METHODS AND COMPOSITIONS FOR EXPRESSION OF POLYPEPTIDES IN A CELL

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of United States provisional patent application No. 62/026,499; filed July 18, 2014; the disclosure of which is hereby incorporated by reference, in its entirety, for all purposes.

STATEMENT REGARDING FEDERAL SUPPORT

Not applicable.

FIELD

The present disclosure is in the field of recombinant protein expression.

BACKGROUND

Controlled production of recombinant polypeptides is useful in the fields of research, diagnostics and therapeutics. For example, purified recombinant proteins can be used as therapeutics, for screening, for lead optimization and for target validation.

Typical expression systems are characterized by a host cell line comprising an expression vector containing a heterologous promoter operatively linked to a cDNA encoding a gene product of interest. See, for example, U.S. Pat. Nos. 5,168,062 and 5,385,839. Additional methods for protein production involve integration of promoters or other regulatory sequences into a chromosome adjacent to a gene whose expression is to be regulated. See, e.g., U.S. Pat. Nos. 5,272,071; 5,641,670; 5,733,761; 5,968,502 and 6,361,972.

Expression systems which yield levels of protein that are higher than those obtained using presently available systems are desirable.

SUMMARY

In a first aspect, the present invention refers to a vector comprising transcriptional regulatory sequences derived from viral genomes comprising a viral promoter and further comprising a transgene, a polyadenylation signal in operative linkage with the transgene and a post-transcriptional regulatory element (PRE) selected from the group consisting of a human hepatitis B virus PRE (HPRE) and a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), and wherein the vector does not comprise a viral untranslated region (UTR) downstream sequence or a viral untranslated region (UTR) upstream sequence. In another aspect, the present invention refers to a cell comprising the vector of the present invention, wherein the cell is selected from the group consisting of a prokaryotic cell and a eukaryotic cell. Also disclosed herein are new methods for expression of polypeptides in cells that utilize new nucleic acid vectors, also disclosed herein. The vectors include

transcriptional regulatory sequences derived from viral genomes, such as the cytomegalovirus (CMV) genome. In some examples, the expression vectors further include a post-transcriptional regulatory element (PRE). In some examples, the expression vectors further include a MAR/SAR sequence. These vectors provide higher expression levels of genes to which they are operatively linked, thus providing higher expression of recombinant proteins, compared to existing recombinant expression systems.

In addition, the vectors disclosed herein provide stable, high-level expression of recombinant polypeptides for up to 50 or more generations, thereby exceeding the expression longevity of currently-existing expression systems.

Thus, the present disclosure provides, *inter alia*, the following examples.

1. A polynucleotide comprising:

(a) a cytomegalovirus (CMV) RC-UTR downstream sequence,

(b) a CMV major immediate early (MIE) promoter,

(c) a CMV UTR upstream sequence,

(d) a CMV Intron A, and

(e) a CMV UTR downstream sequence.

2. The polynucleotide of example 1, wherein the cytomegalovirus is human cytomegalovirus (hCMV).

3. The polynucleotide of example 1, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 1 or a nucleotide sequence that is substantially identical to SEQ ID NO:1 .

4. A polynucleotide having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity to the polynucleotide of example 3.

5. A polynucleotide that is complementary to the polynucleotide of example 3.

6. A polynucleotide that hybridizes under stringent conditions to the polynucleotide of example 3.

7. A nucleic acid vector comprising the polynucleotide of example 1.

8. The vector of example 7, wherein elements (a)-(e) of the CMV sequences are present in upstream-to-downstream order.

9. The vector of example 7, further comprising a polyadenylation signal.

10. The vector of example 9, wherein the polyadenylation signal is selected from the group consisting of the simian virus 40 (SV40) polyadenylation signal and the bovine growth hormone (BGH) polyadenylation signal.

11. The vector of example 9, further comprising a multiple cloning site disposed between the CMV sequences and the polyadenylation signal.

12. The vector of example 9, further comprising a transgene.

13. The vector of example 12, further comprising a post-transcriptional element (PRE).

14. The vector of example 13, wherein the PRE is selected from the group consisting of a woodchuck hepatitis virus PRE (WPRE), a human hepatitis B virus PRE (HPRE), and hybrids thereof.

15. The vector of example 12, further comprising a MAR/SAR sequence.

16. The vector of example 15, wherein the MAR/SAR sequence is selected from the group consisting of a chicken lysozyme MAR/SAR (CLM) sequence, an interferon alpha-2 MAR/SAR (IAM) sequence, an interferon beta MAR/SAR (IBM) sequence, a X29 MAR/SAR sequence, a S4 MAR/SAR sequence and hybrids thereof.

17. The vector of example 11, further comprising:

(a) sequences encoding a first selection marker that functions in eukaryotic cells to provide resistance to a first selective agent;

(b) a promoter operably linked to the sequences encoding the first selection marker; and

(c) a second polyadenylation signal operably linked to the sequences encoding the first selection marker.

18. The vector of example 17, wherein the first selection marker is selected from the group consisting of genes encoding dihydrofolate reductase (DHFR), glutamine synthetase (GS), puromycin-N-acetyl transferase, hygromycin phosphotransferase, aminoglycoside-3-phosphotransferase, ble, and neomycin resistance.

19. The vector of example 17, wherein the first selective agent is selected from the group consisting of methotrexate, neomycin, geneticin, puromycin, bleomycin, Zeocin, blasticidin, hygromycin, methionine sulfoximine and L-glutamine.

20. The vector of example 17, wherein the promoter is selected from the group consisting of the cytomegalovirus (CMV) major immediate early (MIE) promoter, the herpes simplex virus thymidine kinase (TK) promoter, the SV40 early promoter, the SV40 late promoter, the Ubiquitin C promoter, the phosphoglycerate kinase (PGK) promoter and the eukaryotic elongation factor-1 alpha (EF-1α) promoter.

21. The vector of example 17, wherein the second polyadenylation signal is selected from the group consisting of the SV40 polyadenylation signal and the bovine growth hormone (BGH) polyadenylation signal.

22. The vector of example 7 further comprising:

(a) a replication origin that functions in prokaryotic cells; and

(b) a second selection marker that functions in prokaryotic cells to confer resistance to a second selective agent.

23. The vector of example 22, wherein the replication origin is selected from the group consisting of the pBR322 origin (*rep*), *oriC*, the pSC101 origin and the pUC origin.

24. The vector of example 22, wherein the second selection marker is a gene encoding beta-lactamase.

25. The vector of example 22, wherein the second selective agent is selected from the group consisting of ampicillin, kanamycin, chloramphenicol and tetracycline.

26. The vector of example 12, further comprising a second transgene.

27. The vector of example 26, wherein the second transgene is in operative linkage with a second promoter and a third polyadenylation signal.

28. The vector of example 27, wherein the second promoter is selected from the group consisting of SEQ ID NO:1, homologues of SEQ ID NO:1, functional equivalents of SEQ ID NO:1, the cytomegalovirus (CMV) major immediate early (MIE) promoter, the herpes simplex virus thymidine kinase (TK) promoter, the SV40 early promoter, the SV40 late promoter, the Ubiquitin C promoter, the phosphoglycerate kinase (PGK) promoter and the eukaryotic elongation factor-1 alpha (EF-1α) promoter

29. The vector of example 27, wherein the third polyadenylation signal is selected from the group consisting of the SV40 polyadenylation signal and the bovine growth hormone (BGH) polyadenylation signal.

30. The vector of example 26, further comprising an internal ribosome entry site (IRES) disposed between the first transgene and the second transgene.

31. The vector of example 30, wherein the IRES is a picornaviral IRES, an encephalomyocarditis virus (EMCV) IRES, a Chenyl IRES or IRES2A.

32. The vector of example 12, wherein the transgene is a gene that encodes a protein selected from the group consisting a recombinant protein, a fusion protein, an antibody, a cytokine, a hormone, an enzyme and a clotting factor.

33. The vector of example 32, wherein the antibody is a monoclonal antibody, a single chain antibody, a bispecific antibody or an antibody conjugate.

34. The vector of example 33, wherein the antibody is selected from the group consisting of anti-CD20 (Rituximab), anti-CD25 (Daclizumab, Basiliximab), anti-p18Her-2 (Trastuzumab), anti-TNFα (Infliximab, Adalimumab), anti-pF-RSV (Palivizumab), anti-CD52 (Alemtuzumab), anti-IgE-Fc (Omalizumab), anti-erbB1 (Cetuximab), anti-Complement C5 (Eculizumab), anti-alpha 4 integrin (Natalizumab), anti-CDE33 (Gemtuzumab), anti-CTLA-4 (Ipilimumab), anti-HuMax-CD20 (Ofatumumab), anti- ABX-Epidermal Growth Factor (Panitumumab), anti-MK-3475 (Pembrolizumab), and conjugates thereof.

35. The vector of example 32, wherein the cytokine is erythropoietin, granulocyte colony-stimulating factor (G-CSF), filgrastim, or PEGfilgrastim.

36. The vector of example 32, wherein the hormone is human growth hormone, luteinizing hormone (Luveris), or epoetin (Procrit).

37. The vector of example 32, wherein the enzyme is selected from the group consisting of alpha-glucosidase (Myozyme), Laronidase (Aldurazyme), IgG-CTLA4 fusion (Orencia), and N-acetylgactosameine 4-Sulfatase (Nagdazyme).

38. The vector of example 26, wherein the first and second transgenes are an antibody heavy chain and an antibody light chain.

39. A virus comprising the vector of example 7.

40. The vector of example 7, wherein the vector is a plasmid or a cosmid.

41. A cell comprising the vector of example 12, wherein the cell is selected from the group consisting of a prokaryotic cell and a eukaryotic cell.

42. The cell of example 41, wherein the eukaryotic cell is selected from the group consisting of a plant cell, an insect cell, a fungal cell and a mammalian cell.

43. The cell of example 42, wherein the eukaryotic cell is a mammalian cell.

44. The cell of example 43, wherein the cell is selected from the group consisting of CHO, CHOK1, NS0, HEK293, BHK, SP20, PERC.6, CAP®, CAP-T® and derivatives thereof.

45. The cell of example 43, wherein the cell is a myeloma or a hybridoma or a derivative thereof.

46. The cell of example 43, wherein the cell has been adapted to grow in serum-free medium.

47. The cell of example 41, wherein the cell is *E. coli*.

48. A method for expressing a polypeptide in a cell, the method comprising:

(a) introducing the vector of example 12 into a cell; and

(b) culturing the cell under conditions such that the vector is stably maintained in the cell.

49. The method of example 48, wherein the cell is selected from the group consisting of CHO, CHOK1, NS0, HEK293, BHK, SP20, PERC.6, CAP®, CAP-T® and derivatives thereof.

50. The method of example 48, wherein the cell is a myeloma or a hybridoma or a derivative thereof.

51. The method of example 48, wherein the cell is cultured in serum-containing medium.

52. The method of example 48, wherein the cell is cultured in serum-free medium.

53. The method of example 52, wherein the cell is pre-adapted for growth in serum-free medium prior to introducing the vector into the cell.

54. A method for expressing a polypeptide in a population of transfected cells, the method comprising:

transfecting a population of cells with the vector of example 13;

wherein greater than 20% of the cells in the population express the polypeptide.

55. The method of example 54, wherein greater than 40% of the cells in the population express the polypeptide.

56. The method of example 54, further wherein the cells expressing the polypeptide express the polypeptide for at least ten generations after selection.

57. The method of example 54, wherein the cells expressing the polypeptide express the polypeptide for at least twenty-five generations after selection.

58. The method of example 54, wherein the cells expressing the polypeptide express the polypeptide for at least fifty generations after selection.

59. A polynucleotide comprising any one of SEQ ID NOs: 2, 3 4 or 6, a sequence substantially identical to any one of SEQ ID NOs: 2, 3 4 or 6, or any combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram showing an exemplary expression vector containing a glutamine synthetase (GS) selection cassette; an expression cassette that includes the CMV MIE promoter, transgene sequences encoding human growth hormone (hGH), a WPRE, and the BGH polyadenylation signal (BHG polyA); two multiple cloning sites (MCS1 and MCS2); the pBR322 replication origin (ori) and the pBR322 beta-lactamase (bla) gene.

Figure 2 shows the results of a protein blotting assay for human growth hormone expression in CHO-K1 cells transfected with the expression vector shown in Figure 1. Lane 1: markers; Lane 2: hGH standard, 15 pg; Lane 3: hGH standard, 90 pg; Lane 4: CHO-K1 cells transfected with 4 ug of expression vector DNA; Lane 5: CHO-K1 cells transfected with 6 ug of expression vector DNA; Lane 6: untransfected CHO-K1 cells; Lane 7: markers (the three lowest bands represent 20, 30 and 40 KDa, in ascending order).

Figure 3 shows the results of a protein blotting assay for human growth hormone expression in suspension/serum-free adapted CHO-K1 cells (SSA-CHO-K1) and control cells electroporated with the expression vector shown in Figure 1. Lane 1: markers (the

CLAIMS

What is claimed is:

1. A vector comprising transcriptional regulatory sequences derived from viral genomes comprising a viral promoter and further comprising a transgene, a polyadenylation signal in operative linkage with the transgene and a post-transcriptional regulatory element (PRE) selected from the group consisting of a human hepatitis B virus PRE (HPRE) and a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), and wherein the vector does not comprise a viral untranslated region (UTR) downstream sequence or a viral untranslated region (UTR) upstream sequence.

2. The vector of claim 1, wherein the vector does not comprise a viral intron.

3. The vector of claim 1 or 2, wherein the polyadenylation signal is selected from the group consisting of the simian virus 40 (SV40) polyadenylation signal and the bovine growth hormone (BGH) polyadenylation signal.

4. The vector of claim 3, further comprising a multiple cloning site.

5. The vector of any one of claims 1-4, further comprising a MAR/SAR sequence.

6. The vector of claim 5, wherein the MAR/SAR sequence is selected from the group consisting of a chicken lysozyme MAR/SAR (CLM) sequence, an interferon alpha-2 MAR/SAR (IAM) sequence, an interferon beta MAR/SAR (IBM) sequence, a X29 MAR/SAR sequence, a S4 MAR/SAR sequence and hybrids thereof.

7. The vector of any one of claims 1-6, wherein the transgene is a gene that encodes a protein selected from the group consisting of a recombinant protein, a fusion protein, an antibody, a cytokine, a hormone, an enzyme and a clotting factor.

8. A cell comprising the vector of any one of claims 1-7, wherein the cell is selected from the group consisting of a prokaryotic cell and a eukaryotic cell.