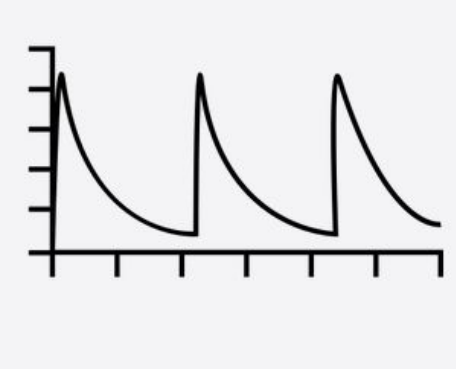
AMIDD 2023 Lecture 5: Five key questions of drug discovery

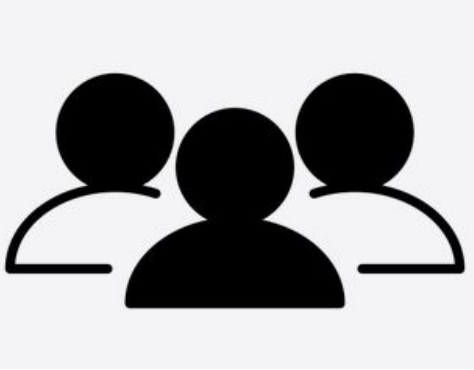
Medical Need

Target(s) & modality



PK/PD

Benefit/risk



Patient stratification

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Today's goals

1. Five questions and a *meta*-question for drug discovery and development
2. Case study: mRNA vaccines for coronavirus

Interests and concerns of companies working on drug discovery: summary of our previous discussions

Interests

- Return of Investment
  - Commercial potential
  - Cycle time
- Good reputation
  - Efficacy of the drug
  - Safety of the drug
  - Market access
- Environmental, social, and governance (e.g. fighting internal corruption, diversity of board members).

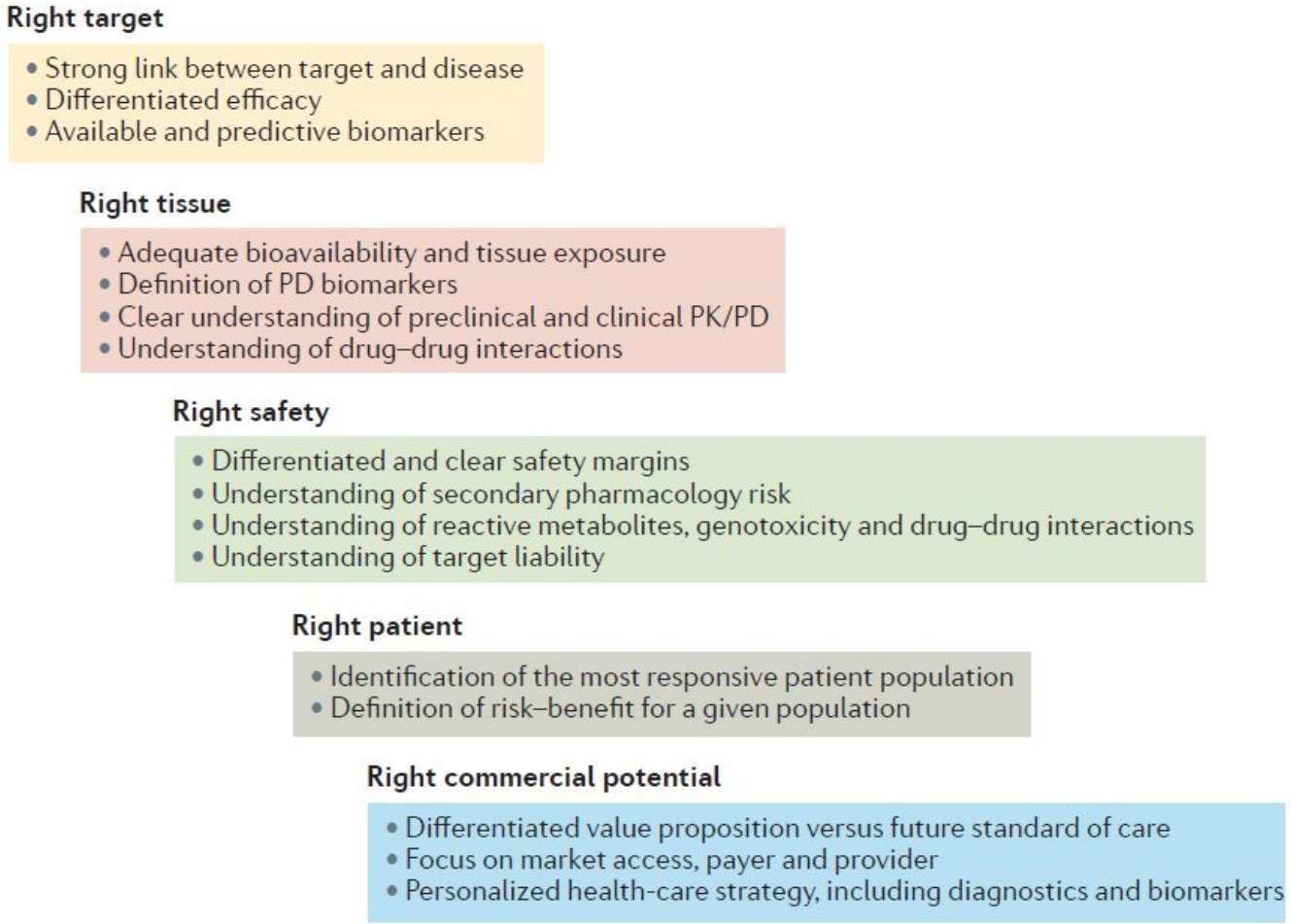
Concerns

- Low or no return of investment
  - Lack of efficacy of drugs
  - Unfavorable benefit/risk profiles of the drug
  - No approval from agency
  - Cost, time, effectiveness of R&D
  - Competitor
  - Poor targets or disease models due to lack of reproducibility of published data
  - Companion diagnostic
- Intellectual property
- Idea and knowledge management
- Acceptance by doctors and patients
- Legal concerns

Five key questions to ask before we start and along the way

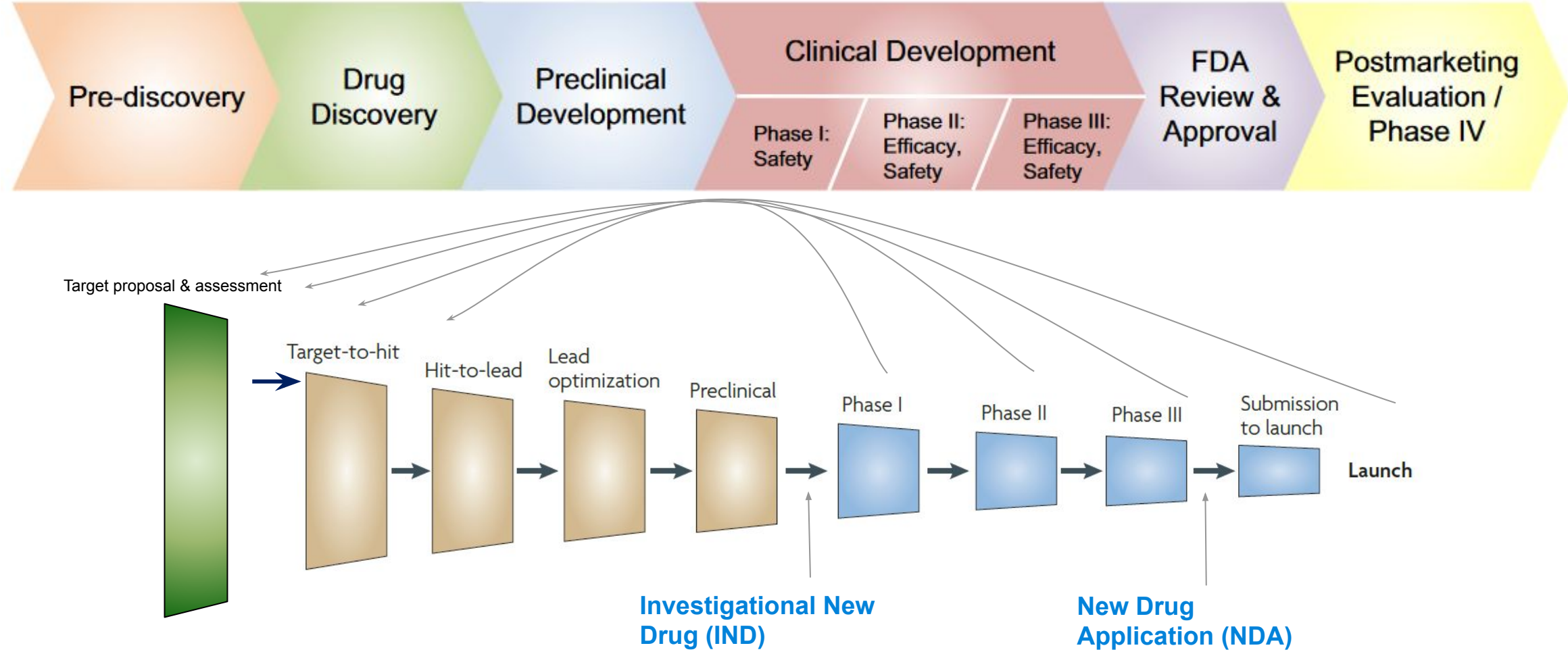
1. What is the unmet medical need to be addressed?
2. What are the target(s) and what is the modality of our drug?
3. Where should the drug go in patient's body, what does body do to the drug, and what does the drug do to the body?
4. What is the safety profile of the drug in light of its benefits?
5. Who are responsive to the drug, or susceptible to adverse events?

The *meta*-question: What knowledge, data, and tools do we have to address these questions?



Morgan, P. et al. Impact of a five-dimensional framework on R&D productivity at AstraZeneca. Nature Reviews Drug Discovery 17, 167–181 (2018).

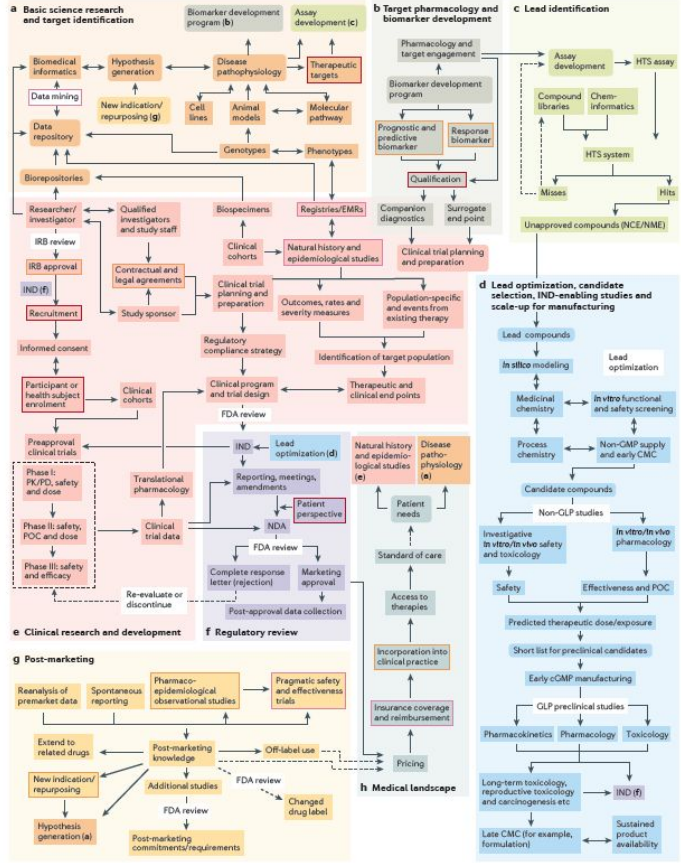
Chevron diagrams as a pipeline view of drug discovery and development



FDA: US Food and Drug Administration. Top: Wagner, J. A. et al. Application of a Dynamic Map for Learning, Communicating, Navigating, and Improving Therapeutic Development. Clinical and Translational Science 11, 166–174 (2018). Bottom: Adapted from Paul et al. How to Improve R&D Productivity: The Pharmaceutical Industry's Grand Challenge. Nature Reviews Drug Discovery, 2010.

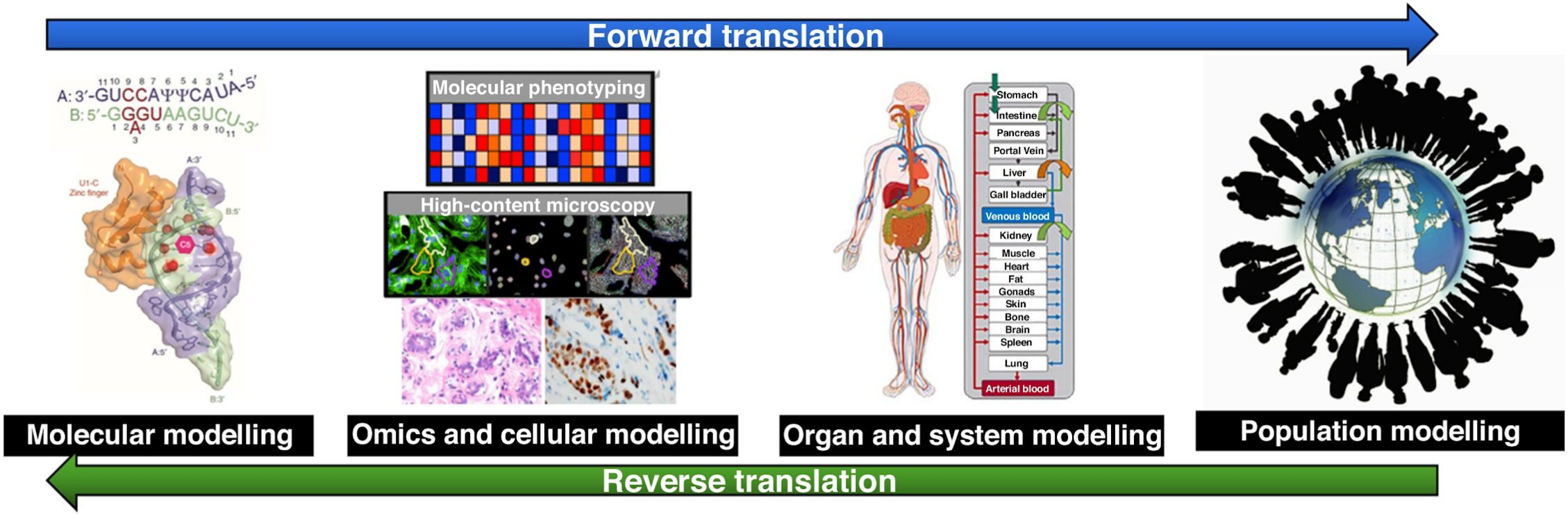
A dynamic map for drug discovery, development, and deployment

1. **Basic science research and target identification.** *What causes the disease? What do we want to achieve? Which protein can I target with which modality?*
2. **Target pharmacology and biomarker development.** *What is the effect of targeting the protein? What we can measure to confirm that the protein is properly targeted?*
3. **Lead identification.** *How can we find a starting point of a new drug?*
4. **Lead optimization and clinical candidate selection**. *What are criteria to define a good drug? How can I improve the starting material?*
5. **Clinical research and development**. *Does it work in human? How about efficacy and safety profiles?*
6. **Regulatory review**. *Should we approve the drug?*
7. **Post marketing**. *How does the drug work in real world?*



Wagner, J. et al. A dynamic map for learning, communicating, navigating and improving therapeutic development. Nat Rev Drug Discov 17, 150–150 (2018).

The multiscale modelling view of drug discovery

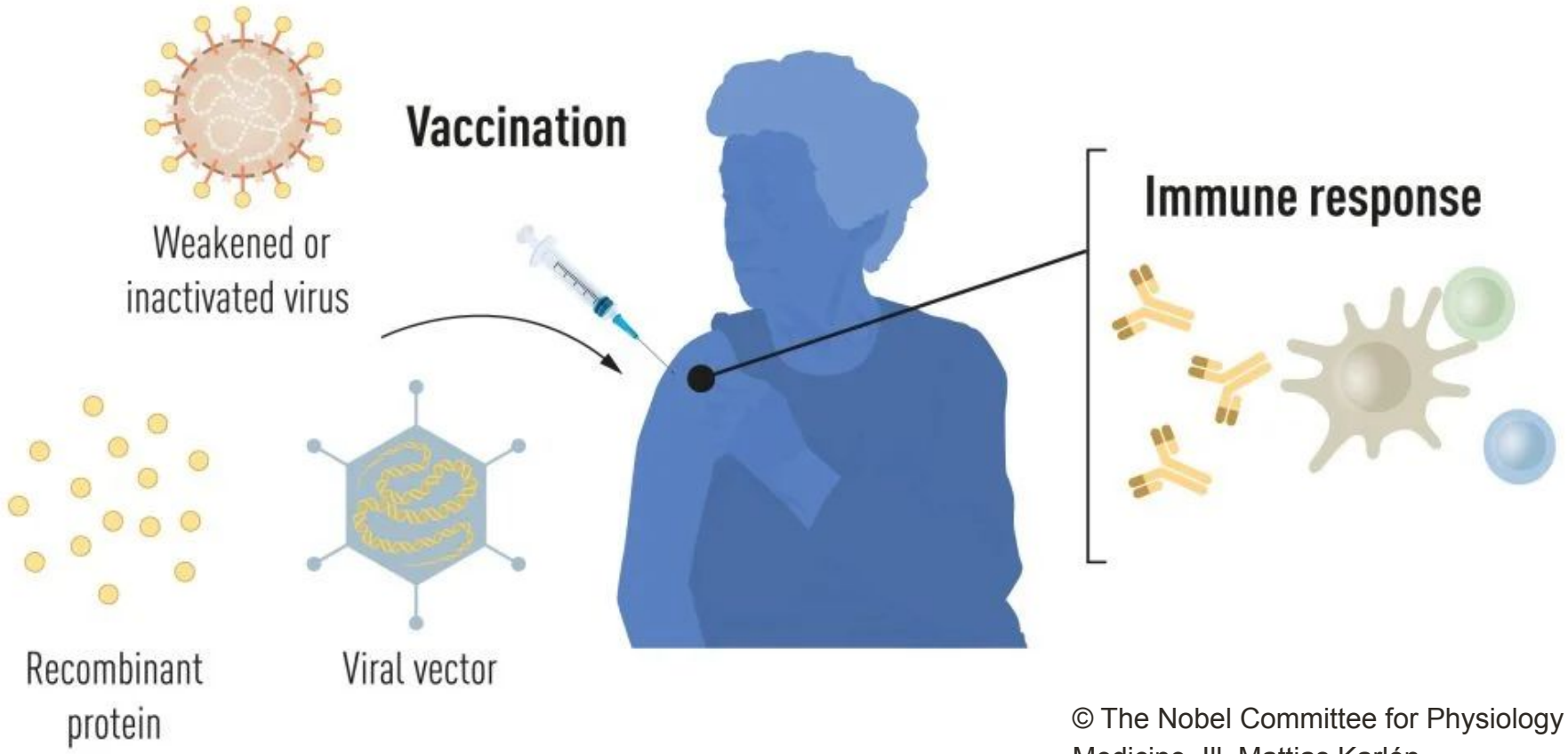


Zhang, Jitao David, Lisa Sach-Peltason, Christian Kramer, Ken Wang, and Martin Ebeling. 2020. “Multiscale Modelling of Drug Mechanism and Safety.” Drug Discovery Today 25 (3): 519–34. https://doi.org/10.1016/j.drudis.2019.12.009.

Nobel Prize in Physiology or Medicine 2023 was awarded to Katalin Karikó and Drew Weissman for “their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19”

Methods for vaccine production before the COVID-19 pandemic.

Issues: large-scale cell culture required, which limits the possibilities for rapid production in response to pandemics.

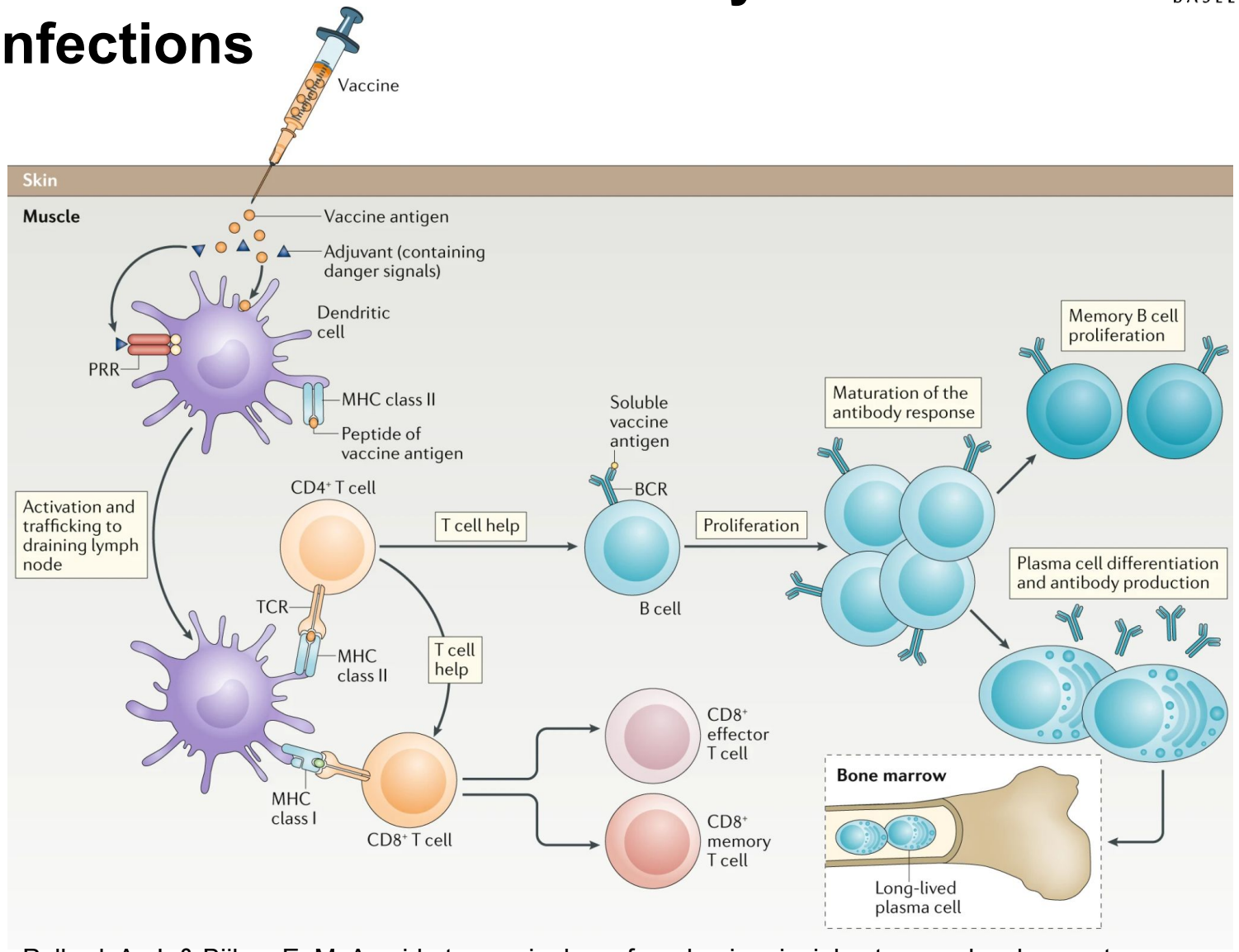


Vaccine mimics viral infection to activate the immune system to protect body from future infections

Vaccine mimics a viral infection to activate innate and adaptive immune system, while minimizing the pathogenic effects.

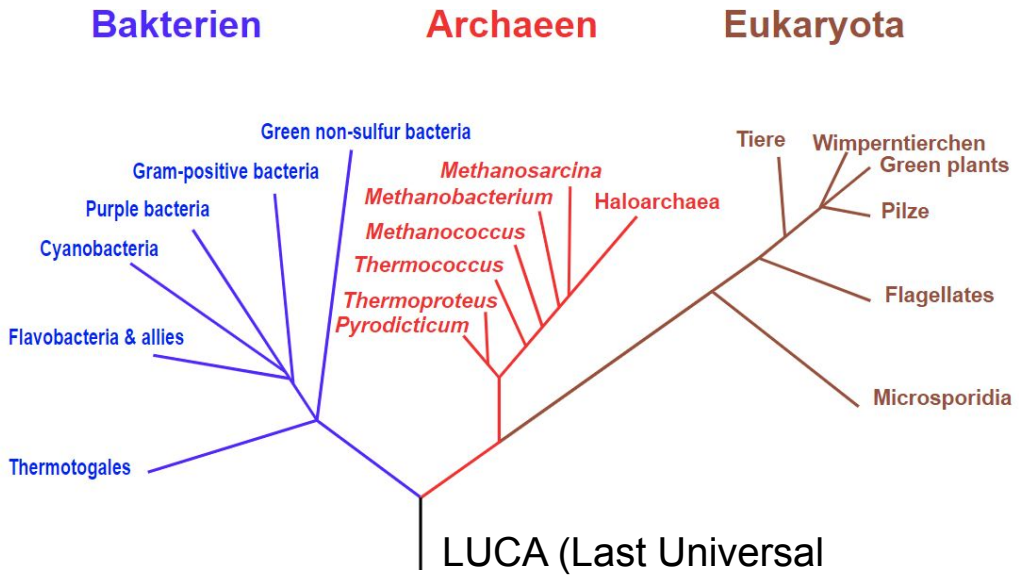
Key players in the game:

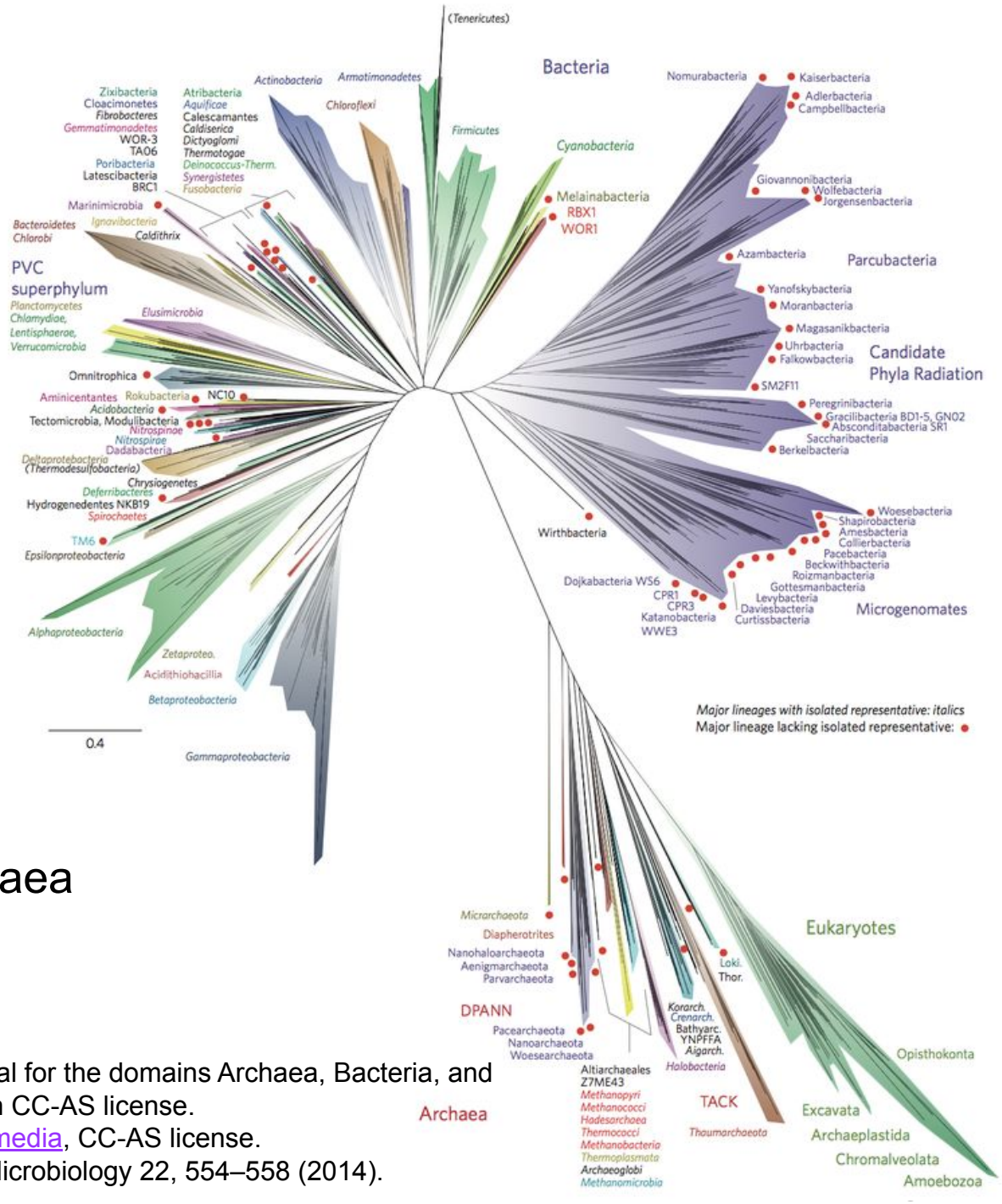
1. Viral proteins as *antigens*
2. Antigen-presenting cells (e.g. dendritic cells)
3. T cells (T comes from Thymus, because they mature there)
4. B cells (B comes from bone marrow).



Pollard, A. J. & Bijker, E. M. A guide to vaccinology: from basic principles to new developments. Nature Reviews Immunology 21, 83–100 (2021).

Virus is special

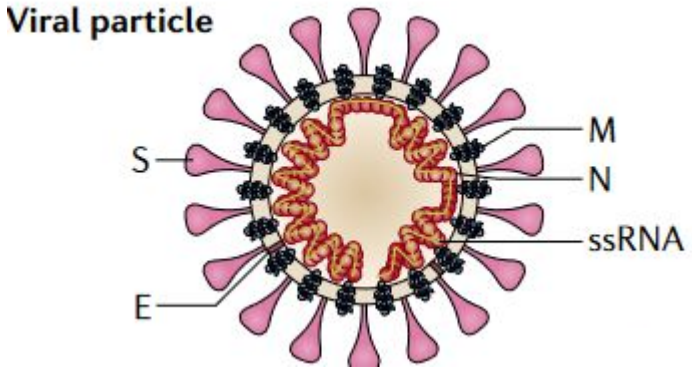




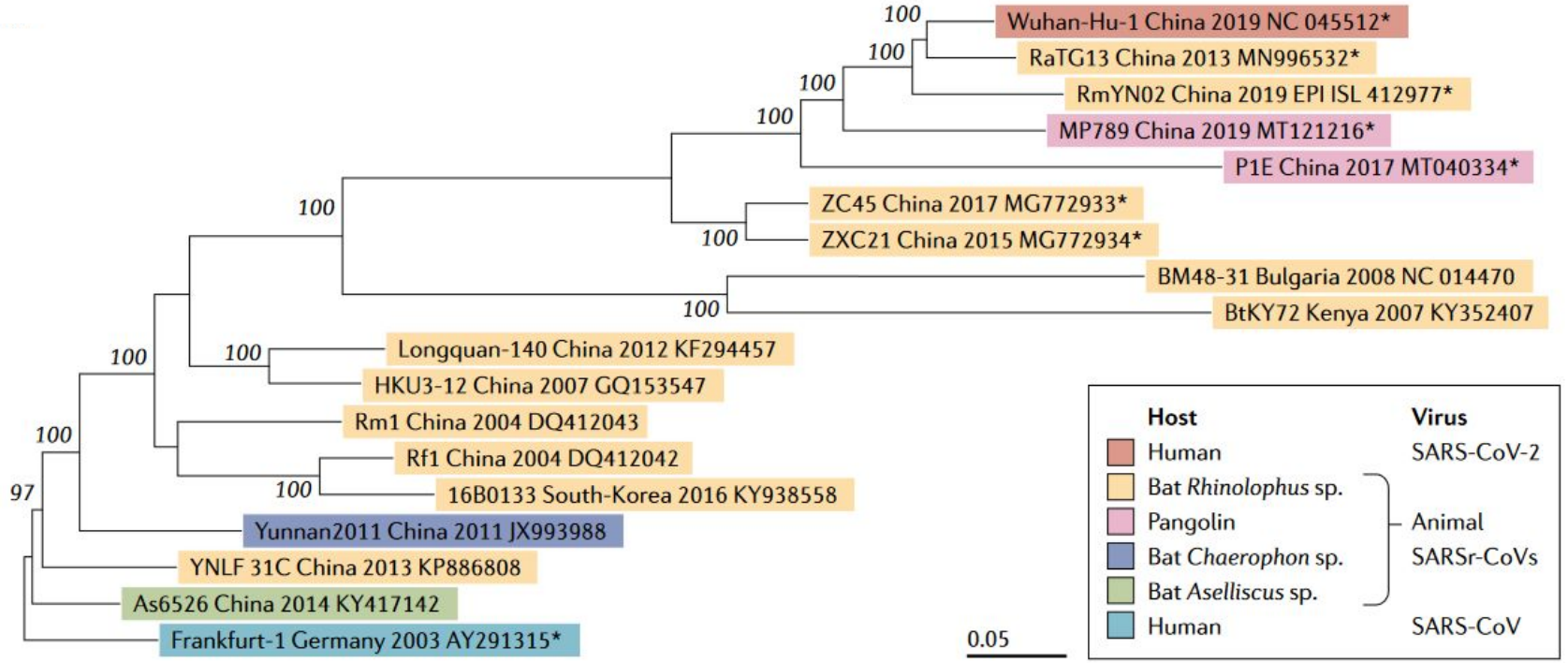
- The three-domain model of *cellular* life: (eu-)bacteria, archaebacteria, and eukaryotes.
- The two-domain model: bacteria as one branch, archaea and eukaryotes as the other.
- Virus fits in no domain of neither models.

1. Woese, C. R., Kandler, O. & Wheelis, M. L. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A 87, 4576–4579 (1990). Figure from Wikimedia, reused with CC-AS license.
1. Hug, L. A. et al. A new view of the tree of life. Nat Microbiol 1, 1–6 (2016). Figure from Wikimedia, CC-AS license.
1. Forterre, P., Krupovic, M. & Prangishvili, D. Cellular domains and viral lineages. Trends in Microbiology 22, 554–558 (2014).

Coronavirus is a RNA virus infecting human and other species



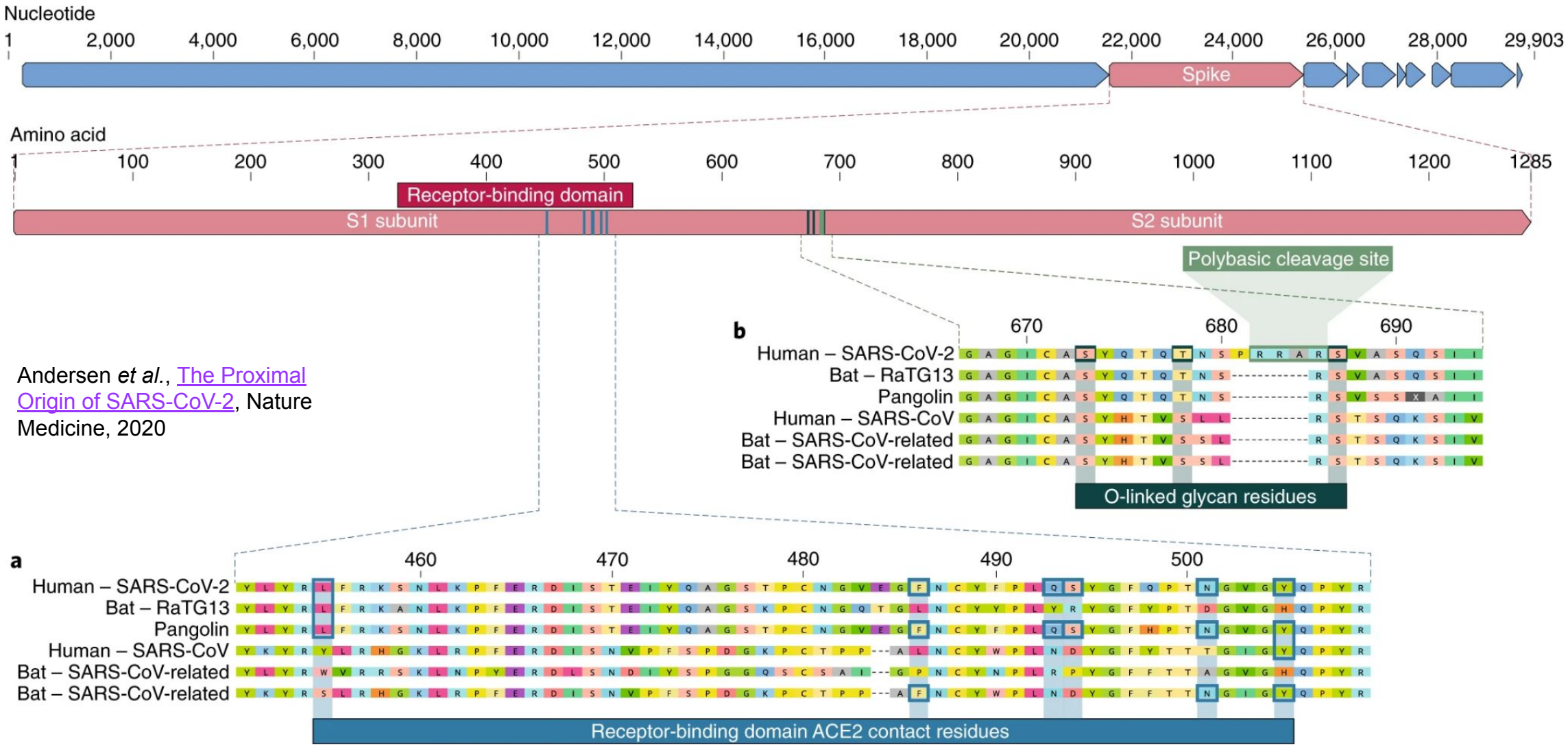
The coronavirus virion consists of structural proteins, namely spike (S), envelope (E), membrane (M), nucleocapsid (N)



Phylogenetic relationships of representative members of the species Severe acute respiratory syndrome-related coronavirus

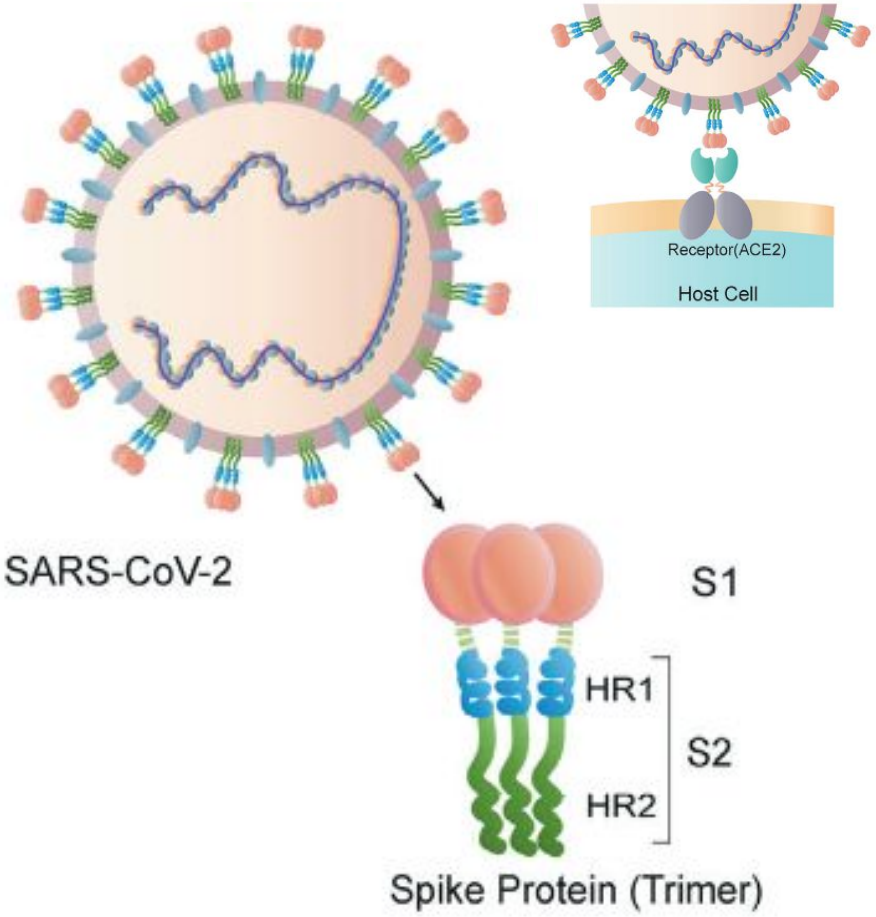
V'kovski, P., Kratzel, A., Steiner, S., Stalder, H. & Thiel, V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol 19, 155–170 (2021).

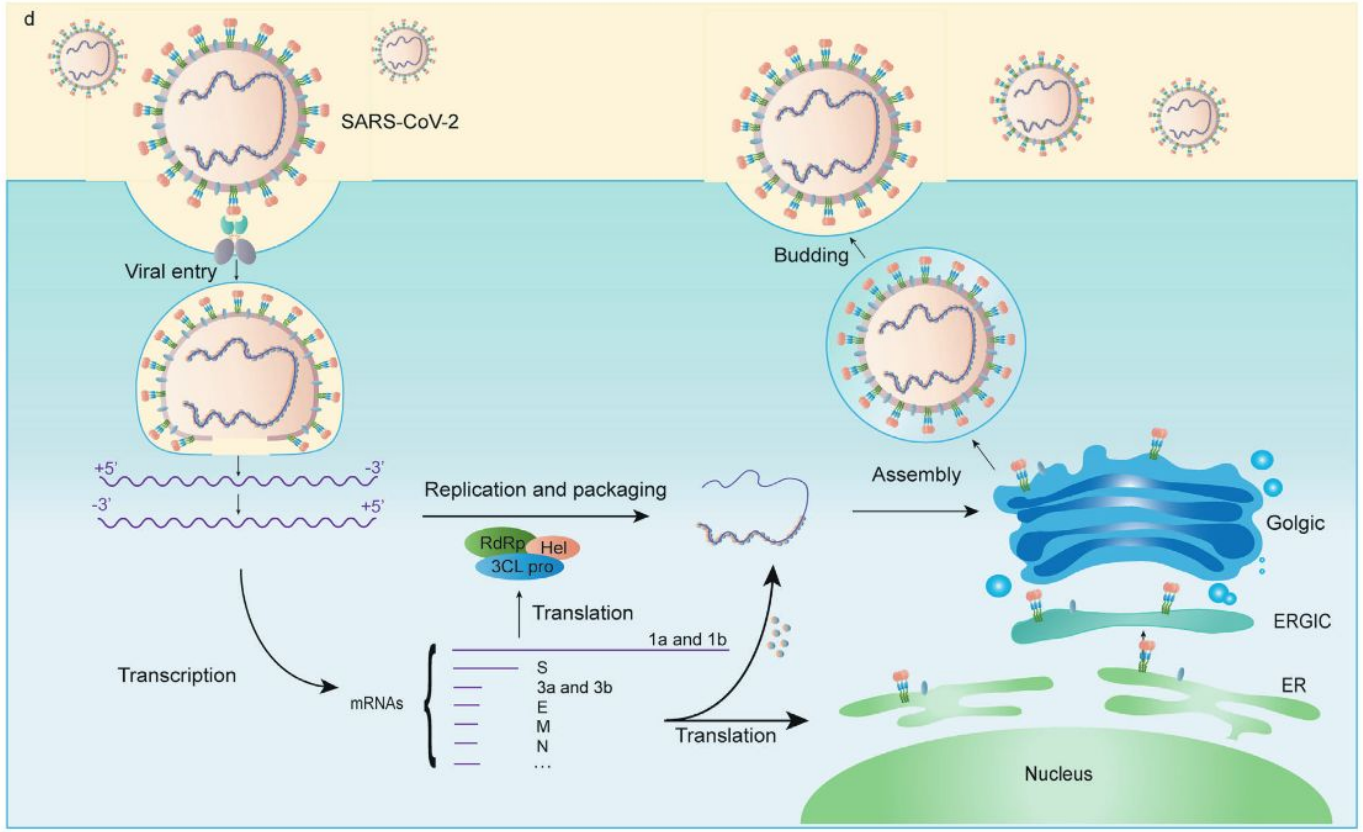
Sequence of the spike protein is largely conserved between corona and related viruses



Andersen *et al.*, The Proximal Origin of SARS-CoV-2, Nature Medicine, 2020

Spike protein of coronavirus is responsible for viral entry into human cells



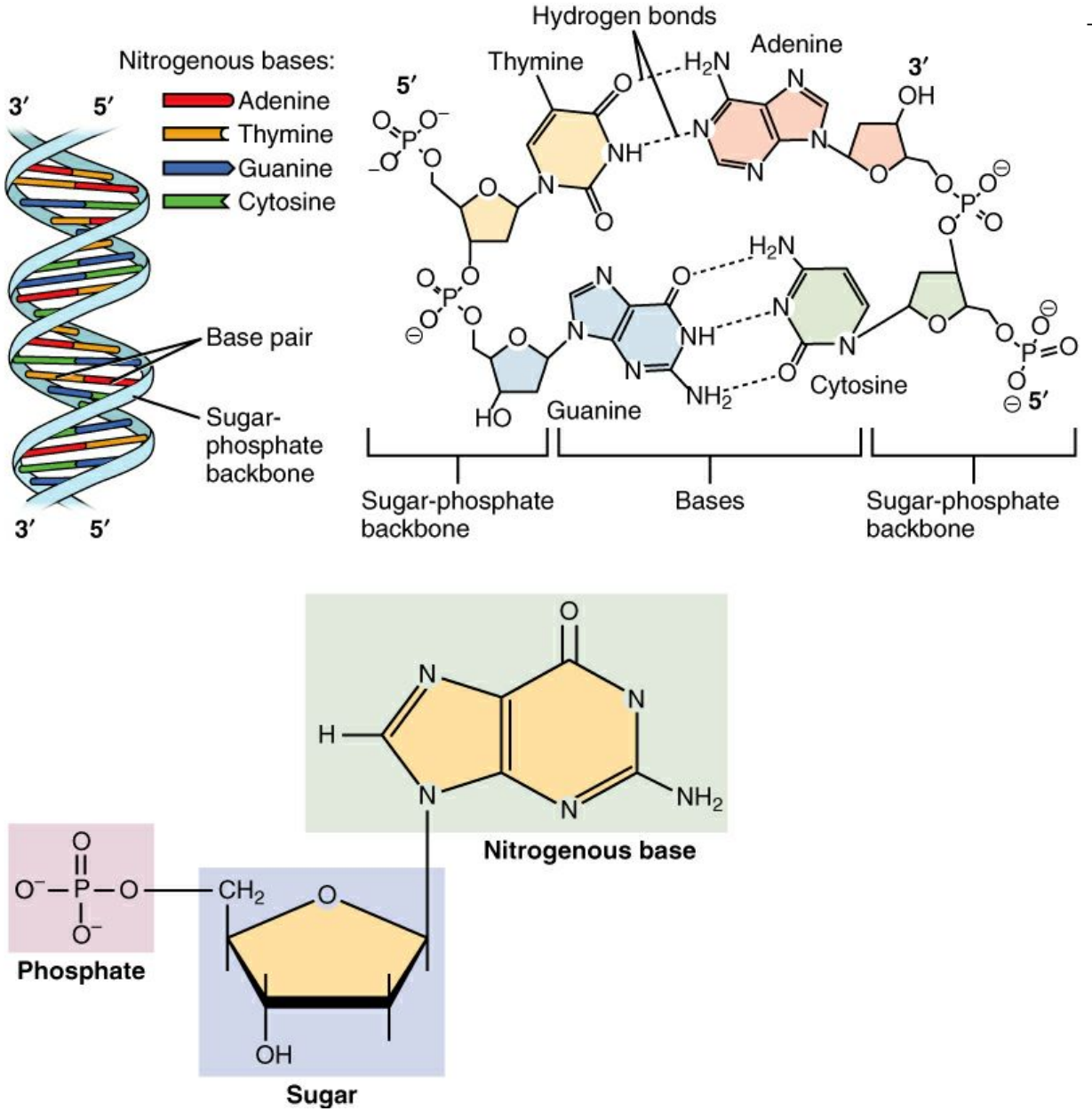


Huang, Y., Yang, C., Xu, X., Xu, W. & Liu, S. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin 41, 1141–1149 (2020).

DNA or RNA encodes genetic information of all life forms that we know, including viruses

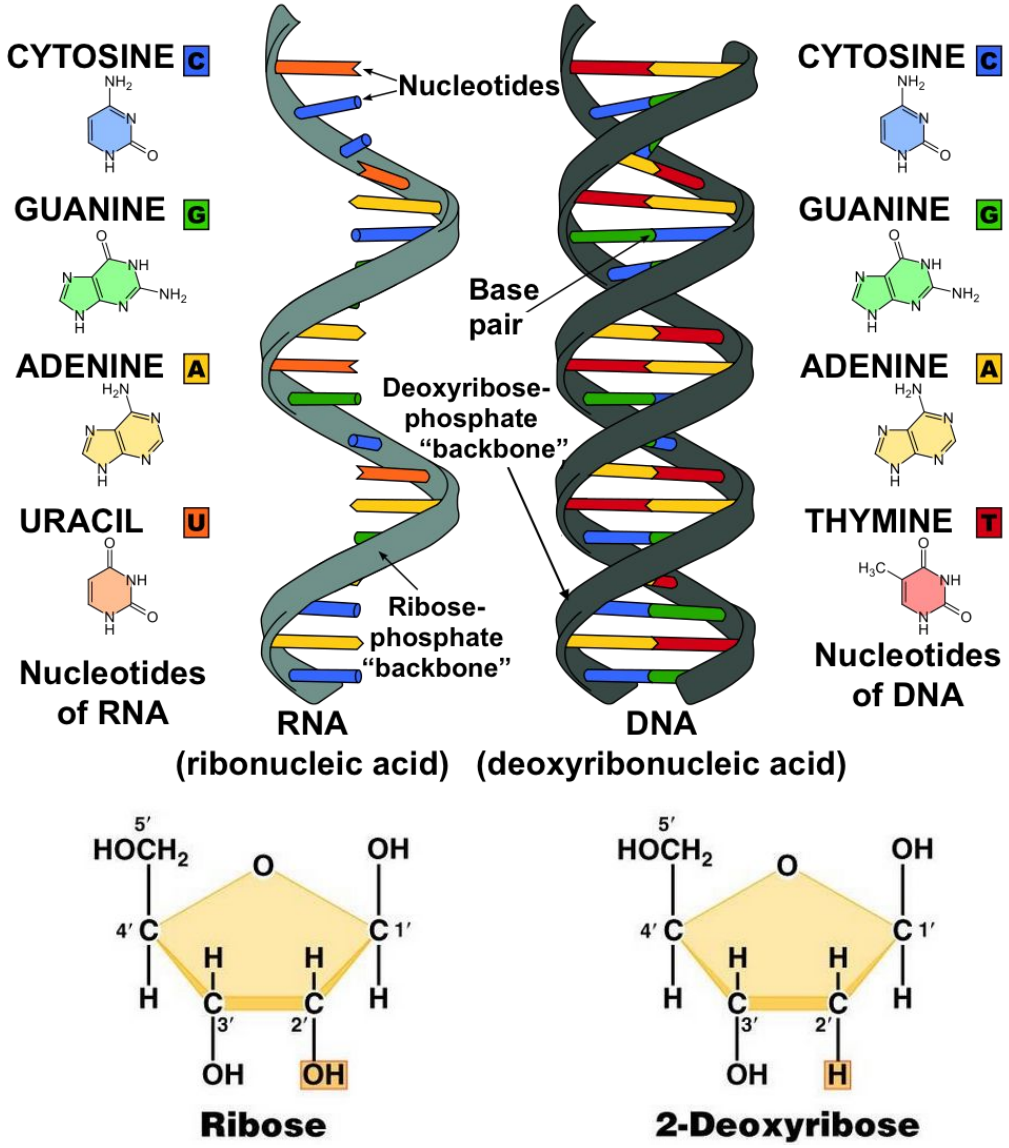
Photo 51, X-ray diffraction image of DNA

Franklin R, Gosling RG (1953) "Molecular Configuration in Sodium Thymonucleate". ***Nature*** 171**: 740–741.**



From the textbook OpenStax Anatomy and Physiology, discovered through Wikimedia, reused under the CC license.

RNA is transcribed from DNA and translated into protein



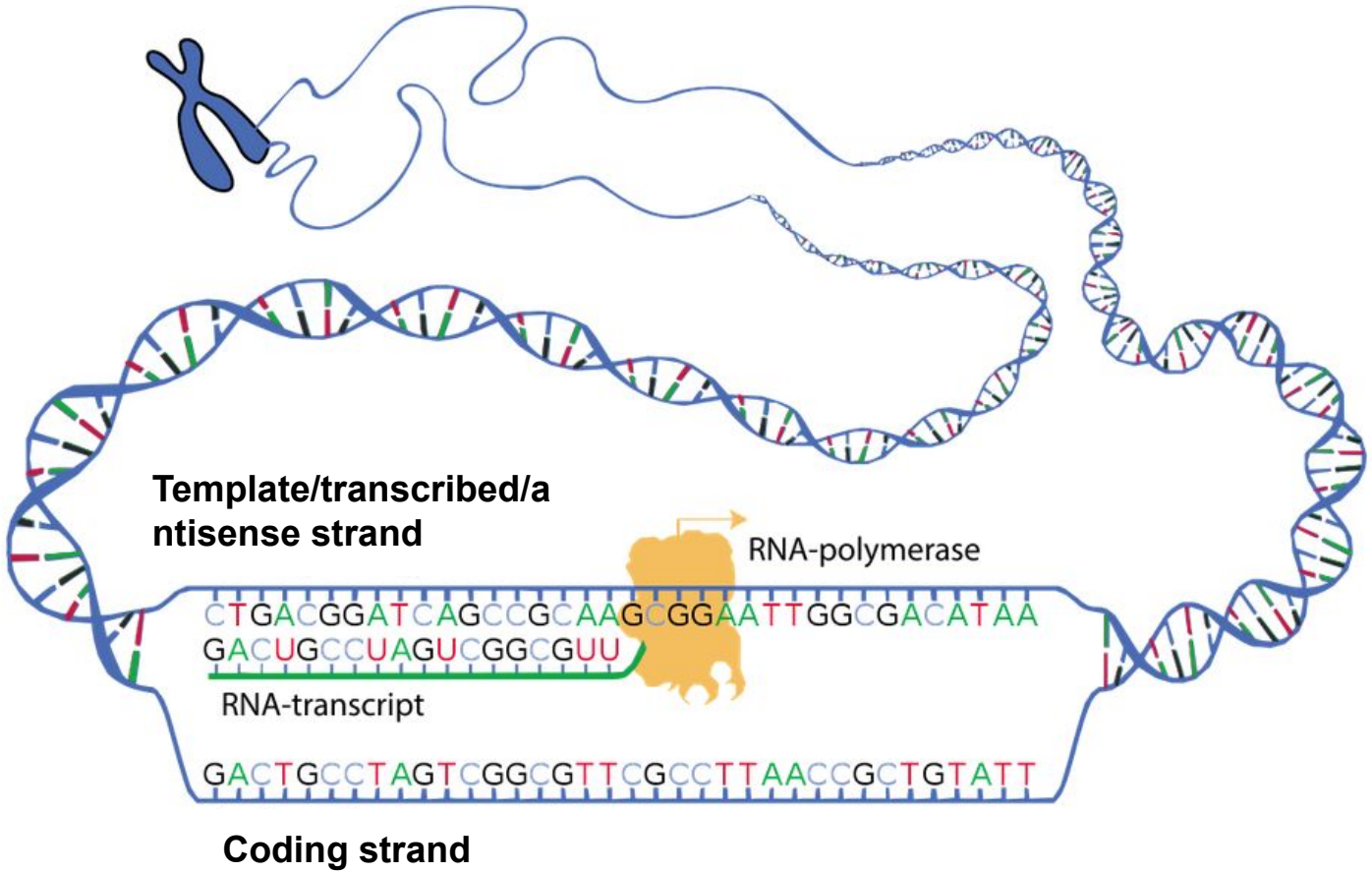
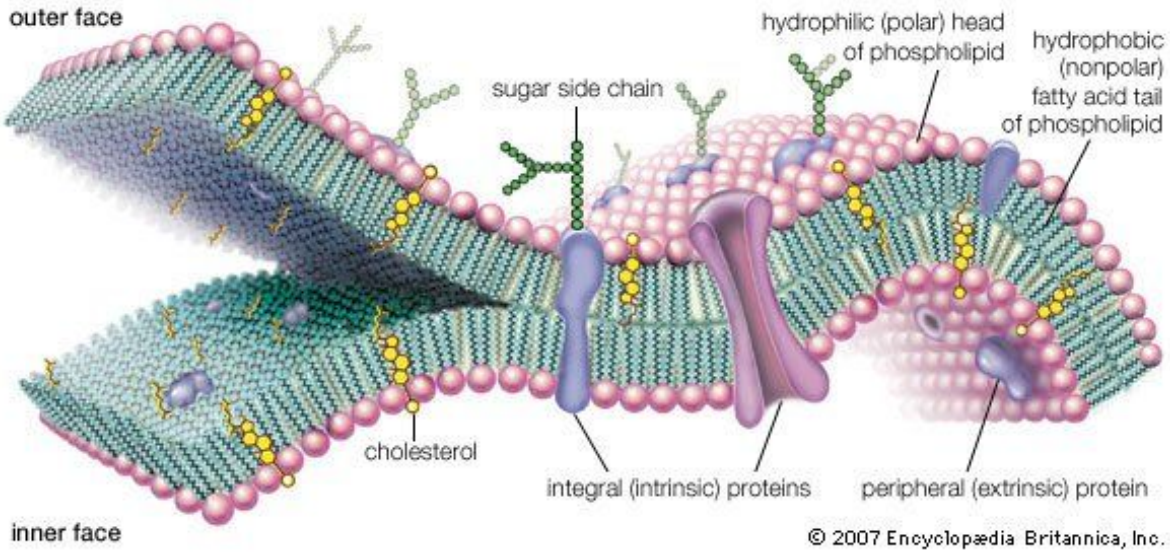


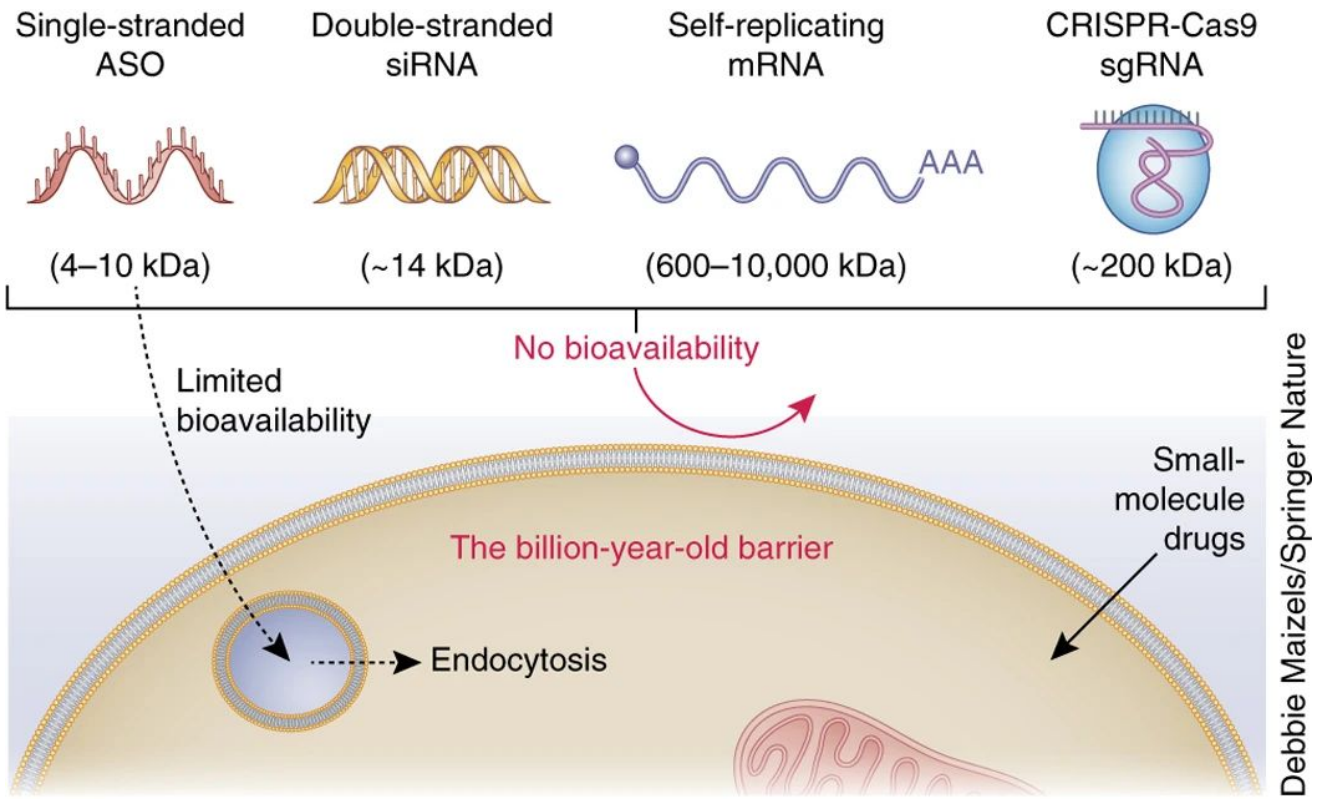
Figure: https://commons.wikimedia.org/wiki/File:DNA_transcriptie.svg and https://en.m.wikipedia.org/wiki/File%3AHAR1F_RF00635_rna_secondary_structure.jpg. Original work by wikipedia user: OrgreBot and user:Ppgardne. Used under CC-SA 3.0 license.

A summary of what we have learned so far in the context of coronavirus

1. What is the unmet medical need to be addressed? We need a vaccine to prevent a large population of individuals from being infected by coronavirus, which have severe consequences.
2. What are the target(s) of our drug? Spike protein is conserved: immune reaction is desired.
3. Where should the drug go in patient's body, what does body do to the drug, and what does the drug do to the body? Due to time constraints, classical vaccine may not meet the need. How about mRNA vaccines?
4. What is the safety profile of the drug in light of its benefits? To be investigated.
5. Who are responsive to the drug, or susceptible to adverse events? To be investigated.

Three essential challenges for mRNA-based therapies: delivery, stability, and *unwanted* immune responses





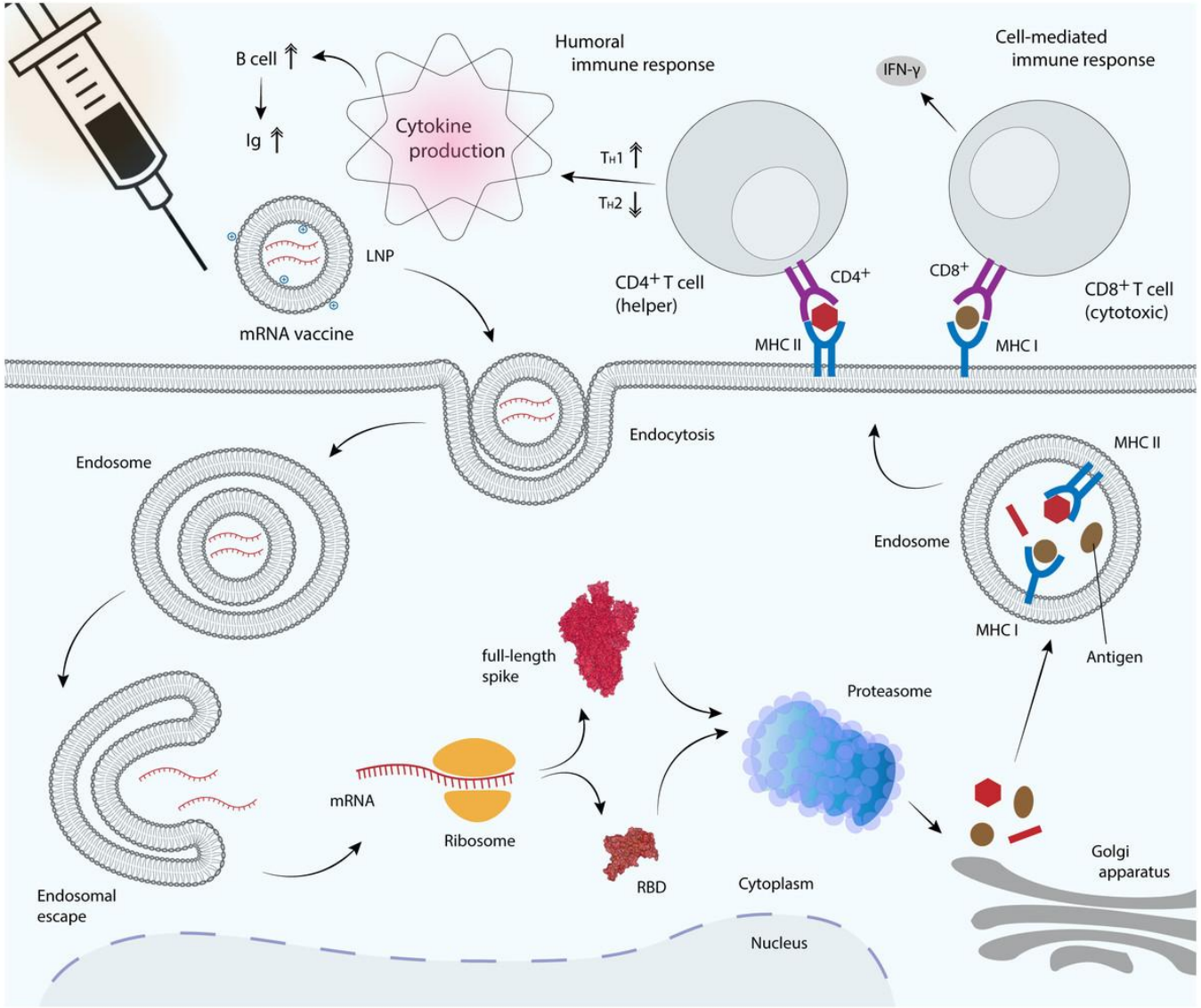
Key challenges:

- mRNAs are too large and charged to pass lipid bilayers.
- mRNAs are readily degraded by ribonucleases.
- *Exogeneous* mRNAs cause *Immunogenicity*.

Left: Cell membrane, copyright of Encylopedia Britannica, Inc. Right: The four-billion-year-old barrier to RNA therapeutic

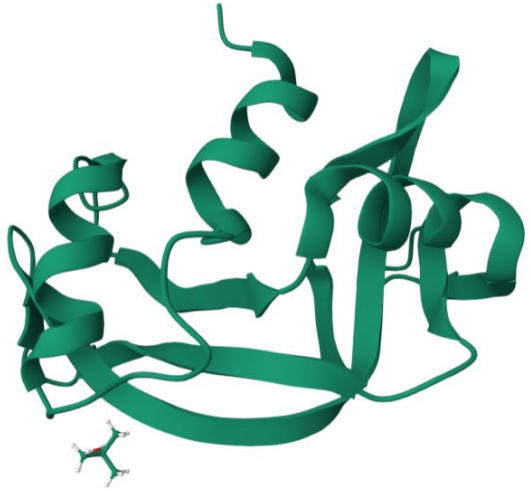
Lipid NanoParticles (LNP) helps delivering RNAs into cells

- Lipid nanoparticles can take mRNA vaccines as largos, and deliver them into human cells.
- In the cell, mRNA encoding the part of the spike protein sequence is translated into proteins with the human protein translation mechanism.
- Synthesized proteins will be degraded and exposed on cell surface, which will be recognized by antigen presenting cells.

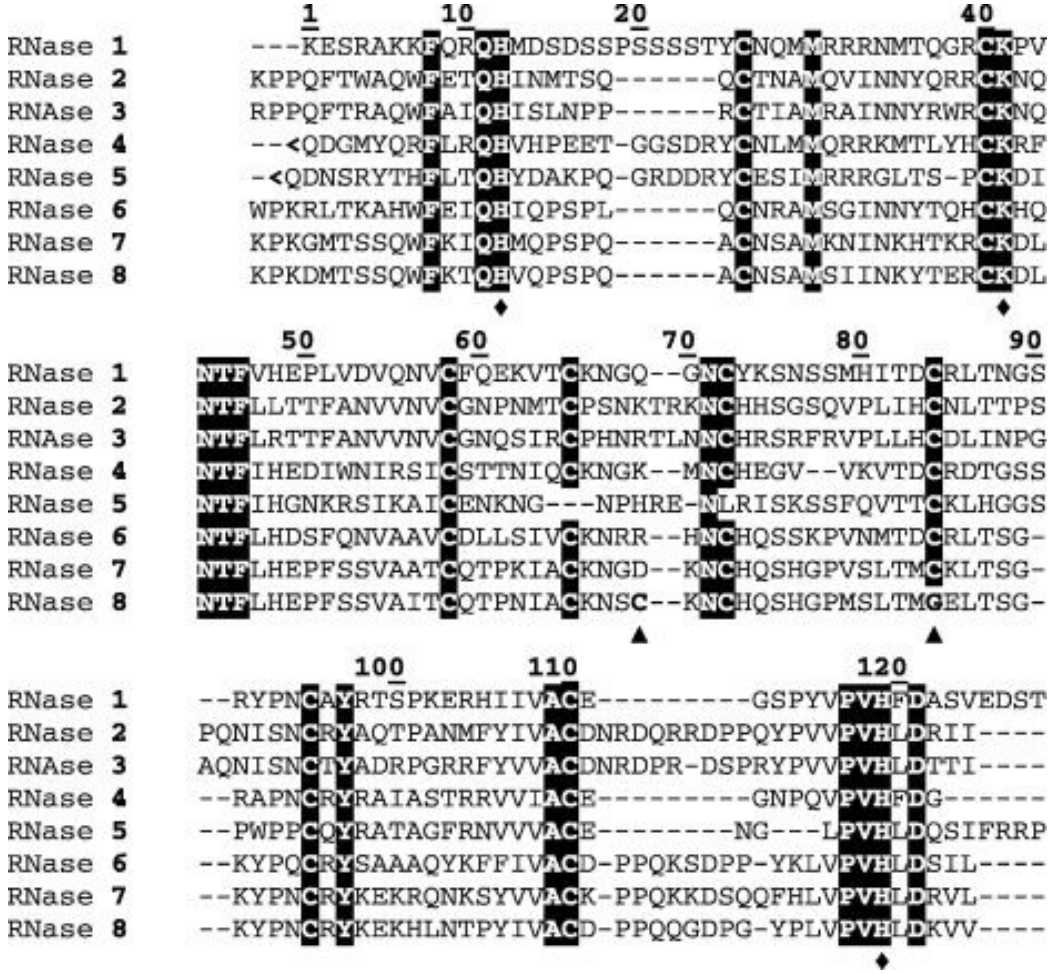


Salleh, Mohd Zulkifli *et al.* "Immunogenicity Mechanism of mRNA Vaccines and Their Limitations in Promoting Adaptive Protection against SARS-CoV-2." PeerJ 10 (March 9, 2022)

RNAs are degraded by ribonucleases (RNAases)



- mRNAs are too large and charged to pass lipid bilayers.
- **mRNAs are degradable by ribonucleases (RNases).**
- mRNAs are rapidly cleared from liver and kidney.
- *Exogeneous* mRNAs induce *immunogenicity*.



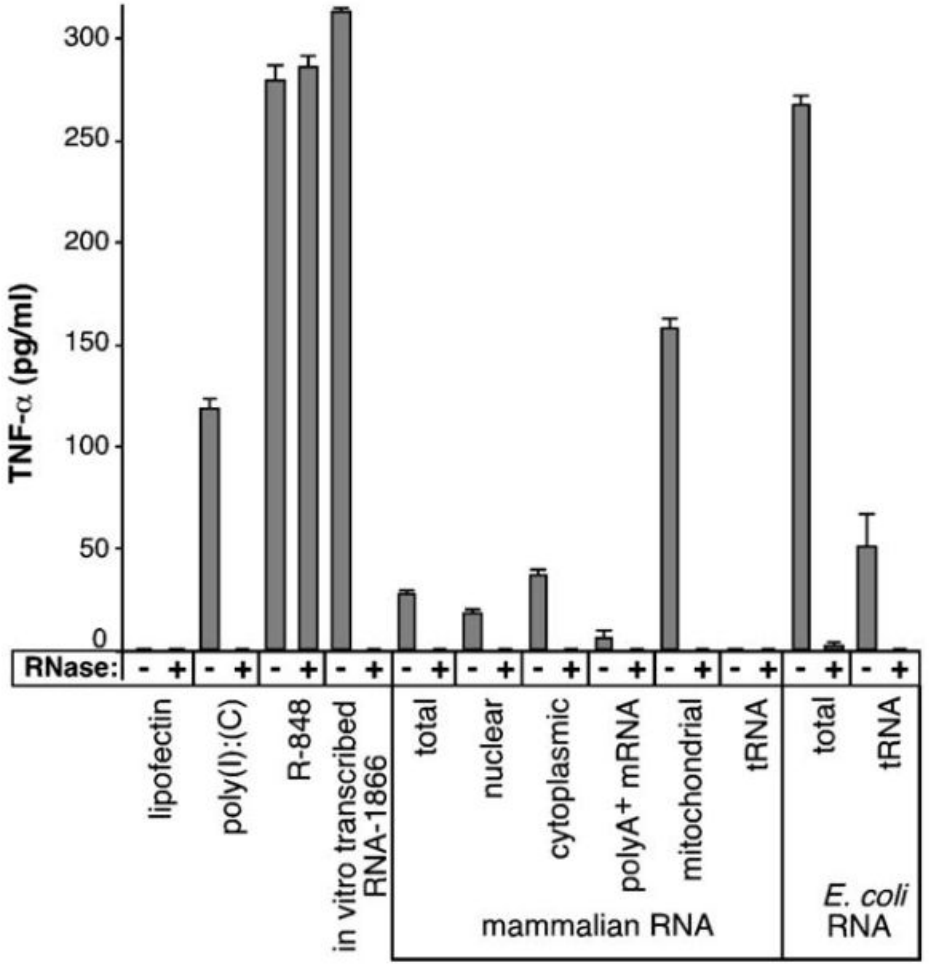
Left: Structure of PDB 7RSA. Right: alignment of protein sequences of 8 canonical human RNases (ribonuclease A family). Sorrentino FEBS Letters, 2010.

Unmodified RNA induces unwanted immune reactions: modified RNAs instead have reduced or little unwanted effect

***Exogeneous* mRNAs induce Immunogenicity.** All RNAs is synthesize from four ribonucleotides: ATP (adenosine triphosphate), CTP (cytidine triphosphate), UTP (uridine triphosphate), and GTP (guanosine triphosphate).

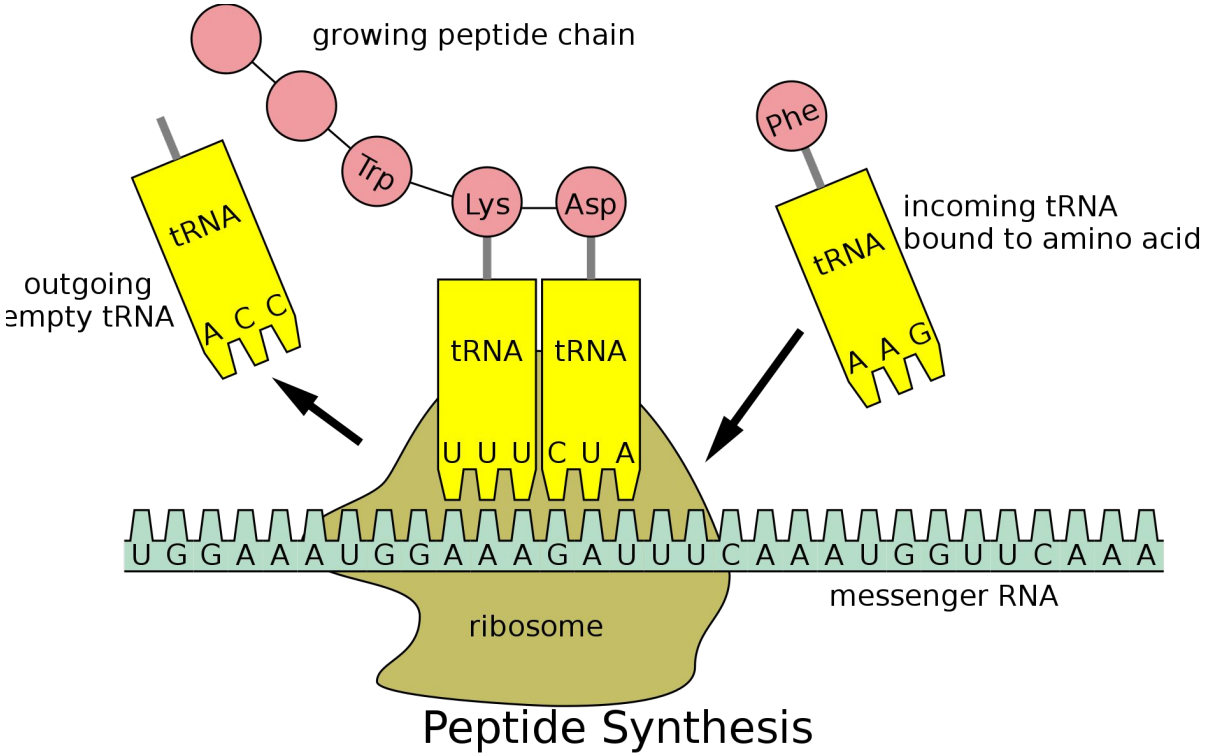
When unmodified RNAs are delivered into cells, they induce unwanted immune reaction, e.g. the release of the tumor necrosis factor alpha (TNF-alpha), by activating the Toll-like receptors (TLRs), which defend against bacterial and viral infections.

Some type of RNA, however, does not induce immunogenicity, for instance human *tRNA*. This finding by Karikó and Drewman made major contributions to successful development of SARS-CoV-2 mRNA vaccines.



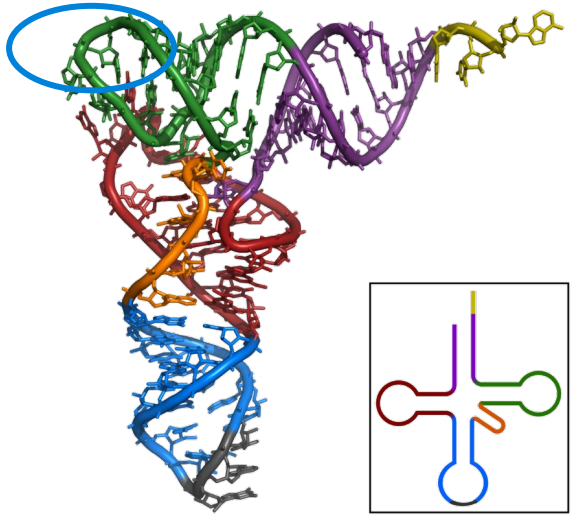
Karikó, K., Buckstein, M., Ni, H. & Weissman, D. Suppression of RNA Recognition by Toll-like Receptors: The Impact of Nucleoside Modification and the Evolutionary Origin of RNA. Immunity 23, 165–175 (2005).

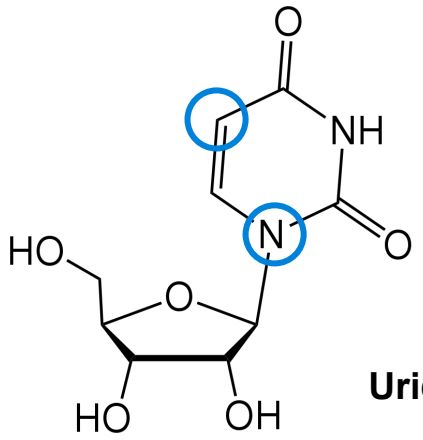
tRNAs transfer amino-acids to ribosome for protein translation, and human tRNA contains *pseudouridine*, a modified uridine



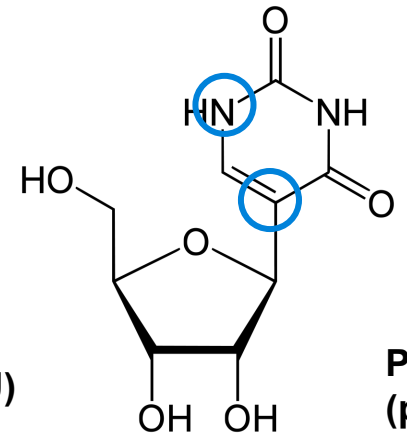
By Boumphreyfr vector conversion by Glrx - File:Peptide syn.png, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=101457889. By Yikrazuul, CC BY-SA 3.0, via Wikimedia Commons.

Left: Role of tRNA in protein translation. Top right: tRNA structure, with the TΨC loop highlighted in the blue ellipse. Below: structures of uridine and pseudouridine.



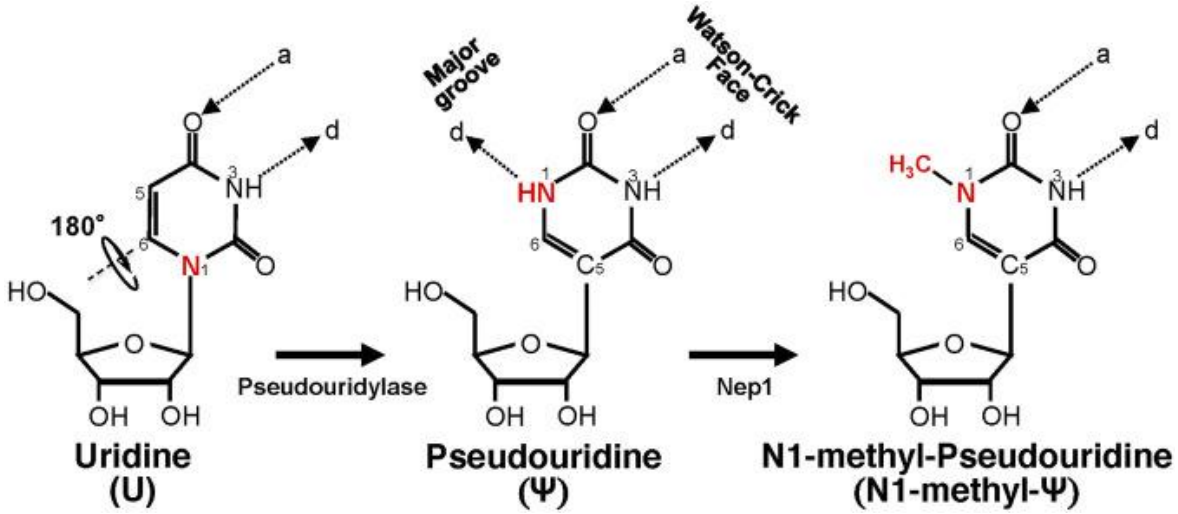


Uridine (U)

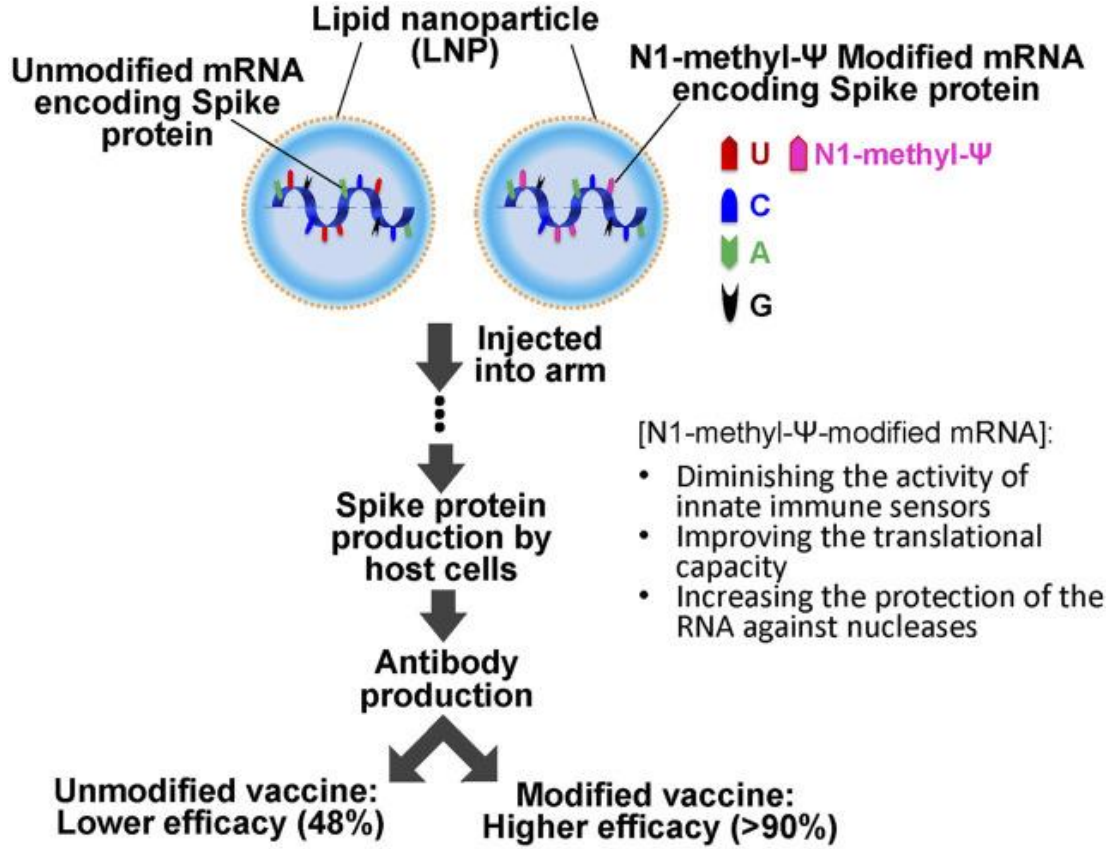


Pseudouridine (psi, Ψ)

Further modification (N1-mythel-Ψ) combined with LNP delivery lead to the currently used vaccines

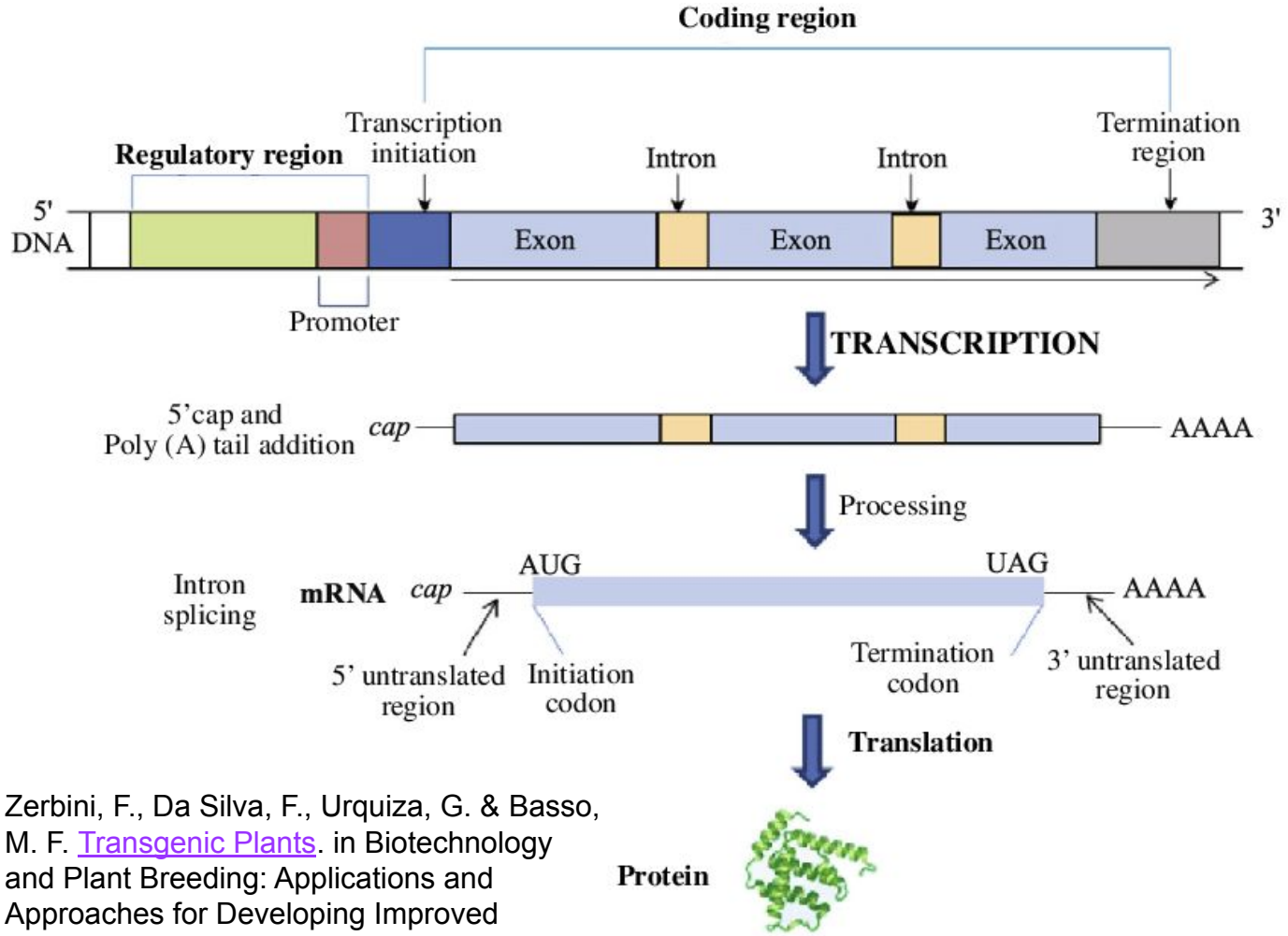


mRNA vaccines against human SARS-Cov-2 viruses, developed in 2020 by Pfizer-BioNTech and Moderna Therapeutics (comirnaty® and spikevax®, respectively), reached clinical efficacies higher than 90%. Both used modified RNA and LNP. Curevac mRNA vaccine (CVnCoV), which used LNP but not modified RNA, reached an efficacy of 48%.



Morais, P., Adachi, H. & Yu, Y.-T. The Critical Contribution of Pseudouridine to mRNA COVID-19 Vaccines. Front Cell Dev Biol 9, 789427 (2021).

Coding sequence of the spike protein along is not enough: mRNA transcription depends on 5’-UTR and 3’-UTR, too

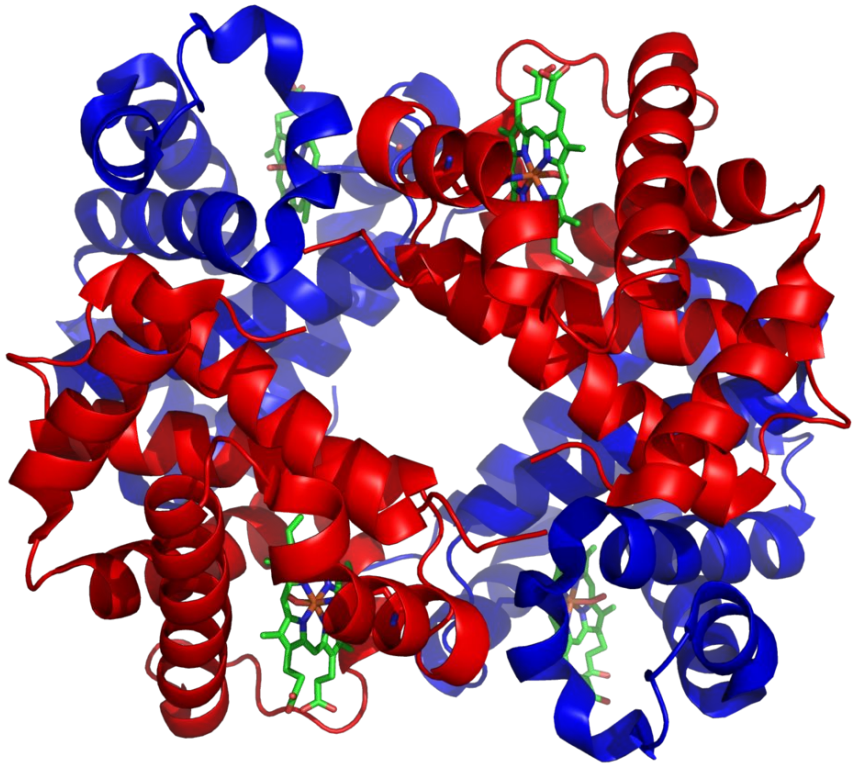


Zerbini, F., Da Silva, F., Urquiza, G. & Basso, M. F. Transgenic Plants. in Biotechnology and Plant Breeding: Applications and Approaches for Developing Improved Cultivars 179–199 (2014).

The structure of a gene and the control of gene expression in eukaryotes:

1. RNA polymerase, an enzyme, binds to the promoter region and reads the DNA from the 5′ UTR to the 3′ UTR.
2. pre-mRNA is synthesized and receives a cap of a modified nucleotide (7-methylguanosine triphosphate) to the 5′ end, and a repeated adenine sequence (a poly-A tail) to the 3′ end.
3. pre-mRNA is spliced to remove introns. Mature mRNA contains the 5’ cap, 5’-untranslated region (5’-UTR), coding sequence, 3’-untranslated region (3’-UTR), and a poly-A tail.
4. mRNAs are transported from the nucleus to the cytoplasm for translation.

5’-UTR of human hemoglobin is a good choice to make sure that the vaccine sequence is stable and highly translated



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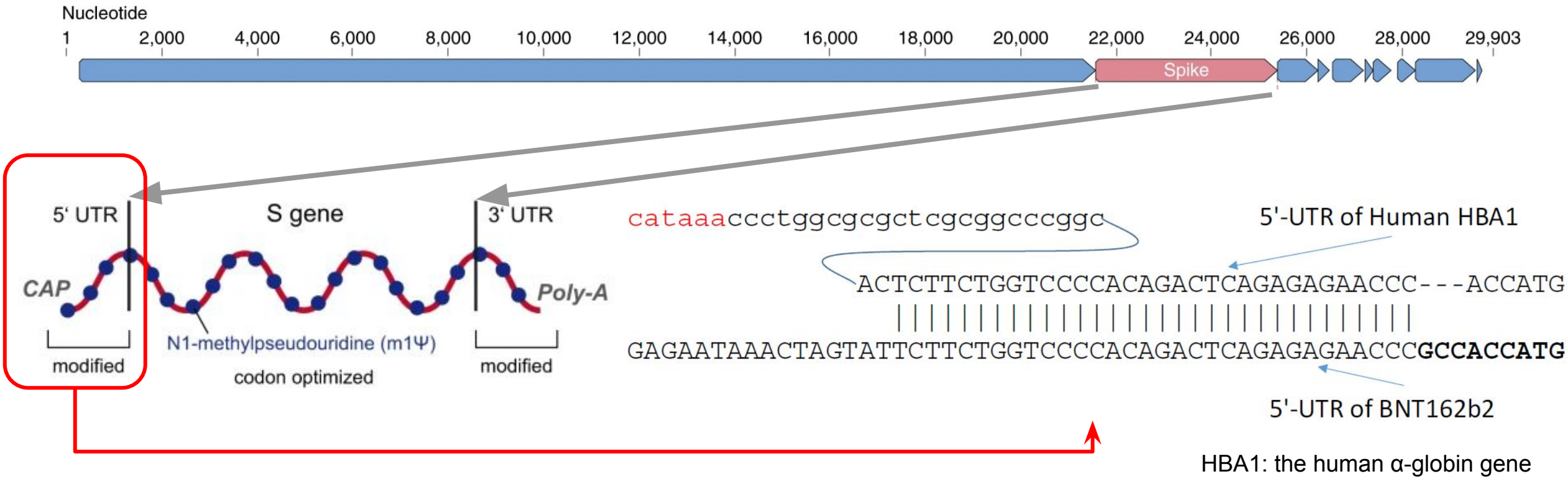
1    -MVLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFD------LSHGS   53  P69905   HBA_HUMAN
1    MVHLTPEEKSAVTALWGKVNV--DEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGN   58  P68871   HBB_HUMAN
1    MVHLTPEEKTAVNALWGKVNV--DAVGGEALGRLLVVYPWTQRFFESFGDLSSPDAVMGN   58  P02042   HBD_HUMAN
      : *:* :*: *.* **** .     *.*** *::: :* *: :*  *          *.

54   AQVKGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAH  113  P69905   HBA_HUMAN
59   PKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHH  118  P68871   HBB_HUMAN
59   PKVKAHGKKVLGAFSDGLAHLDNLKGTFSQLSELHCDKLHVDPENFRLLGNVLVCVLARN  118  P02042   HBD_HUMAN
      :**.*****  *:::.:**:*::  ::: **:**..**:*** **:**.. *: .** .

114  LPAEFTPAVHASLDKFLASVSTVLTSKYR  142  P69905   HBA_HUMAN
119  FGKEFTPPVQAAYQKVVAGVANALAHKYH  147  P68871   HBB_HUMAN
119  FGKEFTPQMQAAYQKVVAGVANALAHKYH  147  P02042   HBD_HUMAN
:  **** ::*: :*.:*.*:..*: **:
```

- Hemoglobin (left) is a iron-containing protein that transports oxygen.
- The protein present in erythrocytes of almost all vertebrates.
- The protein is essential, therefore the mRNA is relatively stable and highly translated.

LNP, modified RNA, and 5'-UTR of HBA are all essential to make effective *and safe* vaccines against coronavirus



HBA1: the human α-globin gene

References: Heinz, Franz X., and Karin Stiasny. "Distinguishing Features of Current COVID-19 Vaccines: Knowns and Unknowns of Antigen Presentation and Modes of Action." Npj Vaccines 6, no. 1 (August 16, 2021): 1–13. https://doi.org/10.1038/s41541-021-00369-6; Assemblies of putative SARS-CoV2-spike-encoding mRNA sequences for vaccines BNT-162b2 and mRNA-1273 (github.com/NAalytics); Xia, Xuhua. "Detailed Dissection and Critical Evaluation of the Pfizer/BioNTech and Moderna MRNA Vaccines." Vaccines 9, no. 7 (July 3, 2021): 734. https://doi.org/10.3390/vaccines9070734.

A summary of what we have learned so far in the context of coronavirus

1. What is the unmet medical need to be addressed? We need a vaccine to prevent a large population of individuals from being infected by coronavirus, which have severe consequences.
2. What are the target(s) of our drug? Spike protein is conserved: immune reaction is desired.
3. Where should the drug go in patient's body, what does body do to the drug, and what does the drug do to the body? Thanks to LNP, N1-mythel-Ψ, and 5'-UTR of HBA1, the mRNA vaccination can enter cells with minimal side effects. In cells, spike protein RNA is synthesized into proteins, which are digested, presented, and elicit immune response.
4. What is the safety profile of the drug in light of its benefits? Initial study: Polack, F. P. et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. New England Journal of Medicine 383, 2603–2615 (2020), and watch this video.
5. Who are responsive to the drug, or susceptible to adverse events? Updated regularly by regulatory agencies, for instance European Medicines Agency.

Conclusions

1. Drug discovery is an interdisciplinary effort to solve medical and technical challenges.
2. Biological understanding, including sequence analysis, is key for indication and target selection.
3. Modern drug discovery needs to address five key questions:
    a. **Unmet medical need**
    b. **Target(s) and modalities**
    c. **Pharmacokinetics** (what body does to the drug) **and pharmacodynamics** (what the drug does to the body)
    d. **Safety** (benefit/risk assessment)
    e. **Patient enrichment/stratification**