mRNA Vaccine: The Starting Point of the Human Body Revolution

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Abstract. In the face of the initially high incidence of allergic reactions to the mRNA vaccines against COVID-19, scientists' explanations fell short. To address this, a study delved into the history of in vitro-transcribed mRNA. The researcher proposes that a more plausible reason lies in +1 ribosomal frameshifting of certain tRNAs during the translation of in vitro transcribed mRNA in vivo. While not the first instance of experiments, the underlying mechanism remains unclear. Based on this, I have conceived an explanatory principle: "tRNA stalling leading to +1 frameshifting due to collisions." Although further experiments are needed to validate, the theoretical explanation holds a certain degree of possibility. This concept may contribute to addressing the issue of "+1 frameshifting," enhancing the functionality of in vitro-transcribed mRNA..

Keywords: mRNA vaccine, base modification, +1 frameshift..

1. Introduction

Infections are unpredictable and can have long-term consequences, even being deadly. So getting vaccinated is safer than getting sick.

Traditional vaccines work by imitating an infection to engage the body’s natural defenses. Traditional vaccines can be broadly classified into two types. The first type is attenuated live vaccines, such as the varicella vaccine, which can provide long-lasting or even lifelong protection. However, it may pose a risk of life-threatening infections for individuals with a weakened or suppressed immune system, such as those with cancer or HIV. The second type is non-live vaccines, like the DTaP vaccine, which is considered safer for individuals with a compromised immune system. Nevertheless, the protection will tend to diminish over time [1]. Moreover, traditional vaccines have two major drawbacks. Firstly, they require two or even more doses to achieve and sustain maximum immunity. Secondly, their production speed is slow and requires large-scale cell cultivation. The recent experience with the COVID-19 pandemic has once again emphasized to humanity:” We will not wait for you.” However, the mRNA vaccine may have told humans what to do.

2. Principles of Operation of mRNA Vaccines

2.1. Body’s Natural Defenses: Immune Regulation

To understand the mechanism of vaccines, it is necessary to first understand the body's immune regulation process. Immune regulation can be divided into two parts [28] [29] [30] :

2.1.1 Humoral Immunity

In this pathway, when antigens (foreign substances, such as pathogenic viruses or bacteria) are detected by phagocytic cells, these cells send signals to T cells. The T cells then secrete lymphokines to activate B cells. B cells differentiate into memory cells and plasma cells. The antibodies produced by plasma cells can specifically bind to the antigen to eliminate it. Memory cells can quickly proliferate and differentiate into plasma cells upon encountering the same antigen again, facilitating faster antigen elimination.

2.1.2 Cellular Immunity

Not all antigens are freely present in body fluids; some antigens hide inside cells. In this case, direct binding of antibodies to antigens is not possible, requiring cellular immunity. In this pathway, T cells differentiate into effector T cells. Effector T cells bind to target cells (cells invaded by the antigen), causing them to lyse and release the antigen.

Please refer to the diagram for further illustrations (Fig. 1).

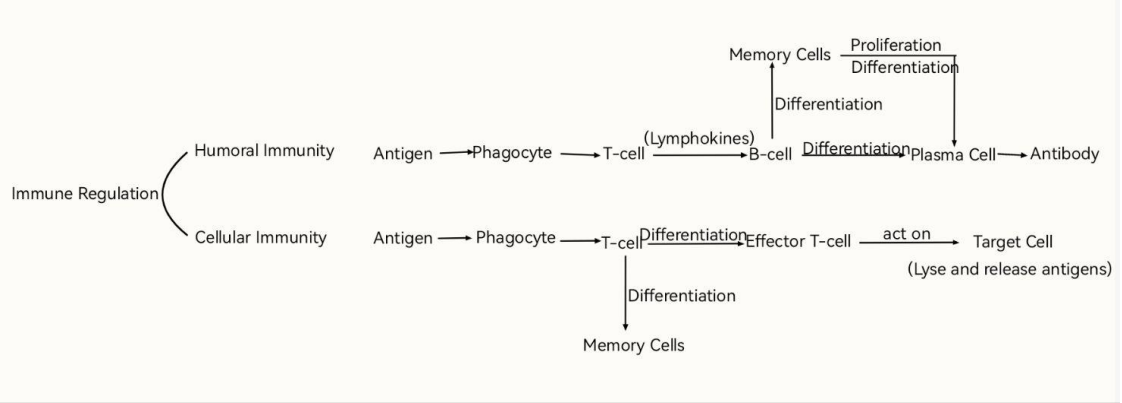

Fig. 1 The process of immune regulation

Here, we need to clarify a few points. Firstly, only plasma cells can produce corresponding antibodies. Unfortunately, plasma cells cannot proliferate, so their numbers will decrease over time once the antigenic stimulation is lost. Secondly, the number of memory cells will decrease over time after losing the stimulation caused by the antigen, which is why traditional vaccines require multiple doses.

2.2. Principles of mRNA Vaccines

If we inject the corresponding mRNA into the human body, allowing it to produce relevant proteins internally, it can indeed achieve the effect of a vaccine. In theory, this mRNA vaccine not only addresses issues of production speed and scale (e.g., a 5-liter bioreactor can produce nearly one million doses of mRNA vaccine in a single reaction [2]), but also enhances the vaccine's effectiveness and durability. Traditional inactive vaccines contain a limited amount of diverse types of proteins, leading to unevenly induced immune responses. In contrast, mRNA in mRNA vaccines can encode only the most effective antigens. After entering the body, it can generate proteins several times its own quantity. To illustrate, if you want to drink milk,traditional vaccines are like raising a herd of cows for milking, while mRNA vaccines are akin to directly acquiring the ability to produce milk on your own.

However, there is a problem: the body's immune system can recognize these foreign mRNAs and trigger rejection. This not only affects the translation efficiency of mRNA, reducing the effectiveness of vaccines, but may also have some negative effects on the body. To enable the mRNA to function inside the body, it is necessary to find ways to disguise it, deceiving the body's defense systems.

2.3. History of the Development of mRNA Vaccines

2.3.1 Base modification of mRNA

Mammalian RNA bases undergo extensive modifications, while bacteial RNA has fewer modifications. Is this difference in nucleotide modifications utilized by the body to distinguish itself from pathogens? Additionally, is externally transcribed RNA lacking modification mechanisms considered pathogenic RNA and subsequently eliminated?

In 2005, Katalin Karikó and others demonstrated the correctness of the idea. They found that vitro-transcribed RNA stimulates human TLR3, TLR7, and TLR8, but most of the nucleoside-modified RNAs are not stimulatory [3].

In 2008, Katalin Karikó experiments revealed the remarkable translation of mRNA containing pseudouridine in cultured cells. They injected modified mRNA into mice, successfully detecting translated products within the mice. The principle involves the 2',5'-oligoadenylate synthetase (OAS), which can recognize conventionally transcribed mRNA outside the body, leading to its degradation by RNaseL. However, base modification (substituting uridine with pseudouridine) suppresses this recognition process, deceiving the body's defense system.

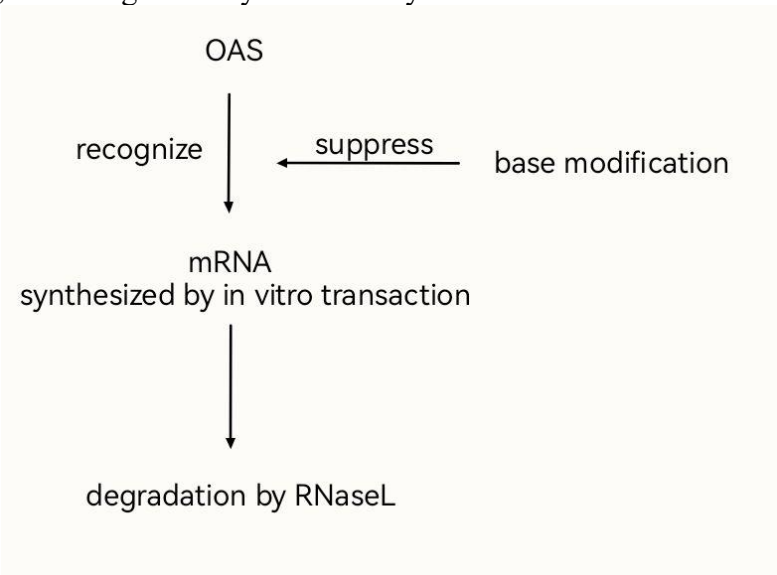

Fig. 2 The mechanism of recognition and degradation of mRNA in vitro

Furthermore, in this experiment, it was unexpectedly discovered that the translation level of in vitro-transcribed messenger RNA (mRNA) is enhanced. That is, under different modifications, it can alter the quantity of translation products, sometimes even severalfold [4].

In 2010, Katalin Karikó and Drew Weissman revealed the principle behind this. She believed that incorporating pseudouridine into mRNA enhances translation by reducing PKR activation. Protein Kinase R (PKR) is an intracellular stress sensor that functions to inhibit protein synthesis in infected cells, thereby hindering viral replication. Katalin Karikó and Drew Weissman's experimental results demonstrate that conventional in vitro transcribed mRNA induces translation inhibition, conventional in vitro transcribed mRNA activates PKR, mRNA containing pseudouridine does not activate PKR in cells, and the translation of unmodified mRNA is enhanced after inhibiting or eliminating PKR. Furthermore, mRNA containing pseudouridine does not bind with PKR [5]. Its operating principle is shown in the figure below.

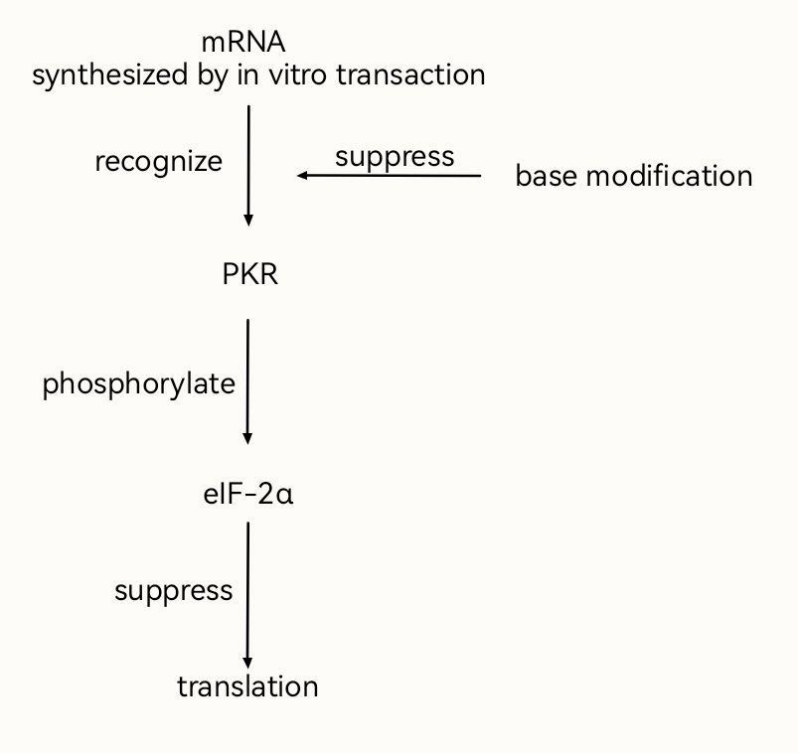

Fig. 3 The mechanism of remarkable translation of mRNA by base modification

Conventional in vitro-transcribed mRNA can activate PKR, leading to eIF-2α phosphorylation and thereby inhibiting the mRNA translation of proteins. However, base modifications can suppress mRNA activation of PKR. Turning a negative into a positive increases protein production. Thus, base modifications not only reduce adverse reactions but also enhance protein yield, eliminating crucial obstacles in the clinical application of mRNA vaccines.

2.3.2 Delivery system of mRNA

The new problem arises. Since base modification can inhibit the interaction between mRNA and PKA in vitro, will it also affect the binding of mRNA to other translation-related substances, thus impacting its function? Unfortunately, the answer seems to be affirmative. In 2015, Thomas Schlake et al. selected mRNA that could be expressed in vivo without modification and found that mRNA modified by the same method showed lower expression levels in vivo, confirming the reasonableness of this speculation [16]. Additionally, the process of cellular uptake of naked nucleic acids is inherently inefficient, and mRNA may also undergo in vivo escape, resulting in mRNA failing to reach the target cells [17]. This requires a delivery system that not only assists in capturing the necessary substances but also aids in mRNA delivery.

In the same year, Drew Weissman and colleagues delivered lipid nanoparticle (LNP)-encapsulated, HPLC-purified mRNA encoding firefly luciferase with 1-methylpseudouridine to cultured cells and mice. Administration of the mRNA-LNP complex resulted in significant protein production in vivo, suggesting that LNPs may be an effective tool for mRNA delivery [18]. In the subsequent year of 2018, researchers injected mRNA encapsulated in lipid nanoparticles (LNPs) into mice and non-human primates, demonstrating its ability to generate effective and long-lasting antibodies. This indicates that mRNA/LNP serves as a versatile platform and can be utilized as a strategy for developing mRNA vaccines [19].

As for lipid nanoparticles (LNPs), they typically consist of the following components [17] [20]:

(1) Glycerophospholipids, which constitute the main framework of LNPs, providing structural support and cell fusion capability;

(2) Cationic lipids, which aid in the capture and stabilization of nucleic acids, promote the formation process of LNPs, and facilitate nucleic acid release in circulation;

(3) Sterol lipids, which enhance the stability and cellular uptake efficiency of LNPs;

(4) Polyethylene glycol (PEG)-modified lipids, which can adjust the surface charge of LNPs, improve their stability and biocompatibility, and reduce the likelihood of clearance by the immune system.

Each component plays a crucial role in LNPs, collectively promoting the stability, delivery, and intracellular release of RNA. Coupled with the preceding base modifications, mRNA vaccines have begun to take shape.

2.3.3 Practice of mRNA vaccines

Afterwards, in 2023, the U.S. FDA approved the Pfizer-BioNTech COVID-19 vaccine (2023-2024 formula), followed by the approval of Moderna's COVID-19 vaccine. Despite being granted emergency use authorization in the context of COVID-19, this approval has not yet received full FDA licensure [22].

mRNA vaccines have shown vast potential in preliminary studies, but their optimization and enhancement still face challenges, including cost, limiting their large-scale deployment. Despite the boost from the COVID-19 pandemic, the development of mRNA vaccines encounters technical and cost issues in preparation, storage, and distribution. Overcoming these challenges is crucial for widespread implementation. Additionally, the long-term application and maturity of traditional vaccine technologies impact the extensive use of mRNA vaccines.

3. The Latest Applications and Prospects of mRNA Vaccines

3.1. The Driving Force of the COVID-19 Pandemic on mRNA Vaccines

The COVID-19 pandemic has spurred the demand for rapidly developing vaccines. Companies like Pfizer-BioNTech and Moderna swiftly introduced highly efficient COVID-19 mRNA vaccines, injecting new momentum into the development of mRNA vaccine technology. Consequently, mRNA vaccines were deployed on a large scale for the first time, showcasing their efficient research and development speed. However, the accompanying challenges have also become more apparent.

3.2. Safety Concerns of mRNA Vaccines

Anaphylactic reactions have been observed in approximately 4.7 per million anti-COVID-19 vaccinations with the Pfizer-BioNTech vaccine and 2.5 per million vaccinations with the Moderna vaccine [6], which is about two- to fourfold more than is typically seen with more traditional vaccines [7]. At that time, scientists explained that patients had pre-existing antibodies to polyethylene glycol (PEG)-modified lipids used in lipid nanoparticles (LNP). These antibodies could form in the body in response to the presence of PEG in various consumer products, such as toothpaste and shampoo [8]. Although PEG is considered safe, there are rumors that it activates the humoral immune response in a subset of the population independently of T-cells. It achieves this by directly crosslinking B-cell receptors and inducing IgM production [9].

However, according to current research, PEG is generally considered to play a supportive role in the immune response in the body rather than directly activating the immune reaction. The immunological effects of PEG are still under investigation, but the current consensus is that PEG in the body does not directly activate the humoral immune system, as rumored [10] [11].

What could be the cause of allergic reactions? If it's an individual's specific bodily reaction, the proportion wouldn't be that high. Unless, after the mRNA vaccine enters the body, some translate into unexpected proteins, it's not entirely random but follows a certain pattern of systematically mistranslating.

3.3. mRNA Vaccine May Produce Unexpected Proteins

In December 2023, James E. D. Thaventhiran and Anne E. Willis found that N1-methylpseudouridylation of mRNA causes +1 ribosomal frameshifting [12].

They artificially synthesized an mRNA capable of encoding the N-terminal and C-terminal of firefly luciferase. This mRNA, when translated normally, would produce an inactive N-terminal. However, if a frameshift occurs, resulting in +1, it simultaneously generates active N-terminal and C-terminal. These two ends undergo complementary pairing, leading to the production of luciferase, which is cleverly detectable through enzyme activity assays. After testing, only mRNA modified with N1-methylpseudouridine could express active luciferase (Fig. 4) [12].

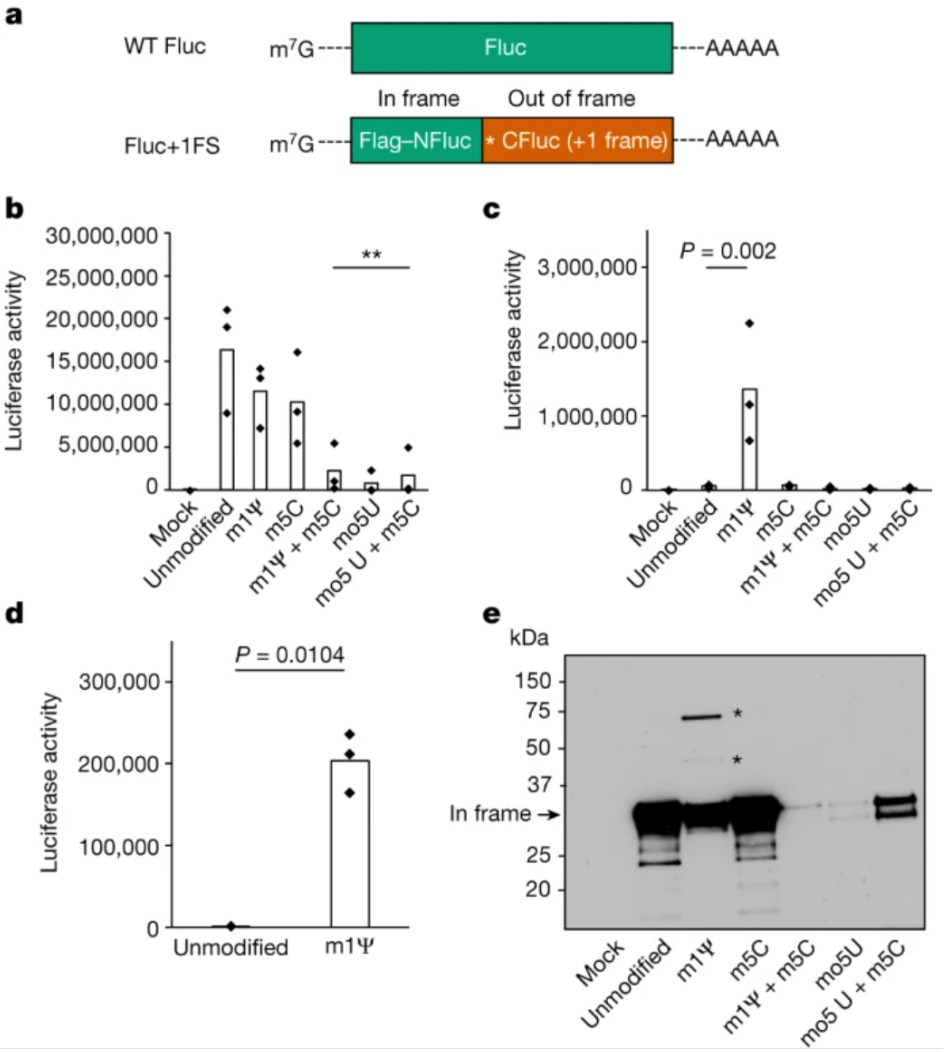

Fig. 4 Translation of 1-methyl Ψ-modified mRNA produces +1 frameshifted polypeptides [12]

If there is no frameshifting, the required protein is expressed normally, serving as an antigen to trigger an immune response. However, if a +1 frameshift occurs, it expresses alternative products (Fig. 5a) [12], causing off-target effects and rendering the vaccine ineffective. They injected Pfizer-BioNTech and AstraZeneca vaccines into mice, both expressing the required protein and eliciting an immune response (Fig. 5c) [12]. In mice not previously immunized or those immunized with the AstraZeneca vaccine, there was no response. The reaction occurred in mice immunized with the Pfizer-BioNTech vaccine because it utilizes N1-methylpseudouridine-modified mRNA, leading to frameshifting (Fig. 5b, f) [12].

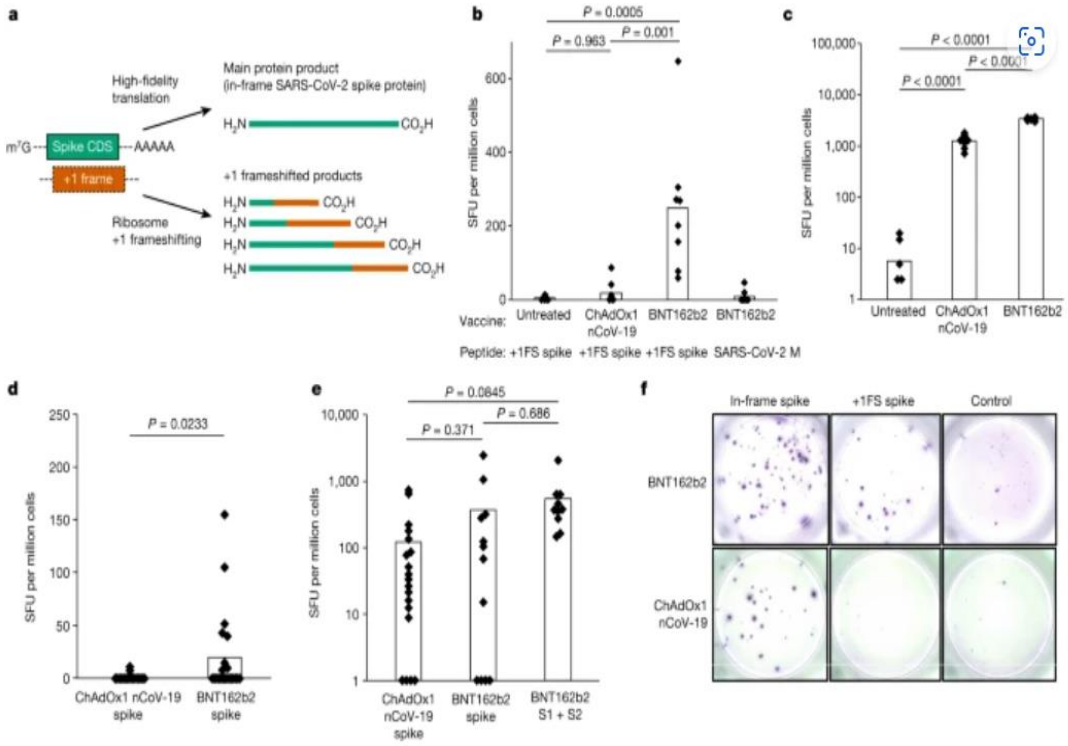

Fig. 5 +1 frameshifted products elicit off-target cellular immune responses following modified mRNA vaccination [12]

mRNA translation occurs in triplets, with each group of three bases encoding a specific amino acid. Therefore, if a displacement occurs that is not a multiple of three, subsequent translations will progressively lead to errors, resulting in significant differences in the expressed proteins. Besides causing off-target effects, could this lead to the production of other proteins the trigger allergic reactions or even the formation of toxic proteins?

The pseudouridine modification, described in research from previous years, has been characterized as significantly reducing the immunogenicity of extracellular transcribed mRNA while enhancing translation efficiency and stability. However, the unexpected result of this modification is a +1 ribosomal frameshift. The position of this shift is determined, allowing the pre-design of mRNA sequences to generate the expected proteins. Yet, if the conditions triggering the frameshift are random, it becomes challenging to accurately determine the product of a single translation. Considering mRNA vaccines contain multiple mRNA molecules, the consequence is the presence of a small amount of unintended by-products among the vast majority of expected products. The defined shift position and uncertain shifting conditions might be the fundamental reasons for the occurrence of allergic reactions.

3.4. Inference of the Principle of +1 Frameshift

The "components" of the human body are extremely precise, and even minor changes may affect the final functional outcome. The modification of pseudouridine indeed allows mRNA to evade the immune system. However, when tRNA binds for translation, some tRNAs may still detect the changes in bases, or the modification of pseudouridine alters the translation environment, potentially causing abnormal function in some tRNAs. Daniel E. Eyler et al. revealed that replacing a single uridine nucleotide with pseudouridine in an mRNA codon impedes amino acid addition and EF-Tu GTPase activation. They also found that the presence of pseudouridine can promote the low-level synthesis of multiple peptide products from a single mRNA sequence in the reconstituted translation system as well as human cells [27]. In simple terms, one consequence of tRNA encountering pseudouridine is a moderate alteration in translation speed, which may also be a self-protective mechanism of the body.

The change in translation speed caused an adaptive stress response, resulting in translation stalling, where tRNA stalled on mRNA cannot be promptly decomposed by the ASC-1 complex (ASCC), potentially affecting mRNA translation rates [13]. This precisely explains the phenomenon observed by James E. D. Thaventhiran, Anne Willis, and others regarding the slow translation rate of pseudouridine-modified mRNA. In 2018, Michael F. Jantsch [28] also mentioned that they unexpectedly discovered in experiments that translation stalling occurs when inosine is present in codons. This proves that changes in translation speed due to base modifications are not exclusive to pseudouridine.

How is the +1 frameshift generated? In 2019, Carrie L. Simms, Liewei L. Yan, Jessica K. Qiu, and others observed a positive correlation between ribosome density and frameshift efficiency [14]. Based on this observation, it is inferred that "tRNA collisions on mRNA may lead to +1 frameshifting." If the stalled tRNA is not disassembled, collision may occur when the next tRNA arrives, resulting in a +1 frameshift.

In summary, a specific tRNA recognizes pseudouridine on the mRNA, causing the translation speed on the mRNA to slow down, thereby triggering adaptive emergency translation, leading to translation stalling. The stalled mRNA cannot be timely degraded, slowing down the translation speed. When the next tRNA collides with it, the preceding stalled tRNA undergoes a +1 frameshift, and the originally stalled tRNA continues translation after displacement, resulting in errors in protein translation.

3.5. The Conceptual Solution Idea for the +1 Frameshift

If the inference based on the principles mentioned is reasonable, then the problem of +1 frameshift can be addressed from the following perspectives, thereby improving the accuracy of in vivo translation of extracellular transcribed mRNA.

3.5.1 Resolution of tRNA Collision Issues

The collision of tRNA may be the direct cause of +1 frameshifting. If we could efficiently degrade stalled tRNA, the problem would be easily resolved. In the translation surveillance mechanism known as ribosome-associated quality control (RQC), the ASC-1 complex (ASCC) plays a crucial role in disassembling stalled ribosomes on mRNA. The key to completing this process lies in the normal synthesis of the ASC-1 complex and methods to enhance its functionality. Strategies to improve the disassembly of stalled ribosomes by the ASC-1 complex (ASCC) involve various approaches, such as enhancing the expression of ASCC subunits, optimizing ASCC's ability to dismantle ribosomes, and regulating interactions with other proteins. Additionally, studying the mechanisms of ribosomal quality control may reveal new regulatory points, ultimately enhancing the efficiency of the ASC-1 complex in dealing with ribosomal stalling [15].

3.5.2 Elimination of the mRNA after a tRNA Collision Occurs

Rather than producing proteins that could be harmful to the body, stifling this translation at its inception is considered a viable solution. According to the research by Carrie L. Simms, Liewei L. Yan, Jessica K. Qiu, and others [14], collisions emit signals for mRNA degradation through a process called No-Go Decay (NGD). When NGD is impaired, stalling affects ribosomal function, leading to +1 frameshifting. Ensuring the proper functioning of this mechanism may potentially eliminate the hazards associated with +1 frameshifting in translation.

3.5.3 Translation of Resolving the tRNA Stalling Issue

RNA modifications can affect the fate and function of all classes of RNAs [23]. Moreover, modifications can affect RNA processing, stability, localization, and translation [24]. The fundamental reason behind all these problems may lie in the potential re-modification of the bases, possibly deceiving this portion of tRNA once again. However, could this lead to recognition by other tRNA molecules, causing different frameshift issues? In comparison to the two aforementioned solutions, this approach may be more challenging, potentially requiring a deeper understanding of the

recognition signals and corresponding functions of various components in the human body. This could lead to the development of a "human body component dictionary," possibly becoming the ultimate goal of human research. The RNA Modification Database, MODOMICS, is a good example. It provides comprehensive information about the chemical structures of modified ribonucleosides, their biosynthetic pathways, RNA modification enzymes, and the positions of modified residues in RNA sequences [25]. Currently, it has cataloged over 150 different types of modifications and continues to be updated [24].

4. Discussion

The impact of in vitro transcribed mRNA is not limited to vaccine development; it spans gene therapy, biomedical research, biosynthesis, and more. Its profound effects are poised to become a central direction in the future development of biotechnology [21]. In the last two decades, in vitro-transcribed mRNA has emerged in intracellular translation, contributing to its deployment in the face of COVID-19.

The challenges observed in this recent deployment may indicate that mRNA research is still in its early stages, with much yet to be understood. The emergence of the "+1 frameshift" issue could pose a new hurdle in mRNA research. In response, I have conceptualized the principle of "collision-induced +1 frameshift." Building upon this theory, I have proposed three solutions. However, due to constraints, this theory remains grounded in previous experimental observations and has not undergone relevant experiments for validation.

5. Conclusion

Despite renewed concerns about the safety of mRNA vaccines, their outstanding performance in swiftly addressing the pandemic, high efficiency, and long-lasting effects keep the outlook for their development optimistic. Perhaps the development of mRNA vaccines is just the beginning of in vitro transcription mRNA technology, which could become a key element in future human enhancement. In comparison to DNA sequence modification, in vitro transcription of mRNA is not only safer but also avoids the unpredicted risks associated with genetics. Looking ahead, it is anticipated that in vitro transcription mRNA technology will play a crucial role in areas such as gene therapy and personalized medicine, driving advancements in medicine and biotechnology.

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