segarini et al., 1987). Labeled AMP ligands are incubated with cells, and the bound ligand is cross-linked to the receptors by disuccinimidyl suberate. The labeled proteins are resolved by SDS-PAGE, and autodiography is used to visualize the cross-linked complex permitting an estimate of the MW of the receptor(s). Synthetic peptide mimics or antagonists permit studies of the cellular receptors, and their properties are reasonably inferred prior to future definitive identification, presumably by cloning techniques.

In addition to crosslinking studies, antibodies, or his6-tagged (SEQ ID NO: 16) AMP-18 or peptides are used to isolate cellular or mucus proteins which bind to AMP-18. As an additional approach, an immobilized AMP-18 affinity matrix can be created by using CNMBr-activated Sepharose. As a simple beginning to the analysis of the signal transduction pathway mediated by any cell receptor, a test to assay protein tyrosine kinase activity in affinity isolates is available (Yarden and Ullrich, 1988; Schlessinger and Ullrich, 1992).

19. **Is AMP-18 Processed to Bioactive Peptides?**

The functional molecular form(s) of AMP-18 is not known. Certainly, the ca.
18 kDa is the protein form which accumulates in antrum mucosal cells, and substantial amounts of polypeptides of lower MW are not detected with the antisera, even though they do react with pepsin fragments down to ca. 10 kDa and also with the bioactive peptide 78-119 (having only 42 amino acids). Having access to labeled or tagged AMP-18 enables a question of whether the protein is processed in antrum mucosal extracts, or by the epithelial cells which respond to it, to be explored.

20. **Genes for AMP-18 in Man and Mouse**

Using PCR techniques employing primers based on the sequence of the human cDNA clone, genomic clones of human and mouse preAMP-18 were obtained. The exon/intron structure (FIG. 13) is complete. Mouse AMP exons are sufficiently similar to those of human and pig to allow a sequence of the mouse gene to be assembled. Human and mouse genes have very similar structures, the mouse gene being slightly smaller. The ORF contained in exons of the mouse gene predicts a protein having 65% identity to the human and pig proteins. A 2 kb of sequence is upstream of the human gene.

21. **Knockout of the AMP-18 Gene in Mouse**

From the mouse map a targeting construct is designed. The construct preferably contains: [5' - TK (a functional thymidine kinase gene) - ca. 5 kb of the 5' end of AMP-18 DNA - the neomycin phospho-transferase (neo) gene under the control of the phosphoglycerate kinase (PGK) promoter -ca. 3 kb of the 3' end of the gene - 3' ]. A considerable length of homology of the construct with the resident AMP-18 gene is required for efficient targeting. Increasing the total homology from 1.7 to 6.8 kb increases the efficiency of homologous targeting into the hrpt gene about 200-fold (Hasty et al., 1991). Beyond that total length, the efficiency increases only slightly.

To facilitate the detection of homologous intergrants by a PCR reaction, it is useful to have the neo gene close to one end of the vector. The resulting transfectants can be provided by PCR with two primers, one in the neo gene and the other in the AMP-18 locus just outside of the targeting vector. Flanks extending 4 kb 5' and 4.5 kb 3' of the mouse gene have been obtained. Through homologous recombination, the coding region will be replaced by the neo gene to ensure a complete knockout of the gene are
already cloned. After trimming off the plasmid sequence, the targeting cassette will be transfected into ES cells and stable transfectants obtained by selection with G418, an analog of neomycin, and ganciclovir (Mansour et al., 1988). Southern blots with the probe from the flanking sequence will be used to screen for targeted homologous recombinants. Correctly targeted ES cell clones will be injected in blastocysts from C57BL/6 mice.

Male offspring obtained from surrogate mothers that have at least 50% agouti coat (embryonic stem cell (ES) cell derived) are bred with C57BL/6 mice. F1 mice that are agouti have the paternal component derived from the ES cells (agouti is dominant over black). 50% of these mice should have the knockout preAMP-18 allele. These hemizygous mice are monitored for any effect of diminished gene dosage. Homozygous knockouts are preferable. If the sole function of AMP-18 is in the stomach following birth, then viable homozygotes are expected. If these cannot be obtained, a fetally lethal defect would be indicated, and the fetal stage of abortion would be ascertained. This result would suggest an unanticipated role of the protein in normal development.

Homozygous AMP-18 knockout mice are useful for investigations of stomach morphology and function. It is expected that such knockouts will show if AMP-18 is essential, and at which stage of gastro-intestinal development it is bioactive. It is possible that the AMP-18 knockout hemizygous mice will already show a phenotype. This could occur if reduced dosage of the protein reduces or eliminates its function, or if parental imprinting or random mono-allelic expression has a significant influence. A range of possible outcomes of the AMP-18 knockout in mice include: i) no viable homozygotes, implying an essential unanticipated developmental role; ii) viable homozygotes, but with obviously impaired gastrointestinal functions; iii) no strong phenotype, i.e. the protein is not important to the development and life of the laboratory mouse. If appropriate, the generation of AMP-18 in overexpressing mice is pursued. A truncated AMP-18 protein produced in the mice could potentially create a dominant negative phenotype; knowledge gained from the experiments will further define the functional domains of the protein.

Abbreviations for amino acids
<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Three-letter abbreviation</th>
<th>One-letter symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>Ala</td>
<td>A</td>
</tr>
<tr>
<td>Arginine</td>
<td>Arg</td>
<td>R</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Asn</td>
<td>N</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>Asp</td>
<td>D</td>
</tr>
<tr>
<td>Asparagine or aspartic acid</td>
<td>Asx</td>
<td>B</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Cys</td>
<td>C</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Gln</td>
<td>Q</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>Glu</td>
<td>E</td>
</tr>
<tr>
<td>Glutamine or glutamic acid</td>
<td>Glx</td>
<td>Z</td>
</tr>
<tr>
<td>Glycine</td>
<td>Gly</td>
<td>G</td>
</tr>
<tr>
<td>Histidine</td>
<td>His</td>
<td>H</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Ile</td>
<td>I</td>
</tr>
<tr>
<td>Leucine</td>
<td>Leu</td>
<td>L</td>
</tr>
<tr>
<td>Lysine</td>
<td>Lys</td>
<td>K</td>
</tr>
<tr>
<td>Methionine</td>
<td>Met</td>
<td>M</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Phe</td>
<td>F</td>
</tr>
<tr>
<td>Proline</td>
<td>Pro</td>
<td>P</td>
</tr>
<tr>
<td>Serine</td>
<td>Ser</td>
<td>S</td>
</tr>
<tr>
<td>Threonine</td>
<td>Thr</td>
<td>T</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Trp</td>
<td>W</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Tyr</td>
<td>Y</td>
</tr>
<tr>
<td>Valine</td>
<td>Val</td>
<td>V</td>
</tr>
</tbody>
</table>
DOCUMENTS CITED


SEQUENCE LISTING

<110> UNIVERSITY OF CHICAGO

<120> CONTROL OF GROWTH AND REPAIR OF GASTRO-INTESTINAL TISSUES BY GASTROKINES AND INHIBITORS

<130> 31737-2030

<140> CA 2,442,684

<141> 2003-09-29

<150> PCT/US02/09885

<151> 2002-03-29

<150> 09/821,726

<151> 2001-03-29

<160> 26

<170> PatentIn Ver. 3.2

<210> 1

<211> 7995

<212> DNA

<213> Homo sapiens

<400> 1

catgatgatc ccatcttagg gtttcaacct atgcagcag gaaatgtt
10
ccagcagaa ttttcaaaagg gaaatattat agagttgtag ttaatgaaat acaagtttcc
20
ctctggtgca attataatta ctatcctcacc tggatatgaa aagcagagaa aaaaatta
30
accaacctc acctaaacct gttgttggct tttactttgt tcgcccacatg atagttctca
40
tttctggtt ctctggtgca attataatta ctatcctcacc tggatatgaa aagcagagaa aaaaatta
50
agggatctcc atacatcatc gattttattt tactactattt cttttttcag gaaagttttg
60
tctcttttt tctctttttt tctcttttttt cttctttttt cttcttttttt cttcttttttt
70
catgatggag cattggttc gagttggtgc ccagcagcag aaaaatta
80
gaggttgctg ccagcagcag aaaaatta
90
tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt
100
tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt
110
tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt
120
tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt
130
tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt
140
tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt
150
tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt
160
tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt
170
tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt
180
tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt
190
tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt
200
tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt tttttttttttt
210

CA 02442684 2007-12-14

8217523.1
31737-2030
cacaattcgtac ctctacgttg ccctctccct gcctcggacc ctcgacccgag cactgtgtgttg 6000
ttgctgtgtga gaggagaaca gctctgctgt caccaatacg aaccttacgat ctctggccaa 6060
ugacatatatg tgaaggtattag tggattagct cagatttttc agtttaaatg atttaaatgaa gcaatggaa 6120
ctccgatgtac tgtggatattg gcaagactgt cagatttttc agtttaaatg atttaaatgaa gcaatggaa 6180
aggctttttg attatacataa aaaaagctca tttgtttactt aaatttcaact taattttactt 6240
attttataatt ttgctctgac atacatcactt aggtgtacag aataagacac tccaatatgt 6300
attttataatt ttgctctgac atacatcactt aggtgtacag aataagacac tccaatatgt 6360
aagttgtaga aataaataata aactgcacatt cagagaagtc tattttcata tgtcatactc 6420
aggaatcattgt gatgacacaaga gtggctcgaag ttgcacagat aaatataagg ccagatctct 6480
tgatcattagat gatgacacaaga gtggctcgaag ttgcacagat aaatataagg ccagatctct 6540
aagatatacaga gataraacgata gaaattacctt ttgctcagct atgtagataa gtaataagtc ctccagcttg 6600
tgagcagcagac ttgctcagct atgtagataa gtaataagtc ctccagcttg 6660
attcccttgc attcccttgc attcccttgc attcccttgc attcccttgc attcccttgc 6720
aggagcagac ttgctcagct atgtagataa gtaataagtc ctccagcttg 6780
gacgctgaca ggtggacacc aacatcagcrt gggacttgat gggtgacacc aacatcagcrt 6840
gctgagagga tggacagcagac ttgctcagct atgtagataa gtaataagtc ctccagcttg 6900
attgctgacag tttcctgtcttt gggtgacacc aacatcagcrt gggacttgat gggtgacacc 6960
aagggcacaaga ttttctcagct gttgctgtgt ttggctgtgt tggctgatcttc ttttctcagct 7020
tgatcattagat gatgacacaaga gtggctcgaag ttgcacagat aaatataagg ccagatctct 7080
aggagagttg gatgacacaaga gtggctcgaag ttgcacagat aaatataagg ccagatctct 7140
agtgctgatgatt gatgacacaaga gtggctcgaag ttgcacagat aaatataagg ccagatctct 7200
tagatcattagat gatgacacaaga gtggctcgaag ttgcacagat aaatataagg ccagatctct 7260
aagttcgattac tctctctctct ttgctcagct atgtagataa gtaataagtc ctccagcttg 7320
ttgctcagct atgtagataa gtaataagtc ctccagcttg 7380
ttgctcagct atgtagataa gtaataagtc ctccagcttg 7440
agggcacaaga ttttctcagct tggagcaagtt gttgctgtgt tggctgatcttc ttttctcagct 7500
bagcttcgctt gttgctgtgt tggctgatcttc ttttctcagct tggagcaagtt gttgctgtgt 7560
cagcggattac tctctctctct ttgctcagct atgtagataa gtaataagtc ctccagcttg 7620
cagcggattac tctctctctct ttgctcagct atgtagataa gtaataagtc ctccagcttg 7680
cagcggattac tctctctctct ttgctcagct atgtagataa gtaataagtc ctccagcttg 7740
acacatcactt acacatcactt acacatcactt acacatcactt acacatcactt acacatcactt 7800
ttgctcagct atgtagataa gtaataagtc ctccagcttg 7860
aggtggactt gttgctgtgt tggctgatcttc ttttctcagct tggagcaagtt gttgctgtgt 7920
acacatcactt acacatcactt acacatcactt acacatcactt acacatcactt acacatcactt 7980
cacgctgactg atttttacttc ctttattccat gcctttgacacc ttgctcttctt cccttacttctt 7955
ctctctctct ctctctctct ctctctctct ctctctctct ctctctctct ctctctctct
<210> 2
<211> 752
<212> DNA
<213> Homo sapiens

<400> 2

cattgctgacc ttttctcctg ttggctgtgt gcctcggacc ctcgacccgag cactgtgtgttg 60
ctttgtgcctga ttttctcctg ttggctgtgt gcctcggacc ctcgacccgag cactgtgtgttg 120
caatgtagac aacaacatctt ctttacttctt gcctcggacc ctcgacccgag cactgtgtgttg 180
caatgtagac aacaacatctt ctttacttctt gcctcggacc ctcgacccgag cactgtgtgttg 240
tgggaatgccc cccgatcctc tcaaatgctg atggatgttg ccacgactcct gcactgctgttgt 300
gagaagaggg gccggatctc ctttacttctt gcctcggacc ctcgacccgag cactgtgtgttg 360
taggggtgacag gacggatcct ctttacttctt gcctcggacc ctcgacccgag cactgtgtgttg 420
cacagattggt gcacaggagac gacggatcct ctttacttctt gcctcggacc ctcgacccgag 480
aacatggtctt ggtgacagac gacggatcct ctttacttctt gcctcggacc ctcgacccgag 540
caagctgactg atggatgttg ccacgactcct gcactgctgttgt 600
aacaatatttt ttttacttctt gcctcggacc ctcgacccgag cactgtgtgttg 660
ggtgacagac gtttttatttt gcacttatttt gcctcggacc ctcgacccgag cactgtgtgttg 720
ctctctctct ctctctctct ctctctctct ctctctctct ctctctctct ctctctctct
<210> 3
<211> 185
<212> PRT
<213> Homo sapiens

<400> 3

8217523.1
31737-2030
Met Lys Phe Thr Ile Val Phe Ala Gly Leu Leu Gly Val Phe Leu Ala
1 5 10 15
Pro Ala Leu Ala Asn Tyr Asn Ile Asn Val Asn Asp Asp Asn Asn
20 25 30
Ala Gly Ser Gly Gln Gln Ser Val Ser Val Asn Asn Glu His Asn Val
35 40 45
Ala Asn Val Asp Asn Asn Asn Gly Trp Asp Ser Trp Asn Ser Ile Trp
50 55 60
Asp Tyr Gly Asn Gly Phe Ala Ala Thr Arg Leu Phe Gln Lys Lys Thr
65 70 75 80
Cys Ile Val His Lys Met Asn Lys Glu Val Met Pro Ser Ile Glu Ser
85 90
Leu Asp Ala Leu Val Lys Glu Leu Gln Gly Lys Gly Pro Gly
100 105 110
Gly Pro Pro Pro Lys Gly Leu Met Tyr Ser Val Asn Pro Asn Lys Val
115 120 125
Asp Leu Ser Lys Phe Gly Lys Asn Ile Ala Asn Met Cys Arg Gly
130 135 140
Ile Pro Thr Tyr Met Ala Glu Glu Met Gln Glu Ala Ser Leu Phe
145 150 155 160
Tyr Ser Gly Thr Cys Tyr Thr Thr Ser Val Leu Trp Ile Val Asp Ile
165 170 175
Ser Phe Cys Gly Asp Thr Val Glu Asn
180 185

<210> 4
<211> 7226
<212> DNA
<213> Mus sp.

<220>
<221> modified_base
<222> (7030)
<223> a, c, t, g, other or unknown

<220>
<221> modified_base
<222> (7084)
<223> a, c, t, g, other or unknown

<400> 4
gaattcaac agcagcccat ctttcaccag cactatccga atctagccat accagcattc 60
tagaagagat gcagggcagtg agctaaacat cagacccctgt cagcctgtta agctccagac
ctggagaag agggaagtgg tgtgttaacc gacgttttca gatgtggaat tcttgtagct 120
gtgattttata aaacacagggt tccccccttta tttgatttaga accacagct tcatggtgtag 180
aagagaccag gttaaaaaat aataagtgtgc atgcagttct cccctaaaac tatattaaac 240
gtttaccttat tttgccaagt gacagtctct gttttcctctc ttaggagaag tttctcttta
300
ttttatatata atatattgaaaa gttttcatat ttcagacagg tggttcttcat cctgtgagggc 360
atgacccccct tggggtgtttt gaacgcacct ttcacagggg tctacctata gctttcctgc 420
attctagcata tttcattttat gattcataac agtacaaaaa ttagtttaga agtaggaaca 480
aaatatcccc atgggtggtgg ttcacctactg ttagaggggt cccgacccatt cagaggttgtg
540
agaactcgtgg tttcagaggg aaataaagag acagagttcc tttgactggg cccagagcag 600
CA 02442684 2007-12-14

-6-

ggggaacctg tgctcctactg tttctgtgtt ttttaataag agttttgcctg ccacgagact 4560
cttcctcag aagtctgcta tggtcagca aagtgaacag catgccatgc ccctccttca 4620
ggcctcgtgat aacaatctcg aacagcaga ggtaaaagcc tcgcctctlct ttgtaggtga 4680
cagagatctg tacaagcgct taccgagact gcaccttac ccctccttcg ttgtagtgag 4740
catcagcagc tggttgcctac cagataatcg aacagcaga tcgccttcca ctctcctgtc 4800
tgatgatcagt ggtgcctcag cttggtcag tgcctgcccc cacacgcctcc tctcgaggtt 4860
ccagtaaacg actgctacat tgcagtcgta cagcacccta aagactttagt ctctcccctc 4920
agctcctgctg atttcagcag cacttcctca cagttgcctg ggttatcagta actacacaca 4980
ataagactcg gttggtcttca tgtgagagat cttcttcttt ctcctttatt cgtctttagt 5040
aatgtttagg atatctccac tatactaatg aatatatatt tcgctctttg gaaacattat 5100
tgtgtaaacc tctccttatg tcgctcctag atgtagacag aagaggagac 5160
cgcctttttt tacctttccc agttgtctct atgtgacact tggtaggtcg tggagagact 5220
acacagactc ctctgtgatg ggtggtcttc tatactaatg attgagctct ggtgaattcg 5280
tcaagacttgg gacagcagc ttccttcttt tcgctcctag cttgcttctc ttcctctttt 5340
cttttcttaa caactataac atttcttcat ctcctaactct ctaaactgcct tgggacactt 5400
agcccatcgc ctagctgacat gtttcctttct ctctttccttt caacacatct taaactcgct 5460
ttgttccccac tcgcccttgta ctctctttcat ctctcttttt ttgcgtgtcg ctgcaggtcg 5520
actctctttt ctttctttcc ctcctactac cagggtaaag ggtcttcaagc acctctctcc 5580
aagagtcttg gaaacatctg aagttttctg cagtttgctg gttggtgcctg atctgacact 5640
aagtctcctg ctacatctag cagttttctc cagtttgtctc ggtggttcttg atctgacact 5700
atcccttctg gcctgcttct cagtttcttc gttggttcctg cagttttctg gttggtgtttg 5760
tggccacagc agcttctcttg ttgatgatcag cttgagctct ttccttttgt ctcctttttt 5820
agccacttgg gacagcagc gtttcttttt tttctttctg cttgcttctc ttccttctttt 5880
acactctctg ctagcttctc ctctgctttc tttctttctt tttctttctt tttctttctt 5940
aaaacacttg ccaacatttc ctctttttct gcctgctttc gtttgctttt ctctttctttc 6000
gtcttttttt gttggtttctg ctctctttct ctcttttttt tttctttcct cttgctttct 6060
agttaagacg caaagctataa aggcttatag tcggctacat cagatcacttg ggtccttttt 6120
ttgtttatcct ctaagcctgtt ctggctttca aagaaagaatg ggcaccttctt cttgcttttt 6180
tcaacagactc caaagctataa aggcttatag tcggctttcct cttgcttttt cttgcttttt 6240
ggaacagtta aagaaagaatg ggcacacata gaaagcctata aagaaagaatg ggcacacata 6300
attataataa ttcacttctg aagttttctc cagttttctt gttggttttt ctcttttttt 6360
ccgctttttt cagttttctt gttggttttt ctcttttttt ctcttttttt ctcttttttt 6420
gctcactctg tccttttttt cttgcttttt tttctttttt tttctttttt tttctttttt 6480
caactctctg ctagcttctc ctctgcttttc tttctttttt tttctttttt tttctttttt 6540
agaagctctg ctagcttctc ctctgcttttc tttctttttt tttctttttt tttctttttt 6600
agccacttgg gacagcagc gtttcttttt tttctttttt tttctttttt tttctttttt 6660
agccacttgg gacagcagc gtttcttttt tttctttttt tttctttttt tttctttttt 6720
agccacttgg gacagcagc gtttcttttt tttctttttt tttctttttt tttctttttt 6780
agccacttgg gacagcagc gtttcttttt tttctttttt tttctttttt tttctttttt 6840
tgaagactctag acaagctctag ggaagctctag ggaagactctag ggaagactctag ggaagactctag 6900
aagaaagcttg ggtgcctcttg aactggtatc gccctttttt ggtgcctcttg aactggtatc 6960
agttaagacg caaagctataa aggcttatag tcggctttcct cttgcttttt cttgcttttt 7020
atcagctctg ctagcttctc ctctgcttttc tttctttttt tttctttttt tttctttttt 7080
cagttttctc catctttttt ctcttttttt tttctttttt tttctttttt tttctttttt 7140
tctatcactg ctagcttctc ctctgcttttc tttctttttt tttctttttt tttctttttt 7200
gttttctttt cagttttctt cttgcttttt cttgcttttt cttgcttttt cttgcttttt 7262

<210> 5
<211> 543
<212> DNA
<213> Mus sp.

<400> 5
atggttctgtcg tgggtctgtcg tgggtctctt gcacgcctcttg gtttttgcctt caggtgctcag 60
atcaaagtgg tgggtctgtcg tgggtctctt gcacgcctcttg gtttttgcctt caggtgctcag 120
tgcctcagac ccacagcagc aacagcagc gtttcttttt tttctttttt tttctttttt 180
gagtggagg cctgtggagg cttgagctct ttcctttttt tttctttttt tttctttttt 240
aggtctgact ctagctgact ctagctgact ctagctgact ctagctgact ctagctgact 300
acagtgtgg ggggttgagg aagttttctc cagttttctt gttggttttt ctcttttttt 360
agactgtgatt ggttttttttt caggtgctcag 320
acgtctgaca tctctgcttg ttcctttttt tttctttttt tttctttttt tttctttttt 420
acagctgacag tctctggtat ggcacctttc gttggttttt ctcttttttt ctcttttttt 480
acagtgtgatt ggttttttttt caggtgctcag 540
acagtgtgatt ggttttttttt caggtgctcag 543

8217525.1
31737-2030
<table>
<thead>
<tr>
<th></th>
<th>Met</th>
<th>Lys</th>
<th>Leu</th>
<th>Thr</th>
<th>Met</th>
<th>Phe</th>
<th>Val</th>
<th>Val</th>
<th>Gly</th>
<th>Leu</th>
<th>Leu</th>
<th>Gly</th>
<th>Leu</th>
<th>Leu</th>
<th>Ala</th>
<th>Ala</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pro</td>
<td>Gly</td>
<td>Phe</td>
<td>Ala</td>
<td>Tyr</td>
<td>Thr</td>
<td>Val</td>
<td>Asn</td>
<td>Ile</td>
<td>Asn</td>
<td>Gly</td>
<td>Asn</td>
<td>Asp</td>
<td>Gly</td>
<td>Asn</td>
<td>Val</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td></td>
<td></td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Asp</td>
<td>Gly</td>
<td>Ser</td>
<td>Gly</td>
<td>Gln</td>
<td>Gln</td>
<td>Ser</td>
<td>Val</td>
<td>Ser</td>
<td>Ile</td>
<td>Asn</td>
<td>Gly</td>
<td>Val</td>
<td>His</td>
<td>Asn</td>
<td>Val</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td></td>
<td></td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Ala</td>
<td>Asn</td>
<td>Ile</td>
<td>Asp</td>
<td>Asn</td>
<td>Asn</td>
<td>Gly</td>
<td>Trp</td>
<td>Asp</td>
<td>Ser</td>
<td>Trp</td>
<td>Asn</td>
<td>Ser</td>
<td>Leu</td>
<td>Trp</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td></td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Asp</td>
<td>Tyr</td>
<td>Glu</td>
<td>Asn</td>
<td>Ser</td>
<td>Phe</td>
<td>Ala</td>
<td>Ala</td>
<td>Thr</td>
<td>Arg</td>
<td>Leu</td>
<td>Phe</td>
<td>Ser</td>
<td>Lys</td>
<td>Lys</td>
<td>Ser</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65</td>
<td></td>
<td></td>
<td>70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Cys</td>
<td>Ile</td>
<td>Val</td>
<td>His</td>
<td>Arg</td>
<td>Met</td>
<td>Asn</td>
<td>Lys</td>
<td>Asp</td>
<td>Ala</td>
<td>Met</td>
<td>Pro</td>
<td>Ser</td>
<td>Leu</td>
<td>Gln</td>
<td>Asp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>85</td>
<td></td>
<td></td>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>Leu</td>
<td>Asp</td>
<td>Thr</td>
<td>Met</td>
<td>Val</td>
<td>Lys</td>
<td>Glu</td>
<td>Gln</td>
<td>Lys</td>
<td>Gly</td>
<td>Lys</td>
<td>Gly</td>
<td>Pro</td>
<td>Gly</td>
<td>Gly</td>
<td>Ala</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td></td>
<td>105</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>Pro</td>
<td>Pro</td>
<td>Lys</td>
<td>Asp</td>
<td>Leu</td>
<td>Met</td>
<td>Tyr</td>
<td>Ser</td>
<td>Val</td>
<td>Asn</td>
<td>Pro</td>
<td>Thr</td>
<td>Arg</td>
<td>Val</td>
<td>Glu</td>
<td>Asp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>115</td>
<td></td>
<td>120</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>Leu</td>
<td>Asn</td>
<td>Thr</td>
<td>Phe</td>
<td>Gly</td>
<td>Pro</td>
<td>Lys</td>
<td>Ile</td>
<td>Ala</td>
<td>Gly</td>
<td>Met</td>
<td>Cys</td>
<td>Arg</td>
<td>Gly</td>
<td>Ile</td>
<td>Pro</td>
</tr>
<tr>
<td></td>
<td></td>
<td>130</td>
<td></td>
<td>135</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Thr</td>
<td>Tyr</td>
<td>Val</td>
<td>Ala</td>
<td>Glu</td>
<td>Ile</td>
<td>Pro</td>
<td>Gly</td>
<td>Pro</td>
<td>Asn</td>
<td>Gln</td>
<td>Pro</td>
<td>Leu</td>
<td>Tyr</td>
<td>Ser</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>145</td>
<td></td>
<td></td>
<td>150</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Lys</td>
<td>Lys</td>
<td>Cys</td>
<td>Tyr</td>
<td>Thr</td>
<td>Ala</td>
<td>Asp</td>
<td>Ile</td>
<td>Leu</td>
<td>Trp</td>
<td>Ile</td>
<td>Leu</td>
<td>Arg</td>
<td>Met</td>
<td>Ser</td>
<td>Phe</td>
</tr>
<tr>
<td></td>
<td></td>
<td>165</td>
<td></td>
<td></td>
<td>170</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>Cys</td>
<td>Gly</td>
<td>Thr</td>
<td>Ser</td>
<td>Val</td>
<td>Glu</td>
<td>Thr</td>
<td>Tyr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>180</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

```
<400> 6
atgcctgacct ttctcacttca ttgcatgggt gaagccaaga tgaagttcac aattgccctt 60
gctggacttc cttggcgctt cctgacactt gcccctgctg actataatct cagttgcaac 120
gacgaacgcga caagtgggtag gaaggggacc cagtcgatgg tggcgcacaa tgaacacaac 180
gtgcccaagcc ttgcaaatata caatgggatgg acactctctga atgcccctctg ggactataag 240
actggtttgt cttgtaaccg accttctgag aagaagttgct gcatggcagc caaaatgataa 300
aagggggccga caggggacc acctcccaag agccctgggt acctgcgtgaa ccccaacaga 360
gttgcaaacatt cttgggcaagtt tggaaatcttc atctctgctc tcgctgacaa gacgccgtaa 420
tctgccgcttg aagagatgtc aagacgccag tctgattcgaag aagccgagcgg aacat gcctg 480
ttgctgctgctgt cttcgcaacat tggccgagtc aagccgagcgg aacat gcctg 540
gcaataatacgtttgttgcttt atacatctttcc ttcgtgcttg gaatagcggga gaacta 597
```
<211> 185
<212> PRT
<213> Sus scrofa

<400> 8
Met Lys Phe Thr Ile Ala Phe Ala Gly Leu Leu Gly Val Phe Leu Thr 1 5 10
Pro Ala Leu Ala Asp Tyr Ser Ile Ser Val Asn Asp Asp Gly Asn Ser 20 25 30
Gly Gly Ser Gly Glu Glu Ser Val Ser Val Asn Asn Glu His Asn Val 35 40 45
Ala Asn Val Asp Asn Asn Gly Trp Asn Ser Trp Asn Ala Leu Trp 50 55 60
Asp Tyr Arg Thr Gly Phe Ala Val Thr Arg Leu Phe Glu Lys Lys Ser 65 70 75 80
Cys Ile Val His Lys Met Lys Lys Ala Met Pro Ser Leu Glu Ala 85 90 95
Leu Asp Ala Leu Val Lys Glu Lys Lys Leu Glu Lys Gly Lys Gly Pro Gly 100 105 110
Gly Pro Pro Pro Pro Pro Lys Ser Leu Arg Tyr Ser Val Asn Pro Asn Arg Val 115 120 125
Asp Asn Leu Asp Lys Phe Gly Ser Ile Val Ala Met Cys Lys Gly 130 135 140
Ile Pro Thr Tyr Met Ala Glu Glu Ile Glu Gly Ala Asn Leu Ile Ser 145 150 155 160
Tyr Ser Glu Lys Cys Ile Ser Ala Asn Ile Leu Trp Ile Leu Asn Ile 165 170 175
Ser Phe Cys Gly Gly Ile Ala Glu Asn 180 185

<210> 9
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 9
Met Arg Gly Ser His His His His His Gly Ser 1 5 10

<210> 10
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Consensus sequence
<211> MOD_RES
<222> (4)
<223> Lys or Gln

<220>
<221> MOD_RES
<222> (6)
<223> Leu or deleted

<220>
<221> MOD_RES
<222> (7)
<223> Gln or deleted

<220>
<221> MOD_RES
<222> (14)
<223> Pro or Ala

<400> 10
Val Lys Glu Xaa Lys Xaa Xaa Gly Lys Gly Pro Gly Gly Xaa Pro
1 5

Lys

<210> 11
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 11
Lys Lys Leu Gln Gly Lys Gly Pro Gly Gly Pro Pro Pro Lys
1 5

<210> 12
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 12
Leu Asp Ala Leu Val Lys Glu Lys Lys Leu Gln Gly Lys Gly Pro Gly
1 5
Gly Pro Pro Pro Lys
10

<210> 13
<211> 25
<212> PRT
<213> Artificial Sequence
Description of Artificial Sequence: Synthetic peptide

Leu Asp Ala Leu Val Lys Glu Lys Lys Leu Gln Gly Lys Gly Pro Gly
1 5 10 15
Gly Pro Pro Pro Lys Gly Leu Met Tyr
20 25

Artificial Sequence

Description of Artificial Sequence: Synthetic peptide

Lys Lys Thr Cys Ile Val His Lys Met Lys Lys
1 5 10

Artificial Sequence

Description of Artificial Sequence: Synthetic peptide

Lys Lys Glu Val Met Pro Ser Ile Gln Ser Leu Asp Ala Leu Val Lys
1 5 10 15
Glu Lys Lys

Artificial Sequence

Description of Artificial Sequence: 6-His tag

His His His His His
1 5

Artificial Sequence

Description of Artificial Sequence: Synthetic peptide

8217523.1
31737-2030
<400> 17
Lys Lys Thr Cys Ile Val His Lys Met Lys Lys Glu Val Met Pro Ser
1 5 10
Ile Gln Ser Leu Asp Ala Leu Val Lys Glu Lys Leu Gln Gly Lys
20 25 30
Gly Pro Gly Gly Pro Pro Pro Lys Gly Leu
35 40

<210> 18
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 18
Lys Lys Leu Gln Gly Lys Gly Pro Gly Gly Pro Pro Pro Lys Gly Leu
1 5 10 15
Met Tyr

<210> 19
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 19
Gly Lys Pro Leu Gly Gln Pro Gly Lys Val Pro Lys Leu Asp Gly Lys
1 5 10 15
Glu Pro Leu Ala Lys
20

<210> 20
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 20
Lys Gly Pro Gly Gly Pro Pro Pro Lys
1 5

<210> 21
<211> 6
<212> PRT
<213> Artificial Sequence
Description of Artificial Sequence: Synthetic peptide

Lys Lys Leu Gln Gly Lys
1

Georgia

Description of Artificial Sequence: Synthetic peptide

Gly Pro Gly Gly
1

Description of Artificial Sequence: Synthetic peptide

Leu Asp Thr Met Val Lys Glu Gln Lys Gly Lys Gly Pro Gly Gly Ala
1 5
Pro Pro Lys Asp Leu Met Tyr
10 15

Description of Artificial Sequence: Glycine-proline synthetic peptide

Gly Pro Gly Gly Pro Pro Pro
1 5

Description of Artificial Sequence: Synthetic peptide
<220>
<221> MOD_RES
<222> (4)
<223> Lys or Gln

<220>
<221> MOD_RES
<222> (14)
<223> Pro or Ala

<400> 25
Val Lys Glu Xaa Lys Leu Gln Gly Lys Gly Pro Gly Gly Xaa Pro Pro
1  5  10  15
Lys

<210> 26
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<220>
<221> MOD_RES
<222> (4)
<223> Lys or Gln

<220>
<221> MOD_RES
<222> (12)
<223> Pro or Ala

<400> 26
Val Lys Glu Xaa Lys Gly Lys Gly Pro Gly Gly Xaa Pro Pro Lys
1  5  10  15
THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A synthetic growth stimulating peptide, consisting of an amino acid sequence from position 78 to position 119 of SEQ ID NO: 3.

2. A synthetic growth stimulating peptide, consisting of an amino acid sequence from position 97 to position 117 of SEQ ID NO: 3.

3. A synthetic growth stimulating peptide, consisting of an amino acid sequence from position 97 to position 121 of SEQ ID NO: 3.

4. A synthetic growth stimulating peptide, consisting of an amino acid sequence from position 104 to position 117 of SEQ ID NO: 3.

5. An isolated bioactive peptide that is: KKLQGKGPGGPPK (SEQ ID NO: 11), LDALVKEKKLQGKGPGGPPK (SEQ ID NO: 12), or LDALVKEKKLQGKGPGGPPKGLMY (SEQ ID NO: 13).

6. A pharmaceutical composition for use in the stimulation of growth of epithelial cells in the gastrointestinal tract of mammals, said pharmaceutical composition comprising the peptide of any one of claims 1 to 5, and a carrier.

7. A pharmaceutical composition for use in the treatment of a gastro-intestinal disorder, said pharmaceutical composition comprising the peptide of any one of claims 1 to 5, and a carrier.

8. A pharmaceutical composition for use in the treatment of a gastro-intestinal disorder, said pharmaceutical composition comprising a carrier and a cell growth stimulating peptide consisting of an amino acid sequence that is KKLQGKGPGGPPK (SEQ ID NO: 11).
9. A pharmaceutical composition for use in the treatment of a gastro-intestinal disorder, said pharmaceutical composition comprising a carrier and a cell growth stimulating peptide consisting of an amino acid sequence that is LDALVKEKKLQGKPGGPPPK (SEQ ID NO: 12).

10. A pharmaceutical composition for use in the treatment of a gastro-intestinal disorder, said pharmaceutical composition comprising a carrier and a cell growth stimulating peptide consisting of an amino acid sequence that is LDALVKEKKLQGKPGGPPPKGLMY (SEQ ID NO: 13).

11. A pharmaceutical composition for use in the stimulation of growth of epithelial cells in the gastrointestinal tract of mammals, said pharmaceutical composition comprising a carrier and a cell growth stimulating peptide consisting of an amino acid sequence that is KKLQGKPGGPPPK (SEQ ID NO: 11).

12. A pharmaceutical composition for use in the stimulation of growth of epithelial cells in the gastrointestinal tract of mammals, said pharmaceutical composition comprising a carrier and a cell growth stimulating peptide consisting of an amino acid sequence that is LDALVKEKKLQGKPGGPPPK (SEQ ID NO: 12).

13. A pharmaceutical composition for use in the stimulation of growth of epithelial cells in the gastrointestinal tract of mammals, said pharmaceutical composition comprising a carrier and a cell growth stimulating peptide consisting of an amino acid sequence that is LDALVKEKKLQGKPGGPPPKGLMY (SEQ ID NO: 13).

14. Use of a peptide consisting of an amino acid sequence that is KKLQGKPGGPPPK (SEQ ID NO: 11), for the preparation of a medicament for the treatment of an injury of the gastrointestinal tract of mammals, wherein the migration of epithelial cells is to be stimulated.

15. Use of a peptide consisting of an amino acid sequence that is LDALVKEKKLQGKPGGPPPK (SEQ ID NO: 12), for the preparation of a medicament
for the treatment of an injury of the gastrointestinal tract of mammals, wherein the migration of epithelial cells is to be stimulated.

16. Use of a peptide consisting of an amino acid sequence that is LDALVKEKKLQGKPGGPPPKGLMY (SEQ ID NO: 13), for the preparation of a medicament for the treatment of an injury of the gastrointestinal tract of mammals, wherein the migration of epithelial cells is to be stimulated.

17. Use of a peptide consisting of an amino acid sequence that is KKLQGKGPGgPPPK (SEQ ID NO: 11), for the preparation of a medicament for the cytoprotection of injured epithelial cells in the gastrointestinal tract of mammals.

18. Use of a peptide consisting of an amino acid sequence that is LDALVKEKKLQGKPGGPPPKGLMY (SEQ ID NO: 12), for the preparation of a medicament for the cytoprotection of injured epithelial cells in the gastrointestinal tract of mammals.

19. Use of a peptide consisting of an amino acid sequence that is LDALVKEKKLQGKGPGGPPPKGLMY (SEQ ID NO: 13), for the preparation of a medicament for the cytoprotection of injured epithelial cells in the gastrointestinal tract of mammals.

20. The use of any one of claims 17 to 19, wherein the injured epithelial cells are an ulcer.

21. Use of a peptide consisting of an amino acid sequence that is KKLQGKGPGGPPPK (SEQ ID NO: 11), for the treatment of an injury of the gastrointestinal tract of mammals, wherein the migration of epithelial cells is to be stimulated.

22. Use of a peptide consisting of an amino acid sequence that is LDALVKEKKLQGKGPGGPPPK (SEQ ID NO: 12), for the treatment of an injury of the gastrointestinal tract of mammals.
gastrointestinal tract of mammals, wherein the migration of epithelial cells is to be stimulated.

23. Use of a peptide consisting of an amino acid sequence that is LDALVKEKKLQGKPGPGLMY (SEQ ID NO: 13), for the treatment of an injury of the gastrointestinal tract of mammals, wherein the migration of epithelial cells is to be stimulated.

24. Use of a peptide consisting of an amino acid sequence that is KKLQGKPGPGLMY (SEQ ID NO: 11), for the cytoprotection of injured epithelial cells in the gastrointestinal tract of mammals.

25. Use of a peptide consisting of an amino acid sequence that is LDALVKEKKLQGKPGPGLMY (SEQ ID NO: 12), for the cytoprotection of injured epithelial cells in the gastrointestinal tract of mammals.

26. Use of a peptide consisting of an amino acid sequence that is LDALVKEKKLQGKPGPGLMY (SEQ ID NO: 13), for the cytoprotection of injured epithelial cells in the gastrointestinal tract of mammals.

27. The use of any one of claims 24 to 26, wherein the injured epithelial cells are an ulcer.
FIG. 1

1 AGCTTTATTA CCATGTGATC CCATCTTTAG GCTTCAACAGC ATGACAGGA

51 GGAANATCTG GGCCAGCAGA TTTCCTAAAGG GAAATAATTAT AGATTTGTTAG

101 TTAATGAAAT ACACTTTTCC TCCCTTGGCRA ATAATTTTTA CTAGCTTTCAC

151 TGATAGGAAA AARAGCAGGAA AAAAAATTAA AACAACCTAC CTCCAACACCT

201 GTTTTGACCT TTTACTTGGC TGCCCAAGTTG ATAGTTTCTA CTCTCTGCTT

251 TTGATGAAAA TATTTTTTAT TATTTTTAATG TATCTTCTGA AAACATAAATT

301 ATCTAGGAGG AAATAAAGAG ATATTGCTTT TATAGTTCCC AGAGAGGAAA

351 AACAAACACT AGGAAGTTCC TATCTATCAG ATGSGGGGAGA TGTGATGAGG

401 GCACTGATAT TTGAGCCTGAG CCTTGAACAA TGACAGAGGG TCTTCCAACGC

451 GAGAGGCTAG CGGGTTGGCCTC TCAAGATAAA ACAACACGACT CTACACAGGSC

501 ATGGAGACAT ACAACTCTTG ACTCTTCCAG GAATCTTTGGG AACGCTGGTG

551 GAGCTTGAAT GTAGGATCAG AGCATAAGGT GCCAGACGGG AGAGCTTGGG

601 AAATCTTTAT ACCATAGGACC CGTGGATGCCA TTCCAATGAC TTTGRATTTT

651 CTGTAAGCCTG CCACGAAAT TTCCAAAGGCT GATAGACTCA TGCTCTCTCTA

701 TGCACTTCCG AAAGACCAACC TCAAGGTTAA TGGAGAAAAT GCATTTGGAAT

751 ATAGAAAGCT GTGGACCCAGA GTGATCAAGT GCTAGACTGT TGTGAAAGTC

801 CAGGTGAGGG GACGCTGGGG CAAGGGTCAA GGTGGAGAGC ATTTCAAGGA

851 TAAATAGACA GTATCTAGT AGATCTCAGG CTGAAGAGAA AGGCTCTGTC

901 CAGATATAGT GTGCTATCTG TAAGTGAGCT CAACATGGCA GAACAGGGGT

951 AGGTTTGGTG GGAGTTGGCTC ACAAAAACNTG TTTAGTCTAA GCAAACACAT

1001 TGCCATGGGC TCAGATAAAA GTTRAGAAGT GGAACCCATT CCTACATTCG

1051 TATAGGAGCT GCTATCTGGA AGGCCCTGTA TACAGGTCGC TTTCACGCTG

1101 TGATTTTGTT TGATTTTGAG GATTATTTCT TTTCGAAATC TGAGCAATGT

1/30
1151 TAGGCTGTAA AATACTCACA CCCACAGCTT TGACCTGGTG AAGAGTTATC
1201 ATAAAYGATA TGGAGTTTGT TGTGATAACCT TGAGCTTCAA CAAATGATGA
1251 GTCAAGTCAA CTCCATGTGA AAGTTTCTTG CTAGACAGTG AAGATAPCTG
1301 AAAGGTTCCT TGCTACACTG GCTCATGCGA CGATAGGAG AATATTGAGGA
1351 AGTIAAGTCT TTGACCCCACT GTGATAACAC CTGATTGAGAG TCAACCTGCT
1401 TAAAGTACAA TATGAGRAGG CTTCTCCTTC AGTTCGATGC TTGGCCATCTC
1451 CTCTGTCACG TGCTTCTCGTG AAGAAGAGAT GAAGTTCAAC GTGAGTAGAT
1501 TTTTCCTTTT GATTTAACCA CCATATAGTT GGAGACTTGC AAAATTCTGA
1551 GATTTTAGGAG GTTGCTCTCT TATGCCCCCA TCATTGGAAG TTGGTTTAAA
1601 AAAAATTTTC TCTTCAAACA CAGGGAACAC GAGAGGGGAA CAACCACACAC
1651 CAGGTCTCTGT TGGGGGGTGG AGASTGAGGG GAGGGAACTT AGAGGACAGG
1701 TCAAATGGGG CAGCAAAACCA CCAAGGCACA CATATACCTA TGTAAAAAAC
1751 CGCAGACGTC TCGACAGTGA TCCCTTTTTT TTAGAGGAG AAATAATGAA
1801 AAAAAACCTT TTTCTATATT ATATAATCAT GSCATTTATA AGCATCTCTA
1851 TTAGAGAGGA TAAATGATGC GTAATTAGAC ATCTGCTCGA GCACCTCACA
1901 CTGACCTATT TTTAAACAAA TGACATTTCCA CATCAGCTGA TTCCGGCTCC
1951 ATGCAGGGTA AGGAGTTCCG AAGGCCTAGA AGGTCGGAGT CATCCTCAT
2001 TCTGCAATTC CTCTCTTTAT TTACAAAAAT TCTGCGACAT GTTCAAGAAA
2051 GATGAAAGGG TTNTATACAA AATTAGTGGG TGAGATAGAC TTCTGTTCAC
2101 ATTTGTACAA GTAAAAATGGG TCTCAAAGAA AGAAGATTT GCTTTGGGCT
2151 CGACAGATGG CATTATATTA AGGAAGCAGT TGAAAACATGA AGCTACCACA
2201 GCACTCCTCC TGTGCTTCCA GACGGAAAGCC TGAGAGCTGA GAGGAAGGTT
2251 GACCGAGAAA CCCTGCCCAA GAAACTAGTA GTGCGGGTTT TCCATCAACA
2301 CGATGCAAGG GGCTGAAAT CAGATGACTA TTTCTATGGT TCAACATACT

FIG. 1 Cont.
ACACACTGGA AAACGTTACG GCAGACTCTA CTTTATATAT GGGCTGCAA
TGAAATAGA CTACTAGAAC TACGCCTCCTT TATAGCCGC RAGGTTTAAA
AGGTCAGGCG GAGACCTCAG ACACAGGTTA ATTTTGATT TTCTCTGATG
TCTGTGCTGA ACAAAGGGTA TAGYTTGCG CAATCTACTT AAACCCCTGAA
GCTCAGTTCG CTATCTGTTA AAATGATTGC ATTTGACTAG GTTTCTGCTA
AAATTCTTC TACCTCTGAC TTTTTAGGAG ACTARATTTT AACTCTATT
TGCAGTATTG GGAGAAAAAT TTARATTTTT TCCAAAGATT ACCAGTTACT
TCTAGAGCAG TTTCTCAAAAC TATTTTGTCC CAGCCTAGGG AAATTAGACT
AGTACCCAG AATGAGGCCG CCTGCAATAA GCTCTGTGCT CTGAAACCCA
ATGTGAGGGA CCTGTTGATA AAATAATTTG ACAAAGTATCC TGGGACACTG
CTAGTGACAT CGCCCTCTGT GATGACCTCTT GCCAGCGGAGC CACCTATAC
TGCAATTCGC ATCATTTGGCA TCCAACACTGC CTACTAATCC ATGCTTTGG
AAGTTTITTT TTAATAAAAA GATTTTTCT ATAGAGAGGA AAACATCCCA
TGTTAAATAG GAAATATNAC TGAAATCATT TGGGATGTTG ATTTTCTAGCA
CTTATAGCCA TTTCATACCA TAGTATGACT TAGTATAGTG CGTATATTG
TAAACTCTCT ATTTTTTC CC AAGGAAAATA AGATAAGTTG CTATTATTA
AAACATAGAC TTTCTATAT TGGGGACTTG ACCRAATTC AATCTGTTA
CAGATATGTT ACCTAGGGGG TCAAAATATG TGCCAGGTTGA ATTTTCTGAA
TTTCTCTAA AAGAGATTTT TAAACCTAT AAAACATTTA GAAACAAGTG
AGTAGAGGT GAGCATCAAAC AACCTGTTGA AGATAAGCCA CAGTACAART
TAAAGGCTAA TACCCCRAGCC ATGTCAGTTA TCCCCATCA TTTTGTTAA
TATAGAGGATACACATA TTTCAATAAA CTAAAAAGTG AATCTTTACT
CTATCTCTTT AATACCTGAA GAAGTATAAC TTTCTCTTTT TACTAGATT
AAATATCCA AATACTACTC CAAAGTAGGA TGCTATGATT AACTATAGCT

FIG. 1 Cont.
GAGTTATCC AARAAATGAAA AATCATGAAG ATTTATAAG CATTTTAAA

ATATTCATT ATAGCAGAAGC CTGGAAGGCT CTAAATAGGA AGGCCAGTTC

CTCCTCCTCC TATAAACACCT ATGGTTTATA TTACTATAATA TAATTCAAAC

AAACAGCAATT CTGACCACTTG ATAAAATTATA GGAAATTCTG TGGGCCAGTA

TATGTTTTAT TATAAAAGTA ATATTTTGAC CAACTCTTAAA AATTTTTAAA

CTCTATTTCTG AGATTCCAG AAGTATTATC TTAGCRAAGTC ATCTTTATGA

TACCACTTAT TAAACTGAAG AGAAACAAGA TGGTACATTC TGGGTTTAC

TTTAAAAGGG ATTTGATTCA ATRAATTGGA TTATCATAC TTGAAATTA

CATTTTCTTC CTCAGACTGG ATGGCAATGA GATGAAAGCA GCTTTCTGG

CTCTCAACTT CCGTCTCTCA TCAATTTTC AGGAGTTTCA TAAAGGCTAC

ACTRAAAATT CTAAAACATAT ATTACACATT AATAPAAATTA CTTATATT

ATCAGCAATT CTCACATATC GAAAAACCT TTATGGTTAA AAATGGAAG

GTAAGAGAAG AAAAARACAC ATTTAAGCTAG AACTGAACAC ATGGGTAARA

TTAGTGAATA CTTTTCATAAA GCTTGAGATAG AGGAAGAAAG AAGACACTAT

TTGGCCATGT AACAGGAGAC CAATGTATTT TGGATTTCA GATYGCTTTT

GCTGGAACCTC TTGGATCTTT TCTAGCTCCT GCCCTAGCTA ACTATGTAAG

TCTGACCTTT TCGAGTTTGC TACCARAATG CATTTGCAAG GAATGAGTAT

ATTAATACAC TCTCAATCTC TTAAAACTTT CAGAATATCA AGGTAATAGA

TGCAACACAC AATGCTGGAA GTGGGCAAGCA GTCAAGTGAAT GTCAACAAATG

AACACAATGT GGCCAATGGT GCAAATACAA AGGGATGGGA CTCCGGAAT

TCCATCTGGG ATATGGAGAA TTATGGTATG CAACGTGCCA TTTTCACTTT

ATGGTTTTAA ANYACGACTT CTTTTTACA AAAAAAGTGGC ATGTTAAACCA

TAAAGAAATT AAAAAATAAT TCTATTACA CTAAGCATAA AGTTAAAAAT

FIG. 1 Cont.
AAAGGTGACC ATTTTGGCTGA TCCGATTTTG TCCGCTAGAG ATAAPCTACTG
TTAATATGTT TGCCATGATC AGTTARAAATT CAANACCAACA AACACTATGT
TCAAGGGATT GTGGGTATAT ACAAAAAATA TGAACATCTC TTGCGCTTGC
CTSCAGATAC CTCATAAAAT GCCTGAAGAC TTATACAAACA TTACTGCTTC
CRAAGCTTAG ACTATCTCAG TTTGTCTTCT AAGGAGGGTT TACGACCTTC
TAAAGAGATT GAAATTGCAG TTTCACCTAA AACCTGGGCA ATGYTRAAATGA
CAATATATAT TGGYRTAGAGA GGAAGGAAAG AAGAAAGGAGGA GAGGGAAGA
AAAGAAAGRAG GAAGAGAGGA AAGAAAGGAA GAAGAAAGGA AGAAGAGAGA
AAAGAAAGRA AAAGAAAGGA AGAAGAGAGG AGAAGGAAAG GAAGAGAGAA
GGAGAGAAAG AGAGAGAAAG AGAAGAGAGG AGAAGAAAGGA AGAAGACCTT
AGCAGTGTTG GTGAGACCACCA ACTCTGGGAAT TATCAAGCTAT ATATPPACA
AACGTATATC TTTTAAATAG CAAACTCTTT ATGTTTTCAA TTTATCTGG
TCAATGGAA AAATATATTT TGCTTTATCT GTCTCCTTGA AATGTGAGGA
TCAAGAGGAA CTAAGACATG ATAGCTTTTA AAGGCTATTT CGTATAACA
GACTTATATA GAGGGTGTPT TATCATGCTG GAACCTGGAA ATAAAGCAA
CCAGTTAGAT GCTCAGTCTC TGCCCTCACA GAATYGCAGT CTGCCAGCAC
AAATGTCAAC AATAGATATG AATGCCAAAGC AGTGGCCCAT CCAGTGCTCT
TATCCAGGCT CATCAGGAGT CTTGGAGTCC CATTTCTCTC TGCCAGTGGA
ACTGACCTCT GATRAGAAAA GCTCCTGGGA GAACACATGC CTCACTATT
GCCATCTACT TTAACAGGGG TTGGCTGCCAA CCAGACTCTT TCAAATAAGAG
ACATGCAATTG TGCACAAAAAT GAACAGGAGA TGTACGCCCC ATCATTACATC
CCTTGATGCA CTGGTCAAGGG AAAAGAAAGGT AAAATAAAAA GCTTTTPTAT
TTTGATCGAG GGGAGGGTTT TTACATCCCT CAGTAAATAA CGAGAGACG
ACAGTCATTC CTCCTTGAGCT AAGTTATGTT GTAGTGTGCA GCGAAAGGG
5901 GGAAGTTATT GGTGATTCGC TGAGGGAAGG CACCTTCTGC CACATCAAAAT
5951 GCTGTTGGCTC ACCCTACCTT CTACACAAGCG TGAGCAGAAGCC ACTTGAAACC
6001 TTGACCTGTTA GAGGAGCAAGA GTCTGTTGCTA CACCAATAGG AGGCTCAGTA
6051 CTTTGCGCAA AGACATTTATC TGCAAGAGTT AGTTAGGGTT ATTAGATTTA
6101 GCAAATGAAA ATAGAAGATA TCCGTTTCTG TTTGAATTCT AGGTTAGCAG
6151 CAGGTCTTTT TAGTATAATA TATCCCTATGC AATATTAGGA ATATACTAAA
6201 AAAAGATCCA TGTCTTATCTG AATTCCAAAAT GGAAGCCTGGG ATGTATATT
6251 TGTCTGCGCC ATACTAATATC AGGGAGTGAG AAAGAAGAGA TCCATAATTG
6301 TTTAAAAATTT TGCTCTGAGT TCATATCTCT ATTACGTATA AATGGGATACC
6351 TTTCATGGAC AGGTTAGGAGA AATTATATAA ACTGCAATTCT CAGAAGATGA
6401 TTATACATA GTCTAATCCA AGGAATCTAT GAGGACCAAA TGAGGTCCAA
6451 GTGCGCAATT AATATAGGCC CTACTGCTCTG GTGTTATAGA GAAGCTGAGG
6501 TTTCAAAACCA GCTAATCCCC TTAGGACACT TAGAAATGCT AAGATATACA
6551 GAATAAGCTA GAAATGGGCTC TTCTTCATCT TGATTATAGA AAAATTAGC
6601 TGAGCACAAC TCACTGTTGG CCTCTGATAC CCCCRAAGTC AACAAAACAC
6651 TTGGCTTGGCC ATTCATTCTC TCCCCATAGTT CTTTTCTACG TTCTTCTCTCC
6701 ACACCTAGCT TCAGGTTAGG GGCACCAGAG GACCCCGCTTC CAGGCGCGTG
6751 ATGGACTCTAG TCAACCACAA CAAAATGCGT GACCTGAGCA AGTTGCGGAA
6801 AAGCATTTGA ACAATGCTGT GGGAGATTCC AACATACATG GCTGAGGAGA
6851 TCAGAGGCTGA GTAGCATCCC TACTGTGCAC CCCAGTAGG TGCTGGTTGGG
6901 ATGTCAGAGC TTACCTCGCG GGTGTTCCATA GTGGGCAACCA GTGATGCAGG
6951 GATGTCATCT AAGGCAAACA TTGGCTGAGT GTGGCTCTGG TGCAGGGGAC
7001 TGTTCTAGTG GCTTTAAGTG TGTTAATCTCG GTTTCTACA GCAATCTTAT
7051 AGTTCTCTAT TTATATCTAC TTTTGAGATG AGGAACCTGA GGTACAGAGA

FIG. 1 Cont.
FIG. 1 Cont.
CATCCTGGCC TACTCCCTCT TCGACTGCTT TCACGTAAGAC AAGATGAACT
TCAATTATG TTTGCTGGA CATTCTGGAG TCTTTTCTGCC TCCGGCCCTA
GCTAATATTT ATATTCRAGT CAATGCATGC AACACACATT CTGAGATGSG
GCAGCAGTCA GTGAGTTGCA ACAATGAACA CAATGTGGCC AATGGTGACA
ATACACACGG ATGGGACCTCC TGGAATTCCA TCTGGGATTTA TGGAATGSGC
TGGCTGCAA CAGACTCTT TCAAAGAAGAC ACATGCTTG TGGCAAAAT
GAACAAGGAA GCATGSCCCT CAATTCAATC CCTTGAAGCA CTCGTCAGGG
AAAAGAAGCT TCAGGTTAGA GGACCAGGAG GACCCACCTCC CAGGGGGCTG
ATGACTGAGTCAAACCCAAA CAAGCTGATG GACCTGAGCA AGTTCCGAAAA
AAACATAGCA AACATGCGTC GTGGGATTCC AACATACATG GCTGAGAGGA
TGCAAGAGCC AAGCCTTTTT TTTTATCTGAG GACGTGCTTA CAGGACAGT
GTACTATGGA TTGTGGACAT TTTCTCTGCT GGGACACGG TGGAGAAACTA
AACAAATTTT TAAGACTACT ATGGATTAG TCATCTGAAAT ATGCGTGCCR
GAAAAATAT ATGCCAGTCT ATGTGATTAC ACCATACATG GAAATTTTTC
TCTACAGTT ATGTTCGATT CTCTAAAGTT TCAAATAAT CATTAGACAT
TG
FIG. 3
1 GAATTCAAC AGCAGGCCAT CTTTCACCAG CACTATCCGA ATCTAGCCAT
51 ACCAGCATC TAGAAGAGAT GCAGGCCAGT AGCTAAGCAT CAGACCCCTG
101 CAGGCCCTGA AGCTCCAGAC CATGGGAGAG AGGAGAGTTG TGGTTCCAAG
151 GAGCCTTTCA GAGTGGAAAAT CTGTGATTCG TGGATTATA AAACCGAGTT
201 TCCGCCCTTA TTGATTTGCA ACCAAGAGCT TCAGTTGAG AAGGAAACAG
251 GTTTAAAAAT AATAAGTGTC ATGCAATTTCT CCTCAAACAC TATTAAAAC
301 GTTTACTTAT TTTGCCAAGT GACGCTCCTG GCTTCCTCTC CTGGAGAAG
351 TCTTCCCTTA TTTTATATA ATATTTGAAA GTTTTACTTA TTGACCAG
401 TGTTTCTCAT CCTGTTGCCC AAGACGCCCT TGGGCGGGTT GAACGACCCT
451 TTCAACAGGG CTCAGTTATCA GATACTTCTG ATCTTACGTA TTTACATTAT
501 GATCCATAC AGTAGGAAAA TTATTTAGGA AGTAGGACCA AATAAAGATT
551 ATGATTGTGG TCAACACTAT GTTAGAGGGT CGGACAGATT CAGAGGGTGG
601 AGAACGTGTG TTCTAGAGGG AAATAAGAAG ACAGAGTTCG TTGAAGGGGC
651 CCAGAGCCAG TGAAAGAAAGT TTCCACGTAG AAAGTGGAAGA AGGCTCTGAG
701 TCCGAAGCAG TGAGAACCTT AAAAAAGAAA AAACAAAAAC ATPSCCAACT
751 AACAGTCCAG GAGAGAGACG GGSCATGAAA GGGTGAGTTC CCATGGGATG
801 CCTTGAATGG AACAGAAGGTG TGGGAAAAAT TGTTGTGCTG CGAGGGAGT
851 GCGGCGCATC TCGAGAGCCT GTAGCTGGGG AAGACGGGAA CCCTTTAGG
901 ATCCCAAGAT GCCATTCCAA TGAGCTTGAG ATTTTCTGCA TGGACTGCACA
951 GTGAATGTCT ATACGCTCCG GAAATTAATG TTTACTATT TTCCATATTCT
1001 TAGGGGAGAAA CCCTGGGAAA AATGGGAGGAC ATCCATGAAA ATATCGAGT
1051 CCTGGGATAA GGCAGGCTTG GTCCTACACG TCTGTAACAA GTCCATCACG
1101 AAGTGCCCTTG ACCAAGGCTG GAAGTGGAAG CGTGTGGAGA GATGTAAGGG

FIG. 4
CAAGGTTAG TGCTAGATA TGTAAGTGGC AAGAPGGTGTC TGCCAAGACG
CCCAGAGCT CTAAACCCACT GAGAAAACCA GGAATGAAGT ATGGGAGATG
GCTTTGGTGC CAGCTGCTAG TGACATGGCT GGAAGCGTCG ACTGGCTGCG
AGGCCAGACA ATTCCTCAAG GAAACATCTG GCCAGGGTGC AAGGCCAGT
TTCTTCTCTT GGAGTTCCTT TCACAGCTAA GACATCATC CCCAACCAC
TGTTTTGGTT AAAAAGTTT TCAATAGACT TGAGCATGGT CAAGAAACAG
AGAGAGGGGG AAATRAGGGT GGAAGAGGCT GGAGAAAGCT TACAAAGAG
CTGGGTAAGAG GGAGGGAGRA GAAACCAATTG CGGATTCAC ATAGGAGCCA
GTACCAGGAA GGGCAGGGTG ATCACAAGAT TCTATCTAAG GGCATGGTTG
GTTTAGGGAAT TACTCTCTCC CGGAATCTGA GCAGCAGCAA TAGTTAAAT
ACCCACRCCC ATGGCTTCCA TATTCGAGAA CTTATCACAAR AGCCTGTGAG
GTITAAGTGAG ATACCTTCGT CAGAGGATGA GTCAGAGGCC TCTGCGCTAA
GGGCCCTACT GAGCAGGCGC CTAARAGGCTT CGGGCCCTCT GCAAGCTCCAC
AGATACAGGA GAGGGAGAGCA GATAAGCCGT GGCATCCACC TGAGCAGCRC
TAGCTTAGGC AAAGCTGCTC AGGTACAARAT AGCAGAGGGC TGAAAGGCTG
TGAGCAGGCC GCTGATGCTC TGCTCCTCACC AACTCCGTCG CCGCATGAAG
CTCAGATGAA GTCAGATCTT CTTTTPCAATG CGAGCCTGATA CAACANTTAT
AGTCAGGGGT GAGGGGGCTG GACCTCTGAC CATAGTTGGA
ATATTCCTCT TCTCTTTCTG GGAATCATGG TGGTTACAG CATGCGCTATA
GAGAAAGAGA ATGCCCCCGG AAGAGGCGCTG CACAGGGCTGA ATACCTGTGA
ATTGCTCTTC ACAGCAACTG TGCCAGGGTT CTACTTAAAG GAGGAGGCTG
CTGGGCTCGCA GAAAGACTGT TTCTTAGGCT TGATCTCCTT CTTATTTCTG
ATTTCTCTCT TTCTTACCGAT GATTTCCACT TTACCAGTT CTGGGCAATG

FIG. 4 Cont.
2301 TCCGTCAGA GTGGAAGATC ACTGCTGTCG AAGACTAGCT TCAACACTCT
2351 TGCGCTTTAA CACGAGAAC ACAGCTCTTC GGACCCTCTG AAGCAATTTCT
2401 TGGAGAAAGT CTCTGGGGAT GAAGCTATCT CAGTTGCCGC ACTGAAAGTCC
2451 TAGGATACAG AGGCTCAAAC AGAGTGACAC TATCTATTTT TGGCATAACTC
2501 TATTTGGCGCT GCTTATGGAAT TCATATGAAT TTATGGGAAT TGGAAATGAA
2551 ACTATGACCA AGAGACGCTCC ACCTCAGAAG CAGTTGGGAG GGAGACCTCA
2601 AGCCACAGGCG AGAGGGCTGC GTTCTCTTTC TAGTCTCTGC TAGGAGRGTG
2651 GTTCTGAGCC TTCTCTATGC TCGAGCCCTT TRATACAGTT CCTCAAGGTTG
2701 TGCTGACCTC CAGGCAATAA ATACCTTTCA TTGCTACTGC ATAACTGTAA
2751 TTTGCTACC ATATTAGAGTT GTAATGAAAAT TATCTGATAT GCAAGATACC
2801 AGATAACCTA AGAAACGGTT GTTTGAACCTT TAAAGGGGTC ACAACCCCA
2851 GGTTGAGAAC TACTGCTCTA GGGTCTTTTA CAGTCCTTTA GCTGCCCCAT
2901 TTAGAGAGAG TACATCTAGC CTCAAAAACC CCCCACCAT TTGGCCTTTT
2951 GGTTGTTTTT GTTTGTGTCTT TCAAGCACAG GTTTTCTCTG GTAGGCTCAG
3001 CTGTCCTGGA ACTCAGCTTT TTAGACACGG CTGGCCTCGA ACTCAGAAAAT
3051 CGGCGTGGCTT CTGCTCCTGG AGCGCTGGGA TTAAGGGCGT GGCCACACCT
3101 GCTGCGTCGA CATCCGGCTT TTTRAGAGAC CGATTTAAAC TTCTGGCATT
3151 GAAATAAAT AACTAGTAAAA TGCTTTACCT ACTAGACAAA TAAAAAACAG
3201 ATTCCTTCTG CTAGAAAGGA CAGCTCCAGC ACTAAGAGAA AAAACCTTTT
3251 CAGGGCTTTG ATTACACTGT GCCATGCACT ATTTTTATGT TTTCTTCAGC
3301 AGTTTTCAGT GTGCTGAAATT CAGTGTCAAAA ATTCCTAGAC TACATAAGAA

FIG. 4 Cont.
TATGATTACA GTAACCTCAGC AATTCCTATGT TACCAATGAA TTTTCTCTGA

GTCTTTAAAAAA AAGGTGGAAG AGAAAGGACG AGATAGTTTA GCACATGGGT

AAGAACAGTA AACTATTCTTG AGAGACCTGGA TGAAGATGCT GTAAACCATG

CGACCACCAG TCCCTGTCCTC TGTCCTTTCA GATGGTCGTC GTGGGTCTGC

TGCGCTCCCT TGCAGCTCCT GATTCTGATT AGCTAAGTCT CATTTTTCTG

AAGTCTAATG TCAAACACTGC ATTACACTGTG AAAATGGATGC TTAGTGCCAC

CTCTGGTCTCT TAGAAGACATT AGACCCTGCAA CTCGATGTGG AATGATGGCR

AGTTAGAACAG AAGTGGAGACG CATCAGGTGA GCATCAATGG TGTCACACAC

GTGCCCATAA TGAGACACAA TAAGGGCTGG GACTCTGGGA ATAGGCTCTG

GGACTTATGA AAGCTATGCT AAGGACACAC AGGCTAAGAA TATGGTGTAAG

CCACCAACCA TTAAAATTTG TGGAGTTGAT TCTAGCCTTT CATGACACATT

AAGAGCTGCC AGTAAAAGTG CCCATCCCAAC TCAAAACAAT TTTACTTTTT

TGCAATAATAT TAGTTGTAAT AAATCTTTCA CAAATGCTGG AAATTCAAGAG

GGATCAATAG TAAGGATAAA AACATATGTAC AAAGACAAAC ACAGCAATCCT

TTGGTCTTCC CTGGCGAGAG TCTCCATGAT GTTAAGAGCT CAAAGTTTTA

TGGAGGCTGA ATGAAATACG AATGCTCCTG TGAAGAAAAG GCCCAACAGAT

CTTATGGAGA ATGAGTGAAG TATGATGCT ATTAGTTGTA AGAGARGGGG

ATGCAAAGCA ACACTTGCGCA CCACTTGCCA ATTACTACTT TCTATTTAA

ATGTAGTTTA AAAAAAGAAG CCTGCTTCC CTGCTCTCTG GAAACAGCTGC

GGATGGAGGT AGACCAAGGT ATGAAAGCTG TTAAAGTTAT GTCAGCAAAA

FIG. 4 Cont.

13/30
FIG. 4 Cont.
FIG. 4 Cont.
FIG. 4 Cont.
FIG. 5
MKLTIFVYGL LGLLAAPGFA YTUNINGHG WYDGSQGQSY SINGVHIVAH

IDININGWspin MSLMIEYSF AAATLSKKS CIVHRMOKA NPSLOQLOTM

VKSOGKGIPI GAPPKOLHYS VNPTRVEDLN TFGPKIAGMC RGITTYVABE

IPQPHQPLYS KKCYTADILM ILRHSFOCTS VETY

FIG. 6

18/30
FIG. 7
Human 1 MKFTIVFAGLLGVFLAPALANYNVNDDNNNAGSGQVSVPNNEHNVAN 50
Pig 1 MKFTIAFAQGLGVLTPALADYSISVNDGNGSSGQVSVPNNEHNVAN 50
51 VDNNGSWNSIWDYGHGFATRFQKTCIVHKMKKEVMPSLQDAL 100
51 VDNNGWSWNASWRTGFATVRFRKKSCIVHMKKEAMPSLQDAL 100
101 VKEKKLGSGPPPKGLMSVNPKNVDDLSKFGKSHVIANMCRGIPTMA 150
101 VKEKKLGSGPPPKLSRLYSVNPNRVDNLDFKFGSKIVAMCGIPTMA 150
151 EEMQESLFFYSGTCYTTSVLWIVDISFCGTVEN 185
151 EEIQGANLISYSEKCSANILWLNISFCGIAEN 185

FIG. 9
<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Pig</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MKPTIVP.AG LLGVPLAPAL ANYHINVN.D DNNNAGSGQQ SVSVNNEHNV</td>
<td>MKPTIAP.AG LLGVFLTPAL ADYSISV.N.D DGNSSGGSGQQ SVSVNNEHNV</td>
<td>MKLT.M.FVG LLGLLAAPGF A.YTVNINGN DGNVDGSGQQ SVSINGVHRN</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>ANVDNNNGWD SWNSIWDDYN GFAATRLFQK KTCIVHMKMN EVMPSIQLD</td>
<td>ANVDNNNGWN SWNALWDDYRT GFAVTRLFEK KSCIVHKMMK EAMPSLQALD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>ALVKEKKLQG KGPGGPMPKG LMYSVNPNKV DDLSKPGKNI ANMCRGIPTY</td>
<td>ALVKEKKLQG KGPGGPMPKS LRYSVNPNRV DNLDKPGKSI VAMCKGIPTY</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>MAEMQEASL FFYSGCYVT SVALWVDISF CGDTVEN</td>
<td>MAEBIQGANL ISYSEKCISA NILWILNISF CCGIAEWF</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>188</td>
</tr>
<tr>
<td></td>
<td>VAEHIGPNQ PLYSKKICYTA DILWILRMSF CGTSVETY</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FIG. 10**
FIG. 111
A

mrgshhhhhhs  21 NYNINVHDDNNNAGSQQSVSVNNEHNVAN

51 VDNNGDWDSWNSIWDYNGFAATRLFQKKTCTIVMKMNKEVMPSIQSLDAL

101 VKEKKLGKGPQPPPKLMSVNPVDKVSLLSKFQKNIANMCRGIPTYMA

151 EEMQEASLFFYGSCTYTTSLWIVDISFCGDTVEN

B

FIG. 13
A

**HUMAN 1**

MKFTIVFAGLLGVFLAPALANYNIMWNDNNAGSGQQSVSNVNEHNVAN 50

**PIG 1**

MKFTIAFAGLLGVFLTPALADYSISVNDGNSGSQSGGQSVSNVNEHNVAN 50

51 VDNNGWDSWSNIFDGYNGPFAATRLFQKKTCTICVHKMNKEVMP81QSLDAL 100

51 VDNNGWNSWNLWYRTGFVALRLEKKSCIIVHMKKRAMPPLQLAL 100

101 VKEKLLQGKPGPPPKLMSVNPKEHLSKFKCNIAHCRGIPTYMA 150

101 VKEKLLQGKPGPPPKLRSYVNPVRDNLDKFGISGVAMCIGIPTYMA 150

151 BEMQESASLFFYSGTCTYDTADLVFDIMSCDVTEN* 186

**=termination**

151 BEIQGANLISYBERKCIANSILWILNISFCGGIAEN* 186

B

**Predicted Structure for the preAMP-18 ORF**

**Human**

![Diagram of protein structure](image)

**FIG. 14**
FIG. 16

28/39
FIG. 17

IEC-18

Cells x 10^6, % control

Peptide 58-99
Peptide 67-85

Peptide 57-85 (μg/ml)

0 0.01 0.025 0.05 0.1 0.25 0.5 1 2
FIG. 19

![Graph showing migration distance in HAE and IEC-18 cells over time.](image)
FIG. 24

[Graph showing % change over hours of exposure to AMP peptide for Occludin and ZO-1]
FIG. 26

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>8hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZO-1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Occludin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>rhAMP-18</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
FIG. 27

DAYS AFTER DSS STOPPED

% CHANGE IN WEIGHT

VEHICLE

PEPTIDE

38/38

HEMOCULT POSITIVE STOOL %

VEHICLE

PEPTIDE

DAYS OF DSS ADMINISTRATION
A

HUMAN 1 MKFTIVFAAQLGFLAPALANYINNVDDNMMAGSGQGSVSVPN5NHEHNVAN 50
51 VDNNGWDSNWSIWDYNGFAPATRLFQKKTIVHKMNHEVMP8ISLQAL 100
51 VDNNGWNSWVNLWDYRTGFAVTRFLPEKSCIVHKMKKBAMPSLQAL 100
101 VKEKLGQKGPAGGPPPKGLMYSVNPKVDDLMSKFGKNIANMCRGIPTYMA 150
101 VKEKLGQKGPAGGPPPKSLRYSVPNPNRVDNLDFKFSIVAMCKGIPTYMA 150
151 EBIQGANLISYSEKCISANILWILNISFCGGAEN* 186

B

Predicted Structure for the preAMP-18 ORF

Human

H2N- signal peptide N-rich W-rich C amphipathic helix

GP turn

helix C amphipathic helix

C hydrophobic C -COOH