AAV2 capsids, and the presence of empty capsids inhibits hepatocyte transduction in vivo following high dose vector administration. The potential adverse effects of the unwanted immunogenicity of empty capsids compromise product safety and efficacy.

Removal of empty capsids that have no therapeutic function by known methods is difficult due to the innate similarity of their particle size, affinity and protein composition to the complete particles containing vector DNA. There have been continuous efforts to separate empty AAV capsids from genome containing complete particles. Empty particle-free AAV2 has been achieved by differential $CHCl_3$. However, there may be problems using this method in scale up production and GMP manufacture. Ion exchange chromatography has also been reported for the separation of empty capsids in AAV2, 4, 5 and 8. However, from 20% up to 30 fold empty capsids remained in the final products.

Therefore, there is need to develop new methods of AAV vector production which decrease or eliminate the presence of empty capsids in the final product. This would improve the safety and efficacy of AAV products. Reduction of empty particles would also overcome the hurdle in high titre production.

SUMMARY

Cellular and viral factors that contribute to the formation of two types of particles in AAV vector production are still largely unknown. In general, viruses package their genomes into protein capsids either via association of structural proteins with the viral genome or via insertion of viral genomes into preassembled capsids. AAVs are known to package their genome into preassembled capsids. It has been reported that specific amino acid interactions are required for efficient insertion of viral DNA and single amino acid mutations and gross conformation change of AAV capsid proteins have been shown to result in deficiency of packaging AAV genomes. There have also been very limited studies on the involvement of cellular proteins in AAV assembly, indicating that total producer cell proteins and DNA helicase are necessitated in AAV assembly. However, the actual mechanism by which AAV DNA is inserted into capsids is unknown.

In order to better understand the role of cellular proteins in AAV assembly and, ultimately improve the safety and quality of AAV products, the present inventors have analysed host cellular proteins co-produced and co-purified in AAV vectors. In particular, the present inventors have conducted the first systematic analysis and comparison of the protein composition between empty capsids and complete vectors in three AAV serotypes: AAV2; AAV5; and AAV8. The inventors have

demonstrated intrinsic similarity among the three AAV serotypes, although there are some notable differences between the different serotypes. Importantly, the inventors demonstrated for the first time that there are significant differences between empty capsids and complete vectors. Finally, the inventors have identified a number of host cell proteins that are inherently associated with AAV products. In particular, the inventors have demonstrated that host cell proteins YB1, NPM1 and NCL are found in AAV products, and that modulating the expression of these host cell proteins impacts the production of AAV vector particles.

Accordingly, in one aspect, the present invention refers to a transgenic producer cell line in which the expression of YB1 is reduced compared with a control producer cell line. In another aspect, the present invention refers to a method for producing an adeno-associated viral (AAV) vector comprising culturing an adeno-associated virus in a producer cell line in which the expression of YB1 is reduced compared with a control producer cell line. Also described herein is a transgenic producer cell line in which the expression of at least one of YB1, NPM1 and NCL is modulated compared with a control producer cell line. Typically, the expression of: (i) NPM1 and NCL; (ii) YB1 and NPM1; (iii) YB1 and NCL; or (iv) YB1, NPM1 and NCL; is modulated in the producer cell line described herein compared with a control producer cell line.

In a preferred example, the expression of at least one of YB1, NPM1 and NCL is reduced compared with a control producer cell line, wherein the expression of YB1, NPM1 and/or NCL may be reduced using CRISPR genome editing, a double stranded RNA (dsRNA), a small interfering RNA (siRNA), a small hairpin RNA (shRNA), a micro RNA or an antisense RNA. Typically the expression of YB1, NPM1 and/or NCL is reduced using a shRNA, and preferably: YB1 expression is reduced using a shRNA comprising the nucleotide sequence SEQ ID NOs: 14 to 18 (Y1 to Y5); NPM1 expression is reduced using a shRNA comprising the nucleotide sequence SEQ ID NOs: 4 to 8 (NPM-N6 to NPM-N10); and/or NCL expression is reduced using a shRNA comprising the nucleotide sequence SEQ ID NOs: 9 to 13 (NCL-N1 to NCL-N5). The expression of YB1, NPM1 and/or NCL may be reduced using CRISPR genome editing. In one example, the expression of YB1 is reduced using a gRNA pair selected from SEQ ID NOs: 33 and 34; SEQ ID NOs: 35 and 36; SEQ ID NOs: 37 and 38; and/or SEQ ID NOs: 39 and 40.

The expression of one or more additional genes and/or proteins listed in Table 2 herein may also be modulated in a producer cell line as described herein compared with a control producer cell line.

In a preferred example, the transgenic producer cell line as described herein is a human embryonic kidney 293T cell line.

Also described herein is a method for producing an adeno-associated viral (AAV) vector comprising culturing an adeno-associated virus in a producer cell line in which the expression of at least one of YB1, NPM1 and NCL is modulated.

Typically the expression of: (i) NPM1 and NCL; (ii) YB1 and NPM1; (iii) YB1 and NCL; or (iv) YB1, NPM1 and NCL; in the producer cell line is modulated according to a method as described herein. Preferably the modulation of YB1, NPM1 and/or NCL expression is a reduction in the expression of YB1, NPM1 and/or NCL, wherein the expression of YB1, NPM1 and/or NCL is reduced using CRISPR genome editing, a double stranded RNA (dsRNA), a small interfering RNA (siRNA), a small hairpin RNA (shRNA),a micro RNA or an antisense RNA. Typically the expression of YB1, NPM1 and/or NCL is reduced using a shRNA, and preferably: YB1 expression is reduced using a shRNA comprising the nucleotide sequence SEQ ID NOs: 14 to 18 (Y1 to Y5); NPM1 expression is reduced using a shRNA comprising the nucleotide sequence SEQ ID NOs: 4 to 8 (NPM-N6 to NPM-N10); and/or NCL expression is reduced using a shRNA comprising the nucleotide sequence SEQ ID NOs: 9 to 13 (NCL-N1 to NCL-N5). The expression of YB1, NPM1 and/or NCL may be reduced using CRISPR genome editing. In one example, the expression of YB1 is reduced using a gRNA pair selected from SEQ ID NOs: 33 and 34; SEQ ID NOs: 35 and 36; SEQ ID NOs: 37 and 38; and/or SEQ ID NOs: 39 and 40.

The expression of one or more of the additional genes and/or proteins listed in Table 2 may also be modulated in the producer cell line used in a method as described herein.

The method as described herein may increase the titre of AAV vector at least 2 fold compared with the titre of AAV vector produced by a control method, and/or the method as described herein may increase the ratio of complete: empty AAV vector by at least 20% compared with the ratio of complete: empty AAV vector produced by a control method.

In a preferred example, the transgenic producer cell line used in a method of the invention is a human embryonic kidney 293T cell line.

Preferably, the method as described herein produces AAV2, AAV5 and/or AAV8 serotype AAV vectors.

Also described herein is a population of adeno-associated viral (AAV) vectors obtainable from a producer cell line as described herein and/or a method as described herein. Preferably, the ratio of complete: empty AAV vectors in the population as described herein is increased by at least 20% compared the ratio of complete: empty AAV vector in a population produced by a control method. Preferably the AAV vector population as described herein comprises AAV2, AAV5 and/or AAV8 serotype vectors.

CLAIMS

1 A transgenic producer cell line in which the expression of YB1 is reduced compared with a control producer cell line.

2 The transgenic producer cell line of claim 1, wherein the expression of: (i) YB1 and NPM1; (ii) YB1 and NCL; or (iii) YB1, NPM1 and NCL; is reduced compared with a control producer cell line.

3 The transgenic producer cell line with reduced YB1 expression of claim 1 or 2, wherein the expression of YB1, NPM1 and/or NCL is reduced using CRISPR genome editing, a double stranded RNA (dsRNA), a small interfering RNA (siRNA), a small hairpin RNA (shRNA),a micro RNA or an antisense RNA.

4 The transgenic producer cell line with reduced YB1 expression of claim 3, wherein the expression of YB1, NPM1 and/or NCL is reduced using a shRNA, and preferably:

(a) YB1 expression is reduced using a shRNA comprising a nucleotide sequence selected from SEQ ID NOs: 14 to 18 (Y1 to Y5);

(b) NPM1 expression is reduced using a shRNA comprising a nucleotide sequence selected from SEQ ID NOs: 4 to 8 (NPM-N6 to NPM-N10); and/or

(c) NCL expression is reduced using a shRNA comprising a nucleotide sequence selected from SEQ ID NOs: 9 to 13 (NCL-N1 to NCL-N5).

5 The transgenic producer cell line with reduced YB1 expression of claim 3, wherein the expression of YB1, NPM1 and/or NCL is reduced using CRISPR genome editing.

6 The transgenic producer cell line of claim 5, wherein the expression of YB1 is reduced using a gRNA pair selected from SEQ ID NOs: 33 and 34; SEQ ID NOs: 35 and 36; SEQ ID NOs: 37 and 38; and/or SEQ ID NOs: 39 and 40.

7 The transgenic producer cell line of any one of claims 1 to 6, wherein the expression of one or more additional genes and/or proteins listed in Table 2 is modulated compared with a control producer cell line.

8 The transgenic producer cell line of any one of claims 1 to 7, which is a human embryonic kidney 293T cell line.

9 A method for producing an adeno-associated viral (AAV) vector comprising culturing an adeno-associated virus in a producer cell line in which the expression of YB1 is reduced compared with a control producer cell line.

10 The method of claim 9, wherein the expression of: (i) YB1 and NPM1; (ii) YB1 and NCL; or (iii) YB1, NPM1 and NCL; in the producer cell line is reduced.

11 The method of claim 9 or 10, wherein the expression of YB1, NPM1 and/or NCL is reduced using CRISPR genome editing, a double stranded RNA (dsRNA), a small interfering RNA (siRNA), a small hairpin RNA (shRNA),a micro RNA or an antisense RNA.

12 The method of claim 11, wherein the expression of YB1, NPM1 and/or NCL is reduced using a shRNA, and preferably:

(a) YB1 expression is reduced using a shRNA comprising a nucleotide sequence selected from SEQ ID NOs: 14 to 18 (Y1 to Y5);

(b) NPM1 expression is reduced using a shRNA comprising a nucleotide sequence selected from SEQ ID NOs: 4 to 8 (NPM-N6 to NPM-N10); and/or

(c) NCL expression is reduced using a shRNA comprising a nucleotide sequence selected from SEQ ID NOs: 9 to 13(NCL-N1 to NCL-N5).

13 The method of claim 11, wherein the expression of YB1, NPM1 and/or NCL is reduced using CRISPR genome editing.

14 The method of claim 13, wherein the expression of YB1 is reduced using a gRNA pair selected from SEQ ID NOs: 33 and 34; SEQ ID NOs: 35 and 36; SEQ ID NOs: 37 and 38; and/or SEQ ID NOs: 39 and 40.

15 The method of any one of claims 9 to 14, wherein the expression of one or more of the additional genes and/or proteins listed in Table 2 is modulated in the producer cell line.

16 The method of any one of claims 9 to 15, wherein the titre of AAV vector is increased at least 2 fold compared with the titre of AAV vector produced by a method using a control producer cell line, and said AAV vector is AAV2 or AAV8.

17 The method of any one of claims 9 to 16, wherein the ratio of complete: empty AAV vector is increased by at least 20% compared with the ratio of complete: empty AAV vector produced by a method using a control producer cell line, and said AAV vector is AAV2 or AAV8.

18 The method of any one of claims 9 to 17, wherein the producer cell line is a human embryonic kidney 293T cell line.