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(54) Benævnelse: **ALFA-CONNEXIN-C-TERMINALE (ACT)-PEPTIDER TIL BEHANDLING AF ALDERSRELATERET MAKULADEGENERATION**

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Fortsættes ...

DESCRIPTION

BACKGROUND

[0001] Macular degeneration is a medical condition predominantly found in elderly adults in which the center of the inner lining of the eye, known as the macula area of the retina, suffers thinning, atrophy, and in some cases bleeding. This can result in loss of central vision, which entails inability to see fine details, to read, or to recognize faces. According to the American Academy of Ophthalmology, it is the leading cause of central vision loss (blindness) and in the United States for those over the age of fifty years. Although some macular dystrophies that affect younger individuals are sometimes referred to as macular degeneration, the term generally refers to age-related macular degeneration (AMD).

[0002] Advanced AMD, which is responsible for profound vision loss, has two forms: dry and wet. Central geographic atrophy, the dry form of advanced AMD, results from atrophy to the retinal pigment epithelial layer below the retina, which causes vision loss through loss of photoreceptors (rods and cones) in the central part of the eye. While no treatment is currently available for this condition, vitamin supplements with high doses of antioxidants, Lutein and Zeaxanthin, have been demonstrated by the National Eye Institute and others to slow the progression of dry macular degeneration and in some patients, improve visual acuity.

[0003] Neovascular or exudative AMD, the wet form of advanced AMD, causes vision loss due to abnormal blood vessel growth in the choriocapillaries, through Bruch's membrane, ultimately leading to blood and protein leakage below the macula. Bleeding, leaking, and scarring from these blood vessels eventually cause irreversible damage to the photoreceptors and rapid vision loss if left untreated.

[0004] Until recently, no effective treatments were known for wet macular degeneration. However, anti-VEGF (anti-Vascular Endothelial Growth Factor) agents, when injected directly into the vitreous humor of the eye using a small, painless needle, can cause contraction of the abnormal blood vessels and improvement of vision. The injections frequently have to be repeated on a monthly or bi-monthly basis. Examples of these agents include Lucentis, Avastin and Macugen. Only Lucentis and Macugen are FDA approved as of April 2007, and only Lucentis and Avastin appear to be able to improve vision, but the improvements are slight and do not restore full vision. Thus, needed in the art are compositions and methods treat or prevent macular degeneration.

BRIEF SUMMARY

[0005] In accordance with the purpose of this invention, as embodied and broadly described herein, this invention relates to compositions for use in methods of treating or preventing macular degeneration. The invention is as defined in the claims.

[0006] Additional advantages of the disclosed method and compositions will be set forth in part in the description which follows, and in part will be understood from the description, or may be learned by practice of the disclosed method and compositions. The advantages of the disclosed method and compositions will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the disclosed method and compositions and together with the description, serve to explain the principles of the disclosed method and compositions.

Figure 1 shows the alpha connexin carboxy-terminal (ACT) polypeptide ACT1 prevents VEGF-induced deterioration of TER in ARPE-19 cells. Trans-epithelial resistance (TER) measurements, using ARPE19 cell (immortalized human RPE cells) monolayers revealed that VEGF leads to rapid deterioration, which was blocked by pretreating the cells with the ACT peptide.

Figure 2 shows by Western blot a concentration series of 25-0.04 ng/µL of pure ACT1 peptide. ACT1 was detectable in the eye anterior chamber fluid at levels in excess of the highest concentration of pure peptide. No peptide was observed in the anterior chamber fluid control eyes receiving the vehicle control 0.05 % Brij-78 solution.

[0008] Disclosed are compositions and methods for treating or preventing macular degeneration in a subject, comprising administering to the subject a polypeptide comprising a carboxy-terminal amino acid sequence of an alpha Connexin (also referred to herein as an alpha Connexin carboxy-Terminal (ACT) polypeptide), or a conservative variant thereof. The invention is as defined in the claims.

[0009] The disclosed method and compositions maybe understood more readily by reference to the following detailed description of particular embodiments and the Example included therein and to the Figures and their previous and following description.

A. Compositions

[0010] Disclosed for use in the disclosed method is an isolated polypeptide comprising a carboxy-terminal amino acid sequence of an alpha Connexin (also referred to herein as an alpha Connexin carboxy-Terminal (ACT) polypeptide), or a conservative variant thereof. The ACT polypeptides of the provided method are disclosed in International Patent Publication WO/2006/069181.

[0011] A variety of sequences are provided herein and these and others can be found in Genbank at www.pubmed.gov. Those of skill in the art understand how to resolve sequence discrepancies and differences and to adjust the compositions and methods relating to a particular sequence to other related sequences. Primers and/or probes can be designed for any sequence given the information disclosed herein and known in the art.

[0012] Connexins are the sub-unit protein of the gap junction channel which is responsible for intercellular communication (Goodenough and Paul, 2003). Based on patterns of conservation of nucleotide sequence, the genes encoding Connexion proteins are divided into two families termed the alpha and beta Connexion genes. The carboxy-terminal-most amino acid sequences of alpha Connexins are characterized by multiple distinctive and conserved features (see Table 2). This conservation of organization is consistent with the ability of ACT peptides to form distinctive 3D structures, interact with multiple partnering proteins, mediate interactions with lipids and membranes, interact with nucleic acids including DNA, transit and/or block membrane channels and provide consensus motifs for proteolytic cleavage, protein cross-linking, ADP-ribosylation, glycosylation and phosphorylation. Thus, the provided polypeptide interacts with a domain of a protein that normally mediates the binding of said protein to the carboxy-terminus of an alpha Connexin. For example, nephroblastoma overexpressed protein (NOV) interacts with a Cx43 c-terminal domain (Fu et al., J Biol Chem. 2004 279(35):36943-50). It is considered that this and other proteins interact with the carboxy-terminus of alpha Connexins and further interact with other proteins forming a macromolecular complex. Thus, the provided polypeptide can inhibit the operation of a molecular machine, such as, for example, one involved in regulating the aggregation of Cx43 gap junction channels.

[0013] As used herein, "inhibit," "inhibiting," and "inhibition" mean to decrease an activity, response, condition, disease, or other biological parameter. This can include, but is not limited to, the complete loss of activity, response, condition, or disease. This can also include, for example, a 10% reduction in the activity, response, condition, or disease as compared to the native or control level. Thus, the reduction can be a 10, 20, 30, 40, 50, 60,70, 80, 90, 100%, or any amount of reduction in between as compared to native or control levels.

[0014] Amino acid sequences for alpha connexins are known in the art and include those identified in Table 1 by accession number.

Table 1: Alpha Connexins

Protein	Accession No.	Protein	Accession No.
mouse Connexin 47	NP_536702	Phodopus Connexin 43	AAR33085
human Connexin 47	AAH89439	Rat Connexin 43	AAH81842
Human Connexin46.6	AAB94511	Sus Connexin 43	AAR33087
Cow Connexin 46.6	XP_582393	Mesocricetus Connexin 43	AAO61857
Mouse Connexin 30.2	NP_848711	Mouse Connexin 43	AAH55375
Rat Connexin 30.2	XP_343966	Cavia Connexin 43	AAU06305
Human Connexin 31.9	AAM18801	Cow Connexin 43	NP_776493
Dog Connexin 31.9	XP_548134	Erinaceus Connexin 43	AAR33083

Protein	Accession No.	Protein	Accession No.
Sheep Connexin 44	AAD56220	Chick Connexin 43	AAA53027
Cow Connexin 44	146053	Xenopus Connexion 43	NP_988856
Rat Connexin 33	P28233	Oryctolagus Connexin 43	AAS89649
Mouse Connexin 33	AAR28037	Cyprinus Connexin 43	AAG17938
Human Connexin 36	Q9UKL4	Zebrafish Connexin 43	CAH69066
mouse Connexin 36	NP_034420	Danio aequipinnatus Connexin 43	AAC19098
rat Connexin 36	NP_062154	Zebrafish Connexin 43.4	NP_571144
dog Connexing 36	XP_544602	Zebrafish Connexin 44.2	AAH45279
chick Connexin 36	NP_989913	Zebrafish Connexin 44.1	NP_571884
zebrafish Connexin 36	NP_919401	human Connexin45	138430
morone Connexin 35	AAC31884	chimp Connexin45	XP_511557
morone Connexin 35	AAC31885	dog Connexin 45	XP_548059
Cynops Connexin 35	BAC22077	mouse Connexin 45	AAH71230
Tetraodon Connexin 36	CAG06428	cow Connexin 45	XP_588395
human Connexin 37	155593	rat Connexin 45	AAN17802
chimp Connexin 37	XP_524658	chick Connexin45	NP_990834
dog Connexin 37	XP_539602	Tetraodon Connexin 45	CAF93782
Cricetulus Connexin 37	AAR98615	chick Connexin 45.6	I50219
Mouse Connexin 37	AAH56613	human Connexin 46	NP_068773
Mesocricetus Connexin37	AAS83433	chimp Connexin 46	XP_522616
Rat Connexin37	AAH86576	mouse Connexin 46	NP_058671
mouse Connexin 39	NP_694726	dog Connexion 46	XP_543178
rat Connexin 39	AAN17801	rat Connexin 46	NP_077352
human Connexin 40.1	NP_699199	Mesocricetus Connexin 46	AAS83437
Xenopus Connexin38	AAH73347	Cricetulus Connexin 46	AAS77618
Zebrafish Connexin 39.9	NP_997991	Chick Connexin 56	A45338
Human Connexin 40	NP_859054	Zebrafish Connexin 39.9	NP_997991
Chimp Connexin 40	XP_513754	cow Connexin 49	XP_602360
dog Connexin 40	XP_540273	human Connexin 50	P48165
cow Connexin 40	XP_587676	chimp Connexin 50	XP_524857
mouse Connexin 40	AAH53054	rat Connexin 50	NP_703195
rat Connexin 40	AAH70935	mouse Connexin 50	AAG59880
Cricetulus Connexin 40	AAP37454	dog Connexin 50	XP_540274
Chick Connexin 40	NP_990835	sheep Connexin 49	AAV01367
human Connexin 43	P17302	Mesocricetus Connexin 50	AAS83438
Cercopithecus Connexin 43	AAR33082	Cricetulus Connexin 50	AAR98618
Oryctolagus Connexin 43	AAR33084	Chick Connexion 50	BAA05381
Spermophilus Connexin 43	AAR33086	human Connexin 59	AAG09406
Cricetulus Connexin 43	AAO61858		

[0015] The 20-30 carboxy-terminal-most amino acid sequence of alpha Connexins are characterized by a distinctive and conserved organization. This distinctive and conserved organization would include a type II PDZ binding motif (Φ-x-Φ; wherein x = any amino acid and Φ = a Hydrophobic amino acid; e.g., Table 2, **BOLD**) and proximal to this motif, Proline (P) and/or Glycine (G) hinge residues; a high frequency phospho-Serine (S) and/or phospho-Threonine (T) residues; and a high frequency of positively

charged Arginine (R), Lysine (K) and negatively charged Aspartic acid (D) or Glutamic acid (E) amino acids. For many alpha Connexins, the P and G residues occur in clustered motifs (e.g., Table 2, *italicized*) proximal to the carboxy-terminal type II PDZ binding motif. The S and T phosphor-amino acids of most alpha Connexins also are typically organized in clustered, repeat-like motifs (e.g., Table 2, underlined). This organization is particularly the case for Cx43, where 90% of 20 carboxyl terminal-most amino acids are comprised of the latter seven amino acids. In a further example of the high conservation of the sequence, ACT peptide organization of Cx43 is highly conserved from humans to fish (e.g., compare Cx43 ACT sequences for humans and zebrafish in Table 2). In another example, the ACT peptide organization of Cx45 is highly conserved from humans to birds (e.g., compare Cx45 ACT sequences for humans and chick in Table 2).). In another example, the ACT peptide organization of Cx36 is highly conserved from primates to fish (e.g., compare Cx36 ACT sequences for chimp and zebrafish in Table 2).

Table 2. Alpha Connexin Carboxy-Terminal (ACT) Amino Acid Sequences

Gene	**Sequence**	**SEQ ID NO**
Human alpha Cx43	P SSRA SSRA SSR *PRP* D **DLEI**	(SEQ ID NO:1)
Chick alpha Cx43	P S RA SSRA SSR *PRP* D **DLEI**	(SEQ ID NO:29)
Zebrafish alpha Cx43	P CSRA SSRM SSRA R P D **DLDV**	(SEQ ID NO:89)
Human alpha Cx45	G SNKS TA SSKS *GDG* KN **SVWI**	(SEQ ID NO:30)
Chick alpha Cx45	G SNKSS A SSKS *GDGKN* **SVWI**	(SEQ ID NO:31)
Human alpha Cx46	**G** RA SKAS RASS ***GRARP*** E **DLAI**	SEQ ID NO:32)
Human alpha Cx46.6	**G** SASS RD *G* K **TVWI**	(SEQ ID NO:33)
Chimp alpha Cx36	P RVSV *PNFG* R TQ SSD **SAYV**	(SEQ ID NO:34)
Chick alpha Cx36	P RMSM *PNFG* R TQ SSD **S AYV**	(SEQ ID NO:35)
Zebrafish alpha Cx36	P RMSM *PNFG* R TQ SSD **S AYV**	(SEQ ID NO:90)
Human alpha Cx47	P RAGSEK G SASS R DG KT **TVWI**	(SEQ ID NO:36)
Human alpha Cx40	G HRL ***PHG*** YHSDKRRL SKASS KARSD **DLSV**	(SEQ ID NO:37)
Human alpha Cx50	P ELTTDDAR P LSRL SKASS RARSD **DLTV**	(SEQ ID NO:38)
Human alpha Cx59	P NHW SLTN NLI *GRRVP* T **DLQI**	(SEQ ID NO:39)
Rat alpha Cx33	P S CV SSS A VLTTIC SS DQVV *PVG* L **SS FYM**	(SEQ ID NO:40)
Sheep alpha Cx44	G R SSKA SKSS *GG* RARAA **DLAI**	(SEQ ID NO:41)
Human beta Cx26	LC YLLIR YCSGK SKKPV	(SEQ ID NO:42)

[0016] Thus, in some aspects, the provided polypeptide comprises one, two, three or all of the amino acid motifs selected from the group consisting of 1) a type II PDZ binding motif, 2) Proline (P) and/or Glycine (G) hinge residues; 3) clusters of phospho-Serine (S) and/or phospho-Threonine (T) residues; and 4) a high frequency of positively charged Arginine (R) and Lysine (K) and negatively charged Aspartic acid (D) and/or Glutamic acid (E) amino acids). In some aspects, the provided polypeptide comprises a type II PDZ binding motif at the carboxy-terminus, Proline (P) and/or Glycine (G) hinge residues proximal to the PDZ binding motif, and positively charged residues (K, R, D, E) proximal to the hinge residues.

[0017] PDZ domains were originally identified as conserved sequence elements within the postsynaptic density protein PSD95/SAP90, the *Drosophila* tumor suppressor dlg-A, and the tight junction protein ZO-1. Although originally referred to as GLGF or DHR motifs, they are now known by an acronym representing these first three PDZ-containing proteins (PSD95/DLG/ZO-1). These 80-90 amino acid sequences have now been identified in well over 75 proteins and are characteristically expressed in multiple copies within a single protein. Thus, in some aspects, the provided polypeptide can inhibit the binding of an alpha Connexin to a protein comprising a PDZ domain. The PDZ domain is a specific type of protein-interaction module that has a structurally well-defined interaction 'pocket' that can be filled by a PDZ-binding motif, referred to herein as a "PDZ motif". PDZ motifs are consensus sequences that are normally, but not always, located at the extreme intracellular carboxyl terminus. Four types of PDZ motifs have been classified: type I (S/T-x-Φ), type II (Φ-x-Φ), type III (Ψ-x-Φ) and type IV (D-x-V), where x is any amino acid, Φ is a hydrophobic residue (V, I, L, A, G,W, C, M, F) and Ψ is a basic, hydrophilic residue (H, R, K). (Songyang, Z., et al. 1997. Science 275, 73-77). Thus, in some aspects, the provided polypeptide comprises a type II PDZ binding motif.

[0018] It is noted that the 18 carboxy-terminal-most amino acid sequence of alpha Cx37 represents an exceptional variation on

the ACT peptide theme. The Cx37 ACT-like sequence is GQKPPSRPSSSASKKQ*YV (SEQ ID NO: 43). Thus the carboxy terminal 4 amino acids of Cx37 conform only in part to a type II PDZ binding domain. Instead of a classical type II PDZ binding domain, Cx37 has a neutral Q* at position 2 where a hydrophobic amino acid would be expected. As such Cx37 comprises what might be termed a type II PDZ binding domain -like sequence. Nonetheless, Cx37 strictly maintains all other aspects of ACT peptide organization including clustered serine residues, frequent R and K residues and a P-rich sequence proximal to the PDZ binding domain-like sequence. Given this overall level of conservation of ACT-like organization in common with the other >70 alpha Connexins listed above, it is understood that the Cx37 ACT-like carboxy terminus functions in the provided capacity.

[0019] For comparison, the beta Connexin Cx26 is shown in Table 2. Cx26 has no carboxyl terminal type II PDZ binding motif; less than 30% of the carboxyl terminal most amino acids comprise S, T, R, D or E residues; it has no evidence of motifs proximal to a type II PDZ binding motif or PDZ binding like motif containing clusters of P and G hinge residues; and no evidence of clustered, repeat-like motifs of serine and threonine phospho-amino acids. Cx26 does have three Lysine (K) residues, clustered one after the other near the carboxy terminus of the sequence. However, no alpha Connexin surveyed in the >70 alpha Connexins listed above was found to display this feature of three repeated K residues domain at carboxy terminus (Cx26 is a beta connexin, thus by definition does not have an ACT domain).

[0020] As provided herein, the unique functional characteristics of this relatively short stretch of amino acids encompass the disclosed roles in treating or preventing pathologies involving epithelial permeablization and/or neovascularization. Thus, in some aspects, the provided polypeptide comprises a type II PDZ binding motif (Φ-x-Φ; wherein x = any amino acid and Φ = a Hydrophobic amino acid). In some aspects, greater than 50%, 60%, 70%, 80%, 90% of the amino acids of the provided ACT polypeptide is comprised one or more of Proline (P), Glycine (G), phospho-Serine (S), phospho-Threonine (T), Arginine (R), Lysine (K), Aspartic acid (D), or Glutamic acid (E) amino acid residues.

[0021] The amino acids Proline (P), Glycine (G), Arginine (R), Lysine (K), Aspartic acid (D), and Glutamic acid (E) are necessary determinants of protein structure and function. Proline and Glycine residues provide for tight turns in the 3D structure of proteins, enabling the generation of folded conformations of the polypeptide required for function. Charged amino acid sequences are often located at the surface of folded proteins and are necessary for chemical interactions mediated by the polypeptide including protein-protein interactions, protein-lipid interactions, enzyme-substrate interactions and protein-nucleic acid interactions. Thus, in some aspects Proline (P) and Glycine (G) Lysine (K), Aspartic acid (D), and Glutamic acid (E) rich regions proximal to the type II PDZ binding motif provide for properties necessary to the provided actions of ACT peptides. In some aspects, the provided polypeptide comprises Proline (P) and Glycine (G) Lysine (K), Aspartic acid (D), and/or Glutamic acid (E) rich regions proximal to the type II PDZ binding motif.

[0022] Phosphorylation is the most common post-translational modification of proteins and is crucial for modulating or modifying protein structure and function. Aspects of protein structure and function modified by phosphorylation include protein conformation, protein-protein interactions, protein-lipid interactions, protein-nucleic acid interactions, channel gating, protein trafficking and protein turnover. Thus, in some aspects the phospho-Serine (S) and/or phospho-Threonine (T) rich sequences are necessary for modifying the function of ACT peptides, increasing or decreasing efficacy of the polypeptides in their provided actions. In some aspects, the provided polypeptide comprise Serine (S) and/or phospho-Threonine (T) rich sequences or motifs.

[0023] In another example, respecting definition of an ACT peptide, it is highly auspicious, in light of the high degree of tissue/organ regeneration potential in lower animals such as fish, that a methionine occurs near the amino terminus of the ACT sequence of zebrafish Cx43 (Table 2). In addition to encoding methionine, the methionine base pair triplet is an alternate translation start site. If translation initiated from this methionine, the sequence SSRARPDDLDV (SEQ ID NO:90), would be produced. This translation product maintains all the conserved and distinctive features of a canonical ACT peptide. Specifically this peptide comprises a carboxy terminal type II PDZ binding domain and has a domain enriched in P, R and D residues proximal to the PDZ binding domain. In addition, the sequence comprises a clustered S motif, with potential to modulate ACT peptide function at its amino terminal. This raises the interesting prospect that animals with high tissue/organ regeneration potential such as fish may translate ACT peptides sequences directly.

[0024] Thus, in some aspects, the provided polypeptide comprises the c-terminal sequence of human Cx43. Thus, the provided polypeptide can comprise the amino acid sequence SEQ ID NO:1 or SEQ ID NO:2. The polypeptide can comprise 9 amino acids of the carboxy terminus of human Cx40. Thus, the polypeptide can comprise the amino acid sequence SEQ ID NO:5. In other aspects, the provided polypeptide does not comprises the c-terminal sequence of human Cx43. Thus, is some aspects, the provided polypeptide does not consist of the amino acid sequence SEQ ID NO:1 or SEQ ID NO:2.

[0025] When specific proteins are referred to herein, variants, derivatives, and fragments are contemplated. Protein variants and derivatives are well understood to those of skill in the art and in can involve amino acid sequence modifications. For example,

amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known and include, for example, M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues. Deletions or insertions preferably are made in adjacent pairs, i.e., a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure unless such a change in secondary structure of the mRNA is desired. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Table 3 and are referred to as conservative substitutions.

TABLE 3: Amino Acid Substitutions

Original Residue	Exemplary Substitutions
Ala	Ser
Arg	Lys
Asn	Gln
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Gln
Ile	Leu; Val
Leu,	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Pro	Gly
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

[0026] For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations shown in Table 3. Conservatively substituted variations of each explicitly disclosed sequence are included within the polypeptides provided herein.

[0027] Typically, conservative substitutions have little to no impact on the biological activity of a resulting polypeptide. In a particular example, a conservative substitution is an amino acid substitution in a peptide that does not substantially affect the biological function of the peptide.

[0028] A polypeptide can be produced to contain one or more conservative substitutions by manipulating the nucleotide sequence that encodes that polypeptide using, for example, standard procedures such as site-directed mutagenesis or PCR. Alternatively, a polypeptide can be produced to contain one or more conservative substitutions by using standard peptide

synthesis methods. An alanine scan can be used to identify which amino acid residues in a protein can tolerate an amino acid substitution. In one example, the biological activity of the protein is not decreased by more than 25%, for example not more than 20%, for example not more than 10%, when an alanine, or other conservative amino acid (such as those listed below), is substituted for one or more native amino acids.

[0029] Further information about conservative substitutions can be found in, among other locations, in Ben-Bassat et al., (J. Bacteriol. 169:751-7, 1987), O'Regan et al., (Gene 77:237-51, 1989), Sahin-Toth et al., (Protein Sci. 3:240-7, 1994), Hochuli et al., (Bio/Technology 6:1321-5, 1988) and in standard textbooks of genetics and molecular biology.

[0030] Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also may be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

[0031] Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco pp 79-86 [1983]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

[0032] It is understood that there are numerous amino acid and peptide analogs which can be incorporated into the disclosed compositions. For example, there are numerous D amino acids or amino acids which have a different functional substituent than the amino acids shown in Table 3. The opposite stereoisomers of naturally occurring peptides are disclosed, as well as the stereoisomers of peptide analogs. These amino acids can readily be incorporated into polypeptide chains by charging tRNA molecules with the amino acid of choice and engineering genetic constructs that utilize, for example, amber codons, to insert the analog amino acid into a peptide chain in a site specific way (Thorson et al., Methods in Molec. Biol. 77:43-73 (1991), Zoller, Current Opinion in Biotechnology, 3:348-354 (1992); Ibba, Biotechnology & Genetics Enginerring Reviews 13:197-216 (1995), Cahill et al., TIBS, 14(10):400-403 (1989); Benner, TIB Tech, 12:158-163 (1994); Ibba and Hennecke, Bio/technology, 12:678-682 (1994).

[0033] Molecules can be produced that resemble polypeptides, but which are not connected via a natural peptide linkage. For example, linkages for amino acids or amino acid analogs can include CH_2NH--, --CH_2S--, -CH_2--CH_2--, --CH=CH- (cis and trans), --$COCH_2$--, --$CH(OH)CH_2$--, and --CHH_2SO-(These and others can be found in Spatola, A. F. in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Spatola, A. F., Vega Data (March 1983), Vol. 1, Issue 3, Peptide Backbone Modifications (general review); Morley, Trends Pharm Sci (1980) pp. 463-468; Hudson, D. et al., Int J Pept Prot Res 14:177-185 (1979) (--CH_2NH--, CH_2CH_2--); Spatola et al. Life Sci 38:1243-1249 (1986) (--CHH_2-S); Hann J. Chem. Soc Perkin Trans. 1307-314 (1982) (--CH--CH--, cis and trans); Almquist et al. J. Med. Chem. 23:1392-1398 (1980) (--$COCH_2$--); Jennings-White et al. Tetrahedron Lett 23:2533 (1982) (--$COCH_2$--); Szelke *et al. European Appln,* EP 45665 CA (1982): 97:39405 (1982) (--$CH(OH)CH_2$--); Holladay et al. Tetrahedron. Lett 24:4401-4404 (1983) (--$C(OH)CH_2$--); and Hruby Life Sci 31:189-199 (1982) (--CH_2--S-). It is understood that peptide analogs can have more than one atom between the bond atoms, such as b-alanine, g-aminobutyric acid, and the like.

[0034] Amino acid analogs and peptide analogs often have enhanced or desirable properties, such as, more economical production, greater chemical stability, enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.), altered specificity (e.g., a broad-spectrum of biological activities), reduced antigenicity, greater ability to cross biological barriers (e.g., gut, blood vessels, blood-brain-barrier), and others.

[0035] D-amino acids can be used to generate more stable peptides, because D Amino acids are not recognized by peptidases and such. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) can be used to generate more stable peptides. Cysteine residues can be used to cyclize or attach two or more peptides together. This can be beneficial to constrain peptides into particular conformations. (Rizo and Gierasch Ann. Rev. Biochem. 61:387 (1992)).

[0036] Thus, the provided polypeptide can comprise a conservative variant of the c-terminus of an alpha Connexion (ACT). As shown in Table 4, an example of a single conservative substitution within the sequence SEQ ID NO:2 is given in the sequence

SEQ ID NO:3. An example of three conservative substitutions within the sequence SEQ ID NO:2 is given in the sequence SEQ ID NO:4. Thus, the provided polypeptide can comprise the amino acid SEQ ID NO:3 or SEQ ID NO:4.

Table 4. ACT Polypeptide Variants

Sequence	SEQ ID NO
RPRPDDLEI	SEQ ID NO:2
RPRPDDLEV	SEQ ID NO:3
RPRPDDVPV	SEQ ID NO:4
SSRASSRASSRPRPDDLEV	SEQ ID NO: 44
RPKPDDLEI	SEQ ID NO: 45
SSRASSRASSRPKPDDLEI	SEQ ID NO: 46
RPKPDDLDI	SEQ ID NO: 47
SSRASSRASSRPRPDDLDI	SEQ ID NO: 48
SSRASTRASSRPRPDDLEI	SEQ ID NO: 49
RPRPEDLEI	SEQ ID NO: 50
SSRASSRASSRPRPEDLEI	SEQ ID NO: 51
GDGKNSVWV	SEQ ID NO: 52
SKAGSNKSTASSKSGDGKNSVWV	SEQ ID NO: 53
GQKPPSRPSSASKKLYV	SEQ ID NO: 54

[0037] It is understood that one way to define any variants, modifications, or derivatives of the disclosed genes and proteins herein is through defining the variants, modification, and derivatives in terms of sequence identity (also referred to herein as homology) to specific known sequences. Specifically disclosed are variants of the nucleic acids and polypeptides herein disclosed which have at least 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 percent sequence identity to the stated or known sequence. Those of skill in the art readily understand how to determine the sequence identity of two proteins or nucleic acids. For example, the sequence identity can be calculated after aligning the two sequences so that the sequence identity is at its highest level.

[0038] Another way of calculating sequence identity can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local sequence identity algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the sequence identity alignment algorithm of Needleman and Wunsch, J. MoL Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

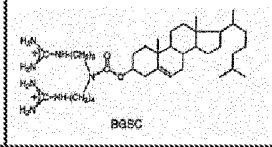
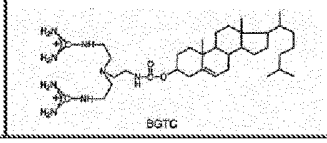
[0039] The same types of sequence identity can be obtained for nucleic acids by, for example, the algorithms disclosed in Zuker, M. Science 244:48-52,1989, Jaeger et al. Proc, Natl. Acad. Sci. USA 86:7706-7710, 989, Jaeger et al. Methods Enzymol. 183:281-306, 1989.

[0040] As an example, provided is a polypeptide (SEQ ID NO:4) having 66% sequence identity to the same stretch of 9 amino acids occurring on the carboxy-terminus of human Cx43 (SEQ ID NO:2).

[0041] The herein provided polypeptides can be added directly to a tissue in a subject. However, efficiency of cytoplasmic localization of the provided polypeptide is enhanced by cellular internalization transporter chemically linked in *cis* or *trans* with the polypeptide. Efficiency of cell internalization transporters are enhanced further by light or co-transduction of cells with Tat-HA peptide.

[0042] Thus, the provided polypeptide can comprise a cellular internalization transported or sequence. The cellular internalization sequence can be any internalization sequence known or newly discovered in the art, or conservative variants thereof. Non-limiting examples of cellular internalization transporters and sequences include Antennapedia sequences, TAT, HIV-Tat, Penetration, Antp-3A (Antp mutant), Buforin II, Transportan, MAP (model amphipathic peptide), K-FGF, Ku70, Prion, pVEC, Pep-1, SynB1, Pep-7, HN-1, BGSC (Bis-Guanidinium-Spermidine-Cholesterol, and BGTC (Bis-Guanidinium-Tren-Cholesterol) (see Table 5).

Table 5: Cell Internalization Transporters

Name	Sequence	SEQ ID NO
Antp	RQPKIWFPNRRKPWKK	(SEQ ID NO:7)
HIV-Tat	GRKKRRQRPPQ	(SEQ ID NO:14)
Penetration	RQIKIWFQNRRMKWKK	(SEQ ID NO:15)
Antp-3A	RQIAIWFQNRRMKWAA	(SEQ ID NO:16)
Tat	RKKRRQRRR	(SEQ ID NO:17)
Buforin II	TRSSRAGLQFPVGRVHRLLRK	(SEQ ID NO:18)
Transportan	GWTLNSAGYLLGKINKALAALA KKIL	(SEQ ID NO:19)
model amphipathic peptide (MAP)	KLALKLALKALKAALKLA	(SEQ ID NO:20)
K-FGF	AAVALLPAVLLALLAP	(SEQ ID NO:21)
Ku70	VPMLK- PMLKE	(SEQ ID NO:22)
Prion	MANLGYWLLALFVTMWTDVGL CKKRPKP	(SEQ ID NO:23)
pVEC	LLIILRRRIRKQAHAHSK	(SEQ ID NO:24)
Pep-1	KETWWETWWTEWSQPKKKRKV	(SEQ ID NO:25)
SynB1	RGGRLSYSRRRFSTSTGR	(SEQ ID NO:26)
Pep-7	SDLWEMMMVSLACQY	(SEQ ID NO:27)
HN-1	TSPLNIHNGQKL	(SEQ ID NO:28)
BGSC (Bis-Guanidinium-Spermidine-Cholesterol)	 BGSC	
BGTC (Bis-Guanidinium-Tren-Cholesterol)	 BGTC	

[0043] Thus, the provided polypeptide can further comprise the amino acid sequence SEQ ID NO:7, SEQ ID NO:14 (Bucci, M. et al. 2000. Nat. Med. 6, 1362-1367), SEQ ID NO:15 (Derossi, D., et al. 1994. Biol.Chem. 269, 10444-10450), SEQ ID NO:16 (Fischer, P.M. et al. 2000. J. Pept. Res. 55, 163-172), SEQ ID NO:17 (Frankel, A. D. & Pabo, C. O. 1988. Cell 55,1189-1193 ; Green, M. & Loewenstein, P. M. 1988. Cell 55, 1179-1188), SEQ ID NO:18 (Park, C. B., et al. 2000. Proc. Natl Acad. Sci. USA 97, 8245-8250), SEQ ID NO:19 (Pooga, M., et al. 1998. FASEB J 12, 67-77), SEQ ID NO:20 (Oehlke, J. et al. 1998. Biochim. Biophys. Acta. 1414,127-139), SEQ ID NO:21 (Lin, Y. Z., et al. 1995. J. Biol. Chem. 270, 14255-14258), SEQ ID NO:22 (Sawada, M., et al.

2003. Nature Cell Biol. 5, 352-357), SEQ ID NO:23 (Lundberg, P. et al. 2002. Biochem. Biophys. Res. Commun. 299, 85-90), SEQ ID NO:24 (Elmquist, A., et al. 2001. Exp. Cell Res. 269, 237-244), SEQ ID NO:25 (Morris, M. C., et al. 2001. Nature Biotechnol. 19, 1173-1176), SEQ ID NO:26 (Rousselle, C. et al. 2000. Mol. Pharmacol. 57,679-686), SEQ ID NO:27 (Gao, C. et al. 2002. Bioorg. Med. Chem. 10, 4057-4065), or SEQ ID NO:28 (Hong, F. D. & Clayman, G. L. 2000. Cancer Res. 60, 6551-6556). The provided polypeptide can further comprise BGSC (Bis-Guanidinium-Spermidine-Cholesterol) or BGTC (Bis-Guanidinium-Tren-Cholesterol) (Vigneron, J.P. et al. 1998. Proc. Natl. Acad. Sci. USA. 93, 9682-9686). Any other internalization sequences now known or later identified can be combined with a peptide of the invention.

[0044] The provided polypeptide can comprise any ACT sequence (e.g, any of the ACT peptides disclosed herein) in combination with any of the herein provided cell internalization sequences. Examples of said combinations are given in Table 6. Thus, the provided polypeptide can comprise an Antennapedia sequence comprising amino acid sequence SEQ ID NO:7. Thus, the provided polypeptide can comprise the amino acid sequence SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

Table 6: ACT Polypeptides with Cell Internalization Sequences (CIS)

CIS/ ACT	Sequence	SEQ ID NO
Antp/ ACT 2	RQPKIWFPNRRKPWKK PSSRASSRASSRPRPDDLEI	SEQ ID NO:8
Antp/ ACT 1	RQPKIWFPNRRKPWKK RPRPDDLEI	SEQ ID NO:9
Antp/ ACT 3	RQPKIWFPNRRKPWKK RPRPDDLEV	SEQ ID NO:10
Antp/ ACT 4	RQPKIWFPNRRKPWKK RPRPDDVPV	SEQ ID NO:11
Antp/ ACT 5	RQPKIWFPNRRKPWKK KARSDDLSV	SEQ ID NO:12
HIV-Tat/ ACT 1	GRKKRRQRPPQ RPRPDDLEI	SEQ ID NO:56
Penetratin/ ACT 1	RQIKIWFQNRRMKWKK RPRPDDLEI	SEQ ID NO57
Antp-3A/ ACT 1	RQIAIWFQNRRMKWAA RPRPDDLEI	SEQ ID NO:58
Tat/ ACT 1	RKKRRQRRR RPRPDDLEI	SEQ ID NO:59
Buforin II/ ACT 1	TRSSRAGLQFPVGRVHRLLRK RPRPDDLEI	SEQ ID NO:60
Transportan/ ACT 1	GWTLNSAGYLLGKINKALAALAKKIL RPRPDDLEI	SEQ ID NO:61
MAP/ ACT 1	KLALKLALKALKAALKLA RPRPDDLEI	SEQ ID NO:62
K-FGF/ ACT 1	AAVALLPAVLLALLAP RPRPDDLEI	SEQ ID NO:63
Ku70/ ACT 1	VPMLKPMLKE RPRPDDLEI	SEQ ID NO:64
Prion/ ACT 1	MANLGYWLLALFVTMWTDVGLCKKRPKP RPRPDDLEI	SEQ ID NO:65
pVEC/ ACT 1	LLIILRRRIRKQAHAHSK RPRPDDLEI	SEQ ID NO:66
Pep-1/ ACT 1	KETWWETWWTEWSQPKKKRKV RPRPDDLEI	SEQ ID NO:67
SynB1/ ACT 1	RGGRLSYSRRRFSTSTGR RPRPDDLEI	SEQ ID NO:68
Pep-7/ ACT 1	SDLWEMMMVSLACQY RPRPDDLEI	SEQ ID NO:69
HN-1/ ACT 1	TSPLNIHNGQKL RPRPDDLEI	SEQ ID NO:70

[0045] Also provided are isolated nucleic acids encoding the polypeptides provided herein. The disclosed nucleic acids are made up of for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for example, when a vector is expressed in a cell, the expressed mRNA will typically be made up of A, C, G, and U.

[0046] By "isolated nucleic acid" or "purified nucleic acid" is meant DNA that is free of the genes that, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector, such as an autonomously replicating plasmid or virus; or incorporated into the genomic DNA of a prokaryote or eukaryote (e.g., a transgene); or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR, restriction endonuclease digestion, or chemical or *in vitro* synthesis). It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence. The term "isolated nucleic acid" also refers to RNA, e.g., an mRNA molecule that is encoded by an isolated DNA molecule, or that is chemically synthesized, or that is separated or substantially free from at least some cellular components, e.g., other types of RNA molecules or polypeptide molecules.

[0047] Thus, provided is an isolated nucleic acid encoding a polypeptide comprising the amino acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

[0048] Thus, the provided nucleic acid can comprise the nucleic acid sequence SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, or SEQ ID NO:89.

[0049] The herein provided nucleic acid can be operably linked to an expression control sequence. Also provided is a vector comprising one or more of the herein provided nucleic acids, wherein the nucleic acid is operably linked to an expression control sequence. There are a number of compositions and methods which can be used to deliver nucleic acids to cells, either *in vitro* or i*in vivo*. These methods and compositions can largely be broken down into two classes: viral based delivery systems and non-viral based delivery systems. For example, the nucleic acids can be delivered through a number of direct delivery systems such as, electroporation, lipofection, calcium phosphate precipitation, plasmids, viral vectors, viral nucleic acids, phage nucleic acids, phages, cosmids, or via transfer of genetic material in cells or carriers such as cationic liposomes. Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., Science, 247, 1465-1468, (1990); and Wolff, J. A. Nature, 352, 815-818, (1991). Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modifed to specifically function with large DNA molecules. Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier.

[0050] Transfer vectors can be any nucleotide construction used to deliver genes into cells (e.g., a plasmid), or as part of a general strategy to deliver genes, e.g., as part of recombinant retrovirus or adenovirus (Ram et al. Cancer Res. 53:83-88, (1993)).

[0051] As used herein, plasmid or viral vectors are agents that transport the disclosed nucleic acids, such as SEQ ID NO:6, into the cell without degradation and include a promoter yielding expression of the gene in the cells into which it is delivered. In some embodiments the promoters are derived from either a virus or a retrovirus. Viral vectors are, for example, Adenovirus, Adeno-associated virus, Herpes virus, Vaccinia virus, Polio virus, AIDS virus, neuronal trophic virus, Sindbis and other RNA viruses, including these viruses with the HIV backbone. Also disclosed are any viral families which share the properties of these viruses which make them suitable for use as vectors. Retroviruses include Murine Maloney Leukemia virus, MMLV, and retroviruses that express the desirable properties of MMLV as a vector. Retroviral vectors are able to carry a larger genetic payload, i.e., a transgene or marker gene, than other viral vectors, and for this reason are a commonly used vector. However, they are not as useful in non-proliferating cells. Adenovirus vectors are relatively stable and easy to work with, have high titers, and can be delivered in aerosol formulation, and can transfect non-dividing cells. Pox viral vectors are large and have several sites for inserting genes, they are thermostable and can be stored at room temperature. Also disclosed is a viral vector which has been engineered so as to suppress the immune response of the host organism, elicited by the viral antigens. Vectors of this type can carry coding regions for Interleukin 8 or 10.

[0052] Viral vectors can have higher transaction (ability to introduce genes) abilities than chemical or physical methods to introduce genes into cells. Typically, viral vectors contain, nonstructural early genes, structural late genes, an RNA polymerase III transcript, inverted terminal repeats necessary for replication and encapsidation, and promoters to control the transcription and replication of the viral genome. When engineered as vectors, viruses typically have one or more of the early genes removed and a gene or gene/promotor cassette is inserted into the viral genome in place of the removed viral DNA. Constructs of this type can carry up to about 8 kb of foreign genetic material. The necessary functions of the removed early genes are typically supplied by cell lines which have been engineered to express the gene products of the early genes in trans.

[0053] A retrovirus is an animal virus belonging to the virus family of Retroviridae, including any types, subfamilies, genus, or tropisms. Retroviral vectors, in general, are described by Verma, I.M., Retroviral vectors for gene transfer. In Microbiology-1985, American Society for Microbiology, pp. 229-232, Washington, (1985). Examples of methods for using retroviral vectors for gene therapy are described in U.S. Patent Nos. 4,868,116 and 4,980,286; PCT applications WO 90/02806 and WO 89/07136; and Mulligan, (Science 260:926-932 (1993).

[0054] A retrovirus is essentially a package which has packed into it nucleic acid cargo. The nucleic acid cargo carries with it a packaging signal, which ensures that the replicated daughter molecules will be efficiently packaged within the package coat. In addition to the package signal, there are a number of molecules which are needed in cis, for the replication, and packaging of the replicated virus. Typically a retroviral genome, contains the gag, pol, and env genes which are involved in the making of the

protein coat. It is the gag, pol, and env genes which are typically replaced by the foreign DNA that it is to be transferred to the target cell. Retrovirus vectors typically contain a packaging signal for incorporation into the package coat, a sequence which signals the start of the gag transcription unit, elements necessary for reverse transcription, including a primer binding site to bind the tRNA primer of reverse transcription, terminal repeat sequences that guide the switch of RNA strands during DNA synthesis, a purine rich sequence 5' to the 3' LTR that serve as the priming site for the synthesis of the second strand of DNA synthesis, and specific sequences near the ends of the LTRs that enable the insertion of the DNA state of the retrovirus to insert into the host genome. The removal of the gag, pol, and env genes allows for about 8 kb of foreign sequence to be inserted into the viral genome, become reverse transcribed, and upon replication be packaged into a new retroviral particle. This amount of nucleic acid is sufficient for the delivery of a one to many genes depending on the size of each transcript.

[0055] Since the replication machinery and packaging proteins in most retroviral vectors have been removed (gag, pol, and env), the vectors are typically generated by placing them into a packaging cell line. A packaging cell line is a cell line which has been transfected or transformed with a retrovirus that contains the replication and packaging machinery, but lacks any packaging signal. When the vector carrying the DNA of choice is transfected into these cell lines, the vector containing the gene of interest is replicated and packaged into new retroviral particles, by the machinery provided in cis by the helper cell. The genomes for the machinery are not packaged because they lack the necessary signals.

[0056] The construction of replication-defective adenoviruses has been described (Berkner et al., J. Virology 61:1213-1220 (1987); Massie et al., Mol. Cell. Biol. 6:2872-2883 (1986); Haj-Ahmad et al., J. Virology 57:267-274 (1986); Davidson et al., J. Virology 61:1226-1239 (1987); Zhang "Generation and identification of recombinant adenovirus by liposome-mediated transfection and PCR analysis" BioTechniques 15:868-872 (1993)). The benefit of the use of these viruses as vectors is that they are limited in the extent to which they can spread to other cell types, since they can replicate within an initial infected cell, but are unable to form new infectious viral particles. Recombinant adenoviruses have been shown to achieve high efficiency gene transfer after direct, i*in vivo* delivery to airway epithelium, hepatocytes, vascular endothelium, CNS parenchyma and a number of other tissue sites (Morsy, J. Clin. Invest. 92:1580-1586 (1993); Kirshenbaum, J. Clin. Invest. 92:381-387 (1993); Roessler, J. Clin. Invest. 92:1085-1092 (1993); Moullier, Nature Genetics 4:154-159 (1993); La Salle, Science 259:988-990 (1993); Gomez-Foix, J. Biol. Chem. 267:25129-25134 (1992); Rich, Human Gene Therapy 4:461-476 (1993); Zabner, Nature Genetics 6:75-83 (1994); Guzman, Circulation Research 73:1201-1207 (1993); Bout, Human Gene Therapy 5:3-10 (1994); Zabner, Cell 75:207-216 (1993); Caillaud, Eur. J. Neuroscience 5:1287-1291 (1993); and Ragot, J. Gen. Virology 74:501-507 (1993)). Recombinant adenoviruses achieve gene transduction by binding to specific cell surface receptors, after which the virus is internalized by receptor-mediated endocytosis, in the same manner as wild type or replication-defective adenovirus (Chardonnet and Dales, Virology 40:462-477 (1970); Brown and Burlingham, J. Virology 12:386-396 (1973); Svensson and Persson, J. Virology 55:442-449 (1985); Seth, et al., J. Virol. 51:650-655 (1984); Seth, et al., Mol. Cell. Biol. 4:1528-1533 (1984); Varga et al., J. Virology 65:6061-6070 (1991); Wickham et al., Cell 73:309-319 (1993)).

[0057] A viral vector can be one based on an adenovirus which has had the E1 gene removed, and these virons are generated in a cell line such as the human 293 cell line. In some aspects, both the E1 and E3 genes are removed from the adenovirus genome.

[0058] Another type of viral vector is based on an adeno-associated virus (AAV). This defective parvovirus can infect many cell types and is nonpathogenic to humans. AAV type vectors can transport about 4 to 5 kb and wild type AAV is known to stably insert into chromosome 19. As an example, this vector can be the P4.1 C vector produced by Avigen, San Francisco, CA, which can contain the herpes simplex virus thymidine kinase gene, HSV-tk, and/or a marker gene, such as the gene encoding the green fluorescent protein, GFP.

[0059] In another type of AAV virus, the AAV contains a pair of inverted terminal repeats (ITRs) which flank at least one cassette containing a promoter which directs cell-specific expression operably linked to a heterologous gene. Heterologous in this context refers to any nucleotide sequence or gene which is not native to the AAV or B19 parvovirus.

[0060] Typically the AAV and B19 coding regions have been deleted, resulting in a safe, noncytotoxic vector. The AAV ITRs, or modifications thereof, confer infectivity and site-specific integration, but not cytotoxicity, and the promoter directs cell-specific expression.

[0061] The disclosed vectors thus provide DNA molecules which are capable of integration into a mammalian chromosome without substantial toxicity.

[0062] The inserted genes in viral and retroviral usually contain promoters, and/or enhancers to help control the expression of

the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and may contain upstream elements and response elements.

[0063] Molecular genetic experiments with large human herpes viruses have provided a means whereby large heterologous DNA fragments can be cloned, propagated and established in cells permissive for infection with herpes viruses (Sun et al., Nature genetics 8: 33-41, 1994; Cotter and Robertson,. Curr Opin Mol Ther 5: 633-644, 1999). These large DNA viruses (herpes simplex virus (HSV) and Epstein-Barr virus (EBV), have the potential to deliver fragments of human heterologous DNA > 150 kb to specific cells. EBV recombinants can maintain large pieces of DNA in the infected B-cells as episomal DNA. Individual clones carried human genomic inserts up to 330 kb appeared genetically stable. The maintenance of these episomes requires a specific EBV nuclear protein, EBNA1, constitutively expressed during infection with EBV. Additionally, these vectors can be used for transfection, where large amounts of protein can be generated transiently *in vitro.* Herpesvirus amplicon systems are also being used to package pieces of DNA > 220 kb and to infect cells that can stably maintain DNA as episomes.

[0064] Other useful systems include, for example, replicating and host-restricted non-replicating vaccinia virus vectors.

[0065] The disclosed compositions can be delivered to the target cells in a variety of ways. For example, the compositions can be delivered through electroporation, or through lipofection, or through calcium phosphate precipitation. The delivery mechanism chosen will depend in part on the type of cell targeted and whether the delivery is occurring for example i*in vivo* or *in vitro.*

[0066] Thus, the compositions can comprise, in addition to the disclosed polypeptides, nucleic acids or vectors, for example, lipids such as liposomes, such as cationic liposomes (e.g., DOTMA, DOPE, DC-cholesterol) or anionic liposomes. Liposomes can further comprise proteins to facilitate targeting a particular cell, if desired. Administration of a composition comprising a compound and a cationic liposome can be administered to the blood afferent to a target organ or inhaled into the respiratory tract to target cells of the respiratory tract. Regarding liposomes, see, e.g., Brigham et al. Am. J. Resp. Cell. Mol. Biol. 1:95-100 (1989); Felgner et al. Proc. Natl. Acad. Sci USA 84:7413-7417 (1987); U.S. Pat. No.4,897,355. Furthermore, the compound can be administered as a component of a microcapsule that can be targeted to specific cell types, such as macrophages, or where the diffusion of the compound or delivery of the compound from the microcapsule is designed for a specific rate or dosage.

[0067] In the methods described above which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), delivery of the compositions to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, MD), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, WI), as well as other liposomes developed according to procedures standard in the art. In addition, the disclosed nucleic acid or vector can be delivered *iin vivo* by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, AZ).

[0068] Nucleic acids that are delivered to cells which are to be integrated into the host cell genome, typically contain integration sequences. These sequences are often viral related sequences, particularly when viral based systems are used. These viral integration systems can also be incorporated into nucleic acids which are to be delivered using a non-nucleic acid based system of deliver, such as a liposome, so that the nucleic acid contained in the delivery system can be come integrated into the host genome.

[0069] Other general techniques for integration into the host genome include, for example, systems designed to promote homologous recombination with the host genome. These systems typically rely on sequence flanking the nucleic acid to be expressed that has enough homology with a target sequence within the host cell genome that recombination between the vector nucleic acid and the target nucleic acid takes place, causing the delivered nucleic acid to be integrated into the host genome. These systems and the methods necessary to promote homologous recombination are known to those of skill in the art.

[0070] The compositions can be delivered to the subject's cells *in vivo* and/or *ex vivo* by a variety of mechanisms well known in the art (e.g., uptake of naked DNA, liposome fusion, intramuscular injection of DNA via a gene gun, endocytosis and the like).

[0071] If *ex vivo* methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art. The compositions can be introduced into the cells via any gene transfer mechanism, such as, for example, calcium phosphate mediated gene delivery, electroporation, microinjection or proteoliposomes. The transduced cells can then be infused (e.g., in a pharmaceutically acceptable carrier) or homotopically transplanted back into the subject per standard methods for the cell or tissue type. Standard methods are known for transplantation or infusion of various cells into a subject.

[0072] The nucleic acids that are delivered to cells typically contain expression controlling systems. For example, the inserted genes in viral and retroviral systems usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and may contain upstream elements and response elements.

[0073] Promoters controlling transcription from vectors in mammalian host cells may be obtained from various sources, for example, the genomes of viruses such as: polyoma, Simian Virus 40 (SV40), adenovirus, retroviruses, hepatitis-B virus, cytomegalovirus, or from heterologous mammalian promoters, e.g. beta actin promoter. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature, 273: 113 (1978)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment (Greenway, P.J. et al., Gene 18: 355-360 (1982)). Of course, promoters from the host cell or related species also are useful herein.

[0074] Enhancer generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' (Laimins, L. et al., Proc. Natl. Acad. Sci.78: 993 (1981)) or 3' (Lusky, M.L., et al., Mol. Cell Bio. 3: 1108 (1983)) to the transcription unit. Furthermore, enhancers can be within an intron (Banerji, J.L. et al., Cell 33: 729 (1983)) as well as within the coding sequence itself (Osborne, T.F., et al., Mol. Cell Bio. 4: 1293 (1984)). They are usually between 10 and 300 bp in length, and they function in cis. Enhancers function to increase transcription from nearby promoters. Enhancers also often contain response elements that mediate the regulation of transcription. Promoters can also contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression of a gene. While many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin), typically one will use an enhancer from a eukaryotic cell virus for general expression. Examples are the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

[0075] The promotor and/or enhancer may be specifically activated either by light or specific chemical events which trigger their function. Systems can be regulated by reagents such as tetracycline and dexamethasone. There are also ways to enhance viral vector gene expression by exposure to irradiation, such as gamma irradiation, or alkylating chemotherapy drugs.

[0076] In certain embodiments the promoter and/or enhancer region can act as a constitutive promoter and/or enhancer to maximize expression of the region of the transcription unit to be transcribed. In certain constructs the promoter and/or enhancer region be active in all eukaryotic cell types, even if it is only expressed in a particular type of cell at a particular time. A promoter of this type is the CMV promoter (650 bases). Other such promoters are SV40 promoters, cytomegalovirus (full length promoter), and retroviral vector LTR.

[0077] It has been shown that all specific regulatory elements can be cloned and used to construct expression vectors that are selectively expressed in specific cell types such as melanoma cells. The glial fibrillary acetic protein (GFAP) promoter has been used to selectively express genes in cells of glial origin.

[0078] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) may also contain sequences necessary for the termination of transcription which may affect mRNA expression. These regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3' untranslated regions also include transcription termination sites. The transcription unit can also contain a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA. The identification and use of polyadenylation signals in expression constructs is well established. Homologous polyadenylation signals can be used in the transgene constructs. In certain transcription units, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists of about 400 bases. Transcribed units an contain other standard sequences alone or in combination with the above sequences improve expression from, or stability of, the construct.

[0079] The viral vectors can include nucleic acid sequence encoding a marker product. This marker product is used to determine if the gene has been delivered to the cell and once delivered is being expressed. Example marker genes are the *E. Coli* lacZ gene, which encodes ß-galactosidase, and green fluorescent protein.

[0080] In some embodiments the marker may be a selectable marker. Examples of suitable selectable markers for mammalian cells are dihydrofolate reductase (DHFR), thymidine kinase, neomycin, neomycin analog G418, hydromycin, and puromycin.

When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell can survive if placed under selective pressure. There are two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to grow independent of a supplemented media. Two examples are: Chinese hamster ovary (CHO) DHFR- cells and mouse LTK- cells. These cells lack the ability to grow without the addition of such nutrients as thymidine or hypoxanthine. Because these cells lack certain genes necessary for a complete nucleotide synthesis pathway, they cannot survive unless the missing nucleotides are provided in a supplemented media. An alternative to supplementing the media is to introduce an intact DHFR or TK gene into cells lacking the respective genes, thus altering their growth requirements. Individual cells which were not transformed with the DHFR or TK gene will not be capable of survival in non-supplemented media.

[0081] The second category is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These schemes typically use a drug to arrest growth of a host cell. Those cells which have a novel gene would express a protein conveying drug resistance and would survive the selection. Examples of such dominant selection use the drugs neomycin, (Southern P. and Berg, P., J. Molec. Appl. Genet. 1:327 (1982)), mycophenolic acid, (Mulligan, R.C. and Berg, P. Science 209: 1422 (1980)) or hygromycin, (Sugden, B. et al., Mol. Cell. Biol. 5: 410-413 (1985)). The three examples employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid) or hygromycin, respectively. Others include the neomycin analog G418 and puramycin.

[0082] Also provided is a cell comprising one or more of the herein provided vectors. As used herein, "cell", "cell line", and "cell culture" may be used interchangeably and all such designations include progeny. The disclosed cell can be any cell used to clone or propagate the vectors provided herein. Thus, the cell can be from any primary cell culture or established cell line. The method may be applied to any cell, including prokaryotic or eukaryotic, such as bacterial, plant, animal, and the like. The cell type can be selected by one skilled in the art based on the choice of vector and desired use.

[0083] Disclosed are animals produced by the process of transfecting a cell within the animal with any of the nucleic acid molecules or vectors disclosed herein. Disclosed are animals produced by the process of transfecting a cell within the animal any of the nucleic acid molecules or vectors disclosed herein, wherein the animal is a mammal. Also disclosed are animals produced by the process of transfecting a cell within the animal any of the nucleic acid molecules or vectors disclosed herein, wherein the mammal is mouse, rat, rabbit, cow, sheep, pig, or primate.

[0084] Provided is a composition comprising one or more of the herein provided polypeptides, nucleic acids, or vectors in a pharmaceutically acceptable carrier. Thus, provided is a composition comprising a combination of two or more of any of the herein provided ACT polypeptides in a pharmaceutically acceptable carrier. For example, provided is a composition comprising SEQ ID NO:1 and SEQ ID NO:5 in a pharmaceutically acceptable carrier.

[0085] By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

[0086] The herein provide composition can further comprise any known or newly discovered substance that can be administered to a tissue of a subject. For example, the provided composition can further comprise one or more of classes of **antibiotics** (e.g. Aminoglycosides, Cephalosporins, Chloramphenicol, Clindamycin, Erythromycins, Fluoroquinolones, Macrolides, Azolides, Metronidazole, Penicillin's, Tetracycline's, Trimethoprim-sulfamethoxazole, Vancomycin), **steroids** (e.g. Andranes (e.g. Testosterone), Cholestanes (e.g. Cholesterol), Cholic acids (e.g. Cholic acid), Corticosteroids (e.g. Dexamethasone), Estraenes (e.g. Estradiol), Pregnanes (e.g. Progesterone), **narcotic and non-narcotic analgesics** (e.g. Morphine, Codeine, Heroin, Hydromorphone, Levorphanol, Meperidine, Methadone, Oxydone, Propoxyphene, Fentanyl, Methadone, Naloxone, Buprenorphine, Butorphanol, Nalbuphine, Pentazocine), **chemotherapy** (e.g. **anti-cancer drugs** such as but not limited to Altretamine, Asparaginase, Bleomycin, Busulfan, Carboplatin, Carmustine, Chlorambucil, Cisplatin, Cladribine, Cyclophosphamide, Cytarabine, Dacarbazine, Diethylstilbesterol, Ethinyl estradiol, Etoposide, Floxuridine, Fludarabine, Fluorouracil, Flutamide, Goserelin, Hydroxyurea, Idarubicin, Ifosfamide, Leuprolide, Levamisole, Lomustine, Mechlorethamine, Medroxyprogesterone, Megestrol, Melphalan, Mercaptopurine, Methotrexate, Mitomycin, Mitotane, Mitoxantrone, Paclitaxel, pentastatin, Pipobroman, Plicamycin, Prednisone, Procarbazine, Streptozocin, Tamoxifen, Teniposide, Vinblastine, Vincristine), **anti-inflammatory agents** (e.g. Alclofenac; Alclometasone Dipropionate; Algestone Acetonide; alpha Amylase; Amcinafal; Amcinafide; Amfenac Sodium; Amiprilose Hydrochloride; Anakinra; Anirolac; Anitrazafen; Apazone; Balsalazide Disodium; Bendazac; Benoxaprofen; Benzydamine Hydrochloride; Bromelains; Broperamole; Budesonide; Carprofen; Cicloprofen; Cintazone; Cliprofen; Clobetasol Propionate; Clobetasone Butyrate; Clopirac; Cloticasone Propionate; Cormethasone Acetate;

Cortodoxone; Decanoate; Deflazacort; Delatestryl; Depo-Testosterone; Desonide; Desoximetasone; Dexamethasone Dipropionate; Diclofenac Potassium; Diclofenac Sodium; Diflorasone Diacetate; Diflumidone Sodium; Diflunisal; Difluprednate; Diftalone; Dimethyl Sulfoxide; Drocinonide; Endrysone; Enlimomab; Enolicam Sodium; Epirizole; Etodolac; Etofenamate; Felbinac; Fenamole; Fenbufen; Fenclofenac; Fenclorac; Fendosal; Fenpipalone; Fentiazac; Flazalone; Fluazacort; Flufenamic Acid; Flumizole; Flunisolide Acetate; Flunixin; Flunixin Meglumine; Fluocortin Butyl; Fluorometholone Acetate; Fluquazone; Flurbiprofen; Fluretofen; Fluticasone Propionate; Furaprofen; Furobufen; Halcinonide; Halobetasol Propionate; Halopredone Acetate; Ibufenac; Ibuprofen; Ibuprofen Aluminum; Ibuprofen Piconol; Ilonidap; Indomethacin; Indomethacin Sodium; Indoprofen; Indoxole; Intrazole; Isoflupredone Acetate; Isoxepac; Isoxicam; Ketoprofen; Lofemizole Hydrochloride; Lomoxicam; Loteprednol Etabonate; Meclofenamate Sodium; Meclofenamic Acid; Meclorisone Dibutyrate; Mefenamic Acid; Mesalamine; Meseclazone; Mesterolone; Methandrostenolone; Methenolone; Methenolone Acetate; Methylprednisolone Suleptanate; Momiflumate; Nabumetone; Nandrolone; Naproxen; Naproxen Sodium; Naproxol; Nimazone; Olsalazine Sodium; Orgotein; Orpanoxin; Oxandrolane; Oxaprozin; Oxyphenbutazone; Oxymetholone ; Paranyline Hydrochloride; Pentosan Polysulfate Sodium; Phenbutazone Sodium Glycerate; Pirfenidone; Piroxicam; Piroxicam Cinnamate; Piroxicam Olamine; Pirprofen; Prednazate; Prifelone; Prodolic Acid; Proquazone; Proxazole; Proxazole Citrate; Rimexolone; Romazarit; Salcolex; Salnacedin; Salsalate; Sanguinarium Chloride; Seclazone; Sermetacin; Stanozolol; Sudoxicam; Sulindac; Suprofen; Talmetacin; Talniflumate; Talosalate; Tebufelone; Tenidap; Tenidap Sodium; Tenoxicam; Tesicam; Tesimide; Testosterone; Testosterone Blends; Tetrydamine; Tiopinac; Tixocortol Pivalate; Tolmetin; Tolmetin Sodium; Triclonide; Triflumidate; Zidometacin; Zomepirac Sodium), or **anti-histaminic agents** (e.g. Ethanolamines (like diphenhydrmine carbinoxamine), Ethylenediamine (like tripelennamine pyrilamine), Alkylamine (like chlorpheniramine, dexchlorpheniramine, brompheniramine, triprolidine), other anti-histamines like astemizole, loratadine, fexofenadine, Bropheniramine, Clemastine, Acetaminophen, Pseudoephedrine, Triprolidine).

[0087] The herein provide composition can further comprise anti-VEGF (anti-Vascular Endothelial Growth Factor) agents. Examples of these agents include Lucentis, Avastin and Macugen.

[0088] The compositions may be administered topically, orally, or parenterally. For example, the compositions can be administered extracorporeally, intracranially, intravaginally, intraanally, subcutaneously, intradermally, intracardiac, intragastric, intravenously, intramuscularly, by intraperitoneal injection, transdermally, intranasally, or by inhalant. As used herein, "intracranial administration" means the direct delivery of substances to the brain including, for example, intrathecal, intracisternal, intraventricular or trans-sphenoidal delivery via catheter or needle.

[0089] Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795.

[0090] As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the nucleic acid or vector. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation.

[0091] The exact amount of the compositions required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the allergic disorder being treated, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

[0092] The materials may be in solution or suspension (for example, incorporated into microparticles, liposomes, or cells). These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et

Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

[0093] Suitable carriers and their formulations are described in Remington: The Science and Practice of Pharmacy (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution can be from about 5 to about 8, from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of composition being administered.

[0094] Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

[0095] Pharmaceutical compositions may include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

[0096] The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration may be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection.

[0097] Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

[0098] Formulations for topical administration may include ointments, lotions, creams, gels (e.g., poloxamer gel), drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. The disclosed compositions can be administered, for example, in a microfiber, polymer (e.g., collagen), nanosphere, aerosol, lotion, cream, fabric, plastic, tissue engineered scaffold, matrix material, tablet, implanted container, powder, oil, resin, wound dressing, bead, microbead, slow release bead, capsule, injectables, intravenous drips, pump device, silicone implants, or any bio-engineered materials.

[0099] In some aspects the provided pharmaceutically acceptable carrier is a poloxamer. Poloxamers, referred to by the trade name Pluronics®, are nonionic surfactants that form clear thermoreversible gels in water. Poloxamers are polyethylene oxide-polypropylene oxide-polyethylene oxide (PEO-PPO-PEO) tri-block copolymers. The two polyethylene oxide chains are hydrophilic but the polypropylene chain is hydrophobic. These hydrophobic and hydrophilic characteristics take charge when placed in aqueous solutions. The PEO-PPO-PEO chains take the form of small strands where the hydrophobic centers would come together to form micelles. The micelle, sequentially, tend to have gelling characteristics because they come together in groups to form solids (gels) where water is just slightly present near the hydrophilic ends. When it is chilled, it becomes liquid, but it hardens when warmed. This characteristic makes it useful in pharmaceutical compounding because it can be drawn into a syringe for accurate dose measurement when it is cold. When it warms to body temperature (when applied to skin) it thickens to a perfect consistency (especially when combined with soy lecithin/isopropyl palmitate) to facilitate proper inunction and adhesion. Pluronic®

F127 (F127) is widely used because it is obtained easily and thus it is used in such pharmaceutical applications. F127 has a EO:PO:EO ratio of 100:65:100, which by weight has a PEO:PPO ratio of 2:1. Pluronic gel is an aqueous solution and typically contains 20-30% F-127. Thus, the provided compositions can be administered in F127.

[0100] Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable.

[0101] Some of the compositions may potentially be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

[0102] Effective dosages and schedules for administering the compositions may be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms disorder are effected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual doctor in the event of any counterindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. The range of dosage largely depends on the application of the compositions herein, severity of condition, and its route of administration.

[0103] For example, in applications as a laboratory tool for research, the ACT peptide compositions can be used in doses as low as 0.01% w/v. The dosage can be as low as 0.02% w/v and possibly as high as 2% w/v in topical treatments. Thus, upper limits of the provided polypeptides may be up to 2-5 % w/v or v/v if given as an initial bolus delivered for example directly into a tumor mass. Recommended upper limits of dosage for parenteral routes of administration for example intramuscular, intracerebral, intracardicardiac and intraspinal could be up to 1 % w/v or v/v. This upper dosage limit may vary by formulation, depending for example on how the polypeptide(s) is combined with other agents promoting its action or acting in concert with the polypeptide(s).

[0104] For continuous delivery of the provided polypeptides, for example, in combination with an intravenous drip, upper limits of 0.01 g /Kg body weight over time courses determined by the doctor based on improvement in the condition can be used. In another example, upper limits of concentration of the provided nucleic acids delivered topically would be 5-10 $\mu g/cm^2$ of tissue depending for example on how the nucleic acid is combined with other agents promoting its action or acting in concert with the nucleic acids. This would be repeated at a frequency determined by the Doctor based on improvement. In another example, upper limits of concentration of the provided nucleic acids delivered internally for example, intramuscular, intracerebral, intracardicardiac and intraspinal would be 50-100 µg/ml of solution. Again, the frequency would be determined by the Doctor based on improvement.

[0105] Viral vectors remain highly experimental tools that nonetheless show considerable potential in clinical applications. As such, caution is warranted in calculation of expected dosage regimes for viral vectors and will depend considerably on the type of vector used. For example, retroviral vectors infect dividing cells such as cancer cells efficiently, intercalating into the host cell genome and continuing expression of encoded proteins indefinitely. Typical dosages of retroviruses in an animal model setting are in the range of 10^7 to 10^9 infectious units per ml. By contrast, adenoviruses most efficiently target post-mitotic cells, but cells are quickly eliminated by the host immune system or virus is eventually lost if infected cells resume proliferation and subsequently dilute the viral episomal DNA. Indeed, this transient time course of infection may be useful for short-term delivery of the composition described herein in certain clinical situations. In animal models, concentrations of 10^8-10^{11} infectious units per ml of adenovirus are typical for uses in research. Dose ranges of vectors based on data derived from animal models would be envisaged to be used eventually in clinical setting(s), pending the development of pharmaceutically acceptable formulation(s).

[0106] Two topical applications of ACT compositions at 0.02 % w/v; one applied acutely and the second applied 24 hours later can be used in treating or preventing pathologies involving epithelial permeablization and/or neovascularization. However, in a clinical setting an increased frequency of up to 3 applications per day topically at a concentration of up to 5 % is recommended until significant improvement is achieved as determined by a Doctor. For internal administration, for example, intravenously, intramuscularly, intracerebral, intracardicardiac and intraspinally and increased frequency of up to 3 dosages of 1 % w/v or v/v per day is recommended until significant improvement is determined by the Doctor.

[0107] Also provided are materials comprising the herein provided compositions (e.g., polypeptides, nucleic acids, or vectors). For example, provided are materials coated with an ACT polypeptide.

[0108] For example, the material can be soaked in the provided polypeptide at a concentration ranging from 10-200 μM. The material can then be dried and sealed in a sterile container. The material can also be immersed in liquid 10-30% pluronic gel at 4 °C containing polypeptide at 10-200 μM concentration. The material can then be brought to approximate room temperature so that the gel polymerizes, leaving a coat of polypeptide-impregnated gel surrounding the material, which can be sealed in a sterile container. The polypeptide can also be incorporated into a cross-linkable hydrogel system, such as the poly(lactic-co-glycolic acid) (PLGA) or polyurethane, which can then be fashioned into materials for treating a desired pathology. Thus, provided are composite hydrogel-peptide materials.

B. Methods of using the compositions

Macular Degeneration

[0109] By "macular degeneration" is meant the degeneration of the center of the inner lining of the eye, known as the macula. In some aspects, the macular degeneration is age-related macular degeneration (AMD). In some aspects, the macular degeneration is neovascular or exudative AMD, the wet form of advanced AMD.

[0110] In some aspects, the subject has been diagnosed with macular degeneration. In some aspects, the subject has been identified as being at risk of developing macular degeneration. Thus, the subject can be anyone over 50, 60, 65, 70, 75 years of age. In some aspects, the subject is known to smoke tobacco. In some aspects, the subject is known to have a relative with macular degeneration. In some aspects, the subject has been identified as having a single nucleotide polymorphism (SNP) associated with macular degeneration. For example, the SNP can be complement system protein factor H (CFH) Tyr402His. As another example, the SNP can be rs11200638 in HTRA1. In some aspects, the subject has been identified as having high blood pressure. In some aspects, the subject has been identified as having high cholesterol. In some aspects, the subject is obese. In some aspects, the subject has been identified as having drusen in the macula. In some aspects, the subject has been identified as having abnormal neovascularization of choriocapillaries through Bruch's membrane.

[0111] Macular degeneration is a medical condition predominantly found in elderly adults in which the center of the inner lining of the eye, known as the macula area of the retina, suffers thinning, atrophy, and in some cases, bleeding. This can result in loss of central vision, which entails inability to see fine details, to read, or to recognize faces. According to the American Academy of Ophthalmology, it is the leading cause of central vision loss (blindness) in the United States today for those over the age of fifty years. Although some macular dystrophies that affect younger individuals are sometimes referred to as macular degeneration, the term generally refers to age-related macular degeneration (AMD or ARMD).

[0112] Age-related macular degeneration begins with characteristic yellow deposits in the macula called drusen between the retinal pigment epithelium and the underlying choroid. Thus, also provided is a method of reducing or preventing drusen in the macula of a subject. Drusen are tiny yellow or white accumulations of extracellular material that build up in Bruch's membrane of the eye. The presence of a few small ("hard") drusen is normal with advancing age, and most people over 40 have some hard drusen. However, the presence of larger and more numerous drusen in the macula is a common early sign of age-related macular degeneration (AMD). Drusen associated with aging and macular degeneration are distinct from optic disc drusen, which are present in the optic nerve head. Both age-related drusen and optic disc drusen can be observed by ophthalmoscopy.

[0113] Most people with these early changes (referred to as age-related maculopathy) have good vision. People with drusen can go on to develop advanced AMD. The risk is considerably higher when the drusen are large and numerous and associated with disturbance in the pigmented cell layer under the macula. Recent research indicates that large and soft drusen are related to elevated cholesterol deposits and can respond to cholesterol lowering agents or the Rheo Procedure.

[0114] Advanced AMD, which is responsible for profound vision loss, has two forms: dry and wet. Central geographic atrophy, the dry form of advanced AMD, results from atrophy to the retinal pigment epithelial layer below the retina, which causes vision loss through loss of photoreceptors (rods and cones) in the central part of the eye. Vitamin supplements with high doses of antioxidants, lutein and zeaxanthin, have been demonstrated by the National Eye Institute and others to slow the progression of dry macular degeneration and in some patients, improve visual acuity.

[0115] Neovascular or exudative AMD, the wet form of advanced AMD, causes vision loss due to abnormal blood vessel growth in the choriocapillaries, through Bruch's membrane, ultimately leading to blood and protein leakage below the macula. Bleeding, leaking, and scarring from these blood vessels eventually cause irreversible damage to the photoreceptors and rapid vision loss if left untreated.

[0116] Anti-angiogenics or anti-VEGF (anti-Vascular Endothelial Growth Factor) agents, when injected directly into the vitreous humor of the eye using a small needle, can cause regression of the abnormal blood vessels and improvement of vision. The injections frequently have to be repeated on a monthly or bi-monthly basis. Examples of these agents include Lucentis, Avastin and Macugen. Only Lucentis and Macugen are FDA approved as of April 2007.

[0117] The Amsler Grid Test is one of the simplest and most effective methods for patients to monitor the health of the macula. The Amsler Grid is essentially a pattern of intersecting lines (identical to graph paper) with a black dot in the middle. The central black dot is used for fixation (a place for the eye to stare at). With normal vision, all lines surrounding the black dot will look straight and evenly spaced with no missing or odd looking areas when fixating on the grid's central black dot. When there is disease affecting the macula, as in macular degeneration, the lines can look bent, distorted and/or missing.

[0118] Macular degeneration by itself will not lead to total blindness. For that matter, only a very small number of people with visual impairment are totally blind. In almost all cases, some peripheral vision remains. Other complicating conditions may possibly lead to such an acute condition (severe stroke or trauma, untreated glaucoma, etc.), but few macular degeneration patients experience total visual loss. The area of the macula comprises about 5% of the retina and is responsible for about 35% of the visual field. The remaining 65% (the peripheral field) remains unaffected by the disease.

[0119] Similar symptoms with a very different etiology and different treatment can be caused by Epiretinal membrane or macular pucker or leaking blood vessels in the eye.

[0120] Fluorescein angiography allows for the identification and localization of abnormal vascular processes. Optical coherence tomography is now used by most ophthalmologists in the diagnosis and the follow-up evaluation of the response to treatment by using either Avastin or Lucentis which are injected into the vitreous of the eye at various intervals.

[0121] Juvenile macular degeneration is not a term in standard usage at this time. The preferred term for conditions that affect the macula in younger individuals related to genetics is macular dystrophy. Examples of these include: Best's disease, Doyne's honeycomb retinal dystrophy, Sorsby's disease, and Stargardt's disease.

[0122] In some aspects, subjects are identified by medical diagnosis. For example, subjects with diabetic retinopathy and macular degeneration can be identified by visualization of excess blood vessels in the eyes. Acute lung injury can be diagnosed by lung edema in the absence of congetive heart failure. Ischemic stroke can be diagnosed by neurologic presentation and imaging (MRI and CT). Other known or newly discovered medical determinations can be used to identify subjects for use in the disclosed methods.

[0123] In addition, subjects can be identified by genetic predisposition. For example, genes that predispose patients to age related macular degeneration have been identified (Klein RJ, et al, 2005; Yang Z, et al. 2006; Dewan A, et al. 2006). Likewise, genetic mutations that predispose patients to vascular malformations in the brain have been identified (Plummer NW, et al., 2005). Other known or newly discovered genetic determinations can be used to identify subjects for use in the disclosed methods.

Administration

[0124] The disclosed compounds and compositions can be administered in any suitable manner. The manner of administration can be chosen based on, for example, whether local or systemic treatment is desired, and on the area to be treated. For example, the compositions can be administered orally, parenterally (e.g., by injection to target specific tissues, organs and parts of the body, intravenous, intraocular, intra-tumor, intra-joint, intracardiac, intraperitoneal, or intramuscular injection), by inhalation, extracorporeally, topically (including transdermally, ophthalmically, vaginally, rectally, intranasally) or the like.

[0125] The route of administration and the dosage regimen will be determined by skilled clinicians, based on factors such as the exact nature of the condition being treated, the severity of the condition, and the age and general physical condition of the patient. Specific routes of administration to the eye may include topical application (such as by eyedrops, creams or erodible

formulations to be placed under the eyelid), intraocular injection into the aqueous or the vitreous humor, injection into the external layers of the eye, such as via subconjunctival injection or subtenon injection, parenteral administration or via oral routes. For example, provided are eye drops comprising one or more of the herein disclosed compositions.

[0126] The herein disclosed compositions, including peptides, can be combined with a targeting protein, compound, nanotechnological device, or cell that can target the peptide to the site of the pathology. For example, provided is an antibody linked to one or more peptides disclosed herein, wherein the antibody selectively homes and binds to tissue at the site of the pathology. Also provided is an aptamer linked to one or more peptides disclosed herein, wherein the aptamer selectively homes and binds to tissue at the site of the pathology. Also provided is a cell linked to one or more peptides disclosed herein, wherein the cell selectively homes and binds to tissue at the site of the pathology.

[0127] Administration can further be by way of intraocular implant. For example, the implant can deliver a regular dose of the ACT peptide to the anterior chamber, posterior chamber, retina, macula, retinal pigment epithelium, choroid, Bruch's membrane, vitreous, cornea, or lens. Thus, provided herein is a method comprising introducing an intraocular implant into the eye of a subject, wherein the intraocular implant releases one or more peptides disclosed herein to the anterior chamber, posterior chamber, retina, macula, retinal pigment epithelium, choroid, Bruch's membrane, vitreous, cornea, or lens.

[0128] Ophthalmic products for topical use may be packaged in multidose form. Preservatives are thus required to prevent microbial contamination during use. Suitable preservatives include: benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, polyquatemium-1, or other agents known to those skilled in the art. Such preservatives are typically employed at a level of from 0.001 to 1.0% weight/volume ("% w/v"). Such preparations may be packaged in dropper bottles or tubes suitable for safe administration to the eye, along with instructions for use.

[0129] When the ophthalmic compositions disclosed herein are administered during intraocular surgical procedures, such as through retrobulbar or periocular injection and intraocular perfusion or injection, the use of balanced salt irrigating solutions as vehicles are most preferred. BSS Sterile Irrigating Solution and BSS Plus.RTM. Sterile Intraocular Irrigating Solution (Alcon Laboratories, Inc., Fort Worth, Tex. USA) are examples of physiologically balanced intraocular irrigating solutions. The latter type of solution is described in U.S. Pat. No. 4,550,022 (Garabedian, et al.). Retrobulbar and periocular injections are known to those skilled in the art and are described in numerous publications including, for example, Ophthalmic Surgery: Principles of Practice, Ed., G. L. Spaeth. W. B. Sanders Co., Philadelphia, Pa., U.S.A., (1990).

[0130] The ophthalmic compositions may also be used as an adjunct to ophthalmic surgery, such as by intra vitreal or subconjunctival injection following ophthalmic surgery. The compounds may be used for acute treatment of temporary conditions, or may be administered chronically, especially in the case of degenerative disease. The ophthalmic compositions may also be used prophylactically, especially prior to ocular surgery or noninvasive ophthalmic procedures or other types of surgery.

[0131] Pharmaceutical compositions (also referred to herein as "ophthalmic compositions") that include a peptide disclosed herein and a pharmaceutically acceptable carrier may be packed with instructions for use of the pharmaceutical composition for treatment and/or prevention of, for example, macular degeneration. The ingredients may be packaged together in the form of a kit.

[0132] As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the nucleic acid or vector. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation.

[0133] Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795.

[0134] The exact amount of the compositions required can vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the macular degeneration, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. Thus, effective dosages and schedules for administering the compositions may be determined empirically, and making such

determinations is within the skill in the art. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms disorder are effected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage can vary with the age, condition, sex and extent of the disease in the patient, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counter indications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products.

[0135] Some of the compositions may potentially be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

[0136] Effective dosages and schedules for administering the compositions may be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms disorder are effected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual doctor in the event of any counterindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. The range of dosage largely depends on the application of the compositions herein, severity of condition, and its route of administration.

[0137] For example, in applications as a laboratory tool for research, the ACT peptide compositions can be used in doses as low as 0.01% w/v. The dosage can be as low as 0.02% w/v and as high as 5% w/v. Significantly higher concentrations of the compositions by themselves or in combination with other compounds may be used in applications. Recommended upper limits of dosage for parenteral routes of administration for example intramuscular, intracerebral, intracardicardiac and intraspinal could be up to 1 % w/v or v/v.This upper dosage limit may vary by formulation, depending for example on how the polypeptide(s) is combined with other agents promoting its action or acting in concert with the polypeptide(s).

[0138] For continuous delivery of the provided polypeptides, for example, in combination with an intravenous drip, upper limits of 0.01g /Kg body weight over time courses determined by the doctor based on improvement in the condition can be used. In another example, upper limits of concentration of the provided nucleic acids delivered topically would be 5-10 $\mu g/cm^2$. This would be repeated at a frequency determined by the Doctor based on improvement. In another example, upper limits of concentration of the provided nucleic acids delivered internally for example, intramuscular, intracerebral, intracardicardiac and intraspinal would be 50-100 µg/ml of solution. Again, the frequency would be determined by the Doctor based on improvement.

[0139] Viral vectors remain highly experimental tools that nonetheless show considerable potential in clinical applications. As such, caution is warranted in calculation of expected dosage regimes for viral vectors and will depend considerably on the type of vector used. For example, retroviral vectors infect dividing cells such as cancer cells efficiently, intercalating into the host cell genome and continuing expression of encoded proteins indefinitely. Typical dosages of retroviruses in an animal model setting are in the range of 10^7 to 10^9 infectious units per ml. By contrast, adenoviruses most efficiently target post-mitotic cells, but cells are quickly eliminated by the host immune system or virus is eventually lost if infected cells resume proliferation and subsequently dilute the viral episomal DNA. Indeed, this transient time course of infection may be useful for short-term delivery of the composition described herein in certain clinical situations. In animal models, concentrations of 10^8-10^{11} infectious units per ml of adenovirus are typical for uses in research. Dose ranges of vectors based on data derived from animal models would be envisaged to be used eventually in clinical setting(s), pending the development of pharmaceutically acceptable formulation(s).

[0140] Following administration of a disclosed composition, such as a polypeptide, the efficacy of the therapeutic composition can be assessed in various ways well known to the skilled practitioner. For instance, one of ordinary skill in the art will understand that a composition, such as a polypeptide, disclosed herein is efficacious in treating or preventing macular degeneration in a subject by observing that the composition can reduce neovascularization, or improve vision. Methods for measuring these criteria are known in the art and discussed herein.

C. Methods of making the compositions

[0141] The compositions disclosed herein and the compositions necessary to perform the disclosed methods can be made using any method known to those of skill in the art for that particular reagent or compound unless otherwise specifically noted.

[0142] For example, the provided nucleic acids can be made using standard chemical synthesis methods or can be produced using enzymatic methods or any other known method. Such methods can range from standard enzymatic digestion followed by nucleotide fragment isolation (see for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edition (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) Chapters 5, 6) to purely synthetic methods, for example, by the cyanoethyl phosphoramidite method using a Milligen or Beckman System 1Plus DNA synthesizer (for example, Model 8700 automated synthesizer of Milligen-Biosearch, Burlington, MA or ABI Model 380B). Synthetic methods useful for making oligonucleotides are also described by Ikuta et al., Ann. Rev. Biochem. 53:323-356 (1984), (phosphotriester and phosphite-triester methods), and Narang et al., Methods Enzymol., 65:610-620 (1980), (phosphotriester method). Protein nucleic acid molecules can be made using known methods such as those described by Nielsen et al., Bioconjug. Chem. 5:3-7 (1994).

[0143] One method of producing the disclosed polypeptides, such as SEQ ID NO:2, is to link two or more peptides or polypeptides together by protein chemistry techniques. For example, peptides or polypeptides can be chemically synthesized using currently available laboratory equipment using either Fmoc (9-fluorenylmethyloxycarbonyl) or Boc (*tert* -butyloxycarbonoyl) chemistry. (Applied Biosystems, Inc., Foster City, CA). One skilled in the art can readily appreciate that a peptide or polypeptide corresponding to the disclosed proteins, for example, can be synthesized by standard chemical reactions. For example, a peptide or polypeptide can be synthesized and not cleaved from its synthesis resin whereas the other fragment of a peptide or protein can be synthesized and subsequently cleaved from the resin, thereby exposing a terminal group which is functionally blocked on the other fragment. By peptide condensation reactions, these two fragments can be covalently joined via a peptide bond at their carboxyl and amino termini, respectively, to form a protein, or fragment thereof (Grant GA (1992) Synthetic Peptides: A User Guide. W.H. Freeman and Co., N.Y. (1992); Bodansky M and Trost B., Ed. (1993) Principles of Peptide Synthesis. Springer-Verlag Inc., NY. Alternatively, the peptide or polypeptide is independently synthesized *in vivo* as described herein. Once isolated, these independent peptides or polypeptides may be linked to form a peptide or fragment thereof via similar peptide condensation reactions.

[0144] For example, enzymatic ligation of cloned or synthetic peptide segments allow relatively short peptide fragments to be joined to produce larger peptide fragments, polypeptides or whole protein domains (Abrahmsen L et al., Biochemistry, 30:4151 (1991)). Alternatively, native chemical ligation of synthetic peptides can be utilized to synthetically construct large peptides or polypeptides from shorter peptide fragments. This method consists of a two step chemical reaction (Dawson et al. Synthesis of Proteins by Native Chemical Ligation. Science, 266:776-779 (1994)). The first step is the chemoselective reaction of an unprotected synthetic peptide-thioester with another unprotected peptide segment containing an amino-terminal Cys residue to give a thioester-linked intermediate as the initial covalent product. Without a change in the reaction conditions, this intermediate undergoes spontaneous, rapid intramolecular reaction to form a native peptide bond at the ligation site (Baggiolini M et al. (1992) FEBS Lett. 307:97-101; Clark-Lewis I et al., J.Biol.Chem., 269:16075 (1994); Clark-Lewis I et al., Biochemistry, 30:3128 (1991); Rajarathnam K et al., Biochemistry 33:6623-30 (1994)).

[0145] Alternatively, unprotected peptide segments are chemically linked where the bond formed between the peptide segments as a result of the chemical ligation is an unnatural (non-peptide) bond (Schnolzer, M et al. Science, 256:221 (1992)). This technique has been used to synthesize analogs of protein domains as well as large amounts of relatively pure proteins with full biological activity (deLisle Milton RC et al., Techniques in Protein Chemistry IV. Academic Press, New York, pp. 257-267 (1992)).

[0146] Disclosed are processes for making the compositions as well as the intermediates leading to the compositions. There are a variety of methods that can be used for making these compositions, such as synthetic chemical methods and standard molecular biology methods. It is understood that the methods of making these and the other disclosed compositions are specifically disclosed. Disclosed are nucleic acid molecules produced by the process comprising linking in an operative way a nucleic acid encoding a polypeptide disclosed herein and a sequence controlling the expression of the nucleic acid. Disclosed are cells produced by the process of transforming the cell with any of the herein disclosed nucleic acids. Disclosed are any of the disclosed peptides produced by the process of expressing any of the herein disclosed nucleic acids. Disclosed are animals produced by the process of transfecting a cell within the animal with any of the nucleic acid molecules disclosed herein. Disclosed are animals produced by the process of transfecting a cell within the animal any of the nucleic acid molecules disclosed herein, wherein the animal is a mammal. Also disclosed are animals produced by the process of transfecting a cell within the animal any of the nucleic acid molecules disclosed herein, wherein the mammal is mouse, rat, rabbit, cow, sheep, pig, or primate. Also disclose are animals produced by the process of adding to the animal any of the cells disclosed herein.

D. Definitions

[0147] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed method and compositions belong. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present method and compositions, the particularly useful methods, devices, and materials are as described. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such disclosure by virtue of prior invention. No admission is made that any reference constitutes prior art. The discussion of references states what their authors assert, and applicants reserve the right to challenge the accuracy and pertinency of the cited documents.

[0148] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a peptide" includes a plurality of such peptides, reference to "the peptide" is a reference to one or more peptides and equivalents thereof known to those skilled in the art, and so forth.

[0149] The term "therapeutically effective" means that the amount of the composition used is of sufficient quantity to ameliorate one or more causes or symptoms of a disease or disorder. Such amelioration only requires a reduction or alteration, not necessarily elimination. The term "carrier" means a compound, composition, substance, or structure that, when in combination with a compound or composition, aids or facilitates preparation, storage, administration, delivery, effectiveness, selectivity, or any other feature of the compound or composition for its intended use or purpose. For example, a carrier can be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject.

[0150] By "treat" or "treatment" is meant a method of reducing the effects of a disease or condition. Treatment can also refer to a method of reducing the underlying cause of the disease or condition itself rather than just the symptoms. The treatment can be any reduction from native levels and can be but is not limited to the complete ablation of the disease, condition, or the symptoms of the disease or condition. For example, a disclosed method for treating macular degeneration is considered to be a treatment if there is a 10% reduction in one or more symptoms of the disease in a subject with the disease when compared to native levels in the same subject or control subjects. Thus, the reduction can be a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between as compared to native or control levels.

[0151] By "prevent" or other forms of prevent means to stop a particular characteristic or condition from developing or from progressing. Prevent does not require comparison to a control as it is typically more absolute than, for example, reduce or inhibit. As used herein, something could be reduced but not inhibited or prevented, but something that is reduced could also be inhibited or prevented. It is understood that where reduce, inhibit or prevent are used, unless specifically indicated otherwise, the use of the other two words is also expressly disclosed. Thus, if inhibition of permeability is disclosed, then reduction and prevention of permeability are also disclosed.

[0152] As used herein, "subject" includes, but is not limited to, animals, plants, bacteria, viruses, parasites and any other organism or entity that has nucleic acid. The subject may be a vertebrate, more specifically a mammal (e.g., a human, horse, pig, rabbit, dog, sheep, goat, non-human primate, cow, cat, guinea pig or rodent), a fish, a bird or a reptile or an amphibian. The subject can be an invertebrate, more specifically an arthropod (e.g., insects and crustaceans). The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered. A patient refers to a subject afflicted with a disease or disorder. The term "patient" includes human and veterinary subjects.

[0153] "Optional" or "optionally" means that the subsequently described event, circumstance, or material may or may not occur or be present, and that the description includes instances where the event, circumstance, or material occurs or is present and instances where it does not occur or is not present.

[0154] Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that when a value is disclosed that "less than or equal to" the value, "greater than or equal to the value" and possible ranges between values are also disclosed, as

appropriately understood by the skilled artisan. For example, if the value "10" is disclosed the "less than or equal to 10"as well as "greater than or equal to 10" is also disclosed. It is also understood that the throughout the application, data is provided in a number of different formats, and that this data, represents endpoints and starting points, and ranges for any combination of the data points. For example, if a particular data point "10" and a particular data point 15 are disclosed, it is understood that greater than, greater than or equal to, less than, less than or equal to, and equal to 10 and 15 are considered disclosed as well as between 10 and 15. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

[0155] Throughout the description and claims of this specification, the word "comprise" and variations of the word, such as "comprising" and "comprises," means "including but not limited to," and is not intended to exclude, for example, other additives, components, integers or steps.

E. Examples

[0156] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the disclosure. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

1. Example 1

[0157] As shown in Fig. 1, the alpha connexin carboxy-terminal (ACT) polypeptide ACT1 prevents VEGF-induced deterioration of TER in ARPE-19 cells. Trans-epithelial resistance (TER) measurements, using ARPE19 cell (immortalized human RPE cells) monolayers revealed that VEGF leads to rapid deterioration, which was blocked by pretreating the cells with the ACT peptide. Thus, while not wishing to be bound by theory, stabilizing the tight junction proteins with the ACT peptide can prevent loss of tight-junction disintegration and thus damage to RPE/Bruch's membrane.

[0158] ACT1 Peptide contains an amino terminal cell internalization sequence. Together with a mild detergent that is used in ocular applications, Brij-78 the antenapedia sequence assists in permeation of ACT1 into interior fluids and tissues of the eye. In some aspects, the ability of ACT1 to enter the internal fluids and tissues of eye is a mode of action of ACT1 in treating diseases of the eye such as macular degeneration.

i. Results

[0159] Application of ACT1 peptide in a solution containing 0.05 % Brij-78 to the cornea of mouse eyes resulted in a detectable level of ACT1 in the internal fluids of the anterior chamber (i.e., the aqueous humor) 20 and 40 minutes post-application (Fig. 2). Lower levels of ACT1 could also be detected by Western blotting in fluid from the posterior chamber of eye 20 and 40 minutes, i.e., the vitreous humor.

[0160] Following application of ACT1 in a solution containing 0.05 % Brij-78 to the cornea of mouse eyes, ACT1 was detectable in the retinal pigment epithelial layer of eye minutes post-application. Moreover, ACT1 was immunohistochemically detected in the retinal pigment epithelial layer of eyes exposed to the peptide, but not to the vehicle control solution via corneal application.

ii. Methods:

[0161] Three CD1 mice were anesthetized by IP injection of 0.2 mL Salazine/ketamine. 10µL of 1mM ACT1 peptide dissolved in a solution containing normal saline and 0.05% Brij-78 was gently dripped onto the corneal surface of both eyes and allowed to permeate for 20 or 40 min. 0.05% Brij-78 in normal saline was used on a control mouse. The mice were sacrificed in a CO_2 chamber and cervically dislocated at 20, 40 min (the control mouse sacrificed at 20 min). The eyes were removed and rinsed in PBS. A small incision was made in the anterior chamber and the aqueous humor (~10 µL) was transferred to tube and flash frozen in a dry ice ethanol bath. The total sample was dissolved in 2x samples loading buffer and loaded on a 10-20% Tris-Tricine gel.

Gel was transferred to a PDVF membrane and stained using RBT Sigma anti-CX43 CT antibody (1:10000) and a goat anti-RBT AP secondary (1:15000) to reveal the ACT1 band at <10kDa.

[0162] Application of ACT1 to the cornea in Brij-78 was the same as described above. After sacrifice the mouse eyes were removed, washed in PBS briefly, and transferred to 5% Paraformaldehyde overnight. The eyes were embedded in paraffin, sectioned, and stained with Sigma Rbt anti-Cx43, streptavidin and Hoeschst stain and placed at 4 degrees overnight. As disclosed herein, ACT1 is detectable in the interior fluids and tissues of the eye following a simple corneal exposure.

F. Sequences

[0163]

SEQ ID NO:1 (ACT 2)
PSSRASSRASSRPRPDDLEI

SEQ ID NO:2 (ACT 1)
RPRPDDLEI

SEQ ID NO:3 (ACT 3)
RPRPDDLEV

SEQ ID NO:4 (ACT 4)
RPRPDDVPV

SEQ ID NO:5 (ACT 5)
KARSDDLSV

SEQ ID NO:6
aga cct cgg cct gat gac ctg gag att

SEQ ID NO:7 (Antp)
RQPKIWFPNRRKPWKK

SEQ ID NO:8 (Antp/ ACT 2)
RQPKIWFPNRRKPWKKPSSRASSRASSRPRPDDLEI

SEQ ID NO:9 (Antp/ ACT 1)
RQPKIWFPNRRKPWKKRPRPDDLEI

SEQ ID NO:10 (Antp/ ACT 3)
RQPKIWFPNRRKPWKKRPRPDDLEV

SEQ ID NO:11 (Antp/ ACT 4)
RQPKIWFPNRRKPWKKRPRPDDVPV

SEQ ID NO:12 (Antp/ ACT 5)
RQPKIWFPNRRKPWKKKARSDDLSV

SEQ ID NO:13 (encodes polypeptide of SEQ ID NO 9)
cgg cag ccc aag atc tgg ttc ccc aac cgg cgg aag ccc tgg aag aag cgg ccc ggc ccg acg acc tgg aga tc

SEQ ID NO:14 (HIV-Tat)
GRKKRRQRPPQ

SEQ ID NO:15 (Penetratin)
RQIKIWFQNRRMKWKK

SEQ ID NO:16 (Antp-3A)
RQIAIWFQNRRMKWAA

SEQ ID NO:17 (Tat)
RKKRRQRRR

SEQ ID NO:18 (Buforin II)
TRSSRAGLQFPVGRVHRLLRK

SEQ ID NO:19 (Transportan)
GWTLNSAGYLLGKINKALAALAKKIL

SEQ ID NO:20 (model amphipathic peptide)
KLALKLALKALKAALKLA

SEQ ID NO:21 (K-FGF)
AAVALLPAVLLALLAP

SEQ ID NO:22 (Ku70)
VPMLK-PMLKE

SEQ ID NO:23 (Prion)
MANLGYWLLALFVTMWTDVGLCKKRPKP

SEQ ID NO:24 (pVEC)
LLIILRRRIRKQAHAHSK

SEQ ID NO:25 (Pep-1)
KETWWETWWTEWSQPKKKRKV

SEQ ID NO:26 (SynB1)
RGGRLSYSRRRFSTSTGR

SEQ ID NO:27 (Pep-7)
SDLWEMMMVSLACQY

SEQ ID NO:28 (HN-1)
TSPLNIHNGQKL

SEQ ID NO:29 (Chick alpha Cx43 ACT)
PSRASSRASSRPRPDDLEI

SEQ ID NO:30 (Human alpha Cx45)
GSNKSTASSKSPDPKNSVWI

SEQ ID NO:31 (Chick alpha Cx45)
GSNKSSASSKSGDGKNSVWI

SEQ ID: 32 (Human alpha Cx46)
GRASKASRASSGRARPEDLAI

SEQ ID: 33 (Human alpha Cx46.6)
GSASSRDGKTVWI

SEQ ID NO:34 (Chimp alpha Cx36)
PRVSVPNFGRTQSSDSAYV

SEQ ID NO:35 (Chick alpha Cx36)
PRMSMPNFGRTQSSDSAYV

SEQ ID NO:36 (Human alpha Cx47)
PRAGSEKGSASSRDGKTTVWI

SEQ ID NO:37 (Human alpha Cx40)
GYHSDKRRLSKASSKARSDDLSV

SEQ ID NO:38 (Human alpha Cx50)
PLSRLSKASSRARSDDLTV

SEQ ID NO:39 (Human alpha Cx59)
PNHVVSLTNNLIGRRVPTDLQI

SEQ ID NO:40 (Rat alpha Cx33)
PSCVSSSAVLTTICSSDQVVPVGLSSFYM

SEQ ID NO:41 (Sheep alpha Cx44)
GRSSKASKSSGGRARAADLAI

SEQ ID NO:42 (Human beta Cx26)
LCYLLIRYCSGKSKKPV

SEQ ID: 43 (Human alpha Cx37)
G QK PP SRPS SSAS K KQ*YV

SEQ ID 44: (conservative Cx43 variant)
SSRASSRASSRPRPDDLEV

SEQ ID 45: (conservative Cx43 variant)
RPKPDDLEI,

SEQ ID 46: (conservative Cx43 variant)
SSRASSRASSRPKPDDLEI,

SEQ ID 47: (conservative Cx43 variant)
RPKPDDLDI

SEQ ID 48: (conservative Cx43 variant)
SSRASSRASSRPRPDDLDI

SEQ ID 49: (conservative Cx43 variant)
SSRASTRASSRPRPDDLEI

SEQ ID 50: (conservative Cx43 variant)
RPRPEDLEI

SEQ ID 51: (conservative Cx43 variant)
SSRASSRASSRPRPEDLEI,

SEQ ID 52: (conservative Cx45 variant)
GDGKNSVWV

SEQ ID 53: (conservative Cx45 variant)
SKAGSNKSTASSKSGDGKNSVWV

SEQ ID 54: (conservative Cx37 variant)
GQKPPSRPSSSASKKLYV

SEQ ID NO: 55 (non-active control peptide)
RQPKIWFPNRRKPWKIELDDPRPR

SEQ ID NO:56 (HIV-Tat/ ACT 1)
GRKKRRQRPPQ RPRPDDLEI

SEQ ID NO:57 (Penetratin/ ACT 1)
RQIKIWFQNRRMKWKK RPRPDDLEI

SEQ ID NO:58 (Antp-3A/ ACT 1)
RQIAIWFQNRRMKWAA RPRPDDLEI

SEQ ID NO:59 (Tat/ ACT 1)
RKKRRQRRR RPRPDDLEI

SEQ ID NO:60 (Buforin II/ ACT 1)
TRSSRAGLQFPVGRVHRLLRK RPRPDDLEI

SEQ ID NO:61 (Transportan/ ACT 1)

GWTLNSAGYLLGKINKALAALAKKIL RPRPDDLEI

SEQ ID NO:62 (MAP/ ACT 1)
KLALKLALKALKAALKLA RPRPDDLEI

SEQ ID NO:63 (K-FGF/ ACT 1)
AAVALLPAVLLALLAP RPRPDDLEI

SEQ ID NO:64 (Ku70/ ACT 1)
VPMLKPMLKE RPRPDDLEI

SEQ ID NO:65(Prion/ ACT 1)
MANLGYWLLALFVTMWTDVGLCKKRPKP RPRPDDLEI

SEQ ID NO:66 (pVEC/ ACT 1)
LLIILRRRIRKQAHAHSK RPRPDDLEI

SEQ ID NO:67 (Pep-1/ ACT 1)
KETWWETWWTEWSQPKKKRKV RPRPDDLEI

SEQ ID NO:68 (SynB1/ ACT 1)
RGGRLSYSRRRFSTSTGR RPRPDDLEI

SEQ ID NO:69 (Pep-7/ ACT 1)
SDLWEMMMVSLACQY RPRPDDLEI

SEQ ID NO:70 (HN-1/ ACT 1)
TSPLNIHNGQKL RPRPDDLEI

SEQ ID NO: 71 (20 to 120 residues flanking amino acid 363 of human Cx43)
KGKSDPYHATSGALSPAKDCGSQKYAYFNGCSSPTAPLSPMSPPGYKLVT
GDRNNSSCRNYNKQASEQNWANYSAEQNRMGQAGSTISNSHAQPFDFPDD
NQNSKKLAAGHELQPLAIVDQR

SEQ ID NO: 72 (20 to 120 residues flanking amino acid 362 of chick Cx43)
KTDPYSHSGTMSPSKDCGSPKYAYYNGCSSPTAPLSPMSPPGYKLVTGDRNNSSCRNYNKQAS
EQNWANYSAEQNRMGQAGSTISNSHAQPFDFADEHQNTKKLASGHELQPLTIVDQRP

SEQ ID NO: 73 (20 to 120 residues flanking amino acid 377 of human Cx45)
LGFGTIRDSLNSKRRELEDPGAYNYPFTWNTPSAPPGYNIAVKPDQIQYTELSNAKIAYKQNKANT
AQEQQYGSHEENLPADLEALQREIRMAQERLDLAVQAYSHQNNPHGPREKKAKV

SEQ ID NO: 74 (20 to 120 residues flanking amino acid 375 of chick Cx45)
GFGTIRDTLNNKRKELEDSGTYNYPFTWNTPSAPPGYNIAVKPDQMQYTELSNAKMAYKQNKANI
AQEQQYGSNEENIPADLENLQREIKVAQERLDMAIQAYNNQNNPGSSSREKKSKA.

SEQ ID NO: 75 (20 to 120 residues flanking amino acid 313 of human Cx37)
PYLVDCFVSRPTEKTIFIIFMLVVGLISLVLNLLELVHLLCRCLSRGMRARQGQDAPPTQGTSSDPY
TDQVFFYLPVGQGPSSPPCPTYNGLSSSEQNWANLTTEERLASSRPPLFLDPP

SEQ ID NO: 76 (20 to 120 residues flanking amino acid 258 of rat Cx33)
CGSKEHGNRKMRGRLLLTYMASIFFKSVFEVAFLLIQWYLYGFTLSAVYICEQSPCPHRVDCFLSR
PTEKTIFILFMLVVSMVSFVLNVIELFYVLFKAIKNHLGNEKEEVYCNPVELQK.

SEQ ID NO: 77 (enhanced green fluorescent protein)
MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICT
TGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIF
FKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHN
VYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNH
YLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK

SEQ ID NO:78 (ACT 2)
CCCTCCTCCCGGGCCTCCTCCCGGGCCTCCTCCCGGCCCCGGCCCGAC GACCTGGAGATC

SEQ ID NO:79(ACT 1)
CGGCCCCGGCCCGACGACCTGGAGATC

SEQ ID NO:80 (ACT 3)
CGGCCCCGGCCCGACGACCTGGAGGTG

SEQ ID NO:81 (ACT 4)

CGGCCCCGGCCCGACGACGTGCCCGTG

SEQ ID NO:82 (ACT 5)
AAGGCCCGGTCCGACGACCTGTCCGTG

SEQ ID NO:83 (Antp)
CGGCAGCCCAAGATCTGGTTCCCCAACCGGCGGAAGCCCTGGAAG AAG

SEQ ID NO:84 (Antp/ ACT 2)
CGGCAGCCCAAGATCTGGTTCCCCAACCGGCGGAAGCCCTGGAAG
AAGCCCTCCTCCCGGGCCTCCTCCCGGGCCTCCTCCCGGCCCCGGCCC
GACGACCTGGAGATC

SEQ ID NO:85 (Antp/ ACT 1)
CGGCAGCCCAAGATCTGGTTCCCCAACCGGCGGAAGCCCTGGAAGAAGCGGCCCCGGCCC
GACGACCTGGAGATC

SEQ ID NO:86 (Antp/ ACT 3)
CGGCAGCCCAAGATCTGGTTCCCCAACCGGCGGAAGCCCTGGAAGAAGCGGCCCCGGCCC
GACGACCTGGAGGTG

SEQ ID NO:87 (Antp/ ACT 4)
CGGCAGCCCAAGATCTGGTTCCCCAACCGGCGGAAGCCCTGGAAGAAGCGGCCCCGGCCC
GACGACGTGCCCGTG

SEQ ID NO:88 (Antp/ ACT 5)
CGGCAGCCCAAGATCTGGTTCCCCAACCGGCGGAAGCCCTGGAAGAAGAAGGCCCGGTCC
GACGACCTGTCCGTG

SEQ ID NO:89 (Zebrafish alpha Cx43)
PCSRASSRMSSRARPDDLDV

SEQ ID NO:90 (Chick alpha Cx36)
PRVSVPNFGRTQSSDSAYV

SEQ ID NO:91 (Zebrafish alpha Cx36)
P RMSM PNFG R TQ SSD S AYV

SEQ ID NO:92 (Cx43 isoleucine deletion)
RQPKIWFPNRRKPWKKRASSRASSRPRPDDLE

SEQUENCE LISTING

[0164]

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<130> SMW FP6670582

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<141> 2008-06-23

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<400> 1
Pro Ser Ser Arg Ala Ser Ser Arg Ala Ser Ser Arg Pro Arg Pro Asp
1 5 10 15
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<400> 2
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1 5

<210> 3
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<400> 3
Arg Pro Arg Pro Asp Asp Leu Glu Val
1 5

<210> 4
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<400> 4
Arg Pro Arg Pro Asp Asp Val Pro Val
1 5

<210> 5
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<400> 5
Lys Ala Arg Ser Asp Asp Leu Ser Val
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<210> 6
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<210> 7
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<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 7
Arg Gln Pro Lys Ile Trp Phe Pro Asn Arg Arg Lys Pro Trp Lys Lys
1 5 10 15

<210> 8
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<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 8
Arg Gln Pro Lys Ile Trp Phe Pro Asn Arg Arg Lys Pro Trp Lys Lys
1 5 10 15
Pro Ser Ser Arg Ala Ser Ser Arg Ala Ser Ser Arg Pro Arg Pro Asp
20 25 30
Asp Leu Glu Ile

35

<210> 9
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<400> 9
Arg Gln Pro Lys Ile Trp Phe Pro Asn Arg Arg Lys Pro Trp Lys Lys
1 5 10 15
Arg Pro Arg Pro Asp Asp Leu Glu Ile
20 25

<210> 10
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Arg Gln Pro Lys Ile Trp Phe Pro Asn Arg Arg Lys Pro Trp Lys Lys
1 5 10 15
Arg Pro Arg Pro Asp Asp Leu Glu Val
20 25

<210> 11
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<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 11

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Arg Gln Pro Lys Ile Trp Phe Pro Asn Arg Arg Lys Pro Trp Lys Lys
 1               5                  10                  15
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            20                  25
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<210> 12

<211> 25

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<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 12

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Arg Gln Pro Lys Ile Trp Phe Pro Asn Arg Arg Lys Pro Trp Lys Lys
 1               5                  10                  15
Lys Ala Arg Ser Asp Asp Leu Ser Val
            20                  25
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<210> 13

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<400> 13

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<210> 14

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<400> 14

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<210> 15

<211> 16

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<400> 15

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Arg Gln Ile Lys Ile Trp Phe Gln Asn Arg Arg Met Lys Trp Lys Lys
 1               5                  10                  15
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<210> 16

<211> 16

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 16

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 1               5                  10                  15
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<210> 17

<211> 9

<212> PRT
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<400> 17
Arg Lys Lys Arg Arg Gln Arg Arg Arg
1 5

<210> 18
<211> 21
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Thr Arg Ser Ser Arg Ala Gly Leu Gln Phe Pro Val Gly Arg Val His
1 5 10 15
Arg Leu Leu Arg Lys
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<210> 19
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<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 19
Gly Trp Thr Leu Asn Ser Ala Gly Tyr Leu Leu Gly Lys Ile Asn Lys
1 5 10 15
Ala Leu Ala Ala Leu Ala Lys Lys Ile Leu
20 25

<210> 20
<211> 18
<212> PRT
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<220>
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<400> 20
Lys Leu Ala Leu Lys Leu Ala Leu Lys Ala Leu Lys Ala Ala Leu Lys
1 5 10 15
Leu Ala

<210> 21
<211> 16
<212> PRT
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<400> 21
Ala Ala Val Ala Leu Leu Pro Ala Val Leu Leu Ala Leu Leu Ala Pro
1 5 10 15

<210> 22
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<400> 22
Val Pro Met Leu Lys Pro Met Leu Lys Glu
1 5 10

<210> 23
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<220>
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<400> 23
Met Ala Asn Leu Gly Tyr Trp Leu Leu Ala Leu Phe Val Thr Met Trp
1 5 10 15
Thr Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro
20 25

<210> 24
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 24
Leu Leu Ile Ile Leu Arg Arg Arg Ile Arg Lys Gln Ala His Ala His
1 5 10 15
Ser Lys

<210> 25
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 25
Lys Glu Thr Trp Trp Glu Thr Trp Trp Thr Glu Trp Ser Gln Pro Lys
1 5 10 15
Lys Lys Arg Lys Val
20

<210> 26
<211> 18
<212> PRT
<213> Artificial Sequence

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<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 26
Arg Gly Gly Arg Leu Ser Tyr Ser Arg Arg Arg Phe Ser Thr Ser Thr
1 5 10 15

Gly Arg

<210> 27
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 27
Ser Asp Leu Trp Glu Met Met Met Val Ser Leu Ala Cys Gln Tyr
1 5 10 15

<210> 28
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<400> 28
Thr Ser Pro Leu Asn Ile His Asn Gly Gln Lys Leu
1 5 10

<210> 29
<211> 19
<212> PRT
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<220>
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<400> 29
Pro Ser Arg Ala Ser Ser Arg Ala Ser Ser Arg Pro Arg Pro Asp Asp
1 5 10 15
Leu Glu Ile

<210> 30
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 30
Gly Ser Asn Lys Ser Thr Ala Ser Ser Lys Ser Pro Asp Pro Lys Asn
1 5 10 15
Ser Val Trp Ile
20

<210> 31
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 31
Gly Ser Asn Lys Ser Ser Ala Ser Ser Lys Ser Gly Asp Gly Lys Asn
1 5 10 15
Ser Val Trp Ile
20

<210> 32
<211> 21
<212> PRT
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<400> 32
Gly Arg Ala Ser Lys Ala Ser Arg Ala Ser Ser Gly Arg Ala Arg Pro
1 5 10 15
Glu Asp Leu Ala Ile
20

<210> 33
<211> 13
<212> PRT
<213> Artificial Sequence

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<400> 33
Gly Ser Ala Ser Ser Arg Asp Gly Lys Thr Val Trp Ile
1 5 10

<210> 34
<211> 19
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<400> 34
Pro Arg Val Ser Val Pro Asn Phe Gly Arg Thr Gln Ser Ser Asp Ser
1 5 10 15
Ala Tyr Val

<210> 35
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<400> 35
Pro Arg Met Ser Met Pro Asn Phe Gly Arg Thr Gln Ser Ser Asp Ser
1 5 10 15
Ala Tyr Val

<210> 36
<211> 21
<212> PRT
<213> Artificial Sequence

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<400> 36
Pro Arg Ala Gly Ser Glu Lys Gly Ser Ala Ser Ser Arg Asp Gly Lys
1 5 10 15
Thr Thr Val Trp Ile
20

<210> 37
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<223> Description of Artificial Sequence: note = Synt hetic Construct

<400> 37
Gly Tyr His Ser Asp Lys Arg Arg Leu Ser Lys Ala Ser Ser Lys Ala
1 5 10 15
Arg Ser Asp Asp Leu Ser Val
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<210> 38
<211> 19
<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 38
Pro Leu Ser Arg Leu Ser Lys Ala Ser Ser Arg Ala Arg Ser Asp Asp
1 5 10 15
Leu Thr Val

<210> 39
<211> 22
<212> PRT
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<400> 39
Pro Asn His Val Val Ser Leu Thr Asn Asn Leu Ile Gly Arg Arg Val
1 5 10 15
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<210> 40
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<400> 40
Pro Ser Cys Val Ser Ser Ser Ala Val Leu Thr Thr Ile Cys Ser Ser
1 5 10 15
Asp Gln Val Val Pro Val Gly Leu Ser Ser Phe Tyr Met
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<210> 41
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<400> 41
Gly Arg Ser Ser Lys Ala Ser Lys Ser Ser Gly Gly Arg Ala Arg Ala
1 5 10 15
Ala Asp Leu Ala Ile
20

<210> 42
<211> 17
<212> PRT
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<400> 42
Leu Cys Tyr Leu Leu Ile Arg Tyr Cys Ser Gly Lys Ser Lys Lys Pro
1 5 10 15
Val

<210> 43
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<400> 43
Gly Gln Lys Pro Pro Ser Arg Pro Ser Ser Ser Ala Ser Lys Lys Gln
1 5 10 15
Tyr Val

<210> 44
<211> 19
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<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 44
Ser Ser Arg Ala Ser Ser Arg Ala Ser Ser Arg Pro Arg Pro Asp Asp
1 5 10 15
Leu Glu Val

<210> 45
<211> 9
<212> PRT
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Arg Pro Lys Pro Asp Asp Leu Glu Ile
1 5

<210> 46
<211> 19
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<400> 46
Ser Ser Arg Ala Ser Ser Arg Ala Ser Ser Arg Pro Lys Pro Asp Asp
1 5 10 15
Leu Glu Ile

<210> 47
<211> 9
<212> PRT
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<400> 47
Arg Pro Lys Pro Asp Asp Leu Asp Ile
1 5

<210> 48
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<400> 48
Ser Ser Arg Ala Ser Ser Arg Ala Ser Ser Arg Pro Arg Pro Asp Asp
1 5 10 15
Leu Asp Ile

<210> 49
<211> 19
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<400> 49
Ser Ser Arg Ala Ser Thr Arg Ala Ser Ser Arg Pro Arg Pro Asp Asp
1 5 10 15
Leu Glu Ile

<210> 50
<211> 9
<212> PRT
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<400> 50
Arg Pro Arg Pro Glu Asp Leu Glu Ile
1 5

<210> 51
<211> 19
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<400> 51
Ser Ser Arg Ala Ser Ser Arg Ala Ser Ser Arg Pro Arg Pro Glu Asp
1 5 10 15
Leu Glu Ile

<210> 52
<211> 9
<212> PRT
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<400> 52
Gly Asp Gly Lys Asn Ser Val Trp Val
1 5

<210> 53
<211> 23
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<400> 53
Ser Lys Ala Gly Ser Asn Lys Ser Thr Ala Ser Ser Lys Ser Gly Asp
1 5 10 15
Gly Lys Asn Ser Val Trp Val
20

<210> 54
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<212> PRT
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<400> 54
Gly Gln Lys Pro Pro Ser Arg Pro Ser Ser Ser Ala Ser Lys Lys Leu
1 5 10 15
Tyr Val

<210> 55
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Arg Gln Pro Lys Ile Trp Phe Pro Asn Arg Arg Lys Pro Trp Lys Ile
1 5 10 15
Glu Leu Asp Asp Pro Arg Pro Arg
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<210> 56
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<400> 56
Gly Arg Lys Lys Arg Arg Gln Arg Pro Pro Gln Arg Pro Arg Pro Asp
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Asp Leu Glu Ile
20

<210> 57
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Arg Gln Ile Lys Ile Trp Phe Gln Asn Arg Arg Met Lys Trp Lys Lys
1 5 10 15
Arg Pro Arg Pro Asp Asp Leu Glu Ile
20 25

<210> 58
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 58
Arg Gln Ile Ala Ile Trp Phe Gln Asn Arg Arg Met Lys Trp Ala Ala
1 5 10 15
Arg Pro Arg Pro Asp Asp Leu Glu Ile
20 25

<210> 59
<211> 18
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 59
Arg Lys Lys Arg Arg Gln Arg Arg Arg Arg Pro Arg Pro Asp Asp Leu
1 5 10 15
Glu Ile

<210> 60
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 60
Thr Arg Ser Ser Arg Ala Gly Leu Gln Phe Pro Val Gly Arg Val His
1 5 10 15
Arg Leu Leu Arg Lys Arg Pro Arg Pro Asp Asp Leu Glu Ile
20 25 30

<210> 61
<211> 35
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 61
Gly Trp Thr Leu Asn Ser Ala Gly Tyr Leu Leu Gly Lys Ile Asn Lys
1 5 10 15
Ala Leu Ala Ala Leu Ala Lys Lys Ile Leu Arg Pro Arg Pro Asp Asp
20 25 30
Leu Glu Ile
35

<210> 62
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 62
Lys Leu Ala Leu Lys Leu Ala Leu Lys Ala Leu Lys Ala Ala Leu Lys
1 5 10 15
Leu Ala Arg Pro Arg Pro Asp Asp Leu Glu Ile
20 25

<210> 63
<211> 25
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 63
Ala Ala Val Ala Leu Leu Pro Ala Val Leu Leu Ala Leu Leu Ala Pro
1 5 10 15
Arg Pro Arg Pro Asp Asp Leu Glu Ile
20 25

<210> 64
<211> 19
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 64
Val Pro Met Leu Lys Pro Met Leu Lys Glu Arg Pro Arg Pro Asp Asp
1 5 10 15
Leu Glu Ile

<210> 65
<211> 37
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 65
Met Ala Asn Leu Gly Tyr Trp Leu Leu Ala Leu Phe Val Thr Met Trp
1 5 10 15

Thr Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Arg Pro Arg Pro
20 25 30
Asp Asp Leu Glu Ile
35

<210> 66
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 66
Leu Leu Ile Ile Leu Arg Arg Arg Ile Arg Lys Gln Ala His Ala His
1 5 10 15
Ser Lys Arg Pro Arg Pro Asp Asp Leu Glu Ile
20 25

<210> 67
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 67
Lys Glu Thr Trp Trp Glu Thr Trp Trp Thr Glu Trp Ser Gln Pro Lys
1 5 10 15
Lys Lys Arg Lys Val Arg Pro Arg Pro Asp Asp Leu Glu Ile
20 25 30

<210> 68
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 68
Arg Gly Gly Arg Leu Ser Tyr Ser Arg Arg Arg Phe Ser Thr Ser Thr
1 5 10 15
Gly Arg Arg Pro Arg Pro Asp Asp Leu Glu Ile
20 25

<210> 69
<211> 24
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 69

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Ser Asp Leu Trp Glu Met Met Met Val Ser Leu Ala Cys Gln Tyr Arg
 1               5                  10                  15
Pro Arg Pro Asp Asp Leu Glu Ile
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<210> 70

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 70

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Thr Ser Pro Leu Asn Ile His Asn Gly Gln Lys Leu Arg Pro Arg Pro
 1               5                  10                  15
Asp Asp Leu Glu Ile
            20
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<210> 71

<211> 122

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 71

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Lys Gly Lys Ser Asp Pro Tyr His Ala Thr Ser Gly Ala Leu Ser Pro
 1               5                  10                  15
Ala Lys Asp Cys Gly Ser Gln Lys Tyr Ala Tyr Phe Asn Gly Cys Ser
            20                  25                  30
Ser Pro Thr Ala Pro Leu Ser Pro Met Ser Pro Pro Gly Tyr Lys Leu
        35                  40                  45
Val Thr Gly Asp Arg Asn Asn Ser Ser Cys Arg Asn Tyr Asn Lys Gln
    50                  55                  60
Ala Ser Glu Gln Asn Trp Ala Asn Tyr Ser Ala Glu Gln Asn Arg Met
65                  70                  75                  80
Gly Gln Ala Gly Ser Thr Ile Ser Asn Ser His Ala Gln Pro Phe Asp
                85                  90                  95
Phe Pro Asp Asp Asn Gln Asn Ser Lys Lys Leu Ala Ala Gly His Glu
            100                 105                 110
Leu Gln Pro Leu Ala Ile Val Asp Gln Arg
        115                 120
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<210> 72

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 72

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Lys Thr Asp Pro Tyr Ser His Ser Gly Thr Met Ser Pro Ser Lys Asp
 1               5                  10                  15
Cys Gly Ser Pro Lys Tyr Ala Tyr Tyr Asn Gly Cys Ser Ser Pro Thr
            20                  25                  30
Ala Pro Leu Ser Pro Met Ser Pro Pro Gly Tyr Lys Leu Val Thr Gly
        35                  40                  45
Asp Arg Asn Asn Ser Ser Cys Arg Asn Tyr Asn Lys Gln Ala Ser Glu
    50                  55                  60
Gln Asn Trp Ala Asn Tyr Ser Ala Glu Gln Asn Arg Met Gly Gln Ala
65                  70                  75                  80
Gly Ser Thr Ile Ser Asn Ser His Ala Gln Pro Phe Asp Phe Ala Asp
                85                  90                  95

Glu His Gln Asn Thr Lys Lys Leu Ala Ser Gly His Glu Leu Gln Pro
            100                 105                 110
Leu Thr Ile Val Asp Gln Arg Pro
        115                 120
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<210> 73

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 73

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Leu Gly Phe Gly Thr Ile Arg Asp Ser Leu Asn Ser Lys Arg Arg Glu
 1               5                  10                  15
Leu Glu Asp Pro Gly Ala Tyr Asn Tyr Pro Phe Thr Trp Asn Thr Pro
            20                  25                  30
Ser Ala Pro Pro Gly Tyr Asn Ile Ala Val Lys Pro Asp Gln Ile Gln
        35                  40                  45
Tyr Thr Glu Leu Ser Asn Ala Lys Ile Ala Tyr Lys Gln Asn Lys Ala
    50                  55                  60
Asn Thr Ala Gln Glu Gln Gln Tyr Gly Ser His Glu Glu Asn Leu Pro
65                  70                  75                  80
Ala Asp Leu Glu Ala Leu Gln Arg Glu Ile Arg Met Ala Gln Glu Arg
                85                  90                  95
Leu Asp Leu Ala Val Gln Ala Tyr Ser His Gln Asn Asn Pro His Gly
            100                 105                 110
Pro Arg Glu Lys Lys Ala Lys Val
        115                 120
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<210> 74

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 74

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Gly Phe Gly Thr Ile Arg Asp Thr Leu Asn Asn Lys Arg Lys Glu Leu
 1               5                  10                  15
Glu Asp Ser Gly Thr Tyr Asn Tyr Pro Phe Thr Trp Asn Thr Pro Ser
            20                  25                  30
Ala Pro Pro Gly Tyr Asn Ile Ala Val Lys Pro Asp Gln Met Gln Tyr
        35                  40                  45
Thr Glu Leu Ser Asn Ala Lys Met Ala Tyr Lys Gln Asn Lys Ala Asn
    50                  55                  60
Ile Ala Gln Glu Gln Gln Tyr Gly Ser Asn Glu Glu Asn Ile Pro Ala
65                  70                  75                  80
Asp Leu Glu Asn Leu Gln Arg Glu Ile Lys Val Ala Gln Glu Arg Leu
                85                  90                  95
Asp Met Ala Ile Gln Ala Tyr Asn Asn Gln Asn Asn Pro Gly Ser Ser
            100                 105                 110
Ser Arg Glu Lys Lys Ser Lys Ala
        115                 120
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<210> 75

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 75

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Pro Tyr Leu Val Asp Cys Phe Val Ser Arg Pro Thr Glu Lys Thr Ile
 1               5                  10                  15
Phe Ile Ile Phe Met Leu Val Val Gly Leu Ile Ser Leu Val Leu Asn
            20                  25                  30
Leu Leu Glu Leu Val His Leu Leu Cys Arg Cys Leu Ser Arg Gly Met
        35                  40                  45
Arg Ala Arg Gln Gly Gln Asp Ala Pro Pro Thr Gln Gly Thr Ser Ser
    50                  55                  60
Asp Pro Tyr Thr Asp Gln Val Phe Phe Tyr Leu Pro Val Gly Gln Gly
65                  70                  75                  80
Pro Ser Ser Pro Pro Cys Pro Thr Tyr Asn Gly Leu Ser Ser Ser Glu
                85                  90                  95
Gln Asn Trp Ala Asn Leu Thr Thr Glu Glu Arg Leu Ala Ser Ser Arg
            100                 105                 110
Pro Pro Leu Phe Leu Asp Pro Pro
        115                 120
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<210> 76

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 76

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Cys Gly Ser Lys Glu His Gly Asn Arg Lys Met Arg Gly Arg Leu Leu
 1               5                  10                  15
Leu Thr Tyr Met Ala Ser Ile Phe Phe Lys Ser Val Phe Glu Val Ala
            20                  25                  30
Phe Leu Leu Ile Gln Trp Tyr Leu Tyr Gly Phe Thr Leu Ser Ala Val
        35                  40                  45
Tyr Ile Cys Glu Gln Ser Pro Cys Pro His Arg Val Asp Cys Phe Leu
    50                  55                  60
Ser Arg Pro Thr Glu Lys Thr Ile Phe Ile Leu Phe Met Leu Val Val
65                  70                  75                  80
Ser Met Val Ser Phe Val Leu Asn Val Ile Glu Leu Phe Tyr Val Leu
                85                  90                  95
Phe Lys Ala Ile Lys Asn His Leu Gly Asn Glu Lys Glu Glu Val Tyr
            100                 105                 110
Cys Asn Pro Val Glu Leu Gln Lys
        115                 120
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<210> 77

<211> 239

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 77

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

Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
65                  70                  75                  80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
                85                  90                  95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
            100                 105                 110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
        115                 120                 125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
    130                 135                 140
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
145                 150                 155                 160
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
                165                 170                 175
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
            180                 185                 190
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
        195                 200                 205
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
    210                 215                 220
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
225                 230                 235
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<210> 78

<211> 60

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 78

ccct cct ccc gggcct cct c ccgggcct cc t cccggcccc ggcccgacga cct ggagat c 60

<210> 79

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 79

cggccccggc ccgacgacct ggagatc c 27

<210> 80
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 80
cggccccggc ccgacgacct ggaggtg 27

<210> 81
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 81
cggccccggc ccgacgacgt gcccgtg 27

<210> 82
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 82
aaggcccggt ccgacgacct gtccgtg 27

<210> 83
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 83
cggcagccca agatctggtt ccccaaccgg cggaagccct ggaagaag 48

<210> 84
<211> 108
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 84
cggcagccca agatctggtt ccccaaccgg cggaagccct ggaagaagcc ctcctcccgg 60
gcctcctccc gggcctcctc ccggccccgg cccgacgacc tggagatc 108

<210> 85
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 85
cggcagccca agatctggtt ccccaaccgg cggaagccct ggaagaagcg gccccggccc 60
gacgacctgg agatc 75

<210> 86
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 86
cggcagccca agatctggtt ccccaaccgg cggaagccct ggaagaagcg gccccggccc 60
gacgacctgg aggtg 75

<210> 87
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 87
cggcagccca agatctggtt ccccaaccgg cggaagccct ggaagaagcg gccccggccc 60
gacgacgtgc ccgtg 75

<210> 88
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 88
cggcagccca agatctggtt ccccaaccgg cggaagccct ggaagaagaa ggcccggtcc 60
gacgacctgt ccgtg 75

<210> 89
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 89
Pro Cys Ser Arg Ala Ser Ser Arg Met Ser Ser Arg Ala Arg Pro Asp
1 5 10 15
Asp Leu Asp Val
20

<210> 90
<211> 19
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 90
Pro Arg Val Ser Val Pro Asn Phe Gly Arg Thr Gln Ser Ser Asp Ser
1 5 10 15
Ala Tyr Val

<210> 91
<211> 19

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 91
Pro Arg Met Ser Met Pro Asn Phe Gly Arg Thr Gln Ser Ser Asp Ser
1 5 10 15
Ala Tyr Val

<210> 92
<211> 32
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note = Synthetic Construct

<400> 92
Arg Gln Pro Lys Ile Trp Phe Pro Asn Arg Arg Lys Pro Trp Lys Lys
1 5 10 15
Arg Ala Ser Ser Arg Ala Ser Ser Arg Pro Arg Pro Asp Asp Leu Glu
20 25 30

REFERENCES CITED IN THE DESCRIPTION

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Patentkrav

1. Isoleret polypeptid omfattende en aminosyresekvens udvalgt fra gruppen bestående af SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 og SEQ ID NO: 5 eller en konservativ variant deraf indeholdende 1 til 5 konservative substitutioner eller en deletion af en aminosyre fra carboxy-terminus, til anvendelse i en fremgangsmåde til behandling eller forebyggelse af makuladegeneration hos et individ, hvilken fremgangsmåde omfatter at indgive polypeptidet til individet.

2. Polypeptid til anvendelse ifølge krav 1, hvor polypeptidet omfatter aminosekvensen ifølge SEQ ID NO: 2.

3. Polypeptid til anvendelse ifølge krav 1, hvor polypeptidet omfatter en aminosyresekvens med mindst 65 % sekvensidentitet med de 9 mest c-terminale aminosyrer ifølge SEQ ID NO:1.

4. Polypeptid til anvendelse ifølge krav 1, hvor polypeptidet omfatter en aminosyresekvens med mindst 75 % sekvensidentitet med de 9 mest c-terminale aminosyrer ifølge SEQ ID NO:1.

5. Polypeptid til anvendelse ifølge krav 1, hvor polypeptidet omfatter en aminosyresekvens med mindst 85 % sekvensidentitet med de 9 mest c-terminale aminosyrer ifølge SEQ ID NO:1.

6. Polypeptid til anvendelse ifølge krav 1, hvor polypeptidet yderligere omfatter en cellulær internaliseringssekvens.

7. Polypeptid til anvendelse ifølge krav 6, hvor den cellulære internaliseringssekvens omfatter en aminosyresekvens af et protein udvalgt fra en gruppe bestående af Antennapedia, TAT, HIV-Tat, Penetratin, Antp-3A (Antp-mutant), Buforin II, Transportan, MAP (model amphipathic peptide), K-FGF, Ku70, Prion, pVEC, Pep-1, SynB1, Pep-7, HN-1, BGSC (Bis-Guanidinium-Spermidin-kolesterol) og BGTC (Bis-Guanidinium-Tren-kolesterol).

8. Polypeptid til anvendelse ifølge krav 7, hvor den cellulære internaliseringssekvens er Antennapedia, og hvor sekvensen omfatter aminosyresekvensen ifølge SEQ ID NO:7.

9. Polypeptid til anvendelse ifølge krav 1, hvor polypeptidet omfatter en aminosyresekvens udvalgt fra gruppen bestående af SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:11 og SEQ ID NO:12.

10. Polypeptid til anvendelse ifølge krav 1, hvor polypeptidet omfatter en aminosyresekvens med mindst 95 % sekvensidentitet med SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO:11 eller SEQ ID NO.12.

DRAWINGS

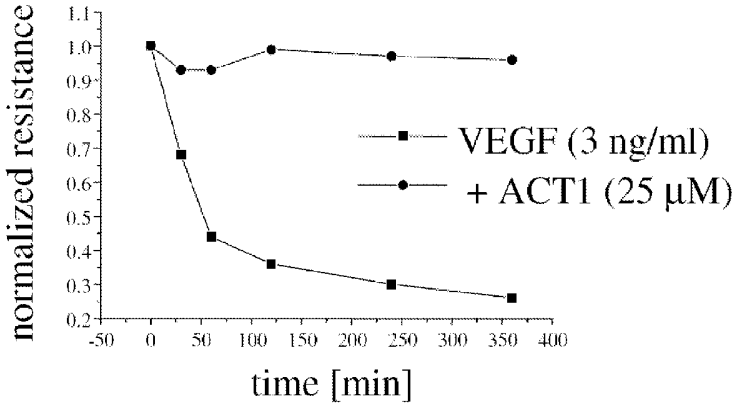

FIG. 1

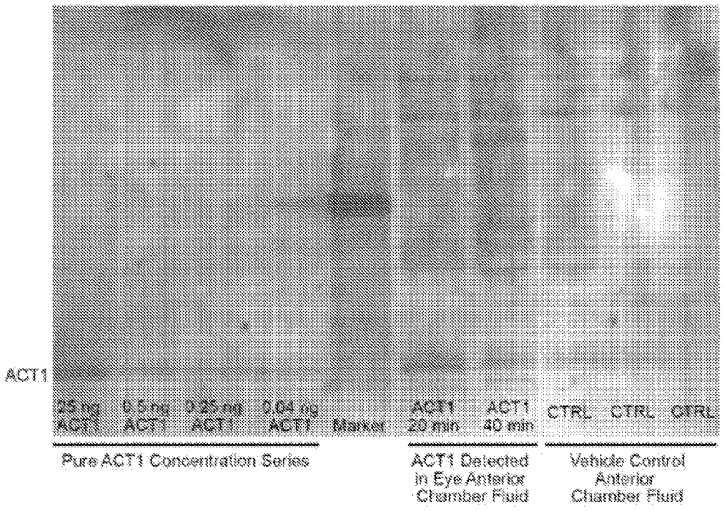

FIG. 2