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(71) Applicant: **EASTERN VIRGINIA MEDICAL SCHOOL** [US/US]; 735 Fairfax Ave., Suite 1008C, Norfolk, Virginia 23507 (US).

(72) Inventors: **BRACHOVA, Pavla**; c/o Eastern Virginia Medical School, 735 Fairfax Ave., Suite 1008C, Norfolk, Virginia 23507 (US). **ALVAREZ, Nehemiah Seth**; c/o Eastern Virginia Medical School, 735 Fairfax Ave., Suite 1008C, Norfolk, Virginia 23507 (US).

(74) Agent: **ANDREANSKY, Eric S.** et al.; Meunier Carlin & Curfman LLC, 999 Peactree Street NE, Suite 1300, Atlanta, Georgia 30309 (US).

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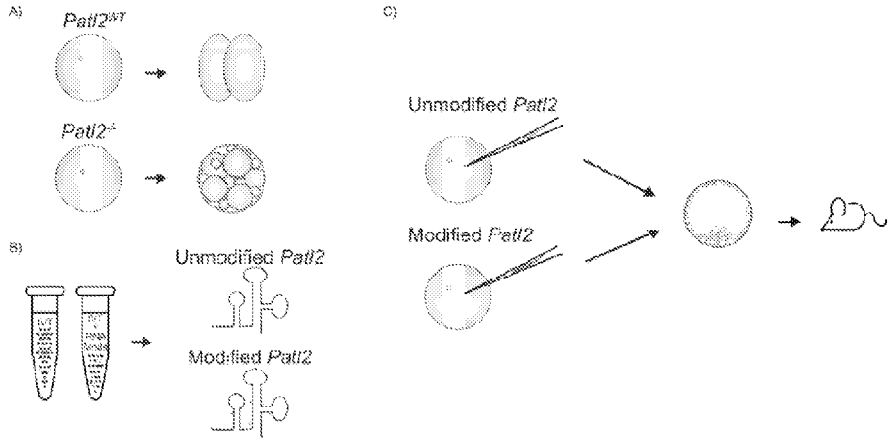


FIG. 1

(57) Abstract: The present disclosure provides synthetic mRNA a coding region encoding a protein involved in oocyte maturation or a variant thereof. Methods of using the disclosed synthetic mRNA in oocyte maturation or in vitro fertilization are also provided.

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MRNA THERAPEUTICS FOR OOCYTE MATURATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to United States Provisional Patent Application No. 63/454,833 filed March 27, 2023, the disclosure of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

This disclosure relates to compositions and methods for fertility treatments, and more particularly to messenger RNA therapeutics for oocyte maturation.

BACKGROUND

Infertility is a major reproductive health issue that affects ~12% of women of reproductive age in the United States. In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) fertility treatments rely on mature oocytes that are retrieved from women and fertilized with sperm in vitro. The ensuing embryos are transplanted into the individual's uterus to establish pregnancy. Unfortunately, some women suffer from recurrent oocyte maturation arrest, and although oocytes can be retrieved, they remain immature and thus, fertilization is not possible. Women who experience oocyte maturation arrest currently have no fertility options for having their own children.

Oocyte maturation occurs when a germinal vesicle (GV) oocyte progresses to metaphase I (MI) and ultimately to metaphase II (MII). Successful oocyte maturation depends on achieving both nuclear and cytoplasmic maturation to support fertilization and development, which occurs in the absence of transcription. Advances of genome sequencing during the last decade have brought insight into the causes of recurrent oocyte arrest, with 14 described pathologic genetic variants leading to female infertility. As might be expected, several genes involved in meiosis result in recurrent oocyte arrest if they are altered, such as SYCP3, TRIP13, MCM8, STAG3, PATL2, TUBB8, AURKC, and WEE2.

RNA drugs make up two general categories of molecules: 1) antisense RNA (RNAi), in which short oligonucleotides bind to complementary sequences in RNA transcripts and typically result in degradation; and 2) mRNA encoding certain peptides or proteins that are transiently expressed in the cytoplasm. The use of RNA therapeutics requires several hurdles to be overcome: 1) avoiding rapid degradation of the RNA via RNases, 2) delivery

of negatively charged RNA molecules that a cannot pass across hydrophobic cell membranes, and 3) strong cellular immunogenicity caused by encountering exogenous RNA.

Microinjection of RNA into oocytes has a long-standing history and is an important research tool to understand oocyte maturation and early embryo development in vitro. The use of RNA as therapeutic faces several roadblocks linked to the susceptibility of RNA to enzymatic degradation, the immunogenicity of in vitro transcribed RNA, and the efficiency of uptake by the recipient cells. Immune activation of in vitro transcribed mRNA was overcome by incorporating modified nucleosides, and the improvement of the delivery of mRNA therapeutics as well as protection from nucleases has greatly accelerated the route of mRNA therapeutics into the clinic. Today, a number of in vitro transcribed mRNA therapeutics are in preclinical and clinical trials, and a few, such as the SARS-CoV-2 mRNA vaccines, have been approved by the FDA. The use of RNA as a therapy has only recently been applied to a clinical in vitro fertilization setting.

The injection of Wee2 in vitro synthesized RNA during ICSI into the oocytes of women with pathogenic Wee2 variants generated successful blastocysts in women with repeated IVF failures. Similar successful results were seen in women with variants in the gene Thyroid hormone receptor interactor 13 (Trip13). Injection of Trip13 RNA occurred at the time of egg collection and was followed by ICSI. 7 out of 22 collected eggs made blastocysts, while none of the uninjected controls even generated pronuclei following ICSI.

There is a clear need for additional therapeutic tools that can be used to induce oocyte maturation and subsequent use of the generated ovum in fertilization methods. This disclosure addresses this as well as other needs.

SUMMARY

The present disclosure provides compositions and methods of making and using said compositions. In particular, the present disclosure provides synthetic mRNA and compositions thereof which may find use in inducing oocyte maturation, further finding applications in various fertility treatments.

In one aspect, a synthetic mRNA is provided. In some aspects, the synthetic mRNA can comprise a coding region encoding a protein involved in oocyte maturation or a variant thereof that is 80% or more homologous. In some aspects, the synthetic mRNA can further comprise at least one chemical modification to a nucleotide. In some aspects, the protein

involved in oocyte maturation can be selected from SYCP3, TRIP13, MCM8, STAG3, PATL2, TUBB8, AURKC, and WEE2. In some aspects, the protein involved in oocyte maturation can be PATL2. In some aspects, the coding region can comprise an RNA sequence encoded by a gene selected from Table 1 below, or a variant thereof that is 80% or more homologous, wherein at least one nucleotide in the RNA sequence has been chemically modified.

In some aspects, the coding region can comprise an RNA sequence selected from SEQ ID NO:1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, and SEQ ID NO: 13, or a variant thereof that is 80% or more homologous, wherein at least one nucleotide the sequence has been chemically modified. In some aspects, the coding region can comprise a sequence of SEQ ID NO: 7, or a variant thereof that is 80% or more homologous, wherein at least one nucleotide the sequence has been chemically modified. In some aspects, the coding region can encode a polypeptide having a sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 14, or a variant thereof that is 80% or more homologous. In some aspects, the coding region can encode a polypeptide having a sequence of SEQ ID NO: 8, or a variant thereof that is 80% or more homologous.

In some aspects, the at least one chemical modification of a nucleoside can comprise: N4-acetylcytidine triphosphate (N4-acetyl-CTP), inosine triphosphate (ITP), 5-methylcytidine-5'-triphosphate (5-methyl-CTP), pseudouridine-5'-triphosphate (Pseudo-UTP), N1-methyladenosine-5'-triphosphate (N1-methyl-ATP), N1-methyl-pseudouridine-5'-triphosphate (N1-methylpseudo-UTP), 2-thiouridine-5'-triphosphate (2-Thio-UTP), 5-methoxyuridine-5'-triphosphate (5-Methoxy-UTP), 7-methylguanosine, 2'-O-7-methylguanosine, or combinations thereof. In some aspects, the synthetic mRNA can further comprise a human-derived or synthetic 5' untranslated region (5' UTR). In some aspects, the 5' UTR can comprise a sequence selected from SEQ ID NO: 16 to SEQ ID NO: 33, or a variant thereof that is 80% or more homologous. In some aspects, the 5' UTR can be operably linked to a 5' end of the coding region. In some aspects, the synthetic mRNA can further comprise a human-derived or synthetic 3' untranslated region (3' UTR). In some aspects, the 3' UTR can comprise a sequence selected from SEQ ID NO: 34 to SEQ ID NO: 51, or a variant thereof that is 80% or more homologous. In some aspects, the 3' UTR can be operably linked to a 3' end of the coding region. In some aspects, the synthetic mRNA can further comprise a 5' terminal cap operably linked to a 5' end of the mRNA. In some

aspects, the 5' terminal cap can have a cap-1 structure. In some aspects, the synthetic mRNA can further comprise a 3' poly(A) tail operably linked to a 3' end of the mRNA.

In another aspect, therapeutic compositions are provided. In some aspects, the therapeutic composition comprises a synthetic mRNA described herein formulated within a delivery vehicle. In some aspects, the delivery vehicle can be a liposome, a lipoplex, a lipid nanoparticle, a polymer, or a polymeric nanoparticle.

In another aspect, a method of inducing maturation in an oocyte is provided. In some aspects, the method can comprises contacting the oocyte with a synthetic mRNA or a therapeutic composition described herein.

In another aspect, a method of fertilization is provided. In some aspects, the method can comprise contacting an oocyte with a synthetic mRNA or a therapeutic composition described herein, whereupon the oocyte undergoes maturation into an ovum. In some aspects, the method can further comprise fertilizing the ovum with a sperm to form a zygote. In some aspects, fertilizing the ovum with the sperm can comprise contacting the ovum with the sperm in a culture medium. In some aspects, fertilizing the ovum can comprise intracytoplasmic injection of the sperm into the ovum.

In some aspects, the oocyte can be collected from an ovary of a first subject. In some aspects, the first subject can be a human. In some aspects, the oocyte is a human oocyte. In some aspects, the method can further comprise the zygote or embryo formed therefrom within a uterus of a second subject. In some aspects, the second subject can be the same as the first subject. In some aspects, the first subject exhibits oocyte maturation arrest.

The details of one or more aspect of the disclosure are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the disclosure will be apparent from the description, the drawings, and the claims.

DESCRIPTION OF DRAWINGS

FIG. 1 depicts the experimental approach for analyzing modification of mRNA on oocyte maturation as described in the examples. A) Oocytes from mice deficient in Patl2 arrest during meiosis in both mice and women. B) Patl2 mRNA generated to rescue oocyte maturation using in vitro transcription (IVT) with canonical RNA bases or modified RNA bases (represented by colored dots). C) Patl2 with unmodified bases or different

combinations of modified bases microinjected into Patl2-/- GV oocytes, which are in vitro fertilized, and blastocysts transferred to recipient moms to test fertility (live birth) rates.

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

The following description of the disclosure is provided as an enabling teaching of the disclosure in its best, currently known aspects. Many modifications and other aspects disclosed herein will come to mind to one skilled in the art to which the disclosed compositions and methods pertain, benefiting from the teachings presented in the descriptions herein and the associated drawings. Therefore, it is understood that the disclosures are not limited to the specific aspects disclosed and that modifications and other aspects are intended to be included within the scope of the appended claims. The skilled artisan will recognize many variants and adaptations of the aspects described herein. These variants and adaptations are intended to be included in the teachings of this disclosure and to be encompassed by the claims herein.

Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

As apparent to those of skill in the art upon reading this disclosure, each of the individual aspects described and illustrated herein has discrete components and features that may be readily separated from or combined with the features of any of the other several aspects without departing from the scope or spirit of the present disclosure.

Any recited method can be carried out in the order of events recited or any other order that is logically possible. Unless otherwise expressly stated, it is in no way intended that any method or aspect set forth herein be construed as requiring that its steps be performed in a specific order. Accordingly, where a method claim does not explicitly state in the claims or descriptions that the steps are to be limited to a particular order, it is in no way intended that an order be inferred in any respect. This holds for any possible non-express basis for interpretation, including logic concerning arrangement of steps or operational flow, meaning derived from grammatical organization or punctuation, or the number or type of aspects described in the specification.

All publications mentioned herein are incorporated by reference to disclose and describe the methods or materials in connection with which the publications are cited. The

publications discussed herein are provided solely for their disclosure before the filing date of the present application. The dates of publication provided herein can be different from the actual publication dates, which can require independent confirmation.

It is also to be understood that the terminology herein describes particular aspects only and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosed compositions and methods belong. It can be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the specification and relevant art and should not be interpreted in an idealized or overly formal sense unless expressly defined herein.

Before describing the various aspects of the present disclosure, the following definitions are provided and should be used unless otherwise indicated. Additional terms may be defined elsewhere in the present disclosure.

Definitions

As used herein, "comprising" is interpreted as specifying the presence of the stated features, integers, steps, or components but does not preclude the presence or addition of one or more features, integers, steps, components, or groups thereof. Moreover, each of the terms "by," "comprising," "comprises," "comprised of," "including," "includes," "included," "involving," "involves," "involved," and "such as" are used in their open, non-limiting sense and may be used interchangeably. Further, the term "comprising" is intended to include examples and aspects encompassed by the terms "consisting essentially of" and "consisting of." Similarly, "consisting essentially of" is intended to include examples encompassed by the term "consisting of."

As used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context dictates otherwise.

Ratios, concentrations, amounts, and other numerical data can be expressed herein in a range format. Further, the endpoints of each of the ranges are significant both in relation to the other endpoint and independently of the other endpoint. There are many values disclosed herein, and each value is also disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. Ranges can be expressed herein as from "about" one particular value and to

"about" another particular value. Similarly, when values are expressed as approximations, using the antecedent "about," the particular value forms a further aspect. For example, if the value "about 10" is disclosed, then "10" is also disclosed.

When a range is expressed, a further aspect includes from the one particular value and to the other particular value. For example, where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure, e.g., the phrase "x to y" includes the range from 'x' to 'y' as well as the range greater than 'x' and less than 'y'. The range can also be expressed as an upper limit, e.g. 'about x, y, z, or less' and should be interpreted to include the specific ranges of 'about x,' 'about y,' and 'about z' as well as the ranges of 'less than x,' 'less than y.' and 'less than z.' Likewise, the phrase 'about x, y, z, or greater' should be interpreted to include the specific ranges of 'about x,' 'about y,' and 'about z' as well as the ranges of 'greater than x,' greater than y,' and 'greater than z.' In addition, the phrase "about 'x' to 'y'," where 'x' and 'y' are numerical values, includes "about 'x' to about 'y'."

Such a range format is used for convenience and brevity and thus, should be interpreted flexibly to include not only the numerical values explicitly recited as the limits of the range but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited. To illustrate, a numerical range of "about 0.1% to 5%" should be interpreted to include not only the explicitly recited values of about 0.1% to about 5%, but also include individual values (e.g., about 1%, about 2%, about 3%, and about 4%) and the sub-ranges (e.g., about 0.5% to about 1.1%; about 5% to about 2.4%; about 0.5% to about 3.2%, and about 0.5% to about 4.4%, and other possible sub-ranges) within the indicated range.

As used herein, the terms "about," "approximate," "at or about," and "substantially" mean that the amount or value in question can be the exact value or a value that provides equivalent results or effects as recited in the claims or taught herein. That is, amounts, sizes, formulations, parameters, and other quantities and characteristics are not and need not be exact but may be approximate, larger or smaller, as desired, reflecting tolerances, conversion factors, rounding, measurement error, and the like, and other factors known to those of skill in the art such that equivalent results or effects are obtained. In some circumstances, the value that provides equivalent results or effects cannot be reasonably determined. In such cases, as used herein, "about" and "at or about" mean the nominal value indicated ±10% variation unless otherwise indicated or inferred. In general, an amount, size,

formulation, parameter, or other quantity or characteristic is "about," "approximate," or "at or about," whether or not expressly stated to be such. Where "about," "approximate," or "at or about" is used before a quantitative value, the parameter also includes the specific quantitative value itself unless expressly stated otherwise.

As used herein, the term "therapeutically effective amount" refers to an amount sufficient to achieve the desired therapeutic result or to have an effect on undesired symptoms but generally insufficient to cause adverse side effects. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors, including the disorder being treated and the severity of the disorder; the specific composition employed; the age, body weight, general health, sex, and diet of the patient; the time of administration; the route of administration; the rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the particular compound employed and like factors within the knowledge and expertise of the health practitioner and which may be well known in the medical arts. In the case of treating a particular disease or condition, in some instances, the desired response can be inhibiting the progression of the disease or condition. This may involve only slowing the progression of the disease temporarily. However, in other instances, it may be desirable to permanently halt the progression of the disease. This can be monitored by routine diagnostic methods known to one of ordinary skill in the art for any particular disease. The desired response to treatment of the disease or condition can also be delaying the onset or even preventing the onset.

For example, it is well within the skill of the art to start doses of a compound at levels lower than those required to achieve the desired therapeutic effect and to increase the dosage gradually until the desired effect is achieved. If desired, the effective daily dose can be divided into multiple doses for administration. Consequently, single dose compositions can contain such amounts or submultiples thereof to make up the daily dose. The individual physician can adjust the dosage in the event of any contraindications. It is generally preferred that a maximum dose of the pharmacological agents of the disclosure (alone or in combination with other therapeutic agents) be used, that is, the highest safe dose according to sound medical judgment. However, a patient may insist on a lower or tolerable dose for medical reasons, psychological reasons, or virtually any other reason.

A response to a therapeutically effective dose of a disclosed compound or composition can be measured by determining the physiological effects of the treatment or

medication, such as the decrease or lack of disease symptoms following the administration of the treatment or pharmacological agent. Other assays will be known to one of ordinary skill in the art and can be employed for measuring the level of the response. The amount of a treatment may be varied, for example, by increasing or decreasing the amount of a disclosed compound or pharmaceutical composition, changing the disclosed compound or pharmaceutical composition administered, changing the route of administration, changing the dosage timing, and so on. Dosage can vary and can be administered in one or more dose administrations daily for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products.

As used herein, "optional" or "optionally" means that the subsequently described event or circumstance can or cannot occur. The description includes instances where said event or circumstance occurs and those where it does not.

As used interchangeably herein, "subject," "individual," or "patient" can refer to a vertebrate organism, such as a mammal (e.g., human). "Subject" can also refer to a cell, a population of cells, a tissue, an organ, or an organism, preferably to a human and constituents thereof.

As used herein, "treating" and "treatment" generally refer to obtaining a desired pharmacological or physiological effect. The effect can be but does not necessarily have to be prophylactic in preventing or partially preventing a disease, symptom, or condition. The effect can be therapeutic regarding a partial or complete cure of a disease, condition, symptom, or adverse effect attributed to the disease, disorder, or condition. The term "treatment" as used herein can include any treatment of a disorder in a subject, particularly a human. It can include any one or more of the following: (a) preventing the disease from occurring in a subject who may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., mitigating or ameliorating the disease or its symptoms or conditions. The term "treatment," as used herein, can refer to both therapeutic treatment alone, prophylactic treatment alone, or both therapeutic and prophylactic treatment. Those in need of treatment (i.e., subjects in need thereof) can include those already with the disorder or those in which the disorder is to be prevented. As used herein, the term "treating" can include inhibiting the disease, disorder, or condition, e.g., impeding its progress; and relieving the disease, disorder, or condition, e.g., causing regression of the disease, disorder, or condition. Treating the disease, disorder, or condition can include ameliorating at least one symptom of

the particular disease, disorder, or condition, even if the underlying pathophysiology is not affected, e.g., such as treating the pain of a subject by administration of an analgesic agent even though such agent does not treat the cause of the pain.

As used herein, "dose," "unit dose," or "dosage" can refer to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of a disclosed compound or a therapeutic composition thereof calculated to produce the desired response or responses in association with its administration.

As used herein, "therapeutic" can refer to treating, healing, or ameliorating a disease, disorder, condition, or side effect or decreasing the rate of advancement of a disease, disorder, condition, or side effect.

As used herein, the term or phrase "effective," "effective amount," or "conditions effective to" refers to such amount or condition that is capable of performing the function or property for which an effective amount or condition is expressed. As will be pointed out below, the exact amount or particular condition required will vary from one aspect to another, depending on recognized variables such as the materials employed and the processing conditions observed. Thus, it is not always possible to specify an exact "effective amount" or "condition effective to." However, it should be understood that an appropriate effective amount will be readily determined by one of ordinary skill in the art using only routine experimentation. Although the operations of exemplary aspects of the disclosed method may be described in a particular sequential order for convenient presentation, it should be understood that disclosed aspects can encompass an order of operations other than the particular sequential order disclosed. For example, operations described sequentially may, in some cases, be rearranged or performed concurrently. Further, descriptions and disclosures provided in association with one particular aspect are not limited to that aspect and may be applied to any aspect disclosed.

As used herein, "homology" refers to overall relatedness between polymeric molecules, e.g. between nucleic acid molecules (e.g. DNA molecules and/or RNA molecules) and/or polypeptide molecules. In some aspects, polymeric molecules are "homologous" to one another if their sequences are at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical or similar. The term "homologous" necessarily refers to a comparison between at least two sequences (polynucleotide or polypeptide sequences). In accordance with the disclosure, two polynucleotide sequences

are considered to be homologous if the polypeptides they encode are at least about 50%, 60%, 70%, 80%, 90%, 95, or even 99% identical or similar for at least about 20 amino acids. In accordance with the present disclosure, two polypeptide sequences are considered to be homologous if the polypeptides are at least about 50%, 60%, 70%, 80%, 90%, 95, or even 99% identical or similar for at least about 20 amino acids.

Another way of calculating homology can be performed by published algorithms. Optimal alignment of protein sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, *J. MoL Biol.* 48: 443 (1970), by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection. The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989. It is understood that the description of conservative mutations and homology can be combined together in any combination, such as aspects that have at least 70% homology to a particular sequence wherein the variants are conservative mutations.

The term "encapsulate" as used herein means to enclose, surround or encase. As it relates to compositions of the present disclosure, encapsulation may be substantial, complete, or partial. The term "substantially encapsulated" means that at least greater than 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99, or greater than 99.999% of the composition may be enclosed, surrounded, or encased within a delivery vehicle. "Partially encapsulated" means that less than 10, 20, 30, 40, 50, or less of the composition may be enclosed, surrounded, or encased within a delivery vehicle. Advantageously, encapsulation may be determined by measuring the escape or the activity of the composition by using fluorescence and/or electron micrography. For example, at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.9, 99.99, or greater than 99.999% of the composition are encapsulated in the delivery vehicle.

The term "messenger RNA" (mRNA) as used herein refers to any polynucleotide which encodes a polypeptide of interest, or variants thereof as described herein, and which

is capable of being translated to produce the encoded polypeptide *in vitro*, *in vivo*, *in situ*, or *ex vivo*.

As used herein, "modified" refers to a changed state or structure of a molecule of the disclosure. Molecules may be modified in many ways including chemically, structurally, and functionally. In one aspect, the polynucleotides of the present disclosure are "chemically modified" by the introduction of non-natural nucleosides and/or nucleotides, e.g., as it relates to the natural ribonucleotides A, U, G, and C. Modifications of the nucleosides and/or nucleotides as used in the present disclosure may be naturally occurring (i.e., comprise a nucleotide and/or nucleoside other than the natural ribonucleotides A, U, G, and C) or may be artificial. Non-canonical nucleotides such as the cap structures are not considered "modified" although they differ from the chemical structure of A, G, C, and U ribonucleotides. As used herein, a "structural" modification is one in which two or more linked nucleosides are inserted, deleted, duplicated, inverted or randomized in a polynucleotide without significant chemical modification to the nucleotides themselves. Because chemical bonds will necessarily be broken and reformed to affect a structural modification, structural modifications are of a chemical nature and hence are chemical modifications. However, structural modifications will result in a different sequence of nucleotides. When the polynucleotides of the present disclosure are chemically and/or structurally modified, the polynucleotides may be referred to as "modified nucleotides".

As used herein, "operably linked," when referring to a first nucleic acid sequence that is operably linked with a second nucleic acid sequence, means a situation when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence.

The term "synthetic" means produced, prepared, and/or manufactured by the hand of man. Synthesis of polynucleotides or other molecules of the present disclosure may be chemical or enzymatic.

As used herein, the term "transfection" refers to methods to introduce exogenous nucleic acids into a cell. Methods of transfection include, but are not limited to, chemical methods, physical treatments, cationic lipids or mixtures, or the like.

As used herein, "translation" is the process by which mRNA is processed by a ribosome or ribosomal-like machinery, e.g., cellular or artificial, to produce a peptide or polypeptide.

As used herein, "unmodified" refers to any substance, compound, or molecule prior to being changed in any way. Unmodified may, but does not always, refer to the wild type or native form of a biomolecule. Molecules may undergo a series of modifications whereby each modified molecule may serve as the "unmodified" starting molecule for a subsequence modification.

Synthetic Messenger RNA (mRNA) Compositions

The present disclosure relates to a method to increase human GV oocyte maturation rates in an assisted reproductive technology setting using a class of RNA therapeutics, called mRNA therapeutics. Oocyte in vitro maturation (IVM) is an assisted reproductive technology designed to obtain mature oocytes following culture of immature GV oocytes or cumulus–oocyte complexes collected from antral follicles. In vitro maturation can be performed clinically for women with or without the ovulatory hCG trigger, by in vitro maturation of GV oocytes prior to hCG treatment, or in vitro maturation of GV oocytes that have failed to mature following the hCG trigger.

The present disclosure relates to an mRNA therapeutic that is an in vitro synthesized wild-type mRNA that may contain all the features of an endogenous RNA which include a 5' and 3' UTR, coding sequence, and poly(A) tail. Additionally, the mRNA therapeutic may contain modified nucleic acids. The codified nucleic acids could occur as a single/multiple type modified base(s) as a specific location or locations within the synthetic mRNA.

The mRNA therapeutic can represent a mixture of modified and unmodified synthetic mRNA molecules, or a pure mixture of modified synthetic mRNA. The modifications to the synthetic mRNA can include: N4-acetyl-CTP, ITP, 5-methyl-CTP, Pseudo-UTP, N1-methyl-ATP, N1-methylpseudo-UTP, 2-Thio-UTP, 5-Methoxy-UTP, 7-methylguanosine, 2`-O-7-methylguanosine.

Many proteins have been experimentally determined to play a role in mammalian oocyte maturation. These include but are not limited to genes listed in Table 1 herein. In humans, the proteins SYCP3, TRIP13, MCM8, STAG3, PATL2, TUBB8, AURKC, and WEE2 have been implicated in oocyte maturation defects, however, oocyte maturation defects are not limited to these proteins. The present disclosure relates to a method to treat oocyte maturation defects with a synthetic mRNA therapeutic containing specific RNA modifications. The mRNA therapeutic can be used to rescue or enhance oocyte maturation

by expressing endogenous proteins necessary for oocyte maturation, which include but are not limited to those proteins disclosed here.

The present disclosure also relates to the specific RNA modification(s) that regulate the stability and translation of synthetic mRNA therapeutics injected into oocytes regardless of the underlying protein the mRNA therapeutic encodes. RNA modifications impact the translation, stability, and immunogenicity of RNA. Oocytes have a unique RNA environment and particular types of RNA modifications may be required for RNA processing in oocytes.

In vitro maturation (IVM) has been practiced for decades as a research tool and is no longer considered experimental, but the uptake of IVM in the clinic is currently limited. In vitro maturation is used clinically in limited locations as a treatment option to reduce the risk of ovarian hyperstimulation syndrome associated with ovarian stimulation, such as for women with polycystic ovary syndrome. IVM can also be used to help eggs mature that have failed to mature following ovarian stimulation. However, the process of IVM is inefficient, with a success rate of around 32%, compared with around 40% for a single cycle of IVF.

Clinically, during IVM, immature eggs are placed in a cell culture and are stimulated with hormones until they reach maturity. New research attempts to replace the hormones with added proteins, such as cumulin and c-AMP until the oocytes reach maturity. However, the rates of oocyte maturation remain low. The use of mRNA therapeutics to promote GV oocyte maturation solves this issue by promoting human GV oocyte maturation by providing oocytes a means to translate proteins necessary for maturation in situations where endogenous mRNA translated products are defective or deficient. The use of mRNA therapeutics is transient; the mRNA is subsequently degraded over the course of hours through natural molecular machinery of the oocyte.

The disclosed compositions and methods can be used in a fertility clinic where assisted reproductive technology (ART) is utilized. ART can be in the form of ICSI or IVF. After oocyte retrieval, immature GV or MII oocytes can be injected with an mRNA therapeutic that enhances maturation rates. This is especially important in clinical situations where patients produce only immature GV oocytes or very few MII oocytes. The use of mRNA therapy can increase the available MII oocytes that can be used in ICSI or IVF, increasing the procedure success rate. There are a few groups of women who could benefit

from using RNA therapeutics for in vitro maturation: women sensitive to hormones or who are prone to ovarian hyperstimulation syndrome, such as with polycystic ovarian syndrome; women who are recovering or in remission from cancer, since hormonal stimulation could stimulate cancer cells; women seeking alternative options due to financial reasons, since IVM is lower cost and has less associated risks than traditional IVF; women who undergo IVF but all collected eggs are immature, including women with genetic causes of oocyte arrest; women with ovulation disorders, premature ovarian failure, or uterine fibroids; couples or women with unexplained infertility; women with blocked, damaged, or removed fallopian tubes; and male factor infertility.

Messenger RNA (mRNA) Architecture

The present disclosure provides messenger RNA (mRNA) which encodes a protein involved in oocyte maturation and natural or artificial variants thereof. The mRNA may have any of the features described herein.

Traditionally, the basic components of an mRNA molecule include at least a coding region, a 5' untranslated region (5' UTR), a 3' untranslated region (3' UTR), a 5' terminal cap and a polyadenylate tail. The mRNA of the present disclosure may include one or more modifications from the naturally occurring mRNA transcript for the polypeptide of interest. The modified mRNA of the present disclosure are distinguished from wild-type mRNA in their functional and/or structural design features, which may serve to overcome existing problems of effective polypeptide production using nucleic-acid based therapeutics, while still maintaining the desired activity in inducing oocyte maturation. It is to be understood that the protein of interest, and natural or artificial variants thereof, may be expressed in a human cell, such as an oocyte, using the modified mRNA as described herein.

The mRNA described herein contains a first region of linked nucleotides that is operably linked to a first flanking region on the 5' end and a second flanking region at the 3' end. In typical aspects, the first region comprises the encoding sequence for the polypeptide of interest. The first flanking region may comprise a region of linked nucleotides comprising one or more 5' UTR sequences. The first flanking region may include at least one nucleic acid sequence including, for example, translation control sequences, for example a Kozak consensus sequence. The first flanking region may also include a 5' terminal cap at the terminal 5' base location. The 5' terminal capping region may include a naturally occurring cap, a synthetic cap, or an optimized cap. In certain aspects, the 5' cap is

enzymatically added. Non-limiting examples of optimized caps include the caps taught by Rhoads in U.S. Pat. No. 7,074,596 and International Patent Publication No. WO2008157668, WO2009149253 and WO2013103659, the contents of each of which are herein incorporated by reference in their entirety. The second flanking region may comprise a region of linked nucleotides comprising one or more 3' UTR sequences. The second flanking region may include at least one nucleic acid sequence including, but not limited to, translation control sequences. The second flanking region may also comprise a 3' tailing sequence. The 3' tailing sequence may contain a polyadenylation motif or short poly A tail, for example less than about 100 nucleotides. Bridging the 5' terminus of the first region and the first flanking region is a first operational region. Traditionally this operational region comprises a Start codon. The operational region may alternatively comprise any translation control sequence, for example a Kozak consensus sequence, or signal including a Start codon.

Bridging the 3' terminus of the first region and the second flanking region is a second operational region. Traditionally this operational region comprises a Stop codon. The operational region may alternatively comprise any translation control sequence or signal including a Stop codon. Multiple serial stop codons may also be used in the polynucleotide. In one aspect, the operational region of the present disclosure may comprise two stop codons. The first stop codon may be "UGA" and the second stop codon may be selected from the group consisting of "UAA," "UGA" or "UAG."

Coding Region

The present disclosure provides an mRNA comprising a coding region encoding a protein involved in oocyte maturation or a variant thereof that is 80% or more homologous.

Representative examples of proteins involved in oocyte maturation as encoded in the coding region of the present disclosure include, but are not limited to: AASS, ABCB1, ABCB4, ABCF3, ABHD13, ABHD4, ACBD3, ACCSL,ACOT9, ACOXL, ACP6, ACVR1B, ACVR2B, ADAR, ADCY9, ADK, ADSS2, AIG1, ALDH9A1, ALKBH5, AMFR, AMN1, AP1M1, APIP, ARAP2, ARCN1, ARFIP1, ARHGEF16, ARHGEF18, ARL4A, ARL6, ARNTL, ARPP19, ARRDC1, ARRDC2, ASF1A, ASF1B, ASPM, ASTL, ASZ1, ATG16L1, ATG5, ATL2, ATP2C1, ATP8A2, ATR, AXIN2, B3GALNT2, B3GNT2, B4GALT4, B4GALT6, BANP, BBS4, BBS5, BCAR3, BCL2L11, BIRC3, BIRC5, BLCAP, BMP15, BMP2K, BMP5, BMS1, BNC1, BNC2, BPGM, BRCA1, BRCA2, BRDT, BTG4, BTK, BUB1, BUB1B-PAK6, C16orf74, C16orf87, C18orf21, C19orf67, C2CD3, C2orf42, C5orf15, C5orf22, C8B, CACHD1, CAMK2G, CAMSAP1,

CAPRIN2, CBX2, CCDC117, CCDC14, CCDC69, CCNO, CCNYL1, CD160, CD164L2, CD177, CD7, CDC42SE2, CDCA5, CDK5RAP1, CDK5RAP2, CDO1, CDR2, CDS1, CDYL, CENPH, CENPN, CEP164, CEP55, CHD9, CHST7, CHSY1, CITED2, CMAS, CMKLR2, CMTM8, CMYA5, CNDP1, CNKSR3, CNOT4, CNOT6L, CNOT7, COPB1, COPS8, CORO2B, CPA1, CPSF4L, CRABP2, CRELD2, CRLF2, CRYL1, CSAD, CSF1, CSMD3, CTBP2, CUL1, CUL4B, CWF19L2, CXCR3, CYP19A1, DAZ1, DAZ2, DAZ3, DAZ4, DAZL, DCLK1, DCLK2, DCLRE1A, DCP1A, DDHD1, DDX19A, DDX4, DEFB110, DEGS1, DEPDC7, DGUOK, DHX57, DISP1, DNAJA3, DNAJB1, DNAJB4, DNMT1, DOCK11, DOCK5, DOCK7, DPY19L1, DPYSL3, DTX2, DUSP7, E2F5, ECHDC3, EDC4, EEF2K, EFHC2, EGFR, EHBP1, EIF1B, EIF2AK4, EIF4ENIF1, ELMOD2, ELOVL5, EMP2, EPC1, EPHA4, ERMP1, EVX1, EXOC1, EXOC2, EXOC3, FA2H, FAM107B, FAM110C, FAM161A, FAM50A, FBXL6, FBXO21, FBXO28, FBXO30, FBXO34, FBXO38, FBXW12, FH, FKBP5, FMN2, FNBP1L, FNDC3B, FOXO1, FOXRED2, FPGT, FXR1, FYN, GALM, GALNT9, GBX2, GDA, GDPD1, GIPC2, GJA4, GLCE, GMCL1, GMCL2, GMDS, GNPDA2, GORASP2, GPN2, GPR149, GPR3, GPX6, GRM2, GSDMC, GSPT2, GSTM3, GTDC1, GTF2A1L, GTF2H2, GTF2H2C, GTPBP1, GTSF1, GUCY2C, GYG1, HERPUD2, HIF1A, HMG20A, HOXA7, HPS3, HS2ST1, HS3ST5, HSD17B7, HSD3B7, HUS1, ICA1, IFITM1, IFITM2, IFITM3, IGBP1, IGBP1P2, IGF2BP2, IL17RB, IPO8, IPP, IQCA1, IQCB1, ITGA8, ITPK1, ITPR1, ITPR2, ITSN2, JAG1, JAK2, KALRN, KBTBD6, KBTBD7, KCNH1, KCNN2, KCNQ1, KIAA0586, KIT, KLF17, KLHL13, KLHL8, KTN1, LHFPL2, LHX8, LIG4, LMBR1, LMO1, LNX2, LPCAT1, LPIN2, LRRC17, LRRC28, LRRC36, LRRC8D, LRRC8E, LSM10, LYSMD4, MACROD2, MANSC1, MAP2K1, MAP2K4, MAP4K5, MAPK3, MAPK8, MAPKAP1, MAPRE1, MAPRE2, MASTL, MAT2B, MBL2, MCM3AP, MCM9, MDM4, MED22, MED28, MED30, MED7, MEIS2, MELK, MESP2, MIER2, MIOX, MKNK1, MLLT10, MLLT3, MMADHC, MOCOS, MOSPD2, MPHOSPH10, MRPL9, MS4A1, MSH3, MSH4, MTMR14, MTMR4, MTMR7, MTSS1, MYO5A, MYO5B, MYT1, N4BP1, N4BP2, NAT14, NCAPH, NDFIP1, NDFIP2, NEDD9, NEIL3, NEO1, NEXN, NFKBIZ, NFXL1, NFYA, NGLY1, NHEJ1, NIN, NINJ1, NINJ2, NIT2, NKD1, NLRP14, NLRP4, NLRP4, NLRP5, NLRP9, NOBOX, NOC4L, NR2C1, NR2E1, NSMCE2, NUDT14, NUDT5, NUDT7, NUF2, NUP214, NUP35, NUP37, NUP88, NUP93, NXT1, OAS1, OFD1, OMA1, OOEP, OOSP1, OPHN1, OSBPL11, PACSIN2, PADI6, PAIP1, PAIP2, PAK1IP1, PAN2, PAPSS1, PARP12, PATL2, PCBD1, PCBD2, PCDH9, PCGF6, PDCD7, PDE6B, PDE6D, PDE8A, PDHX, PDK3, PECR, PEX26, PEX7, PFDN4, PGS1, PHC2, PHF14, PHOSPHO2, PHTF2, PIGA, PLA2G12A, PLAT, PLD1, PLD2, PLEKHG1, PLRG1, POFUT2, POLE3, POLR2D, POLR2G, POLR3A, POMZP3, POU2F1, POU4F1, PPA1, PPARG, PPP1R10, PPP1R3D, PRAMEF1, PRAMEF10, PRAMEF11, PRAMEF12, PRAMEF13, PRAMEF14, PRAMEF15, PRAMEF17, PRAMEF18, PRAMEF19, PRAMEF2, PRAMEF20, PRAMEF25, PRAMEF26, PRAMEF27, PRAMEF33, PRAMEF4, PRAMEF5, PRAMEF6, PRAMEF7, PRAMEF8, PRAMEF9, PRDM16, PRDX2, PRICKLE1,

PRICKLE2, PRKD1, PRPF6, PRR14, PSMD10, PSMD5, PSMD9, PSMG1, PSTPIP1, PTPN21, PTTG1, PTTG2, QARS1, RAB11A, RAB38, RAB3B, RAB3D, RAB8B, RAD51C, RALBP1, RAPGEF5, RASA2, RASA4, RASA4B, RB1, RBM18, RBM22, RBM38, RBM7, RBPMS2, RCHY1, RCN2, RDH10, RDH12, RDX, RFC3, RFPL4A, RFPL4AL1, RFX5, RGN, RGS17, RHCE, RHD, RHOH, RIMS1, RIOK1, RIPK2, RNASEH1, RNF114, RNF141, RNF185, RNF34, RNF38, RPA1, RPA2, RPH3AL, RPS6KA6, RPUSD4, RRAGC, RSPO2, RTTN, RUFY1, RUNX1T1, SAP30, SASH1, SBDS, SCML2, SDF2, SENP8, SEPSECS, SERPINC1, SERPINE2, SETD4, SF3A3, SH3BP2, SHB, SHCBP1, SHPRH, SIAH1, SIAH2, SIN3A, SKI, SLAIN1, SLC10A6, SLC15A4, SLC23A2, SLC25A14, SLC25A15, SLC25A17, SLC30A5, SLC31A2, SLC35A1, SLC35B3, SLC39A10, SLC39A6, SLC44A2, SLC45A3, SLC5A12, SLC6A15, SLC6A20, SLC7A11, SLC7A2, SLC7A6OS, SLCO3A1, SMAD2, SMAD3, SMAD4, SMARCAL1, SNX9, SOCS7, SPAG1, SPAST, SPATS2, SPECC1L, SPECC1L-ADORA2A, SPIN1, SPIRE1, SPRY1, SRD5A3, SRPK2, SSPN, ST6GAL1, ST7, STARD13, STARD7, STAU1, STK31, STRADB, STX5, STX6, STYK1, SUFU, SUSD3, SYCE2, SYCP2, SYTL2, SYTL4, TAFAZZIN, TAPT1, TBC1D14, TBC1D15, TBC1D19, TBC1D8, TBP, TBX4, TCL1A, TCL1B, TDRD12, TDRD3, TERF2, TERT, TESC, TGFB2, TGFB3, TGFBR3, THAP11, TIAM1, TIAM2, TIMELESS, TIPARP, TM2D1, TM9SF2, TMCC2, TMCC3, TMCO3, TMEM108, TMEM109, TMEM128, TMEM14C, TMEM161B, TMEM199, TMEM39B, TMEM60, TOB2, TPD52L2, TPMT, TRAFD1, TRAK1, TRAM1, TRIM11, TRIM60, TRIT1, TRPS1, TSC22D1, TSHZ1, TSPAN14, TSPAN5, TTK, TTLL11, TTYH3, TUBB6, TUBGCP5, TXNIP, TXNRD3, TYMS, TYW1, TYW1B, UBASH3B, UBE2C, UBE2G1, UBE2G2, UBE2K, UBE2T, UCHL1, UHRF1, UNC13B, UNC50, USP15, USP19, USP2, USP7, UXS1, VPS13A, VPS26A, VPS72, WDR36, WDR76, WDR82, WEE2, WFDC3, WNK2, XRCC1, XRCC5, ZBED3, ZBTB2, ZBTB8B, ZC3H6, ZCCHC10, ZCCHC3, ZCRB1, ZNF233, ZNF394, ZNF451, ZNF473, ZNF655, ZNF750, ZP3, ZSCAN21, and ZSWIM3.

In some aspects, the coding region comprises an RNA sequence encoded by a gene selected from Table 1 below, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous, wherein at least one nucleotide in the RNA sequence has been chemically modified.

Table 1. Genes Associated with Oocyte Maturation

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000010592	Dazl	ENSG00000205916	DAZ4
ENSMUSG00000010592	Dazl	ENSG00000187191	DAZ3

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000010592	Dazl	ENSG00000188120	DAZ1
ENSMUSG00000010592	Dazl	ENSG00000205944	DAZ2
ENSMUSG00000025665	Rps6ka6	ENSG00000072133	RPS6KA6
ENSMUSG00000022913	Psmg1	ENSG00000183527	PSMG1
ENSMUSG00000024563	Smad2	ENSG00000175387	SMAD2
ENSMUSG00000022948	Setd4	ENSG00000185917	SETD4
ENSMUSG00000009293	Ube2g2	ENSG00000184787	UBE2G2
ENSMUSG00000021947	Cryl1	ENSG00000165475	CRYL1
ENSMUSG00000039616	Mocos	ENSG00000075643	MOCOS
ENSMUSG00000031221	Igbp1	ENSG00000089289	IGBP1
ENSMUSG00000037697	Ddhd1	ENSG00000100523	DDHD1
ENSMUSG00000020482	Ccdc117	ENSG00000159873	CCDC117
ENSMUSG00000031482	Slc25a15	ENSG00000102743	SLC25A15
ENSMUSG00000039178	Tbc1d19	ENSG00000109680	TBC1D19
ENSMUSG00000025817	Nudt5	ENSG00000165609	NUDT5
ENSMUSG00000024270	Slc39a6	ENSG00000141424	SLC39A6
ENSMUSG00000025544	Tm9sf2	ENSG00000125304	TM9SF2
ENSMUSG00000022404	Slc25a17	ENSG00000100372	SLC25A17
ENSMUSG00000000037	Scml2	ENSG00000102098	SCML2
ENSMUSG00000006418	Rnf114	ENSG00000124226	RNF114
ENSMUSG00000001150	Mcm3ap	ENSG00000160294	MCM3AP
ENSMUSG00000060445	Sycp2	ENSG00000196074	SYCP2
ENSMUSG00000054520	Sh3bp2	ENSG00000087266	SH3BP2
ENSMUSG00000037347	Chst7	ENSG00000147119	CHST7
ENSMUSG00000039063	Echdc3	ENSG00000134463	ECHDC3
ENSMUSG00000027487	Cdk5rap1	ENSG00000101391	CDK5RAP1

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000033467	Crlf2	ENSG00000205755	CRLF2
ENSMUSG00000055717	Slain1	ENSG00000139737	SLAIN1
ENSMUSG00000066359	Tcl1b1	ENSG00000213231	TCL1B
ENSMUSG00000020260	Pofut2	ENSG00000186866	POFUT2
ENSMUSG00000020454	Eif4enif1	ENSG00000184708	EIF4ENIF1
ENSMUSG00000024273	2700062C07Rik	ENSG00000141428	C18orf21
ENSMUSG00000036435	Exoc1	ENSG00000090989	EXOC1
ENSMUSG00000027340	Slc23a2	ENSG00000089057	SLC23A2
ENSMUSG00000020415	Pttg1	ENSG00000250254	PTTG2
ENSMUSG00000033970	Rfc3	ENSG00000133119	RFC3
ENSMUSG00000049999	Ppp1r3d	ENSG00000132825	PPP1R3D
ENSMUSG00000022010	Tsc22d1	ENSG00000102804	TSC22D1
ENSMUSG00000056162	Cndp1	ENSG00000150656	CNDP1
ENSMUSG00000027276	Jag1	ENSG00000101384	JAG1
ENSMUSG00000029223	Uchl1	ENSG00000154277	UCHL1
ENSMUSG00000052033	Pfdn4	ENSG00000101132	PFDN4
ENSMUSG00000010505	Myt1	ENSG00000196132	MYT1
ENSMUSG00000016664	Pacsin2	ENSG00000100266	PACSIN2
ENSMUSG00000031429	Psmd10	ENSG00000101843	PSMD10
ENSMUSG00000029192	Tbc1d14	ENSG00000132405	TBC1D14
ENSMUSG00000024096	Ralbp1	ENSG00000017797	RALBP1
ENSMUSG00000067787	Blcap	ENSG00000166619	BLCAP
ENSMUSG00000035696	Rnf38	ENSG00000137075	RNF38
ENSMUSG00000061778	Mospd2	ENSG00000130150	MOSPD2
ENSMUSG00000024277	Mapre2	ENSG00000166974	MAPRE2
ENSMUSG00000036782	Klhl13	ENSG00000003096	KLHL13

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000048349	Pou4f1	ENSG00000152192	POU4F1
ENSMUSG00000021009	Ptpn21	ENSG00000070778	PTPN21
ENSMUSG00000025038	Efhc2	ENSG00000183690	EFHC2
ENSMUSG00000025885	Myo5b	ENSG00000167306	MYO5B
ENSMUSG00000042197	Zfp451	ENSG00000112200	ZNF451
ENSMUSG00000047446	Arl4a	ENSG00000122644	ARL4A
ENSMUSG00000033444	Specc1l	ENSG00000100014	SPECC1L
ENSMUSG00000033444	Specc1l	ENSG00000258555	SPECC1L-ADORA2A
ENSMUSG00000062995	Ica1	ENSG00000003147	ICA1
ENSMUSG00000067825	Pex26	ENSG00000288683	
ENSMUSG00000023066	Rttn	ENSG00000176225	RTTN
ENSMUSG00000030559	Rab38	ENSG00000123892	RAB38
ENSMUSG00000027998	Plrg1	ENSG00000171566	PLRG1
ENSMUSG00000024789	Jak2	ENSG00000096968	JAK2
ENSMUSG00000036943	Rab8b	ENSG00000166128	RAB8B
ENSMUSG00000024791	Cdca5	ENSG00000146670	CDCA5
ENSMUSG00000044167	Foxo1	ENSG00000150907	FOXO1
ENSMUSG00000028496	Mllt3	ENSG00000171843	MLLT3
ENSMUSG00000032899	Styk1	ENSG00000060140	STYK1
ENSMUSG00000032340	Neo1	ENSG00000067141	NEO1
ENSMUSG00000056124	B4galt6	ENSG00000118276	B4GALT6
ENSMUSG00000039536	Stau1	ENSG00000124214	STAU1
ENSMUSG00000040667	Nup88	ENSG00000108559	NUP88
ENSMUSG00000027210	Meis2	ENSG00000134138	MEIS2
ENSMUSG00000026972	Arrdc1	ENSG00000197070	ARRDC1
ENSMUSG00000009545	Kcnq1	ENSG00000053918	KCNQ1

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000042535	Gtpbp1	ENSG00000100226	GTPBP1
ENSMUSG00000001473	Tubb6	ENSG00000176014	TUBB6
ENSMUSG00000035683	Melk	ENSG00000165304	MELK
ENSMUSG00000021365	Nedd9	ENSG00000111859	NEDD9
ENSMUSG00000041729	Coro2b	ENSG00000103647	CORO2B
ENSMUSG00000028152	Tspan5	ENSG00000168785	TSPAN5
ENSMUSG00000001962	Fam50a	ENSG00000071859	FAM50A
ENSMUSG00000029203	Ube2k	ENSG00000078140	UBE2K
ENSMUSG00000036565	Ttyh3	ENSG00000136295	TTYH3
ENSMUSG00000037966	Ninj1	ENSG00000131669	NINJ1
ENSMUSG00000024515	Smad4	ENSG00000141646	SMAD4
ENSMUSG00000009995	Taz	ENSG00000102125	TAFAZZIN
ENSMUSG00000044447	Dock5	ENSG00000147459	DOCK5
ENSMUSG00000028078	Dclk2	ENSG00000170390	DCLK2
ENSMUSG00000005580	Adcy9	ENSG00000162104	ADCY9
ENSMUSG00000021376	Tpmt	ENSG00000137364	TPMT
ENSMUSG00000036158	Prickle1	ENSG00000139174	PRICKLE1
ENSMUSG00000002489	Tiam1	ENSG00000156299	TIAM1
ENSMUSG00000031093	Dock11	ENSG00000147251	DOCK11
ENSMUSG00000021384	Susd3	ENSG00000157303	SUSD3
ENSMUSG00000031214	Ophn1	ENSG00000079482	OPHN1
ENSMUSG00000027797	Dclk1	ENSG00000133083	DCLK1
ENSMUSG00000040029	Ipo8	ENSG00000133704	IPO8
ENSMUSG00000024533	Spire1	ENSG00000134278	SPIRE1
ENSMUSG00000030138	Bms1	ENSG00000165733	BMS1
ENSMUSG00000024052	Lpin2	ENSG00000101577	LPIN2

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000027737	Slc7a11	ENSG00000151012	SLC7A11
ENSMUSG00000048546	Tob2	ENSG00000183864	TOB2
ENSMUSG00000038372	Gmds	ENSG00000112699	GMDS
ENSMUSG00000021428	Riok1	ENSG00000124784	RIOK1
ENSMUSG00000030970	Ctbp2	ENSG00000175029	CTBP2
ENSMUSG00000046982	Tshz1	ENSG00000179981	TSHZ1
ENSMUSG00000040084	Bub1b	ENSG00000259288	BUB1B-PAK6
ENSMUSG00000045822	Zswim3	ENSG00000132801	ZSWIM3
ENSMUSG00000074682	Zcchc3	ENSG00000247315	ZCCHC3
ENSMUSG00000068250	Amn1	ENSG00000151743	AMN1
ENSMUSG00000031601	Cnot7	ENSG00000198791	CNOT7
ENSMUSG00000046230	Vps13a	ENSG00000197969	VPS13A
ENSMUSG00000032329	Hmg20a	ENSG00000140382	HMG20A
ENSMUSG00000026768	Itga8	ENSG00000077943	ITGA8
ENSMUSG00000050232	Cxcr3	ENSG00000186810	CXCR3
ENSMUSG00000031381	Piga	ENSG00000165195	PIGA
ENSMUSG00000040586	Ofd1	ENSG00000046651	OFD1
ENSMUSG00000028032	Papss1	ENSG00000138801	PAPSS1
ENSMUSG00000037824	Tspan14	ENSG00000108219	TSPAN14
ENSMUSG00000021253	Tgfb3	ENSG00000119699	TGFB3
ENSMUSG00000068205	Macrod2	ENSG00000172264	MACROD2
ENSMUSG00000039431	Mtmr7	ENSG00000003987	MTMR7
ENSMUSG00000032718	Mansc1	ENSG00000111261	MANSC1
ENSMUSG00000026933	Camsap1	ENSG00000130559	CAMSAP1
ENSMUSG00000005102	Eif2ak4	ENSG00000128829	EIF2AK4
ENSMUSG00000031095	Cul4b	ENSG00000158290	CUL4B

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000027173	Depdc7	ENSG00000121690	DEPDC7
ENSMUSG00000025747	Tyms	ENSG00000176890	TYMS
ENSMUSG00000023272	Creld2	ENSG00000184164	CRELD2
ENSMUSG00000021357	Exoc2	ENSG00000112685	EXOC2
ENSMUSG00000041857	Oosp1	ENSG00000284873	OOSP1
ENSMUSG00000029204	Rhoh	ENSG00000168421	RHOH
ENSMUSG00000020847	Rph3al	ENSG00000181031	RPH3AL
ENSMUSG00000057963	Itpk1	ENSG00000100605	ITPK1
ENSMUSG00000032000	Birc3	ENSG00000023445	BIRC3
ENSMUSG00000029321	Slc10a6	ENSG00000145283	SLC10A6
ENSMUSG00000040396	Abhd13	ENSG00000139826	ABHD13
ENSMUSG00000032252	Glce	ENSG00000138604	GLCE
ENSMUSG00000038485	Socs7	ENSG00000274211	SOCS7
ENSMUSG00000000827	Tpd52l2	ENSG00000101150	TPD52L2
ENSMUSG00000055421	Pcdh9	ENSG00000184226	PCDH9
ENSMUSG00000032320	Rcn2	ENSG00000117906	RCN2
ENSMUSG00000022105	Rb1	ENSG00000139687	RB1
ENSMUSG00000029330	Cds1	ENSG00000163624	CDS1
ENSMUSG00000021843	Ktn1	ENSG00000126777	KTN1
ENSMUSG00000022019	Tdrd3	ENSG00000083544	TDRD3
ENSMUSG00000021608	Lpcat1	ENSG00000153395	LPCAT1
ENSMUSG00000076434	Wfdc3	ENSG00000124116	WFDC3
ENSMUSG00000059288	Cdyl	ENSG00000153046	CDYL
ENSMUSG00000021395	Spin1	ENSG00000106723	SPIN1
ENSMUSG00000058624	Gda	ENSG00000119125	GDA
ENSMUSG00000033790	Tubgcp5	ENSG00000275835	TUBGCP5

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000016128	Stard13	ENSG00000133121	STARD13
ENSMUSG00000021611	Tert	ENSG00000164362	TERT
ENSMUSG00000001403	Ube2c	ENSG00000175063	UBE2C
ENSMUSG00000042650	Alkbh5	ENSG00000091542	ALKBH5
ENSMUSG00000071723	Gspt2	ENSG00000189369	GSPT2
ENSMUSG00000039994	Timeless	ENSG00000111602	TIMELESS
ENSMUSG00000038696	Mapkap1	ENSG00000119487	MAPKAP1
ENSMUSG00000032349	Elovl5	ENSG00000012660	ELOVL5
ENSMUSG00000032179	Bmp5	ENSG00000112175	BMP5
ENSMUSG00000041644	Slc5a12	ENSG00000148942	SLC5A12
ENSMUSG00000041359	Tcl1	ENSG00000100721	TCL1A
ENSMUSG00000027479	Mapre1	ENSG00000101367	MAPRE1
ENSMUSG00000037795	N4bp2	ENSG00000078177	N4BP2
ENSMUSG00000023279	Bmp15	ENSG00000130385	BMP15
ENSMUSG00000048626	Klf17	ENSG00000171872	KLF17
ENSMUSG00000002688	Prkd1	ENSG00000184304	PRKD1
ENSMUSG00000054446	Cpa1	ENSG00000091704	CPA1
ENSMUSG00000029491	Pde6b	ENSG00000133256	PDE6B
ENSMUSG00000000751	Rpa1	ENSG00000132383	RPA1
ENSMUSG00000037989	Wnk2	ENSG00000165238	WNK2
ENSMUSG00000002455	Prpf6	ENSG00000101161	PRPF6
ENSMUSG00000036992	Nxt1	ENSG00000132661	NXT1
ENSMUSG00000036270	Edc4	ENSG00000038358	EDC4
ENSMUSG00000038497	Tmco3	ENSG00000150403	TMCO3
ENSMUSG00000001228	Uhrf1	ENSG00000276043	UHRF1
ENSMUSG00000034486	Gbx2	ENSG00000168505	GBX2

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000039396	Neil3	ENSG00000109674	NEIL3
ENSMUSG00000016626	Nlrp14	ENSG00000158077	NLRP14
ENSMUSG00000038160	Atg5	ENSG00000057663	ATG5
ENSMUSG00000021758	Ddx4	ENSG00000152670	DDX4
ENSMUSG00000041147	Brca2	ENSG00000139618	BRCA2
ENSMUSG00000035069	Oma1	ENSG00000162600	OMA1
ENSMUSG00000006586	Runx1t1	ENSG00000079102	RUNX1T1
ENSMUSG00000034659	Tmem109	ENSG00000110108	TMEM109
ENSMUSG00000028563	Tm2d1	ENSG00000162604	TM2D1
ENSMUSG00000029312	Klhl8	ENSG00000145332	KLHL8
ENSMUSG00000043987	Cep164	ENSG00000110274	CEP164
ENSMUSG00000049717	Lig4	ENSG00000174405	LIG4
ENSMUSG00000038683	Pak1ip1	ENSG00000111845	PAK1IP1
ENSMUSG00000004069	Dnaja3	ENSG00000103423	DNAJA3
ENSMUSG00000021496	Pcbd2	ENSG00000132570	PCBD2
ENSMUSG00000033106	Slc7a6os	ENSG00000103061	SLC7A6OS
ENSMUSG00000045312	Lhfpl2	ENSG00000145685	LHFPL2
ENSMUSG00000025935	Tram1	ENSG00000067167	TRAM1
ENSMUSG00000015804	Med28	ENSG00000118579	MED28
ENSMUSG00000004952	Rasa4	ENSG00000170667	RASA4B
ENSMUSG00000029173	Sepsecs	ENSG00000109618	SEPSECS
ENSMUSG00000026885	Ttll11	ENSG00000175764	TTLL11
ENSMUSG00000071662	Polr2g	ENSG00000168002	POLR2G
ENSMUSG00000030491	Tdrd12	ENSG00000173809	TDRD12
ENSMUSG00000024501	Dpysl3	ENSG00000113657	DPYSL3
ENSMUSG00000033294	Noc4l	ENSG00000184967	NOC4L

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000034761	Map4k5	ENSG00000012983	MAP4K5
ENSMUSG00000021820	Camk2g	ENSG00000148660	CAMK2G
ENSMUSG00000027680	Fxr1	ENSG00000114416	FXR1
ENSMUSG00000025077	Dclre1a	ENSG00000198924	DCLRE1A
ENSMUSG00000027242	Wdr76	ENSG00000092470	WDR76
ENSMUSG00000051920	Rspo2	ENSG00000147655	RSPO2
ENSMUSG00000019843	Fyn	ENSG00000010810	FYN
ENSMUSG00000030788	Rnf141	ENSG00000110315	RNF141
ENSMUSG00000030020	Prickle2	ENSG00000163637	PRICKLE2
ENSMUSG00000039987	Phtf2	ENSG00000006576	PHTF2
ENSMUSG00000028902	Sf3a3	ENSG00000183431	SF3A3
ENSMUSG00000004948	Zp3	ENSG00000188372	ZP3
ENSMUSG00000022487	Gtsf1	ENSG00000170627	GTSF1
ENSMUSG00000036275	9530068E07Rik	ENSG00000113583	C5orf15
ENSMUSG00000048485	Zbtb8b	ENSG00000273274	ZBTB8B
ENSMUSG00000048485	Zbtb8b	ENSG00000254553	
ENSMUSG00000003411	Rab3b	ENSG00000169213	RAB3B
ENSMUSG00000029629	Phf14	ENSG00000106443	PHF14
ENSMUSG00000001855	Nup214	ENSG00000126883	NUP214
ENSMUSG00000047419	Cmya5	ENSG00000164309	CMYA5
ENSMUSG00000053490	Trim60	ENSG00000176979	TRIM60
ENSMUSG00000027510	Rbm38	ENSG00000132819	RBM38
ENSMUSG00000026999	Nup35	ENSG00000163002	NUP35
ENSMUSG00000029359	Tesc	ENSG00000088992	TESC
ENSMUSG00000030282	Cmas	ENSG00000111726	CMAS
ENSMUSG00000025231	Sufu	ENSG00000107882	SUFU

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000040935	Padi6	ENSG00000276747	PADI6
ENSMUSG00000029032	Arhgef16	ENSG00000130762	ARHGEF16
ENSMUSG00000022613	Miox	ENSG00000100253	MIOX
ENSMUSG00000034906	Ncaph	ENSG00000121152	NCAPH
ENSMUSG00000021361	Tmem14c	ENSG00000111843	TMEM14C
ENSMUSG00000030543	Mesp2	ENSG00000188095	MESP2
ENSMUSG00000032044	Rpusd4	ENSG00000165526	RPUSD4
ENSMUSG00000036136	Fam110c	ENSG00000184731	FAM110C
ENSMUSG00000022559	Fbxl6	ENSG00000182325	FBXL6
ENSMUSG00000049649	Gpr3	ENSG00000181773	GPR3
ENSMUSG00000054477	Kcnn2	ENSG00000080709	KCNN2
ENSMUSG00000023403	Stk31	ENSG00000196335	STK31
ENSMUSG00000047799	Oog4	ENSG00000279804	PRAMEF18
ENSMUSG00000047799	Oog4	ENSG00000120952	PRAMEF2
ENSMUSG00000047799	Oog4	ENSG00000243073	PRAMEF4
ENSMUSG00000047799	Oog4	ENSG00000279169	PRAMEF13
ENSMUSG00000047799	Oog4	ENSG00000187545	PRAMEF10
ENSMUSG00000047799	Oog4	ENSG00000239810	PRAMEF11
ENSMUSG00000047799	Oog4	ENSG00000116721	PRAMEF1
ENSMUSG00000047799	Oog4	ENSG00000232423	PRAMEF6
ENSMUSG00000047799	Oog4	ENSG00000270601	PRAMEF5
ENSMUSG00000047799	Oog4	ENSG00000237700	PRAMEF33
ENSMUSG00000047799	Oog4	ENSG00000204510	PRAMEF7
ENSMUSG00000047799	Oog4	ENSG00000182330	PRAMEF8
ENSMUSG00000022311	Csmd3	ENSG00000164796	CSMD3
ENSMUSG00000026779	Mastl	ENSG00000120539	MASTL

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000048012	Zfp473	ENSG00000142528	ZNF473
ENSMUSG00000044813	Shb	ENSG00000255872	
ENSMUSG00000044813	Shb	ENSG00000107338	SHB
ENSMUSG00000019803	Nr2e1	ENSG00000112333	NR2E1
ENSMUSG00000010110	Stx5a	ENSG00000162236	STX5
ENSMUSG00000038622	Med30	ENSG00000164758	MED30
ENSMUSG00000021123	Rdh12	ENSG00000139988	RDH12
ENSMUSG00000030754	Copb1	ENSG00000129083	COPB1
ENSMUSG00000022722	Arl6	ENSG00000113966	ARL6
ENSMUSG00000029233	Srd5a3	ENSG00000128039	SRD5A3
ENSMUSG00000029233	Srd5a3	ENSG00000288695	
ENSMUSG00000021432	Slc35b3	ENSG00000124786	SLC35B3
ENSMUSG00000029534	St7	ENSG00000288635	
ENSMUSG00000029534	St7	ENSG00000004866	ST7
ENSMUSG00000026187	Xrcc5	ENSG00000079246	XRCC5
ENSMUSG00000043687	1190005I06Rik	ENSG00000154102	C16orf74
ENSMUSG00000007812	Zfp655	ENSG00000197343	ZNF655
ENSMUSG00000033579	Fa2h	ENSG00000103089	FA2H
ENSMUSG00000033022	Cdo1	ENSG00000129596	CDO1
ENSMUSG00000026655	Fam107b	ENSG00000065809	FAM107B
ENSMUSG00000039910	Cited2	ENSG00000164442	CITED2
ENSMUSG00000030521	Mphosph10	ENSG00000124383	MPHOSPH10
ENSMUSG00000017715	Pgs1	ENSG00000087157	PGS1
ENSMUSG00000049588	Ccdc69	ENSG00000198624	CCDC69
ENSMUSG00000033316	Galnt9	ENSG00000182870	GALNT9
ENSMUSG00000038507	Parp12	ENSG00000059378	PARP12

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000028646	Rragc	ENSG00000116954	RRAGC
ENSMUSG00000020630	Rnaseh1	ENSG00000286905	
ENSMUSG00000020630	Rnaseh1	ENSG00000171865	RNASEH1
ENSMUSG00000037617	Spag1	ENSG00000104450	SPAG1
ENSMUSG00000025451	Paip1	ENSG00000172239	PAIP1
ENSMUSG00000049811	Fam161a	ENSG00000170264	FAM161A
ENSMUSG00000031596	Slc7a2	ENSG00000003989	SLC7A2
ENSMUSG00000028035	Dnajb4	ENSG00000162616	DNAJB4
ENSMUSG00000072889	Nfxl1	ENSG00000170448	NFXL1
ENSMUSG00000026743	Mllt10	ENSG00000078403	MLLT10
ENSMUSG00000024863	Mbl2	ENSG00000165471	MBL2
ENSMUSG00000029050	Ski	ENSG00000157933	SKI
ENSMUSG00000026889	Rbm18	ENSG00000119446	RBM18
ENSMUSG00000029695	Aass	ENSG00000008311	AASS
ENSMUSG00000025050	Pcgf6	ENSG00000156374	PCGF6
ENSMUSG00000014959	Gorasp2	ENSG00000115806	GORASP2
ENSMUSG00000031255	Sytl4	ENSG00000102362	SYTL4
ENSMUSG00000037536	Fbxo34	ENSG00000178974	FBXO34
ENSMUSG00000027552	E2f5	ENSG00000133740	E2F5
ENSMUSG00000015776	Med22	ENSG00000148297	MED22
ENSMUSG00000039286	Fndc3b	ENSG00000075420	FNDC3B
ENSMUSG00000019775	Rgs17	ENSG00000091844	RGS17
ENSMUSG00000034087	Nlrp4b	ENSG00000160505	NLRP4
ENSMUSG00000039220	Ppp1r10	ENSG00000204569	PPP1R10
ENSMUSG00000051934	Spats2	ENSG00000123352	SPATS2
ENSMUSG00000014850	Msh3	ENSG00000113318	MSH3

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000056293	Gsdmc2	ENSG00000147697	GSDMC
ENSMUSG00000030287	Itpr2	ENSG00000123104	ITPR2
ENSMUSG00000041135	Ripk2	ENSG00000104312	RIPK2
ENSMUSG00000063065	Mapk3	ENSG00000102882	MAPK3
ENSMUSG00000051768	Xrcc1	ENSG00000073050	XRCC1
ENSMUSG00000020078	Vps26a	ENSG00000122958	VPS26A
ENSMUSG00000016520	Lnx2	ENSG00000139517	LNX2
ENSMUSG00000052212	Cd177	ENSG00000204936	CD177
ENSMUSG00000030556	Lrrc28	ENSG00000168904	LRRC28
ENSMUSG00000028865	Cd164l2	ENSG00000174950	CD164L2
ENSMUSG00000034601	2700049A03Rik	ENSG00000100578	KIAA0586
ENSMUSG00000046985	Tapt1	ENSG00000169762	TAPT1
ENSMUSG00000024240	Epc1	ENSG00000120616	EPC1
ENSMUSG00000059586	Nsmce2	ENSG00000156831	NSMCE2
ENSMUSG00000039883	Lrrc17	ENSG00000128606	LRRC17
ENSMUSG00000036840	Siah1a	ENSG00000196470	SIAH1
ENSMUSG00000031221	Igbp1	ENSG00000266826	IGBP1P2
ENSMUSG00000031105	Slc25a14	ENSG00000102078	SLC25A14
ENSMUSG00000024222	Fkbp5	ENSG00000096060	FKBP5
ENSMUSG00000028970	Abcb1b	ENSG00000085563	ABCB1
ENSMUSG00000039131	Gipc2	ENSG00000137960	GIPC2
ENSMUSG00000020089	Ppa1	ENSG00000180817	PPA1
ENSMUSG00000018239	Zcchc10	ENSG00000155329	ZCCHC10
ENSMUSG00000024604	Rbm22	ENSG00000086589	RBM22
ENSMUSG00000050234	Gja4	ENSG00000187513	GJA4
ENSMUSG00000025584	Pde8a	ENSG00000073417	PDE8A

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000021639	Gtf2h2	ENSG00000145736	GTF2H2
ENSMUSG00000066152	Slc31a2	ENSG00000136867	SLC31A2
ENSMUSG00000042396	Rbm7	ENSG00000255663	
ENSMUSG00000042396	Rbm7	ENSG00000076053	RBM7
ENSMUSG00000037159	Wee2	ENSG00000214102	WEE2
ENSMUSG00000002365	Snx9	ENSG00000130340	SNX9
ENSMUSG00000031767	Nudt7	ENSG00000140876	NUDT7
ENSMUSG00000024154	Gtf2a1l	ENSG00000242441	GTF2A1L
ENSMUSG00000032640	Chsy1	ENSG00000131873	CHSY1
ENSMUSG00000045693	Nlrp4e	ENSG00000160505	NLRP4
ENSMUSG00000034663	Bmp2k	ENSG00000138756	BMP2K
ENSMUSG00000042289	Hsd3b7	ENSG00000099377	HSD3B7
ENSMUSG00000025921	Rdh10	ENSG00000121039	RDH10
ENSMUSG00000041012	Cmtm8	ENSG00000170293	CMTM8
ENSMUSG00000045435	Tmem60	ENSG00000135211	TMEM60
ENSMUSG00000036432	Siah2	ENSG00000181788	SIAH2
ENSMUSG00000020124	Usp15	ENSG00000135655	USP15
ENSMUSG00000041995	Zbed3	ENSG00000132846	ZBED3
ENSMUSG00000025790	Slco3a1	ENSG00000176463	SLCO3A1
ENSMUSG00000020448	Rnf185	ENSG00000138942	RNF185
ENSMUSG00000021983	Atp8a2	ENSG00000132932	ATP8A2
ENSMUSG00000025287	Acot9	ENSG00000123130	ACOT9
ENSMUSG00000035473	Galm	ENSG00000143891	GALM
ENSMUSG00000032387	Rbpms2	ENSG00000166831	RBPMS2
ENSMUSG00000032020	Ubash3b	ENSG00000154127	UBASH3B
ENSMUSG00000019806	Aig1	ENSG00000146416	AIG1

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000029627	Zkscan14	ENSG00000160908	ZNF394
ENSMUSG00000021068	Nin	ENSG00000100503	NIN
ENSMUSG00000042417	Ccno	ENSG00000152669	CCNO
ENSMUSG00000031609	Sap30	ENSG00000164105	SAP30
ENSMUSG00000034432	Cops8	ENSG00000198612	COPS8
ENSMUSG00000038679	Trps1	ENSG00000104447	TRPS1
ENSMUSG00000016552	Foxred2	ENSG00000100350	FOXRED2
ENSMUSG00000053253	Ndfip2	ENSG00000102471	NDFIP2
ENSMUSG00000028653	Trit1	ENSG00000043514	TRIT1
ENSMUSG00000045273	Cenph	ENSG00000153044	CENPH
ENSMUSG00000038871	Bpgm	ENSG00000172331	BPGM
ENSMUSG00000042476	Abcb4	ENSG00000005471	ABCB4
ENSMUSG00000029209	Gnpda2	ENSG00000163281	GNPDA2
ENSMUSG00000031751	Amfr	ENSG00000159461	AMFR
ENSMUSG00000042032	Mat2b	ENSG00000038274	MAT2B
ENSMUSG00000017146	Brca1	ENSG00000012048	BRCA1
ENSMUSG00000022793	B4galt4	ENSG00000121578	B4GALT4
ENSMUSG00000032939	Nup93	ENSG00000102900	NUP93
ENSMUSG00000028121	Bcar3	ENSG00000137936	BCAR3
ENSMUSG00000020003	Pex7	ENSG00000112357	PEX7
ENSMUSG00000041992	Rapgef5	ENSG00000136237	RAPGEF5
ENSMUSG00000021109	Hif1a	ENSG00000100644	HIF1A
ENSMUSG00000000094	Tbx4	ENSG00000121075	TBX4
ENSMUSG00000032050	Rdx	ENSG00000137710	RDX
ENSMUSG00000031652	N4bp1	ENSG00000102921	N4BP1
ENSMUSG00000020415	Pttg1	ENSG00000164611	PTTG1

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000043881	Kbtbd7	ENSG00000165572	KBTBD6
ENSMUSG00000043881	Kbtbd7	ENSG00000120696	KBTBD7
ENSMUSG00000027999	Pla2g12a	ENSG00000123739	PLA2G12A
ENSMUSG00000022322	Shcbp1	ENSG00000171241	SHCBP1
ENSMUSG00000026189	Pecr	ENSG00000115425	PECR
ENSMUSG00000004771	Rab11a	ENSG00000103769	RAB11A
ENSMUSG00000039410	Prdm16	ENSG00000142611	PRDM16
ENSMUSG00000059811	Atl2	ENSG00000119787	ATL2
ENSMUSG00000024425	Ndfip1	ENSG00000131507	NDFIP1
ENSMUSG00000032322	Pstpip1	ENSG00000140368	PSTPIP1
ENSMUSG00000023070	Rgn	ENSG00000130988	RGN
ENSMUSG00000035232	Pdk3	ENSG00000067992	PDK3
ENSMUSG00000015202	Cnksr3	ENSG00000153721	CNKSR3
ENSMUSG00000022751	Nit2	ENSG00000114021	NIT2
ENSMUSG00000007646	Rad51c	ENSG00000108384	RAD51C
ENSMUSG00000039197	Adk	ENSG00000156110	ADK
ENSMUSG00000038379	Ttk	ENSG00000112742	TTK
ENSMUSG00000008429	Herpud2	ENSG00000122557	HERPUD2
ENSMUSG00000028487	Bnc2	ENSG00000173068	BNC2
ENSMUSG00000032999	Nlrp4f	ENSG00000160505	NLRP4
ENSMUSG00000067825	Pex26	ENSG00000215193	PEX26
ENSMUSG00000026869	Psmd5	ENSG00000095261	PSMD5
ENSMUSG00000028394	Pole3	ENSG00000148229	POLE3
ENSMUSG00000040624	Plekhg1	ENSG00000120278	PLEKHG1
ENSMUSG00000029440	Psmd9	ENSG00000110801	PSMD9
ENSMUSG00000029440	Psmd9	ENSG00000256950	

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000031264	Btk	ENSG00000010671	BTK
ENSMUSG00000020397	Med7	ENSG00000155868	MED7
ENSMUSG00000051232	Tmem199	ENSG00000244045	TMEM199
ENSMUSG00000015023	Ddx19a	ENSG00000168872	DDX19A
ENSMUSG00000025986	Slc39a10	ENSG00000196950	SLC39A10
ENSMUSG00000036934	4921524J17Rik	ENSG00000155330	C16orf87
ENSMUSG00000035351	Nup37	ENSG00000075188	NUP37
ENSMUSG00000036442	Thap11	ENSG00000168286	THAP11
ENSMUSG00000020023	Tmcc3	ENSG00000057704	TMCC3
ENSMUSG00000029416	Slc15a4	ENSG00000139370	SLC15A4
ENSMUSG00000041837	Pdcd7	ENSG00000090470	PDCD7
ENSMUSG00000028556	Dock7	ENSG00000116641	DOCK7
ENSMUSG00000059108	Ifitm6	ENSG00000288681	
ENSMUSG00000059108	Ifitm6	ENSG00000185885	IFITM1
ENSMUSG00000002804	Nudt14	ENSG00000183828	NUDT14
ENSMUSG00000055116	Arntl	ENSG00000133794	ARNTL
ENSMUSG00000007656	Arpp19	ENSG00000128989	ARPP19
ENSMUSG00000037211	Spry1	ENSG00000164056	SPRY1
ENSMUSG00000006941	Eif1b	ENSG00000114784	EIF1B
ENSMUSG00000028532	Cachd1	ENSG00000158966	CACHD1
ENSMUSG00000035151	Elmod2	ENSG00000179387	ELMOD2
ENSMUSG00000024989	Cep55	ENSG00000138180	CEP55
ENSMUSG00000074513	Arfip1	ENSG00000164144	ARFIP1
ENSMUSG00000022807	Osbpl11	ENSG00000144909	OSBPL11
ENSMUSG00000047648	Fbxo30	ENSG00000118496	FBXO30
ENSMUSG00000042557	Sin3a	ENSG00000169375	SIN3A

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000020455	Trim11	ENSG00000154370	TRIM11
ENSMUSG00000022635	Zcrb1	ENSG00000139168	ZCRB1
ENSMUSG00000025337	Sbds	ENSG00000126524	SBDS
ENSMUSG00000022837	Iqcb1	ENSG00000173226	IQCB1
ENSMUSG00000067773	Defb41	ENSG00000203970	DEFB110
ENSMUSG00000032010	Usp2	ENSG00000036672	USP2
ENSMUSG00000035064	Eef2k	ENSG00000103319	EEF2K
ENSMUSG00000042851	Zc3h6	ENSG00000188177	ZC3H6
ENSMUSG00000010911	Apip	ENSG00000149089	APIP
ENSMUSG00000037999	Arap2	ENSG00000047365	ARAP2
ENSMUSG00000003134	Tbc1d8	ENSG00000204634	TBC1D8
ENSMUSG00000053870	Fpgt	ENSG00000254685	FPGT
ENSMUSG00000039103	Nexn	ENSG00000162614	NEXN
ENSMUSG00000038633	Degs1	ENSG00000143753	DEGS1
ENSMUSG00000029474	Rnf34	ENSG00000170633	RNF34
ENSMUSG00000049314	Fbxw13	ENSG00000164049	FBXW12
ENSMUSG00000056608	Chd9	ENSG00000177200	CHD9
ENSMUSG00000052298	Cdc42se2	ENSG00000158985	CDC42SE2
ENSMUSG00000028796	Phc2	ENSG00000134686	PHC2
ENSMUSG00000046079	Lrrc8d	ENSG00000171492	LRRC8D
ENSMUSG00000020828	Pld2	ENSG00000129219	PLD2
ENSMUSG00000035051	Dhx57	ENSG00000163214	DHX57
ENSMUSG00000036890	Gtdc1	ENSG00000121964	GTDC1
ENSMUSG00000024258	Polr2d	ENSG00000144231	POLR2D
ENSMUSG00000000532	Acvr1b	ENSG00000135503	ACVR1B
ENSMUSG00000057193	Slc44a2	ENSG00000129353	SLC44A2

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000054387	Mdm4	ENSG00000198625	MDM4
ENSMUSG00000042211	Fbxo38	ENSG00000145868	FBXO38
ENSMUSG00000042302	Ehbp1	ENSG00000115504	EHBP1
ENSMUSG00000010914	Pdhx	ENSG00000110435	PDHX
ENSMUSG00000029397	Rchy1	ENSG00000163743	RCHY1
ENSMUSG00000025577	Cbx2	ENSG00000173894	CBX2
ENSMUSG00000022885	St6gal1	ENSG00000073849	ST6GAL1
ENSMUSG00000004341	Gpx6	ENSG00000198704	GPX6
ENSMUSG00000027695	Pld1	ENSG00000075651	PLD1
ENSMUSG00000001168	Oas1h	ENSG00000089127	OAS1
ENSMUSG00000042066	Tmcc2	ENSG00000133069	TMCC2
ENSMUSG00000026435	Slc45a3	ENSG00000158715	SLC45A3
ENSMUSG00000025898	Cwf19l2	ENSG00000152404	CWF19L2
ENSMUSG00000026301	Iqca	ENSG00000132321	IQCA1
ENSMUSG00000004568	Arhgef18	ENSG00000104880	ARHGEF18
ENSMUSG00000039298	Cdk5rap2	ENSG00000136861	CDK5RAP2
ENSMUSG00000032056	Btg4	ENSG00000137707	BTG4
ENSMUSG00000040997	Abhd4	ENSG00000100439	ABHD4
ENSMUSG00000026565	Pou2f1	ENSG00000143190	POU2F1
ENSMUSG00000030822	Prr14	ENSG00000156858	PRR14
ENSMUSG00000027381	Bcl2l11	ENSG00000153094	BCL2L11
ENSMUSG00000032346	Ooep	ENSG00000203907	OOEP
ENSMUSG00000028848	Gpn2	ENSG00000142751	GPN2
ENSMUSG00000046324	Ermp1	ENSG00000099219	ERMP1
ENSMUSG00000039354	Smarcal1	ENSG00000138375	SMARCAL1
ENSMUSG00000030255	Sspn	ENSG00000123096	SSPN

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000059108	Ifitm6	ENSG00000142089	IFITM3
ENSMUSG00000059108	Ifitm6	ENSG00000185201	IFITM2
ENSMUSG00000005483	Dnajb1	ENSG00000132002	DNAJB1
ENSMUSG00000026249	Serpine2	ENSG00000135919	SERPINE2
ENSMUSG00000020375	Rufy1	ENSG00000176783	RUFY1
ENSMUSG00000038236	Hoxa7	ENSG00000122592	HOXA7
ENSMUSG00000054320	Lrrc36	ENSG00000159708	LRRC36
ENSMUSG00000040151	Hs2st1	ENSG00000153936	HS2ST1
ENSMUSG00000040151	Hs2st1	ENSG00000267561	
ENSMUSG00000038304	Cd160	ENSG00000117281	CD160
ENSMUSG00000023044	Csad	ENSG00000139631	CSAD
ENSMUSG00000027233	Patl2	ENSG00000229474	PATL2
ENSMUSG00000039239	Tgfb2	ENSG00000092969	TGFB2
ENSMUSG00000021785	Ngly1	ENSG00000151092	NGLY1
ENSMUSG00000015961	Adss	ENSG00000035687	ADSS2
ENSMUSG00000028825	Rhd	ENSG00000187010	RHD
ENSMUSG00000028825	Rhd	ENSG00000188672	RHCE
ENSMUSG00000026235	Epha4	ENSG00000116106	EPHA4
ENSMUSG00000039238	Zfp750	ENSG00000141579	ZNF750
ENSMUSG00000014599	Csf1	ENSG00000184371	CSF1
ENSMUSG00000022833	Ccdc14	ENSG00000175455	CCDC14
ENSMUSG00000026675	Hsd17b7	ENSG00000132196	HSD17B7
ENSMUSG00000039242	B3galnt2	ENSG00000162885	B3GALNT2
ENSMUSG00000021639	Gtf2h2	ENSG00000183474	GTF2H2C
ENSMUSG00000001166	Oas1c	ENSG00000089127	OAS1
ENSMUSG00000032570	Atp2c1	ENSG00000017260	ATP2C1

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000003033	Ap1m1	ENSG00000072958	AP1M1
ENSMUSG00000041377	Ninj2	ENSG00000171840	NINJ2
ENSMUSG00000063145	Bbs5	ENSG00000163093	BBS5
ENSMUSG00000026429	Ube2t	ENSG00000077152	UBE2T
ENSMUSG00000021629	Slc30a5	ENSG00000145740	SLC30A5
ENSMUSG00000030768	Disp1	ENSG00000154309	DISP1
ENSMUSG00000051705	Senp8	ENSG00000166192	SENP8
ENSMUSG00000005672	Kit	ENSG00000157404	KIT
ENSMUSG00000026526	Fh1	ENSG00000091483	FH
ENSMUSG00000033581	Igf2bp2	ENSG00000073792	IGF2BP2
ENSMUSG00000040614	Nlrp9c	ENSG00000185792	NLRP9
ENSMUSG00000010796	Asz1	ENSG00000154438	ASZ1
ENSMUSG00000002910	Arrdc2	ENSG00000105643	ARRDC2
ENSMUSG00000025163	Cd7	ENSG00000173762	CD7
ENSMUSG00000046679	C87436	ENSG00000115998	C2orf42
ENSMUSG00000021962	Dcp1a	ENSG00000272886	DCP1A
ENSMUSG00000032096	Arcn1	ENSG00000095139	ARCN1
ENSMUSG00000038784	Cnot4	ENSG00000080802	CNOT4
ENSMUSG00000021936	Mapk8	ENSG00000107643	MAPK8
ENSMUSG00000096225	Lhx8	ENSG00000162624	LHX8
ENSMUSG00000035191	Rfpl4	ENSG00000229292	RFPL4AL1
ENSMUSG00000035191	Rfpl4	ENSG00000223638	RFPL4A
ENSMUSG00000028093	Acp6	ENSG00000162836	ACP6
ENSMUSG00000035762	Tmem161b	ENSG00000164180	TMEM161B
ENSMUSG00000039735	Fnbp1l	ENSG00000137942	FNBP1L
ENSMUSG00000026499	Acbd3	ENSG00000182827	ACBD3

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000000440	Pparg	ENSG00000132170	PPARG
ENSMUSG00000005774	Rfx5	ENSG00000143390	RFX5
ENSMUSG00000061393	Acvr2b	ENSG00000114739	ACVR2B
ENSMUSG00000005682	Pan2	ENSG00000135473	PAN2
ENSMUSG00000056310	Tyw1	ENSG00000277149	TYW1B
ENSMUSG00000056310	Tyw1	ENSG00000198874	TYW1
ENSMUSG00000029736	Nobox	ENSG00000106410	NOBOX
ENSMUSG00000046408	1700067K01Rik	ENSG00000188032	C19orf67
ENSMUSG00000066867	Oas1e	ENSG00000089127	OAS1
ENSMUSG00000036111	Lmo1	ENSG00000166407	LMO1
ENSMUSG00000062604	Srpk2	ENSG00000135250	SRPK2
ENSMUSG00000010592	Dazl	ENSG00000092345	DAZL
ENSMUSG00000032413	Rasa2	ENSG00000155903	RASA2
ENSMUSG00000005161	Prdx2	ENSG00000167815	PRDX2
ENSMUSG00000042638	Gucy2c	ENSG00000070019	GUCY2C
ENSMUSG00000028884	Rpa2	ENSG00000117748	RPA2
ENSMUSG00000043067	Dpy19l1	ENSG00000173852	DPY19L1
ENSMUSG00000046856	Gpr1	ENSG00000183671	CMKLR2
ENSMUSG00000026239	Pde6d	ENSG00000156973	PDE6D
ENSMUSG00000023800	Tiam2	ENSG00000146426	TIAM2
ENSMUSG00000026111	Unc50	ENSG00000115446	UNC50
ENSMUSG00000026766	Mmadhc	ENSG00000168288	MMADHC
ENSMUSG00000019528	Gyg	ENSG00000163754	GYG1
ENSMUSG00000025235	Bbs4	ENSG00000140463	BBS4
ENSMUSG00000022195	6030458C11Rik	ENSG00000082213	C5orf22
ENSMUSG00000023994	Nfya	ENSG00000001167	NFYA

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000042757	Tmem108	ENSG00000144868	TMEM108
ENSMUSG00000004947	Dtx2	ENSG00000091073	DTX2
ENSMUSG00000028354	Fmn2	ENSG00000155816	FMN2
ENSMUSG00000029287	Tgfbr3	ENSG00000069702	TGFBR3
ENSMUSG00000026715	Serpinc1	ENSG00000117601	SERPINC1
ENSMUSG00000028708	Mknk1	ENSG00000079277	MKNK1
ENSMUSG00000030269	Mtmr14	ENSG00000163719	MTMR14
ENSMUSG00000044499	Hs3st5	ENSG00000249853	HS3ST5
ENSMUSG00000028456	Unc13b	ENSG00000198722	UNC13B
ENSMUSG00000042570	Mier2	ENSG00000105556	MIER2
ENSMUSG00000026027	Stradb	ENSG00000082146	STRADB
ENSMUSG00000029686	Cul1	ENSG00000055130	CUL1
ENSMUSG00000006676	Usp19	ENSG00000172046	USP19
ENSMUSG00000037058	Paip2	ENSG00000120727	PAIP2
ENSMUSG00000038393	Txnip	ENSG00000265972	TXNIP
ENSMUSG00000038299	Wdr36	ENSG00000134987	WDR36
ENSMUSG00000057363	Uxs1	ENSG00000115652	UXS1
ENSMUSG00000027615	Hps3	ENSG00000163755	HPS3
ENSMUSG00000030309	Caprin2	ENSG00000110888	CAPRIN2
ENSMUSG00000034724	Cnot6l	ENSG00000138767	CNOT6L
ENSMUSG00000020122	Egfr	ENSG00000146648	EGFR
ENSMUSG00000034152	Exoc3	ENSG00000180104	EXOC3
ENSMUSG00000014767	Tbp	ENSG00000112592	TBP
ENSMUSG00000034640	Tiparp	ENSG00000163659	TIPARP
ENSMUSG00000000142	Axin2	ENSG00000168646	AXIN2
ENSMUSG00000024068	Spast	ENSG00000021574	SPAST

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000022505	Emp2	ENSG00000213853	EMP2
ENSMUSG00000014554	Dguok	ENSG00000114956	DGUOK
ENSMUSG00000032409	Atr	ENSG00000175054	ATR
ENSMUSG00000005503	Evx1	ENSG00000106038	EVX1
ENSMUSG00000031756	Cenpn	ENSG00000166451	CENPN
ENSMUSG00000047799	Oog4	ENSG00000116726	PRAMEF12
ENSMUSG00000047799	Oog4	ENSG00000204501	PRAMEF15
ENSMUSG00000047799	Oog4	ENSG00000204481	PRAMEF14
ENSMUSG00000047799	Oog4	ENSG00000204480	PRAMEF19
ENSMUSG00000047799	Oog4	ENSG00000204479	PRAMEF17
ENSMUSG00000047799	Oog4	ENSG00000204478	PRAMEF20
ENSMUSG00000047799	Oog4	ENSG00000274764	PRAMEF27
ENSMUSG00000047799	Oog4	ENSG00000229571	PRAMEF25
ENSMUSG00000047799	Oog4	ENSG00000204505	PRAMEF9
ENSMUSG00000047799	Oog4	ENSG00000280267	PRAMEF26
ENSMUSG00000001157	Gmcl1	ENSG00000244234	GMCL2
ENSMUSG00000001157	Gmcl1	ENSG00000087338	GMCL1
ENSMUSG00000004032	Gstm5	ENSG00000134202	GSTM3
ENSMUSG00000032898	Fbxo21	ENSG00000135108	FBXO21
ENSMUSG00000090112	Shprh	ENSG00000146414	SHPRH
ENSMUSG00000027380	Acoxl	ENSG00000153093	ACOXL
ENSMUSG00000020098	Pcbd1	ENSG00000166228	PCBD1
ENSMUSG00000025280	Polr3a	ENSG00000148606	POLR3A
ENSMUSG00000032274	Cyp19a1	ENSG00000137869	CYP19A1
ENSMUSG00000018401	Mtmr4	ENSG00000108389	MTMR4
ENSMUSG00000026162	Nhej1	ENSG00000187736	NHEJ1

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000003824	Syce2	ENSG00000161860	SYCE2
ENSMUSG00000053716	Dusp7	ENSG00000164086	DUSP7
ENSMUSG00000003234	Abcf3	ENSG00000161204	ABCF3
ENSMUSG00000027951	Adar	ENSG00000160710	ADAR
ENSMUSG00000030102	Itpr1	ENSG00000150995	ITPR1
ENSMUSG00000035285	Nat14	ENSG00000090971	NAT14
ENSMUSG00000043441	Gpr149	ENSG00000174948	GPR149
ENSMUSG00000025316	Banp	ENSG00000172530	BANP
ENSMUSG00000061666	Gdpd1	ENSG00000153982	GDPD1
ENSMUSG00000030486	Zfp108	ENSG00000159915	ZNF233
ENSMUSG00000032536	Trak1	ENSG00000182606	TRAK1
ENSMUSG00000022710	Usp7	ENSG00000187555	USP7
ENSMUSG00000026289	Atg16l1	ENSG00000085978	ATG16L1
ENSMUSG00000034593	Myo5a	ENSG00000197535	MYO5A
ENSMUSG00000023192	Grm2	ENSG00000164082	GRM2
ENSMUSG00000004885	Crabp2	ENSG00000143320	CRABP2
ENSMUSG00000015966	Il17rb	ENSG00000056736	IL17RB
ENSMUSG00000019066	Rab3d	ENSG00000105514	RAB3D
ENSMUSG00000017716	Birc5	ENSG00000089685	BIRC5
ENSMUSG00000004099	Dnmt1	ENSG00000130816	DNMT1
ENSMUSG00000058248	Kcnh1	ENSG00000284299	
ENSMUSG00000058248	Kcnh1	ENSG00000283952	
ENSMUSG00000058248	Kcnh1	ENSG00000143473	KCNH1
ENSMUSG00000035356	Nfkbiz	ENSG00000144802	NFKBIZ
ENSMUSG00000074061	Fbxw19	ENSG00000164049	FBXW12
ENSMUSG00000032402	Smad3	ENSG00000166949	SMAD3

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000032604	Qars	ENSG00000172053	QARS1
ENSMUSG00000019894	Slc6a15	ENSG00000072041	SLC6A15
ENSMUSG00000061751	Kalrn	ENSG00000160145	KALRN
ENSMUSG00000033352	Map2k4	ENSG00000065559	MAP2K4
ENSMUSG00000028293	Slc35a1	ENSG00000164414	SLC35A1
ENSMUSG00000030878	Cdr2	ENSG00000140743	CDR2
ENSMUSG00000024673	Ms4a1	ENSG00000156738	MS4A1
ENSMUSG00000047539	Fbxo28	ENSG00000143756	FBXO28
ENSMUSG00000026683	Nuf2	ENSG00000143228	NUF2
ENSMUSG00000041670	Rims1	ENSG00000079841	RIMS1
ENSMUSG00000043831	Lysmd4	ENSG00000183060	LYSMD4
ENSMUSG00000050468	Astl	ENSG00000188886	ASTL
ENSMUSG00000010721	Lmbr1	ENSG00000105983	LMBR1
ENSMUSG00000051650	B3gnt2	ENSG00000170340	B3GNT2
ENSMUSG00000015305	Sash1	ENSG00000111961	SASH1
ENSMUSG00000067365	Tmem128	ENSG00000132406	TMEM128
ENSMUSG00000036814	Slc6a20a	ENSG00000163817	SLC6A20
ENSMUSG00000029656	C8b	ENSG00000021852	C8B
ENSMUSG00000042726	Trafd1	ENSG00000135148	TRAFD1
ENSMUSG00000027367	Stard7	ENSG00000084090	STARD7
ENSMUSG00000020130	Tbc1d15	ENSG00000121749	TBC1D15
ENSMUSG00000008958	Vps72	ENSG00000163159	VPS72
ENSMUSG00000031921	Terf2	ENSG00000132604	TERF2
ENSMUSG00000027088	Phospho2	ENSG00000144362	PHOSPHO2
ENSMUSG00000005897	Nr2c1	ENSG00000120798	NR2C1
ENSMUSG00000033952	Aspm	ENSG00000066279	ASPM

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000027379	Bub1	ENSG00000169679	BUB1
ENSMUSG00000058298	Mcm9	ENSG00000111877	MCM9
ENSMUSG00000005470	Asf1b	ENSG00000105011	ASF1B
ENSMUSG00000031538	Plat	ENSG00000104368	PLAT
ENSMUSG00000029279	Brdt	ENSG00000137948	BRDT
ENSMUSG00000050188	Lsm10	ENSG00000181817	LSM10
ENSMUSG00000028140	Mrpl9	ENSG00000143436	MRPL9
ENSMUSG00000022353	Mtss1	ENSG00000170873	MTSS1
ENSMUSG00000025105	Bnc1	ENSG00000169594	BNC1
ENSMUSG00000020640	Itsn2	ENSG00000198399	ITSN2
ENSMUSG00000053730	Tmem39b	ENSG00000121775	TMEM39B
ENSMUSG00000004948	Zp3	ENSG00000146707	POMZP3
ENSMUSG00000030616	Sytl2	ENSG00000137501	SYTL2
ENSMUSG00000037017	Zscan21	ENSG00000166529	ZSCAN21
ENSMUSG00000075327	Zbtb2	ENSG00000181472	ZBTB2
ENSMUSG00000046589	Lrrc8e	ENSG00000171017	LRRC8E
ENSMUSG00000015721	Nlrp5	ENSG00000171487	NLRP5
ENSMUSG00000020794	Ube2g1	ENSG00000132388	UBE2G1
ENSMUSG00000002064	Sdf2	ENSG00000132581	SDF2
ENSMUSG00000047248	C2cd3	ENSG00000168014	C2CD3
ENSMUSG00000005493	Msh4	ENSG00000057468	MSH4
ENSMUSG00000018727	Cpsf4l	ENSG00000187959	CPSF4L
ENSMUSG00000004936	Map2k1	ENSG00000169032	MAP2K1
ENSMUSG00000028696	Ipp	ENSG00000197429	IPP
ENSMUSG00000075023	Accsl	ENSG00000205126	ACCSL
ENSMUSG00000026687	Aldh9a1	ENSG00000143149	ALDH9A1

Mouse Gene ID	Mouse Gene Name	Human Gene ID	Human Gene Name
ENSMUSG00000000811	Txnrd3	ENSG00000197763	TXNRD3
ENSMUSG00000031661	Nkd1	ENSG00000140807	NKD1
ENSMUSG00000020413	Hus1	ENSG00000136273	HUS1
ENSMUSG00000070871	Ccnyl1	ENSG00000163249	CCNYL1
ENSMUSG00000026470	Stx6	ENSG00000135823	STX6
ENSMUSG00000004952	Rasa4	ENSG00000105808	RASA4
ENSMUSG00000019857	Asf1a	ENSG00000111875	ASF1A
ENSMUSG00000020257	Wdr82	ENSG00000164091	WDR82

In some aspects, the protein involved in oocyte maturation is selected from SYCP3, TRIP13, MCM8, STAG3, PATL2, TUBB8, AURKC, and WEE2.

In some aspects, the protein involved in oocyte maturation is SYCP3. In some aspects, the coding region comprises an RNA sequence of SEQ ID NO: 1, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous, wherein at least one nucleotide in the RNA sequence has been chemically modified:

SEQ ID NO: 1

AUGGUGUCCUCCGGAAAAAAGUAUUCCAGGAAAUCUGGGAAGCCGUCUGUGG
AAGAUCAGUUUACGAGAGCCUAUGACUUUGAGACUGAAGAUAAGAAAGAUC
UGAGUGGAUCAGAGGAAGAUGUUAUUGAAGGGAAGACUGCAGUCAUUGAGA
AACGUAGGAAGAAAAGGUCUUCUGCAGGAGUAGUUGAAGAUAUGGGGGGUG
AAGUGCAGAAUAUGCUGGAAGGAGUUGGAGUUGACAUUAACAAGGCUCUUC
UUGCCAAGAGAAAGAGACUAGAAAUGUAUACCAAGGCUUCUCUCAAAACUAG
UAACCAGAAAAUUGAACAUGUUUGGAAAACACAACAAGAUCAAAGGCAGAA
GCUUAACCAAGAAUAUUCUCAGCAGUUUCUGACUUUGUUUCAGCAGUGGGAU
UUAGAUAUGCAGAAAGCUGAGGAACAAGAAGAAAAAAUACUUAAUAUGUUU
CGACAGCAACAAAAGAUUCUUCAACAAUCUAGAAUUGUUCAGAGCCAGAGAU
UGAAAACAAUUAAACAGUUAUAUGAGCAGUUCAUAAAGAGUAUGGAAGAGU
UGGAGAAGAAUCAUGAUAAUCUACUUACUGGUGCACAAAAUGAAUUUAAAA

AAGAAAUGGCUAUGUUGCAAAAAAAAAUUAUGAUGGAAACUCAGCAGCAAG
AGAUAGCAAGUGUUCGGAAGUCUCUUCAAUCCAUGUUAUUCUGA

In some aspects, the coding region encodes a polypeptide having a sequence of SEQ ID NO: 2, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous:

SEQ ID NO: 2

MVSSGKKYSRKSGKPSVEDQFTRAYDFETEDKKDLSGSEEDVIEGKTAVIEKRRKK
RSSAGVVEDMGGEVQNMLEGVGVDINKALLAKRKRLEMYTKASLKTSNQKIEHV
WKTQQDQRQKLNQEYSQQFLTLFQQWDLDMQKAEEQEEKILNMFRQQQKILQQS
RIVQSQRLKTIKQLYEQFIKSMEELEKNHDNLLTGAQNEFKKEMAMLQKKIMMET
QQQEIASVRKSLQSMLF

In some aspects, the protein involved in oocyte maturation is TRIP13. In some aspects, the coding region comprises an RNA sequence of SEQ ID NO: 3, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous, wherein at least one nucleotide in the RNA sequence has been chemically modified:

SEQ ID NO: 3

AUGGACGAGGCCGUGGGCGACCUGAAGCAGGCGCUUCCCUGUGUGGCCGAGU
CGCCAACGGUCCACGUGGAGGUGCAUCAGCGCGGCAGCAGCACUGCAAAGAA
AGAAGACAUAAACCUGAGUGUUAGAAAGCUACUCAACAGACAUAAUAUUGU
GUUUGGUGAUUACACAUGGACUGAGUUUGAUGAACCUUUUUUGACCAGAAA
UGUGCAGUCUGUGUCUAUUAUUGACACAGAAUUAAAGGUUAAAGACUCACA
GCCCAUCGAUUUGAGUGCAUGCACUGUUGCACUUCACAUUUUCCAGCUGAAU
GAAGAUGGCCCCAGCAGUGAAAAUCUGGAGGAAGAGACAGAAAACAUAAUU
GCAGCAAAUCACUGGGUUCUACCUGCAGCUGAAUUCCAUGGGCUUUGGGACA
GCUUGGUAUACGAUGUGGAAGUCAAAUCCCAUCUCCUCGAUUAUGUGAUGAC
AACUUUACUGUUUUCAGACAAGAACGUCAACAGCAACCUCAUCACCUGGAAC
CGGGUGGUGCUGCUCCACGGUCCUCCUGGCACUGGAAAAACAUCCCUGUGUA
AAGCGUUAGCCCAGAAAUUGACAAUUAGACUUUCAAGCAGGUACCGAUAUGG
CCAAUUAAUUGAAAUAAACAGCCACAGCCUCUUUUCUAAGUGGUUUUCGGAA
AGUGGCAAGCUGGUAACCAAGAUGUUUCAGAAGAUUCAGGAUUUGAUUGAU
GAUAAAGACGCCCUGGUGUUCGUGCUGAUUGAUGAGGUGGAGAGUCUCACA
GCCGCCCGAAAUGCCUGCAGGGCGGGCACCGAGCCAUCAGAUGCCAUCCGCG

UGGUCAAUGCUGUCUUGACCCAAAUUGAUCAGAUUAAAAGGCAUUCCAAUGU
UGUGAUUCUGACCACUUCUAACAUCACCGAGAAGAUCGACGUGGCCUUCGUG
GACAGGGCUGACAUCAAGCAGUACAUUGGGCCACCCUCUGCAGCAGCCAUCU
UCAAAAUCUACCUCUCUUGUUUGGAAGAACUGAUGAAGUGUCAGAUCAUAU
ACCCUCGCCAGCAGCUGCUGACCCUCCGAGAGCUAGAGAUGAUUGGCUUCAU
UGAAAACAACGUGUCAAAAUUGAGCCUUCUUUUGAAUGACAUUUCAAGGAA
GAGCGAGGGCCUCAGCGGCCGGGUCCUGAGAAAACUCCCCUUUCUGGCUCAU
GCGCUGUAUGUCCAGGCCCCCACCGUCACCAUAGAGGGGUUCCUCCAGGCCC
UGUCUCUGGCAGUGGACAAGCAGUUUGAAGAGAGAAAGAAGCUUGCAGCUU
ACAUCUGA

In some aspects, the coding region encodes a polypeptide having a sequence of SEQ ID NO: 4, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous:

SEQ ID NO: 4

MDEAVGDLKQALPCVAESPTVHVEVHQRGSSTAKKEDINLSVRKLLNRHNIVFGDY
TWTEFDEPFLTRNVQSVSIIDTELKVKDSQPIDLSACTVALHIFQLNEDGPSSENLEEE
TENIIAANHWVLPAAEFHGLWDSLVYDVEVKSHLLDYVMTTLLFSDKNVNSNLIT
WNRVVLLHGPPGTGKTSLCKALAQKLTIRLSSRYRYGQLIEINSHSLFSKWFSESGKL
VTKMFQKIQDLIDDKDALVFVLIDEVESLTAARNACRAGTEPSDAIRVVNAVLTQID
QIKRHSNVVILTTSNITEKIDVAFVDRADIKQYIGPPSAAAIFKIYLSCLEELMKCQIIY
PRQQLLTLRELEMIGFIENNVSKLSLLLNDISRKSEGLSGRVLRKLPFLAHALYVQAP
TVTIEGFLQALSLAVDKQFEERKKLAAYI

In some aspects, the protein involved in oocyte maturation is MCM8. In some aspects, the coding region comprises an RNA sequence of SEQ ID NO: 5, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous, wherein at least one nucleotide in the RNA sequence has been chemically modified:

SEQ ID NO: 5

AUGAAUGGAGAGUAUAGAGGCAGAGGAUUUGGACGAGGAAGAUUUCAAAGC
UGGAAAAGGGGAAGAGGUGGUGGGAACUUCUCAGGAAAAUGGAGAGAAAGA
GAACACAGACCUGAUCUGAGUAAAACCACAGGAAAACGUACUUCUGAACAAA
CCCCACAGUUUUUGCUUUCAACAAAGACCCCACAGUCAAUGCAGUCAACAUU
GGAUCGAUUCAUACCAUAUAAAGGCUGGAAGCUUUAUUUCUCUGAAGUUUA

CAGCGAUAGCUCUCCUUUGAUUGAGAAGAUUCAAGCAUUUGAAAAAUUUUU
CACAAGGCAUAUUGAUUUGUAUGACAAGGAUGAAAUAGAAAGAAAGGGAAG
UAUUUUGGUAGAUUUUAAAGAACUGACAGAAGGUGGUGAAGUAACUAACUU
GAUACCAGAUAUAGCAACUGAACUAAGAGAUGCACCUGAGAAAACCUUGGCU
UGCAUGGGUUUGGCAAUACAUCAGGUGUUAACUAAGGACCUUGAAAGGCAU
GCAGCUGAGUUACAAGCCCAGGAAGGAUUGUCUAAUGAUGGAGAAACAAUG
GUAAAUGUGCCACAUAUUCAUGCAAGGGUGUACAACUAUGAGCCUUUGACAC
AGCUCAAGAAUGUCAGAGCAAAUUACUAUGGAAAAUACAUUGCUCUAAGAG
GGACAGUGGUUCGUGUCAGUAAUAUAAAGCCUCUUUGCACCAAGAUGGCUUU
UCUUUGUGCUGCAUGUGGAGAAAUUCAGAGCUUUCCUCUUCCAGAUGGAAAA
UACAGUCUUCCCACAAAGUGUCCUGUGCCUGUGUGUCGAGGCAGGUCAUUUA
CUGCUCUCCGCAGCUCUCCUCUCACAGUUACGAUGGACUGGCAGUCAAUCAA
AAUCCAGGAAUUGAUGUCUGAUGAUCAGAGAGAAGCAGGUCGGAUUCCACG
AACAAUAGAAUGUGAGCUUGUUCAUGAUCUUGUGGAUAGCUGUGUCCCGGG
AGACACAGUGACUAUUACUGGAAUUGUCAAAGUCUCAAAUGCGGAAGAAGG
UUCUCGAAAUAAGAAUGACAAGUGUAUGUUCCUUUUGUAUAUUGAAGCAAA
UUCUAUUAGUAAUAGCAAAGGACAGAAAACAAAGAGUUCUGAGGAUGGGUG
UAAGCAUGGAAUGUUGAUGGAGUUCUCACUUAAAGACCUUUAUGCCAUCCAA
GAGAUUCAAGCUGAAGAAAACCUGUUUAAACUCAUUGUCAACUCGCUUUGCC
CUGUCAUUUUUGGUCAUGAACUUGUUAAAGCAGGUUUGGCAUUAGCACUCU
UUGGAGGAAGCCAGAAAUACGCAGAUGACAAAAACAGAAUUCCAAUUCGGG
GAGACCCCCACAUCCUUGUUGUUGGAGAUCCAGGCCUAGGAAAAAGUCAAAU
GCUACAGGCAGCGUGCAAUGUUGCCCCACGUGGCGUGUAUGUUUGUGGUAAC
ACCACGACCACCUCUGGUCUGACGGUAACUCUUUCAAAAGAUAGUUCCUCUG
GAGAUUUUGCUUUGGAAGCUGGUGCCCUGGUACUUGGUGAUCAAGGUAUUU
GUGGAAUCGAUGAAUUUGAUAAGAUGGGGAAUCAACAUCAAGCCUUGUUGG
AAGCCAUGGAGCAGCAAAGUAUUAGUCUUGCUAAGGCUGGUGUGGUUUGUA
GCCUUCCUGCAAGAACUUCCAUUAUUGCUGCUGCAAAUCCAGUUGGAGGACA
UUACAAUAAAGCCAAAACAGUUUCUGAGAAUUUAAAAAUGGGGAGUGCACU
ACUAUCCAGAUUUGAUUUGGUCUUUAUCCUGUUAGAUACUCCAAAUGAGCAU
CAUGAUCACUUACUCUCUGAACAUGUGAUUGCAAUAAGAGCUGGAAAGCAGA
GAACCAUUAGCAGUGCCACAGUAGCUCGUAUGAAUAGUCAAGAUUCAAAUAC
UUCCGUACUUGAAGUAGUUUCUGAGAAGCCAUUAUCAGAAAGACUAAAGGU
GGUUCCUGGAGAAACAAUAGAUCCCAUUCCCCACCAGCUAUUGAGAAAGUAC

AUUGGCUAUGCUCGGCAGUAUGUGUACCCAAGGCUAUCCACAGAAGCUGCUC
GAGUUCUUCAAGAUUUUUACCUUGAGCUCCGGAAACAGAGCCAGAGGUUAAA
UAGCUCACCAAUCACUACCAGGCAGCUGGAAUCUUUGAUUCGUCUGACAGAG
GCACGAGCAAGGUUGGAAUUGAGAGAGGAAGCAACCAAAGAAGACGCUGAG
GAUAUAGUGGAAAUUAUGAAAUAUAGCAUGCUAGGAACUUACUCUGAUGAA
UUUGGGAACCUAGAUUUUGAGCGAUCCCAGCAUGGUUCUGGAAUGAGCAACA
GGUCAACAGCGAAAAGAUUUAUUUCUGCUCUCAACAACGUUGCUGAAAGAAC
UUAUAAUAAUAUAUUUCAAUUUCAUCAACUUCGGCAGAUUGCCAAAGAACU
AAACAUUCAGGUUGCUGAUUUUGAAAAUUUUAUUGGAUCACUAAAUGACCA
GGGUUACCUCUUGAAAAAAGGCCCAAAAGUUUACCAGCUUCAAACUAUGUAA

In some aspects, the coding region encodes a polypeptide having a sequence of SEQ ID NO: 6, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous:

SEQ ID NO: 6

MNGEYRGRGFGRGRFQSWKRGRGGGNFSGKWREREHRPDLSKTTGKRTSEQTPQ
FLLSTKTPQSMQSTLDRFIPYKGWKLYFSEVYSDSSPLIEKIQAFEKFFTRHIDLYDK
DEIERKGSILVDFKELTEGGEVTNLIPDIATELRDAPEKTLACMGLAIHQVLTKDLER
HAAELQAQEGLSNDGETMVNVPHIHARVYNYEPLTQLKNVRANYYGKYIALRGTV
VRVSNIKPLCTKMAFLCAACGEIQSFPLPDGKYSLPTKCPVPVCRGRSFTALRSSPLT
VTMDWQSIKIQELMSDDQREAGRIPRTIECELVHDLVDSCVPGDTVTITGIVKVSNA
EEGSRNKNDKCMFLLYIEANSISNSKGQKTKSSEDGCKHGMLMEFSLKDLYAIQEIQ
AEENLFKLIVNSLCPVIFGHELVKAGLALALFGGSQKYADDKNRIPIRGDPHILVVGD
PGLGKSQMLQAACNVAPRGVYVCGNTTTTSGLTVTLSKDSSSGDFALEAGALVLGD
QGICGIDEFDKMGNQHQALLEAMEQQSISLAKAGVVCSLPARTSIIAAANPVGGHY
NKAKTVSENLKMGSALLSRFDLVFILLDTPNEHHDHLLSEHVIAIRAGKQRTISSATV
ARMNSQDSNTSVLEVVSEKPLSERLKVVPGETIDPIPHQLLRKYIGYARQYVYPRLS
TEAARVLQDFYLELRKQSQRLNSSPITTRQLESLIRLTEARARLELREEATKEDAEDI
VEIMKYSMLGTYSDEFGNLDFERSQHGSGMSNRSTAKRFISALNNVAERTYNNIFQF
HQLRQIAKELNIQVADFENFIGSLNDQGYLLKKGPKVYQLQTM

In some aspects, the protein involved in oocyte maturation is PATL2. In some aspects, the coding region comprises an RNA sequence of SEQ ID NO: 7, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous, wherein at least one nucleotide in the RNA sequence has been chemically modified:

SEQ ID NO: 7

AUGAAUUGCCUUGAAGGGCCAGGUAAGACCUGUGGCCCCUUGGCUUCUGAGG
AGGAGCUGGUGUCUGCCUGCCAGUUGGAAAAAGAAGAAGAGAAUGAAGGGG
AGGAGGAGGAAGAGGAGGAGGACGAGGAGGAUCUGGACCCAGAUCUGGACC
CAGACCUAGAGGAGGAAGAGAAUGAUCUUGGGGAUCCAGCUGUACUUGGUG
CUGUCCACAACACCCAGAGAGCUCUGCUUAGCUCCCCUGGAGUCAAGGCCCC
UGGUAUGCUGGGAAUGUCACUUGCCUCCUUGCAUUUUCUGUGGCAGACCUUG
GACUACCUGUCGCCCAUCCCUUUCUGGCCUACAUUUCCCAGCACCAGCUCUCC
AGCACAGCACUUUGGACCUCGGCUGCCCUCACCAGACCCAACUCUCUUCUGC
AGCCUGCUGACCUCGUGGCCCCCUAGGUUCAGUCAUCUGACCCAGCUCCACCC
UCGGCACCAACGAAUCUUGCAGCAGCAGCAGCAUAGUCAAACACCAAGUCCC
CCAGCCAAGAAGCCUUGGUCUCAGCAGCCAGACCCCUAUGCUAACCUCAUGA
CCAGAAAAGAGAAGGACUGGGUGAUAAAAGUGCAGAUGGUGCAGCUGCAGA
GUGCAAAACCCCGCCUGGAUGACUACUAUUACCAGGAAUAUUACCAGAAGCU
AGAGAAGAAGCAGGCAGACGAAGAGCUACUUGGACGAAGAAACCGGGUUGA
GUCCCUCAAGCUGGUAACGCCUUACAUUCCGAAGGCAGAGGCUUAUGAGUCC
GUGGUCCGAAUCGAGGGUUCCCUGGGCCAGGUAGCUGUGUCGACAUGCUUCA
GCCCUCGCCGAGCUAUUGAUGCGGUACCCCAUGGAACUCAAGAGCAGGAUAU
AGAAGCUGCAAGCAGUCAGAGGCUUCGGGUAUUAUACCGGAUUGAGAAGAU
GUUCCUUCAGUUACUAGAAAUAGAGGAAGGCUGGAAGUAUAGGCCUCCACCG
CCCUGCUUUUCUGAGCAGCAAAGCAACCAGGUUGAGAAGCUCUUCCAGACCU
UAAAGACCCAGGAGCAGAACAACCUGGAAGAGGCAGCAGAUGGCUUCCUGCA
GGUGCUCUCUGUGAGGAAGGGGAAGGCCCUGGUGGCCCGGCUGCUCCCCUUC
CUGCCCCAGGAUCAGGCUGUUACCAUUCUUUUGGCUAUCACCCACCAUCUGC
CCCUCCUGGUCCGGAGGGAUGUGGCUGAUCAGGCCCUACAAAUGUUAUUCAA
ACCUCUGGGCAAAUGUAUUAGUCACUUGACCCUCCACGAACUCCUCCAAGGA
CUUCAGGGAUUAACGCUGUUGCCACCUGGCUCCUCAGAGCGGCCAGUCACCG
UGGUGCUUCAGAAUCAGUUUGGAAUAUCUUUGCUCUAUGCCCUGCUGAGCCA
UGGGGAGCAACUGGUAUCGCUGCAUUCUUCCCUAGAGGAACCCAACAGUGAC
CAUACAGCUUGGACAGACAUGGUGGUUCUGAUUGCCUGGGAGAUAGCCCAAA
UGCCUACAGCCUCUCUGGCAGAACCCCUAGCUUUCCCCAGCAACCUACUUCCC
CUGUUCUGUCACCACGUGGACAAACAAUUGGUUCAGCAGCUGGAGGCCAGGA
UGGAGUUUGCCUGGAUUUACUGA

In some aspects, the coding region encodes a polypeptide having a sequence of SEQ ID NO: 8, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous:

SEQ ID NO: 8

MNCLEGPGKTCGPLASEEELVSACQLEKEEENEGEEEEEEEDEEDLDPDLDPDLEEE
ENDLGDPAVLGAVHNTQRALLSSPGVKAPGMLGMSLASLHFLWQTLDYLSPIPFWP
TFPSTSSPAQHFGPRLPSPDPTLFCSLLTSWPPRFSHLTQLHPRHQRILQQQQHSQTPS
PPAKKPWSQQPDPYANLMTRKEKDWVIKVQMVQLQSAKPRLDDYYYQEYYQKLE
KKQADEELLGRRNRVESLKLVTPYIPKAEAYESVVRIEGSLGQVAVSTCFSPRRAIDA
VPHGTQEQDIEAASSQRLRVLYRIEKMFLQLLEIEEGWKYRPPPPCFSEQQSNQVEK
LFQTLKTQEQNNLEEAADGFLQVLSVRKGKALVARLLPFLPQDQAVTILLAITHHLP
LLVRRDVADQALQMLFKPLGKCISHLTLHELLQGLQGLTLLPPGSSERPVTVVLQNQ
FGISLLYALLSHGEQLVSLHSSLEEPNSDHTAWTDMVVLIAWEIAQMPTASLAEPLAF
PSNLLPLFCHHVDKQLVQQLEARMEFAWIY

In some aspects, the protein involved in oocyte maturation is TUBB8. In some aspects, the coding region comprises an RNA sequence of SEQ ID NO: 9, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous, wherein at least one nucleotide in the RNA sequence has been chemically modified:

SEQ ID NO: 9

AUGAGGGAGAUCGUGCUCACGCAGAUCGGGCAGUGCGGGAAUCAGAUCGGCG
CCAAGUUCUGGGAGGUGAUCUCUGAUGAACAUGCCAUCGACUCCGCUGGCAC
CUACCACGGGGACAGCCACCUGCAGCUGGAGCGCAUCAACGUGUACUACAAC
GAGGCCAGCGGUGGCAGGUACGUGCCCCGCGCUGUGCUCGUGGAUCUGGAGC
CGGGCACCAUGGACUCUGUGCGCUCGGGGCCCUUCGGGCAGGUCUUCAGGCC
AGACAACUUCAUCUUCGGUCAGUGUGGGGCCGGAAACAACUGGGCCAAGGGA
CACUACACCGAAGGCGCGGAGCUGAUGGAGUCAGUGAUGGACGUUGUCAGAA
AGGAGGCUGAGAGCUGUGACUGCCUGCAGGGUUUCCAGCUGACCCACUCCCU
GGGUGGGGGGACUGGGUCUGGGAUGGGUACCCUUCUGCUCAGUAAGAUCCGG
GAGGAGUACCCAGACAGGAUCAUAAACACAUUCAGCAUCCUGCCCUCGCCCA
AGGUGUCGGACACCGUGGUGGAGCCCUACAACGCCACCCUCUCAGUCCACCA
GCUCAUAGAAAACGCAGAUGAGACCUUUUGCAUAGAUAACGAAGCUCUGUAU
GACAUAUGUUCCAAGACCCUAAAACUGCCCACACCCACCUAUGGUGACCUGA

ACCACCUGGUGUCUGCUACCAUGAGUGGGGUCACCACGUGCCUGCGCUUCCC
GGGCCAGCUGAAUGCUGACCUGCGGAAGCUGGCCGUGAACAUGGUCCCGUUU
CCCCGGCUGCAUUUCUUCAUGCCCGGCUUUGCCCCACUGACCAGCCGGGGCA
GCCAGCAGUACCGGGCCUUGACUGUGGCUGAGCUUACCCAGCAGAUGUUUGA
UGCUAAGAACAUGAUGGCUGCCUGUGACCCCCGUCACGGCCGCUACCUAACG
GCGGCUGCCAUUUUCAGGGGUCGCAUGCCCAUGAGGGAGGUGGAUGAACAAA
UGUUCAACAUUCAAGAUAAGAACAGCAGUUACUUUGCUGACUGGCUCCCCAA
CAACGUAAAAACAGCCGUCUGUGACAUCCCACCCCGGGGGCUAAAAAUGUCA
GCCACCUUCAUUGGGAAUAAUACGGCCAUCCAGGAACUCUUCAAGCGUGUCU
CAGAGCAGUUUACAGCAAUGUUCAGGCGCAAGGCCUUCCUCCACUGGUACAC
GGGCGAGGGCAUGGAUGAGAUGGAAUUCACCGAGGCCGAGAGCAACAUGAAC
GACCUGGUGUCUGAAUAUCAGCAAUAUCAGGAUGCCACGGCCGAGGAGGAGG
AGGAUGAGGAGUAUGCCGAGGAGGAGGUGGCCUAG

In some aspects, the coding region encodes a polypeptide having a sequence of SEQ ID NO: 10, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous:

SEQ ID NO: 10

MREIVLTQIGQCGNQIGAKFWEVISDEHAIDSAGTYHGDSHLQLERINVYYNEASG
GRYVPRAVLVDLEPGTMDSVRSGPFGQVFRPDNFIFGQCGAGNNWAKGHYTEGAE
LMESVMDVVRKEAESCDCLQGFQLTHSLGGGTGSGMGTLLLSKIREEYPDRIINTFS
ILPSPKVSDTVVEPYNATLSVHQLIENADETFCIDNEALYDICSKTLKLPTPTYGDLN
HLVSATMSGVTTCLRFPGQLNADLRKLAVNMVPFPRLHFFMPGFAPLTSRGSQQYR
ALTVAELTQQMFDAKNMMAACDPRHGRYLTAAAIFRGRMPMREVDEQMFNIQDK
NSSYFADWLPNNVKTAVCDIPPRGLKMSATFIGNNTAIQELFKRVSEQFTAMFRRKA
FLHWYTGEGMDEMEFTEAESNMNDLVSEYQQYQDATAEEEEDEEYAEEEVA

In some aspects, the protein involved in oocyte maturation is AURKC. In some aspects, the coding region comprises an RNA sequence of SEQ ID NO: 11, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous, wherein at least one nucleotide in the RNA sequence has been chemically modified:

SEQ ID NO: 11

AUGAGCUCCCCCAGAGCUGUGGUGCAGCUGGGCAAAGCUCAACCUGCA
GGCGAAGAGUUGGCUACAGCAAACCAAACAGCCCAGCAGCCCAGCAGCCCAG

CCAUGCGGCGCCUCACAGUCGAUGACUUUGAAAUCGGGCGUCCCCUGGGCAA
GGGGAAAUUUGGGAAUGUGUACCUGGCUCGGCUCAAGGAAAGCCAUUUCAU
UGUGGCCCUGAAGGUUCUCUUCAAGUCGCAGAUAGAGAAGGAAGGACUGGA
GCACCAGCUGCGCCGGGAAAUUGAGAUCCAGGCUCAUCUACAACACCCCAAU
AUCCUGCGCCUGUAUAACUAUUUCCAUGAUGCACGCCGGGUGUACCUGAUUC
UGGAAUAUGCUCCAAGGGGUGAGCUCUACAAGGAGCUGCAGAAAAGCGAGA
AAUUAGAUGAACAGCGCACAGCCACGAUAAUAGAGGAGUUGGCAGAUGCCCU
GACCUACUGCCAUGACAAGAAAGUGAUUCACAGAGAUAUUAAGCCAGAGAAC
CUGCUGCUGGGGUUCAGGGGUGAGGUGAAGAUUGCAGAUUUUGGCUGGUCU
GUGCACACCCCCUCCCUGAGGAGGAAGACAAUGUGUGGGACACUGGACUACU
UGCCGCCAGAAAUGAUUGAGGGGAGAACAUAUGAUGAAAAGGUGGAUUUGU
GGUGCAUUGGAGUGCUCUGCUAUGAGCUGCUGGUGGGAUAUCCACCCUUUGA
GAGCGCCUCCCACAGUGAGACUUACAGACGCAUCCUCAAGGUAGAUGUGAGG
UUUCCACUAUCAAUGCCUCUGGGGGCCCGGGACUUGAUUUCCAGGCUUCUCA
GAUACCAGCCCUUGGAGAGACUGCCCCUGGCCCAGAUCCUGAAGCACCCCUG
GGUUCAGGCCCACUCCCGAAGGGUGCUGCCUCCCUGUGCUCAGAUGGCUUCC
UGA

In some aspects, the coding region encodes a polypeptide having a sequence of SEQ ID NO: 12, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous:

SEQ ID NO: 12

MSSPRAVVQLGKAQPAGEELATANQTAQQPSSPAMRRLTVDDFEIGRPLGKGKFGN
VYLARLKESHFIVALKVLFKSQIEKEGLEHQLRREIEIQAHLQHPNILRLYNYFHDAR
RVYLILEYAPRGELYKELQKSEKLDEQRTATIIEELADALTYCHDKKVIHRDIKPENL
LLGFRGEVKIADFGWSVHTPSLRRKTMCGTLDYLPPEMIEGRTYDEKVDLWCIGVL
CYELLVGYPPFESASHSETYRRILKVDVRFPLSMPLGARDLISRLLRYQPLERLPLAQ
ILKHPWVQAHSRRVLPPCAQMAS

In some aspects, the protein involved in oocyte maturation is WEE2. In some aspects, the coding region comprises an RNA sequence of SEQ ID NO: 13, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous, wherein at least one nucleotide in the RNA sequence has been chemically modified:

SEQ ID NO: 13

AUGGAUGACAAAGAUAUUGACAAAGAACUAAGGCAGAAAUUAAACUUUUCC
UAUUGUGAGGAGACUGAGAUUGAAGGGCAGAAGAAAGUAGAAGAAAGCAGG
GAGGCUUCGAGCCAAACCCCAGAGAAGGGUGAAGUGCAGGAUUCAGAGGCAA
AGGGUACACCACCUUGGACUCCCCUUAGCAACGUGCAUGAGCUCGACACAUC
UUCGGAAAAAGACAAAGAAAGUCCAGAUCAGAUUUUGAGGACUCCAGUGUC
ACACCCUCUCAAAUGUCCUGAGACACCAGCCCAACCAGACAGCAGGAGCAAG
CUGCUGCCCAGUGACAGCCCCUCUACUCCCAAAACCAUGCUGAGCCGGUUGG
UGAUUUCUCCAACAGGGAAGCUUCCUUCCAGAGGCCCUAAGCAUUUGAAGCU
CACACCUGCUCCCCUCAAGGAUGAGAUGACCUCAUUGGCUCUGGUCAAUAUU
AAUCCCUUCACUCCAGAGUCCUAUAAAAAAUUAUUUCUUCAAUCUGGUGGCA
AGAGGAAAAUAAGAGGAGAUCUUGAGGAAGCUGGUCCAGAGGAAGGCAAGG
GAGGGCUGCCUGCCAAGAGAUGUGUUUUACGAGAAACCAACAUGGCUUCCCG
CUAUGAAAAAGAAUUCUUGGAGGUUGAAAAAAUUGGGGUUGGCGAAUUUGG
UACAGUCUACAAGUGCAUUAAGAGGCUGGAUGGAUGUGUUUAUGCAAUAAA
GCGCUCUAUGAAAACUUUUACAGAAUUAUCAAAUGAGAAUUCGGCUUUGCA
UGAAGUUUAUGCUCACGCAGUGCUUGGGCAUCACCCCCAUGUGGUACGUUAC
UAUUCCUCAUGGGCAGAAGAUGACCACAUGAUCAUUCAGAAUGAAUACUGCA
AUGGUGGGAGUUUGCAAGCUGCUAUAUCUGAAAACACUAAGUCUGGCAAUC
AUUUUGAAGAGCCAAAACUCAAGGACAUCCUUCUACAGAUUUCCCUUGGCCU
UAAUUACAUCCACAACUCUAGCAUGGUACACCUGGACAUCAAACCUAGUAAU
AUAUUCAUUUGUCACAAGAUGCAAAGUGAAUCCUCUGGAGUCAUAGAAGAA
GUUGAAAAUGAAGCUGAUUGGUUUCUCUCUGCCAAUGUGAUGUAUAAAAUU
GGUGACCUGGGCCACGCAACAUCAAUAAACAAACCCAAAGUGGAAGAAGGAG
AUAGUCGCUUCCUGGCUAAUGAGAUUUUGCAAGAGGAUUACCGGCACCUUCC
CAAAGCAGACAUAUUUGCCUUGGGAUUAACAAUUGCAGUGGCUGCAGGAGCA
GAGUCAUUGCCCACCAAUGGUGCUGCAUGGCACCAUAUCCGCAAGGGUAACU
UUCCGGACGUUCCUCAGGAGCUCUCAGAAAGCUUUUCCAGUCUGCUCAAGAA
CAUGAUCCAACCUGAUGCCGAACAGAGACCUUCUGCAGCAGCUCUGGCCAGA
AAUACAGUUCUCCGGCCUUCCCUGGGAAAAACAGAAGAGCUCCAACAGCAGC
UGAAUUUGGAAAAGUUCAAGACUGCCACACUGGAAAGGGAACUGAGAGAAG
CCCAGCAGGCCCAGUCACCCCAGGGAUAUACCCAUCAUGGUGACACUGGGGU
CUCUGGGACCCACACAGGAUCAAGAAGCACAAAACGCCUGGUGGGAGGAAAG
AGUGCAAGGUCUUCAAGCUUUACCUCAGGAGAGCGUGAGCCUCUGCAUUAA

In some aspects, the coding region encodes a polypeptide having a sequence of SEQ ID NO: 14, or a variant thereof that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous:

SEQ ID NO: 14

MDDKDIDKELRQKLNFSYCEETEIEGQKKVEESREASSQTPEKGEVQDSEAKGTPP
WTPLSNVHELDTSSEKDKESPDQILRTPVSHPLKCPETPAQPDSRSKLLPSDSPSTPK
TMLSRLVISPTGKLPSRGPKHLKLTPAPLKDEMTSLALVNINPFTPESYKKLFLQSGG
KRKIRGDLEEAGPEEGKGGLPAKRCVLRETNMASRYEKEFLEVEKIGVGEFGTVYK
CIKRLDGCVYAIKRSMKTFTELSNENSALHEVYAHAVLGHHPHVVRYYSSWAEDDH
MIIQNEYCNGGSLQAAISENTKSGNHFEEPKLKDILLQISLGLNYIHNSSMVHLDIKP
SNIFICHKMQSESSGVIEEVENEADWFLSANVMYKIGDLGHATSINKPKVEEGDSRF
LANEILQEDYRHLPKADIFALGLTIAVAAGAESLPTNGAAWHHIRKGNFPDVPQELS
ESFSSLLKNMIQPDAEQRPSAAALARNTVLRPSLGKTEELQQQLNLEKFKTATLERE
LREAQQAQSPQGYTHHGDTGVSGTHTGSRSTKRLVGGKSARSSSFTSGEREPLH

Chemical Modification of Nucleotides

The mRNA of the present disclosure includes at least one chemical modification. As used herein, the terms "chemical modification" or "chemically modified" refer to modification with respect to adenosine (A), guanosine (G), uridine (U), or cytidine (C) ribonucleosides in one or more of their position, pattern, percent, or population. Generally, these terms are not intended to refer to modifications in naturally occurring 5 '-terminal mRNA cap moieties.

The chemical modifications may be various distinct modifications. In some aspects, the mRNA may contain one, two, or more of the same or different nucleoside or nucleotide chemical modifications. In some aspects, a modified mRNA may exhibit reduced degradation in the cell, as compared to an unmodified polynucleotide. Chemical modifications to the nucleosides as used in the present disclosure may be naturally occurring or may be artificial, i.e. not found in nature and synthesized by man.

In some aspects, the one or more chemical modifications include modifications to an adenosine ribonucleoside within the mRNA. Representative examples of adenosine ribonucleoside modifications include, but are not limited to 2-methylthio-N6-(cishydroxyisopentenyl)adenosine (ms2i6A), 2-methylthio-N6-methyladenosine (ms2m6A),

2-methylthio-N6-threonylcarbamoyladenosine (ms2t6A), N6-glycinylcarbamoyladenosine (g6A), N6-isopentenyladenosine (i6A), N6-methyladenosine (m6A), N6-threonylcarbamoyladenosine (t6A), 1,2'-O-dimethyladenosine (mlAm), 1-methyladenosine (mlA), 2'-O-methyladenosine (Am), 2'-O-ribosyladenosine (phosphate) (Ar(p)), 2-methyladenosine (m2A), 2-methylthio-N6-isopentenyladenosine (ms2i6A), 2-methylthio-N6-hydroxynorvalylcarbamoyladenosine (ms2hn6A), 2'-O-methyladenosine (m6A), isopentenyladenosine (Iga), N6-(cis-hydroxyisopentenyl)adenosine (io6A), N6,2'-O-dimethyladenosine (m6Am), N6,N6,2'-O-trimethyladenosine (m62Am), N6,N6-dimethyladenosine (m62A), N6-acetyladenosine (ac6A), N6-hydroxynorvalylcarbamoyladenosine (hn6A), N6-methyl-N6-threonylcarbamoyladenosine (m6t6A), 2-methyladenosine (m2A), 2-methylthio-N-isopentenyladenosine (ms2i6A), 7-deaza-adenosine, N1-methyl-adenosine, N6,N6(dimethyl)adenine, N6-cis-hydroxy-isopentenyl-adenosine, α-thio-adenosine, 2(amino)adenine, 2(aminopropyl)adenine, 2(methylthio)N6(isopentenyl)adenine, 2-(alkyl)adenine, 2-(aminoalkyl)adenine, 2-(aminopropyl)adenine, 2-(halo)adenine, 2-(propyl)adenine, 2'-amino-2'-deoxy-ATP, 2'-azido-2'-deoxy-ATP, 2'-deoxy-2'-α-aminoadenosine TP, 2'-deoxy-2'-α-azidoadenosine TP, 6(alkyl)adenine, 6(methyl)adenine, 6-(alkyl)adenine, 6-(methyl)adenine, 7(deaza)adenine, 8(alkenyl)adenine, 8(alkynyl)adenine, 8(amino)adenine, 8(thioalkyl)adenine, 8-(alkenyl)adenine, 8-(alkyl)adenine, 8-(alkynyl)adenine, 8-(amino)adenine, 8-(halo)adenine, 8-(hydroxyl)adenine, 8-(thioalkyl)adenine, 8-(thiol)adenine, 8-azido-adenosine, azaadenine, deazaadenine, N6(methyl)adenine, N6-(isopentyl)adenine, 7-deaza-8-aza-adenosine, 7-methyladenine, 1-deazaadenosine TP, 2'-fluoro-N6-Bz-deoxyadenosine TP, 2'-OMe-2-Amino-ATP, 2'-O-methyl-N6-Bz-deoxyadenosine TP, 2'-α-ethynyladenosine TP, 2-aminoadenine, 2-aminoadenosine TP, 2-amino-ATP, 2'-α-trifluoromethyladenosine TP, 2-azidoadenosine TP, 2'-β-ethynyladenosine TP, 2-bromoadenosine TP, 2'-β-trifluoromethyladenosine TP, 2-chloroadenosine TP, 2'-deoxy-2',2'-difluoroadenosine TP, 2'-deoxy-2'-α-mercaptoadenosine TP, 2'-deoxy-2'-α-thiomethoxyadenosine TP, 2'-deoxy-2'-β-aminoadenosine TP, 2'-deoxy-2'-β-azidoadenosine TP, 2'-deoxy-2'-β-bromoadenosine TP, 2'-deoxy-2'-β-chloroadenosine TP, 2'-deoxy-2'-β-fluoroadenosine TP, 2'-deoxy-2'-β-iodoadenosine TP, 2'-deoxy-2'-β-mercaptoadenosine TP, 2'-deoxy-2'-β-thiomethoxyadenosine TP, 2-fluoroadenosine TP, 2-iodoadenosine TP, 2-mercaptoadenosine TP, 2-methoxy-adenine, 2-methylthio-adenine, 2-trifluoromethyladenosine TP, 3-deaza-3-bromoadenosine TP, 3-Deaza-3-chloroadenosine TP, 3-Deaza-3-fluoroadenosine TP, 3-Deaza-3-iodoadenosine TP, 3-deazaadenosine TP, 4'-azidoadenosine TP, 4'-carbocyclic

adenosine TP, 4'-ethynyladenosine TP, 5'-homo-adenosine TP, 8-aza-ATP, 8-bromo-adenosine TP, 8-trifluoromethyladenosine TP, 9-deazaadenosine TP, 2-aminopurine, 7-deaza-2,6-diaminopurine, 7-deaza-8-aza-2,6-diaminopurine, 7-deaza-8-aza-2- aminopurine, 2,6-diaminopurine, 7-deaza-8-aza-adenine, and 7-deaza-2-aminopurine.

In some aspects, from about 5% to about 100% of the adenosine ribonucleosides within the mRNA are modified. In some aspects, from about 5%, about 10%, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, bout 80%, about 90%, or about 100% of the adenine nucleosides within the mRNA are modified.

In some aspects, the one or more chemical modifications include modifications to a cytidine ribonucleoside within the mRNA. Representative examples of cytidine ribonucleoside modifications include, but are not limited to, 2-thiocytidine (s2C), 3-methylcytidine (m3C), 5-formylcytidine (f5C), 5-hydroxymethylcytidine (hm5C), 5-methylcytidine (m5C), N4-acetylcytidine (ac4C), 2'-O-methylcytidine (Cm), 5,2'-O-dimethylcytidine (m5Cm), 5-formyl-2'-O-methylcytidine (f5Cm), lysidine (k2C), N4,2'-O-dimethylcytidine (m4Cm), N4-acetyl-2'-O-methylcytidine (ac4Cm), N4-methylcytidine (m4C), N4,N4-dimethyl-2'-OMe-Cytidine TP, 4-methylcytidine, 5-aza-cytidine, pseudo-iso-cytidine, pyrrolo-cytidine, α-thio-cytidine, 2-(thio)cytosine, 2'-amino-2'-deoxy-CTP, 2'-azido-2'-deoxy-CTP, 2'-deoxy-2'-α-aminocytidine TP, 2'-deoxy-2'-α-azidocytidine TP, 3(deaza)5(aza)cytosine, 3(methyl)cytosine, 3-(alkyl)cytosine, 3-(deaza)5(aza)cytosine, 3-(methyl)cytidine, 4,2'-O-dimethylcytidine, 5(halo)cytosine, 5(methyl)cytosine, 5(propynyl)cytosine, 5(trifluoromethyl)cytosine, 5-(alkyl)cytosine, 5-(alkynyl)cytosine, 5-(halo)cytosine, 5-(propynyl)cytosine, 5-(trifluoromethyl)cytosine, 5-bromo-cytidine, 5-iodo-cytidine, 5-propynylcytosine, 6-(azo)cytosine, 6-aza-cytidine, azacytosine, deazacytosine, N4(acetyl)cytosine, 1-methyl-1-deaza-pseudoisocytidine, 1-methyl-pseudoisocytidine, 2-methoxy-5-methyl-cytidine, 2-methoxy-cytidine, 2-thio-5-methyl-cytidine, 4-methoxy-1-methyl-pseudoisocytidine, 4-methoxy-pseudoisocytidine, 4-thio-1-methyl-1-deaza-pseudoisocytidine, 4-thio-1-methyl-pseudoisocytidine, 4-thio-pseudoisocytidine, 5-aza-zebularine, 5-methyl-zebularine, pyrrolo-pseudoisocytidine, zebularine, (E)-5-(2-bromo-vinyl)cytidine TP, 2,2'-anhydro-cytidine TP hydrochloride, 2'-fluor-N4-Bz-cytidine TP, 2'-fluoro-N4-Acetyl-cytidine TP, 2'-O-methyl-N4-acetyl-cytidine TP, 2'-O-methyl-N4-Bz-cytidine TP, 2'-α-ethynylcytidine TP, 2'-α-trifluoromethylcytidine TP, 2'-β-ethynylcytidine TP, 2'-β-trifluoromethylcytidine TP, 2'-deoxy-2',2'-difluorocytidine TP, 2'-deoxy-2'-α-mercaptocytidine TP, 2'-deoxy-2'-α-

thiomethoxycytidine TP, 2'-deoxy-2'-β-aminocytidine TP, 2'-deoxy-2'-β-azidocytidine TP, 2'-deoxy-2'-β-bromocytidine TP, 2'-deoxy-2'-β-chlorocytidine TP, 2'-deoxy-2'-β-fluorocytidine TP, 2'-deoxy-2'-β-iodocytidine TP, 2'-deoxy-2'-β-mercaptocytidine TP, 2'-deoxy-2'-β-thiomethoxycytidine TP, 2'-O-methyl-5-(l-propynyl)cytidine TP, 3'-ethynylcytidine TP, 4'-szidocytidine TP, 4'-carbocycliccytidine TP, 4'-ethynylcytidine TP, 5-(l-propynyl)ara-cytidine TP, 5-(2-chloro-phenyl)-2-thiocytidine TP, 5-(4-amino-phenyl)-2-thiocytidine TP, 5-aminoallyl-CTP, 5-cyanocytidine TP, 5-ethynylara-cytidine TP, 5-ethynylcytidine TP, 5'-homo-cytidine TP, 5-methoxycytidine TP, 5-trifluoromethyl-cytidine TP, N4-amino-cytidine TP, N4- benzoyl-cytidine TP, and pseudoisocytidine.

In some aspects, from about 5% to about 100% of the cytidine ribonucleosides within the mRNA are modified. In some aspects, from about 5%, about 10%, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, bout 80%, about 90%, or about 100% of the cytidine nucleosides within the mRNA are modified.

In some aspects, one or more chemical modifications include modifications to a guanosine ribonucleoside within the mRNA. Representative examples of guanosine ribonucleoside modifications include, but are not limited to, 7-methylguanosine (m7G), N2,2'-O-dimethylguanosine (m2Gm), N2-methylguanosine (m2G), wyosine (imG), 1,2'-O-dimethylguanosine (mlGm), 1-methylguanosine (mlG), 2'-O-methylguanosine (Gm), 2'-O-ribosylguanosine (phosphate) (Gr(p)), 7-aminomethyl-7-deazaguanosine (preQ1), 7-cyano-7-deazaguanosine (preQ0), archaeosine (G+), methylwyosine (mimG), N2,7-dimethylguanosine (m2,7G), N2,N2,2'-O-trimethylguanosine (m22Gm), N2,N2,7-trimethylguanosine (m2,2,7G), N2,N2-dimethylguanosine (m22G), N2,7,2'-O-trimethylguanosine (m2,7Gm), 6-thio-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, N1-methyl-guanosine, α-thio-guanosine, 2(propyl)guanine, 2-(alkyl)guanine, 2'-amino-2'-deoxy-GTP, 2'-azido-2'-deoxy-GTP, 2'-deoxy-2'-α-aminoguanosine TP, 2'-deoxy-2'-α-azidoguanosine TP, 6(methyl)guanine, 6-(alkyl)guanine, 6-(methyl)guanine, 6-methyl-guanosine, 7(alkyl)guanine, 7(deaza)guanine, 7(methyl)guanine, 7-(alkyl)guanine, 7-(deaza)guanine, 7-(methyl)guanine, 8(alkyl)guanine, 8(alkynyl)guanine, 8(halo)guanine, 8(thioalkyl)guanine, 8-(alkenyl)guanine, 8-(alkyl)guanine, 8-(alkynyl)guanine, 8-(amino)guanine, 8-(halo)guanine, 8-(hydroxyl)guanine, 8-(thioalkyl)guanine, 8-(thiol)guanine, aza guanine, deaza guanine, N-(methyl)guanine, l-methyl-6-thio-guanosine, 6-methoxy-guanosine, 6-thio-7-deaza-8-aza-guanosine, 6-thio-7-deaza-guanosine, 6-thio-7-methyl-guanosine, 7-deaza-8-aza-guanosine, 7-methyl-8-oxo-guanosine, N2,N2-dimethyl-

6-thio-guanosine, N2-methyl-6-thio-guanosine, 1-Me-GTP, 2'-fluoro-N2-isobutyl-guanosine TP, 2'-O-methyl-N2-isobutyl-guanosine TP, 2'-α-ethynylguanosine TP, 2'-α-trifluoromethylguanosine TP, 2'-β-ethynylguanosine TP, 2'-β-trifluoromethylguanosine TP, 2'-deoxy-2',2'-difluoroguanosine TP, 2'-deoxy-2'-α-mercaptoguanosine TP, 2'-deoxy-2'-α-thiomethoxyguanosine TP, 2'-deoxy-2'-β-aminoguanosine TP, 2'-deoxy-2'-β-azidoguanosine TP, 2'-deoxy-2'-β-bromoguanosine TP, 2'-deoxy-2'-β-chloroguanosine TP, 2'-deoxy-2'-β-fluoroguanosine TP, 2'-deoxy-2'-β-iodoguanosine TP, 2'-deoxy-2'-β-mercaptoguanosine TP, 2'-deoxy-2'-β-thiomethoxyguanosine TP, 4'-azidoguanosine TP, 4'-carbocyclicguanosine TP, 4'-ethynylguanosine TP, 5'-homo-guanosine TP, 8-bromo-guanosine TP, 9-deazaguanosine TP, and N2-isobutyl-guanosine TP.

In some aspects, from about 5% to about 100% of the guanosine ribonucleosides within the mRNA are modified. In some aspects, from about 5%, about 10%, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, bout 80%, about 90%, or about 100% of the guanosine nucleosides within the mRNA are modified.

In some aspects, the one or more chemical modifications include modifications to a uridine ribonucleoside within the mRNA. Representative examples of uridine ribonucleoside modifications include, but are not limited to, 2-thiouridine (s2U), 3-methyluridine (m3U), 5-carboxymethyluridine (cm5U), 5-hydroxyuridine (ho5U), 5-methyluridine (m5U), 5-taurinomethyl-2-thiouridine (τm5s2U), 5-taurinomethyluridine (τm5U), Dihydrouridine (D), Pseudouridine (Ψ), (3-(3-amino-3-carboxypropyl)uridine (acp3U), 1-methyl-3-(3-amino-5-carboxypropyl)pseudouridine (m1acp3Ψ), 1-methylpseudouridine (ihIΨ), 2'-O-methyluridine (Um), 2'-O-methylpseudouridine (Ψm), 2-thio-2'-O-methyluridine (s2Um), 3-(3-amino-3-carboxypropyl)uridine (acp3U), 3,2'-O-dimethyluridine (m3Um), 3-methyl-pseudo-Uridine TP, 4-thiouridine (s4U), 5-(carboxyhydroxymethyl)uridine (chm5U), 5-(carboxyhydroxymethyl)uridine methyl ester (mchm5U), 5,2'-O-dimethyluridine (m5Um), 5,6-dihydro-uridine, 5-aminomethyl-2-thiouridine (nm5s2U), 5-carbamoylmethyl-2'-O- methyluridine (ncm5Um), 5-carbamoylmethyluridine (ncm5U), 5-carboxyhydroxymethyluridine, 5-carboxyhydroxymethyluridine methyl ester, 5-carboxymethylaminomethyl-2'-O-methyluridine (cmnm5Um), 5-carboxymethylaminomethyl-2-thiouridine (cmnm5s2U), 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluridine (cmnm5U), 5-carboxymethylaminomethyluridine, 5-carbamoylmethyluridine TP, 5-methoxycarbonylmethyl-2'-O-methyluridine (mcm5Um), 5-methoxycarbonylmethyl-2-

thiouridine (mcm5s2U), 5-methoxycarbonylmethyluridine (mcm5U), 5-methoxyuridine (mo5U), 5-methyl-2-thiouridine (m5s2U), 5-methylaminomethyl-2-selenouridine (mnm5se2U), 5-methylaminomethyl-2-thiouridine (mnm5s2U), 5-methylaminomethyluridine (mnm5U), 5-methyldihydrouridine, 5-oxyacetic acid-uridine TP, 5-oxyacetic acid-methyl ester-uridine TP, N1-methyl-pseudo-uridine, uridine 5-oxyacetic acid (cmo5U), uridine 5-oxyacetic acid methyl ester (mcmo5U), 3-(3-Amino-3-carboxypropyl)-Uridine TP, 5-(iso-pentenylaminomethyl)-2-thiouridine TP, 5-(iso-pentenylaminomethyl)-2'-O-methyluridine TP, 5-(iso-pentenylaminomethyl)uridine TP, 5-propynyluracil, α-thio-uridine, l(aminoalkylamino-carbonylethylenyl)- 2(thio)-pseudouracil, 1-(aminoalkylaminocarbonylethylenyl)-2,4-(dithio)pseudouracil, 1-(aminoalkylaminocarbonylethylenyl)-4(thio)pseudouracil, 1-(aminoalkylaminocarbonylethylenyl)-pseudouracil, 1-(aminocarbonylethylenyl)-2(thio)-pseudouracil, 1-(aminocarbonylethylenyl)-2,4-(dithio)pseudouracil, 1-(aminocarbonylethylenyl)-4-(thio)pseudouracil, l-(aminocarbonylethylenyl)-pseudouracil, 1-substituted 2(thio)-pseudouracil, 1-substituted 2,4-(dithio)pseudouracil, 1-substituted 4(thio)pseudouracil, 1-substituted pseudouracil, l-(aminoalkylamino-carbonylethylenyl)-2-(thio)-pseudouracil, l-methyl-3-(3-amino-3- carboxypropyl)pseudouridine TP, 1-methyl-3-(3-amino-3- carboxypropyl)pseudo-UTP, 1-methyl-pseudo-UTP, 2-(thio)pseudouracil, 2'-deoxyuridine, 2'-fluorouridine, 2-(thio)uracil, 2,4-(dithio)psuedouracil, 2'-methyl-2'-amino-2'-azido-2'-fluoro-guanosine, 2'-Amino-2'-deoxy-UTP, 2'-azido-2'-deoxy-UTP, 2'-azido-deoxyuridine TP, 2'-O-methylpseudouridine, 2'-deoxyuridine, 2'-fluorouridine, 2'-deoxy-2'-α-aminouridine TP, 2'-deoxy-2'-α-azidouridine TP, 2-methylpseudouridine (S), 3-(3-amino-3-carboxypropyl)uracil, 4-(thio)pseudouracil, 4-(thio)uracil, 5-(l,3-diazole-l-alkyl)uracil, 5(2-aminopropyl)uracil, 5(aminoalkyl)uracil, 5(dimethylaminoalkyl)uracil, 5-(guanidinium alkyl)uracil, 5-(methoxycarbonylmethyl)-2-(thio)uracil, 5-(methoxycarbonyl-methyl)uracil, 5-(methyl)-2-(thio)uracil, 5-(methyl)-2,4-(dithio)uracil, 5-(methyl)-4-(thio)uracil, 5-(methylaminomethyl)-2-(thio)uracil, 5-(methylaminomethyl)-2,4-(dithio)uracil, 5-(methylaminomethyl)-4-(thio)uracil, 5-(propynyl)uracil, 5-(trifluoromethyl)uracil, 5-(2-aminopropyl)uracil, 5-(alkyl)-2-(thio)pseudouracil, 5-(alkyl)-2,4(dithio)pseudouracil, 5-(alkyl)-4(thio)pseudouracil, 5-(alkyl)pseudouracil, 5-(alkyl)uracil, 5-(alkynyl)uracil, 5-(allylamino)uracil, 5-(cyanoalkyl)uracil, 5-(dialkylaminoalkyl)uracil, 5-(dimethylaminoalkyl)uracil, 5-(guanidinium alkyl)uracil, 5-(halo)uracil, 5-(l,3-diazole-l-alkyl)uracil, 5-(methoxy)uracil, 5-(methoxycarbonylmethyl)-2-(thio)uracil, 5-(methoxycarbonyl-methyl)uracil, 5-(methyl)2(thio)uracil, 5-

(methyl)2,4(dithio)uracil, 5-(methyl)4(thio)uracil, 5-(methyl)-2-(thio)pseudouracil, 5-(methyl)-2,4(dithio)pseudouracil, 5-(methyl)-4(thio)pseudouracil, 5-(methyl)pseudouracil, 5-(methylaminomethyl)-2(thio)uracil, 5-(methylaminomethyl)-2,4(dithio)uracil, 5-(methylaminomethyl)-4-(thio)uracil, 5-(propynyl)uracil, 5-(trifluoromethyl)uracil, 5-aminoallyl-uridine, 5-bromo-uridine, 5-iodo-uridine, 5-uracil, 6(azo)uracil, 6-(azo)uracil, 6-aza-uridine, allyamino-uracil, azauracil, deazauracil, N3-(methyl)uracil, pseudo-UTP-1-2-ethanoic acid, pseudouracil, 4-thio-pseudo-UTP, 1-carboxymethyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 1 -propynyl-uridine, 1-taurinomethyl-1-methyl-uridine, 1-taurinomethyl-4-thio-uridine, 1-taurinomethyl-pseudouridine, 2-methoxy-4-thio-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-l-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-l-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, (±)l-(2-hydroxypropyl)pseudouridine TP, (2R)-l-(2-hydroxypropyl)pseudouridine TP, (2S)-l-(2-hydroxypropyl)pseudouridine TP, (E)-5-(2-bromo-vinyl)ara-uridine TP, (E)-5-(2-bromo-vinyl)uridine TP, (Z)-5-(2-bromo-vinyl)ara-uridine TP, (Z)-5-(2-bromo-vinyl)uridine TP, l-(2,2,2-trifluoroethyl)-pseudo-UTP, 1-(2,2,3,3,3-pentafluoropropyl)pseudouridine TP, 1-(2,2-diethoxyethyl)pseudouridine TP, 1-(2,4,6-trimethylbenzyl)pseudouridine TP, l-(2,4,6-trimethyl-benzyl)pseudo-UTP, l-(2,4,6-trimethyl-phenyl)pseudo-UTP, 1-(2-amino-2-carboxyethyl)pseudo-UTP, 1-(2-amino-ethyl)pseudo-UTP, l-(2-hydroxyethyl)pseudouridine TP, l-(2-methoxyethyl)pseudouridine TP, l-(3,4-bis-trifluoromethoxybenzyl)pseudouridine TP, 1-(3,4-dimethoxybenzyl)pseudouridine TP, l-(3-amino-3-carboxypropyl)pseudo-UTP, 1-(3-amino-propyl)pseudo-UTP, 1-(3-cyclopropyl-prop-2-ynyl)pseudouridine TP, l-(4-amino-4-carboxybutyl)pseudo-UTP, l-(4-amino-benzyl)pseudo-UTP, 1-(4-amino-butyl)pseudo-UTP, l-(4-amino-phenyl)pseudo-UTP, l-(4-azidobenzyl)pseudouridine TP, l-(4-bromobenzyl)pseudouridine TP, l-(4-chlorobenzyl)pseudouridine TP, l-(4-fluorobenzyl)pseudouridine TP, l-(4-iodobenzyl)pseudouridine TP, l-(4-methanesulfonylbenzyl)pseudouridine TP, l-(4-methoxybenzyl)pseudouridine TP, l-(4-methoxy-benzyl)pseudo-UTP, l-(4-methoxy-phenyl)pseudo-UTP, l-(4-methylbenzyl)pseudouridine TP, l-(4-methyl-benzyl)pseudo-UTP, l-(4-nitrobenzyl)pseudouridine TP, l-(4-nitro-benzyl)pseudo-UTP, l(4-nitro-phenyl)pseudo-UTP, l-(4-thiomethoxybenzyl)pseudouridine TP, 1-(4-trifluoromethoxybenzyl)pseudouridine TP, l-(4-trifluoromethylbenzyl)pseudouridine TP, l-(5-amino-pentyl)pseudo-UTP, l-(6-amino-hexyl)pseudo-UTP, l,6-dimethyl-pseudo-UTP, l-[3-(2-{2-[2-(2-aminoethoxy)-ethoxy]-

ethoxy}-ethoxy)-propionyl]pseudouridine TP, 1-{3-[2-(2-aminoethoxy)-ethoxy]- propionyl} pseudouridine TP, 1-acetylpseudouridine TP, 1-alkyl-6-(l-propynyl)-pseudo-UTP, 1-alkyl-6-(2-propynyl)-pseudo-UTP, 1-alkyl-6-allyl-pseudo-UTP, 1-alkyl-6-ethynyl-pseudo-UTP, 1-alkyl-6-homoallyl-pseudo-UTP, 1-alkyl-6-vinyl-pseudo-UTP, 1-allylpseudouridine TP, 1-aminomethyl-pseudo-UTP, 1-benzoylpseudouridine TP, 1-benzyloxymethylpseudouridine TP, 1-benzyl-pseudo-UTP, 1-biotinyl-PEG2-pseudouridine TP, 1-biotinylpseudouridine TP, 1-butyl-pseudo-UTP, 1-cyanomethylpseudouridine TP, 1-cyclobutylmethyl-pseudo-UTP, 1-cyclobutyl-pseudo-UTP, 1-cycloheptylmethyl-pseudo-UTP, 1-cycloheptyl-pseudo-UTP, 1-cyclohexylmethyl-pseudo-UTP, 1-cyclohexyl-pseudo-UTP, 1-cyclooctylmethyl-pseudo-UTP, 1-cyclooctyl-pseudo-UTP, 1-cyclopentylmethyl-pseudo-UTP, 1-cyclopentyl-pseudo-UTP, 1-cyclopropylmethyl-pseudo-UTP, 1-cyclopropyl-pseudo-UTP, 1-ethyl-pseudo-UTP, 1-hexyl-pseudo-UTP, 1-homoallylpseudouridine TP, 1-hydroxymethylpseudouridine TP, 1-iso-propyl-pseudo-UTP, 1-Me-2-thio-pseudo-UTP, 1-Me-4-thio-pseudo-UTP, 1-Me-alpha-thio-pseudo-UTP, 1-methanesulfonylmethylpseudouridine TP, 1-methoxymethylpseudouridine TP, 1-methyl-6-(2,2,2-Trifluoroethyl)pseudo-UTP, 1-methyl-6-(4-morpholino)-pseudo-UTP, 1-methyl-6-(4-thiomorpholino)-pseudo-UTP, 1-methyl-6-(substituted phenyl)pseudo-UTP, 1-methyl-6-amino-pseudo-UTP, 1-methyl-6-azido-pseudo-UTP, 1-methyl-6-bromo-pseudo-UTP, 1-methyl-6-butyl-pseudo-UTP, 1-methyl-6-chloro-pseudo-UTP, 1-methyl-6-cyano-pseudo-UTP, 1-methyl-6-dimethylamino-pseudo-UTP, 1-methyl-6-ethoxy-pseudo-UTP, 1-methyl-6-ethylcarboxylate-pseudo-UTP, 1-methyl-6-ethyl-pseudo-UTP, 1-methyl-6-fluoro-pseudo-UTP, 1-methyl-6-formyl-pseudo-UTP, 1-methyl-6-hydroxyamino-pseudo-UTP, 1-methyl-6-hydroxy-pseudo-UTP, 1-methyl-6-iodo-pseudo-UTP, 1-methyl-6-iso-propyl-pseudo-UTP, 1-methyl-6-methoxy-pseudo-UTP, 1-methyl-6-methylamino-pseudo-UTP, 1-methyl-6-phenyl-pseudo-UTP, 1-methyl-6-propyl-pseudo-UTP, 1-methyl-6-tert-butyl-pseudo-UTP, 1-methyl-6-trifluoromethoxy-pseudo-UTP, 1-methyl-6-trifluoromethyl-pseudo-UTP, 1-morpholinomethylpseudouridine TP, 1-pentyl-pseudo-UTP, 1-phenyl-pseudo-UTP, 1-pivaloylpseudouridine TP, 1-propargylpseudouridine TP, 1-propyl-pseudo-UTP, 1-propynyl-pseudouridine, 1-p-tolyl-pseudo-UTP, 1-tert-butyl-pseudo-UTP, 1-thiomethoxymethylpseudouridine TP, 1-thiomorpholinomethylpseudouridine TP, 1-trifluoroacetylpseudouridine TP, 1-trifluoromethyl-pseudo-UTP, 1-vinylpseudouridine TP, 2,2' -anhydro-uridine TP, 2'-bromo-deoxyuridine TP, 2-F-5-Methyl-2'-deoxy-UTP, 2'-OMe-5-Me-UTP, 2-OMe-pseudo-UTP, 2'-α-ethynyluridine TP, 2'-α-trifluoromethyluridine TP, 2'-β-ethynyluridine TP, 2'-β-trifluoromethyluridine TP, 2'-deoxy-2',2'-difluorouridine TP, 2'-deoxy-2'-α-mercaptouridine TP, 2'-deoxy-2'-α-thiomethoxyuridine TP, 2'-deoxy-2'-

β-aminouridine TP, 2'-deoxy-2'-β-azidouridine TP, 2'-deoxy-2'-β-bromouridine TP, 2'-deoxy-2'-β-chlorouridine TP, 2'-deoxy-2'-β-fluorouridine TP, 2'-deoxy-2'-β-iodouridine TP, 2'-deoxy-2'-β-mercaptouridine TP, 2'-deoxy-2'-β-thiomethoxyuridine TP, 2-methoxy-4-thio-uridine, 2-methoxyuridine, 2'-O-methyl-5-(1-propynyl)uridine TP, 3-alkyl-pseudo-UTP, 4'-azidouridine TP, 4'-carbocyclic uridine TP, 4' -ethynyluridine TP, 5-(1-propynyl)ara-uridine TP, 5-(2-furanyl)uridine TP, 5-cyanouridine TP, 5-dimethylaminouridine TP, 5'-homo-uridine TP, 5-iodo-2'-fluoro-deoxyuridine TP, 5-phenylethynyluridine TP, 5-trideuteromethyl-6-deuterouridine TP, 5-trifluoromethyl-Uridine TP, 5-vinylarauridine TP, 6-(2,2,2-trifluoroethyl)-pseudo-UTP, 6-(4-morpholino)-pseudo-UTP, 6-(4-thiomorpholino)-pseudo-UTP, 6-(substituted-phenyl)-pseudo-UTP, 6-amino-pseudo-UTP, 6-azido-pseudo-UTP, 6-bromo-pseudo-UTP, 6-butyl-pseudo-UTP, 6-chloro-pseudo-UTP , 6-cyano-pseudo-UTP, 6-dimethylamino-pseudo-UTP, 6-ethoxy-pseudo-UTP, 6-ethylcarboxylate-pseudo-UTP, 6-ethyl-pseudo-UTP, 6-fluoro-pseudo-UTP, 6-formyl-pseudo-UTP, 6-hydroxyamino-pseudo-UTP, 6-hydroxy-pseudo-UTP, 6-iodo-pseudo-UTP, 6-iso-propyl-pseudo-UTP, 6-methoxy-pseudo-UTP, 6-methylamino-pseudo-UTP, 6-methyl-pseudo-UTP, 6-phenyl-pseudo-UTP , 6-propyl-pseudo-UTP, 6-tert-butyl-pseudo-UTP, 6-trifluoromethoxy-pseudo-UTP, 6-trifluoromethyl-pseudo-UTP, alpha-thio-pseudo-UTP, pseudouridine-1-(4-methylhenzenesulfonic acid) TP, pseudouridine 1-(4-methylbenzoic acid) TP, pseudouridine TP 1-[3-(2- ethoxy)]propionic acid, pseudouridine TP 1-[3-{2-(2-[2-(2-ethoxy)-ethoxy]-ethoxy)-ethoxy}]propionic acid, pseudouridine TP 1-[3-{2-(2-[2-{2(2-ethoxy)-ethoxy}-ethoxy]-ethoxy)-ethoxy}]propionic acid, pseudouridine TP 1-[3-{2-(2-[2-ethoxy]-ethoxy)-ethoxy}]propionic acid, pseudouridine TP 1-[3-{2-(2-ethoxy)-ethoxy}]propionic acid, pseudouridine TP 1-methylphosphonic acid, pseudouridine TP 1-methylphosphonic acid diethyl ester, pseudo-UTP-N1-3-propionic acid, pseudo-UTP-N1-4-butanoic acid, pseudo-UTP-N1-5-peritanoic acid, pseudo-UTP-N1-6-hexanoic acid, pseudo-UTP-N1-7-heptanoic acid, pseudo-UTP-N1-methyl-p-benzoic acid, and pseudo-UTP-N1-p-benzoic acid.

In some aspects, from about 5% to about 100% of the uridine ribonucleosides within the mRNA are modified. In some aspects, from about 5%, about 10%, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, bout 80%, about 90%, or about 100% of the uridine nucleosides within the mRNA are modified.

5' Untranslated Regions (5' UTR) and 3' Untranslated Regions (3' UTR)

The mRNA of the present disclosure may comprise one or more regions or parts, which act or function as an untranslated region. By definition, wild type untranslated

regions (UTRs) of a gene are transcribed but not translated. In mRNA, the 5' UTR starts at the transcription start site and continues to the start codon but does not include the start codon. The 3' UTR starts immediately following the stop codon and continues until the transcriptional termination signal. There is increasing evidence that UTRs play a regulatory role in terms of stability of the polynucleotide and translation. The regulatory features of a UTR can be incorporated into the mRNA of the present disclosure to enhance the stability of the molecule, for example. Specific features can also be incorporated to ensure controlled down-regulation of transcription when present in undesired locations, for example miRNA binding sites and RNA binding protein (RBP) binding sites.

Specific 5' UTR and 3 UTR for use in the present disclosure can be any suitable UTR sequence, for example, a natural UTR sequence, a derivatized naturally occurring UTR, or a synthetic UTR. In some aspects, the 5' UTR and/or the 3' UTR is a naturally occurring human UTR or a human-derived UTR. The use of human-derived UTRs may facilitate the expression of the polypeptide encoded by the coding region in human cells. In other aspects, the 5' UTR and/or the 3' UTR are synthetic, i.e. not completely homologous with a UTR found in any species. The 5' UTR is operably linked to the 5' end of the coding region. The 3' UTR is operably linked to the 3' end of the coding region.

Natural 5' UTRs have features which play roles in translation initiation. They can harbor, for example, Kozak consensus sequences which are known to be involved in the process by which the ribosome initiates translation. The Kozak consensus has the sequence GCCNCCAUGG (SEQ ID NO: 15), where N is a purine (adenine or guanine) three nucleobases upstream from the start codon AUG. 5' UTRs have also been known to form secondary structures which are involved in elongation factor binding.

By using the polynucleotide sequence features found in abundantly expressed genes of target cells, one can enhance the stability and protein production of the mRNA. Untranslated regions useful in the design and manufacture of mRNA include, but are not limited to, those disclosed in International Application Publication No. WO2014164253, incorporated herein by reference in its entirety.

Other non-UTR sequences may be also used as regions or subregions within the mRNA. Combinations of features may be included in regions flanking the coding region and may be contained within other features. For example, the coding region may be flanked by a 5' UTR which may contain a strong Kozak consensus sequence. WO2014164253

provides a list of exemplary UTRs which may be used as flanking regions and is incorporated herein by reference. Variants of 5' or 3' UTRs may be used wherein one or more nucleotides are added or removed at the termini.

Any UTR may be incorporated into the mRNA. For example, multiple wild-type UTRs may be used. Alternatively, UTRs derivatized from a wild-type UTR may be used. In addition, artificial UTRs may be used that are not variants of wild-type regions. These UTRs or portions thereof may be placed in the same orientation as the transcript from which they were selected or may be altered in orientation or location. A 5' or 3' UTR may be shortened, lengthened, or made from one or more other 5' or 3' UTRs. A UTR may be "altered", meaning that the UTR has been changed in some way relative to the reference sequence. For example, a 5' or 3' UTR may be altered relative to the native UTR by a change in orientation or location, by the inclusion of additional nucleotides, deletion of nucleotides, or by swapping or transposing nucleotides.

In one aspect, a double, triple, or quadruple UTR such as a 5' or 3' UTR may be used. A double UTR is one in which two copies of the UTR are encoded in series or substantially in series. In another aspect, a patterned 3' or 5' UTR may be used. A patterned UTR are those which reflect a repeating or alternative pattern, such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times. In these patterns, each letter A, B, or C represents a different UTR at the nucleotide level.

In one aspect, flanking regions are selected from a family of transcripts whose proteins share a common function, structure, feature, or property. The UTRs of any of these genes may be swapped for any other UTRs of the same or different family of proteins to create a new mRNA.

The untranslated region may also include translation enhancer elements (TEEs). As a non-limiting example, the TEE may include those described in U.S. Patent Publication No. 20090226470, which is incorporated herein by reference.

Native 3' UTRs are known to have stretches of adenosines and uridines embedded in them. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, these AU rich elements (AREs) can be separated into three classes. Class I AREs contain several dispersed copies of an AUUUA motif within uridine-rich regions. Class II AREs contain two or more

UUAUUUA(U/A)(U/A) nonamers. Class III AREs are less well defined; these uridine-rich regions do not contain an AUUUA motif. Most proteins binding to AREs are known to destabilize mRNA, wherein members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3' UTR of the mRNA of the present disclosure will lead to HuR binding and subsequent stabilization of the mRNA in vivo.

Introduction, removal, or modification of 3' UTR AU rich elements (AREs) can be used to modify the stability of the mRNA of the present disclosure. One or more copies of an ARE can be introduced into the 3 'UTR of the mRNA to make it less stable, leading to lowered translation and decreased production of the resultant protein. Alternatively, AREs can be identified and removed or mutated to increase the intracellular stability, increasing translation and production of the resultant protein.

MicroRNAs (or miRNA) are 19-25 nucleotide long noncoding RNAs that bind to the 3' UTR of nucleic acid molecules and down-regulate gene expression either by reducing nucleic acid molecule stability or by inhibiting translation. The mRNA of the disclosure may comprise one or more microRNA target or binding sequences. microRNA target or binding sequences are well known in the art.

A microRNA sequence comprises a "seed" region, i.e., a sequence in the region of positions 2-8 of the mature microRNA, which sequence has perfect Watson-Crick complementarity to the miRNA target sequence. A microRNA seed may comprise positions 2-8 or 2-7 of the mature microRNA. In some aspects, a microRNA seed may comprise 7 nucleotides (e.g., nucleotides 2-8 of the mature microRNA), wherein the seed-complementary site in the corresponding miRNA target is flanked by an adenine (A) opposed to microRNA position 1. In some aspects, a microRNA seed may comprise 6 nucleotides (e.g., nucleotides 2-7 of the mature microRNA), wherein the seed-complementary site in the corresponding miRNA target is flanked by an adenine (A) opposed to microRNA position 1. See for example, Grimson A, Farh K, Johnston W K, Garrett-Engele P, Lim L P, Bartel D P; Mol Cell. 2007 Jul. 6; 27(l):91-l05, which is incorporated herein by reference. The bases of the microRNA seed have complete complementarity with the target sequence. By engineering microRNA target sequences into the polynucleotides (e.g., in a 3'UTR like region or other region) of the disclosuree one can target the molecule for degradation or reduced translation, provided the microRNA in question is available. This process will reduce the hazard of off target effects upon nucleic

acid molecule delivery. Identification of microRNA, microRNA target regions, and their expression patterns and role in biology have been reported (Bonauer et ah, Curr Drug Targets 2010 11 :943-949; Anand and Cheresh Curr Opin Hematol 2011 18: 171-176; Contreras and Rao Leukemia 2012 26:404-413 (2011 Dec. 20. doi: 10. 1038/leu.201 1.356); Bartel Cell 2009 136:215-233; Landgraf et al, Cell, 2007 129: 1401-1414, which are incorporated herein by reference). Introduction of one or multiple binding sites for different microRNA can be engineered to further decrease the longevity, stability, and protein translation of polynucleotides.

As used herein, the term "microRNA target sequence" or "microRNA binding sequence" refers to a microRNA target site or a microRNA recognition site, or any nucleotide sequence to which a microRNA binds or associates. It should be understood that "binding" may follow traditional Watson-Crick hybridization rules or may reflect any stable association of the microRNA with the target sequence at or adjacent to the microRNA site.

Conversely, for the purposes of the mRNA of the present disclosure, microRNA binding sites can be engineered out of (i.e. removed from) sequences in which they occur, e.g., in order to increase protein expression in specific tissues. Regulation of expression in multiple tissues can be accomplished through introduction or removal or one or several microRNA binding sites.

In the mRNA of the present disclosure, binding sites for microRNAs that are involved in such processes may be removed or introduced, in order to tailor the expression of the polynucleotides to biologically relevant cell types or to the context of relevant biological processes.

Examples of use of microRNA to drive tissue or disease-specific gene expression are described in, for example, Getner and Naldini, Tissue Antigens. 2012, 80:393-403, herein incorporated by reference in its entirety. In addition, microRNA binding sequences can be incorporated into mRNA to decrease expression in certain cells which results in a biological improvement.

Lastly, through an understanding of the expression patterns of microRNA in different cell types, mRNA can be engineered for more targeted expression in specific cell types or only under specific biological conditions. Through introduction of tissue-specific microRNA binding sites, polynucleotides can be designed that would be optimal for protein expression in a tissue or in the context of a biological condition.

Table 2 below provides exemplary 5' UTRs that may be used in the mRNA s of the present disclosure. Variations of these 5' UTRs may be used wherein one or more nucleotides are added to or removed from the termini, including A, U, C, or G.

Table 2. Exemplary 5' Untranslated Regions

SEQ ID NO:	Sequence
16	GGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACC
17	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACC
18	GGGAGAUCAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACC
19	GGAAUAAAAGUCUCAACACAACAUAUACAAAACAAACGAAUCUCAAGCAAU CAAGCAUUCUACUUCUAUUGCAGCAAUUUAAAUCAUUUCUUUUAAAGCAAA AGCAAUUUUCUGAAAAUUUUCACCAUUUACGAACGAUAGCAAC
20	GGGAGACAAGCUUGGCAUUCCGGUACUGUUGGUAAAGCCACC
21	UCAAGCUUUUGGACCCUCGUACAGAAGCUAAUACGACUCACUAUAGGGAAA UAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACC
22	AUGGCCGGACCCGCGACUCAGAGCCCAAUGAAGCUGAUGGCUCUUCA
23	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACC
24	GGGAAUUAACAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACC
25	GGGAAAUUAGACAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACC
26	GGGAAAUAAGAGAGUAAAGAACAGUAAGAAGAAAUAUAAGAGCCACC
27	GGGAAAAAAGAGAGAAAAGAAGACUAAGAAGAAAUAUAAGAGCCACC
28	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAUAUAUAAGAGCCACC
29	GGGAAAUAAGAGACAAAACAAGAGUAAGAAGAAAUAUAAGAGCCACC
30	GGGAAAUUAGAGAGUAAAGAACAGUAAGUAGAAUUAAAAGAGCCACC
31	GGGAAAUAAGAGAGAAUAGAAGAGUAAGAAGAAAUAUAAGAGCCACC
32	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAAUUAAGAGCCACC
33	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUUAAGAGCCACC

Table 3 below provides exemplary 3' UTRs that may be used in the mRNA of the present disclosure. Variations of these 3' UTRs may be used wherein one or more nucleotides are added to or removed from the termini, including A, U, C, or G.

Table 3. Exemplary 3' Untranslated Regions

SEQ ID NO:	Sequence
34	GCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUC UCCCUUGCACCUGUACCUCUUGGUCUUUGAAUAAAGCCUGAGUA GGAAGGCGGCCGCAAAAA
35	GCGCCUGCCCACCUGCCACCGACUGCUGGAACCCAGCCAGUGGG AGGGCCUGGCCCACCAGAGUCCUGCUCCCUCACUCCUCGCCCCGC CCCCUGUCCCAGAGUCCCACCUGGGGGCUCUCUCCACCCUUCUCA GAGUUCCAGUUUCAACCAGAGUUCCAACCAAUGGGCUCCAUCCU CUGGAUUCUGGCCAAUGAAAUAUCUCCCUGGCAGGGUCCUCUUC UUUUCCCAGAGCUCCACCCCAACCAGGAGCUCUAGUUAAUGGAG AGCUCCCAGCACACUCGGAGCUUGUGCUUUGUCUCCACGCAAAG CGAUAAAUAAAAGCAUUGGUGGCCUUUGGUCUUUGAAUAAAGC CUGAGUAGGAAGUCUAGA
36	GCCCCUGCCGCUCCCACCCCCACCCAUCUGGGCCCCGGGUUCAAG AGAGAGCGGGGUCUGAUCUCGUGUAGCCAUAUAGAGUUUGCUU CUGAGUGUCUGCUUUGUUUAGUAGAGGUGGGCAGGAGGAGCUG AGGGGCUGGGGCUGGGGUGUUGAAGUUGGCUUUGCAUGCCCAG CGAUGCGCCUCCCUGUGGGAUGUCAUCACCCUGGGAACCGGGAG UGGCCCUUGGCUCACUGUGUUCUGCAUGGUUUGGAUCUGAAUUA AUUGUCCUUUCUUCUAAAUCCCAACCGAACUUCUUCCAACCUCC AAACUGGCUGUAACCCCAAAUCCAAGCCAUUAACUACACCUGAC AGUAGCAAUUGUCUGAUUAAUCACUGGCCCCUUGAAGACAGCAG AAUGUCCCUUUGCAAUGAGGAGGAGAUCUGGGCUGGGCGGGCCA GCUGGGGAAGCAUUUGACUAUCUGGAACUUGUGUGUGCCUCCUC AGGUAUGGCAGUGACUCACCUGGUUUUAAUAAAACAACCUGCAA CAUCUCAUGGUCUUUGAAUAAAGCCUGAGUAGGAAGUCUAGA
37	ACACACUCCACCUCCAGCACGCGACUUCUCAGGACGACGAAUCU UCUCAAUGGGGGGGCGGCUGAGCUCCAGCCACCCCGCAGUCACU

SEQ ID NO:	Sequence
	UUCUUUGUAACAACUUCCGUUGCUGCCAUCGUAAACUGACACAG UGUUUAUAACGUGUACAUACAUUAACUUAUUACCUCAUUUUGU UAUUUUUCGAAACAAAGCCCUGUGGAAGAAAAUGGAAAACUUG AAGAAGCAUUAAAGUCAUUCUGUUAAGCUGCGUAAAUGGUCUU UGAAUAAAGCCUGAGUAGGAAGUCUAGA
38	CAUCACAUUUAAAAGCAUCUCAGCCUACCAUGAGAAUAAGAGAA AGAAAAUGAAGAUCAAAAGCUUAUUCAUCUGUUUUUCUUUUUC GUUGGUGUAAAGCCAACACCCUGUCUAAAAAACAUAAAUUUCUU UAAUCAUUUUGCCUCUUUUCUCUGUGCUUCAAUUAAUAAAAAA UGGAAAGAAUCUAAUAGAGUGGUACAGCACUGUUAUUUUUCAA AGAUGUGUUGCUAUCCUGAAAAUUCUGUAGGUUCUGUGGAAGU UCCAGUGUUCUCUCUUAUUCCACUUCGGUAGAGGAUUUCUAGUU UCUUGUGGGCUAAUUAAAUAAAUCAUUAAUACUCUUCUAAUGG UCUUUGAAUAAAGCCUGAGUAGGAAGUCUAGA
39	GCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUC UCCCUUGCACCUGUACCUCUUGGUCUUUGAAUAAAGCCUGAGUA GGAAGGCGGCCGCUCGAGCAUGCAUCUAGA
40	GCCAAGCCCUCCCCAUCCCAUGUAUUUAUCUCUAUUUAAUAUUU AUGUCUAUUUAAGCCUCAUAUUUAAAGACAGGGAAGAGCAGAA CGGAGCCCCAGGCCUCUGUGUCCUUCCCUGCAUUUCUGAGUUUC AUUCUCCUGCCUGUAGCAGUGAGAAAAAGCUCCUGUCCUCCCAU CCCCUGGACUGGGAGGUAGAUAGGUAAAUACCAAGUAUUUAUU ACUAUGACUGCUCCCCAGCCCUGGCUCUGCAAUGGGCACUGGGA UGAGCCGCUGUGAGCCCCUGGUCCUGAGGGUCCCCACCUGGGAC CCUUGAGAGUAUCAGGUCUCCCACGUGGGAGACAAGAAAUCCCU GUUUAAUAUUUAAACAGCAGUGUUCCCCAUCUGGGUCCUUGCAC CCCUCACUCUGGCCUCAGCCGACUGCACAGCGGCCCCUGCAUCCC CUUGGCUGUGAGGCCCCUGGACAAGCAGAGGUGGCCAGAGCUGG GAGGCAUGGCCCUGGGGUCCCACGAAUUUGCUGGGGAAUCUCGU UUUUCUUCUUAAGACUUUUGGGACAUGGUUUGACUCCCGAACAU CACCGACGCGUCUCCUGUUUUUCUGGGUGGCCUCGGGACACCUG CCCUGCCCCCACGAGGGUCAGGACUGUGACUCUUUUUAGGGCCA

SEQ ID NO:	Sequence
	GGCAGGUGCCUGGACAUUUGCCUUGCUGGACGGGGACUGGGGAU GUGGGAGGGAGCAGACAGGAGGAAUCAUGUCAGGCCUGUGUGU GAAAGGAAGCUCCACUGUCACCCUCCACCUCUUCACCCCCCACUC ACCAGUGUCCCCUCCACUGUCACAUUGUAACUGAACUUCAGGAU AAUAAAGUGUUUGCCUCCAUGGUCUUUGAAUAAAGCCUGAGUA GGAAGGCGGCCGCUCGAGCAUGCAUCUAGA
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46	CCCACCCUGCACGCCGGCACCAAACCCUGUCCUCCCACCCCUCCC CACUCAUCACUAAACAGAGUAAAAUGUGAUGCGAAUUUUCCCGA CCAACCUGAUUCGCUAGAUUUUUUUUAAGGAAAAGCUUGGAAA GCCAGGACACAACGCUGCUGCCUGCUUUGUGCAGGGUCCUCCGG GGCUCAGCCCUGAGUUGGCAUCACCUGCGCAGGGCCCUCUGGGG CUCAGCCCUGAGCUAGUGUCACCUGCACAGGGCCCUCUGAGGCU CAGCCCUGAGCUGGCGUCACCUGUGCAGGGCCCUCUGGGGCUCA GCCCUGAGCUGGCCUCACCUGGGUUCCCCACCCCGGGCUCUCCU GCCCUGCCCUCCUGCCCGCCCUCCCUCCUGCCUGCGCAGCUCCUU CCCUAGGCACCUCUGUGCUGCAUCCCACCAGCCUGAGCAAGACG

SEQ ID NO:	Sequence
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SEQ ID NO:	Sequence
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51	GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCCAGCCCCUCCUCC CCUUCCUGCACCCGUACCCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC

5' Terminal Cap

The 5' terminal cap structure of natural mRNA is involved in nuclear transport, increasing mRNA stability and binds the mRNA Cap Binding Protein (CBP), which is responsible for mRNA stability in the cell and translation competency through the association of CBP with poly(A) binding protein to form the mature cyclic mRNA species. The 5' terminal cap is operably linked to the 5' end of the mRNA as described herein.

Endogenous mRNA molecules may be 5'-end capped generating a 5'-ppp-5'-triphosphate linkage between a terminal guanosine cap residue and the 5'-terminal transcribed sense nucleotide of the mRNA molecule. This 5'-guanylate cap may then be

methylated to generate an N7-methyl-guanylate residue. The ribose sugars of the terminal and/or anteterminal transcribed nucleotides of the 5' end of the mRNA may optionally also be 2'-O-methylated. 5'-decapping through hydrolysis and cleavage of the guanylate cap structure may target a nucleic acid molecule, such as an mRNA molecule, for degradation.

In some aspects, mRNA of the present disclosure may be designed to incorporate a cap moiety. Modifications to the polynucleotides of the present disclosure may generate a non-hydrolyzable cap structure preventing decapping and thus increasing mRNA half-life. Because cap structure hydrolysis requires cleavage of 5'-ppp-5' phosphorodiester linkages, modified nucleotides may be used during the capping reaction. For example, a Vaccinia Capping Enzyme from New England Biolabs (Ipswich, Mass.) may be used with α-thio-guanosine nucleotides according to the manufacturer's instructions to create a phosphorothioate linkage in the 5'-ppp-5' cap. Additional modified guanosine nucleotides may be used such as α-methyl-phosphonate and seleno-phosphate nucleotides.

Additional modifications include, but are not limited to, 2'-O-methylation of the ribose sugars of 5'-terminal and/or 5'-anteterminal nucleotides of the polynucleotide (as mentioned above) on the 2'-hydroxyl group of the sugar ring. Multiple distinct 5'-cap structures can be used to generate the 5'-cap of a nucleic acid molecule, such as a polynucleotide which functions as an mRNA molecule.

Cap analogs, which herein are also referred to as synthetic cap analogs, chemical caps, chemical cap analogs, or structural or functional cap analogs, differ from natural (i.e., endogenous, wild-type or physiological) 5'-caps in their chemical structure, while retaining cap function. Cap analogs may be chemically (i.e., non-enzymatically) or enzymatically synthesized and/or linked to the polynucleotides of the disclosure.

For example, the Anti-Reverse Cap Analog (ARCA) cap contains two guanines linked by a 5'-5'-triphosphate group, wherein one guanine contains an N7 methyl group as well as a 3'-O-methyl group (i.e., N7,3'-O-dimethyl-guanosine-5'-triphosphate-5'-guanosine (m7G-3'mppp-G; which may equivalently be designated 3'-O-Me-m7G(5')ppp(5')G). The 3'-O atom of the other, unmodified, guanine becomes linked to the 5 '-terminal nucleotide of the capped polynucleotide. The N7- and 3'-O-methlyated guanine provides the terminal moiety of the capped polynucleotide.

Another exemplary cap is mCAP, which is similar to ARCA but has a 2'-O-methyl group on guanosine (i.e., N7,2'-O-dimethyl-guanosine-5 '-triphosphate-5 '-guanosine, m7Gm-ppp-G).

In one aspect, the cap is a dinucleotide cap analog. As a non-limiting example, the dinucleotide cap analog may be modified at different phosphate positions with a boranophosphate group or a phosphoroselenoate group such as the dinucleotide cap analogs described in U.S. Patent No. 8,519,110, the contents of which are herein incorporated by reference in its entirety.

In another aspect, the cap is a N7-(4-chlorophenoxyethyl)substituted dinucleotide form of a cap analog known in the art and/or described herein. Non-limiting examples of a N7- (4-chlorophenoxyethyl) substituted dinucleotide form of a cap analog include a N7-(4-chlorophenoxyethyl)-G(5')ppp(5')G and a N7-(4-chlorophenoxyethyl)-m3-O G(5')ppp(5')G cap analog (See e.g., the various cap analogs and the methods of synthesizing cap analogs described in Kore et al. Bioorganic & Medicinal Chemistry 2013 21 :4570-4574, herein incorporated by reference in its entirety). In another aspect, a cap analog of the present disclosure is a 4- chloro/bromophenoxyethyl analog.

While cap analogs allow for the concomitant capping of a polynucleotide or a region thereof, in an in vitro transcription reaction up to 20% of transcripts can remain uncapped. This, as well as the structural differences of a cap analog from an endogenous 5'-cap structures of nucleic acids produced by the endogenous, cellular transcription machinery, may lead to reduced translational competency and reduced cellular stability.

mRNA of the disclosure may also be capped post-manufacture (whether by IVT or chemical synthesis), using enzymes, in order to generate more authentic 5'-cap structures, for example to closely mirror or mimic, either structurally or functionally, an endogenous or wild type feature. Non-limiting examples of such 5' cap structures of the present disclosure are those which, among other things, have enhanced binding of cap binding proteins, increased half-life, reduced susceptibility to 5' endonucleases and/or reduced 5' decapping, as compared to synthetic 5' cap structures known in the art (or to a wild-type, natural or physiological 5' cap structure). For example, recombinant Vaccinia Virus Capping Enzyme and recombinant 2'-O-methyltransferase enzyme can create a canonical 5'-5'-triphosphate linkage between the 5'-terminal nucleotide of a polynucleotide and a guanine cap nucleotide wherein the cap guanine contains an N7 methylation and the 5'-terminal nucleotide of the

mRNA contains a 2'-O-methyl. Such a structure is termed the Cap1 structure. This cap results in a higher translational-competency and cellular stability and a reduced activation of cellular pro-inflammatory cytokines, as compared, e.g., to other 5' cap analog structures known in the art. Cap structures include, but are not limited to, 7mG(5')ppp(5')N,pN2p (cap 0), 7mG(5')ppp(5')NlmpNp (cap 1), and 7mG(5')-ppp(5')NlmpN2mp (cap 2).

5'-terminal caps may include endogenous caps or cap analogs. A 5' terminal cap may comprise a guanine analog. Useful guanine analogs include, but are not limited to, inosine, Nl-methyl-guanosine, 2'-fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

3' Tailing Sequences

During RNA processing, a long chain of adenine nucleotides, known as the poly(A) tail, may be added to the mRNA molecule in order to increase stability. Immediately after transcription, the 3' end of the transcript may be cleaved to free a 3' hydroxyl. Then poly(A) polymerase adds a chain of adenine nucleotides to the RNA. The process, called polyadenylation, adds a poly(A) tail that can be between, for example, approximately 80 to approximately 250 residues long, including approximately 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240 or 250 residues long. The poly(A) tail is operably linked to 3' end of the mRNA as described herein. Poly(A) tails may also be added after the construct is exported from the nucleus.

Terminal groups on the poly(A) tail may be incorporated for stabilization into mRNA of the present disclosure. Polynucleotides of the present disclosure may include des-3' hydroxyl tails. They may also include structural moieties or 2'-O-methyl modifications as taught by Junjie Li, et al. (Current Biology, Vol. 15, 1501-1507, Aug. 23, 2005, herein incorporated by reference in its entirety).

The mRNA of the present disclosure may be designed to encode transcripts with alternative poly(A) tail structures including histone mRNA. According to Norbury, terminal uridylation has also been detected on human replication-dependent histone mRNA. The turnover of these mRNA is thought to be important for the prevention of potentially toxic histone accumulation following the completion or inhibition of chromosomal DNA replication. These mRNA are distinguished by their lack of a 3' poly(A) tail, the function of which is instead assumed by a stable stem-loop structure and its cognate stem-loop binding protein (SLBP); the latter carries out the same functions as those of PABP on

polyadenylated mRNA" (Norbury, "Cytoplasmic RNA: a case of the tail wagging the dog," Nature Reviews Molecular Cell Biology; AOP, published online 29 Aug. 2013; doi: 10. 1038/nrm3645, herein incorporated by reference in its entirety).

Unique poly(A) tail lengths provide certain advantages to the mRNA of the present disclosure. Generally, the length of a poly(A) tail, when present, is greater than 30 nucleotides in length. In another aspect, the poly(A) tail is greater than 35 nucleotides in length (e.g., at least or greater than about 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1,000, 1, 100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, 2,000, 2,500, and 3,000 nucleotides). In some aspects, the polynucleotide or region thereof includes from about 30 to about 3,000 nucleotides.

In one aspect, the poly(A) tail is designed relative to the length of the overall polynucleotide or the length of a particular region of the polynucleotide. This design may be based on the length of the coding region, the length of a particular feature or region or based on the length of the ultimate product expressed.

In this context the poly(A) tail may be 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% greater in length than the polynucleotide or feature thereof. The poly(A) tail may also be designed as a fraction of the polynucleotides to which it belongs. In this context, the poly(A) tail may be 10, 20, 30, 40, 50, 60, 70, 80, or 90% or more of the total length of the construct, a construct region or the total length of the construct minus the poly(A) tail. Further, engineered binding sites and conjugation of polynucleotides for Poly(A) binding protein may enhance expression.

Additionally, multiple distinct polynucleotides may be linked together via the PABP (Poly-A binding protein) through the 3 '-end using modified nucleotides at the 3 '-terminus of the poly(A) tail.

In one aspect, the mRNA of the present disclosure is designed to include a poly(A) G Quartet region. The G-quartet is a cyclic hydrogen bonded array of four guanine nucleotides that can be formed by G-rich sequences in both DNA and RNA. In this aspect, the G-quartet is incorporated at the end of the poly(A) tail. The resultant polynucleotide is assayed for stability, protein production and other parameters including half-life at various time points. The poly(A) G quartet results in protein production from an mRNA equivalent to at least 75% of that seen using a poly(A) tail of 120 nucleotides alone.

Start Codon Region

In some aspects, the mRNA of the present disclosure may have regions that are analogous to or function like a start codon region. In one aspect, the translation of the mRNA may initiate on a codon which is not the start codon AUG. Translation of the polynucleotide may initiate on an alternative start codon such as, but not limited to, ACG, AGG, AAG, CUG, GUG, AUA, AUU, UUG (see Touriol et al. Biology of the Cell 95 (2003) 169-178 and Matsuda and Mauro PLoS ONE, 2010 5: 11, herein incorporated by reference in its entirety). As a non-limiting example, the translation of a polynucleotide begins on the alternative start codon ACG. As another non-limiting example, polynucleotide translation begins on the alternative start codon CUG. As yet another non-limiting example, the translation of a polynucleotide begins on the alternative start codon GUG.

Stop Codon Region

In one aspect, the mRNA of the present disclosure may include at least two stop codons before the 3 ' untranslated region (UTR). The stop codon may be selected from UGA, UAA and UAG. In one aspect, the polynucleotides of the present disclosure include the stop codon UGA and one additional stop codon. In a further aspect, the additional stop codon may be UAA. In another aspect, the polynucleotides of the present disclosure include three stop codons.

Codon Optimization

The coding region of the mRNA of the present disclosure and their regions or parts or subregions may be codon optimized. Codon optimization methods are known in the art and may be useful in efforts to achieve one or more of several goals. These goals include to match codon frequencies in target and host organisms to ensure proper folding, to bias GC content to increase mRNA stability or reduce secondary structures, to minimize tandem repeat codons or base runs that may impair gene construction or expression, to customize translational control regions, to insert or remove protein trafficking sequences, to remove/add post translation modification sites in encoded protein (e.g. glycosylation sites), to add or remove or shuffle protein domains, to insert or delete restriction sites, to modify ribosome binding sites and mRNA degradation sites, to adjust translational rates to allow the various domains of the protein to fold properly, or to reduce or eliminate problematic secondary structures within the polynucleotide. Codon optimization tools, algorithms and services are known in the art, non-limiting examples of which include services from

GeneArt (Life Technologies), DNA2.0 (Menlo Park Calif.) and/or proprietary methods. In one aspect, the coding region sequence is optimized using optimization algorithms. mRNA Codon options for each amino acid are given in Table 4.

Table 4. Codon Options for Each Amino Acid

Amino Acid	Single Letter Code	Codon Options
Isoleucine	I	AUU, AUC, AUA
Leucine	L	CUU, CUC, CUA, CUG, UUA, UUG
Valine	V	GUU, GUC, GUA, GUG
Phenylalanine	F	UUU, UUC
Methionine	M	AUG
Cysteine	C	UGU, UGC
Alanine	A	GCU, GCC, GCA, GCG
Glycine	G	GGU, GGC, GGA, GGG
Proline	P	CGU, CCC, CCA, CCG
Threonine	T	ACU, ACC, ACA, ACG
Serine	S	UCU, UCC, UCA, UCG, AGU, AGC
Tyrosine	Y	UAU, UAC
Tryptophan	W	UGG
Glutamine	Q	CAA, CAG
Asparagine	N	AAU, AAC
Histidine	H	CAU, CAC
Glutamic Acid	E	GAA, GAG
Aspartic Acid	D	GAU, GAC
Lysine	K	AAA, AAG

Amino Acid	Single Letter Code	Codon Options
Arginine	R	CGU, CGC, CGA, CGG, AGA, AGG
Selenocysteine	Sec	UGA in mRNA in presence of SECIS
Stop Codons	Stop	UAA, UAG, UGA

Protein Variants

In some aspects, variants of the proteins encoded by the mRNA described herein are also contemplated. Protein variants and derivatives are well understood to those of skill in the art and can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with Tables 5 and 6 below and are referred to as conservative substitutions.

TABLE 5: Amino Acid Abbreviations

Amino Acid	Abbreviations	
Alanine	Ala	A
alloisoleucine	AIle	

Arginine	Arg	R
asparagine	Asn	N
aspartic acid	Asp	D
Cysteine	Cys	C
glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
phenylalanine	Phe	F
proline	Pro	P
pyroglutamic acid	pGlu	
Serine	Ser	S
Threonine	Thr	T
Tyrosine	Tyr	Y
Tryptophan	Trp	W
Valine	Val	V

TABLE 6: Amino Acid Substitutions

Original Residue Exemplary Conservative Substitutions, others are known in the art.

Ala	Ser
Arg	Lys; Gln
Asn	Gln; His

Asp	Glu
Cys	Ser
Gln	Asn, Lys
Glu	Asp
Gly	Pro
His	Asn;Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Substantial changes in function are made by selecting substitutions that are less conservative than those in Table 6, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side

chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of the proteins encoded by the mRNA described herein are each encompassed by the present disclosure.

Therapeutic Compositions

The synthetic mRNA described herein can be formulated using one or more excipients to increase stability, increase cell transfection, permit sustained or delayed release, alter biodistribution, increase *in vivo* translation of the encoded protein, and/or alter the *in vivo* release profile of the encoded protein. In addition to traditional excipients such as any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, and preservatives, excipients of the present disclosure can include, without limitation, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, and combinations thereof.

Accordingly, the therapeutic compositions of the present disclosure can include one or more excipients, each in an amount that may increase the stability of the polynucleotide, increase cell transfection by the polynucleotide, increase the expression of the encoded protein, or alter the release profile of the encoded protein.

The synthetic mRNA described herein can be administered by any suitable method and technique presently or prospectively known to those skilled in the art. For example, the active components described herein can be formulated in a physiologically- or pharmaceutically-acceptable form and administered by any suitable route known in the art including, for example, oral and parenteral routes of administering. As used herein, the term "parenteral" includes subcutaneous, intradermal, intravenous, intramuscular, intraperitoneal, and intrasternal administration, such as by injection. Administration of the active components of their compositions can be a single administration, or at continuous and distinct intervals as can be readily determined by a person skilled in the art.

Compositions, as described herein, comprising a synthetic mRNA and a pharmaceutically acceptable carrier or excipient of some sort may be useful in a variety of medical and non-medical applications. For example, therapeutic compositions comprising an active compound and an excipient may be useful for fertilization.

"Pharmaceutically acceptable carrier" (sometimes referred to as a "carrier") means a carrier or excipient that is useful in preparing a therapeutic composition that is generally safe and non-toxic and includes a carrier that is acceptable for veterinary and/or human pharmaceutical or therapeutic use. The terms "carrier" or "pharmaceutically acceptable carrier" can include, but are not limited to, phosphate buffered saline solution, water, emulsions (such as an oil/water or water/oil emulsion) and/or various types of wetting agents. As used herein, the term "carrier" encompasses, but is not limited to, any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, lipid, stabilizer, or other material well known in the art for use in pharmaceutical formulations and as described further herein.

"Excipients" include any and all solvents, diluents or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. General considerations in formulation and/or manufacture can be found, for example, in Remington's Pharmaceutical Sciences, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980), and Remington: The Science and Practice of Pharmacy, 21st Edition (Lippincott Williams & Wilkins, 2005).

Exemplary excipients include, but are not limited to, any non-toxic, inert solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials which can serve as excipients include, but are not limited to, sugars such as lactose, glucose, and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols such as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; detergents such as Tween 80; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming

agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator. As would be appreciated by one of skill in this art, the excipients may be chosen based on what the composition is useful for. For example, the choice of the excipient will depend on the route of administration, the agent being delivered, time course of delivery of the agent.

Exemplary diluents include calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, etc., and combinations thereof.

Exemplary granulating and/or dispersing agents include potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (Veegum), sodium lauryl sulfate, quaternary ammonium compounds, etc., and combinations thereof.

Exemplary surface active agents and/or emulsifiers include natural emulsifiers (e.g. acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (e.g. bentonite [aluminum silicate] and Veegum [magnesium aluminum silicate]), long chain amino acid derivatives, high molecular weight alcohols (e.g. stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (e.g. carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxy vinyl polymer), carrageenan, cellulosic derivatives (e.g. carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (e.g. polyoxyethylene sorbitan monolaurate [Tween 20], polyoxyethylene sorbitan [Tween 60], polyoxyethylene sorbitan monooleate [Tween 80], sorbitan monopalmitate [Span 40], sorbitan monostearate [Span 60], sorbitan tristearate [Span 65], glyceryl monooleate, sorbitan monooleate [Span 80]), polyoxyethylene esters

(e.g. polyoxyethylene monostearate [Myrj 45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and Solutol), sucrose fatty acid esters, polyethylene glycol fatty acid esters (e.g. Cremophor), polyoxyethylene ethers, (e.g. polyoxyethylene lauryl ether [Brij 30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, Pluronic F 68, Poloxamer 188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, etc. and/or combinations thereof. Exemplary binding agents include starch (e.g. cornstarch and starch paste), gelatin, sugars (e.g. sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol, etc.), natural and synthetic gums (e.g. acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl-pyrrolidone), magnesium aluminum silicate (Veegum), and larch arabogalactan), alginates, polyethylene oxide, polyethylene glycol, inorganic calcium salts, silicic acid, polymethacrylates, waxes, water, alcohol, etc., and/or combinations thereof.

Exemplary preservatives include antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and other preservatives.

Exemplary antioxidants include alpha tocopherol, ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and sodium sulfite.

Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA) and salts and hydrates thereof (e.g., sodium edetate, disodium edetate, trisodium edetate, calcium disodium edetate, dipotassium edetate, and the like), citric acid and salts and hydrates thereof (e.g., citric acid monohydrate), fumaric acid and salts and hydrates thereof, malic acid and salts and hydrates thereof, phosphoric acid and salts and hydrates thereof, and tartaric acid and salts and hydrates thereof. Exemplary antimicrobial preservatives include benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and thimerosal.

Exemplary antifungal preservatives include butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and sorbic acid.

Exemplary alcohol preservatives include ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and phenylethyl alcohol.

Exemplary acidic preservatives include vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and phytic acid. Other preservatives include tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, Glydant Plus, Phenonip, methylparaben, Germall 115, Germaben II, Neolone, Kathon, and Euxyl. In certain aspects, the preservative is an anti-oxidant. In other aspects, the preservative is a chelating agent.

Exemplary buffering agents include citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, D-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen- free water, isotonic saline, Ringer's solution, ethyl alcohol, etc., and combinations thereof.

Exemplary lubricating agents include magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behanate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, etc., and combinations thereof.

Exemplary natural oils include almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, chamomile, canola, caraway, carnauba, castor, cinnamon,

cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mink, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils. Exemplary synthetic oils include, but are not limited to, butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and combinations thereof.

Additionally, the composition may further comprise a polymer. Exemplary polymers contemplated herein include, but are not limited to, cellulosic polymers and copolymers, for example, cellulose ethers such as methylcellulose (MC), hydroxyethylcellulose (HEC), hydroxypropyl cellulose (HPC), hydroxypropyl methyl cellulose (HPMC), methylhydroxyethylcellulose (MHEC), methylhydroxypropylcellulose (MHPC), carboxymethyl cellulose (CMC) and its various salts, including, e.g., the sodium salt, hydroxyethylcarboxymethylcellulose (HECMC) and its various salts, carboxymethylhydroxyethylcellulose (CMHEC) and its various salts, other polysaccharides and polysaccharide derivatives such as starch, dextran, dextran derivatives, chitosan, and alginic acid and its various salts, carageenan, varoius gums, including xanthan gum, guar gum, gum arabic, gum karaya, gum ghatti, konjac and gum tragacanth, glycosaminoglycans and proteoglycans such as hyaluronic acid and its salts, proteins such as gelatin, collagen, albumin, and fibrin, other polymers, for example, polyhydroxyacids such as polylactide, polyglycolide, polyl(lactide-co-glycolide) and poly(.epsilon.-caprolactone-co-glycolide)-, carboxyvinyl polymers and their salts (e.g., carbomer), polyvinylpyrrolidone (PVP), polyacrylic acid and its salts, polyacrylamide, polyacrylic acid/acrylamide copolymer, polyalkylene oxides such as polyethylene oxide, polypropylene oxide, poly(ethylene oxide-propylene oxide), and a Pluronic polymer, polyoxy ethylene (polyethylene glycol), polyanhydrides, polyvinylalchol, polyethyleneamine and polypyrridine, polyethylene glycol (PEG) polymers, such as PEGylated lipids (e.g., PEG-stearate, 1,2-Distearoyl-sn-glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-1000], 1,2-Distearoyl-sn-glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000], and 1,2-Distearoyl-sn-

glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-5000]), copolymers and salts thereof.

Additionally, the composition may further comprise an emulsifying agent. Exemplary emulsifying agents include, but are not limited to, a polyethylene glycol (PEG), a polypropylene glycol, a polyvinyl alcohol, a poly-N-vinyl pyrrolidone and copolymers thereof, poloxamer nonionic surfactants, neutral water-soluble polysaccharides (e.g., dextran, Ficoll, celluloses), non-cationic poly(meth)acrylates, non-cationic polyacrylates, such as poly (meth) acrylic acid, and esters amide and hydroxy alkyl amides thereof, natural emulsifiers (e.g. acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (e.g. bentonite [aluminum silicate] and Veegum [magnesium aluminum silicate]), long chain amino acid derivatives, high molecular weight alcohols (e.g. stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (e.g. carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxy vinyl polymer), carrageenan, cellulosic derivatives (e.g. carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (e.g. polyoxyethylene sorbitan monolaurate [Tween 20], polyoxyethylene sorbitan [Tween 60], polyoxyethylene sorbitan monooleate [Tween 80], sorbitan monopalmitate [Span 40], sorbitan monostearate [Span 60], sorbitan tristearate [Span 65], glyceryl monooleate, sorbitan monooleate [Span 80]), polyoxyethylene esters (e.g. polyoxyethylene monostearate [Myrj 45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and Solutol), sucrose fatty acid esters, polyethylene glycol fatty acid esters (e.g. Cremophor), polyoxyethylene ethers, (e.g. polyoxyethylene lauryl ether [Brij 30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, Pluronic F 68, Poloxamer 188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, etc. and/or combinations thereof. In certain aspects, the emulsifying agent is cholesterol.

Liquid compositions include emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the active compound, the liquid composition may contain inert diluents commonly used in the art such as, for example, water or other solvents,

solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the liquid compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents.

Injectable compositions, for example, injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a injectable solution, suspension, or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents for pharmaceutical or cosmetic compositions that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. Any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables. In certain aspects, the particles are suspended in a carrier fluid comprising 1% (w/v) sodium carboxymethyl cellulose and 0.1% (v/v) Tween 80. The injectable composition can be sterilized, for example, by filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

Lipidoid Formulations

The synthesis of lipidoids has been extensively described and formulations containing these compounds are particularly suited for delivery of polynucleotides (see Mahon et ak, Bioconjug Chem. 2010 21 : 1448-1454; Schroeder et ak, J Intern Med. 2010 267:9-21; Akinc et ak, Nat Biotechnol. 2008 26:561-569; Love et ak, Proc Natl Acad Sci USA. 2010 107: 1864-1869; Siegwart et ak, Proc Natl Acad Sci USA. 2011 108: 12996-3001, and US 2016/0317647, herein incorporated by reference in their entirety).

While these lipidoids have been used to effectively deliver double stranded small interfering RNA molecules in rodents and non-human primates (see Akinc et ak, Nat Biotechnol. 2008 26:561-569; Frank-Kamenetsky et ak, Proc Natl Acad Sci USA. 2008 105: 11915-11920; Akinc et al., Mol Ther. 2009 17:872-879; Love et al., Proc Natl Acad Sci

USA. 2010 107: 1864- 1869; Leuschner et al., Nat Biotechnol. 2011 29: 1005-1010, herein incorporated by reference in their entirety), the present disclosure describes their formulation and use in delivering mRNA contained therein.

Complexes, micelles, liposomes or particles can be prepared containing these lipidoids and therefore, can result in an effective delivery of the polynucleotide, as judged by the production of an encoded protein, following the injection of a lipidoid formulation via localized and/or systemic routes of administration. Lipidoid complexes of polynucleotides can be administered by various means including, but not limited to, intravenous, intramuscular, or subcutaneous routes.

In vivo delivery of nucleic acids may be affected by many parameters, including, but not limited to, the formulation composition, nature of particle PEGylation, degree of loading, polynucleotide to lipid ratio, and biophysical parameters such as, but not limited to, particle size (Akinc et al., Mol Ther. 2009 17:872-879, herein incorporated by reference in its entirety). As an example, small changes in the anchor chain length of polyethylene glycol) (PEG) lipids may result in significant effects on in vivo efficacy. Formulations with the different lipidoids, including, but not limited to penta[3-(l-laurylaminopropionyl)]-triethylenetetramine hydrochloride (TETA-5LAP; aka 98N12-5, see Murugaiah et al., Analytical Biochemistry, 401 :61 (2010), herein incorporated by reference in its entirety, and MD1, can be tested for in vivo activity.

The lipidoid referred to herein as "98N12-5" is disclosed by Akinc et al., Mol Ther. 2009 17:872-879, herein incorporated by reference in its entirety. The lipidoid referred to herein as "02-200" is disclosed by Love et al., Proc Natl Acad Sci USA. 2010 107: 1864-1869 and Liu and Huang, Molecular Therapy. 2010 669-670, herein incorporated by reference in their entirety. The lipidoid formulations can include particles comprising either 3 or 4 or more components in addition to polynucleotides. As an example, formulations with certain lipidoids, include, but are not limited to, 98N12-5 and may contain 42% lipidoid, 48% cholesterol and 10% PEG (Cl 4 alkyl chain length). As another example, formulations with certain lipidoids, include, but are not limited to, C12-200 and may contain 50% lipidoid, 10% disteroylphosphatidyl choline, 38.5% cholesterol, and 1.5% PEG-DMG.

Combinations of different lipidoids may be used to improve the efficacy of polynucleotides directed protein production as the lipidoids may be able to increase cell transfection by the mRNA; and/or increase the translation of encoded protein (see

Whitehead et al., Mol. Ther. 2011, 19: 1688- 1694, herein incorporated by reference in its entirety).

Liposomal, Lipoplex, and Lipid Nanoparticle Formulations

The mRNA of the present disclosure can be formulated using one or more liposomes, lipoplexes, or lipid nanoparticles. In one aspects, the therapeutic compositions described herein include liposomes. Liposomes are artificially prepared vesicles which may primarily be composed of a lipid bilayer and may be used as a delivery vehicle for the administration of nutrients and pharmaceutical formulations. Liposomes can be of different sizes such as, but not limited to, a multilamellar vesicle (MLV) which may be hundreds of nanometers in diameter and may contain a series of concentric bilayers separated by narrow aqueous compartments, a small unilamellar vesicle (SUV) which may be smaller than 50 nm in diameter, and a large unilamellar vesicle (LUV) which may be between 50 and 500 nm in diameter. Liposome design may include, but is not limited to, opsonins or ligands in order to improve the attachment of liposomes to unhealthy tissue or to activate events such as, but not limited to, endocytosis. Liposomes may contain a low or a high pH in order to improve the delivery of the pharmaceutical formulations.

The formation of liposomes may depend on the physicochemical characteristics such as, but not limited to, the pharmaceutical formulation entrapped and the liposomal ingredients, the nature of the medium in which the lipid vesicles are dispersed, the effective concentration of the entrapped substance and its potential toxicity, any additional processes involved during the application and/or delivery of the vesicles, the optimization size, polydispersity and the shelf-life of the vesicles for the intended application, and the batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products. As a non-limiting example, liposomes such as synthetic membrane vesicles may be prepared by the methods, apparatus and devices described in US Patent Publication No. US20130177638, US20130177637, US20130177636, US20130177635, US20130177634, US20130177633, US20130183375, US20130183373 and US20130183372, the contents of each of which are herein incorporated by reference in its entirety.

In one aspect, therapeutic compositions described herein may include, without limitation, liposomes such as those formed from 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA) liposomes, DiLa2 liposomes from Marina Biotech (Bothell, Wash.), 1,2-

dilinoleyloxy-3-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[l,3]-dioxolane (DLin-KC2-DMA), and MC3 (US20100324120; herein incorporated by reference in its entirety) and liposomes which may deliver small molecule drugs such as, but not limited to, DOXIL® from Janssen Biotech, Inc. (Horsham, Pa.).

In one aspect, therapeutic compositions described herein may include, without limitation, liposomes such as those formed from the synthesis of stabilized plasmid-lipid particles (SPLP) or stabilized nucleic acid lipid particle (SNALP) that have been previously described and shown to be suitable for oligonucleotide delivery in vitro and in vivo (see Wheeler et al. Gene Therapy. 1999 6:271-281; Zhang et al. Gene Therapy. 1999 6: 1438-1447; Jeffs et al. Pharm Res. 2005 22:362-372; Morrissey et al., NatBiotechnol. 2005 2: 1002-1007; Zimmermann et al., Nature. 2006 441:111-114; Heyes et al. J Contr Rel. 2005 107:276-287; Semple et al. Nature Biotech. 2010 28: 172-176; Judge et al. J Clin Invest. 2009 119:661-673; deFougerolles Hum Gene Ther. 2008 19: 125-132; U.S. Patent Publication No US20130122104, herein incorporated by reference in their entirety). The original manufacture method by Wheeler et al. was a detergent dialysis method, which was later improved by Jeffs et al. and is referred to as the spontaneous vesicle formation method. The liposome formulations are composed of 3 to 4 lipid components in addition to the polynucleotide. As an example a liposome can contain, but is not limited to, 55% cholesterol, 20% distcroylphosphatidyl choline (DSPC), 10% PEG-S-DSG, and 15% l,2-dioleyloxy-N,N- dimethylaminopropane (DODMA), as described by Jeffs et al. As another example, certain liposome formulations may contain, but are not limited to, 48% cholesterol, 20% DSPC, 2% PEG- c-DMA, and 30% cationic lipid, where the cationic lipid can be l,2-distearloxy-N,N-dimethylaminopropane (DSDMA), DODMA, DLin-DMA, or l,2-dilinolenyloxy-3- dimethylaminopropane (DLenDMA), as described by Heyes et al.

In some aspects, liposome formulations may comprise from about 25.0% cholesterol to about 40.0% cholesterol, from about 30.0% cholesterol to about 45.0% cholesterol, from about 35.0% cholesterol to about 50.0% cholesterol and/or from about 48.5% cholesterol to about 60% cholesterol. In some aspects, formulations may comprise a percentage of cholesterol selected from the group consisting of 28.5%, 31.5%, 33.5%, 36.5%, 37.0%, 38.5%, 39.0% and 43.5%. In some aspects, formulations may comprise from about 5.0% to about 10.0% DSPC and/or from about 7.0% to about 15.0% DSPC.

In one aspect, therapeutic compositions may include liposomes which may be formed to deliver mRNA of the present disclosure. The polynucleotide may be encapsulated by the liposome and/or it may be contained in an aqueous core which may then be encapsulated by the liposome (see International Pub. Nos. WO2012031046, WO2012031043, WO2012030901 and W02012006378 and US Patent Publication No. US20130189351, US20130195969 and US20130202684; the contents of each of which are herein incorporated by reference in their entirety).

In another aspect, liposomes may be formulated for targeted delivery. The liposome used for targeted delivery may include, but is not limited to, the liposomes described in and methods of making liposomes described in US Patent Publication No. US20130195967, the contents of which are herein incorporated by reference in its entirety.

In another aspect, the mRNA of the present disclosure may be formulated in a cationic oil-in-water emulsion where the emulsion particle comprises an oil core and a cationic lipid which can interact with the polynucleotide anchoring the molecule to the emulsion particle (see International Pub. No. W02012006380; herein incorporated by reference in its entirety).

In one aspect, the mRNA of the present disclosure may be formulated in a water-in-oil emulsion comprising a continuous hydrophobic phase in which the hydrophilic phase is dispersed. As a non-limiting example, the emulsion may be made by the methods described in International Publication No. WO201087791, the contents of which are herein incorporated by reference in its entirety.

In another aspect, the lipid formulation may include at least a cationic lipid, a lipid which may enhance transfection and a least one lipid which contains a hydrophilic head group linked to a lipid moiety (International Pub. No. WO2011076807 and U.S. Pub. No. 20110200582; the contents of each of which is herein incorporated by reference in their entirety). In another aspect, the mRNA of the present disclosure may be formulated in a lipid vesicle which may have crosslinks between functionalized lipid bilayers (see U.S. Pub. No. 20120177724, the contents of which is herein incorporated by reference in its entirety).

In one aspect, the polynucleotides may be formulated in a liposome as described in International Patent Publication No. WO2013086526, the contents of which is herein incorporated by reference in their entirety. The mRNA may be encapsulated in a liposome using reverse pH gradients and/or optimized internal buffer compositions as described in

International Patent Publication No. WO2013086526, the contents of which is herein incorporated by reference in its entirety.

In one aspect, the mRNA therapeutic compositions may be formulated in liposomes such as, but not limited to, DiLa2 liposomes (Marina Biotech, Bothell, Wash.), SMARTICLES® (Marina Biotech, Bothell, Wash.), neutral DOPC (1,2-dioleoyl-sn-glycero-3- phosphocholine) based liposomes (e.g., siRNA delivery for ovarian cancer (Landen et al. Cancer Biology & Therapy 2006 5(12)1708-1713, herein incorporated by reference in its entirety) and hyaluronan-coated liposomes (Quiet Therapeutics, Israel).

In one aspect, the cationic lipid may be a low molecular weight cationic lipid such as those described in US Patent Application No. 20130090372, the contents of which are herein incorporated by reference in its entirety.

In one aspect, the mRNA may be formulated in a lipid vesicle which may have crosslinks between functionalized lipid bilayers.

In one aspect, the mRNA may be formulated in a liposome comprising a cationic lipid. The liposome may have a molar ratio of nitrogen atoms in the cationic lipid to the phosphates in the RNA (N:P ratio) of between 1:1 and 20:1 as described in International Publication No. W02013006825, herein incorporated by reference in its entirety. In another aspect, the liposome may have a N:P ratio of greater than 20:1 or less than 1:1.

In one aspect, the mRNA may be formulated in a lipid-polycation complex. The formation of the lipid-polycation complex may be accomplished by methods known in the art and/or as described in U.S. Pub. No. 20120178702, herein incorporated by reference in its entirety. As a non-limiting example, the polycation may include a cationic peptide or a polypeptide such as, but not limited to, polylysine, polyornithine and/or polyarginine and the cationic peptides described in International Pub. No. WO2012013326 or US Patent Pub. No. US20130142818; each of which is herein incorporated by reference in its entirety. In another aspect, the mRNA may be formulated in a lipid-polycation complex which may further include a non-cationic lipid such as, but not limited to, cholesterol or dioleoyl phosphatidylethanolamine (DOPE).

In one aspect, the mRNA may be formulated in an aminoalcohol lipidoid. Aminoalcohol lipidoids which may be used in the present disclosure may be prepared by the methods described in U.S. Pat. No. 8,450,298, herein incorporated by reference in its entirety.

The liposome formulation may be influenced by, but not limited to, the selection of the cationic lipid component, the degree of cationic lipid saturation, the nature of the PEGylation, ratio of all components and biophysical parameters such as size. In one example by Semple et al. (Nature Biotech. 2010 28: 172-176, herein incorporated by reference in its entirety), the liposome formulation was composed of 57.1% cationic lipid, 7.1% dipalmitoylphosphatidylcholine, 34.3% cholesterol, and 1.4% PEG-c-DMA. As another example, changing the composition of the cationic lipid could more effectively deliver siRNA to various antigen presenting cells (Basha et al. Mol Ther. 2011 19:2186-2200, herein incorporated by reference in its entirety). In some aspects, liposome formulations may comprise from about 35 to about 45% cationic lipid, from about 40% to about 50% cationic lipid, from about 50% to about 60% cationic lipid and/or from about 55% to about 65% cationic lipid. In some aspects, the ratio of lipid to mRNA in liposomes may be from about 5: 1 to about 20: 1, from about 10: 1 to about 25: 1, from about 15: 1 to about 30: 1 and/or at least 30: 1.

In some aspects, the ratio of PEG in the lipid nanoparticle (LNP) formulations may be increased or decreased and/or the carbon chain length of the PEG lipid may be modified from C14 to C18 to alter the pharmacokinetics and/or biodistribution of the LNP formulations. As a non-limiting example, LNP formulations may contain from about 0.5% to about 3.0%, from about 1.0% to about 3.5%, from about 1.5% to about 4.0%, from about 2.0% to about 4.5%, from about 2.5% to about 5.0% and/or from about 3.0% to about 6.0% of the lipid molar ratio of PEG-c-DOMG (R-3-[(co-methoxy-poly(ethyleneglycol)2000)carbamoyl)]-l,2-dimyristyloxypropyl-3- amine) (also referred to herein as PEG-DOMG) as compared to the cationic lipid, DSPC and cholesterol. In another aspect, the PEG-c-DOMG may be replaced with a PEG lipid such as, but not limited to, PEG-DSG (l,2-Distearoyl-sn-glycerol, methoxypolyethylene glycol), PEG-DMG (l,2-Dimyristoyl-sn-glycerol) and/or PEG-DPG (l,2-Dipalmitoyl-sn-glycerol, methoxypolyethylene glycol). The cationic lipid may be selected from any lipid known in the art such as, but not limited to, DLin-MC3-DMA, DLin-DMA, C12-200 and DLin-KC2-DMA.

In one aspect, the mRNA may be formulated in a lipid nanoparticle such as those described in International Publication No. W02012170930, the contents of which are herein incorporated by reference in its entirety.

In one aspect, the mRNA formulation is a nanoparticle which may comprise at least one lipid. The lipid may be selected from, but is not limited to, DLin-DMA, DLin-K-DMA, 98N12-5, C12-200, DLin-MC3-DMA, DLin-KC2-DMA, DODMA, PLGA, PEG, PEG-DMG, PEGylated lipids and amino alcohol lipids. In another aspect, the lipid may be a cationic lipid such as, but not limited to, DLin-DMA, DLin-D-DMA, DLin-MC3-DMA, DLin-KC2-DMA, DODMA and amino alcohol lipids. The amino alcohol cationic lipid may be the lipids described in and/or made by the methods described in US Patent Publication No. US20130150625, herein incorporated by reference in its entirety. As a non-limiting example, the cationic lipid may be 2-amino-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-2-{[(9Z,2Z)-octadeca-9,12-dien-1-yloxy]methyl}propan-l-ol (Compound 1 in US20130150625); 2-amino-3-[(9Z)-octadec-9-en-l-yloxy]-2-{[(9Z)-octadec-9-en-1-yloxy]methyl}propan-l-ol (Compound 2 in US20130150625); 2-amino-3-[(9Z,l2Z)-octadeca-9,l2-dien-l-yloxy]-2-[(octyloxy)methyl]propan-l-ol (Compound 3 in US20130150625); and 2-(dimethylamino)-3-[(9Z,l2Z)-octadeca-9,12-dien-l-yloxy]-2-{[(9Z,l2Z)-octadeca-9,12-dien-l-yloxy]methyl}propan-l-ol (Compound 4 in US20130150625); or any pharmaceutically acceptable salt or stereoisomer thereof.

Lipid nanoparticle formulations typically comprise a lipid, in particular, an ionizable cationic lipid, for example, 2,2-dilinoleyl-4-dimethylaminoethyl-[l,3]-dioxolane (DLin-KC2- DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), or di((Z)-non-2-en-l-yl)9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319), and further comprise a neutral lipid, a sterol and a molecule capable of reducing particle aggregation, for example a PEG or PEG- modified lipid.

In one aspect, the lipid nanoparticle formulation consists essentially of (i) at least one lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[l,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-l-yl)-9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319); (ii) a neutral lipid selected from DSPC, DPPC, POPC, DOPE and SM; (iii) a sterol, e.g., cholesterol; and (iv) a PEG-lipid, e.g., PEG-DMG or PEG-cDMA, in a molar ratio of about 20-60% cationic lipid:5-25% neutral lipid:25-55% sterol; 0.5-15% PEG-lipid.

In one aspect, the formulation includes from about 25% to about 75% on a molar basis of a cationic lipid selected from 2,2-dilinoleyl-4-dimethylaminoethyl-[l,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-l-yl)-9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319), e.g.,

from about 35 to about 65%, from about 45 to about 65%, about 60%, about 57.5%, about 50% or about 40% on a molar basis.

In one aspect, the formulation includes from about 0.5% to about 15% on a molar basis of the neutral lipid, e.g., from about 3 to about 12%, from about 5 to about 10% or about 15%, about 10%, or about 7.5% on a molar basis. Exemplary neutral lipids include, but are not limited to, DSPC, POPC, DPPC, DOPE and SM. In one aspect, the formulation includes from about 5% to about 50% on a molar basis of the sterol (e.g., about 15 to about 45%, about 20 to about 40%, about 40%, about 38.5%, about 35%, or about 31% on a molar basis. An exemplary sterol is cholesterol. In one aspect, the formulation includes from about 0.5% to about 20% on a molar basis of the PEG or PEG-modified lipid (e.g., about 0.5 to about 10%, about 0.5 to about 5%, about 1.5%, about 0.5%, about 1.5%, about 3.5%, or about 5% on a molar basis. In one aspect, the PEG or PEG modified lipid comprises a PEG molecule of an average molecular weight of 2,000 Da. In other aspects, the PEG or PEG modified lipid comprises a PEG molecule of an average molecular weight of less than 2,000, for example around 1,500 Da, around 1,000 Da, or around 500 Da. Exemplary PEG-modified lipids include, but are not limited to, PEG-distearoyl glycerol (PEG-DMG) (also referred herein as PEG-C14 or C14-PEG) and PEG-cDMA (further discussed in Reyes et al. J. Controlled Release, 107, 276-287 (2005), herein incorporated by reference in its entirety).

In one aspects, the formulations described herein include about 57.5% of a cationic lipid selected from the PEG lipid is PEG-cDMA (PEG-cDMA is further discussed in Reyes et al. (J. Controlled Release, 107, 276-287 (2005), the contents of which are herein incorporated by reference in its entirety), about 7.5% of the neutral lipid, about 31.5% of the sterol, and about 3.5% of the PEG or PEG-modified lipid on a molar basis.

In some aspects, lipid nanoparticle formulation consists essentially of a lipid mixture in molar ratios of about 20-70% cationic lipid: 5-45% neutral lipid: 20-55% cholesterol: 0.5-15% PEG-modified lipid; more preferably in a molar ratio of about 20-60% cationic lipid: 5- 25% neutral lipid: 25-55% cholesterol: 0.5-15% PEG-modified lipid.

In some aspects, the molar lipid ratio is approximately 50/10/38.5/1.5 mol % cationic lipid/neutral lipid, e.g., DSPC/Chol/PEG-modified lipid, e.g., PEG-DMG, PEG-DSG or PEG-DPG, 57.2/7.1134.3/1.4 mol % cationic lipid/neutral lipid, e.g., DPPC/Chol/PEG-modified lipid, e.g., PEG-cDMA, 40/15/40/5 mol % cationic lipid/neutral lipid, e.g., DSPC/Chol/PEG-modified lipid, e.g., PEG-DMG, 50/10/35/4.5/0.5 mol %

cationic lipid/neutral lipid, e.g., DSPC/Chol/PEG-modified lipid, e.g., PEG-DSG, 50/10/35/5 cationic lipid/neutral lipid, e.g., DSPC/Chol/PEG-modified lipid, e.g., PEG-DMG, 40/10/40/10 mol % cationic lipid/neutral lipid, e.g., DSPC/Chol/PEG-modified lipid, e.g., PEG-DMG or PEG-cDMA, 35/15/40/10 mol % cationic lipid/neutral lipid, e.g., DSPC/Chol/PEG-modified lipid, e.g., PEG-DMG or PEG-cDMA or 52/13/30/5 mol % cationic lipid/neutral lipid, e.g., DSPC/Chol/PEG-modified lipid, e.g., PEG- DMG or PEG-cDMA.

Exemplary lipid nanoparticle compositions and methods of making same are described, for example, in Semple et al. (2010) Nat. Biotechnol. 28: 172-176; Jayarama et al. (2012), Angew. Chem. Int. Ed., 51 : 8529-8533; and Maier et al. (2013) Molecular Therapy 21, 1570-1578, herein incorporated by reference in its entirety.

In one aspect, the lipid nanoparticle formulations described herein may comprise a cationic lipid, a PEG lipid and a structural lipid and optionally comprise a non-cationic lipid. As a non-limiting example, the lipid nanoparticle may comprise about 40-60% of cationic lipid, about 5-15% of a non-cationic lipid, about 1-2% of a PEG lipid and about 30-50% of a structural lipid. As another non-limiting example, the lipid nanoparticle may comprise about 50% cationic lipid, about 10% non-cationic lipid, about 1.5% PEG lipid and about 38.5% structural lipid. As yet another non-limiting example, the lipid nanoparticle may comprise about 55% cationic lipid, about 10% non-cationic lipid, about 2.5% PEG lipid and about 32.5% structural lipid. In one aspect, the cationic lipid may be any cationic lipid described herein such as, but not limited to, DLin-KC2-DMA, DLin-MC3-DMA and L319.

In one aspect, the lipid nanoparticle formulations described herein may be 4 component lipid nanoparticles. The lipid nanoparticle may comprise a cationic lipid, a non-cationic lipid, a PEG lipid and a structural lipid. As a non-limiting example, the lipid nanoparticle may comprise about 40-60% of cationic lipid, about 5-15% of a non-cationic lipid, about 1-2% of a PEG lipid and about 30-50% of a structural lipid. As another non-limiting example, the lipid nanoparticle may comprise about 50% cationic lipid, about 10% non-cationic lipid, about 1.5% PEG lipid and about 38.5% structural lipid. As yet another non-limiting example, the lipid nanoparticle may comprise about 55% cationic lipid, about 10% non-cationic lipid, about 2.5% PEG lipid and about 32.5% structural lipid. In one aspect, the cationic lipid may be any cationic lipid described herein such as, but not limited to, DLin-KC2-DMA, DLin-MC3-DMA and L319.

In one aspect, the lipid nanoparticle formulations described herein may comprise a cationic lipid, a non-cationic lipid, a PEG lipid and a structural lipid. As a non-limiting example, the lipid nanoparticle comprise about 50% of the cationic lipid DLin-KC2-DMA, about 10% of the non-cationic lipid DSPC, about 1.5% of the PEG lipid PEG-DOMG and about 38.5% of the structural lipid cholesterol. As a non-limiting example, the lipid nanoparticle comprise about 50% of the cationic lipid DLin-MC3-DMA, about 10% of the non-cationic lipid DSPC, about 1.5% of the PEG lipid PEG-DOMG and about 38.5% of the structural lipid cholesterol. As a non-limiting example, the lipid nanoparticle comprise about 50% of the cationic lipid DLin-MC3-DMA, about 10% of the non-cationic lipid DSPC, about 1.5% of the PEG lipid PEG-DMG and about 38.5% of the structural lipid cholesterol. As yet another non-limiting example, the lipid nanoparticle comprise about 55% of the cationic lipid L319, about 10% of the non-cationic lipid DSPC, about 2.5% of the PEG lipid PEG-DMG and about 32.5% of the structural lipid cholesterol.

In one aspect, the cationic lipid may be selected from, but not limited to, a cationic lipid described in International Publication Nos. W02012040184, W02011153120, WO2011149733, WO2011090965, WO2011043913, WO2011022460, WO2012061259, WO2012054365, WO2012044638, W02010080724, W0201021865, W02008103276, WO2013086373 and WO2013086354, U.S. Pat. Nos. 7,893,302, 7,404,969, 8,283,333, and 8,466,122 and US Patent Publication Nos. US20100036115, US20120202871, US20130064894, US20130129785, US20130150625, US20130178541 and US20130225836; the contents of each of which are herein incorporated by reference in their entirety. In another aspect, the cationic lipid may be selected from, but not limited to, formula A described in International Publication Nos. W02012040184, WO2011153120, WO2011149733, WO2011090965, WO2011043913, WO2011022460, WO2012061259, WO2012054365, WO2012044638 and WO2013116126 or US Patent Publication Nos. US20130178541 and US20130225836; the contents of each of which is herein incorporated by reference in their entirety. In yet another aspect, the cationic lipid may be selected from, but not limited to, formula CLI-CLXXIX of International Publication No. W02008103276, formula CLI-CLXXIX of U.S. Pat. No. 7,893,302, formula CLI-CLXXXXII of U.S. Pat. No. 7,404,969 and formula I- VI of US Patent Publication No. US20100036115, formula I of US Patent Publication No US20130123338; each of which is herein incorporated by reference in their entirety. As a non-limiting example, the cationic lipid may be selected from (20Z,23Z)-N,N-dimethylnonacosa-20,23-dien-10-amine, (17Z,20Z)-N,N-

dimemylhexacosa-17,20-dien-9-amine, (1Z,19Z)-N5N-dimethylpentacosa-l6,19-dien-8-amine, (13Z,16Z)-N,N-dimethyldocosa-13,16-dien-5-amine, (12Z,15Z)-N,N-dimethylhenicosa-12,15-dien-4-amine, (14Z,17Z)-N,N-dimethyltricosa-14,17-di-1-en-6-amine, (15Z,18Z)-N,N-dimethyltetracosa-15,18-dien-7-amine, (18Z,21Z)-N,N-dimethylheptacosa-18,21-dien-10-amine, (15Z,18Z)-N,N-dimethyltetracosa-15,18-dien-5-amine, (14Z,17Z)-N,N-dimethyltricosa-14,17-dien-4-amine, (19Z,22Z)-N,N-dimethyloctacosa-19,22-dien-9-amine, (18Z,21Z)-N,N-dimethylheptacosa-18,21-dien-8-amine, (17Z,20Z)-N,N-dimethylhexacosa-17,20-dien-7-amine, (16Z,19Z)-N,N-dimethylpentacosa-16,19-dien-6-amine, (22Z,25Z)-N,N-dimethylhentriaconta-22,25-dien-10-amine, (21Z,24Z)-N,N-dimethyltriaconta-21,24-dien-9-amine, (18Z)-N,N-dimetylheptacos-18-en-10-amine, (17Z)-N,N-dimethylhexacos-17-en-9-amine, (19Z,22Z)-N,N-dimethyloctacosa-19,22-dien-7-amine, N,N-dimethylheptacosan-l0-amine, (20Z,23Z)-N-ethyl-N-methylnonacosa-20,23-dien-10-amine, 1-[(11Z,14Z)-1-nonylicosa-11,14-dien-l-yl]pyrrolidine, (20Z)-N,N-dimethylheptacos-20-en-10-amine, (15Z)-N,N-dimethylheptacos-15-en-10-amine, (14Z)-N,N-dimethylnonacos-14-en-10-amine, (17Z)-N,N-dimethylnonacos-17-en-10-amine, (24Z)-N,N-dimethyltritriacont-24-en-10-amine, (20Z)-N,N-dimethylnonacos-20-en-10-amine, (22Z)-N,N-dimethylhentriacont-22-en-10-amine, (16Z)-N,N-dimethylpentacos-16-en-8-amine, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, (13Z,16Z)-N,N-dimethyl-3-nonyldocosa-13,16-dien-1-amine, N,N-dimethyl-l-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, l-[(1S,2R)-2-hexylcyclopropyl]-N,N-dimethylnonadecan-10-amine, N,N-dimethyl-1-[(1S,2R)-2-octyl cyclopropyl-nonadecan-10-amine, N,N-dimethyl-21-[(1S,2R)-2-octylcyclopropyl]henicosan-10-amine,N,N-dimethyl-l-[(1S,2S)-2-{[(1R,2R)-2-pentylcyclopropyl]methyl}cyclopropyl]nonadecan-10-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]hexadecan-8-amine, N,N-dimethyl-[(1R,2S)-2-undecylcyclopropyl]tetradecan-5-amine, N,N-dimethyl-3-{7-[(1S,2R)-2-octylcyclopropyl]heptyl}dodecan-1-amine, 1-[(1R,2S)-2-heptylcyclopropyl]-N,N-dimethyloctadecan-9-amine, 1-[(1S,2R)-2-decylcyclopropyl]-N,N-dimethylpentadecan-6-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]pentadecan-8-amine, R-N,N-dimethyl-l-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-3-(octyloxy)propan-2-amine, S-N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-3-(octyloxy)propan-2-amine, 1-{2-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-1-[(octyloxy)methyl]ethyl}pyrrolidine, (2S)-N,N-dimethyl-1-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-3-[(5Z)-oct-5-en-1-yloxy]propan-2-amine, 1-{2-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-1-[(octyloxy)methyl]ethyl}azetidine, (2S)-1-

(hexyloxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, (2S)-1-(heptyloxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, N,N-dimethyl-1-(nonyloxy)-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, N,N-dimethyl-1-[(9Z)-octadec-9-en-1-yloxy]-3-(octyloxy)propan-2-amine; (2S) N,N-dimethyl-1-[(6Z,9Z,12Z)-octadeca-6,9,12-trien-1-yloxy]-3-(octyloxy)propan-2-amine, (2S)-1-[(11Z,14Z)-icosa-11,14-dien-1-yloxy]-N,N-dimethyl-3-(pentyloxy)propan-2-amine, (2S)-1-(hexyloxy)-3-[(11,14Z)-icosa-11,14-dien-l-yloxy]-N,N-dimethylpropan-2-amine, 1-[(11Z,14Z)-icosa-11,14-dien-1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, 1-[(13Z,16Z)-docosa-13,16-dien-1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, (2S)-1-[(13Z,16Z)-docosa-13,16-dien-1-yloxy]-3-(hexyloxy)-N,N-dimethylpropan-2-amine, (2S)-1-[(13Z)-docos-13-en-1-yloxy]-3-(hexyloxy)-N,N-dimethylpropan-2-amine, 1-[(13Z)-docos-13-en-1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, 1-[(9Z)-hexadec-9-en-1-yloxy]-N,N-dimethyl-3-(octyloxy)propan-2-amine, (2R)-N,N-dimethyl-H(1-etoyloctyl)oxy]-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, (2R)-1-[(3,7-dimethyloctyl)oxy]-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-2-amine, N,N-dimethyl-1-(octyloxy)-3-({8-[(1S,2S)-2-{[(1R,2R)-2-pentylcyclopropyl]methyl}cyclopropyl]octyl}oxy)propan-2-amine, N,N-dimethyl-1-{[8-(2-octylcyclopropyl)octyl]oxy}-3-(octyloxy)propan-2-amine and (11E,20Z,23Z)-N,N-dimethylnonacosa-11,20,2-trien-l0-amine or a pharmaceutically acceptable salt or stereoisomer thereof.

In one aspect, the lipid may be a cleavable lipid such as those described in International Publication No. WO2012170889, herein incorporated by reference in its entirety.

In another aspect, the lipid may be a cationic lipid such as, but not limited to, Formula (I) of U.S. Patent Application No. US20130064894, the contents of which are herein incorporated by reference in its entirety.

In one aspect, the cationic lipid may be synthesized by methods known in the art and/or as described in International Publication Nos. W02012040184, WO2011153120, WO2011149733, WO2011090965, WO2011043913, WO2011022460, WO2012061259, WO2012054365, WO2012044638, W02010080724, W0201021865, WO2013086373 and WO2013086354; the contents of each of which are herein incorporated by reference in their entirety.

In another aspect, the cationic lipid may be a trialkyl cationic lipid. Non-limiting examples of trialkyl cationic lipids and methods of making and using the trialkyl cationic lipids are described in International Patent Publication No. WO2013126803, the contents of which are herein incorporated by reference in its entirety.

In one aspect, the lipid nanoparticle formulations may contain PEG-c-DOMG at 3% lipid molar ratio. In another aspect, the LNP formulations may contain PEG-c-DOMG at 1.5% lipid molar ratio.

In one aspect, the therapeutic compositions may include at least one of the PEGylated lipids described in International Publication No. WO2012099755, the contents of which is herein incorporated by reference in its entirety.

In one aspect, the lipid nanoparticle formulation may contain PEG-DMG 2000 (1,2-dimyristoyl-sn-glycero-3-phophoethanolamine-N-[methoxy(polyethylene glycol)-2000). In one aspect, the LNP formulation may contain PEG-DMG 2000, a cationic lipid known in the art and at least one other component. In another aspect, the LNP formulation may contain PEG- DMG 2000, a cationic lipid known in the art, DSPC and cholesterol. As a non-limiting example, the LNP formulation may contain PEG-DMG 2000, DLin-DMA, DSPC and cholesterol. As another non-limiting example, the LNP formulation may contain PEG-DMG 2000, DLin-DMA, DSPC and cholesterol in a molar ratio of 2:40: 10:48 (see e.g., Geall et al., Nonviral delivery of self-amplifying RNA vaccines, PNAS 2012; PM ID 22908294, herein incorporated by reference in its entirety).

In one aspect, the lipid nanoparticle formulation may be formulated by the methods described in International Publication Nos. WO2011127255 or W02008103276, the contents of each of which is herein incorporated by reference in their entirety. As a non-limiting example, the mRNA described herein may be encapsulated in LNP formulations as described in WO2011127255 and/or W02008103276; each of which is herein incorporated by reference in their entirety.

In one aspect, the mRNA described herein may be formulated in a nanoparticle to be delivered by a parenteral route as described in U.S. Pub. No. US20120207845; the contents of which are herein incorporated by reference in its entirety.

In one aspect, the mRNA may be formulated in a lipid nanoparticle made by the methods described in US Patent Publication No. US20130156845 or International

Publication Nos. WO2013093648 or WO2012024526, each of which is herein incorporated by reference in its entirety.

The lipid nanoparticles described herein may be made in a sterile environment by the system and/or methods described in US Patent Publication No. US20130164400, herein incorporated by reference in its entirety.

In one aspect, the mRNA may be formulated in a nanoparticle such as a nucleic acid- lipid particle described in U.S. Pat. No. 8,492,359, the contents of which are herein incorporated by reference in its entirety. As a non-limiting example, the lipid particle may comprise one or more active agents or therapeutic agents; one or more cationic lipids comprising from about 50 mol % to about 85 mol % of the total lipid present in the particle; one or more non-cationic lipids comprising from about 13 mol % to about 49.5 mol % of the total lipid present in the particle; and one or more conjugated lipids that inhibit aggregation of particles comprising from about 0.5 mol % to about 2 mol % of the total lipid present in the particle. The nucleic acid in the nanoparticle may be the polynucleotides described herein and/or are known in the art. In one aspect, the lipid nanoparticle formulation may be formulated by the methods described in International Publication Nos. WO2011127255 or W02008103276, the contents of each of which are herein incorporated by reference in their entirety. As a non-limiting example, modified RNA polynucleotide described herein may be encapsulated in lipid nanoparticle formulations as described in WO2011127255 and/or W02008103276; the contents of each of which are herein incorporated by reference in their entirety.

In one aspect, lipid nanoparticle formulations described herein may comprise a polycationic composition. As a non-limiting example, the polycationic composition may be selected from formula 1-60 of US Patent Publication No. US20050222064; the content of which is herein incorporated by reference in its entirety. In another aspect, the LNP formulations comprising a polycationic composition may be used for the delivery of the modified RNA described herein in vivo and/or in vitro.

In one aspect, the LNP formulations described herein may additionally comprise a permeability enhancer molecule. Non-limiting permeability enhancer molecules are described in US Patent Publication No. US20050222064; the content of which is herein incorporated by reference in its entirety.

In one aspect, the mRNA therapeutic compositions may be formulated in liposomes such as, but not limited to, DiLa2 liposomes (Marina Biotech, Bothell, Wash.), SMARTICLES® (Marina Biotech, Bothell, Wash.), neutral DOPC (1,2-dioleoyl-sn-glycero-3- phosphocholine) based liposomes (e.g., siRNA delivery for ovarian cancer as described in Landen et al. Cancer Biology & Therapy 2006 5(12): 1708-1713, herein incorporated by reference in its entirety) and hyaluronan-coated liposomes (Quiet Therapeutics, Israel).

In one aspect, the mRNA may be formulated in a lyophilized gel-phase liposomal composition as described in US Publication No. US2012060293, herein incorporated by reference in its entirety.

The nanoparticle formulations may comprise a phosphate conjugate. The phosphate conjugate may increase in vivo circulation times and/or increase the targeted delivery of the nanoparticle. Phosphate conjugates for use with the present disclosure may be made by the methods described in International Application No. WO2013033438 or US Patent Publication No. US20130196948, the contents of each of which are herein incorporated by reference in its entirety. As a non-limiting example, the phosphate conjugates may include a compound of any one of the formulas described in International Application No. WO2013033438, herein incorporated by reference in its entirety.

The nanoparticle formulation may comprise a polymer conjugate. The polymer conjugate may be a water-soluble conjugate. The polymer conjugate may have a structure as described in U.S. Patent Publication No. US20130059360, the contents of which are herein incorporated by reference in its entirety. In one aspect, polymer conjugates with the polynucleotides of the present disclosure may be made using the methods and/or segmented polymeric reagents described in U.S. Patent Publication No. US20130072709, herein incorporated by reference in its entirety. In another aspect, the polymer conjugate may have pendant side groups comprising ring moieties such as, but not limited to, the polymer conjugates described in U.S. Patent Publication No. US20130196948, the contents of which is herein incorporated by reference in its entirety.

In another aspect, therapeutic compositions comprising the polynucleotides of the present disclosure and a conjugate which may have a degradable linkage. Non-limiting examples of conjugates include an aromatic moiety comprising an ionizable hydrogen atom, a spacer moiety, and a water-soluble polymer. As a non-limiting example, therapeutic compositions comprising a conjugate with a degradable linkage and methods for delivering

such therapeutic compositions are described in US Patent Publication No. US20130184443, the contents of which are herein incorporated by reference in its entirety.

The nanoparticle formulations may be a carbohydrate nanoparticle comprising a carbohydrate carrier and an mRNA. As a non-limiting example, the carbohydrate carrier may include, but is not limited to, an anhydride-modified phytoglycogen or glycogen-type material, phytoglycogen octenyl succinate, phytoglycogen beta-dextrin, anhydride-modified phytoglycogen beta-dextrin. (See e.g., International Publication No. W02012109121; the contents of which are herein incorporated by reference in its entirety).

Nanoparticle formulations of the present disclosure may be coated with a surfactant or polymer in order to improve the delivery of the particle. In one aspect, the nanoparticle may be coated with a hydrophilic coating such as, but not limited to, PEG coatings and/or coatings that have a neutral surface charge. The hydrophilic coatings may help to deliver nanoparticles with larger payloads such as, but not limited to, the polynucleotides within the central nervous system. As a non-limiting example nanoparticles comprising a hydrophilic coating and methods of making such nanoparticles are described in U.S. Patent Publication No. US20130183244, the contents of which are herein incorporated by reference in its entirety.

In one aspect, the lipid nanoparticles of the present disclosure may be hydrophilic polymer particles. Non-limiting examples of hydrophilic polymer particles and methods of making hydrophilic polymer particles are described in U.S. Patent Publication No. US20130210991, the contents of which are herein incorporated by reference in its entirety.

In another aspect, the lipid nanoparticles of the present disclosure may be hydrophobic polymer particles.

Lipid nanoparticle formulations may be improved by replacing the cationic lipid with a biodegradable cationic lipid which is known as a rapidly eliminated lipid nanoparticle (reLNP). Ionizable cationic lipids, such as, but not limited to, DLinDMA, DLin-KC2-DMA, and DLin- MC3-DMA, have been shown to accumulate in plasma and tissues over time and may be a potential source of toxicity. The rapid metabolism of the rapidly eliminated lipids can improve the tolerability and therapeutic index of the lipid nanoparticles by an order of magnitude from a 1 mg/kg dose to a 10 mg/kg dose in rat. Inclusion of an enzymatically degraded ester linkage can improve the degradation and metabolism profile of the cationic component, while still maintaining the activity of the

reLNP formulation. The ester linkage can be internally located within the lipid chain or it may be terminally located at the terminal end of the lipid chain. The internal ester linkage may replace any carbon in the lipid chain.

In one aspect, the internal ester linkage may be located on either side of the saturated carbon.

Lipid nanoparticles may be engineered to alter the surface properties of particles so the lipid nanoparticles may penetrate the mucosal barrier. Mucus is located on mucosal tissue such as, but not limited to, oral (e.g., the buccal and esophageal membranes and tonsil tissue), ophthalmic, gastrointestinal (e.g., stomach, small intestine, large intestine, colon, rectum), nasal, respiratory (e.g., nasal, pharyngeal, tracheal and bronchial membranes), genital (e.g., vaginal, cervical and urethral membranes). Nanoparticles larger than 10-200 nm which are preferred for higher drug encapsulation efficiency and the ability to provide the sustained delivery of a wide array of drugs have been thought to be too large to rapidly diffuse through mucosal barriers. Mucus is continuously secreted, shed, discarded or digested and recycled so most of the trapped particles may be removed from the mucosal tissue within seconds or within a few hours. Large polymeric nanoparticles (200 nm-500 nm in diameter) which have been coated densely with a low molecular weight polyethylene glycol (PEG) diffused through mucus only 4 to 6-fold lower than the same particles diffusing in water (Lai et al. PNAS 2007 104(5): 1482-487; Lai et al. Adv Drug Deliv Rev. 2009 61(2): 158-171, herein incorporated by reference in their entirety). The transport of nanoparticles may be determined using rates of permeation and/or fluorescent microscopy techniques including, but not limited to, fluorescence recovery after photobleaching (FRAP) and high resolution multiple particle tracking (MPT). As a non-limiting example, compositions which can penetrate a mucosal barrier may be made as described in U.S. Patent No. 8,241,670 or International Publication No. WO2013110028, the contents of each of which are herein incorporated by reference in its entirety.

The lipid nanoparticle engineered to penetrate mucus may comprise a polymeric material (i.e. a polymeric core) and/or a polymer-vitamin conjugate and/or a tri-block co-polymer. The polymeric material may include, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, poly(styrenes), polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. The polymeric material may be biodegradable and/or biocompatible. Non-

limiting examples of biocompatible polymers are described in International Publication No. WO2013116804, the contents of which are herein incorporated by reference in its entirety. The polymeric material may additionally be irradiated. As a non-limiting example, the polymeric material may be gamma irradiated (See e.g., International Publication No. WO201282165, herein incorporated by reference in its entirety). Non-limiting examples of specific polymers include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacrylate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as polyethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as polyethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly (isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fumarate, polyoxymethylene, poloxamers, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), PEG- PLGA-PEG and trimethylene carbonate, polyvinylpyrrolidone. The lipid nanoparticle may be coated or associated with a co-polymer such as, but not limited to, a block co-polymer (such as a branched polyether-polyamide block copolymer described in International Publication No. WO2013012476, herein incorporated by reference in its entirety), and (poly(ethylene glycol))-(poly(propylene oxide))-(poly(ethylene glycol)) triblock copolymer (see e.g., ETS Publication 20120121718 and US Publication No. 20100003337 and U.S. Patent No.

8,263,665; each of which is herein incorporated by reference in their entirety). The co-polymer may be a polymer that is generally regarded as safe (GRAS) and the formation of the lipid nanoparticle may be in such a way that no new chemical entities are created. For example, the lipid nanoparticle may comprise poloxamers coating PLGA nanoparticles without forming new chemical entities which are still able to rapidly penetrate human mucus (Yang et al. Angew. Chem. Int. Ed. 2011 50:2597-2600, herein incorporated by reference in its entirety). A non-limiting scalable method to produce nanoparticles which can penetrate human mucus is described by Xu et al. (J Control Release 2013, 170(2):279-86, herein incorporated by reference in its entirety).

The vitamin of the polymer-vitamin conjugate may be vitamin E. The vitamin portion of the conjugate may be substituted with other suitable components such as, but not limited to, vitamin A, vitamin E, other vitamins, cholesterol, a hydrophobic moiety, or a hydrophobic component of other surfactants (e.g., sterol chains, fatty acids, hydrocarbon chains and alkylene oxide chains). The lipid nanoparticle engineered to penetrate mucus may include surface altering agents such as, but not limited to, polynucleotides, anionic proteins (e.g., bovine serum albumin), surfactants (e.g., cationic surfactants such as for example dimethyldioctadecyl-ammonium bromide), sugars or sugar derivatives (e.g., cyclodextrin), nucleic acids, polymers (e.g., heparin, polyethylene glycol and poloxamer), mucolytic agents (e.g., N-acetylcysteine, mugwort, bromelain, papain, clerodendrum, acetylcysteine, bromhexine, carbocisteine, eprazinone, mesna, ambroxol, sobrerol, domiodol, letosteine, stepronin, tiopronin, gelsolin, thymosin b4 dornase alfa, neltenexine, erdosteine) and various DNases including rhDNase. The surface altering agent may be embedded or enmeshed in the particle's surface or disposed (e.g., by coating, adsorption, covalent linkage, or other process) on the surface of the lipid nanoparticle (see e.g., U.S. Publication Nos. 20100215580, US20080166414, and US20130164343; the contents of each of which is herein incorporated by reference in their entirety).

In one aspect, the mucus penetrating lipid nanoparticles may comprise at least one polynucleotide described herein. The polynucleotide may be encapsulated in the lipid nanoparticle and/or disposed on the surface of the particle. The polynucleotide may be covalently coupled to the lipid nanoparticle. Formulations of mucus penetrating lipid nanoparticles may comprise a plurality of nanoparticles. Further, the formulations may contain particles which may interact with the mucus and alter the structural and/or adhesive

properties of the surrounding mucus to decrease mucoadhesion which may increase the delivery of the mucus penetrating lipid nanoparticles to the mucosal tissue.

In another aspect, the mucus penetrating lipid nanoparticles may be a hypotonic formulation comprising a mucosal penetration enhancing coating. The formulation may be hypotonic for the epithelium to which it is being delivered. Non-limiting examples of hypotonic formulations may be found in International Patent Publication No. WO2013110028, the contents of which are herein incorporated by reference in its entirety.

In one aspect, in order to enhance the delivery through the mucosal barrier the polynucleotide formulation may comprise or be a hypotonic solution, see e.g., Ensign et al. Biomaterials 2013 34(28):6922-9, herein incorporated by reference in its entirety.

In one aspect, the mRNA is formulated as a lipoplex, such as, without limitation, the ATUPLEX™ system, the DACC system, the DBTC system and other siRNA-lipoplex technology from Silence Therapeutics (London, ETnited Kingdom), STEMFECT™ from STEMGENT® (Cambridge, Mass.), and polyethyleneimine (PEI) or protamine-based targeted and non-targeted delivery of nucleic acids (Aleku et al. Cancer Res. 2008 68:9788-9798; Strumberg et al. Int J Clin Pharmacol Ther 2012 50:76-78; Santel et al., Gene Ther 2006 13 : 1222-1234; Santel et al., Gene Ther 2006 13 : 1360-1370; Gutbier et al., Pulm Pharmacol. Ther. 2010 23 :334-344; Kaufmann et al. Microvasc Res 2010 80:286-293 Weide et al. J Immunother. 2009 32:498-507; Weide et al. J Immunother. 2008 31 : 180-188; Pascolo Expert Opin. Biol. Ther. 4: 1285-1294; Fotin-Mleczek et al., 2011 J. Immunother. 34: 1-15; Song et al., Nature Biotechnol. 2005, 23 :709-717; Peer et al., Proc Natl Acad Sci USA. 2007 6; 104:4095-4100; deFougerolles Hum Gene Ther. 2008 19: 125- 132, herein incorporated by reference in their entirety).

Formulations can also be selectively targeted through expression of different ligands on their surface as exemplified by, but not limited by, folate, transferrin, N-acetylgalactosamine (GalNAc), and antibody targeted approaches (Kolhatkar et al., Curr Drug Discov Technol. 2011 8: 197-206; Musacchio and Torchilin, Front Biosci. 2011 16: 1388-1412; Yu et al., Mol Membr Biol. 2010 27:286-298; Patil et al., Crit Rev Ther Drug Carrier Syst. 2008 25: 1-61; Benoit et al., Biomacromolecules. 2011 12:2708-2714; Zhao et al., Expert Opin Drug Deliv. 2008 5:309-319; Akinc et al., Mol Ther. 2010 18: 1357-1364; Srinivasan et al., Methods Mol Biol. 2012 820: 105-116; Ben-Arie et al., Methods Mol Biol. 2012 757:497- 507; Peer 2010 J Control Release. 20:63-68; Peer et al., Proc Natl Acad Sci

USA. 2007 104:4095- 4100; Kim et al., Methods Mol Biol. 2011 721 :339-353; Subramanya et al., Mol Ther. 2010 18:2028-2037; Song et al., Nat Biotechnol. 2005 23 :709-717; Peer et al., Science. 2008 319:627- 630; Peer and Lieberman, Gene Ther. 2011 18: 1127-1133, herein incorporated by reference in its entirety).

In one aspect, the mRNA is formulated as a solid lipid nanoparticle. A solid lipid nanoparticle (SLN) may be spherical with an average diameter between 10 to 1000 nm. SLN possess a solid lipid core matrix that can solubilize lipophilic aspect and may be stabilized with surfactants and/or emulsifiers. In a further aspect, the lipid nanoparticle may be a self- assembly lipid-polymer nanoparticle (see Zhang et al., ACS Nano, 2008, 2 (8), pp 1696-1702, herein incorporated by reference in its entirety). As a non-limiting example, the SLN may be the SLN described in International Patent Publication No. WO2013105101, the contents of which are herein incorporated by reference in its entirety. As another non-limiting example, the SLN may be made by the methods or processes described in International Patent Publication No. W02013105101, the contents of which are herein incorporated by reference in its entirety.

Liposomes, lipoplexes, or lipid nanoparticles may be used to improve the efficacy of polynucleotides directed protein production as these formulations may be able to increase cell transfection by the mRNA; and/or increase the translation of encoded protein. One such example involves the use of lipid encapsulation to enable the effective systemic delivery of polyplex plasmid DNA (Heyes et al., Mol Ther. 2007 15:713-720; herein incorporated by reference in its entirety). The liposomes, lipoplexes, or lipid nanoparticles may also be used to increase the stability of the polynucleotide.

In one aspect, the mRNA of the present disclosure can be formulated for controlled release and/or targeted delivery. As used herein, "controlled release" refers to a therapeutic composition or compound release profile that conforms to a particular pattern of release to effect a therapeutic outcome. In one aspect, the polynucleotide may be encapsulated into a delivery agent described herein and/or known in the art for controlled release and/or targeted delivery. As used herein, the term "encapsulate" means to enclose, surround or encase. As it relates to the formulation of the synthetic mRNA of the disclosure, encapsulation may be substantial, complete or partial. The term "substantially encapsulated" means that at least greater than 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.9 or greater than 99.999% of the therapeutic composition or synthetic mRNA of the disclosure may be enclosed, surrounded or encased within the delivery agent. "Partially encapsulated"

means that less than 10, 10, 20, 30, 40 50 or less of the therapeutic composition or synthetic mRNA of the disclosure may be enclosed, surrounded or encased within the delivery agent. Advantageously, encapsulation may be determined by measuring the escape or the activity of the therapeutic composition or synthetic mRNA of the disclosure using fluorescence and/or electron micrograph. For example, at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the therapeutic composition or synthetic mRNA of the disclosure are encapsulated in the delivery agent.

In one aspect, the controlled release formulation may include, but is not limited to, tri-block co-polymers. As a non-limiting example, the formulation may include two different types of tri-block co-polymers (International Publication Nos. W02012131104 and W02012131106; the contents of each of which is herein incorporated by reference in its entirety).

In another aspect, the mRNA may be encapsulated into a lipid nanoparticle or a rapidly eliminated lipid nanoparticle and the lipid nanoparticles or a rapidly eliminated lipid nanoparticle may then be encapsulated into a polymer, hydrogel and/or surgical sealant described herein and/or known in the art. As a non-limiting example, the polymer, hydrogel or surgical sealant may be PLGA, ethylene vinyl acetate (EVAc), poloxamer, GELSITE® (Nanotherapeutics, Inc. Alachua, Fla.), HYLENEX® (Halozyme Therapeutics, San Diego Calif.), surgical sealants such as fibrinogen polymers (Ethicon Inc. Cornelia, Ga.), TISSELL@ (Baxter International, Inc Deerfield, Ill.), PEG-based sealants, and COSEAL® (Baxter International, Inc Deerfield, Ill.).

In another aspect, the lipid nanoparticle may be encapsulated into any polymer known in the art which may form a gel when injected into a subject. As another non-limiting example, the lipid nanoparticle may be encapsulated into a polymer matrix which may be biodegradable.

In one aspect, the mRNA formulation for controlled release and/or targeted delivery may also include at least one controlled release coating. Controlled release coatings include, but are not limited to, OPADRY®, polyvinylpyrrolidone/vinyl acetate copolymer, polyvinylpyrrolidone, hydroxypropyl methylcellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, EUDRAGIT RL®, EUDRAGIT RS® and cellulose derivatives such as ethylcellulose aqueous dispersions (AQUACOAT® and SURELEASE®).

In one aspect, the mRNA controlled release and/or targeted delivery formulation may comprise at least one degradable polyester which may contain polycationic side chains. Degradeable polyesters include, but are not limited to, poly(serine ester), poly(L-lactide-co-L- lysine), poly(4-hydroxy-L-proline ester), and combinations thereof. In another aspect, the degradable polyesters may include a PEG conjugation to form a PEGylated polymer.

In one aspect, the mRNA controlled release and/or targeted delivery formulation comprising at least one polynucleotide may comprise at least one PEG and/or PEG related polymer derivatives as described in U.S. Patent No. 8,404,222, herein incorporated by reference in its entirety.

In another aspect, the mRNA controlled release delivery formulation comprising at least one polynucleotide may be the controlled release polymer system described in U.S. Patent Publication No. US20130130348, herein incorporated by reference in its entirety.

In one aspect, the mRNA of the present disclosure may be encapsulated in a therapeutic nanoparticle, referred to herein as "therapeutic nanoparticles." Therapeutic nanoparticles may be formulated by methods described herein and known in the art such as, but not limited to, International Publication Nos. W02010005740, W02010030763, W02010005721, W02010005723, WO2012054923, US Patent Publication Nos. US20110262491, US20100104645, US20100087337, US20100068285, US20110274759, US20100068286, US20120288541, US20130123351 and US20130230567 and U.S. Patent Nos. 8,206,747, 8,293,276, 8,318,208 and 8,318,211; the contents of each of which are herein incorporated by reference in their entirety. In another aspect, therapeutic polymer nanoparticles may be identified by the methods described in U.S. Publication No. US20120140790, the contents of which is herein incorporated by reference in its entirety.

In one aspect, the therapeutic nanoparticles may be formulated for sustained release. As used herein, "sustained release" refers to a therapeutic composition or synthetic mRNA that conforms to a release rate over a specific period of time. The period of time may include, but is not limited to, hours, days, weeks, months and years. As a non-limiting example, the sustained release nanoparticle may comprise a polymer and a therapeutic agent such as, but not limited to, the polynucleotides of the present disclosure (see International Pub No. 2010075072 and US Pub No. US20100216804, US20110217377 and US20120201859, each of which is herein incorporated by reference in their entirety). In another non-limiting example, the sustained release formulation may comprise agents which

permit persistent bioavailability such as, but not limited to, crystals, macromolecular gels and/or particulate suspensions (see US Patent Publication No US20130150295, the contents of which is herein incorporated by reference in its entirety).

In one aspect, the therapeutic nanoparticles may be formulated to be target specific. As a non-limiting example, the therapeutic nanoparticles may include a corticosteroid (see International Publication No. WO2011084518; herein incorporated by reference in its entirety). As a non-limiting example, the therapeutic nanoparticles may be formulated in nanoparticles described in International Publication Nos. WO2008121949, W02010005726, W02010005725, WO2011084521 and US Publication Nos. US20100069426, US20120004293 and US20100104655, each of which is herein incorporated by reference in their entirety.

In one aspect, the nanoparticles of the present disclosure may comprise a polymeric matrix. As a non-limiting example, the nanoparticle may comprise two or more polymers such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polylysinc, polyethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy- L-proline ester) or combinations thereof.

In one aspect, the therapeutic nanoparticle comprises a diblock copolymer. In one aspect, the diblock copolymer may include PEG in combination with a polymer such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polylysine, polyethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester) or combinations thereof. In yet another aspect, the diblock copolymer may be a high-X diblock copolymer such as those described in International Patent Publication No. WO2013120052, the contents of which are herein incorporated by reference in its entirety.

As a non-limiting example, the therapeutic nanoparticle comprises a PLGA-PEG block copolymer (see U.S. Publication No. US20120004293 and U.S. Patent No. 8,236,330,

each of which is herein incorporated by reference in their entirety). In another non-limiting example, the therapeutic nanoparticle is a stealth nanoparticle comprising a diblock copolymer of PEG and PLA or PEG and PLGA (see U.S. Patent No. 8,246,968 and International Publication No. WO2012166923, the contents of each of which are herein incorporated by reference in its entirety). In yet another non-limiting example, the therapeutic nanoparticle is a stealth nanoparticle or a target-specific stealth nanoparticle as described in US Patent Publication No. US20130172406, the contents of which are herein incorporated by reference in its entirety.

In one aspect, the therapeutic nanoparticle may comprise a multiblock copolymer (See e.g., U.S. Patent Nos. 8,263,665 and 8,287,910 and US Patent Publication No. US20130195987; the contents of each of which are herein incorporated by reference in its entirety).

In yet another non-limiting example, the lipid nanoparticle comprises the block copolymer PEG-PLGA-PEG (see e.g., the thermosensitive hydrogel (PEG-PLGA-PEG) was used as a TGF- betal gene delivery vehicle in Lee et al. Thermosensitive Hydrogel as a Tgf-b î Gene Delivery Vehicle Enhances Diabetic Wound Healing. Pharmaceutical Research, 2003 20(12): 1995-2000; as a controlled gene delivery system in Li et al. Controlled Gene Delivery System Based on Thermosensitive Biodegradable Hydrogel. Pharmaceutical Research 2003 20(6):884-888; and Chang et al., Non-ionic amphiphilic biodegradable PEG-PLGA-PEG copolymer enhances gene delivery efficiency in rat skeletal muscle. J Controlled Release. 2007 118:245-253, herein incorporated by reference in their entirety). The mRNA of the present disclosure may be formulated in lipid nanoparticles comprising the PEG-PLGA-PEG block copolymer.

In one aspect, the therapeutic nanoparticle may comprise a multiblock copolymer (See e.g., U.S. Patent Nos. 8,263,665 and 8,287,910 and U.S. Patent Publication No. US20130195987; the contents of each of which are herein incorporated by reference in its entirety).

In one aspect, the block copolymers described herein may be included in a polyion complex comprising a non-polymeric micelle and the block copolymer. (See e.g., U.S. Publication No. 20120076836; herein incorporated by reference in its entirety).

In one aspect, the therapeutic nanoparticle may comprise at least one acrylic polymer. Acrylic polymers include but are not limited to, acrylic acid, methacrylic acid,

acrylic acid and methacrylic acid copolymers, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, amino alkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), polycyanoacrylates and combinations thereof.

In one aspect, the therapeutic nanoparticles may comprise at least one poly(vinyl ester) polymer. The poly(vinyl ester) polymer may be a copolymer such as a random copolymer. As a non-limiting example, the random copolymer may have a structure such as those described in International Publication No. WO2013032829 or US Patent Publication No. US20130121954, the contents of which are herein incorporated by reference in its entirety. In one aspect, the poly (vinyl ester) polymers may be conjugated to the polynucleotides described herein.

In one aspect, the therapeutic nanoparticle may comprise at least one diblock copolymer. The diblock copolymer may be, but it not limited to, a poly(lactic) acid-poly(ethylene)glycol copolymer (see e.g., International Patent Publication No. WO2013044219; herein incorporated by reference in its entirety).

In one aspect, the therapeutic nanoparticles may comprise at least one cationic polymer described herein and/or known in the art. In one aspect, the therapeutic nanoparticles may comprise at least one amine- containing polymer such as, but not limited to polylysine, polyethylene imine, poly(amidoamine) dendrimers, poly(beta-amino esters) (see e.g., U.S. Patent No. 8,287,849; herein incorporated by reference in its entirety) and combinations thereof.

In another aspect, the nanoparticles described herein may comprise an amine cationic lipid such as those described in International Patent Application No. WO2013059496, the contents of which are herein incorporated by reference in its entirety. In one aspect, the cationic lipids may have an amino-amine or an amino-amide moiety.

In one aspect, the therapeutic nanoparticles may comprise at least one degradable polyester which may contain poly cationic side chains. Degradable polyesters include, but are not limited to, poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), and combinations thereof. In another aspect, the degradable polyesters may include a PEG conjugation to form a PEGylated polymer.

In one aspect, the therapeutic nanoparticles may be formulated using the methods described by Podobinski et al in U.S. Patent No. 8,404,799, the contents of which are herein incorporated by reference in its entirety.

In one aspect, the mRNA may be encapsulated in, linked to and/or associated with synthetic nanocarriers. Synthetic nanocarriers include, but are not limited to, those described in International Publication Nos. WQ2010005740, WQ2010030763, W0201213501, WO2012149252, WO2012149255, WO2012149259, WO2012149265, WO2012149268, WO2012149282, W02012149301, WO2012149393, WO2012149405, WO2012149411, WO2012149454 and WO2013019669, and U.S. Publication Nos. US20110262491, US20100104645, US20100087337 and US20120244222, each of which is herein incorporated by reference in their entirety. The synthetic nanocarriers may be formulated using methods known in the art and/or described herein. As a non-limiting example, the synthetic nanocarriers may be formulated by the methods described in International Publication Nos. WO2010005740, WO2010030763 and WO201213501 and US Publication Nos. US20110262491, US20100104645, US20100087337 and US2012024422, each of which is herein incorporated by reference in their entirety. In another aspect, the synthetic nanocarrier formulations may be lyophilized by methods described in International Publication No. WO2011072218 and U.S. Patent No. 8,211,473; the content of each of which is herein incorporated by reference in their entirety. In yet another aspect, formulations of the present disclosure, including, but not limited to, synthetic nanocarriers, may be lyophilized or reconstituted by the methods described in U.S. Patent Publication No. US20130230568, the contents of which are herein incorporated by reference in its entirety.

In one aspect, the synthetic nanocarriers may contain reactive groups to release the polynucleotides described herein (see International Pub. No. WO20120952552 and US Pub No. US20120171229, each of which is herein incorporated by reference in their entirety).

In one aspect, the synthetic nanocarriers may be formulated for targeted release. In one aspect, the synthetic nanocarrier is formulated to release the polynucleotides at a specified pH and/or after a desired time interval. As a non-limiting example, the synthetic nanoparticle may be formulated to release the mRNA after 24 hours and/or at a pH of 4.5 (see International Publication Nos. W02010138193 and W02010138194 and U.S. Publication Nos. US20110020388 and US20110027217, each of which is herein incorporated by reference in their entireties).

In one aspect, the synthetic nanocarriers may be formulated for controlled and/or sustained release of the polynucleotides described herein. As a non-limiting example, the synthetic nanocarriers for sustained release may be formulated by methods known in the art,

described herein and/or as described in International Publication No. W02010138192 and U.S. Publication No. 20100303850, each of which is herein incorporated by reference in their entirety. In one aspect, the mRNA may be formulated for controlled and/or sustained release wherein the formulation comprises at least one polymer that is a crystalline side chain (CYSC) polymer. CYSC polymers are described in U.S. Patent No. 8,399,007, herein incorporated by reference in its entirety.

In one aspect, the synthetic nanocarrier may include at least one adjuvant. As non limiting example, the adjuvant may comprise dimethyldioctadecylammonium-bromide, dimethyldioctadecylammonium-chloride, dimethyldioctadecylammonium-phosphate or dimethyldioctadecylammonium-acetate (DDA) and an apolar fraction or part of said apolar fraction of a total lipid extract of a mycobacterium (See e.g, U.S. Patent No. 8,241,610; herein incorporated by reference in its entirety). In another aspect, the synthetic nanocarrier may comprise at least one polynucleotide and an adjuvant. As a non-limiting example, the synthetic nanocarrier comprising and adjuvant may be formulated by the methods described in International Publication No. WO2011150240 and U.S. Publication No. US20110293700, each of which is herein incorporated by reference in its entirety.

In one aspect, the mRNA may be encapsulated in, linked to and/or associated with zwitterionic lipids. Non-limiting examples of zwitterionic lipids and methods of using zwitterionic lipids are described in U.S. Patent Publication No. US20130216607, the contents of which are herein incorporated by reference in its entirety. In one aspect, the zwitterionic lipids may be used in the liposomes and lipid nanoparticles described herein.

In one aspect, the mRNA may be formulated in colloid nanocarriers as described in U.S. Patent Publication No. US20130197100, the contents of which are herein incorporated by reference in its entirety.

In some aspect, lipid nanoparticles comprise the lipid KL52 (an amino-lipid disclosed in U.S. Patent Publication No. 2012/0295832 expressly incorporated herein by reference in its entirety). Activity and/or safety (as measured by examining one or more of ALT/AST, white blood cell count and cytokine induction) of lipid nanoparticle administration may be improved by incorporation of such lipids. Lipid nanoparticles comprising KL52 may be administered intravenously and/or in one or more doses. In some aspect, administration of lipid nanoparticles comprising KL52 results in equal or improved mRNA and/or protein expression as compared to LNPs comprising MC3. In some aspects,

the mRNA may be delivered using smaller lipid nanoparticles. Such particles may comprise a diameter from below 0.1 um up to 100 nm such as, but not limited to, less than 0.1 um, less than 1.0 um, less than 5 um, less than 10 um, less than 15 um, less than 20 um, less than 25 um, less than 30 um, less than 35 um, less than 40 um, less than 50 um, less than 55 um, less than 60 um, less than 65 um, less than 70 um, less than 75 um, less than 80 um, less than 85 um, less than 90 um, less than 95 um, less than 100 um, less than 125 um, less than 150 um, less than 175 um, less than 200 um, less than 225 um, less than 250 um, less than 275 um, less than 300 um, less than 325 um, less than 350 um, less than 375 um, less than 400 um, less than 425 um, less than 450 um, less than 475 um, less than 500 um, less than 525 um, less than 550 um, less than 575 um, less than 600 um, less than 625 um, less than 650 um, less than 675 um, less than 700 um, less than 725 um, less than 750 um, less than 775 um, less than 800 um, less than 825 um, less than 850 um, less than 875 um, less than 900 um, less than 925 um, less than 950 um, less than 975 um,

In another aspect, the mRNA may be delivered using smaller lipid nanoparticles which may comprise a diameter from about 1 nm to about 100 nm, from about 1 nm to about 10 nm, about 1 nm to about 20 nm, from about 1 nm to about 30 nm, from about 1 nm to about 40 nm, from about 1 nm to about 50 nm, from about 1 nm to about 60 nm, from about 1 nm to about 70 nm, from about 1 nm to about 80 nm, from about 1 nm to about 90 nm, from about 5 nm to about from 100 nm, from about 5 nm to about 10 nm, about 5 nm to about 20 nm, from about 5 nm to about 30 nm, from about 5 nm to about 40 nm, from about 5 nm to about 50 nm, from about 5 nm to about 60 nm, from about 5 nm to about 70 nm, from about 5 nm to about 80 nm, from about 5 nm to about 90 nm, about 10 to about 50 nM, from about 20 to about 50 nm, from about 30 to about 50 nm, from about 40 to about 50 nm, from about 20 to about 60 nm, from about 30 to about 60 nm, from about 40 to about 60 nm, from about 20 to about 70 nm, from about 30 to about 70 nm, from about 40 to about 70 nm, from about 50 to about 70 nm, from about 60 to about 70 nm, from about 20 to about 80 nm, from about 30 to about 80 nm, from about 40 to about 80 nm, from about 50 to about 80 nm, from about 60 to about 80 nm, from about 20 to about 90 nm, from about 30 to about 90 nm, from about 40 to about 90 nm, from about 50 to about 90 nm, from about 60 to about 90 nm and/or from about 70 to about 90 nm.

In some aspect, such lipid nanoparticles are synthesized using methods comprising microfluidic mixers. Exemplary microfluidic mixers may include, but are not limited to a slit interdigitial micromixer including, but not limited to those manufactured by

Microinnova (Allerheiligen bei Wildon, Austria) and/or a staggered herringbone micromixer (SHM) (Zhigaltsev, I. V. et al., Bottom-up design and synthesis of limit size lipid nanoparticle systems with aqueous and triglyceride cores using millisecond microfluidic mixing have been published (Langmuir. 2012. 28:3633-40; Belliveau, N. M. et al., Microfluidic synthesis of highly potent limit-size lipid nanoparticles for in vivo delivery of siRNA. Molecular Therapy-Nucleic Acids. 2012. 1 :e37; Chen, D. et al., Rapid discovery of potent siRNA-containing lipid nanoparticles enabled by controlled microfluidic formulation. J Am Chem Soc. 2012. 134(16):6948-51). In some aspects, methods of lipid nanoparticle generation comprising SHM, further comprise the mixing of at least two input streams wherein mixing occurs by microstructure-induced chaotic advection (MICA). According to this method, fluid streams flow through channels present in a herringbone pattern causing rotational flow and folding the fluids around each other. This method may also comprise a surface for fluid mixing wherein the surface changes orientations during fluid cycling. Methods of generating lipid nanoparticles using SHM include those disclosed in U.S. Patent Publication Nos. 2004/0262223 and 2012/0276209, each of which is expressly incorporated herein by reference in their entirety.

In one aspect, the mRNA of the present disclosure may be formulated in lipid nanoparticles created using a micromixer such as, but not limited to, a Slit Interdigital Microstructured Mixer (SIMM-V2) or a Standard Slit Interdigital Micro Mixer (SSIMM) or Caterpillar (CPMM) or Impinging-jet (IJMM) from the Institut fur Mikrotechnik Mainz GmbH, Mainz Germany).

In one aspect, the mRNA of the present disclosure may be formulated in lipid nanoparticles created using microfluidic technology (see Whitesides, George M. The Origins and the Future of Microfluidics. Nature, 2006 442: 368-373; and Abraham et al. Chaotic Mixer for Microchannels. Science, 2002 295: 647-651, herein incorporated by reference in their entirety). As a non-limiting example, controlled microfluidic formulation includes a passive method for mixing streams of steady pressure-driven flows in micro channels at a low Reynolds number (See e.g., Abraham et al. Chaotic Mixer for Microchannels. Science, 2002 295: 647-651, herein incorporated by reference in its entirety).

In one aspect, the mRNA of the present disclosure may be formulated in lipid nanoparticles created using a micromixer chip such as, but not limited to, those from Harvard Apparatus (Holliston, Mass.) or Dolomite Microfluidics (Royston, UK). A

micromixer chip can be used for rapid mixing of two or more fluid streams with a split and recombine mechanism.

In one aspect, the mRNA of the disclosure may be formulated for delivery using the drug encapsulating microspheres described in International Patent Publication No. WO2013063468 or U.S. Patent No. 8,440,614, each of which is herein incorporated by reference in its entirety. The microspheres may comprise a compound of the formula (I), (II), (III), (IV), (V) or (VI) as described in International Patent Publication No. WO2013063468, the contents of which are herein incorporated by reference in its entirety. In another aspect, the amino acid, peptide, polypeptide, and lipids (APPL) are useful in delivering the mRNA of the disclosure to cells as described in International Patent Publication No. WO2013063468, the contents of which is herein incorporated by reference in its entirety.

In one aspect, the mRNA of the disclosure may be formulated in lipid nanoparticles having a diameter from about 10 to about 100 nm such as, but not limited to, about 10 to about 20 nm, about 10 to about 30 nm, about 10 to about 40 nm, about 10 to about 50 nm, about 10 to about 60 nm, about 10 to about 70 nm, about 10 to about 80 nm, about 10 to about 90 nm, about 20 to about 30 nm, about 20 to about 40 nm, about 20 to about 50 nm, about 20 to about 60 nm, about 20 to about 70 nm, about 20 to about 80 nm, about 20 to about 90 nm, about 20 to about 100 nm, about 30 to about 40 nm, about 30 to about 50 nm, about 30 to about 60 nm, about 30 to about 70 nm, about 30 to about 80 nm, about 30 to about 90 nm, about 30 to about 100 nm, about 40 to about 50 nm, about 40 to about 60 nm, about 40 to about 70 nm, about 40 to about 80 nm, about 40 to about 90 nm, about 40 to about 100 nm, about 50 to about 60 nm, about 50 to about 70 nm about 50 to about 80 nm, about 50 to about 90 nm, about 50 to about 100 nm, about 60 to about 70 nm, about 60 to about 80 nm, about 60 to about 90 nm, about 60 to about 100 nm, about 70 to about 80 nm, about 70 to about 90 nm, about 70 to about 100 nm, about 80 to about 90 nm, about 80 to about 100 nm and/or about 90 to about 100 nm. In one aspect, the lipid nanoparticles may have a diameter from about 10 to 500 nm.

In one aspect, the lipid nanoparticle may have a diameter greater than 100 nm, greater than 150 nm, greater than 200 nm, greater than 250 nm, greater than 300 nm, greater than 350 nm, greater than 400 nm, greater than 450 nm, greater than 500 nm, greater than 550 nm, greater than 600 nm, greater than 650 nm, greater than 700 nm, greater than 750

nm, greater than 800 nm, greater than 850 nm, greater than 900 nm, greater than 950 nm or greater than 1000 nm.

In one aspect, the lipid nanoparticle may be a limit size lipid nanoparticle described in International Patent Publication No. WO2013059922, the contents of which are herein incorporated by reference in its entirety. The limit size lipid nanoparticle may comprise a lipid bilayer surrounding an aqueous core or a hydrophobic core; where the lipid bilayer may comprise a phospholipid such as, but not limited to, diacylphosphatidylcholine, a diacylphosphatidylethanolamine, a ceramide, a sphingomyelin, a dihydrosphingomyelin, a cephalin, a cerebroside, a C8-C20 fatty acid diacylphophatidylcholine, and 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC). In another aspect, the limit size lipid nanoparticle may comprise a polyethylene glycol-lipid such as, but not limited to, DLPE-PEG, DMPE-PEG, DPPC-PEG and DSPE-PEG.

In one aspect, the mRNA may be delivered, localized and/or concentrated in a specific location using the delivery methods described in International Patent Publication No. W02013063530, the contents of which are herein incorporated by reference in its entirety. As a non-limiting example, a subject may be administered an empty polymeric particle prior to, simultaneously with or after delivering the mRNA to the subject. The empty polymeric particle undergoes a change in volume once in contact with the subject and becomes lodged, embedded, immobilized or entrapped at a specific location in the subject.

In one aspect, the mRNA may be formulated in an active substance release system (See e.g., ET.S. Patent Publication No. ETS20130102545, the contents of which is herein incorporated by reference in its entirety). The active substance release system may comprise 1) at least one nanoparticle bonded to an oligonucleotide inhibitor strand which is hybridized with a catalytically active nucleic acid and 2) a compound bonded to at least one substrate molecule bonded to a therapeutically active substance (e.g., polynucleotides described herein), where the therapeutically active substance is released by the cleavage of the substrate molecule by the catalytically active nucleic acid.

In one aspect, the mRNA may be formulated in a nanoparticle comprising an inner core comprising a non-cellular material and an outer surface comprising a cellular membrane. The cellular membrane may be derived from a cell or a membrane derived from a virus. As a non limiting example, the nanoparticle may be made by the methods described

in International Patent Publication No. WO2013052167, herein incorporated by reference in its entirety. As another non limiting example, the nanoparticle described in International Patent Publication No. WO2013052167, herein incorporated by reference in its entirety, may be used to deliver the mRNA described herein.

In one ASPECT, the mRNA may be formulated in porous nanoparticle-supported lipid bilayers (protocells). Protocells are described in International Patent Publication No. WO2013056132, the contents of which are herein incorporated by reference in its entirety.

In one aspect, the mRNA described herein may be formulated in polymeric nanoparticles as described in or made by the methods described in U.S. Patent Nos. 8,420,123 and 8,518,963 and European Patent No. EP2073848B1, the contents of each of which are herein incorporated by reference in their entirety. As a non-limiting example, the polymeric nanoparticle may have a high glass transition temperature such as the nanoparticles described in or nanoparticles made by the methods described in Ei.S. Patent No. 8,518,963, the contents of which are herein incorporated by reference in its entirety. As another non-limiting example, the polymer nanoparticle for oral and parenteral formulations may be made by the methods described in European Patent No. EP2073848B 1, the contents of which are herein incorporated by reference in its entirety.

In another aspect, the mRNA described herein may be formulated in nanoparticles used in imaging. The nanoparticles may be liposome nanoparticles such as those described in EiS Patent Publication No US20130129636, herein incorporated by reference in its entirety. As a non limiting example, the liposome may comprise gadolinium(III) 2-{4,7-bis-carboxymethyl-10-[(N,N-distearylamidomethyl-N-amido-methyl]-1,4,7,10-tetra-azacyclododec-1-yl}-acetic acid and a neutral, fully saturated phospholipid component (see e.g., US Patent Publication No E1S20130129636, the contents of which is herein incorporated by reference in its entirety).

In one aspect, the nanoparticles which may be used in the present disclosure are formed by the methods described in U.S. Patent Publication No. US20130130348, the contents of which is herein incorporated by reference in its entirety.

The nanoparticles of the present disclosure may further include nutrients. As a non-limiting example, the nutrient may be iron in the form of ferrous, ferric salts or elemental iron, iodine, folic acid, vitamins or micronutrients. In one aspect, the mRNA of the present disclosure may be formulated in a swellable nanoparticle. The swellable nanoparticle may

be, but is not limited to, those described in U.S. Patent No. 8,440,231, the contents of which is herein incorporated by reference in its entirety. As a non-limiting aspect, the swellable nanoparticle may be used for delivery of the mRNA of the present disclosure to the pulmonary system (see e.g., U.S. Patent No. 8,440,231, the contents of which is herein incorporated by reference in its entirety).

The mRNA of the present disclosure may be formulated in polyanhydride nanoparticles such as, but not limited to, those described in U.S. Patent No. 8,449,916, the contents of which is herein incorporated by reference in its entirety.

The nanoparticles and microparticles of the present disclosure may be geometrically engineered to modulate macrophage and/or the immune response. In one aspect, the geometrically engineered particles may have varied shapes, sizes and/or surface charges in order to incorporated the polynucleotides of the present disclosure for targeted delivery (see e.g., International Publication No. W02013082111, the contents of which is herein incorporated by reference in its entirety). Other physical features the geometrically engineering particles may have include, but are not limited to, fenestrations, angled arms, asymmetry and surface roughness, charge which can alter the interactions with cells and tissues. As a non-limiting example, nanoparticles of the present disclosure may be made by the methods described in International Publication No W02013082111, the contents of which is herein incorporated by reference in its entirety.

In one aspect, the nanoparticles of the present disclosure may be water soluble nanoparticles such as, but not limited to, those described in International Publication No. W02013090601, the contents of which is herein incorporated by reference in its entirety. The nanoparticles may be inorganic nanoparticles which have a compact and zwitterionic ligand in order to exhibit good water solubility. The nanoparticles may also have small hydrodynamic diameters (HD), stability with respect to time, pH, and salinity and a low level of non-specific protein binding.

In one aspect, the nanoparticles of the present disclosure may be developed by the methods described in U.S. Patent Publication No. US20130172406, the contents of which are herein incorporated by reference in its entirety. In one aspect, the nanoparticles of the present disclosure are stealth nanoparticles or target-specific stealth nanoparticles such as, but not limited to, those described in U.S. Patent Publication No. US20130172406; the contents of which is herein incorporated by reference in its entirety. The nanoparticles of the

present disclosure may be made by the methods described in U. S. Patent Publication No. US20130172406, the contents of which are herein incorporated by reference in its entirety.

In another aspect, the stealth or target-specific stealth nanoparticles may comprise a polymeric matrix. The polymeric matrix may comprise two or more polymers such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polyesters, poly anhydrides, polyethers, polyurethanes, polymethacrylates, polyacrylates, polycyanoacrylates or combinations thereof.

In one aspect, the nanoparticle may be a nanoparticle-nucleic acid hybrid structure having a high-density nucleic acid layer. As a non-limiting example, the nanoparticle-nucleic acid hybrid structure may made by the methods described in U.S. Patent Publication No. US20130171646, the contents of which are herein incorporated by reference in its entirety. The nanoparticle may comprise a nucleic acid such as, but not limited to, polynucleotides described herein and/or known in the art.

At least one of the nanoparticles of the present disclosure may be embedded in the core a nanostructure or coated with a low density porous 3-D structure or coating which is capable of carrying or associating with at least one payload within or on the surface of the nanostructure. Non-limiting examples of the nanostructures comprising at least one nanoparticle are described in International Patent Publication No. WO2013123523, the contents of which are herein incorporated by reference in its entirety.

Polymer and Polymeric Nanoparticle Formulations

The mRNA of the disclosure can be formulated using natural and/or synthetic polymers. Non-limiting examples of polymers which may be used for delivery include, but are not limited to, DYNAMIC POLYCONJUGATE® (Arrowhead Research Corp., Pasadena, Calif.) formulations from MIRUS® Bio (Madison, Wis.) and Roche Madison (Madison, Wis.), PHASERX™ polymer formulations such as, without limitation, SMARTT POLYMER TECHNOLOGY™ (PHASERX®, Seattle, Wash), DMRI/DOPE, poloxamer, VAXFECTIN® adjuvant from Vical (San Diego, Calif.), chitosan, cyclodextrin from Calando Pharmaceuticals (Pasadena, Calif.), dendrimers and poly(lactic-co-glycolic acid) (PLGA) polymers. RONDEL™ (RNAi/Oligonucleotide Nanoparticle Delivery) polymers

(Arrowhead Research Corporation, Pasadena, Calif.) and pH responsive co-block polymers such as, but not limited to, PHASERX® (Seattle, Wash.).

A non-limiting example of a chitosan formulation includes a core of positively charged chitosan and an outer portion of negatively charged substrate (ET.S. Publication No. 20120258176; herein incorporated by reference in its entirety). Chitosan includes, but is not limited to N-trimethyl chitosan, mono-N-carboxymethyl chitosan (MCC), N-palmitoyl chitosan (NPCS), EDTA- chitosan, low molecular weight chitosan, chitosan derivatives, or combinations thereof.

In one aspect, the polymers used in the present disclosure have undergone processing to reduce and/or inhibit the attachment of unwanted substances such as, but not limited to, bacteria, to the surface of the polymer. The polymer may be processed by methods known and/or described in the art and/or described in International Publication No. WO2012150467, herein incorporated by reference in its entirety.

A non-limiting example of PLGA formulations include, but are not limited to, PLGA injectable depots (e.g., ELIGARD® which is formed by dissolving PLGA in 66% N-methyl-2- pyrrolidone (NMP) and the remainder being aqueous solvent and leuprolide. Once injected, the PLGA and leuprolide peptide precipitates into the subcutaneous space).

Many of these polymer approaches have demonstrated efficacy in delivering oligonucleotides in vivo into the cell cytoplasm (reviewed in deFougerolles Hum Gene Ther. 2008 19: 125-132). Two polymer approaches that have yielded robust in vivo delivery of nucleic acids, in this case with small interfering RNA (siRNA), are dynamic polyconjugates and cyclodextrin- based nanoparticles (see e.g., U.S. Patent Publication No. US20130156721, herein incorporated by reference in its entirety). The first of these delivery approaches uses dynamic poly conjugates and has been shown in vivo in mice to effectively deliver siRNA and silence endogenous target mRNA in hepatocytes (Rozema et ak, Proc Natl Acad Sci USA. 2007 104: 12982-12887, herein incorporated by reference in its entirety). This particular approach is a multicomponent polymer system whose key features include a membrane-active polymer to which nucleic acid, in this case siRNA, is covalently coupled via a disulfide bond and where both PEG (for charge masking) and N-acetylgalactosamine (for hepatocyte targeting) groups are linked via pH-sensitive bonds (Rozema et ah, Proc Natl Acad Sci USA. 2007 104: 12982-12887, herein incorporated by reference in its entirety). On binding to the hepatocyte and entry into the endosome, the

polymer complex disassembles in the low-pH environment, with the polymer exposing its positive charge, leading to endosomal escape and cytoplasmic release of the siRNA from the polymer. Through replacement of the N-acetylgalactosamine group with a mannose group, it was shown one could alter targeting from asialoglycoprotein receptor-expressing hepatocytes to sinusoidal endothelium and Kupffer cells. Another polymer approach involves using transferrin-targeted cyclodextrin- containing polycation nanoparticles. These nanoparticles have demonstrated targeted silencing of the EWS-FLI1 gene product in transferrin receptor-expressing Ewing's sarcoma tumor cells (Hu- Lieskovan et ah, Cancer Res. 2005 65: 8984-8982, herein incorporated by reference in its entirety) and siRNA formulated in these nanoparticles was well tolerated in non-human primates (Heidel et ah, Proc Natl Acad Sci USA 2007 104:5715-21, herein incorporated by reference in its entirety). Both of these delivery strategies incorporate rational approaches using both targeted delivery and endosomal escape mechanisms.

The polymer formulation can permit the sustained or delayed release of polynucleotides. The altered release profile for the polynucleotide can result in, for example, translation of an encoded protein over an extended period of time. The polymer formulation may also be used to increase the stability of the polynucleotide. Biodegradable polymers have been previously used to protect nucleic acids other than polynucleotide from degradation and been shown to result in sustained release of payloads in vivo (Rozema et ah, Proc Natl Acad Sci USA. 2007 104: 12982- 12887; Sullivan et ah, Expert Opin Drug Deliv. 2010 7: 1433-1446; Convertine et ah, Biomacromolecules. 2010 Oct. 1; Chu et al., Acc Chem Res. 2012 Jan. 13; Manganiello et ah, Biomaterials. 2012 33 :2301-2309; Benoit et al., Biomacromolecules. 2011 12:2708-2714; Singha et al., Nucleic Acid Ther. 2011 2: 133-147; deFougerolles Hum Gene Ther. 2008 19: 125-132; Schaffert and Wagner, Gene Ther. 2008 16: 1131-1138; Chaturvedi et al., Expert Opin Drug Deliv. 2011 8: 1455-1468; Davis, Mol Pharm. 2009 6:659-668; Davis, Nature 2010 464: 1067-1070, herein incorporated by reference in their entirety). In one aspect, the mRNA therapeutic compositions may be sustained release formulations. Sustained release formulations may include, but are not limited to, PLGA microspheres, ethylene vinyl acetate (EVAc), poloxamer, GELSITE® (Nanotherapeutics, Inc. Alachua, Fla.), HYLENEX® (Halozyme Therapeutics, San Diego Calif.), surgical sealants such as fibrinogen polymers (Ethicon Inc. Cornelia, Ga.), TISSELL® (Baxter International, Inc Deerfield, Ill.), PEG-based sealants, and COSEAL® (Baxter International, Inc Deerfield, Ill.).

As a non-limiting example, the mRNA may be formulated in PLGA microspheres by preparing the PLGA microspheres with tunable release rates (e.g., days and weeks) and encapsulating the modified mRNA in the PLGA microspheres while maintaining the integrity of the modified mRNA during the encapsulation process. EVAc are non-biodegradable, biocompatible polymers which are used extensively in pre-clinical sustained release implant applications (e.g., extended release products Ocusert a pilocarpine ophthalmic insert for glaucoma or progestasert a sustained release progesterone intrauterine device; transdermal delivery systems Testoderm, Duragesic and Selegiline; catheters). Poloxamer F-407 NF is a hydrophilic, non-ionic surfactant triblock copolymer of polyoxyethylene-polyoxypropylene-polyoxyethylene having a low viscosity at temperatures less than 5° C and forms a solid gel at temperatures greater than 15° C. PEG-based surgical sealants comprise two synthetic PEG components mixed in a delivery device which can be prepared in one minute, seals in 3 minutes and is reabsorbed within 30 days. GELSITE® and natural polymers are capable of in-situ gelation at the site of administration. They have been shown to interact with protein and peptide therapeutic candidates through ionic interaction to provide a stabilizing effect.

Polymer formulations can also be selectively targeted through expression of different ligands as exemplified by, but not limited by, folate, transferrin, and N-acetylgalactosamine (GalNAc) (Benoit et ak, Biomacromolecules. 201 1 12:2708-2714; Rozema et ah, Proc Natl Acad Sci USA. 2007 104: 12982-12887; Davis, Mol Pharm. 2009 6:659-668; Davis, Nature 2010 464: 1067-1070, herein incorporated by reference in their entirety).

The mRNA of the disclosure may be formulated with or in a polymeric compound. The polymer may include at least one polymer such as, but not limited to, polyethenes, polyethylene glycol (PEG), poly(l-lysine)(PLL), PEG grafted to PLL, cationic lipopolymer, biodegradable cationic lipopolymer, polyethyleneimine (PEI), cross-linked branched poly(alkylene imines), a polyamine derivative, a modified poloxamer, a biodegradable polymer, elastic biodegradable polymer, biodegradable block copolymer, biodegradable random copolymer, biodegradable polyester copolymer, biodegradable polyester block copolymer, biodegradable polyester block random copolymer, multiblock copolymers, linear biodegradable copolymer, poly[a-(4- aminobutyl)-L-glycolic acid) (PAGA), biodegradable cross-linked cationic multi-block copolymers, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides,

polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polylysine, poly(ethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), acrylic polymers, amine-containing polymers, dextran polymers, dextran polymer derivatives or combinations thereof.

As a non-limiting example, the mRNA of the disclosure may be formulated with the polymeric compound of PEG grafted with PLL as described in U.S. Patent No. 6, 177,274; herein incorporated by reference in its entirety. The formulation may be used for transfecting cells in vitro or for in vivo delivery of polynucleotides. In another example, the polynucleotide may be suspended in a solution or medium with a cationic polymer, in a dry therapeutic composition or in a solution that is capable of being dried as described in U.S. Publication Nos. 20090042829 and 20090042825; each of which are herein incorporated by reference in their entireties.

As another non-limiting example, the mRNA of the disclosure may be formulated with a PLGA-PEG block copolymer (see U.S. Publication No. US20120004293 and U.S. Patent No. 8,236,330, herein incorporated by reference in their entireties) or PLGA-PEG-PLGA block copolymers (See U.S. Patent No. 6,004,573, herein incorporated by reference in its entirety). As a non-limiting example, the mRNA of the disclosure may be formulated with a diblock copolymer of PEG and PLA or PEG and PLGA (see U.S. Patent No. 8,246,968, herein incorporated by reference in its entirety).

A polyamine derivative may be used to deliver nucleic acids or to treat and/or prevent a disease or to be included in an implantable or injectable device (U.S. Publication No. 20100260817 (now U.S. Patent No. 8,460,696) the contents of each of which is herein incorporated by reference in its entirety). As a non-limiting example, a therapeutic composition may include the mRNA and the polyamine derivative described in U.S. Publication No. 20100260817 (now U.S. Patent No. 8,460,696; the contents of which are incorporated herein by reference in its entirety. As a non-limiting example, the mRNA of the present disclosure may be delivered using a polyamide polymer such as, but not limited to, a polymer comprising a 1,3 -dipolar addition polymer prepared by combining a carbohydrate diazide monomer with a dilkyne unite comprising oligoamines (U.S. Patent No. 8,236,280; herein incorporated by reference in its entirety).

The mRNA of the disclosure may be formulated with at least one acrylic polymer. Acrylic polymers include but are not limited to, acrylic acid, methacrylic acid, acrylic acid and methacrylic acid copolymers, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, amino alkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), polycyanoacrylates and combinations thereof.

In one aspect, the mRNA of the present disclosure may be formulated with at least one polymer and/or derivatives thereof described in International Publication Nos. WO2011115862, WO2012082574 and WO2012068187 and U.S. Publication No. 20120283427, each of which are herein incorporated by reference in their entireties.

In another aspect, the mRNA of the present disclosure may be formulated with a polymer of formula Z as described in International Patent Publication No. WO2011115862, herein incorporated by reference in its entirety. In yet another aspect, the mRNA may be formulated with a polymer of formula Z, Z^7 or Z' as described in International Publication Nos. WO2012082574 or WO2012068187 and U.S. Publication No. 2012028342, each of which are herein incorporated by reference in their entireties. The polymers formulated with the modified RNA of the present disclosure may be synthesized by the methods described in International Publication Nos. WO2012082574 or WO2012068187, each of which are herein incorporated by reference in their entireties.

The mRNA of the disclosure may be formulated with at least one acrylic polymer. Acrylic polymers include but are not limited to, acrylic acid, methacrylic acid, acrylic acid and methacrylic acid copolymers, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, amino alkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), polycyanoacrylates and combinations thereof.

Formulations of the mRNA of the disclosure may include at least one amine-containing polymer such as, but not limited to polylysine, polyethylene imine, poly(amidoamine) dendrimers, poly(amine-co-esters) or combinations thereof. As a non-limiting example, the poly(amine-co- esters) may be the polymers described in and/or made by the methods described in International Publication No. WO2013082529, the contents of which are herein incorporated by reference in its entirety.

For example, the mRNA of the disclosure may be formulated in a pharmaceutical compound including a poly(alkylene imine), a biodegradable cationic lipopolymer, a biodegradable block copolymer, a biodegradable polymer, or a biodegradable random

copolymer, a biodegradable polyester block copolymer, a biodegradable polyester polymer, a biodegradable polyester random copolymer, a linear biodegradable copolymer, PAGA, a biodegradable cross- linked cationic multi-block copolymer or combinations thereof. The biodegradable cationic lipopolymer may be made by methods known in the art and/or described in U.S. Patent No. 6,696,038, U.S. Publication Nos. 20030073619 and 20040142474 each of which is herein incorporated by reference in their entireties. The poly(alkylene imine) may be made using methods known in the art and/or as described in U.S. Publication No. 20100004315, herein incorporated by reference in its entirety. The biodegradable polymer, biodegradable block copolymer, the biodegradable random copolymer, biodegradable polyester block copolymer, biodegradable polyester polymer, or biodegradable polyester random copolymer may be made using methods known in the art and/or as described in U.S. Patent Nos. 6,517,869 and 6,267,987, the contents of which are each incorporated herein by reference in their entirety. The linear biodegradable copolymer may be made using methods known in the art and/or as described in U.S. Patent No. 6,652,886. The PAGA polymer may be made using methods known in the art and/or as described in U.S. Patent No. 6,217,912 herein incorporated by reference in its entirety. The PAGA polymer may be copolymerized to form a copolymer or block copolymer with polymers such as but not limited to, poly-L4ysine, polyargine, polyomithine, histones, avidin, protamines, polylactides and poly(lactide-co-glycolides). The biodegradable cross-linked cationic multi-block copolymers may be made my methods known in the art and/or as described in U.S. Patent Nos. 8,057,821, 8,444,992 or U.S. Publication No. 2012009145 each of which are herein incorporated by reference in their entireties. For example, the multi-block copolymers may be synthesized using linear polyethyleneimine (LPEI) blocks which have distinct patterns as compared to branched polyethyleneimines. Further, the composition or therapeutic composition may be made by the methods known in the art, described herein, or as described in U.S. Publication No. 20100004315 or U.S. Patent Nos. 6,267,987 and 6,217,912 each of which are herein incorporated by reference in their entireties. The mRNA of the disclosure may be formulated with at least one degradable polyester which may contain polycationic side chains. Degradable polyesters include, but are not limited to, poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), and combinations thereof. In another aspect, the degradable polyesters may include a PEG conjugation to form a PEGylated polymer.

The mRNA of the disclosure may be formulated with at least one crosslinkable polyester. Crosslinkable polyesters include those known in the art and described in ET.S. Publication No. 20120269761, the contents of which is herein incorporated by reference in its entirety.

The mRNA of the disclosure may be formulated in or with at least one cyclodextrin polymer. Cyclodextrin polymers and methods of making cyclodextrin polymers include those known in the art and described in ET.S. Publication No. 20130184453, the contents of which are herein incorporated by reference in its entirety.

In one aspect, the mRNA of the disclosure may be formulated in or with at least one crosslinked cation-binding polymers. Crosslinked cation-binding polymers and methods of making crosslinked cation-binding polymers include those known in the art and described in International Patent Publication Nos. WO2013106072, WO2013106073 and WO2013106086, the contents of each of which are herein incorporated by reference in its entirety.

In one aspect, the mRNA of the disclosure may be formulated in or with at least one branched polymer. Branched polymers and methods of making branched polymers include those known in the art and described in International Patent Publication No. WO2013113071, the contents of each of which are herein incorporated by reference in its entirety.

In one aspect, the mRNA of the disclosure may be formulated in or with at least PEGylated albumin polymer. PEGylated albumin polymer and methods of making PEGylated albumin polymer include those known in the art and described in U.S. Patent Publication No. US20130231287, the contents of each of which are herein incorporated by reference in its entirety.

In one aspect, the polymers described herein may be conjugated to a lipid terminating PEG. As a non-limiting example, PLGA may be conjugated to a lipid-terminating PEG forming PLGA-DSPE-PEG. As another non-limiting example, PEG conjugates for use with the present disclosure are described in International Publication No. W02008103276, herein incorporated by reference in its entirety. The polymers may be conjugated using a ligand conjugate such as, but not limited to, the conjugates described in U.S. Pat. No. 8,273,363, herein incorporated by reference in its entirety.

In one aspect, the mRNA disclosed herein may be mixed with the PEGs or the sodium phosphate/sodium carbonate solution prior to administration. In another aspect, polynucleotides encoding the protein of interest may be mixed with the PEGs and also mixed with the sodium phosphate/sodium carbonate solution.

In one aspect, the mRNA described herein may be conjugated with another compound. Non-limiting examples of conjugates are described in ET.S. Patent Nos. 7,964,578 and 7,833,992, each of which are herein incorporated by reference in their entireties. In another aspect, the mRNA of the present disclosure may be conjugated with conjugates of formula 1-122 as described in ET.S. Patent Nos. 7,964,578 and 7,833,992, each of which are herein incorporated by reference in their entireties. The mRNA described herein may be conjugated with a metal such as, but not limited to, gold. (See e.g., Giljohann et al. Joum. Amer. Chem. Soc. 2009 131(6): 2072-2073). In another aspect, the mRNA described herein may be conjugated and/or encapsulated in gold-nanoparticles. (International Publication No. WO201216269 and ET.S. Publication No. 20120302940 and ETS20130177523; the contents of each of which is herein incorporated by reference in its entirety).

In one aspect, the polymer formulation of the present disclosure may be stabilized by contacting the polymer formulation, which may include a cationic carrier, with a cationic lipopolymer which may be covalently linked to cholesterol and polyethylene glycol groups. The polymer formulation may be contacted with a cationic lipopolymer using the methods described in ET.S. Publication No. 20090042829 herein incorporated by reference in its entirety. The cationic carrier may include, but is not limited to, polyethylenimine, poly(trimethylenimine), poly(tetramethylenimine), polypropylenimine, aminoglycoside-polyamine, dideoxy-diamino-b- cyclodextrin, spermine, spermidine, poly(2-dimethylamino)ethyl methacrylate, poly(lysine), poly(histidine), poly(arginine), cationized gelatin, dendrimers, chitosan, 1,2-Dioleoyl-3- Trimethylammonium-Propane(DOTAP), N-[l-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), 1-[2-(oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTIM), 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-l-propanaminium trifluoroacetate (DOSPA), 3B-[N-(N',N'-Dimethylaminoethane)-carbamoyl]Cholesterol Hydrochloride (DC- Cholesterol HC1), diheptadecylamidoglycyl spermidine (DOGS), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE), N,N-dioleyl-N,N-

dimethylammonium chloride DODAC) and combinations thereof. As a non-limiting example, the mRNA may be formulated with a cationic lipopolymer such as those described in U.S. Patent Publication No. 20130065942, the contents of which are herein incorporated by reference in its entirety.

The mRNA of the disclosure may be formulated in a polyplex of one or more polymers (See e.g., U.S. Patent No. 8,501,478, U.S. Publication Nos. 20120237565, 20120270927 and 20130149783 and International Patent Publication No. W02013090861; the contents of each of which is herein incorporated by reference in its entirety). As a non-limiting example, the polyplex may be formed using the noval alpha-aminoamidine polymers described in International Publication No. WO2013090861, the contents of which are herein incorporated by reference in its entirety. As another non-limiting example, the polyplex may be formed using the click polymers described in U.S. Patent No. 8,501,478, the contents of which is herein incorporated by reference in its entirety. In one aspect, the polyplex comprises two or more cationic polymers. The cationic polymer may comprise a polyethylene imine) (PEI) such as linear PEI. In another aspect, the polyplex comprises p(TETA/CBA) its PEGylated analog p(TETA/CBA)-g-PEG2k and mixtures thereof (see e.g., US Patent Publication No. US20130149783, the contents of which are herein incorporated by reference in its entirety.

The mRNA of the disclosure can also be formulated as a nanoparticle using a combination of polymers, lipids, and/or other biodegradable agents, such as, but not limited to, calcium phosphate. Components may be combined in a core-shell, hybrid, and/or layer-by-layer architecture, to allow for fine-tuning of the nanoparticle so to delivery of the polynucleotide, may be enhanced (Wang et ah, Nat Mater. 2006 5:791-796; Fuller et ah, Biomaterials. 2008 29: 1526- 1532; DeKoker et ah, Adv Drug Deliv Rev. 2011 63 :748-761; Endres et ah, Biomaterials. 2011 32:7721-7731; Su et al., Mol Pharm. 2011 Jun. 6; 8(3):774-87, herein incorporated by reference in its entirety). As a non-limiting example, the nanoparticle may comprise a plurality of polymers such as, but not limited to hydrophilic-hydrophobic polymers (e.g., PEG-PLGA), hydrophobic polymers (e.g., PEG) and/or hydrophilic polymers (International Publication No. WO20120225129; the contents of which is herein incorporated by reference in its entirety). As another non-limiting example, the nanoparticle comprising hydrophilic polymers for the mRNA may be those described in or made by the methods described in International Patent Publication No. WO2013119936, the contents of which are herein incorporated by reference in its entirety.

In one aspect, the biodegradable polymers which may be used in the present disclosure are poly(ether-anhydride) block copolymers. As a non-limiting example, the biodegradable polymers used herein may be a block copolymer as described in International Patent Publication No. W02006063249, herein incorporated by reference in its entirety, or made by the methods described in International Patent Publication No. W02006063249, herein incorporated by reference in its entirety.

In another aspect, the biodegradable polymers which may be used in the present disclosure are alkyl and cycloalkyl terminated biodegradable lipids. As a non-limiting example, the alkyl and cycloalkyl terminated biodegradable lipids may be those described in International Publication No. WO2013086322 and/or made by the methods described in International Publication No. WO2013086322; the contents of which are herein incorporated by reference in its entirety.

In yet another aspect, the biodegradable polymers which may be used in the present disclosure are cationic lipids having one or more biodegradable group located in a lipid moiety. As a non-limiting example, the biodegradable lipids may be those described in U.S. Patent Publication No. US20130195920, the contents of which are herein incorporated by reference in its entirety.

Biodegradable calcium phosphate nanoparticles in combination with lipids and/or polymers have been shown to deliver polynucleotides in vivo. In one aspect, a lipid coated calcium phosphate nanoparticle, which may also contain a targeting ligand such as anisamide, may be used to deliver the mRNA of the present disclosure. For example, to effectively deliver siRNA in a mouse metastatic lung model a lipid coated calcium phosphate nanoparticle was used (Li et ah, J Contr Rel. 2010 142: 416-421; Li et al., J Contr Rel. 2012 158: 108-114; Yang et ah, Mol Ther. 2012 20:609-615, herein incorporated by reference in their entirety). This delivery system combines both a targeted nanoparticle and a component to enhance the endosomal escape, calcium phosphate, in order to improve delivery of the siRNA. In one aspect, calcium phosphate with a PEG-polyanion block copolymer may be used to deliver the mRNA (Kazikawa et al., J Contr Rel. 2004 97:345-356; Kazikawa et ah, J Contr Rel. 2006 111 :368-370, herein incorporated by reference in their entirety).

In one aspect, a PEG-charge-conversional polymer (Pitella et al., Biomaterials. 2011 32:3106-3114, herein incorporated by reference in their entirety) may be used to form a

nanoparticle to deliver the mRNA of the present disclosure. The PEG-charge-conversional polymer may improve upon the PEG-polyanion block copolymers by being cleaved into a polycation at acidic pH, thus enhancing endosomal escape.

In one aspect, a polymer used in the present disclosure may be a pentablock polymer such as, but not limited to, the pentablock polymers described in International Patent Publication No. WO2013055331, herein incorporated by reference in its entirety. As a non-limiting example, the pentablock polymer comprises PGA-PCL-PEG-PCL-PGA, wherein PEG is polyethylene glycol, PCL is poly(E-caprolactone), PGA is poly(glycolic acid), and PLA is poly(lactic acid). As another non-limiting example, the pentablock polymer comprises PEG-PCL-PLA-PCL-PEG, wherein PEG is polyethylene glycol, PCL is poly(E-caprolactone), PGA is poly(glycolic acid), and PLA is poly(lactic acid).

In one aspect, a polymer which may be used in the present disclosure comprises at least one diepoxide and at least one aminoglycoside (See e.g., International Patent Publication No. WO2013055971, the contents of which are herein incorporated by reference in its entirety). The diepoxide may be selected from, but is not limited to, 1,4 butanediol diglycidyl ether (1,4B),1,4-cyclohexanedimethanol diglycidyl ether (1,4C),4-vinylcyclohexene diepoxide (4VCD), ethyleneglycol diglycidyl ether (EDGE), glycerol diglycidyl ether (GDE), neopentylglycol diglycidyl ether (NPDGE), poly(ethyleneglycol) diglycidyl ether (PEGDE), poly(propyleneglycol) diglycidyl ether (PPGDE) and resorcinol diglycidyl ether (RDE). The aminoglycoside may be selected from, but is not limited to, streptomycin, neomycin, framycetin, paromomycin, ribostamycin, kanamycin, amikacin, arbekacin, bekanamycin, dibekacin, tobramycin, spectinomycin, hygromycin, gentamicin, netilmicin, sisomicin, isepamicin, verdamicin, astromicin, and apramycin. As a non-limiting example, the polymers may be made by the methods described in International Patent Publication No. WO2013055971, the contents of which are herein incorporated by reference in its entirety. As another non-limiting example, compositions comprising any of the polymers comprising at least one least one diepoxide and at least one aminoglycoside may be made by the methods described in International Patent Publication No. WO2013055971, the contents of which are herein incorporated by reference in its entirety.

In one aspect, a polymer which may be used in the present disclosure may be a cross- linked polymer. As a non-limiting example, the cross-linked polymers may be used to form a particle as described in U.S. Patent No. 8,414,927, the contents of which are herein incorporated by reference in its entirety. As another non-limiting example, the cross-linked

polymer may be obtained by the methods described in U.S. Patent Publication No. US20130172600, the contents of which are herein incorporated by reference in its entirety.

In another aspect, a polymer which may be used in the present disclosure may be a cross-linked polymer such as those described in U.S. Patent No. 8,461,132, the contents of which are herein incorporated by reference in its entirety. As a non-limiting example, the cross-linked polymer may be used in a therapeutic composition for the treatment of a body tissue. The therapeutic composition may be administered to damaged tissue using various methods known in the art and/or described herein such as injection or catheterization.

In one aspect, a polymer which may be used in the present disclosure may be a di-aliphatic substituted pegylated lipid such as, but not limited to, those described in International Patent Publication No. WO2013049328, the contents of which are herein incorporated by reference in its entirety.

In one aspect, a block copolymer is PEG-PLGA-PEG (see e.g., the thermosensitive hydrogel (PEG-PLGA-PEG) was used as a gene delivery vehicle in Lee et al. Pharmaceutical Research, 2003 20(12): 1995-2000; as a controlled gene delivery system in Li et al. Controlled Gene Delivery System Based on Thermo sensitive Biodegradable Hydrogel. Pharmaceutical Research 2003 20(6):884-888; and Chang et al., Non-ionic amphiphilic biodegradable PEG-PLGA-PEG copolymer enhances gene delivery efficiency in rat skeletal muscle. J Controlled Release. 2007 118:245-253), herein incorporated by reference in their entirety, may be used in the present disclosure.

In another aspect, the PEG-PLGA-PEG block copolymer is used in the present disclosure to develop a biodegradable sustained release system. In one aspect, the mRNA of the present disclosure is mixed with the block copolymer prior to administration. In another aspect, the mRNA of the present disclosure is co-administered with the block copolymer.

In one aspect, the polymer used in the present disclosure may be a multi-functional polymer derivative such as, but not limited to, a multi-functional N-maleimidyl polymer derivatives as described in U.S. Patent No. 8,454,946, the contents of which are herein incorporated by reference in its entirety.

The use of core-shell nanoparticles has additionally focused on a high-throughput approach to synthesize cationic cross-linked nanogel cores and various shells (Siegwart et ah, Proc Natl Acad Sci USA. 2011 108: 12996-13001, herein incorporated by reference in its entirety). The complexation, delivery, and internalization of the polymeric nanoparticles

can be precisely controlled by altering the chemical composition in both the core and shell components of the nanoparticle.

In one aspect, a hollow lipid core comprising a middle PLGA layer and an outer neutral lipid layer containing PEG may be used to delivery of the mRNA of the present disclosure. As a non-limiting example, in mice bearing a luciferease-expressing tumor, it was determined that the lipid-polymer-lipid hybrid nanoparticle significantly suppressed luciferase expression, as compared to a conventional lipoplex (Shi et al, Angew Chem Int Ed. 2011 50:7027-7031; herein incorporated by reference in its entirety).

In one aspect, the lipid nanoparticles may comprise a core of the mRNA disclosed herein and a polymer shell. The polymer shell may be any of the polymers described herein and are known in the art. In an additional aspect, the polymer shell may be used to protect the polynucleotides in the core.

Core-shell nanoparticles for use with the mRNA of the present disclosure are described and may be formed by the methods described in U.S. Patent No. 8,313,777 or International Patent Publication No. WO2013124867, the contents of each of which are herein incorporated by reference in their entirety.

In one aspect, the polymer used with the formulations described herein may be a modified polymer (such as, but not limited to, a modified polyacetal) as described in International Publication No. WO2011 120053, the contents of which are herein incorporated by reference in its entirety. In one aspect, the formulation may be a polymeric carrier cargo complex comprising a polymeric carrier and at least one nucleic acid molecule. Non-limiting examples of polymeric carrier cargo complexes are described in International Patent Publications Nos. WO2013113326, WO2013113501, WO2013113325, WO2013113502 and WO2013113736 and European Patent Publication No. EP2623121, the contents of each of which are herein incorporated by reference in their entireties. In one aspect, the polymeric carrier cargo complexes may comprise a negatively charged nucleic acid molecule such as, but not limited to, those described in International Patent Publication Nos. WO2013113325 and WO2013113502, the contents of each of which are herein incorporated by reference in its entirety.

In one aspect, a therapeutic composition may comprise the mRNA of the disclosure and a polymeric carrier cargo complex.

In one aspect, the polymer used with the formulations described herein may be a modified polymer (such as, but not limited to, a modified polyacetal) as described in International Publication No. WO2011 120053, the contents of which are herein incorporated by reference in its entirety.

Methods of Use

The synthetic mRNA or therapeutic compositions described may find use in inducing maturation of oocytes. Thus, in one aspect, a method is provided for inducing maturation in an oocyte. In some aspects, the method comprises contacting the oocyte with a synthetic mRNA or therapeutic composition described herein.

As used herein, an "oocyte" refers to a female germ cell arrested in prophase of meiosis I of oogenesis. "Maturation," as used herein in reference to an oocyte, refers to the oocyte attaining the competence to be fertilized and undergo embryogenesis. Maturation allows the formation of an oocyte into an "ovum," or egg. An "ovum," as used herein, is a cell that can be fertilized to produce a normal embryo, and, in typical aspects, is arrested in metaphase of meiosis II. An oocyte and ovum are also distinct in several ways. An oocyte is found in the ovary in vivo, has an intact nucleus, an interphase microtubule network, and minimal pervitelline space. In contrast, an ovum is found in the oviduct in vivo, lacks a nucleus, has a visible first polar body and an enlarged perivitelline space in its vicinity, and have a meiotic spindle.

In some aspects, the oocyte as used in the methods herein may be of any mammalian origin. In some aspects, the oocyte is selected from a human oocyte, a bovine oocyte, a porcine oocyte, an equine oocyte, a canine oocyte, a feline oocyte, a murine oocyte (such as a mouse or rate oocyte), an ovine oocyte, and a non-human primate oocyte.

As used herein, reference to an oocyte encompasses an oocyte devoid of companion cells or inclusive of companion cells. For example, the oocyte may be a denuded oocyte where the somatic cell layers (e.g., cumulus cells) that surround the oocyte have been removed. The oocyte may also be part of a follicle, or may be part of a cumulus oocyte complex (COC) in which the cumulus vestments remain intact.

The synthetic mRNA and therapeutic compositions thereof may find use in in vitro maturation (IVM) methods. The particulars of IVM methods are known in the art. First, the oocyte needs to be obtained from a subject. An oocyte can be harvested or collected from an ovary according to standard techniques long known in the art. For example, see Textbook of Assisted Reproduction: Laboratory and Clinical Perspectives (2003, supra). Most oocyte

collection techniques involve the insertion of an aspirating needle into an ovarian follicle using transvaginal ultrasound. The aspirating needle is connected by tubing to a material collection trap and the collection trap, in turn, is connected to a suction source such as a manually operated syringe or an electromechanical vacuum source. Oocytes are typically isolated from multiple follicles. As such, harvested oocytes represent a heterogeneous population with regard to their maturity and therefore developmental potential.

In some aspects, the oocytes may be primed (such as with follicle-stimulating hormone (FSC) or human chorionic gonadotrophin (hCG)) prior to retrieval. However, in other aspects, priming may not be necessary. In some aspects, the oocytes are classified depending on their condition, with the best oocytes chosen to be maturated.

In some aspects, the oocyte is contacts with the synthetic mRNA or therapeutic composition described herein while present in a culture medium. The base medium to which the synthetic mRNA or therapeutic composition thereof is added may be any medium which supports and maintains the viability of an oocyte cultured in the medium in vitro. A suitable base medium for example may include, but is not limited to, Tissue Culture Medium 199 (also known as Media 199, TCM199, and M199) (ThermoFisher Scientific), Minimum Essential Medium Eagle (also known as Eagles' Minimum Essential Medium, EMEM and MEM)(Sigma-Aldrich), Minimum Essential Medium Eagle Alpha Modifications (also known as α-MEM)(Sigma-Aldrich), Dulbecco's Modified Eagle Medium (also known as DMEM or D-MEM)(ThermoFisher Scientific), Ham's F12 (also known as F-12 Ham, Ham's F12 Medium, and F12 Nutrient Mixture)(ThermoFisher Scientific), RPMI Medium 1640 (ThermoFisher Scientific), Isocove's Modified Dulbecco's Medium (also known as IMDM)(ThermoFisher Scientific), Waymouth's MB 752/1 Medium (also known as Waymouth or Waymouth Medium)(Sigma-Aldrich), Chang's Medium (Irvine Scientific), HTF Medium (Irvine Scientific), Dulbecco's Modified Eagle's Medium/Nutrient F-12 Ham (also known as DMEM/F-12 and DME F12)(ThermoFisher Scientific), Vitromat (IVF Vet Solutions, Robinson Research Institute, University of Adelaide, SA, Australia) and ART-1600-B (Origio, Denmark). For the maturation of human oocytes, for example, companies such as Origio (Denmark) provide appropriate base media, such as ART-1600-B medium. Despite the inclusion of the list above, other base media are contemplated, as would be understood by a person skilled in the art, provided that the base media supports and maintains the viability of an oocyte cultured in the medium in vitro.

The base medium to which the synthetic mRNA or therapeutic composition thereof is added may also be supplemented with additional components. For example, see Culture

Media, Solutions, and Systems in Human ART (2014), Editor Patrick Quinn, Cambridge University Press, ISBN 978-1-107-61953-1. Examples of additional components include, but are not limited to, inorganic ions (such as cations and anions, e.g., Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, PO_4^{2-}, and HCO_3^-), energy substrates (such as glucose, lactate, pyruvate, amino acids), nitrogen sources (such as essential and non-essential amino acids), vitamins, fatty acids or precursors, nucleic acid precursors, chelators (such as EDTA), antioxidants, proteins or macromolecules (such as HSA and hyaluronate), other growth factors or hormones (such as insulin-transferrin-selenium, insulin-like growth factor, epidermal growth factor, follicle stimulating hormone and human chorionic gonadotropin), buffers to maintain a physiological pH, antibiotics, pH indicators, and combinations of any one or more of the above. It is to be made clear that certain base media may already contain one or more of the additional components listed above. Preferred amounts and ranges of these additional components can be found in standard text books known in the art. For example, see Culture Media, Solutions, and Systems in Human ART (2014, supra), and Textbook of Assisted Reproduction: Laboratory and Clinical Perspectives (2003) Editors Gardner, D. K., Weissman, A., Howles, C. M., Shoham, Z. Martin.

Once the oocyte has sufficiently maturate, it can be fertilized in vitro. Thus, in another aspect, a method of fertilization is also provided. In some aspects, the method comprises maturing the oocyte into an ovum using the synthetic mRNA or therapeutic composition thereof as described herein. In further aspects, the method comprises fertilizing the ovum with a sperm to form a zygote.

Methods of in vitro fertilization are known in the art. In some aspects, fertilizing the ovum with the sperm comprises contacting the ovum with the sperm in a culture medium. In other aspects, fertilizing the ovum comprises intracytoplasmic injection of the sperm into the ovum.

In some aspects, the oocyte is collected from an ovary of a first subject. The first subject from which the oocyte is obtained may be an otherwise healthy subject but for their reduced capacity or inability to conceive and/or carry a pregnancy to term. Alternatively, the first subject may be aged and/or obese, thereby also having a reduced capacity or inability to conceive and/or carry a pregnancy to term due to the effect of age and/or obesity on oocyte developmental competence. An aged subject would be considered a subject who is older than the peak fertility age range of the particular species. For example, an otherwise healthy human subject would be expected to have a peak fertility age range between 23 and 35, and thus an aged human subject would be considered a subject older than 35. However,

the peak fertility age range in humans may vary depending on a number of factors, such as genetic background.

The ovum produced by the aforementioned methods in vitro may form part of an assisted reproductive technology. The term "assisted reproductive technology" as used throughout the specification is to be understood to mean any laboratory or clinical technology applied to isolated gametes (oocyte/ovum or sperm) and/or embryos for the purposes on reproduction.

Such technologies include in vitro fertilization (IVF; aspiration of an oocyte/ovum, fertilization in the laboratory and transfer of the embryo into a recipient), gamete intrafallopian transfer (GIFT; placement of oocyte/ovum and sperm into the fallopian tube), zygote intrafallopian transfer (ZIFT; placement of fertilized ovum into the fallopian tube), tubal embryo transfer (TET; the placement of cleaving embryos into the fallopian tube), peritoneal oocyte and sperm transfer (POST; the placement of ovum and sperm into the pelvic cavity), intracytoplasmic sperm injection (ICSI), testicular sperm extraction (TESE), and microsurgical epididymal sperm aspiration (MESA); or any other in vitro technique for producing embryos in humans and/or animals, such as nuclear transfer, parthenogenic activation, embryonic stem cell production, and the use of totipotent cells.

In one aspect, the assisted reproductive technology comprises in vitro fertilization (IVF). IVF relates to the fertilization of an ovum in vitro, wherein an oocyte is isolated from the subject and matured into an ovum as described herein to allow fertilization. As indicated above, methods are well known in the art for collecting oocytes from suitable females and fertilizing in vitro. It is contemplated that fertilization will ideally occur no less than 24 hours, but no later than 60 hours, after collection and maturation as described herein. For in vitro fertilization, the sperm may be incubated with the ovum for a period of between 1 to 60 hours.

In circumstances where it is desired to accomplish fertilization by other than natural interaction of sperm and ovum, such as where the sperm is unable to fertilize the ovum due to a thickened zona pellucida surrounding the ovum, or where the sperm is from a male-factor patient, the sperm may be transported into the ovum by a technique called intracytoplasmic sperm injection (ICSI). Accordingly, in some aspects, the assisted reproductive technology or the method of assisted reproduction is ICSI. When the ICSI technique is used, the cumulus cells are removed, and sperm is injected into the interior of the ovum using a glass pipette.

With respect to any of the aforementioned assisted reproductive technologies, the collected sperm may be maintained in a medium prior to fertilization. A suitable medium would be known in the art and is set out in standard texts, such as the Textbook of Assisted Reproduction: Laboratory and Clinical Perspectives (2003, supra). The medium containing the sperm may be of a constitution so as to minimize any stress placed on the ovum when transferred from the maturation medium to the medium containing the sperm. Accordingly, in some aspects, the medium housing the sperm may have a similar or identical composition of ions and non-essential amino acids as the maturation medium.

With respect to the fertilization process, a suitable medium in which this is conducted (i.e. a fertilization medium) would be known in the art and is set out in standard texts, such as the Textbook of Assisted Reproduction: Laboratory and Clinical Perspectives (2003, supra). The fertilization medium may be of a constitution so as to promote sperm function and fertilization. For example, the fertilization medium may comprise an elevated concentration of sodium and/or phosphate compared to the maturation medium. The fertilization medium may also be supplemented with carbohydrates such as glucose, lactate and pyruvate. Specific formulations may involve supplementation of the medium with one or more of bicarbonate, glutathione to promote sperm head decondensation, non-essential amino acids, HSA, hyaluronate, and antibiotics such as penicillin and streptomycin.

Alternatively, the collected sperm may be transferred directly into the maturation medium which contains the ovum (for in vitro fertilisation) or may be injected directly into an ovum that is present in the maturation medium (for ICSI).

With respect to the ICSI technique, an alternative arrangement would be to use a single medium (an ICSI medium) that can be used to culture the oocyte and can also serve as a carrier for the sperm as it is transported by injection into the ovum. The ICSI medium should preferably be highly compatible with the interior and exterior of the ovum. The ICSI media may be a base medium as described above and may comprise ionic constituents similar to those found in the oocyte maturation medium described herein. In one aspect, phosphate may be omitted to avoid metabolic and homeostatic stress on the ovum. Because ICSI is a clinical procedure performed outside the incubator, a buffering system that is effective in a normal atmosphere is typically used. MOPS and HEPES are accordingly preferred buffers for the ICSI medium. Because the cumulus cells have been removed from the ovum, and the sperm is at the conclusion of its independent life, glucose (the main energy source for cumulus cells and sperm, but not the ovum) may be omitted from the ICSI medium. In order to nourish the ovum, non-essential amino acids most abundant in the

ovum (e.g. glycine, proline, serine and taurine) and glutamine can be included in the ICSI medium to avoid osmotic and pH stress. The ICSI medium may also include hyaluronate or polyvinylpyrollidone (PVP) to immobilize or slow the sperm so that they may be captured in the ICSI pipette.

Following fertilization, the zygote may be incubated in a medium which supports development of the embryo (an embryo culture medium). The embryo culture medium may be a base medium as described above and may comprise ionic constituents similar to those found in the oocyte maturation medium. In one aspect, the embryo culture medium may comprise EDTA which is believed to bind and disable toxins that may be deleterious to the early embryo. The embryo culture medium may also comprise HSA and hyaluronate. Furthermore, alanyl-glutamine may be substituted for glutamine to reduce ammonium build up within the medium.

In some particular aspects, the first subject is a human. Thus, in some aspects, the oocyte is a human oocyte. In some particular aspects, the method may further comprise implanting the zygote or an embryo formed therefrom within a uterus of a second subject. The second subject may be the same individual as or a different individual from the first subject.

In some aspects, the first subject exhibits oocyte maturation arrest. In some aspects, the oocyte maturation arrest is the result of a genetic anomaly or disorder. In some aspects, the first subject has polycystic ovary syndrome. In some aspects, the first subject exhibits high antral follicle counts. In some aspects, the first subject exhibits ovarian hyper-responsiveness. In some aspects, the first subject may have empty follicle syndrome (EFS).

The active ingredient may be administered in such amounts, time, and route deemed necessary in order to achieve the desired result. The exact amount of the active ingredient will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the medical disorder, the particular active ingredient, its mode of administration, its mode of activity, and the like. The active ingredient, whether the active compound itself, or the active compound in combination with an agent, is preferably formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the active ingredient will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the active ingredient employed; the specific composition employed; the age, body weight,

general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific active ingredient employed; the duration of the treatment; drugs used in combination or coincidental with the specific active ingredient employed; and like factors well known in the medical arts.

The exact amount of an active ingredient required to achieve a therapeutically or prophylactically effective amount will vary from subject to subject, depending on species, age, and general condition of a subject, severity of the side effects or disorder, identity of the particular compound(s), mode of administration, and the like. The amount to be administered to, for example, a child or an adolescent can be determined by a medical practitioner or person skilled in the art and can be lower or the same as that administered to an adult.

Useful dosages of the active agents and therapeutic compositions disclosed herein can be determined by comparing their in vitro activity, and in vivo activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art.

The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms or disorder are affected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counterindications. Dosage can vary and can be administered in one or more dose administrations daily, for one or several days.

Additional Aspects

In view of the described compounds, compositions, and methods, hereinbelow are described certain more particular aspects of the disclosure. These particularly recited aspects should not, however, be interpreted to have any limiting effect on any different claims containing different or more general teachings described herein, or that the "particular" aspects are somehow limited in some way other than the inherent meanings of the language and formulae literally used therein.

Aspect 1. A synthetic mRNA comprising a coding region encoding a protein involved in oocyte maturation or a variant thereof that is 80% or more homologous, wherein the synthetic mRNA further comprises at least one chemical modification to a nucleotide.

Aspect 2. The synthetic mRNA of aspect 1, wherein the protein involved in oocyte maturation is selected from SYCP3, TRIP13, MCM8, STAG3, PATL2, TUBB8, AURKC, and WEE2.

Aspect 3. The synthetic mRNA of aspect 1 or aspect 2, wherein the protein involved in oocyte maturation is PATL2.

Aspect 4. The synthetic mRNA of aspect 3, wherein the coding region comprises an RNA sequence encoded by a gene selected from Table 1, or a variant thereof that is 80% or more homologous, wherein at least one nucleotide in the RNA sequence has been chemically modified.

Aspect 5. The synthetic mRNA of aspect 1, wherein the coding region comprises an RNA sequence selected from SEQ ID NO:1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, and SEQ ID NO: 13, or a variant thereof that is 80% or more homologous, wherein at least one nucleotide the sequence has been chemically modified.

Aspect 6. The synthetic mRNA of aspect 1, wherein the coding region comprises a sequence of SEQ ID NO: 7, or a variant thereof that is 80% or more homologous, wherein at least one nucleotide the sequence has been chemically modified.

Aspect 7. The synthetic mRNA of aspect 1, wherein the coding region encodes a polypeptide having a sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 14, or a variant thereof that is 80% or more homologous.

Aspect 8. The synthetic mRNA of aspect 1, wherein the coding region encodes a polypeptide having a sequence of SEQ ID NO: 8, or a variant thereof that is 80% or more homologous.

Aspect 9. The synthetic mRNA of any one of aspects 1-8, wherein the at least one chemical modification of a nucleoside comprises: N4-acetylcytidine triphosphate (N4-acetyl-CTP), inosine triphosphate (ITP), 5-methylcytidine-5'-triphosphate (5-methyl-CTP), pseudouridine-5'-triphosphate (Pseudo-UTP), N1-methyladenosine-5'-triphosphate (N1-methyl-ATP), N1-methyl-pseudouridine-5'-triphosphate (N1-methylpseudo-UTP), 2-thiouridine-5'-triphosphate (2-Thio-UTP), 5-methoxyuridine-5'-triphosphate (5-Methoxy-UTP), 7-methylguanosine, 2'-O-7-methylguanosine, or combinations thereof.

Aspect 10. The synthetic mRNA of any one of aspects 1-9, further comprising a human-derived or synthetic 5' untranslated region (5' UTR).

Aspect 11. The synthetic mRNA of aspect 10, wherein the 5' UTR comprises a sequence selected from SEQ ID NO: 16 to SEQ ID NO: 33, or a variant thereof that is 80% or more homologous.

Aspect 12. The synthetic mRNA of aspect 10 or aspect 11, wherein the 5' UTR is operably linked to a 5' end of the coding region.

Aspect 13. The synthetic mRNA of any one of aspects 1-12, further comprising a human-derived or synthetic 3' untranslated region (3' UTR).

Aspect 14. The synthetic mRNA of aspect 13, wherein the 3' UTR comprises a sequence selected from SEQ ID NO: 34 to SEQ ID NO: 51, or a variant thereof that is 80% or more homologous.

Aspect 15. The synthetic mRNA of aspect 13 or aspect 14, wherein the 3' UTR is operably linked to a 3' end of the coding region.

Aspect 16. The synthetic mRNA of any one of aspects 1-15, further comprising a 5' terminal cap operably linked to a 5' end of the mRNA.

Aspect 17. The synthetic mRNA of aspect 16, wherein the 5' terminal cap has a cap-1 structure.

Aspect 18. The synthetic mRNA of any one of aspects 1-17, further comprising a 3' poly(A) tail operably linked to a 3' end of the mRNA.

Aspect 19. A therapeutic composition comprising a synthetic mRNA of any one of aspects 1-18 formulated within a delivery vehicle.

Aspect 20. The therapeutic composition of aspect 19, wherein the delivery vehicle is a liposome, a lipoplex, a lipid nanoparticle, a polymer, or a polymeric nanoparticle.

Aspect 21. A method of inducing maturation in an oocyte, comprising contacting the oocyte with a synthetic mRNA of any one of aspects 1-18 or a therapeutic composition of any one of aspects 19-20.

Aspect 22. A method of fertilization comprising:

a) contacting an oocyte with a synthetic mRNA of any one of aspects 1-18 or a therapeutic composition of any one of aspects 19-20, whereupon the oocyte undergoes maturation into an ovum;

b) fertilizing the ovum with a sperm to form a zygote.

Aspect 23. The method of aspect 22, wherein fertilizing the ovum with the sperm comprises contacting the ovum with the sperm in a culture medium.

Aspect 24. The method of aspect 22, wherein fertilizing the ovum comprises intracytoplasmic injection of the sperm into the ovum.

Aspect 25. The method of any one of aspects 21-24, wherein the oocyte is collected from an ovary of a first subject.

Aspect 26. The method of aspect 25, wherein the first subject is a human.

Aspect 27. The method of any one of aspects 21-26, wherein the oocyte is a human oocyte.

Aspect 28. The method of any one of aspects 25 or 26, further comprising implanting the zygote or an embryo formed therefrom within a uterus of a second subject.

Aspect 29. The method of aspect 28, wherein the second subject is the same as the first subject.

Aspect 30. The method of any of aspects 25 or 26, wherein the first subject exhibits oocyte maturation arrest.

A number of aspects of the disclosure have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the disclosure. Accordingly, other aspects are within the scope of the following claims.

By way of non-limiting illustration, examples of certain aspects of the present disclosure are given below.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated and are intended to be purely exemplary and are not intended to limit the disclosure. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

The non-limiting exemplary approach described herein involves generating in vitro transcribed Patl2 without RNA modifications (unmodified), and with combinations of RNA modifications (modified). In vitro transcribed unmodified and modified Patl2 are microinjected into GV oocytes, and rates of oocyte maturation are measured. In vitro

fertilization can be performed and embryo development assessed of oocytes injected with unmodified and modified Patl2, and the live birth rates tested following embryo transfer.

Combinations of RNA modifications can enhance oocyte maturation due to increased translatability and RNA stability

Design and testing scheme of in vitro transcribed Patl2 RNA therapeutic molecules: We can test the impact of 5 different RNA modifications (inosine, N4-acetylcytidine, N6-methyladenosine, 5-methylcytidine, and pseudouridine) singly, and in combination, on the stability of in vitro synthesized and microinjected Patl2 mRNA therapeutic. In vitro transcribed Patl2 (unmodified, and modified) is screened for "activity" using an oocyte maturation assay. The rate of oocyte maturation after microinjection of unmodified in vitro transcribed Patl2 into Patl2-/- oocytes is the baseline level of "activity" in our oocyte maturation assay. Transcripts with single types of RNA modifications or multiple types of RNA modifications are microinjected (31 different combinations). For example, Patl2 modified with inosine only vs Patl2 modified with a combination of inosine and pseudouridine. Combinations that promote oocyte maturation are labeled as "active" and further tested. By altering only the RNA modifications, it is possible to study RNA modifications in isolation, without confounding factors within the synthetic transcript. We can in vitro synthesize unmodified Patl2 mRNA and all the combinations of modification types of modified Patl2 mRNA.

Patl2 deficient mouse model: We purchased Patl2 FL/FL mice from the EUCOMM Consortium. After crossing to CMVcre+ recombinase mice, the Patl2 gene is truncated at exon 7, so even if a protein product is generated from the modified gene, it would be deficient in the topoisomerase II-associated protein PAT1 domain and not be functional. The resulting mice (Patl2fl/fl:CMVCre+) here are called Patl2-/- mice. This same Patl2 deficient mouse model was previously reported by Christou-Kent et al., who demonstrated female infertility due to meiotic arrest in oocytes. Knockout females had a similar number of follicles as control animals but following stimulation with pregnant mare serum gonadotropin (PMSG), GV oocytes had significant defects during in vitro maturation, and only 27.2% reached blastocyst stage 22, in contrast to 87.1% of wild-type embryos. Thus, the Patl2 deficient mice recapitulate the human condition of oocyte arrest.

GV oocyte collection: Cumulus-oocyte-complexes are released from large antral follicles into flushing and handling medium (FHM) supplemented with 4 mg/ml BSA (mFHM) and

2.5 μM milrinone to prevent germinal vesicle breakdown using previously established procedures. GV oocytes within enclosed cumulus complexes undergo oocyte microinjection, followed by IVM and are collected at 16 hours after Milrinone removal at MII stage. Confirmation of MII stage is performed using live cell imaging documented polar body extrusion using Hoechst 33342 imaging. Oocytes at all stages GV, MI, MII are quantified.

Oocyte microinjection: Synthetic mRNA is injected into 40 GV oocytes for each injection paradigm outlined below. Injections are performed on an inverted Nikon Eclipse TE2000-S with a Sutter microinjection system and 0.3 mm ID EggJek microinjection needles (MicroJek, Kansas City, KS). GV oocytes are allotted into three groups: noninjected, injected with either unmodified or modified synthetic mRNA. Oocytes are transferred to Terasaki plates and cultured for 4–5 hr in KSOMaa supplemented with milrinone to maintain GV arrest and to allow for protein expression to occur. GV oocytes are then washed and cultured without milrinone in a live cell EVOS culture system to monitor viability until they reach the MII stage. After incubation, oocytes are washed in milrinone-free M16 medium, and cultured for 3 hours to observe meiotic resumption (GVBD) and 14 hours to detect the first polar body extrusion. We collect the oocytes after milrinone removal for further analysis at various time points.

Statistical power analyses: Based on the published oocyte maturation rates of 27.2% in Patl2-/- GV oocytes, our power analysis takes this into account as a level of baseline maturation. To test the difference in oocyte maturation microinjection of unmodified or modified Patl2 mRNA, we microinject at least 40 Patl2-/- GV oocytes per RNA and experiment. Each RNA microinjection group contains a minimum of 40 GV oocytes, to achieve a power of 90% at $\alpha = 0.05$ to detect a 20% difference in oocyte maturation. We assume a two-tailed test since outcomes could increase, decrease, or not change. Subsequent statistical analyses are performed using one-way ANOVA tests. Microinjection experiments are performed blinded to decrease potential bias.

Determine absolute abundance of endogenous Patl2 during oocyte maturation. We determine the mass of Patl2 in GV oocytes and MII eggs using digital PCR, allowing us to understand the dynamics of Patl2 mRNA decay under normal conditions. Briefly, we isolate RNA from GV oocytes and MII eggs (21-day old females stimulated for 46 hours with PMSG; 6 GV oocytes/MII eggs from 5 different females). Complementary DNA (cDNA) is generated from single GV oocytes after injection and single MII eggs in a reverse

transcription (RT) reaction. Digital PCR (dPCR) is performed using a QuantStudio™ Absolute Q Digital PCR system using gene target probes for Patl2. Fluorescent signals are imaged and analyzed using QuantStudio™ Absolute Q software. We use the average abundance of Patl2 from 40 GV oocytes to inform the amount of in vitro transcribed Patl2 to microinject into Patl2-/- GV oocytes.

Generate in vitro transcribed unmodified and modified Patl2 RNA therapies. Patl2 mRNA therapeutic are generated by first designing a synthetic dsDNA geneBlock (IDT) that contains a T7 promoter at the 5' end Patl2 mRNA. An IVT reaction is carried out using T7 RNA polymerase (NEB) and either unmodified or in combination with modified nucleotides spiked at a molar ratio of 1:20 (5% modified) to generate RNA that are 10% modified. A 5% incorporation rate is a standard in RNA therapeutics applications. The modified nucleotides (Jena Biosciences) used in the IVT reactions are; inosine, N4-acetylcytidine, N6-methyladenosine, 5-methylcytidine, and pseudouridine. Following IVT, a 7-methylguanosine (m7G) Cap-1 structure is added to the IVT using the Vaccinia Capping System (NEB) in combination with mRNA Cap 2′-O-Methyltransferase (NEB). The Cap-1 structure has been demonstrated to enhance translation and reduce innate cellular immune response in eukaryotic cells. Following cap addition, IVT reactions a poly(A) tail are added using poly(A) polymerase (NEB). After poly(A) tail addition, reactions are treated with XRN1 (NEB), a 5'-3' exoribonuclease that degrades uncapped RNA. Following XRN1 treatment, the RNA is assessed for purity (UV spectrophotometry) and size (electrophoresis) followed by HPLC size selection purification. The sequence composition of the final purified and size-selected mRNA is verified by next generation sequencing (iSeq 100, Illumina). Additionally, the proportion of modifications and position of modifications, and poly(A) tail length are determined by direct RNA sequencing (Oxford Nanopore). After verification of mRNA purity and composition final Patl2 RNA therapies are stored at -80°C.

Determine the time necessary to allow maximal PATL2 protein translation. Patl2-/- GV oocytes are micro-injected with an unmodified fluorescent reporter Patl2 mRNA (Patl2-eGFP) at physiological abundance, as determined above, to allow the monitoring of adequate PATL2 translation following microinjection. Unmodified Patl2 mRNA fused to eGFP is microinjected into 40 different Patl2-/- GV oocytes and incubated in milrinone in a live cell EVOS culture system to monitor eGFP fluorescence for 24 hours to determine the time at which maximal translation occurs (eGFP fluorescence). This time is the incubation period used in all subsequent experiments.

Identify in vitro unmodified and modified Patl2 molecules that promote oocyte maturation. 21–23-day old female mice are injected with 5 IU of PMSG (Lee Biosolutions) to synchronize estrous cycles and enable collection of GV oocytes after 44 hours. Oocytes at the GV stage with distinct GV nuclei are cultured as previously described. In Patl2 mRNA therapeutic is microinjected into Patl2-/- GV oocytes at physiological abundance as determined above. Oocytes are incubated in milrinone to allow for translation, and then entered into an oocyte maturation assay. The maturation rates of Patl2-/- GV oocytes microinjected with unmodified Patl2 are the baseline activity. All modifications and combinations of modifications with significantly improved oocyte maturation rates are designated as "active". Microinjected Patl2-/- GV oocytes are in vitro matured and assessed for standard oocyte quality measurements. Briefly, groups of 20 oocytes are cultured in mini-drops of M16 medium (M7293; Sigma-Aldrich) covered in mineral oil (M5310; Sigma-Aldrich) at 37°C in a 5% CO2 atmosphere. Eggs are fixed, labeled for tubulin, f-actin, and DNA, and imaged by an EVOS imaging system to assess meiotic maturation (metaphase-II spindle and polar body extrusion) as previously described. All eggs are collected at the end of the oocyte maturation assay to test how the microinjected Patl2 mRNA therapeutic corresponds to translated PATL2 and Patl2 mRNA therapeutic abundance.

Measure absolute copy number of microinjected Patl2 RNA molecules. Digital PCR measures the amount of Patl2 mRNA therapeutic that was injected into GV oocytes and then measures the rate of decay at the MII stage in order to understand how RNA modifications impact the RNA decay of injected Patl2 molecules. We perform digital PCR as described above.

Determine the translational capacity of modified Patl2 mRNA therapies. We monitor protein abundance using live cell imaging of our Patl2-eGFP mRNA reporter microinjected into Patl2-/- GV oocytes, at physiological abundance as determined above. After release from milrinone, oocytes are monitored during in vitro maturation and GFP fluorescence is recorded. We correlate the eGFP fluorescence signal to modified Patl2 mRNA transcripts with oocyte maturation rates and RNA stability rates to get a multifaceted understanding of the relationship between RNA modifications, translation, and RNA decay. Digital PCR and oocyte maturation, comparisons between groups undergo ANOVA tests with Bonferroni-Dunn correction for single-cell RNA-sequencing, after quality control and normalization,

cell markers are identified using a Wilcoxon test and differentially expressed genes with an adjusted p value < 0.5 are retained for further analysis.

Determine efficacy of Patl2 mRNA therapy to restore fertility.

Combinations of RNA modifications will restore fertility in a Patl2 deficient mouse model

The impact of RNA modifications on fertility is tested by measuring live birth rates of microinjected oocytes that undergo in vitro maturation, fertilization, embryo culture, and blastocyst transfer to recipient moms. Patl2-/- GV oocytes microinjected with unmodified or modified Patl2 undergo in vitro maturation, are in vitro fertilized and cultured until the blastocyst stage. Briefly, cauda epididymal sperm from C57Bl/6J adult males is released into mTyrode's solution containing 4 mg/mL BSA. Capacitation is performed for 90 minutes in a swim up column of mTyrodes at 37°C. Groups of hyaluronidase treated MII eggs, are transferred to an IVF culture dish with 500 µL KSOMaa media with 4 mg/mL BSA under mineral oil. Capacitated sperm ($2x10^5$/ mL) are added for 4.5 hours, after which inseminated eggs are washed in KSOMaa. Embryos are cultured, and blastocysts are transferred into pseudopregnant females at 2.5 days postcoitus, and in vivo developmental potential measured.

To test if the embryonic genome is altered by microinjection of an RNA therapeutic, we perform short-read Illumina RNA-seq of early embryos that were microinjected with RNA therapeutic along with 4-thiouridine, which labels de novo transcription by the generation of T-to-C mismatches, thus allowing for differentiation of the embryonic genome from the abundant maternal transcriptome. This method, called SLAM-seq, is a similar approach to the identification of inosine RNA modifications, which appear as A-to-G mismatches. Because embryonic genome activation is rapid and transient, beginning at the 1-cells stage, and continuing during the early and late 2-cell stage, we collect embryos at 1-cell, early 2-cell, late 2-cell, 4-cell, and morula, thus fully encompassing embryonic genome activation. We document rates of cleavage, and embryos at specific stages are picked off for RNA isolation.

Determine the efficacy of active combinations of Patl2 mRNA therapeutics to rescue embryogenesis and birth following microinjection into Patl2-/- oocytes. Patl2-/- GV oocytes are microinjected with unmodified or modified Patl2 mRNA therapeutic, undergo in vitro fertilization, and are cultured to blastocyst stage. Embryos that fail to progress, exhibit excessive blastomere fragmentation, or lose cellular morphology during in vitro culture are

counted as arrested and removed from the culture. Blastocyst stage embryos are transferred to pseudopregnant female mice, and the number of born pups are recorded. 8-10 blastocysts are transferred to the uterine horns of 5 different 2.5 days post coitus pseudopregnant females per experimental RNA, and live birth rates are counted. To prepare pseudopregnant surrogates, C57Bl/6J female mice (8-12 weeks old) at the estrus stage are mated with vasectomized C57Bl/6J male mice.

Determine the impact of RNA modifications in Patl2 mRNA therapeutic on embryonic genome activation. Standard RNA-seq approaches are unable to directly distinguish between maternal RNA and embryonic RNA following embryo genome activation. SLAM-seq, an approach that uses thiol-(SH)-linked alkylation of RNA for metabolic labeling sequencing, was developed to label newly synthesized RNA within a cell. De novo transcription is labeled through the incorporation of a modified ribonucleotide base, 4-thiouridine (4sU). In RNA-seq library preparations, the reverse transcription step causes misincorporation of cytosine (C) at 4sU bases within the RNA. SLAM-seq has been adopted to mouse single cells, (scSLAM-seq), as well as early embryonic development in zebrafish 33,34. To assess the impact of a Patl2 mRNA therapeutic on embryonic genome activation, we utilize scSLAM-seq on embryos generated from microinjected Patl2-/-

GV oocytes. Patl2-/- GV oocytes are coinjected with 4-thiouridine and unmodified or modified Patl2 mRNA therapeutic. Following in vitro fertilization of MII eggs, embryos are collected at the 1-cell, early 2-cell, late 2-cell, 4-cell, and morula stages (n=6 embryos per stage). Once embryos reach the desired point of development, zona pellucida are removed using acidified Tyrode's buffer, followed by a brief wash in M2 medium. Embryos are directly lysed in reverse transcription buffer using a Low-input Ovation Solo Total RNA sequencing kit (Tecan) with ERCC spike-in. RNA sequencing is performed using our NextSeq 2000 system. Raw reads are processed to identify T-to-C substitutions in RNA utilizing our previously published approach. Briefly our analysis pipeline is as follows: quality filtered and trimmed reads are aligned to a dbSNP annotated reference genome with ERCC spike in contigs using HISAT2. The elprep software is then used to identify high confidence mismatches between the genome reference and cDNA using known genetic sites of variation identified multiple mouse strains from the Mouse Genomes Project database, Wellcome Trust Sanger Institute mouse strains. Further filtering is performed to remove low converge mismatches. Ensembl Variant Effect Predictor (VEP) is used to assign mismatches to the reference transcriptome and ERCC spike-in.: Nucleotide substitution positions

identified from RNA-seq undergo Wilcoxon test, with Bonferroni-Dunn corrected p value < 0.5 retained for further analysis.

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The compositions and methods of the appended claims are not limited in scope by the specific compositions and methods described herein, which are intended as illustrations of a few aspects of the claims and any compositions and methods that are functionally equivalent are intended to fall within the scope of the claims. Various modifications of the compositions and methods in addition to those shown and described herein are intended to fall within the scope of the appended claims. Further, while only certain representative compositions and method steps disclosed herein are specifically described, other combinations of the compositions and method steps also are intended to fall within the scope of the appended claims, even if not specifically recited. Thus, a combination of steps, elements, components, or constituents may be explicitly mentioned herein; however, other combinations of steps, elements, components, and constituents are included, even though not explicitly stated.

WHAT IS CLAIMED IS:

1. A synthetic mRNA comprising a coding region encoding a protein involved in oocyte maturation or a variant thereof that is 80% or more homologous, wherein the synthetic mRNA further comprises at least one chemical modification to a nucleotide.

2. The synthetic mRNA of claim 1, wherein the protein involved in oocyte maturation is selected from SYCP3, TRIP13, MCM8, STAG3, PATL2, TUBB8, AURKC, and WEE2.

3. The synthetic mRNA of claim 1 or claim 2, wherein the protein involved in oocyte maturation is PATL2.

4. The synthetic mRNA of claim 3, wherein the coding region comprises an RNA sequence encoded by a gene selected from Table 1, or a variant thereof that is 80% or more homologous, wherein at least one nucleotide in the RNA sequence has been chemically modified.

5. The synthetic mRNA of claim 1, wherein the coding region comprises an RNA sequence selected from SEQ ID NO:1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, and SEQ ID NO: 13, or a variant thereof that is 80% or more homologous, wherein at least one nucleotide in the sequence has been chemically modified.

6. The synthetic mRNA of claim 1, wherein the coding region comprises a sequence of SEQ ID NO: 7, or a variant thereof that is 80% or more homologous, wherein at least one nucleotide in the sequence has been chemically modified.

7. The synthetic mRNA of claim 1, wherein the coding region encodes a polypeptide having a sequence selected from SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 14, or a variant thereof that is 80% or more homologous.

8. The synthetic mRNA of claim 1, wherein the coding region encodes a polypeptide having a sequence of SEQ ID NO: 8, or a variant thereof that is 80% or more homologous.

9. The synthetic mRNA of any one of claims 1-8, wherein the at least one chemical modification of a nucleoside comprises: N4-acetylcytidine triphosphate (N4-acetyl-CTP), inosine triphosphate (ITP), 5-methylcytidine-5'-triphosphate (5-methyl-CTP), pseudouridine-5'-triphosphate (Pseudo-UTP), N1-methyladenosine-5'-triphosphate (N1-methyl-ATP), N1-methyl-pseudouridine-5'-triphosphate (N1-methylpseudo-UTP), 2-

thiouridine-5'-triphosphate (2-Thio-UTP), 5-methoxyuridine-5'-triphosphate (5-Methoxy-UTP), 7-methylguanosine, 2'-O-7-methylguanosine, or combinations thereof.

10. The synthetic mRNA of any one of claims 1-9, further comprising a human-derived or synthetic 5' untranslated region (5' UTR).

11. The synthetic mRNA of claim 10, wherein the 5' UTR comprises a sequence selected from SEQ ID NO: 16 to SEQ ID NO: 33, or a variant thereof that is 80% or more homologous.

12. The synthetic mRNA of claim 10 or claim 11, wherein the 5' UTR is operably linked to a 5' end of the coding region.

13. The synthetic mRNA of any one of claims 1-12, further comprising a human-derived or synthetic 3' untranslated region (3' UTR).

14. The synthetic mRNA of claim 13, wherein the 3' UTR comprises a sequence selected from SEQ ID NO: 34 to SEQ ID NO: 51, or a variant thereof that is 80% or more homologous.

15. The synthetic mRNA of claim 13 or claim 14, wherein the 3' UTR is operably linked to a 3' end of the coding region.

16. The synthetic mRNA of any one of claims 1-15, further comprising a 5' terminal cap operably linked to a 5' end of the mRNA.

17. The synthetic mRNA of claim 16, wherein the 5' terminal cap has a cap-1 structure.

18. The synthetic mRNA of any one of claims 1-17, further comprising a 3' poly(A) tail operably linked to a 3' end of the mRNA.

19. A therapeutic composition comprising a synthetic mRNA of any one of claims 1-18 formulated within a delivery vehicle.

20. The therapeutic composition of claim 19, wherein the delivery vehicle is a liposome, a lipoplex, a lipid nanoparticle, a polymer, or a polymeric nanoparticle.

21. A method of inducing maturation in an oocyte, comprising contacting the oocyte with a synthetic mRNA of any one of claims 1-18 or a therapeutic composition of any one of claims 19-20.

22. A method of fertilization comprising:

a) contacting an oocyte with a synthetic mRNA of any one of claims 1-18 or a therapeutic composition of any one of claims 19-20, whereupon the oocyte undergoes maturation into an ovum;

b) fertilizing the ovum with a sperm to form a zygote.

23. The method of claim 22, wherein fertilizing the ovum with the sperm comprises contacting the ovum with the sperm in a culture medium.

24. The method of claim 22, wherein fertilizing the ovum comprises intracytoplasmic injection of the sperm into the ovum.

25. The method of any one of claims 21-24, wherein the oocyte is collected from an ovary of a first subject.

26. The method of claim 25, wherein the first subject is a human.

27. The method of any one of claims 21-26, wherein the oocyte is a human oocyte.

28. The method of any one of claims 25 or 26, further comprising implanting the zygote or an embryo formed therefrom within a uterus of a second subject.

29. The method of claim 28, wherein the second subject is the same as the first subject.

30. The method of any of claims 25 or 26, wherein the first subject exhibits oocyte maturation arrest.

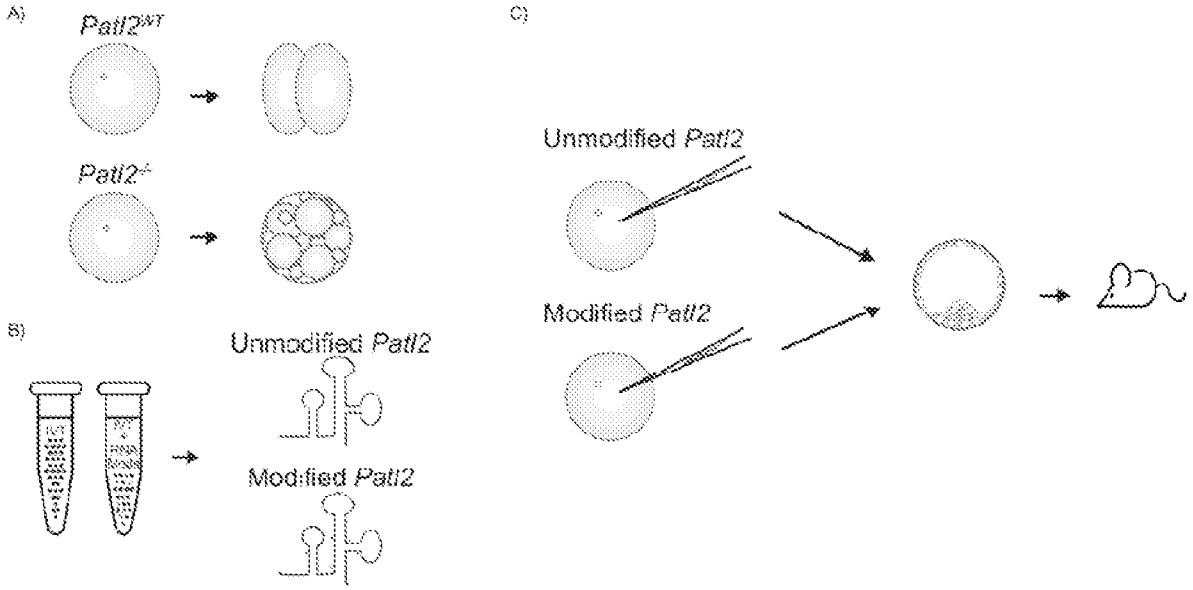

A)
Patl2WT
Patl2-/-
B)
Unmodified Patl2
Modified Patl2
C)
Unmodified Patl2
Modified Patl2

FIG. 1