[0012] A "transcription regulatory element" refers to nucleotide sequences of a gene involved in regulation of genetic transcription including a promoter, plus response elements, activator and enhancer sequences for binding of transcription factors to aid RNA polymerase binding and promote expression, and operator or silencer sequences to which repressor proteins bind to block RNA polymerase attachment and prevent expression. The term "liver specific transcription regulatory element" refers to a regulatory element that modulates gene expression specifically in the liver tissue. Examples of liver specific regulatory elements include, but are not limited to, the mouse thyretin promoter (mTTR), the endogenous human factor VIII promoter (F8), human alpha-1-antitrypsin promoter (hAAT) and active fragments thereof, human albumin minimal promoter, and mouse albumin promoter. Enhancers derived from liver specific transcription factor binding sites are also contemplated, such as EBP, DBP, HNF1, HNF3, HNF4, HNF6, with Enh1.

[0012a] In one aspect, there is provided an adeno-associated virus (AAV) vector comprising an AAV2 5' inverted terminal repeat (ITR), a liver-specific transcription regulatory region, a functionally active FVIII coding region, a polyadenylation sequence, an AAV2 3' ITR, and optionally one or more introns, wherein said AAV vector is less than 5.0 kb in length.

[0013] In one example, the AAV vector of the disclosure comprises a nucleic acid encoding functionally active FVIII having the B domain replaced by the 14 amino acid SQ sequence, i.e., encoding FVIII SQ. The SQ sequence is disclosed in Ward et al., Blood, 117:798-807, 2011, and McIntosh et al., Blood 121:3335-3344, 2013. The FVIII coding region sequence is a codon-optimized sequence (see Nathwani et al., US Pat. App. Pub. No. 2013/0024960A1, published January 24, 2013, which is incorporated herein by reference in its entirety, and McIntosh et al., Blood 121:3335-3344, 2013). This sequence is referred herein as the "UCL SQ FVIII."

[0014] In one example, the AAV vector of the disclosure comprises Proto 1, which is depicted schematically in Figure 2A, and comprises the nucleic acid sequence set forth in SEQ ID NO: 1.

[0015] In one example, the AAV vector of the disclosure comprises Proto 1S, which is depicted schematically in Figure 2B, and comprises the nucleic acid sequence set forth in SEQ ID NO: 2.

[0016] In one example, the AAV vector of the disclosure comprises Proto 2S, which is depicted schematically in Figure 2C, and comprises the nucleic acid sequence set forth in SEQ ID NO: 3.

more different orientation(s). The present disclosure is also directed to the above described constructs in an opposite orientation.

[0062] The AAV vectors of the disclosure in single strand is less than about 7.0 kb in length, or is less than 6.5kb in length, or is less than 6.4 kb in length, or is less than 6.3 kb in length, or is less than 6.2 kb in length, or is less than 6.0 kb in length, or is less than 5.8 kb in length, or is less than 5.6 kb in length, or is less than 5.5 kb in length, or is less than 5.4 kb in length, or is less than 5.4 kb in length, or is less than 5.2 kb in length or is less than 5.0 kb in length. The AAV vectors of the invention in single strand ranges from about 5.0 kb to about 6.5 kb in length, or ranges from about 4.8 kb to about 5.2 k in length, or 4.8 kb to 5.3 kb in length, or ranges from about 4.9 kb to about 5.5 kb in length, or about 4.8 kb to about 6.0 kb in length, or about 5.0 kb to 6.2 kb in length or about 5.1 kb to about 6.3 kb in length ,or about 5.2 kb to about 6.4 kb in length, or about 5.5 kb to about 6.5 kb in length.

[0062a] In another aspect, there is provided a method of producing a recombinant adeno-associated virus (AAV) particle comprising A) culturing a cell that has been transfected with an AAV vector as described herein; and B) recovering recombinant AAV particle from the supernatant of the transfected cell.

[0063] In another example, the disclosure provides for methods of producing a recombinant adeno-associated virus (AAV) particle comprising any of the AAV vectors of the invention. The methods comprise the steps of culturing a cell that has been transfected with any of the AAV vectors of the invention and recovering recombinant AAV from the supernatant of the transfected cell.

[0064] The cells of the disclosure are any cell type are susceptible to baculovirus infection, including insect cells such as High Five, Sf9, Se301, SeIZD2109, SeUCR1, Sf9, Sf900+, Sf21, BTI-TN-5B1-4, MG-1, Tn368, HzAm1, BM-N, Ha2302, Hz2E5 and Ao38. Preferred mammalian cells used can be HEK293, HeLa, CHO, NS0, SP2/0, PER.C6, Vero, RD, BHK, HT 1080, A549, Cos-7, ARPE-19 and MRC-5 cells, and including mammalian cells such as HEK293, HeLa, CHO, NS0, SP2/0, PER.C6, Vero, RD, BHK, HT 1080, A549, Cos-7, ARPE-19 and MRC-5 cells.

[0064a] In yet another aspect, there is provided a viral particle comprising the viral vector as described herein.

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.

[0067] The invention also provides for cells comprising any of the AAV vectors of the invention, and viral particles produced by these cells of the invention.

[0068] In another example, the disclosure provides for methods of treating a patient suffering from hemophilia A comprising administering to the patient an effective amount of any of the AAV vectors of the disclosure, or a viral particle of the invention or a viral particles produced by a method of the disclosure.

[0068a] In yet another aspect, there is provided use of an AAV FVIII vector as described herein for preparation of a medicament for treatment of hemophilia A.

[0069] In a further example, the disclosure provides for a use of any of the AAV vectors of the disclosure for preparation of a medicament for the treatment of hemophilia A. In one example, the medicament comprises an amount of AAV vector that expresses human FVIII in an amount effective to treat hemophilia A.

[0069a] In yet another aspect, there is provided a composition comprising an AAV FVIII vector as described herein for use in the treatment of hemophilia A.

[0070] In another example, the disclosure provides for a composition comprising any of the AAV vectors of the disclosure for the treatment of hemophilia A. In one example, the composition comprises an amount of AAV vector that expresses human FVIII in an amount effective to treat hemophilia A.

[0071] In another example, the AAV vectors of the invention are used to produce AAV viral particles that are useful to treat a patient suffering from Hemophilia A.

DESCRIPTION OF DRAWINGS

[0072] Figure 1 provides a schematic of the UCL SQ vector. From left to right, the UCL SQ vector comprises the AAV2 5' ITR, wild-type AAV2 viral sequence, the 34 base human ApoE/C1 enhancer, the 32 base human AAT promoter distal X region, the 186 base human AAT promoter, including 42 bases of 5' UTR sequence, the codon-optimized human FVIII SQ sequence (see Nathwani et al., US Pat. App. Pub. No. 2013/0024960A1, published January 24,

What is Claimed:

1. An adeno-associated virus (AAV) vector comprising an AAV2 5' inverted terminal repeat (ITR), a liver-specific transcription regulatory region, a functionally active FVIII coding region, a polyadenylation sequence, an AAV2 3' ITR, and optionally one or more introns, wherein said AAV vector is less than 5.0 kb in length.

2. The AAV vector of Claim 1, wherein said liver-specific transcriptional regulatory region is located immediately 3' to said AAV2 5 ' ITR.

3. The AAV vector of Claim 1 or 2, wherein said liver-specific regulatory region comprises nucleotides 146-397 of SEQ ID NO:1.

4. The AAV vector of any one of Claims 1-3, which comprises nucleotides 1-397 of SEQ ID NO:1.

5. The AAV vector of any one of Claims 1-4, wherein said functionally active FVIII coding region comprises nucleotides 923-5296 of SEQ ID NO: 9.

6. The AAV vector of any one of claims 1 – 5,
comprising the nucleotide sequence of SEQ ID NO: 1.,

7. The AAV vector of Claim 1, comprising the nucleotide sequence set forth in SEQ ID NO: 2.

8. The AAV vector of Claim 1, comprising the nucleotide sequence set forth in SEQ ID NO: 3.

9. The AAV vector of Claim 1, comprising the nucleotide sequence set forth in SEQ ID NO: 4.

10. The AAV vector of Claim 1, comprising the nucleotide sequence set forth in SEQ ID NO: 5.

11. The AAV vector of Claim 1, comprising the nucleotide sequence set forth in SEQ ID NO: 6.

12. The AAV vector of Claim 1, comprising the nucleotide sequence set forth in SEQ ID NO: 7.

13. The AAV vector of Claim 1, comprising the nucleotide sequence set forth in SEQID NO: 8.

14. A method of producing a recombinant adeno-associated virus (AAV) particle comprising
A) culturing a cell that has been transfected with an AAV vector of any one of claim 1 - 13; and
B) recovering recombinant AAV particle from the supernatant of the transfected cell.

15. A viral particle comprising an AAV vector of any one of claims 1-13.

16. A cell comprising an AAV vector of any one of claims 1-13.

17. Use of an AAV vector of any one of claims 1-13 for preparation of a medicament for treatment of hemophilia A.

18. A composition comprising an AAV vector of any one of claims 1-13 for use in the treatment of hemophilia A.