METHODS OF REGULATING ADENO-ASSOCIATED VIRUS PRODUCTION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 63/350,849, filed on June 9, 2022, and to U.S. Provisional Application No. 63/209,735, filed on June 11, 2021, both of which are hereby incorporated by reference in their entireties, and to which priority is claimed.

1. FIELD OF INVENTION

The presently disclosed subject matter relates to compositions and methods for the regulation of recombinant adeno-associated virus (rAAV) production in cell culture. In particular, the presently disclosed subject matter relates to strategies to overcome AAV Rep protein-mediated cytotoxicity by reversible post-translational regulation of the expression of AAV Rep protein, resulting in regulated rAAV production.

2. BACKGROUND

There are a variety of AAV production systems used to produce rAAV in cell culture. These include plasmid transient transfection of human embryonic kidney (HEK) 293 cells, Hela producer cell lines, BHK21-based platforms, and baculovirus-based production systems. Each of these systems has strengths and weaknesses. For example, given the importance of the adenovirus E1a protein in initiating the production of rAAV, E1a-expressing cells, e.g., HEK293 cells, are attractive to produce rAAV as they eliminate the need to otherwise introduce an E1a gene into the host cell genome. E1a-expressing cells, e.g., HEK293 cells, can also offer ease of growth and adaptability to growth in suspension. However, efforts to create stable and passagable rAAV producing E1a-expressing cell lines have been hampered by cellular toxicity caused by E1a-induced accumulation of AAV Rep protein. In view of the foregoing, there is a need in the art for new rAAV production strategies where accumulation of Rep protein can be regulated to avoid Rep-mediated cytotoxicity, resulting in regulated rAAV production.

3. SUMMARY OF THE INVENTION

In one aspect, provided herein is a method of regulating the production of recombinant adeno-associated virus (rAAV) vector particles, the method comprising: a) introducing into a cell: (i) an rAAV comprising a gene of interest; and (ii) a nucleic acid

encoding a fusion protein, wherein the fusion protein comprises an AAV Rep protein, and a degradation ligand-dependent degradation domain, b) culturing the cell under conditions suitable for producing the rAAV vector particles; and c) contacting the cell with a degradation ligand, wherein the degradation ligand binds to the degradation domain to regulate the expression of the AAV protein and thereby regulate the production of rAAV vector particles.

In another aspect, provided herein is a rAAV producing cell, wherein the cell comprises a nucleic acid encoding a fusion protein comprising an AAV Rep protein, and a degradation ligand-dependent degradation domain.

In certain embodiments, the present disclosure is directed to methods of regulating the production of recombinant adeno-associated virus (rAAV) vector particles, the method comprising: introducing into a cell: an rAAV comprising a gene of interest and

WHAT IS CLAIMED IS:

1. A method of regulating the production of recombinant adeno-associated virus (rAAV) vector particles, the method comprising:

a) introducing into a cell:

(i). an rAAV comprising a gene of interest; and

(ii). a nucleic acid encoding a fusion protein, wherein the fusion protein comprises an AAV Rep protein, and a degradation ligand-dependent degradation domain,

b) culturing the cell under conditions suitable for producing the rAAV vector particles; and

c) contacting the cell with a degradation ligand, wherein the degradation ligand binds to the degradation domain to regulate the expression of the AAV protein and thereby regulate the production of rAAV vector particles.

2. The method of claim 1, wherein the nucleic acid encoding the fusion protein further encodes a linker, operably linking the AAV protein to the degradation ligand-dependent degradation domain.

3. The method of claim 1 or 2, wherein the degradation ligand-dependent degradation domain:

(a) is a FKBP12 domain (FKBP), optionally comprising an L106P and/or a F36V amino acid substitution;

(b) is dihydrofolate reductase (DHFR); or

(c) is an auxin induced degradation domain.

4. The method of claim 1, wherein the Rep protein is Rep78, Rep68, Rep52, or Rep40 protein.

5. The method of any one of claims 1-4, wherein:

(a) the degradation ligand-dependent degradation domain is fused to the C-terminal end of the Rep protein; or

(b) the degradation ligand-dependent degradation domain is fused to the N-terminal end

of the Rep protein.

6. The method of any one of claims 1-5, comprising introducing into the cell a nucleic acid encoding a Cap protein, wherein:

(a) the nucleic acid encoding the fusion protein and the nucleic acid encoding a Cap protein, are introduced into the cell using the same plasmid; or

(b) the nucleic acid encoding the fusion protein and the nucleic acid encoding a Cap protein, are introduced into the cell using separate plasmids.

7. The method of any one of claims 1-6, wherein the rep, cap, or helper genes are under the control of a regulatory element.

8. The method of claim 7, wherein said regulatory element comprises a Tet response binding element.

9. The method of claim 1, wherein the animal cell is a mammalian cell.

10. The method of any one of claims 1-9, wherein:

(a) two or more different AAV proteins are independently fused to degradation ligand-dependent degradation domains; or

(b) each different AAV protein is independently fused to a different degradation ligand-dependent degradation domain.

11. A rAAV producing cell, wherein the cell comprises a nucleic acid encoding a fusion protein comprising an AAV Rep protein, and a degradation ligand-dependent degradation domain.

12. The rAAV producing cell of claim 11, wherein the nucleic acid encoding a fusion protein comprises the AAV protein, a linker, and a degradation ligand-dependent degradation domain.

13. The rAAV producing cell of claim 11 or 12, wherein the degradation ligand-dependent degradation domain :

(a) is a FKBP12 domain (FKBP), optionally comprising an L106P and/or a F36V amino acid substitution;

(b) is dihydrofolate reductase (DHFR); or

(c) is an auxin induced degradation domain.

14. The rAAV producing cell of 11, wherein the animal cell is a mammalian cell.

15. The rAAV producing cell of any one of claims 11-14, wherein:

(a) the degradation ligand-dependent degradation domain is fused via the linker to the C-terminal end of the Rep protein; or

(b) the cell is a mammalian cell and the degradation ligand-dependent degradation domain is fused via the linker to the N- terminal end of the Rep protein.

16. The rAAV producing cell of claim 14 or claim 15, further comprising introducing into the cell a nucleic acid encoding a Cap protein, wherein:

(a) the cell is a mammalian cell and the nucleic acid encoding the fusion protein and the nucleic acid encoding a Cap protein are introduced into the cell using the same plasmid; or

(b) the cell is a mammalian cell and the nucleic acid encoding the fusion protein and the nucleic acid encoding a Cap protein are introduced into the cell using separate plasmids.

17. The rAAV producing cell of any one of claims 14-16, wherein the cell is a mammalian cell and the rep, cap, or helper genes are under the control of a regulatory element.

18. The rAAV producing cell of any one of claims 11-17 wherein:

(a) two or more different AAV proteins are independently fused to degradation ligand-dependent degradation domains; or

(b) each different AAV protein is independently fused to a different degradation ligand-dependent degradation domain.