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(54) Title: MODIFIED NUCLEOSIDE, NUCLEOTIDE, AND NUCLEIC ACID COMPOSITIONS

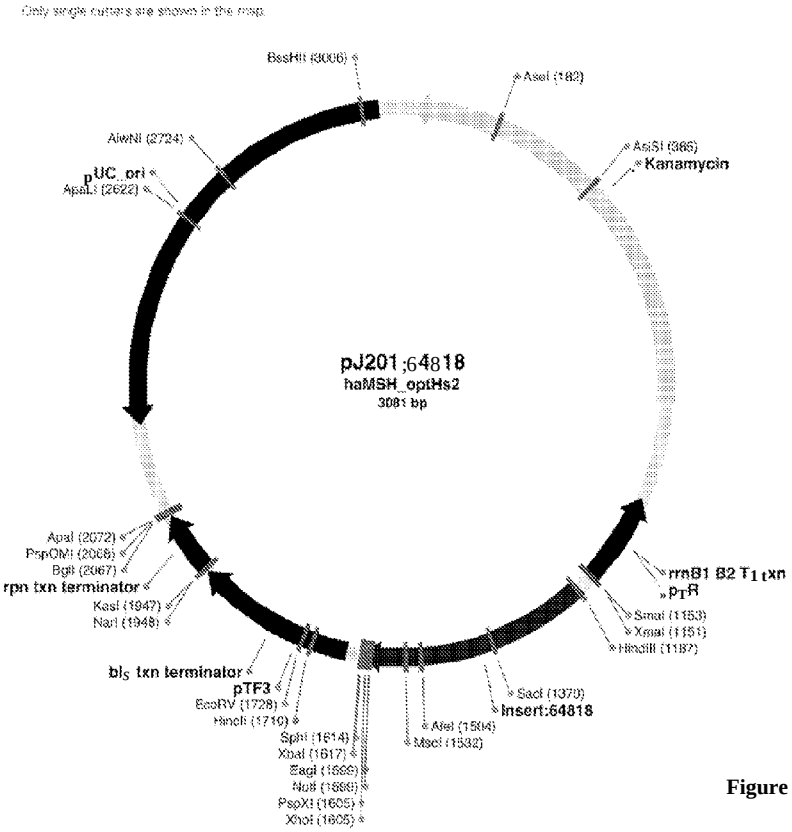

Figure 2

(57) Abstract: The present disclosure provides, *inter alia*, formulation compositions comprising modified nucleic acid molecules which may encode a protein, a protein precursor, or a partially or fully processed form of the protein or a protein precursor. The formulation composition may further include a modified nucleic acid molecule and a delivery agent. The present invention further provides nucleic acids useful for encoding polypeptides capable of modulating a cell's function and/or activity.

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MODIFIED NUCLEOSIDE, NUCLEOTIDE, AND NUCLEIC ACID COMPOSITIONS

REFERENCE TO SEQUENCE LISTING

[0001] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing file, entitled M1 lPCTSQLST.txt, was created on December 14, 2012 and is 25,579 bytes in size. The information in electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Provisional Patent Application No. 61/576,705, filed December 16, 201 1, entitled Modified Nucleoside, Nucleotide, and Nucleic Acid Compositions, U.S. Provisional Patent Application No. 61/618,957, filed April 2, 2012, entitled Modified Nucleoside, Nucleotide, and Nucleic Acid Compositions, U.S. Provisional Patent Application No. 61/648,244, filed May 17, 2012, entitled Modified Nucleoside, Nucleotide, and Nucleic Acid Compositions, U.S. Provisional Patent Application No. 61/681,712, filed August 10,2012, entitled Modified Nucleoside, Nucleotide, Nucleic Acid Compositions and U.S. Provisional Patent Application No. 61/696,381 filed September 4, 2012, entitled Modified Nucleoside, Nucleotide and Nucleic Acid Compositions, and Nucleic Acid Compositions, U.S. Provisional Patent Application No. 61/709,303 filed October 3, 2012, entitled Modified Nucleoside, Nucleotide and Nucleic Acid Compositions, U.S. Provisional Patent Application No. 61/712,490 filed October 11, 2012, entitled Modified Nucleoside, Nucleotide and Nucleic Acid Compositions and International Pub. No. PCT/US2012/058519 filed October 3, 2012 Modified Nucleosides, Nucleotides, and Nucleic Acids, And Uses Thereof, the contents of which are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

[0003] In general, exogenous unmodified nucleic acid molecules, particularly viral nucleic acids, introduced into the cell induce an innate immune response which results in cytokine and interferon (IFN) production and ultimately cell death. It is of great interest for therapeutics, diagnostics, reagents and for biological assays to be able to deliver a nucleic acid, *e.g.,* a ribonucleic acid (RNA), into a cell, such as to cause intracellular translation of the nucleic acid and production of the encoded

protein instead of generating an innate immune response. Thus, there is a need to develop formulation compositions comprising a delivery agent that can effectively facilitate the *in vivo* delivery of nucleic acids to targeted cells without generating an innate immune response.

SUMMARY OF THE INVENTION

[0004] The present disclosure provides, *inter alia,* formulation compositions comprising modified nucleic acid molecules which may encode a protein, a protein precursor, or a partially or fully processed form of the protein or a protein precursor. The formulation compositions may further include a modified nucleic acid molecule and a delivery agent. The present invention further provides nucleic acids useful for encoding polypeptides capable of modulating a cell's function and/or activity.

[0005] In one aspect a method of producing a polypeptide of interest in a mammalian cell or tissue is described. The method comprises contacting the mammalian cell or tissue with a formulation comprising a modified mRNA encoding a polypeptide of interest. The formulation may be, but is not limited to, nanoparticles, poly(lactic-co-glycolic acid)(PLGA) microspheres, lipidoids, lipoplex, liposome, polymers, carbohydrates (including simple sugars), cationic lipids, fibrin gel, fibrin hydrogel, fibrin glue, fibrin sealant, fibrinogen, thrombin, rapidly eliminated lipid nanoparticles (reLNPs) and combinations thereof. The modified mRNA may comprise a purified IVT transcript.

[0006] In one embodiment, the formulation comprising the modified mRNA 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 and PEGylated 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 and DODMA.

[0007] The lipid to modified mRNA ration in the formulation may be between 10:1 and 30:10. The mean size of the nanoparticle formulation may comprise the modified mRNA between 60 and 225 nm. The PDI of the nanoparticle formulation comprising the modified mRNA is between 0.03 and 0.15. The zeta potential of the lipid may be from -10 to +10 at a pH of 7.4

[0008] The formulations of modified mRNA may comprise a fusogenic lipid, cholesterol and a PEG lipid. The formulation may have a molar ratio 50:10:38.5:1.5-3.0 (cationic lipid:fusogenic lipid: cholesterol: PEG lipid). The PEG lipid may be selected from, but is not limited to PEG-c-DOMG, PEG-DMG. The fusogenic lipid may be DSPC.

[0009] The mammalian cell or tissue may be contacted using a device such as, but not limited to, a syringe pump, internal osmotic pump and external osmotic pump.

[0010] The formulation of modified mRNA may be a PLGA microsphere which may be between 4 and 20 μιη in size. The modified mRNA may be released from the formulation at less than 50% in a 48 hour time period. The PLGA microsphere formulation may be stable in serum. Stability may be determined relative to unformulated modified mRNA in 90%.

[0011] The loading weight percent of the modified mRNA PLGA microsphere may be at least 0.05%, at least 0.1%, at least 0.2%, at least 0.3%, at least 0.4% or at least 0.5%. The encapsulation efficiency of the modified mRNA in the PLGA microsphere may be at least 50%, at least 70%, at least 90% or at least 97%.

[0012] A lipid nanoparticle of the present invention may be formulated in a sealant such as, but not limited to, a fibrin sealant.

[0013] The mammalian cells or tissues may be contacted by a route of administration such as, but not limited to, intravenous, intramuscular, intravitreal, intrathecal, intratumoral, pulmonary and subcutaneous. The mammalian cells or tissues may be contacted using a split dosing schedule. The mammalian cell or tissue may be contacted by injection. The injection may be made to tissue selected from the group consisting of intradermal space, epidermis, subcutaneous tissue and muscle. The polypeptide of interest may be produced in the cell or tissue in a location systemic from the location of contacting.

[0014] The polypeptide of interest may be detectable in serum for up to 72 hours after contacting. The level of the polypeptide of interest can be higher than the levels prior to dosing. The level of the polypeptide of interest may be greater in the serum of female subjects than in the serum of male subjects.

[0015] The formulation of modified mRNA may comprise more than one modified mRNA. The formulation may have two or three modified mRNA.

[0016] The formulation comprising the modified mRNA may comprise a rapidly eliminated lipid nanoparticle (reLNP) which may comprise a reLNP lipid, fusogenic lipid, cholesterol and a PEG lipid at a molar ratio of 50: 10: 38.5: 1.5 (reLNP lipid:fusogenic lipid: cholesterol: PEG lipid). The fusogenic lipid may be DSPC and the PEG lipid may be PEG-c-DOMG. The reLNP lipid may be DLin-DMA with an internal or terminal ester or DLin-MC3-DMA with an internal or terminal ester. The total lipid to modified mRNA weight ration may be between 10:1 and 30:1.

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