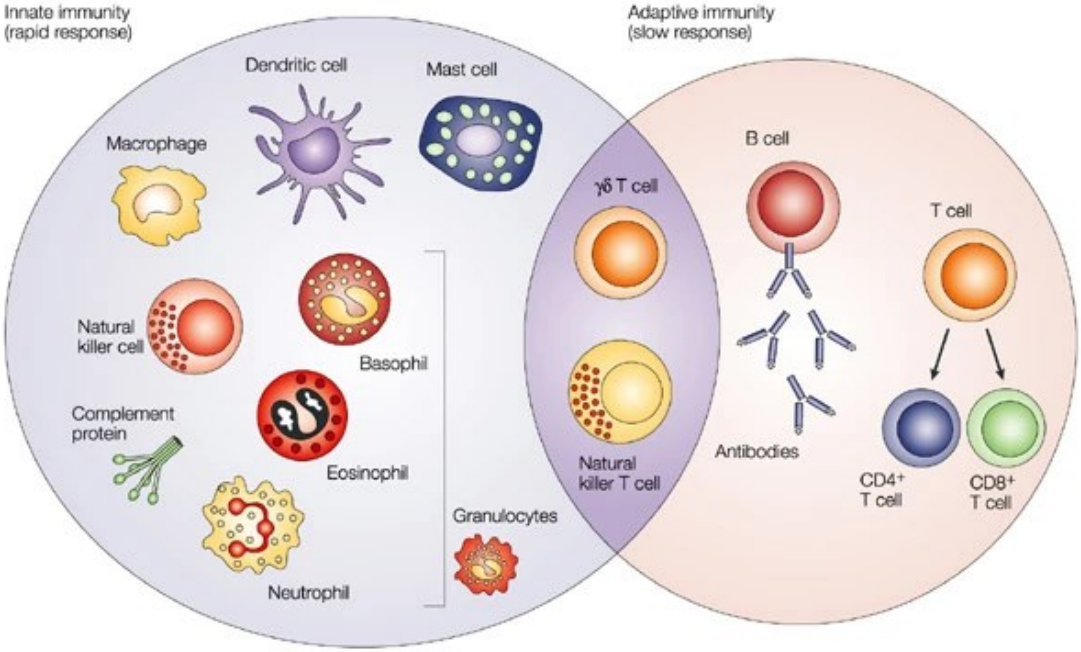
RECOMBINANT VACCINES

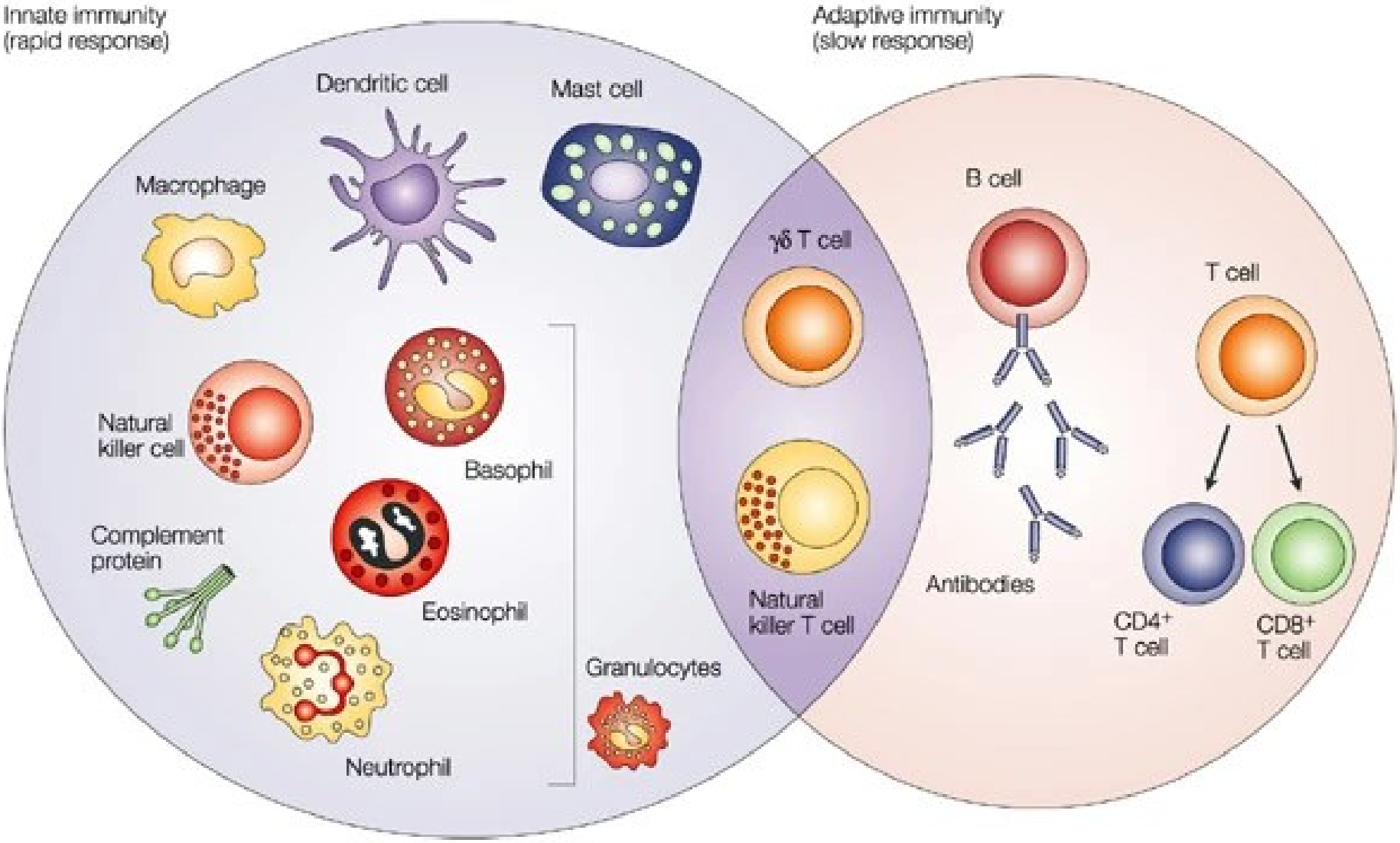
IMMUNITY

Innate:	Adaptive
Primitive	Evolutionarily recent
Non-specific	Antigen specific
Pattern recognition	Define epitope targets
First line of defense	Second line of defense
Necessary for proper function of adaptive immunity	Response improves upon repeated infection



Dranoff G. Cytokines in cancer pathogenesis and cancer therapy. Nat Rev Cancer. 2004 Jan;4(1):11-22. doi: 10.1038/nrc1252.

IMMUNITY



Dranoff G. Cytokines in cancer pathogenesis and cancer therapy. Nat Rev Cancer. 2004 Jan;4(1):11-22. doi: 10.1038/nrc1252.

IMMUNITIES

Natural Active Immunity

Naturally acquired active immunity occurs when a person is exposed to a live pathogen, develops the disease, and then develops immunity.

Natural Passive Immunity

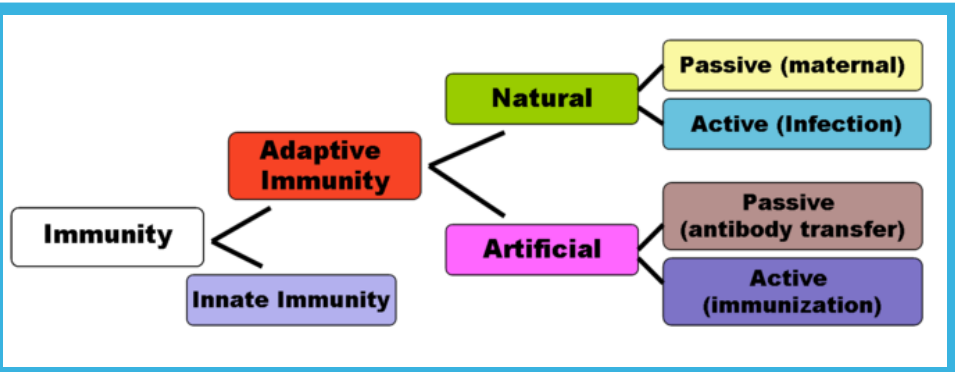
Naturally acquired passive immunity occurs during pregnancy, when antibodies are passed from the maternal blood into the fetal bloodstream.

Artificial Immunity

Artificial immunity is a mean by which the body is given immunity to a disease by intentional exposure to small quantities of it.

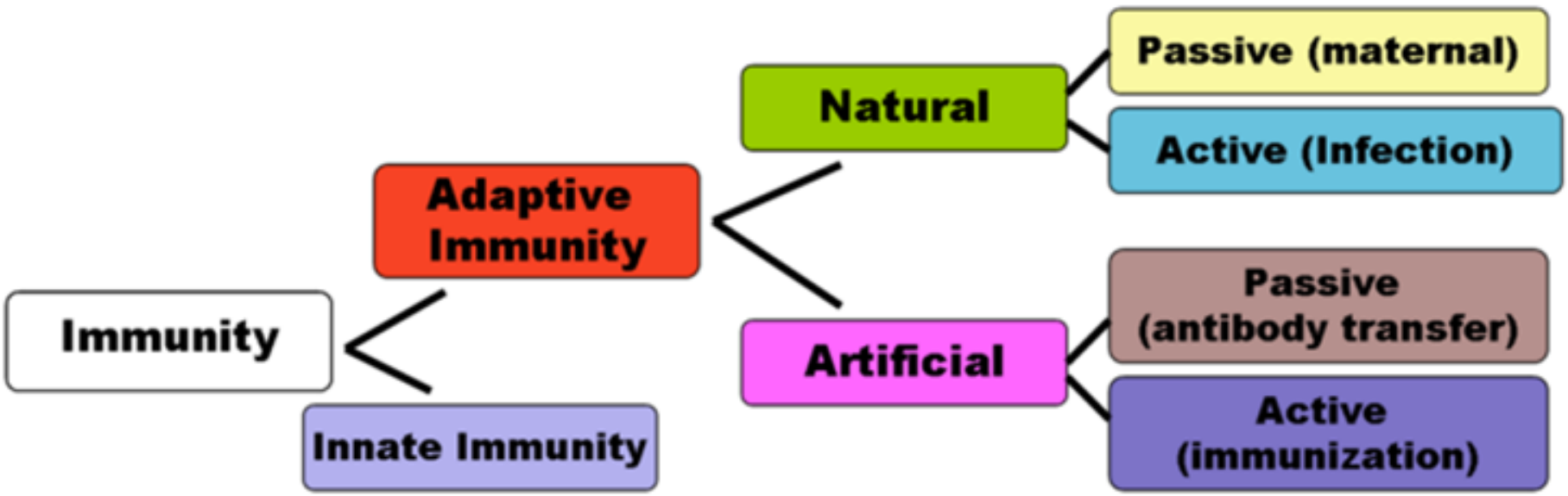
The passive form involves introducing an antibody into the system once a person has already been infected with a disease, ultimately relieving the present symptoms of the sickness and preventing re-occurrence.

The most common form of artificial immunity is classified as active and comes in the form of vaccinations, typically given to children and young adults.



https://courses.lumenlearning.com/boundless-microbiology/chapter/classifying-immunities/

IMMUNITIES



https://courses.lumenlearning.com/boundless-microbiology/chapter/classifying-immunities/

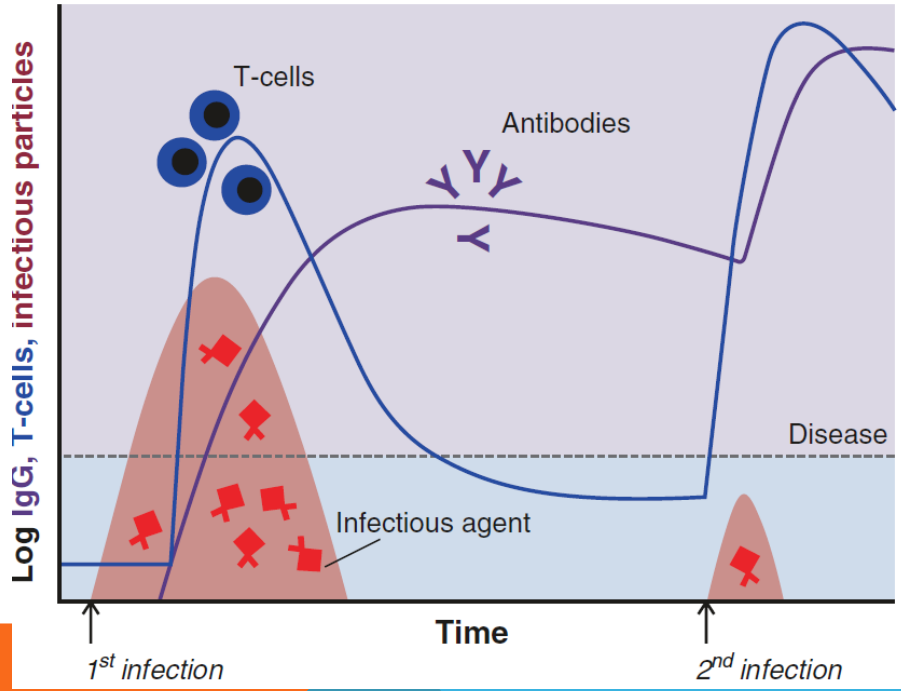
IMPORTANT IMMUNE PRODUCTS PROTECTING AGAINST INFECTIOUS DISEASES (HUMORAL – CELL MEDIATED)

Immune response	Immune product	Accessory factors	Infectious agents
Humoral	IgG	Complement, neutrophils	Bacteria and viruses
	IgA	Alternative complement Pathway	Microorganisms causing respiratory and enteric infections
	IgM	Complement, macrophages	(Encapsulated) bacteria
	IgE	Mast cells	Extracellular parasites
Cell mediated	CTL	Cytolytic proteins	Viruses,mycobacteria, Intracellular Parasites
	Th1	Macrophages	Mycobacteria, treponema (syphilis), fungi

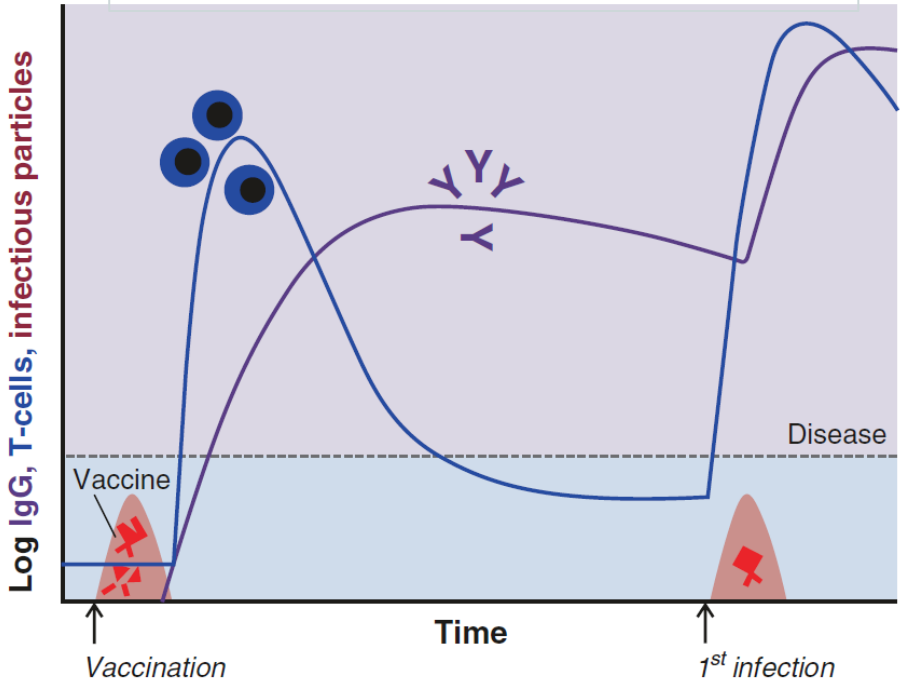
ISBN 978-3-030-00709-6 - ISBN 978-3-030-00710-2 (eBook)
https://doi.org/10.1007/978-3-030-00710-2 -

PRINCIPLE OF ADAPTIVE IMMUNE RESPONSES FOLLOWING INFECTION AND VACCINATION.

primary and secondary infection

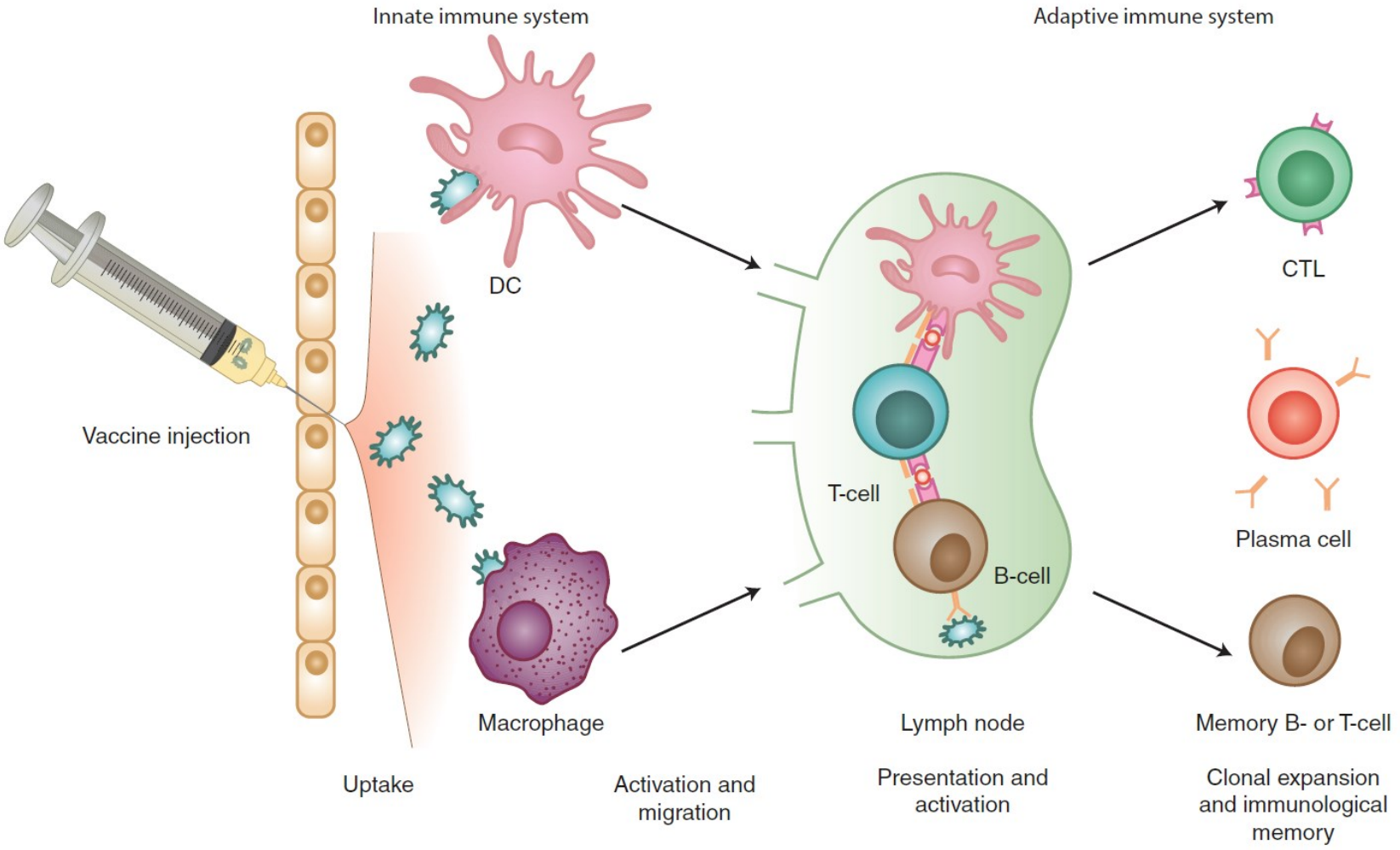


vaccine that induces an adaptive immune response



ISBN 978-3-030-00709-6 - ISBN 978-3-030-00710-2 (eBook)
https://doi.org/10.1007/978-3-030-00710-2 -

OVERVIEW OF THE STEPS LEADING TO IMMUNITY AFTER ADMINISTRATION OF A VACCINE



ISBN 978-3-030-00709-6 - ISBN 978-3-030-00710-2 (eBook)
https://doi.org/10.1007/978-3-030-00710-2 -

GENERATION OF AN IMMUNE RESPONSE AND IMMUNOLOGICAL MEMORY

The generation of the immune response by vaccination

→ lead to a potent effector response and/ or long-lasting memory.

Every immune reaction against a pathogen or a vaccine starts with the activation of the innate immune system

→ first step is uptake by professional **antigen-presenting cells (APCs)** at the site of application.
APCs shuttle the vaccine components to secondary lymphoid organs and present the antigens to T- and B-lymphocytes (activation).

→ the **innate response** itself does not lead to immunological memory, it **is instrumental in activating and educating the adaptive immune system**.

ISBN 978-3-030-00709-6 - ISBN 978-3-030-00710-2 (eBook)
https://doi.org/10.1007/978-3-030-00710-2 -

NAÏVE AND MEMORY T CELLS EXHIBIT QUALITATIVELY DIFFERENT RESPONSES TO ANTIGEN

It has been evident for many years that memory-phenotype T cells respond to antigen in a qualitatively different way from naïve-phenotype T cells.

- **Memory T cells appear to have less stringent requirements for activation than naïve T cells.** This may include an ability to respond to lower concentrations of antigen than naïve T cells,
- **Memory T cells are less dependent on costimulatory signals than naïve T cells**, and do not require as long a duration of antigenic stimulation.
- There is some evidence that **memory T cells proliferate faster and reach higher numbers in vivo than naïve T cells following antigenic stimulation**
- While activation of naïve T cells is strictly dependent on antigen presentation by dendritic cells (DCs), **memory T cells respond to antigen presented on other APCs, including resting B cells.**

It is a general finding that **memory T cells display effector functions sooner** after activation than naïve T cells.

Immunology 2002 Jun; 106(2): 127–138.

NB:
THE SPECIFICS OF INNATE IMMUNE MEMORY

One of the most important traits of immune host defense against pathogens is memory, which improves survival if the same pathogen is reencountered.

→immune memory can also be deleterious, driving autoimmune diseases and the rejection of transplanted organs.

→Memory characteristics have been considered a fundamental property of adaptive immune cells such as T and B lymphocytes.

→However, **innate immune cells such as myeloid cells and natural killer (NK)** cells can **also adapt to previous encounters with pathogens** through **epigenetic, transcriptional, and functional reprogramming, called trained immunity**

→The discovery of this **innate immune memory** emerged from studies with live vaccines and was described as being largely nonspecific

Science 05 Jun 2020: Vol. 368, Issue 6495, pp. 1052-1053
DOI: 10.1126/science.abc2660

VACCINE CATEGORIES

VACCINE CATEGORIES

Vaccines can be classified based on whether they are aimed to

Prevent (prophylactic) a disease

Cure (therapeutic) a disease

→ **the type of disease** to treat (infectious diseases, allergy, autoimmune disease, cancer, etc.),

→ **the antigen source** used for vaccination (e.g., whole pathogens, subunits, peptides, or nucleic acids)

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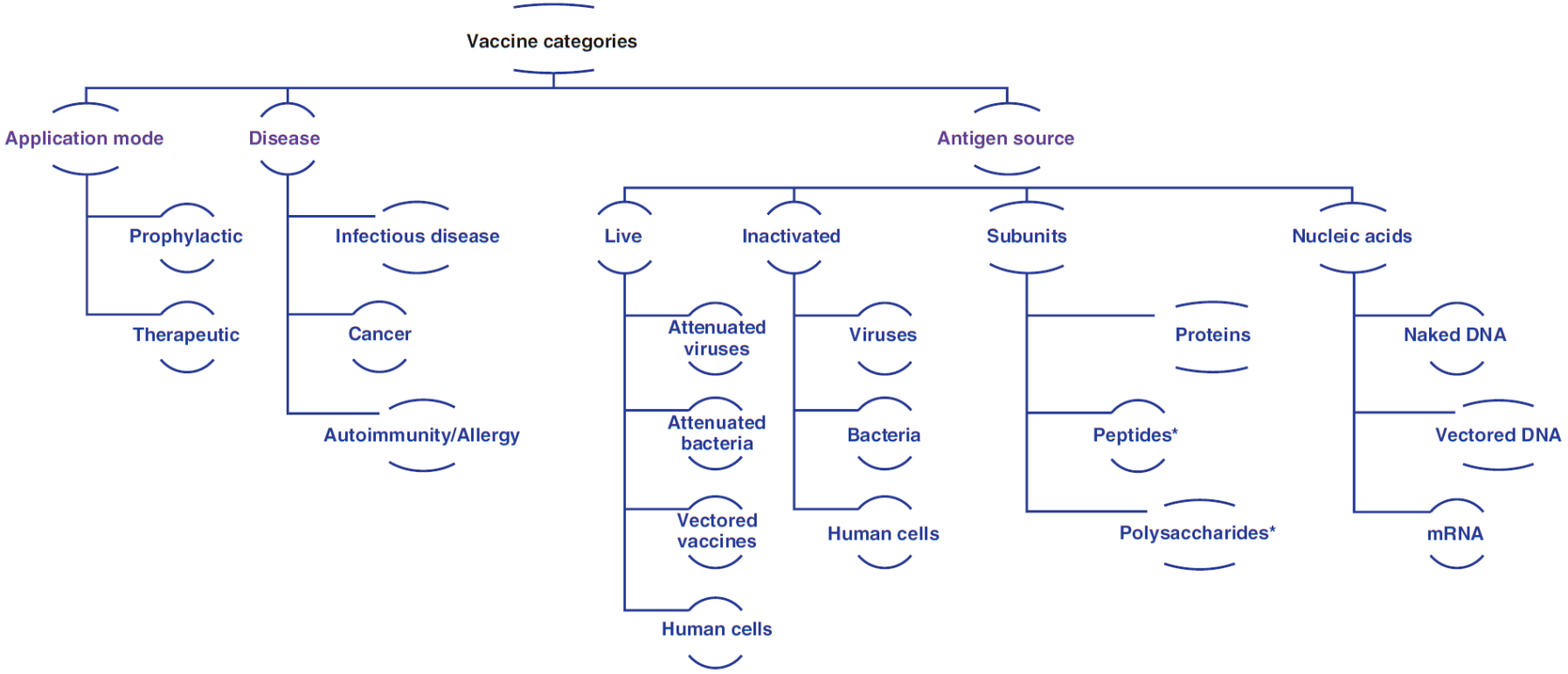
https://doi.org/10.1007/978-3-030-00710-2 -

VACCINE CATEGORIES



*either free or conjugated to a protein carrier

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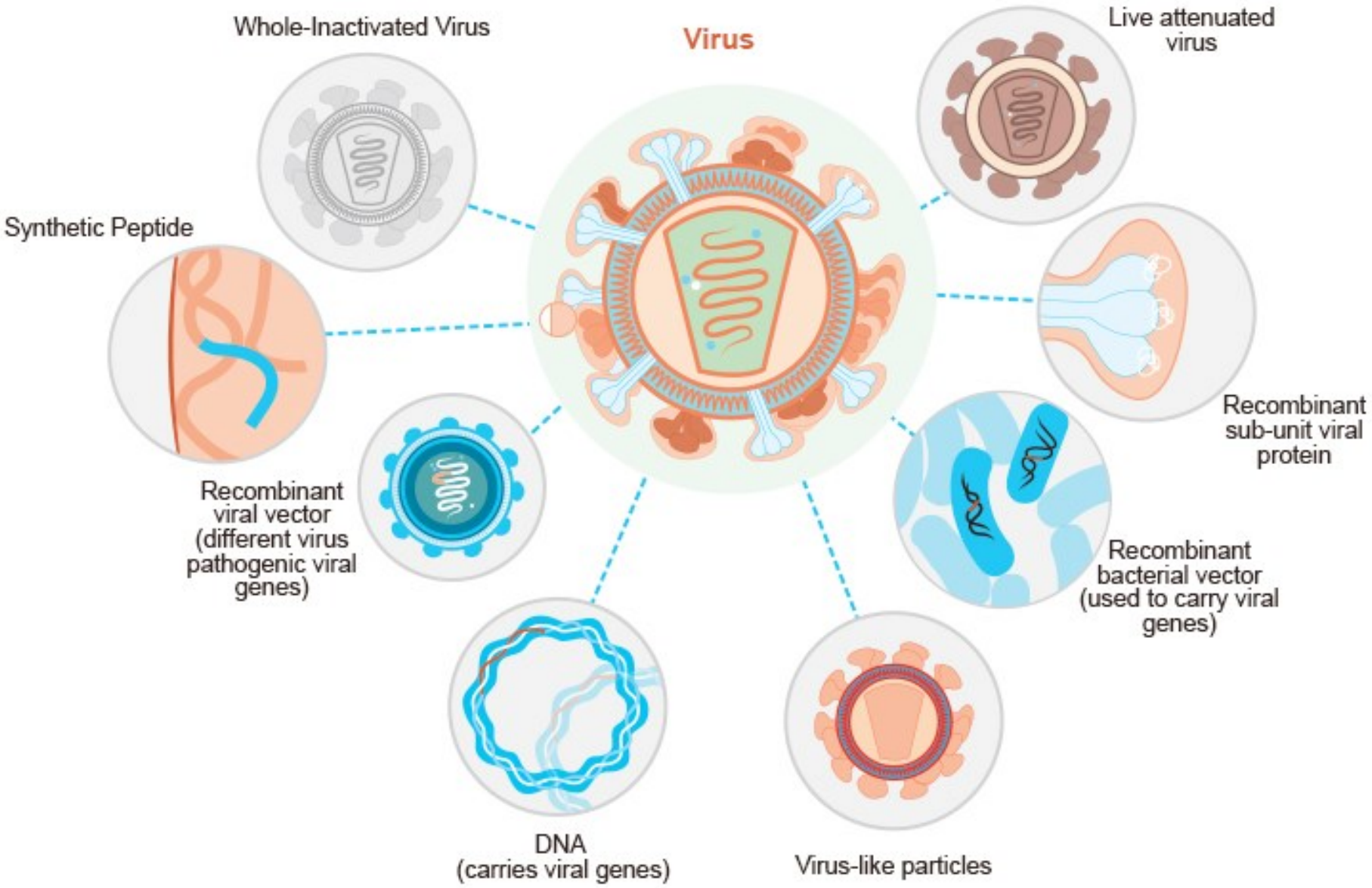
https://doi.org/10.1007/978-3-030-00710-2 -

VARIOUS APPROACHES FOR VACCINE DEVELOPMENT



https://www.genscript.com/recombinant-vaccine.html

WHAT IS A RECOMBINANT VACCINE?

Vaccine generated using recombinant DNA technology is called recombinant vaccine.

While there are various types of vaccines made possible by recombinant DNA technology, recombinant vaccines can be classified into two major categories.

- Recombinant (protein subunit) vaccines
- DNA vaccines
- mRNA vaccines

https://www.genscript.com/recombinant-vaccine.html

RECOMBINANT (PROTEIN SUBUNIT) VACCINES

Subunit vaccines containing only a fraction of the pathogenic organism.

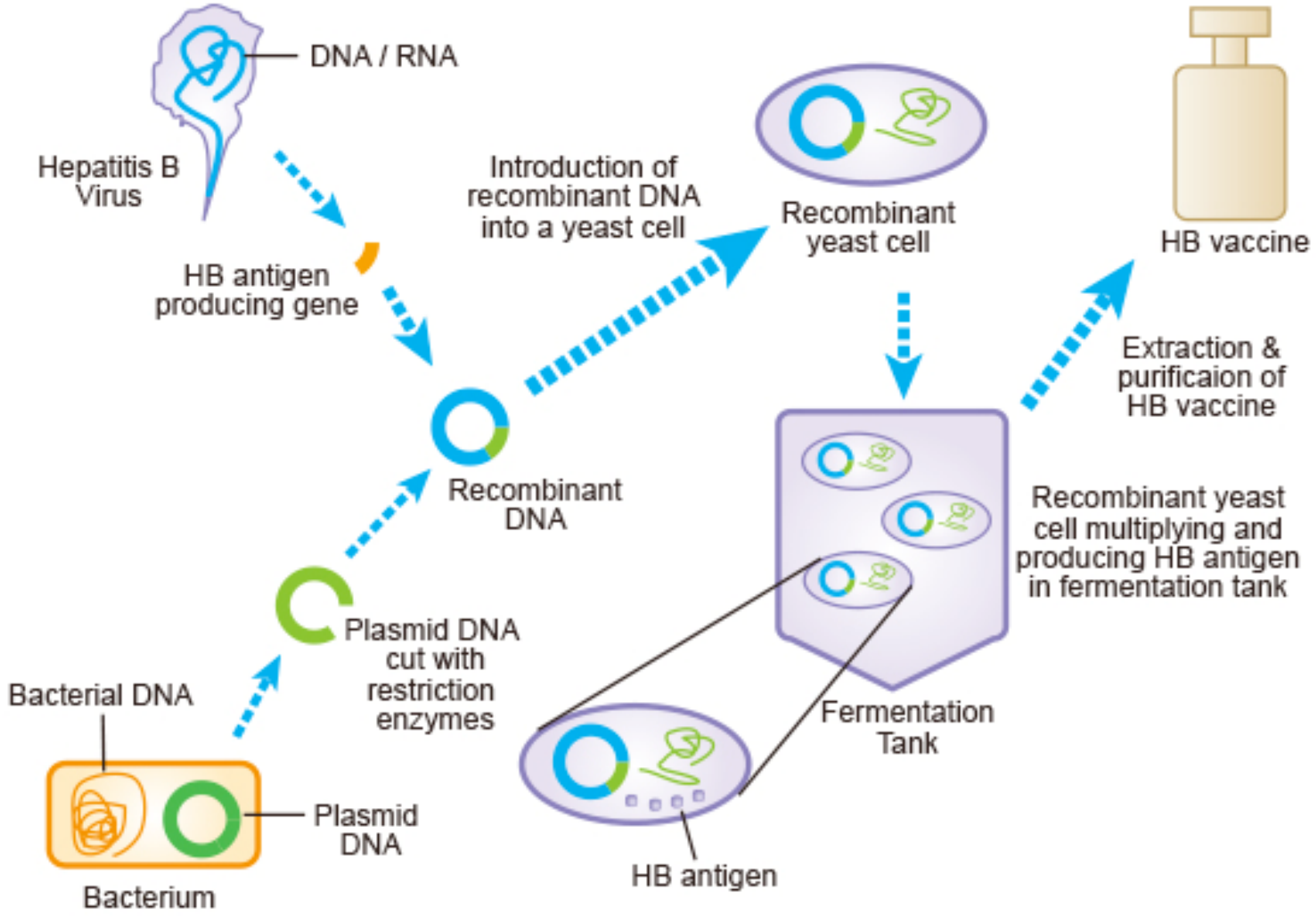
Often these are **synthetic peptides** that represent the protein component that induces an immune response.

Or consist of protein subunits (antigens) expressed in a heterologous expression system (*E. coli*, yeast, insect etc.) using recombinant protein expression technologies.

Most of the vaccines under investigation today are based on such purified **recombinant proteins or subunits of antigens**.

https://www.genscript.com/recombinant-vaccine.html

RECOMBINANT (PROTEIN SUBUNIT) VACCINES



Recombinant Hepatitis B Vaccine Production Summary

https://www.genscript.com/recombinant-vaccine.html

RECOMBINANT (PROTEIN SUBUNIT) VACCINES

The current vaccine for Hepatitis B Virus is produced by expressing the HBV surface antigen (HBsAg) using yeast expression system.

Yeast secretes the antigen into culture supernatant facilitating purification.

Yeast post translational machinery is suitable for this purpose as it renders the Antigen with the necessary glycosylation patterns.

Upon recombinant expression, the HBsAg assembles into virus-like particles (VLPs) which are extremely immunogenic, making it a very effective vaccine.

https://www.genscript.com/recombinant-vaccine.html

RECOMBINANT (PROTEIN SUBUNIT) VACCINES

The vaccine against Human Papilloma Virus (HPV).

There are currently two vaccines against HPV infection, both of which have been developed based on VLPs assembled from recombinant HPV coat proteins.

These vaccines utilize the L1 recombinant capsid protein of the virus subtype produced either in insect or yeast-expression system.

https://www.genscript.com/recombinant-vaccine.html

RECOMBINANT VACCINES

Bacterial expression systems are widely used due to the easy of handling and their capacity for high level expression.

For antigens in which post-translational modifications are necessary, the use of mammalian, yeast or insect cells is considered.

Although vaccines based on recombinant proteins offer several advantages such as safety and economy of production,

most of them suffer from poor immunogenicity when administered alone.

And hence they require the use of adjuvants to elicit a longer-lasting immune response.

https://www.genscript.com/recombinant-vaccine.html

DNA VACCINES

These vaccines usually consist of synthetic DNA containing the gene that encodes the disease-agent protein.

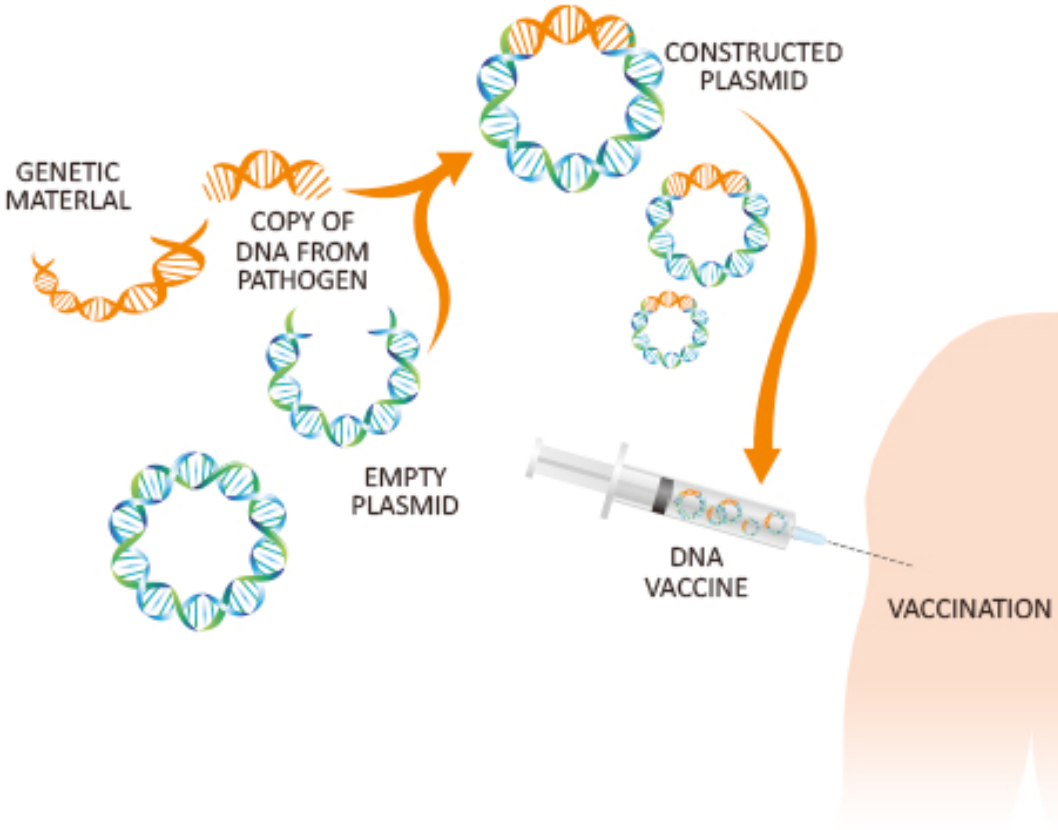
Usually, the plasmid DNA used as vaccine is propagated in bacteria such as *E. coli* and they are isolated and purified for injection.

This "naked" DNA is usually injected intramuscularly or intradermally.

The principle behind a DNA vaccine is that the antigen can be expressed directly by host cells in a way that simulates viral infection and invokes an immune response from the host.

https://www.genscript.com/recombinant-vaccine.html

DNA VACCINES



A DNA vaccine usually consists:

- Origin of replication for plasmid amplification in E. coli
- Strong promoter (generally CMV)
- Multiple cloning sites into which gene is inserted
- Antibiotic selection marker

https://www.genscript.com/recombinant-vaccine.html

DNA VACCINES

The promoter drives the expression of the gene encoding antigenic protein, when introduced into the target.

The protein antigens can be processed in the cytoplasm and the fragmented peptides presented to the immune system by class I MHC molecules.

In addition, if the protein is exported or secreted, it can be processed by class II MHC molecules and, as a result, mount a specific antibody response.

https://www.genscript.com/recombinant-vaccine.html

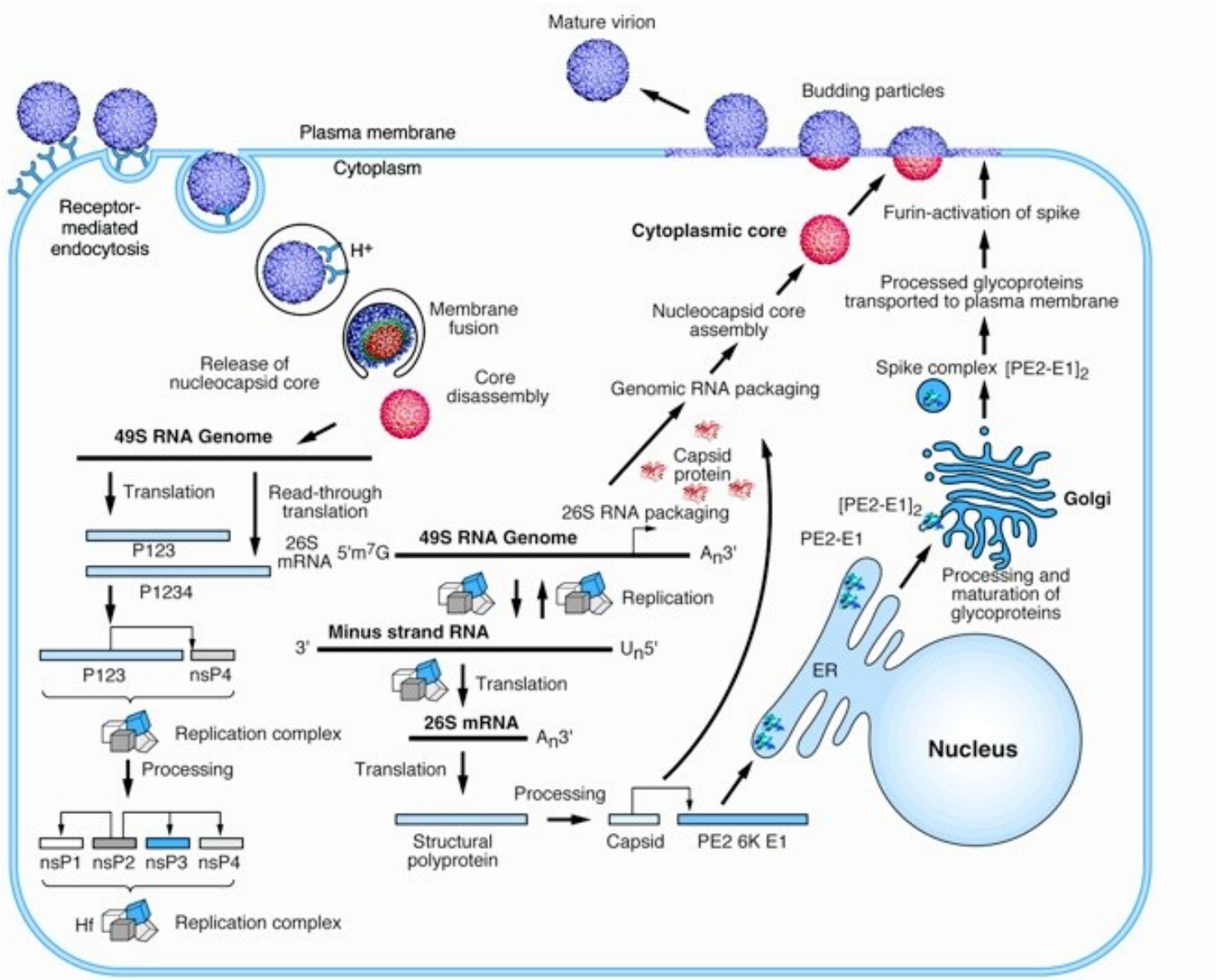
SELF-AMPLIFYING MRNA-BASED VACCINE TECHNOLOGY AND ITS MODE OF ACTION

Maruggi G., Ulmer J.B., Rappuoli R., Yu D. (2021)
Self-amplifying mRNA-Based Vaccine Technology and Its Mode of Action.
In: . Current Topics in Microbiology and Immunology. Springer, Berlin, Heidelberg. https://doi.org/10.1007/82_2021_233

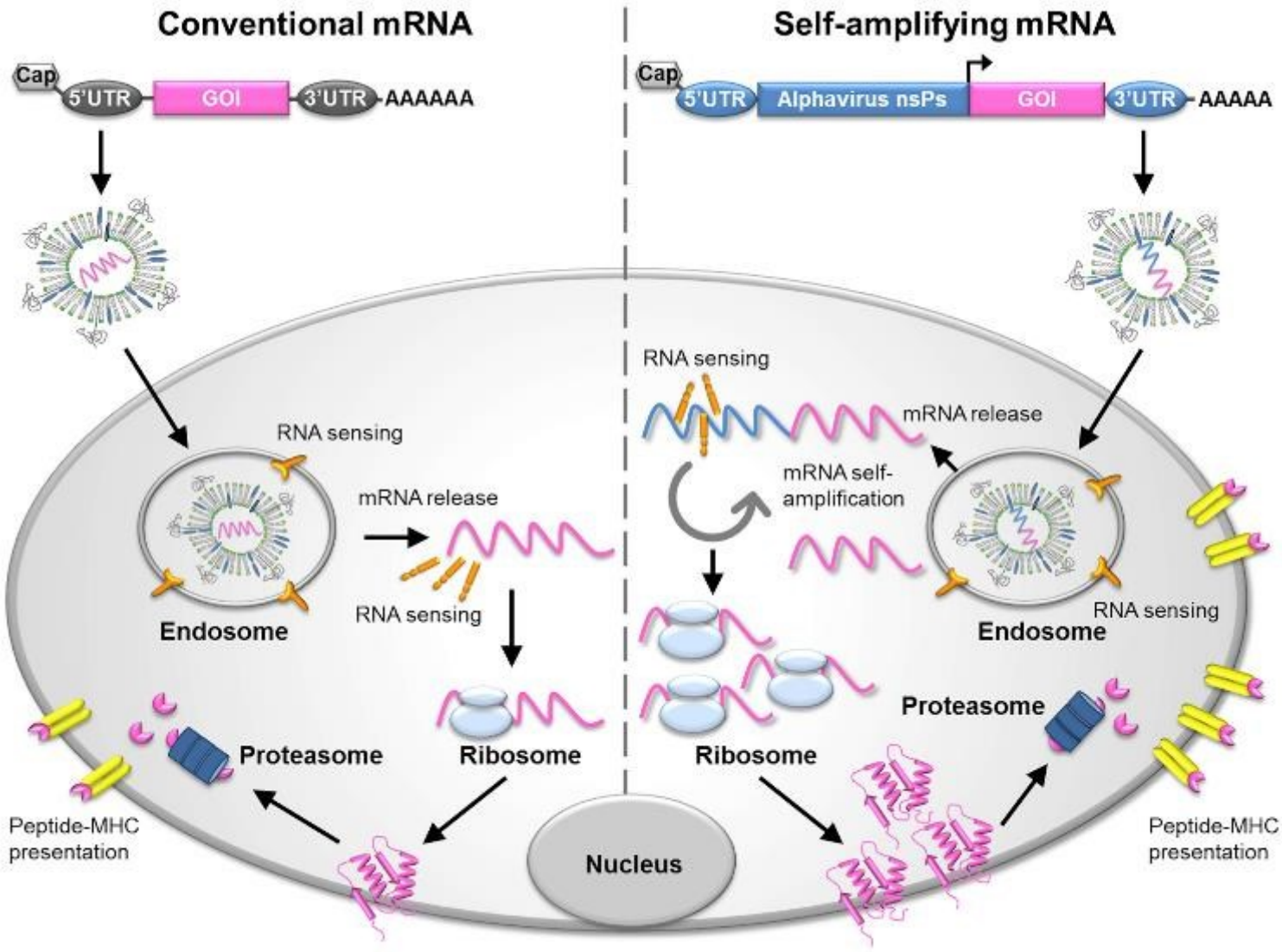
Self-amplifying mRNAs derived from the genomes of positive-strand RNA viruses have recently come into focus as a promising technology platform for vaccine development.

Non-virally delivered self-amplifying mRNA vaccines have the potential to be highly versatile, potent, streamlined, scalable, and inexpensive.

ALPHAVIRUSES: SINDBIS VIRUS, CHIKUNGUNYA VIRUS



https://www.bio.purdue.edu/lab/kuhn/research.html



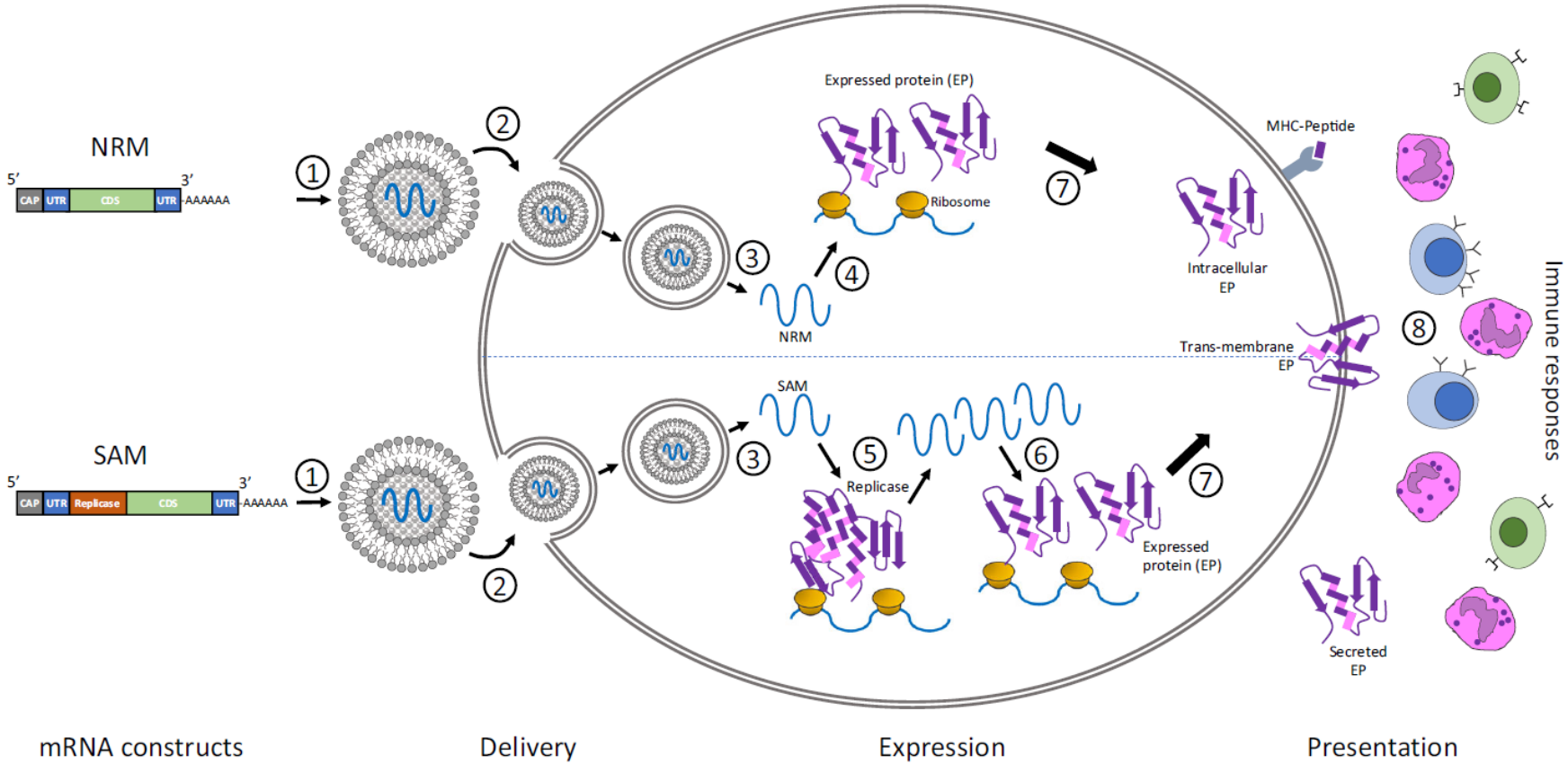
The self-amplifying mRNA is often **derived from the genome of positive-sense single-stranded RNA viruses, such as alphaviruses.**

→ It encodes both the antigen of interest and viral nonstructural proteins (nsPs) required for intracellular RNA amplification and high levels of antigen expression

Maruggi G, Zhang C, Li J, Ulmer JB, Yu D. mRNA as a Transformative Technology for Vaccine Development to Control Infectious Diseases. Mol Ther. 2019 Apr 10;27(4):757-772. doi: 10.1016/j.ymthe.2019.01.020.

The promise of mRNA vaccines: a biotech and industrial perspective

Two categories of mRNA constructs are being actively evaluated.



Non-replicating mRNA (NRM) constructs encode the coding sequence (CDS) and are flanked by 5 ′and 3 ′untranslated regions (UTRs), a 5 ′-cap structure and a 3 ′-poly-(A) tail.
The self-amplifying mRNA (SAM) construct **encodes additional replicase components** able to direct intracellular mRNA amplification.

npj Vaccines (2020) 5:11 ; https://doi.org/10.1038/s41541-020-0159-8

The promise of mRNA vaccines: a biotech and industrial perspective

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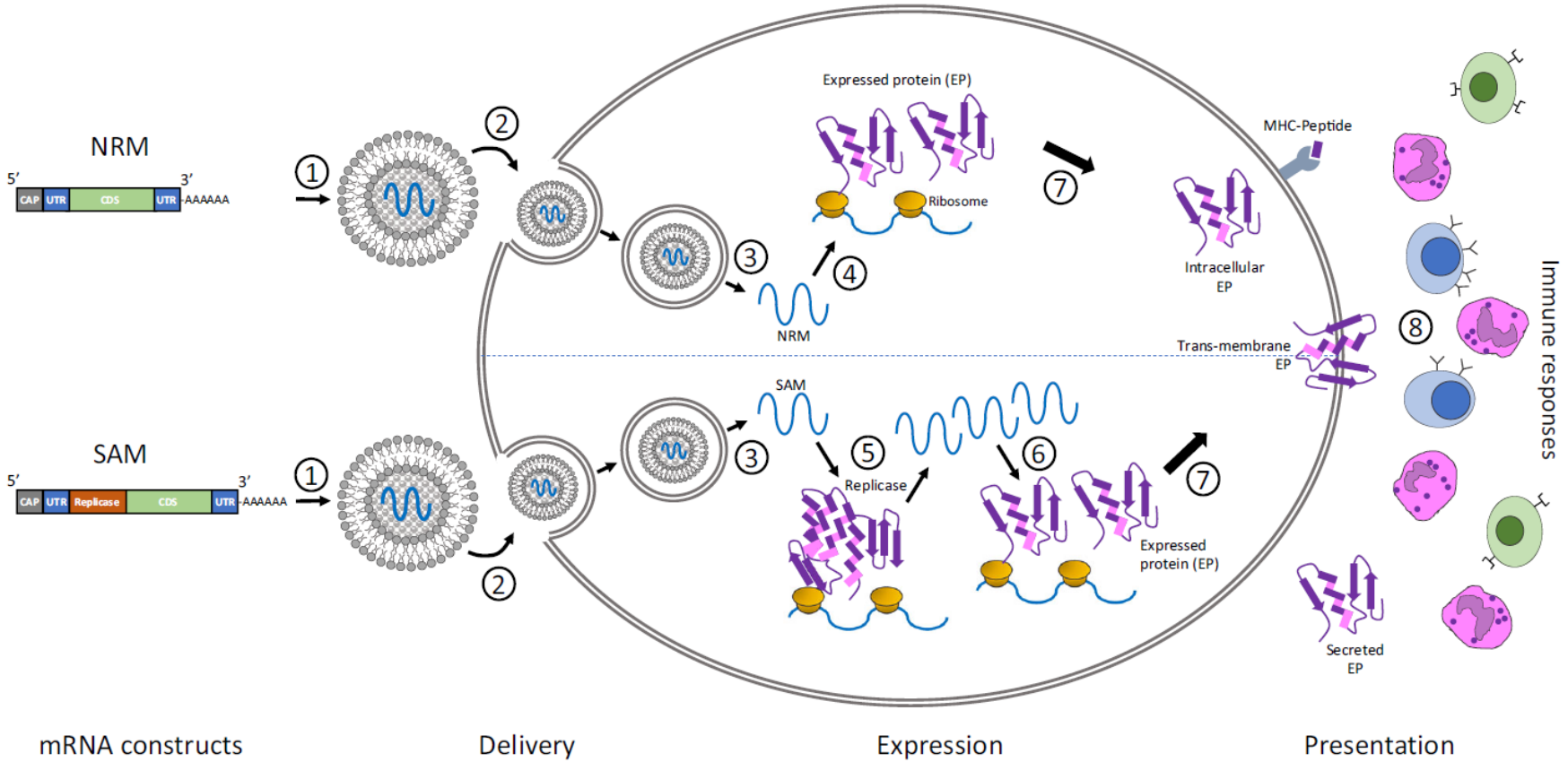

(1) NRM and SAM are formulated in **in lipid nanoparticles (LNPs)** that encapsulate the mRNA constructs to protect them from degradation and promote cellular uptake.

(2) Cellular uptake of the **mRNA with its delivery system typically exploits membrane-derived endocytic pathways.**

(3) **Endosomal escape allows release of the mRNA into the cytosol.**

npj Vaccines (2020) 5:11 ; https://doi.org/10.1038/s41541-020-0159-8

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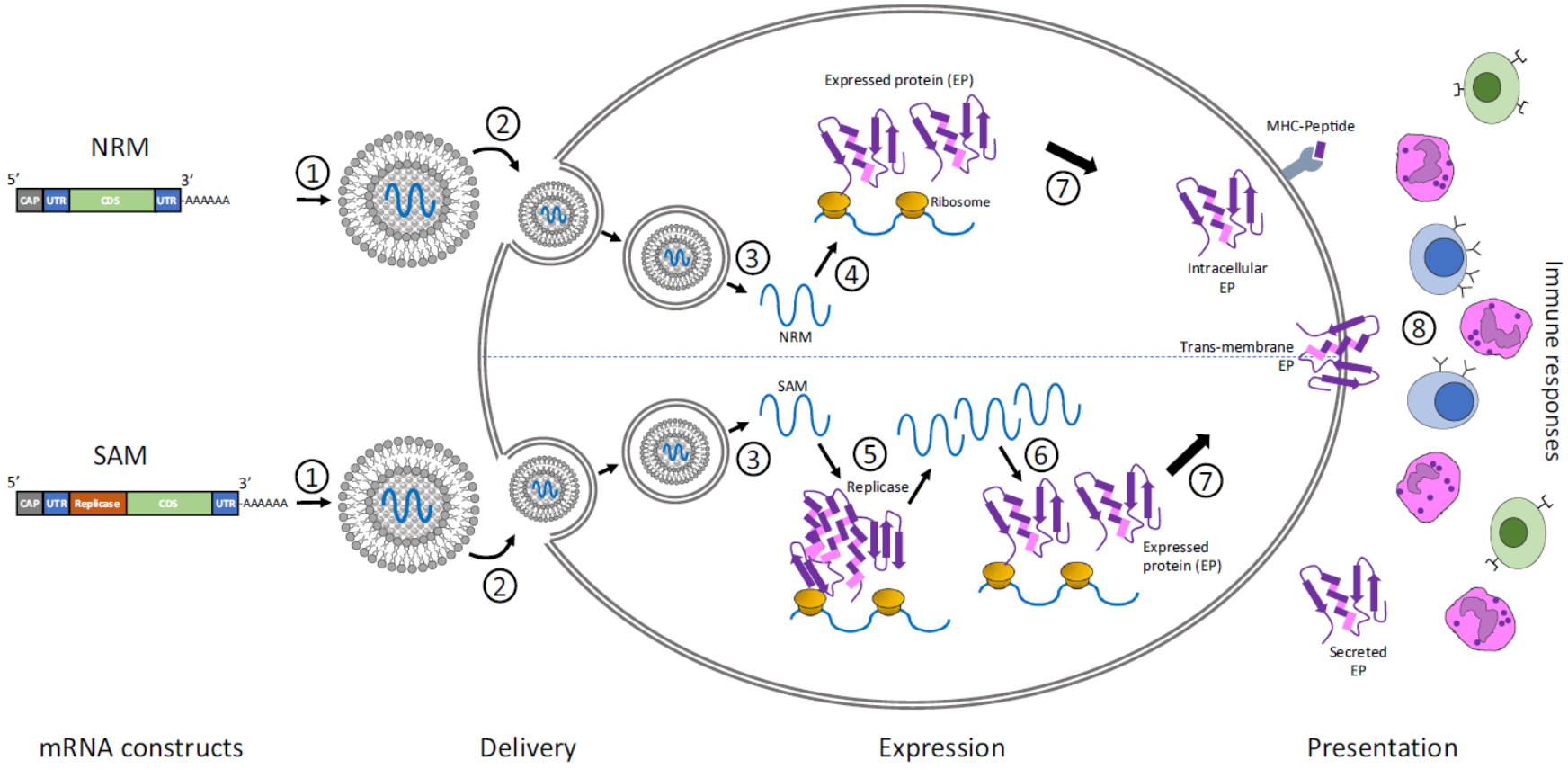

(4) Cytosol-located **NRM constructs are immediately translated** by ribosomes to produce the protein of interest, which undergoes subsequent post-translational modification.

npj Vaccines (2020) 5:11 ; https://doi.org/10.1038/s41541-020-0159-8

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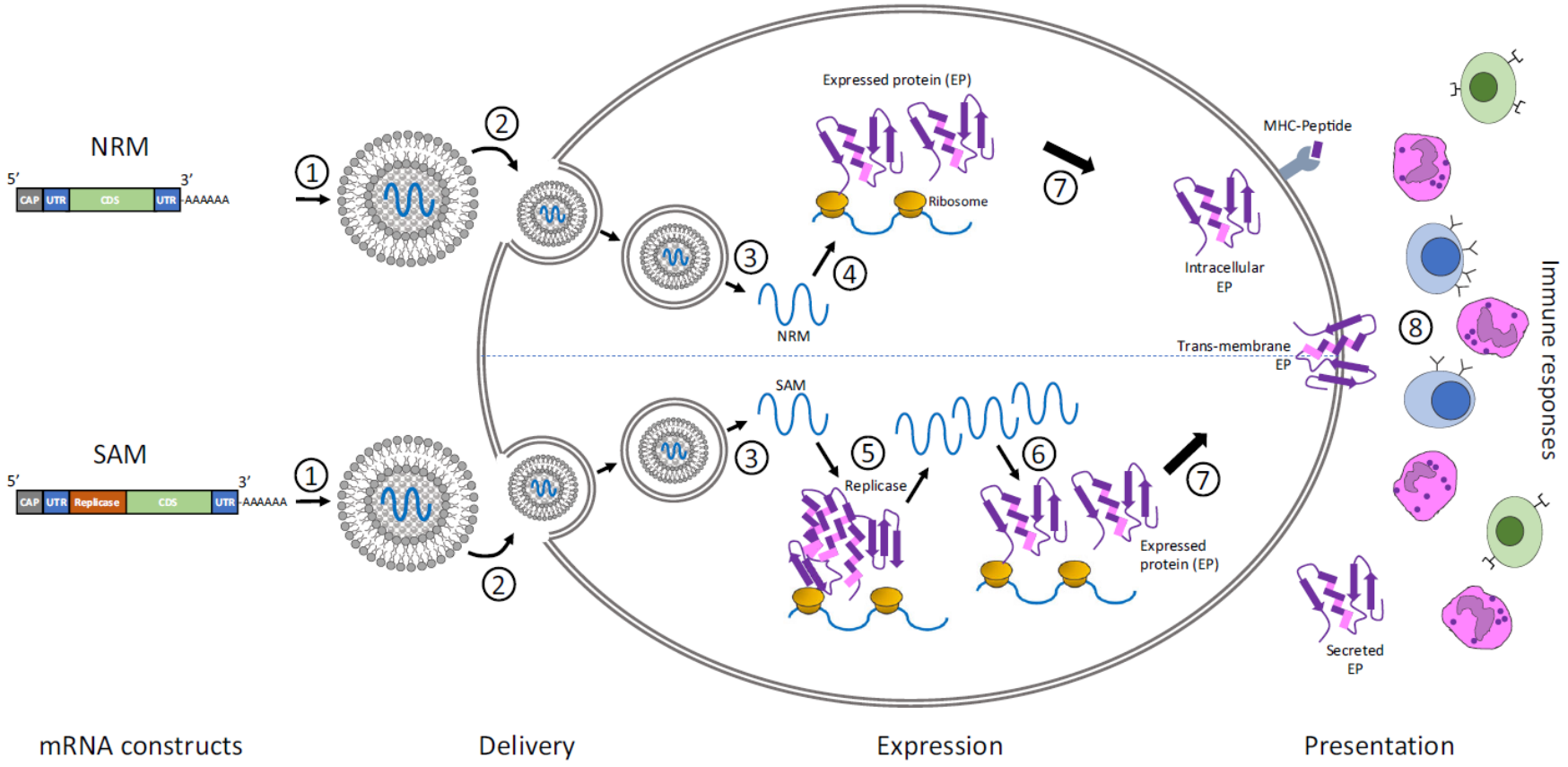


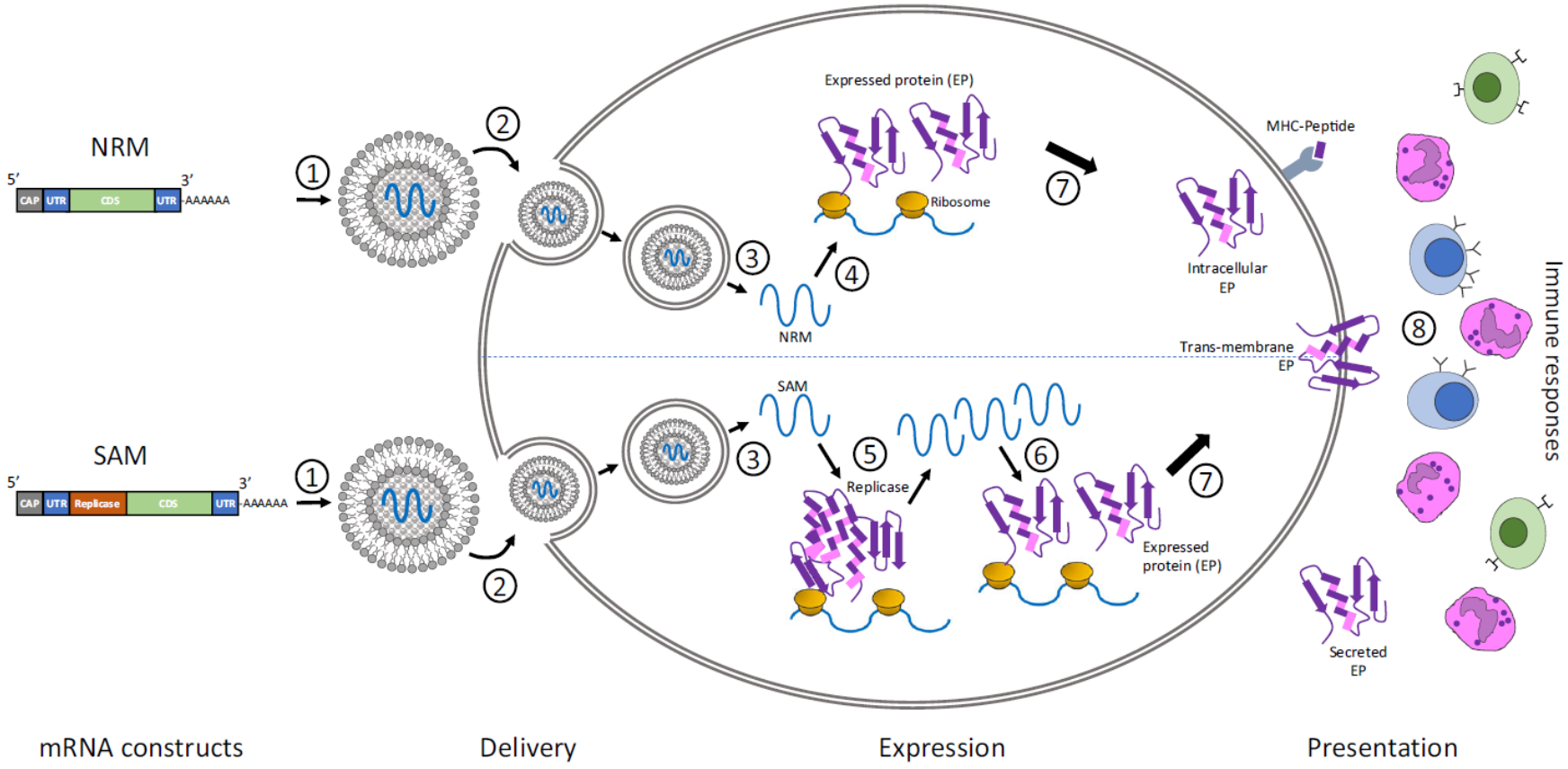
(5) **SAM constructs** can also be immediately translated by ribosomes to **produce the replicase machinery necessary for self-amplification of the mRNA**.

(6) **Self-amplified mRNA constructs are translated by ribosomes** to produce the protein of interest, which undergoes subsequent post-translational modification

npj Vaccines (2020) 5:11 ; https://doi.org/10.1038/s41541-020-0159-8

The promise of mRNA vaccines: a biotech and industrial perspective

Two categories of mRNA constructs are being actively evaluated.



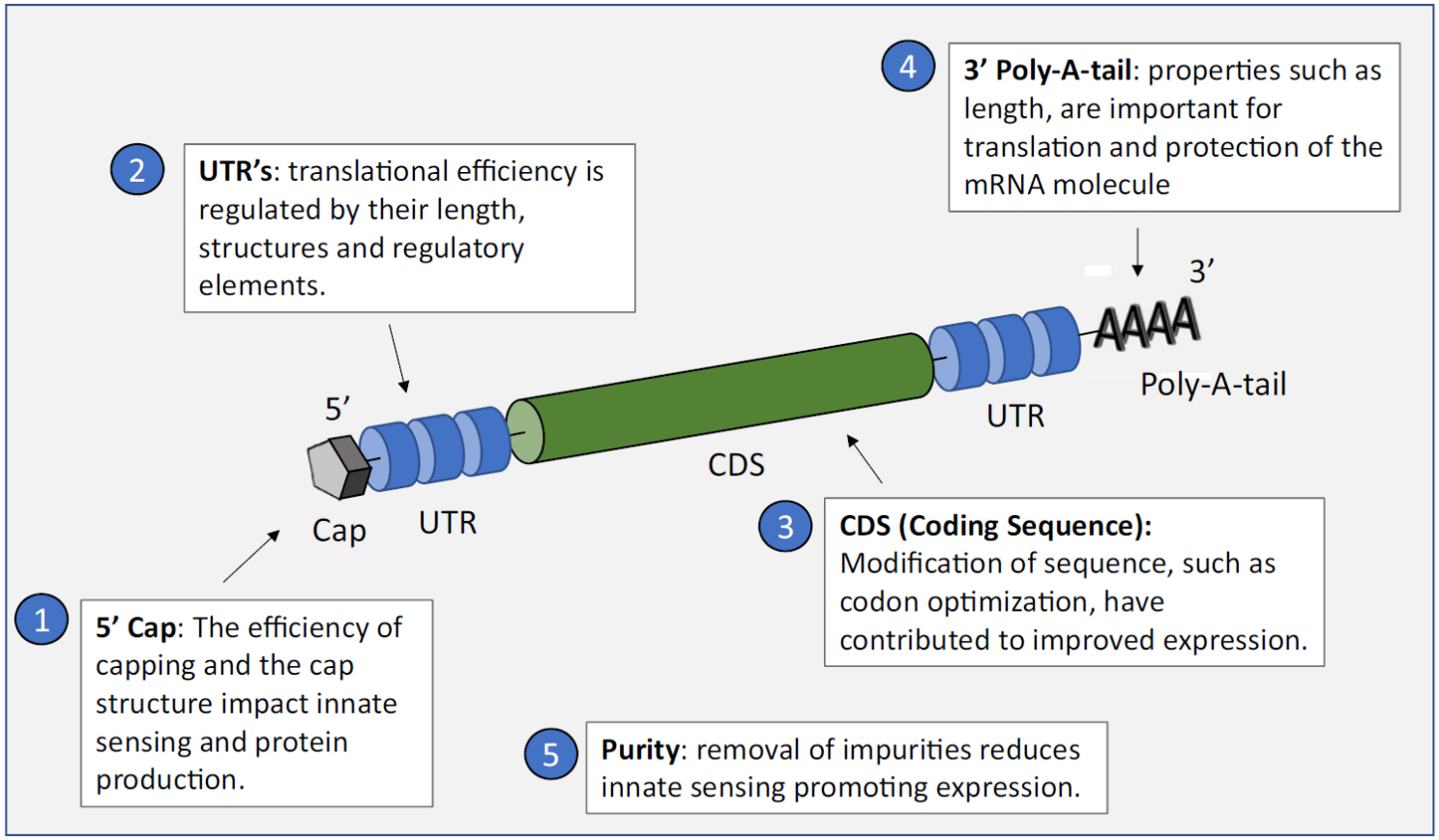
(7) The expressed proteins of interest are generated **as secreted, trans-membrane, or intracellular protein.**

(8) The innate and **adaptive immune responses detect the protein of interest**.

npj Vaccines (2020) 5:11 ; https://doi.org/10.1038/s41541-020-0159-8

The promise of mRNA vaccines: a biotech and industrial perspective

Two categories of mRNA constructs are being actively evaluated.

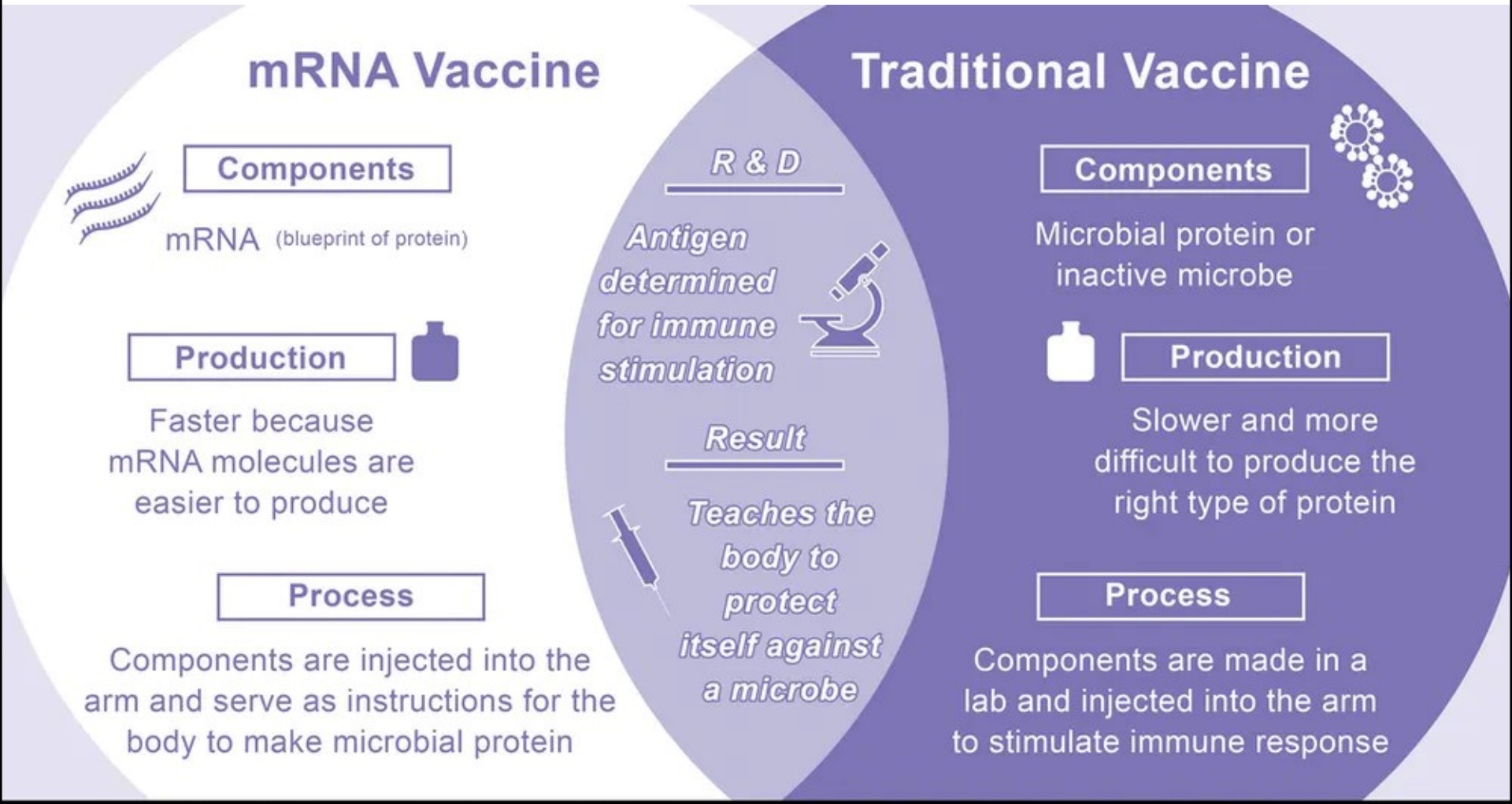


Critical quality attributes (CQAs)

have been identified that dictate the performance of the mRNA construct to express the gene of interest efficiently. Five principal CQAs include

5 'capping efficiency and structure; **UTR structure**, length, and regulatory elements; **modification of coding sequence**; **poly-A-tail** properties; **mRNA purity**.

npj Vaccines (2020) 5:11 ; https://doi.org/10.1038/s41541-020-0159-8



https://theconversation.com/how-mrna-vaccines-from-pfizer-and-moderna-work-why-theyre-a-breakthrough-and-why-they-need-to-be-kept-so-cold-150238

WHAT ARE PROBLEMS WITH MRNA?

mRNA

- is notoriously unstable and easy to degrade into smaller components,
- is also easily destroyed by the human body's immune defenses, which make delivering it to the target very in efficient.

- The most important **challenge** for development of a **mRNA vaccine remains its inherent instability,** because it is more likely to break apart above freezing temperatures.
- It is called synthetic messenger RNA, an ingenious variation on the natural substance that directs protein production in cells throughout the body.

https://theconversation.com/how-mrna-vaccines-from-pfizer-and-moderna-work-why-theyre-a-breakthrough-and-why-they-need-to-be-kept-so-cold-150238

WHAT ARE PROBLEMS WITH MRNA?

mRNA

is also easily destroyed by the human body's immune defenses, which make delivering it to the target very in efficient.

Solution

- It is called **synthetic messenger RNA**, an ingenious variation on the natural substance that directs protein production in cells throughout the body.
- But in **its altered, synthetic form, one of those building blocks,** like a misaligned wheel on a car, was throwing everything off by signaling the immune system

https://theconversation.com/how-mrna-vaccines-from-pfizer-and-moderna-work-why-theyre-a-breakthrough-and-why-they-need-to-be-kept-so-cold-150238

WHAT ARE PROBLEMS WITH mRNA?

synthetic messenger RNA, an ingenious variation on the natural substance that directs protein production in cells throughout the body.

One way to alter RNA secondary structure is:

change the primary sequence.

In the CDS, however, primary sequence changes necessarily alter codon usage, confounding any effects that might be attributable to changes in mRNA structure alone.

affect secondary structure without changing codons

is to incorporate modified nucleotides (nt) that maintain the same Watson–Crick base-pairing relationships (**e.g., pseudouridine [Ψ] forU**)

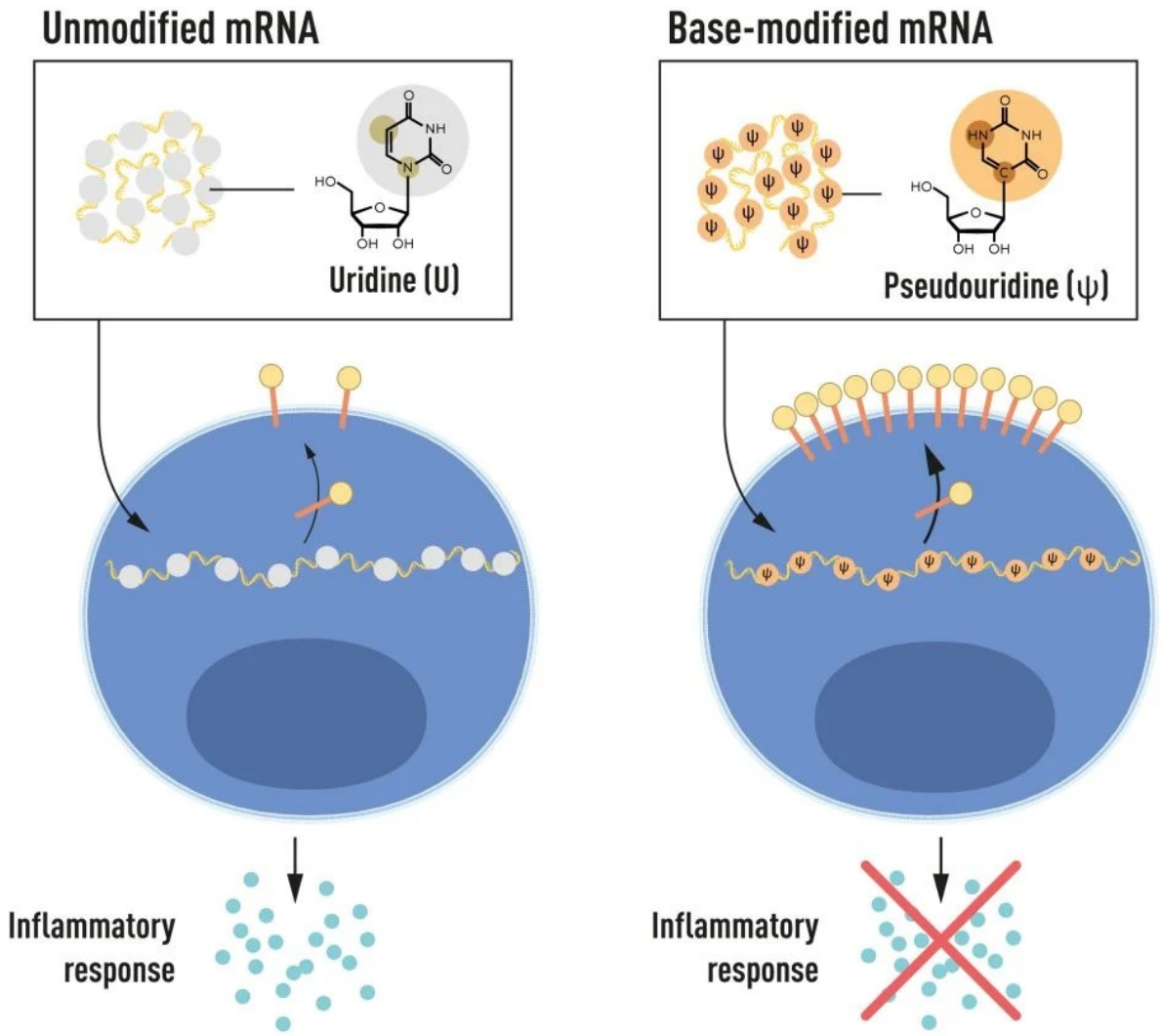
→ but have small effects on local secondary structure.

Such modified nucleotides can either stabilize (**14**) or destabilize (**15**) base pairs and hence overall mRNA structure.

PNAS November 26, 2019 116 (48) 24075-24083; first published November 11, 2019; https://doi.org/10.1073/pnas.1908052116

The Nobel Laureates discovered that

base-modified mRNA can be used to block activation of inflammatory reactions (secretion of signaling molecules) and increase protein production when mRNA is delivered to cells.



The Nobel Prize in Physiology or Medicine 2023

https://www.nobelprize.org/prizes/medicine/2023/summary/

Katalin Karikó

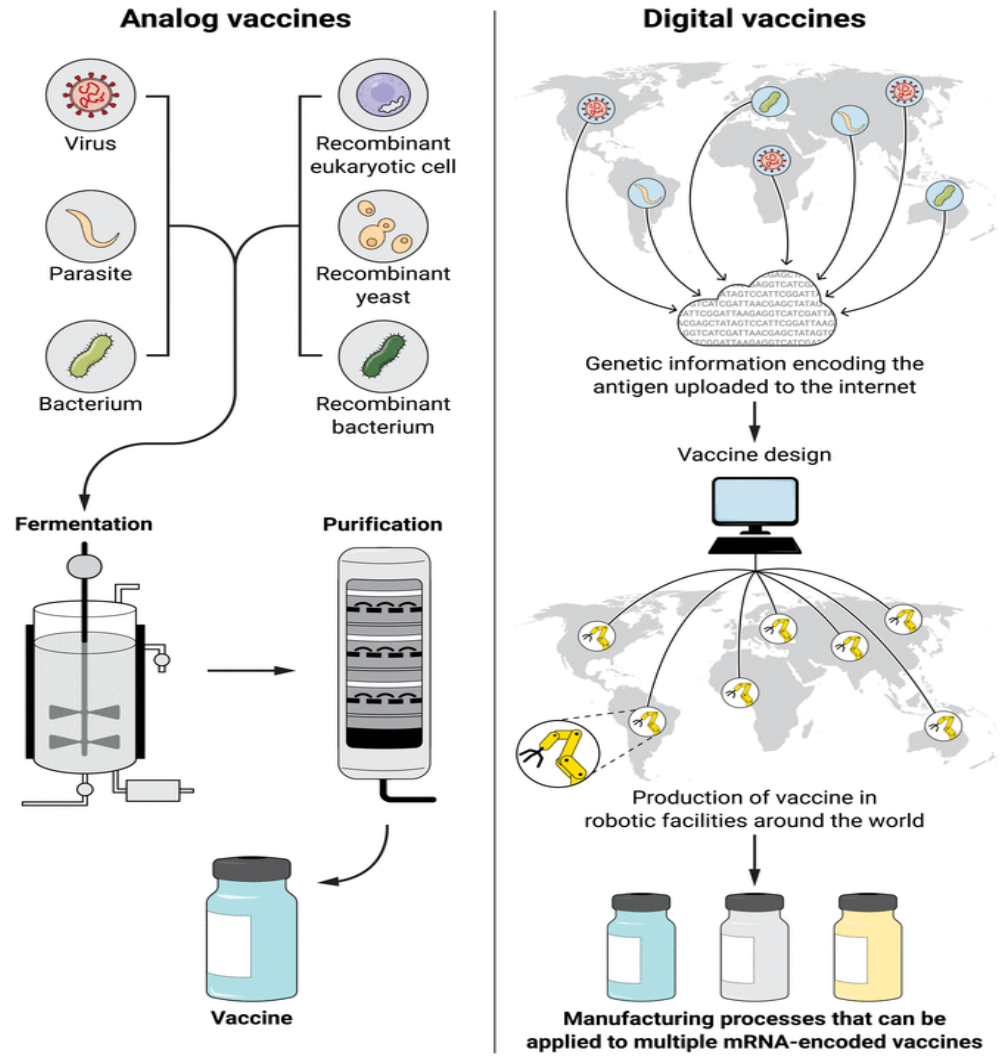
Prize share: ½

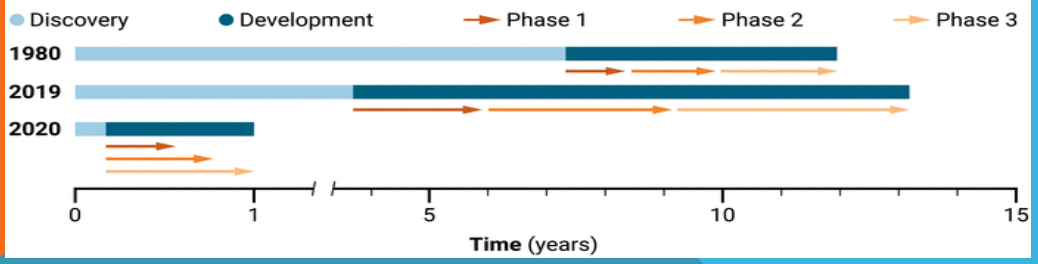
Ill. Niklas Elmehed © Nobel Prize Outreach

Drew Weissman

Prize share: ½

Ill. Niklas Elmehed © Nobel Prize Outreach





Vaccines 2020: The era of the digital vaccine is here,

Volume: 13, Issue: 624, DOI: (10.1126/scitranslmed.abm3249)

RNA THERAPY

The biggest barrier to RNA therapy has long been delivering RNA to the correct place in the correct cells.

The past several years have seen a flurry of advances that have improved researchers' ability to get such drugs into liver cells — an important development because so many proteins implicated in diseases are made in the liver.

Nature 574, S2-S3 (2019)
doi: 10.1038/d41586-019-03068-4

RNA THERAPY

Path to the clinic

Messenger RNA was discovered in 1961, but RNA therapies only took off in the 1990s.

1990

A study in mice shows that injecting mRNA into skeletal muscle leads to production of the protein encoded by the RNA1. This result lays the groundwork for treatments based on mRNA.

1993

Injection of mRNA from the influenza virus induces an immune response in mice2, providing a proof of concept for RNA-based vaccines.

1998

A team led by Andrew Fire at the Carnegie Institution for Science, Washington DC, and Craig Mello at the University of Massachusetts, Worcester, show that short interfering RNAs (siRNA) can suppress gene activity in Caenorhabiditis elegans3.

The first RNA therapy, fomivirsen, is approved by the US Food and Drug Administration (FDA). The antisense oligonucleotide (ASO) drug tackles cytomegalovirus retinitis, inflammation of the retina.

Nature 574, S2-S3 (2019)
doi: 10.1038/d41586-019-03068-4

RNA THERAPY

2001

Researchers demonstrate that RNA interference (RNAi), a mechanism for gene silencing underpinned by siRNA, occurs not only in plants and invertebrates, but also in mammalian cells4, suggesting its potential for targeting harmful genes.

2002

Researchers led by Mark Kay at Stanford University, California, use RNAi to target a sequence in the hepatitis C virus for destruction in mice5, highlighting the technology's therapeutic potential.

2003

Judith Lieberman and her colleagues at Harvard Medical School in Boston, Massachusetts, demonstrate that RNAi can suppress HIV replication in macrophages6

Nature 574, S2-S3 (2019)
doi: 10.1038/d41586-019-03068-4

RNA THERAPY

2004

Pegaptanib becomes the first RNA aptamer that targets proteins to be approved by the FDA.

2006

Fire and Mello receive the Nobel Prize in Physiology or Medicine for their work on RNAi. Meanwhile, Kay and his team report that long-term siRNA expression can cause liver damage and even death in mice7, temporarily halting progress on RNAi-based therapies.

2010

The first use of RNAi-mediated gene silencing in humans is reported in a phase I trial in people with the skin cancer melanoma8. The therapy reduced expression of a gene needed for tumour cells to multiply.

RNA THERAPY

2018

Approval to market patisiran and inotersen for hereditary ATTR amyloidosis is granted in the United States and Europe.
Two disease-modifying drugs, inotersen (an antisense oligonucleotide) and patisiran (a small interfering RNA agent), were recently approved for the treatment of hATTR polyneuropathy.

- Conceição I. Novel RNA-targeted therapies for hereditary ATTR amyloidosis and their impact on the autonomic nervous system. Clin Auton Res. 2019 Sep;29(Suppl1):11-17. doi: 10.1007/s10286-019-00626-8.; J Manag Care Spec Pharm, 2019 Jan;25(1):10-15. - https://doi.org/10.18553/jmcp.2019.25.1.010; Benson MD, Waddington-Cruz M, Berk JL, et al. Inotersen treatment for patients with hereditary transthyretin amyloidosis. *New Engl J Med*. 2018;379(1):22-31.

Hereditary (familial) ATTR amyloidosis. In this form, there is a change (mutation) in the DNA that is inherited and can be passed from one generation to the next. This makes the TTR protein more unstable and more likely to form amyloid fibrils. Different mutations lead to different symptoms — some may affect the nerves; some may affect the heart; and some may affect both.

2020

Zolgensma 2 x 10Exp13 vector genomes/mL solution for infusion (Novartis)
Onasemnogene abeparvovec is a gene therapy medicinal product that expresses the human survival motor neuron (SMN) protein. It is a non-replicating recombinant adeno-associated virus serotype 9 (AAV9) based vector containing the cDNA of the human SMN gene under the control of the cytomegalovirus enhancer/chicken-β-actin-hybrid promoter.
treatment of:
- patients with 5q **spinal muscular atrophy (SMA)** with a bi-allelic mutation in the SMN1 gene and a clinical diagnosis of SMA Type 1, or
- patients with 5q SMA with a bi-allelic mutation in the SMN1 gene and up to 3 copies of the SMN2 gene.
https://www.medicines.org.uk/emc/product/11572/smpc#gref

→mRNA vaccines for Sars-CoV-2

Nature 574, S2-S3 (2019)
doi: 10.1038/d41586-019-03068-4

mRNA's NEXT CHALLENGE: WILL IT WORK AS A DRUG?

Message in a bottle

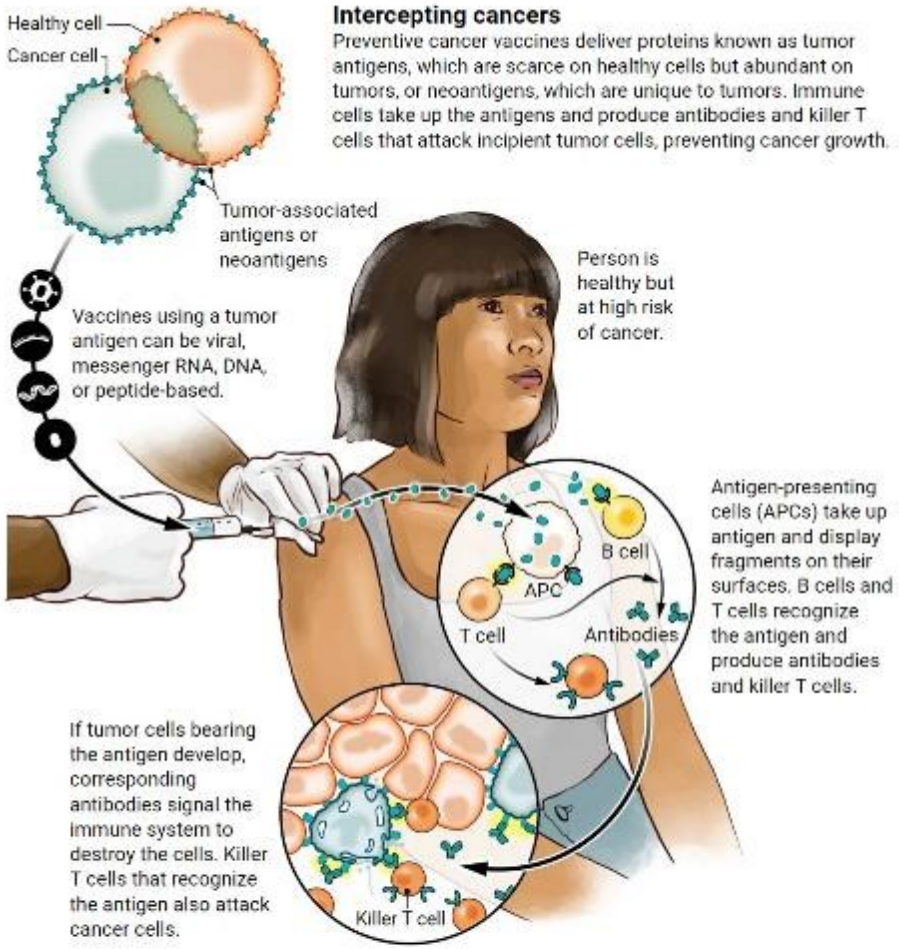
Although dozens of trials are testing messenger RNA to arm the immune system against viruses or cancer, only a few companies have launched small clinical trials of other therapies—such as mRNA to replace missing or defective proteins. Here are some.

CONDITION	PROTEIN ENCODED BY mRNA	ROUTE OF ADMINISTRATION	SPONSORING COMPANY
Cystic fibrosis	CFTR, which maintains fluid balance across membranes	Inhaled	Translate Bio
Heart failure	VEGF-A, which stimulates blood vessel growth	Epicardial injection	AstraZeneca
Ornithine transcarbamylase deficiency	OTC, which helps remove nitrogen from the body	Intravenous	Arcturus Therapeutics
Propionic acidemia	propionyl-CoA carboxylase, needed for normal metabolism	Intravenous	Moderna
Transthyretin amyloidosis	Cas9, which cuts DNA to remove a defective gene	Intravenous	Intellia Therapeutics

Science 18 Dec 2020:Vol. 370, Issue 6523, pp. 1388-1389
DOI: 10.1126/science.370.6523.1388

Cancer and Vaccines

doi: 10.1126/science.abq3411 - Science (2022)



Researchers are trying out several vaccine strategies. Some use so-called tumor antigens, molecular markers that are scarce on healthy cells but plentiful on cancer cells. The Lynch vaccine instead targets "neoantigens," a potent type of antigen only found on tumor cells. Some deploy just a single antigen whereas others use a large number, in a bid to broadly shield against cancer. The best approach is unclear, and developers also face the difficult challenge of measuring success without waiting decades for healthy people to develop cancers.

Cancer and Vaccines

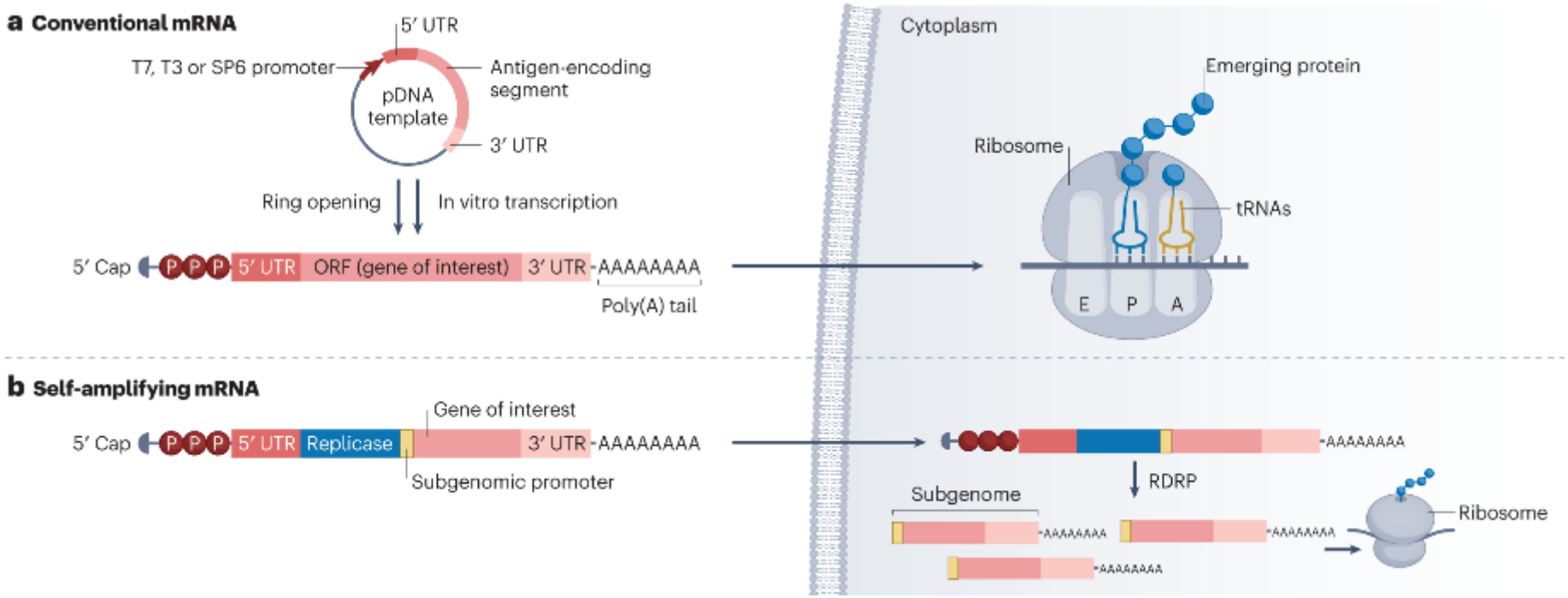
Cancer prevention vaccines on trial

Planned and in-progress clinical tests of vaccines to prevent cancer include the following:

TARGET CANCERS	Participants	Number of participants	Start date	Antigens	Vaccine type
Breast, ovarian, prostate	People with *BRCA1* or *BRCA2* mutations who have never had cancer or are in remission	44	April 2021	hTERT, PMSA, WNT1	DNA
Triple negative breast	People in remission after treatment for triple negative breast cancer	24	October 2021	Alpha-lactalbumin	Protein
Pancreatic	People with an inherited mutation or family history that puts them at high risk for pancreatic cancer	25	May 2022	KRAS	Peptide
Colon, endometrial, others	People with Lynch syndrome who have never had cancer or are in remission	45	June 2022	Suite of 209 frameshift neoantigens	Viral vector

doi: 10.1126/science.abq3411 - Science (2022)

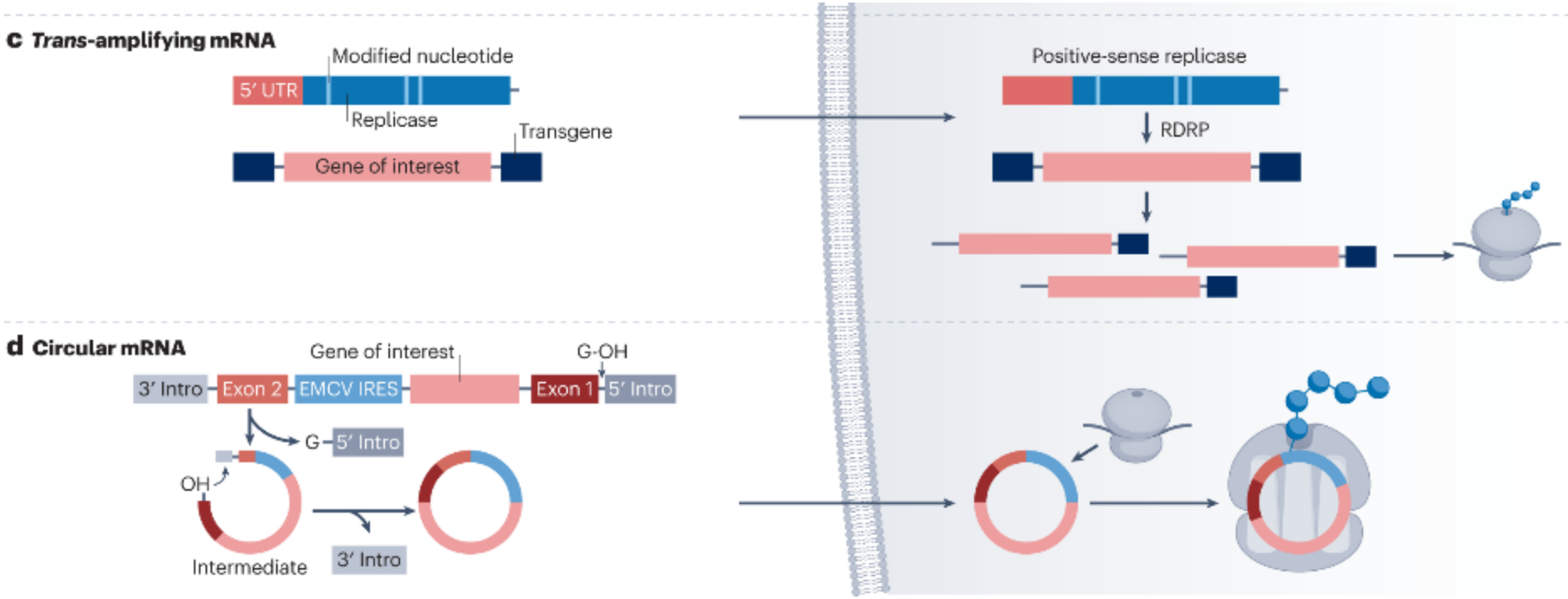
IN VITRO TRANSCRIBED mRNA FOR CANCER THERAPEUTICS.



Liu, C., Shi, Q., Huang, X. *et al.* mRNA-based cancer therapeutics.
Nat Rev Cancer **23**, 526–543 (2023). https://doi.org/10.1038/s41568-023-00586-2

IN VITRO TRANSCRIBED mRNA FOR CANCER THERAPEUTICS.

Replicase produces RNA-dependent RNA polymerase (RDRP) and mediates the replication of protein-encoding RNA



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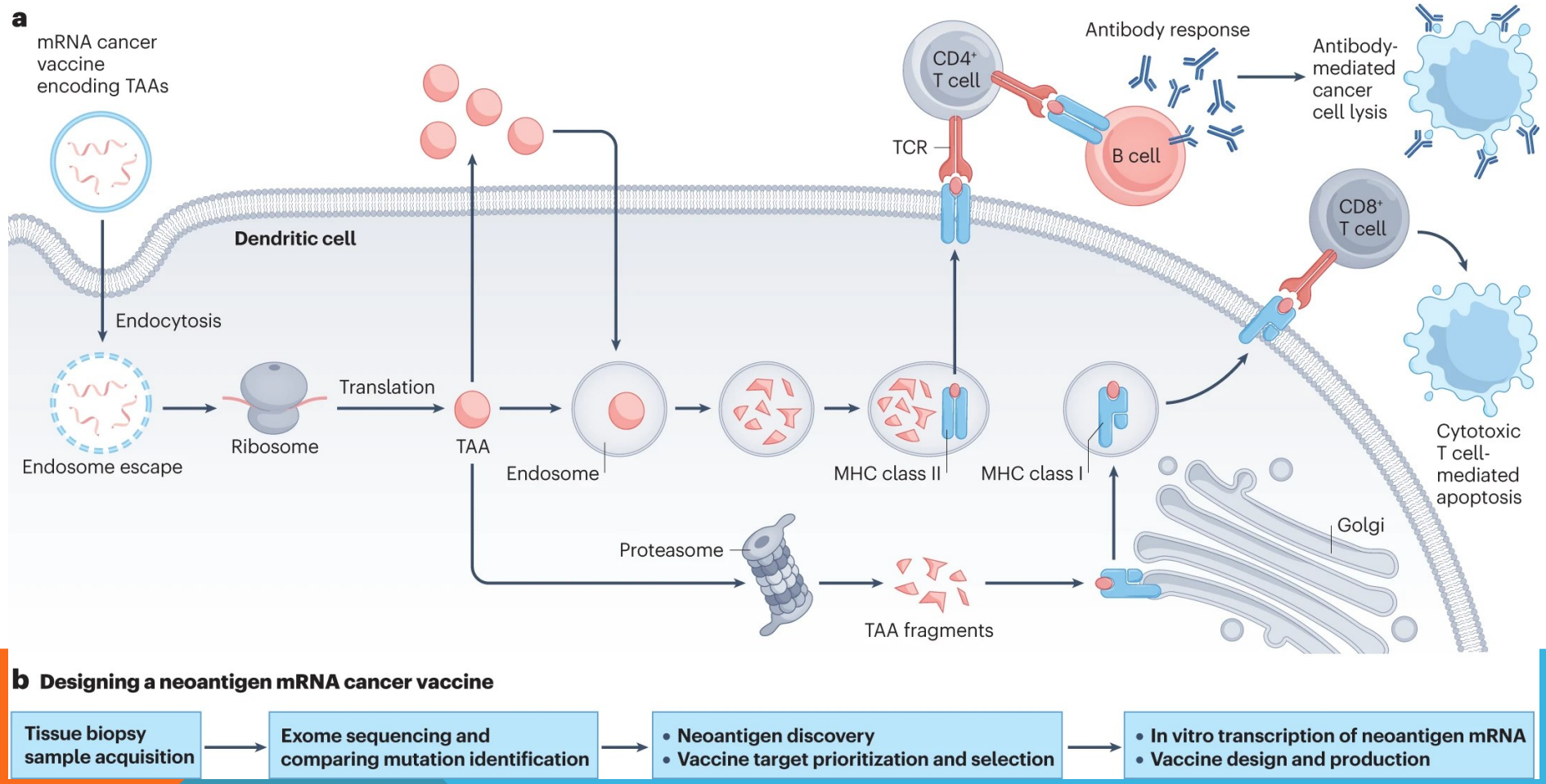
THE DEVELOPMENT OF mRNA CANCER VACCINE ENCODING TUMOUR-ASSOCIATED ANTIGENS OR NEOANTIGENS.



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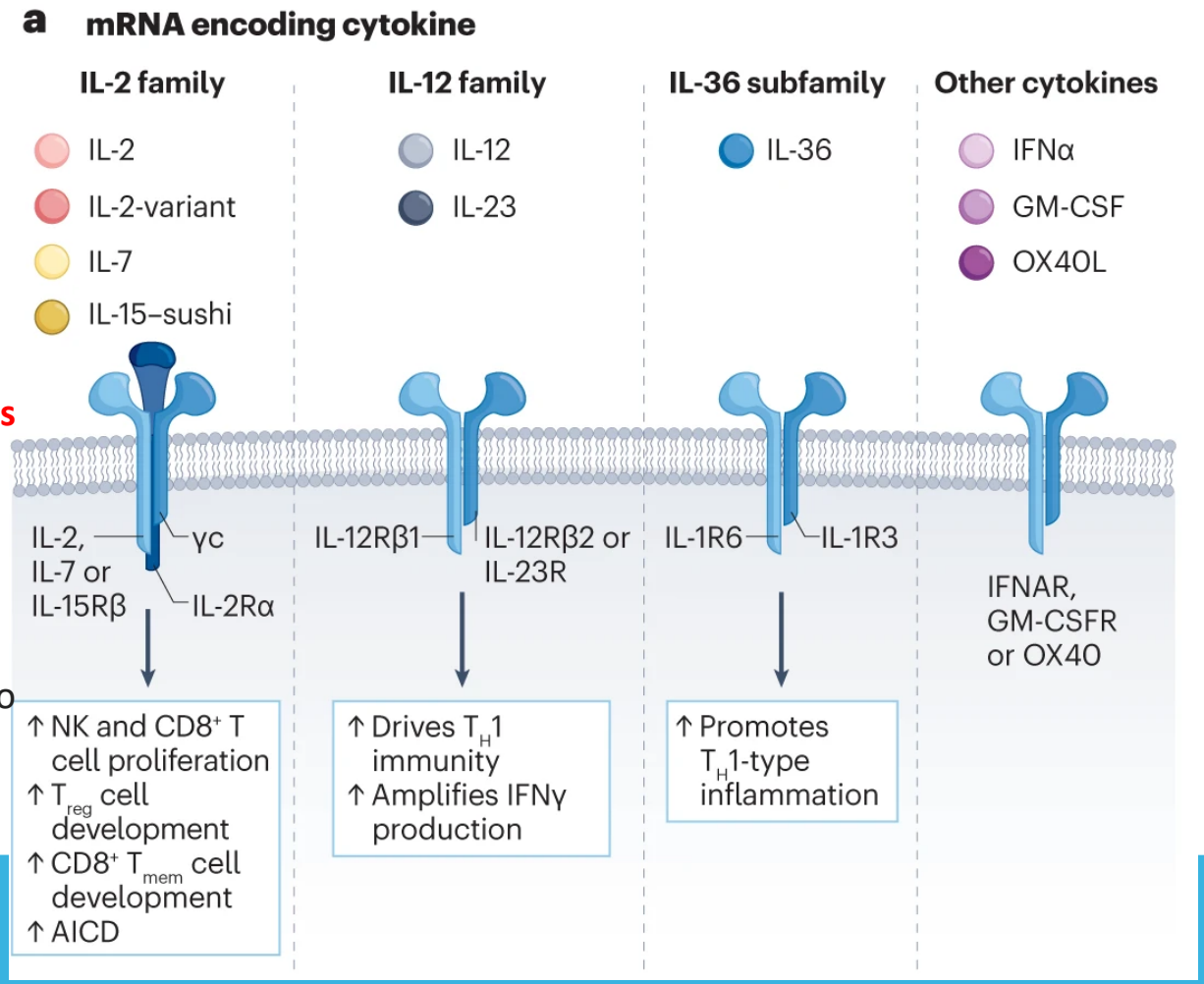
mRNA ENCODING CYTOKINES AND TUMOUR SUPPRESSORS FOR CANCER THERAPY.

Gene therapy can enable the direct expression of cytokines, especially interleukins, in tumour tissue.

mRNA encoding these cytokines leads to cytokine expression within the tumour microenvironment. These cytokines can be expressed on the cancer cell membrane surface or released into the cellular microenvironment, where they bind to specific receptors on the surface of T cells to **activate antitumour immune responses**



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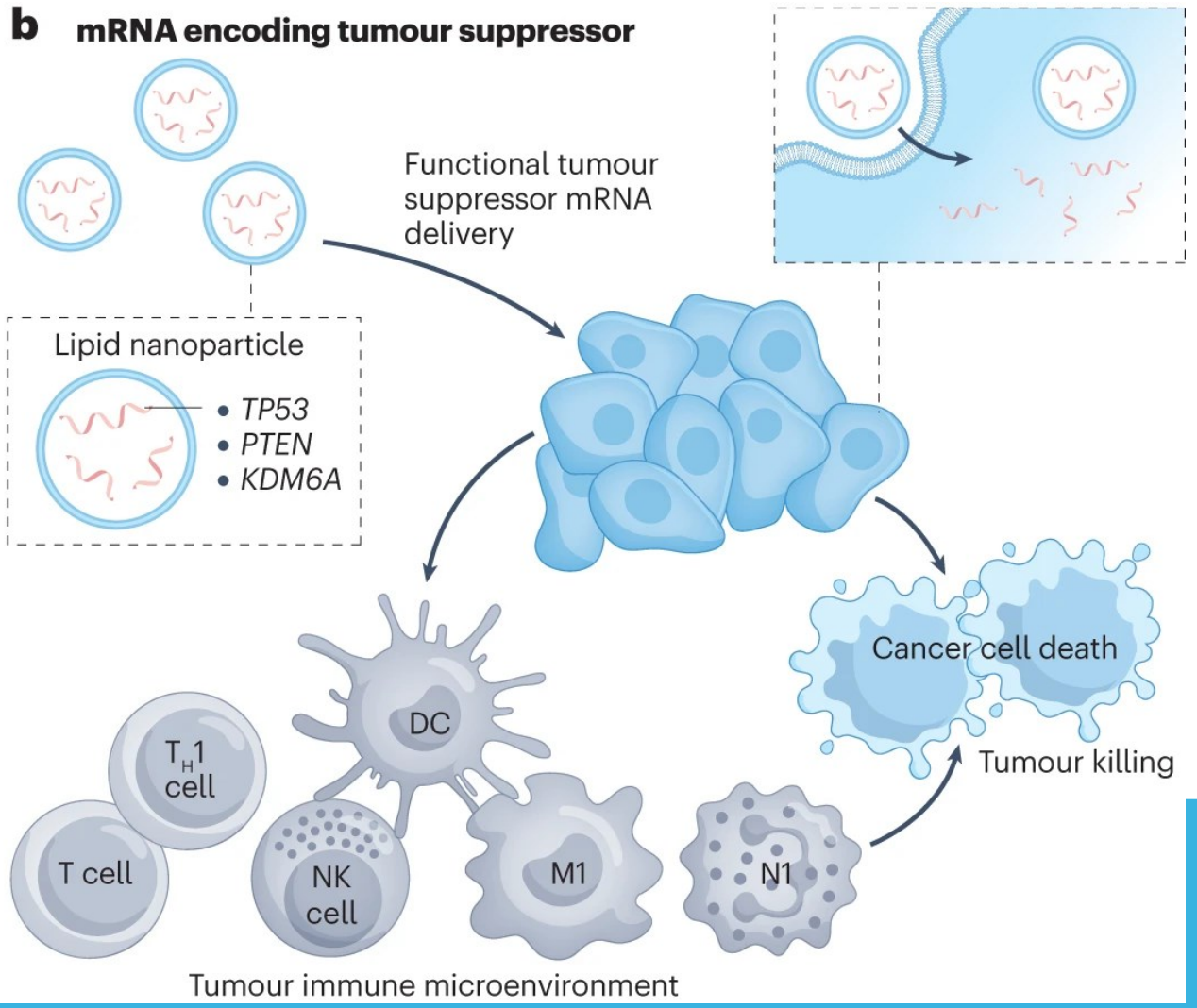
mRNA ENCODING CYTOKINES AND TUMOUR SUPPRESSORS FOR CANCER THERAPY.

mRNA encoding tumour suppressors within lipid nanoparticles for cancer therapy. After transfection of tumour cells with mRNA encoding a tumour suppressor, the **sensitivity of tumour cells to apoptosis is restored**



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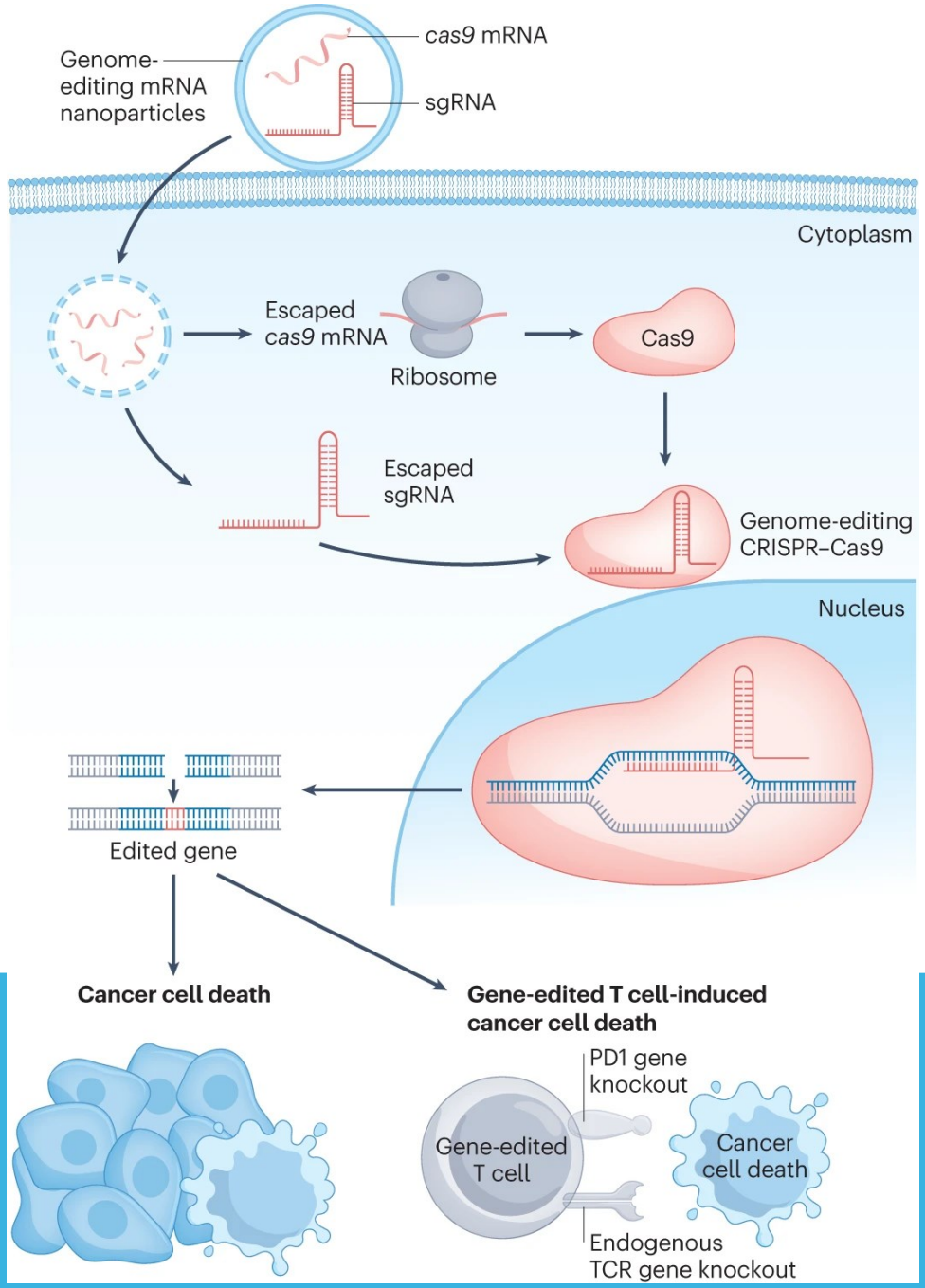
mRNA ENCODING CAS9 MEDIATED GENOME EDITING FOR CANCER IMMUNOTHERAPY

CRISPR-associated protein 9 (Cas9) protein, which then complexes with the escaped sgRNA from the nanoparticles to form ribonucleoproteins with an affinity for specific DNA sequences.

knockout of the gene encoding programmed cell death 1 (PD1) or endogenous T cell receptor (TCR) to induce apoptosis in tumour cells.



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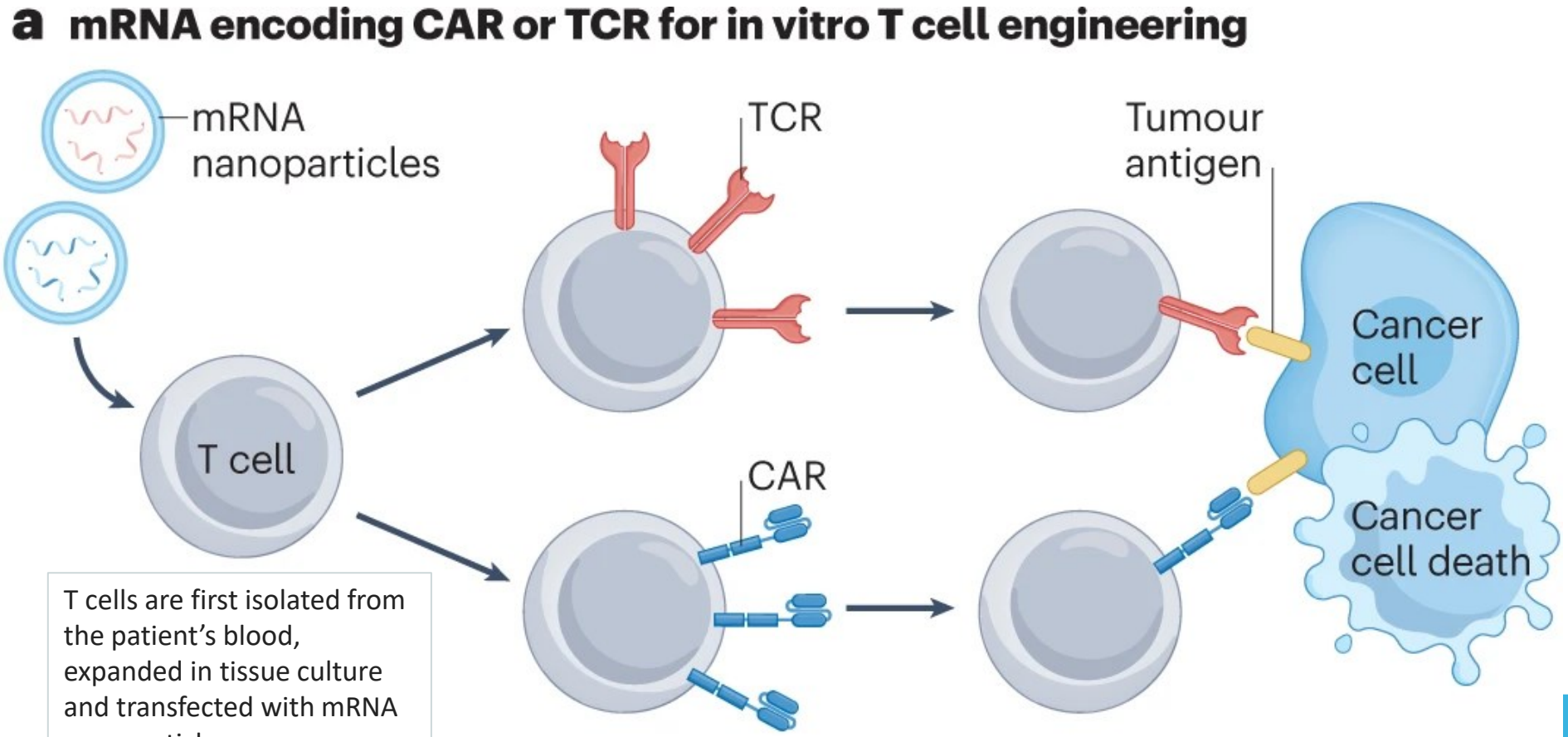
IN VITRO AND IN VIVO DELIVERY OF mRNA ENCODING CAR AND TCR FOR T CELL ENGINEERING.



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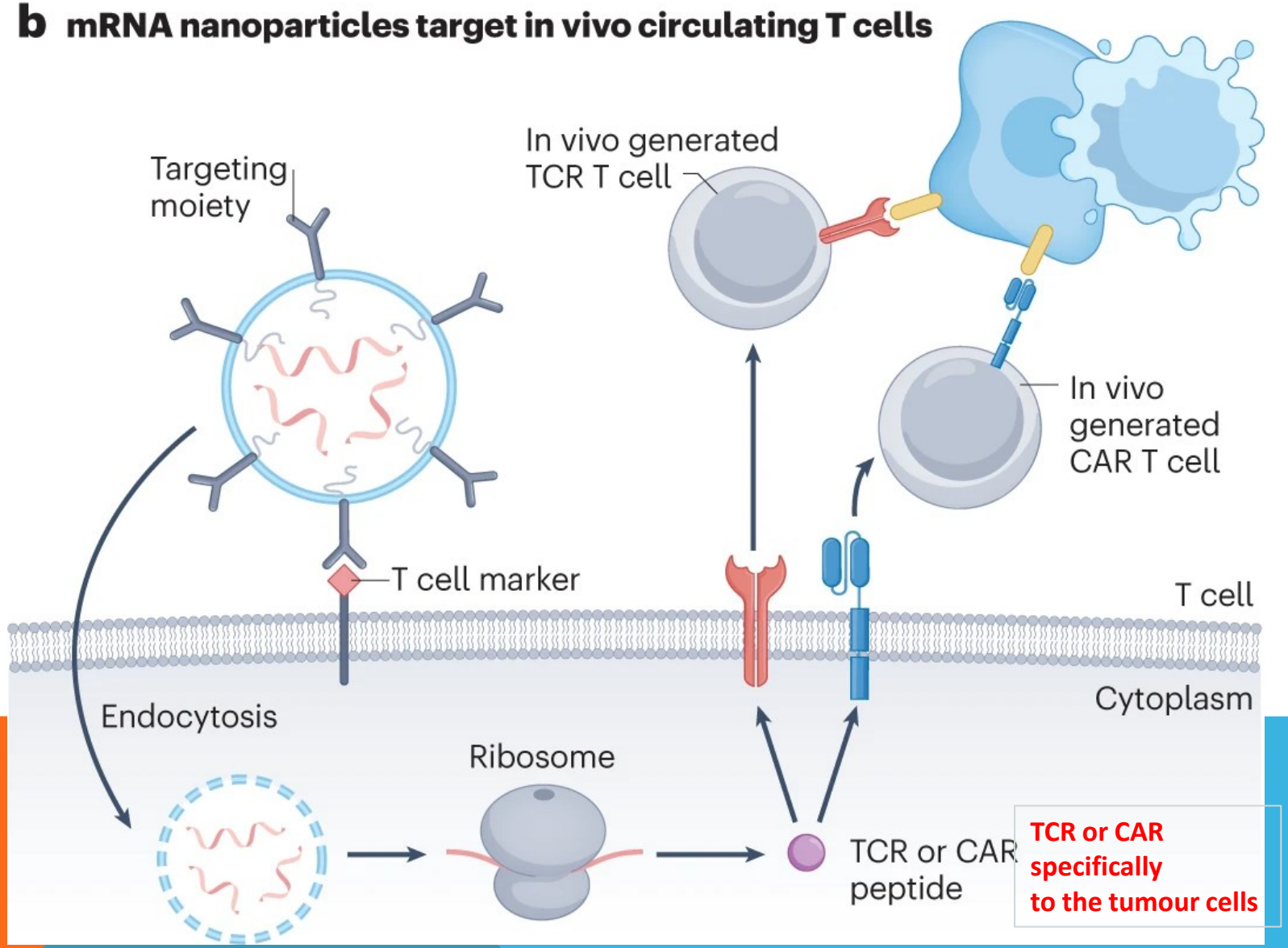

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Technological advancements expected to unlock more promises of mRNA-based cancer therapeutics soon.

- **The selection of suitable and effective tumour antigens is a key step in the design and development of mRNA cancer vaccines**, and therefore more efforts should be devoted to identifying new tumour antigens. It will be important to optimize the screening and identification steps necessary for tumour antigens, especially neoantigens, to help in the development of systematic methods for inexpensive and rapid preparation of new tumour antigen-based vaccines. This will require further development of high-throughput deep gene sequencing technologies in combination with advanced data analysis
- The targeted delivery nanoplatforms could be greatly expanded to **improve the delivery efficiency of mRNA to desired organs and tissues** (for example, spleen, brain, lung, lymph node, kidney) instead of the sites where particles are typically trapped (for example, liver and other organs)

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- Candidates in mRNA design should continue to be investigated, for example, **self-amplifying mRNA (saRNA), trans-amplifying mRNA (taRNA) and circular mRNA (circRNA),** which can increase expression time and efficacy even with a minimal dose, to avoid repeated administration of mRNA nanoparticles.
- For mRNA cancer therapeutics, saRNAs, taRNAs and circRNA might be more appropriate for applications that require the long-term expression of target proteins.
- The administration of **mRNA encoding CAR or TCR in T cell-targeting nanoparticles could enable in vivo transfection of circulating T cells**.
  → For instance, this strategy has shown efficacy to cause tumour regression in mouse models of human leukaemia, prostate cancer and hepatitis B virus-induced hepatocellular carcinoma.

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Technological advancements expected to unlock more promises of mRNA-based cancer therapeutics soon.

- Additionally, in vitro **CRISPR–Cas9 gene-edited T cells (PD1 and TCR-encoding genes simultaneous knockout)** from patients with advanced and refractory tumours have shown safety and feasibility in the phase I clinical trial. Therefore, the use of T cell-targeted mRNA nanoparticles encoding genome-editing protein for the in vivo production of gene-edited T cells could be highly attractive for cancer treatment.

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