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DELIVERY OF MULTIPLE GENES TO CELLS USING PLURAL ADENO-ASSOCIATED VIRAL VECTORS



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INTRODUCTION

Adeno-associated viruses (AAV's) were discovered as satellite viruses present in preparations of adenovirus. AAV isolates (human, simian, and avian) cause no apparent disease and are not related etiologically with cancer or any other adverse effect. In some studies, AAV was associated with a protective effect on carcinogenesis mediated by other viruses, such as tumorigenic strains of adenovirus and bovine papilloma virus.

Recombinant AAV (rAAV) vectors have been used as vectors for expressing gene products in vitro (usually in tissues culture) and in animals, see, for example, U.S. Pat. No. 5,193,941 and WO 94/13788, generally in the lungs (Afione et al., J. Virol. (1996) 70:3235-3241) or oral mucosa, the normal sites of AAV infection, but also, for example, in spinal cord neurons (Peel et al., Gene Ther. (1997) 4:16-24); liver (Snyder et al., Nat. Genet. (1997) 16:270-276; Koeberl et al., Proc. Natl. Acad. Sci (1997) 94:1426-1431); retina (Ali et al., Hum. Mol. Genet. (1996) 5:591-594; Zolotukhin et al., J. Virol. (1996) 70:4646-4654); bone marrow cells (Goodman et al., Blood (1994) 84:1492-1500); brain (Kaplitt et al., Nat. Genet. (1994) 8:148-154; McCown et al., Brain Res. (1996) 713:99-107); cardiac muscle (Kaplitt et al., Ann. Thorac. Surg. (1996) 62:1669-1676); and

muscle (Kessler et al., Proc. Natl. Acad. Sci. (1996) 93:14082-14087; Xiao et al., J. Virol. (1996) 70:8098-8108; Fisher et al., Nature Med. (1997) 3:306-312; and Snyder et al., Hum. Gene Ther. (1997) 8:1891-1900).

Transduction with recombinant AAV vectors has been attempted, for example, in developing a treatment for cystic fibrosis. The AAV vectors in those experiments were administered by intraperitoneal injection, intratracheal injection and direct endobronchial instillation in animals, see Flotte & Carter, Gene Ther. (1995) 2:357-362. The methods of delivering the AAV were focused on providing the recombinant AAV vector at a site closest to the lung. However, intraperitoneal, intratracheal and endobronchial injection are undesirable for routine use in humans due in part to the invasiveness of the procedures and the dangers of producing infection and adhesions, see Goodman & Gilman, "The Pharmacological Basis of Therapeutics" pp. 1-32, 8th ed., 1993; and "Remington's Pharmaceutical Sciences" p. 1686, 18th ed., 1990.

There are several scenarios where it would be desirable to introduce two or more genes into a single cell or to introduce into a host a plurality of vectors carrying different genes wherein those different expressed by different cells express gene products which interact structurally or functionally. Thus, one gene may encode a cofactor required for the proper functioning of a second gene. For example, tyrosine hydroxylase requires tetrahydrobiopterin to function. Genes encoding tyrosine hydroxylase and tetrahydrobiopterin may be cloned in separate vectors and used to transform single cells or different cells, preferably in proximity so that the gene products can be received by the respective cells, to obtain proper tyrosine hydroxylase activity.

In another scenario, the plural genes may be related as a means for coordinated regulation or enhanced regulation of a foreign gene. Thus, a first gene product may be a

regulator in the expression of a second gene product. Thus, well-controlled expression of the second gene product by the first gene product can be obtained.

Another reason for transferring multiple genes would be where the individual gene products associate to form a functional molecule. Thus, the plural vectors may express the various subunits which require association to form a functional composite molecule. See for example, Kaufman, Curr. Op. Biotech. (1990) 1:141-150.

Yet another scenario for transforming with plural vectors would be to introduce multiple gene products required in a metabolic pathway. Thus, the gene products could be two different enzymes required in the synthesis of a final end product.

In another example, the plural vectors are configured to carry the various elements required for expression of a nucleic acid encoding a polypeptide or polynucleotide of interest. Thus, while one vector may carry the above-mentioned nucleic acid without any regulatory elements required for expression, a second vector would carry a promoter which when operably linked to said first nucleic acid encoding a polypeptide or polynucleotide of interest would obtain expression of same. Moreover, there may be a third vector carrying an enhancer sequence operable with the transgenes of the first and second vectors, and so on. The vectors alone are incapable of expression but once placed in operable linkage, proper expression of the gene of interest is obtained.

Clearly, there are other scenarios where the transfer or plural genes by plural vectors is desirable, the above is merely a non-exhaustive listing of several of such possibilities.

As an example of the second scenario described hereinabove, the tTA or tet gene regulation system is comprised of the DNA binding domain of the E. coli tet repressor fused to the activation domain of the herpes simplex VP16 protein. The result is a chimeric tetracycline-controlled transactivator (tTA) which regulates expression of a second gene under

the control of the tet operator. The regulation is rapid, reversible and occurs over several orders of magnitude. Efficient induction of expression occurs in mammalian cell clones stably transduced with both constructs after withdrawal of tetracycline. Gene expression quickly decreases in the presence of tetracycline, which disrupts the binding of the transactivator to the operator sequences (Gossen & Bujard, Proc. Natl. Acad. Sci. (1992) 89:5547-5551).

Dhawan et al. (Somat. Cell Mol. Genet. (199) 21:233-240) demonstrated that the tet regulated system is efficient following direct injection of plasmid DNA into the skeletal muscle of adult mice. When the transactivator and inducible target plasmids were co-injected into mouse muscle, expression levels were two orders of magnitude higher than in control animals injected with the target plasmid alone. The expression from the inducible marker plasmid alone was close to baseline levels. The oral administration of tetracycline efficiently suppressed gene expression, which was dose dependent.

Erythropoietin (Epo) is the hormone which stimulates erythrocyte production (Naffakh & Danos, Mol. Med. Today (1996) 2:343-348). The production of erythrocytes can be monitored by periodically measuring the hematocrit. Alternatively, Epo can be measured directly in the circulation, such as be ELISA or RIA.

Bohl et al. (Nat. Med. (1997) 3:299-305) showed that primary myoblasts could be transduced in culture with retroviral vectors encoding a tetracycline regulatable system controlling Epo expression and that regulation could be achieved in culture. To demonstrate regulation of a therapeutic gene in an animal, the cells then were implanted into regenerating anterior tibialis muscles of the adult mouse. Control animals received myoblasts singly transduced with the inducible Epo vector alone. Doxycycline then was iteratively administered to, and withdrawn from the experimental animals over the course of 5 months. Because a reverse tet binding mutant was used, on administration of doxycycline, gene

expression should increase. After doxycycline administration, serum Epo levels were seen to rise in about 4 days, as assayed by RIA, and hematocrit increased at a slower rate (about two weeks). Epo levels declined significantly by four days post drug withdrawal and hematocrit declined to baseline levels over 7 or 8 weeks. Control animals showed no significant induction of Epo expression in the presence of doxycycline by either RIA or hematocrit.

The production of L-dopa (Figure 1) requires the activity of the enzyme tyrosine hydroxylase (TH). TH requires the co-factor, tetrahydrobiopterin (BH4), for activity. The rate-limiting enzyme in the production of BH4 is GTP cyclohydrolase I (GTPCH). Bencsics et al. (J. Neurosci. (1996) 16:4449-4456) demonstrated that transduction of rat fibroblasts with separate retroviral vectors encoding human TH and rat GTPCH resulted in the production of L-dopa in culture and after implantation of the modified fibroblasts into the striatum.

Leff et al. ("In Vivo L-Dopa Production Via Intrastriatal Recombinant AAV Injections in a Rat Model of Parkinson's Disease (PD)" in The 6th International Neural Transplantation Meeting, San Diego, CA, 13-16 February 1997) reported that direct transduction of rat striatal neurons with separate AAV vectors encoding human TH and human GTPCH, resulted in the production of L-dopa in vivo.

A goal of gene therapy is to provide long-term, therapeutically effective levels of expression of a particular gene of interest, in or adjacent to the tissues where administered or at distal sites following systemic transport of the vector or the gene product of the vector to the tissue or cells where such gene expression is therapeutically beneficial. That may require obtaining regulated expression of the gene of interest in the targeted tissues, using for example, a promoter in the recombinant AAV vector that operates in the targeted tissues,

possibly requiring a mode of administration that delivers the recombinant AAV vector to the appropriate tissues or both.

Muscle is a suitable tissue target, whether the target tissue is the muscle or some other tissue accessible by the circulation, because of accessibility and high vascularization. Muscle also may be a suitable target because it is a multinucleate cell (Hall & Ralston, Cell (1989) 59:771-772).

SUMMARY OF THE INVENTION

The instant invention demonstrates that two or more AAV-derived vectors may be used in combination to deliver separate exogenous or foreign DNA's to the same cell or to multiple cells for effective expression of plural genes of interest in animals, including humans.

The exogenous DNA's preferably comprise genes which encode any diffusible polypeptide, ribozyme, nucleic acid or antisense oligonucleotide for therapy. One of the exogenous DNA's can comprise a gene encoding an effector molecule required for the expression of the other gene. The effector molecule may be an enzyme, a cofactor or a part of a composite molecule comprised of plural polypeptides, plural nucleotides or a combination of both, wherein function requires assemblage of the composite by the components thereof. The instant invention provides a means for delivering AAV vectors to obtain regulated and long-term expression in regions proximal to or distal from the transformed cell or cells.

The discovery that the delivery of plural, different recombinant AAV vectors for gene therapy can result in the production of molecules which are therapeutic is surprising because

there has been no demonstration yet that plural gene expression can be achieved from two separate AAV vectors, and particularly from a single cell transformed in the absence of selection. Several examples of delivering and expressing two genes (using a promoter in combination with an internal ribosomal entry site (IRES) or using two separate promoters) contained in a single rAAV vector have been described (Zolotukhin et al., J. Virol. (1996) 70:4646-4654; Bertran et al., J. Virol. (1996) 70:6759-6766; Chen et al., Hum. Gen. Ther. (1996) 7:1515-1525; and Okada et al., Gene Ther. (1996) 3:957-964). However, the use of plural rAAV vectors practicing the methods and recombinant AAV vectors described herein provides a significant development in the field of recombinant AAV vector gene therapy.

It is an object of the present invention to provide a method for expressing a recombinant protein in a mammalian cell population by transduction comprising: exposing said cell population to at least two different types of recombinant adeno-associated virus (rAAV), wherein each of said different types of rAAV carries a different foreign gene, the expression products of which interact to produce said recombinant protein.

Yet a further object of the present invention is to provide a method of transduction, comprising administering to a host in need of treatment a plurality of types of recombinant adeno-associated virus (rAAV), wherein each of said types of rAAV carries a different expressed foreign gene.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of the dopamine biosynthesis pathway.

Figure 2 is a schematic representation of the rAAV-MD-TH vector. ITR: AAV inverted terminal repeat; CMV: Cytomegalovirus immediate early promoter; ß-gb IVS: ß-globin mRNA splice donor/splice acceptor; human TH: human tyrosine hydroxylase cDNA; ß-gb pA: ß-globin polyadenylation site.

Figure 3 is a schematic representation of the rAAV-MD-GTPCH vector. ITR: AAV inverted terminal repeat; CMV: Cytomegalovirus immediate early promorter; ß-

gb IVS: ß-globin mRNA splice donor/splice acceptor; human GTP-CH: human GTP cyclohydrolase I cDNA; ß-gb pA: ß-globin polyadenylation site.

Figures 4A and 4B are schematic representations of the synthesis of L-dopa from muscle transduced with rAAV-MD-TH and rAAV-MD-GTPCH viruses. Figure 4A represents production of L-dopa when both vectors infect the same multinucleated muscle cell. Figure 4B represents production of L-dopa when each vector infects a separate multinucleated muscle cell. Tetrahydrobiopterin (BH4) diffuses transcellularly from the cell infected with rAAV-MD-GTP-CH to the cell infected with rAAV-MD-TH.

Figure 5 is a graph showing production of L-dopa in vivo following intramuscular injections of mice with rAAV-MD-TH and rAAV-MD-GTPCH viruses.

Figure 6 is a schematic representation of the rAAV-tetO7 minCMV-mEPO vector. ITR: AAV inverted terminal repeat; tetO7 minCMV: tetracycline responsive element reiterated seven times regulating the minimal Cytomegalovirus immediate early promoter; ß-gb IVS: ß-globin mRNA splice donor/splice acceptor; murine EPO: mouse erythropoietin cDNA; bGH pA: bovine growth hormone polyadenylation site.

Figure 7 is a schematic representation of the rAAV-CMV-tTA vector. ITR: AAV inverted terminal repeat; CMV: Cytomegalovirus immediate early promoter; tTA: tetracycline responsive transactivator comprised of the tetracycline DNA binding domain and the activator domain of VP16; mP1 IVS/pA: mouse protamine polyadenylation site and mRNA splice donor/splice acceptor.

Figure 8 is a schematic representation of the tetracycline-regulated gene expression system.

Figure 9 is a bar graph showing regulated expression of murine erythropoietin in vitro following transfection of tissue culture cells with plasmids SSV9/reg.mEPO of Figure 19 separately or in combination with S/CMV-tTA-mP of Figure 20, in the presence and absence of tetracycline (tet). Epo is produced when the cells are infected with both SSV9/reg.mEPO and S/CMV-tTA-mP vectors and tetracycline is not present.

Figures 10A and 10B are schematic representations of the synthesis of erythropoietin (Epo) from muscle transduced with rAAV-tetO7 minCMV-mEPO and rAAV-CMV-tTA viruses. Figure 10A represents production of Epo when both vectors infect the same multinucleated muscle cell and no tetracycline is administered. Figure 10B represents the inhibition of production of Epo when both vectors infect the same multinucleated muscle cell and tetracycline is administered.

Figures 11A and 11B are graphs of Epo expression. Figure 11A is a graph showing tetracycline regulated expression of murine erythropoietin in vivo following intramuscular injections of mice with rAAV-MD-tTA virus in combination with rAAV-tetO7 minCMV-mEPO virus. Expression of Epo protein is modulated by tetracycline. Figure 11B depicts Epo expression as determined by ELISA. The x axis represents time in weeks. The error ranges indicate the standard error of the mean. Animals receiving the Epo vector alone are depicted in the graph with the triangles; the tTA vector alone with diamonds; both vectors and with tetracycline, but with placebo at 10 weeks, with the circles; and those that received both vectors with placebo, but with tetracycline at 10 weeks, are represented by the squares.

Figure 12 is a schematic representation of the rAAV-MD-mEPO vector. ITR: AAV inverted terminal repeat; CMV: Cytomegalovirus immediate early promoter; ß-gb IVS: ß-globin mRNA splice donor/splice acceptor; mouse EPO: murine erythropoietin cDNA; ß-gb pA: ß-globin polyadenylation site.

Figure 13 is a schematic representation of the rAAV-CMV-lacZ vector, pdx-31-lacZ, described previously by McCown et al., infra.

Figures 14A and 14B are graphs depicting Epo expression in control animals. Figure 14A is a graph showing expression of murine erythropoietin in vivo following intramuscular injections of mice with rAAV-MD-EPO or rAAV-CMV-LacZ viruses as determined by the hematocrit. Expression of the Epo gene under the control of the MD promoter is not modulated by tetracycline. The experiments served as controls for the experiment described in Figure 11. Figure 14B is a graph of plasma Epo levels, as determined using a commercially available RIA kit, in controls injected with rAAV-MD-EPO and administered tetracycline four weeks post virus injection, circles; injected with rAAV-CMV-lacZ without tetracycline, squares; and with rAAV-CMV-tTA, triangles.

Figure 15 is a diagram of the SSV9/MD2-TH2' vector.

Figure 16 is a diagram of the AAVLTRhTH2' vector.

Figure 17 is a diagram of the SSV9/reg.TH2 vector.

Figure 18 is a diagram of the SSV9-MD2-GTPCH vector.

Figure 19 is a diagram of the SSV9/reg.mEPO vector.

Figure 20 is a diagram of the S/CMV-tTA-mP vector.

Figure 21 is a diagram of the S/MD2-mEPO vector.

Figure 22 is a diagram of the SSV9/MD2 vector.

DESCRIPTION OF SPECIFIC EMBODIMENTS

Different vectors, such as naked DNA, adenovirus and retrovirus, have been utilized to deliver various transgenes directly into various tissues, including muscle. However, such systems cannot offer both high efficiency and long term expression. For naked plasmid DNA directly delivered into muscle tissue, the efficiency is low. There are only a few cells near the injection site that can maintain transgene expression. Furthermore, the plasmid DNA in the cells remains as non-replicating episomes, i.e. in the unintegrated form. Therefore, eventually the plasmid will be lost.

Adenovirus vectors can infect non-dividing cells, and therefore, can be delivered directly into mature tissue, such as muscle. However, the transgenes delivered by adenovirus vectors are not useful for long term expression for a variety of reasons. First, adenovirus vectors retain most of the viral genes and thus pose potential problems, i.e. safety. Expression of the adenovirus genes can cause the immune system to destroy the cells

containing the vectors (see, for example, Yang et al., Proc. Natl Acad. Sci. (1994) 91:4407-4411). Since adenovirus is not an integration virus, the vector eventually will be diluted or degraded in the cells. Also, because of the immune response, adenovirus vectors cannot be delivered repeatedly. In the case of lifetime disease, that will be a major limitation.

For retrovirus vectors, although stable integration into the host chromosomes can be achieved, the use thereof is restricted because currently-used vectors only can infect dividing cells, a large majority of target cells being non-dividing.

AAV vectors have certain advantages over the above-mentioned vector systems. First, like adenovirus, AAV infects non-dividing cells. Second, all the AAV viral genes are eliminated in the vector. Since the viral gene expression-induced immune reaction is no longer a concern, AAV vectors are safer than adenovirus vectors. As AAV is an integration virus, integration into the host chromosome will maintain the transgene in the cells. AAV is an extremely stable virus, resistant to many detergents, pH changes and heat (stable at 56°C for about an hour). AAV can be lyophilized and redissolved without losing significant activity. Finally, AAV causes no known diseases or pathogenic symptoms in humans. Therefore, AAV is a very promising delivery vehicle for gene therapy.

Two recent review articles provide an overview of the recent status on the use of AAV vectors and include a collection of additional recent scientific publications in the field: Samulski, "Adeno-associated Viral Vectors", Chap. 3 in "Viruses in Human Gene Therapy", Chapman & Hall, Vos ed., 1994; and Samulski, "Adeno-associated Virus-based Vectors for Human Gene Therapy", Chap. 11 in "Gene Therapy: From Laboratory to the Clinic", World Scientific, Hui ed., 1994.

Since AAV has in the past been shown to have a broad host range (for example, for

pulmonary expression); can be administered by a variety of routes, including intramuscular injection; and are operable in different cells types, such as liver, retina, neurons and so on, there are no known limits of the host in which the herein described methods of delivery can take place, particularly in mammals, birds, fish and reptiles, especially domesticated mammals and birds, such as cattle, sheep, pigs, horses, dogs, cats, chickens and turkeys. Both human and veterinary uses particularly are preferred.

The recombinant vectors of the invention comprise: (1) a promoter derived from a non-AAV source operable in AAV, (2) optionally, an mRNA splice donor/splice acceptor, (3) a gene of interest that encodes a polypeptide or a nucleic acid, (4) a polyadenylation site and (5) two AAV inverted terminal repeats flanking the components (1)-(4) above.

The gene being expressed can be either a DNA segment encoding a protein, with whatever control elements (e.g., promoters and operators) are desired by the user, or a non-coding DNA segment, the transcription of which produces all or part of some RNA-containing molecule, such as a transcription control element or a ribozyme. Since the instant invention is directed to the vector and use thereof rather than to the material being delivered, there are no limitations on the foreign DNA (or heterologous gene, heterologous DNA or transgene, which is not of AAV origin) being delivered by the vector so long as the gene products are related in some coordinated fashion, that is to say, that the gene products operate together to achieve a desired end result. The gene may be one which acts locally or which can be transported systemically to act at a site distal to the cell producing the gene product.

A preferred gene of interest is a polynucleotide that encodes a diffusible polypeptide, i.e. a polypeptide that can diffuse from the site of administration to effect systemic delivery, or a diffusible nucleic acid. For example, diffusible polypeptides that may be useful for gene

therapy include insulin, Factor VIII and cytokines, including interferons (IFN-α, IFN-ß and IFN-γ), interleukins, GM-CSF (granulocyte-macrophage colony-stimulating factor), M-CSF (macrophage colony-stimulating factor), tumor necrosis factors, growth factors (TGF-ß (transforming growth factor-ß) and PDGF (platelet-derived growth factor)) or neurotrophic factors (CNTF (ciliary neurotrophic factor) and GDNF (glial cell line derived neurotrophic factor)). It will be readily apparent to one of ordinary skill in the art that the methods described herein can be used to express systemically or locally via, for example, intramuscular injection, any diffusible polypeptide or polynucleotide of therapeutic interest. Local delivery includes intracellular administration of a virus, vector or gene.

The promoter can be any of the many characterized viral (e.g., thymidine kinase or Rous sarcoma virus) or cellular promoters (e.g. neuron-specific enolase, vimentin or fibronectin) so long as the promoter is operable in AAV. The accompanying regulatory signals also can be used for expression of transgenes in the vector backbone. Regulated gene expression from an inducible promoter (e.g. metallothionein etc.) also can direct expression of transgenes in the recombinant AAV vectors of the instant invention. Elements of the CMV promoter also can be used for controlled gene expression.

As to the optional mRNA splice donor/splice acceptor site, any such donor/acceptor site compatible with and operable in AAV can be used. Particular, non-limiting examples of suitable donor/acceptor sites are taught hereinbelow.

As to the polyadenylation site, any such polyadenylation site compatible with and operable in AAV can be used. Particular, non-limiting examples of suitable polyadenylation sites are taught hereinbelow.

As to the AAV inverted terminal repeats, any such ITR's or derivatives thereof, which contain nucleotide substitutions, deletions, inversions and/or insertions yet retain the

requisite biological activities required of an AAV vector carrying a foreign gene, can be used. Also, ITR's from different AAV serotypes can be used.

The use of plural rAAV's to transduce single cells may depend on whether the host cell is multinucleate, polysomic or polyploid. Thus, other suitable target cells may be cells of a syncytium, hepatocytes, muscle cells, germ cells, marrow cells, megakaryocytes and so on. However, the instant invention is not to be construed to be limited to such cells and may be practiced on normal, diploid, somatic, mononucleate cells as well.

As used herein, a multinucleate cell is one which contains more than one nucleus. A multinucleate cell may be polyploid or polysomic, in whole or in part. The development of more than one nucleus in a cell is not necessarily dependent on having multiple, complete diploid sets of chromosomes. Often, however, a multinucleate cell contains multiple, complete diploid sets of chromosomes.

It has been reported that delivery of two separate rAAV vectors (one encoding the human tyrosine hydroxylase gene and the other encoding the human GTP cyclohydrolase I gene) to the striatum of the rat brain resulted in production of L-dopa. The tyrosine hydroxylase enzyme requires the cofactor, tetrahydrobiopterin (which is made by the GTP cyclohydrolase I) to synthesize L-dopa. The two vectors did not have to be expressed in the same cell because, first, the product of GTP-cyclohydrolase I activity, tetrahydrobiopterin, is able to diffuse transcellularly and, second, particularly in view of viral immunity, a phenomenon wherein a cell infected with a virus becomes refractory to infection by another virus.

As discussed hereinabove, delivery of two or more separate rAAV vectors to the cells of the muscle can result in the expression of the two different gene products in one cell. The two genes can be selected to be interdependent on one another, for example, to provide

greater and more efficient regulation of expression of the foreign gene product, or for the gene products, to interact to produce a therapeutic effect. Thus, for example, one gene product may be an enzyme which acts on the second gene product, the first gene product is a cofactor essential for the expression of the second gene product or the two gene products may combine to form a composite molecule with biologic activity.

For example, recombinant AAV vector particles encoding mouse erythropoietin under the control of a tetracycline responsive element were prepared, along with a vector expressing tTA, and injected intramuscularly in immunocompetent BALB/c mice. The gene of interest was syngeneic with the host animal. Expression of mouse erythropoietin was monitored in serum samples collected over time after a single intramuscular injection. Expression of mouse erythropoietin was assayed by monitoring the hematocrit and by directly measuring plasma Epo by RIA or ELISA. Tightly regulated expression of Epo and of the resulting hematocrit were observed with the addition or withdrawal of tetracycline.

AAV tends to transduce cells in a fashion wherein the vectors are inserted into the host genome as multiple, tandemly repeated copies. Moreover, the copies are in the same orientation, that is to say, there is head-to-tail concatamerization of the copies. Based on that means of integration into the host genome, it is possible to configure tightly regulated gene expression using plural rAAV's by the specific activation of genes of interest when vectors carrying all of the essential regulatory elements for the expression thereof are used to transduce a cell in combination with a vector carrying a transgene.

Thus, if two types of vectors integrate in alternating fashion, it can be seen that vectors carrying an operable promoter would intersperse with vectors carrying expressible transgenes. A promoter then would be integrated operably upstream from an expressible transgene.

Alternatively, the transgene can be configured so that the promoter element is downstream of the gene of interest. It can be seen that on transduction and concatamerization, the promoter sequences will be positioned operably upstream from the gene of interest.

Such vectors also enable delivery of larger genes of interest as the regulatory elements thereof can be carried on a separate vector. A suitable third vector is one carrying a suitable enhancer sequence.

Actual delivery of the recombinant AAV vectors is accomplished by using any physical method that will transport the AAV recombinant vector into the target cell, such as the muscular regions of a host. As used herein, "AAV vector", means both a bare recombinant AAV vector and recombinant AAV vector polynucleotides packaged into viral coat proteins, as is well known for AAV administration. Simply formulating an AAV vector in Hanks' balanced saline solution or phosphate buffered saline is sufficient to provide a solution useful for administration. There are no known restrictions on the carriers or other components that can be coadministered with the vector (although compositions that degrade the virion or polynucleotides thereof should be avoided in the normal manner with vectors).

A preferred delivery mode employs encapsidated vector because, for example, of the stability of the particles.

Intramuscular administration is a very attractive mode for gene delivery because the muscle is highly vascularized and is very easy to access. If a disease is caused by a defective gene product which is required to be produced and/or secreted, such as in hemophilia, diabetes, Gaucher's disease etc., intramuscular delivery will be a good candidate mode of delivering the gene product if the appropriate gene product can be delivered effectively into the cells. In addition, the cells of the muscle have multiple nuclei.

However, any suitable means for delivery of AAV can be employed, as known in the art. Thus, any intravenous means of delivery can be practiced. Because AAV is stable, mode of administration is not limited to the known parenteral routes but can include, for example, oral and topical delivery.

Pharmaceutical compositions can be prepared as injectable formulations for administration as known in the art, including the use of implantable pumps (known by those of skill in the art and described, for example, in U.S. Pat. No. 5,474,552). Numerous formulations for oral or parenteral administration are known and can be used in the practice of the instant invention. The vectors can be used with any pharmaceutically acceptable carrier for ease of administration and handling.

For parenteral administration, solutions in an adjuvant such as sesame or peanut oil or in aqueous propylene glycol can be employed, as well as sterile aqueous solutions. Such aqueous solutions can be buffered, if desired, and the liquid diluent first rendered isotonic with saline or glucose. Solutions of the AAV vector as a free acid (DNA contains acidic phosphate groups) or a pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. A dispersion of AAV viral particles also can be prepared in glycerol, liquid polyethylene glycols and mixtures thereof and in oils. Under ordinary conditions of storage and use, the preparations contain a preservative to prevent the growth of microorganisms. The sterile aqueous media employed are obtainable by standard techniques well-known to those skilled in the art.

The pharmaceutical forms suitable for parenteral administration include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that parenteral administration is possible. The formulation must be stable

under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by use of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile parenteral formulations are prepared by incorporating the AAV vector in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying which yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

The preparation of interest may be administered topically, for example, application to a skin site following dermabrasion. For purposes of topical administration, dilute sterile, aqueous solutions (usually in about 0.1% to 5% concentration), otherwise similar to the

above parenteral solutions, are prepared in containers suitable for delivery, for example, by a transdermal patch, and can include known carriers, such as pharmaceutical grade dimethylsulfoxide (DMSO), or other known topical applications, such as, gels, ointments, foams, lotions, drops and so on.

The instant recombinant vectors also can be administered orally. Such preparations can be formulated as known in the art and can include, for example, flavorants, odorants, colorings and the like to facilitate presentation.

The therapeutic compounds of the instant invention may be administered to a host alone or in combination with pharmaceutically acceptable carriers. As noted above, the relative proportions of active ingredient and carrier are determined by the solubility and chemical nature of the compound, chosen route of administration and standard pharmaceutical practice.

The dosage of the instant therapeutic agents which will be most suitable for prophylaxis or treatment will vary with the form of administration, the particular recombinant AAV vector chosen and the physiological characteristics of the particular patient under treatment. Generally, small dosages will be used initially and, if necessary, will be increased by small increments until the optimum effect under the circumstances is reached. Exemplary dosages are within the range of 10^8 up to approximately 5×10^{15} particles in a total volume of 3-10 ml.

The invention now being described will be exemplified in the following non-limiting examples.

EXAMPLES

EXAMPLE 1. PRODUCTION OF L-DOPA IN MUSCLE CELLS

The biochemical pathway for L-dopa production is shown in Figure 1. The production of L-dopa requires the activity of the enzyme tyrosine hydroxylase (TH). TH activity requires the co-factor, tetrahydrobiopterin (BH4). The rate-limiting enzyme in the production of BH4 is GTP cyclohydrolase or GTP cyclohydrolase I (GTPCH or GTP-CH or GTPCHI). Figures 4A and 4B are schematic diagrams showing the production of L-dopa in muscle cells following infection with rAAV-MD-TH (Figure 2) and rAAV-MD-GTPCH (Figure 3). In Figure 4A, L-dopa is produced from single cells infected with both vectors. In Figure 4B, L-dopa is produced from single cells infected with either vector but preferably in cells infected with a TH-producing vector, and BH4 diffuses from the cell in which it is made to other cells capable of expressing TH.

EXAMPLE 2. RECOMBINANT AAV VECTOR: RAAV-MD-TH

The vector plasmid SSV9/MD2-TH2' plasmid (Figure 15) was constructed with an expression cassette comprised of the CMV immediate early promotor (-607 to +72), human ß-globin intervening sequence (nucleotides (nt) 62613-62720 (exon 2 splice/donor site) and 63088-63532) and ß-globin exon 3 and the poly A site (nucleotides 63532-64297) inserted between the AAV inverted terminal repeats of psub201 (Samulski et al., J. Virol. (1987) 61:3096-3101). The SSV9/MD2-TH2' plasmid was made by digesting SSV9/MD-2 (Figure 22) with BglII and HindIII and inserting the PmlI to HindIII fragment from the same plasmid together with the TH gene in two fragments (PmlI to XhoI and XhoI to BamHI from MFG-S-hu-TH (Bankiewicz et al., Exp. Neuro. (1997) 144:147) in a four fragment ligation.

EXAMPLE 3. RECOMBINANT AAV VECTOR: RAAV-MD-GTPCH

To construct the SSV9-MD2-GTPCH vector (Figure 18), the full-length cDNA encoding an active isoform of human GTP cyclohydrolase I was isolated using PCR amplification from a 'Quick clone' (Clontech) liver cDNA library. The primers (GTPCH1.1, 5'TCCATGGAGAAGGGCCCTGTGC3' (SEQ ID NO:___), Genbank accession No. S44053, nt 65-86; and GTPCHa2.2, 5'CTGATCAAATCTGGCAGTACGATCGGCAACC3' (SEQ ID NO:___) Genbank accession no. S44049, complement to nt 771-751 plus linker sequence) were based on the published sequence and included sequences encoding NcoI restriction sites at the N-terminal encoding end and BclI and BglII sites at the end closest to the C-terminus of the encoded protein. Fifty base pairs (bp) of 3' non-coding sequence are included in the clone. The cDNA is 810 bp in length. The construct was sequenced in entirety in both strands after cloning into the "TA" vector, pCRII (Invitrogen). An NcoI-BglII fragment of the cDNA was cloned into the SSV9/MD-2 vector (Figure 22) at the NcoI and BamHI sites.

EXAMPLE 4. PACKAGING OF THE RECOMBINANT AAV VECTORS

The recombinant AAV vectors were packaged as described by Snyder et al., "Production of recombinant adeno-associated viral vectors," in Current Protocols in Human Genetics, pp. 12.1.1-12.1.24, Dracopoli et al., eds., John Wiley & Sons, New York, 1996. Briefly, subconfluent 293 cells were cotransfected with the vector plasmid and the AAV helper plasmid, pACG2-1 (Li et al., J. Virol. (1997) 71:5236-5243) using the calcium phosphate method. Cells then were infected with adenovirus Ad5dl312 (an E1A$^-$ mutant) at an multiplicity of infection (moi) of 2 and the infection was allowed to proceed for 72 hours. Cells were harvested and three freeze/thaw cycles were carried out to lyse the cells. The nucleic acid in the lysate was digested with 250 U/ml Benzonase (Nycomed) at 37°C for

10 minutes and then the lysate was centrifuged at 1500g to pellet the cellular debris. The cell lysate then was fractionated by ammonium sulfate precipitation and the rAAV virions were isolated on two sequential CsCl gradients. The gradient fractions containing rAAV were dialyzed against sterile PBS, heated for 45 minutes at 56°C and stored at -80°C.

EXAMPLE 5. L-DOPA PRODUCTION IN THE MUSCLE OF IMMUNOCOMPETENT MICE

Recombinant AAV vectors rAAV-MD-TH (Figure 2) and rAAV-MD-GTPCH (Figure 3) were packaged into virions as described in Example 4. The graph of Figure 5 shows the mean plasma L-dopa levels (ng/ml; x-axis) vs. time in days (y-axis) post virus injection in animals bilaterally injected in the quadriceps muscle with 2.0 x 10^{10} particles of rAAV-MD-TH (-♦-), 1.4 x 10^{9} particles of rAAV-MD-GTPCH (-■-), a mixture of both viruses (-▲-) or a control group which received no virus (-□-).

Four groups of six BALB/c female mice (Taconic) were injected with 40 μl of virus in HBSS at 1 μl/sec using a 30g needle connected to a pump (KD Scientific). Plasma was collected on the day of virus injection and at weekly intervals thereafter. Three hours prior to plasma collection, all animals were injected IP with 100 mg/kg of buffered 3-hydroxybenzylhydrazine NSD-1015, an L-aromatic amino acid decarboxylase inhibitor which prevents the breakdown of L-dopa. Animals were anesthetized with isofluorane, bled by the retro-orbital technique and plasma collected using heparin-coated capillary tubes in microtainer tubes.

L-dopa levels were analyzed by isocratic reverse-phase HPLC using a C-18, 3 mm x 150 mm Hypersil ODS column (Keystone Scientific) and an ESA Coulochem II electrochemical detector. Plasma was extracted with perchloric acid (PCA) at a final concentration of 0.2 M and EDTA at 5 mM. Twenty nanograms of

3,4,-dihydroxybenzylamine (DHBA) were added as an internal standard. After filtration through a 0.2 μm RPM spin filter tubes (BIO 101, Inc.), 20 μl samples were injected at 4°C using a Waters 717 autosampler and run using a mobile phase comprised of 3% methanol, 50 mM sodium phosphate, 1 mM heptane sulfonic acid, 0.01% triethylamine and 0.2 mM EDTA (final pH 2.5). The flow rate was 0.6 ml/min. Peaks were integrated using a commercially available program (MacIntegrator II, Rainin).

EXAMPLE 6. REGULATED PRODUCTION OF ERYTHROPOIETIN IN MUSCLE CELLS

A schematic representation of the tetracycline regulated expression of the mouse erythropoietin gene is shown in Figure 8. The transactivator protein tTA is made from one vector and binds to the tet operators of the other vector to induce transcription from the minimal CMV promoter which drives expression of the mouse Epo gene. The activation by tTA is inhibited by tetracycline which prevents the tTA protein from binding to the tet operators. Figures 10A and 10B are schematics of the tetracycline regulated expression of the mouse erythropoietin gene in muscle following infection with rAAV-tetO7 minCMV-mEPO (Figure 6) and rAAV-CMV-tTA (Figure 7). In Figure 10A, Epo is produced only from single cells which have been infected with both vectors. The tTA protein does not act transcellularly because it is contained in the muscle cell in which it is made. In Figure 10B, the production of Epo is inhibited by tetracycline.

EXAMPLE 7. RAAV VECTOR: RAAV-TETO7 MINCMV-MEPO VECTOR

The SSV9/reg.mEPO plasmid (Figure 19) was derived from SSV9/reg.TH2 (Figure 17). To construct the plasmid SSV9/reg.TH2 (Figure 17), the CMV minimal promotor and heptamerized operator sequences from the plasmid pUHC13-3 (Gossen &

Bujard, Proc. Natl. Acad. Sci. (1992) 89:5547-5551) were PCR amplified and the ends of the fragment modified to contain restriction sites. The oligonucleotides used were 5'CTAGCTAGCCTCGAGTTTACCACT3' (SEQ ID NO:___) and 5'AAGTTTGATATCGAGCTCGAATTCGGG3'(SEQ ID NO:__). The fidelity of the PCR was verified by sequencing. The resulting 473 bp fragment was cloned into the pCR™ II vector (Invitrogen) and cut with NheI and HindIII. The AAVLTRhTH2' plasmid (Figure 16; also know as pSSV9-MFG-hTH, the construction of which is described in Snyder et al., Nat. Genet. (1997) 16:270-276) was digested with NheI and MluI to generate a 2500 bp fragment containing the SSV9 backbone, the 3' end of the hTH2 cDNA and the bovine growth hormone polyA sequence. A 1574 bp fragment containing the ß-globin intervening sequence and 5' portion of the hTH2 cDNA sequence was cut from the SSV9/MD2-TH2' plasmid (Figure 15) with HindIII and MluI. The above three fragments were joined to generate the plasmid SSV9/reg.TH2 (Figure 17). The hTH2 cDNA then was replaced by the murine erythropoietin (mEpo) cDNA fragment isolated from plasmid S/MD2-mEPO (Figure 21) following cutting with PmlI and BglII. The fragment then was cloned into the PmlI and BamHI cut SSV9/reg.TH2. A three-way ligation was performed as the 544 bp BamHI to PmlI ß-globin intervening sequence fragment from SSV9/reg.TH2 which was removed in the process had to be reintroduced into the plasmid.

EXAMPLE 8. RECOMBINANT AAV VECTOR: RAAV-CMV-TTA VECTOR

The plasmid S/CMV-tTA-mP (Figure 20) was constructed using the CMV immediate early promotor and tet-VP16 transactivator sequences from the plasmid pUHD15-1 (Gossen & Bujard, Proc. Natl. Acad. Sci. (1992) 89:5547-5551). A 1.79 kilobase (kb) fragment was created by digestion with XhoI, followed by blunt ending and digestion with BamHI. The

mouse protamine splice donor/splice acceptor and polyA sequences were PCR amplified from the pAB11 plasmid (Goodman et al., Blood (1994) 84:1492-500) and the ends of the fragment were modified to contain additional restriction sites. The oligonucleotides used were 5'AAGCTTCACGTGAGATCTAATAACCATGGCCAGATACCGATGCTG-3' (SEQ ID NO:___) and 5'CCCGTCGACGTACAGGTGGCTTGGTAGTCA3' (SEQ ID NO:___). The resulting fragment was cloned into the pCR™ II vector (Invitrogen), sequenced to verify accuracy and cut with SalI and BglII to generate a 540 bp fragment. The above fragments were ligated to the 4005 bp SSV9 backbone sequence from the SSV9/MD2-TH2' plasmid (Figure 15), which had been cut with XbaI, then blunt ended.

EXAMPLE 9. REGULATED ERYTHROPOIETIN EXPRESSION IN TISSUE CULTURE CELLS

Erythropoietin (Epo) expression was obtained in tissue culture media after transient transfection of Hela cells (Figure 9) with the plasmids SSV9/reg.mEPO (Figure 19) and/or S/CMV-tTA-mP (Figure 20). The x axis indicates the relevant plasmids while the y axis represents Epo levels in mIU (international units) per milliliter. Co-transfection with both plasmids, in the absence of tetracycline, yielded 69-fold higher levels of Epo than in the presence of the drug.

Hela cells were transfected transiently by standard methods using lipofectamine reagent (Gibco BRL) and 0.5 μg of each DNA. Twenty hours prior to transfection, 3.5 x 10^5 Hela cells were seeded per 35 mm well in DMEM media containing 10% fetal bovine serum. Cells were transfected with either no DNA (mock), the inducible SSV9/reg.mEPO plasmid (Figure 19) alone (ind. epo) or SSV9/reg.mEPO (Figure 19) together with the S/CMV-tTA-mP plasmid (Figure 20), regulated by (ind. epo+tTA) the presence or absence of 1 μg/ml tetracycline. Epo levels in the tissue culture media between

24 and 48 hours after transfection were evaluated with a commercially available ELISA kit (Quantikine IVD™) using a 1:100 dilution of antiserum. The optical density of the samples was read using a Molecular Devices plate reader and the data analyzed using Softmax version 2.02 software.

EXAMPLE 10. REGULATED EPO EXPRESSION IN MUSCLE OF IMMUNOCOMPETENT MICE

Recombinant AAV vectors rAAV-tetO7-minCMV-mEPO (Figure 6) and rAAV-CMV-tTA (Figure 7) were packaged into virions as described in Example 4. The graph in Figure 11A shows the hematocrit values for animals injected unilaterally in the right quadriceps muscle with rAAV. The x axis represents time (in days) and the y axis is percent hematocrit. The groups in the study include rAAV-tet07 minCMV-mEPO plus rAAV-CMV-tTA, administered tetracycline at 4 weeks post virus injection (-♦-; n = 4); rAAV-tet07 minCMV-mEPO plus rAAV-CMV-tTA, given a placebo pellet at 4 weeks post-injection (-■-; n = 4); rAAV-tet07 minCMV-mEPO alone (-▲-; n = 3) and rAAV-CMV-tTA alone (-x-; n = 3).

Female BALB/c mice were injected with 1 x 10^{10} particles each of rAAV at time = 0 days. Each virus stock was diluted to 20 μl in HBSS and was injected using a 30g needle connected to a pump (KD Scientific) at 1 μl/sec. Retro-orbital sinus bleeds were performed weekly under isofluorane anesthesia using heparin-coated capillary tubes. The blood was collected into EDTA-coated tubes. Hematocrits were performed by standard methods. At four and seven weeks post-virus injection, the appropriate animals were anesthetized with sodium pentobarbital and subcutaneously implanted at a mid-dorsal site with 15 mg, 21-day timed-release tetracycline or placebo pellets (Innovative Research of America).

The graph of Figure 11B shows Epo protein expression as revealed by plasma Epo levels determined by ELISA using a commercially available kit. Animals receiving only a single vector demonstrated baseline levels of Epo (≤ 30 mU/ml). One group receiving both vectors was administered tetracycline at 4 weeks after receiving virus. Epo was expressed at baseline levels, O in the graph. Tetracycline was withdrawn at 10 weeks and an increase in Epo expression was observed thereafter. Another group of mice receiving both vectors were implanted with a placebo pellet at 4 weeks following virus administration. High levels of Epo were observed, see □ in the graph. Tetracycline was administered at 10 weeks resulting in a decrease in Epo expression.

EXAMPLE 11. RECOMBINANT AAV VECTOR: RAAV-MD-MOUSE EPO

The recombinant AAV vector S/MD2-mEPO (Figure 21) was constructed by insertion of the muEpo cDNA between the PmlI and BglII cloning sites of pSSV9/MD-2 (Figure 22). Plasmid SSV9/MD-2 (Figure 22) was constructed by subcloning a 2.35 kb XbaI fragment from pMDG (Naldini et al., Sci. (1996) 272:263-267) into the XbaI sites of plasmid SSV9 (also known as psub201; Samulski et al., J. Virol. (1987) 61:3096-3101). pSSV9-MD-2 contains the cytomegalovirus (CMV) immediate early gene promoter/enhancer (nucleotide positions 195-993), exons 2 and 3 and intervening sequence 2 (IVS2) (nucleotide positions 1036-1561) and a polyadenylation signal (nucleotide positions 1573-2355) derived from the human ß-globin gene. The fragment was modified by PCR to contain restriction sites for PmlI, EcoRI and BglII at nucleotide positions 1562, 1567 and 1573, respectively, for subcloning of selected transgenes. The accuracy of the construct was verified by DNA sequencing. In S/MD2-mEPO, the mEPO gene is flanked by the CMV promoter, ß-globin intron and the ß-globin poly(A) sequence. The 579 base pair mEpo coding region was

generated by PCR using multiple overlapping oligonucleotides (Dillion & Rosen, Biotechniq. (1990) 9:298-299) and contained compatible PmlI and BglII sites for subcloning in the vector backbone.

EXAMPLE 12. RECOMBINANT AAV VECTOR: RAAV-CMV-LACZ

The rAAV-CMV-lacZ vector, pdx-31-lacZ, was described previously (McCown et al., Brain Res. (1996) 713:99-107), Figure 13.

EXAMPLE 13. CONTROLS FOR EPO EXPRESSION IN IMMUNOCOMPETENT MICE

Recombinant AAV vectors were packaged into virions as described in Example 4. Hematocrit values for control animals (Figure 14A) were used for the data shown in Example 10. The x axis represents time (in days) and the y axis is percent hematocrit. The control groups received rAAV-MD-mEPO (Figure 12) with tetracycline administered at 4 weeks post virus injection (-▲-; n = 4); or rAAV CMV-lacZ (Figure 13) (-■- ; n = 3) and no tetracycline pellet. The mice were injected and assayed as in Example 10. Plasma Epo expression as determined by RIA for controls is depicted in Figure 14B.

All references mentioned in the specification are herein incorporated in entirety by reference to the same extent as if each individual reference was specifically and individually indicated to be incorporated by reference.

The invention now being described fully, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention described in the appended claims.

CLAIMS

1. A method for expressing a recombinant protein in a mammalian cell population by transduction comprising: exposing said cell population to at least two different types of recombinant adeno-associated virus (rAAV), wherein each of said different types of rAAV carries a different foreign gene, the expression products of which interact to produce said recombinant protein.

2. The method of claim 1, wherein a portion of said cell population comprises polysomic, polyploid or multinucleate cells.

3. The method of claim 1, wherein said cell population comprises monocytes, neurons, fibroblasts, retinal cells, pneumocytes, bone marrow cells, hepatocytes or muscle cells.

4. The method of claim 3, wherein said cell population comprises a muscle cell.

5. The method of claim 3, wherein said cell population comprises a liver cell.

6. The method of claim 1, wherein a cell is transduced with at least two types of rAAV.

7. The method of claim 1, wherein a first rAAV contains a promoter and expression of a second gene in a second rAAV is controlled by said promoter.

8. The method of claim 1, wherein said first and second rAAV are physically joined.

9. The method of claim 1, wherein a first type of rAAV comprises a first foreign gene encoding a gene product required for expression of a second foreign gene in a second type of rAAV.

10. The method of claim 9, wherein a first foreign gene encodes an enzyme and substrate of said enzyme is encoded by a second foreign gene.

11. The method of claim 9, wherein said first foreign gene encodes a cofactor.

12. The method of claim 9, wherein a first foreign gene encodes a subunit which combines with one or more other types of rAAV to produce a biologically active molecule.

13. A mammalian cell comprising a plurality of types of recombinant adeno-associated virus (rAAV), wherein each type carries a different expressed foreign gene.

14. The cell of claim 13, which is a monocyte, neuron, fibroblast, retinal cell, pneumocyte, bone marrow cell, hepatocyte or muscle cell.

15. The cell of claim 13, which is a muscle cell.

16. The cell of claim 13, which is a liver cell.

17. A method of transduction, comprising administering to a host in need of treatment a plurality of types of recombinant adeno-associated virus (rAAV), wherein each of said types of rAAV carries a different expressed foreign gene.