IMMUNOGENIC COMPOSITIONS AND USES THEREOF

BACKGROUND

Vaccination has made an enormous contribution to both human and animal health. Since the invention of the first vaccine in 1796, vaccines have come to be considered the most successful method for preventing many infectious diseases by provoking an immune response in a subject. According to the World Health Organization, immunization currently prevents 2-3 million deaths every year across all age groups. Today, vaccines have been developed to prevent and control the spread of more than 20 infectious diseases, including diphtheria, tetanus, pertussis, influenza, and measles, and have led to the complete eradication of smallpox. There remains a need to develop new and improved immunogenic compositions and uses thereof.

SUMMARY

In an example, the disclosure provides a circular polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) one or more expression augmenting element; (c) a polyribonucleotide cargo encoding an immunogen; and (d) a second post-circularization element; wherein the first post-circularization element and the second post-circularization element together form a circularization junction.

In another example, the disclosure provides circular polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) a polyribonucleotide cargo encoding an immunogen; (c) one or more expression augmenting element; and (d) a second post-circularization element; wherein the first post-circularization element and the second post-circularization element together form a circularization junction.

In some embodiments, the one or more expression augmenting element comprises a fusion of a translation enhancer and a spacer element comprising a polyA region. In some embodiments, the spacer element comprises from 5 to 200 ribonucleotides in length (e.g., from 5 to 150, 5 to 100, 5 to 50, 5 to 20, 20 to 200, 50 to 200, 100 to 200, 150 to 200, and 50 to 150 ribonucleotides in length). In some embodiments, the one or more expression augmenting element comprises a translation enhancer. In some embodiments, the translation enhancer is from a plant virus. In some embodiments, the translation enhancer is from a mammalian virus. In some embodiments, the translation enhancer is a human translation enhancer. In some embodiments, the translation enhancer is selected from the group comprising a BYDV like-element (BTE), a translation enhancer element (TED), a PMV/PEMV-like translation enhancer (PTE), an I-shaped structure (ISS), a Y-shaped structure (YSS), a t-shaped structure (TSS), dumbbell shaped structure, viral RNA UTRs (including Dengue, West Nile, Zika, Rotavirus), EMCV, CVB3, hepatitis B virus posttranscriptional regulatory element, human genomic fragments, a histone mRNA sequence, a cyclin D mRNA sequence, or an eIF4g aptamer sequence. In some embodiments, the translation enhancer binds to eIF4g.

In some embodiments, the circular polyribonucleotide further comprises one or more spacer elements comprising a polyA region. In some embodiments, the spacer element has a length of between 50 to 500 (e.g., 50 to 450, 50 to 400, 50 to 350, 50 to 300, 50 to 250, 50 to 200, 50 to 150, 50 to 100, 50 to 80, 55 to 500, 60 to 500, 70 to 500, 80 to 500, 90 to 500, 110 to 500, 115 to 500, 120 to 500, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, 450 to 500, 50 to 300, 50 to 200, 60 to 300, and 60 to 200) ribonucleotides. In some embodiments, the spacer element has a length of between 100 to 500 (e.g., 100 to 450, 100 to 400, 100 to 350, 100 to 300, 100 to 250, 100 to 200, 100 to 150, 110 to 500, 115 to 500, 120 to 500, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, 450 to 500, 100 to 300, 100 to 200, 110 to 300, and 110 to 200) ribonucleotides. In some embodiments, the spacer element has a length of between 110 to 500 (e.g., 110 to 450, 110 to 400, 110 to 350, 110 to 300, 110 to 250, 110 to 200, 110 to 150, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the spacer element has a length of between 120 to 500 (e.g., 120 to 450, 120 to 400, 120 to 350, 120 to 300, 120 to 250, 120 to 200, 120 to 150, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the spacer element has a length of between 100 to 300 (e.g., 100 to 280, 100 to 260, 100 to 240, 100 to 220, 100 to 200, 100 to 180, 100 to 160, 100 to 140, 100 to 120, 110 to 300, 115 to 300, 120 to 300, 140 to 300, 160 to 300, 180 to 300, 200 to 300, 220 to 300, 240 to 300, 260 to 300, 110 to 200, and 280 to 300) ribonucleotides. In some embodiments, the spacer has a length of between 10 and 200 (e.g., between 10 and 175, 10 and 150, 10 and 125, 10 and 100, 10 and 75, 10 and 50, 10 and 25, 25 and 200, 50 and 200, 75 and 200, 100 and 200, 150 and 200, 175 and 200, and 25 and 100) ribonucleotides. In some embodiments, the spacer element has a length of about 120 ribonucleotides. In some embodiments, the spacer element has a length of about 50 ribonucleotides.

In another example, the disclosure provides a circular polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) a first expression augmenting element; (c) a polyribonucleotide cargo encoding an immunogen; (d) a second expression augmenting element; and (e) a second post-circularization element; wherein the first post-circularization element and the second post-circularization element together form a circularization junction. In some embodiments, the first expression augmenting element comprises a fusion of a translation enhancer and a spacer element. In some embodiments, the spacer element comprises from 5 to 200 ribonucleotides in length (e.g., from 5 to 150, 5 to 100, 5 to 50, 5 to 20, 20 to 200, 50 to 200, 100 to 200, 150 to 200, and 50 to 150 ribonucleotides in length). In some embodiments, the second expression augmenting element comprises a fusion of a translation enhancer and a spacer element. In some embodiments, the spacer element comprises from 5 to 200 ribonucleotides in length (e.g., from 5 to 150, 5 to 100, 5 to 50, 5 to 20, 20 to 200, 50 to 200, 100 to 200, 150 to 200, and 50 to 150 ribonucleotides in length). In some embodiments, the first expression augmenting element comprises a translation enhancer. In some embodiments, the second expression augmenting element comprises a translation enhancer. In some embodiments, the translation enhancer is from a plant virus. In some embodiments, the translation enhancer is from a mammalian virus. In some embodiments, the translation enhancer is a human translation enhancer. In some embodiments, the

comparison to a polyribonucleotide without a stability element after the circular polyribonucleotide is administered to a subject. In some embodiments, the polynucleotide cargo encodes an immunogen, wherein the immunogen has at least 2-fold greater expression in comparison to a polyribonucleotide without a stability element.

In some embodiments, the circular polyribonucleotide further comprises one or more spacer elements comprising a polyA region. In some embodiments, the spacer element has a length of between 100 to 500 (e.g., 100 to 450, 100 to 400, 100 to 350, 100 to 300, 100 to 250, 100 to 200, 100 to 150, 120 to 500, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the spacer element has a length of between 120 to 500 (e.g., 120 to 450, 120 to 400, 120 to 350, 120 to 300, 120 to 250, 120 to 200, 120 to 150, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the spacer element has a length of between 100 to 300 (e.g., 100 to 280, 100 to 260, 100 to 240, 100 to 220, 100 to 200, 100 to 180, 100 to 160, 100 to 140, 100 to 120, 120 to 300, 140 to 300, 160 to 300, 180 to 300, 200 to 300, 220 to 300, 240 to 300, 260 to 300, and 280 to 300) ribonucleotides.

In another example, the disclosure provides a circular polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) a spacer element; (c) a polyribonucleotide cargo encoding an immunogen; (d) an expression augmenting element; and (e) a second post-circularization element and wherein the first post-circularization element and the second post-circularization element together form a circularization junction.

In another example, the disclosure provides a circular polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) an expression augmenting element; (c) a polyribonucleotide cargo encoding an immunogen; (d) a spacer element; and (e) a second post-circularization element and wherein the first post-circularization element and the second post-circularization element together form a circularization junction.

In another example, the disclosure provides a circular polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) a first expression augmenting element; (c) a polyribonucleotide cargo encoding an immunogen; (d) a second expression augmenting element; and (e) a second post-circularization element and wherein the first post-circularization element and the second post-circularization element together form a circularization junction.

In an aspect, the disclosure provides a circular polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) a first spacer element having a length of 110 to 500 ribonucleotides; (c) a polyribonucleotide cargo encoding an immunogen; (d) a second spacer element having a length of 110 to 500 ribonucleotides; and (e) a second post-circularization element; and wherein the first post-circularization element and the second post-circularization element together form a circularization junction.

In another aspect, the disclosure provides a circular polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) a first spacer element having a length of 50 to 500 ribonucleotides; (c) a polyribonucleotide cargo encoding an immunogen; (d) a second spacer element having a length of 110 to 500

ribonucleotides; and (e) a second post-circularization element; and wherein the first post-circularization element and the second post-circularization element together form a circularization junction, wherein the polyribonucleotide comprises an miRNA binding site.

In some embodiments, the first spacer element has a length of between 50 to 500 (e.g., 50 to 450, 50 to 400, 50 to 350, 50 to 300, 50 to 250, 50 to 200, 50 to 150, 50 to 100, 50 to 80, 55 to 500, 60 to 500, 70 to 500, 80 to 500, 90 to 500, 110 to 500, 115 to 500, 120 to 500, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, 450 to 500, 50 to 300, 50 to 200, 60 to 300, and 60 to 200) ribonucleotides. In some embodiments, the first spacer element has a length of between 100 to 500 (e.g., 100 to 450, 100 to 400, 100 to 350, 100 to 300, 100 to 250, 100 to 200, 100 to 150, 110 to 450, 110 to 400, 110 to 350, 110 to 300, 110 to 250, 110 to 200, 110 to 150, 120 to 500, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the second spacer element has a length of between 50 to 500 (e.g., 50 to 450, 50 to 400, 50 to 350, 50 to 300, 50 to 250, 50 to 200, 50 to 150, 50 to 100, 50 to 80, 55 to 500, 60 to 500, 70 to 500, 80 to 500, 90 to 500, 110 to 500, 115 to 500, 120 to 500, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, 450 to 500, 50 to 300, 50 to 200, 60 to 300, and 60 to 200) ribonucleotides. In some embodiments, the second spacer element has a length of between 100 to 500 (e.g., 100 to 450, 100 to 400, 100 to 350, 100 to 300, 100 to 250, 100 to 200, 100 to 150, 110 to 450, 110 to 400, 110 to 350, 110 to 300, 110 to 250, 110 to 200, 110 to 150, 120 to 500, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each comprise 110 to 500 (e.g., 110 to 450, 110 to 400, 110 to 350, 110 to 300, 110 to 250, 110 to 200, 110 to 150, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each comprise 120 to 500 (e.g., 120 to 450, 120 to 400, 120 to 350, 120 to 300, 120 to 250, 120 to 200, 120 to 150, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each have a length of 150 to 500 (e.g., 150 to 450, 150 to 400, 150 to 350, 150 and 300, 150 and 250, 150 and 200, 200 500, 250 and 500, 300 and 500, 350 and 500, 400 and 500, or 450 and 500) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each have a length of 200 to 500 (e.g., 200 to 450, 200 to 400, 200 to 350, 200 to 300, 200 to 250, 250 to 500, 300 to 500, 350 to 500, 400 to 500, or 450 to 500) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each have a length of 100 to 300 (e.g., 100 to 280, 100 to 260, 100 to 240, 100 to 220, 100 to 200, 100 to 180, 100 to 160, 100 to 140, 100 to 120, 120 to 300, 140 to 300, 160 to 300, 180 to 300, 200 to 300, 220 to 300, 240 to 300, 260 to 300, and 280 to 300) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each have a length of 100 to 200 (e.g., 100 to 190, 100 to 180, 100 to 170, 100 to 160, 100 to 150, 100 to 140, 100 to 130, 100 to 120, 100 to 110, 110 to 200, 120 to 200, 130 to 200, 140 to 200, 150 to 200, 160 to 200, 170 to 200, 180 to 200, and 90 to 200) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each have a length of 110 to 300 (e.g., 110 to 280, 110 to 260, 110 to 240, 110 to 220, 110 to 200, 110 to 180, 110 to 160, 110 to 140, 140 to 300, 160 to

expression sequence further comprises a 3' untranslated region or a 5' untranslated region. In some embodiments, the expression sequence encodes an immunogen.

In some embodiments, the polyribonucleotide comprises between 500 and 20,000 (e.g., between 500 to 15,000, 500 to 10,000, 500 to 5,000, 500 to 1,000, 1,000 to 20,000, 5,000 to 20,000, 10,000 to 20,000, and 15,000 to 20,000) ribonucleotides. In some embodiments, the polyribonucleotide comprises between 2,000 and 20,000 (e.g., 2,000 to 5,000, 2,000 to 7,500, 2,000 to 10,000, 2,000 to 12,500, 2,000 to 15,000, 2,000 to 17,500, 5,000 to 20,000, 7,500 to 20,000, 10,000 to 20,000, 12,500 to 20,000, 15,000 to 20,000, and 17,500 to 20,000) ribonucleotides.

In some embodiments, the circularization junction comprises a splice junction. In some embodiments, the first post-circularization element comprises a first exon fragment and the second post-circularization element comprises a second exon fragment, and wherein the first exon fragment and the second exon fragment are joined by the splice junction. In some embodiments, the circularization junction comprises an oligonucleotide splint that is hybridized to the first post-circularization element and to the second post-circularization element. In some embodiments, the oligonucleotide splint is a DNA splint or an RNA splint. In some embodiments, the first post-circularization element comprises a region that is capable of hybridizing to a first region of the oligonucleotide splint and the second post-circularization element comprises a region that is capable of hybridizing to a second region of the oligonucleotide splint.

In an example, the disclosure provides a linear polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) one or more expression augmenting element; (c) a polyribonucleotide cargo encoding an immunogen; and (d) a second post-circularization element; wherein the first post-circularization element and the second post-circularization element together form a circularization junction.

In an example, the disclosure provides a linear polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) a polyribonucleotide cargo encoding an immunogen; (c) one or more expression augmenting element; and (d) a second post-circularization element; wherein the first post-circularization element and the second post-circularization element together form a circularization junction.

In some embodiments, the one or more expression augmenting element comprises a fusion of a translation enhancer and a spacer element comprising a polyA region. In some embodiments, the spacer element comprises from 5 to 200 ribonucleotides in length (e.g., from 5 to 150, 5 to 100, 5 to 50, 5 to 20, 20 to 200, 50 to 200, 100 to 200, 150 to 200, and 50 to 150 ribonucleotides in length). In some embodiments, the one or more expression augmenting element comprises a translation enhancer. In some embodiments, the translation enhancer is from a plant virus. In some embodiments, the translation enhancer is from a mammalian virus. In some embodiments, the translation enhancer is a human translation enhancer. In some embodiments, the translation enhancer is selected from the group comprising a BTE, a TED, a PTE, an ISS, a YSS, a TSS, dumbbell shaped structure, viral RNA UTRs (including Dengue, West Nile, Zika, Rotavirus), EMCV, CVB3, hepatitis B virus posttranscriptional regulatory element, human genomic fragments, a histone mRNA sequence, a cyclin D mRNA sequence, or an eIF4g aptamer sequence. In some

to 150, 110 to 500, 120 to 500, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the spacer element has a length of between 110 to 500 (e.g., 110 to 450, 110 to 400, 110 to 350, 110 to 300, 110 to 250, 110 to 200, 110 to 150, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the spacer element has a length of between 120 to 500 (e.g., 120 to 450, 120 to 400, 120 to 350, 120 to 300, 120 to 250, 120 to 200, 120 to 150, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the spacer element has a length of between 100 to 300 (e.g., 100 to 280, 100 to 260, 100 to 240, 100 to 220, 100 to 200, 100 to 180, 100 to 160, 100 to 140, 100 to 120, 110 to 300, 120 to 300, 140 to 300, 160 to 300, 180 to 300, 200 to 300, 220 to 300, 240 to 300, 260 to 300, and 280 to 300) ribonucleotides.

In another example, the disclosure provides a linear polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) a first expression augmenting element; (c) a polyribonucleotide cargo encoding an immunogen; (d) a second expression augmenting element; and (e) a second post-circularization element; wherein the first post-circularization element and the second post-circularization element together form a circularization junction. In some embodiments, the first expression augmenting element comprises a fusion of a translation enhancer and a spacer element. In some embodiments, the spacer element comprises from 5 to 200 ribonucleotides in length (e.g., from 5 to 150, 5 to 100, 5 to 50, 5 to 20, 20 to 200, 50 to 200, 100 to 200, 150 to 200, and 50 to 150 ribonucleotides in length). In some embodiments, the second expression augmenting element comprises a fusion of a translation enhancer and a spacer element. In some embodiments, the spacer element comprises from 5 to 200 ribonucleotides in length (e.g., from 5 to 150, 5 to 100, 5 to 50, 5 to 20, 20 to 200, 50 to 200, 100 to 200, 150 to 200, and 50 to 150 ribonucleotides in length). In some embodiments, the first expression augmenting element comprises a translation enhancer. In some embodiments, the second expression augmenting element comprises a translation enhancer. In some embodiments, the translation enhancer is from a plant virus. In some embodiments, the translation enhancer is from a mammalian virus. In some embodiments, the translation enhancer is a human translation enhancer. In some embodiments, the translation enhancer is selected from the group comprising a BTE, a TED, a PTE, an ISS, a YSS, a TSS, dumbbell shaped structure, viral RNA UTRs (including Dengue, West Nile, Zika, Rotavirus), EMCV, CVB3, hepatitis B virus posttranscriptional regulatory element, human genomic fragments, a histone mRNA sequence, a cyclin D mRNA sequence, or an eIF4g aptamer sequence. In some embodiments, the translation enhancer binds to eIF4g.

In some embodiments, the translation enhancer comprises between 50 and 2000 (e.g., 50 to 1500, 50 to 1000, 50 to 500, 50 to 100, 50 to 1250, 50 to 1250, 50 to 750, 100 to 2000, 500 to 2000, 750 to 2000, 1000 to 2000, 1250 to 2000, 1500 to 2000, 1750 to 2000, 100 to 1000, and 500 to 1500) ribonucleotides. In some embodiments, the translation enhancer comprises between 60 and 800 (e.g., 60 to 700, 60 to 600, 60 to 500, 60 to 400, 60 to 400, 60 to 300, 60 to 300, 60 to 200, 60 to 100, 100 to 800, 200 to 800, 300 to 800, 400 to 800, 500 to 800, 600 to 800, 700 to 800, and 200 to 500) ribonucleotides.

(e.g., 120 to 450, 120 to 400, 120 to 350, 120 to 300, 120 to 250, 120 to 200, 120 to 150, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the spacer element has a length of between 100 to 300 (e.g., 100 to 280, 100 to 260, 100 to 240, 100 to 220, 100 to 200, 100 to 180, 100 to 160, 100 to 140, 100 to 120, 120 to 300, 140 to 300, 160 to 300, 180 to 300, 200 to 300, 220 to 300, 240 to 300, 260 to 300, and 280 to 300) ribonucleotides.

In another example, the disclosure provides a linear polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) a spacer element; (c) a polyribonucleotide cargo encoding an immunogen; (d) an expression augmenting element and (e) a second post-circularization element; and wherein the first post-circularization element and the second post-circularization element together form a circularization junction.

In another example, the disclosure provides a linear polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) an expression augmenting element; (c) a polyribonucleotide cargo encoding an immunogen; (d) a spacer element; and (e) a second post-circularization element, and wherein the first post-circularization element and the second post-circularization element together form a circularization junction.

In another example, the disclosure provides a linear polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) a first expression augmenting element; (c) a polyribonucleotide cargo encoding an immunogen; (d) a second expression augmenting element; and (e) a second post-circularization element, and wherein the first post-circularization element and the second post-circularization element together form a circularization junction.

In another aspect, the disclosure provides a linear polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) a first spacer element having a length of 110 to 500 ribonucleotides; (c) a polyribonucleotide cargo encoding an immunogen; (d) a second spacer element having a length of 110 to 500 ribonucleotides; and (e) a second post-circularization element; and wherein the first post-circularization element and the second post-circularization element together form a circularization junction.

In another example, the disclosure provides a linear polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) a first spacer element having a length of at least 100 ribonucleotides; (c) a polyribonucleotide cargo encoding an immunogen; (d) a second spacer element and (e) a second post-circularization element; and wherein the first post-circularization element and the second post-circularization element together form a circularization junction.

In another aspect, the disclosure provides a linear polyribonucleotide comprising, in the following order from 5' to 3': (a) a first post-circularization element; (b) a first spacer element having a length of 50 and 500 ribonucleotides; (c) a polyribonucleotide cargo encoding an immunogen; (d) a second spacer element having a length of 110 to 500 ribonucleotides; and (e) a second post-circularization element; and wherein the first post-circularization element and the second post-circularization element together form a circularization junction

, wherein the first spacer or second spacer comprises an miRNA binding site.

In some embodiments, the first spacer element has a length of between 100 to 500 (e.g., 100 to 450, 100 to 400, 100 to 350, 100 to 300, 100 to 250, 100 to 200, 100 to 150, 110 to 500, 110 to 450, 110 to 400, 110 to 350, 110 to 300, 110 to 250, 110 to 200, 110 to 150, 120 to 500, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the first spacer element has a length of between 100 and 300 (e.g., 100 to 250, 100 to 200, 100 to 150, 110 to 300, 120 to 300, 150 to 300, 200 to 300, and 250 to 300). In some embodiments, the second spacer element has a length of between 100 to 500 (e.g., 100 to 450, 100 to 400, 100 to 350, 100 to 300, 100 to 250, 100 to 200, 100 to 150, 110 to 500, 120 to 500, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each comprise 110 to 500 (e.g., 110 to 450, 110 to 400, 110 to 350, 110 to 300, 110 to 250, 110 to 200, 110 to 150, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each comprise 120 to 500 (e.g., 120 to 450, 120 to 400, 120 to 350, 120 to 300, 120 to 250, 120 to 200, 120 to 150, 150 to 500, 200 to 500, 250 to 500, 300 to 500, 350 to 500, 400 to 500, and 450 to 500) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each have a length of 150 to 500 (e.g., 150 to 450, 150 to 400, 150 to 350, 150 and 300, 150 and 250, 150 and 200, 200 500, 250 and 500, 300 and 500, 350 and 500, 400 and 500, or 450 and 500) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each have a length of 200 to 500 (e.g., 200 to 450, 200 to 400, 200 to 350, 200 to 300, 200 to 250, 250 to 500, 300 to 500, 350 to 500, 400 to 500, or 450 to 500) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each have a length of 100 to 300 (e.g., 100 to 280, 100 to 260, 100 to 240, 100 to 220, 100 to 200, 100 to 180, 100 to 160, 100 to 140, 100 to 120, 120 to 300, 140 to 300, 160 to 300, 180 to 300, 200 to 300, 220 to 300, 240 to 300, 260 to 300, and 280 to 300) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each have a length of 100 to 200 (e.g., 100 to 190, 100 to 180, 100 to 170, 100 to 160, 100 to 150, 100 to 140, 100 to 130, 100 to 120, 100 to 110, 110 to 200, 120 to 200, 130 to 200, 140 to 200, 150 to 200, 160 to 200, 170 to 200, 180 to 200, and 90 to 200) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each have a length of 110 to 300 (e.g., 110 to 280, 110 to 260, 110 to 240, 110 to 220, 110 to 200, 110 to 180, 110 to 160, 110 to 140, 140 to 300, 160 to 300, 180 to 300, 200 to 300, 220 to 300, 240 to 300, 260 to 300, and 280 to 300) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each have a length of 120 to 300 (e.g., 120 to 280, 120 to 260, 120 to 240, 120 to 220, 120 to 200, 120 to 180, 120 to 160, 120 to 140, 140 to 300, 160 to 300, 180 to 300, 200 to 300, 220 to 300, 240 to 300, 260 to 300, and 280 to 300) ribonucleotides. In some embodiments, the first spacer element and the second spacer element each have a length of 150 to 300 (e.g., 150 to 290, 150 to 280, 150 to 270, 150 to 260, 150 to 250, 150 to 240, 150 to 230, 150 to 220, 150 to 210, 150 to 200, 150 to 190, 150 to 180, 150 to 170, 150 to 160, 160 to 300, 170 to 300, 180 to 300, 190 to 300, 200 to 300, 210 to 300, 220 to 300, 230 to 300, 240 to 300, 250 to 300, 260 to 300, 270 to 300, 280 to 300, and 290 to 300) ribonucleotides. In some embodiments, the difference between the length of the first spacer element

circularization junction comprises an oligonucleotide splint that is hybridized to the first post-circularization element and to the second post-circularization element. In some embodiments, the oligonucleotide splint is a DNA splint or an RNA splint. In some embodiments, the first post-circularization element comprises a region that is capable of hybridizing to a first region of the oligonucleotide splint and the second post-circularization element comprises a region that is capable of hybridizing to a second region of the oligonucleotide splint. In some embodiments, the first circularization element comprises a first catalytic intron fragment, a first splice site dinucleotide, and a first exon fragment; and the second circularization element comprises a second catalytic intron fragment, a second splice site dinucleotide, and a second exon fragment. In some embodiments, the first catalytic intron fragment and the second catalytic intron fragment, together, are capable of self-splicing thereby covalently joining the first exon region and the second exon region to produce a circular polyribonucleotide. In some embodiments, the first catalytic intron fragment and the second catalytic intron fragment are from a cyanobacterium Anabaena pre-tRNA-Leu gene or a Tetrahymena pre-rRNA. In some embodiments, the first circularization element comprises a region that is capable of hybridizing to a first region of an oligonucleotide splint and the second circularization element comprises a region that is capable of hybridizing to a second region of the oligonucleotide splint. In another aspect the disclosure provides a DNA vector comprising an RNA polymerase promoter operably linked to a sequence that encodes any one of the linear polyribonucleotides described herein.

In another aspect the disclosure provides a circular polyribonucleotide produced from any one of the linear polyribonucleotides or from the DNA vector described herein. In another example the disclosure provides a pharmaceutical composition comprising any one of the linear polyribonucleotides, circular polyribonucleotides, or the DNA vector described herein and a pharmaceutically acceptable excipient.

In another example the disclosure provides a method of expressing an immunogen in a cell or a subject, the method comprising providing to the cell or the subject any one of the linear polyribonucleotides, the circular polyribonucleotides, the DNA vector, or the pharmaceutical composition described herein.

In another aspect the disclosure provides a method of producing a circular polyribonucleotide from any one of the linear polyribonucleotides described herein, the method comprising providing the linear polyribonucleotide under conditions suitable for self-splicing of the linear polyribonucleotide to produce a circular polyribonucleotide.

In another aspect, the disclosure provides an immunogenic composition comprising: (a) the circular polyribonucleotide of the disclosure, or the linear polyribonucleotide of the disclosure, and (b) a diluent, a carrier, an adjuvant, or any combination thereof.

Definitions

To facilitate the understanding of this disclosure, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the disclosure. Terms such as "a", "an," and "the" are not intended to refer to only a singular entity but include the general class of which a specific example may be used for illustration. The term "or" is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only