IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

How Chemical Modifications In Mrna Influence Vaccine Stability And Immune Response

Tara Zende

Student, Greenwood High International School
Bangalore, Karnataka, India

Abstract:

mRNA - or messenger RNA - vaccines have been a work in progress for several decades, often overshadowed by developments in DNA vaccines due to the safety and efficacy concerns that come with mRNA vaccines. mRNA vaccines function by delivering the coding for an antigen into the host cells. The mRNA is then translated into a protein and processed so that it elicits an immune response. mRNA is a highly favourable vaccination method due to several factors, including the rapid production process, high safety, and non- integrating nature of the mRNA.

The three primary struggles of mRNA vaccines are: stability, efficacy, and immunogenicity. These can all be addressed via three highlighted chemical modifications that have shown promise in their ability to improve the functionality of mRNA vaccines. The first modification reduces innate immune sensing upon the reception of an mRNA vaccine by modifying the nucleosides so they are able to bypass the bodies defensive mechanisms and prevent the vaccine from being identified as a threat. Furthermore, 5' cap modifications work as a protective structure that shields RNA from exonuclease cleavage, which is necessary for effective mRNA translation. Lastly, UTR (untranslated regions) and poly(A) tail modifications can extend the lifespan of mRNA and maintains intracellular stability.

This literature review aims to summarise the purposes and benefits of these modifications in the future development of mRNA vaccines, and examines the three aforementioned key chemical modifications.

1. Introduction

The rise of mRNA vaccines has been revolutionary for the modern field of vaccinology. Traditional methods—like live-attenuated, inactivated, and subunit vaccines—have done a solid job controlling many infectious diseases, but they often take a long time to develop and have challenges with scaling up production. On the flip side, mRNA vaccines provide a flexible, programmable option that can be quickly designed and produced when new pathogens emerge. The success of mRNA vaccines against COVID-19 has really showcased this technology and opened the door to applying it to other diseases, cancer treatments, and even protein replacement therapies.

At a molecular level, mRNA vaccines work by delivering synthetic messenger RNA that codes for specific proteins. After getting injected—usually in lipid nanoparticle (LNP) formulations—the mRNA makes its way into the host cells and takes over their machinery to produce the target protein. This natural production of antigens allows for presentation through both MHC class I and class II pathways, triggering responses from CD8+ T cells and antibodies, which is something that has been tough to achieve with traditional protein-based vaccines.

Despite this, naked mRNA is not without its own challenges that limit its effectiveness at first. Unmodified mRNA breaks down much faster due to RNases throughout the host, and this can provoke strong innate immune reactions through pattern recognition receptors like Toll-like receptors (TLRs) and RIG-I-like receptors. While some immune response can be beneficial, too much can reduce translation and cause side effects. These issues led to the need for creative chemical modification strategies to boost mRNA stability, enhance translation efficiency, and fine-tune immune responses.

This review will focus on three key types of chemical modifications that have been crucial for the success of mRNA vaccines: nucleoside modifications that help reduce recognition by the innate immune system, 5' cap modifications that protect mRNA and boost translation, and changes to the UTR and poly(A) tail that help regulate mRNA stability and how well ribosomes can bind. By looking into the evidence for each of these modifications, this review aims to give a thorough understanding of how chemical engineering has made mRNA a strong candidate for a viable vaccine platform.

2. Background

2. 1 Understanding mRNA Vaccines

mRNA vaccines work by producing antigens inside the host, only in the target region. These vaccines deliver mRNA, which is transcribed in a lab, into the host cells. This mRNA is then translated into proteins, which the immune system recognizes to trigger both antibody and T cell responses. This method has some key advantages over traditional vaccines. Firstly, it allows for quick design and scalable production. After a pathogen is identified, it can take as little as a few weeks to develop a scalable vaccine. Additionally, these vaccines don't use live or replicating vectors, which is essential in eliminating the risks linked to incomplete inactivation or returning to a harmful state. Because mRNA expression is temporary, it is also safer since it does not integrate into the host's DNA and is naturally broken down after its job is done.

In their review, Chaudhary, Weissman, and Whitehead point out two main types of mRNA vaccine platforms: conventional (non-replicating) mRNA and self-amplifying mRNA. Conventional mRNA vaccines only include the antigen and the necessary elements for translation. On the other hand, self-amplifying mRNA, which comes from alphavirus genomes, has genes for a viral replicase that replicates the mRNA within the cell. This could mean that lower doses are possible, but it requires larger constructs. Most mRNA vaccines being used, including those approved for COVID-19, are conventional.

2. 2 Design Principles for mRNA

The effectiveness of mRNA vaccines hinges on the careful optimization of various sequence elements. Things like proper capping with a 5' cap structure and the right length of the poly(A) tail are crucial for mRNA stability and efficiency in translation; these are fundamental design parameters. Choosing suitable 5' and 3' UTRs and optimizing codons enhances ribosome functioning, which leads to more proteins being produced. Additionally, incorporating modified nucleosides helps avoid innate immune detection and improves translation by making it easier to bypass RNA sensors, which is essential for a balanced immune response

Another extremely important aspect is the purity of the material. It is essential to eliminate double-stranded RNA (dsRNA) contaminants through chromatographic techniques to prevent excessive activation of the innate immune system and to enhance potency. dsRNA is an unwanted byproduct of lab transcription and can trigger strong type I interferon responses that interfere with translation and reduce the production of the antigen.

2. 3 Production and Delivery

mRNA vaccines are created using bacteriophage RNA polymerases like T7, SP6, or T3. Following that, they undergo purification, through enzymatic capping. This purification can also be done via the use of co- transcriptional cap analogs. This production method makes it possible to scale production up at a high rate and avoids the complexities of cell based manufacturing that is used for other types of vaccines, such as protein based vaccines.

mRNA vaccines depend greatly on the method of delivery of the genetic material inside the host body. Non viral LNPs (lipid nanoparticles) protect the mRNA from breaking down or being harmed outside the host/target cells. This ensures the safe passage of the mRNA, and allows for easy translation in the host cell cytoplasm. A typical LNP formulation features an ionizable lipid, cholesterol, a structural phospholipid, and a PEGylated lipid, all of which work together to optimize encapsulation efficiency, facilitate endosomal escape, and maximize circulation time. Ionizable lipids gain a positive charge in the acidic environment of endosomes, which disrupts the membranes and allows the mRNA to be released into the cytoplasm, which is a critical step for effectiveness.

2. 4 Immune Responses

mRNA vaccines stimulate both innate and adaptive immune responses. The innate immune system provides a kind of built-in boost by recognizing the mRNA through pattern recognition receptors, but too much sensing can actually reduce how much antigen is expressed. Using modified nucleosides and maintaining a high purity standard allows the mRNA to be accepted by the host. The natural expression of the antigen allows for MHC class I cross-presentation for CD8+ T cell responses and MHC class II presentation for CD4+ T helper cells and antibody responses, which supports the wide range of immune responses seen in clinical trials.

The fast rollout of mRNA vaccines during the COVID-19 pandemic showed that well-optimized mRNA sequences paired with LNP delivery can lead to quick development times and high efficacy.

3. Types of Chemical Modifications

3. 1 Nucleoside Modifications

mRNA contains 4 kinds of nucleosides: adenosine, guanosine, cytidine, and uridine, which, when unmodified in a vaccine, can trigger the bodies innate immune response, and this causes the host to reject the mRNA. Modifying the uridine base and replacing it with pseudouridine or N1-methylpseudouridine allows it to bypass the innate immune sensors such as TLR3, TLR7, and TLR8 by modifying the mRNAs backbone to reduce recognition. Furthermore, nucleoside modifications also allow for enhanced translation, which is inhibited when unmodified mRNA enters a host. This is because the unmodified genetic material - if transferred in vitro - can trigger an inflammatory response that hinders protein production. By modifying the mRNA, it is far less likely that the innate immune response will be triggered, and the mRNA becomes less immunogenic.

3.2 5 ’Cap Modifications

The 5 ’cap is a modified structure of guanosine, also called m7GpppN, which can be added to the 5 ’end of a eukaryotic mRNA strand. It serves many purposes in enhancing the functionality of mRNA vaccines. Firstly, it prevents exonucleases from degrading the 5 ’end of the mRNA, which prevents proper gene expression. The cap also maintains and oversees ribosome recruitment by allowing the mRNA to interact with other cap-binding proteins. This cap can either be added during the transcription process, or after, using capping enzymes.

Phosphorothioate-modified cap analogs like beta-S-ARCA provide higher levels of resistance to decapping enzymes and allow for more efficient translation. These modifications can be stereoselective, with different diastereomers showing distinct effects on translation in different cell types.

3.3 UTR and Poly(A) Tail Modifications

Untranslated regions (UTRs) are found on either side of the coding part of mRNA and are really important for regulating its function. The 5' UTR helps with ribosome attachment and scanning, while the 3' UTR is key for mRNA stability, where it ends up in the cell, and how well it's translated. Both regions have elements that interact with proteins that bind to RNA and microRNAs. Choosing the right UTR sequences can really affect how well mRNA vaccines work.

Then there's the poly(A) tail, which is basically a long chain of adenosine added to the 3' end of mRNA, helping to make it more stable and easier to translate. The poly(A) tail's length and composition play a big role in how long the mRNA lasts and how efficiently it gets translated. Recent studies have looked into using different kinds of non-adenosine nucleotides in these tails to better optimize mRNA performance for vaccines.

4. Impact on Vaccine Performance

4.1 Nucleoside Modifications: Enhanced Immunogenicity and Antigen Production

A seminal study by Pardi et al. showed that nucleoside-modified mRNA vaccines enhance both T helper cells and germinal center B cell responses, which are responsible for the development of long-lasting and permanent antibody responses. They compared these LNP-formulated, nucleoside-modified mRNA vaccines against adjuvanted protein and inactivated virus vaccines across a number of antigens and animal species.

Key takeaways included the impressive generation of antigen-specific Tfh cells, critical for forming germinal centers and refining antibody affinity. Moreover, the vaccines induced a profound germinal center B cell response and large quantities of plasma cells, consistent with eliciting high-affinity, long-lived neutralizing antibodies. Upon intradermal administration, nucleoside-modified mRNA-LNPs consistently elicited multifunctional antigen-specific CD4+ T cell responses. In head-to-head comparisons, these mRNA-LNP vaccines eclipsed both adjuvanted protein vaccines and inactivated virus preparations in terms of the elicitation of potent neutralizing antibody responses and durable protection.

These improved responses are a function of the increased antigen production that the nucleoside modification allows for. Utilizing non-inflammatory modified nucleosides means a large amount of antigen is able to be made because the modified mRNA does not get sensed by innate immune sensors, which restrict translation. The decreased innate sensing of the mRNA makes the mRNA less inflammatory, allowing higher protein production instead of being restricted due to the suppression caused by innate immunity. This high antigen production capability is directly related to the strong humoral responses observed post-vaccination with modified mRNA-LNPs.

Although the study describes the immunological advantages from nucleoside modifications, it lacks direct biochemical measurements of mRNA molecular stability, such as nuclease resistance or exact intracellular half-life. Therefore, with regard to this study, claims about changes in molecular stability are not supported. Rather than the stabilization of the molecule per se, it would appear that the major benefit is functional: strong antigen expression through minimal interference by the innate immune system.

4. 2 5' Cap Modifications: Translation Enhancers in Certain Cell Types

The study conducted by Kuhn et al. (2010) brought valuable insights into the fact that specific 5' cap modifications greatly enhance mRNA stability and translation efficiency in cell types relevant for vaccines. They had tested phosphorothioate beta-S-ARCA cap analogs, in particular the D1 diastereomer, for its effects on protein expression and immune responses.

Beta-S-ARCA-capped mRNAs demonstrated a remarkably higher translation efficiency and faster protein expression than unmodified caps in immature dendritic cells, the key players in vaccine responses. Importantly, this translated benefit was cell type-specific: whereas highly significant in immature dendritic cells, it did not appear to be present in mature dendritic cells, indicating that the effects of caps depend on the cellular context. Indeed, enhanced translation goes along with increased RNA stability in immature dendritic cells, which can support longer availability for translation and antigen presentation.

RNAs capped with beta-S-ARCA(D1) outperformed the standard caps in antigen expression and in effectively priming and expanding naive antigen-specific T cells when tested in living organisms. Beta-S-ARCA(D1)-capped RNA delivery led to higher protein expression levels, consistent with improved cellular stability and translation seen in the lab. These improvements resulted in enhanced priming and expansion of antigen-specific T cells, which prove that the advantages of cap-mediated stability and translation have concrete effects on vaccine effectiveness.

The authors found that selection of the appropriate 5' cap analog can be a personalized strategy to enhance the immunobiological availability of antigens encoded by vaccines and pointed out phosphorothioate cap analogs as a practical strategy for fine-tuning RNA vaccine translation, stability, and immune responses. Their work has provided evidence that cap chemistry does not merely protect mRNA but functionally impacts vaccine potency and can be optimized for defined cell targets.

4. 3′ UTR and Poly(A) Tail Modifications: Fine-Tuning Expression

The recent work of Medjmedj et al. (2025) systematically investigates how variations in the sequences of the 5' UTR, 3' UTR, and poly(A) tail modulate mRNA vaccine performance. The authors investigated multiple UTR combinations and introduced a novel heterologous A/G poly(A) tail in both luciferase reporters and vaccine-targeting SARS-CoV-2 spike mRNA formulated with LNPs.

The investigators tested six 5′ UTRs (representing both cap-dependent and cap-independent elements) and nine 5′–3′ UTR combinations for sequences that conferred enhanced translation in both cells and in vivo in mice. They identified the human α-globin 5′ UTR as significantly enhancing translation, while VP6 and SOD 3′ UTRs demonstrated the potential to enhance mRNA performance when combined with specific 5′ UTRs. Mechanistically, the enhanced protein expression associated with specific UTRs was correlated with an increased ribosome load and changed mRNA half-life, demonstrating the interplay of UTR selection with both translation efficiency and stability.

A further significant innovation was the use of a mixed A/G poly(A) tail instead of the standard tails. The heterologous A/G tail in this paper was compared side by side to the poly(A) tail used in the Pfizer-BioNTech COVID-19 vaccine and proved equally effective in both in vitro and in vivo assays. Changes to the tail composition correlated with findings of mRNA half-life and ribosome load, thus linking the tail structure with both persistence and translation efficiency. Of particular interest, these results suggested that the chosen 3' UTRs could add or act in synergy with the heterologous tail to yield an even better expression and stability profile. Importantly, the

chosen 3' UTRs and the heterologous A/G tail were verified through SARS-CoV-2 spike mRNA formulated in LNPs and tested in immunization in mice, making them more relevant to real-life applications of mRNA vaccines. The study showed that careful pairing of 5' UTR, 3' UTR, and custom poly(A) or A/G tails, together with the assessment of ribosome load and half-life, is a sound approach toward the improvement of mRNA vaccine potency and durability. The authors propose using the human α-globin 5' UTR and studying VP6 or SOD 3' UTR, in combination with alternative tail chemistries to enhance translation and stability for vaccine candidates. In vivo mouse validation is included in the paper; however, a substantial amount of testing is still required to build up these findings in humans and with different antigens.

5. Future Uses

As we learn more about how chemical tweaks affect the performance of mRNA vaccines, a lot of exciting possibilities for future developments are coming to light. There are a few key areas that seem to be taking priority in this field.

5.1 Personalized and Combinatorial Optimization

Most mRNA vaccines today use a standard mix of modifications that work for a wide range. In the future, we may see these modifications being tailored more specifically to certain antigens, tissues, or patient groups. For instance, different cap analogs could be better suited for vaccines aimed at varying types of antigen-presenting cells, and we could even customize UTR choices based on how quickly we want certain antigens expressed. By testing several modification strategies at the same time, we could speed up finding the best formulations for specific vaccine uses.

5.2 Expansion to Non-Infectious Disease Applications

So far, the push for mRNA vaccines has mainly focused on infectious diseases, but the strategies we've talked about here could also work well in therapeutic contexts. For example, cancer vaccines that need strong CD8+ T cell responses against tumor targets might benefit from tweaks that improve MHC class I presentation. Genetic disease treatments could use modifications that boost translation efficiency and prolong expression. In the context of autoimmune diseases, we might see adjustments that help fine-tune immune responses instead of just ramping them up.

5. 3 Next-Generation Modification Chemistries

The three types of modifications we've covered are pretty much the best we have right now, but new chemical modifications keep popping up. There are some exciting newcomers, like more nucleoside analogs beyond just pseudouridine, next-gen cap structures that offer better stability or are more specific to certain cell types, and even engineered UTRs with regulatory elements that can be triggered or are specific to certain tissues. With computational design and high-throughput screening, we could really speed up the discovery of these new and improved modifications.

5. 4 Thermostability and Global Access

One of the big hurdles for rolling out mRNA vaccines, especially in areas with limited resources, is the need for ultra-cold storage. If we can develop chemical modifications that boost thermostability, it would allow mRNA vaccines to be stored and shipped at higher temperatures, making them much more accessible worldwide. Researching ways to increase resistance to hydrolysis and thermal breakdown is crucial for ensuring vaccines can be distributed fairly.

5. 5 Self-Adjuvanting Vaccines

Currently, mRNA vaccines are trying to strike a balance between keeping innate immune activation low (to boost translation) while still retaining some adjuvant effect. Future modifications might let us fine-tune immune responses more precisely, leading to self-adjuvanting vaccines that wouldn't need extra adjuvants or complex formulations. This could involve creating mRNA constructs that activate helpful innate pathways without triggering negative responses.

5. 6 Dose Reduction and Safety Optimization

Chemical modifications that enhance translation efficiency and stability could allow for much lower doses, which would lower costs and might reduce side effects. By optimizing all modification components together—like nucleosides, caps, UTRs, and tails—we could achieve some pretty impressive results, making effective vaccination possible with far less mRNA than we currently need.

6. Conclusion

Chemical modifications have transformed mRNA from an unstable, immunogenic molecule into a powerful vaccine platform with rapid development and deployment against emerging infectious diseases. This review has discussed three critical categories of modifications: nucleoside modifications, 5' cap modifications, and UTR and poly(A) tail modifications, in relation to vaccine stability, translation, and immune responses.

These modifications, especially incorporation of pseudouridine or N1-methylpseudouridine, minimize innate immune sensing and allow high antigen expression, which subsequently results in strong Tfh and GC B cell responses that produce long-lived, high-affinity antibodies. These modifications represented a key advance enabling clinical mRNA vaccines.

Among these, 5' cap modifications, in particular phosphorothioate-modified cap analogs, confer translation and stability improvements in a cell type-specific manner; the beta-S-ARCA(D1) cap was superior in immature dendritic cells and gave higher T cell priming in vivo. Cell-type specificity of cap effects underscores the importance of matching modifications to target cell populations.

Moreover, UTR and poly(A) tail optimization affords further levers for the fine-tuning of mRNA vaccine performance: with particular 5' UTRs such as human α-globin, 3' UTRs such as VP6 and SOD, and novel heterologous A/G tails yielding improvements in translation efficiency, stability, and immunogenicity. Finally, a systematic approach to UTR and tail optimization from recent work provides a roadmap toward rational vaccine design.

The aforementioned modification strategies together address the basic limitations that initially faced mRNA therapeutics: instability, poor translation, and excessive innate immune activation. Clinical successes of mRNA vaccines against COVID-19 have validated these approaches and illustrated their potential for rapid responses to emerging pathogens. Further refinement in the chemistry of modifications, in combination with ongoing advances in technologies of vaccine delivery and manufacturing, will broaden mRNA vaccine applications to a wide array of infectious diseases, cancers, and therapeutic protein delivery. The field is poised for further innovation as researchers explore novel chemistries of modification, personalized optimization approaches, and strategies to enhance thermostability and expand global access.

References

- Chaudhary, N., Weissman, D. & Whitehead, K.A. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. Nat Rev Drug Discov 20, 817–838 (2021). https://doi.org/10.1038/s41573-021-00283-5
- Pardi, N., Hogan, M. J., Naradikian, M. S., Parkhouse, K., Cain, D. W., Jones, L., Moody, M. A., Verkerke, H. P., Myles, A., Willis, E., LaBranche, C. C., Montefiori, D. C., Lobby, J. L., Saunders, K. O., Liao, H.-X., Korber, B. T., Sutherland, L. L., Scearce, R. M., Hraber, P. T., & Tombácz, I. (2018). Nucleoside-modified mRNA vaccines induce potent T follicular helper and germinal center B cell responses. The Journal of Experimental Medicine, 215(6), 1571–1588. https://doi.org/10.1084/jem.20171450
- Kuhn, A. N., Diken, M., Kreiter, S., Selmi, A., Kowalska, J., Jemielity, J., Darzynkiewicz, E., Huber, C., Türeci, O., & Sahin, U. (2010). Phosphorothioate cap analogs increase stability and translational efficiency of RNA vaccines in immature dendritic cells and induce superior immune responses in vivo. Gene Therapy, 17(8), 961–971. https://doi.org/10.1038/gt.2010.52
- Ayoub Medjmedj, Genon, H., Dounia Hezili, Loth, A. N., Clemençon, R., Guimpied, C., Mollet, L., Bigot, A., Wien, F., Hamacek, J., Clément Chapat, & Perche, F. (2025). Evaluation of synthetic mRNA with selected UTR sequences and alternative Poly(A) tail, in vitro and in vivo. Molecular Therapy — Nucleic Acids, 36(3), 102648–102648. https://doi.org/10.1016/j.omtn.2025.102648