

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 a recombinant adeno-associated virus (AAV) comprising: ~~a capsid protein;~~ (a) a capsid comprising: (i) a VP1 capsid protein comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of a VP1 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) a VP2 capsid protein comprising an amino acid sequence

that is at least 95% identical to [a VP2 region of the amino acid sequence](#) ~~a VP2 region~~ of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (iii) [a VP3 capsid protein comprising](#) an amino acid sequence that is at least 95% identical to [a VP3 region of the amino acid sequence](#) ~~a VP3 region~~ of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (b) a transgene comprising a heterologous gene operably linked to [a regulatory sequence that controls](#) expression of the heterologous gene in a host cell.

[0006b] In another aspect, the present disclosure refers to a 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 an *in vitro* 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 that comprises:~~ (i) [an adeno-associated virus \(AAV\) VP1 capsid protein comprising](#) an amino acid sequence that is at least 95% identical to [the amino acid sequence](#) ~~a VP1 region~~ of any one of SEQ ID NOS: ~~16~~, 28, 29, 30, ~~and~~ 31, ~~and~~ 16, (ii) [an AAV VP2 capsid protein comprising](#) an amino acid sequence that is at least 95% identical to [a VP2 region of the amino acid sequence](#) ~~a VP2 region~~ of any one of SEQ ID NOS: ~~16~~, 28, 29, 30, ~~and~~ 31, ~~and~~ 16, and (iii) [an AAV VP3 capsid protein comprising](#) an amino acid sequence that is at least 95% identical to [a VP3 region of the amino acid sequence](#) ~~a VP3 region~~ of any one of SEQ ID NOS: ~~16~~, 28, 29, 30, ~~and~~ 31, ~~and~~ 16.

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

[0006f] In yet another aspect, the present disclosure refers to use of ~~an~~ [the](#) rAAV as disclosed herein in the preparation of a medicament for the treatment of a disorder or disease, wherein the disorder or disease is muscle-related disorder or disease.

[0006g] In yet another aspect, the present disclosure refers to a composition comprising ~~an~~ [the](#) rAAV as disclosed herein, wherein the transgene encodes a therapeutic protein, 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 [in cell culture](#) a cell to produce the AAV, the cell comprising a nucleotide sequence encoding an AAV ~~capsid protein~~ [VP1 capsid protein, an AAV VP2 capsid protein, and an AAV VP3 capsid protein](#), a nucleotide sequence encoding an AAV rep protein, and a nucleotide sequence comprising a transgene flanked by AAV inverted terminal repeats, wherein the transgene comprises a heterologous gene operably linked to a regulatory sequence that controls expression of the heterologous gene in a host cell, and wherein ~~the capsid protein comprises:~~ (i) [the VP1 capsid protein comprises](#) an amino acid sequence that is at least 95% ~~sequence~~-identical to [the amino acid sequence of](#) ~~a VP1 region of~~ any one of SEQ ID NOS: ~~16~~, 28, 29, 30, ~~and~~ 31, ~~and~~ 16, (ii) [the VP2 capsid protein comprising](#) an amino acid sequence that is at least 95% ~~sequence~~-identical to [a VP2 region of the amino acid sequence](#) ~~a VP2 region~~ of any one of SEQ ID NOS: ~~16~~, 28,

29, 30, ~~and 31, and 16~~, and (iii) the VP3 capsid protein comprising an amino acid sequence that is at least 95% ~~sequence~~-identical to a VP3 region of the amino acid sequence of a VP3 region of any one of SEQ ID NOS: ~~16~~, 28, 29, 30, ~~and 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

What is claimed:

1. A recombinant adeno-associated virus (AAV) comprising: ~~a capsid protein,~~
(a) a capsid comprising: (i) a VP1 capsid protein comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of a VP1 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) a VP2 capsid protein comprising an amino acid sequence that is at least 95% identical to a VP2 region of the amino acid sequence a VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (iii) a VP3 capsid protein comprising an amino acid sequence that is at least 95% identical to a VP3 region of the amino acid sequence a VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16; ~~and~~
(b) a transgene comprising a heterologous gene operably linked to a regulatory sequence that controls expression of the heterologous gene in a host cell.
2. The rAAV of claim 1, wherein (i) the VP1 capsid protein comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) the VP2 capsid protein comprises an amino acid sequence that is at least 98% identical to the VP2 region of the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (iii) the VP3 capsid protein comprises an amino acid sequence that is at least 98% identical to the VP3 region of the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16 ~~the capsid protein comprises: (i) an amino acid sequence that is at least 98% identical to a VP1 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) an amino acid sequence that is at least 98% identical to a VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31 and 16, and (iii) an amino acid sequence that is at least 98% identical to a VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16.~~
3. The rAAV of claim 1, wherein (i) the VP1 capsid protein comprises the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) the VP2 capsid protein comprises the VP2 region of the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (iii) the VP3 capsid protein comprises the VP3 region of the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16 ~~the capsid protein comprises: (i) an~~

~~amino acid sequence of a VP1 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) an amino acid sequence of a VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (iii) an amino acid sequence of a VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16.~~

4. The rAAV of claim 1, wherein (i) the VP1 capsid protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 28, (ii) the VP2 capsid protein comprises an amino acid sequence that is at least 95% identical to the VP2 region of the amino acid sequence of SEQ ID NO: 28, and (iii) the VP3 capsid protein comprises an amino acid sequence that is at least 95% identical to the VP3 region of the amino acid sequence of SEQ ID NO: 28.

5. The rAAV of claim 1, wherein (i) the VP1 capsid protein comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 28, (ii) the VP2 capsid protein comprises an amino acid sequence that is at least 98% identical to the VP2 region of the amino acid sequence of SEQ ID NO: 28, and (iii) the VP3 capsid protein comprises an amino acid sequence that is at least 98% identical to the VP3 region of the amino acid sequence of SEQ ID NO: 28.

6. The rAAV of claim 1, wherein (i) the VP1 capsid protein comprises the amino acid sequence of SEQ ID NO: 28, (ii) the VP2 capsid protein comprises the VP2 region of the amino acid sequence of SEQ ID NO: 28, and (iii) the VP3 capsid protein comprises the VP3 region of the amino acid sequence of SEQ ID NO: 28.

47. The rAAV of any one of claims 1 to 36, further comprising an AAV inverted terminal repeats.

58. The rAAV of any one of claims 1 to 6, wherein the transgene is flanked by a 5' AAV ITR and a 3' AAV ITR.

59. A composition comprising the rAAV of any one of claims 1 to 48, and a physiologically compatible carrier.

610. A method of delivering a transgene to an *in vitro* cell, comprising contacting the cell with the rAAV of any one of claims 1 to 48.

711. The method of claim 610, wherein the cell is a muscle cell.

812. The method of claim 6-10 or 711, wherein the transgene encodes a microdystrophin protein.

913. A vector comprising a nucleic acid sequence encoding ~~an adeno-associated virus (AAV) capsid protein that comprises~~: (i) an adeno-associated virus (AAV) VP1 capsid protein comprising an amino acid sequence that is at least 95% identical to the amino acid sequence a VP1 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) an AAV VP2 capsid protein comprising an amino acid sequence that is at least 95% identical to a VP2 region of the amino acid sequence a VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (iii) an AAV VP3 capsid protein comprising an amino acid sequence that is at least 95% identical to a VP3 region of the amino acid sequence a VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16.

~~1014~~. The vector of claim 913, wherein: (i) the VP1 capsid protein comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) the VP2 capsid protein comprises an amino acid sequence that is at least 98% identical to the VP2 region of the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (iii) the VP3 capsid protein comprises an amino acid sequence that is at least 98% identical to the VP3 region of the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16~~the AAV capsid protein comprises: (i) an amino acid sequence that is at least 98% identical to a VP1 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) an amino acid sequence that is at least 98% identical to a VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31 and 16, and (iii) an amino acid sequence that is at least 98% identical to a VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16.~~

~~1415.~~ The vector of claim ~~913~~, wherein: (i) the VP1 capsid protein comprises the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) the VP2 capsid protein comprises the VP2 region of the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (iii) the VP3 capsid protein comprises the VP3 region of the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16~~the AAV capsid protein comprises: (i) an amino acid sequence of a VP1 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) an amino acid sequence of a VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (iii) an amino acid sequence of a VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16.~~

16. The vector of claim 13, wherein (i) the VP1 capsid protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 28, (ii) the VP2 capsid protein comprises an amino acid sequence that is at least 95% identical to the VP2 region of the amino acid sequence of SEQ ID NO: 28, and (iii) the VP3 capsid protein comprises an amino acid sequence that is at least 95% identical to the VP3 region of the amino acid sequence of SEQ ID NO: 28.

17. The vector of claim 13, wherein (i) the VP1 capsid protein comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 28, (ii) the VP2 capsid protein comprises an amino acid sequence that is at least 98% identical to the VP2 region of the amino acid sequence of SEQ ID NO: 28, and (iii) the VP3 capsid protein comprises an amino acid sequence that is at least 98% identical to the VP3 region of the amino acid sequence of SEQ ID NO: 28.

18. The vector of claim 13, wherein (i) the VP1 capsid protein comprises the amino acid sequence of SEQ ID NO: 28, (ii) the VP2 capsid protein comprises the VP2 region of the amino acid sequence of SEQ ID NO: 28, and (iii) the VP3 capsid protein comprises the VP3 region of the amino acid sequence of SEQ ID NO: 28.

~~1419.~~ The vector of any one of claims ~~913~~ to ~~1418~~, wherein the nucleic acid sequence is operably linked to a heterologous regulatory element that controls expression of the capsid protein in a host cell.

~~1320~~. An *in vitro* cell comprising the vector of any one of claims ~~9-13~~ to ~~1219~~.

~~1421~~. Use of ~~anthe~~ rAAV according to any one of claims 1 ~~to -38~~, in the preparation of a medicament for the treatment of a disorder or disease, wherein the disorder or disease is muscle-related disorder or disease.

~~1522~~. The use of claim ~~1421~~, wherein the muscle-related disorder or disease is associated with ~~abnormal activity of~~ an endogenous protein not being expressed or being expressed at less than normal levels in a subject, and wherein the transgene encodes a functional gene product.

~~1623~~. The use of claim ~~14-21~~ or ~~1522~~, wherein the muscle-related disorder or disease is Duchenne muscular dystrophy and the transgene encodes a microdystrophin protein.

~~1724~~. A composition comprising ~~anthe~~ rAAV of any one of claims 1 to ~~48~~, wherein the transgene encodes a therapeutic protein, for use in medicine.

~~1825~~. A method of producing an adeno-associated virus (AAV), the method comprising the steps of:

(a) culturing in cell culture a cell to produce the AAV, the cell comprising a nucleotide sequence encoding an AAV ~~capsid protein~~ VP1 capsid protein, an AAV VP2 capsid protein, and an AAV VP3 capsid protein, a nucleotide sequence encoding an AAV rep protein, and a nucleotide sequence comprising a transgene flanked by AAV inverted terminal repeats, wherein the transgene comprises a heterologous gene operably linked to a regulatory sequence that controls expression of the heterologous gene in a host cell, and wherein ~~the capsid protein comprises~~: (i) the VP1 capsid protein comprises an amino acid sequence that is at least 95% ~~sequence~~-identical to the amino acid sequence of a VP1 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) the VP2 capsid protein comprising an amino acid sequence that is at least 95% ~~sequence~~-identical to a VP2 region of the amino acid sequence a VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (iii) the VP3 capsid protein comprising an amino acid sequence that is at least 95% ~~sequence~~-identical to a VP3 region of the amino acid sequence of a VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16; and

(b) collecting the AAV from the cell culture.

1926. The method of claim 1825, wherein: (i) the VP1 capsid protein comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) the VP2 capsid protein comprises an amino acid sequence that is at least 98% identical to the VP2 region of the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (iii) the VP3 capsid protein comprises an amino acid sequence that is at least 98% identical to the VP3 region of the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16 ~~the capsid protein comprises: (i) an amino acid sequence that is at least 98% identical to a VP1 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) an amino acid sequence that is at least 98% identical to a VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (iii) an amino acid sequence that is at least 98% identical to a VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16.~~

2027. The method of claim 1825, wherein: (i) the VP1 capsid protein comprises the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, (ii) the VP2 capsid protein comprises the VP2 region of the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16, and (iii) the VP3 capsid protein comprises the VP3 region of the amino acid sequence of any one of SEQ ID NOS: 28, 29, 30, 31, and 16 ~~the capsid protein comprises: (i) an amino acid sequence of a VP1 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16; (ii) an amino acid sequence of a VP2 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16; and (iii) an amino acid sequence of a VP3 region of any one of SEQ ID NOS: 28, 29, 30, 31, and 16.~~

28. The method of claim 25, wherein (i) the VP1 capsid protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO: 28, (ii) the VP2 capsid protein comprises an amino acid sequence that is at least 95% identical to the VP2 region of the amino acid sequence of SEQ ID NO: 28, and (iii) the VP3 capsid protein comprises an amino acid sequence that is at least 95% identical to the VP3 region of the amino acid sequence of SEQ ID NO: 28.

29. The method of claim 25, wherein (i) the VP1 capsid protein comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 28, (ii) the VP2 capsid protein comprises an amino acid sequence that is at least 98% identical to the VP2

region of the amino acid sequence of SEQ ID NO: 28, and (iii) the VP3 capsid protein comprises an amino acid sequence that is at least 98% identical to the VP3 region of the amino acid sequence of SEQ ID NO: 28.

30. The method of claim 25, wherein (i) the VP1 capsid protein comprises the amino acid sequence of SEQ ID NO: 28, (ii) the VP2 capsid protein comprises the VP2 region of the amino acid sequence of SEQ ID NO: 28, and (iii) the VP3 capsid protein comprises the VP3 region of the amino acid sequence of SEQ ID NO: 28.

2431. The method of any one of claims 18-25 to 2030, wherein the cell is selected from an invertebrate cell, an insect cell, or a mammalian cell.

2232. The method of claim 2431, wherein the mammalian cell is selected from HEK293, HeLa, CHO, NS0, SP2/0, PER.C6, Vero, RD, BHK, HT-1080, A549, Cos-7, ARPE-19, or MRC-5, ~~or any combination thereof.~~

2333. The method of claim 2431, wherein the insect cell is selected from High Five, Sf9, Se301, SeIZD2109, SeUCR1, Sf900+, Sf21, Bti-tn-5b1-4, MG-1, Tn368, HzAm1, BM-N, Ha2302, Hz2E5, or Ao38, ~~or any combination thereof.~~