Understanding mRNA Technology and Vaccines

- WHEN
- HOW
- WHY

MELVIN SANICAS
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DISCLOSURE

Senor Global Medical Director
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Global Assessor
Royal Society of Tropical Medicine & Hygiene

Health Data Expert Group
Facebook

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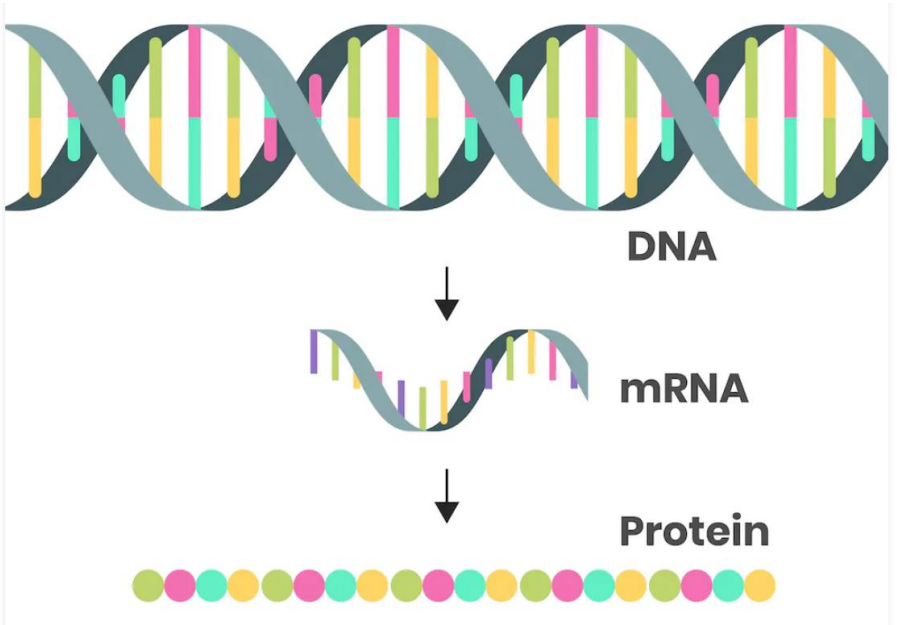
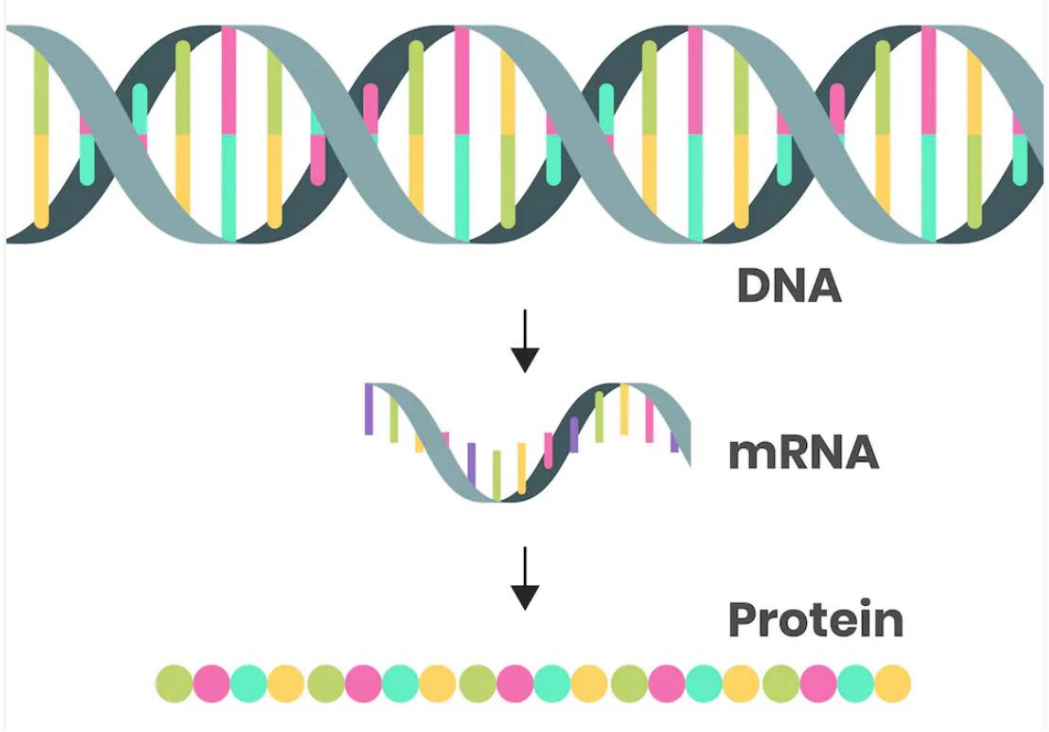
TED

@Vaccinologist

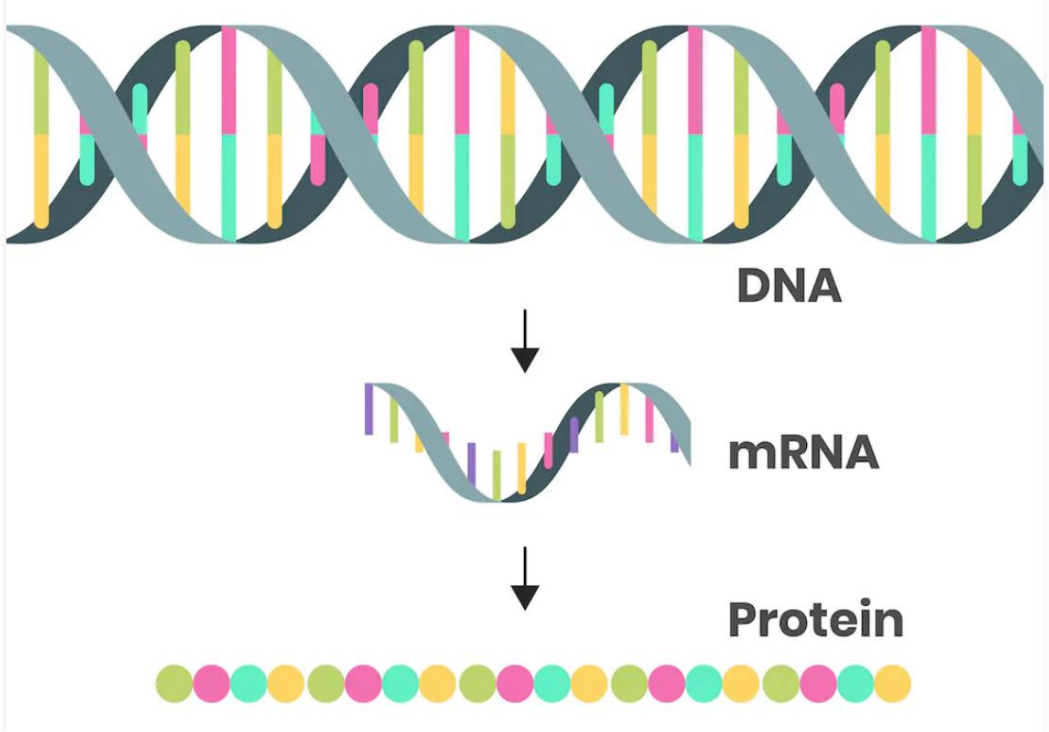




WHEN

HOW

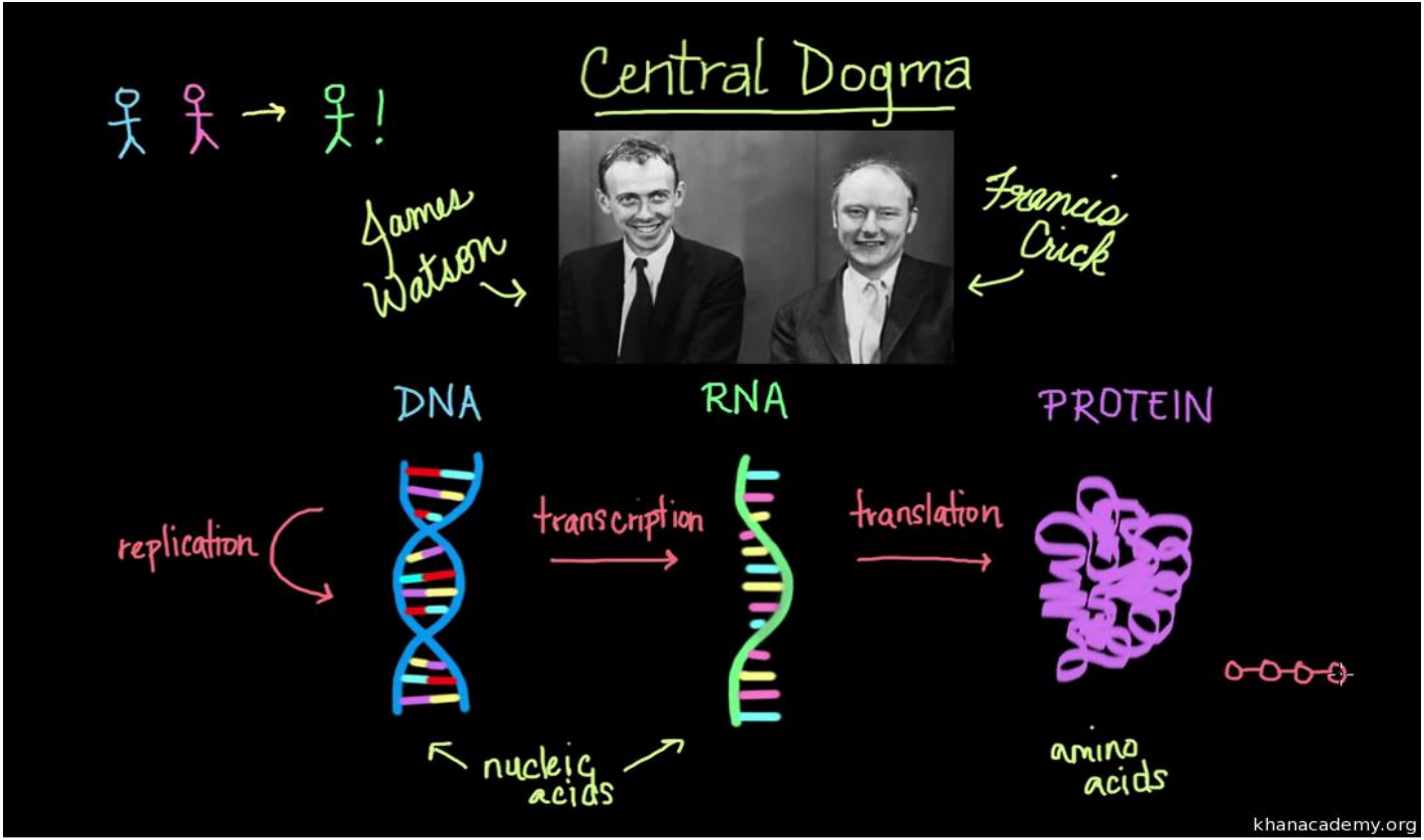
WHY



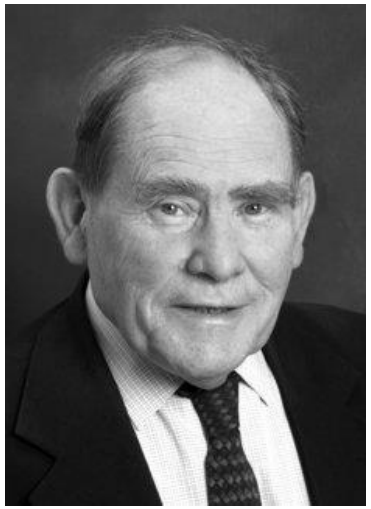
WHEN did mRNA research start?

HOW

WHY


Central Dogma
James Watson
Francis Crick
DNA
RNA
PROTEIN
replication
transcription
translation
nucleic acids
amino acids
khanacademy.org

AN UNSTABLE INTERMEDIATE CARRYING INFORMATION FROM GENES TO RIBOSOMES FOR PROTEIN SYNTHESIS



By DR. S. BRENNER

Medical Research Council Unit for Molecular Biology, Cavendish Laboratory, University of Cambridge

DR. F. JACOB

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AND

DR. M. MESELSON

Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, California

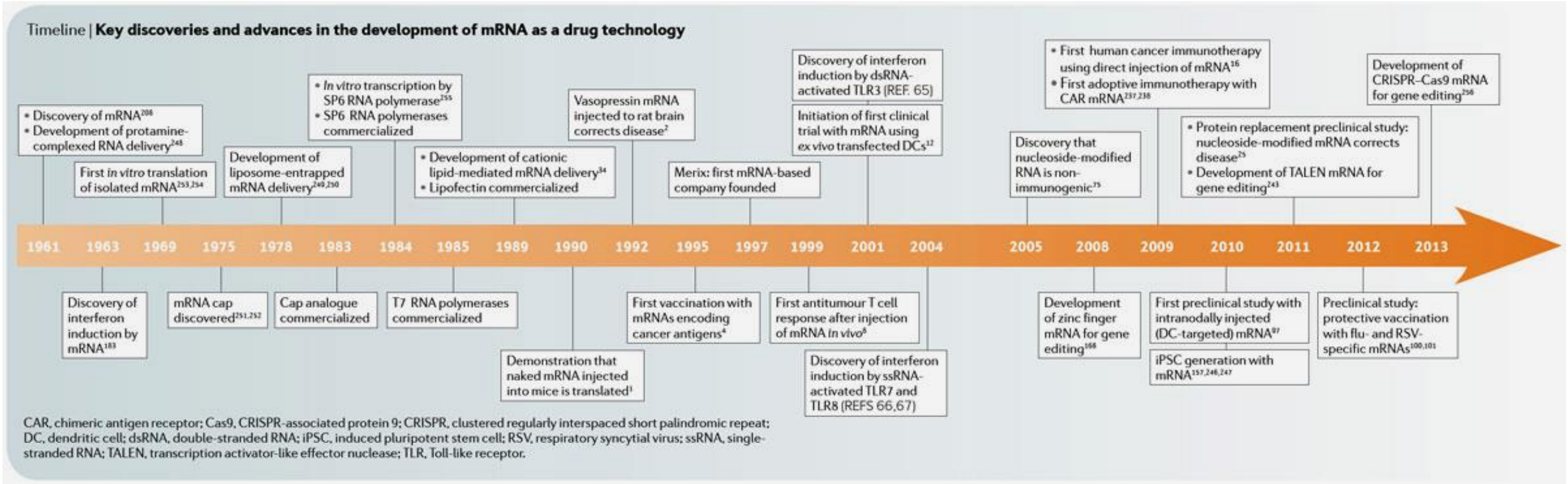
Sydney Brenner shared in the **2002 Nobel Prize in Physiology or Medicine** for deciphering the genetics of programmed cell death and animal development, including how the nervous system forms.

A LARGE amount of evidence suggests that genetic information for protein structure is encoded in deoxyribonucleic acid (DNA) while the actual assembling of amino-acids into proteins occurs in cytoplasmic ribonucleoprotein particles called ribosomes. The fact that proteins are not synthesized directly on genes demands the existence of an intermediate information carrier. This intermediate

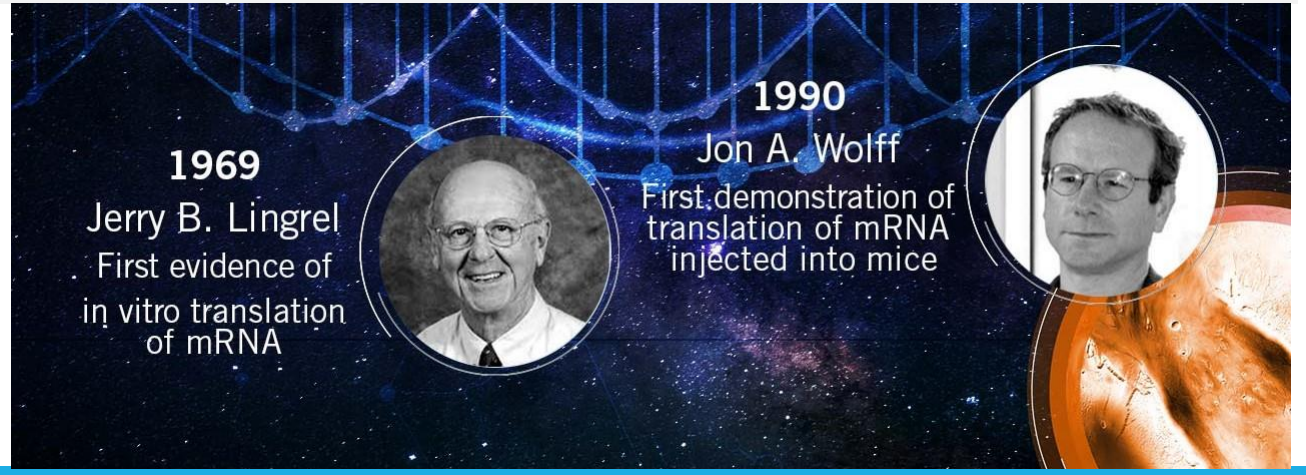
RNA is not the intermediate carrier of information from gene to protein, but rather that ribosomes are non-specialized structures which receive genetic information from the gene in the form of an unstable intermediate or 'messenger'. We present here the results of experiments on phage-infected bacteria which give direct support to this hypothesis.

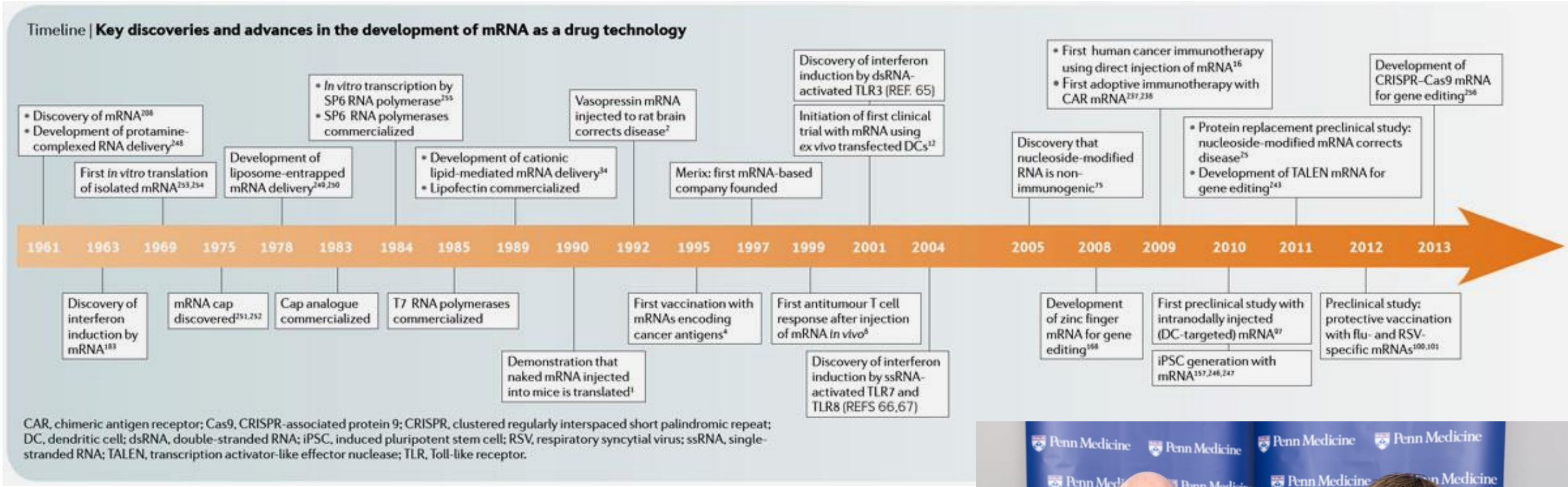
	RNA	mRNA
Definition	RNA is a type of nucleic acid containing ribose and uracil.	mRNA is a type of RNA, which encodes for a particular amino acid sequence of a protein.
Significance	Messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA) are the three major types of RNA found in the cell.	The mRNA is a type of RNA.
Function	RNA is involved in mediating biological processes of the cell such as protein expression and cell signaling.	The mRNA is encoded for a particular protein. The message of a protein is sent for the translation from the nucleus via mRNA.

RNA and mRNA are two types of nucleic acids, mediating the protein synthesis in the cell. Both RNA and mRNA contain ribose and uracil in their structure. The three major types of RNA are mRNA, tRNA, and rRNA.



1961: discovery (isolation) of messenger RNA (mRNA)
1969: first proteins produced from isolated mRNA
1970: advances in liposomes
1984: mRNA synthesized in the lab
1990: translation of mRNA injected into mice





1961: discovery (isolation) of messenger RNA (mRNA)
1969: first proteins produced from isolated mRNA
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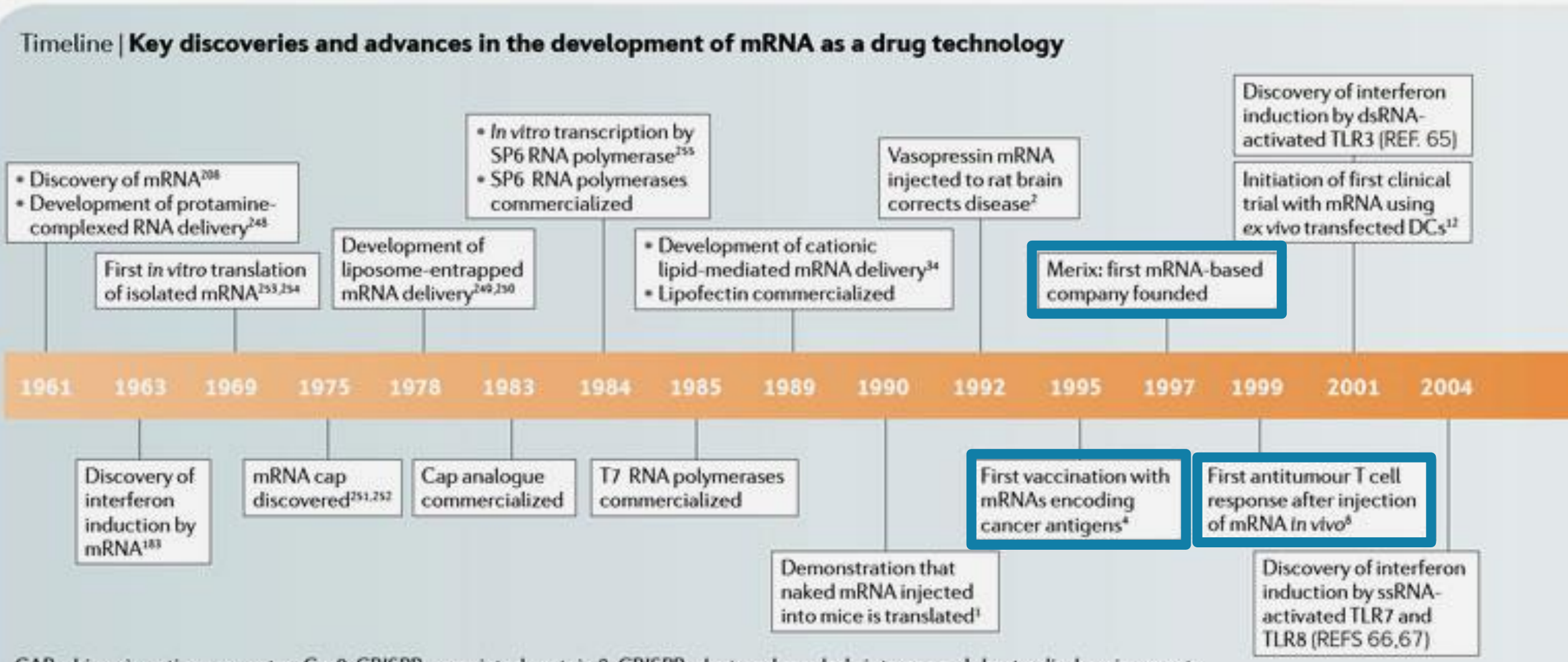
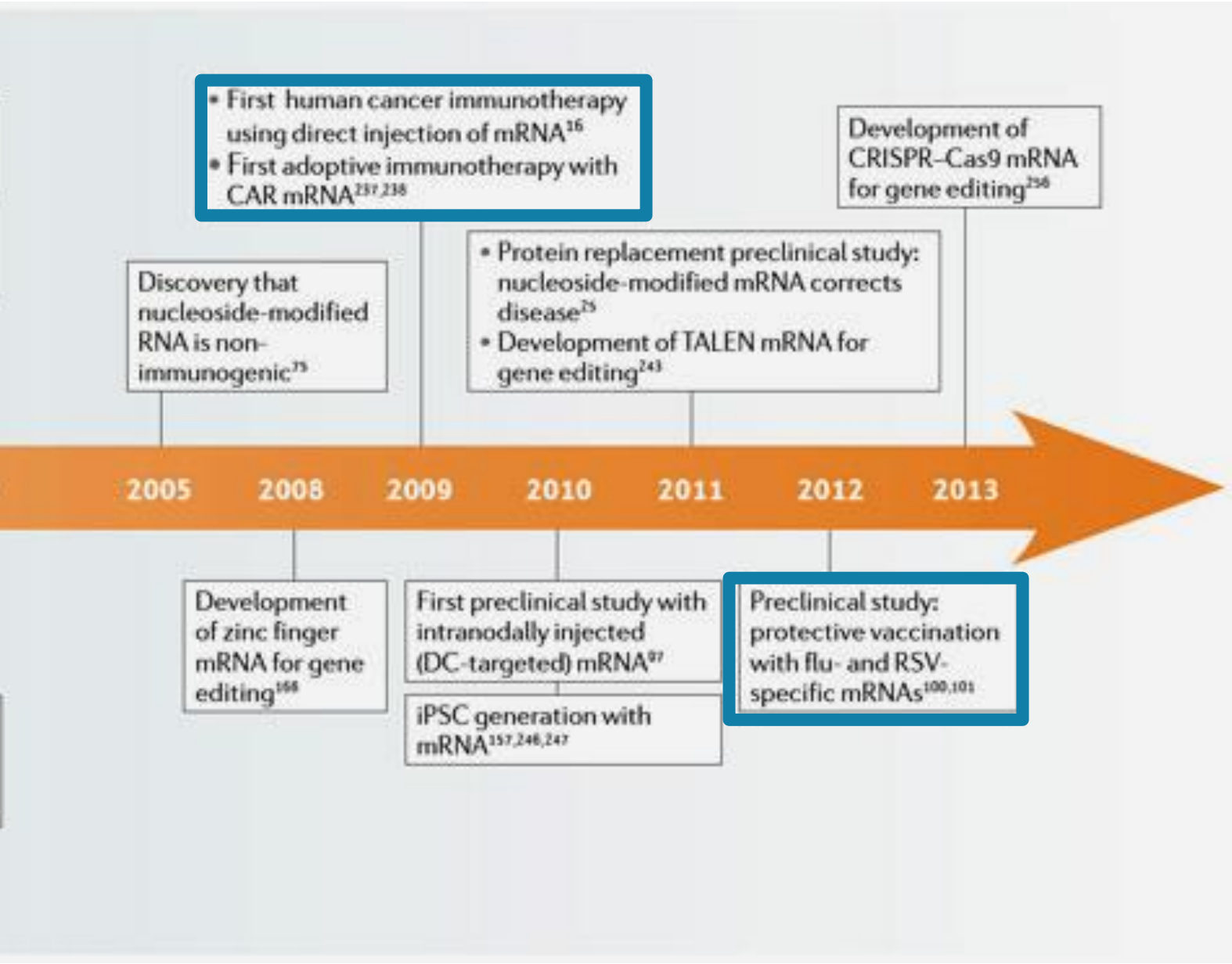
1990: translation of mRNA injected into mice

Several years after no significant developments

Timeline | **Key discoveries and advances in the development of mRNA as a drug technology**



CAR, chimeric antigen receptor; Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeat; DC, dendritic cell; dsRNA, double-stranded RNA; iPSC, induced pluripotent stem cell; RSV, respiratory syncytial virus; ssRNA, single-stranded RNA; TALEN, transcription activator-like effector nuclease; TLR, Toll-like receptor.



2018: Patisiran approved by US FDA

ONPATTRO™ (Patisiran) is used to treat **polyneuropathy (nerve disease)** caused by **hereditary transthyretin-mediated amyloidosis**.

BIONTECH

JOHANNES GUTENBERG UNIVERSITÄT MAINZ

JG|U

- Improving mRNA stability and translation in human dendritic cells (DC)
- Selective mRNA delivery to DCs in lymphoid tissues in vivo
- Exploitation of inherent adjuvant activity for stimulating Th1 immunity

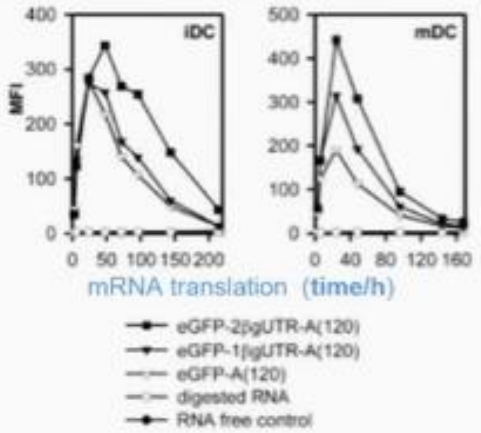
Sebastian Kreiter

Raouf Selmi

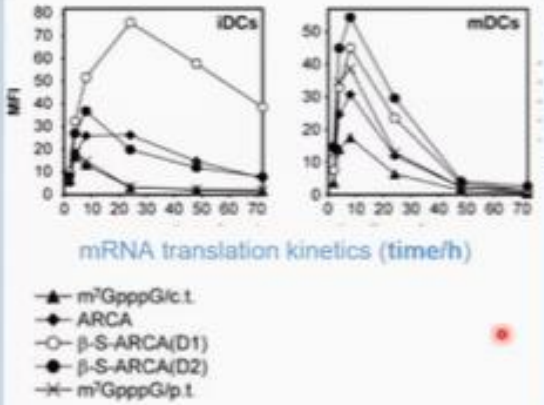
Mustafa Diken

Johannes Gutenberg University Mainz

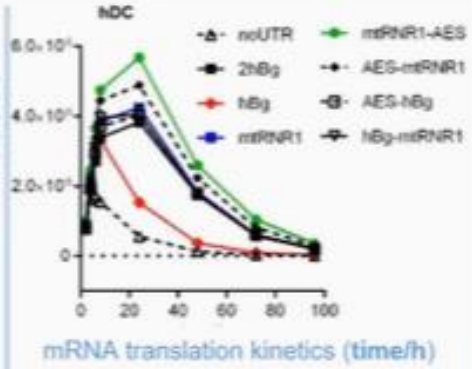
Improving mRNA translation and stability in DC



Holtkamp S et al., Modification of antigen-encoding RNA increases stability, translational efficacy, and T-cell stimulatory capacity of dendritic cells. ***Blood*** **108, 2006.**

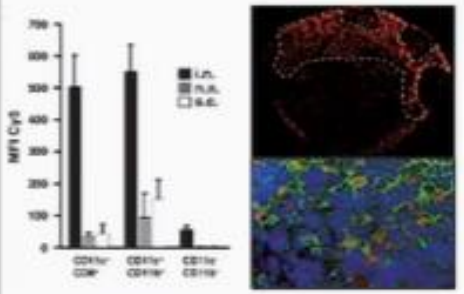


Kuhn et al. Phosphorothioate cap analogs increase stability and translational efficiency of RNA vaccines in immature dendritic cells and induce superior immune responses *in vivo*, ***Gene Therapy, 2010***



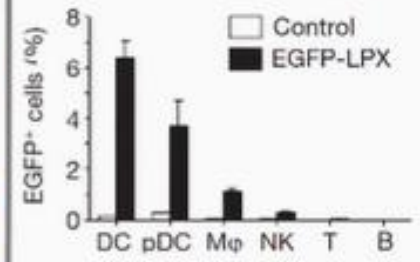
Orlandini et al . Improving mRNA-based therapeutic gene delivery by expression-augmenting 3' UTRs identified by cellular library screening, **Molecular Therapy, 2019**

Selective *in vivo* mRNA targeting to DCs



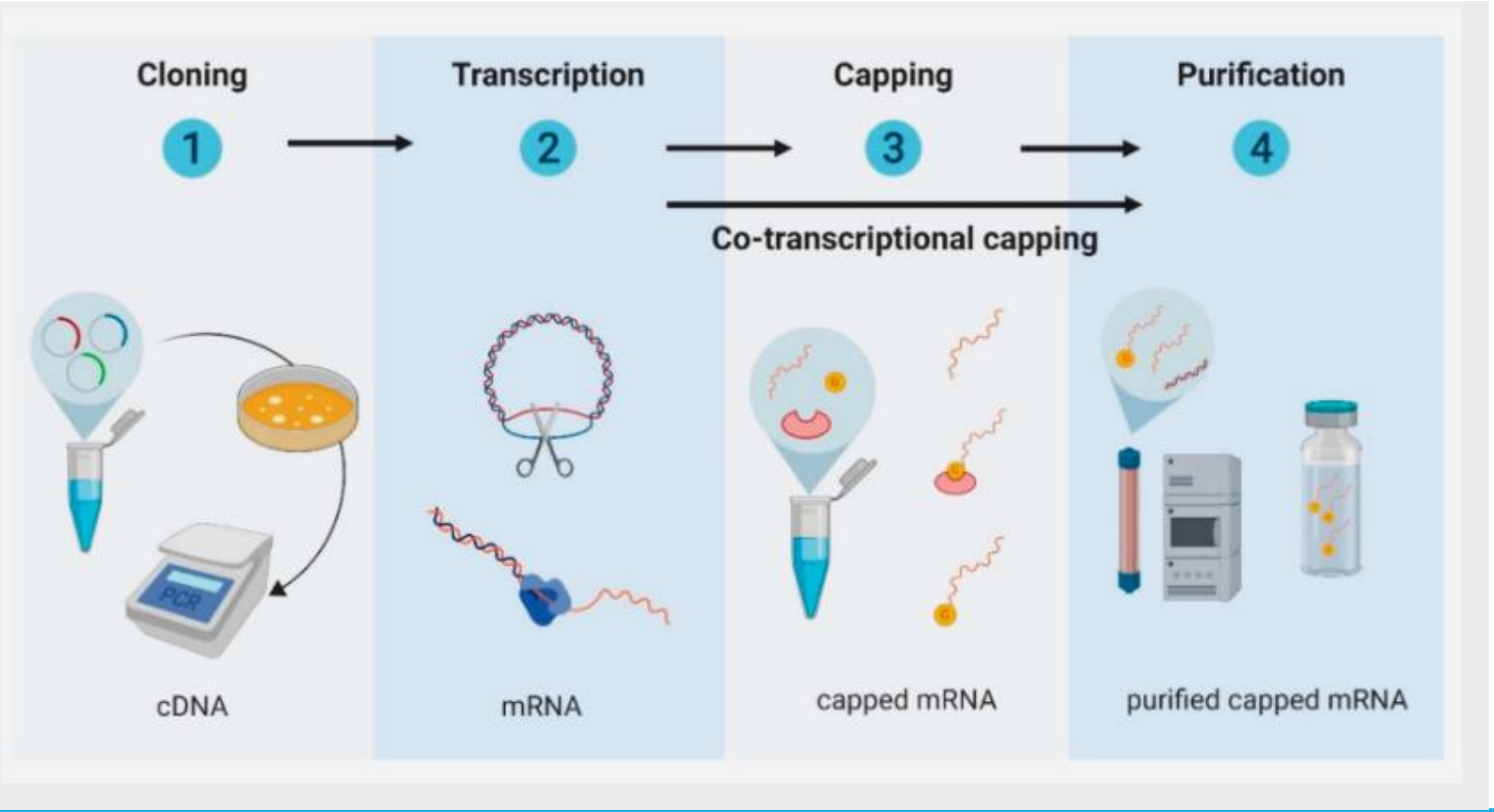
Targeting lymp node DCs

Kreiter, S. Intranodal vaccination with naked antigen-encoding RNA elicits potent prophylactic and therapeutic antitumoural immunity. ***Cancer Res.*** **2010.**



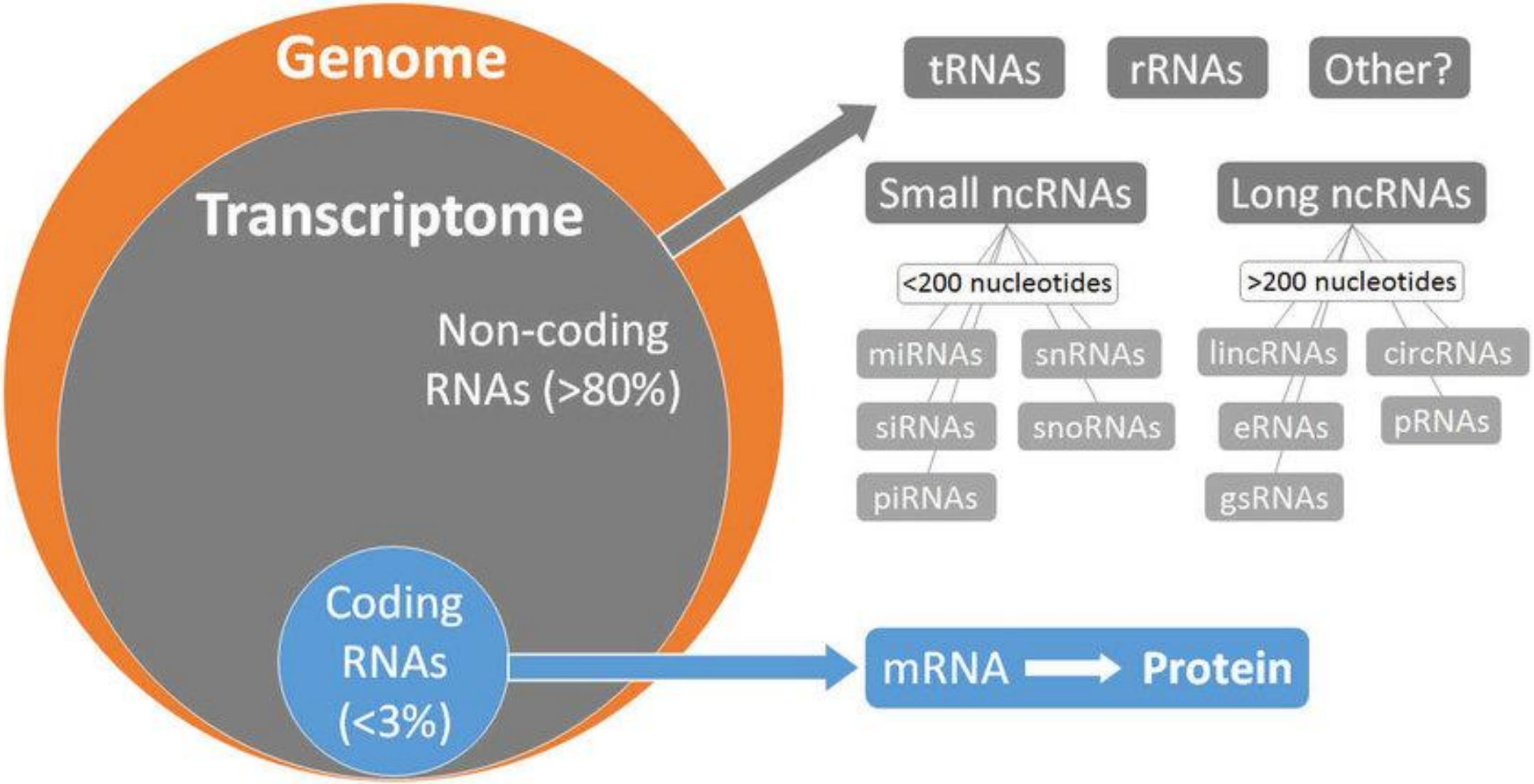
Targeting spleen DCs

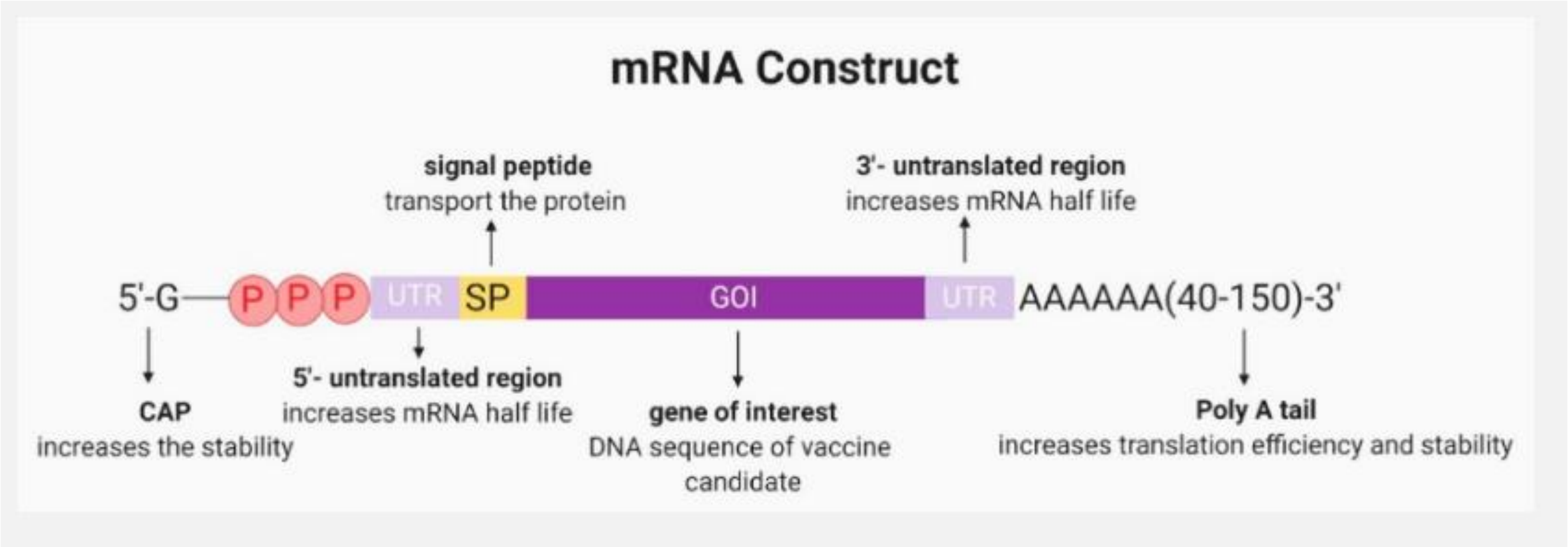
Kranz, L. et al., Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. ***Nature,*** **2016.**



Enlisting the mRNA Vaccine Platform to Combat Parasitic Infections (https://www.mdpi.com/2076-393X/7/4/122/htm)

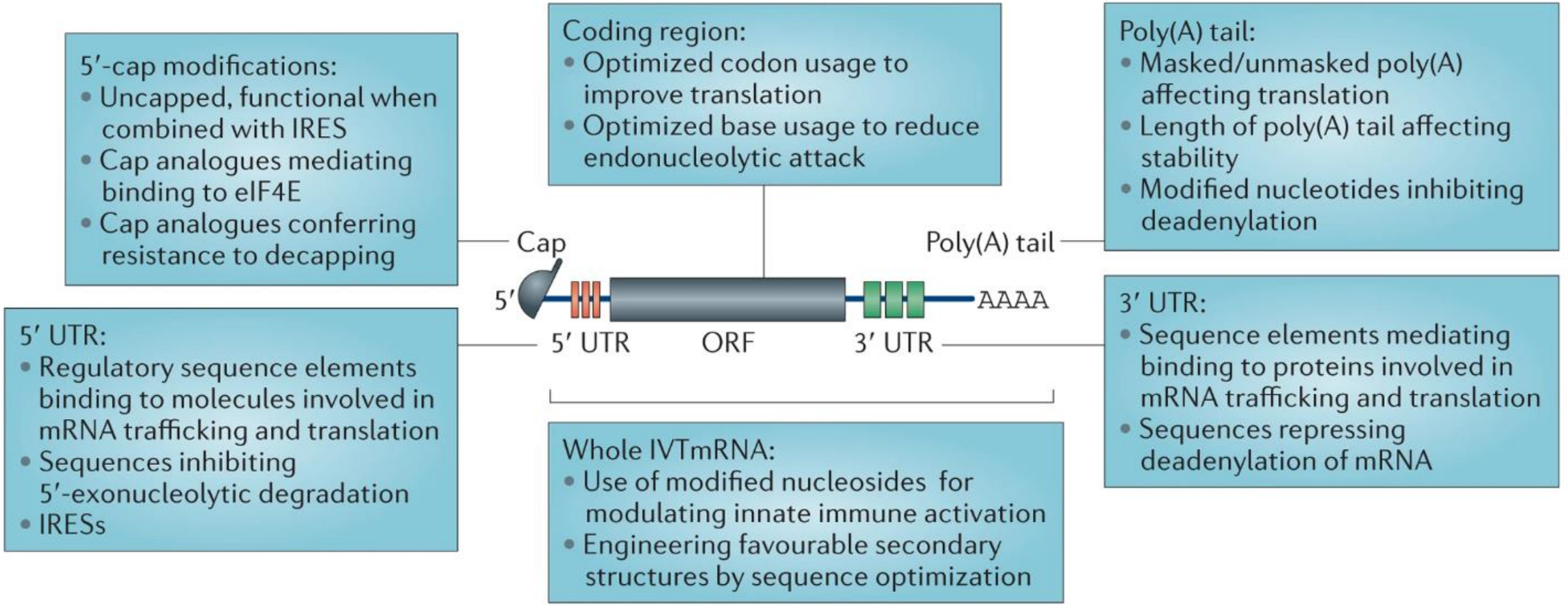
There are coding and non-coding RNAs



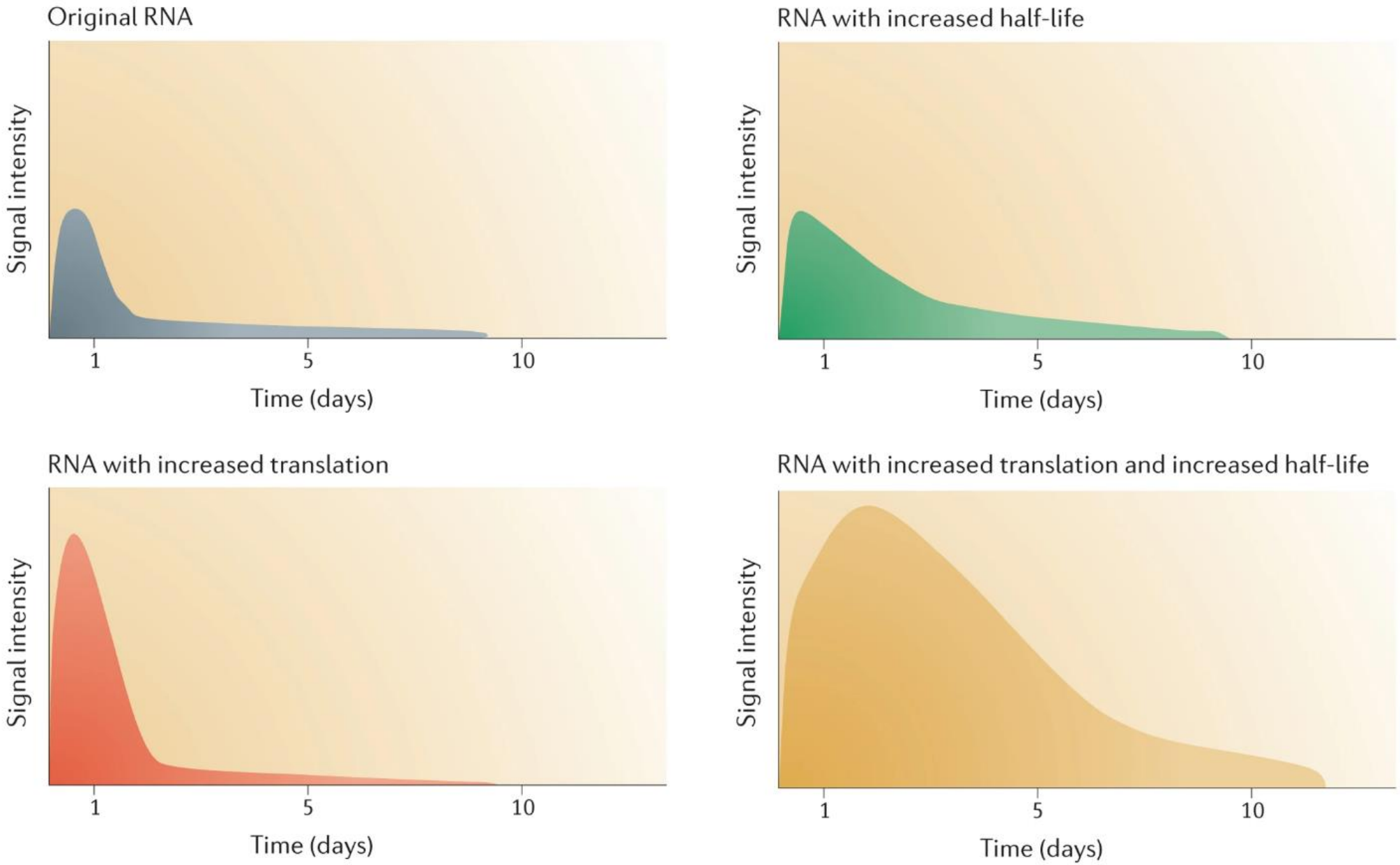


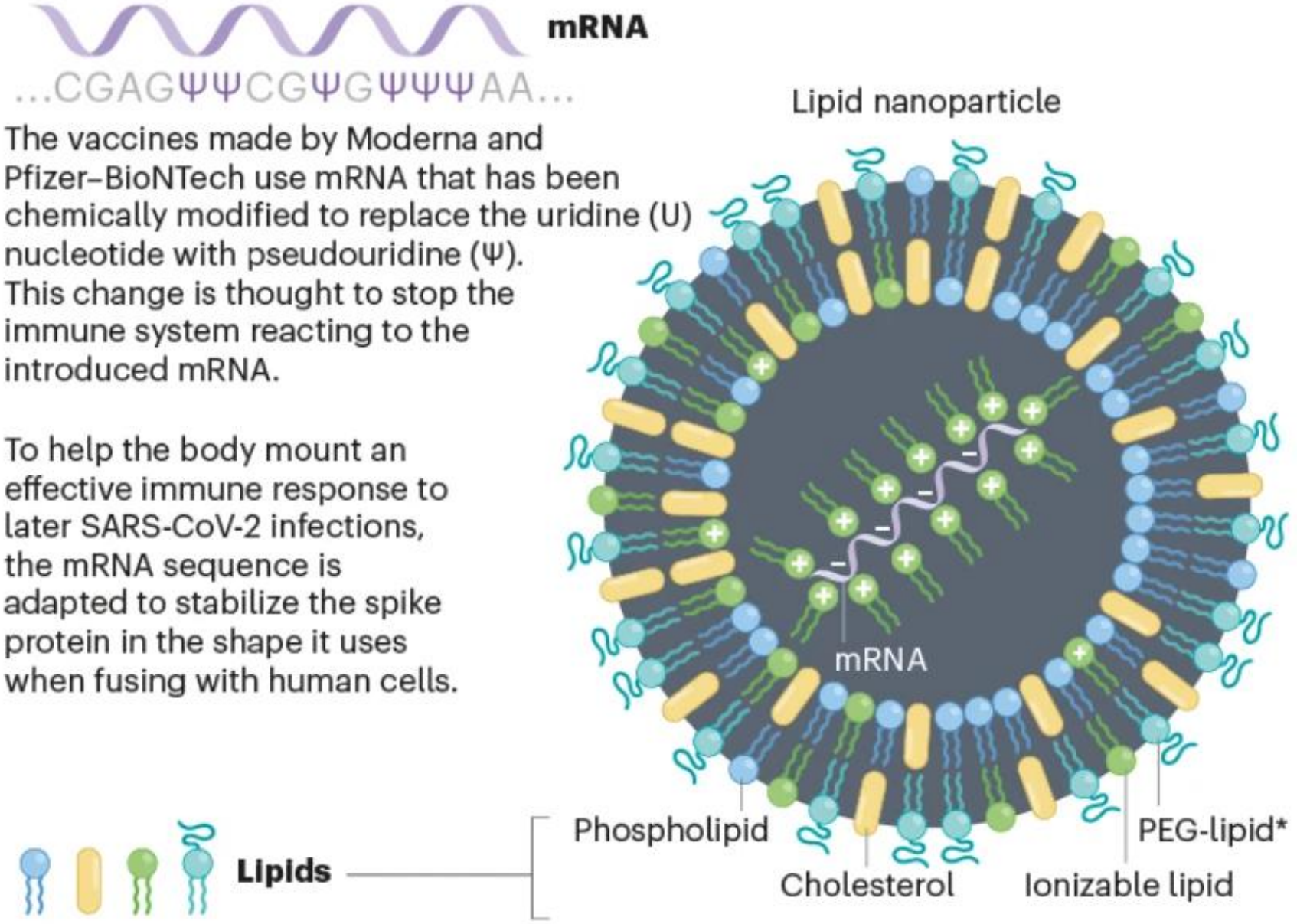
A typical mRNA construct with supporting untranslated regions, poly(A) tail, and an optional signal peptide sequence attached to the coding sequence.

a Structural modifications for tuning mRNA pharmacokinetics

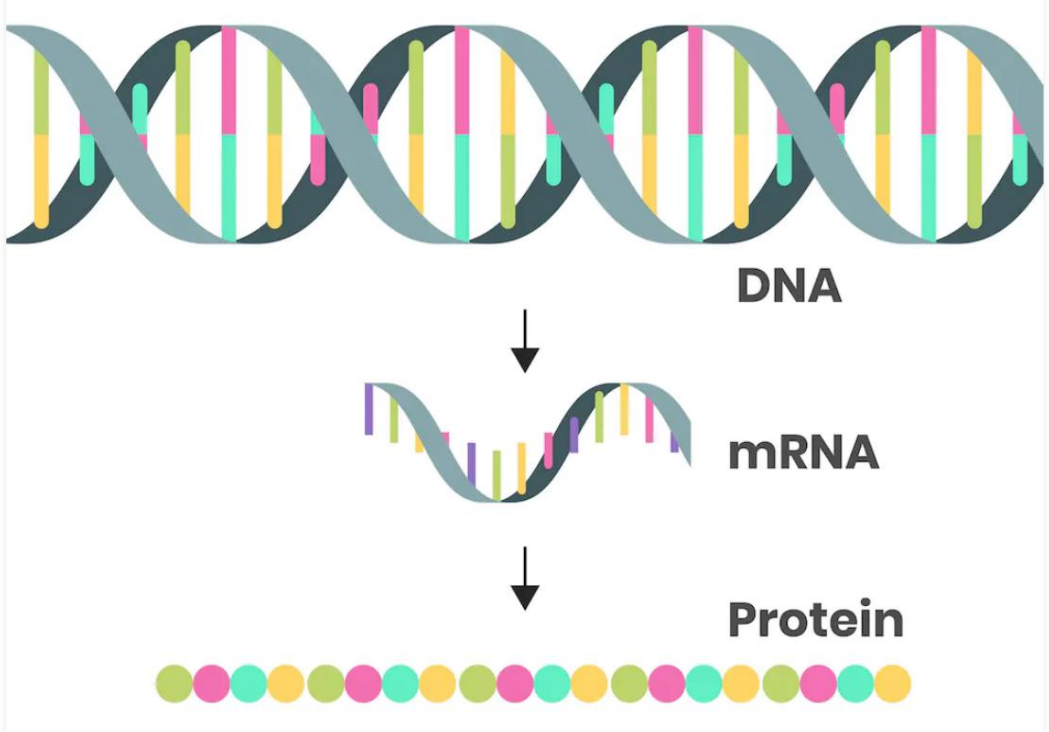


eIF4E, eukaryotic translation initiation factor 4E; IRES, internal ribosome entry site; ORF, open reading frame


Original RNA
Signal intensity
1
5
10
Time (days)
RNA with increased half-life
Signal intensity
1
5
10
Time (days)
RNA with increased translation
Signal intensity
1
5
10
Time (days)
RNA with increased translation and increased half-life
Signal intensity
1
5
10
Time (days)



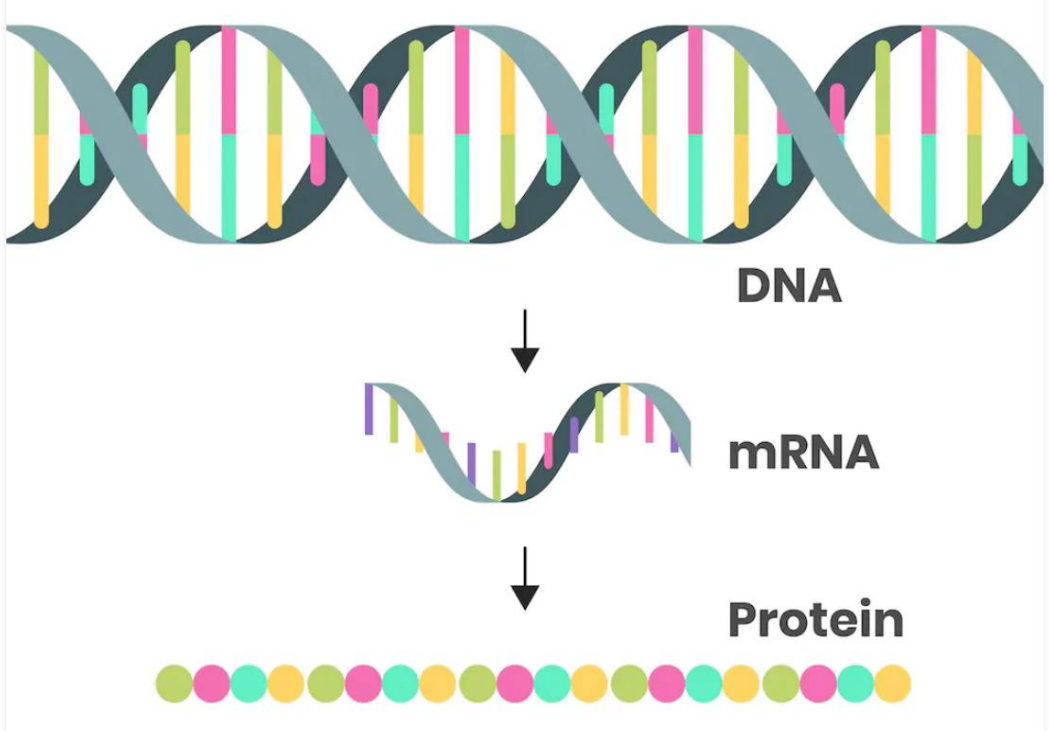
Nik Spencer/Nature; Adapted from M. D. Buschmann et al. Vaccines 9, 65 (2021)



WHEN

HOW

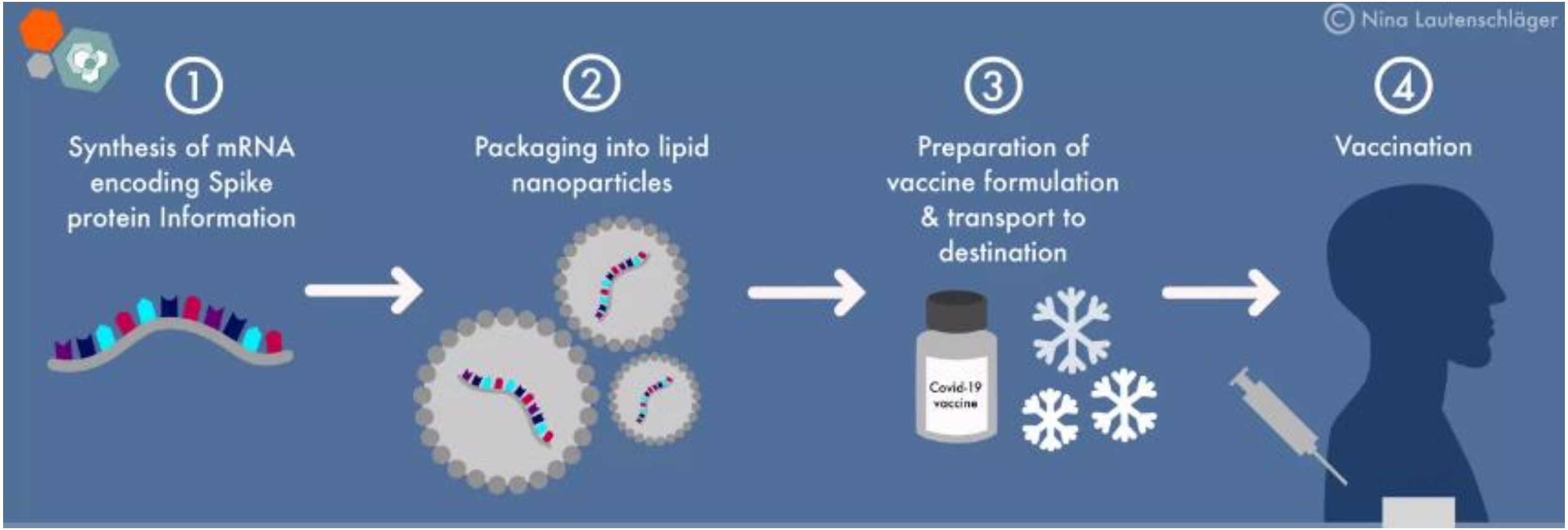
WHY



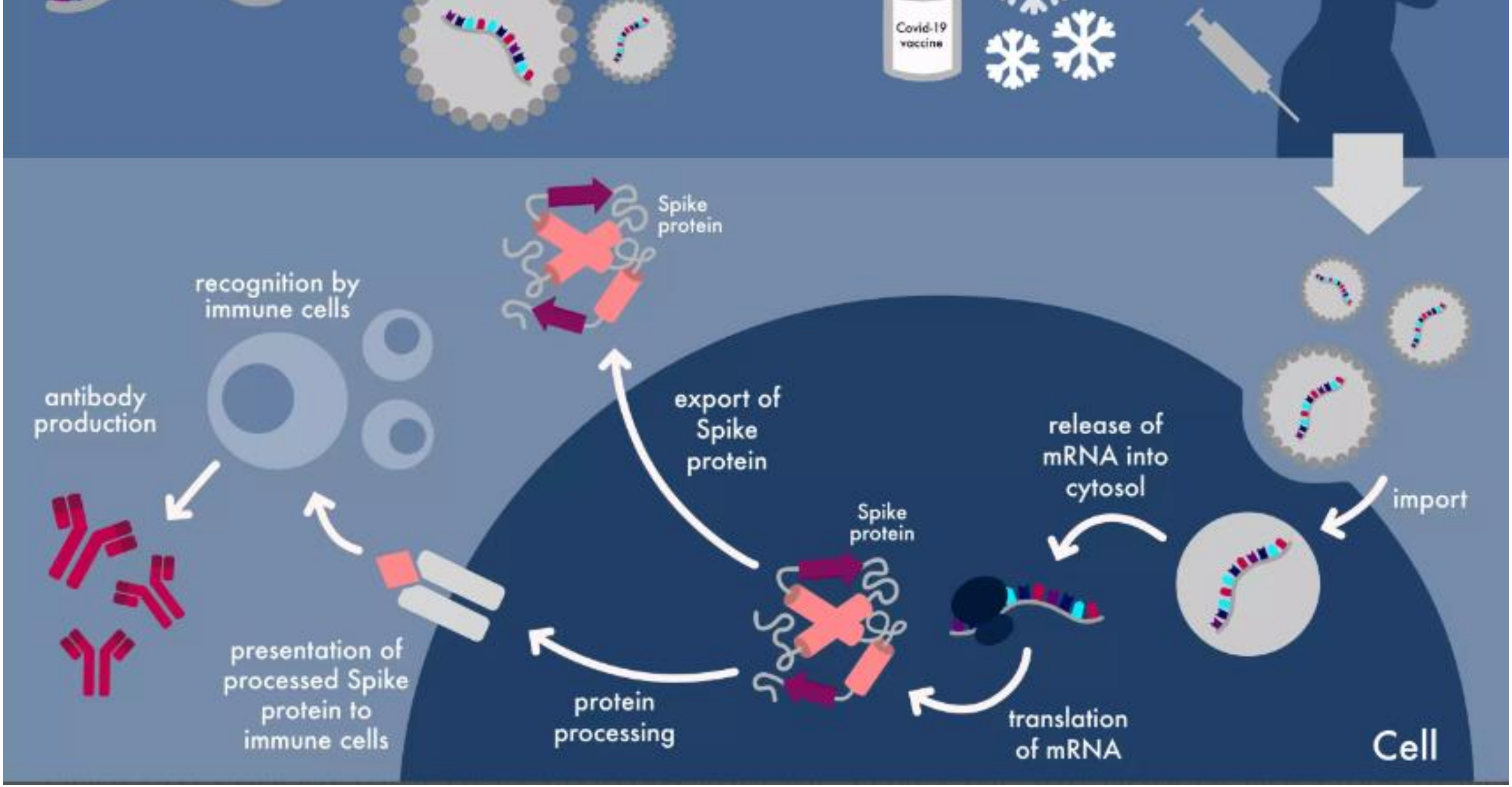
WHEN did mRNA research start?

HOW do they work? HOW are they produced?

WHY



The mRNA Covid-19 vaccine pathway https://www.phdnet.mpg.de/offspring/Covid-19_vaccines)

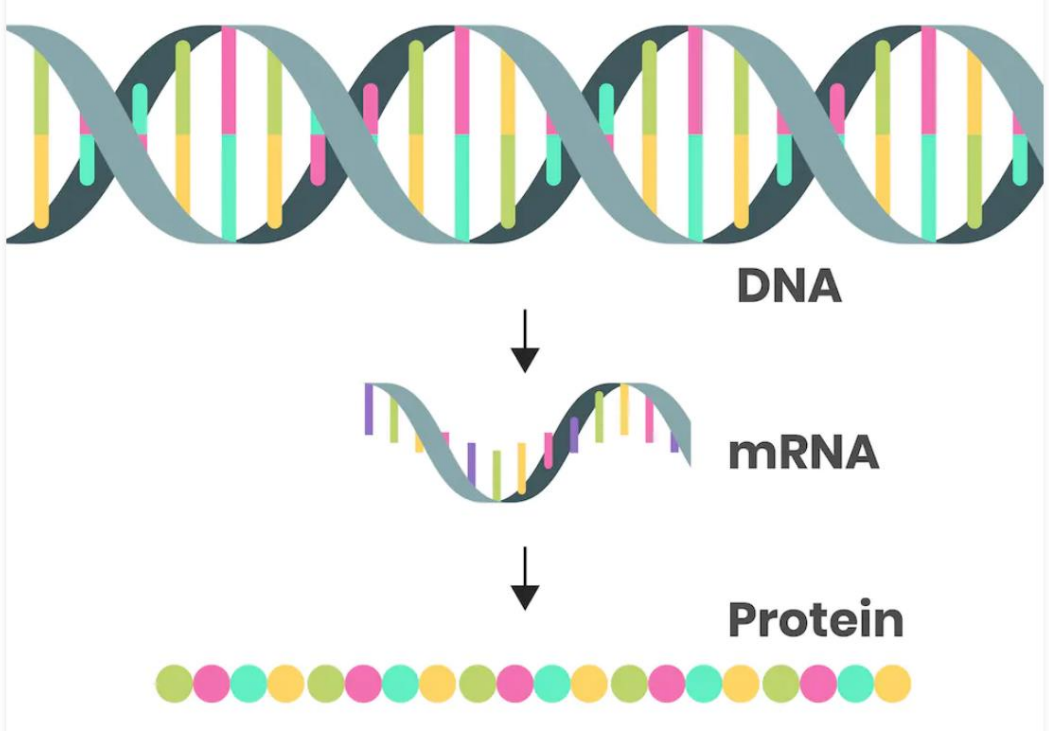


The mRNA Covid-19 vaccine pathway https://www.phdnet.mpg.de/offspring/Covid-19_vaccines)

How do you know mRNA vaccines will not alter your DNA?

- ✓ mRNA is chemically and structurally different from DNA, mRNA is located in a different cellular compartment.
  - mRNA is *produced* in the nucleus but is quickly exported to the cytoplasm with a one-way ticket: it does not come back.
- ✓ Only specific proteins carry “nuclear localization signals” can migrate from the cytoplasm into the nucleus
  - mRNA vaccines do not include such molecular instruction.
- ✓ RNA molecule is charged and carries the same charge as the nucleus, like charges repel.
  - RNA molecule is physically repelled by the nucleus.

The mRNA Covid-19 vaccine pathway https://www.phdnet.mpg.de/offspring/Covid-19_vaccines)



WHEN did mRNA research start?

HOW are the different from traditional vaccines?

WHY

mRNA Vaccine

Components

mRNA (blueprint of protein)

Production

Faster because mRNA molecules are easier to produce

Process

Components are injected into the arm and serve as instructions for the body to make microbial protein

R & D

Antigen determined for immune stimulation

Result

Teaches the body to protect itself against a microbe

Traditional Vaccine

Components

Microbial protein or inactive microbe

Production

Slower and more difficult to produce the right type of protein

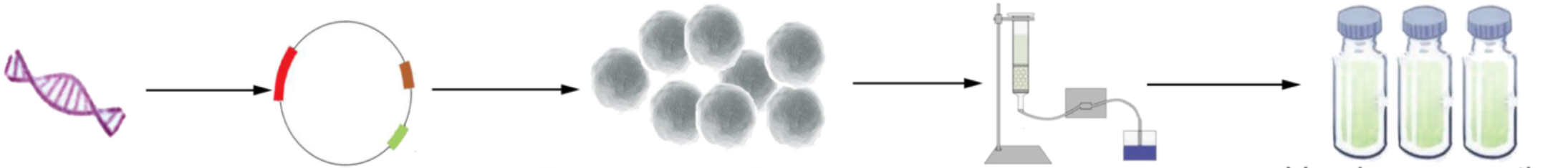
Process

Components are made in a lab and injected into the arm to stimulate immune response

Quality-control methods for different vaccines

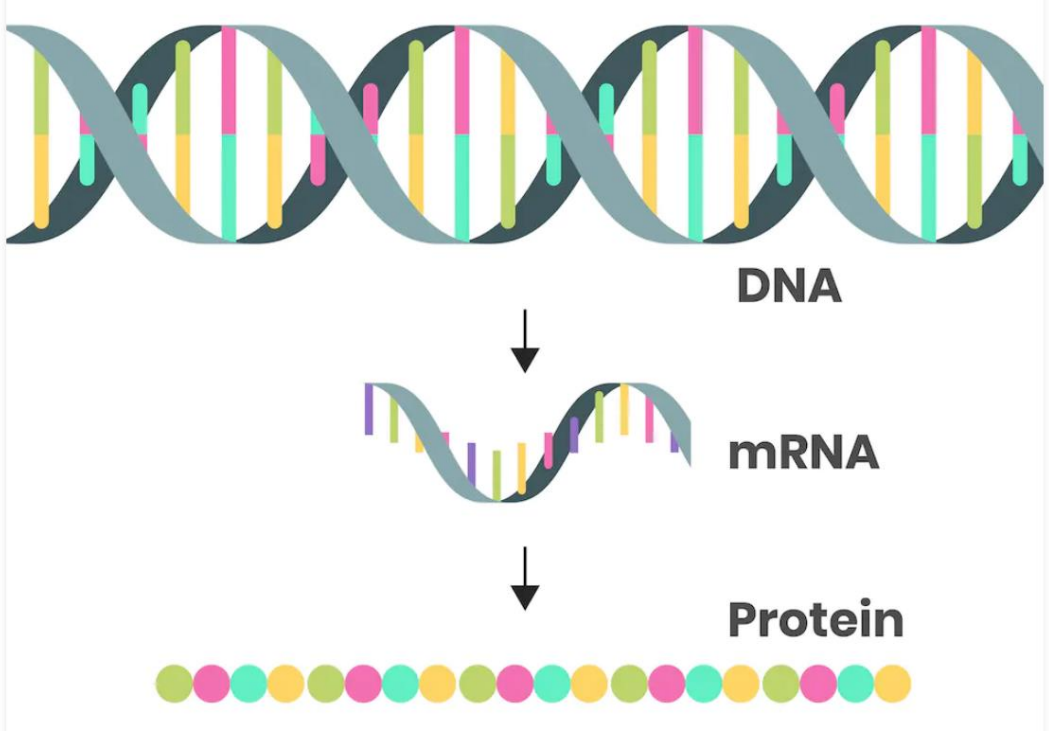
Recombinant protein vaccine

Target antigen	Identification, Content, Purity, Process-related impurities (Host protein residue, Host nucleic acid residue), Endotoxin, pH, etc.
Adjuvant	Adjuvant content, Antigen adsorption rate, pH, etc.
Final products	Identification, Potency *in vitro* and *in vivo,* Endotoxin, pH, Sterility test, Abnormal toxicity, Process related impurities (e.g. Antibiotic residues), etc.



mRNA vaccine

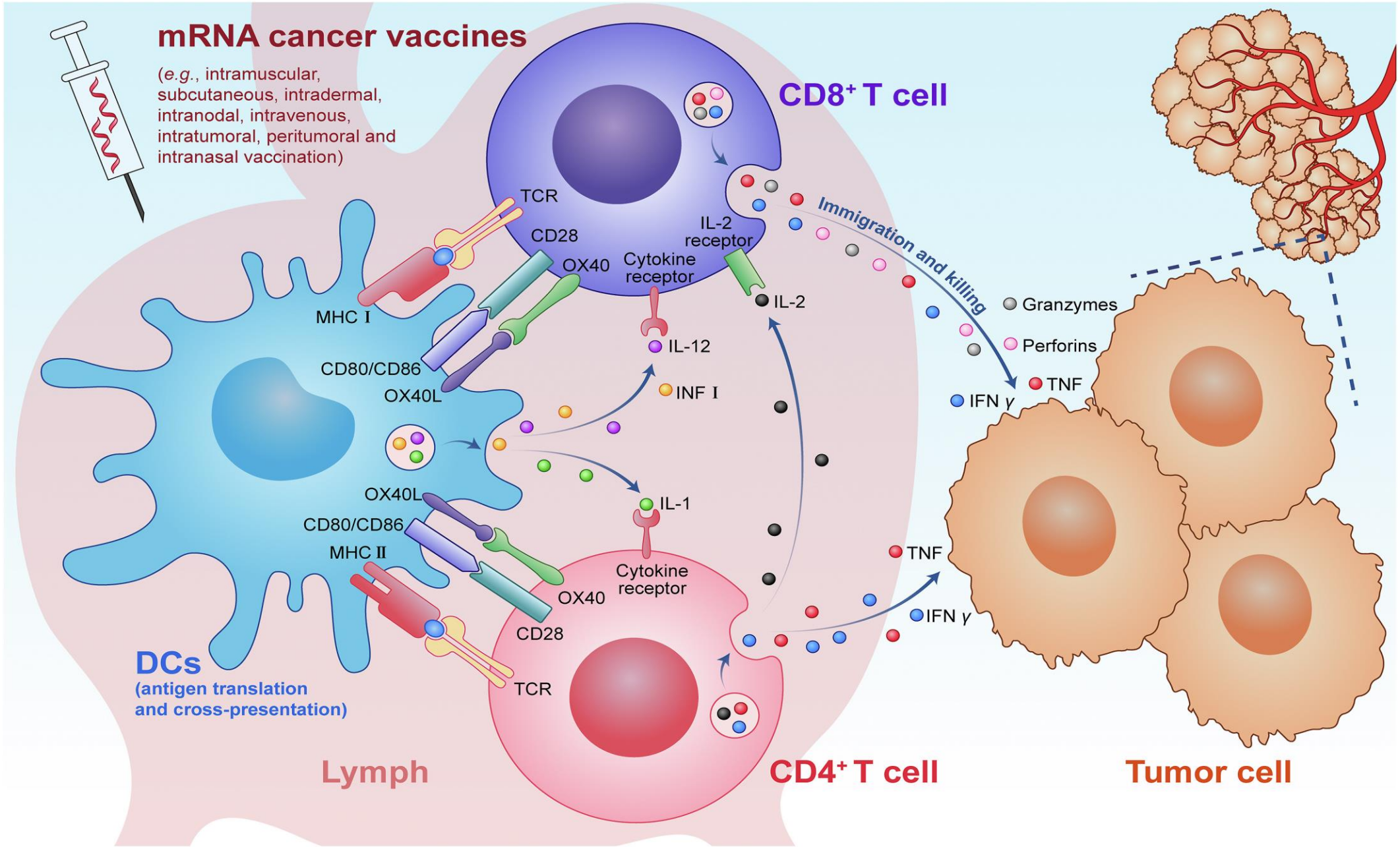
mRNA bulk	mRNA identification, mRNA sequence length, Sequence integrity and Accuracy, etc.
Nanoparticle carrier	Optimization of encapsulation rate, Particle size distribution, Surface charge, and Stability, etc.
Final products	Identification, Potency *in vitro* and *in vivo,* Endotoxin, pH, Sterility test, Abnormal toxicity, Process related impurities (e.g. Antibiotic residues), etc.



WHEN did mRNA research start?

HOW can we maximize the use of the mRNA tech?

WHY



mRNA cancer vaccines: Advances, trends and challenges https://www.sciencedirect.com/science/article/pii/S2211383522001198

Journal of *Clinical Medicine*

MDPI

Review

Advancements in Nucleic Acid Based Therapeutics against Respiratory Viral Infections

Kumari Asha [1,*], Prashant Kumar [2], Melvin Sanicas [3], Clement A. Meseko [4], Madhu Khanna [5] and Binod Kumar [1,*]

Received: 26 November 2018; Accepted: 19 December 2018; Published: 20 December 2018

Abstract: Several viruses cause pulmonary infections due to their shared tropism with cells of the respiratory tract. These respiratory problems due to viral infection become a public health concern due to rapid transmission through air/aerosols or via direct-indirect contact with infected persons. In addition, the cross-species transmission causes alterations to viral genetic makeup thereby increasing the risk of emergence of pathogens with new and more potent infectivity. With the introduction of effective nucleic acid-based technologies, post translational gene silencing (PTGS) is being increasingly used to silence viral gene targets and has shown promising approach towards management of many viral infections. Since several host factors are also utilized by these viruses during various stages of infection, silencing these host factors can also serve as promising therapeutic tool. Several nucleic acid-based technologies such as short interfering RNAs (siRNA), antisense oligonucleotides, aptamers, deoxyribozymes (DNAzymes), and ribozymes have been studied and used against management of respiratory viruses. These therapeutic nucleic acids can be efficiently delivered through the airways. Studies have also shown efficacy of gene therapy in clinical trials against respiratory syncytial virus (RSV) as well as models of respiratory diseases including severe acute respiratory syndrome (SARS), measles and influenza. In this review, we have summarized some of the recent advancements made in the area of nucleic acid based therapeutics and highlighted the emerging roles of nucleic acids in the management of some of the severe respiratory viral infections. We have also focused on the methods of their delivery and associated challenges.

With the introduction of effective nucleic acid-based technologies, **post translational gene silencing (PTGS) is being increasingly used to silence viral gene targets** and has shown promising approach towards management of many viral infections.

Nucleic acid-based molecules have shown tremendous potential to block gene expression either during the transcriptional or post-transcriptional level. These nucleic acid-based molecules have also been shown to have **potential applications in cancer, neurological disorders, cardiovascular, inflammatory disorders**, and **infectious diseases.**

Advancements in Nucleic Acid Based Therapeutics against Respiratory Viral Infections - PubMed (nih.gov)

Future / potential mRNA applications

	Pre-clinical
Cancer immunotherapy	Melanoma, prostate cancer, haematological malignancies, ovarian cancer, lymphoma, leukaemia, mesothelioma
Infectious diseases	Influenza, tuberculosis, respiratory tract infection, tick-borne encephalitis
Allergy tolerization	Allergies for peanut, egg white, grass pollen and dust mite
Protein replacement	Diabetes insipidus, anaemia, congenital lung disease, asthma, myocardial infarction, melanoma, autoimmune myocarditis, inflammation

Sahin, U., Karikó, K. & Türeci, Ö. mRNA-based therapeutics — developing a new class of drugs. *Nat Rev Drug Discov* 13, 759–780 (2014). https://doi.org/10.1038/nrd4278


Rhesus Macaque
Pig-tailed Macaque
Cynomolgus Monkey


The Collaboration for TB Vaccine Discovery
Launch Meeting - 2015
CTVD

Future / potential mRNA applications

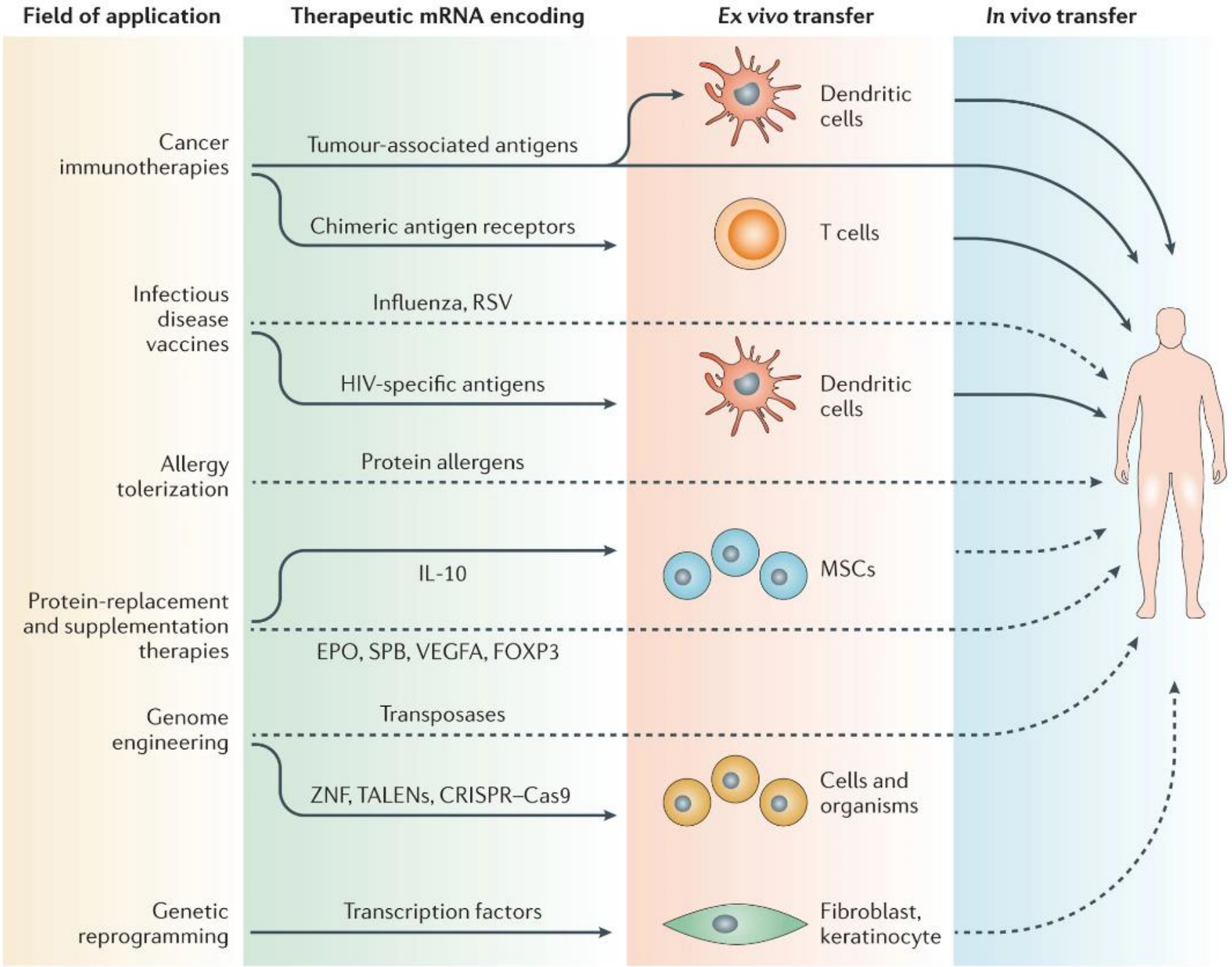
	Pre-clinical	Clinical
Cancer immunotherapy	Melanoma, prostate cancer, haematological malignancies, ovarian cancer, lymphoma, leukaemia, mesothelioma	Melanoma, renal cell carcinoma, prostate cancer, pancreatic cancer, metastatic malignancies, colon cancer, leukaemia
Infectious diseases	Influenza, tuberculosis, respiratory tract infection, tick-borne encephalitis	HIV
Allergy tolerization	Allergies for peanut, egg white, grass pollen and dust mite	None, for now
Protein replacement	Diabetes insipidus, anaemia, congenital lung disease, asthma, myocardial infarction, melanoma, autoimmune myocarditis, inflammation	None, for now

Sahin, U., Karikó, K. & Türeci, Ö. mRNA-based therapeutics — developing a new class of drugs. *Nat Rev Drug Discov* 13, 759–780 (2014). https://doi.org/10.1038/nrd4278

Future / potential mRNA applications

	Pre-clinical	Clinical
Genome engineering, gene editing	Gene editing, engineered animal models: engineered mice, engineered rats, engineered rabbits, engineered macaques	None, for now
Genetic reprogramming of cells, tissue engineering	Transcription factors, progerin (biomarker used in studies on natural aging) for modeling of Parkinson's disease, generating induced pluripotent stem cells	None, for now

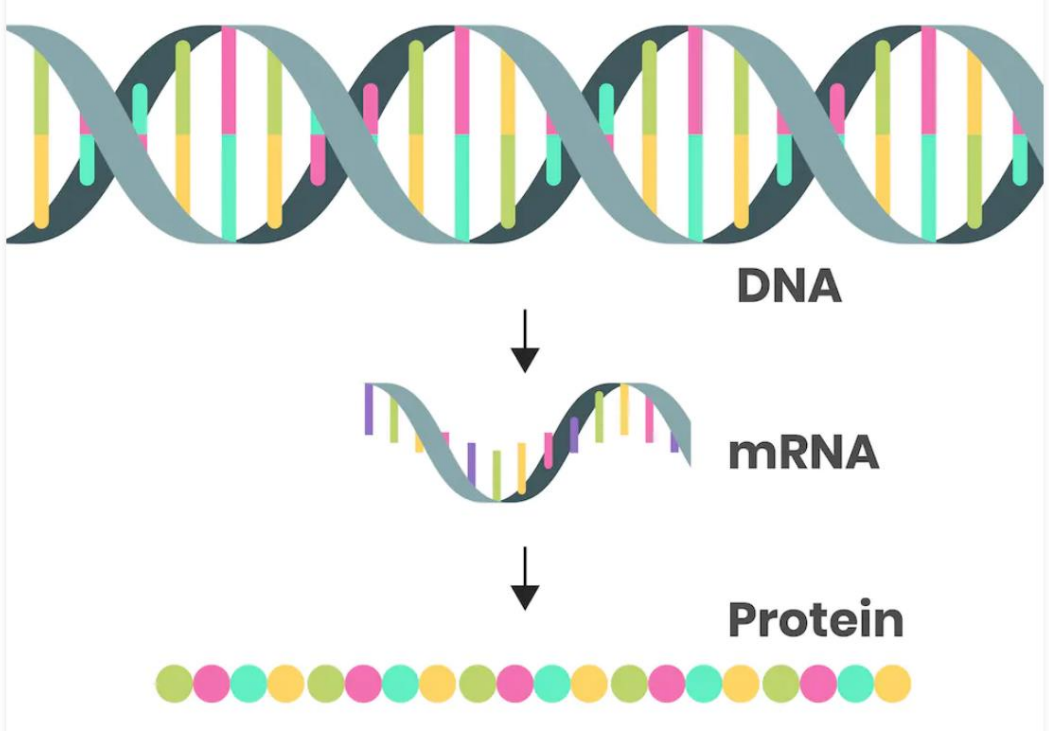
Sahin, U., Karikó, K. & Türeci, Ö. mRNA-based therapeutics — developing a new class of drugs. *Nat Rev Drug Discov* 13, 759–780 (2014). https://doi.org/10.1038/nrd4278



The solid arrows pointing in the right-hand column denote applications that are in the clinic, whereas stippled arrows refer to preclinical applications.

Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeat; EPO, erythropoietin; FOXP3, forkhead box P3; IL-10, interleukin-10; MSC, mesenchymal stem cell; RSV, respiratory syncytial virus; SPB, surfactant protein B; TALEN, transcription activator-like effector nuclease; VEGFA, vascular endothelial growth factor A; ZNF, zinc finger nuclease.

https://www.nature.com/articles/nrd4278/figures/6



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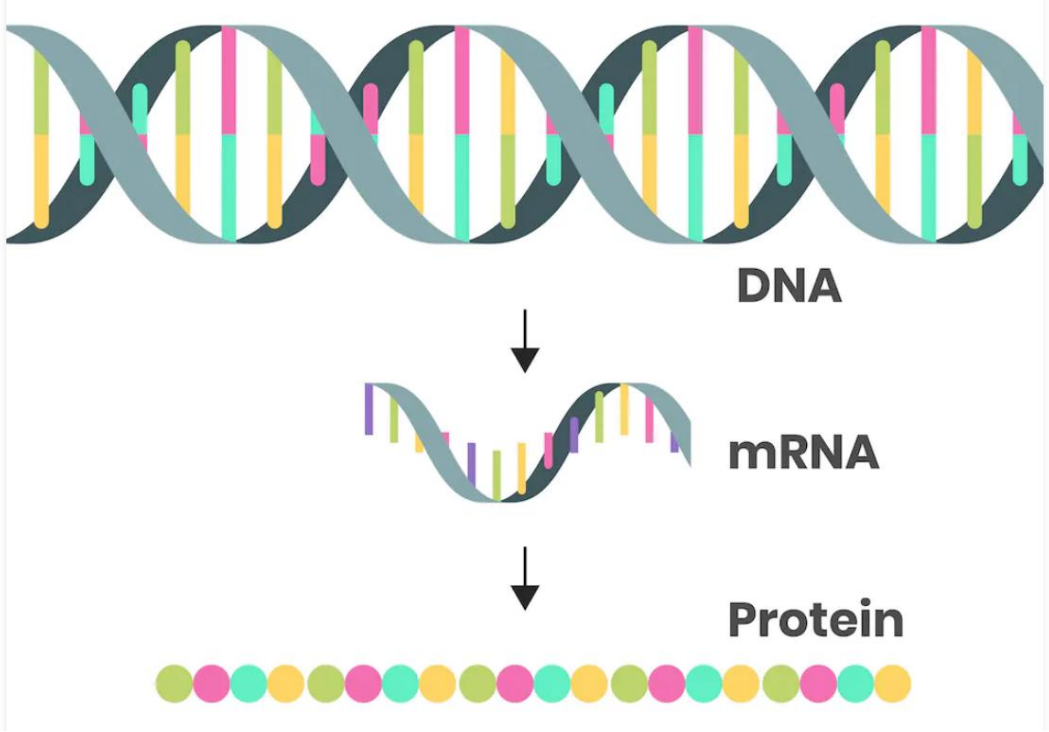
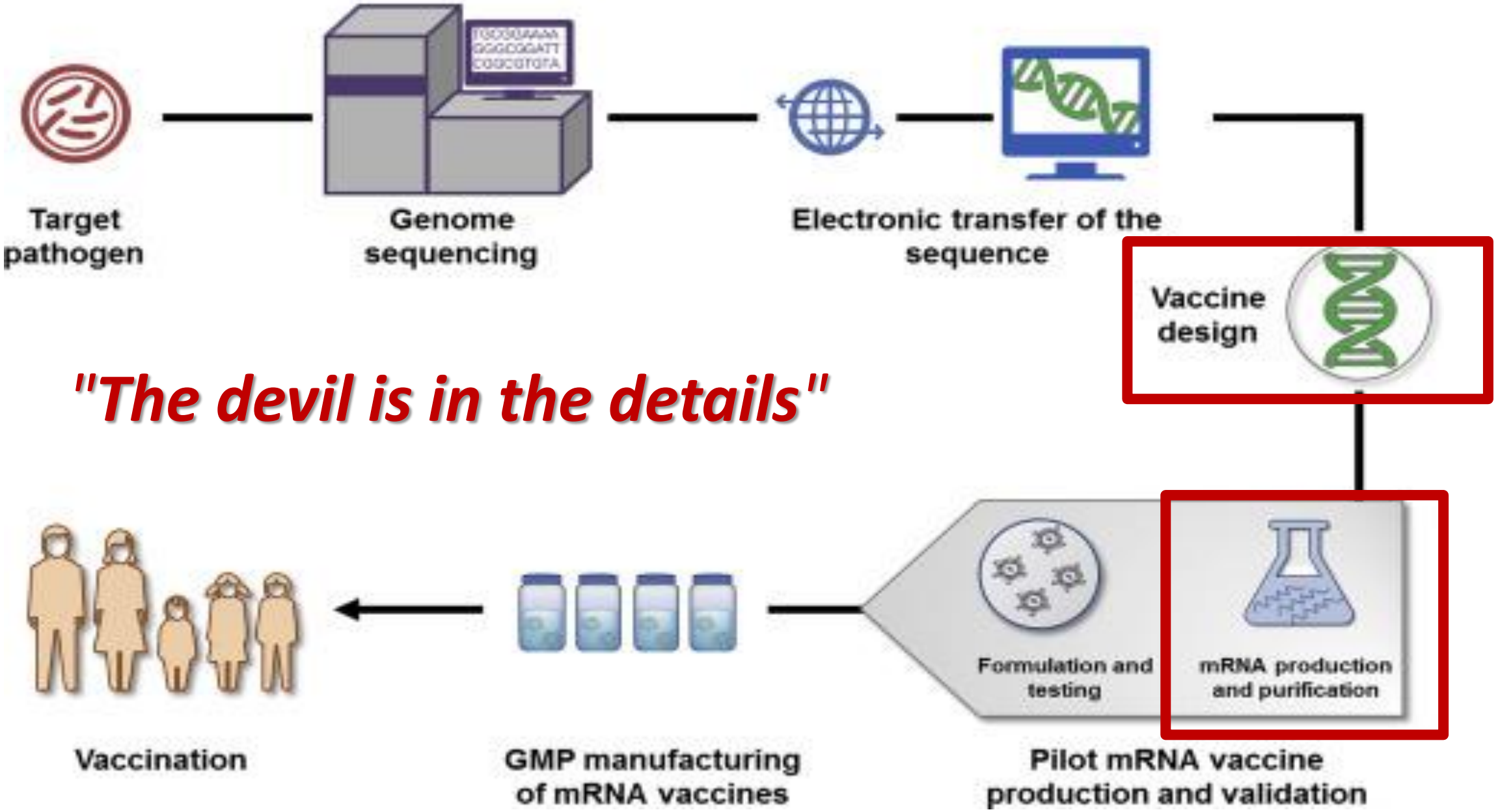
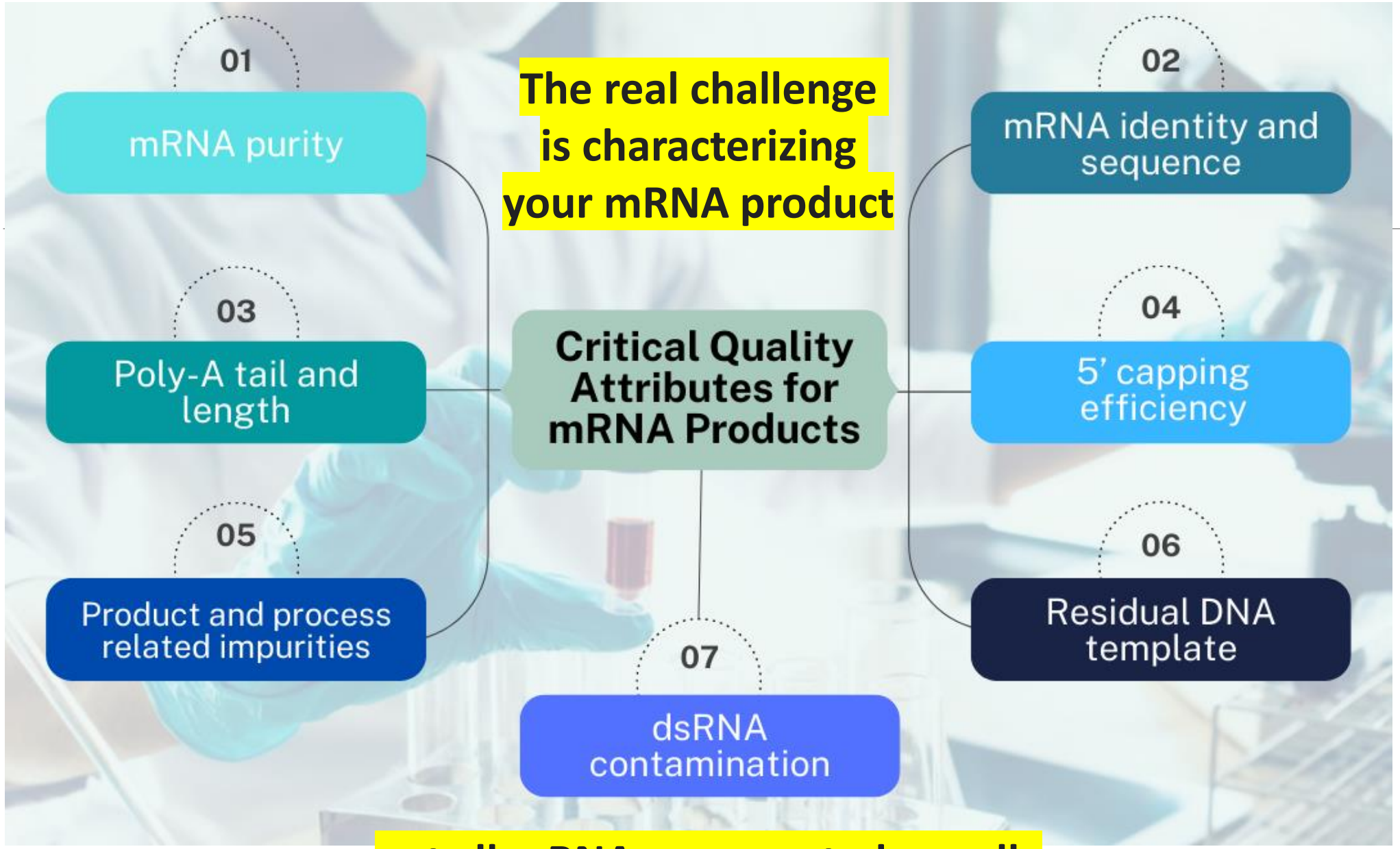


WHEN did mRNA research start?

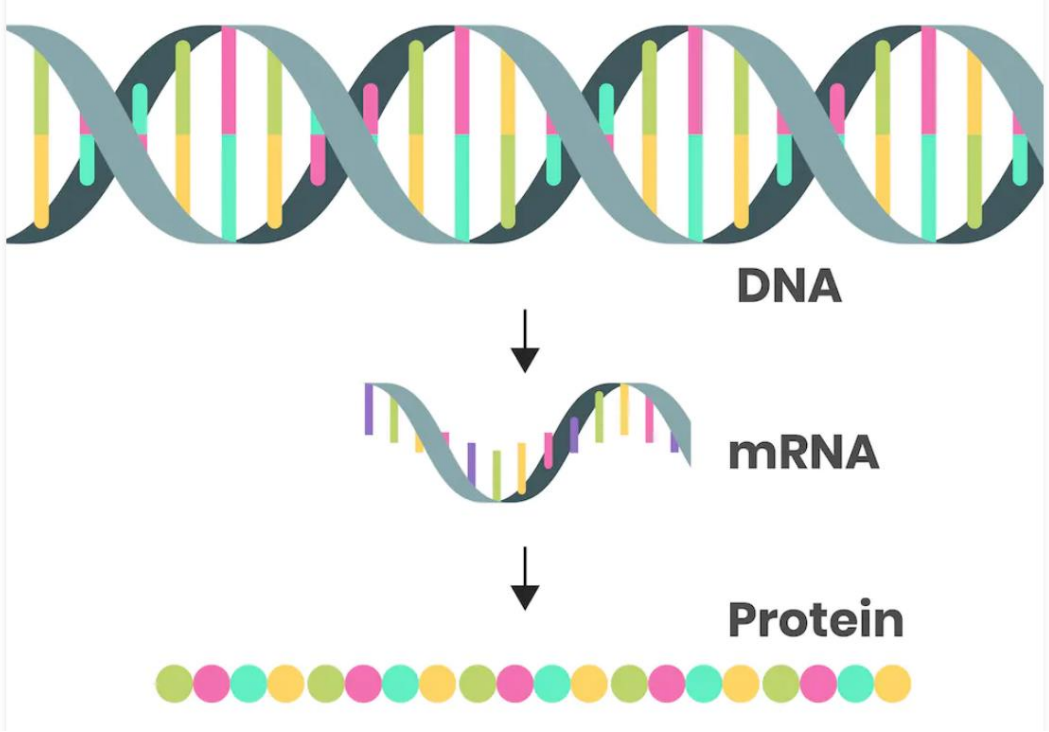
HOW do they work? HOW are they produced?

WHY can’t everyone just make mRNA vaccines?


Target pathogen
Genome sequencing
Electronic transfer of the sequence
Vaccine design
"The devil is in the details"
Formulation and testing
mRNA production and purification
Pilot mRNA vaccine production and validation
GMP manufacturing of mRNA vaccines
Vaccination



not all mRNAs are created equally



WHEN did mRNA research start?

HOW do they work? HOW are they produced?

WHY don’t we have our own vaccine manufacturer?

CAIXIN

How a Chinese mRNA COVID vaccine was approved in Indonesia

Southeast Asian country aims to be more self-sufficient in dealing with pandemic

WHO's African mRNA Hub to Begin Animal Trial on COVID Vaccine

Drug & Diagnostics Development 05/10/2022 · Megha Kaveri

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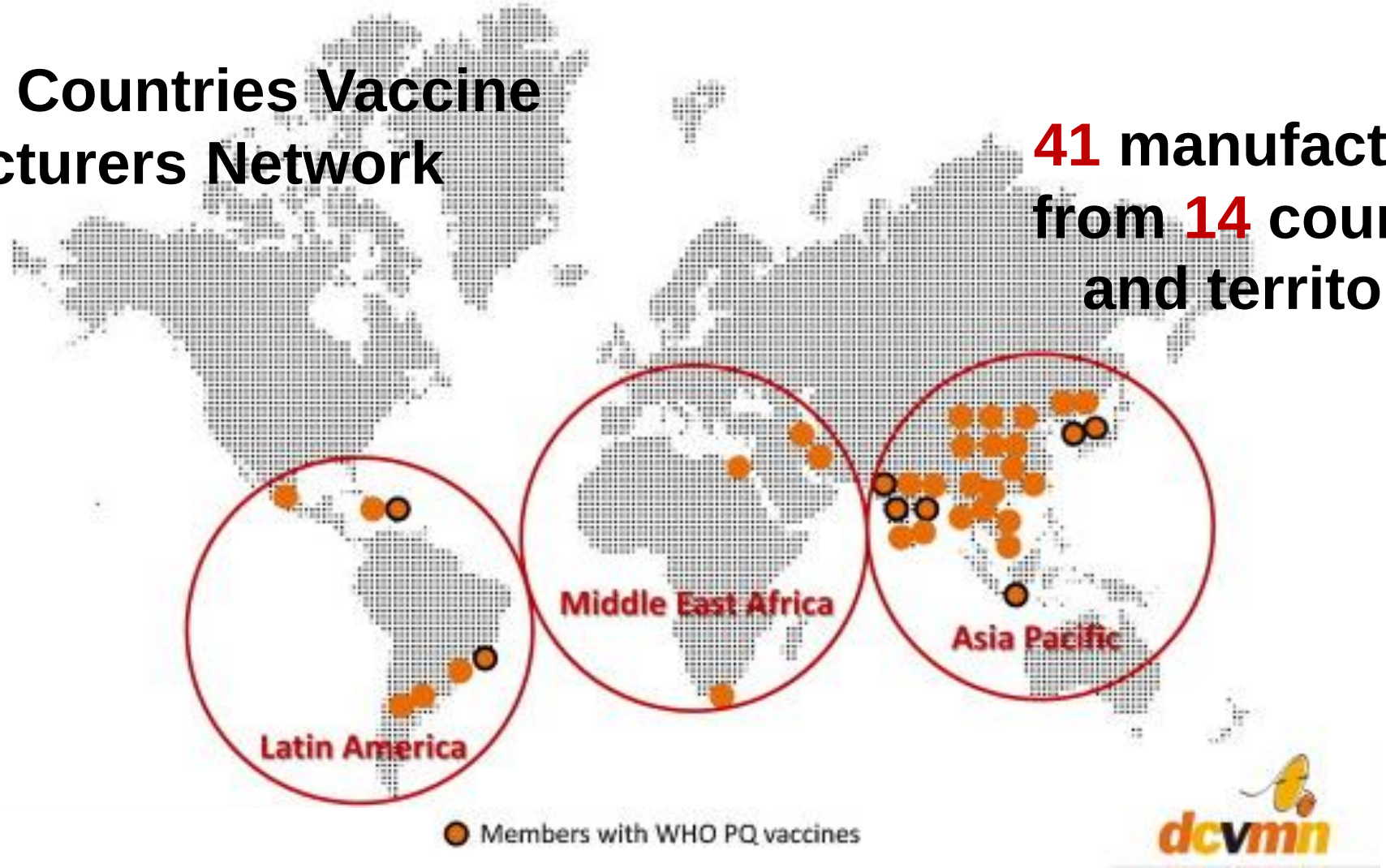
Thailand Targets Homegrown mRNA Vaccine Roll-Out by Year-End

By Thomas Kutty Abraham +Follow
May 8, 2022 at 3:51 AM GMT+2

Over 40 vaccine manufacturers in LMIC

Developing Countries Vaccine Manufacturers Network

41 manufacturers from 14 countries and territories

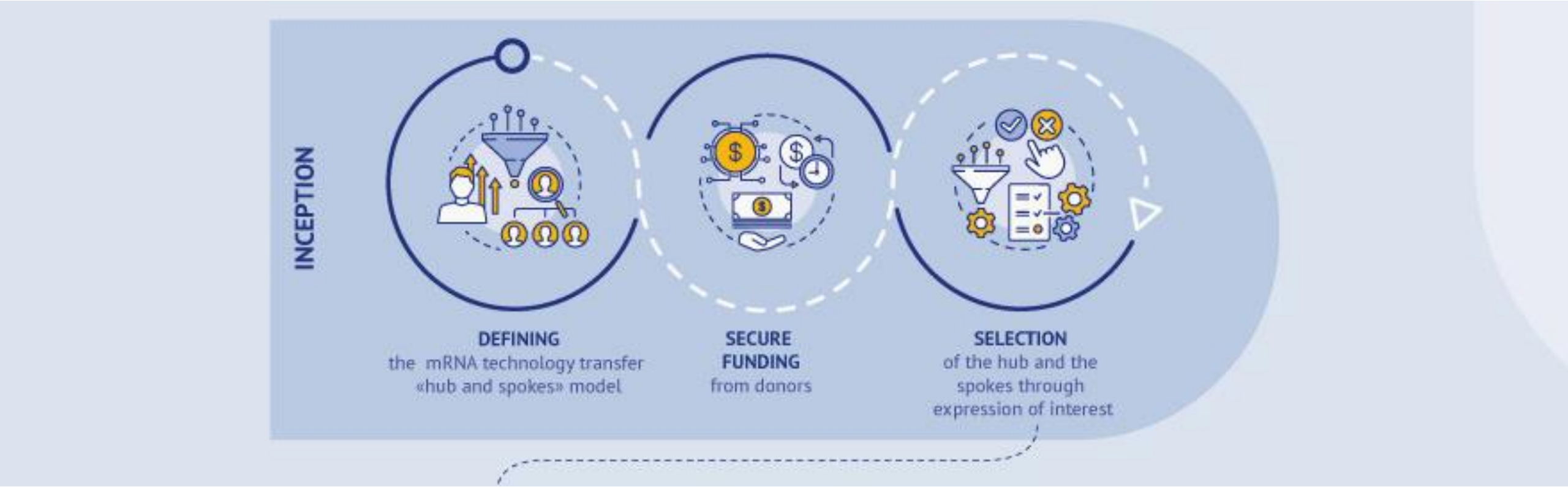


Over 40 vaccine manufacturers in LMIC

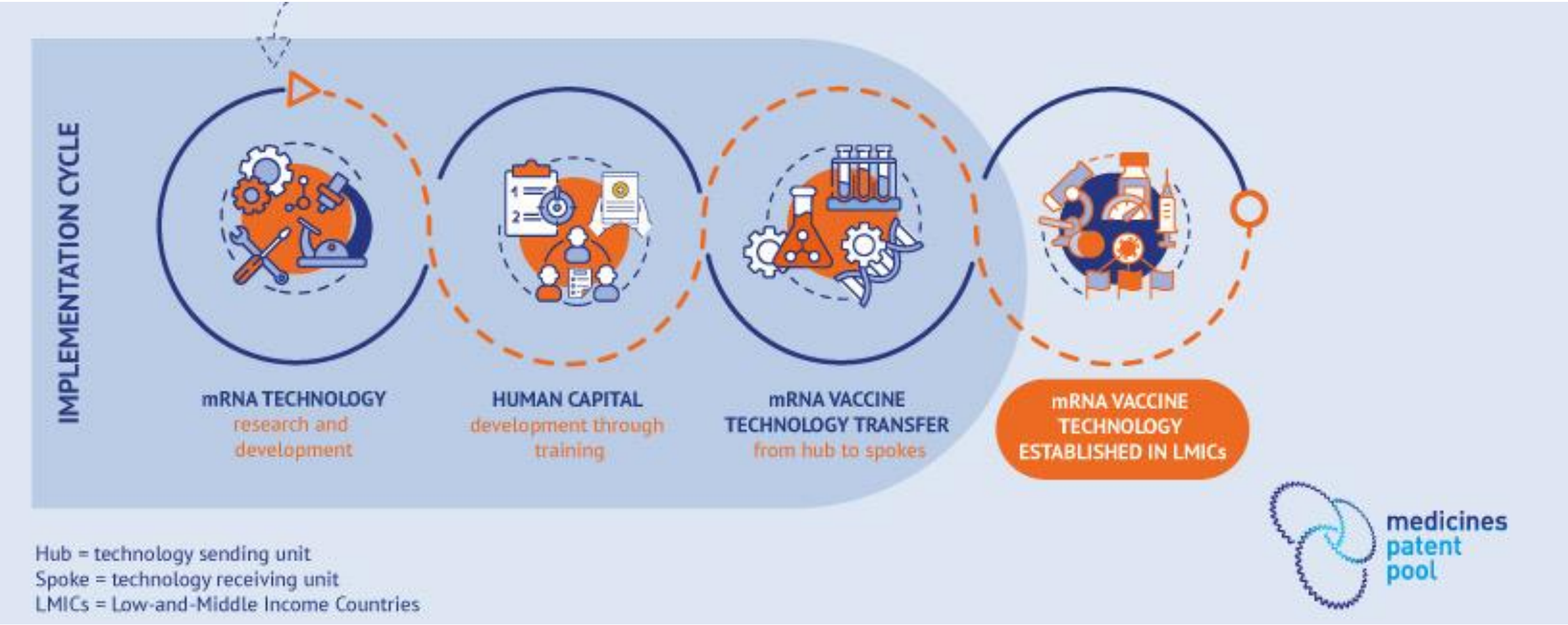
Rank	Country	Population in million	GDP Nominal millions of USD
—	ASEAN	654.306	3,173,141
1	Indonesia	266.998	1,088,768
2	Thailand	67.913	509,200
3	Philippines	108.307	377,362
4	Vietnam	96.801	340,602
5	Singapore	5.670	337,451
6	Malaysia	32.801	336,300
7	Myanmar	53.019	71,690
8	Cambodia	16.494	26,316
9	Laos	7.163	18,653
10	Brunei	0.447	13,469

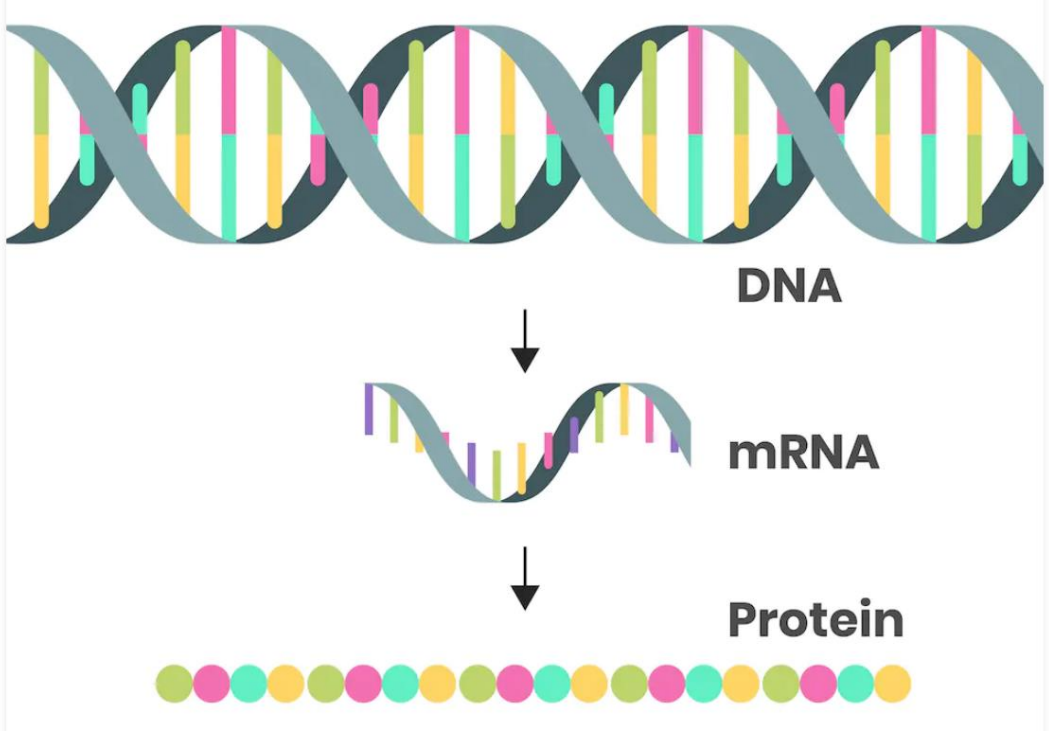
The mRNA technology transfer programme is a global initiative that aims to improve health and health security by establishing sustainable, locally owned mRNA manufacturing capabilities in and for low- and middle-income countries (LMICs).

mRNA Tech Transfer: Inception and Implementation

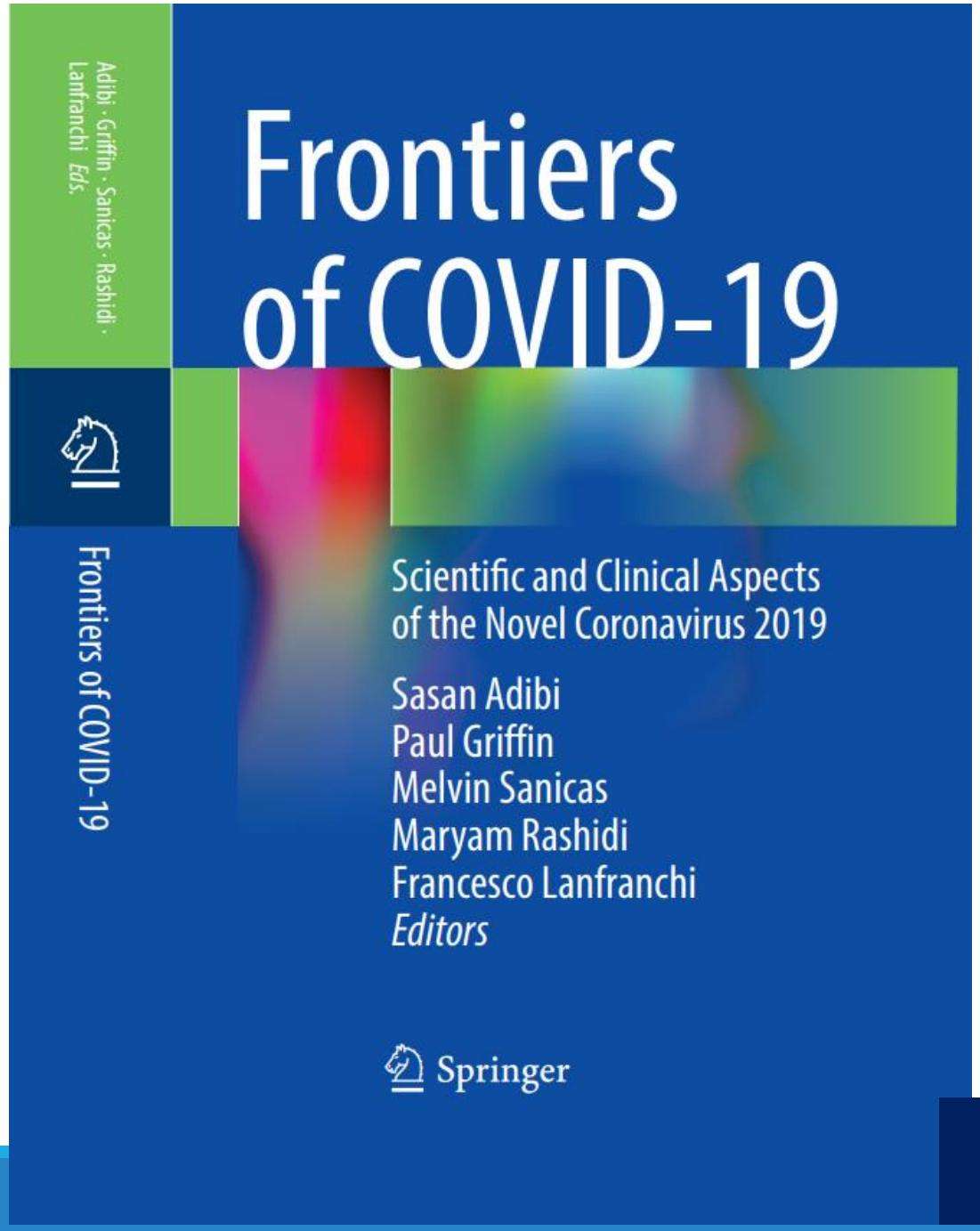


mRNA Tech Transfer: Inception and Implementation





- ✓ 1960s: mRNA tech started, 2005: tech maturity
- ✓ Central dogma, not all mRNAs are created equally
- ✓ LMICs can have mRNA tech transfer



Frontiers of COVID-19
Scientific and Clinical Aspects of the Novel Coronavirus 2019

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https://link.springer.com/book/10.1007/978-3-031-08045-6

Thank you for your attention.


backup

Characteristics of COVID-19-associated myocarditis & myocarditis post COVID-19 mRNA vaccination

Myocarditis type	Incidence	Survival (%)	Potential mechanisms
'Common' viral myocarditis	1–10 per 100,000 people per year	>80	Myocardial injury Genetic (variants in genes encoding HLA, desmosomal, cytoskeletal or sarcomeric proteins) Immune crossreactivity Sex-related factors
COVID-19-associated myocarditis and cardiac injury	1,000–4,000 per 100,000 people with SARS-CoV-2 infection	30–80	Endothelial injury and microthrombosis Genetic (variants in genes encoding HLA, desmosomal, cytoskeletal or sarcomeric proteins) Sepsis and shock
Myocarditis after COVID-19 mRNA vaccination	0.3–5.0 per 100,000 vaccinated people	>99	Hypersensitivity reaction Genetic (variants in genes encoding HLA, desmosomal, cytoskeletal or sarcomeric proteins) Immune crossreactivity Sex-related factors

https://www.nature.com/articles/s41569-021-00662-w/tables/1

siRNA name	Mechanism of Action
Patisiran	The agent is a double-stranded siRNA formulated as a lipid nanoparticle (LNP) taken up by hepatocytes once bound to apolipoprotein E (APOE) receptors. The RNAi of the TTR mRNA decreases the production of TTR protein in circulation and its deposition in tissues and organs.
Givosiran	The agent is a double-stranded siRNA conjugated with *N*-acetylgalactosamine (GalNAc) ligand for intake by hepatocytes. Once taken up by hepatocytes and degrading its target mRNA, it decreases aminolevulinic acid (ALA) and porphobilinogen (PBG) levels in the blood, further limiting AHP disease characteristics.
Lumasiran	The agent is a double-stranded siRNA conjugated with GalNAc ligand for effective uptake by hepatocytes. HAO1 produces glycolate oxidase (GO), an enzyme responsible for producing glyoxylate, a substrate for the further synthesis of oxalate. The inhibition of the GO enzyme results in decreased oxalate precursor levels which in turn reduces the production of the enzyme alanine glyoxylate aminotransferase (AGT) that is mutated in PH1.
Inclisiran	The sense strand of the siRNA is bound with GalNAc ligand, allowing hepatocytes to efficiently uptake the siRNA agent and target PCSK9. PCSK9 internalizes and breaks down the hepatic LDL receptors once attached. Inclisiran inhibits this action of PCSK9, further promoting the expression of LDL-C receptors on the cell's surface and facilitating receptor cycling. Once LDL-C is bound to its receptor, it is subject to degradation by lysosomal enzymes and recycled back to the cell's surface. This results in a raised uptake of LDL-C and reduces its levels in the blood.

Small Interfering RNA (siRNA) Based Therapy - StatPearls - NCBI Bookshelf (nih.gov)

Some important references on mRNA quality control

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