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(54) Title: DOUBLE-STRANDED/SELF-COMPLEMENTARY VECTORS WITH A TRUNCATED CBA PROMOTER AND METHODS OF GENE DELIVERY

(57) Abstract: The present invention concerns vectors carrying a truncated chimeric CMV- chicken β -actin (smCBA) promoter in which the hybrid chicken β -actin/rabbit β -globin intron is greatly shortened, and their use to deliver to an operatively linked polynucleotide to host cells in vitro or in vivo, resulting in expression of the polynucleotide in the host cells. In one embodiment, the vector carrying the smCBA promoter is administered to the eye. In another embodiment, the vector carrying the smCBA promoter is a self-complementary adeno-associated virus (AAV). The AAV vector may be of any serotype (e.g., type 1, type 2, type 3, type 4, type 5, type 6, type 7, type 8, type 9, type 10). In another embodiment, a self-complementary vector carrying the smCBA promoter is administered to the eye. Another aspect of the invention concerns host cells carrying a vector of the invention. Another aspect of the invention concerns pharmaceutical composition comprising the vectors or host cells of the invention, and a pharmaceutically acceptable carrier.



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5 DOUBLE-STRANDED/SELF-COMPLEMENTARY VECTORS WITH A
 TRUNCATED CBA PROMOTER AND METHODS OF GENE DELIVERY

CROSS-REFERENCE TO RELATED APPLICATION

 The present application claims the benefit of U.S. Provisional Application Serial
No. 60/795,759, filed April 28, 2006, which is hereby incorporated by reference herein in
10 its entirety, including any figures, tables, nucleic acid sequences, amino acid sequences,
and drawings.

BACKGROUND OF THE INVENTION

 Recombinant adeno-associated virus (rAAV) has widely been used as a vehicle
15 for the delivery of genetic material to target cells. AAV has several advantages over
other viral vectors. For example, wild-type AAV has not been associated with any
pathological human condition, rAAV does not contain native viral coding sequence and
persistent expression of delivered transgenes has been observed in many applications.

 Under normal circumstances, AAV packages a single-stranded DNA molecule of
20 up to 4800 nucleotides in length. Following infection of cells by the virus, the intrinsic
molecular machinery of the cell is required for conversion of single-stranded DNA into
double stranded form. The double-stranded form is then capable of being transcribed,
thereby allowing expression of the delivered gene to commence. It has been shown in a
number of cell and tissue types that second strand synthesis of DNA by the host cell is the
25 rate-limiting step in expression. By virtue of already being packaged as a double stranded
DNA molecule, self-complementary AAV (scAAV) bypasses this step, thereby greatly
reducing the time to onset of gene expression.

 Self-complementary AAV is generated through the use of vector plasmid with a
mutation in one of the terminal resolution sequences of the AAV virus. This mutation
30 leads to the packaging of a self-complementary, double-stranded DNA molecule
covalently linked at one end. Vector genomes are required to be approximately half

genome size (2.4KB) in order to package effectively in the normal AAV capsid. Because of this size limitation, large promoters are unsuitable for use with scAAV. Most broad applications to date have used the cytomegalovirus immediate early promoter (CMV) alone for driving transgene expression. However, it has been shown by others that
5 transgene expression with CMV markedly drops off in certain tissue types, such as eye and liver, sometimes as early as two weeks post-injection. A long acting, ubiquitous promoter of small size would be very useful in a scAAV system.

The chimeric CMV-chicken β -actin promoter (CBA) has been utilized extensively as a promoter that supports expression in a wide variety of cells when in rAAV vectors
10 delivered to retina. In addition to broad tropism, the present inventors have observed that CBA also has the capacity to promote expression for long periods post infection (Acland, G.M. *et al. Mol Ther.*, 2005, 12(6):1072-1082). CBA is ~1700 base pairs in length, too large in most cases to be used in conjunction with scAAV to deliver cDNAs (over 300 bps pairs in length).

BRIEF SUMMARY OF THE INVENTION

The present invention concerns vectors carrying a truncated chimeric CMV-chicken β -actin (smCBA) promoter in which the hybrid chicken β -actin/rabbit β -globin intron is greatly shortened, and their use to deliver to host cells *in vitro* or *in vivo* a
20 polynucleotide operatively linked to the truncated promoter, resulting in expression of the polynucleotide in the host cells. Use of the smCBA promoter has several advantages: (1) the promoter can be used with various serotypes of adeno-associated virus (AAV); (2) expression of the polynucleotide (*e.g.*, transgene) is initiated sooner than with other promoters; (3) expression of the polynucleotide is maintained for a longer period of time,
25 compared to expression using other promoters; and (4) because the promoter is truncated, it permits packaging of more polynucleotide(s) into the vector, which is particularly relevant for double-stranded viral vectors.

In one embodiment, the vector carrying the smCBA promoter is administered to the eye. In another embodiment, the vector carrying the smCBA promoter is a self-
30 complementary AAV. The AAV vector may be of any serotype (*e.g.*, type 1, type 2, type 3, type 4, type 5, type 6, type 7, type 8, type 9, type 10). In another embodiment, a self-complementary vector carrying the smCBA promoter is administered to the eye. Another

aspect of the invention concerns host cells carrying a vector of the invention. Another aspect of the invention concerns a pharmaceutical composition comprising the vectors or host cells of the invention and a pharmaceutically acceptable carrier.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows images of whole mount mouse retina after single subretinal injection of type 2 self complimentary AAV (scAAV) or type 2 standard AAV expressing green fluorescent protein under the control of smCBA.

10 **Figure 2** shows transduction of heart and liver following para-cardial or intra-cardial injection (respectively) of type I scAAV-sm CBA-GFP or standard AAV-CBA-GFP.

Figure 3 shows versions of the chimeric CMV-chicken β -actin promoter (CBA).

15 **Figures 4A-4C** show smCBA AAV constructs. **Figure 4A** shows an AAV construct of the invention with smCBA driving green fluorescent protein (GFP) expression. **Figure 4B** shows an AAV construct of the invention with smCBA driving expression of bovine Guanylate Cyclase (bGC1). **Figure 4C** shows an AAV construction of the invention with smCBA driving mouse beta subunit of phosphodiesterase (β -PDE).

20 **Figures 5A-1, 5A-2, 5B-1, 5B-2, 5C-1, 5C-2, 5D-1, and 5D-2** show neonate C57Black6 mice subretinally injected with AAV2-smCBA-GFP or AAV2-CBA-GFP with equal amounts (1:1) AAV2-CMV-LacZ. Whole mount retinas stained for LacZ and anti-GFP.

25 **Figures 6A-1, 6A-2, 6B-1, and 6B-2** show adult C57Black6 mouse subretinally injected with AAV2-smCBA-GFP (left eye) or AAV2-CBA-GFP (right eye) with equal amount (1:1) AAV2-CMV-LacZ. Whole mount retina stained for LacZ and anti-GFP.

Figure 7 shows immunolocalization of GC1 (red) and cone arrestin (green) in age matched Guanylate cyclase (GC1) knockout (left), wild type (middle) and AAV5-smCBA-GC1-treated GC1 KO mouse (right) retinas. Images taken at 8 weeks of age.

30 **Figure 8** shows full-field scotopic flash ERG responses at 10cds/m² recorded from injected (R) and uninjected (L) eyes of an rd10 mouse 3 weeks post-subretinal injection with AAV5-smCBA-mPDEbeta.

Figure 9 shows representative sections of AAV-smCBA-GFP serotype 2 and 5 at 5 weeks post-injection in C57Black6 mice, stained with DAPI and anti-GFP.

Figure 10 shows adult C57Black6 mouse 7 months post-subretinal injection with AAV2-smCBA-GFP, Whole mount retina stained for anti-GFP.

5 **Figures 11A and 11B** show self-complementary adeno-associated (scAAV) vector constructs of the invention.

Figures 12A-12C show adult mouse retina after subretinal injection with scAAV5-smCBA-GFP. Sections stained with anti-GFP show intense RPE signal with less intense photoreceptor signal.

10 **Figure 13** shows whole mount mouse retina after subretinal injection of type 2 self complimentary AAV (scAAV) or type 2 standard AAV expressing green fluorescent protein under the control of smCBA.

Figure 14 shows adult mouse retina after subretinal injection with either scAAV1-smCBA or AAV1-CBA-GFP. Sections stained with anti-GFP and
15 counterstained with DAPI. The RPE supports strong GFP expression with weaker photoreceptor expression also evident.

Figure 15 shows whole mount adult mouse retina after intravitreal injection with scAAV2-smCBA-GFP. Staining with anti-GFP antibody shows rapid, intense ganglion cell signal. 40X magnification.

20 **Figure 16** shows whole mount adult mouse retina stained with anti-GFP after subretinal injection with scAAV5-MOPS-GFP.

DETAILED DESCRIPTION OF THE INVENTION

The present inventors have made a truncated chimeric cytomegalovirus (CMV)-
25 chicken β -actin (CBA) promoter (smCBA) in which the hybrid chicken β -actin/rabbit β -globin intron is greatly shortened (Mah, C. *et al. Hum. Gene Ther.*, 2003, 14(2):143-152). Full CBA was first cut with the restriction endonuclease Bsp 120I and treated with exonuclease to polish the end, subsequently the DNA was cut with Xba I and treated with DNA polymerase to fill the restricted end. A ligation of the blunt ends resulted in the
30 formation of smCBA. The total size of smCBA is 953 bps versus 1714 bps for full length CBA.

The smCBA promoter is described in Mah, C. *et al.*, *Hum. Gene Ther.*, 2003, 14(2):143-152 and Haire, S.E. *et al. Invest. Ophthalmol. Vis. Sci.*, 2006, 47(9):3745-3753, which are each incorporated herein by reference in its entirety. The nucleic acid sequence of the smCBA promoter is:

5 aattcgggtaccctagttattaatagtaataacgacgggtcattagttcatagcccatatatggagttccgcgttacataacttacggt
 aaatggcccgctgggtgaccgcccacgacccccgccattgacgtcaataatgacgtatgttcccatagtaacgccaatagg
 gactttccattgacgtcaatgggtggactatttacggtaaactgccacttggcagtacatcaagtgtatcatatgccaagtacgcc
 ccctattgacgtcaatgacggtaaatggcccgctggcattatgccagttacatgaccttatgggactttcctacttggcagtacat
 ctacgtattagtcacgctattaccatgggtcgaggtgagccccacgttctgcttactctccccatctccccccctccccacccccca
 10 atttgtatttatttttttaatttttgtgcagcgatgggggccccggggggggggggggggcgcgcgccaggcggggccccg
 gggcgaggggccccgccccgagggcgagggcgagggcgagggcgagggcgagggcgagggcgagggcgagggcgagggcgagggcg
 atggcgagggcgggcgggcgggcgggcgccctataaaaagcgaagcgcgcgggcgggcgaggagtcgctgacgctgccttcgc
 cccgtgccccgctccgccgcccgcctcgcgcccgccccggctctgactgaccgcgttactcccacaggtgagcgggcg
 gacggcccttctcctccgggctgtaattagcgttgggttaatgacggctgtttcttttctgtggctgcgtgaaagccttgaggggct
 15 ccgggagctagagcctctgctaaccatgttcattccttcttttctacagctcctgggcaacgtgctggtattgtgtgtctcat
 cattttggcaaag (SEQ ID NO:1).

Experiments evaluating tropism (cell type targeting) and extent and duration of transgene expression by smCBA relative to full CBA in mouse retina resulted in the two promoters being interchangeable. As such, the present inventors have created a scAAV
 20 construct with smCBA driving expression of humanized green fluorescent protein (GFP) and analyzed the progression of gene expression relative to the matched standard AAV construct with full length CBA (CBA-GFP). These comparisons have been done with AAV packaged in capsid serotypes 1, 2 and 5. The present inventors have also evaluated transgene expression when virus is delivered to the vitreous, as well as subretinally.

25 Results with serotype 2 in mouse retina indicate onset of transgene expression is markedly faster with scAAV2-smCBA-GFP than with the corresponding AAV2-CBA-GFP (see Figure 1). Typically, expression of GFP with AAV2-CBA-GFP is first apparent at 15 days post-injection whereas expression from scAAV-smCBA-GFP was seen as early as two days post-injection, with robust expression at day 8.

30 Results of scAAV1-smCBA-GFP (serotype I) injected into the paracardial space of mice indicate efficient expression of GFP in myocytes 5 days post-injection. In addition, efficient transduction of liver was achieved by intracardial injection of the same

(type I) virus at day 5 post-injection (see Figure 2). Standard AAV1-CBA-GFP controls for both heart and liver were negative at 10 days post injection.

Self-complementary AAV has been established as a faster system of transgene expression than standard AAV, yet it retains the same advantages of AAV over other gene delivery viral vectors. The broad tropism and small size of the smCBA coupled to scAAV create a gene delivery system that can target a number of tissue types faster than the current conventional AAV constructs. In addition, smCBA supports persistent expression, and given its smaller size can accommodate larger gene constructs in the scAAV system. In the Examples, the smCBA promoter is compared to the CMV promoter (U.S. Patent Nos. 5,168,062 and 5,385,839, which are each incorporated herein by reference in their entirety).

The present invention concerns vectors carrying a truncated chimeric CMV-chicken β -actin (smCBA) promoter in which the hybrid chicken β -actin/rabbit β -globin intron is greatly shortened, and their use to deliver to an operatively linked polynucleotide to host cells *in vitro* or *in vivo*, resulting in expression of the polynucleotide in the host cells. Preferably, the vector carrying the smCBA promoter is a self-complementary adeno-associated virus (AAV); however, other viral or non-viral vectors such as lentivirus or liposomes can be utilized.

The vector carrying the smCBA promoter can be administered to any anatomical site on or in the subject. In one embodiment, the vector carrying the smCBA promoter is administered to the eye. For example, the vector can be administered to the eye by subretinal or intravitreal injection. In another embodiment, a self-complementary AAV vector carrying the smCBA promoter is administered to the eye. Examples of polynucleotides and vectors useful for treating ocular disorders, and methods for their administration, are described in U.S. patent publication 20040022766 (Acland, G.M. *et al.*), filed November 20, 2002, and Acland G.M. *et al.*, *Molecular Therapy*, 2005 Dec., 12(6):1072-1082, which are each incorporated herein by reference in its entirety. The smCBA promoter can be placed in a vector described in the aforementioned publications. The smCBA promoter can be operably-linked with a polynucleotide described in the aforementioned publications. The smCBA promoter can be placed in a vector and be operably-linked to a polynucleotide to treat an ocular disorder described in the aforementioned publications.

In those embodiments in which an AAV vector (e.g., a self-complementary AAV vector) is utilized to carry the smCBA promoter, the AAV vector may be of any serotype (e.g., type 1, type 2, type 3, type 4, type 5, type 6, type 7, type 8, type 9, type 10, or others). Examples of AAV serotypes that may be utilized in the present invention
5 include, but are not limited to, those described in Gao G. *et al.*, *Curr. Gene Ther.*, 2005, 5(3):285-297, which is incorporated herein by reference in its entirety.

One aspect of the invention provides a method for delivering a polynucleotide to a host cell *in vitro* or *in vivo*, comprising administering a vector containing the polynucleotide operably-linked to a smCBA promoter to the host cell, wherein the
10 polynucleotide is expressed in the host cell. The polynucleotide may be one that is found naturally within the host cell and/or encodes a polypeptide that is found naturally within the host cell (*i.e.*, that is endogenous to the host cell). Alternatively, the polynucleotide may be one that is not found naturally in the host cell and/or encodes a polypeptide that is not found naturally in the host cell (*i.e.*, is heterologous to the host cell).

15 According to the *in vivo* methods of the present invention, a vector containing a polynucleotide operably-linked to a smCBA promoter can be administered to a human or non-human subject. The vector may be administered, for example, in order to alleviate (e.g., reduce or eliminate) one or more symptoms associated with a pathologic disorder. Treatment with the vector is intended to include prophylactic intervention to prevent or
20 reduce onset of the symptoms associated with the disorder. The vectors, host cells, and pharmaceutical compositions containing them can be co-administered (concurrently or consecutively) to a subject with other therapeutic agents, for example. Examples of pathologic disorders that may be treated using the vectors of the invention, and corresponding therapeutic polynucleotides useful for treating the disorders, include (but
25 are not limited to) those described in Warrington, K.H. and Herzog, R.W. *Hum. Genet.*, 2006 Apr 13, Epub ahead of print, and Mah, C. *et al. Human Gene Therapy*, 2003, 14:143-152, which are each incorporated herein by reference in its entirety. The vectors, host cells, and compositions of the invention can be administered to the subject systemically (e.g., orally, intravenously) or locally at a target anatomical site.

30 The methods of the invention may include further steps in addition to administration of the vector, host cell, or pharmaceutical composition. In some embodiments, a subject with the disorder is identified or a subject at risk for the disorder

is identified. A subject may be someone who has not been diagnosed with the disease or condition (diagnosis, prognosis, and/or staging) or someone diagnosed with the disease or condition (diagnosis, prognosis, monitoring, and/or staging), including someone treated for the disease or condition (prognosis, staging, and/or monitoring). Optionally, diagnosis can include identifying a subject suffering from or at risk for developing the disorder. Alternatively, the subject may not have been diagnosed with the disorder but suspected of having the disorder based either on patient history or family history, or the exhibition or observation of characteristic symptoms, for example.

Optionally, the methods of the invention can include a step of analyzing expression of the polynucleotide delivered *in vitro* or *in vivo* to the host cells. The step of analyzing the expression of a polynucleotide can be performed in a variety of different ways. Numerous suitable techniques are known for analyzing protein expression. For example, protein expression can be determined directly by assessing protein expression of cells or fluid of a biological sample. Protein expression can be detected using immunological techniques, *e.g.*, using antibodies that specifically bind the protein (*e.g.*, a therapeutic protein) in assays such as immunofluorescence or immunohistochemical staining and analysis, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoblotting (*e.g.*, Western blotting), and like techniques. Expression of a polynucleotide can also be determined by directly or indirectly measuring the amount of mRNA encoded by the polynucleotide in a cellular sample using known techniques such as Northern blotting and PCR-based methods such as competitive quantitative reverse transcriptase PCR (Q-RT-PCR).

Vectors

Any of a variety of nucleic acid vectors may be used to deliver and express a polynucleotide operatively linked to the truncated chimeric CMV-chicken β -actin promoter in accordance with the methods of the invention, *e.g.*, recombinant viruses, such as recombinant adeno-associated virus (AAV), lentivirus, recombinant adenoviruses, recombinant retroviruses, recombinant poxviruses, recombinant baculovirus, and other viruses known in the art, as well as plasmids, cosmids and phages, *etc.* A wealth of publications known to those of skill in the art discusses the use of a variety of such vectors for delivery of genes (see, *e.g.*, Ausubel *et al.*, Current Protocols in Molecular

Biology, John Wiley & Sons, New York, 1989; Kay, M. A. *et al*, 2001 *Nat. Medic.*, 7(1):33-40; and Walther W. and Stein U., *Drugs*, 2000, 60(2):249-71, which are each incorporated herein by reference in their entirety).

In one embodiment, the recombinant AAV vector is a self-complementary AAV.

5 In one embodiment of the invention, the vector is a recombinant self-complementary AAV carrying a wild-type (*i.e.*, normal) version of a selected transgene-encoding cDNA driven by a truncated chimeric CMV-chicken β -actin promoter that expresses the product of the wild-type cDNA in selected ocular cells of the affected subject. To exemplify the methods and compositions of this invention, the presently preferred vector, a recombinant
10 AAV is described in detail.

Adeno-associated viruses are small, single-stranded DNA viruses which require helper virus to facilitate efficient replication (K. I. Berns, *Parvoviridae: the viruses and their replication*, p. 1007-1041, in F. N. Fields *et al.*, *Fundamental Virology*, 3rd ed., vol. 2, (Lippencott-Raven Publishers, Philadelphia, Pa.) (1995)). The 4.7 kb genome of AAV
15 is characterized by two inverted terminal repeats (ITR) and two open reading frames which encode the Rep proteins and Cap proteins, respectively. The Rep reading frame encodes four proteins of molecular weight 78 kD, 68 kD, 52 kD and 40 kD. These proteins function mainly in regulating AAV replication and rescue and integration of the AAV into a host cell's chromosomes. The Cap reading frame encodes three structural
20 proteins of molecular weight 85 kD (VP 1), 72 kD (VP2) and 61 kD (VP3) which form the virion capsid. More than 80% of total proteins in AAV virion comprise VP3.

Flanking the rep and cap open reading frames at the 5' and 3' ends are 145 bp inverted terminal repeats (ITRs), the first 125 bp of which are capable of forming Y- or T-shaped duplex structures. The two ITRs are the only cis elements essential for AAV
25 replication, rescue, packaging and integration of the AAV genome. There are two conformations of AAV ITRs called "flip" and "flop". These differences in conformation originated from the replication model of adeno-associated virus which uses the ITR to initiate and reinitiate the replication (R. O. Snyder *et al.*, 1993, *J Virol.*, 67:6096-6104 (1993); K. I. Berns, 1990 *Microbiological Reviews*, 54:316-329, which are incorporated
30 herein by reference in their entirety). The entire rep and cap domains can be excised and replaced with a therapeutic or reporter transgene (B. J. Carter, in "Handbook of Parvoviruses", ed., P. Tijsser, CRC Press, pp. 155-168 (1990)).

AAVs have been found in many animal species, including primates, canine, fowl and human (F. A. Murphy *et al.*, "The Classification and Nomenclature of Viruses: Sixth Report of the International Committee on Taxonomy of Viruses", Archives of Virology, (Springer-Verlag, Vienna) (1995)). Several primate serotypes have been reported (*e.g.*,
5 AAV1, AAV2, AAV3, AAV4, AAV5 and AAV6). The AAV ITR sequences and other AAV sequences employed in constructs used in the present invention may be obtained from a variety of sources. For example, the sequences may be provided by AAV type 5, AAV type 2, AAV type 1, AAV type 3, AAV type 4, AAV type 6, or other AAV serotypes or other densoviruses, including presently identified human AAV types and
10 AAV serotypes yet to be identified. Similarly, AAVs known to infect other animals may also provide these ITRs employed in the molecules or constructs of this invention. Similarly, the capsids from a variety of serotypes of AAV may be "mixed and matched" with the other vector components. See, *e.g.*, International Patent Publication No. WO 01/83692, published Nov. 8, 2001, which is incorporated herein by reference in its
15 entirety. A variety of these viral serotypes and strains are available from the American Type Culture Collection, Manassas, Va., or are available from a variety of academic or commercial sources. Alternatively, it may be desirable to synthesize sequences used in preparing the vectors and viruses of the invention using known techniques, which may utilize AAV sequences that are published and/or available from a variety of databases.
20 The source of the sequences utilized in preparation of the constructs of the invention, is not a limitation of the present invention. Similarly, the selection of the species and serotype of AAV that provides these sequences is within the skill of the artisan and does not limit the following invention.

Examples of self-complementary AAV vectors that may be used in the vectors,
25 host cells, and methods of the invention are described, for example, in Han Z. *et al.*, *Mitochondrion*, 2007, Feb. 20, Epub ahead of print; Wu J. *et al.*, *Hum Gene Ther.*, 2007, 18(2):171-182; Nathwani A.C. *et al.*, *Blood*, 2007, 109(4):1414-1421; Nathwani A.C. *et al.*, *Blood*, 2006, 107(7):2653-2661, Epub 2005 Dec. 1; Choi V.W. *et al.*, *J. Virol.*, 2005, 79(11):6801-6807; Xu D. *et al.*, 2005, 11(4):523-530; Zhong L. *et al.*, *Mol. Ther.*, 2004,
30 10(5):950-957; Fu H. *et al.*, *Mol. Ther.*, 2003, 8(6):911-917; McCarty D.M. *et al.*, *Gene Ther.*, 2003, 10(26):2112-2118; Ding W. *et al.*, *J. Virol.*, 77(13):7361-7366; and McCarty

D.M. *et al.*, *Gene Ther.*, 2001, 8(16):1248-1254, which are each incorporated herein by reference in its entirety.

Host Cells

5 Examples of target cells to which the vectors of the present invention can be administered *in vitro* or *in vivo* include, but are not limited to, those listed in Table 1. As will be understood by one of skill in the art, there are over 200 cell types in the human body. It is believed that the methods of the subject invention can be used to deliver polynucleotides to any of these cell types for therapeutic or other purposes. For example,
10 any cell arising from the ectoderm, mesoderm, or endoderm germ cell layers can be a target cell for administration of the vectors of the invention. In one embodiment, the host cell is a liver cell. In another embodiment, the host cell is a non-liver cell. In another embodiment, the host cells are ocular cells, such as cells of the retina (*e.g.*, retinal pigment epithelial cells). In another embodiment, the host cells are muscle cells. In
15 another embodiment, the host cells are heart cells.

 The terms “recombinant host cells”, “host cells”, “genetically modified host cells” “cells”, “cell lines”, “cell cultures”, and other such terms denoting microorganisms or higher eukaryotic cells refer to cells that can be, or have been, used as recipients for recombinant vectors or other transfer DNA, immaterial of the method by which the DNA
20 is introduced into the cell or the subsequent disposition of the cell. The terms include the progeny of the original cell that has been transfected. Cells in primary culture can also be used as recipients. Host cells can range in plasticity and proliferation potential.

 Host cells can be differentiated cells, progenitor cells, or stem cells, for example. Thus, the cells can range in plasticity from totipotent or pluripotent stem cells (*e.g.*, adult
25 or embryonic), precursor or progenitor cells, to highly specialized cells, such as those of the central nervous system (*e.g.*, neurons and glia). Stem cells can be obtained from a variety of sources, including embryonic tissue, fetal tissue, adult tissue, umbilical cord blood, peripheral blood, bone marrow, and brain, for example.

 Host cells can be genetically modified with the vectors of the present invention.
30 The vector may be in the form of a plasmid, a virus, (*e.g.*, AAV, lentivirus, or other virus), a viral particle, a phage, *etc.* The genetically modified host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting

transformants/transfectants or amplifying the subunit-encoding polynucleotide. The culture conditions, such as temperature, pH and the like, generally are similar to those previously used with the host cell selected for expression, and will be apparent to those of skill in the art.

5 In one embodiment, the host cell is a human cell. In another embodiment, the host cell is a non-human animal cell. In another embodiment, the host cell is a vertebrate cell. In another embodiment, the host cell is a mammalian cell. It will be understood by one of skill in the art that the methods of the present invention are also applicable for veterinary purposes. For example, host cells of non-human animals can find application either in
10 human or animal patients (*e.g.*, veterinary uses).

Both prokaryotic and eukaryotic host cells may be used for expression of desired coding sequences when appropriate control sequences (*e.g.*, promoter sequences) that are compatible with the designated host are used. For example, among prokaryotic hosts, *Escherichia coli* may be used. Transfer vectors compatible with prokaryotic hosts can be
15 derived from, for example, the plasmid pBR322 that contains operons conferring ampicillin and tetracycline resistance, and the various pUC vectors, that also contain sequences conferring antibiotic resistance markers. These markers may be used to obtain successful transformants by selection. Eukaryotic hosts include yeast and mammalian cells in culture systems. *Pichia pastoris*, *Saccharomyces cerevisiae* and *S. carlsbergensis*
20 are commonly used yeast hosts. Yeast-compatible vectors carry markers that permit selection of successful transformants by conferring protrophy to auxotrophic mutants or resistance to heavy metals on wild-type strains. Yeast compatible vectors may employ the 2- μ origin of replication (Broach *et al. Meth. Enzymol.* 101:307, 1983), the combination of CEN3 and ARS1 or other means for assuring replication, such as
25 sequences that will result in incorporation of an appropriate fragment into the host cell genome.

The invention encompasses the host cells transformed by a vector according to the invention. These cells may be obtained by introducing (*in vitro* or *in vivo*) into host cells a nucleotide sequence inserted into a vector as defined above. Optionally, if the cells are
30 isolated, the cells can be cultured under conditions allowing the replication and/or the expression of the polynucleotide sequences carried by the vector.

Host cells useful for expression of the polynucleotides may be primary cells or cells of cell lines. The host cells may be tumor cells or non-tumor cells. Mammalian cell lines available as hosts for expression are known in the art and are available from depositories such as the American Type Culture Collection. These include but are not limited to HeLa cells, human embryonic kidney (HEK) cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, and others.

Reporter Polypeptides

The polynucleotide carried by the vector of the present invention can encode a reporter polypeptide (also referred to herein interchangeably as the “reporter gene” or “marker gene”). The polynucleotide encoding the reporter polypeptide may be the only transgene carried by the vector; alternatively, other polynucleotides may be present in addition to the reporter gene such as those encoding useful polypeptides (*e.g.*, therapeutic polypeptides) or useful inhibitory nucleic acids (such as antisense oligonucleotides or interfering RNA). Reporter polypeptides such as Beta-globin, CAT, luciferase, and beta-gal may be used. Preferably, the reporter polypeptide is one whose production can be detected (and, optionally, measured qualitatively, quantitatively, and/or semi-quantitatively) in living, intact cells. Examples of such reporter polypeptides include fluorescent polypeptides (also referred to herein as fluorescent proteins (FP)) such as the green fluorescent proteins (GFP), and variants of GFP such as yellow fluorescent proteins (YFP), *etc.*, for example, PS-FP (Yang F. *et al.*, *Nat. Biotechnol.*, 1996, 10:1246-1251; Cubitt A.B. *et al.*, “Understanding Structure-Function Relationships in the *Aequorea victoria* Green Fluorescent Protein, in *Methods in Cell Biology*, Vol. 58, Green Fluorescent Protein, Academic Press, 1999:19-29; Kain S.R., “Enhanced Variants of the Green Fluorescent Protein for Greater Sensitivity, Different Colours and Detection of Apoptosis”, in *Fluorescent and Luminescent Probes*, 2nd Edition, 1999, Chapter 19:284-292; Tsien R.Y., *Annu. Rev. Biochem.*, 1998, 67:509-544; Eisenstein, M., *Nature Methods*, January 2005, Research Highlights, 2(1):8-9; each of which are incorporated herein by reference in their entirety). As used herein, “variants of GFP” include, but are not limited to, polypeptides known in the art as green fluorescent protein-like proteins, GFP-like chromoproteins, green fluorescent protein fragments, red fluorescent proteins, and orange fluorescent proteins.

In addition, the vector of the present invention may optionally include another marker gene such as an antibiotic resistance gene and the fluorescent protein is used here as a visualization marker gene for example, FP/PS1/Ble, to aid visualization and fluorescent quantitation of the protein. Many FPs, originally isolated from the jellyfish *Aequorea Victoria* (for example, GFP) retain their fluorescent properties when expressed in heterologous cells, thereby providing a powerful tool as fluorescent recombinant probes to monitor cellular events or functions (see, for example, Chalfie *et al.*, *Science*, 1994, 263(5148):802-805; Prasher, *Trends Genet.*, 1995, 11(8):320-3; and PCT publication no. WO 95/07463, each of which are incorporated herein by reference in their entirety).

Several spectral and mutational variants of GFP proteins have since been isolated, for example, the naturally occurring blue-fluorescent variant of GFP (Heim *et al.*, *Proc. Natl. Acad. Sci. USA*, 1994, 91(26):12501-4; U.S. Patent No. 6,172,188, both of which are incorporated herein by reference), the yellow-fluorescent protein variant of GFP (Miller *et al.*, *J. Mol. Biol.*, 1999, 288:975-987; Weiss, *et al.*, *Proc. Natl. Acad. Sci. USA*, 2001, 98(26):14961-62001; Majoul, *et al.*, *Dev. Cell.*, 2001, 1(1):139-53; Laird *et al.*, *Microsc. Res. Tech.*, 2001;52(3):263-72; Daabrowski *et al. Protein Expr. Purif.*, 1999, 16(2):315-23, and more recently the red fluorescent protein isolated from the coral *Discosoma* (Fradkov *et al.*, *FEBS Lett.*, 2000, 479(3):127-30; Miller *et al.*, *J. Mol. Biol.*, 1999, 288:975-987), which allows the use of fluorescent probes having different excitation and emission spectra permitting the simultaneous monitoring of more than one process. GFP proteins provide non-invasive assays that allow detection of cellular events in intact, living cells. The skilled artisan will recognize that the invention is not limited to the fluorescent polypeptides explicitly described herein and one may use any other spectral or mutational variant or derivative in accordance with the present invention.

Several methods to identify and quantitate cells that are fluorescently tagged with fluorescent gene products are well known in the art and may be used in the context of the present invention. One example is the use of fluorescent activated cell sorting (FACS); flow cytometry or flow microfluorometry provides the means of scanning individual cells for the presence of a fluorescent protein. The method employs instrumentation that is capable of activating, and detecting the excitation emissions of cells that express a fluorescent marker in a liquid medium. FACS is unique in its ability to provide a rapid,

reliable, quantitative, and multi-parameter analysis on either living or fixed cells in culture or *in vivo*. Other methods to measure fluorescent markers are also well known.

Formulations and Therapeutic/Prophylactic Administration Thereof

5 Mammalian species which benefit from the disclosed methods of polynucleotide delivery include, and are not limited to, apes, chimpanzees, orangutans, humans, monkeys; domesticated animals (*e.g.*, pets) such as dogs, cats, guinea pigs, hamsters, Vietnamese pot-bellied pigs, rabbits, and ferrets; domesticated farm animals such as cows, buffalo, bison, horses, donkey, swine, sheep, and goats; exotic animals typically
10 found in zoos, such as bear, lions, tigers, panthers, elephants, hippopotamus, rhinoceros, giraffes, antelopes, sloth, gazelles, zebras, wildebeests, prairie dogs, koala bears, kangaroo, opossums, raccoons, pandas, hyena, seals, sea lions, elephant seals, otters, porpoises, dolphins, and whales. The terms "patient" and "subject" are used interchangeably herein are intended to include such human and non-human mammalian
15 species. According to the *in vivo* methods of the present invention, an effective amount of a viral or non-viral vector carrying a polynucleotide operatively linked to the smCBA promoter is administered to cells of a subject, wherein the polynucleotide is then expressed in the cells. The polynucleotide may encode a polypeptide that is naturally occurring within the subject's species or a different mammalian species. The expression
20 vectors used in the subject invention can comprise nucleic acid sequences encoding any human or non-human mammalian polypeptide. In instances where host cells carrying the vectors of the invention are administered to a subject, the cells may be autogenic, allogeneic, or xenogeneic, for example.

The pharmaceutical compositions of the present invention can be formulated
25 according to known methods for preparing pharmaceutically useful compositions. Furthermore, as used herein, the phrase "pharmaceutically acceptable carrier" includes any of the standard pharmaceutically acceptable carriers. The pharmaceutically acceptable carrier can include diluents, adjuvants, and vehicles, as well as implant carriers, and inert, non-toxic solid or liquid fillers, diluents, or encapsulating material that
30 does not react with the active ingredients of the invention. Examples include, but are not limited to, phosphate buffered saline, physiological saline, water, and emulsions, such as oil/water emulsions. The carrier can be a solvent or dispersing medium containing, for

example, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. Formulations containing pharmaceutically acceptable carriers are described in a number of sources which are well known and readily available to those skilled in the art. For example,
5 *Remington's Pharmaceutical Sciences* (Martin E.W., 1995, Easton Pennsylvania, Mack Publishing Company, 19th ed.), which is incorporated herein by reference in its entirety, describes formulations that can be used in connection with the subject invention.

Pharmaceutical compositions of the present invention useful for parenteral injection can include pharmaceutically acceptable sterile aqueous or nonaqueous
10 solutions, dispersions, suspensions or emulsions, as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil),
15 and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. Formulations suitable for parenteral administration include, for example, aqueous injectable solutions that may contain antioxidants, buffers, and solutes which render the
20 formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions, which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the condition of the sterile liquid carrier, for example, water for
25 injections, prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powder, granules, tablets, *etc.* It should be understood that, in addition to the ingredients particularly mentioned above, the formulations of the subject invention can include other agents conventional in the art having regard to the type of formulation in question.

30 The pharmaceutical compositions used in the methods of the present invention may also contain adjuvants such as preservatives, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by the

inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents that delay absorption, such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of the active agent (*e.g.*, a polypeptide encoded by the polynucleotide to be administered), it is desirable to slow the absorption from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility.

Injectable depot forms are made by forming microencapsule matrices of the agent (*e.g.*, vectors or host cells of the invention) in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of active agent (*e.g.*, polynucleotide or encoded polypeptide) to polymer and the nature of the particular polymer employed, the rate of release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active agents (*e.g.*, vectors or host cells of the invention) are mixed with at least one pharmaceutically acceptable excipient or carrier such as sodium nitrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia; c) humectants such as glycerol; d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; e) solution retarding agents such as paraffin; f) absorption accelerators such as quaternary ammonium compounds; g) wetting agents such as, for example, cetyl alcohol and

glycerol monostearate; h) absorbents such as kaolin and bentonite clay; and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

5 Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. 10 Optionally, the solid dosage forms contain opacifying agents, and can be of a composition that releases the vector or host cells at a target anatomical site. Examples of embedding compositions that can be used include polymeric substances and waxes. The active agents (*e.g.*, vectors or host cells of the invention) can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

15 Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the vectors or host cells of the invention, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, 20 benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and 25 perfuming agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, and tragacanth, and mixtures thereof.

30 Topical administration includes administration to the skin or mucosa, including surfaces of the lung, eye, *etc.* Compositions for topical administration, including those for inhalation, may be prepared as a dry powder, which may be pressurized or non-

pressurized. In non-pressurized powder compositions, the active ingredients in finely divided form may be used in admixture with a larger-sized pharmaceutically acceptable inert carrier comprising particles having a size, for example, of up to 100 μm in diameter. Suitable inert carriers include sugars such as lactose. Desirably, at least 95% by weight
5 of the particles of the active ingredient have an effective particle size in the range of 0.01 to 10 μm .

The pharmaceutical composition of the present invention can include a liposome component. According to the present invention, a liposome comprises a lipid composition that is capable of fusing with the plasma membrane of a cell, thereby
10 allowing the liposome to deliver a construct of the invention into the cell. Some preferred liposomes include those liposomes commonly used in gene delivery methods known to those of skill in the art. Some preferred liposome delivery vehicles comprise multilamellar vesicle (MLV) lipids and extruded lipids, although the invention is not limited to such liposomes. Methods for preparation of MLVs are well known in the art.
15 "Extruded lipids" are also contemplated. Extruded lipids are lipids that are prepared similarly to MLV lipids, but which are subsequently extruded through filters of decreasing size, as described in Templeton *et al.*, *Nature Biotech.*, 1997, 15:647-652, which is incorporated herein by reference in its entirety. Small unilamellar vesicle (SUV) lipids can also be used in the compositions and methods of the present invention. Other
20 preferred liposome delivery vehicles comprise liposomes having a polycationic lipid composition (*i.e.*, cationic liposomes). For example, cationic liposome compositions include, but are not limited to, any cationic liposome complexed with cholesterol, and without limitation, include DOTMA and cholesterol, DOTAP and cholesterol, DOTIM and cholesterol, and DDAB and cholesterol. Liposomes utilized in the present invention
25 can be any size, including from about 10 to 1000 nanometers (nm), or any size in between.

A liposome delivery vehicle can be modified to target a particular site in a mammal, thereby targeting and making use of a polynucleotide carried by a vector of the present invention at that site. Suitable modifications include manipulating the chemical
30 formula of the lipid portion of the delivery vehicle. Manipulating the chemical formula of the lipid portion of the delivery vehicle can elicit the extracellular or intracellular targeting of the delivery vehicle. For example, a chemical can be added to the lipid

formula of a liposome that alters the charge of the lipid bilayer of the liposome so that the liposome fuses with particular cells having particular charge characteristics. In one embodiment, other targeting mechanisms, such as targeting by addition of exogenous targeting molecules to a liposome (*i.e.*, antibodies) may not be a necessary component of the liposome of the present invention, since effective immune activation at immunologically active organs can already be provided by the composition when the route of delivery is intravenous or intraperitoneal, without the aid of additional targeting mechanisms. However, in some embodiments, a liposome can be directed to a particular target cell or tissue by using a targeting agent, such as an antibody, soluble receptor or ligand, incorporated with the liposome, to target a particular cell or tissue to which the targeting molecule can bind. Targeting liposomes are described, for example, in Ho *et al.*, *Biochemistry*, 1986, 25: 5500-6; Ho *et al.*, *J Biol Chem*, 1987a, 262: 13979-84; Ho *et al.*, *J Biol Chem*, 1987b, 262: 13973-8; and U.S. Patent No. 4,957,735 to Huang *et al.*, each of which is incorporated herein by reference in its entirety). In one embodiment, if avoidance of the efficient uptake of injected liposomes by reticuloendothelial system cells due to opsonization of liposomes by plasma proteins or other factors is desired, hydrophilic lipids, such as gangliosides (Allen *et al.*, *FEBS Lett*, 1987, 223: 42-6) or polyethylene glycol (PEG)-derived lipids (Klibanov *et al.*, *FEBS Lett*, 1990, 268: 235-7), can be incorporated into the bilayer of a conventional liposome to form the so-called sterically-stabilized or "stealth" liposomes (Woodle *et al.*, *Biochim Biophys Acta*, 1992, 1113: 171-99). Variations of such liposomes are described, for example, in U.S. Patent No. 5,705,187 to Unger *et al.*, U.S. Patent No. 5,820,873 to Choi *et al.*, U.S. Patent No. 5,817,856 to Tirosh *et al.*; U.S. Patent No. 5,686,101 to Tagawa *et al.*; U.S. Patent No. 5,043,164 to Huang *et al.*, and U.S. Patent No. 5,013,556 to Woodle *et al.*, all of which are incorporated herein by reference in their entireties).

The methods of this invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any mode that produces effective levels of response without causing clinically unacceptable adverse effects. Preferred modes of administration include parenteral, injection, infusion, deposition, implantation, anal or vaginal supposition, oral ingestion, inhalation, and topical administration. Injections can be intravenous, intradermal, subcutaneous, intramuscular, or intraperitoneal. For example, the pharmaceutical composition comprising the vectors

or host cells of the invention can be injected or topically applied directly into or on to a target anatomical site. In some embodiments, the doses can be given at multiple locations. Implantation includes inserting implantable drug delivery systems, *e.g.*, microspheres, hydrogels, polymeric reservoirs, cholesterol matrixes, polymeric systems, *e.g.*, matrix erosion and/or diffusion systems and non-polymeric systems, *e.g.*, compressed, fused, or partially fused pellets. Inhalation includes administering the pharmaceutical composition with an aerosol in an inhaler, either alone or attached to a carrier that can be absorbed. For systemic administration, it may be preferred that the pharmaceutical composition is encapsulated in liposomes. The term "parenteral" includes subcutaneous injections, intravenous, intramuscular, intraperitoneal, intrasternal injection or infusion techniques. In certain preferred embodiments of the invention, the administration can be designed so as to result in sequential exposure of the pharmaceutical composition over some period of time, *e.g.*, hours, days, weeks, months or years. This can be accomplished by repeated administrations of the pharmaceutical composition, by one of the methods described above, or alternatively, by a sustained-release delivery system in which the pharmaceutical composition is delivered to the subject for a prolonged period without repeated administrations. By sustained-release delivery system, it is meant that total release of the pharmaceutical composition does not occur immediately upon administration, but rather is delayed for some period of time. Release can occur in bursts or it can occur gradually and continuously. Administration of such a system can be, *e.g.*, by long-lasting oral dosage forms, bolus injections, transdermal patches, and subcutaneous implants.

The vectors and host cells of the invention can be incorporated in a physiologically acceptable carrier or salt, suitable for topical application to the affected area, or for direct injection into the affected areas, or for diffusion from an implanted device, for example. Topical applications include use of gels, creams, lotions, suppositories, and use of devices and dressings such as dissolving patches and bandages impregnated with the vectors or host cells of the invention prior to use. Additional routes of delivery include oral, and injection or infusion that is intramuscular, intravenous, subcutaneous, intraperitoneal, intraspinal, and epidural.

Definitions

As used herein, the term “viral vector” and equivalent terms refer to viruses that are utilized for transferring selected DNA or RNA sequences into a host cell. The vectors may be utilized for the purpose of transferring nucleic acids into cells either *in vitro* or *in vivo*. Viruses that have been commonly used for the transfer of genetic material *in vivo* include the retroviruses, adenoviruses (AV), adeno-associated viruses (AAV), lentiviruses, parvoviruses, and herpes viruses.

As used herein, the term “expression vector”, “construct”, “expression cassette”, and comparable terms refer to a vector that is capable of inducing the expression of nucleic acids that have been cloned into it after transformation into a host cell. Expression cassettes can be placed in viral vectors (*e.g.*, self-complementary AAV vectors, lentiviral vectors) and non-viral vectors (*e.g.*, plasmids, liposomes). The cloned genetic material is usually placed under the control of (*i.e.*, operably linked to) certain regulatory sequences such as promoters or enhancers. Promoter sequences may be constitutive, inducible, or repressible. Any prokaryotic or eukaryotic cell that is the recipient of a vector is the host for that vector. The term encompasses prokaryotic or eukaryotic cells that have been engineered to incorporate a gene in their genome. Cells that can serve as hosts are well known in the art as are techniques for cellular transformation (see *e.g.*, Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor (1989)).

The term “promoter” as used herein refers to any sequence that regulates the expression of a coding sequence, such as a gene. Promoters may be constitutive, inducible, repressible, or tissue-specific, for example. A “promoter” is a control sequence that is a region of a polynucleotide sequence at which initiation and rate of transcription are controlled. It may contain genetic elements at which regulatory proteins and molecules may bind such as RNA polymerase and other transcription factors. The phrases “operable linked”, “operatively positioned”, “operatively linked”, “under control”, and “under transcriptional control” mean that a promoter is in a correct functional location and/or orientation in relation to a nucleic acid sequence to control transcriptional initiation and/or expression of that sequence. A promoter may or may not be used in conjunction with an “enhancer,” which refers to a cis-acting regulatory sequence involved in the transcriptional activation of a nucleic acid sequence. The

vectors of the invention utilize a truncated chimeric CMV-chicken β -actin (smCBA) promoter operatively linked to a polynucleotide of interest.

A promoter may be one naturally associated with a gene or sequence, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment and/or exon. Such a promoter can be referred to as "endogenous". Similarly, an enhancer may be one naturally associated with a nucleic acid sequence, located either downstream or upstream of that sequence. Alternatively, certain advantages will be gained by positioning the coding nucleic acid segment under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with a nucleic acid sequence in its natural environment. A recombinant or heterologous enhancer refers also to an enhancer not normally associated with a nucleic acid sequence in its natural environment. Such promoters or enhancers may include promoters or enhancers of other genes, and promoters or enhancers isolated from any other prokaryotic, viral, or eukaryotic cell, and promoters or enhancers not "naturally occurring," *i.e.*, containing different elements of different transcriptional regulatory regions, and/or mutations that alter expression. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including PCR, in connection with the compositions disclosed herein (see U.S. Patent No. 4,683,202, U.S. Patent No. 5,928,906, each incorporated herein by reference). Furthermore, it is contemplated the control sequences that direct transcription and/or expression of sequences within non-nuclear organelles such as mitochondria, chloroplasts, and the like, can be employed as well.

As used herein, the terms "substantially pure" or "purified" mean that the desired product (*e.g.*, polynucleotide) is essentially free from contaminating cellular components. Contaminants may include, but are not limited to, proteins, carbohydrates and lipids. One method for determining the purity of a protein or nucleic acid is by electrophoresis in a matrix such as polyacrylamide or agarose. Purity is evidenced by the appearance of a single band after staining.

As indicated above, a nucleic acid sequence that initiates the transcription of a gene is a promoter. Promoters are typically found 5' to the gene and located proximal to the start codon. If a promoter is of the inducible type, then the rate of transcription

increases in response to an inducing agent. Expression is the process by which a polypeptide is produced from a nucleic acid sequence. The process involves the transcription of the gene or coding sequence into mRNA and the translation of this mRNA into a polypeptide. Depending on the context in which it is used, the term
5 “expression” may refer to the production of RNA, protein, or both.

As used herein, the term “recombinant” refers to nucleic acid that is formed by combining nucleic acid sequences and sequence elements. A recombinant host is any host receiving a recombinant nucleic acid and the term “recombinant protein” refers to protein produced by such a host.

10 As used herein, the term “polynucleotide” (*e.g.*, polynucleotide encoding a polypeptide) can include a nucleic acid sequence encoding a polypeptide and additional nucleic acid sequences that are coding or non-coding sequences. The term “polynucleotide” is inclusive of the terms “gene”, “transgene”, and “coding sequence”, for example. The term “polynucleotide” can include nucleic acid sequences useful in the
15 constructs of the present invention. For example, a regulatory component that may be used in the vectors of the invention is an internal ribosome entry site (IRES). An IRES sequence, or other suitable systems may be used to produce more than one gene product (*e.g.*, polypeptide) from a single gene transcript. An IRES (or other suitable sequence) is used to produce a polypeptide that contains more than one polypeptide chain (*e.g.*, a
20 fusion protein or multimer) or to express two different proteins from or within the same cell. An exemplary IRES is the poliovirus internal ribosome entry sequence, which supports transgene expression in photoreceptors, RPE and ganglion cells. Preferably, the IRES is located 3' to the transgene in the rAAV vector. In one embodiment, an IRES element is used to produce a reporter polypeptide such as GFP.

25 As used herein, the terms “gene”, “transgene”, and “coding sequence” refer to the nucleic acid sequence that undergoes transcription as the result of promoter activity. A gene may code for a particular polypeptide or for an RNA sequence that is of interest in itself, *e.g.* because it acts as an antisense oligonucleotide or interfering RNA. The gene(s) carried by the vector of the invention can encode any substance that is desired to be
30 delivered to a cell, including, without limitation, a polypeptide, protein, enzyme, carbohydrate, chemical moiety, or nucleic acid sequences which may include oligonucleotides, RNA, and/or DNA. Typically, a gene carried by a vector of the

invention refers to a nucleic acid sequence heterologous to the vector, encoding a desired product, *e.g.*, a polypeptide or RNA of interest, and the regulatory sequences which direct transcription and/or translation thereof in a host cell, and permit expression of the encoded product in a host cell. In one embodiment, the gene may be a nucleic acid molecule that introduces specific genetic modifications into human chromosomes, *e.g.*,
5 for correction of mutated genes. See, *e.g.*, D. W. Russell & R. K. Hirata, 1998, *Nat. Genet.*, 18:325-330, which is incorporated herein by reference in its entirety.

Polynucleotides carried by the vectors of the invention may encode polypeptides or other molecules (*e.g.*, interfering RNA) useful in treating a disorder, such as enzymes,
10 cytokines, hormones, structural proteins, antigens to induce an immunogenic response, *etc.* The term "treating" or "treatment" as used herein encompasses both prophylactic and therapeutic treatment. Treatment includes alleviation of one or more symptoms of the disorder and may correct the disorder. Prophylaxis includes delaying onset of the disorder (*e.g.*, delaying onset of an infection) and may include prevention of the disorder.

15 The terms "operatively linked" and "operably linked" are used interchangeably herein to refer to the functional relationship between a promoter sequence (*e.g.*, a truncated chimeric CMV-chicken β -actin promoter) and a polynucleotide, such that the promoter sequence drives expression of the polynucleotide.

The term "protein", "polypeptide", and "peptide" are used herein interchangeably
20 to refer to an amino acid sequence of any length. The polypeptide encoded by the polynucleotide carried by the vector of the invention may be a multimeric construct (*e.g.*, a fusion protein). Multimeric constructs of the subject invention typically comprise a series of repeating elements, optionally interspersed with other elements. As would be appreciated by one skilled in the art, the order in which the repeating elements occur in
25 the multimeric polypeptide is not critical and any arrangement of the repeating elements as set forth herein can be provided by the subject invention. Thus, a "multimeric construct" according to the subject invention can provide a multimeric polypeptide comprising a series of polypeptides, polypeptide fragments, or epitopes that are, optionally, joined together by linker elements (either chemical linker elements or amino
30 acid linker elements).

The terms "cell" and "cells" are used interchangeably herein to refer to a single cell or plurality of cells (*i.e.*, at least one cell). Host cells can be isolated or present in a subject *in vivo*.

Methods involving conventional molecular biology techniques are described
5 herein. Such techniques are generally known in the art and are described in detail in methodology treatises such as Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, ed. Sambrook *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989; and Current Protocols in Molecular Biology, ed. Ausubel *et al.*, Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates). Various techniques
10 using polymerase chain reaction (PCR) are described, *e.g.*, in Innis *et al.*, PCR Protocols: A Guide to Methods and Applications, Academic Press: San Diego, 1990. Methods for chemical synthesis of nucleic acids are discussed, for example, in Beaucage and Carruthers, *Tetra. Letts.*, 1981, 22:1859-1862, and Matteucci *et al.*, *J. Am. Chem. Soc.*, 1981, 103:3185. Chemical synthesis of nucleic acids can be performed, for example, on
15 commercial automated oligonucleotide synthesizers. Immunological methods (*e.g.*, preparation of antigen-specific antibodies, immunoprecipitation, and immunoblotting) are described, *e.g.*, in Current Protocols in Immunology, ed. Coligan *et al.*, John Wiley & Sons, New York, 1991; and Methods of Immunological Analysis, ed. Masseyeff *et al.*, John Wiley & Sons, New York, 1992. Conventional methods of gene transfer and gene
20 therapy can also be adapted for use in the present invention. See, *e.g.*, Gene Therapy: Principles and Applications, ed. T. Blackenstein, Springer Verlag, 1999; Gene Therapy Protocols (Methods in Molecular Medicine), ed. P. D. Robbins, Humana Press, 1997; and Retro-vectors for Human Gene Therapy, ed. C. P. Hodgson, Springer Verlag, 1996.

Following are examples that illustrate materials, methods, and procedures for
25 practicing the invention. The examples are illustrative and should not be construed as limiting.

Example 1—Transduction and Tropism of an Abbreviated Form of CMV-Chicken β -actin Promoter (CBA) with AAV in Mouse Retina

30 The chimeric CMV-chicken β -actin promoter (CBA) has been utilized extensively as a promoter of expression in a wide variety of cell types when delivered by recombinant adeno-associated virus (rAAV) vectors. Besides broad tropism, CBA has the capacity to

promote expression for long periods post-infection, in our experience up to 5 years in retina (Acland *et al.*, *Mol. Ther.*, 2005, 12(6):1072-1082, which is incorporated herein by reference in its entirety). Another commonly used broad tropism promoter, cytomegalovirus immediate early promoter (CMV), while much smaller in size, has shown a tendency to 'shutdown' in relatively quick fashion (Kachi *et al.*, *Gene Ther.*, 2006, 13(9):798-804, which is incorporated herein by reference in its entirety). Because of the restrictive packaging capacity of AAV (4.8 kb), the large size of CBA (1.7 kb) greatly limits the size of a cDNA that can be expressed in a rAAV construct. The development of self-complementary AAV vectors with even less packaging capacity than standard AAV has further highlighted the utility of a smaller version of CBA that still retains broad tropism and sustained expression.

A. CBA vs. smCBA comparison

The present inventors directly compared the expression pattern of full-length CBA (1720 bases) to a smaller version, smCBA (953 bases), in which the hybrid chicken β -actin/rabbit β -globin intron is greatly shortened (shown in Figure 3). Neonate and adult C57Black6 mice were injected subretinally with 1×10^{10} vg/ml of AAV serotype 2 containing smCBA driving green fluorescent protein (AAV2-smCBA-GFP) or the same construct with the full CBA promoter (AAV2-CBA-GFP). Each injection contained an equal amount of AAV2-CMV-LacZ as internal standard, thereby accounting for injection variation. Whole mount retinas were examined 3-4 weeks post injection by both LacZ staining and anti-GFP antibody staining. SmCBA AAV constructs are shown in Figures 4A-4C. Results are shown in Figures 5-10.

B. Long-term persistence of smCBA driven expression and evaluation of rescue in retinal degenerative animal models

AAV-smCBA constructs driving transgene expression were evaluated for rescue in two separate retinal degenerative mouse models, the RD10 mutant and the GC1 knock-out. Control injections of AAV2-smCBA-GFP or AAV5-smCBA-GFP vectors were used to evaluate persistence of smCBA expression. Rescue was determined using ERG analysis for the RD10 model and restoration of protein translocation (as measured by

immunohistochemistry) in the GC1 knock-out. GFP and transgene expression was evaluated up to 6 months post-injection.

C. Cell specificity/targeting of smCBA

5 Injected eyecups were enucleated, sectioned and immunostained with antibodies against GFP. Sections were counterstained with DAPI and visualized with fluorescent and confocal microscopy.

D. Conclusions

10 Results of AAV mediated transgene expression following subretinal delivery to mouse retina indicate that the smCBA promoter is equivalent to the full-length CBA promoter. smCBA allows the incorporation of larger cDNAs into AAV vectors. Recent advances with self-complementary AAV (scAAV) vectors highlight the need for small promoters capable of driving sustained transgene expression that exhibit broad tropism,
15 such as smCBA.

Example 2— Self-complementary AAV Vectors Promote Fast and Efficient Transduction of Mouse Retina

20 Adeno-associated virus packages a single-stranded DNA molecule of up to 4800 nucleotides in length. Following infection of cells by virus, intrinsic molecular machinery of the cell is required for conversion of single stranded DNA into double stranded form. This second strand synthesis is the rate limiting step to transduction by AAV. Recently, self-complementary (double-stranded) adeno-associated virus (scAAV) has been shown to promote faster transduction than matched standard AAV in selected
25 mouse tissues (McCarty *et al.*, *Gene Ther.*, 2003, Dec,10(26):2112-2118, which is incorporated herein by reference in its entirety). The goal in these experiments was to compare the temporal and cell-type transduction properties of scAAV vectors when delivered to the mouse retina to those already established for standard AAV vectors.

30 A. AAV vectors

AAV vectors with the mouse opsin promoter (MOPS) (Flannery *et al.*, *Proc Natl Acad Sci U S A*, 1997, 94(13):6916-21, which is incorporated herein by reference in its entirety) and an abbreviated version of the chimeric CMV-chicken β -actin promoter

(smCBA) (described in Example 1) driving humanized green fluorescent protein (GFP) were already available in the inventors' laboratory. Matching scAAV vectors were made from vector plasmid with a mutation in one of the terminal resolution sequences of the AAV virus (McCarty *et al.*, *Gene Ther.*, 2003, 10(26):2112-2118, which is incorporated
5 herein by reference in its entirety) (shown in Figures 11A and 11B).

B. Comparison of scAAV to AAV Vectors in Mouse Retina

Subretinal or intravitreal injections of serotype 1, 2 and 5 scAAV and AAV vectors with matching promoters expressing green fluorescent protein (GFP) were
10 performed in adult mice. In some cases, injections with matching standard AAV vectors were done for direct comparison. GFP expression was evaluated in retinal whole mounts and in sections by direct GFP fluorescence or GFP immunohistochemistry. Results are shown in Figures 12-16.

15 C. Conclusions

Self-complimentary AAV vectors support onset of strong passenger gene expression in RPE cells, retinal ganglion cells and photoreceptors within 2 days. The rapidity of expression onset is independent of the AAV vector serotype tested: 1, 2 and 5. In side-by-side experiments, self-complimentary AAV vector expresses passenger genes
20 significantly more rapidly than conventional single-stranded AAV vectors, and at potentially higher levels.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and
25 tables, to the extent they are not inconsistent with the explicit teachings of this specification.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and
30 purview of this application.

Table 1. Examples of Host Cells	
Keratinizing Epithelial Cells	
keratinocyte of epidermis	
basal cell of epidermis	
keratinocyte of fingernails and toenails	
basal cell of nail bed	
hair shaft cells	
medullary	
cortical	
cuticular	
hair-root sheath cells	
cuticular	
of Huxley's layer	
of Henle's layer	
external	
hair matrix cell	
Cells of Wet Stratified Barrier Epithelia	
surface epithelial cell of stratified squamous epithelium of cornea tongue, oral cavity, esophagus, anal canal, distal urethra, vagina	
basal cell of these epithelia	
cell of urinary epithelium	
Epithelial Cells Specialized for Exocrine Secretion	
cells of salivary gland	
mucous cell	
serous cell	
cell of von Ebner's gland in tongue	
cell of mammary gland, secreting milk	
cell of lacrimal gland, secreting tears	
cell of ceruminous gland of ear, secreting wax	
cell of eccrine sweat gland, secreting glycoproteins	
cell of eccrine sweat gland, secreting small molecules	
cell of apocrine sweat gland	
cell of gland of Moll in eyelid	
cell of sebaceous gland, secreting lipid-rich sebum	
cell of Bowman's gland in nose	
cell of Brunner's gland in duodenum, secreting alkaline solution of mucus and enzymes	
cell of seminal vesicle, secreting components of seminal fluid, including fructose	
cell of prostate gland, secreting other components of seminal fluid	
cell of bulbourethral gland, secreting mucus	
cell of Bartholin's gland, secreting vaginal lubricant	
cell of gland of Littre, secreting mucus	
cell of endometrium of uterus, secreting mainly carbohydrates	
isolated goblet cell of respiratory and digestive tracts, secreting mucus	
mucous cell of lining of stomach	
zymogenic cell of gastric gland, secreting pepsinogen	
oxyntic cell of gastric gland, secreting HCl	

Table 1. Examples of Host Cells	
acinar cell of pancreas, secreting digestive enzymes and bicarbonate	
Paneth cell of small intestine, secreting lysozyme	
type II pneumocyte of lung, secreting surfactant	
Clara cell of lung	
Cells Specialized for Secretion of Hormones	
cells of anterior pituitary, secreting	
growth hormone	
follicle-stimulating hormone	
luteinizing hormone	
prolactin	
adrenocorticotrophic hormone	
thyroid-stimulating hormone	
cell of intermediate pituitary, secreting melanocyte-stimulating hormone	
cells of posterior pituitary, secreting	
oxytocin	
vasopressin	
cells of gut and respiratory tract, secreting	
serotonin	
endorphin	
somatostatin	
gastrin	
secretin	
cholecystokinin	
insulin	
glucagons	
bombesin	
cells of thyroid gland, secreting	
thyroid hormone	
calcitonin	
cells of parathyroid gland, secreting	
parathyroid hormone	
oxyphil cell	
cells of adrenal gland, secreting	
epinephrine	
norepinephrine	
steroid hormones	
mineralocorticoids	
glucocorticoids	
cells of gonads, secreting	
testosterone	
estrogen	
progesterone	
cells of juxtaglomerular apparatus of kidney	
juxtaglomerular cell	
macula densa cell	
peripolar cell	

Table 1. Examples of Host Cells	
mesangial cell	
Epithelial Absorptive Cells in Gut, Exocrine Glands, and Urogenital Tract	
brush border cell of intestine	
striated duct cell of exocrine glands	
gall bladder epithelial cell	
brush border cell of proximal tubule of kidney	
distal tubule cell of kidney	
nonciliated cell of ductulus efferens	
epididymal principal cell	
epididymal basal cell	
Cells Specialized for Metabolism and Storage	
hepatocyte	
fat cells (<i>e.g.</i> , adipocyte)	
white fat	
brown fat	
lipocyte of liver	
Epithelial Cells Serving Primarily a Barrier Function, Lining the Lung, Gut, Exocrine Glands, and Urogenital Tract	
type I pneumocyte	
pancreatic duct cell	
nonstriated duct cell of sweat gland, salivary gland, mammary gland, <i>etc.</i>	
parietal cell of kidney glomerulus	
podocyte of kidney glomerulus	
cell of thin segment of loop of Henle	
collecting duct cell	
duct cell of seminal vesicle, prostate gland, <i>etc.</i>	
Epithelial Cells Lining Closed Internal Body Cavities	
vascular endothelial cells of blood vessels and lymphatics (<i>e.g.</i> , microvascular cell)	
fenestrated	
continuous	
splenic	
synovial cell	
serosal cell	
squamous cell lining perilymphatic space of ear	
cells lining endolymphatic space of ear	
squamous cell	
columnar cells of endolymphatic sac	
with microvilli	
without microvilli	
“dark” cell	
vestibular membrane cell	
stria vascularis basal cell	
stria vascularis marginal cell	
cell of Claudius	
cell of Boettcher	
choroid plexus cell	

Table 1. Examples of Host Cells	
squamous cell of pia-arachnoid	
cells of ciliary epithelium of eye	
pigmented	
nonpigmented	
corneal "endothelial" cell	
Ciliated Cells with Propulsive Function	
of respiratory tract	
of oviduct and of endometrium of uterus	
of rete testis and ductulus efferens	
of central nervous system	
Cells Specialized for Secretion of Extracellular Matrix	
epithelial:	
ameloblast	
planum semilunatum cell of vestibular apparatus of ear	
interdental cell of organ of Corti	
nonepithelial:	
fibroblasts	
pericyte of blood capillary (Rouget cell)	
nucleus pulposus cell of intervertebral disc	
cementoblast/cementocyte	
odontoblast/odontocyte	
chondrocytes	
of hyaline cartilage	
of fibrocartilage	
of elastic cartilage	
osteoblast/osteocyte	
osteoprogenitor cell	
hyalocyte of vitreous body of eye	
stellate cell of perilymphatic space of ear	
Contractile Cells	
skeletal muscle cells	
red	
white	
intermediate	
muscle spindle-nuclear bag	
muscle spindle-nuclear chain	
satellite cell	
heart muscle cells	
ordinary	
nodal	
Purkinje fiber	
Cardiac valve tissue	
smooth muscle cells	
myoepithelial cells:	
of iris	
of exocrine glands	

Table 1. Examples of Host Cells	
Cells of Blood and Immune System	
red blood cell (erythrocyte)	
megakaryocyte	
macrophages	
monocyte	
connective tissue macrophage	
Langerhan's cell	
osteoclast	
dendritic cell	
microglial cell	
neutrophil	
eosinophil	
basophil	
mast cell	
plasma cell	
T lymphocyte	
helper T cell	
suppressor T cell	
killer T cell	
B lymphocyte	
IgM	
IgG	
IgA	
IgE	
killer cell	
stem cells and committed progenitors for the blood and immune system	
Sensory Transducers	
photoreceptors	
rod	
cones	
blue sensitive	
green sensitive	
red sensitive	
hearing	
inner hair cell of organ of Corti	
outer hair cell of organ of Corti	
acceleration and gravity	
type I hair cell of vestibular apparatus of ear	
type II hair cell of vestibular apparatus of ear	
taste	
type II taste bud cell	
smell	
olfactory neuron	
basal cell of olfactory epithelium	
blood pH	
carotid body cell	

Table 1. Examples of Host Cells	
type I	
type II	
touch	
Merkel cell of epidermis	
primary sensory neurons specialized for touch	
temperature	
primary sensory neurons specialized for temperature	
cold sensitive	
heat sensitive	
pain	
primary sensory neurons specialized for pain	
configurations and forces in musculoskeletal system	
proprioceptive primary sensory neurons	
Autonomic Neurons	
cholinergic	
adrenergic	
peptidergic	
Supporting Cells of Sense Organs and of Peripheral Neurons	
supporting cells of organ of Corti	
inner pillar cell	
outer pillar cell	
inner phalangeal cell	
outer phalangeal cell	
border cell	
Hensen cell	
supporting cell of vestibular apparatus	
supporting cell of taste bud	
supporting cell of olfactory epithelium	
Schwann cell	
satellite cell	
enteric glial cell	
Neurons and Glial Cells of Central Nervous System	
neurons	
glial cells	
astrocyte	
oligodendrocyte	
Lens Cells	
anterior lens epithelial cell	
lens fiber	
Pigment Cells	
melanocyte	
retinal pigmented epithelial cell	
iris pigment epithelial cell	
Germ Cells	
oogonium/oocyte	

Table 1. Examples of Host Cells	
spermatocyte	
Spermatogonium	
blast cells	
fertilized ovum	
Nurse Cells	
ovarian follicle cell	
Sertoli cell	
thymus epithelial cell (<i>e.g.</i> , reticular cell)	
placental cell	

CLAIMS

We claim:

1. A vector comprising a truncated chimeric CMV-chicken β -actin promoter.
2. The vector of claim 1, wherein said truncated chimeric CMV-chicken β -actin promoter consists of the nucleic acid sequence of SEQ ID NO:1.
3. The vector of claim 1 or 2, further comprising a heterologous polynucleotide operably linked to the truncated chimeric CMV-chicken β -actin promoter.
4. The vector of claim 3, wherein the polynucleotide encodes a polypeptide.
5. The vector of claim 3, wherein the polynucleotide encodes an interfering RNA molecule or antisense oligonucleotide.
6. The vector of any preceding claim, wherein the vector is a viral vector.
7. The vector of any preceding claim, wherein the vector is a self-complementary adeno-associated virus (scAAV).
8. An isolated host cell comprising the vector of any of claims 1-7.
9. The isolated cell of claim 8, wherein the cell is a human cell.
10. The isolated cell of claim 8, wherein the cell is a non-human animal cell.
11. The isolated cell of any of claims 8-10, wherein the cell is of a type selected from the group consisting of brain cell, liver cell, lung cell, cardiac cell, ocular cell, and smooth muscle cell.

12. The isolated cell of any of claims 8-10, wherein the cell is a neuron.
13. A pharmaceutical composition comprising the vector of any of claims 1-7 or the isolated host cell of any of claims 8-13; and a pharmaceutically acceptable carrier.
14. A method for delivering a polynucleotide to a cell, comprising administering an effective amount of the vector of any of claims 3-7 to the cell, wherein the polynucleotide is expressed in the cell.
15. The method of claim 14, wherein said administering is carried out *in vitro*.
16. The method of claim 14, wherein said administering is carried out *in vivo*.
17. The method of any of claims 14-16, wherein the cell is a human cell.
18. The method of any of claims 14-16, wherein the cell is a non-human animal cell.
19. The method of any of claims 14-18, wherein the cell is of a type selected from the group consisting of brain cell, liver cell, lung cell, cardiac cell, ocular cell, and smooth muscle cell.
20. The method of any of claims 14-18, wherein the cell is a neuron.
21. The method of claim 16, wherein said administering comprising administering an effective amount of the vector to a human or animal systemically.
22. The method of claim 16, wherein said administering comprises administering an effective amount of the vector to a human or animal locally at the site of the cell.
23. The method of claim 16, wherein the vector is administered to cells of the eye.

24. The method of claim 23, wherein the vector is administered to the vitreous or subretinally.

25. The method of any of claims 14-24, wherein the polynucleotide is heterologous to the cell.

26. The method of any of claims 14-24, wherein the polynucleotide is native to the cell.

27. The method of any of claims 14-26, wherein the polynucleotide encodes a polypeptide.

28. The method of any of claims 14-25 or 27, wherein the polynucleotide encodes an interfering RNA molecule or antisense oligonucleotide.

29. The method of any of claims 16-28, wherein the vector is administered to a human or animal suffering from, or at risk of developing, a pathologic condition, and the vector is administered to treat or delay onset of the pathologic condition.

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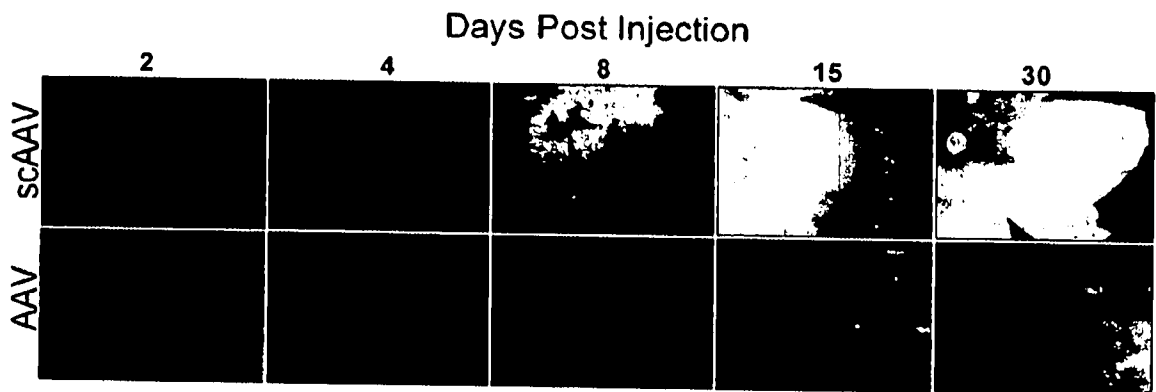


FIG. 1

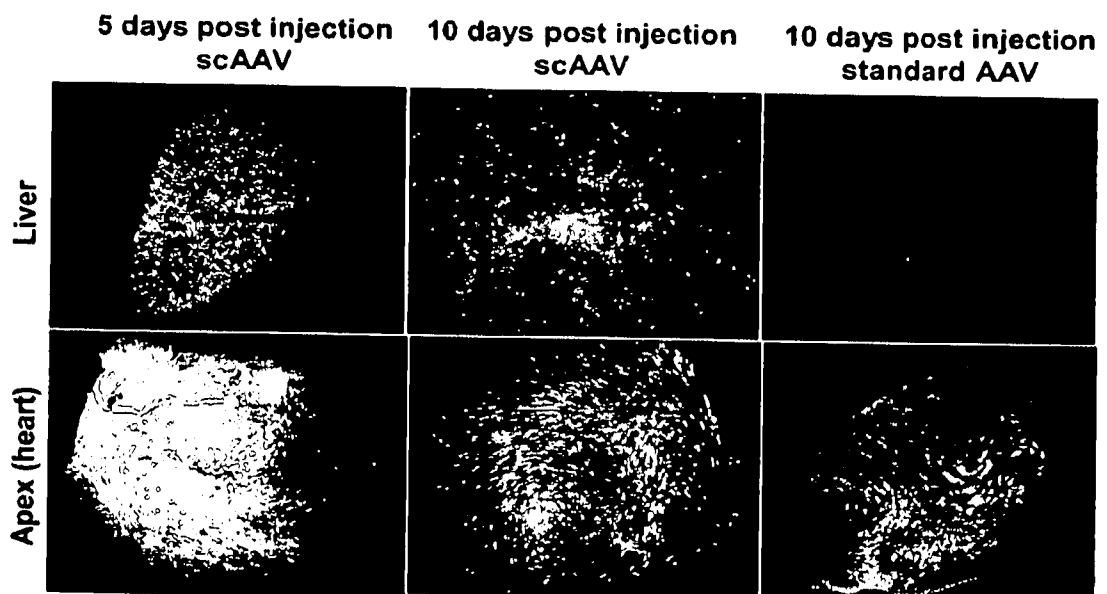


FIG. 2

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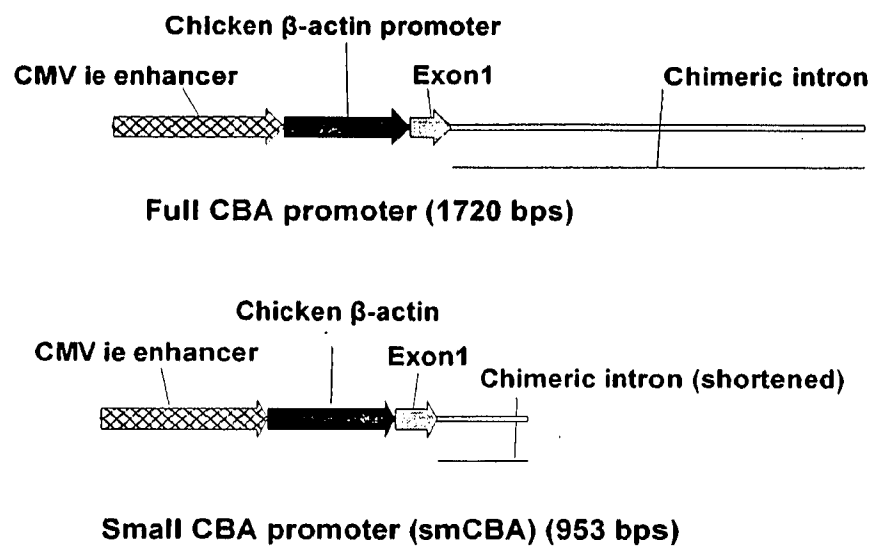


FIG. 3

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FIG. 4A

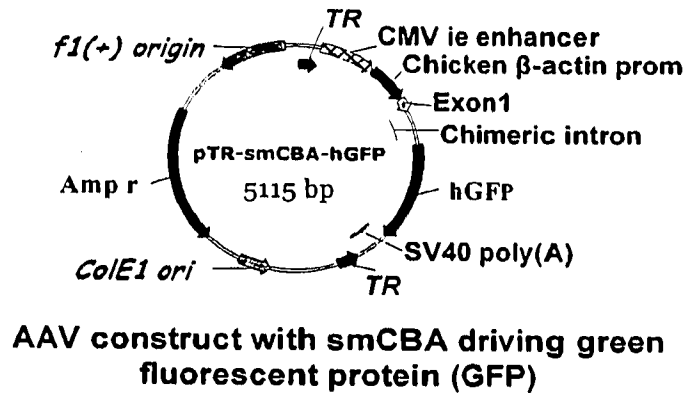


FIG. 4B

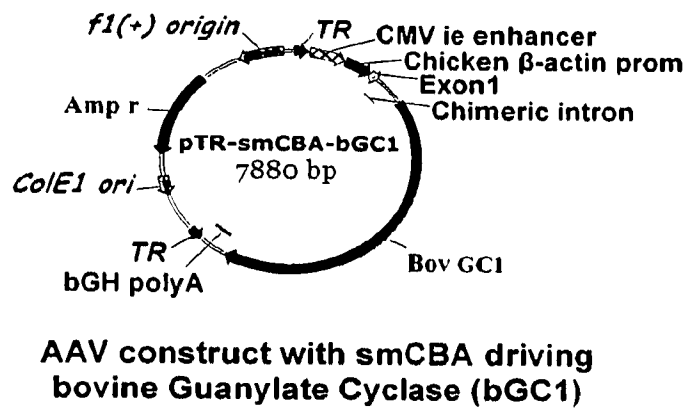


FIG. 4C

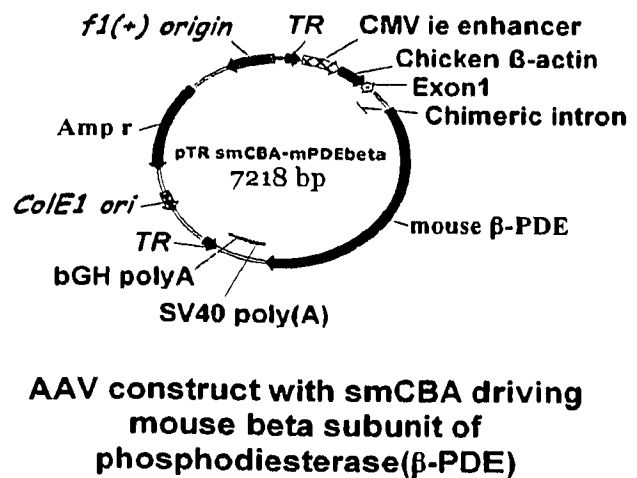


FIG. 5A-1

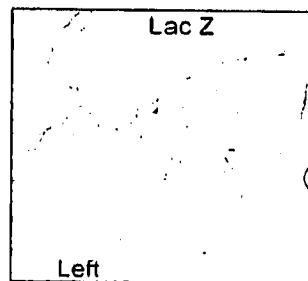


FIG. 5A-2

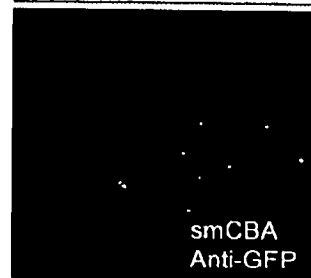


FIG. 5B-1

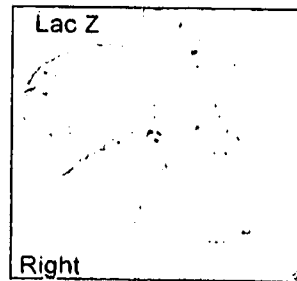


FIG. 5B-2



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FIG. 5C-1

FIG. 5C-2

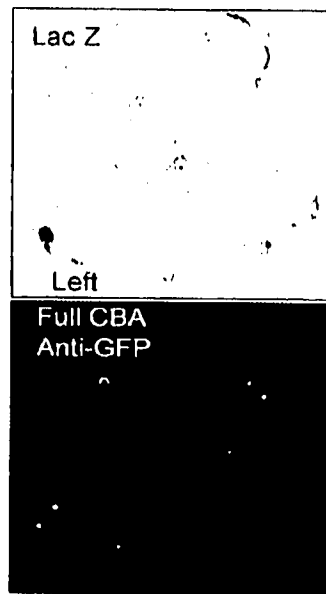
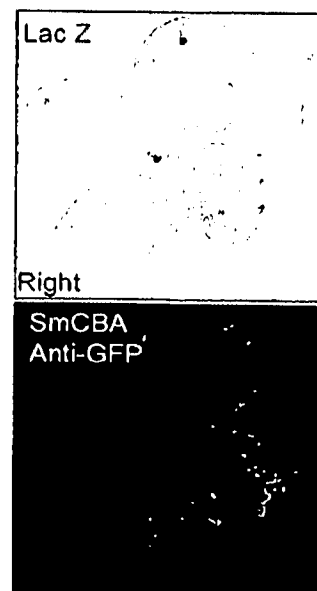


FIG. 5D-1

FIG. 5D-2



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FIG. 6A-1

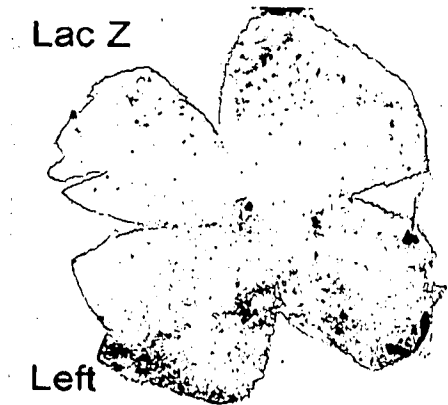


FIG. 6A-2

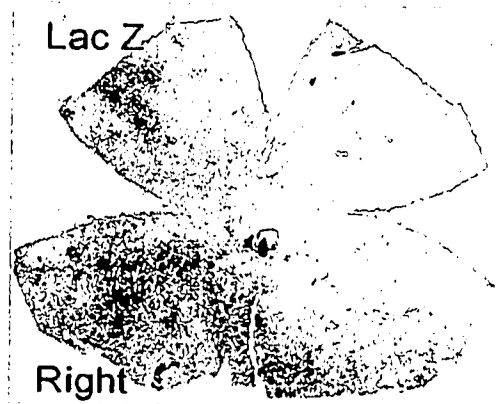
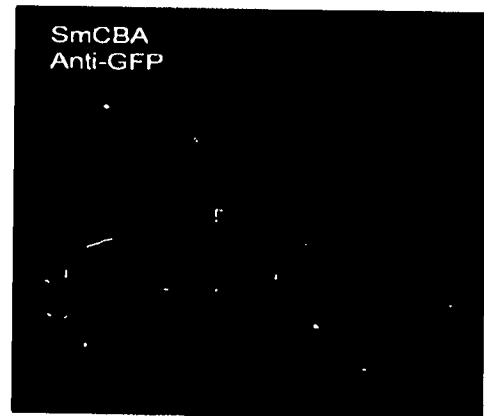


FIG. 6B-2



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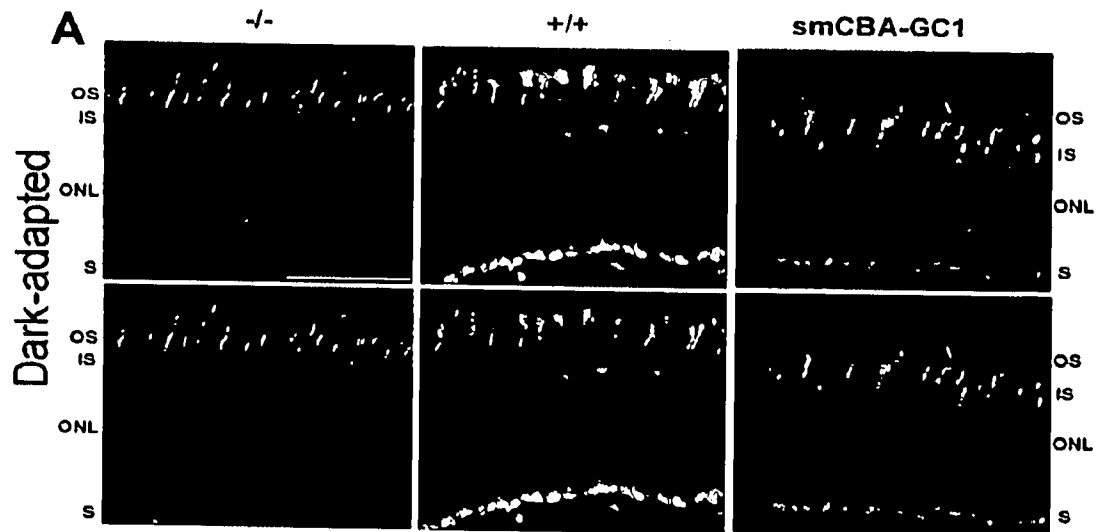


FIG. 7

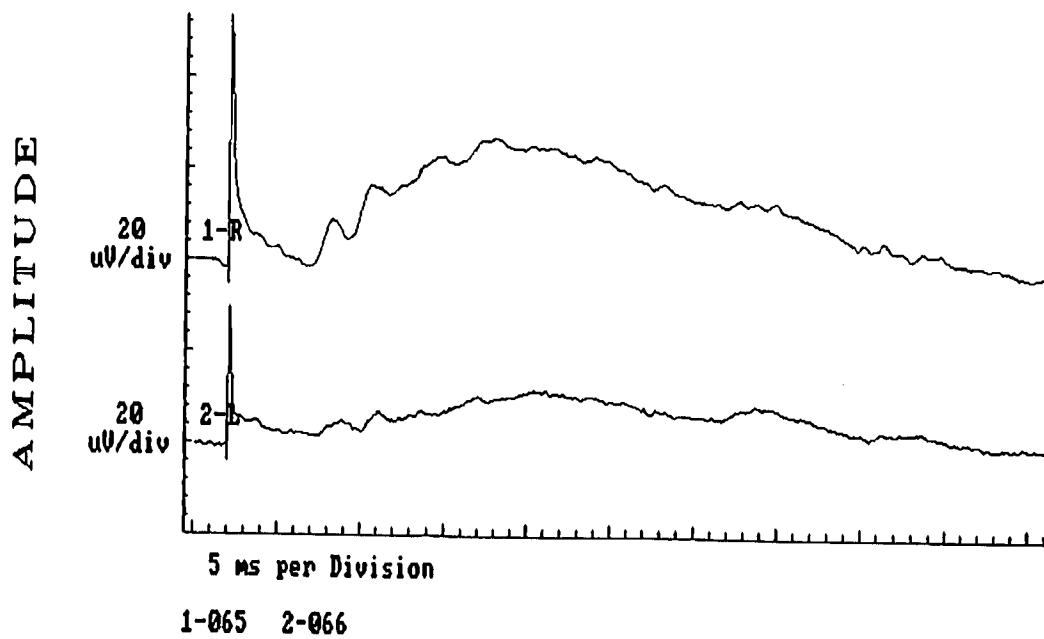


FIG. 8

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FIG. 9

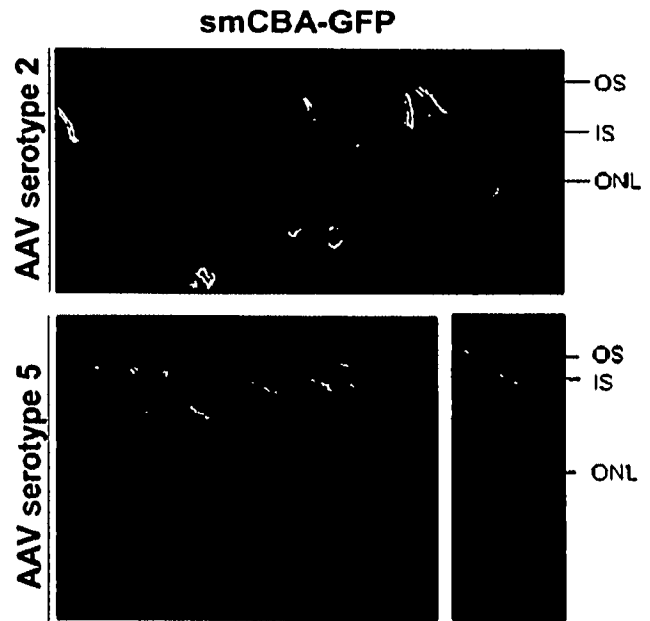


FIG. 10



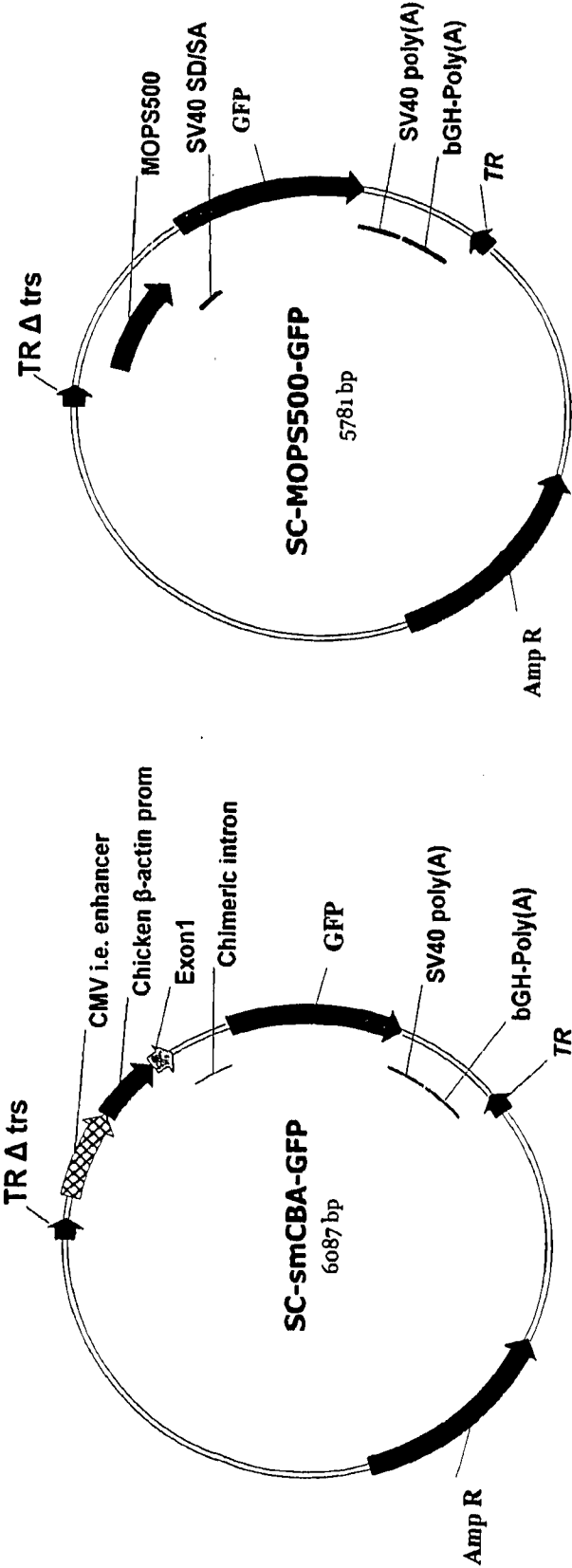


FIG. 11A

FIG. 11B

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FIG. 12A

FIG. 12B

FIG. 12C

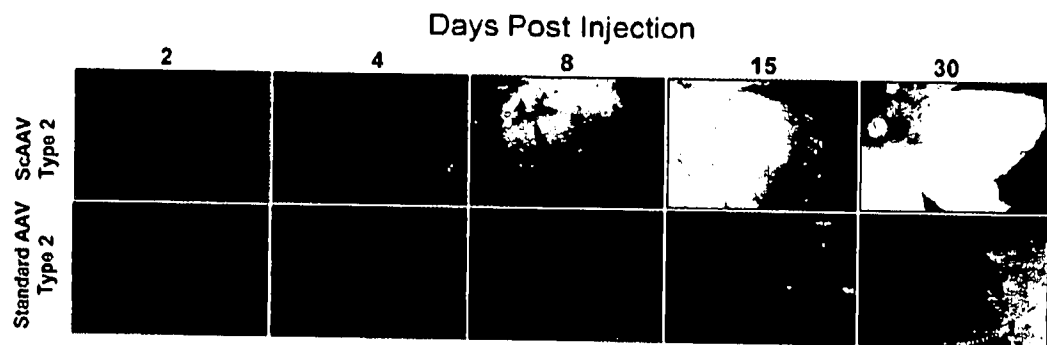


FIG. 13

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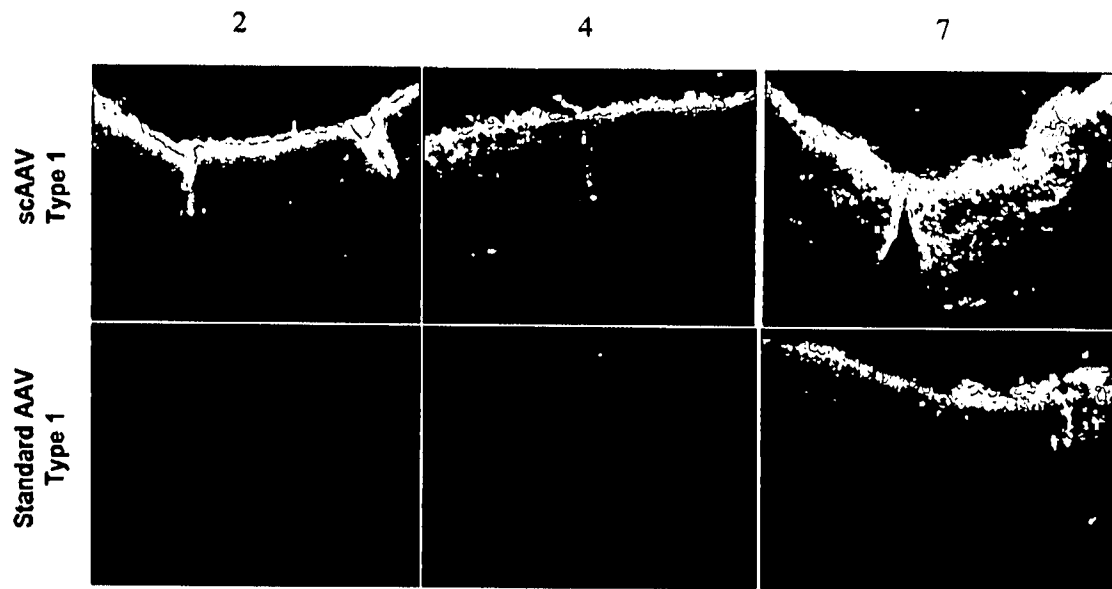


FIG. 14

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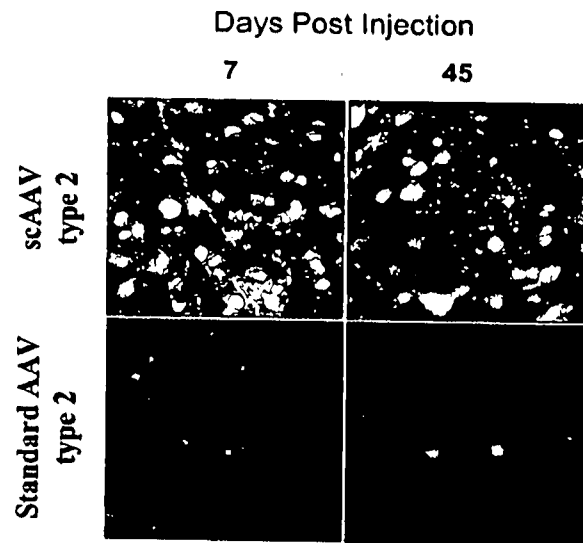


FIG. 15

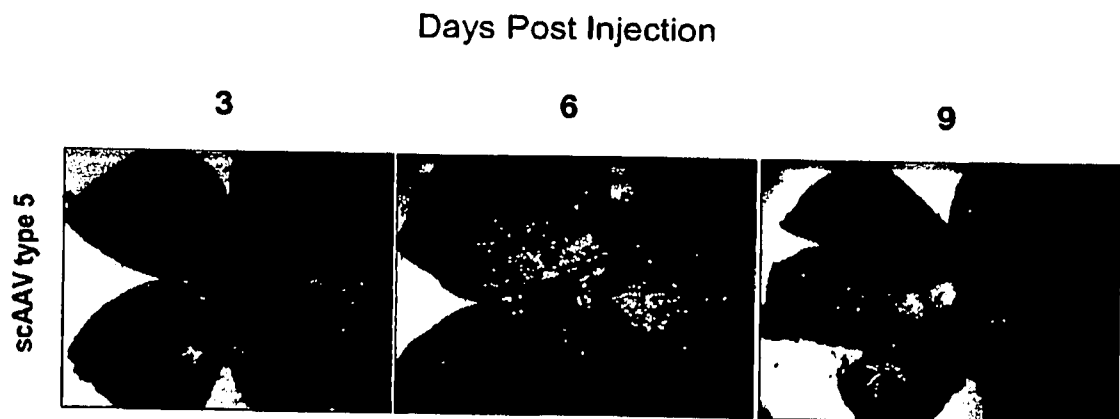


FIG. 16