MODIFIED RIBONUCLEIC ACIDS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application 63/185,925 filed on May 7, 2021, and U.S. Provisional Patent Application 63/273,031 filed on October 28, 2021, the entire contents of each of which is hereby incorporated by reference.

BACKGROUND

[0002] RNA therapeutics is a new and emerging field.

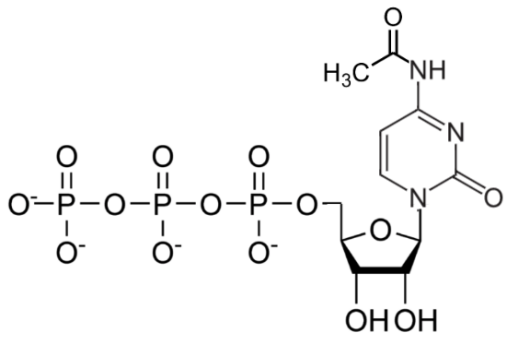
SUMMARY

[0002a] In an aspect of the present disclosure, there is provided a polyribonucleotide encoding a payload comprising a polypeptide, wherein the polyribonucleotide comprises a modified ribonucleotide having a nucleoside comprising an acetyl group, wherein the nucleoside is N4-acetylcytidine and the modified ribonucleotide has:

(a) a 5’ monophosphate;

(b) a 5’ diphosphate; or

(c) a 5’ triphosphate and a structure of:



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wherein the polyribonucleotide comprises cytidine residues and

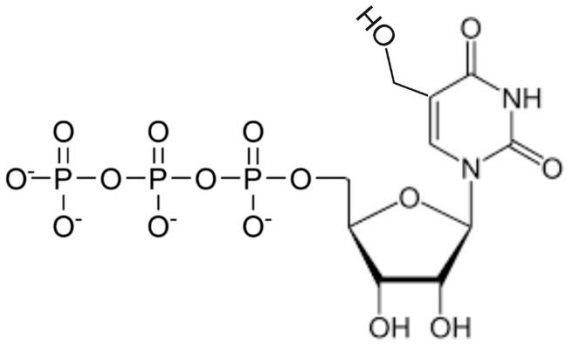
at least 25% of the cytidine residues comprise N4-acetylcytidine.

[0002b] In another aspect of the present disclosure, there is provided a polyribonucleotide encoding a payload comprising a polypeptide, wherein the polyribonucleotide comprises a modified ribonucleotide having a nucleoside comprising a hydroxymethyl group, wherein the nucleoside is 5-hydroxymethyluridine and the modified ribonucleotide has:

(a) a 5’ monophosphate;

(b) a 5’ diphosphate; or

(c) a 5’ triphosphate and a structure of:



wherein the polyribonucleotide comprises uridine residues and

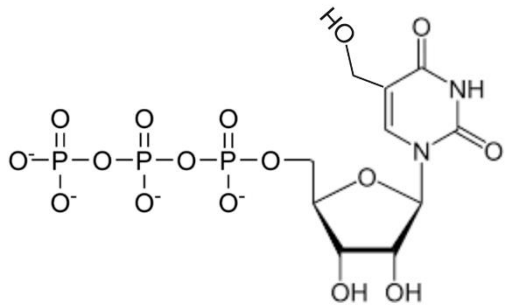
at least 25% of the uridine residues comprise 5-hydroxymethyluridine.

[0002c] In a further aspect of the present disclosure, there is provided a composition comprising the polyribonucleotide as disclosed herein.

[0002d] In another aspect of the present disclosure, there is provided a cell comprising a polyribonucleotide as disclosed herein.

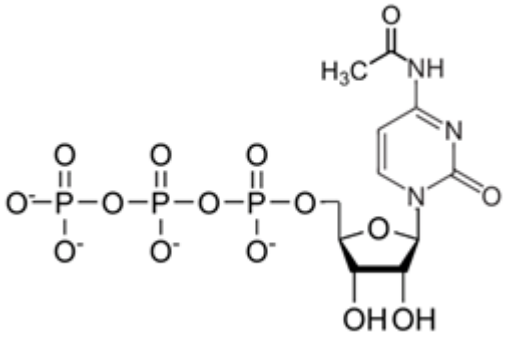
[0002e] In a further aspect of the present disclosure, there is provided a polyribonucleotide encoding a payload comprising a polypeptide, wherein the polyribonucleotide comprises a modified ribonucleotide comprising a nucleoside comprising:

(I) a hydroxymethyl group, wherein the nucleoside is 5-hydroxymethyluridine and the modified ribonucleotide has: (a) a 5’ monophosphate; (b) a 5’ diphosphate; or (c) a 5’ triphosphate and a structure of:



and,

(II) an acetyl group, wherein the nucleoside is N4-acetylcytidine and the modified ribonucleotide has:(a) a 5’ monophosphate; (b) a 5’ diphosphate; or (c) a 5’ triphosphate and a structure of:



wherein the polyribonucleotide comprises:

(i) cytidine residues and at least 25% of the cytidine residues comprise N4-acetylcytidine; and

(ii) uridine residues and at least 25% the uridine residues comprise 5-hydroxymethyluridine.

[0002f] In another aspect of the present disclosure, there is provided a composition comprising the polyribonucleotide as disclosed herein, wherein the composition is a pharmaceutical

composition comprising an immunogenic composition; a vaccine, a gene therapy, a chemotherapy, a protein replacement therapy, an immunotherapy, a cell engineering therapy, or a combination thereof.

[0003] The present disclosure identifies certain challenges with the use of RNA as therapeutics. For example, in some embodiments, the present disclosure identifies certain problems that can be encountered with immunogenicity caused by administration of RNA therapeutics, which can hamper efforts in using higher doses or repeated dosing of RNA therapeutics. Immunogenicity caused by administration of RNA therapeutics in response to the RNA molecules themselves should be contrasted with immunogenicity caused by, e.g., polypeptides encoded by the RNA molecules, which may be desirable as a result of, e.g., an RNA vaccine. The present disclosure also identifies challenges related to payload expression that can be encountered by administration of RNA therapeutics. For example, currently used RNA therapeutic modalities typically do not provide high and/or sustained expression of payloads encoded by the RNA.

[0004] Among other things, the present disclosure provides technologies for reducing immunogenicity of RNA therapeutics and/or increasing payload expression, by providing a polyribonucleotide comprising a modified ribonucleotide, e.g., as disclosed herein. In some embodiments, a modified ribonucleotide comprises a ribonucleotide comprising N4-acetylcytidine and/or a ribonucleotide comprising 5-hydroxymethyluridine. Without wishing to be bound by theory, the present disclosure proposes that a polyribonucleotide comprising N4-acetylcytidine and/or 5-hydroxymethyluridine can achieve reduced immunogenicity when

CLAIMS

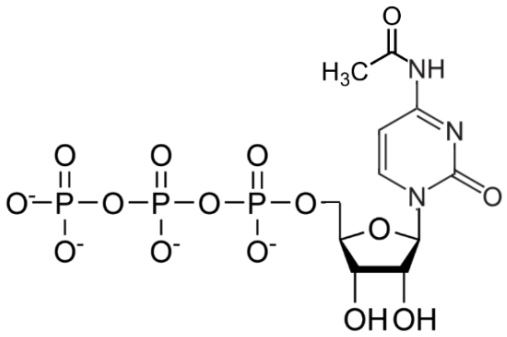
What is claimed is:

1. A polyribonucleotide encoding a payload comprising a polypeptide, wherein the polyribonucleotide comprises a modified ribonucleotide having a nucleoside comprising an acetyl group, wherein the nucleoside is N4-acetylcytidine and the modified ribonucleotide has:

(a) a 5’ monophosphate;

(b) a 5’ diphosphate; or

(c) a 5’ triphosphate and a structure of:



,

wherein the polyribonucleotide comprises cytidine residues and

at least 25% of the cytidine residues comprise N4-acetylcytidine.

2. The polyribonucleotide of claim 1, wherein:

less than 100% of cytidine residues in the polyribonucleotide comprise N4-acetylcytidine.

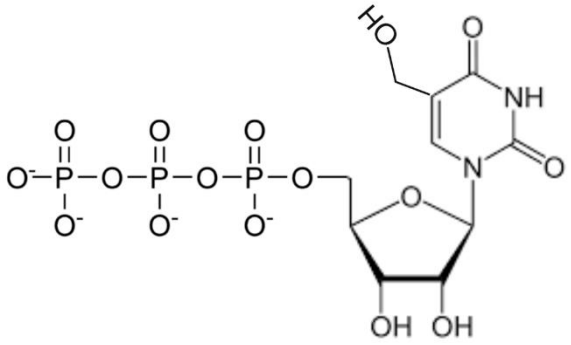
3. The polyribonucleotide of claim 1, wherein the polyribonucleotide further comprises one or more modified ribonucleotides other than N4-acetylcytidine.

4. The polyribonucleotide of claim 3, wherein the one or more modified ribonucleotides is 5-hydroxymethyluridine, and the modified ribonucleotide has:

(a) a 5’ monophosphate;

(b) a 5’ diphosphate; or

(c) a 5’ triphosphate and a structure of:



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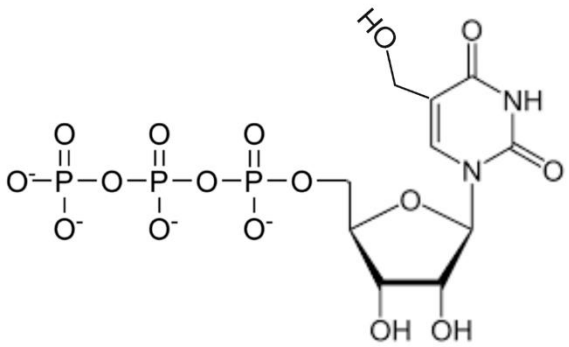
5. The polyribonucleotide of claim 1, characterized in that when assessed in a cell, tissue or an organism that has been administered the polyribonucleotide:
(a) reduced immunogenicity is observed relative to an appropriate reference comparator,
(b) increased cell viability is observed relative to an appropriate reference comparator, or
(c) both (a) and (b).

6. A polyribonucleotide encoding a payload comprising a polypeptide, wherein the polyribonucleotide comprises a modified ribonucleotide having a nucleoside comprising a hydroxymethyl group, wherein the nucleoside is 5-hydroxymethyluridine and the modified ribonucleotide has:

(a) a 5’ monophosphate;

(b) a 5’ diphosphate; or

(c) a 5’ triphosphate and a structure of:



wherein the polyribonucleotide comprises uridine residues and

at least 25% of the uridine residues comprise 5-hydroxymethyluridine.

7. The polyribonucleotide of claim 6, wherein:

(i) less than 100% of uridine residues in the polyribonucleotide comprise 5-hydroxymethyluridine;

(ii) more than 60% of uridine residues in the polyribonucleotide comprise 5-hydroxymethyluridine; or

(iii) a combination thereof.

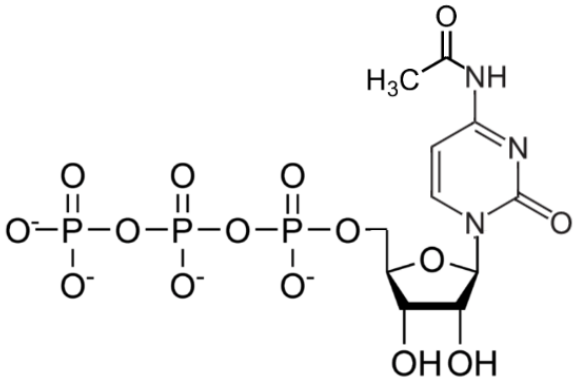
8. The polyribonucleotide of claim 6, wherein the polyribonucleotide further comprises one or more modified ribonucleotides other than 5-hydroxymethyluridine.

9. The polyribonucleotide of claim 8, wherein the one or more modified ribonucleotides is N4-acetylcytidine and the modified ribonucleotide has:

(a) a 5’ monophosphate;

(b) a 5’ diphosphate; or

(c) a 5’ triphosphate and a structure of:



.

10. The polyribonucleotide of claim 6, characterized in that when assessed in a cell, tissue, or organism that has been administered the polyribonucleotide:

(a) reduced immunogenicity is observed relative to an appropriate reference comparator,

(b)increased cell viability is observed relative to an appropriate reference comparator,

(c) increased expression of the payload is observed relative to an appropriate reference comparator, or

(d) a combination of (a)-(c).

11. The polyribonucleotide of claim 10, wherein reduced immunogenicity comprises:

(i) reduced activation of pathways of NFkb, IRF, or other cytokines resulting from inflammation in the cell, tissue or organism; or

(ii) reduced detection of uncapped RNA by a molecular sensor, wherein the molecular sensor is or comprises RIG-I;

(iii) both (i) and (ii).

12. The polyribonucleotide of claim 10, wherein reduced immunogenicity allows for:
(i) repeated dosing of the polyribonucleotide;
(ii) administration of a higher dose of the polyribonucleotide as compared to an appropriate reference comparator; or
(iii) both (i) and (ii).

13. The polyribonucleotide of claim 12, wherein the reference comparator comprises an otherwise similar cell, tissue or organism that has been administered a comparable polyribonucleotide that includes:
(i) fewer N4-acetylcytidine nucleosides ;
(ii) fewer 5-hydroxymethyluridine nucleosides ; or
(iii) or both (i) and (ii).

14. The polyribonucleotide of claim 10, wherein the increase in expression of the payload is about 1.2-fold to about 20-fold compared to the reference comparator.

15. A composition comprising the polyribonucleotide of claim 1.

16. The composition of claim 15, wherein the composition is a pharmaceutical composition

comprising an immunogenic composition; a vaccine, a gene therapy, a chemotherapy, a protein replacement therapy, an immunotherapy, a cell engineering therapy, or a combination thereof.

17. A cell comprising a polyribonucleotide according to claim 1.

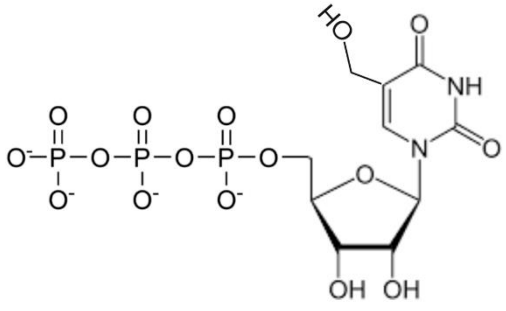
18. A composition comprising the polyribonucleotide of claim 6.

19. The composition of claim 18, wherein the composition is a pharmaceutical composition comprising an immunogenic composition; a vaccine, a gene therapy, a chemotherapy, a protein replacement therapy, an immunotherapy, a cell engineering therapy, or a combination thereof.

20. A cell comprising a polyribonucleotide according to claim 6.

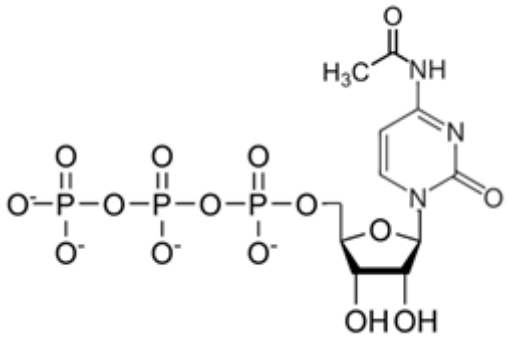
21. A polyribonucleotide encoding a payload comprising a polypeptide, wherein the polyribonucleotide comprises a modified ribonucleotide comprising a nucleoside comprising:

(I) a hydroxymethyl group, wherein the nucleoside is 5-hydroxymethyluridine and the modified ribonucleotide has: (a) a 5’ monophosphate; (b) a 5’ diphosphate; or (c) a 5’ triphosphate and a structure of:



and,

(II) an acetyl group, wherein the nucleoside is N4-acetylcytidine and the modified ribonucleotide has:(a) a 5’ monophosphate; (b) a 5’ diphosphate; or (c) a 5’ triphosphate and a structure of:



wherein the polyribonucleotide comprises:

(i) cytidine residues and at least 25% of the cytidine residues comprise N4-acetylcytidine; and

(ii) uridine residues and at least 25% of the uridine residues comprise 5-hydroxymethyluridine.

22. The polyribonucleotide of claim 21, wherein:

(a) the polyribonucleotide comprises cytidine residues and at least 50% of cytidine residues in the polyribonucleotide comprise N4-acetylcytidine;

(b) the polyribonucleotide comprises uridine residues and at least 50% of uridine residues in the polyribonucleotide comprise 5-hydroxymethyluridine; or

(c) both (a) and (b).

23. The polyribonucleotide of claim 21, characterized in that when assessed in a cell, tissue, or organism that has been administered the polyribonucleotide:

(a) reduced immunogenicity is observed relative to an appropriate reference comparator,

(b) increased cell viability is observed relative to an appropriate reference comparator,

(c) increased expression of the payload is observed relative to an appropriate reference comparator, or

(d) a combination of (a)-(c).

24. The polyribonucleotide of claim 1, wherein at least 50% of the cytidine residues comprise N4-acetylcytidine.

25. The polyribonucleotide of claim 1, wherein at least 75% of the cytidine residues comprise N4-acetylcytidine.

26. The polyribonucleotide of claim 6, wherein at least 50% of the uridine residues comprise 5-hydroxymethyluridine.

27. The polyribonucleotide of claim 6, wherein at least 75% of the uridine residues comprise 5-hydroxymethyluridine.

28. The polyribonucleotide of claim 21, wherein:

(a) the polyribonucleotide comprises cytidine residues and at least 90% of cytidine residues in the polyribonucleotide comprise N4-acetylcytidine;

(b) the polyribonucleotide comprises uridine residues and at least 90% of uridine residues in the polyribonucleotide comprise 5-hydroxymethyluridine; or

(c) both (a) and (b).

29. The polyribonucleotide of claim 21, wherein 100% of the cytidine residues comprise N4-acetylcytidine and 100% of the uridine residues comprise 5-hydroxymethyluridine.

30. A composition comprising the polyribonucleotide of claim 21, wherein the composition is a pharmaceutical composition comprising an immunogenic composition; a vaccine, a gene therapy, a chemotherapy, a protein replacement therapy, an immunotherapy, a cell engineering therapy, or a combination thereof.