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News & Views

How much genuine is mRNA vaccine: Transferring message of small size grabbed Nobel Prize

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Unfeasible to visible

Since the appearance of corona pandemic in early 2020 a lot of debates are going on regarding efficacy of mRNA vaccine among scientific fraternity. Once we talk about mRNA vaccine, it delivers some kind of unfamiliar messages, and the scientific community is skeptical about its effectiveness. However, the intellectual groups are very much aware that as early as 1990, *in vivo* protein synthesis due to the direct introduction of a mRNA reporter gene in the skeletal muscle of mice has established the proof of the concept (Wolff *et al*., 1990). Within two years of this initial report, researchers reported that intra-hypothalamic injection of synthetic copies of mRNA encoding the hormone vasopressin was able to translate functionally active hormone in rats genetically deficient to synthesize vasopressin; thus, reversing diabetes insipidus for transitory period (Jirikowski *et al*., 1992). From 1796, the first clinical trial of smallpox vaccine, the scientific development in vaccine designing has marched a long journey and is no more confined to Louis Pasteur's "3i"s (isolation, inactivation, and injection) principle, rather genetic engineering has taken center stage in vaccine production (Plotkin and Plotkin, 2011). To design a safe vaccine that can elicit protective immunity for an extended period, the modern vaccine manufacturing process is blending structural biology and systems biology information for quality assurance. *In vitro* synthesized short stretch of single-strand mRNA molecules that carry information contained in a gene is called transcript. Once the transcript gets access to enter the cell cytoplasm, it can direct the ribosome to synthesize the protein as per the message written on it. The message inscribed in the transcript may be of small size, but the intricate science behind it to translate the letters in amino acid language to generate an immunogenic life-saving protein has been recognized for the Nobel Prize in Physiology and Medicine for the year 2023. On 2nd October 2023, the Nobel Committee at Karolinska Institute, Sweeden, announced the Nobel Prize in Physiology or Medicine to be shared jointly by Katalin Karikó and Drew Weissman for their groundbreaking discoveries that made it possible to develop effective mRNA vaccines against COVID-19 at a breakneck speed amid COVID crisis that saved millions of lives on our earth. Across the world, several types of vaccines are used to prevent infectious diseases in humans and animals. Those are live-attenuated vaccines, inactivated vaccines, subunit (recombinant, conjugate, and polysaccharide vaccines), toxoid vaccines, viral vaccines, and recent mRNA vaccines. Except for mRNA vaccines, all these vaccines contain proteinaceous components as an immunizing agent; in contrast, the mRNA vaccine comprises nucleotides that instruct the host cells to make desired proteins that induce protective immune responses. In simpler language, the lab-grown mRNA, when introduced to hosts as a vaccine agent, instructs the ribosome of cells to manufacture immunogenic protein under the supervision of instructor (Dolgin, 2021a). Way back in 2012, Andy Geall's team at Novartis's laboratory in Massachusetts made a crucial attempt to pack a small strand of RNA bases inside a minuscule of the fat droplets to use that greasy RNA to vaccinate rats against the respiratory virus (Geall *et al.*, 2012). There are currently two different types of synthetic RNA vaccines: conventional mRNA and self-amplifying mRNA (Bloom *et al*., 2021). Pharma giants were very much cognizant that the mRNA vaccine manufacturing process is scalable, devoid of potential risk of infection or insertional mutagenesis, yet with all these wonderful attributes, they never took it seriously to develop mRNA therapeutics due to the highly unstable character of mRNA. Additionally, lab-grown mRNA has inherent potentially harmful immune responses and lacks adequate *in vivo* delivery techniques, which are its limitations. Toll-like receptors expressed on immune cells are smart enough to recognize *in vitro* transcribed synthetic mRNA as foreign and initiate an unwanted troublesome inflammatory response in the recipient. TLRs bearing immune cells are cruel enough to destroy foreign mRNA in a rapid manner. Initially, researchers were not so confident to rely on the efficacy of mRNA

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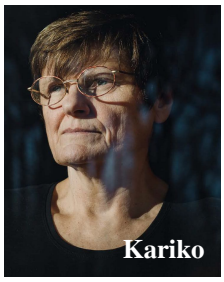
for its use as a vaccine candidate, but Karikó and Weissman from the University of Pennsylvania demonstrated that exchanging the uridine base of synthetic mRNA with another base pseudouridine (ψ) structurally like uridine is escaped from immune cell recognition, resulting no untoward reaction in vaccinee. This innovative idea of nucleoside swapping resulting in the suppression of RNA recognition by Toll-like receptors has revolutionized RNA biology for the human benefit (Karikó *et al*., 2005). This critical part made it possible to bring success in the mRNA vaccine. All these downsides of mRNA vaccines have now been wiped out with technical inputs, and ultimately, the pioneers of mRNA COVID vaccines won the Nobel in medical science. The mRNA vaccines have saved millions of lives and prevented COVID-19 globally, said the Nobel Committee (Callaway and Naddaf 2023). During March 2020, Dr. Mike Ryan, an executive director of WHO's health emergencies program, once said "Bad vaccine is more dangerous than a bad virus". Two RNA vaccine candidates, one from US pharmaceutical giant Pfizer and BioNTech in Mainz, Germany, and another from Moderna in Cambridge, Massachusetts, got emergency approval from regulators to be used against COVID-19 (Ledford 2020). Thirty-one-year-old Sean Doyle, a medical student and PhD candidate at Emory University Hospital Emory School of Medicine in Atlanta, decided to volunteer for an experimental mRNA COVID-19 vaccine that has ultimately been found to be safe for millions of recipients (Gupta, 2020). If we look back at the history of the mRNA vaccine, since the discovery of mRNA in 1961 (Brenner *et al*., 1961), ceaseless effort is going on use it for the benefit of human beings. The earliest clinical trial of mRNA vaccine against rabies for human was initiated during 2013 (Alberer *et al*., 2017). However, the mRNA vaccine against COVID-19 has received global celebration.

Scientific carrier of Nobel Prize winners


Kariko

Katalin Karikó was born in Hungary in 1955, graduated in biology from the University of Szeged, and received her PhD in biochemistry in 1982. She then conducted postdoctoral research at Temple University, Philadelphia, and the University of Health Science, Bethesda. In 1985, Karikó emigrated from Hungary to the US, and in 1989, she started working as an Assistant Professor at the University of Pennsylvania. At the time of the award, Kariko was affiliated with Szeged University, Hungary, as well as the University of Pennsylvania, Philadelphia, and as an adjunct professor at Perelman School of Medicine. She is the 13th woman in history to be a recipient of the Nobel Prize in Medicine. As she failed to secure a routine research grant to pursue her idea on mRNA molecules of her interest, so in 2013, she left for Mainz, Germany, where she joined BioNTech, a little-known biotech firm specializing in RNA pharmaceuticals that has produced COVID-19 vaccine (Nair, 2021).


Weissman

Weissman was born on September 7, 1959, in Lexington, Massachusetts, to a Jewish father and Italian mother. After graduating in 1977, he received master degree in 1981 from Brandeis University. He became a doctor of medicine and received his doctorate in immunology and microbiology from the University of Boston in 1987. Weissman moved to the Perelman School of Medicine at the University of Pennsylvania to start his laboratory to study RNA and innate immune system biology (Jewish library, https://www.jewishvirtuallibrary.org/drew-weissman). Presently, Karikó is senior vice president at BioNTech RNA Pharmaceuticals (Mainz, Germany), and Weissman, is director of vaccine research at the University of Pennsylvania, USA.

Pride of nucleotide

DNA is the blueprint of life that encodes protein molecules, which are the "workhorses" of the cell, carrying out all the functions necessary for a living organism (Clancy and Brown, 2008). DNA needs to be transferred to messenger RNA (mRNA) that acts as a template for protein production. According to the template, the protein structure is designed for smooth production inside protein synthesis workshop using host cell machinery tools. Therefore, mRNA represents the intermediate stage of translation of mRNA letter to the final product in the form of amino acid, the unit of protein blocks. Scientists were curious about synthesizing mRNA in cell free system to probe whether the synthetic mRNA can do the same job like that of naturally produced conventional mRNA if introduced in host cell or not. Since 1980, once cell free *in vitro* production of mRNA became available in the form of *in vitro* transcript, it has been employed for its therapeutic application (Sahin *et al*., 2014). Paul Krieg, a developmental biologist at the University of Arizona, was able to synthesize microgram quantities of eucaryotic mRNAs

and subsequently, when injected with wheat germ extract into the cytoplasm of frog oocytes, it exhibited functionality of mRNAs (Krieg and Melton, 1984). Unlike DNA, putting RNA inside a host cell is not so easy. However, in 1987, a breakthrough experiment was conducted by Robert Malone by mixing mRNA molecules with fat droplets to create one type of molecular soup, which was happily taken up by human cells and initiated creating protein out of it (Malone *et al*., 1989). According to an excerpt from Robert Malone's lab notebooks, what he wrote on 11th January 1988, is like this "if cells could create proteins from mRNA delivered into them, it might be possible to "treat RNA as a drug" (Dolgin, 2021b). For many years after Malone's experiments, mRNA received a bad reputation for unbelievable instability. For mRNA vaccine production, a uniform comprehensive framework has been prepared that includes design, synthesis, and delivery technique.

Decision with precision

Once the researchers could find that Spike protein of coronavirus can elicit protective antibody, they targeted the spike protein gene as vaccine candidate. Cell-free synthesis of mRNA can be made from a linear stretch of DNA template using DNA-dependant RNA polymerase enzyme derived from T7 bacteriophage (Pardi *et al*., 2013). Therefore, in the next step, the spike protein gene already inserted in the plasmid is used as a template to create a newly synthesize single-strand mRNA molecule using RNA polymerase enzyme.

An ideal mRNA vaccine agent should carry a nucleotide cap (methylated 5' guanosine monophosphate) at 5' and a short sequence of the untranslated region both 5' and 3' (5' and 3' UTR), an open reading frame that has to be read and translated into amino acids and finally a tail piece comprises of multiple adenine molecules (Poly A tail). Self-amplifying mRNA molecules have all these five elements, additionally a replica unit that supports self-amplification of mRNA construct (Jackson *et al.*, 2020). Optimization in each of these five elements is needed for translation escalation (Chaudhary *et al*., 2021). The mRNA is engineered in such a manner that it should be like fully functional mature mRNA molecules, which naturally occur in the cytoplasm of eukaryotic cells. Thus, lab-grown mRNA is supposed to be good enough to instruct recipient cells to synthesize the desired proton as per the encoded message written on it, but all is not good. The naked mRNA does not enter the nucleus of the host cells, and mRNA is cleaved by extracellular RNAase. Concurrently, mRNA is not easily internalised by recipient cells, so some modification is needed to achieve this (Tsui *et al*., 2002). Once the synthetic mRNA is delivered inside the cytosol by an improved delivery system, the ribosome can utilise the mRNA for protein synthesis. The expressed protein may prefer to reside intracellularly or be expressed in transmembrane or secretory form. The recipient's immune system is capable enough to detect the novel protein available at any of these locations to mount an immune response against it (Jackson *et al*., 2020). Earlier reports have shown that RNA acts as an agonist for several TLRs, such as TLR3, TLR7 and TLR8 (Anderson *et al*., 2010). Expression of protein has been detected within 12-24 hours of intramuscular injection of naked mRNA that persisted up to 6 days before it is catabolised (Vogel *et al*., 2018). Nucleoside modifications, mRNA formulation, route of administration, and sequence optimization can influence the kinetics and magnitude of protein expression (Pardi *et al*., 2015).

New swing in mRNA string

As early as 2000, Weissman observed that RNA could activate human dendritic cells for its maturation (Weissman *et al*., 2000). Contaminating the double-stranded region of RNA might be the cause of the activation of DC through TLR3, as was predicted (Kariko *et al*., 2004). Interestingly, *in-vitro* transcribed mRNA or total RNA derived from bacteria can induce dendritic cells for upregulation of IL-12 secretion, but this was not the case with mRNA from eukaryotic cells (Koski *et al*., 2004). Fundamentally, mRNA produced within nuclei of live cells completes its job within cell cytoplasm and is never released out of its residence, so a chance to encounter immune cells never happens, but that is not the case once mRNA is introduced through a systemic route. Karikó and Weissman speculated that contrary to natural mRNA, something unusual is there in synthetic mRNA that might be the cause of immune activation. Universally, DNA is composed of four bases: A, T, G, and C, whereas RNA contains four bases, abbreviated A, U, G, and C; hence, U is the differentiating point between DNA and RNA. Drew and Kariko realised that the mRNA on which they were working was no good for the therapeutic applications because of its labile and immunogenic nature, so they designed different variants of mRNA, each with unique chemical alterations in their bases. Once they modified RNA base just swapping uridine base, pseudouridine (ψ) or 2-thiouridine (s2U), the altered mRNA was saved from the immune cells' attack (Fig. 1). Pseudouridine (5-ribosyluracil) is considered to be the "fifth nucleoside" in RNA. Dendritic cells (DCs) exposed to such modified RNA expressed

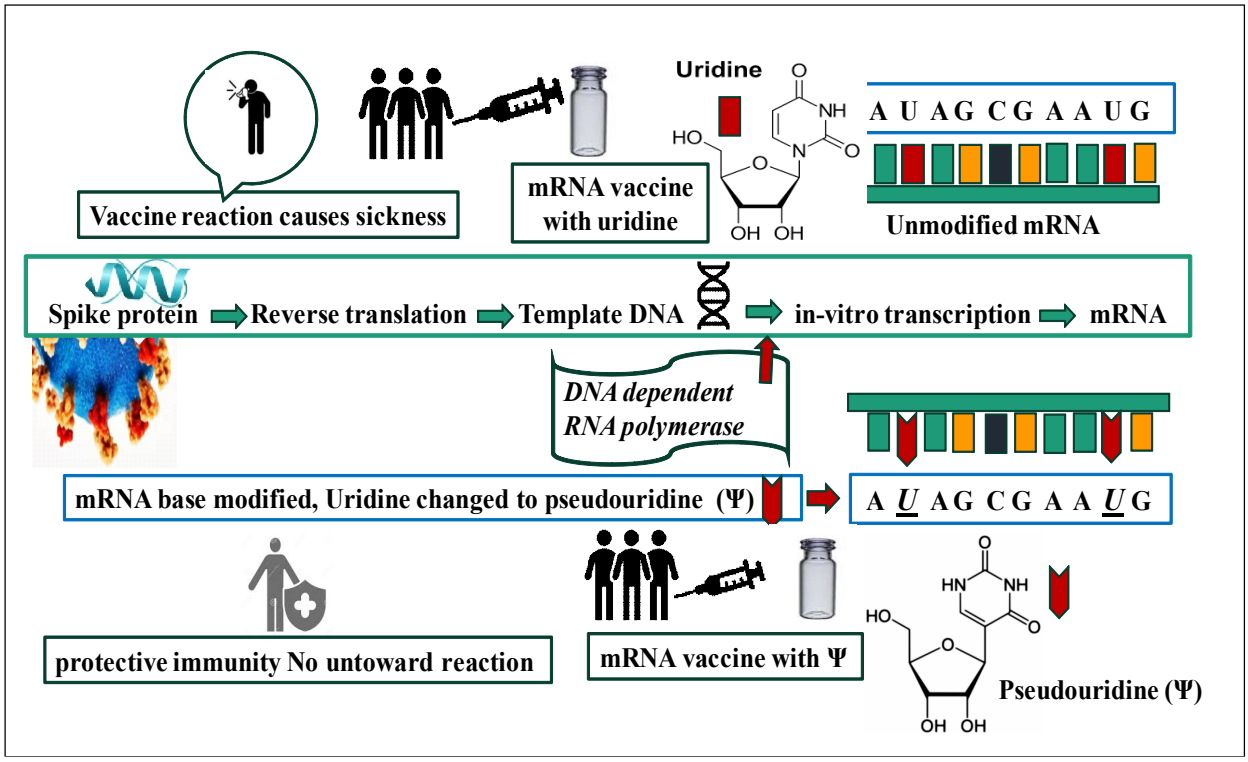


Fig. 1. Safety of COVID-19 mRNA vaccine with modified Uridine base (Kariko *et al*, 2005)

significantly fewer cytokines and activation markers as compared to unaltered RNA (Kariko *et al*., 2005; Fig. 1). This was the groundbreaking work which they published jointly in 2005, making them renowned for being crowned with the Nobel Prize in medicine for 2023 (Callaway and Naddaf, 2023). This was the turning point in their scientific career. In their subsequent experiment using *in vitro* cell lines and mice model, Kariko group found that mRNAs containing pseudouridines have a higher translational capacity than unmodified mRNAs (Kariko *et al*., 2008). Subsequently, they observed that pseudouridine (ψ) containing mRNA having reduced ability to activate RNA-dependent protein kinase enzyme is the underlying mechanism of enhanced protein translation (Anderson *et al*., 2010). Similarly, incorporation of 5-methylcytidine (m5C), N6-methyladenosine (m6A), 5-methyluridine (m5U) or 2-thiouridine (s2U) in synthetic mRNA was unable to induce inflammatory cytokine response in dendritic cell under *in vitro* condition (Kariko *et al*., 2005).

Next part was how to deliver the mRNA effectively inside a living cell so that mRNA can be translated in the form of protein. Use of lipid nanoparticles as vehicle to carry the mRNA for cytosolic delivery was adapted by several workers (Callaway and Naddaf, 2023). Nanoparticles are synthetically formulated with a combination of four different types of lipid components, which are invariably present on the eukaryotic membrane so that the fusion of both can be accelerated for early delivery of cargo. The positively charged cationic lipid of the nanoparticle can entrap negatively charged mRNA due to electrostatic interaction as well as efficiently bind with negatively charged membranes. Cytosolic pH favours its disruption to release the entrapped mRNA (Burki, 2021). For *in vitro* transfection of mRNA, cationic lipids or polymers based transfecting agents such as TransIT-mRNA (Mirus Bio LLC) or Lipofectamine (Invitrogen) are commercially available that work well in many primary cells and cancer cell lines (Kariko *et al*., 2008). The improved form of liposome as a transfecting agent is composed of four units viz, (i) ionizable cationic lipid, (ii) a helper lipid, (iii) cholesterol and (iv) polyethylene glycol (Jeffs *et al*., 2005).

Drugs to be sure when too many cures

When COVID-19 outbreak appeared during December 2019, BioNTech and Moderna came forward to manufacture mRNA vaccine as per the technical knowhow published by Karikó and Weissmann (Mulligan *et al*., 2020). The first regulatory authorizations for mRNA vaccines were given in December 2020 to Pfizer and BioNTech (The Lancet, 2021). During the initial trial, vaccine efficacy of both the Pfizer/ BioNTech and Moderna vaccines against

COVID-19 was found to be more than 90% in the older population (Anderson *et al.*, 2020). The vaccine used by Pfizer/BioNTech and Moderna both having mRNA in which base uridine is replaced with N1-methylpseudouridine (m1ψ) to avoid any unpleasant inflammatory responses in recipients (Fig. 1). Both the vaccine produced protective antibody and memory B and T cell response, but the duration of the antibody was short-lived, therefore, booster immunization was mandatory (Husby and Kober, 2022).

Conclusion

Scientific efforts to modify the nucleoside base have made it possible to develop an effective vaccine against COVID-19 that has been recognised with the Nobel Prize in Medicine for 2023. Due to the labile character of mRNA, it is a delicate issue and seems to be unlikely to be used as therapeutics initially. Kariko and Weissman, both scientists who already had expertise in mRNA biology, took a bold step to exchange one of the nucleotide base uridines with pseudouridine (ψ), resulting it being safe for human use without any untoward immune reaction. The production of vaccine has been achieved in less than one year that has saved countless lives. The technology has made it possible to develop COVID-19 shots vaccine at unprecedented speed. Thereby, the world has recovered from the worst pandemic. With this new idea, scientific groups are confident to face any other severe disease, if at all, appears in future.

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