

cell is infected with a helper virus (for example, adenovirus or herpesvirus). In the case of adenovirus, genes E1A, E1B, E2A, E4 and VA provide helper functions. Upon infection with a helper virus, the AAV provirus is rescued and amplified, and both AAV and adenovirus are produced.

[0005] AAV possesses unique features that make it attractive as a vaccine vector for expressing immunogenic peptides/polypeptides and as a vector for delivering foreign DNA to cells, for example, in gene therapy. AAV infection of cells in culture is non-cytopathic, and natural infection of humans and other animals is silent and asymptomatic. Moreover, AAV infects many mammalian cells allowing the possibility of targeting many different tissues *in vivo*. The AAV proviral genome is infectious as cloned DNA in plasmids which makes construction of recombinant genomes feasible. Furthermore, because the signals directing AAV replication, genome encapsidation and integration are contained within the ITRs of the AAV genome, some or all of the internal approximately 4.3 kb of the genome (encoding replication and structural capsid proteins, rep-cap) may be replaced with foreign DNA such as a gene cassette containing a promoter, a DNA of interest and a polyadenylation signal. The rep and cap proteins may be provided in trans. Another significant feature of AAV is that it is an extremely stable and hearty virus. It easily withstands the conditions used to inactivate adenovirus (56° to 65°C for several hours), making cold preservation of rAAV-vectors less critical. AAV may even be lyophilized. Finally, AAV-infected cells are not resistant to superinfection.

[0006] AAV vectors find use in numerous mammalian gene therapy applications and there is a need for new and/or modified AAV vectors and associated virus that find use in gene therapy applications. The present invention provides for novel AAV vectors expressing the novel AAV capsid proteins or the functional chimeric engineered AAV capsid proteins of the present invention, and novel, non-naturally occurring AAV virions comprising those vectors or capsid proteins.

SUMMARY

[0006a] In one aspect, the present disclosure refers to an adeno-associated virus (AAV) comprising a capsid protein, comprising an amino acid sequence that is at least 95% identical to: (i) a VP1 region of any one of SEQ ID NOS:28, 29, 30, 31, and 16, (ii) a VP2 region of any one

of SEQ ID NOS: 28, 29, 30, 31, and 16, or (iii) a VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and a transgene comprising a heterologous gene operably linked to regulatory sequence that control expression of the heterologous gene in a host cell.

[0006b] In another aspect, the present disclosure refers to composition comprising the AAV as disclosed herein and a physiologically compatible carrier.

[0006c] In yet another aspect, the present disclosure refers to a method of delivering a transgene to a cell comprising contacting the cell with the AAV as disclosed herein.

[0006d] In yet another aspect, the present disclosure refers to a vector comprising a nucleic acid sequence encoding an adeno-associated virus (AAV) capsid protein having an amino acid sequence that is at least 95% identical to (i) any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) the VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, or (iii) the VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16.

[0006e] In yet another aspect, the present disclosure refers to a cell comprising the vector as disclosed herein.

[0006f] In yet another aspect, the present disclosure refers to use of an AAV as disclosed herein in the preparation of a medicament for the treatment of a disorder or disease, wherein the disorder or disease is Duchenne muscular dystrophy and the transgene encodes a microdystrophin protein.

[0006g] In yet another aspect, the present disclosure refers to a composition comprising an AAV as disclosed herein for use in medicine.

[0006h] In yet another aspect, the present disclosure refers to a method of producing an adeno-associated virus (AAV), the method comprising the steps of: (a) culturing a cell to produce the AAV, the cell comprising a nucleotide sequence encoding a capsid protein, wherein the capsid protein comprises an amino acid sequence that is at least 95% sequence identical to a VP1 region, VP2 region, or VP3 region from one of SEQ ID NOS: 28, 29, 30, 31, and 16; and (b) collecting the AAV from the cell culture.

[0007] The disclosure provides for novel AAV capsid proteins, which may be novel VP1, VP2 or VP3 capsid proteins, non-naturally occurring AAV virus comprising any of these capsid proteins, and use of such AAV virus for gene therapy applications and for use in the preparation

of medicaments for gene therapy applications. In some examples, the AAV capsid proteins were isolated and identified from various mammalian tissues. The amino acid sequences of certain novel mammalian-derived AAV capsid VP1 proteins are set out as SEQ ID NOS:15-89, and the associated locations of the respective VP2 and VP3 sequences are also herein described. In addition, the disclosure provides for novel engineered chimeric AAV capsid proteins which have a backbone amino acid sequence derived from one AAV capsid sequence and fragments of capsid protein sequence derived from at least one different AAV capsid sequence. The amino acid sequences of exemplary engineered chimeric AAV capsid VP1 proteins are set out as SEQ ID NOS:90-157. Collectively, the novel capsid proteins are referred to herein as "AAV capsid proteins of the disclosure." The term "non-naturally occurring" when used in regards to any composition of matter described herein means that the composition is not a product of nature, but rather is artificially synthesized by recombinant or other means.

[0008] In one example the disclosure provides a vector and an adeno-associated virus (AAV) having a capsid protein having an amino acid sequence that is at least 95% identical to (i) any one of SEQ ID NOS:15-89, (ii) the VP2 region of any one of SEQ ID NOS:15-89, or (iii) the VP3 region of any one of SEQ ID NOS:15-89, and further having a transgene where the transgene is composed of a heterologous gene operably linked to regulatory sequences that control expression of the heterologous gene in a host cell. In another example the capsid protein has the amino acid sequence of i) any one of SEQ ID NOS:15-89, (ii) the VP2 region of any one of SEQ ID NOS:15-89, or (iii) the VP3 region of any one of SEQ ID NOS:15-89. In yet another example the AAV has an AAV inverted terminal repeat sequence. In further examples the AAV are mixed with a physiologically compatible carrier.

[0009] In another example the disclosure provides a vector and an AAV having a chimeric capsid protein where the chimeric capsid protein has a VP1 amino acid sequence of a recipient backbone AAV capsid having variable regions I, II, III, IV, V, VI, VII, VIII, and IX, except where one or more of the variable regions I, II, III, IV, V, VI, VII, VIII, and IX is replaced by the corresponding variable region from one or more donor AAV capsids. In another example, only one variable region of the recipient capsid is replaced by the corresponding variable region from the donor capsid. In a further example, two or more variable regions of the recipient capsid are replaced by the corresponding variable regions from a single donor AAV capsid. In yet another example, two or more variable regions of the recipient AAV capsid are replaced

by the corresponding variable regions from two or more donor AAV capsids. In another example, all nine variable regions of the recipient AAV capsid are replaced by the corresponding variable regions from a single donor capsid. In yet another example the recipient AAV capsid has a GBS region or a GH loop region and the GBS region or the GH loop region is replaced by the corresponding region from one or more donor AAV capsids. In a further example all nine variable regions and the GBS region of the recipient AAV capsid are replaced by the corresponding variable regions and GBS region from one or more donor AAV capsids. In yet another example all nine variable regions and the GBS region of the recipient AAV capsid are replaced by the corresponding regions and GBS region from two or more donor AAV capsids. In another example the GH loop of the recipient AAV capsid is replaced by the corresponding GH loop region from a donor AAV capsid. In a further example all nine variable regions and the GH loop region of the recipient AAV capsid are replaced by the corresponding variable regions and GH loop region from one or more donor AAV capsids. In one example the recipient AAV capsid sequence is any one of SEQ ID NOS:1-14 and the donor AAV capsid sequences are selected from any one of SEQ ID NOS:1-14 and where the recipient AAV capsid and the donor AAV capsid are different. In another example the recipient AAV capsid sequence is any one of SEQ ID NOS:1-89 and the donor AAV capsid sequences are selected from any one of SEQ ID NOS:1-89 and where the recipient AAV capsid and the donor AAV capsid are different. In yet another example the chimeric capsid has the amino acid sequence of any one of SEQ ID NOS:90-157.

[0010] In another example, the disclosure provides a method of delivering a transgene to a cell involving the step of contacting the cell with any AAV disclosed herein. In another example, the disclosure provides a method of treating a subject from a disorder or disease associated with abnormal activity of an endogenous protein involving the step of administering to the subject an effective amount of an AAV disclosed herein where the AAV has a transgene that encodes a biologically active copy of the protein. In yet another example, the methods involve delivering a transgene to a muscle cell. In a further example, the disease is Duchenne muscular dystrophy and the transgene encodes a microdystrophin.

[0011] In an example, the disclosure provides a composition comprising a vector or AAV disclosed herein for delivery of a transgene to a cell. In another example, the disclosure provides a composition comprising an effective amount of a vector or AAV disclosed herein for

the treatment of a disorder or disease associated with abnormal activity of an endogenous protein, wherein the vector of AAV has a transgene that encodes a biologically active copy of the protein. In yet another example, the composition delivers a transgene to a muscle cell. In a further example, the disease is Duchenne muscular dystrophy and the transgene encodes a microdystrophin.

[0012] The disclosure also provides use of a vector or AAV disclosed herein for the preparation of a medicament effective to treat a subject suffering from a disorder or disease associated with abnormal activity of an endogenous protein, wherein the vector or AAV has a transgene that encodes a biologically active copy of the protein. In yet another example, the medicament delivers a transgene to a muscle cell. In a further example the disease is Duchenne muscular dystrophy and the transgene encodes a microdystrophin.

[0013]

[0014] The disclosure provides for fragments of any of the AAV capsid proteins of the disclosure that retain a biological activity of an AAV capsid protein. Exemplary fragments include VP2 and VP3 spliced variants of the capsid proteins, and fragments comprising one or more of the variable regions (VR) of the capsid protein and/or the glycan binding sequence (GBS) of a capsid protein and/or the GH loop. The disclosure also provides for novel, non-naturally occurring AAV particles comprising a capsid protein fragment and those comprising a capsid protein fragment having at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% sequence identity to a specifically defined capsid protein fragment.

[0015] In one example, the disclosure provides for an isolated adeno-associated virus (AAV) capsid protein, wherein the capsid protein comprises (i) an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the VP1 amino acid sequence of any one of SEQ ID NOS:15-89 or the VP2 or VP3 region of any one of SEQ ID NOS:15-89 or (ii) a VP1 amino acid sequence comprising any one of SEQ ID NOS:15-89 or the VP2 or VP3 region of any one of SEQ ID NOS:15-89. In certain examples, the capsid protein is linked to a heterologous amino acid sequence. The disclosure also provides for non-naturally occurring AAV particles having or comprising any of these capsid proteins. In certain examples, the non-naturally occurring AAV particle comprising any of the above described VP1, VP2 or VP3 capsid proteins comprises a nucleic acid having AAV inverted terminal repeats and a transgene comprising a

heterologous gene operably linked to regulatory sequences which direct expression of the heterologous gene in a host cell. In other examples, the non-naturally occurring AAV particle comprising any of the VP1, VP2 or VP3 capsid sequences described herein comprises a heterologous transgene operably linked to regulatory sequences that control transgene expression in a host cell. As used herein, the terms "heterologous gene" or "heterologous regulatory sequence" means that the referenced gene or regulatory sequence is not naturally present in the AAV vector or particle and is artificially introduced therein. The term "transgene" refers to a nucleic acid that comprises both a heterologous gene and regulatory sequences that are operably linked to the heterologous gene that control expression of that gene in a host cell.

[0016] The disclosure also provides for a polynucleotide comprising a nucleotide sequence encoding an adeno-associated virus (AAV) capsid protein, wherein the capsid protein comprises (i) an amino acid sequence that is at least 95%, 96%, 97%, 98% or 99% identical to the VP1 amino acid sequence of any one of SEQ ID NOS:15-89 or the VP2 or VP3 region of any one of SEQ ID NOS:15-89 or (ii) a VP1 amino acid sequence comprising any one of SEQ ID NOS:15-89 or the VP2 or VP3 region of any one of SEQ ID NOS:15-89, wherein the polynucleotide is operatively linked to a heterologous regulatory control sequence. As such, it is understood that the polynucleotides of the present disclosure are non-naturally occurring. The disclosure also provides for AAV vectors comprising any of these polynucleotide sequences operably linked to a heterologous regulatory sequence and compositions comprising these AAV vectors, including pharmaceutical compositions.

[0017] In another example, the disclosure provides for an isolated adeno-associated virus (AAV) vector comprising a polynucleotide sequence encoding a capsid protein and a heterologous transgene sequence, wherein the capsid protein comprises (i) an amino acid sequence that is at least 95%, 96%, 97%, 98% or 99% identical to the VP1 amino acid sequence of any one of SEQ ID NOS:15-89 or the VP2 or VP3 region of any one of SEQ ID NOS:15-89 or (ii) a VP1 amino acid sequence comprising any one of SEQ ID NOS:15-89 or the VP2 or VP3 region of any one of SEQ ID NOS:15-89. The disclosure also provides for compositions comprising these AAV vectors, including pharmaceutical compositions.

[0018] The present disclosure is also based upon the novel finding described herein that AAV VP1 capsid sequences comprise nine different variable regions, a GBS region and a GH loop

region, and that replacing one or more of these regions in one AAV VP1 capsid sequence with the corresponding region(s) from an at least second, different AAV VP1 capsid sequence can generate novel chimeric AAV capsids whose associated AAVs are functional, are capable of transducing cells and delivering heterologous transgenes, and that have unique properties that may be recombinantly engineered into the chimeric AAV. In regards to the various variable regions and the GBS and GH loop regions, the term "corresponding" means the same region between two different AAV capsid sequences. For example, the region "corresponding" to VR I in a first AAV capsid sequence is the same region (i.e., the VR I region) in a second different AAV capsid sequence. The term "chimeric" in relation to an AAV capsid sequence refers to the fact that the AAV capsid sequence of interest comprises amino acid sequences derived from two or more different AAV capsid sequences.

[0019] As such, the present disclosure also provides for an isolated, non-naturally occurring chimeric adeno-associated virus (AAV) capsid protein, wherein the chimeric capsid protein comprises an amino acid sequence derived from a first AAV capsid sequence having at least one variable region substituted with a variable region from a second AAV capsid sequence that is different from the first AAV capsid sequence. The first AAV capsid sequence (referred to herein as the "recipient") provides the backbone amino acid sequence into which one or more variable regions are swapped or substituted by one or more variable regions from the second AAV capsid sequence (referred to herein as the "donor"). The second AAV capsid sequence is different from the first AAV capsid sequence and will provide the sequence of the variable region(s) which is/are substituted or inserted into the sequence of the backbone or recipient capsid sequence. The disclosure also provides for non-naturally occurring AAV virus or AAV particles that comprise any of the chimeric capsid proteins herein described. In certain examples, the non-naturally occurring AAV particles that comprise any of the chimeric capsid proteins herein described also comprise a heterologous transgene operably linked to regulatory sequences that control transgene expression in a host cell.

[0020] The "variable regions" that may be swapped from a donor AAV capsid sequence into a recipient backbone capsid sequence refer to the nine variable regions within the VP1 sequence of an AAV capsid protein. The variable regions (VR) are referred to herein as VR I, VR II, VR III, VR IV, VR V, VR VI, VR VII, VR VIII and VR IX and their respective locations in various VP1 sequences are herein described. The VR exhibit the highest sequence and structural variation

within the AAV VP1 capsid sequence and may also have roles in receptor attachment, transcriptional activation of transgenes, tissue transduction and antigenicity.

[0021] The “glycan binding sequence (GBS)” or “GBS domain” or “GBS region” refer to the amino acid sequence located between VR IV and VR V that governs the glycan binding specificity of the viral capsid. The locations of the GBS regions in various AAV VP1 amino acid sequences are herein described, and those from other AAV VP1 amino acid sequences are known in the art and/or may be routine identified.

[0022] The “GH loop” refers to a loop sequence that is flanked by β -strand G and β -strand H within the internal β -barrel of the capsid protein. The “GH loop” sequence comprises variable region VR IV through VR VIII, including the encompassed GBS sequence and all interspersed conserved backbone sequence from the donor. The locations of the GH loop regions in various AAV VP1 amino acid sequences are herein described and those from other AAV VP1 amino acid sequences may be routinely identified.

[0023] In regard to the herein described locations of the VR, GBS and GH loop regions, it is noted that the location of the N-terminal and/or C-terminal ends of those regions may vary by from up to 1 amino acid, 2 amino acids, 3 amino acids, 4 amino acids or 5 amino acids from the amino acid locations of those regions as they are explicitly described herein (particularly in Table 2). Novel capsid sequence comprising substituted VR, GBS and/or GH loop region(s) that vary from up to 5 amino acids on the N-terminal and/or C-terminal end as herein defined are encompassed by the present disclosure.

[0024] In one example, the disclosure provides for an isolated, non-naturally occurring, chimeric adeno-associated virus (AAV) capsid protein, wherein the chimeric capsid protein comprises an amino acid sequence derived from a first AAV capsid sequence having at least one variable region substituted by a variable region derived from an at least second different AAV capsid sequence. In certain examples, the non-naturally occurring capsid protein is a VP1 capsid protein. In other examples, the chimeric capsid protein further comprises a GBS domain and/or a GH loop region from an AAV capsid sequence differing from the first recipient AAV capsid sequence. For example, the chimeric AAV capsid proteins of the disclosure have a backbone sequence derived from a first AAV capsid sequence (recipient) and at least one substituted variable region from a second different AAV capsid sequence (donor). In certain

examples, the chimeric AAV capsid proteins of the present disclosure have one, two, three, four, five, six, seven, eight or all nine variable regions substituted by the respective variable region(s) from one or more donor AAV capsid sequence(s) that differ from the first recipient capsid sequence. In other examples, the AAV capsid proteins of the disclosure have a GBS domain or GH loop region sequence derived from a donor capsid sequence that differs from the recipient capsid sequence. Alternatively, the chimeric AAV capsids of the disclosure have at least one substitute variable region and a GBS from the same AAV capsid sequence which differs from the first AAV capsid sequence. The disclosure also provides non-naturally occurring AAV particles comprising any of the chimeric AAV capsid proteins described herein. Such AAVs may also comprise a heterologous transgene operably linked to a regulatory sequence controlling expression of the transgene in a host cell.

[0025] In addition, the disclosure provides for isolated AAV capsid proteins, wherein the capsid protein comprises an amino acid sequence from a first AAV capsid sequence which has two variable regions substituted with the respective variable regions from at least one AAV capsid sequence that differs from the first AAV capsid sequence, or least three variable regions substituted with the respective variable regions from at least one AAV capsid sequence that differs from the first AAV capsid sequence, or least four variable regions substituted with the respective variable regions from at least one AAV capsid sequence that differs from the first AAV capsid sequence, or least five variable regions substituted with the respective variable regions from at least one AAV capsid sequence that differs from the first AAV capsid sequence, or least six variable regions substituted with the respective variable regions from at least one AAV capsid sequence that differs from the first AAV capsid sequence, or least seven variable regions substituted with the respective variable regions from at least one AAV capsid sequence that differs from the first AAV capsid sequence, or least eight variable regions substituted with the respective variable regions from at least one AAV capsid sequence that differs from the first AAV capsid sequence, or all nine of the variable regions substituted with the respective variable regions from at least one AAV capsid sequence that differs from the first AAV capsid sequence. For example, the substituted variable region(s) are from the same AAV capsid sequence or the substituted variable regions are from two or more different AAV capsid sequences that differ from the first AAV capsid sequence. In addition, in any of these AAV capsid proteins of the

disclosure, the GBS and/or the GH loop are also substituted and may be derived from any AAV donor capsid sequence that differs from the first AAV capsid sequence.

[0026] In any of the chimeric AAV capsids of the disclosure, the first/recipient AAV capsid sequence and the second/donor AAV capsid sequence can be any known or herein described AAV capsid sequence including, for example, capsid sequences associated with the following AAV sequences: AAV-1, AAV-2, AAV-3, AAV-3B, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13, AAVbo, AAVmo, AAV6.2, AAVRH.8, AAV4.10, AAVanc80L65 or AAVanc110, or any of the other AAV serotypes or capsid sequences herein described. Production of pseudotyped rAAV is disclosed in, for example, WO 01/83692. Other types of rAAV variants, for example rAAV with capsid mutations, are also contemplated. See, for example, Marsic et al., *Molecular Therapy*, 22(11): 1900-1909 (2014).

[0027] In certain examples, the chimeric AAV capsid proteins of the disclosure may comprise the amino acid sequence of any one of SEQ ID NOS:90-157, each of which have at least one variable region from a donor AAV serotype swapped for the respective variable region(s) in the recipient backbone sequence.

[0028] In any of the chimeric AAV capsid proteins of the disclosure, the backbone sequence or the amino acid sequence from the first AAV capsid sequence derives from the amino acid sequence of any of SEQ ID NO:1-73. In addition, in any of the chimeric AAV capsid proteins of the disclosure, the donor sequence or the amino acid sequence from the second AAV serotype derives from the amino acid sequence of one or more variable regions, GBS domain and/or GH loop of any of SEQ ID NO:1-73.

[0029] In another example, the disclosure provides for an isolated polynucleotide sequence comprising a nucleotide sequence encoding any of the engineered chimeric AAV capsid proteins of the disclosure. In addition, the disclosure provides for isolated AAV vectors comprising these polynucleotide sequences and AAV vectors comprising a polynucleotide sequence encoding any of the chimeric AAV capsid proteins of the disclosure. The disclosure also provides for compositions comprising these AAV vectors, including pharmaceutical compositions. The disclosure also provides for AAV virus comprising any of the herein described non-naturally occurring chimeric AAV capsid proteins.

[0030] The disclosure provides for methods of producing a recombinant adeno-associated virus (AAV) particle comprising the steps of: culturing a cell that has been transfected with any of the AAV vectors of the disclosure and recovering recombinant AAV particle from the supernatant of the transfected cell. In addition, the disclosure provides for viral particles comprising any of the viral vectors or capsid proteins of the disclosure and cells comprising these viral vectors.

[0031] One example of the disclosure provides a method of producing any of the recombinant AAV described herein by culturing a viral production cell into which has been introduced a first nucleic acid vector having 5' and 3' AAV inverted terminal repeat sequences flanking a transgene having a heterologous gene operably linked to regulatory sequences that control expression of the heterologous gene in a host cell, and a second nucleic acid vector having AAV rep and cap nucleic acids sequences, wherein said cap nucleic acid sequence encodes an AAV capsid that is at least 95% identical to any of SEQ ID NOS:15-157; and recovering the AAV from the supernatant of the viral production cell culture. In another example the viral production cell is an insect cell. In a preferred example the insect cell is an Sf9 cell. In a further example the first nucleic acid vector is introduced into the viral production cell by infection of the viral production cell by a baculovirus containing the first nucleic acid vector. In yet another example the first and second nucleic acid vectors are introduced into the viral production cell by infection of the viral production cell by a first baculovirus containing the first nucleic acid vector and a second baculovirus containing the second nucleic acid vector. In further examples the disclosure AAV produced by the production methods provided herein.

[0032] In another example, the disclosure provides for methods of treating a patient suffering from a disorder or disease comprising administering to the patient an effective amount of any of the AAV vectors or virus of the disclosure.

[0033] In a further example, the disclosure provides for use of any of the AAV vectors or virus of the disclosure for preparation of a medicament for the treatment of a disorder or disease. The disclosure also provides for compositions comprising any of the AAV vectors or virus of the disclosure for the treatment of a disease or disorder.

[0034] In yet another example, the disease or disorder in a subject is associated with abnormal activity of an endogenous protein. As used herein “endogenous protein” means a protein or gene product encoded by the genome of the subject suffering from the disease or disorder.

[0035] An "AAV virion" or "AAV viral particle" or "AAV vector particle" or "AAV virus" refers to a viral particle composed of at least one AAV capsid protein and an encapsidated polynucleotide AAV vector. If the particle comprises a heterologous polynucleotide (i.e. a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a mammalian cell), it is typically referred to as an "AAV vector particle" or simply an "AAV vector". Thus, production of AAV vector particle necessarily includes production of AAV vector, as such a vector is contained within an AAV vector particle.

[0036] The disclosure also provides for cells comprising any of the AAV vectors of the disclosure, and viral particles produced by these cells of the disclosure.

[0037] The term “inverted terminal repeat (ITR)” as used herein refers to the art-recognized regions found at the 5’ and 3’ termini of the AAV genome which function in cis as origins of DNA replication and as packaging signals for the viral genome. AAV ITRs, together with the AAV rep coding region, provide for efficient excision and rescue from a plasmid vector, and integration of a nucleotide sequence interposed between two flanking ITRs into a host cell genome. Sequences of certain AAV-associated ITRs are disclosed by Yan et al., *J. Virol.* 79(1):364-379 (2005) which is herein incorporated by reference in its entirety.

[0038] The phrase “helper functions for generating a productive AAV infection” as used herein refers to AAV-derived coding sequences that can be expressed to provide AAV gene products that, in turn, function in trans for productive AAV replication. Thus, AAV helper functions include the rep and cap regions. The rep expression products have been shown to possess many functions, including, among others: recognition, binding and nicking of the AAV origin of DNA replication; DNA helicase activity; and modulation of transcription from AAV (or other heterologous) promoters. The cap expression products supply necessary packaging functions. AAV helper functions are used herein to complement AAV functions in trans that are missing from AAV vectors. Helper functions for generating a productive AAV infection also

may include certain helper functions from baculovirus, herpes virus, adenovirus, or vaccinia virus.

[0039] In some examples, the viral construct comprises a nucleotide sequence encoding AAV rep and cap genes.

[0040] The term “AAV rep gene” as used herein refers to the art-recognized region of the AAV genome which encodes the replication proteins of the virus which are required to replicate the viral genome and to insert the viral genome into a host genome during latent infection. For a further description of the AAV rep coding region, see, e.g., Muzyczka et al., *Current Topics in Microbiol. and Immunol.* 158:97-129 (1992); Kotin et al., *Human Gene Therapy* 5:793-801 (1994), the disclosures of which are incorporated herein by reference in their entireties. The rep coding region, as used herein, can be derived from any viral serotype, such as the AAV serotypes described above. The region need not include all of the wild-type genes but may be altered, e.g., by the insertion, deletion or substitution of nucleotides, so long as the rep genes retain the desired functional characteristics when expressed in a suitable recipient cell.

[0041] The term “AAV cap gene” as used herein refers to the art-recognized region of the AAV genome which encodes the coat proteins of the virus which are required for packaging the viral genome. For a further description of the cap coding region, see, e.g., Muzyczka et al., *Current Topics in Microbiol. and Immunol.* 158:97-129 (1992); Kotin et al., *Human Gene Therapy* 5:793-801 (1994), the disclosures of which are incorporated herein by reference in their entireties. The AAV cap coding region, as used herein, can be derived from any AAV serotype, as described above. The region need not include all of the wild-type cap genes but may be altered, e.g., by the insertion, deletion or substitution of nucleotides, so long as the genes provide for sufficient packaging functions when present in a host cell along with an AAV vector.

[0042] The term “transfection” is used to refer to the uptake of foreign DNA by a cell. A cell has been “transfected” when exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are generally known in the art. See, e.g., Graham et al., *Virology* 52:456 (1973); Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, New York (1989); Davis et al., Basic Methods in Molecular Biology, Elsevier (1986); Chu et al., *Gene* 13:197 (1981), the disclosures of which are incorporated herein by reference in their entireties. Such techniques can be used to introduce one or more exogenous

DNA moieties, such as a nucleotide integration vector and other nucleic acid molecules, into suitable host cells. The term captures chemical, electrical, and viral-mediated transfection procedures.

[0043] The viral construct is, in some examples, in the form of a baculoviral vector capable of productive transformation, transfection or infection in any cell type. In some examples, the viral construct comprises at least one nucleotide sequence encoding a heterologous protein.

[0044] In yet another example, described herein is an AAV particle produced by a method described herein. In some examples, the AAV particle comprises in its genome at least one nucleotide encoding a heterologous protein.

[0045] The term “heterologous proteins or peptides” refer to any protein that is not expressed by wild type AAV including tags such as hexahistidine, FLAG, myc, polyhistidine, or labels or immunogens, adjuvants, selection markers, therapeutic proteins or targeting proteins or peptides, to name a few.

[0046] Exemplary heterologous protein described herein includes, but is not limited to, β -globin, hemoglobin, tissue plasminogen activator, and coagulation factors; colony stimulating factors (CSF); interleukins, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, etc.; growth factors, such as keratinocyte growth factor (KGF), stem cell factor (SCF), fibroblast growth factor (FGF, such as basic FGF and acidic FGF), hepatocyte growth factor (HGF), insulin-like growth factors (IGFs), bone morphogenetic protein (BMP), epidermal growth factor (EGF), growth differentiation factor-9 (GDF-9), hepatoma derived growth factor (HDGF), myostatin (GDF-8), nerve growth factor (NGF), neurotrophins, platelet-derived growth factor (PDGF), thrombopoietin (TPO), transforming growth factor alpha (TGF- α), transforming growth factor beta (TGF- β), and the like; soluble receptors, such as soluble TNF- α receptors, soluble interleukin receptors (e.g., soluble IL-1 receptors and soluble type II IL-1 receptors), soluble γ/Δ T cell receptors, ligand-binding fragments of a soluble receptor, and the like; enzymes, such as α -glucosidase, imiglucrase, β -glucocerebrosidase, and alglucerase; enzyme activators, such as tissue plasminogen activator; chemokines, such as 1P-10, monokine induced by interferon-gamma (Mig), Gro α /IL-8, RANTES, MIP-1 α , MIP-1 β , MCP-1, PF-4, and the like; angiogenic agents, such as vascular endothelial growth factors (VEGFs, e.g., VEGF121, VEGF165, VEGF-

What is claimed:

1. An adeno-associated virus (AAV) comprising a capsid protein, comprising an amino acid sequence that is at least 95% identical to: (i) a VP1 region of any one of SEQ ID NOS:28, 29, 30, 31, and 16, (ii) a VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, or (iii) a VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and a transgene comprising a heterologous gene operably linked to regulatory sequence that control expression of the heterologous gene in a host cell.
2. The AAV of claim 1, wherein the capsid protein comprises the amino acid sequence that is at least 98% identical to: (i) a VP1 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) a VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31 and 16, or (iii) a VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16.
3. The AAV of claim 1, wherein the capsid protein comprises the amino acid sequence of (i) a VP1 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) a VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, or (iii) a VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16.
4. The AAV of claim 1 or 3 further comprising AAV inverted terminal repeats.
5. A composition comprising the AAV of any one of claims 1 to 4 and a physiologically compatible carrier.
6. A method of delivering a transgene to a cell comprising contacting the cell with the AAV of any one of claims 1 to 4.
7. The method of claim 6, wherein the cell is a muscle cell.
8. The method of claim 6 or 7, wherein the transgene encodes a microdystrophin protein.
9. A vector comprising a nucleic acid sequence encoding an adeno-associated virus (AAV) capsid protein having an amino acid sequence that is at least 95% identical to (i) any one of SEQ

ID NOS: 28, 29, 30, 31, and 16, (ii) the VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, or (iii) the VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16.

10. The vector of claim 9, wherein the nucleic acid sequence is operably linked to a heterologous regulatory element that controls expression of the capsid protein in a host cell.

11. A cell comprising the vector of claim 9 or 10.

12. Use of an AAV according to any one of claims 1-3 in the preparation of a medicament for the treatment of a disorder or disease, wherein the disorder or disease is Duchenne muscular dystrophy and the transgene encodes a microdystrophin protein.

13. A composition comprising an AAV of any one of claims 1 to 4 for use in medicine.

14. A method of producing an adeno-associated virus (AAV), the method comprising the steps of:

(a) culturing a cell to produce the AAV, the cell comprising a nucleotide sequence encoding a capsid protein, wherein the capsid protein comprises an amino acid sequence that is at least 95% sequence identical to a VP1 region, VP2 region, or VP3 region from one of SEQ ID NOS: 28, 29, 30, 31, and 16; and

(b) collecting the AAV from the cell culture.

15. The method of claim 14, wherein the nucleic acid sequence encodes a capsid protein that is at least 98% identical to any one of SEQ ID NOS: 28, 29, 30, 31, and 16.

16. The method of claim 14, wherein the nucleic acid sequence encodes a capsid protein to any one of SEQ ID NOS: 28, 29, 30, 31, and 16.