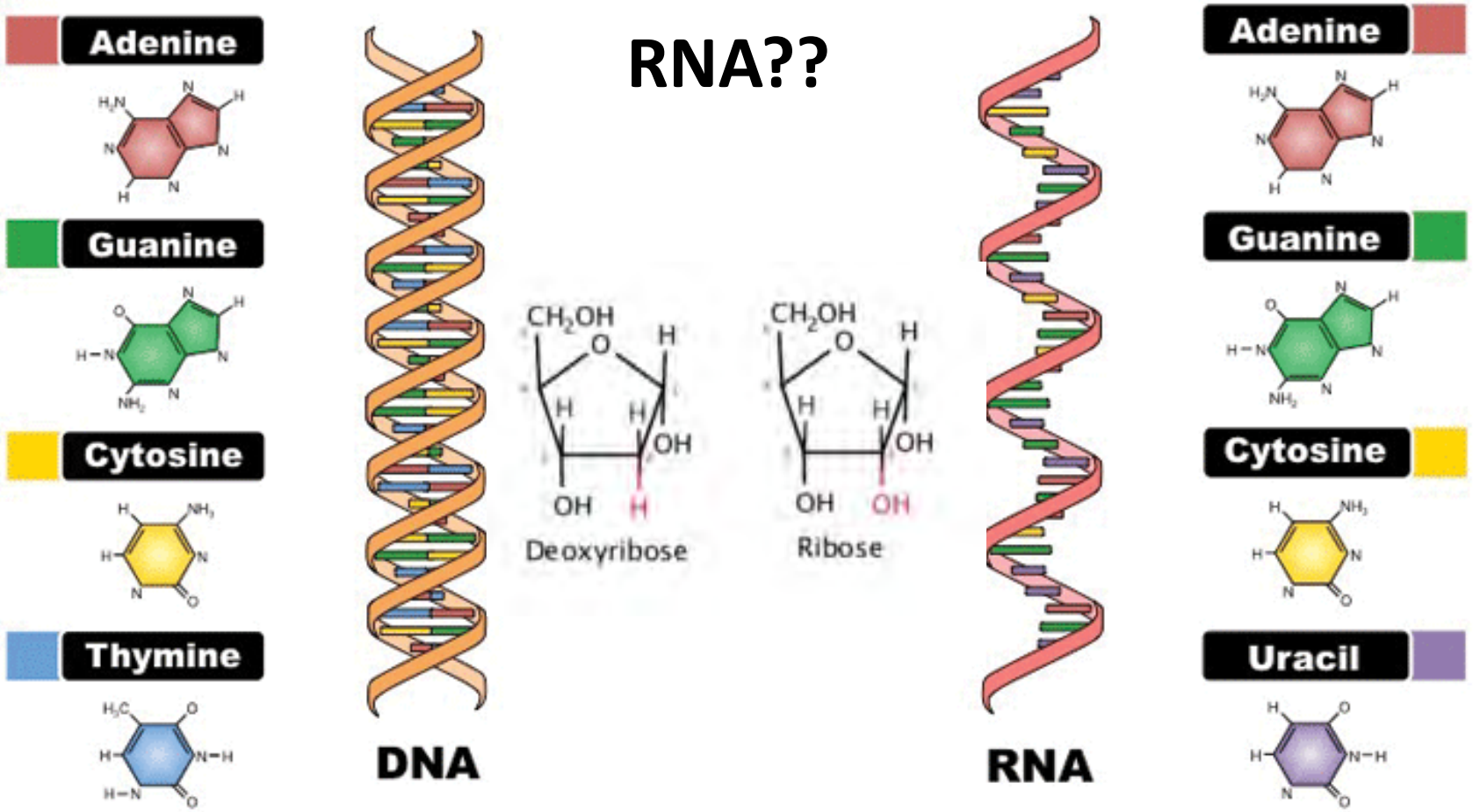
mRNA vaccines

The science and the difficulties in the creation of a miracle.

Here's what we'll cover:

- What is mRNA (messenger RNA)?
- How does the immune system work to attack viruses?
- How does mRNA work to stimulate the immune system?
- Origins of mRNA technology.
- What problems had to be overcome in the development of an mRNA vaccine for SARS-CoV-2?
  - Technical issues.
  - Practical issues.
- Different types of mRNA vaccines.
- Other COVID vaccines.
- Future uses of mRNA vaccines.

To begin, What *is* RNA??

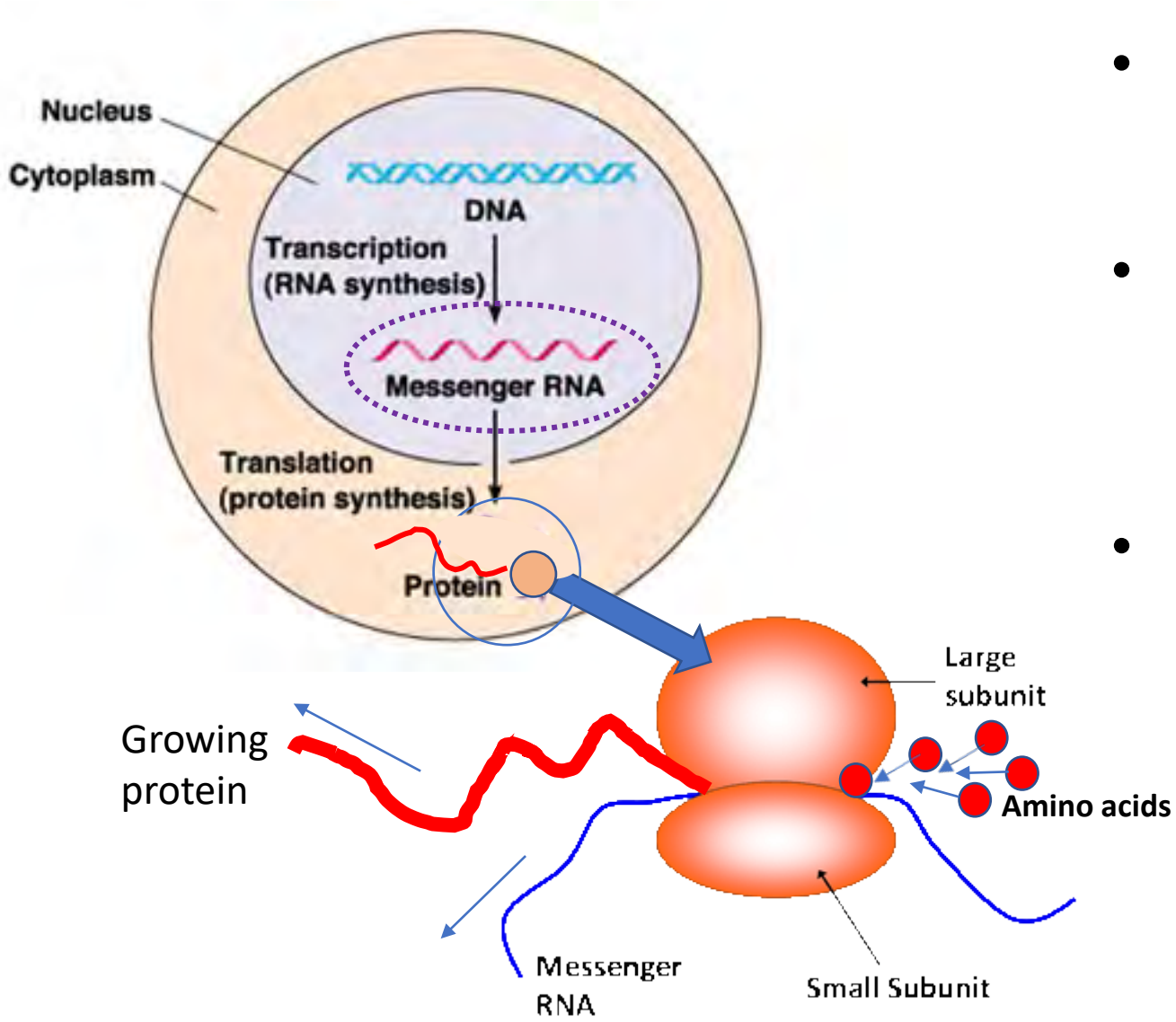


DNA is a double-stranded helix made up of two sugar-based (**d**eoxy**r**ibose) bands bridged by pairs of **n**ucleotide bases. It is very stable, and is used to contain genetic information in the nucleus of the cell

RNA is a single-stranded sugar-based strand (**r**ibose). It is less stable and shorter-lived than DNA and has a variety of purposes in the cell. It uses the same bases (**n**ucleotides) as DNA except that it uses Uracil instead of Thymine.

Cells use 3 kinds of RNA: messenger (mRNA), transfer (tRNA) and ribosomal (rRNA). Each plays a different role in protein synthesis.

Ok, then what is *mRNA*?

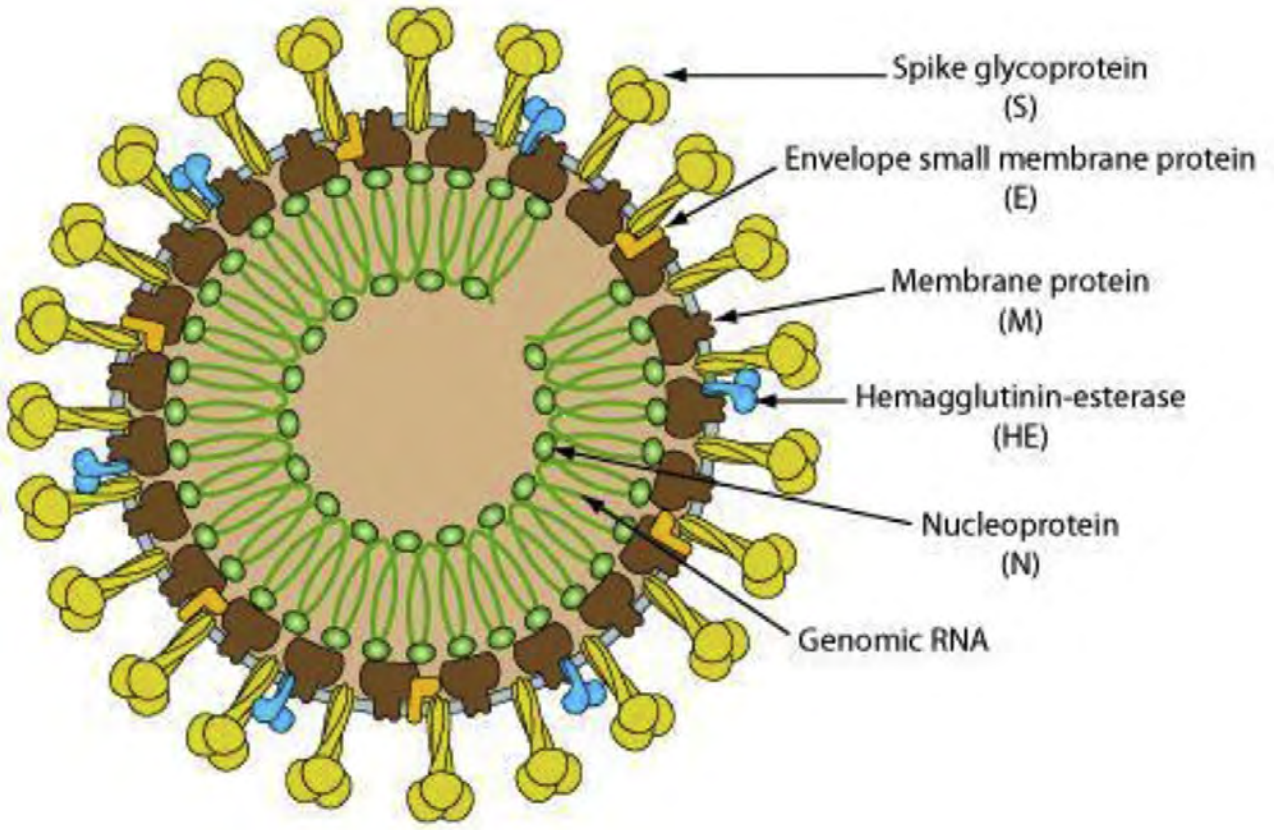


- Normal cells have machinery for making new materials (e.g., proteins) in order to replace worn out materials or to grow or to reproduce.
- The blueprints for proteins are found encoded in **genes**, which are sections of DNA, kept protected in the nucleus.
- The information contained in a gene is transcribed in the nucleus onto an RNA copy of the gene, called **messenger RNA** or mRNA and transported out into the cell.
- Out in the cytoplasm, the messenger RNA attaches to a **ribosome**, providing the ribosome with the information it needs to produce the desired protein.

RNA viruses (like coronaviruses) bring their own messenger RNA into the cell and thus use the cell's machinery to produce their own proteins.

Anatomy of a killer: SARS-CoV-2

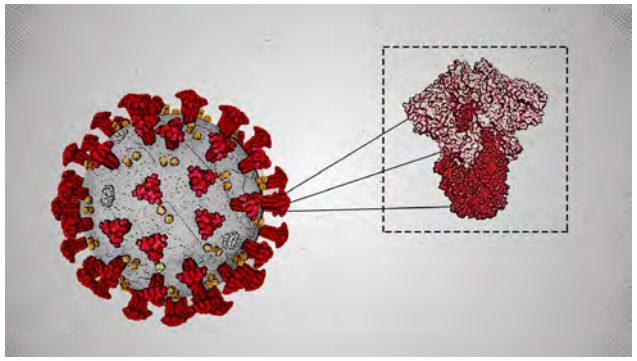
Severe Acute Respiratory Syndrome Coronavirus 2

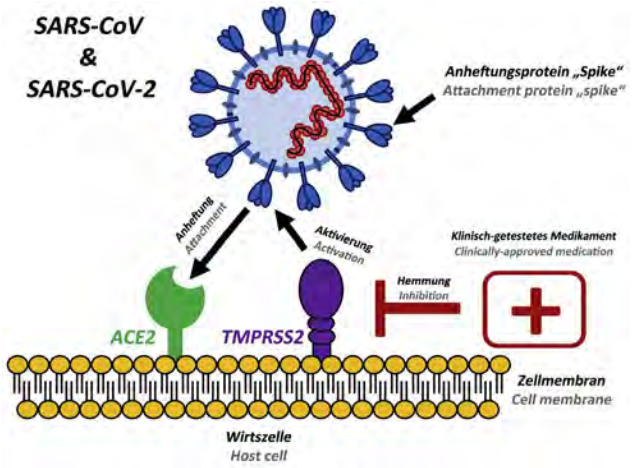


Genomic RNA:

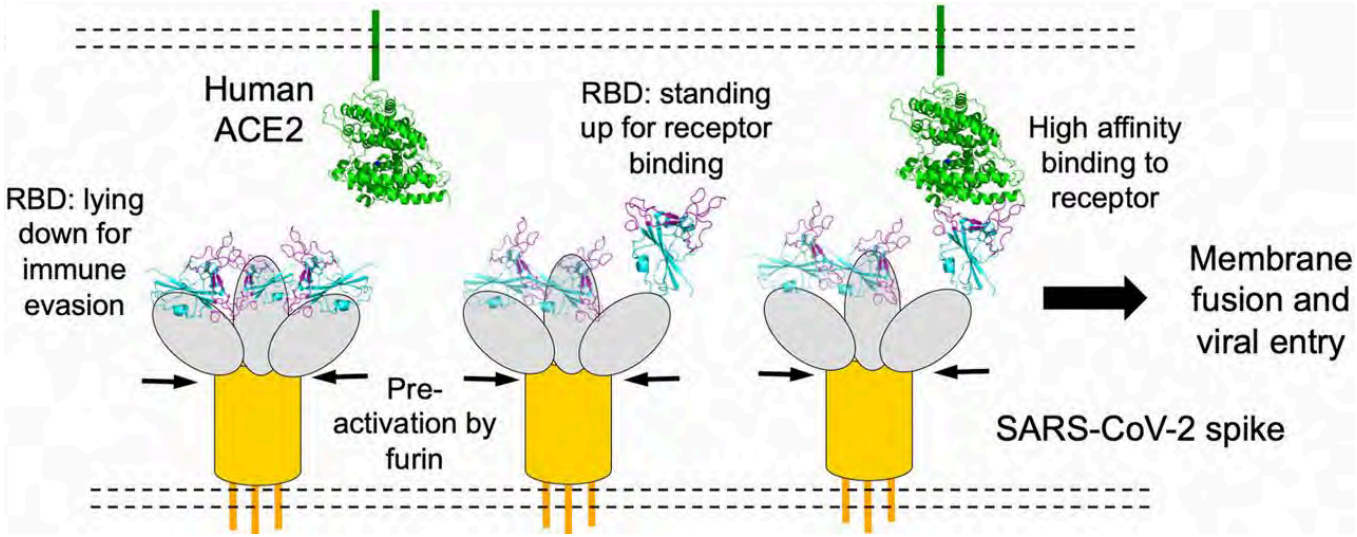
- Single-stranded positive sense RNA
  - Largest genome of all RNA viruses (26-32 kb vs 13kb for Influenza A (the 1918 Spanish Flu virus)
  - Has code for six viral proteins:
    - S (Spike)
    - E (Envelope)
    - M (Membrane)
    - N (Nucleoprotein or Nucleocapsid)
    - HE (Hemagglutin-esterase)
    - 7a
  - These proteins have multiple roles, including interacting with the host cell proteins to advance the virus' reproduction

The spike protein





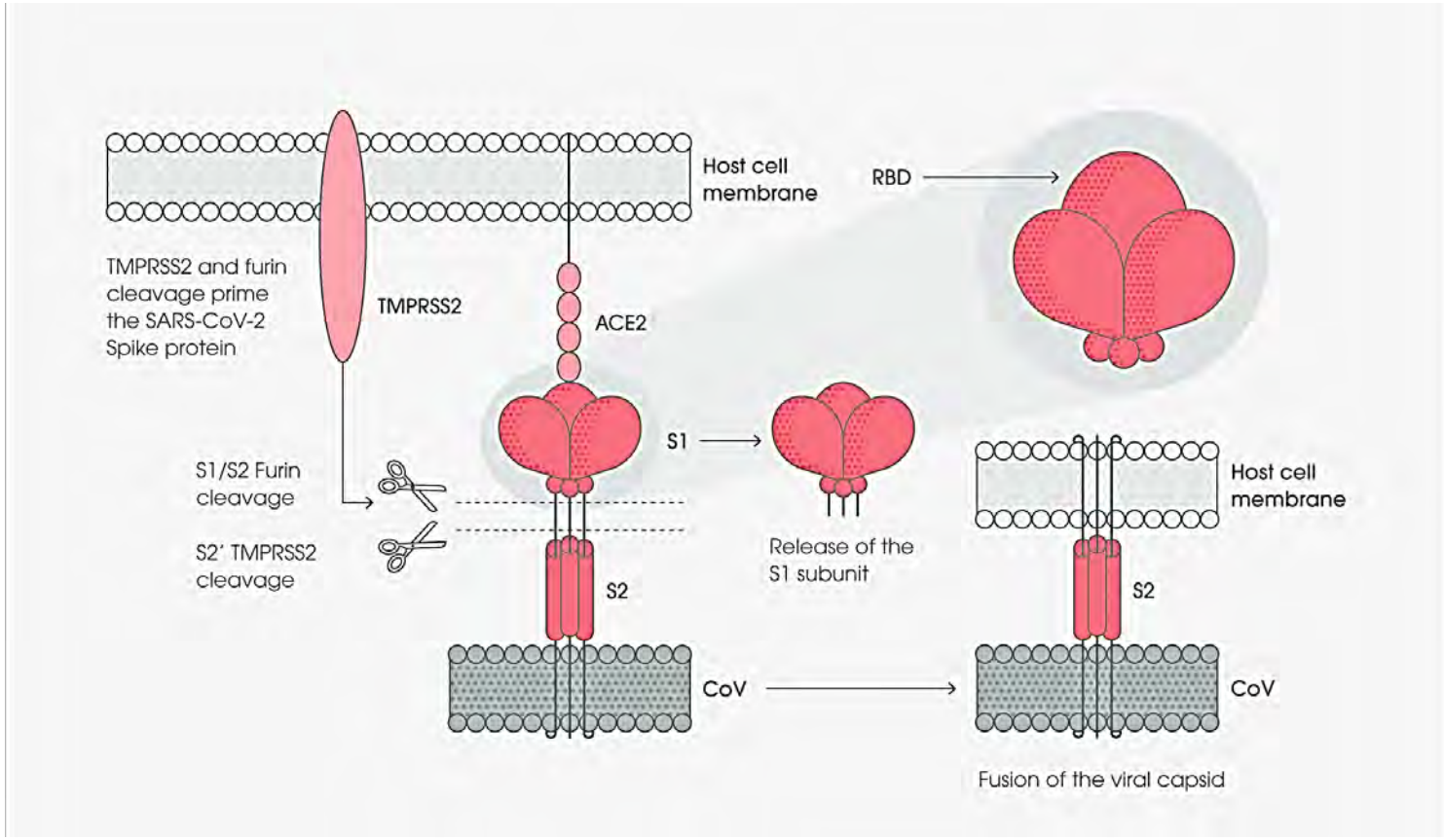
- S (Spike): the S protein contains the RBD (receptor binding domain) that attaches to the hACE2 (human Angiotensin Cleavage enzyme) found on the surface of many cells in the body. hACE2 is present in arterial and venous endothelial cells and arterial smooth muscle cells in all organs studied, and abundantly present in humans in the epithelia of the lung and small intestine.



The RBD is the most immunoreactive site on the spike protein. SARS-CoV2 protects that site from the immune system by keeping it hidden until the spike encounters a cell membrane which expresses proteases such as furin or TMPRSS2*, which cleaves a site near the RBD and allows it to stand up and bind the hACE2 receptor.

*Transmembrane serine protease 2 (TMPRSS2) is a cell surface protein primarily expressed by endothelial cells across the respiratory and digestive tracts.

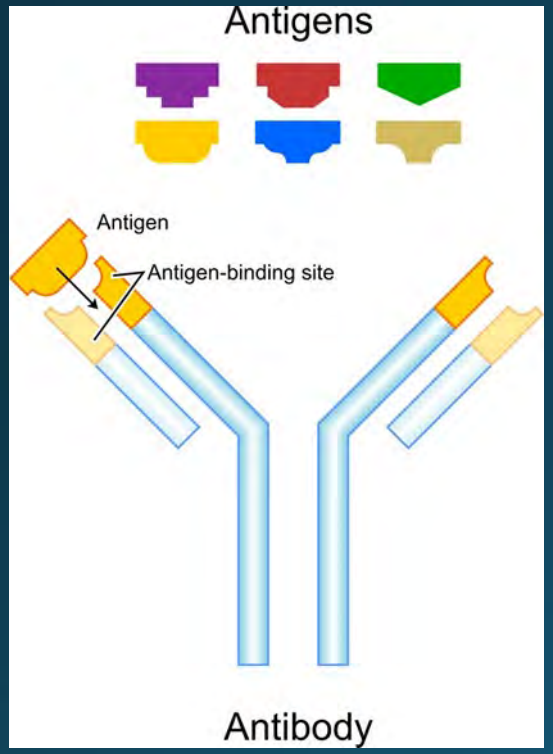
The initial attack: binding



- SARS-CoV-2 utilizes the spike glycoprotein S to promote entry into the host cell. S has two functional domains
  - S1 receptor-binding domain (RBD)
  - S2 mediates the fusion of the viral and host cell membranes.

- The S protein binds to the ACE2 receptor on the host cell through the S1 receptor binding domain. The S1 domain is then shed from the viral surface, allowing the S2 domain to fuse to the host cell membrane.
- This process is dependent upon activation of the S protein, by cleavage at two sites by cell surface proteolytic enzymes.

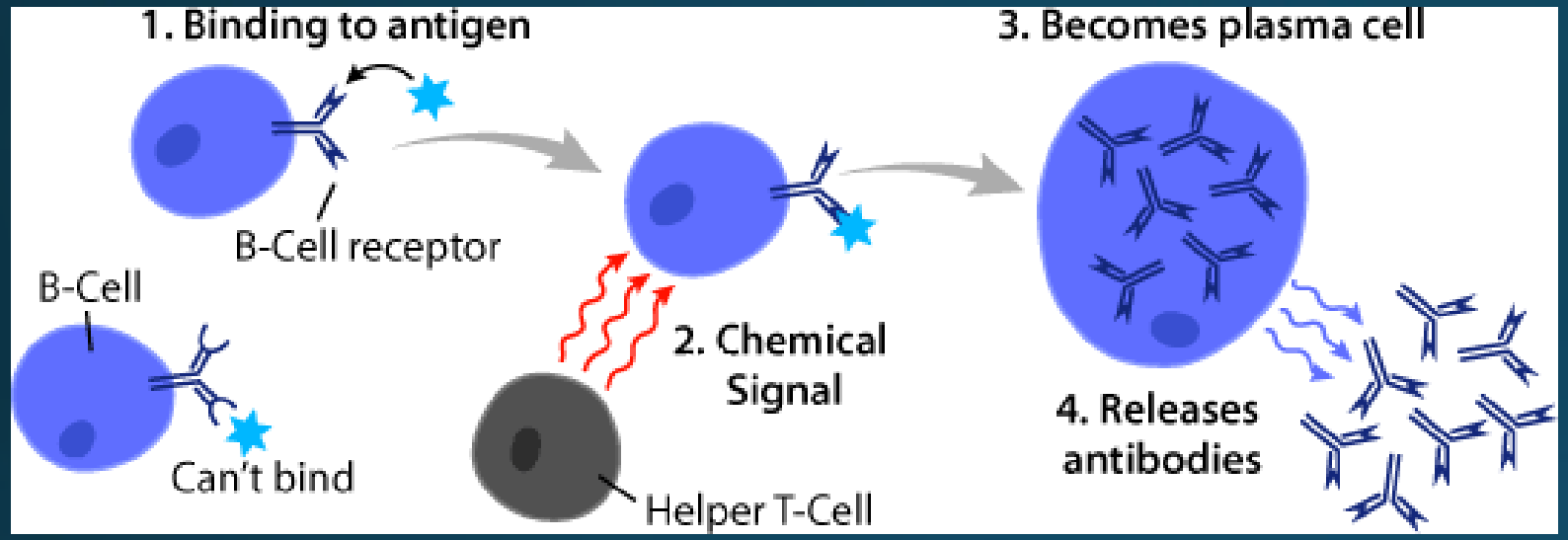
First line of defence: Antibodies

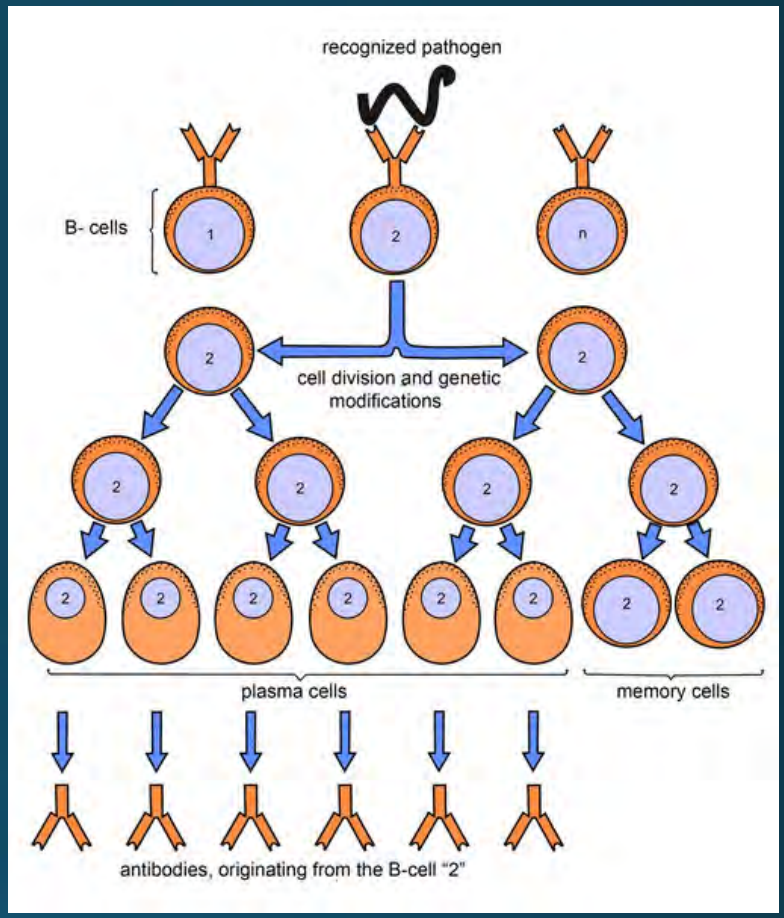


- Antibodies are innate proteins (either free in serum or on the surface of B-cells) that attach to a specific antigen .
  - Antibodies consist of two heavy and two light chains .
  - The heavy chain determines the action of the antibody.
  - Humans have five types ("isotypes") of heavy chains: IgA, IgD, IgE, IgG and IgM. IgM is the primary defense against invading pathogens. IgE is involved in allergic reactions.
  - Both heavy and light chains each have a constant and a variable region. The constant region in the heavy chain determines the isotype. The variable regions in both chains determine the antigen specificity.
- If the antigen is free in the serum IgG antibodies inactivate it by agglutination and mark it for later elimination.
- If the antigen is found on a cell surface such as a bacterium, they mark the cell for attack by macrophages or killer T-cells.

Both SARS-CoV-2 IgM and IgG antibodies may be detected around the same time after infection. However, while IgM is most useful for determining recent infection, it usually becomes undetectable weeks to months following infection; in contrast, IgG is usually detectable for longer periods. (CDC guidelines)

The Immune System Responds!





The process begins when a B-cell binds to an antigen. The binding site is a membrane-bound immunoglobulin molecule attached to a cell-activating mechanism. The antigen, a protein, may be on the surface of a bacterium or virus, may be free-floating, or may be expressed on the surface of a phagocyte or infected cell.

Once the B-cell is activated (a process involving Helper T-cells), it produces antibodies specific to the antigen (thousands a second!). The B-cell divides and gives rise to more antigen-producing daughters ("plasma cells") and to long-lived "memory cells" ready to fight the next assault by the same antigen, even years later. This latter is what vaccines work on.

Traditional Vaccines

The earliest vaccine (smallpox) worked by introducing a related but less harmful virus (cowpox) into the body. The immune system makes antibodies to a variety of portions of the virus' proteins. It works if one such portion is the same in the more lethal virus.

Later vaccines used a weakened form of the virus (or bacteria) to introduce the immune system to its proteins.

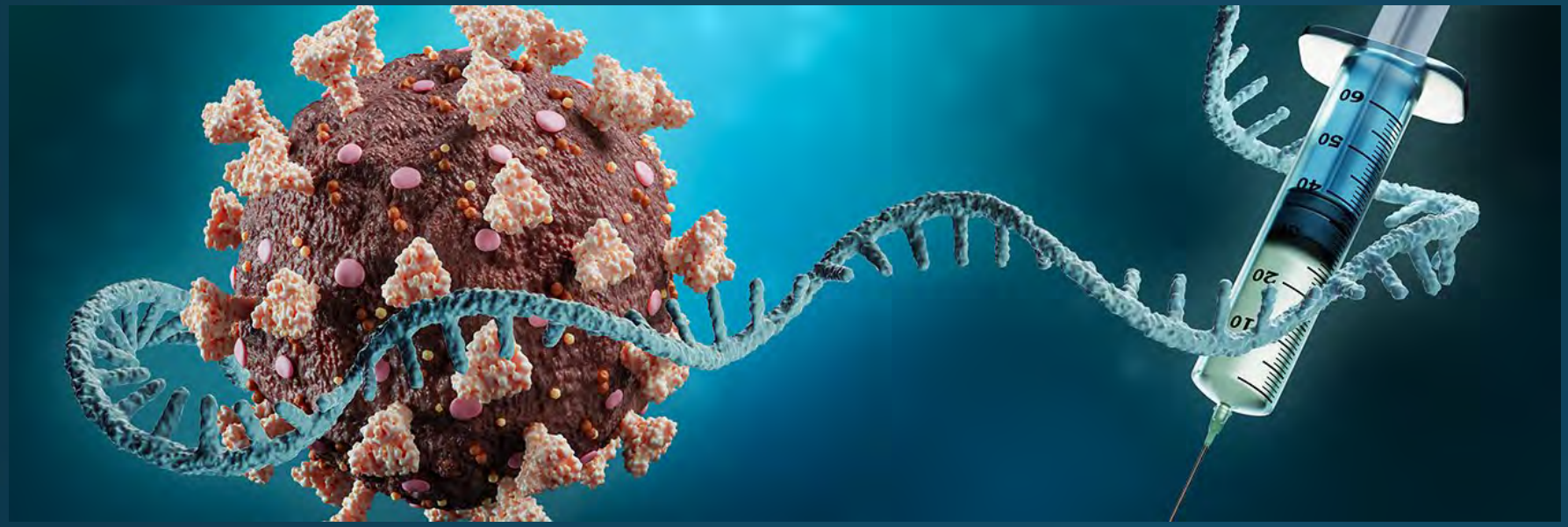
Most modern (traditional) vaccines inject only a single or a few viral or bacterial proteins to train the immune system to recognize the actual virus.

Early Glimmerings of mRNA vaccines

- 1984 Gayle Smith inserted the DNA coding for interferon into insect cells using an insect virus, causing the cells to produce interferon for use as a drug.
- Later he used the same insect cell system with the AIDS virus gp160 envelope protein gene to make a vaccine for HIV. He founded a company, MicroGeneSys. For lack of funding for trials, the company folded. MicroGeneSys was resurrected as Protein Sciences which used Smith's insect-cell system to produce a flu vaccine. Protein Sciences was bought by the French drug giant Sanofi ($650 million).
- Smith left after a few years and founded a new company, Novavax in 1987.
- Novavax uses Smith's insect-cell system. Prior to 2020, company scientists developed experimental vaccines for Ebola, influenza, respiratory syncytial virus (RSV), and other emerging infectious diseases.
- During 2020, the company redirected its efforts to focus on development and approval of its NVX-CoV2373 vaccine for COVID-19 which as of January 2022 is authorized for emergency use by the WHO and in the U.K, Australia, South Korea and India.

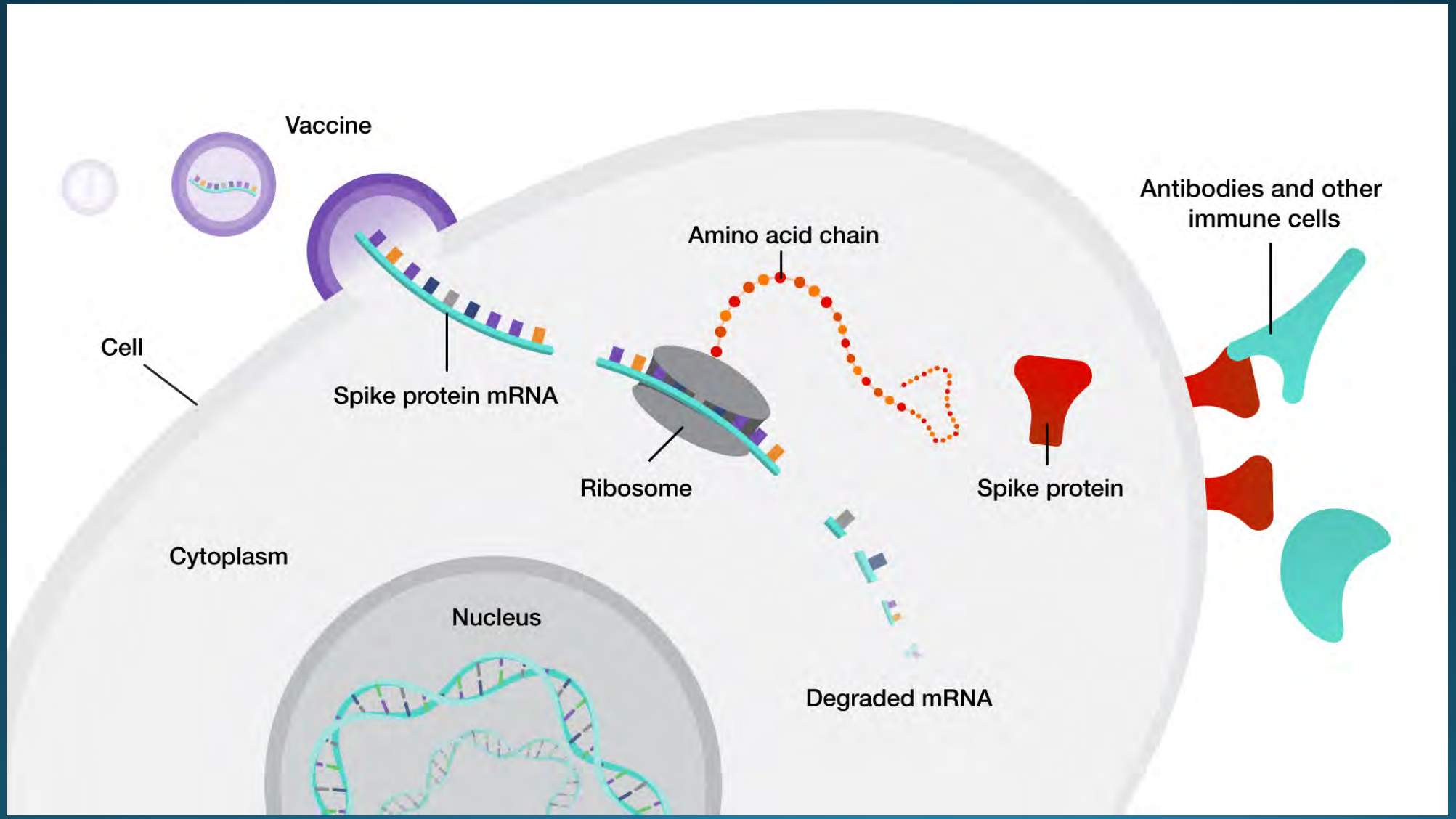
Early Glimmerings

- In 1998 Merck was working toward an HIV vaccine. They decided on the strategy of inserting an HIV gene in people's own cells and having them manufacture that protein in their own bodies, which the immune system would recognize.
- They used an adenovirus to carry the gene into cells.
  - The adenovirus was modified so that it would infect cells and insert the desired DNA but not be able to replicate.
- They ran clinical trials in 2004. It was a disaster. The adenovirus they were using turned out to be a very common one to which most people had already been exposed to and hence to which they were already immune.
- This method is today the basis of the Johnson and Johnson vaccine for covid.



Enter the mRNA vaccines

How do mRNA vaccines work?



Sounds simple enough. What could possibly go wrong?

Some Corporate and Financial Problems

- To make a vaccine and have it approved by various health agencies you need to have proof that it is not harmful and yet is effective.
- This requires several kinds of clinical trials:
  - Phase 1: small-scale, to show that the vaccine is safe.
  - Phase 2: moderate size to produce data indicating that the vaccine is effective.
  - Phase 3: large-scale trials of thousands of patients to verify that there are no low-probability side effects and to quantify the protective value of the vaccine.
- All this requires huge amounts of money for the manufacture of the vaccine and to administer the trials. Usually only large corporations can afford the gamble, and they tend to be quite conservative. Small, innovative companies must scramble for financing, if they can get it.

Some Scientific problems

- mRNA is an unstable molecule. How will you make a stable vaccine?
  - In addition, there are RNAses all over the place, including skin and breath. Glassware must be heated to 500°C. Facilities handling RNA must be super-sterile.
- mRNA ***itself*** provokes a strong cellular immune response.
  - Cells 'think' its an RNA virus and shut down all RNA processes in the cell; with prolonged exposure affected cells self-destruct.
- How will you get it into the cell?
- How long should it remain active in the cell?
  - You want it to last long enough to produce a reasonable supply of target proteins and then be destroyed by the cell.
- Which protein of the many on the surface of the SARS-CoV-2 virus is most effective in provoking the immune system ("immunogenic")?
  - The chosen protein must not itself be harmful.
  - Should you use the whole protein or just a part? Which part?
- The spike protein of SARS-CoV-2 changes shape as soon as it reaches a cell.
  - What form of the spike protein are you going to code for with your mRNA?
  - How will you make your spike protein stable enough for the desired immune response?

Early Glimmerings

- In 2006 Shinya Yamanaka was able to use retroviruses to transform adult cells into “stem”, or pluripotent cells*.
  - Retrovirus genomes are RNA rather than DNA based. After they enter the cell their RNA makes a DNA copy which is incorporated into the host’s DNA causing the cell to make more virus proteins.
- In 2007, Derrick Rossi and Luigi Warren of Harvard’s Stem Cell and Regenerative Biology Department extended Yamanaka’s work using mRNA rather than retroviruses to reprogram cells.
- In 2010, Rossi and Timothy A. Springer solicited investment from a variety of venture capitalists and founded "Moderna Therapeutics“ (from "modified" and "RNA" that luckily happens to contain "modern“).

* Nobel Prize, 2007

Early Glimmerings

- Beginning in 1998, Drew Weissman at U. Penn had been trying to use naked mRNA as a vaccine rather than using a modified virus to introduce DNA into the cells. He had been unsuccessful because when he injected the mRNA in animals it engendered a severe cellular immune response.
  - (Retroviruses use RNA as their genome, so cells have developed defences against it.)
- In 2005 Weissman encountered Katalin Karikó while at the office copy machine. Kariko who had been working with artificial RNA had recently lost her grant.
- Weissman offered her a position.
- Together they found that altering one of the bases in the mRNA allowed it to evade the cell's immune system.

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Early Glimmerings

- Weissman and Karikó published their results in 2005. It was ignored by the scientific community.
- Weissman and Karikó on their own secured patents on their work and in 2006 launched a company called RNARx that focused on developing mRNA therapeutics for a wide range of diseases. But eventually funding ran out and the company shut down.
- Five years after they published their groundbreaking findings, their discovery caught the attention of two biotech newcomers, Moderna of Cambridge, Mass., and Germany's BioNTech. Both companies eventually licensed Weissman and Karikó's patents.

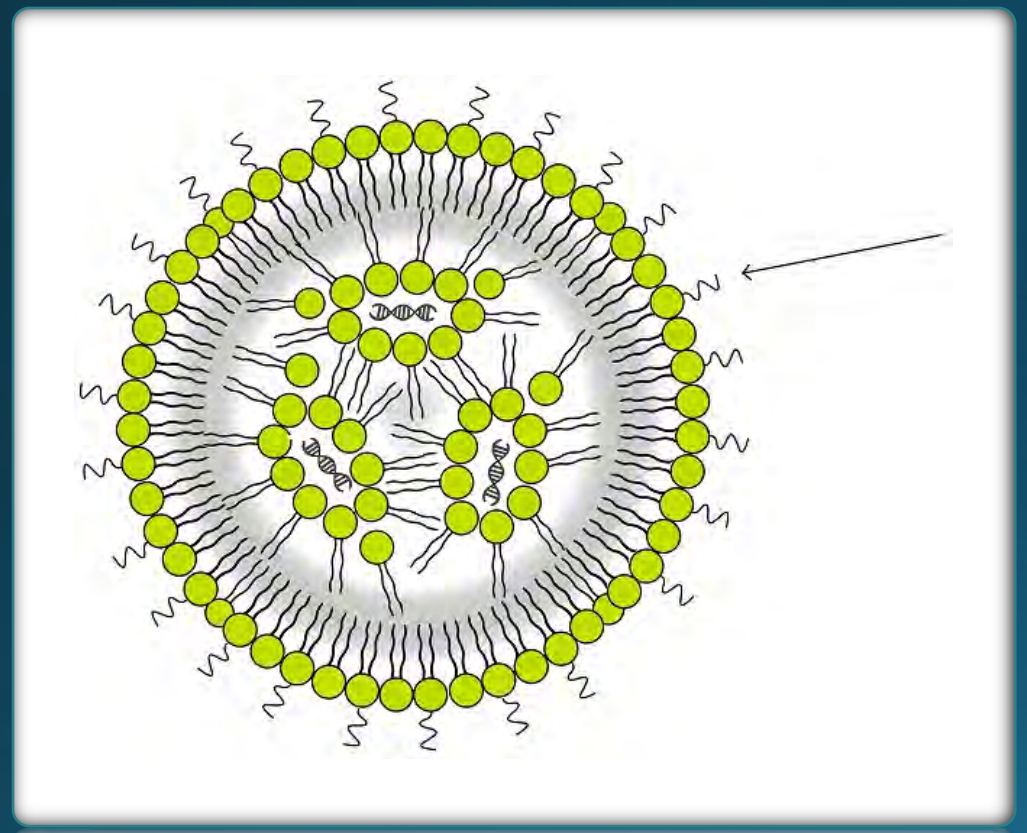
Another Problem: coating the RNA

- Weissman and Karikó found that naked RNA, even the modified RNA, could not be directly injected; it was destroyed before it could reach the cells.
- What was needed was an envelope to protect the RNA until it was absorbed into the cell.
- They hit upon the idea of using lipid nanoparticles (LNPs), such as the body makes to transport fatty acids produced by digestion to the liver.

Early Glimmerings

- Other scientists had been working on the lipid problem since the 1980s.
- Pieter Cullis of Columbia, a nanoparticle scientist, had been developing LNPs to safely deliver otherwise toxic drugs directly to tumor cells (tumors have notably leaky vasculature).
- What was needed was a LNP that was positively charged to stably contain the negatively charged RNA but that would present a neutral surface to the body (cationic lipids are generally toxic).
- In 2005 companies were driven to research LNPs to deliver siRNA, which can selectively silence genes, a useful therapy.
- The first commercially useful LNP for siRNA was patented in 2018 by a company called Alnylam.
- This LNP is the basis for Moderna's vaccine, though Moderna had to further develop it to handle its much larger RNA molecule.

Pegylated Lipid Nanoparticle



- Coating the LNP with polyethylene glycol (PEG) both stabilizes the LNP and provides a way of targeting it to specific cells (like muscle cells at the site of the injection) rather than having them accumulate in the liver.

Different types of vaccines currently approved by the CDC

- mRNA vaccines (Pfizer-BioNTech or Moderna): mRNA inserted into muscle cells causes them to produce the desired immunogenic protein.
- Vector vaccines (Johnson & Johnson's Janssen) Recombinant, replication-incompetent adenovirus* (Ad26) vector, with DNA encoding a stabilized variant of the SARS-CoV-2 spike protein.
- Protein subunit vaccines (currently under development) include harmless pieces (proteins) of the SARS-CoV-2 virus instead of the entire virus.

*Adenoviruses infect host cells, but their DNA is not incorporated into the cell's genetic material. It remains in a transient extrachromosomal state within the nucleus. The adenovirus DNA is transcribed just like any other gene. It is not replicated when the cell undergoes normal division.

The Future of mRNA technology

- Moderna is already testing in human studies potential vaccines against the flu, cytomegalovirus, respiratory syncytial virus, and Zika. Vaccines against the mononucleosis-causing Epstein-Barr virus, the Nipah virus, and HIV are still in the earliest stages of research. The Massachusetts biotech is also developing mRNA treatments for the Chikungunya virus and is exploring research into auto-immune disorders and cancer.

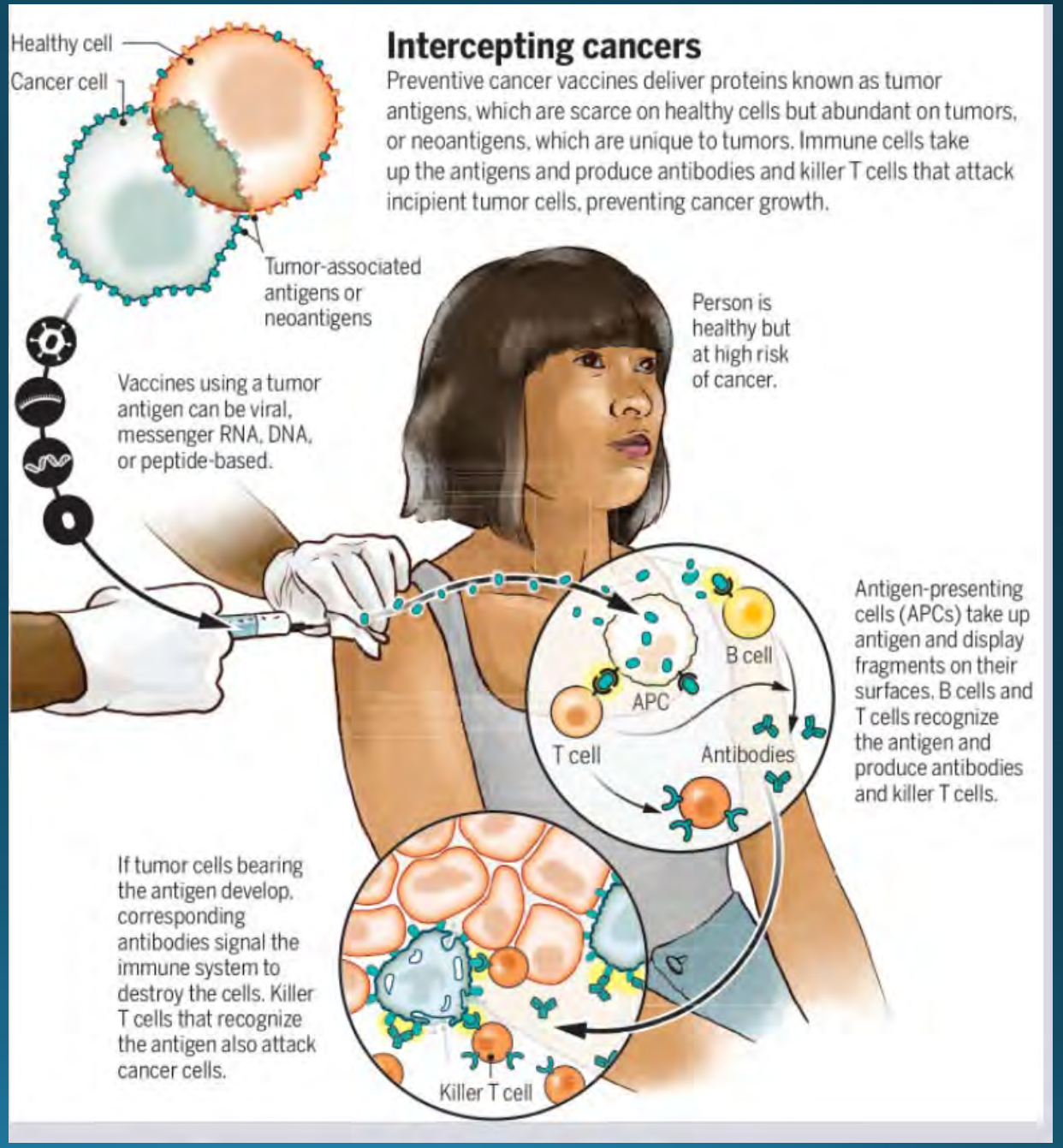
- The German biotech BioNTech originally focused on fighting cancer with mRNA. That's still reflected in its pipeline, where the bulk of the research is in oncology. The biotech is in the early stages of human studies testing its mRNA technology against a range of cancer types, including melanoma, prostate cancer, breast cancer, and ovarian cancer among others.

- BioNTech also has some additional vaccine research. In partnership with Pfizer, it's working on a flu vaccine that has yet to start human testing. BioNTech also partnered with the University of Pennsylvania in 2018 to work on vaccines for infectious diseases.

https://www.businessinsider.com/future-of-healthcare-mrna-uses-beyond-covid-19-vaccines-2021-9

Taking a Shot at Cancer

Science 376 (6589), 8 April 2022, p126



A Small Miracle in a Bottle

I continue to be amazed that all the background work on mRNA, lipid nanoparticles and virus research came to a head just in time for SARS-CoV-2!