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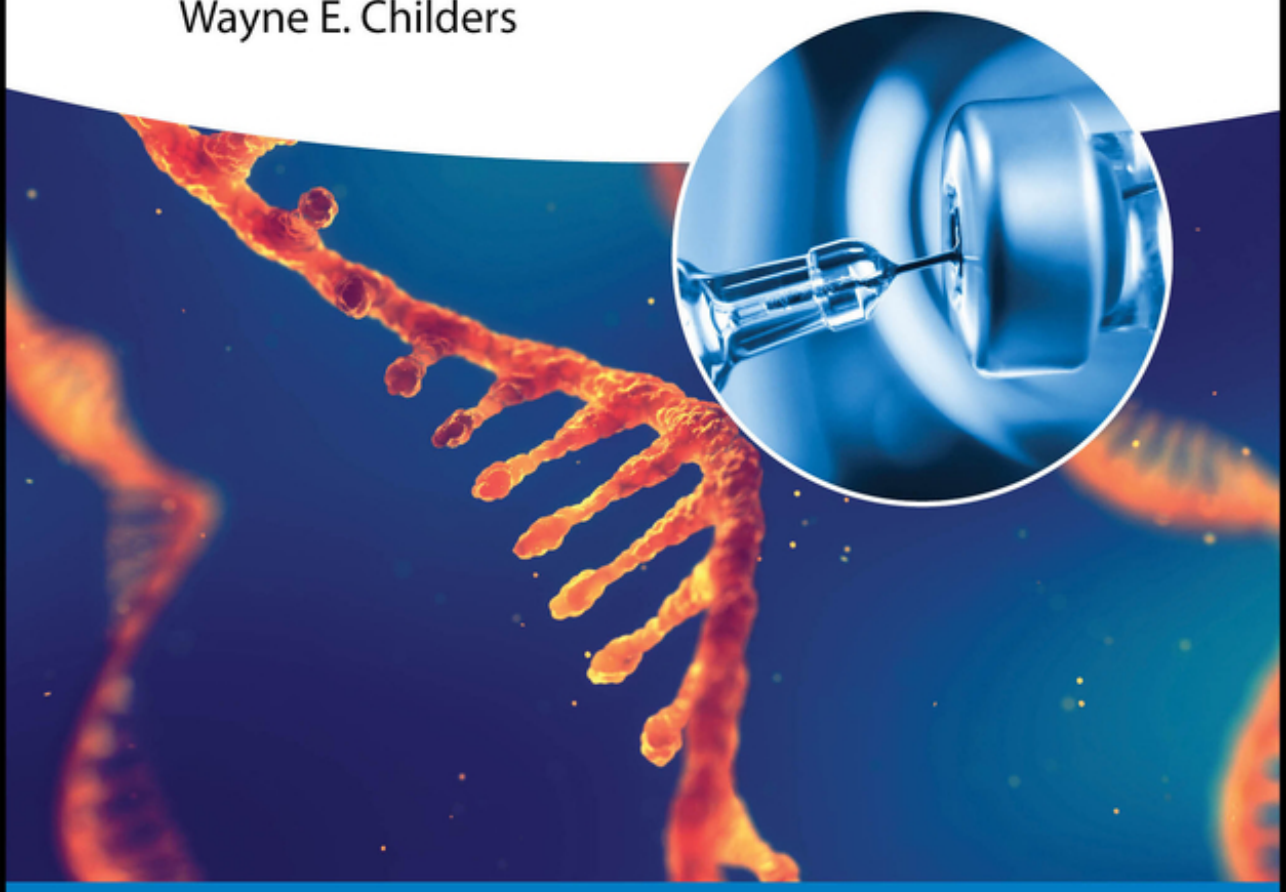
Trends in mRNA Vaccine Research

Volume 1

Series Editors: János Fischer, Christian Klein, Wayne E. Childers



TRENDS IN DRUG DISCOVERY

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Preface

The **Trends in Drug Discovery** book series focuses on recent drug discoveries, including preclinical studies, successful drug development leading to newly approved drugs in major therapeutic fields. The scope of this series includes both small molecule and large molecule drugs including biologics.

Each volume gives an overview of a specific therapeutic field, discusses concepts and technologies, and describes the discoveries of recently approved drugs.

The **series editors** are introduced as follows:

János Fischer (Emeritus Fellow of IUPAC, research advisor at Richter, Hungary) participated in the editing of two book series of Wiley-VCH: Analogue-based Drug Discovery (Vol. 1–3, 2006–2011) and Successful Drug Discovery (Vol. 1–5, 2015–2021).

Christian Klein (Department Head Cancer Immunotherapy and Site Head at the Roche Innovation Center, Zurich, Switzerland) was a coeditor for Successful Drug Discovery, Vol. 3–5.

Wayne E. Childers (Professor of Pharmaceutical Sciences at Temple University School of Pharmacy, Philadelphia, Pennsylvania, United States) was a coeditor for Successful Drug Discovery, Vol. 2–5.

The goals of this book series are to provide the drug discovery community with reference books and to support teaching in the field of drug discovery.

The first volume has the title: **Trends in mRNA Vaccine Research.** Katalin Karikó and Drew Weissman have been recognized with the 2023 Nobel Prize in Physiology or Medicine for their discoveries leading to the development of mRNA vaccines against COVID-19. The book focuses on the promising trends in the field of mRNA vaccine research.

Preface from the Volume Editors

mRNA vaccines received broad attention during the SARS-CoV-2 pandemic, especially after COVID-19 vaccines developed by Moderna and Pfizer-BioNTech demonstrated high effectivity for decreasing hospital admissions and serious COVID-19. The interest towards the mRNA technology was further intensified in 2023 after Katalin Karikó and Drew Weissman received the Nobel Prize in Physiology or Medicine for their discoveries on nucleoside base modifications that enabled the development of safe and effective mRNA vaccines against SARS-CoV-2. mRNA vaccines represent a revolutionary advancement with enormous potential in medicine to target very different diseases by sending information encoded on mRNA to the cells that take up the nucleic acid. To date, the technology demonstrated its viability for infectious disease vaccine development during the SARS-CoV-2 pandemic, and promising results were obtained in the fight against other pathogens, and other indications including cancer or chronic diseases. Publisher Wiley aimed at summarizing the current knowledge and developments on mRNA vaccines in a book as the first volume in a new series focusing on advanced pharmacology therapy developments.

The editors of this volume are grateful for participating in the collection of the valuable chapters written by outstanding researchers of the mRNA vaccine field. The chapters are divided into two main sections.

The first section contains seven chapters summarizing the general knowledge about mRNA vaccines.

Verbeke and colleagues provide a historical overview about the key discoveries during the course of mRNA vaccine development. The chapter focuses on three main topics: first, the discovery, production, and refinement of mRNA as a therapeutic modality; second, the path to find a suitable vehicle to facilitate the safe and efficient delivery of intact mRNA into the target cells; and finally the clinical advancements of mRNA vaccines.

The immune response induced by an optimal mRNA vaccine provide a balanced activation of the innate and the adaptive immune system. The chapter by Exposito and colleagues gives a detailed summary about the immune response induced by the elements of the mRNA vaccine, including the protein-encoding mRNA, the byproducts generated in the *in vitro* transcription reaction and the components of the formulation, as well.

Morais and Yu focus on the use of modified nucleosides in therapeutic mRNA and summarize their effects on mRNA vaccine immunogenicity and mRNA stability extended with a discussion on the main differences between nucleoside-modified and unmodified mRNA vaccines. Features of self-amplifying mRNA (saRNA) vaccines are presented in the chapter written by Lundstrom, focusing on the history of development, the advantages of the platform, and summarizing the current promising developments with saRNA. Circular RNAs (circRNAs) are a type of RNA with increased stability due to their unique closed structure. Synthetic circRNAs have been developed as a novel RNA platform and have demonstrated their potential as new preclinical vaccine candidates. Current achievements with this new platform are summarized by Liu and Zhu.

Upscaling of mRNA production became a hot topic during the SARS-CoV-2 pandemic, as it was critical to produce large amounts of vaccine in a short timeframe. Manufacturing of large amounts of mRNA had challenges and required investment in the technology as described in the chapter written by Stamoula and colleagues.

mRNA vaccines have the potential to induce immunotolerance, and, thus, opening up new indications such as the treatment of autoimmune diseases, allergies, or transplant rejection. This topic is detailed in the chapter written by Gissler and colleagues.

The landscape of mRNA vaccines is continuously and rapidly evolving: the second section of the volume gives examples about some of the novel developments.

The development and approval of the two mRNA COVID-19 vaccines represented a major breakthrough in the fight against the SARS-CoV-2 pandemic. The section starts with a valuable comparison of the performance of the approved COVID-19 mRNA vaccines written by Istvan Tombacz.

Despite significant progress in the prevention and treatment of HIV-1 infection, the virus continues to pose a significant public health threat. A successful vaccine would be a powerful tool for the reduction of the overall burden of the disease. In this section, the current status of the development of mRNA-based preventive or therapeutic vaccines against HIV is described by Paolo Lusso.

Most mRNA vaccines were developed against viruses but vaccine development against other type of pathogens is also important. While Arora and Fikrig summarized mRNA vaccine studies against tick-borne diseases, Versteeg and Pollet detailed the current status and future outlook of mRNA vaccine development against parasites. Aernout and colleagues gave insights into the challenges of mRNA vaccine generation against bacteria through the example of *Mycobacterium tuberculosis*.

Besides mRNA vaccine development for infectious disease indications, mRNA cancer vaccines represent an exciting area of research with promising results in the fight against cancer. Mey and colleagues summarized the recent achievements in this topic in the last chapter.

The current volume is an ideal source for researchers, medical specialists, healthcare workers, public health officers, students, and anyone interested in understanding the trends in mRNA vaccine research.

April 23, 2024

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Part I

How mRNA Vaccines Work

1

A Historical Overview on mRNA Vaccine Development

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1.1 Introduction

In less than one year after the COVID-19 pandemic outbreak, two mRNA vaccines received the first emergency use authorization from the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), i.e. BNT162b2 (Comirnaty) from Pfizer/BioNTech and mRNA-1273 (Spikevax) from Moderna. In Phase 3 clinical trials, these mRNA vaccines were found to be generally safe and up to 95% efficacious after the second dose of vaccination in preventing symptomatic SARS-CoV-2 infection [1, 2]. The outstanding efficacy and unprecedented speed with which these mRNA vaccines were produced and distributed, strongly helped to curtail the burden of the pandemic and prevented millions of deaths [3, 4].

Now proven against COVID-19, there is explosive growth in research and investments in mRNA technology. Most notably, the platform of nucleoside-modified mRNA encapsulated in lipid nanoparticles (LNPs) that is utilized in today's COVID-19 mRNA vaccines is poised to have a rapid transformative effect on the future of medicine. Vaccines based on this platform are now being tested in Phase 3 clinical trials for several viral diseases other than COVID-19, such as against influenza (BNT161 and mRNA-1010), cytomegalovirus (mRNA-1647), and respiratory syncytial virus (mRNA-1345), while many other mRNA vaccines are being (pre)clinically studied to target diseases, such as bacterial and parasitic infections, cancer, and autoimmune diseases. In addition, the ability of the mRNA–LNP platform to deliver genetic information for the temporal production of proteins inside cells makes it a potential key technology to enable gene editing, protein replacement, and other immunotherapeutic approaches [5].

Trends in mRNA Vaccine Research, First Edition. Edited by Gabor Tamas Szabo and Norbert Pardi.

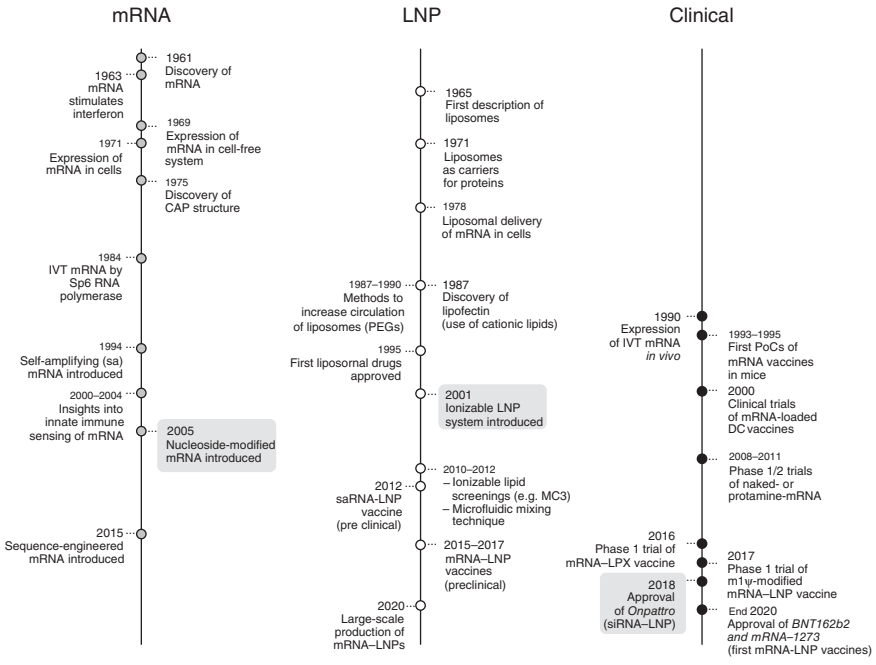

Figure 1.1 Discoveries and milestones in the development of mRNA-based vaccines are subdivided into three parallel timelines of mRNA-, LNP design, and clinical development.

It may seem that the COVID-19 mRNA vaccines came out of the blue, but in fact, decades of research were needed to develop this novel vaccine technology. To understand why it took so long for mRNA vaccines to breakthrough, we need to appreciate the collective efforts made by many scientists and the various problems they have tackled and solved that ultimately led to the development of this first generation of mRNA vaccines. As elucidated by Dowdy, the fundamental problem is that a billion years of evolutionary defenses need to be tackled to successfully deliver RNA. This includes both cellular barriers that have kept foreign RNAs on the outside of cells from invading the inside of cells, as well as the many innate immune defense mechanisms evolved to recognize and destroy foreign RNA [6]. Nonetheless, mRNA represents a most excellent vaccine modality to mimic viral infections, this is to trick the immune system to develop memory against the encoded, pathogen antigen. Indeed, when successfully delivered, mRNA has the potential to process and present encoded antigens through the same cellular machinery as occurring during viral infections, while it may also benefit from the specifically designed immune mechanisms against viruses to prime and promote durable adaptive immune responses, i.e. the immune adjuvant potential of mRNA.

In this first chapter section, a short historical overview is given of the development of mRNA vaccines (Figure 1.1).

1.2 The Path of mRNA as an Unstable and Toxic Product to a New Class of Medicine

1.2.1 The Discovery and *In Vitro* Production of mRNA

In 2015, Cobb wrote an essay that addressed the question of who discovered mRNA. By reconstructing the collective insights and different kinds of evidence gathered during the 1950s through the 1960s, Cobb concluded that mRNA was the product of many years of work by a community of researchers [7]. Ultimately, this research process gained momentum in the summer of 1961, when the nature and properties of mRNA were for the first time described in a theoretical model by Jacob and Monod [8]. In this review article on genetic regulation of protein synthesis, they proposed the existence of an intermediate molecule, or "messenger ribonucleotide" that is produced from DNA and that brings the genetic information to the ribosomes for protein synthesis. At about the same time, experimental support for the existence of mRNA was provided by two different research teams [9, 10]. Both research teams had succeeded in isolating mRNA and demonstrating its association with "pre-existing" ribosomes. This replaced the prevailing theory of the time that new specialized ribosomes are synthesized from the gene, and that these ribosomes are specific for the production of the corresponding protein, i.e. the "one gene – one ribosome – one protein" hypothesis. For detailed information about the remarkable series of events and some of the outstanding experiments that led to the discovery of mRNA, see reference [11].

In 1969, protein synthesis from mRNA inside ribosomes was first demonstrated in cell-free systems. In these experiments, RNA fractions purified from reticulocytes dictated the synthesis of globin when incubated with ribosomes obtained from *Escherichia coli* [12] or a different mammalian species [13]. Later on, the translation of mRNA into hemoglobin was also proven in living cells after the microinjection of 9 s RNA from rabbit reticulocytes into frog oocytes [14, 15]. While these studies might have sparked one's imagination to use mRNA for therapeutic applications, at the time, the focus was solely on understanding its biological function.

In 1984, a simple and efficient method was established for *in vitro* mRNA synthesis using template DNA and a bacteriophage SP6 polymerase that initiates transcription at an SP6 promoter located upstream of the gene of interest [16]. In the following years, T7 and T3 RNA polymerases were also reported for successful *in vitro* transcribed (IVT) RNA synthesis [17–20]. These methods still represent the foundation of how mRNA is manufactured in today's COVID-19 mRNA vaccines. However, a limitation of IVT mRNA production using phage polymerases is that it can give rise to multiple contaminants in the form of short and long double-stranded RNA (dsRNA) strands [21, 22]. These dsRNA byproducts have been shown to be largely responsible for the innate immune response to IVT mRNA, and when not controlled, have the potential to jeopardize the safety and functionality of mRNA vaccines (discussed in more detail below). There is, therefore, continuing interest in optimizing

the IVT process of mRNA in order to reduce the formation of dsRNA byproducts, as well as in finding (more) cost-effective purification methods [23–25].

Decades of basic research into the structural characteristics of mRNA and the biological interactions of mRNA with numerous proteins inside the cell not only brought new insights into mRNA metabolism and mRNA translation process but also leveraged IVT mRNA to reach a more optimal design [26]. The genetic information in mRNA is encoded in a codon sequence, where a triplet of adjacent nucleotides specifies an amino acid to be incorporated in a protein, also referred to as the open reading frame (ORF). The ORF is flanked at the 5′ and 3′ positions with start and stop codons. Because most amino acids are encoded by more than one codon, the codon usage in the ORF can be varied, also referred to as synonymous codon usage. Over the years, several strategies have been proposed to optimize codons so as to improve the translation and half-life of the mRNA, including methods of adjusting codons to match host transfer RNA abundances [27] for the enrichment of GC content [28, 29], and to optimize mRNA folds in the construct [30, 31]. It is important, however, to consider that these methods may have unintended effects on the performance of mRNA vaccines, such as altering protein folding and changing the sites of posttranslational modifications that may affect the immunogenicity and function of the encoded antigen, reviewed in [32].

Different nontranslated structural elements are present in eukaryotic mRNA, which were found to have essential roles in different stages of the mRNA life cycle. In 1975, the cap structure was discovered, which is an N7-methylated guanosine linked to the first nucleotide of the RNA via a reverse 5′ to 5′ triphosphate linkage [33–36]. By binding to the eukaryotic initiation factor (eIF) 4E, the cap structure enables the recruitment of translating ribosomes to mRNA. The cap structure and its methylation state are also significant determinants of how the host cell distinguishes itself from nonself RNA, as well as they are important for mRNA stability. At the 3′ end of mRNA, a poly(A) tail made up of a long stretch of repeating adenosine nucleotides was found [37, 38], which functions synergistically with the cap structure to promote translation and regulate mRNA stability [39]. Furthermore, directly upstream and downstream of the ORF, two untranslated regions (UTRs) are positioned, which contain multiple regulatory elements [40]. Since their discovery, continuous efforts and progress have been made in optimizing these structural elements for improved stability and expression of IVT mRNA through empirical screening approaches and computational models [30, 31, 41–43].

Alternative forms of RNA have also been investigated as a therapeutic modality, such as those based on self-amplifying RNA (saRNA) and circular RNA (circRNA). saRNA includes the genetic information for a viral replicase in addition to the vaccine antigen or gene of interest in the ORF sequence and was already introduced in the mid-1990s for vaccine development [44, 45]. This replicase complex typically derived from alphaviruses allows the intracellular amplification of RNA and, in theory, can significantly increase and/or prolong the antigen expression capacity. The replication kinetics of saRNA may provide potential dose-sparing effects, while more prolonged antigen exposure may benefit the quality and duration of vaccine-induced immunity. Another more recent research avenue is the exploration of circRNA. This form of RNA characterized by its closed-ring structure was first reported in 1976

as an independent plant pathogens known as viroids, and later on also found to be prevalent in eukaryotic cells [46, 47]. Due to its unique structure, circRNA is protected from exonuclease-mediated degradation, which may confer improved stability compared to linear mRNA counterparts. Since circRNA lacks a cap and poly(A) tail structure, translation from circRNA into proteins is typically enabled through the insertion of an internal ribosome entry site [48].

1.2.2 The Inflammatory Nature of mRNA

A key bottleneck impeding the therapeutic use of RNA has been its inflammatory capacity. In the early years, it was already demonstrated that mRNA can induce antiviral innate immunity in mammalian cells. In 1957, it was found that adding heat-inactivated influenza virus to cells makes a protein substance that interferes with the replication of viruses, which was called "interferon" [49]. A few years later, it became clear that IFN must act by interfering with the viral RNA metabolism in virus-infected cells [50] and that the viral RNA itself was the component responsible for this antiviral immune reaction [51]. More than 30 years later, the discovery of pathogen recognition receptors by Hoffman and Beutler laid the groundwork for further studies into how mRNA triggers an innate immune response. Several intracellular sensors that are activated by structural features of mRNA were identified. As an example, dsRNA species were shown to be recognized by Toll-like receptor (TLR) 3 in the endosomes [52], and upon arrival in the cytosol by receptors such as Retinoic acid-inducible gene I (RIG-I) [53] and Melanoma differentiation-associated gene 5 (MDA5) [54]. TLR7 and TLR8 were identified as receptors for viral and synthetic single-stranded RNAs and their degradative products [55–58], while the cap1 structure (m7GpppNm) on mRNA was found to be critical to avoid recognition by RIG-I [59, 60]. These discoveries brought explanations of how mammalian cells are capable of distinguishing nonself from self RNA. The reader should refer to the following reviews [61, 62] on this topic for a comprehensive list of RNA sensors, the involved signal transduction pathways, and more details on the molecular basis of mRNA recognition.

The immune recognition of mRNA activates downstream signaling pathways, which leads to the production of inflammatory cytokines, including type I IFNs, inducing in the cells an antiviral state. This has some very important implications for the design of mRNA vaccines and therapeutics. First of all, the immune recognition of mRNA is considered as one of the mechanisms underlying the cause of reactogenicity symptoms, and thus, to a large extent, will determine the safety of mRNA therapeutics. Second, the innate immune response to mRNA can drastically impair the translation of IVT mRNA, thereby reducing the antigen availability for T-cell and B-cell recognition. For example, dsRNA elements in mRNA products are sensed by intracellular enzymes, such as protein kinase R (PKR) and $2'-5'$-oligoadenylate synthetase (OAS), which in turn activate other proteins that cause translational shutdown and cell death [63, 64]. Yet, on the upside, the innate immune response to mRNA has the potential to provide an important adjuvant effect on vaccine potency, and it can be argued, maybe even play a crucial role in the success of mRNA vaccines. Indeed, downstream signaling from the RNA sensing receptors also activates the expression of several genes involved in the mobilization

and activation of antigen-presenting cells (APCs), while RNA-driven cytokines are capable of exerting direct immune-stimulatory effects on T cells and B cells. Therefore, the most challenging task with mRNA vaccines was to find an optimal mRNA design that could minimize the problems posed by its immune recognition on mRNA translation and vaccine safety while still providing enough adjuvant properties to drive strong vaccine responses against the encoded antigen.

At least part of this problem was overcome by the work of Karikó, Weissman, and colleagues at the University of Pennsylvania (UPenn), who were studying the immune-stimulatory effects of IVT mRNA on dendritic cells (DCs) *in vitro*, i.e. the innate immune cell type specialized in antigen presentation and T-cell activation [65]. By comparing the immune-stimulatory activity of IVT mRNA with RNA types derived from different sources, they found that both bacterial (total) RNA, mammalian mitochondrial RNA, and IVT mRNA primed the DCs to produce high levels of tumor necrosis factor-alpha (TNF-α). In contrast, they found that total-, nuclear-, and cytoplasmic RNAs from mammalian sources were less potent inducers of DC activation, while mammalian transfer RNA was noninflammatory. This finding led the researchers to hypothesize that posttranscriptional modifications commonly present in mammalian RNA, such as pseudouridine and methylated nucleosides, might serve as another molecular signature by which cellular RNA sensors discriminate self-RNA from foreign RNA. When applied to IVT mRNA, modified nucleotides, in particular pseudouridines and their derivatives, indeed protected IVT mRNA from activating the aforementioned RNA sensors; TLR3, TLR7, TLR8, PKR, OAS, RIG-I, and MDA5 [22, 63–66]. Consequently, the use of modified uridines strongly improved the translation and safety profile of IVT mRNA products [67, 68]. It is noteworthy that the following studies also evidenced that the use of modified uridines in the IVT reaction reduces the formation of dsRNA byproducts, which might at least in part explain the reduced immunogenicity of uridine-modified mRNA [22]. In addition, it was shown that pseudouridine not only acts as a major controller of the innate immune activity of IVT mRNA but can also contribute to the intrinsic stability and the dynamics of mRNA molecules with ribosomes, reviewed in [69]. Taken together, the use of modified uridines was a great leap forward for producing more translatable, less immunogenic, and safer IVT mRNA products. Note that for the COVID-19 mRNA vaccines BNT162b2 and mRNA-1273, every uridine residue in the mRNA is replaced with N1-methylpseudouridine (m1ψ).

1.3 How Studying Lipid Bilayer Structures in Cell Membranes Gave Rise to the Eventual Development of Lipid Nanoparticles for RNA Delivery

1.3.1 From Biological Cell Membranes to Liposomal Drugs

RNAs are inherently unstable as they are easily degraded by ribonucleases (RNases), which act as the first line of defense against foreign RNAs as well as allow our cells to regulate RNA metabolism. In addition, the lipid bilayer structure that forms

the foundation of cell membranes is uniquely designed to prevent the permeation of such large, negatively charged RNA molecules, protecting the cells from exogenous RNA entering them. Ironically, long-standing efforts to understand the physical properties and functional roles of lipids in cell membranes led scientists to model lipid membrane systems, which have been instrumental in the development of the delivery technology enabling intact nucleic acids to cross these biological barriers; LNPs containing ionizable cationic lipids.

The history of LNPs began in 1964 when Bangham and Horne first described electron microscopy observations of dispersed lecithin (phosphatidylcholine) in water, showing the formation of lamellar "spherulites" structures [70]. In follow-on papers, Bangham and his colleagues described the bimolecular leaflet structure of lecithin vesicles and proved the dispersed phospholipids were spontaneously forming a closed membrane system, i.e. a lipid bilayer completely enclosing an aqueous space [71]. It was Weissmann who named these lipid vesicles "liposomes." Subsequently, the development and characterization of the physical properties of lipids and liposomes quickly followed [72–76]. These studies on the structures that lipids adopt [72] bilayer permeability [77], and membrane fusion [78] helped lay down the cornerstone for the understanding of the biophysical properties of liposomes [79, 80].

In parallel to understanding the membrane biophysics of liposomes, liposome research has extended into areas of therapeutic application. In 1970, Sessa and Weissman first demonstrated that these liposomes have the ability to entrap lysozyme [81], followed by others that started to explore the therapeutic potential of liposomes as drug carriers for enzymes and proteins in rodent models [82–84]. With the experimental methods available at the time, these studies demonstrated that liposomes were removed from the blood within minutes and accumulated in the liver and spleen of the rats, in which liposome-entrapped protein eventually localized in the lysosomes of cells. However, the major initial advances of liposomes as drug delivery systems came with applications for the delivery of anticancer drugs. Two important advances made in the 1980s were the development of the extrusion process for the rapid production of unilamellar vesicles with diameters in the 100 nm size range [85] and the "remote loading" pH gradient technique for efficiently loading liposomal systems with cancer drugs [86, 87].

The third advance involved the development of techniques to increase the circulation lifetime of liposomes following i.v. administration. The incorporation of cholesterol in phosphatidylcholine liposomes reduced bilayer permeability and increased the stability of these systems *in vitro* and *in vivo* [88, 89]. The presence of gangliosides and sphingomyelin reduced liposome clearance rates, which led to the concept of "stealth" liposomes [90]. Incorporation of polyethylene glycol (PEG) lipids further improved the blood circulation half-life of liposomes [91]. PEGylation quickly became popular to improve pharmacokinetics and biodistribution of liposomes, which along with the improvement of liposomal generation (by extrusion) and drug-loading procedures (using pH gradients) have led to the first liposomal drugs for systemic delivery of small molecule drugs to treat fungal infections and a variety of cancers.

With regard to nucleic acid-based drugs, the idea of using liposomes to deliver DNA and RNA into host cells was introduced simultaneously by Dimitriadis and Ostro [92, 93]. Both studies demonstrated the encapsulation and delivery of rabbit globin mRNA and rabbit reticulocyte 9S mRNA into eukaryotic cells by using large unilamellar liposomes. However, these studies were not pursued due to limited transfection potency, lack of scalable methods of manufacture, and poor-encapsulation efficiencies. The introduction of cationic lipids such as 1,2-di-*O*-octadecenyl-3-trimethylammonium propane (DOTMA) rekindled interest in the late 1980s [94, 95]. The electrostatic interaction between the positively charged quaternary ammonium head group of the lipid and the negatively charged phosphate backbone of nucleic acids leads to the formation of "complexes" with high-encapsulation efficiencies. In addition, the cationic lipid has membrane destabilization properties enabling the delivery of nucleic acid payloads across cellular barriers [96, 97]. In 2001, Hafez et al. proposed that the membrane destabilization properties of cationic lipids were related to their ability to form disruptive nonbilayer structures in combination with the negatively charged lipids found in biological membranes ([96]). This research resulted in products such as Lipofectin™, a liposomal composition of the cationic lipid DOTMA and 1,2-dioleoyl-*sn*-glycero-3-phosphatidyl-ethanolamine (DOPE), followed by other polyvalent cationic lipid containing liposomal formulations such as Lipofectamine™ and Transfectam™. These lipofection reagents are commonly used in biochemistry and molecular biology research for the *in vitro* delivery of DNA and RNA in eukaryotic cells.

1.3.2 Ionizable Lipid Nanoparticles for Systemic Delivery of Nucleic Acids

Delivery of nucleic acid-based drugs presents significantly more problems than the delivery of small molecular drugs. In addition to reaching target tissue, the delivery system has to protect the cargo from degradation, facilitate uptake into target cells, and subsequently deliver the cargo into the cytoplasm of target cells. Complexes of DNA or RNA with cationic liposomes result in a heterogeneous mixture of complexes that are relatively unstable and can change properties such as size over time [98]. In addition, cationic lipids can be toxic and cause membrane disruption, hemolysis and induce inflammatory responses limiting therapeutic applications [99, 100].

The major breakthrough for delivering nucleic acid was the introduction of ionizable cationic lipids. In 1994, Bailey and Cullis synthesized 1,2-dioleoyl-3-dimethylammonium propane (DODAP), an ionizable version of a cationic lipid 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) to study the influence of lipid asymmetry on the fusion of liposomal systems [101]. Subsequently, Cullis and colleagues used lipid-based systems containing ionizable lipids to encapsulate nucleic acids [102]. They showed that DODAP had an apparent pK (pK_a) of approximately 6.5 and that these systems could be used to encapsulate nucleic acids at pH values where the lipids are protonated and positively charged (e.g. pH 4) and that the nucleic acid polymers were retained when the pH was raised to physiological values (pH 7.4). Further investigations showed that these lipid systems had a "solid