Background

[0004] Viral vectors can provide an effective delivery system for transferring genetic material into a cell. Nucleic acids within many viral vectors, such as gamma-retroviral and lentiviral vectors, can integrate into the genome of a host cell. Cis-acting elements, such as viral post-transcriptional regulatory elements (PREs), including hepatitis virus PREs and variants thereof, have been used in viral vectors to enhance gene expression. Nucleic acids, expression cassettes, and vectors containing such elements are available. There is a need for improved PRE-containing nucleic acids, cassettes and vectors. Provided are embodiments that address such needs.

Summary

[0004A] Disclosed is a polynucleotide, comprising: a nucleic acid encoding a recombinant protein; a modified post-transcriptional regulatory element (PRE) operably linked to said nucleic acid, said modified PRE comprising a variant of a wild-type hepatitis virus X gene, said variant X gene comprising a stop codon not present in the wild-type X gene.

[0005] Provided are polynucleotides containing a modified post-transcriptional regulatory element (PRE) that contains a variant X gene that includes a stop codon and/or post-translational degradation sequence that is not present in an unmodified or wild-type hepatitis virus X gene, such as not present in an X gene from a wild-type mammalian hepatitis virus, such as a WHV or HBV. Among the provided polynucleotides are those that contain a modified WPRE containing a variant X gene that includes a stop codon and/or post-translational degradation sequence that is not present in an unmodified or wild-type WHV X gene. The provided polynucleotides include expression cassettes and viral vectors containing such modified PREs. In particular examples, the modified PRE is operably linked to a nucleic acid encoding a recombinant protein, e.g. a heterologous protein.

[0006] Also disclosed is a polynucleotide, comprising a modified post-transcriptional regulatory element (PRE), said modified PRE comprising a variant of

stop codons present in the variant X gene, begins at a position within 36 or 24 nucleotides in the 3' direction from a position corresponding to residue 411 of WHV PRE sequence set forth in SEQ ID NO:1 or SEQ ID NO:125 and/or residue 1503 of the sequence set forth in SEQ ID NO:2. In some such examples, the variant X gene does not contain an open reading frame of greater than at or about 39 nucleotides in length or greater than at or about 27 nucleotides in length.

[0007] In yet another aspect, the present invention provides a polynucleotide, comprising a modified post-transcriptional regulatory element (PRE), said modified PRE comprising a variant of a wild-type hepatitis virus X gene, said variant X gene comprising a plurality of stop codons not present in the wild-type X gene. Provided are polynucleotides containing a modified PRE that contains a variant of a wild-type hepatitis virus X gene in which the variant X gene contains a stop codon, such as at least one stop codon, not present in the wild-type X gene and beginning at a position within 32 or 30 nucleotides of a start codon of the X gene. In some examples, the at least one stop codon, e.g. the first stop codon or one or more of a plurality of stop codons present in the variant X gene, begins at a position within 32 or 30 nucleotides in the 3' direction from a position in the variant X gene corresponding to the 5' position of a start codon of the wild-type X protein open reading frame. In some examples, the at least one stop codon, e.g. the first stop codon or one or more of a plurality of stop codons present in the variant X gene, begins at a position within 32 or 30 nucleotides in the 3' direction from a position corresponding to residue 411 of WHV PRE sequence set forth in SEQ ID NO:1 or SEQ ID NO:125 and/or residue 1503 of the sequence set forth in SEQ ID NO:2. In some such examples, the variant X gene has an open reading frame of less than or equal to 33 nucleotides in length.

[0008] Also disclosed is a polynucleotide, comprising a modified post-transcriptional regulatory element (PRE), said modified PRE comprising a variant of a wild-type hepatitis virus X gene, said variant X gene comprising a sequence encoding a post-translational modification signal not present in the wild-type hepatitis virus X gene. In any of such examples of a modified PRE, the at least one stop codon not present in the wild-type X gene includes a plurality of stop codons. Provided are polynucleotides comprising a modified PRE that contains a variant of a wild-type hepatitis virus X gene in which the variant X gene

[0049] In some examples, any of the provided polynucleotides can further contain viral nucleic acid containing a variant Flap, wherein the variant Flap contains a deletion of all or a portion of the nucleotides corresponding to the central polypurine tract (cPPT) and/or the central termination sequence (CTS) regions of a wild-type or unmodified Flap sequence. In such examples, the polynucleotides contain a modified PRE and a variant Flap. In some examples, such elements are operably linked to nucleic acid encoding a recombinant protein and/or result in an increase in efficiency of gene transfer of a heterologous nucleic acid encoding a recombinant protein compared to the absence of such elements.

[0050] Also disclosed is a polynucleotide, comprising a viral nucleic acid comprising a variant Flap, wherein the variant Flap contains a deletion of all or a portion of the nucleotides corresponding to the central polypurine tract (cPPT) and/or the central termination sequence (CTS) regions of a wild-type or unmodified Flap sequence. Also provided are polynucleotides containing a variant Flap, wherein the variant Flap contains a deletion of all or a portion of the nucleotides corresponding to the central polypurine tract (cPPT) and/or the central termination sequence (CTS) regions of a wild-type or unmodified Flap sequence. In some examples, the polynucleotide containing a variant Flap can further contain nucleic acid encoding a recombinant protein. In some examples, the presence of a polynucleotide containing a variant Flap, while deleted for all or a portion of the cPPT and CTS that are involved in forming the Flap structure, nevertheless can successfully transduce or transfer into cells with the nucleic acid encoding the recombinant protein.

[0051] In some examples of any of the provided polynucleotides containing a variant Flap, the variant Flap contains a deletion of all or a portion of the nucleotide corresponding to the cPPT and the CTS. In some examples, all of the nucleotide residues corresponding to cPPT and all of the nucleotide residues corresponding to the CTS are deleted and/or the variant Flap does not contain nucleotide residues corresponding to the cPPT or nucleotide residues corresponding to the CTS. In some examples of any of the provided polynucleotides, the variant Flap contains deletion of all or a contiguous portion of nucleotides corresponding to nucleotides in the cPPT region set forth in SEQ ID NO: 123. In some

[0058] In yet another aspect, the present invention provides a virus particle, comprising the vector as described herein. In some embodiments, also provided is a virus particle that contains any of the provided vectors.

[0059] In yet another aspect, the present invention provides an *in vitro* method, comprising introducing the expression cassette as described herein, the vector as described herein, or the virus particle as described herein to a cell, under conditions whereby expression of the recombinant protein is effected in the cell. In some examples, also provided are methods that include introducing any of the provided polynucleotides, expression cassettes, vectors, or virus particles into a cell, under conditions whereby expression of the recombinant protein is effected in the cell. In some examples of any of the provided methods, the introduction is effected by transducing the cell with the vector or virus particle. In some examples, the introduction is effected by transfecting the cell with the vector; and/or the introduction is effected by electroporation of the cell with the

vector. In some examples of any of the provided methods, the recombinant protein is expressed at a level that is increased compared to that achieved by introducing the vector in the absence of the modified PRE or a corresponding vector that does not contain the modified PRE or a PRE.

[0060] In yet another aspect, the present invention provides a cell produced by the method as described herein. Also provided is a cell or cells produced by any of the provided methods.

[0061] In yet another aspect, the present invention provides a pharmaceutical composition comprising the cell as described herein, and a pharmaceutically effective carrier. Also provided is a pharmaceutical composition that contains a cell of any of the provided embodiments and a pharmaceutically effective carrier.

[0062] In yet another aspect, the present invention provides a pharmaceutical composition as described herein for treating a disease or condition. In yet another aspect, the present invention provides use of a pharmaceutical composition as described herein for use in the preparation of a medicament for treating a disease or condition, wherein the disease or condition is a cancer, an autoimmune disorder or an infectious disease. Also provided are methods of treatment that include administering to a subject having a disease or condition any of the provided polynucleotides, vectors, virus particles, cells, or pharmaceutical compositions.

[0063] In some examples of any of the provided methods of treatment, the method results in expression of the recombinant protein encoded by the polynucleotide. In some examples, the recombinant protein comprises a recombinant receptor that specifically binds to a ligand expressed by the disease or condition or a cell or tissue thereof. In some examples of any of the provided methods, the receptor is an antigen receptor and the ligand is an antigen specific for and/or associated with the disease or condition. In some examples of any of the provided methods, the disease or condition is a cancer, and autoimmune disorder, or an infectious disease.

CLAIMS

1. A polynucleotide, comprising a modified post-transcriptional regulatory element (PRE), said modified PRE comprising a variant of a wild-type hepatitis virus X gene, said variant X gene comprising a plurality of stop codons not present in the wild-type X gene.

2. The polynucleotide of claim 1, wherein the plurality of stop codons comprises at least one stop codon selected from among:

a stop codon beginning at a position within 36, 30 or 24 nucleotides in the 3' direction from a position in the variant X gene corresponding to residue 411 of WHV post-transcriptional regulatory element (WPRE) sequence set forth in SEQ ID NO: 1 and/or residue 1503 of the WHV sequence set forth as SEQ ID NO: 2.

3. The polynucleotide of claim 1 or claim 2, wherein the variant X gene comprises at least 2 stop codons, at least 3 stop codons or at least 4 stop codons.

4. The polynucleotide of any of claims 1-3, wherein the variant X gene comprises a stop codon in each reading frame present in said variant X gene.

5. The polynucleotide of any of claims 1-4, wherein the variant X gene comprises at least two stop codons in the same reading frame.

6. The polynucleotide of any of claims 1-5, wherein the stop codon comprises at least one stop codon selected from among:

a stop codon beginning at a position within or within at least 9, 12, 15, or 18 nucleotides in the 3' direction from a position in the variant X gene corresponding to residue 411 of WHV post-transcriptional regulatory element (WPRE) sequence set forth in SEQ ID NO: 1 and/or residue 1503 of the WHV sequence set forth as SEQ ID NO: 2.

7. The polynucleotide of any of claims 1-6, wherein said stop codon is selected from among:

a stop codon beginning at a nucleotide position corresponding to position 420 in the sequence set forth in SEQ ID NO:1;

a stop codon beginning at a nucleotide position corresponding to position 424 in the sequence set forth in SEQ ID NO:1;

a stop codon beginning at a nucleotide position corresponding to position 428 in the sequence set forth in SEQ ID NO:1; and

a stop codon beginning at a nucleotide position corresponding to position 432 in the sequence set forth in SEQ ID NO:1.

8. The polynucleotide of claim 7, wherein said stop codon is an amber (TAG) or opal (TGA) stop codon.

9. The polynucleotide of any of claims 1-8, wherein the variant X gene further comprises a sequence encoding a post-translational modification signal not present in the wild-type hepatitis virus X gene.

10. The polynucleotide of claim 9, wherein said post-translational modification signal comprises a ubiquitination site.

11. The polynucleotide of claim 9, wherein said post-translational modification signal comprises one or more PEST sequences.

12. The polynucleotide of any of claims 1-11, further comprising a nucleic acid encoding a recombinant protein operably linked to the modified PRE.

13. The polynucleotide of any of claims 1-12, wherein said variant X gene is a variant of a wild-type mammalian hepatitis virus X gene.

14. The polynucleotide of claim 13, wherein said wild-type mammalian hepatitis X gene is a wild-type woodchuck hepatitis virus (WHV) X gene.

15. The polynucleotide of any of claims 1-14, wherein the modified PRE comprises nucleotide modifications compared to a wild-type or unmodified hepatitis virus PRE that is a mammalian hepatitis PRE.

16. The polynucleotide of claim 15, wherein said wild-type or unmodified mammalian hepatitis PRE is a wild-type woodchuck hepatitis virus PRE (WPRE).

17. The polynucleotide of claim 15 or 16, wherein the wild-type or unmodified hepatitis virus PRE comprises:

a) the sequence of nucleotides set forth in SEQ ID NO:1 or 125 or a sequence of nucleotides that exhibits at least 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:1 or 125; or

b) a portion of the sequence of nucleotides of a), wherein the portion exhibits post-transcriptional activity.

18. The polynucleotide of any of claims 1-17, wherein the modified PRE is selected from among:

a) a modified PRE comprising a sequence of nucleotides that exhibits at least 85% sequence identity to SEQ ID NO:1 or 125, said modified PRE containing a variant X gene comprising at least one stop codon not present in SEQ ID NO:1 or 125; and

b) a modified PRE comprising a portion of the sequence of nucleotides of a), said portion comprising a variant X gene comprising the at least one stop codon, wherein the portion exhibits post-transcriptional activity.

19. The polynucleotide of any of claims 1-18, wherein the variant X gene comprises the sequence of nucleotides set forth in any of SEQ ID NOS: 44-54 or 141-151 and/or the modified PRE comprises the sequence of nucleotides set forth in any of SEQ ID NOS:29-39 or 126-136.

20. The polynucleotide of any of claims 1-19, wherein said variant X gene further comprises a variant of a start codon comprising one or more nucleotide differences compared to a wild-type hepatitis virus X gene start codon and/or compared to the start codon corresponding to nucleotide positions 411-413 of SEQ ID NO: 1, wherein the one or more nucleotide differences results in restricted or prevented translation initiation from said start codon.

21. The polynucleotide of any of claims 1-20, wherein said variant X gene comprises a variant promoter operably linked to said variant X gene, said variant promoter comprising one or more nucleotide differences compared to a wild-type hepatitis virus X gene promoter and/or compared to a promoter of SEQ ID NO: 11, wherein said one or more differences results in restricted or prevention of transcription from said promoter.

22. The polynucleotide of any of claims 1-21, wherein:

upon introduction into a eukaryotic cell, no polypeptide of a length greater than 12, 11, 10, 9, or 8 amino acids in length encoded by said variant X gene is produced; and/or

said polynucleotide is incapable of producing a polypeptide of a length greater than 12, 11, 10, 9, or 8 amino acids in length encoded by said variant X gene.

23. The polynucleotide of any of claims 1-22, wherein said modified PRE encodes an RNA that promotes nuclear RNA export and/or increases mRNA stability.

24. The polynucleotide of any of claims 1-23, further comprising a viral nucleic acid comprising a variant Flap, wherein the variant Flap contains a deletion of all or a portion of the

nucleotides corresponding to the central polypurine tract (cPPT) and/or the central termination sequence (CTS) regions of a wild-type or unmodified Flap sequence.

25. The polynucleotide of claim 24, wherein the wild-type or unmodified Flap sequence comprises from or from about 80 to 200 contiguous nucleotides comprising the cPPT or CTS regions of a retrovirus, which optionally is a lentivirus, optionally HIV-1.

26. The polynucleotide of claim 24 or claim 25, wherein the variant Flap comprises deletion of all or a contiguous portion of nucleotides corresponding to nucleotides in the cPPT region set forth in SEQ ID NO:123 and comprises deletion of all or a contiguous portion of nucleotides corresponding to nucleotides in the CTS region set forth in SEQ ID NO:124, and/or

wherein the viral nucleic acid comprising the variant Flap comprises a sequence of nucleotides that exhibits at least 70% sequence identity to SEQ ID NO:121 , said variant Flap lacking all or a portion of the cPPT and CTS regions.

27. The polynucleotide of any of claims 24-26, comprising:

a variant Flap comprising the sequence of SEQ ID NO: 122 or a sequence having at least at or about 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:122; and

a modified PRE comprising the sequence of SEQ ID NO:29 or 126 or a sequence having at least at or about 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:29 or 126.

28. The polynucleotide of any of claims 12-27, wherein the recombinant protein comprises a recombinant receptor.

29. The polynucleotide of claim 28, wherein the recombinant receptor is an antigen receptor and/or a chimeric receptor.

30. An expression cassette comprising the polynucleotide of any of claims 12-29 and a promoter operably linked to the nucleic acid encoding the recombinant protein.

31. A vector comprising the polynucleotide of any of claims 1-29 or the expression cassette of claim 30, optionally wherein the vector is a viral vector.

32. A cell comprising the expression cassette of claim 30 or the vector of claim 31.

33. A virus particle, comprising the vector of claim 31.

34. An *in vitro* method, comprising introducing the expression cassette of claim 30, the vector of claim 31, or the virus particle of claim 33 to a cell, under conditions whereby expression of the recombinant protein is effected in the cell.

35. A cell produced by the method of claim 34.

36. A pharmaceutical composition comprising the cell of claim 32 or claim 35, and a pharmaceutically effective carrier.

37. A pharmaceutical composition of claim 36 for use in treating a disease or condition.

38. The pharmaceutical composition of claim 37, wherein the disease or condition is a cancer, an autoimmune disorder or an infectious disease.

39. Use of a pharmaceutical composition of claim 36 in the preparation of a medicament for treating a disease or condition, wherein the disease or condition is a cancer, an autoimmune disorder or an infectious disease.