mRNA: gene therapy or vaccine?

SAVIMS (South Africa Vaccine Injury Medicolegal Study group)
April 16, 2023

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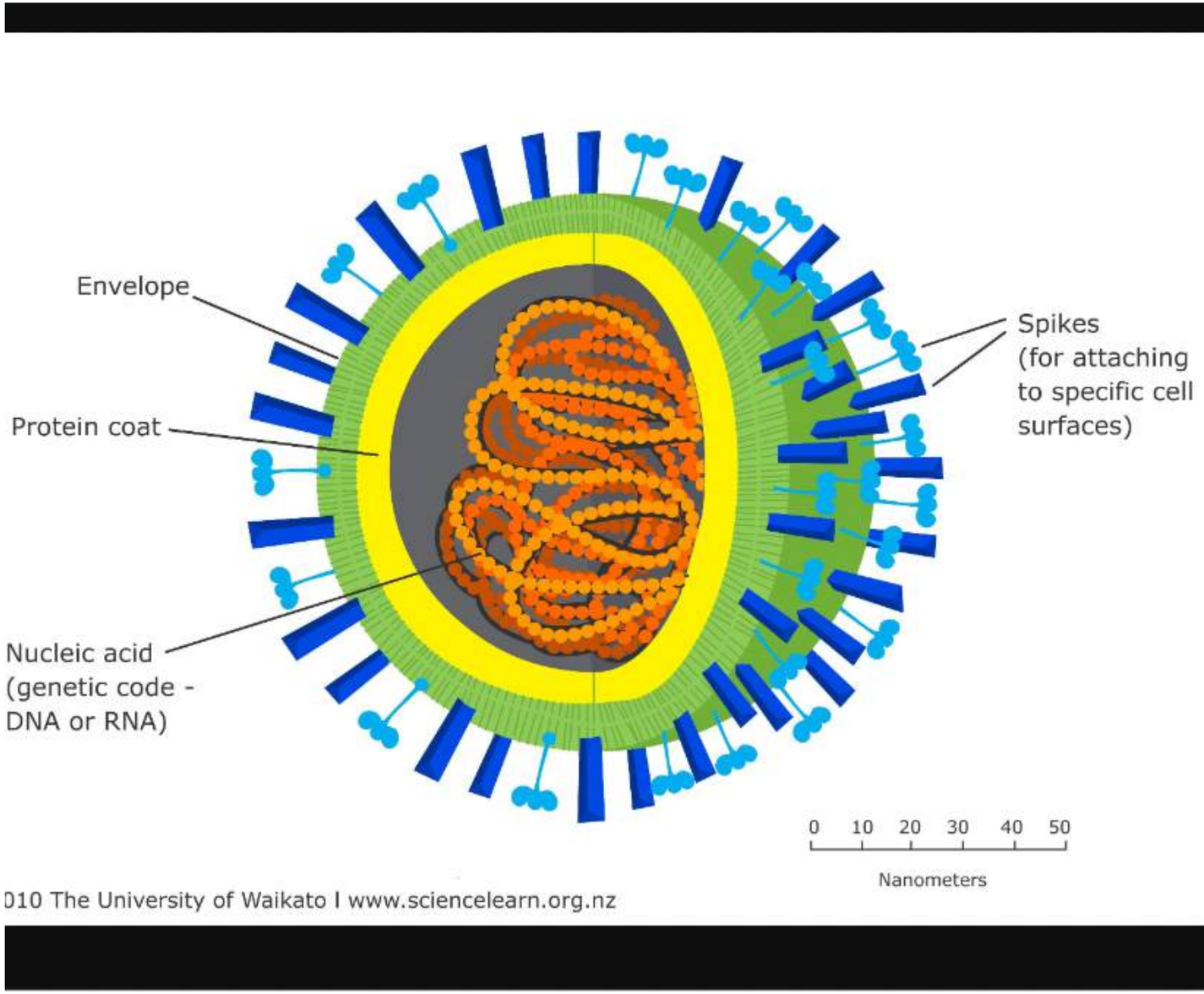
here my publications and authenticated peer-review
https://www.webofscience.com/wos/author/record/2107575

What is a virus

It is a complete parasite
It is formed of nucleic acid (DNA or RNA) and proteins that spontaneously assemble to form a nucleocapsid
It is enveloped or not
It needs a living organism (bacteria, plant, animal) for the synthesis of its proteins, energy and precursor molecules of its nucleic acid



RNA viruses replicate their genomes using virally encoded RNA-dependent RNA polymerase

There are at least three types of RNA that must be synthesized:
the genome,
a copy of the genome (copy genome),
and mRNAs

Schematic representation of replication of positive-strand RNA virus genomes. The genome of a positive-strand RNA virus is an mRNA that is translated, upon entry into the cells, to produce proteins needed for transcription and genome replication (for example, RdRp). After initial rounds of translation, the genome serves as the template for synthesis of copy RNA. The copy RNA is the template for synthesis of additional genomes and subgenomic mRNAs.

RNA viruses replicate their genomes using virally encoded RNA-dependent RNA polymerase = RdRp

The genome is translated after entry in the cell to produce the RdRp and other viral proteins required for additional viral RNAs

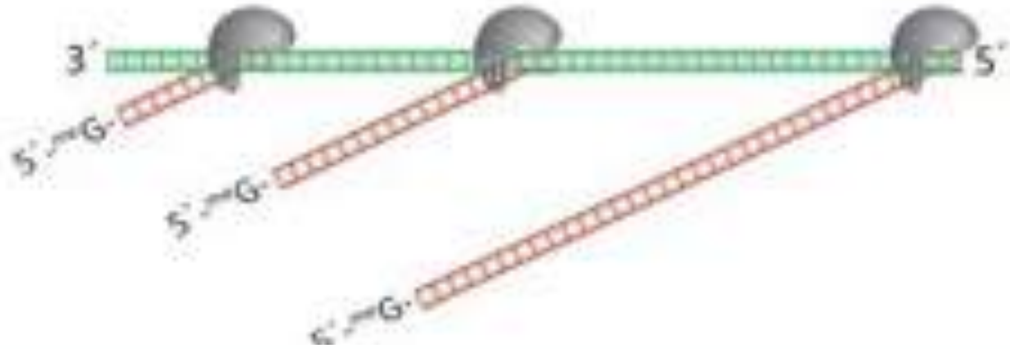
RdRp synthesis a complementary copy of the viral genome

Thus the infecting genome has two functions:
It *is* an mRNA and also serves as the template for synthesis of additional viral RNAs

The copy genome (cRNA in green) serves as a template for the synthesis of additional mRNAs and genomes (red)

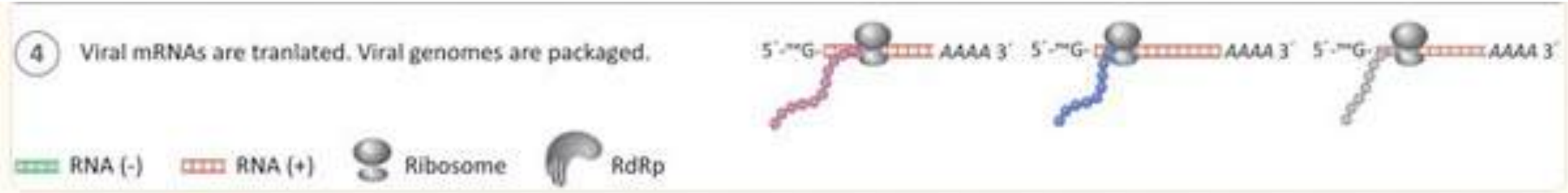
Coronaviruses are positive single strand RNA which is functional as mRNA (messenger RNA to be translated in proteins)

The copy (c) genome or cRNA (green) serves as a template for the synthesis of additional mRNAs and genomes (red).



Viral mRNAs are translated in proteins by the host cell ribosomes

Viral genomes are then packaged with proteins to form the nucleocapsid



The immune system

Classically, the immune system is divided into several branches:

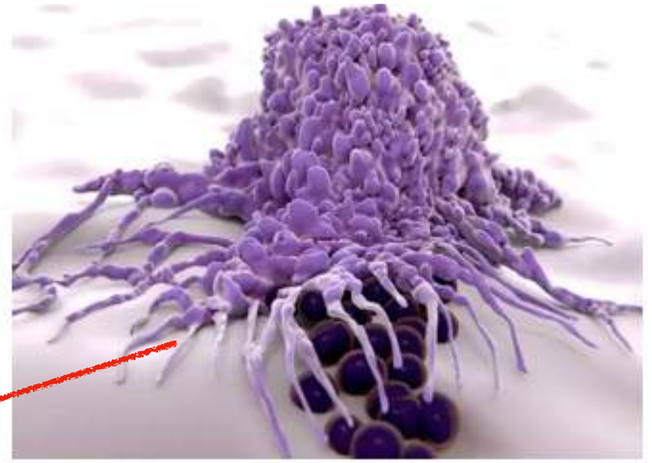
On the one hand, **innate immunity** (non-specific and without memory of the pathogens) is differentiated from **adaptive immunity**.

This adaptive immunity is specific to the pathogens and is composed of cells that keep the memory of them;

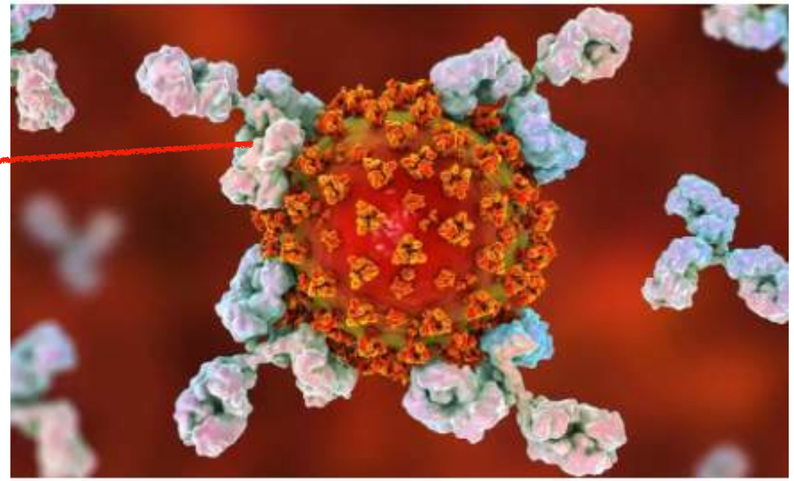
It is divided into 2 branches: cellular immunity (whose effectors are cells -T cells) and humoral immunity (whose effectors are the antibody molecules -produced by B cells).

But all these systems cooperate and could not act alone

- Macrophage



Macrophages are types of white blood cells that play a critical role in both innate and adaptive immune systems. (Image: Juan Gaertner/Shutterstock)



bodies are one of the main components of the adaptive immune system
ge: Kateryna Kon/Shutterstock)

The phenomenon of facilitation of viral infections by antibodies (ADE antibody dependent enhancement) is in contradiction with the protective role of antibodies affirmed by classical immunology

Hélène Banoun (2021).
The role of antibodies in the light of the theory of evolution.
African Journal of Biological Sciences. 3(3), 1-9.
https://www.afjbs.com/files/1627021759_(1)_AFJBS20210145_(p_1-9).pdf

- The phenomenon of antibody facilitation of viral infections has recently been re-discussed in relation to the clinical aspect of Covid-19

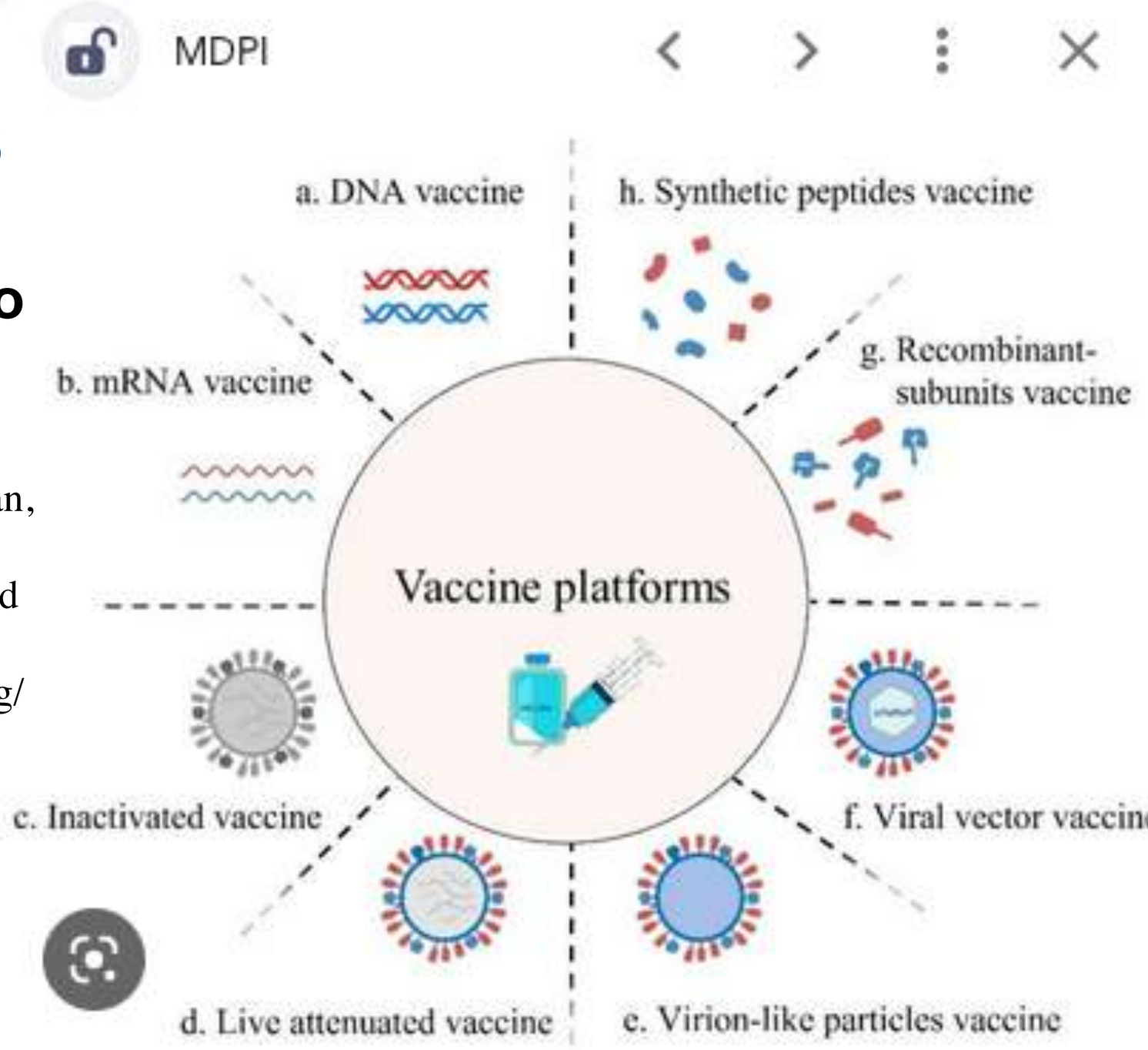
- Antibody dependent enhancement is the accepted mechanism to explain severe reinfections due to dengue virus—among others—as well as the higher occurrence of severe dengue in vaccinated (compared to unvaccinated)

- This effect of antibodies appears to contradict immunological theory, which states that the “role” of antibodies is to protect organisms against pathogens, including viruses.

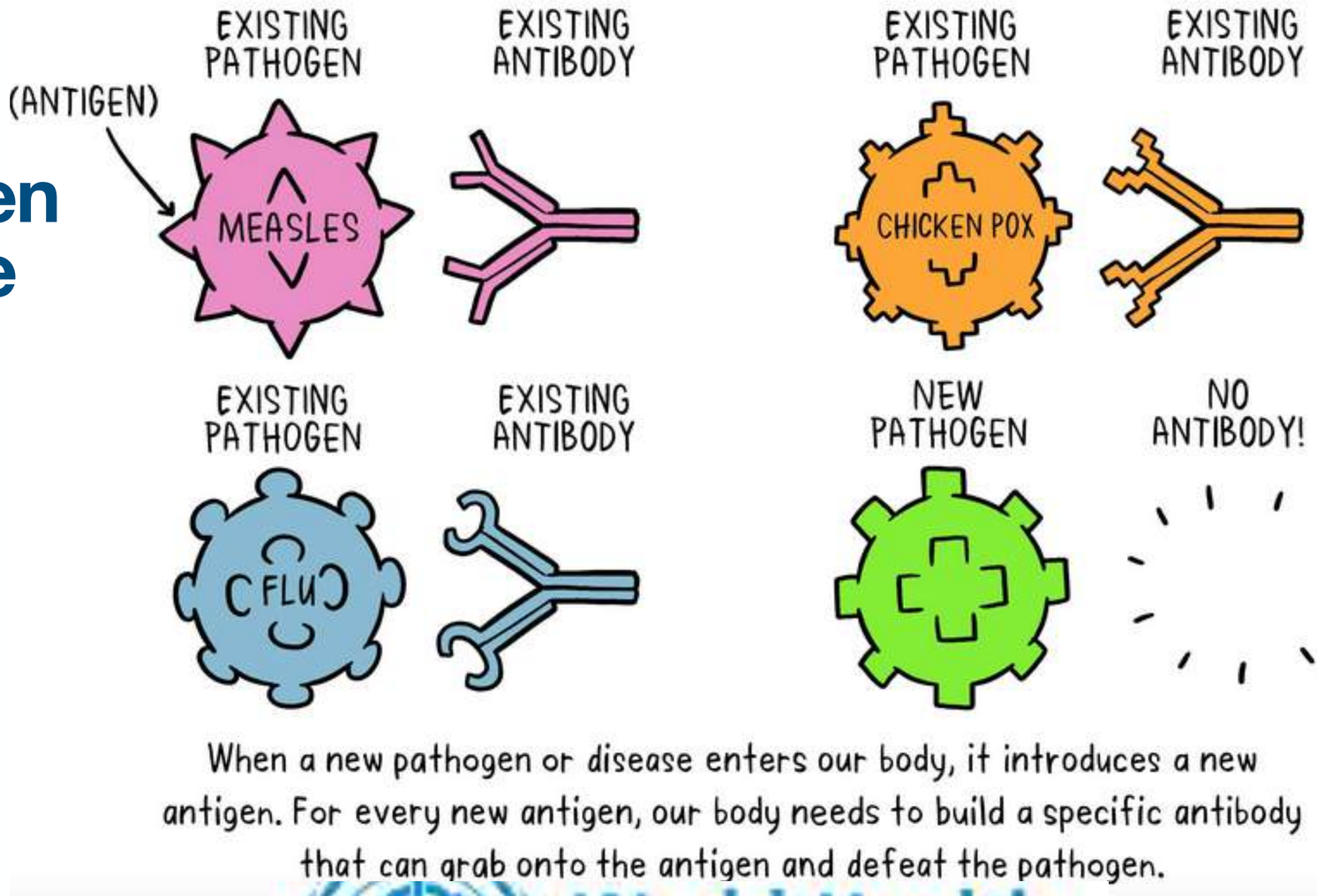
The different types of vaccines

From live attenuated virus to mRNA vaccines

Deng, S.; Liang, H.; Chen, P.; Li, Y.; Li, Z.; Fan, S.; Wu, K.; Li, X.; Chen, W.; Qin, Y.; Yi, L.; Chen, J. Viral Vector Vaccine Development and Application during the COVID-19 Pandemic. *Microorganisms* **2022**, *10*, 1450. https://doi.org/10.3390/microorganisms10071450



Since the beginning of the history of vaccinology and immunology almost only antibodies have been taken into account in the immune response



On this WHO diagram only the antibodies are represented and not the T cells of cellular immunity nor the cells of innate immunity
For coronaviruses, respiratory viruses with a nasal entry point, innate immunity is essential!

https://www.who.int/news-room/feature-stories/detail/how-do-vaccines-work

World Health Organization

Historical evolution of the vaccines

From live attenuated virus to mRNA vaccines injected in the muscle

- This limited view of the immune response has led to the historical evolution of vaccines: from the live attenuated virus (variolization of the 18th century) to a single protein antigen produced in the vaccinee by translation of an mRNA molecule injected into the muscle

- We have forgotten that the body reacts to a living and whole pathogen introduced by a natural way: the upper respiratory tract in the case of a coronavirus for example

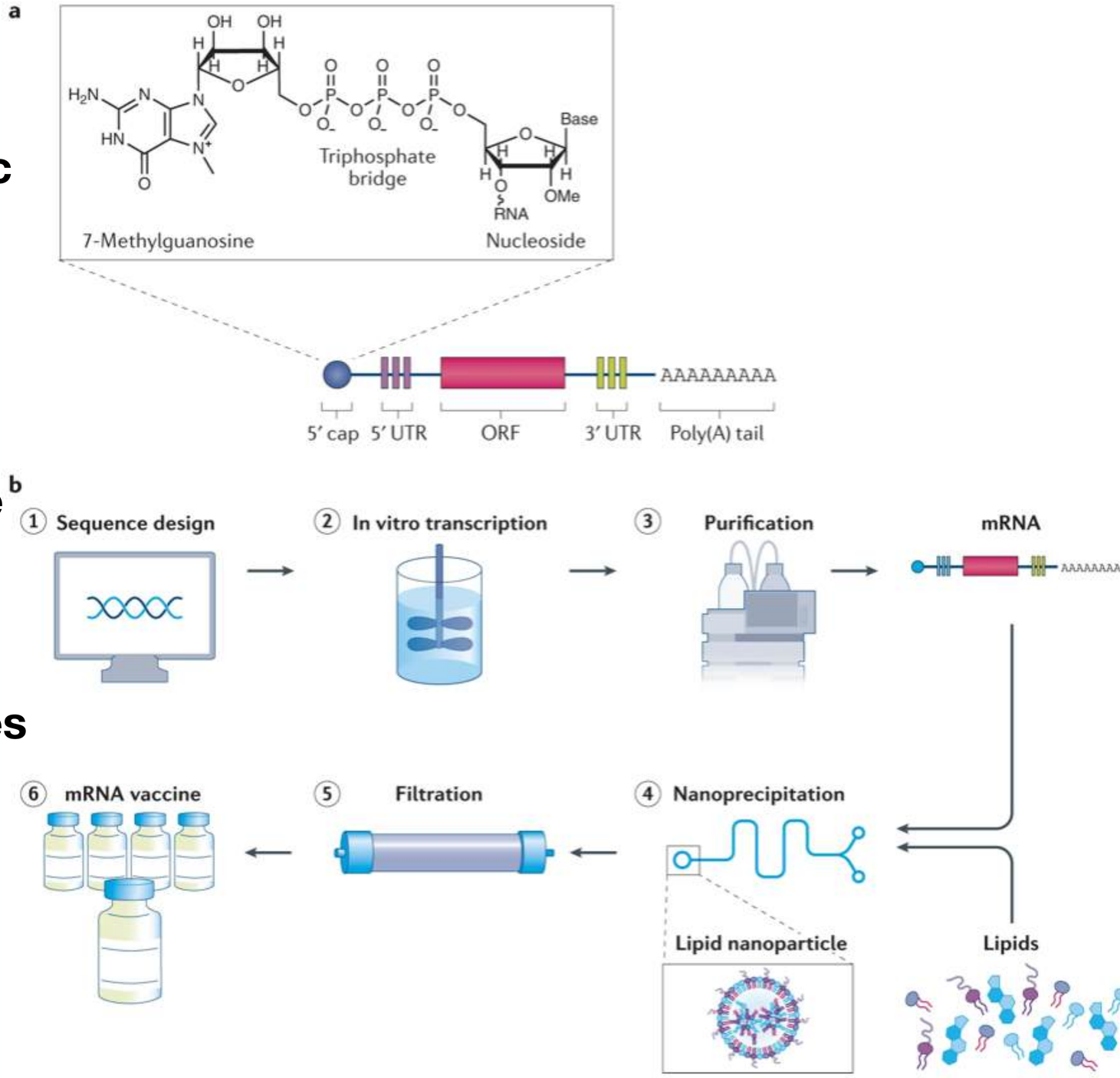
Principles of mRNA design and synthesis

mRNA vaccines comprise synthetic mRNA molecules that direct the production of the antigen that will generate an immune response

mRNA is transcribed in vitro (IVT) from DNA template and mimics the structure of endogenous mRNA

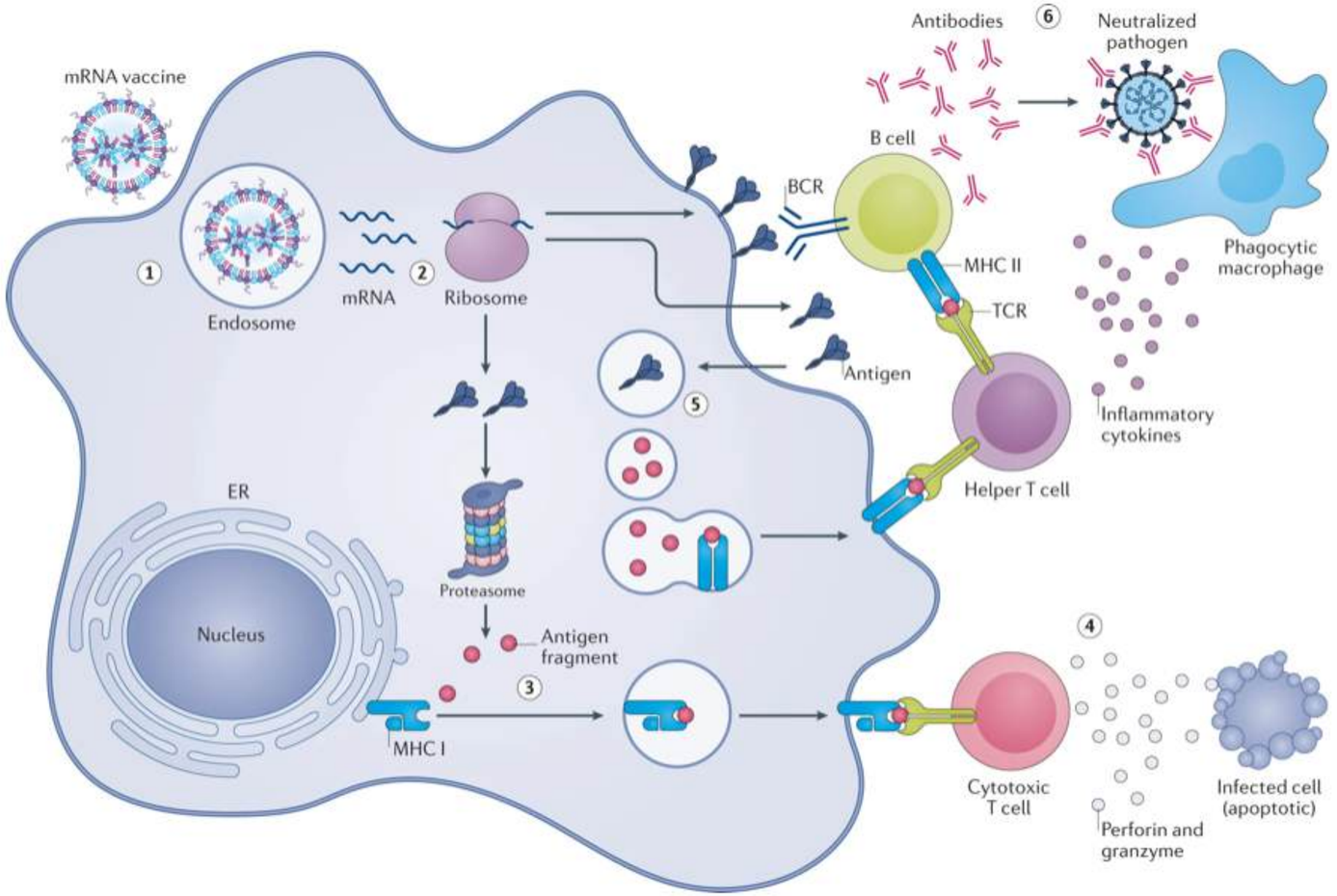
Contaminating DNA from this template is found in mRNA vaccines

Many new steps not mastered by the subcontractors involved in the manufacturing process



Theoretical mode of action of mRNAs

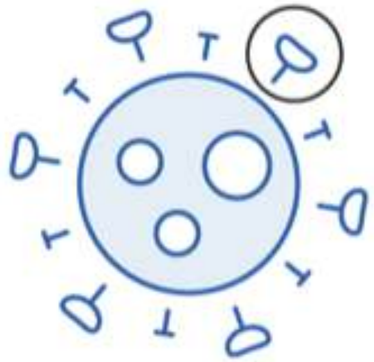
The designers of mRNA vaccines are only interested in the fate of this product in specialized immune cells called « antigen-presenting (APC) » but we now know that mRNA circulates throughout the body and can be translated into spike protein by many cell types (Plus LNP toxicity)



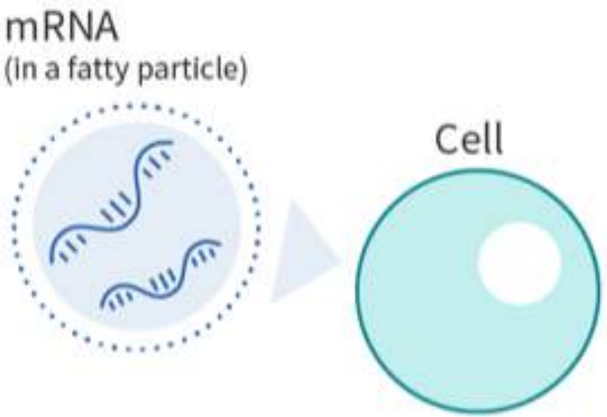
Chaudhary, N., Weissman, D. & Whitehead, K.A. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. Nat Rev Drug Discov 20, 817–838 (2021). https://doi.org/10.1038/s41573-021-00283-5

How does the mRNA coronavirus vaccine work?

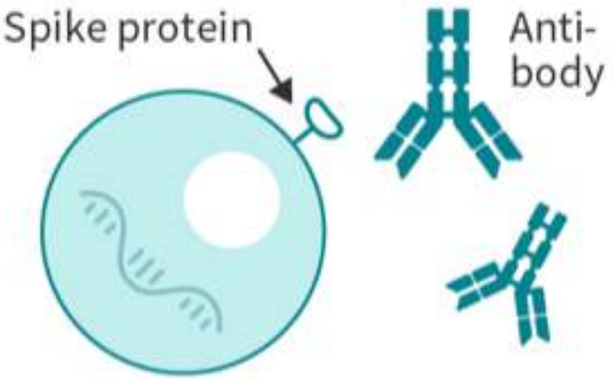
thl



The RNA vaccine contains messenger RNA, which contains an instruction to make a SARS-CoV-2 spike protein.



For messenger RNA (mRNA) to enter the muscle cell at the injection site, it is packaged inside a very small fatty particle.



Messenger RNA instructs cells to produce a coronavirus spike protein.

The body's defence system recognises the spike protein as foreign and begins to protect itself against it.

#coronavirus

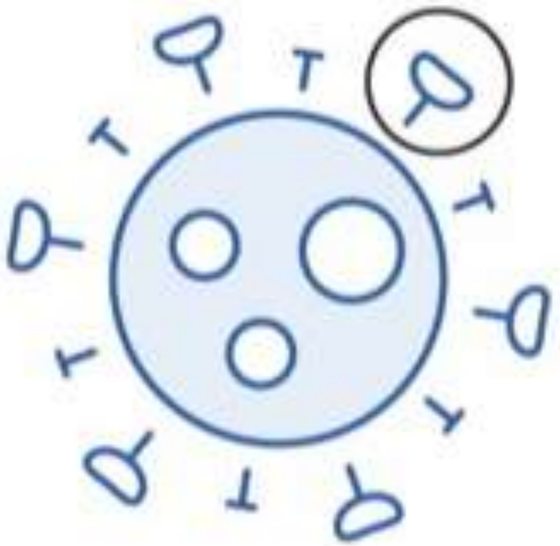
Source: Finnish Institute for Health and Welfare 2020

Principle of action of the mRNA vaccine according to the official discourse

The vaccine mRNA contains the MODIFIED code of the MODIFIED spike protein of the virus

Virtually all of the pathogenic effects of COVID-19 SARS-CoV-2 are due to spike toxicity

Vaccine spike is MORE toxic than viral spike

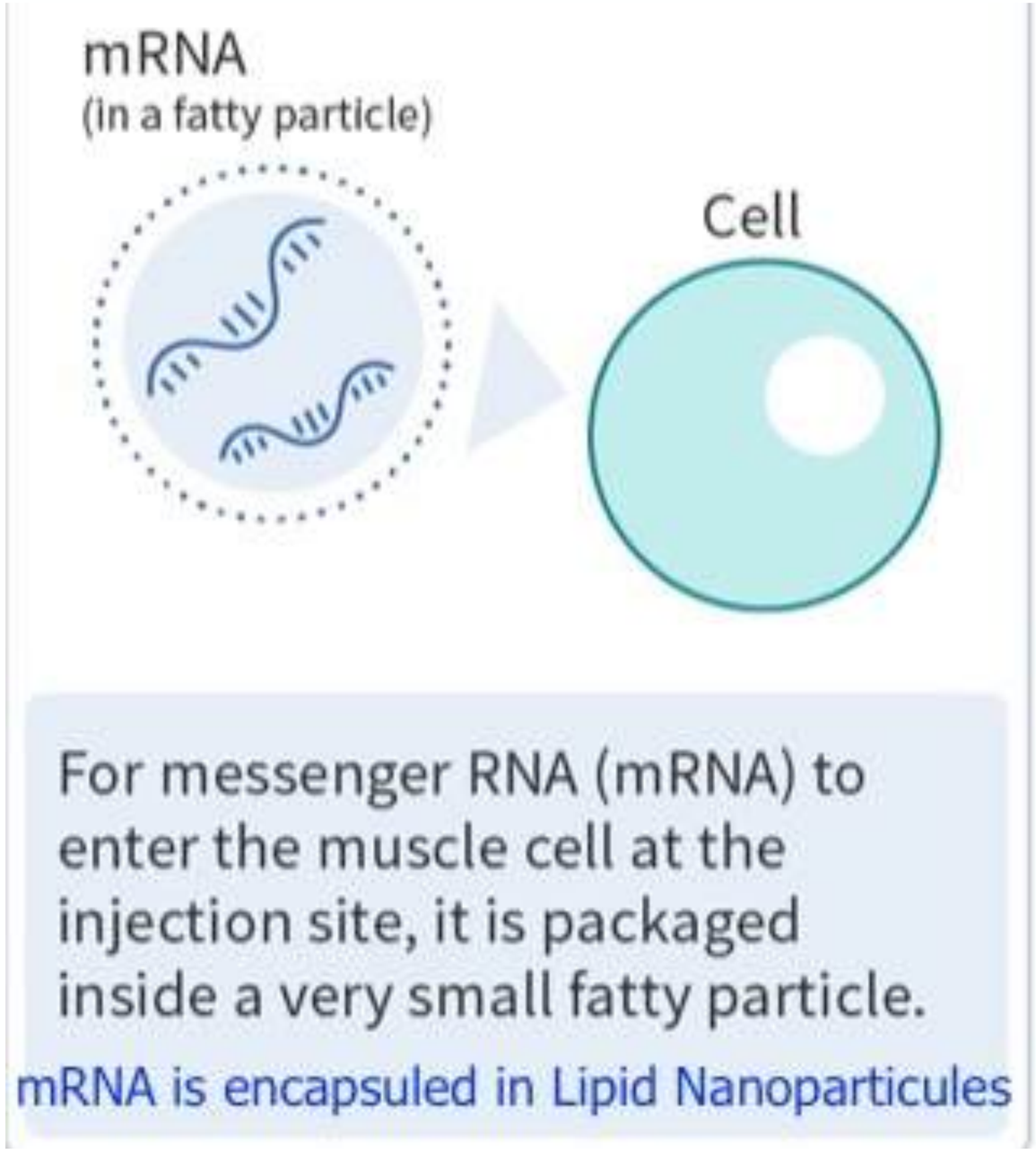


The RNA vaccine contains messenger RNA, which contains an instruction to make a SARS-CoV-2 spike protein.

LNPs, which serve as a vector and protection for mRNA, penetrate ALL the body and many cell types. These LNPs are also toxic

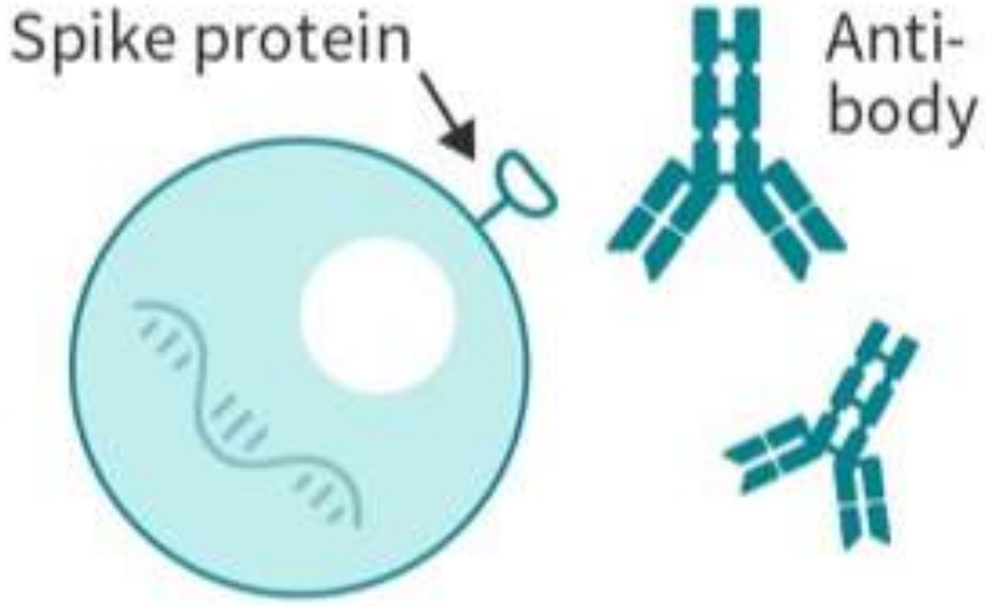
The modified vaccine mRNA and the modified vaccine spike produced by the vaccinee can persist for months in the body

Banoun H. Current state of knowledge on the excretion of mRNA and spike produced by anti-COVID-19 mRNA vaccines; possibility of contamination of the entourage of those vaccinated by these products. *Infect Dis Res*. 2022;3(4):22. https://doi.org/10.53388/IDR20221125022



The transfected cells are those in which the mRNA has penetrated and is translated into spike protein

These cells express spike on their surface: they induce the synthesis of anti-spike antibodies but can also be destroyed because they are recognized as foreign by the immune system



Messenger RNA instructs cells to produce a coronavirus spike protein.

The body's defence system recognises the spike protein as foreign and begins to protect itself against it.

According to this principle of action, mRNA vaccines are gene therapies = GTP

According to FDA

- "*Gene therapy products are all products that mediate their effects by transcription and/or translation of transferred genetic material and/or by integrating into the host genome and that are administered as nucleic acids, viruses, or genetically engineered microorganisms.* »

- FDA Design and Analysis of Shedding Studies for Virus or Bacteria-Based Gene Therapy and Oncolytic Products

Guidance for Industry August 2015 https://www.fda.gov/regulatory-information/search-fda-guidance-documents/design-and-analysis-shedding-studies-virus-or-bacteria-based-gene-therapy-and-oncolytic-products current as of 05/16/2019

According to EMA, mRNA vaccine are also GTP

EMA, 2001 Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use, updated on 16.11.2012
https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32001L0083

- A GTP

(a) contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence;

and

(b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence. Gene therapy medicinal products shall not include vaccines against infectious diseases.

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Vaccine clinical trials

Typical vaccine development timeline takes 5 to 10 years and sometimes longer regulatory approval process takes 2-4 years

https://coronavirus.jhu.edu/vaccines/timeline Vaccine Research&Development John Hopkins University

- Chinese scientists sequenced officially the first **SARS-CoV-2 virus** on Jan 2, 2020 : the sample is labelled Wuhan-Hu-1, the sequencing is finished on Jan 5, 2020 and the **whole genome is released** by Zhang **on Jan 11 2020**

https://www.latimes.com/science/story/2021-11-18/a-timeline-of-covid-19s-first-weeks-in-wuhan A timeline of COVID-19's first weeks in Wuhan, Los Angeles Time, Nov 18, 2021

° The **first COVID-19 vaccine candidate** entered human clinical testing with unprecedented rapidity on 16 March 2020

https://www.nature.com/articles/d41573-020-00073-5 The COVID-19 vaccine development landscape

Pfizer vaccine trial

The development of BNT162b2 was initiated on January 10, 2020

- Pre-clinical studies on Wistar rats were approved by regulatory authorities on 17-Dec-2020 (first report submission, 13 Nov 2020) Study Initiation Date (date protocol signed) 23 Jun 2020 (page 11) : so they started AFTER phase 1 on humans!!

- For mRNA vaccines Phases 1/2/3 were conducted simultaneously
  Phase 1 :to identify the preferred vaccine candidate and dose level (3 dose levels, 15 individuals in each cohort from 18 to 55 years) , safety and immunogenicity. The results give BNT162b2 30 micrograms 2 doses https://www.nejm.org/doi/full/10.1056/NEJMoa2027906

- Between May 4, 2020, and June 22, 2020, a total of 332 healthy adults (men and nonpregnant women) underwent screening at four sites in the United States (two sites per vaccine candidate)

Pfizer clinical trial (continued)

Phase 1/2/3 conducted simultaneously

- Phase 2/3 is conducted on expanded cohort
- Phase 2/3 :Safety and immunogenicity analysis of Phase 2 data (first 360 participants) by unblinded team (these participants will also be included in Phase 3 analyses, (n~21,999 per group, total n~43.998)
- The BMJ published documents provided by whistleblower Brook Jackson regarding data integrity issues in Pfizer's COVID-19 vaccine clinical trial, "Covid-19: Researcher blows the whistle on data integrity issues in Pfizer's vaccine trial"

BMJ 2021 ; 375 doi : https://doi.org/10.1136/bmj.n2635

Moderna clinical trials

Moderna went to work on a Covid-19 vaccine on January 13, 2020 (full genome released Jan 11, 2020)

- Moderna had started early-stage human trial data from 2019.
- In fact, that data was so encouraging that Bancel (CEO) was set to announce in a few days' time that the company would be doubling down on its vaccine-development program in 2020
- https://www.bostonmagazine.com/health/2020/06/04/moderna-coronavirus-vaccine/ The Untold Story of Moderna's Race for a COVID-19 Vaccine 6/4/2020, Boston

Moderna clinical trials (continued)

Preclinical studies on non human primates were conducted by NIH/NIAID researchers for Moderna mRNA-1273 and published in July 2020 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7449230/

- Phase 3 started on July 27, 2020
- (ClinicalTrials.gov Identifier: NCT04470427 A Study to Evaluate Efficacy, Safety, and Immunogenicity of mRNA-1273 Vaccine in Adults Aged 18 Years and Older to Prevent COVID-19)
- Protocol of Phase 3 was amended 20 August 2020
- https://docs.house.gov/meetings/IF/IF02/20200930/111063/HHRG-116-IF02-20200930-SD008.pdf ModernaTX, Inc. 20 Aug 2020 Protocol mRNA-1273-P301, Amendment 3
- No mention of phase 2; phase 3 started juste after phase 1

History of gene therapy versus vaccine regulation

2005 : WHO grants nucleic acids vaccines statut of vaccines

2007 : EMA defines nucleic acids for prophylactic purposes as GTP

2007 (FDA) :DNA plasmid vaccines subject to controls inspired by GTP

2008 (EMA) : DNA plasmid vaccines subject to GTP regulation

Year	Regulatory agency	Rule	Comment
2005	WHO [11]	WHO grant nucleic-acid based vaccines the status of vaccines	Vaccines must comply with GMP In case of new formulations : distribution studies and toxicology studies for new additive are required
2007	EMA [5]	*"Advanced therapy medicinal product' means any of the following medicinal products for human use: a gene therapy medicinal product as defined in Part IV of Annex I to Directive 2001/83/EC"*	A GTP *(a) contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, replacing, adding or deleting a genetic sequence;* and *(b) its therapeutic, prophylactic or diagnostic effect relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence. Gene therapy medicinal products shall not include vaccines against infectious diseases.*
2007	FDA [12]	Manufacturing issues and preclinical required studies for DNA plasmids as vaccine to prevent infectious diseases	DNA plasmids are subject to controls inspired by those for GTP
2008	EMA [21]	DNA plasmids vaccines are subject to GTP products regulation without specifying whether they are intended for use against an infectious disease or not	Does not include mRNAs but the definition provided is not exhaustive
2012	EMA [1], confirmed in 2015 [15]	Vaccines against infectious diseases are not classified as gene therapy products	No ethical or scientific justification is provided
2013	FDA [13,14]	Regulation of gene therapy products did not apply to vaccines against infectious diseases	Apply to DNA plasmids

Table 1 : History of gene therapy vs vaccine regulation

History of gene therapy versus vaccine regulation (continued)

2012 : for the EMA vaccine against infectious disease cannot be classified as GTP

2013 : for the FDA, regulation of GTP do not apply to vaccine against infectious diseases

2007	FDA [12]	Manufacturing issues and preclinical required studies for DNA plasmids as vaccine to prevent infectious diseases	DNA plasmids are subject to controls inspired by those for GTP
2008	EMA [21]	DNA plasmids vaccines are subject to GTP products regulation without specifying whether they are intended for use against an infectious disease or not	Does not include mRNAs but the definition provided is not exhaustive
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of gene therapy vs vaccine regulation

So until 2008 for all regulatory agencies nucleic acid based vaccines had to follow the GTP guidelines

What happened between 2008 and 2012?

- 2010 : After the 2009-2010 H1N1 pandemic, A Fauci is looking for solutions for a universal flu vaccine. In November 2010, there is talk of a DNA vaccine but not yet of RNA vaccines.
- 2011 : Curevac and Sanofi (european companies) start the collaboration with DARPA (US-Defense Advanced Research Projects Agency) to develop RNAm vaccines
- 2013 : DARPA Awards Moderna Therapeutics A Grant For Up To $25 Million To Develop Messenger RNA Therapeutic against infectious diseases.

https://www.nejm.org/doi/full/10.1056/NEJMra1002842
Lambert, L.C. & Fauci, A.S. Influenza vaccines for the future. *N. Engl. J. Med.* 363, 2036–2044 (2010)

https://www.genengnews.com/topics/drug-discovery/curevac-sanofi-pasteur-and-in-cell-art-collaborate-on-e33-1m-darpa-supported-vaccine-program/
CureVac, Sanofi Pasteur, and In-Cell-Art Collaborate on €33.1M DARPA-Supported Vaccine Program
NOV 15,2011

https://www.prnewswire.com/news-releases/darpa-awards-moderna-therapeutics-a-grant-for-up-to-25-million-to-develop-messenger-rna-therapeutics-226115821.html

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I have submitted a preprint about this regulation of mRNA vaccines

https://doi.org/10.32388/WW4UEN.2

mRNA vaccines should follow the GTP regulation:
their supposed principle of action is indeed that of a GTP.
Moreover, an mRNA with antitumor aim is considered as a GTP
This exclusion cannot be justified

mRNA vaccines must follow regulations of human medicines for EMA

EMA, 2001 Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use, **updated on 16.11.2012**
https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32001L0083

- Depending on whether the same molecule (mRNA) is an anti-cancer therapy for example or a vaccine against an infectious disease, it will not be subject to the same controls
- The EMA also requires additional studies for vaccines using new formulations: these have not been conducted.
- Vaccines have been exempt from some of these controls for a long time without any real scientific justification. However, mRNA vaccines, which represent a new class of vaccine, should be subject to more controls than conventional vaccines because they are based on several new technologies
- Moreover, according to the EMA, "*Since vaccines in most cases are given to large numbers of healthy individuals, there is a need for a solid nonclinical safety evaluation*. »
(EMA 2016, 21 July 2016 EMA/CHMP/SWP/242917/2016 Committee for Medicinal Products for Human Use (CHMP) Questions and answers on the withdrawal of the CPMP Note for guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465) https://www.ema.europa.eu/en/documents/other/questions-answers-withdrawal-cpmp-note-guidance-preclinical-pharmacological-toxicological-testing/swp/465_en.pdf

What regulations are mRNA vaccines subject to?

Controls for new vaccines by regulatory agencies

- Demonstration of the purity and quality of the raw material (as for all human medicine)
- In the case of new formulation (new excipient), pharmacodynamic studies (bio distribution) of the product and of the adjuvant
- Toxicological studies of new additive
- Pharmacokinetics includes the study of absorption, distribution, biotransformation and excretion; *"Pharmacokinetic studies are usually not required for vaccines. However, such studies might be applicable when new delivery systems are employed or when the vaccine contains novel adjuvants or excipients."* (according to EMA EMEA/CHMP/VWP/164653/2005)

Regulatory studies for GTMPs were thus avoided for mRNA vaccines

With reference to the rules applying to GTPs for the FDA and the EMA

- **Extensive studies on both the nucleic acid and the vector particle/delivery system that include**
- biodistribution,
- dose study,
- potential target toxicity,
- identification of the target organ to obtain biological activity,
- research into integration in the genome ("even if this integration is unlikely")
- transmission in the germ line,
- toxicity linked to the expression of structurally altered proteins,
- reproductive toxicity,
- repeated toxicity,
- excretion in the environment

The anti-COVID-19 mRNA vaccines have escaped all these controls, although they are essential for a new formulation and a new principle of action.

Why did the EMA give emergency approval despite "specific obligations" not being met?
Why hasn't the FDA really evaluated these vaccines unlike the EMA?

- In 2021, senior FDA officials resigned because they felt excluded from key decisions on Covid vaccines
- According to leaked EMA documents, by the end of 2020, U.S. and EU government officials pressured European drug regulators to quickly approve Pfizer-BioNTech's COVID-19 vaccine, despite safety concerns

Conclusion

- In the future, it should be discussed whether all mRNA-based products should be subject to the same regulations and controls, whether or not they are considered vaccines:
- it is not justifiable to subject therapeutic mRNAs to strict controls when they are intended for patients representing a small proportion of the human population, and to exclude mRNA vaccines intended for the majority of the human population in good health.