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.