

Overview of mRNA-Vaccine Technologies

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Abstract

mRNA immunizations join attractive immunological properties with an extraordinary wellbeing profile and the neglected adaptability of hereditary antibodies. In light of in situ protein articulation, mRNA antibodies are equipped for actuating a reasonable resistant reaction containing both cell and humeral insusceptibility while not expose to MHC haplotype limitation. Moreover, mRNA is a characteristically sheltered vector as it is a negligible and just transient bearer of data that doesn't associate with the genome. Since any protein can be communicated from mRNA without the need to alter the creation procedure, mRNA immunizations additionally offer greatest adaptability concerning advancement. Taken together, mRNA presents a promising vector that may very much turn into the premise of a game-changing immunization innovation stage. Here, we plot the present information with respect to various angles that ought to be viewed as when building up an mRNA-based immunization innovation.

Key words. Overview, mRNA-Vaccine Technologies

Introduction

RNA is considered as famously precarious creation its restorative utilize a provocative thought. In spite of the affectability of the particle to the for all intents and purposes inescapable ribonucleases (RNases), 1 mRNA as a remedial was first advanced in 1989 after the improvement of a comprehensively relevant in vitro transfection technique [1]. Only two or after three years, mRNA was supported as an immunization stage, may be being perfect as in it unites the immunological highlights of live lessened antibodies, for example, endogenous antigen articulation and T cell enlistment with those of murdered or subunit immunizations like characterized arrangement and safety [2-5].

Especially contrasted and DNA as a remedial or all the more explicitly as an immunization, mRNA offers solid wellbeing advantages [6]. As the negligible hereditary build, it harbors just the components legitimately required for articulation of the encoded protein [7]. Additionally, while recombination between single-stranded RNA atoms may happen in uncommon cases, mRNA doesn't associate with the genome [8]. In this manner, possibly adverse genomic mix is avoided. At long last, this absence of genomic joining in mix with mRNA being non-replicative just as metabolically rotting inside a couple days makes mRNA an only transient transporter of data [9].

mRNA as the innovative premise of therapeutics and antibodies is portrayed by an extraordinary adaptability as for creation and application [10]. Any protein can be encoded and communicated by mRNA, on a basic level empowering the improvement of prophylactic and helpful immunizations battling maladies as assorted as contaminations and malignancy just as protein substitution treatments [11]. Since changes of the encoded protein simply modify the arrangement of the RNA atom, leaving its physico-substance attributes generally unaffected, different items can be made utilizing the equivalent set up creation process with no modification, sparing time and lessening cost contrasted and other immunization stages [12]. As far as adequacy, mRNA-based therapeutics benefit from the way that they don't have to cross the atomic envelope instead of DNA. As opposed to peptides, mRNA antibodies need MHC haplotype limitation. Also, mRNA ties to design acknowledgment receptors and mRNA antibodies might be intended to act naturally adjuvanting, a property which peptide-and protein-based immunizations need [13].

With everything taken into account, mRNA presents a promising, regardless of whether testing, class of helpful particles that can possibly turn into the premise of a "troublesome technology." In the accompanying we are illuminating what must be viewed as when building up a mRNA-immunization innovation contacting significant themes, for example, mRNA assembling and quality, mRNA organization and definition just as antigen/protein articulation and immunological properties of mRNA-antibodies [14].

mRNA synthesis

Practical manufactured mRNA might be acquired by in vitro translation of a cDNA format, normally plasmid DNA (pDNA), utilizing a bacteriophage RNA polymerase [15]. Hence, the planning of pDNA is the initial phase

in the creation of mRNA [16]. Assembling of mRNA may in this way seem to require more exertion than production of pDNA. In any case, unpolished pDNA contains hints of bacterial genomic DNA and three types of pDNA (supercoiled, loosened up circle or straight) in factor extents [17]. Henceforth, the reproducible arrangement of unadulterated and invariant pDNA, as required for an antibody, is requesting. Stays of bacterial DNA and the heterogeneity of pDNA are not a worry, then again, whenever linearized pDNA is interpreted utilizing bacteriophage RNA polymerase, on the grounds that all DNA is expelled during further handling advances (see underneath) [18].

Manufactured mRNA contains a protein-encoding open understanding casing (ORF) flanked at the base by two components basic for the capacity of develop eukaryotic mRNA: a "top," i.e., a -methyl-guanosine buildup joined to the 5'-end through a 5'-5' triphosphate, and a poly(A) tail at the 3'-end. Accordingly, a pDNA format for in vitro interpretation contains at any rate a bacteriophage advertiser, an ORF, alternatively a poly(d(A/T)) succession deciphered into poly(A) and a one of a kind limitation site for linearization of the plasmid to guarantee characterized end of translation (the top isn't encoded by the layout) [19].

The linearized pDNA format is deciphered into mRNA in a blend containing recombinant RNA polymerase (T7, T3 or SP6) and nucleoside triphosphates [20]. It is conceivable to acquire topped mRNA by interpretation. To this end, a top simple like the dinucleotide m7G (5')- ppp-(5')G (called "standard top simple" in the accompanying) might be remembered for the reaction. If the top simple is in overabundance of GTP, translation starts with the top simple as opposed to GTP, yielding topped mRNA [21]. Alternatively, the top might be included enzymatically post interpretation. A poly (A) tail may likewise be included post interpretation on the off chance that it isn't given by the pDNA layout. Following interpretation, the pDNA format just as tainting bacterial DNA is processed by DNase [22].

mRNA purification

Now, the example contains the ideal mRNA transcript inside a perplexing blend including different nucleotides, oligodeoxynucleotides, and short failed transcript from fruitless cycling during initiation, just as protein. These contaminants might be expelled from the example by a blend of precipitation and extraction steps [23].

In any case, the example incorporates extra sullying RNA species that can't be isolated from the right transcript by straightforward methods: Shorter than assigned transcripts emerge from untimely end during extension [24]. Longer than assigned transcripts emerge from layout DNA linearized with a catalyst that leaves a 3'-overhang or from hints of nonlinearized format DNA. Unwanted transcripts are additionally delivered because of the RNA-subordinate RNA polymerase movement of bacteriophage polymerases [25]. Accordingly, to be utilized as a medication substance, mRNA should be refined further to evacuate such debasing transcripts [26].

A solitary chromatographic advance that isolates mRNA as indicated by size evacuated both shorter and longer transcripts, yielding an unadulterated single mRNA product [27]. Implementation of such a chromatographic refinement inside a GMP creation process for mRNA expanded the action of mRNA atoms a few crease as far as protein articulation in vivo [28].

Expanded protein articulation because of stringent sanitization of mRNA was additionally seen when transcripts coding for luciferase or erythropoietin were filtered by HPLC [29]. The expansion in protein articulation was a lot higher than would be normal just dependent on the expulsion of off base transcript. The creators exhibited that expanded protein articulation after HPLC sanitization was additionally because of the expulsion of sullying, e.g., twofold stranded, RNA that actuates inborn insusceptible sensors, in this manner lessening protein articulation [30].

Detailing of mRNA

mRNA is undermined by quick corruption by omnipresent extracellular ribonucleases before being taken up by cells. Thus, the viability of mRNA immunizations may profit fundamentally from complexing operators which shield RNA from debasement. Complexation may likewise upgrade take-up by cells or potentially improve conveyance to the interpretation apparatus in the cytoplasm. To this end, mRNA is regularly complexed with either lipids or polymers [31].

Significantly, not all complexing specialists that advance transfection of DNA are reasonable for complexation of mRNA. Distinctive enormous polycations, all demonstrated DNA transfection reagents, were appeared to unequivocally repress interpretation of mRNA in sans cell interpretation frameworks just as inside cells [32]. Just a lot littler polycations took into consideration productive interpretation. Likely, mRNA isn't discharged in the cytosol whenever bound to huge polycations. Interestingly, DNA might be discharged in the cytosol from enormous polycations by endogenous RNA [33].

In accordance with the general origination that mRNA ought to be ensured and take-up upgraded, the principal report exhibiting the enlistment of a resistant reaction upon direct infusion of mRNA *in vivo* utilized mRNA epitomized in liposomes [34]. Common is the utilization of cationic lipids, for example utilized for the intradermal and intravenous infusion of antigen-encoding mRNA. However, complexation of mRNA with protamine, a little arginine-rich atomic protein which settles DNA during spermatogenesis, was appeared to likewise effectively balance out mRNA against corruption by serum components [35].

What's more, complexing specialists objectively intended to additionally improve conveyance of nucleic acids to the cytosol have been utilized for detailing of mRNA antibodies [7]. Hemagglutinating infection of Japan (HVJ)-liposomes have been accounted for to convey their payload legitimately into the cytoplasm of host cells *in vivo* by methods for an infection cell combination system [13]. Such liposomes were utilized to infuse mRNA (recreating right now) melanoma antigen gp100 into the spleen of mice. Alternatively, vectors improving cytosolic nucleic corrosive conveyance by methods for penetration of endosomal films because of their high histidine content have been utilized to define antigen-encoding mRNA [10].

Complexing operators may must be custom fitted to the particular course of conveyance. Because of the wealth of expert antigen introducing cells in the skin, this organ might be especially reasonable for inoculation [19]. In any case, conveyance of a DNA immunization into mouse skin by inking bombed when detailed into cationic nanoparticles however was endless supply of the nanoparticles to shield their surface charge. Likely, adsorption of the cationic nanoparticles to the adversely charged extracellular framework in the skin forestalled their take-up by cells. Likewise, the utilization of complexing specialists *in vivo* is frequently hampered by lethality, especially for high sub-atomic weight compounds [12]. Still, progress in the medication conveyance field is steady, including inventive ways to deal with focusing of medications to specific cell types. Looking forward, improved conveyance is sure to add to expanded adequacy of mRNA antibodies [14].

Adjuvency of mRNA (vaccine)

To be proficient, immunizations ought to contain a solid adjuvant providing a peril signal for the commencement and backing of the versatile safe reaction notwithstanding a suitable antigen. The immunostimulatory properties of RNA were first found by the perception of interferon endless supply of cells to exogenous RNA separated from viruses [1]. Further help originated from manufactured twofold stranded RNA prompting interferon upon intravenous infusion into rabbits. Nonetheless, serious symptoms of these early RNA adjuvants before long restricted their further use. The possibility of engineered RNA, fundamentally delivered by *in vitro* interpretation, as immunostimulant was then re-animated especially during the most recent decade delivering a plenty of studies [3].

In 2004, *in vitro* translated mRNA was appeared to fill in as an adjuvant, in the event that it was balanced out by either complexation or synthetic modification. One year later, a solid peril signal was attributed to protamine-consolidated mRNA prompting TNF α and IFN α emission by different cells [11]. An exhaustive examination of buildings of single-stranded RNA and protamine demonstrated that phone actuation as far as cell selectivity and incited cytokine example may rely upon molecule size. As of late, explore on protamine-complexed RNA finished in a rearranged immunization approach, joining stripped and protamine-detailed mRNA [17]. The subsequent mRNA antibody comprises of two segments supplementing one another; while antigen supply is for the most part determined by the exposed mRNA, the protamine edifices contribute a solid immunostimulatory signal. Of note, protamine-figured RNA can likewise give adjuvency to, e.g., protein vaccines [31].

Among powerful adjuvant targets, RNA-detecting receptors are an especially different class of particles developed to distinguish and balance viral contaminations by coordinating the intrinsic and versatile arms of the invulnerable system [36]. Single- and twofold stranded RNAs are perceived by cost like receptors (TLR) and 3, individually, in the endosome. Nonetheless, TLR3 isn't just actuated by twofold stranded RNA, a mediator for some infections, yet in addition by mRNA either discharged from cells or created by *in vitro* transcription. Protamine-complexation of RNA seems to safeguard its association with TLRs as showed by the incitement of a few platelet types. For mRNA immunizations it was exhibited that enactment of TLR7 and possibly TLR3 is basic for preparing resistant responses. Outstandingly, ideal succession themes for receptor restricting could be recognized for single-stranded RNA [37].

Other example acknowledgment receptors might be significant for the usefulness of a RNA-based adjuvant (and antibody) too. The cytosolic helicase Apparatus I perceives uncapped RNA atoms harboring a 5'-triphosphate moiety [1]. Together with the homologous proteins MDA5 and LGP2, Apparatus I shapes a receptor family whose individuals would all be able to tie twofold stranded RNA, however have extra acknowledgment designs. For instance, MDA5 is associated with the segregation of RNAs dependent on the ribose 2'-O-methylation status of the top structure. For culmination, the cytoplasmic RNA sensors PKR and 2'-5'-oligoadenylate synthetase, hindering interpretation by phosphorylation of eIF-2 α and actuating RNase L, individually, ought to be referenced here as well. In any case, the commitment of these non-TLR RNA-sensors to the immunostimulation by RNA-

based adjuvants (and antibodies) is as yet a matter of discussion [38]. Strikingly, to the extent researched, the cooperation between endosomal just as cytoplasmic receptors and RNA is hindered if the RNA harbors particular nucleotide modifications. As an outcome, such altered RNA debilitates the structure of self-adjuvanting mRNA-antibodies [39].

mRNA-Based Immunizations

After in vivo organization of mRNA was demonstrated to be feasible, the idea of utilizing mRNA as a reason for immunizations was sought after very quickly. First achievement was accounted for in 1993 when subcutaneous infusion of liposome-exemplified mRNA encoding the nucleoprotein (NP) of flu infection was shown to inspire NP-explicit cytotoxic White blood cells (CTLs). On the other hand, stripped mRNA neglected to bring explicit CTLs up in this setting. Without further ado a while later, the utilization of exposed mRNA set off the acceptance of antigen-explicit antibodies in light of a heterologous prime-help plan (rehashed intramuscular RNA inoculation, challenge with tumor cells) [40]. Notwithstanding, none of the creatures was ensured against tumor challenge. An antigen-explicit immunizer reaction incited exclusively with mRNA was shown first utilizing molecule interceded mRNA conveyance into mouse epidermis [41].

In 2000, the field of mRNA immunizations was progressed by presenting another convention for inoculation permitting the organization of stripped mRNA by means of intradermal injection. This fundamental inoculation configuration didn't require any transfection reagents, exceptional gear or heterologous lift, yet could inspire a total versatile invulnerable reaction comprising of antigen-explicit antibodies and White blood cells with lytic action against the model antigen β -galactosidase [42]. Legitimately from that point, intradermal infusion of complete RNA confined from the Stumor cell line was appeared to actuate invulnerability to a consequent test with the tumor. Tumor development hindrance was accomplished by intradermal just as intravenous infusion of in vitro translated and lipid-complexed mRNA encoding the model antigen ovalbumin (OVA). In any case, similar to immunization with mRNA coding for a model tumor/self-antigen was not adequate to break resistance to this self-antigen in TRAMP mice [43]. A comparable methodology utilizing histidylated lipopolyplexes for fundamental infusion uncovered that MART1 mRNA couldn't just forestall B16 melanoma from movement yet in addition from metastasis [44].

In an examination of various organization courses for the conveyance of stripped mRNA antibodies great immunogenicity against ovalbumin and flu an infection hemagglutinin could be shown after rehashed and incessant infusions into the lymph node. So as to advance the immunization's power, the creators designed the antigen by including a MHC class I atom dealing signal for expanded antigen presentation [45]. Not at all like with intranodal infusion, couldn't the creators inspire such insusceptible reactions upon perinodal, subcutaneous or intradermal injections. As of late, another option, streamlined methodology was portrayed prompting effective vaccination by intradermal injection [4]. Joining exposed mRNA with protamine-figured mRNA brings about a two-segment antibody fit for initiating solid invulnerable reactions and tumor assurance in prophylactic just as helpful settings in mice. Right now, two segments satisfy integral capacities: while the stripped mRNA presents ideal antigen articulation, the protamine-complexed mRNA contributes solid immunostimulatory impacts. Prominently, for tumor treatment this new kind of mRNA antibody can be joined with other, standard, treatments, for example, chemotherapy, subsequently accomplishing improved impacts as contrasted and every treatment alone [46].

As a choice to coordinate infusion of mRNA, a resistant reaction may likewise be initiated by immunization with pAPCs transfected with mRNA ex vivo. mRNA-transfected murine dendritic cells (DCs) were appeared to evoke hostile to tumor insusceptibility in EG.7-OVA and B16 melanoma models [47]. Tumor development was likewise essentially endless supply of epidermal cells improved for Langerhans cells, which have a place with the gathering of pAPCs, that had been transfected with complete RNA got from tumor cells. Utilizing human DCs, transfection with mRNA encoding CEA or the E6 antigen of human papillomavirus type 16 actuated an essential CTL reaction in vitro [48]. Today, ex vivo mRNA transfection of pAPCs is the most every now and again utilized methodology for mRNA inoculation in the facility. For example, a clinical preliminary using telomerase mRNA-transfected DCs exhibited the ability of such applications to animate antigen-explicit cell invulnerable responses. Be that as it may, the basic technique is very tedious, difficult and needs persistent explicit (autologous) cell arrangements [49-51].

Not many clinical investigations of the immediate organization of mRNA-based antibodies have been distributed. The primary preliminary sent autologous mRNA libraries got from melanoma injuries, though in a later report a mixed drink of protamine-complexed mRNAs encoding six distinct antigens was given intradermally utilizing an increased treatment regimen. A further clinical preliminary with patients with renal cell carcinoma organize IV incorporated the organization of GM-CSF as adjuvant 24 h after immunization with six antigens [50].

Conclusion

Around two decades after the main fruitful organization of mRNA in vivo, mRNA-based immunizations guarantee to turn into a game-changing antibody innovation stage for restorative just as prophylactic applications. Today, main stream researchers is anxiously hanging tight for first clinical viability information. Be that as it may, there is as yet a wide field for additional turn of events/upgrades of mRNA-based immunizations.

The incorporation of frill mRNA atoms into a mRNA antibody might be a fascinating choice for accomplishing ideal impacts if there should be an occurrence of especially testing medicines. Besides, the blend with other enemy of tumor treatments will doubtlessly yield the best power. In any case, this would expand the unpredictability of the immunization and additionally the treatment routine creation the improvement all the more testing. Taken together, mRNA offers a promising immunization vector in the light of being adaptable, successful and safe. Thus, it could turn into a "problematic innovation" for malignancy immunotherapy, yet additionally for immunization, either prophylactic or helpful, against irresistible illnesses.

References

- [1] Sorrentino S. Human extracellular ribonucleases: multiplicity, molecular diversity and catalytic properties of the major R Nase types. *Cell Mol Life Sci.* 1998; 54:785–94.
- [2] Malone RW, Felgner PL, Verma IM. Cationic liposome-mediated RNA transfection. *Proc Natl Acad Sci U S A.* 1989; 86:6077–81.
- [3] Hilleman MR. Recombinant vector vaccines in vaccinology. *Dev Biol Stand.* 1994; 82:3–20.
- [4] Liu MA. Immunologic basis of vaccine vectors. *Immunity.* 2010; 33:504–15.
- [5] Pascolo S. Vaccination with messenger RNA. *Methods Mol Med.* 2006; 127:23–40.
- [6] Jäschke A, Helm M. RNA sex. *Chem Biol.* 2003; 10:1148–50.
- [7] Chetverin AB. Replicable and recombinogenic RNAs. *FEBS Lett.* 2004; 567:35–41.
- [8] Probst J, Weide B, Scheel B, Pichler BJ, Hoerr I, Rammensee HG, *et al.* Spontaneous cellular uptake of exogenous messenger RNA in vivo is nucleic acid-specific, saturable and ion dependent. *Gene Ther.* 2007; 14:1175–80.
- [9] Fotin-Mlecsek M, Duchardt KM, Lorenz C, Pfeiffer R, Ojkić-Zrna S, Probst J, *et al.* Messenger RNA-based vaccines with dual activity induce balanced TLR-7 dependent adaptive immune responses and provide antitumor activity. *J Immunotherapy.* 2011; 34:1–15.
- [10] Kaslow DC. A potential disruptive technology in vaccine development: gene-based vaccines and their application to infectious diseases. *Trans R Soc Trop Med Hyg.* 2004; 98:593–601.
- [11] Krieg PA, Melton DA. Functional messenger RNAs are produced by SP6 in vitro transcription of cloned cDNAs. *Nucleic Acids Res.* 1984; 12:7057–70.
- [12] Banerjee AK. 5'-terminal cap structure in eucaryotic messenger ribonucleic acids. *Microbiol Rev.* 1980; 44:175–205.
- [13] Wickens M. How the messenger got its tail: addition of poly (A) in the nucleus. *Trends Biochem Sci.* 1990; 15:277–81.
- [14] Konarska MM, Padgett RA, Sharp PA. Recognition of cap structure in splicing in vitro of mRNA precursors. *Cell.* 1984; 38:731–6.
- [15] Munroe D, Jacobson A. mRNA poly(A) tail, a 3' enhancer of translational initiation. *Mol Cell Biol.* 1990; 10:3441–55.
- [16] Gong P, Martin CT. Mechanism of instability in abortive cycling by T7 RNA polymerase. *J Biol Chem.* 2006; 281:23533–44.
- [17] Schenborn ET, Mierendorf RC., Jr. A novel transcription property of SP6 and T7 RNA polymerases: dependence on template structure. *Nucleic Acids Res.* 1985; 13:6223–36.
- [18] Arnaud-Barbe N, Cheynet-Sauvion V, Oriol G, Mandrand B, Mallet F. Transcription of RNA templates by T7 RNA polymerase. *Nucleic Acids Res.* 1998; 26:3550–4.
- [19] Pascolo S. Messenger RNA-based vaccines. *Expert Opin Biol Ther.* 2004; 4:1285–94.
- [20] Karikó K, Muramatsu H, Ludwig J, Weissman D. Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. *Nucleic Acids Res.* 2011; 39:e142.
- [21] Gallie DR. The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. *Genes Dev.* 1991; 5:2108–16.
- [22] Parker R, Song H. The enzymes and control of eukaryotic mRNA turnover. *Nat Struct Mol Biol.* 2004; 11:121–7.
- [23] Yamashita A, Chang T-C, Yamashita Y, Zhu W, Zhong Z, Chen C-YA, *et al.* Concerted action of poly(A) nucleases and decapping enzyme in mammalian mRNA turnover. *Nat Struct Mol Biol.* 2005.
- [24] Kozak M. Circumstances and mechanisms of inhibition of translation by secondary structure in eukaryotic mRNAs. *Mol Cell Biol.* 1989; 9:5134–42.
- [25] Kozak M. A short leader sequence impairs the fidelity of initiation by eukaryotic ribosomes. *Gene Expr.* 1991; 1:111–5.
- [26] Wilkie GS, Dickson KS, Gray NK. Regulation of mRNA translation by 5'- and 3'-UTR-binding factors. *Trends Biochem Sci.* 2003; 28:182–8.
- [27] Wang Z, Day N, Trifillis P, Kiledjian M. An mRNA stability complex functions with poly (A)-binding protein to stabilize mRNA in vitro. *Mol Cell Biol.* 1999; 19:4552–60.
- [28] Ross J. mRNA stability in mammalian cells. *Microbiol Rev.* 1995; 59:423–50.
- [29] Barreau C, Paillard L, Osborne HB. AU-rich elements and associated factors: are there unifying principles? *Nucleic Acids Res.* 2005; 33:7138–50.
- [30] Eulalio A, Huntzinger E, Nishihara T, Rehwinkel J, Fauser M, Izaurralde E. Deadenylation is a widespread effect of miRNA regulation. *RNA.* 2009; 15:21–32.
- [31] Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. *Nature.* 2010; 466:835–40.
- [32] Fang Z, Rajewsky N. The impact of miRNA target sites in coding sequences and in 3'UTRs. *PLoS One.* 2011; 6:e18067.
- [33] Pasquinelli AE, Dahlberg JE, Lund E. Reverse 5' caps in RNAs made in vitro by phage RNA polymerases. *RNA.* 1995; 1:957–67.
- [34] Stepinski J, Waddell C, Stolarski R, Darzynkiewicz E, Rhoads RE. Synthesis and properties of mRNAs containing the novel "anti-reverse" cap analogs 7-methyl(3'-O-methyl)GpppG and 7-methyl (3'-deoxy)GpppG. *RNA.* 2001; 7:1486–95.
- [35] Venkatesan S, Gershowitz A, Moss B. Modification of the 5' end of mRNA. Association of RNA triphosphatase with the RNA guanylyltransferase-RNA (guanine-7)-methyltransferase complex from vaccinia virus. *J Biol Chem.* 1980; 255:903–8.
- [36] Grudzien E, Kalek M, Jemielity J, Darzynkiewicz E, Rhoads RE. Differential inhibition of mRNA degradation pathways by novel cap analogs. *J Biol Chem.* 2006; 281:1857–67.
- [37] Zohra FT, Chowdhury EH, Tada S, Hoshiba T, Akaike T. Effective delivery with enhanced translational activity synergistically accelerates mRNA-based transfection. *Biochem Biophys Res Commun.* 2007; 358:373–8.

- [38] Zhao Y, Moon E, Carpenito C, Paulos CM, Liu X, Brennan AL, et al. Multiple injections of electroporated autologous T cells expressing a chimeric antigen receptor mediate regression of human disseminated tumor. *Cancer Res.* 2010; 70:9053–61.
- [39] Rydzik AM, Kulis M, Lukaszewicz M, Kowalska J, Zuberek J, Darzynkiewicz ZM, et al. Synthesis and properties of mRNA cap analogs containing imidodiphosphate moiety--fairly mimicking natural cap structure, yet resistant to enzymatic hydrolysis. *Bioorg Med Chem.* 2012; 20:1699–710.
- [40] Grudzien-Nogalska E, Jemielity J, Kowalska J, Darzynkiewicz E, Rhoads RE. Phosphorothioate cap analogs stabilize mRNA and increase translational efficiency in mammalian cells. *RNA.* 2007; 13:1745–55.
- [41] Peng J, Schoenberg DR. mRNA with a <20-nt poly(A) tail imparted by the poly(A)-limiting element is translated as efficiently in vivo as long poly(A) mRNA. 2005; 11:1131-40.
- [42] Elango N, Elango S, Shivshankar P, Katz MS. Optimized transfection of mRNA transcribed from a d(A/T)100 tail-containing vector. *Biochem Biophys Res Commun.* 2005; 330:958–66.
- [43] Carralot J-P, Weide B, Schoor O, Probst J, Scheel B, Teufel R, et al. Production and characterization of amplified tumor-derived cRNA libraries to be used as vaccines against metastatic melanomas. *Genet Vaccines Ther.* 2005; 3:6.
- [44] Kreiter S, Selmi A, Diken M, Sebastian M, Osterloh P, Schild H, et al. Increased antigen presentation efficiency by coupling antigens to MHC class I trafficking signals. *J Immunol.* 2008; 180:309–18.
- [45] Kreiter S, Selmi A, Diken M, Koslowski M, Britten CM, Huber C, et al. Intranodal vaccination with naked antigen-encoding RNA elicits potent prophylactic and therapeutic antitumoral immunity. *Cancer Res.* 2010; 70:9031–40.
- [46] Gallie DR, Tanguay RL, Leathers V. The tobacco etch viral 5' leader and poly(A) tail are functionally synergistic regulators of translation. *Gene.* 1995;165:233–8.
- [47] Karikó K, Muramatsu H, Keller JM, Weissman D. Increased erythropoiesis in mice injected with submicrogram quantities of pseudouridine-containing mRNA encoding erythropoietin. *Mol Ther.* 2012; 20:948–53.
- [48] Vivinus S, Baulande S, van Zanten M, Campbell F, Topley P, Ellis JH, et al. An element within the 5' untranslated region of human Hsp70 mRNA which acts as a general enhancer of mRNA translation. *Eur J Biochem.* 2001; 268:1908–17.
- [49] Yakubov E, Rechavi G, Rozenblatt S, Givol D. Reprogramming of human fibroblasts to pluripotent stem cells using mRNA of four transcription factors. *Biochem Biophys Res Commun.* 2010; 394:189–93/
- [50] Tan X, Wan Y. Enhanced protein expression by internal ribosomal entry site-driven mRNA translation as a novel approach for in vitro loading of dendritic cells with antigens. *Hum Immunol.* 2008; 69:32–40.
- [51] Goodarzi H, Najafabadi HS, Oikonomou P, Greco TM, Fish L, Salavati R, et al. Systematic discovery of structural elements governing stability of mammalian messenger RNAs. *Nature.* 2012; 485:264–8.