CLAIMS

What is claimed is:

1. A method of rolling circle translation of one or more expression sequences comprising

expressing the one or more expression sequences from a circular polyribonucleotide via rolling circle translation, wherein the circular polyribonucleotide comprises the one or more expression sequences, a stagger element at a 3' end of at least one of the expression sequences, and lacks a termination element.

2. The method of claim 1, wherein the stagger element is configured to stall a ribosome during the rolling circle translation of the circular polyribonucleotide.

3. The method of claim 1, wherein the stagger element encodes a sequence with a C-terminal consensus sequence that is D(V/I)ExNPGP, where x= any amino acid.

4. The method of claim 1, wherein expressing the one or more expression sequences generates at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% discrete polypeptides of total polypeptides (molar/molar) generated during the rolling circle translation of the circular polyribonucleotide, and wherein each of the discrete polypeptides is generated from a single expression sequence.

5. The method of claim 1, wherein at least one of the expression sequences encodes a secreted protein.

6. The method of claim 1, wherein the circular polyribonucleotide lacks an internal ribosomal entry site.

7. The method of claim 1, wherein the one or more expression sequences comprise a Kozak initiation sequence.

8. The method of claim 1, wherein the circular polyribonucleotide further comprises at least one structural element selected from:

(a) an encryptogen;

(b) a regulatory element;

(c) a replication element; and

(d) quasi-double-stranded secondary structure.

9. The method of claim 1, wherein the circular polyribonucleotide comprises at least one functional characteristic selected from:

(i) greater translation efficiency than a linear counterpart;

(ii) a stoichiometric translation efficiency of multiple translation products;

(iii) less immunogenicity than a counterpart lacking an encryptogen;

(iv) increased half-life over a linear counterpart; and

(v) persistence during cell division.

10. The method of claim 1, wherein the expressing the one or more expression sequences generates at least 5 fold greater expression product than a linear counterpart.

11. The method of claim 1, wherein the expressing the one or more expression sequences comprises expressing the one or more expression sequences in a cell comprising the circular polyribonucleotide.

12. The method of claim 11, wherein the circular polyribonucleotide persists during cell division.

13. The method of claim 11, wherein at least about 60% of an amount of the circular polyribonucleotide persists for at least about 7 days in the cell.

14. The method of claim 11, wherein:

(a) expression of the one or more expression sequences in the cell at a later time point is equal to or higher than an earlier time point; or

(b) expression of the one or more expression sequences in the cell over a time period of at least about 7 days does not decrease by 40%; or

(c) expression of the one or more expression sequences in the cell is maintained at a level that does not vary by more than 40% for at least about 7 days.

15. The method of claim 1, wherein the expressing the one or more expression sequences comprises expressing the expression sequences in a mammalian subject.

16. The method of claim 1, wherein the termination element comprises a stop codon.

17. The method of claim 11, wherein the circular polyribonucleotide further comprises a replication domain configured to mediate self-replication of the circular polyribonucleotide.

18. A pharmaceutical composition comprising a circular polyribonucleotide comprising

(a) a circular polyribonucleotide comprising one or more expression sequences and a stagger element

at a 3’ end of at least one of the one or more expression sequences, wherein the circular polyribonucleotide is competent for rolling circle translation and lacks a termination element; and (b) a pharmaceutically acceptable excipient.

19. A cell comprising a circular polyribonucleotide that comprises one or more expression sequences and a stagger element at a 3’ end of at least one of the one or more expression sequences, wherein the circular polyribonucleotide is competent for rolling circle translation and lacks a termination element.

20. A method of *in vivo* expression of one or more expression sequences in a subject, comprising:

administering a circular polyribonucleotide to a cell of the subject wherein the circular polyribonucleotide comprises the one or more expression sequences; and

expressing the one or more expression sequences from the circular polyribonucleotide in the cell, wherein the circular polyribonucleotide is configured such that expression of the one or more expression sequences in the cell at a later time point is equal to or higher than an earlier time point.

21. A method of *in vivo* expression of one or more expression sequences in a subject, comprising:

administering a circular polyribonucleotide to a cell of the subject wherein the circular polyribonucleotide comprises the one or more expression sequences; and

expressing the one or more expression sequences from the circular polyribonucleotide in the cell, wherein the circular polyribonucleotide is configured such that expression of the one or more expression sequences in the cell over a time period of at least 7, 8, 9, 10, 12, 14, or 16 days does not decrease by greater than about 40%.

22. A method of *in vivo* expression of one or more expression sequences in a subject, comprising:

administering a circular polyribonucleotide to a cell of the subject wherein the circular polyribonucleotide comprises the one or more expression sequences; and

expressing the one or more expression sequences from the circular polyribonucleotide in the cell, wherein the circular polyribonucleotide is configured such that expression of the one or more expression sequences in the cell is maintained at a level that does not vary by more than about 40% for at least 7, 8, 9, 10, 12, 14, or 16 days.

23. A pharmaceutical composition comprising a circular polyribonucleotide that comprises at least one structural element selected from:

a) an encryptogen;

b) a stagger element;

c) a regulatory element;

d) a replication element;

f) quasi-double-stranded secondary structure; and

g) expression sequence;

and at least one functional characteristic selected from:

a) greater translation efficiency than a linear counterpart;

b) a stoichiometric translation efficiency of multiple translation products;

c) less immunogenicity than a counterpart lacking an encryptogen;

d) increased half-life over a linear counterpart; and

e) persistence during cell division.

24. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or excipient and a circular polyribonucleotide that comprises one or more expression sequences, wherein the circular polyribonucleotide is competent for rolling circle translation.

25. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or excipient and a circular polyribonucleotide that comprises one or more expression sequences and is competent for rolling circle translation, wherein the circular polyribonucleotide is configured such that at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of total polypeptides (molar/molar) generated during the rolling circle translation of the circular polyribonucleotide are discrete polypeptides, and wherein each of the discrete polypeptides is generated from a single round of translation or less than a single round of translation of the one or more expression sequences.

26. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or excipient and a circular polyribonucleotide that comprises one or more expression sequences and a stagger element at 3’ end of at least one of the one or more expression sequences, wherein the stagger element is configured to stall a ribosome during rolling circle translation of the circular polyribonucleotide.

27. A method of producing the composition of any one of claims 18, or 23-26, comprising combining the circular polyribonucleotide of any one of claims 18, or 23-26 and the pharmaceutically acceptable carrier or excipient of any one of claims 18, or 23-26

28. A method of treatment, comprising administering the composition of any one of claims 18, or 23-26.

29. A method for protein expression, comprising translating at least a region of the circular polyribonucleotide of any one of claims 18, or 23-26.

30. The method of claim 29, wherein the translation of the at least a region of the circular polyribonucleotide takes place *in vitro*.

31. The method of claim 29, wherein the translation of the at least a region of the circular polyribonucleotide takes place *in vivo*.

32. A polynucleotide encoding the circular polyribonucleotide of any one of claims 18, or 23-26.

33. A method of producing the circular polyribonucleotide of any one of claims 18, or 23-26.

CLAIMS

What is claimed is:

1. A method of making a pharmaceutical composition, comprising:

providing a linear polyribonucleotide that comprises: (a) an internal ribosome entry site (IRES) element, (b) an expression sequence encoding a therapeutic polypeptide and lacking a 3′ poly-A sequence, and (c) a termination element;

circularizing the linear polyribonucleotide to produce a circular polyribonucleotide preparation comprising a circular polyribonucleotide;

enriching for the circular polyribonucleotide from the circular polyribonucleotide preparation to form an enriched preparation; and

formulating the enriched preparation with a pharmaceutically acceptable carrier or excipient, thereby producing the pharmaceutical composition.

2. The method of claim 1, further comprising measuring circularization efficiency of the circular polyribonucleotide preparation.

3. The method of claim 1, further comprising one or both of: (i) measuring an amount of one or more impurities in the circular polyribonucleotide preparation, and (ii) measuring an amount of one or more impurities in the pharmaceutical composition.

4. The method of claim 1, wherein the linear polyribonucleotide lacks a 5′ cap.

5. The method of claim 1, wherein the linear polyribonucleotide lacks a pair of sequences that are complementary to each other at a 5′ end and a 3′ end of the linear polyribonucleotide, respectively.

6. The method of claim 1, wherein the circular polyribonucleotide further comprises at least one element selected from:

(i) an encryptogen;

(ii) a regulatory element; and

(iii) a quasi-double-stranded secondary structure.

7. The method of claim 1, wherein the internal ribosome entry site element comprises a

sequence derived from picornavirus complementary DNA, encephalomyocarditis virus (EMCV) complementary DNA, poliovirus complementary DNA, or an Antennapedia gene from *Drosophila melanogaster.*

8. The method of claim 1, wherein the termination element comprises a stop codon.

9. The method of claim 1, wherein the circular polyribonucleotide comprises two or more of the expression sequence encoding the therapeutic polypeptide.

10. The method of claim 1, wherein the circular polyribonucleotide further comprises a second expression sequence encoding a second polypeptide.

11. The method of claim 1, wherein the circularizing comprises splint ligation-mediated circularization of the linear polyribonucleotide.

12. The method of claim 1, wherein the circularizing does not involve self-splicing of the linear polyribonucleotide.

13. The method of claim 1, wherein the enriching comprises purifying the circular polyribonucleotide via column chromatography, gel excision, size exclusion, or any combination thereof.

14. The method of claim 1, wherein the enriching comprises removing one or more of: free ribonucleic acids, linear RNA, nicked RNA, DNA, or proteins from the circular polyribonucleotide preparation.

15. The method of claim 1, wherein the formulating the enriched preparation comprises formulating the circular polyribonucleotide with an exosome.

16. The method of claim 1, wherein the formulating the enriched preparation comprises formulating the circular polyribonucleotide with a lipid carrier.

17. The method of claim 1, wherein the formulating the enriched preparation comprises formulating the circular polyribonucleotide with a lipid nanoparticle.

18. The method of claim 1, wherein the therapeutic polypeptide comprises at least 150 amino acids.

19. The method of claim 1, wherein the therapeutic polypeptide is an epigenetic modifying agent.

20. The method of claim 1, wherein the therapeutic polypeptide is an antigen.

21. The method of claim 20, wherein the antigen is a viral antigen, a bacterial antigen or a tumor antigen.

22. The method of claim 1, wherein the therapeutic polypeptide is at least a portion of a viral envelope protein.

23. The method of claim 1, wherein the therapeutic polypeptide is an anti-microbial peptide.

24. The method of claim 1, wherein the therapeutic polypeptide is an epigenetic enzyme.

25. The method of claim 1, wherein the therapeutic polypeptide is a nuclease.

26. The method of claim 1, wherein the therapeutic polypeptide is a component of a CRISPR system.

27. The method of claim 1, wherein the therapeutic polypeptide is a pore forming peptide.

28. The method of claim 1, wherein the therapeutic polypeptide is a cytotoxic peptide.

29. The method of claim 1, wherein the therapeutic polypeptide is a nuclease and the circular polyribonucleotide further comprises a guide RNA sequence.

30. A method of making a pharmaceutical composition, comprising:

providing a linear polyribonucleotide that comprises (a) an internal ribosome entry site (IRES) element, (b) an expression sequence encoding a therapeutic polypeptide and lacking a 3′ poly-A sequence, and (c) a termination element;

circularizing the linear polyribonucleotide to produce a circular polyribonucleotide preparation comprising a circular polyribonucleotide;

removing from the circular polyribonucleotide preparation one or more of: free ribonucleic acids, linear RNA, nicked RNA, DNA, or proteins, to form an enriched preparation comprising the circular polyribonucleotide; and

formulating the enriched preparation with a pharmaceutically acceptable carrier or excipient.