genes, including the majority of the viral capsid proteins, are expressed only after significant processing of a single primary transcript issued by the major late promoter (MLP). The MLP is particularly efficient during the late phase of infection, and all the mRNAs issued from this promoter possess a 5'-tripartite leader (TPL) sequence which makes them preferred mRNAs for translation.

[0013] For successful gene therapy, without position effects of integration and transcriptional silencing, the transferred gene must be expressed at high levels in desired tissues or cells. A locus control region (LCR) is particularly suitable for this task as an LCR is characterized by its ability to enhance the expression of linked genes to physiological levels in a tissue-specific and copy number-dependent manner at ectopic chromatic sites. The components of an LCR commonly colocalize to sites of DNAse I hypersensitivity (HS) in the chromatin of expressing cells. The core determinants at individual HSs are composed of arrays of multiple ubiquitous and lineage-specific transcription factor–binding sites.

SUMMARY

[0014] The present disclosure includes, among other things, adenoviral vectors and adenoviral genomes, systems including two or more adenoviral vectors and/or adenoviral genomes of the present disclosure, and uses of such adenoviral vectors, adenoviral genomes, and systems. In certain examples, the present disclosure includes adenoviral vectors and/or adenoviral genomes that include a transposon payload of, *e.g.*, 1 kb to 40 kb. In certain examples of the present disclosure, a transposase can cause integration of a transposon payload of, *e.g.*, up to 40 kb into the genome of a target cell. Thus, the present disclosure includes, among other things vectors, genomes, and systems that enable integration of a payload of up to 40 kb present in an adenoviral donor vector into a target cell genome. As those of skill in the art will appreciate, vector integration capacity, in and of itself, is one critically important feature a gene therapy system, at least in part because integration capacity limits the length and/or complexity of therapeutic payloads.

[0015] Certain examples of long and/or complex nucleic acid payloads recognized in the present disclosure include payloads that include a Long Locus Control Region. Due to their length, Long Locus Control Regions have been historically unsuitable for inclusion in adenoviral payloads, but long and/or complex nucleic acid payloads including without limitation long and/or complex nucleic acid payloads including Long Locus Control Regions, can be integrated into target cell genomes in accordance with vectors, genomes, and systems disclosed herein.

[0016] Thus there is provided in one example an adenoviral donor vector including: (a) an adenoviral capsid; and (b) a linear, double-stranded DNA genome including: (i) a transposon payload of at least 10 kb; (ii) transposon inverted repeats (IRs) that flank the transposon payload; and (iii) recombinase direct repeats (DRs) that flank the transposon inverted repeats.

[0017] Another example is an adenoviral donor genome including: (a) a transposon payload of at least 10 kb; (b) transposon inverted repeats (IRs) that flank the transposon payload; and (c) recombinase direct repeats (DRs) that flank the transposon inverted repeats.

[0018] Also provided is an adenoviral transposition system including: (a) an adenoviral donor vector as described herein; and (b) an adenoviral support vector including (i) the adenoviral capsid; and (ii) an adenoviral support genome including a nucleic acid sequence encoding a transposase.

[0019] Yet another example is an adenoviral transposition system including: (a) an adenoviral donor genome as described herein; and (b) an adenoviral support genome including a nucleic acid sequence encoding a transposase.

[0020] Further, there is provided an adenoviral production system including: (a) a nucleic acid including an adenoviral donor genome as described herein; and (b) a nucleic acid including an adenoviral helper genome including a conditional packaging element.

[0021] Further examples are cells (for instance, a hematopoietic stem cell) that include a vector, genome, or system according to any one of the various examples described herein.

[0022] Also described are cell(s) (for instance, a hematopoietic stem cell) including in its genome the transposon payload of any example described herein, wherein the transposon payload present in the genome of the cell is flanked by the transposon inverted repeats.

[0023] Yet another example is an adenovirus-producing cell including an adenoviral production system according to any one of the examples described herein, optionally wherein the cell is a HEK293 cell.

[0024] A method of modifying a cell, the method including contacting the cell with a vector, genome, or system according to any one of the examples described herein.

[0025] Another example is a method of modifying a cell of a subject, the method including administering to the subject a vector, genome, or system according to any one of the examples described herein.

[0026] Another example is a method of modifying a cell of a subject without isolation of the cell from the subject, the method including administering to the subject a vector, genome, or system according to any one of the examples described herein.

[0027] Also provided are methods of treating a disease or condition in a subject in need thereof, the method including administering to the subject a vector, genome, or system according to any one of the examples described herein.

[0028] In one aspect, the present disclosure provides an adenoviral donor vector comprising: (a) an adenoviral capsid; and (b) a linear, double-stranded DNA genome comprising: (i) a transposon payload of at least 15 kb; (ii) transposon inverted repeats (IRs) that flank the transposon payload; and (iii) recombinase direct repeats (DRs) that flank the transposon inverted repeats. In at least one example, the present disclosure provides an adenoviral donor vector including: (a) an adenoviral capsid; and (b) a linear, double-stranded DNA genome including: (i) a transposon payload of at least 10 kb; (ii) transposon inverted repeats (IRs) that flank the transposon payload; and (iii) recombinase direct repeats (DRs) that flank the transposon inverted repeats.

[0029] In another aspect, the present disclosure provides an adenoviral donor genome comprising: (a) a transposon payload of at least 15 kb; (b) transposon inverted repeats (IRs) that flank the transposon payload; and (c) recombinase direct repeats (DRs) that flank the transposon inverted repeats. In at least one example, the present disclosure provides an adenoviral donor genome including: (a) a transposon payload of at least 10 kb; (b) transposon inverted repeats (IRs) that flank the transposon payload; and (c) recombinase direct repeats (DRs) that flank the transposon inverted repeats.

[0030] In another aspect, the present disclosure provides an adenoviral transposition system comprising: (I) (a) the adenoviral donor vector as disclosed herein; and (b) an adenoviral support vector comprising (i) the adenoviral capsid; and (ii) an adenoviral support genome comprising a nucleic acid sequence encoding a transposase; or (II) (a) the adenoviral donor genome as disclosed herein; and (b) an adenoviral support genome comprising a nucleic acid sequence encoding a transposase. In at least one example, the present disclosure provides an adenoviral transposition system including: (a) the adenoviral donor vector of example 1; and (b) an adenoviral support vector including (i) the adenoviral capsid; and (ii) an adenoviral support genome including a nucleic acid sequence encoding a transposase.

[0031] In another aspect, the present disclosure provides an adenoviral production system comprising: (a) a nucleic acid comprising the adenoviral donor genome as disclosed herein; and (b) a nucleic acid comprising an adenoviral helper genome comprising a conditional packaging element. In at least one example, the present disclosure provides an adenoviral transposition system including: (a) the adenoviral donor genome of example 2; and (b) an adenoviral support genome including a nucleic acid sequence encoding a transposase.

[0032] In at least one example, the present disclosure provides an adenoviral production system including: (a) a nucleic acid including the adenoviral donor genome of example 2; and (b) a nucleic acid including an adenoviral helper genome including a conditional packaging element.

[0033] In another aspect, the present disclosure provides a cell comprising a vector, genome, or system as disclosed herein, optionally wherein the cell is a hematopoietic stem cell. In another aspect, the present disclosure provides a cell comprising in its genome the transposon payload as disclosed herein, wherein the transposon payload present in the genome of the cell is flanked by the transposon inverted repeats, optionally wherein the cell is a hematopoietic stem cell. In another aspect, the present disclosure provides an adenovirus-producing cell comprising an adenoviral production system as disclosed herein, optionally wherein the cell is a HEK293 cell. In another aspect, the present disclosure provides an *in vitro* or *ex vivo* method of modifying a cell, the method comprising contacting the cell with a vector, genome, or system as disclosed herein. In another aspect, the present disclosure provides the use of the vector, genome, or system as disclosed herein in the manufacture of a medicament for: (a) modifying a cell of a subject; (b) modifying a cell of a subject without isolation of the cell from the subject; or (c) treating a disease or condition in a subject in need thereof; optionally wherein the adenoviral donor vector is to be administered to the subject intravenously. In another aspect, the present disclosure provides the vector, genome, or system as disclosed herein for use in therapy. In various examples, the transposon payload includes a Long LCR, optionally where the Long LCR is a β-globin Long LCR including β-globin LCR HS1 to HS5. In various examples, the Long LCR has a length of at least 27 kb. In various examples, the transposon payload includes an LCR set forth in Table 1. In various examples, the transposon payload has a length of at least 15 kb, at least 16 kb, at least 17 kb, at least 18 kb, at least 19 kb, at least 20 kb, at least 21 kb, at least 22 kb, at least 23 kb, at least 24 kb, at least 25 kb, at least 30 kb, at least 35 kb, at least 38 kb, or at least 40 kb. In various examples, the transposon payload has a length of 10 kb-35 kb, 10 kb-30 kb, 15 kb-35 kb, 15 kb-30 kb, 20 kb-35 kb, or 20 kb-30 kb. In various examples, the transposon payload has a length of 10 kb-32.4 kb, 15 kb-32.4 kb, or 20 kb-32.4 kb.

[0034] In various examples, the transposon payload includes a nucleic acid sequence that encodes a protein, optionally where the protein is a therapeutic protein. In various examples, the protein is selected from the group consisting of a β globin replacement protein and a γ-globin replacement protein. In various examples, the protein is a Factor VIII replacement protein. In various examples, the nucleic acid sequence that encodes the protein is operably linked with a promoter, optionally where the promoter is a β globin promoter.

[0035] In various examples, the transposon inverted repeats are Sleeping Beauty (SB) inverted repeats, optionally where the SB inverted repeats are pT4 inverted repeats. In various examples, the transposase is a Sleeping Beauty (SB) transposase, optionally where the transposase is Sleeping Beauty 100x (SB100x). In various examples, the recombinase direct repeats are FRT sites. In various examples, the adenoviral support genome includes a nucleic acid encoding a recombinase. In various examples, the recombinase is a FLP recombinase. In various examples, the transposon payload includes a β-globin long LCR, the transposon payload includes a nucleic acid sequence that encodes β-globin operably linked with

a β-globin promoter, the inverted repeats are SB inverted repeats, and the recombinase direct repeats are FRT sites.

[0036] In various examples, the transposon payload includes a selection cassette, optionally where the selection cassette includes a nucleic acid sequence that encodes $mgmt^{P140K}$.

[0037] In various examples, the adenoviral capsid is modified for increased affinity to CD46, optionally where the adenoviral capsid is an Ad35++ capsid.

[0038] In various examples, the adenoviral helper genome conditional packaging element includes a packaging sequence flanked by recombinase direct repeats.

[0039] In various examples, the recombinase direct repeats that flank the packaging sequence of the conditional packaging element are LoxP sites.

[0040] In various examples, the present disclosure provides a cell including a vector, genome, or system according to the present disclosure.

[0041] In various examples, the present disclosure provides a cell including in its genome the transposon payload according to the present disclosure, where the transposon payload present in the genome of the cell is flanked by the transposon inverted repeats.

[0042] In various examples, the cell is a hematopoietic stem cell.

[0043] In various examples, the present disclosure provides an adenovirus-producing cell including an adenoviral production system according to the present disclosure, optionally where the cell is a HEK293 cell.

[0044] In various examples, the present disclosure provides a method of modifying a cell, the method including contacting the cell with a vector, genome, or system according to the present disclosure.

[0045] In various examples, the present disclosure provides a method of modifying a cell of a subject, the method including administering to the subject a vector, genome, or system according to the present disclosure.

[0046] In various examples, the present disclosure provides a method of modifying a cell of a subject without isolation of the cell from the subject, the method including administering to the subject a vector, genome, or system according to the present disclosure.

[0047] In various examples, the present disclosure provides a method of treating a disease or condition in a subject in need thereof, the method including administering to the subject a vector, genome, or system according to the present disclosure.

[0048] In various examples, the adenoviral donor vector is administered to the subject intravenously.

[0049] In various examples, the method includes administering to the subject a mobilization agent, optionally where the mobilization agent includes one or more of granulocyte-colony stimulating factor (G-CSF), a CXCR4 antagonist, and a CXCR2 agonist. In various examples, the CXCR4 antagonist is AMD3100. In various examples, the CXCR2 agonist is GRO-β.

[0050] In various examples, the transposon payload includes a selection cassette and the method includes administering a selection agent to the subject. In various examples, the selection cassette encodes $mgmt^{P140K}$ and the selection agent is O^6BG/BCNU.

[0051] In various examples, the method causes integration and/or expression of at least one copy of the transposon payload in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of cells expressing CD46. In various examples, the method causes integration and/or expression of at least one copy of the transposon payload in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of hematopoietic stem cells and/or erythroid $Ter119^+$ cells. In various examples, the method causes integration of an average of at least 2 copies of the transposon payload in the genomes of cells including at least 1 copy of the transposon payload. In various examples, the method causes integration of an average of at least 2.5 copies of the transposon payload in the genomes of cells including at least 1 copy of the transposon payload. In various examples, the method causes expression of a protein encoded by the transposon payload at a level that is at least about 20% of the level of reference, optionally where the reference is expression of an endogenous reference protein in the subject or in a reference population. In various examples, the method causes expression of a protein encoded by the transposon payload at a level that is at least about 25% of the level of reference, optionally where the reference is expression of an endogenous reference protein in the subject or in a reference population.

[0052] In various examples, the subject is a subject suffering from thalassemia intermedia, where the transposase payload includes a β-globin Long LCR including β-globin LCR HS1 to HS5 and a nucleic acid sequence encoding a β globin replacement protein and/or γ-globin replacement protein operably linked with a β globin promoter. In various examples, the subject is a subject suffering from hemophilia, where the transposase payload includes a β-globin Long LCR including β-globin LCR HS1 to HS5 and a nucleic acid sequence encoding a Factor VIII replacement protein operably linked with a β globin promoter. In various examples, expression of the protein in the subject reduces at least one symptom of thalassemia intermedia and/or treats thalassemia intermedia.

DEFINITIONS

[0053] ***A, An, The*****:** As used herein, “a”, “an”, and “the” refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” discloses embodiments of exactly one element and embodiments including more than one element.

[0054] ***About****:* As used herein, term “about”, when used in reference to a value, refers to a value that is similar, in context to the referenced value. In general, those skilled in the art, familiar with the context, will appreciate the relevant degree of variance encompassed by “about” in that context. For example, in some embodiments, the term “about” may encompass a range of values

CLAIMS

What is claimed is:

1. An adenoviral donor vector comprising:
   (a) an adenoviral capsid; and
   (b) a linear, double-stranded DNA genome comprising:
      (i) a transposon payload of at least 15 kb;
      (ii) transposon inverted repeats (IRs) that flank the transposon payload; and
      (iii) recombinase direct repeats (DRs) that flank the transposon inverted repeats.

2. An adenoviral donor genome comprising:
   (a) a transposon payload of at least 15 kb;
   (b) transposon inverted repeats (IRs) that flank the transposon payload; and
   (c) recombinase direct repeats (DRs) that flank the transposon inverted repeats.

3. An adenoviral transposition system comprising:
   (I) (a) the adenoviral donor vector of claim 1; and
      (b) an adenoviral support vector comprising
         (i) the adenoviral capsid; and
         (ii) an adenoviral support genome comprising a nucleic acid sequence encoding a transposase; or
   (II) (a) the adenoviral donor genome of claim 2; and
      (b) an adenoviral support genome comprising a nucleic acid sequence encoding a transposase.

4. An adenoviral production system comprising:
   (a) a nucleic acid comprising the adenoviral donor genome of claim 2; and
   (b) a nucleic acid comprising an adenoviral helper genome comprising a conditional packaging element.

5. The vector, genome, or system of any one of claims 1-4, wherein one or more of:
(a) the transposon payload comprises a Long LCR;
(b) the transposon payload comprises a Long LCR, wherein the Long LCR is a β-globin Long LCR comprising β-globin LCR HS1 to HS5;
(c) the transposon payload comprises a Long LCR, wherein the Long LCR has a length of at least 27 kb;
(d) the transposon payload comprises an LCR set forth in Table 1;

(e) the transposon payload has a length of at least 16 kb, at least 17 kb, at least 18 kb, at least 19 kb, at least20 kb, at least 21 kb, at least 22 kb, at least 23 kb, at least 24 kb, at least 25 kb, at least 30 kb, at least 35 kb, at least 38 kb, or at least 40 kb;

(f) the transposon payload has a length of 15 kb-35 kb, 15 kb-30 kb, 20 kb-35 kb, or 20 kb-30 kb; and

(g) the transposon payload has a length of 15 kb-32.4 kb or 20 kb-32.4 kb.

6. The vector, genome, or system of any one of claims 1-5, wherein the transposon payload comprises a nucleic acid sequence that encodes a protein,

optionally wherein the protein is a therapeutic protein;

further optionally wherein:

(a) the protein is selected from the group consisting of a β globin replacement protein and a γ-globin replacement protein; or

(b) the protein is a Factor VIII replacement protein; and

further optionally wherein the nucleic acid sequence that encodes the protein is operably linked with a promoter, even further optionally wherein the promoter is a β globin promoter.

7. The vector, genome, or system of any one of claims 1-6, wherein:

(a) the transposon inverted repeats are Sleeping Beauty (SB) inverted repeats, optionally wherein the SB inverted repeats are pT4 inverted repeats; and/or

(b) the recombinase direct repeats are FRT sites.

8. The vector, genome, or system of any one of claims 3-6, wherein:

(a) the transposase is a Sleeping Beauty (SB) transposase, optionally wherein the transposase is Sleeping Beauty 100x (SB100x); and/or

(b) the adenoviral support genome comprises a nucleic acid encoding a recombinase, optionally wherein the recombinase is a FLP recombinase.

9. The vector, genome, or system of any one of claims 1-8, wherein one or more of:

(a) the transposon payload comprises a β-globin long LCR, the transposon payload comprises a nucleic acid sequence that encodes β-globin operably linked with a β-globin promoter, the inverted repeats are SB inverted repeats, and the recombinase direct repeats are FRT sites;

(b) the transposon payload comprises a selection cassette, optionally wherein the selection cassette comprises a nucleic acid sequence that encodes $mgmt^{P140K}$; and/or

(c) the adenoviral capsid is modified for increased affinity to CD46, optionally wherein the adenoviral capsid is an Ad35++ capsid.

10. The vector, genome, or system of any one of claims 1-9, wherein the vector is an Ad5/35++ vector.

11. The adenoviral production system of any one of claims 4-10, wherein the adenoviral helper genome conditional packaging element comprises a packaging sequence flanked by recombinase direct repeats, optionally wherein the recombinase direct repeats that flank the packaging sequence of the conditional packaging element are LoxP sites.

12. A cell comprising a vector, genome, or system according to any one of claims 1-11, optionally wherein the cell is a hematopoietic stem cell.

13. A cell comprising in its genome the transposon payload of any one of claims 1-11, wherein the transposon payload present in the genome of the cell is flanked by the transposon inverted repeats, optionally wherein the cell is a hematopoietic stem cell.

14. An adenovirus-producing cell comprising an adenoviral production system according to any one of claims 4-11, optionally wherein the cell is a HEK293 cell.

15. An *in vitro* or *ex vivo* method of modifying a cell, the method comprising contacting the cell with a vector, genome, or system according to any one of claims 1-11.

16. Use of the vector, genome, or system according to any one of claims 1-11 in the manufacture of a medicament for:

(a) modifying a cell of a subject;

(b) modifying a cell of a subject without isolation of the cell from the subject; or

(c) treating a disease or condition in a subject in need thereof;

optionally wherein the adenoviral donor vector is to be administered to the subject intravenously.

17. The use according to claim 16, wherein the use comprises that a mobilization agent is to be administered to the subject, optionally wherein the mobilization agent comprises one or more of granulocyte-colony stimulating factor (G-CSF), a CXCR4 antagonist, and a CXCR2 agonist, further optionally wherein (i) the CXCR4 antagonist is AMD3100 and/or (ii) the CXCR2 agonist is GRO-β.

18. The use according to claim 16 or 17, wherein the transposon payload comprises a selection cassette and

the use comprises that a selection agent is to be administered to the subject, optionally wherein the selection cassette encodes $mgmt^{P140K}$ and the selection agent is O^6GB/BCNU.

19. The use according to any one of claims 16-18, wherein one or more of:

(a) the use causes integration and/or expression of at least one copy of the transposon payload in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of cells expressing CD46;

(b) the use causes integration and/or expression of at least one copy of the transposon payload in at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% of hematopoietic stem cells and/or erythroid Ter119+ cells;

(c) the use causes integration of an average of at least 2 copies of the transposon payload in the genomes of cells comprising at least 1 copy of the transposon payload;

(d) the use causes integration of an average of at least 2.5 copies of the transposon payload in the genomes of cells comprising at least 1 copy of the transposon payload;

(e) the use causes expression of a protein encoded by the transposon payload at a level that is at least about 20% of the level of reference, optionally wherein the reference is expression of an endogenous reference protein in the subject or in a reference population;

(f) the use causes expression of a protein encoded by the transposon payload at a level that is at least about 25% of the level of reference, optionally wherein the reference is expression of an endogenous reference protein in the subject or in a reference population;

(g) the subject is a subject suffering from thalassemia intermedia, wherein the transposase payload comprises a β-globin Long LCR comprising β-globin LCR HS1 to HS5 and a nucleic acid sequence encoding a β globin replacement protein and/or γ-globin replacement protein operably linked with a β globin promoter;

(h) the subject is a subject suffering from hemophilia, wherein the transposase payload comprises a β-globin Long LCR comprising β- globin LCR HS1 to HS5 and a nucleic acid sequence encoding a Factor VIII replacement protein operably linked with a β globin promoter; and/or

(i) the subject is a subject suffering from hemophilia, wherein the transposase payload comprises a β-globin Long LCR comprising β- globin LCR HS1 to HS5 and a nucleic acid sequence encoding a Factor VIII replacement protein operably linked with a β globin promoter and expression of the protein in the subject reduces at least one symptom of thalassemia intermedia and/or treats thalassemia intermedia.

20. The vector, genome, or system according to any one of claims 1-11 for use in therapy.