[0004a] In one aspect, there is provided 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) adjacent to each other or separated by one or more spacer sequences of 1 to 100 nucleotides in length, said at least two TEEs consisting of the sequence $R_5M_3S_2CS_3GCN_2GM_4WR_6$ (SEQ ID NO. 4) wherein:

R_5 is absent, or A if present;

M_3 is absent, or C or A if present;

S_2 is G;

S_3 is G or C, and S_2 and S_3 can be the same or different;

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,

wherein the said at least two TEEs are located within 1-50 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] Also disclosed are~~In one aspect, 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 MSCSGCNGMWA (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~~examples, 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.

[0009a] In another aspect, there is provided a DNA vaccine comprising the cloning or expression vector as defined above.

[0010] In ~~a related aspect~~one 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 yet another aspect, there is provided a eukaryotic cell transfected with the cloning or expression vector as defined above, optionally wherein the host cell is a Chinese Hamster Ovary (CHO) cell.

[0014b] In another aspect, there is provided a method for recombinantly producing a polypeptide comprising:

(i) constructing the cloning or expression vector as defined above;

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

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

[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., 1, 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.

[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.

WHAT IS CLAIMED IS:

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) adjacent to each other or separated by one or more spacer sequences of 1 to 100 nucleotides in length, said at least two TEEs consisting of the sequence $R_5M3S2CS3GCN_2GM_4WR6$ (SEQ ID NO. 4) wherein:

R_5 is absent, or A if present;

M_3 is absent, or C or A if present;

S_2 is G;

S_3 is G or C, and S_2 and S_3 can be the same or different;

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,

wherein the said at least two TEEs are located within 1-50 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 sequences selected from one or more of the following sequences:

5'-CGCGGCTGA-3' (SEQ ID NO: 31) or a full-length sequence that is at least 90% identical thereto;

5'AGCCGCCGCA-3' (SEQ ID NO:34) or a full-length sequence that is at least 90% identical thereto; or

5'ACGCCGCCGA-3' (SEQ ID NO:35) or a full-length sequence that is at least 90% identical thereto.

3. 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 one or more of SEQ ID NO: 31, SEQ ID NO:34 or SEQ IDNO:35.

4. 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: 31, SEQ ID NO: 34or SEQIDNO: 35.

5. 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).

6. 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).

7. The cloning or expression vector of any one of claims 1-4, wherein the said at least two TEEs are identical to each other.

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

9. A eukaryotic host cell transfected with the cloning or expression vector of any one of claims 1-7, optionally 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 -7;
(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.

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) adjacent to each other or separated by one or more spacer sequences of 1 to 100 nucleotides in length, said at least two TEEs consisting of the sequence $R_5M_3S_2CS_3GCN_2GM_4WR_6$ (SEQ ID NO. 4) wherein:

R_5 is absent, or A if present;

M_3 is absent, or C or A if present;

S_2 is G;

S_3 is G or C, and S_2 and S_3 can be the same or different;

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,

wherein the said at least two TEEs are located within 1-50 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 sequences selected from one or more of the following sequences:

5'-CGCGGCTGA-3' (SEQ ID NO: 31) or a full-length sequence that is at least 90% identical thereto;

5'AGCCGCCGCA-3' (SEQ ID NO:34) or a full-length sequence that is at least 90% identical thereto; or

5'-ACGCCGCCGA-3' (SEQ ID NO:35) or a full-length sequence that is at least 90% identical thereto.

3. 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 one or more of SEQ ID NO: 31, SEQ ID NO:34 or SEQ ID NO:35.

4. 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: 31, SEQ ID NO:34 or SEQ ID NO:35.

5. 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).

6. 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).

7. The cloning or expression vector of any one of claims 1-4, wherein the said at least two TEEs are identical to each other.

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

9. A eukaryotic host cell transfected with the cloning or expression vector of any one of claims 1-7, optionally 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 1-7;
(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.