**Field of Materials and Production**

**Achievement : For pioneering research contributing to the development of mRNA vaccines**

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## The role of mRNA in protein synthesis

Vaccines are medicines that remind the immune system to attack proteins particular to specific pathogens in order to gain immunity (i.e., resistance) to external pathogens such as viruses and bacteria.

COVID-19 infects human cells by attaching to them using spike proteins (the red, club-shaped elements seen in Figure 1.) Previous coronavirus research has shown that fragments of those spike proteins can act as antigens and activate the immune system to produce antibodies.

The problem with using spike proteins in vaccines is how to get them into the body. It is possible to administer them directly, as is done with conventional vaccines, but mRNA vaccines harness mRNA, which serves as a blueprint for cells to synthesize spike proteins, and which can be synthesized artificially before being administered.

Our bodies know which proteins to make by using our genetic blueprint or DNA. The information on DNA needed for protein synthesis is copied over to the mRNA, whereupon the ribosomes in the cells read that information and begin synthesizing protein. This biological function is used to effectively produce spike proteins within the body.

## How the COVID-19 vaccine works

What mechanisms do COVID-19 vaccines harness to prevent viral infection and reduce symptom severity?

First, vaccination allows the spike protein-coded mRNA to enter the body. That mRNA uses the body's protein synthesis ability to produce spike proteins. Since these proteins are recognized as coming from outside of the body, also known as "non-self" proteins, the immune system is activated to produce antibodies (left image in Fig. 2). Once the immune system has made a record of spike proteins, if COVID-19 virions enter the body, a rapid immune response is induced (right image in Fig. 2).

Vaccines were conventionally made using attenuated versions of a virus or even the viral pathogens themselves, so it took time to guarantee a vaccine's safety, i.e., to guarantee that the vaccine would not cause illness, and to confirm its effectiveness in granting immunity to the disease being targeted. On the other hand, the induced production of spike proteins in the body involves no use of an actual virus, so there is no risk of infection, and the antigenic efficiency of spike proteins can be easily shown, so such vaccines can be made highly effective.

This is the basic mechanism through which the mRNA COVID-19 vaccine works.

## Paving the way to mRNA drugs with pseudouridine

The reason why mRNA had not been used in pharmaceutical products was because it degrades quickly in vivo and, particularly when administered from outside the body, it is recognized as abnormal by the body thereby triggering an inflammatory immune response. As a result, cells administered with mRNA were dying before they could produce the amount of protein needed to acquire immunity, and vaccines were causing fevers and other issues in recipients.

Professors Katalin Karikó and Drew Weissman began collaborating in 1998 when they were both at the Perelman School of Medicine at the University of Pennsylvania in the hopes of conducting research into the potential of harnessing mRNA in drug creation. In 2005, they discovered that they could look at mRNA as a single substance, and that they could replace its constituent uridine with a modified version called pseudouridine, thereby suppressing any undesired immune response (see Fig. 3).

At the time, it was known that immune response can be caused when mRNA introduced from outside the body binds to Toll-like receptors on the cell membrane, but it was not yet known that this binding occurs by way of uridine. After repeated experimentation, Karikó and Weissman learned that mRNA with pseudouridine does not bind to those receptors.

## After years of mRNA research and related technological development, a vaccine is born

The mRNA vaccine may seem to have appeared all of a sudden, but in reality, mRNA first began to be considered for use in medical applications more than 30 years ago. Research has continued since then, even though it did not lead to development of any drugs. It wasn't until 2020 and the urgent need presented by the COVID-19 pandemic arrived that a practical mRNA vaccine was successfully made.

Bolstering the seemingly short-term development of the mRNA vaccine was the accumulated wisdom of the entire body of molecular biology

Figure 1: The role of mRNA in protein synthesis

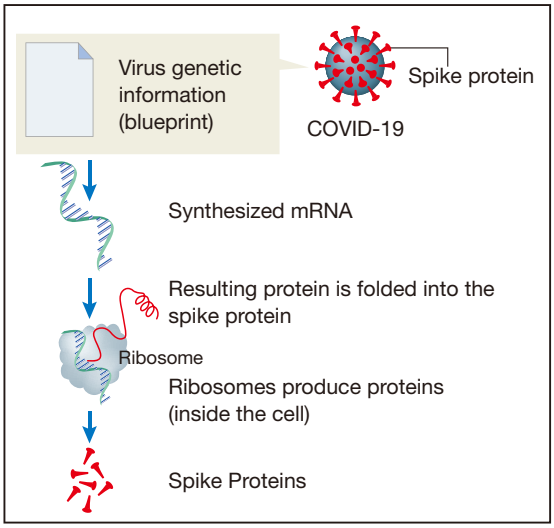
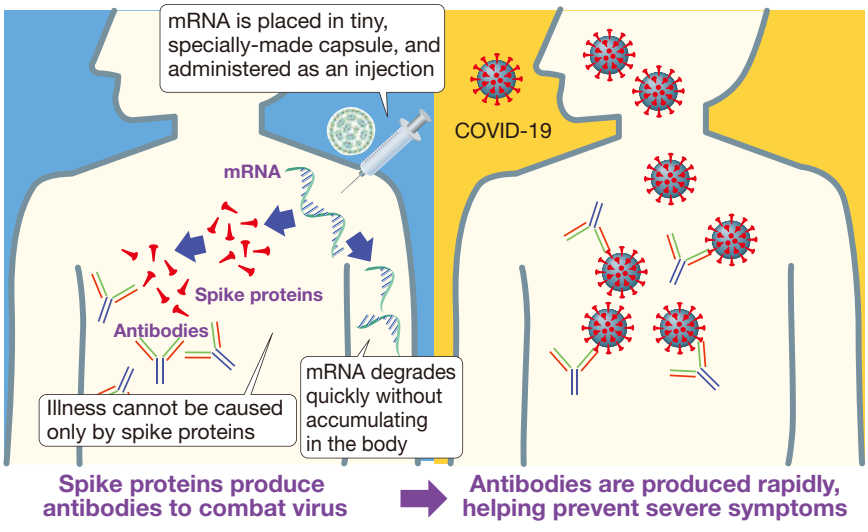



Figure 2：How the COVID-19 vaccine works



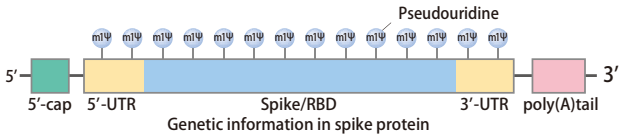
research. Particularly important was Karikó and Weissman's 2005 discovery that pseudouridine could replace uridine, which made it possible to administer mRNA from external sources. Moreover, they showed in 2008 that pseudouridine-mRNA efficiently produces proteins in vivo, and in 2012, they successfully achieved highly-efficient protein production within mice. These fundamentally important findings were the result of the collaboration between the two researchers.

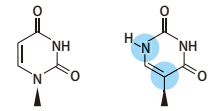
The most important characteristic of mRNA is that it can be artificially designed to make cells produce the specific protein desired. Harnessing this feature not only allows for the creation of vaccines to use against other infectious diseases, but it can also be used to create cancer treatments that produce antibody proteins effective in treating cancer, and can be used in regenerative medicine to treat heart failure and other ailments. Clinical trials are already underway for applications like these.

The development/manufacture of new drugs that use mRNA is accelerating and expanding, leaving us poised on the edge of a revolution in the field of medicine.

It is all thanks to the research of Professor Katalin Karikó and Professor Drew Weissman.

**Figure 3：Paving the way to mRNA drugs with pseudouridine**




In addition to the genetic information on the spike protein, the mRNA used to produce spike proteins is composed of 5'-cap, a poly(A) tail, and more, which prevents the mRNA from degrading too quickly. Inflammation due to immune response can be reduced by replacing uridine, which contains the spike protein genetic information, with pseudouridine.

**Figure 4：The development of mRNA vaccines**

| Year | | Details | |
|---|---|---|---|
| 1960s | 1961 | Discover of mRNA (F. Jacob, J. Monod) | |
| | 1969 | Laboratory synthesis of protein using mRNA isolated from living creature | |
| 1970s | 1971 | Drug transport using liposomes achieved | |
| 1980s | 1984 | mRNA synthesized | |
| | 1989 | mRNA in cationic liposomes administered to human cells and frog embryos | |
| 1990s | 1990 | mRNA injected into mouse muscle and proteins synthesized (Wolff et al) | |
| | 1992 | Trial of mRNA as therapeutic drug for hereditary disease (in rats) | |
| | 1995 | Trial of mRNA in cancer treatment (in mice) | |
| | 1998 | **Professors Karikó and Weissman begin research collaboration** | ① |
| 2000s | 2004 | **Inflammatory response from mRNA administration is discovered to be due to TLRs** | |
| | 2005 | **Modified mRNA found to suppress unwanted TLR immune response *1** | |
| | 2008 | **Modified mRNA found to increase protein expression efficiency *2** | |
| 2010s | 2011 | **Development of mRNA purification method (Karikó et al)** | ② |
| | 2012 | **In vivo protein production using modified mRNA achieved (mice)*3** | |
| | 2012 | Self-amplified mRNA vaccine created | ③ |
| | 2017 | **Beginning of clinical trials of Zika virus vaccine using modified mRNA (in mice and primates). Development of HIV-1 vaccine using modified mRNA (in mice)** | |
| 2020s | 2020 | **Creation of COVID-19 vaccines using modified mRNA (BioNTech/Pfizer, Moderna)** | |

●Blue text: Accomplishments of Profs. Karikó and Weissman

①Research into the medical applications of mRNA was conducted in the 1990s, but it was deemed too difficult due to mRNA instability and inflammatory response.

②Basic lipid nanoparticle model for mRNA transport developed

③Revival of mRNA vaccine research

Refs: *Molecular Therapy*, **27**(4) (2019) ( Special Issue )； E. Dolgen, *Nature* , **597**,318(2021)

*1 Karikó, Katalin; Buckstein, Michael; Ni, Houping; Weissman, Drew; "Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA". *Immunity*. **23**(2), 165-175 (2005).

*2 Karikó, Katalin; Muramatsu, Hiromi; Welsh, Frank A.; Ludwig, János; Kato, Hiroki; Akira, Shizuo; Weissman, Drew (November 2008)."Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability". *Molecular Therapy*, **16** (11): 1833-1840.

*3 Kariko, Katalin; Muramatsu, Hiromi; Keller, Jason M.; Weissman, Drew; "Increased Erythropoiesis in Mice Injected With Submicrogram Quantities of Pseudouridine-containing mRNA Encoding Erythropoietin", *Molecular Therapy*, **20**(5), 948-953(2012)