[0014A] In one aspect, provided is a cloning or expression vector comprising the following elements: (1) a transcriptional promoter, (2) a polynucleotide sequence having a eukaryotic translation initiation codon and encoding a polypeptide operably linked to the transcriptional promoter, (3) a 5' untranslated sequence located between the transcriptional promoter and the eukaryotic translation initiation codon, and (4) a translational enhancer polynucleotide located within the 5' untranslated sequence, wherein the translational enhancer polynucleotide consists of at least two translational enhancer elements (TEEs) and one or more spacer sequences of 1 to 100 nucleotides in length, wherein said at least two TEEs consists of at least two full-length nucleotide sequences that are at least 90% identical to 5'- GCCGAGAGA-3' (SEQ ID NO: 17), wherein the said at least two TEEs are located within about 1-500 nucleotides 5' of the eukaryotic translation initiation codon, and wherein the cloning or expression vector exhibits increased cap-dependent translation compared to the same vector lacking the translational enhancer polynucleotide.

[0005] In one ~~aspect~~example, provided are isolated or synthetic polynucleotide sequences which function as translation enhancers and include at least one mRNA translational enhancer element (TEE). The TEE present in the polynucleotide sequences consists of RNSGAGMGRMR (SEQ ID NO: 1) or MSCSGCNGM WA (SEQ ID NO:2), or a substantially identical sequence thereof. Also, more than one copy (e.g., 2, 5, 10, 25, 50 or more copies) of each TEE can be present in these polynucleotide sequences.

[0006] In an ~~embodiment~~example, the TEE consists Of $R_1N_1S_1GAGM_1GR_2M_2R_2R_3R_4$ (SEQ ID NO:3) or $R_5M_3S_2CS_3GCN_2GM_4WR_6$ (SEQ ID NO:4), or a substantially identical sequence thereof. In these sequences R_1 is absent, or A or G if present; N_1 is absent, or A,C or G if present, preferably A,C or G; S_1 is absent, or C or G if present, preferably C or G; M_1 is A or C; R_2 is absent, or A or G if present, preferably A or G, and R_1 and R_2 can be the same or different; M_2 is absent, or A or C if present, preferably A or C; R_3 is absent, or A or G if present, preferably absent; R_4 is absent, or C if present, preferably absent; and R_5 is absent, or A if present, preferably absent; M_3 is absent, or C or A if present, preferably C or A; S_2 is absent, or C or G if present, preferably C or G; S_3 is G or C; N_2 is G, C or T; M_4 is A or C; W is absent, or A or T if present and R_6 is absent, or A if present.

[0007] In another ~~embodiment~~example, the TEE consists of $R_1N_1S_1GAGM_1GR_2M_2R_2R_3R_4$. In this sequence R_1, R_3 and R_4 are absent; N_1 is A, C or G; S_1 is C or G; M_1 is A or C; R_2 is A or G and M_2 is A or C. In another ~~embodiment~~example, the

TEE consists of $R_5M_3S_2CS_3GCN_2GM_4WR_6$. In this sequence R_5 and R_6 are absent; M_3 is absent, or A or C if present; S_2 is C or G; S_3 is C or G and S_2 and S_3 can be the same or different; N_2 is G, C or T; M_2 is A or C; W is absent, or A if present.

[0008] In some ~~embodiments~~examples, the polynucleotide sequences include a TEE having a sequence that is at least 90% identical to a sequence from SEQ ID NOs: 5-35. In some embodiments, the polynucleotide sequences include a TEE having a sequence that is identical to one of the sequences of SEQ ID NOs: 5-35.

[0009] In some of the polynucleotide sequences, at least 2 copies of the TEE are present. In some of the polynucleotide sequences, more than 2 copies of the TEE are present. In some other ~~embodiments~~examples, the polynucleotide sequences include at least 5 copies of the TEE, at least 10 copies, at least 25 or more copies of the TEE.

[0010] In ~~a related aspect~~another example, provided are cloning or expression vectors for recombinantly expressing a polypeptide in a eukaryotic cell. The vectors contain a eukaryotic promoter which is operably-linked to a polynucleotide sequence that includes at least one mRNA translational enhancer element (TEE) disclosed herein. The TEE present in the vectors consists of RNSGAGMGRMR (SEQ ID NO: 1) or MSCSGCNGMWA (SEQ ID NO:2), or a substantially identical sequence thereof.

[0011] In an ~~embodiment~~example, the TEE present in the vectors consists of $R_1N_1S_1GAGM_1GR_2M_2R_2R_3R_4$ (SEQ ID NO:3) or $R_5M_3S_2CS_3GCN_2GM_4WR_6$ (SEQ ID NO:4), or a substantially identical sequence thereof. In these sequences R_1 is absent, or A or G if present; N_1 is absent, or A,C or G if present, preferably A,C or G; S_1 is absent, or C or G if present, preferably C or G; M_1 is A or C; R_2 is absent, or A or G if present, preferably A or G, and R_1 and R_2 can be the same or different; M_2 is absent, or A or C if present, preferably A or C; R_3 is absent, or A or G if present, preferably absent; R_4 is absent, or C if present, preferably absent; and R_5 is absent, or A if present, preferably absent; M_3 is absent, or C or A if present, preferably C or A; S_2 is absent, or C or G if present, preferably C or G; S_3 is G or C; N_2 is G, C or T; M_4 is A or C; W is absent, or A or T if present and R_6 is absent, or A if present.

[0012] In another ~~embodiment~~example, the TEE present in the vector consists of $R_1N_1S_1GAGM_1GR_2M_2R_2R_3R_4$. In this sequence R_1, R_3 and R_4 are absent; N_1 is A, C or G; S_1 is C or G; M_1 is A or C; R_2 is A or G and M_2 is A or C. In another ~~embodiment~~example, the TEE present in the vector consists Of $R_5M_3S_2CS_3GCN_2GM_4WR_6$. In this sequence R_5 and R_6 are absent; M_3 is absent, or A or C if present; S_2 is C or G; S_3 is C or G and S_2 and S_3 can be the same or different; N_2 is G, C or T; M_2 is A or C; W is absent, or A if present.

[0013] In some of the vectors, the TEEs consist of a sequence that is substantially identical to a sequence selected from SEQ ID NOs: 5-35. In some other vectors, the TEEs consist of a sequence that is identical to a sequence selected from SEQ ID NOs: 5-35.

[0014] In some vectors, the mRNA translational enhancer element is located 3' to the promoter and adapted for directional ligation of a second polynucleotide sequence encoding a polypeptide of interest. Some of these vectors further include a second polynucleotide sequence encoding a polypeptide of interest which is operably-linked to the mRNA

translational enhancer element. In some vectors, the mRNA translational enhancer element is located in the 5' leader of the second polynucleotide sequence. Further provided are DNA vaccines which include a vector expressing an antigen of interest as disclosed herein. Further provided are host cells (*e.g.*, eukaryotic host cells such as CHO cells) which are transfected with the vectors.

[0014A] In another aspect, provided is a DNA vaccine comprising the cloning or expression vector as defined herein.

[0014B] In another aspect, provided is a eukaryotic host cell transfected with the cloning or expression vector as defined herein.

[0015] In ~~a further aspect~~another example, provided are methods for recombinantly producing a polypeptide of interest. These methods entail constructing an expression vector which includes a eukaryotic promoter and at least one copy (e.g., I5 2, 3, 4, 5, 10, 25, 50 or more copies) of a mRNA translational enhancer element that are each operably-linked to a polynucleotide encoding a polypeptide of interest. This is followed by transfecting the expression vector into a eukaryotic host cell (e.g., CHO cell), and then culturing the host cell transfected with the expression vector. In an ~~embodiment~~example, the mRNA translational enhancer element (TEE) present in the vectors consists of RNSGAGMGRMR (SEQ ID NO:1) or MSCSGCNGMWA (SEQ ID NO:2), or a substantially identical sequence thereof.

[0015A] In another aspect, provided is a method for recombinantly producing a polypeptide comprising: (i) constructing the cloning or expression vector as defined herein; (ii) transfecting said vector into a eukaryotic host cell; and (iii) culturing the host cell transfected with said vector; thereby producing the polypeptide.

[0016] In an ~~embodiment~~example, the TEE present in the vectors consists of $R_1N_1S_1GAGM_1GR_2M_2R_2R_3R_4$ (SEQ ID NO:3) or $R_5M_3S_2CS_3GCN_2GM_4WR_6$ (SEQ ID NO:4), or a substantially identical sequence thereof. In these sequences R_1 is absent, or A or G if present; N_1 is absent, or A,C or G if present, preferably A,C or G; S_1 is absent, or C or G if present, preferably C or G; M_1 is A or C; R_2 is absent, or A or G if present, preferably A or G, and R_1 and R_2 can be the same or different; M_2 is absent, or A or C if present, preferably A or C; R_3 is absent, or A or G if present, preferably absent; R_4 is absent, or C if present, preferably absent; and R_5 is absent, or A if present, preferably absent; M_3 is absent, or C or A if present, preferably C or A; S_2 is absent, or C or G if present, preferably C or G;

S_3 is G or C; N_2 is G, C or T; M_4 is A or C; W is absent, or A or T if present and R_6 is absent, or A if present.

[0017] In another ~~embodiment~~example, the TEE present in the vector consists of $R_1N_1S_1GAGM_1GR_2M_2R_2R_3R_4$. In this sequence R_1, R_3 and R_4 are absent; N_1 is A, C or G; S_1 is C or G; M_1 is A or C; R_2 is A or G and M_2 is A or C. In another ~~embodiment~~example, the TEE present in the vector consists Of $R_5M_3S_2CS_3GCN_2GM_4WR_6$. In this sequence R_5 and R_6 are absent; M_3 is absent, or A or C if present; S_2 is C or G; S_3 is C or G and S_2 and S_3 can be the same or different; N_2 is G, C or T; M_2 is A or C; W is absent, or A if present.

CLAIMS

WHAT IS CLAIMED IS:

1. An isolated polynucleotide sequence comprising at least one mRNA translational enhancer element (TEE), wherein the TEE consists of $R_1N_1S_1GAGM_1GR_2M_2R_2R_3R_4$ (SEQ ID NO:3) or $R_5M_3S_2CS_3GCN_2GM_4WR_6$ (SEQ ID NO:4), or a substantially identical sequence thereof, wherein:

R_1 is absent, or A or G if present; N_1 is absent, or A, C or G if present, preferably A, C or G; S_1 is absent, or C or G if present, preferably C or G; M_1 is A or C; R_2 is absent, or A or G if present, preferably A or G, and R_1 and R_2 can be the same or different; M_2 is absent, or A or C if present, preferably A or C; R_3 is absent, or A or G if present, preferably absent; R_4 is absent, or C if present, preferably absent; and

R_5 is absent, or A if present, preferably absent; M_3 is absent, or C or A if present, preferably C or A; S_2 is absent, or C or G if present, preferably C or G; S_3 is G or C; N_2 is G, C or T; M_4 is A or C; W is absent, or A or T if present and R_6 is absent, or A if present.

2. The isolated polynucleotide sequence of claim 1, wherein the TEE consists of $R_1N_1S_1GAGM_1GR_2M_2R_2R_3R_4$, wherein R_1, R_3 and R_4 are absent; N_1 is A, C or G; S_1 is C or G; M_1 is A or C; R_2 is A or G and M_2 is A or C.

3. The isolated polynucleotide sequence of claim 1, wherein the TEE consists of $R_5M_3S_2CS_3GCN_2GM_4WR_6$, wherein R_5 and R_6 are absent; M_3 is absent, or A or C if present; S_2 is C or G; S_3 is C or G and S_2 and S_3 can be the same or different; N_2 is G, C or T; M_2 is A or C; W is absent, or A if present.

4. The isolated polynucleotide sequence of claim 1, 2 or 3, wherein the TEE consists of a sequence that is at least 90% identical to a sequence selected from the group consisting of SEQ ID NOs: 5-35.

~~5. The isolated polynucleotide sequence of claim 1, 2 or 3, wherein the TEE consists of a~~ sequence that is identical to a sequence selected from the group consisting of SEQ ID NOs: 5-35.

6. The polynucleotide sequence of claim 1, 2 or 3 which comprises at least 2 copies of the TEE.

7. The polynucleotide sequence of claim 1, 2 or 3 which comprises at least 5 copies of the TEE.

8. The polynucleotide sequence of claim 1, 2 or 3 which comprises at least 10 copies of the TEE.

9. A vector for recombinantly expressing a polypeptide in a eukaryotic cell, comprising a eukaryotic promoter operably linked to a polynucleotide sequence comprising at least one mRNA translational enhancer element (TEE), wherein the TEE is selected from the TEEs of claims 1, 2 or 3.

10. The vector of claim 9, wherein the mRNA translational enhancer element consists of a sequence that is substantially identical to a sequence selected from the group consisting of SEQ ID NOs: 5-35.

11. The vector of claim 9, wherein the mRNA translational enhancer element consists of a sequence that is identical to a sequence selected from the group consisting of SEQ ID NOs: 5-35.

12. The vector of claim 9, wherein the mRNA translational enhancer element is located 3' to the promoter and adapted for directional ligation of a second polynucleotide sequence encoding a polypeptide of interest.

13. The vector of claim 9, further comprising a second polynucleotide sequence encoding a polypeptide of interest which is operably linked to the mRNA translational enhancer element.

14. The vector of claim 13, wherein the mRNA translational enhancer element is located in the 5' leader sequence of the second polynucleotide sequence.

~~15. A DNA vaccine comprising the vector of claim 9.~~

16. A eukaryotic host cell transfected with the vector of claim 9.

17. The host cell of claim 16, wherein the host cell is a CHO cell.

18. A method for recombinantly producing a polypeptide comprising:

(i) constructing an expression vector which comprises a eukaryotic promoter and a mRNA translational enhancer element that are each operably linked to a polynucleotide encoding the polypeptide, wherein the TEE is selected from the TEEs of any one of claims 1, 2 or 3;

(ii) transfecting the expression vector into a eukaryotic host cell; and

(iii) culturing the host cell transfected with the expression vector; thereby producing the polypeptide.

19. The method of claim 18, wherein the mRNA translational enhancer element consists of a sequence that is substantially identical to a sequence selected from the group consisting of SEQ ID NOs: 5-35.

20. The method of claim 18, wherein the mRNA translational enhancer element consists of a sequence that is identical to a sequence selected from the group consisting of SEQ ID NOs: 5-35.

21. The method of claim 18, further comprising purifying the recombinant polypeptide.

22. The method of claim 18, wherein the host cell is CHO cell.

23. The method of claim 18, wherein the polypeptide is a therapeutic protein.

CLAIMS

1. A cloning or expression vector comprising the following elements: (1) a transcriptional promoter, (2) a polynucleotide sequence having a eukaryotic translation initiation codon and encoding a polypeptide operably linked to the transcriptional promoter, (3) a 5' untranslated sequence located between the transcriptional promoter and the eukaryotic translation initiation codon, and (4) a translational enhancer polynucleotide located within the 5' untranslated sequence,

wherein the translational enhancer polynucleotide consists of at least two translational enhancer elements (TEEs) and one or more spacer sequences of 1 to 100 nucleotides in length, wherein said at least two TEEs consists of at least two full-length nucleotide sequences that are at least 90% identical to 5'- GCCGAGAGA-3' (SEQ ID NO: 17),

wherein the said at least two TEEs are located within about 1-500 nucleotides 5' of the eukaryotic translation initiation codon, and

wherein the cloning or expression vector exhibits increased cap-dependent translation compared to the same vector lacking the translational enhancer polynucleotide.

2. The cloning or expression vector of claim 1, wherein said at least two TEEs consists of at least two full-length nucleotide sequences that are at least 95% identical to SEQ ID NO: 17.

3. The cloning or expression vector of claim 1, wherein said at least two TEEs consists of at least two full-length sequences that are identical to SEQ ID NO: 17.

4. The cloning or expression vector of claim 1, wherein said translational enhancer polynucleotide consists of at least five copies of the same translational enhancer element (TEE).

5. The cloning or expression vector of claim 1, wherein said translational enhancer polynucleotide consists of at least ten copies of the same translational enhancer element (TEE).

6. The cloning or expression vector of any one of claims 1-3, wherein the said at least two TEEs are identical to each other.

7. A DNA vaccine comprising the cloning or expression vector of any one of claims 1-6.

8. A eukaryotic host cell transfected with the cloning or expression vector of any one of claims 1-6.

9. The eukaryotic host cell of claim 8, wherein the host cell is a Chinese Hamster Ovary (CHO) cell.

10. A method for recombinantly producing a polypeptide comprising:

(i) constructing the cloning or expression vector of any one of claims 1-6;

(ii) transfecting said vector into a eukaryotic host cell; and

(iii) culturing the host cell transfected with said vector; thereby producing the polypeptide.

11. The method of claim 10,

(i) further comprising purifying the recombinant polypeptide, or

(ii) wherein the host cell is CHO cell, or

(iii) wherein the polypeptide is a therapeutic protein.