The legacy of mRNA engineering: A lineup of pioneers for the Nobel Prize

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mRNA is like Hermes, delivering the genetic code to cellular construction sites, so it has long been of interest, but only to a small group of scientists, and only demonstrating its remarkable efficacy in coronavirus disease 2019 (COVID-19) vaccines allowed it to go out into the open. Therefore, now is the right timing to delve into the stepping stones that underpin this success and pay tribute to the underlying scientists. From this perspective, advances in mRNA engineering have proven crucial to the rapidly growing role of this molecule in healthcare. Development of consecutive generations of cap analogs, including anti-reverse cap analogs (ARCAs), has significantly boosted translation efficacy and maintained an enthusiasm for mRNA research. Nucleotide modification to protect mRNA molecules from the host's immune system, followed by finding appropriate purification and packaging methods, were other links in the chain enabling medical breakthroughs. Currently, vaccines are the central area of mRNA research, but it will reach far beyond COVID-19. Supplementation of missing or abnormal proteins is another large field of mRNA research. *Ex vivo* cell engineering and genome editing have been expanding recently. Thus, it is time to recognize mRNA pioneers while building upon their legacy.

INTRODUCTION TO NUCLEIC ACID RESEARCH
DNA discovery
The previous two centuries were marked by elucidation of the structure and function of nucleic acids (Figure 1).[1] Friedrich Miescher, a Swiss physician and biologist, isolated nuclein in 1869. The name nucleic acid was coined, and at the end of the 19th century, Albrecht Kossel, a German physician and biochemist, divided this into five bases: adenine, cytosine, guanine, thymine, and uracil. Another half century passed before Oswald Avery, an American bacteriologist, discovered that deoxyribonucleic acid (DNA) is responsible for heredity, thus laying the foundation for molecular biology genetics.[2] Erwin Chargaff's biochemistry discovery of the fixed ratio of certain bases, along with the crystallography studies of Rosalind Franklin, led to discovery of the structure and function of DNA in 1953 by Watson and Crick.[3] However, the role of RNA and its link to DNA and proteins remained unclear.

mRNA discovery
Nuclear transfer in amebas by Goldstein and Plaut[4] proved the location of RNA synthesis in the mid-1950s, followed by identification of ribosomal[5] and transfer[6] RNA. However, it was not until the early 1960s that an avalanche of work demonstrated a third type of RNA: soluble and short-lived messenger RNA (mRNA). Two publications distinguished mRNA from other kinds of cellular RNA.[7,8] Others demonstrated similar-sized RNA molecules attached to ribosomes at that time.[9] Steps toward solving the puzzle of code transfer from DNA to proteins was further facilitated by an article demonstrating the complementarity of DNA and transitory RNA.[10] In 1964, the collinearity of gene structure and protein structure was reported by Charles Yanofsky, who later received the Lasker Award for his work.[11] Ultimately, Nirenberg cracked the genetic code in 1966,[12–14] and he was awarded the Nobel Prize 2 years later. In this way, the era of mRNA discovery unfolded.

Oligonucleotide synthesis
The discovery that nucleic acids are the source of genetic information rapidly fueled scientific investigation to produce synthetic code, starting with Michelson and Todd[15] in 1955 with chemical synthesis of dinucleotide (Figure 2). Synthesis of longer chains of oligonucleotides was made feasible by a new phosphodiester method introduced by Rammler and Khorana et al.[16] in the early 1960s, including protection of 2′- and 5′-hydroxyl groups in ribonucleosides and subsequent condensation. The focus was on oligodeoxyribonucleotides because of their stability and known role in storage of genetic information. Synthetic oligonucleotides were critical for cracking the genetic code and resulted in the Nobel Prize for Khorana and Nirenberg (see above). This method also proved robust enough to produce synthetic oligodeoxyribonucleotides, which could then be joined to double-stranded DNA to serve as a template for the first biologically meaningful molecule of RNA, alanine transfer ribonucleic acid, in 1970.[17] However, the lack of phosphate protection contributed to the branching of oligonucleotide chains, which required a laborious

NUCLEIN ISOLATION

DNA
AS HEREDITY AGENT

DNA

Albrecht
Kossel
1901

Watson
and Crick
1953

DNA discovery

1869
Friedrich
Miescher

NUCLEIC ACID COMPOSITION
– NITROGEN BASES

1944
Oswald
Avery

STRUCTURE AND
FUNCTION OF DNA

RNA SYNTHESIS
IN NUCLEUS

GENE-PROTEIN
COLINEARITY

Synthesis

Export

**early
1960s**

Gene
sequence

Mutation

Protein
sequence

Marshall
Nirenberg
1966

RNA discovery

mid-1950s
Lester Goldstein
and Walter Plaut

1964
Charles
Yanofsky

U-U-U

C-C-C

Phe

Pro

and remaining 62 triplets

THREE TYPES OF RNA –
mRNA IDENTIFICATION

DECIPHERING GENETIC CODE
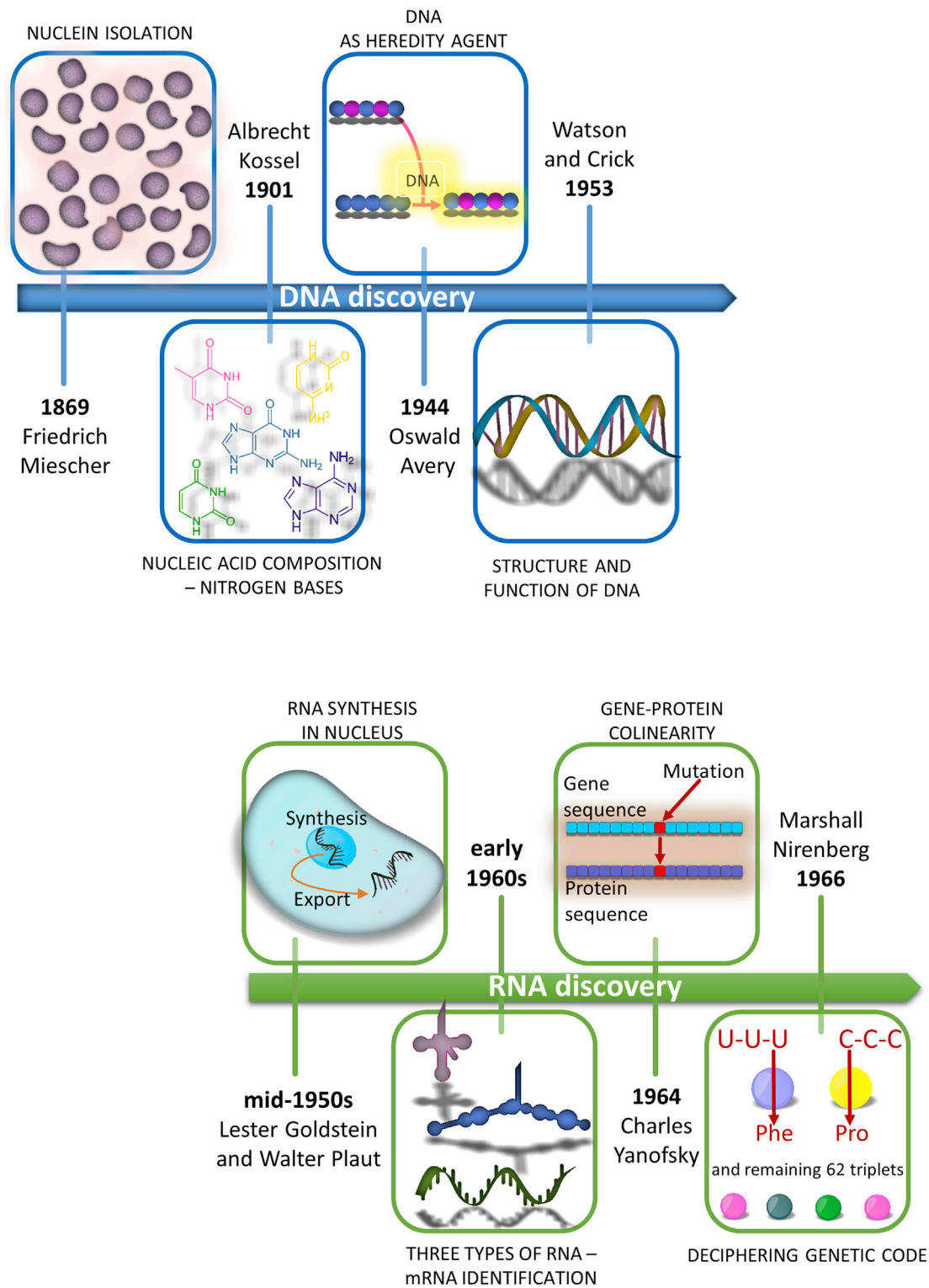
Figure 1. Key events in the history of the discovery of nucleic acids

DINUCLEOTIDE
SYNTHESIS

SOLID PHASE SYNTHESIS OF
OLIGONUCLEOTIDES

Solid-phase

Har G.
Khorana
1970

Marvin H.
Caruthers
1983

DMTrO — B1

Repeat

Oligonucleotide synthesis

Deprotection

DMTrO — B2

HO — B1

1955
Michelson
and Todd

1960/70s
Robert L.
Letsinger

RO — P=O

DMTrO — B2

B1

Condensation

RO — P

Capping

SYNTHESIS OF FIRST TRANSFER
RIBONUCLEIC ACID

Oxidation

B1

PHOSPHORAMIDITE METHOD

ENZYMATIC SYNTHESIS
OF OLIGORIBONUCLEOTIDES

FUNCTIONAL mRNA
SYNTHESIS

Sp6
polymerase

Sp6   cDNA

OH
3'

CAP

mRNA

Shatkin
1975

Protein

1950 - Today

Toward mRNA synthesis

O
HN   CH₃
           N⁺
H₂N   N   OH OH

1950s / 1970s

O=P-O⁻
O=P-O⁻
O=P-O⁻

1983
M.R Green,
T. Maniatis,
D.A. Melton

CAP

ψ

m⁷A

m⁵C

I

m⁶A

CAP DISCOVERY

NUCLEOTIDE AND NUCLEOSIDE
MODIFICATIONS

Figure 2. Stages of scientific and technological development leading to the synthesis of functional mRNA
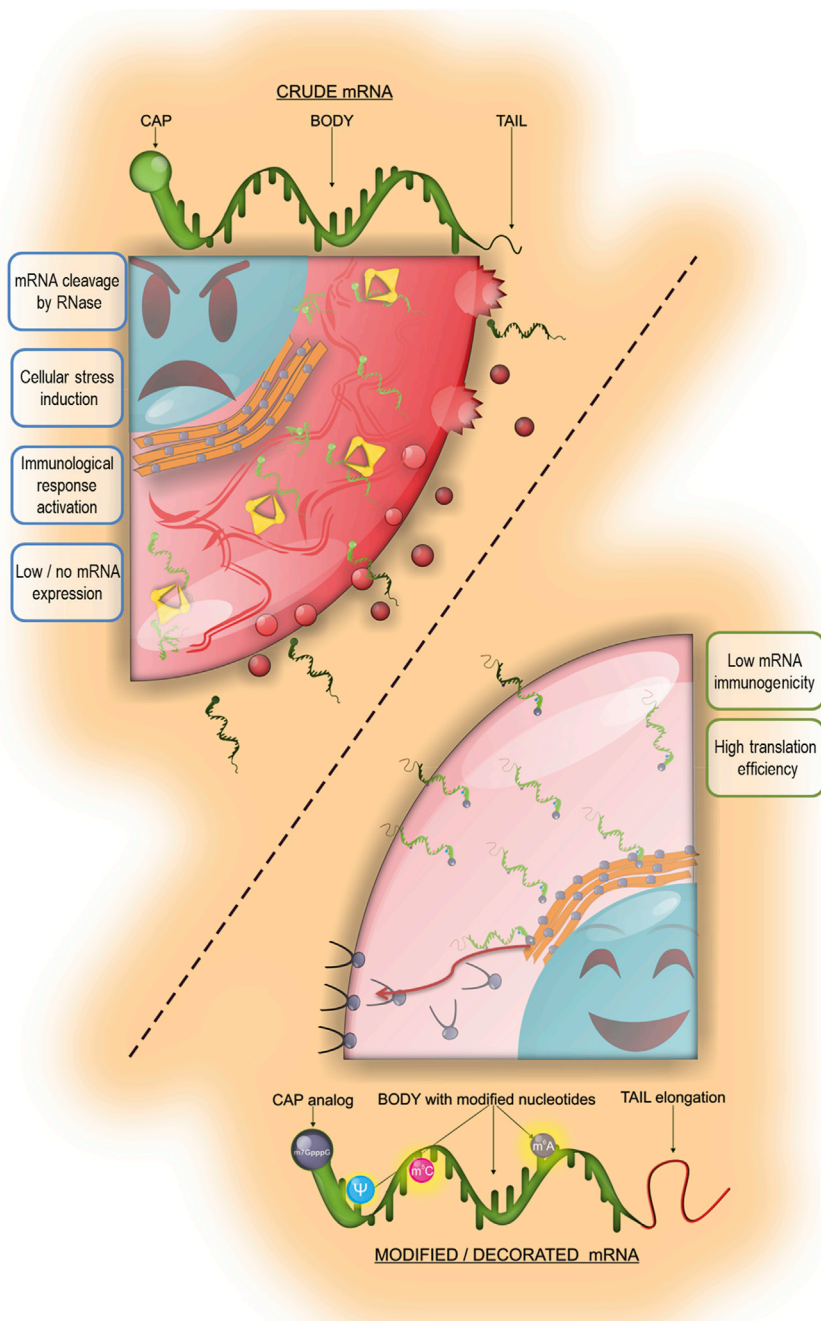
Figure 3. Differences in cell response to transfection with crude and modified mRNA

facilitate large-scale nucleic acid because phosphoramidites could be prepared in advance and then stored and easily activated before use.[20,21]

Toward mRNA production

Enzymatic synthesis of oligoribonucleotides using polynucleotide phosphorylase and RNAse A was achieved as early as the 1950s[22] and further developed in the 1960s.[23] Chemical synthesis of oligoribonucleotide from nucleoside 2′-O-benzyl ethers offered another option.[24] Application of ribonuclease T1 in 1969 allowed enzymatic synthesis of oligoribonucleotides of a defined base sequence.[25] In the same year, a method of DNA-dependent RNA polymerase isolation was described.[26] *In vitro* production of short ribonucleotides based on a DNA template was demonstrated in 1973.[27] Stepwise enzymatic oligoribonucleotide synthesis, including modified nucleotides, was described 2 years later.[28] However, in this era before critical *in vitro* protein synthesis discoveries, none of these ribonucleotides were capable of protein production. In parallel, studies on mRNA structure were undertaken, which, together with the synthetic developments described above, enabled mRNA production. Single-stranded, adenine-rich RNAs were discovered during studies of reoviruses to correspond to the poly(A) tail of mRNA.[29] Then it was determined that a 5′-terminal 7-methylguanosine cap is necessary for translation of eukaryotic mRNA,[30] and the translation initiation region of eukaryotic mRNA was characterized.[31] Finally, all of the components needed to produce functional mRNA were available, culminating in 1983 with the first *in vitro* production of functional mRNA using SP6 bacteriophage promoter fused to the human gene as a template and SP6 polymerase.[32] Nearly a decade later, direct mRNA-based *in vivo* gene transfer to mouse muscle was reported.[33] However, the developments have been hampered by the short duration of protein production and elicitation of the immune response.[34–37]

Nucleotide and nucleoside modifications

Initial studies on nucleoside modifications were undertaken to search for therapeutic targets of alkylating agents.[38,39] Subsequent research in 1964 revealed selective modification of the cytidine residue in RNA by semicarbazide.[40] An avalanche of similar studies followed.[41–45] However, the majority of these research efforts were

purification task at each step, proving impractical for longer oligonucleotides. The drawbacks of Khorana's approach have been addressed by the phosphotriester, solid-state approach of Letsinger introduced in the 1960s and 1970s.[18–20] This method was sufficiently simple to quickly reproduce and served as a basis for the first oligonucleotide synthesizers. However, stepwise efficiency and a long coupling time made extending a chain of nucleotides beyond 20 bases complex. Fine tuning via replacement of the trouble-making polymer with inorganic support and a chloride group by amine was necessary to

directed toward rRNA.[46] Thus, the artificial modifications ran in parallel with the investigation of naturally occurring RNA modifications, which were most frequently detected in tRNA (79), rRNA (28), mRNA (12), small nuclear RNA (snRNA; 11), and other small RNAs (3), until 1994.[47] tRNA nucleotide modifications contribute to the speed of codon-dependent nucleotide polymerization, which is another source of biological variability and a mechanism for regulating cell function, providing an opportunity for mRNA codon optimization to accelerate protein production.[48] Therefore, knowledge of artificial and naturally occurring nucleotide and nucleoside modifications accumulated very quickly.[49]

Transformative biological roles of nucleotide modifications

Nucleotide and nucleoside modifications were a driving force in overcoming challenges preventing the widespread utility of mRNA as a therapeutic agent. They also constitute the most important events in the history of mRNA development. The two most important aspects to be addressed were translational efficacy and immunogenicity. Pioneering work in these fields contributed the most to mRNA's tremendous success, including highly effective coronavirus disease 2019 (COVID-19) vaccines. However, none of the discoveries in the field of mRNA have so far been recognized with the Nobel Prize.

The efficacy of mRNA translation is essential for a number of reasons. Higher efficacy translates to a lower load of mRNA and, thus, decreased immunogenicity. The flanking mRNA regions are the most critical regulators of translational efficacy. As early as 1981, Darzynkiewicz, who worked with Shatkin, showed that methyl esterification of $m^7G^{5'}p$ reversibly blocks its activity as an analog of eukaryotic mRNA 5'-caps.[50] Then Darzynkiewicz contributed a series of cap analogs over the next two decades.[51–55] However, in 1995, it was demonstrated that up to half of the traditional m7GpppG cap is incorporated into mRNA in reverse orientation during *in vitro* transcription, which leads to loss of functionality.[56] In 2001, Darzynkiewicz, in collaboration with Rhoads, published an antidote in the form of the anti-reverse cap analog (ARCA), a new analog that overcomes this limitation and is always incorporated in the proper orientation.[57] ARCA was patented and licensed; it has been available through multiple manufacturers and used widely for the last two decades, showing outstanding results. Initially, it was thought to just double translation efficiency, but in a study of lipofection of dendritic cells, it was shown that ARCA increases translation efficiency 20-fold and acts synergistically with elongation of a poly(A) tail from 64–100 adenosines, which further increases 35-fold, so altogether, the production of reporter genes jumped 700-fold.[58] ARCA has also been astoundingly more effective for stem cell modifications.[59] ARCA served as an inspiration and basis for the next series of cap analogs designed over the next two decades.[60–71] Phosphorothioate cap analogs based on the ARCA concept were patented and exclusively acquired by BioNTech, the designer of the anti-COVID-19 vaccine, and used in their research.[72] Recently, Cap 1 mRNA has been synthesized by TriLink with the co-transcriptional CleanCap analog with a higher efficiency of reaction than enzymatic reaction, which is particularly compelling when a great deal of mRNA needs to be produced in a very short time.[73] Therefore, four decades of research on cap analogs led to mRNA molecules' outstanding efficiency and stability, enabling their vast therapeutic use. It was also a source of motivation dearly needed to advance science. Considering the essential role of the cap in mRNA function, it seems highly justifiable to award a Nobel Prize for cap analog discoveries, and Darzynkiewicz, through his continuous contributions to cap analog design, including ARCA, over the last 40 years, is undoubtedly worthy of such consideration.

In addition to problems with translation efficacy, mRNA immunogenicity quickly dampened enthusiasm for swift translation of mRNA-based medicines to clinical practice. However, the subsequent extraordinary diligence of Karikó and Weissman provided essential insight into the fact that the majority of natural mRNA molecules include modified nucleotides, which may shield them from the cellular innate immune system.[74] Indeed, Karikó et al.[68] demonstrated that incorporation of pseudouridine into mRNA prevented an immediate immune response in the form of a systemic interferon-alpha (IFN-alpha) spike after intravenous administration. Pseudouridine also enhances translation by less pronounced activation of RNA-dependent protein kinase (PKR), which then phosphorylates translation initiation factor 2-alpha (eIF-2α) and inhibits translation of uridine-containing transcripts.[75] Nucleoside modifications in RNA also limit activation of 2'-5'-oligoadenylate synthetase and increase resistance to cleavage by RNase L, another cellular sensing system.[76]

Further lowering of the cellular immune response has been achieved by improving mRNA purification using high-performance liquid chromatography (HPLC).[77] Such prepared mRNA proved highly effective with submicrogram quantities *in vivo* for erythropoietin supplementation in mice and monkeys.[78] In this way, mRNA proved to be fulfilling the promise of gene therapy.[79] Therefore, Karikó also appears to be a certain candidate for the Nobel Prize.

Efforts to improve mRNA quality and biocompatibility continue. Modifications in the bioprocess of pseudouridine production decreased the immune-activating profile of therapeutic mRNA through further elimination of impurities.[80] Incorporation of pseudouridine and ARCA significantly increases the yield per reaction, improves modRNA (chemically modified mRNA) translation, and reduces its immunogenicity *in vitro*. It seems to be an ideal solution to address clinical challenges.[81] Therefore, it appears that decorating crude mRNA with modified nucleotides ultimately made it highly welcome by living organisms (Figure 3). We may have reached a plateau in nucleotide modifications to make mRNA a very attractive medicine applicable to several fields, including vaccines, protein supplementation, and stem cell engineering. All RNA modification pathways are stored in a MODOMICS database, which is an excellent source for advancing research on this topic.[82]

Biological roles of mRNA-based medicines
Vaccines

Vaccines are an indispensable tool for public health,[83] preventing loss of many lives and occupying a prominent place in medicine.
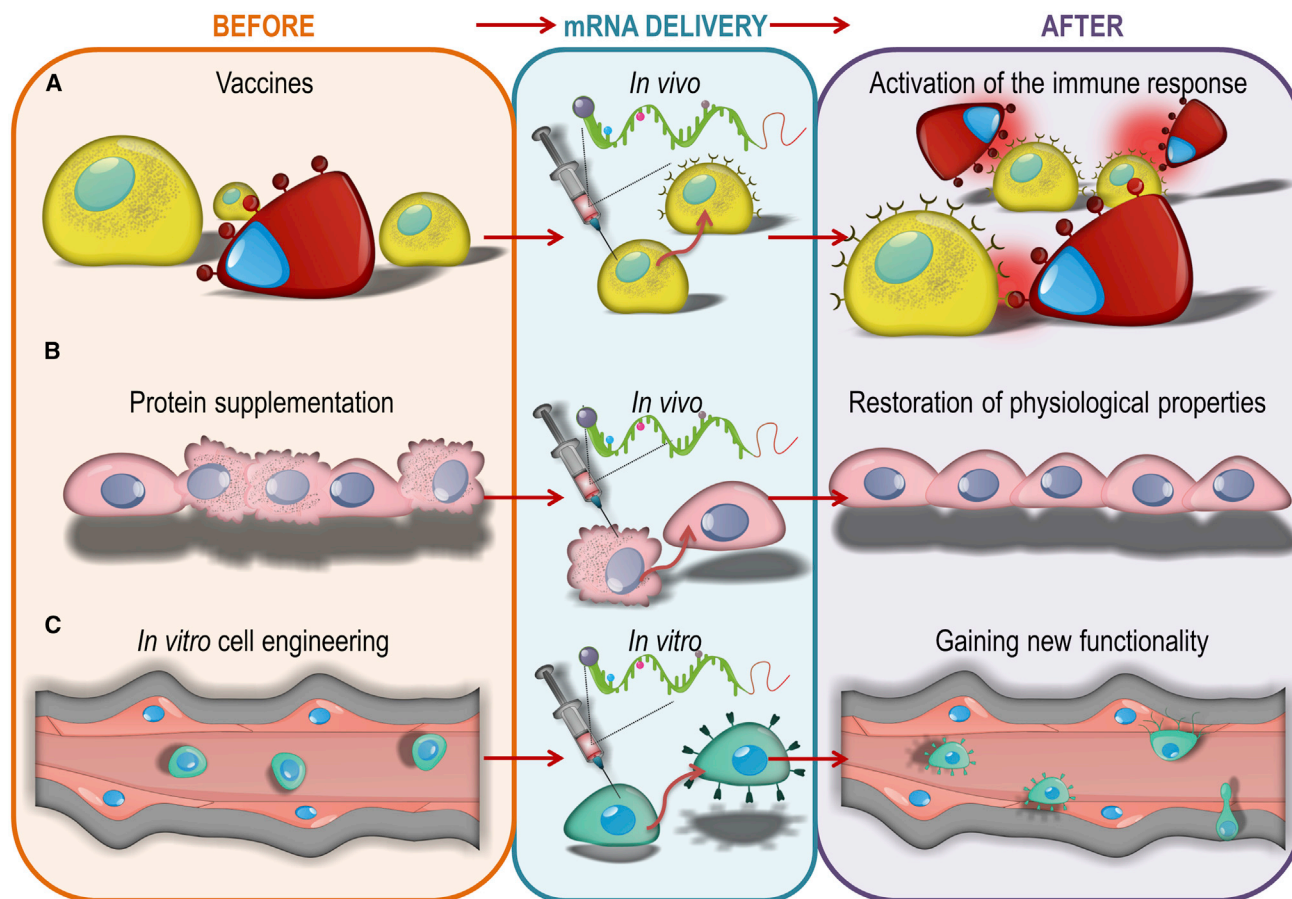
Figure 4. Recent advances in medicine and science because of the introduction of mRNA-based technologies
(A) vaccines, (B) protein supplementation and (C) cell engineering.

However, their efficacy and safety requirements are rigorous because typically they are applied to vast populations of healthy people. The early approaches with attenuated or inactivated microbes were quite effective, but the issue of safety was debatable, and production, purification, and quality control were cumbersome and expensive.[84] Protein-based vaccines have fewer drawbacks but require immune adjuvants, which may involve awakening dormant autoimmune processes.[85] In contrast, DNA-based vaccines are plagued by ineffective cellular and nuclear entry and the need for additional devices to support this process.[86] This challenge has been addressed recently by packing DNA into a harmless virus, but it can still evoke bad associations.[87] Regardless, nearly all of these approaches require living or structurally intact organisms to produce, making development slower and costlier. Against this backdrop, the concept of using mRNA seems to be the holy grail in vaccine development (Figure 4A). mRNA vaccines can be designed within days by simply mimicking the nucleotide sequence of receptor binding domains (RBDs) after sequencing microbe genetic material and then synthetically producing them in robotized factories. Therefore, the timespan from microbe discovery to clinical-grade vaccine candidate is extremely short. The simplicity and speed of the entire procedure also allow

fast reaction and facile tweaking of the primary vaccine to respond to the genetic drift of microbes and formation of variants. There is also no risk of contamination with xenogeneic material, which is reassuring to the public. It seems that these features favor mRNA as an ideal vaccine solution.[88]

When the COVID-19 pandemic emerged, mRNA vaccines already had been tried against previous threats. The first mRNA vaccines took shape in the field of oncology.[89–91] A few years later, they began to be used for treatment of infectious diseases, especially influenza.[92,93] Then the preclinical efficacy of the mRNA vaccine was demonstrated against rabies.[94] Subsequently, it has been shown that a single dose of a mRNA vaccine is sufficient to induce durable protection of mice and monkeys against the Zika virus.[95] However, this virus has never gone global and become a pandemic, so further effort toward vaccine development did not materialize. It has also been shown to be effective in the guinea pig model of another potential global threat: the Ebola virus.[96] mRNA vaccines were proposed to combat outbreaks even before the coronavirus pandemic.[97] Other preclinical targets for mRNA vaccines include cytomegalovirus,[98] tick-transmitted flavivirus,[99] human immunodeficiency virus

(HIV),[100] dengue virus,[101] Crimean-Congo hemorrhagic fever virus (CCHFV),[102] herpes simplex virus 2 (HSV-2),[102] Nipah virus,[103] and respiratory syncytial virus (RSV).[104] The outstanding safety of mRNA vaccines even prompted the scientific community to propose its use to treat allergies.[105] Therefore, it was not surprising that mRNA vaccines were also immediately designed against the novel virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In mice, a single immunization with a nucleoside-modified mRNA vaccine elicited strong cellular and humoral immune responses against SARS-CoV-2.[106] Because of the advantages mentioned above, mRNA vaccines have been found to be not only effective, but they were developed the fastest and received emergency use authorization first. Arriving in huge quantities, they were true stars, and they had a huge impact on taming the COVID-19 pandemic in many geographical regions.[107]

Protein supplementation

Because proteins are critical molecules of intracellular and extracellular compartments of all organisms, their loss or abnormality usually leads to pathological consequences. Therefore, protein supplementation is a valid therapeutic approach. Although there are various approaches to delivering proteins, such as virus-based gene replacement or direct protein delivery, there is also a growing interest in using mRNA to achieve this goal (Figure 4B). There is a specific advantage to this approach. As opposed to DNA, mRNA does not integrate with a genome, obviating many safety concerns. Physicochemically, all mRNA molecules are relatively similar, so the same formulation may serve many genes. However, considerable differences in protein properties cause the formulation and delivery of every protein to be separately developed. The therapeutic effects of mRNA-based protein delivery in an animal model have been demonstrated as far back as nearly three decades ago.[108] In comparison with vaccines, preparation of mRNA for protein supplementation is more demanding. Although induction of the host immune response is welcome in vaccine applications, it is of utmost importance to prevent immunogenicity of mRNA delivered for protein supplementation, especially because protein supplementation may be given throughout the entire lifespan, and immunogenic material may cause chronic inflammation.

Pulmonary diseases are particularly compelling applications because of the potential for mRNA to be administered easily and locally through inhalation.[109] Along this line, it has been demonstrated in a mouse model of asthma that inhalation of mRNA for Foxp3 reduces pulmonary inflammation by increasing the presence of T regulatory (Treg) cells in the lungs.[110] Local delivery of mRNA for Foxp3 may avoid systemic activation of Treg cells, which, in turn, could facilitate tumor formation[111] and the spread of infection.[112] Non-fenestrated endothelial cells in pulmonary capillaries are an additional obstacle when systemically delivering mRNA to the lungs.[113] mRNA-mediated gene supplementation of Toll-like receptors is another strategy investigated in the mouse model of asthma.[114] Inhalation of mRNA for the CFTR gene proved effective in a small animal model of monogenetic lung diseases such as cystic fibrosis.[115] Translate Bio (acquired recently by Sanofi) is currently running a clinical trial to cure cystic

fibrosis with a drug composed of mRNA holding the CFTR gene. Positive effects of mRNA-based therapeutic agents in an animal model of another monogenetic disorder, a1-antitrypsin deficiency, has also been demonstrated recently.[116] Metabolic diseases are another group of illnesses potentially curable by mRNA encoding missing or abnormal enzymes.[117] Methylmalonic aciduria is addressed by mRNA encoding methylmalonyl-coenzyme A (CoA) mutase.[118] mRNA also has been tried as a drug in the context of regenerative medicine to improve revascularization of the heart[119] and skin[120] as well as in cancer immunotherapy.[121]

mRNA-based cell engineering

Stem and immune cell therapies are becoming an important field of medical research.[122–124] Cell engineering is frequently used to increase therapeutic potential. Several cellular functions can be achieved through administration of mRNA. This technology is essential for transient cellular modifications (Figure 4C).

Cellular trafficking and migration can be particularly well addressed by mRNA-based engineering. Indeed, it has been shown that adhesion molecule integrin alpha 4 (ITGA4) can be expressed on the surface of mesenchymal stem cells (MSCs) after administration of encoding mRNA,[59] acquiring their function in vitro,[125] whereas the process of in vivo diapedesis was independent.[126] MSC migration has also been increased in vitro by administration of mRNA encoding CXCR4.[127] The same strategy is also capable of enhancing the homing of natural killer cells.[128]

The expression of active immune molecules is another large field of mRNA-based cell engineering. Administration of mRNA for chimeric antigen receptor (CAR) or T cell receptor (TCR) to lymphocytes produces effective immune cells. The transient nature may be overcome with multiple infusions, whereas short-term expression can prevent severe complications because of immune cell autoaggression.[129] mRNA-based programming of dendritic cells to provide relevant instructions to lymphocytes is another attractive therapeutic strategy to reach clinical trials.[130]

mRNA-based technology is also perfectly suitable for genome editing. The mRNA encoding genome-integrating enzyme transposase can be co-injected with the transposon to deliver a DNA payload.[131] Recent addition of site specificity to transposase through CRISPR technology has finally allowed editing of the genome precisely and efficiently with large DNA payloads.[132] The Cas9 enzyme can also be delivered to cells in the form of mRNA along with relevant guide RNA (gRNA) sequences.[133] mRNA is also a viable strategy for induction of pluripotency in somatic cells.[134]

mRNA packaging and delivery

mRNA is a fragile and negatively charged molecule; therefore, appropriate packaging and delivery systems are critical to fully exploit its potential. There are many approaches to accomplish this task. PEGylated lipid nanoparticles (LNPs) are currently a mainstay of mRNA enveloping, mainly because of their success in delivering

COVID-19 vaccines. However, besides LNPs, there are many other ways to deliver mRNA, such as polymers, inorganic structures, proteins, hydrogels, etc.

Lipid-based carriers have a very long history. Liposomes have been used to deliver a variety of therapeutic compounds. Thus, they were the first obvious choice for nucleic acid delivery. However, they ultimately failed in *in vivo* small interfering RNA (siRNA) delivery and became a motivation to further develop carriers, which resulted in LNPs.[135] Therefore, the rising star of mRNA therapeutic agents immediately enjoyed a fertile ground of powerful carriers capable of effective *in vivo* payload delivery.

Inorganic components such as apatite have been shown to increase the efficacy of mRNA.[136,137] Silica nanoparticles are another option to facilitate nucleic acid delivery[138,139] and are considered an emerging vaccine delivery system for COVID-19.[140] Silica is a relatively low-cost and easy-to-scale solution for safe mRNA delivery and enjoys an extensive functionalization portfolio with a very encouraging storage profile.[141] Recently, room-temperature synthesis of dendritic mesoporous silica nanoparticles with small sizes and large pores enhanced mRNA delivery performance.[142]

Polymers are an extensive group of natural and synthetic compounds. They are characterized by their high versatility in terms of nucleic acid binding and release as well as relative stability. Cationic polymers such as polyethyleneimine-stearic acid (PSA) can self-assemble and encapsulate nucleic acids.[143] Ionizable amino-polyesters synthesized via ring-opening polymerization of tertiary amino alcohols is another group of exciting polymers for mRNA administration.[144] They are devoid of a strong positive charge and, thus, are more neutral for cells. Amphiphilic polyhydrazones are another type of polymer candidate for mRNA delivery.[145] A microneedle patch (RNApatch) composed of low-molecular-weight polyvinylpyrrolidone (PVP) can address the essential aspect of very low mRNA stability by preserving mRNA functionality for up to 2 weeks under ambient conditions.[146] Lipid polymer hybrids are another approach, capitalizing on the best features of lipids and polymers.[147]

Hydrogels are somewhat related to polymers, which are the main components, although with hydrogels, different properties are used as carriers. Hydrogels are typically characterized by a highly tunable structure capable of meeting various requirements. Hydrogels' structure and injectability allow localized deployment of mRNA to body cavities or other relevant locations. However, hydrogel particles can also be used as any other nucleic acid carrier. The tunable size of such particles could perfectly suit the inhalation route. Therefore, hydrogels are a highly versatile carrier. Typically, mRNA can be entrapped within hydrogels and then slowly released over a relatively long period. Different release rates characterize various hydrogel systems, and blending multiple hydrogels presents an opportunity to discover optimal release conditions. One group revealed that chitosan-alginate hybrids encourage local and sustained expression of exogenous proteins in cells.[148] Hydrogels from graphene oxide and polyethylenimine (PEI) can deliver mRNA to tissues, and such mRNA remains functional for up to a month.[149] DNA hydrogels are another concept for small interfering mRNA production, but they could potentially be adapted to mRNA production as well.[149] Because hydrogels can be labeled and imaged relatively quickly, they offer seamless precision for procedures.[150]

RNA viruses capitalize on the ability of nucleoproteins to package mRNA. It has been shown that, under some circumstances, transmembrane proteins can interact and package other mRNA-bearing RNA-packaging signals in the form of virus-like particles.[151] There are also other endogenous proteins, which selectively bind to their mRNA and protect it. One of these proteins (polietylenoglikol 10, PEG10) has been engineered to selectively wrap and package other mRNAs. It is accomplished by flanking genes of interest with PEG10 untranslated regions.[152] Such mRNA-PEG10 complexes can be shuttled between cells as virus-like particles (VLPs). The high modularity of this platform to fit a variety of mRNAs and the endogenous character of proteins to avoid immune activation are advantages that may lead to the rapid adoption of this approach to achieve highly effective and non-toxic mRNA delivery.

Summary and future outlook

The history of nucleic acid discoveries makes for one of the most fabulous stories ever. The chemical findings and physical discoveries applied to a biological context contributed to the revelation of the greatest secret of life. It, in turn, launched an era of miraculous medical discoveries. For decades, stable and easy-to-handle DNA was the focus of research, whereas in recent years, mRNA came into the spotlight. As always, the devil is in the details, and the tireless efforts of scientists allowed us to uncover the intricacies of mRNA molecules. Engineering of cap analogs and nucleotide modification was a recipe for potent mRNA, which became the basis for a new generation of powerful medicines. The entire mRNA orchestra became harmonious when it was most needed to tackle the worst pandemic in 100 years. Therefore, there is a strong need to pay tribute to the early pioneers of mRNA and those still alive, such as Darzynkiewicz, Karikó, and others, and recognize them with a Nobel Prize.

We expect such remarkable mRNAs to prompt further breakthroughs in medicine in the near future. We anticipate other advancements in mRNA technology and continuous contributions to healthcare. Purely chemical, DNA template-free synthesis of mRNA may be another step toward widespread use of mRNA.[153] The alternative genetic code may inflate the potency of mRNA therapeutic agents.[154] Unnatural base pairs are another biotechnological frontier.[155] Artificial intelligence adds yet another dimension to RNA research.[156] Thus, our supply of mRNA-related ingredients is rapidly expanding to meet the needs of successive healthcare developments. the discoveries made in genetics could move outside the box and be applied in other fields, such as computing, information storage, sensors, robotics, and more.

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