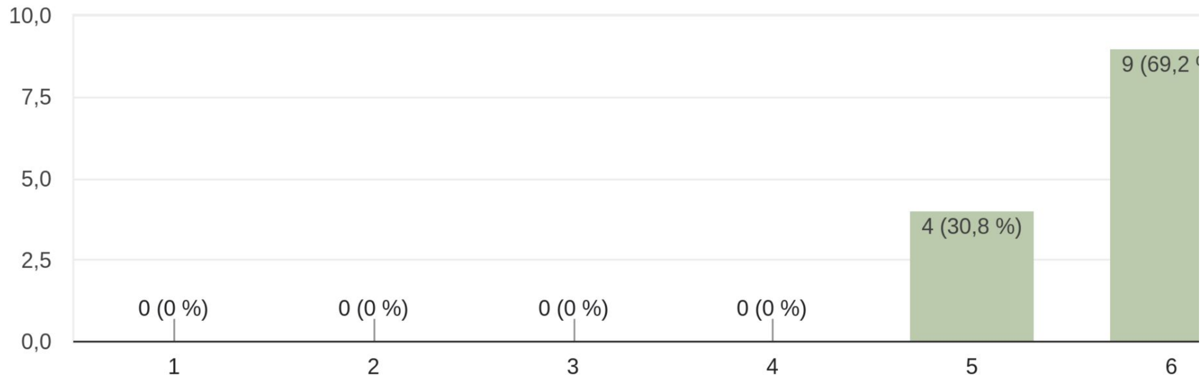
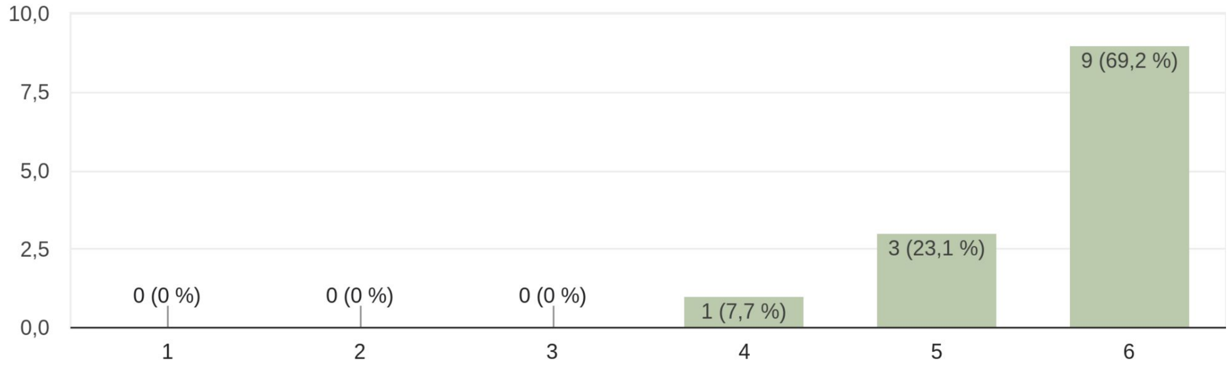
Thank you for the feedback - keep doing it and remain critical!

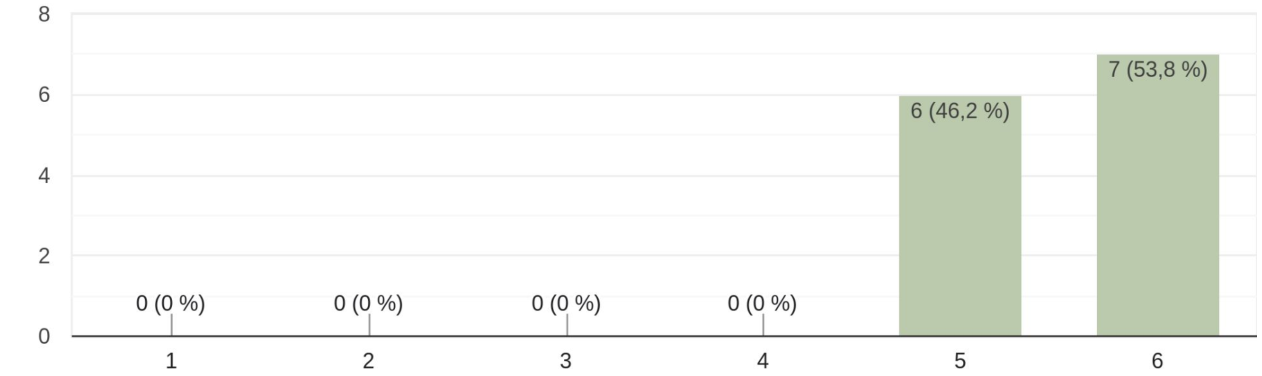
How was your overall impression of the third lecture?
13 Antworten



How well could you understand and follow David (the lecturer)?
13 Antworten



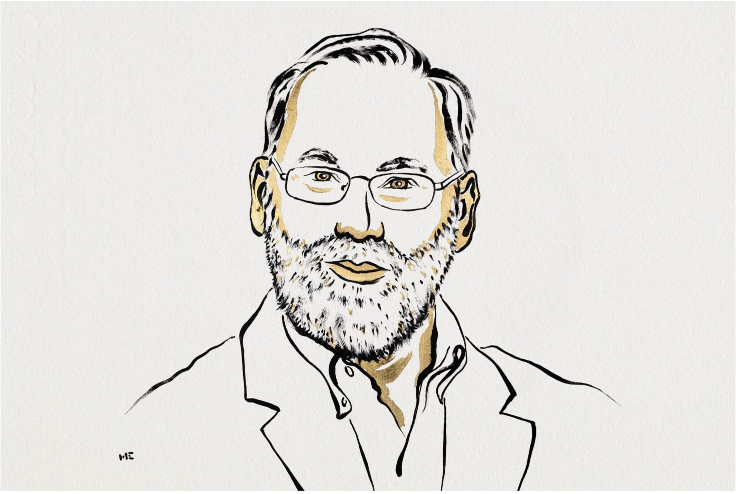
How did you experience the interactions between your peers and David, and among the peers?
13 Antworten



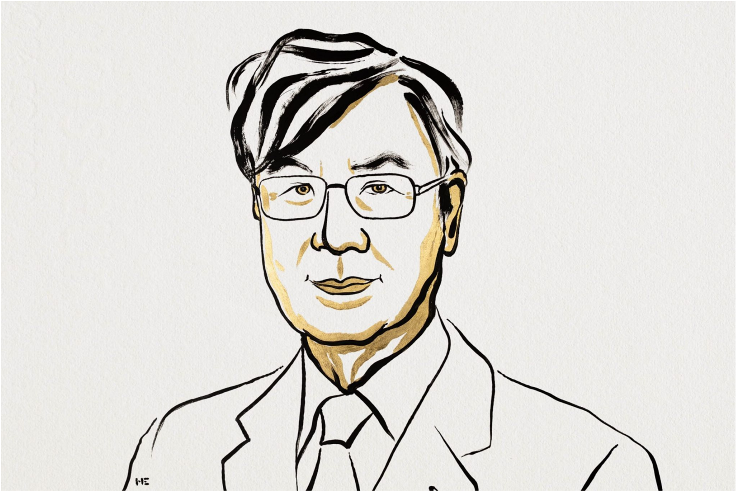
- + Teamwork and interaction wished
- + So far easy to follow and understandable
- + Good peer and lecture interaction
- + Team discussions are highly valuable
- \- Role play can be shorter but was interesting
- \- More insights on how decisions are made

AMIDD Lecture 4: Biological foundation of drug discovery

Mary E. Brunkow



Fred Ramsdell



Shimon Sakaguchi

Nobel Prize in Physiology or Medicine 2025 acknowledges *groundbreaking discoveries concerning peripheral immune tolerance that prevents the immune system from harming the body*.

Dr. Jitao David Zhang, Computational Biologist

[1] *Pharmaceutical Sciences, Pharma Research and Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche*

[2] *Department of Mathematics and Informatics, University of Basel*

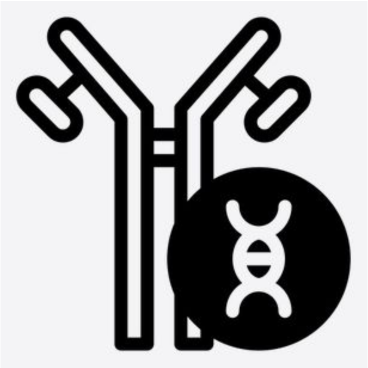
Topics of lecture 4

- **Key questions of drug discovery and the biological foundations in the context of Covid-19 vaccine**

Five key questions in drug discovery

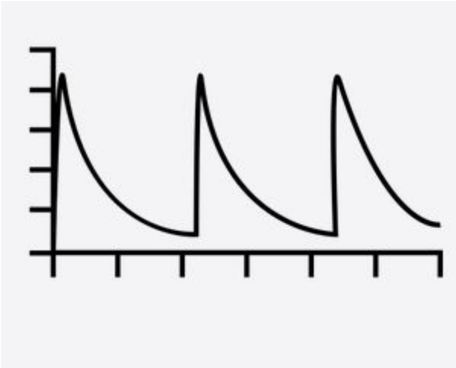
Medical Need

What is the unmet medical need to be addressed?



Target & modality

What is the target? What is the modality?

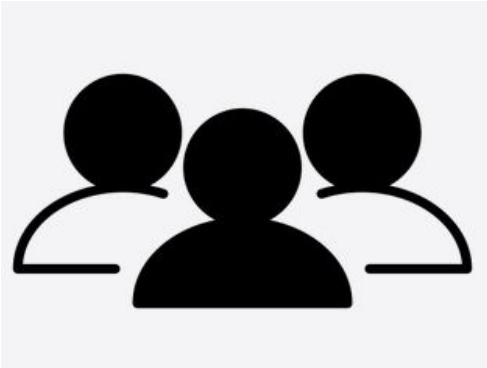


PK/PD

How much drug reach which body part? What does body do to the drug (PK)? What does the drug do to the body (PD)?

Benefit/risk

What is the toxicity of the drug? Is it justifiable given the benefits?



Patient stratification

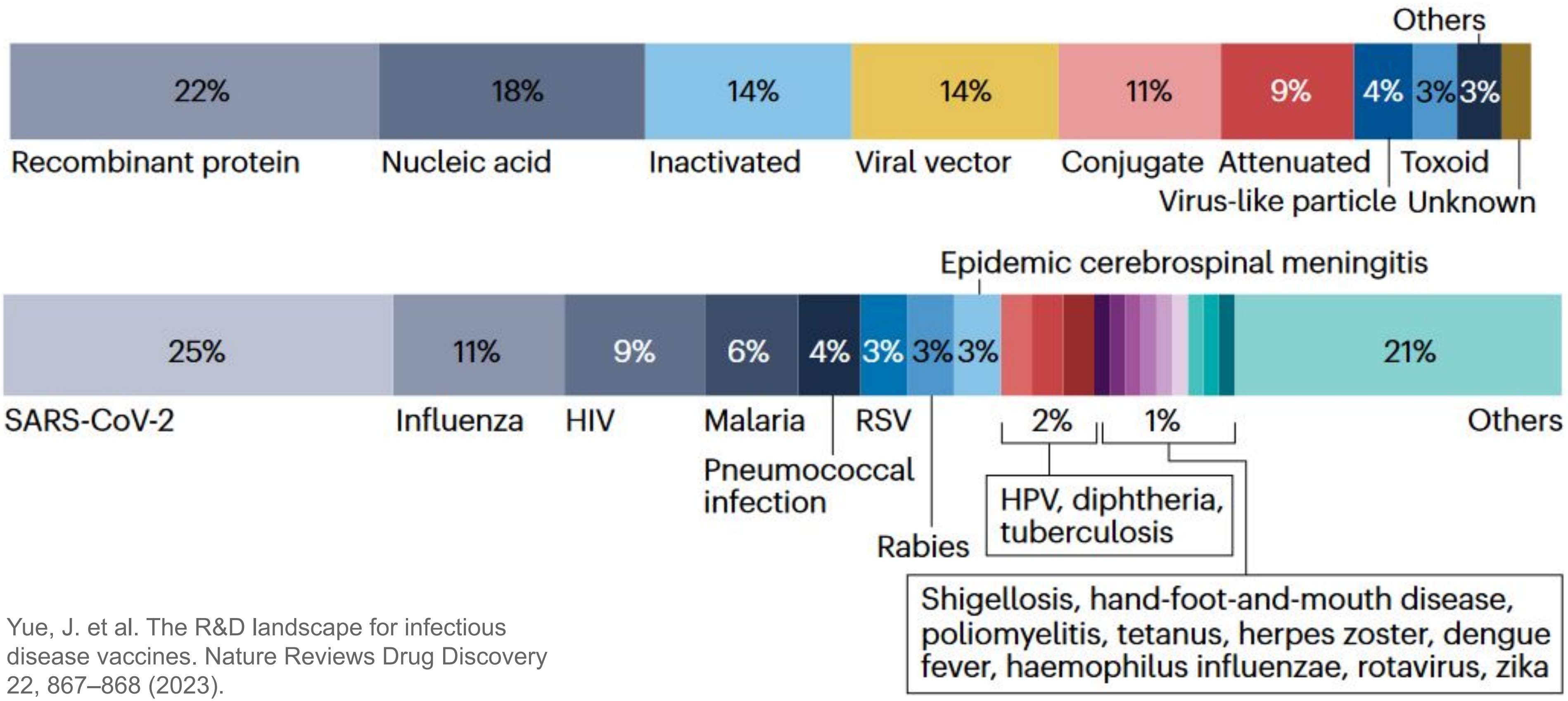
Who are responsive to the drug? Who are susceptible to adverse events?

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”

Main methods for vaccine production before the COVID-19 pandemic:

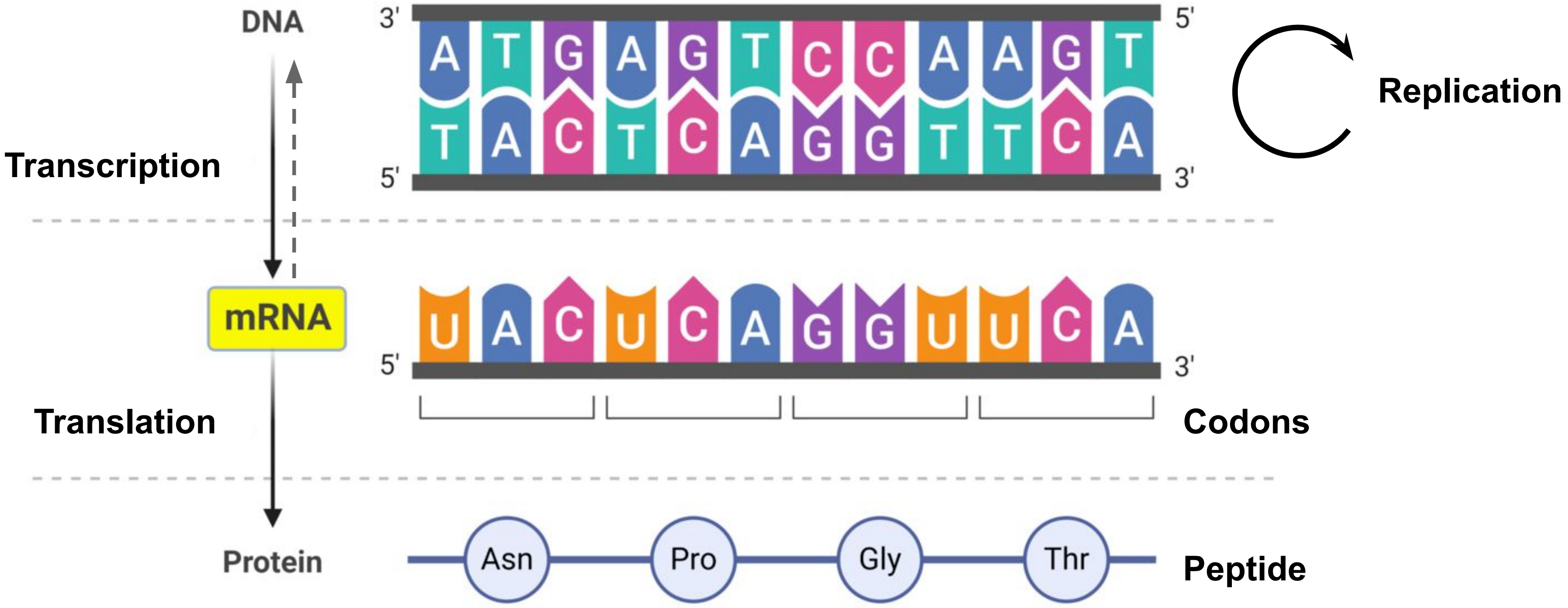
- **Recombinant protein** (e.g. HBV)
- **Inactivated viruses** (e.g. Influenza and Polio)
- **viral vectors** (e.g. HIV).

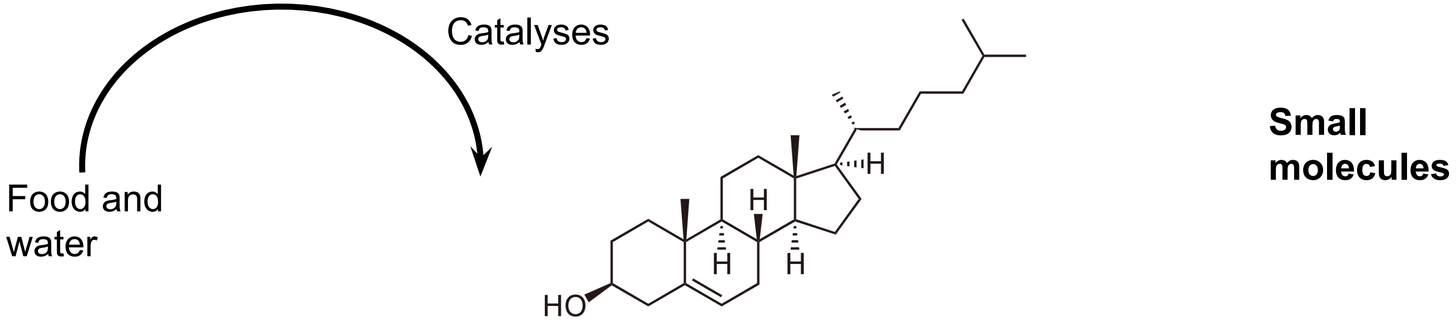
Issues: large-scale cell culture is required, which limits the possibilities for rapid production in response to pandemics.



Yue, J. et al. The R&D landscape for infectious disease vaccines. Nature Reviews Drug Discovery 22, 867–868 (2023).

The central dogma applies to human and viruses including SARS-CoV-2





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

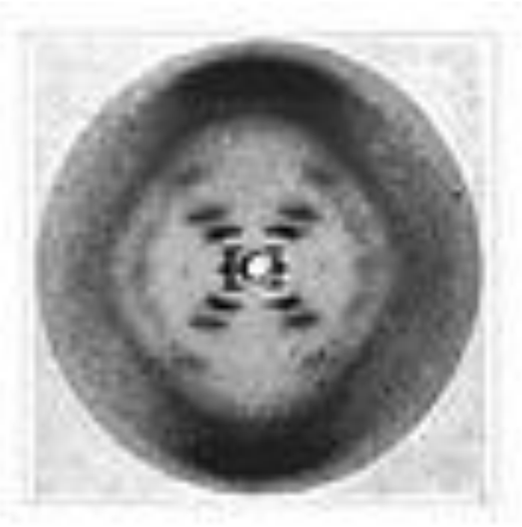
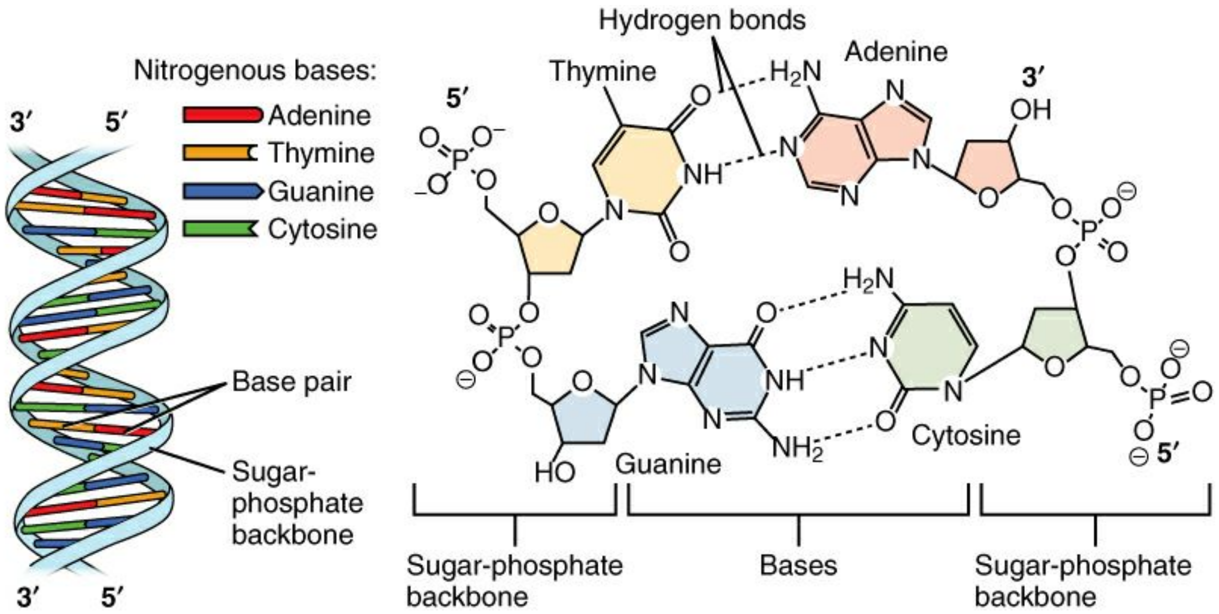
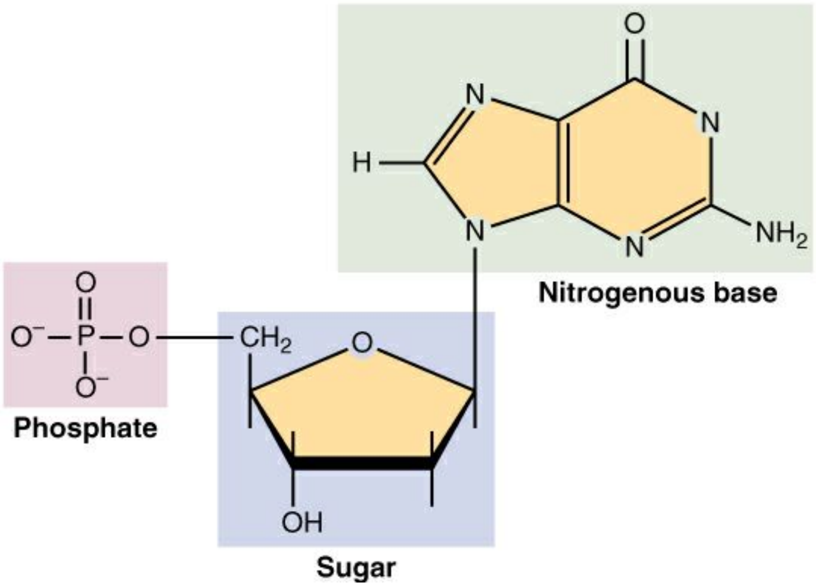


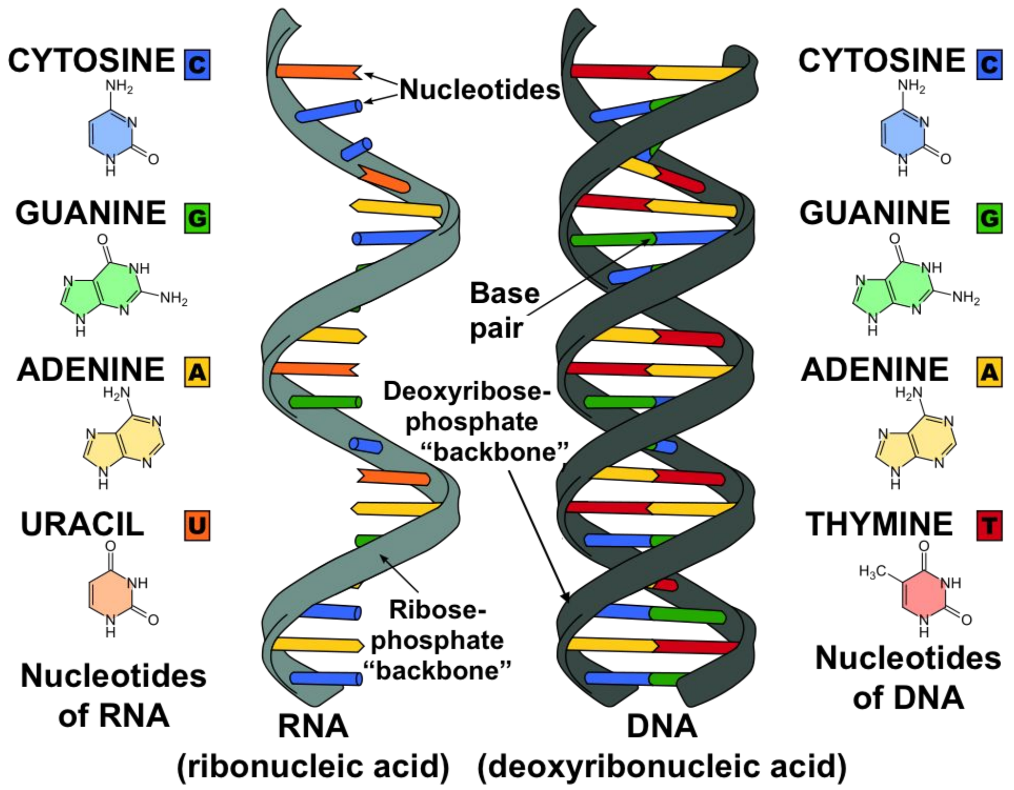
Photo 51, X-ray diffraction image of DNA

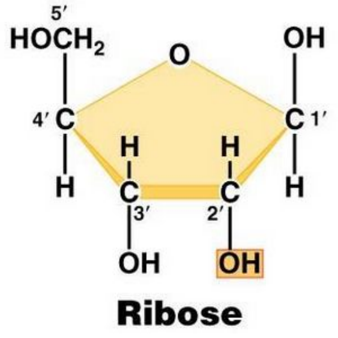
Franklin R, Gosling RG (1953) "Molecular Configuration in Sodium Thymonucleate". ***Nature*** 171**: 740–741.**

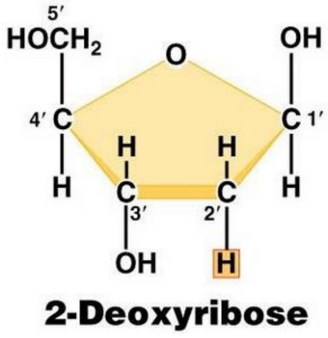




RNA is transcribed from DNA







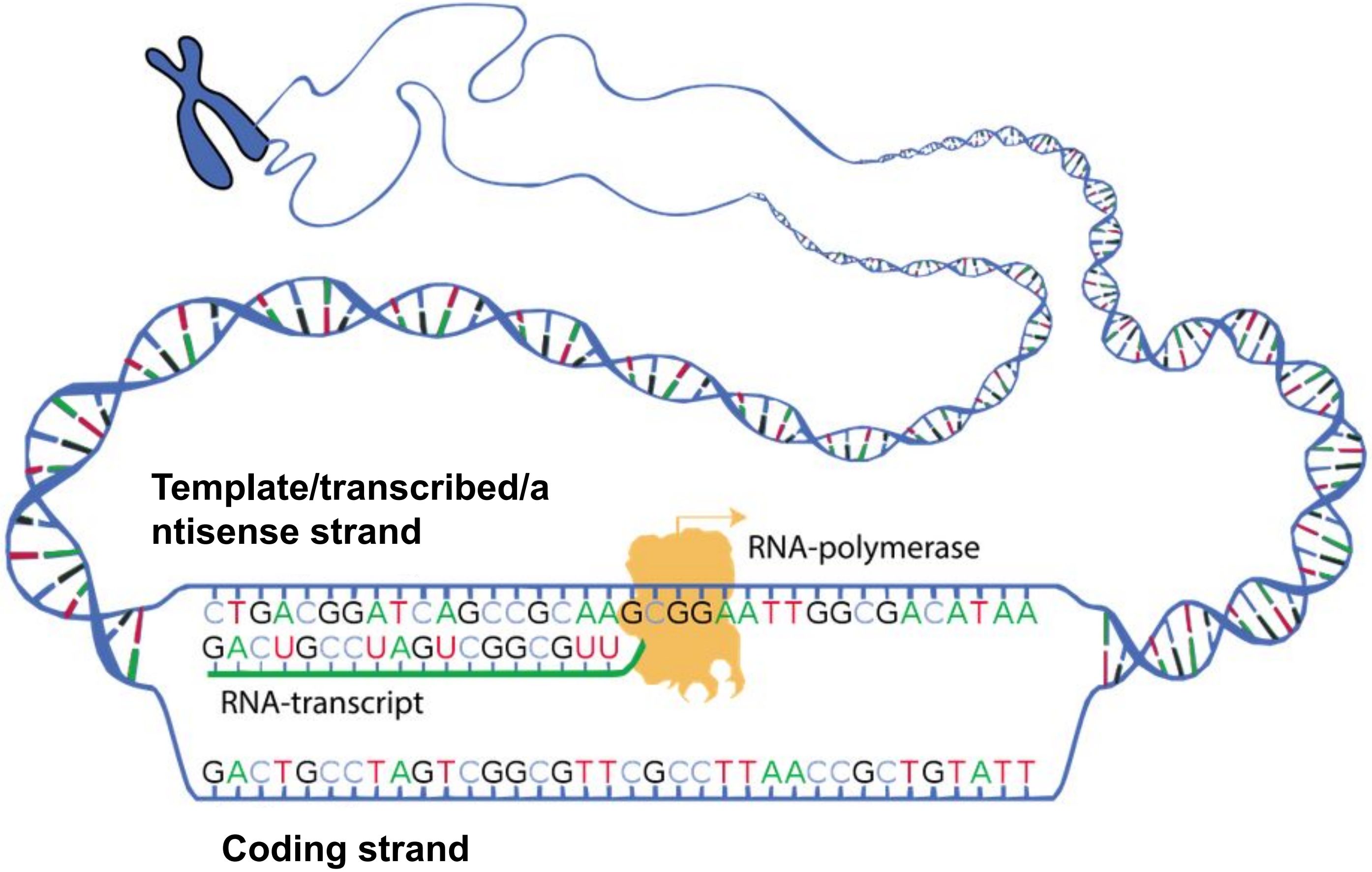


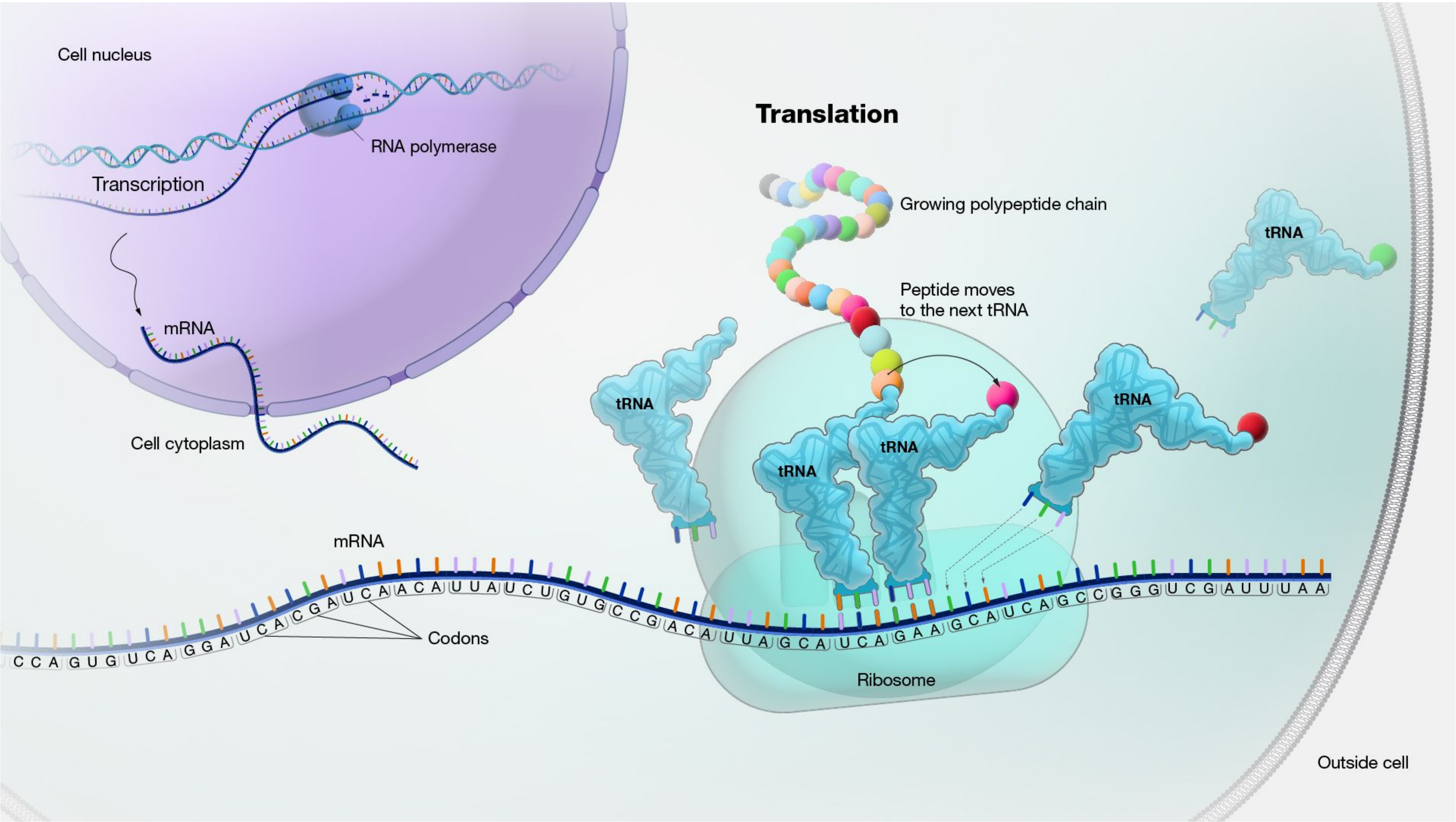
Figure: https://commons.wikimedia.org/wiki/File:DNA_transcriptie.svg and https://en.m.wikipedia.org/wiki/File%3AHAR1F_RF00635_rna_secondary_structure.jpg. Original work by wikipedia user: OrgreBot and user:Ppgardne. Used under CC-SA 3.0 license.

mRNA is translated into proteins

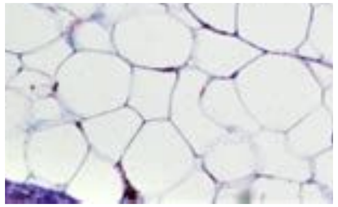
Information encoded in messenger RNA directs the addition of amino acids during protein synthesis.

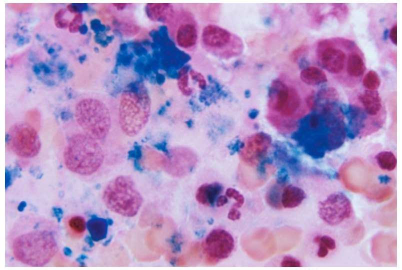
The process, known as *translation*, takes place on ribosomes in the cell cytoplasm.

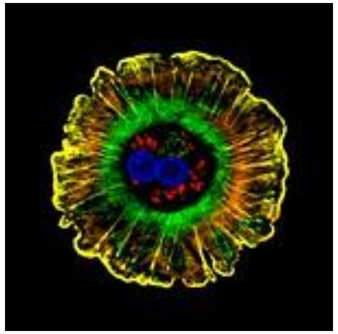
Different cells have different DNA segments ‘active’ and different RNAs/proteins expressed.

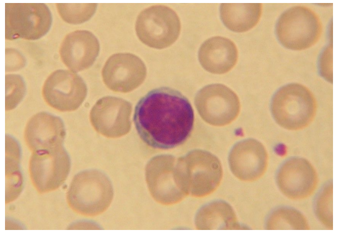


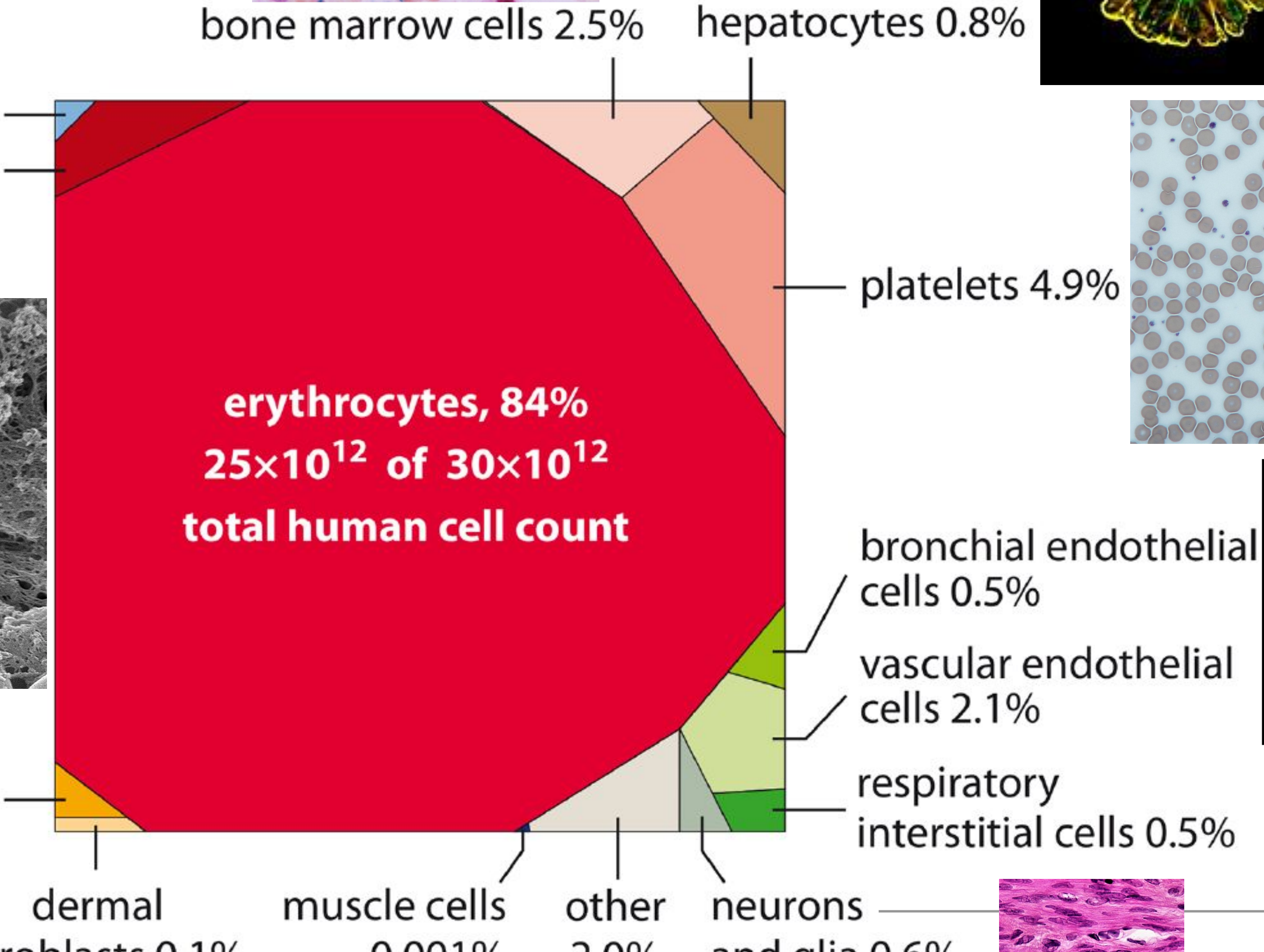
Our body contains a variety of cells

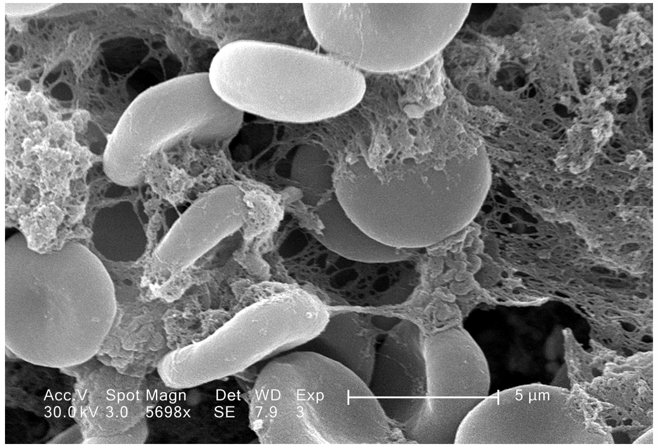


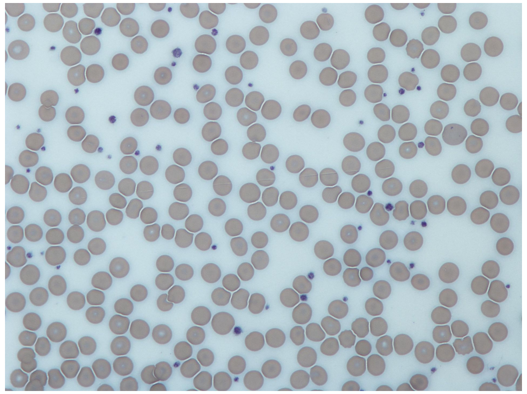


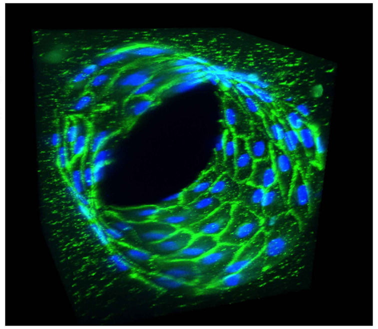


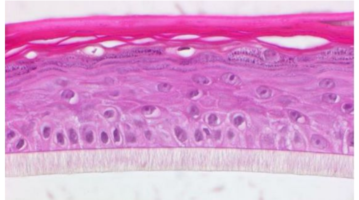


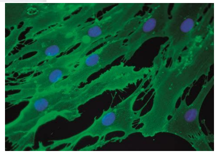


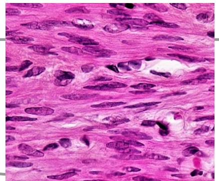


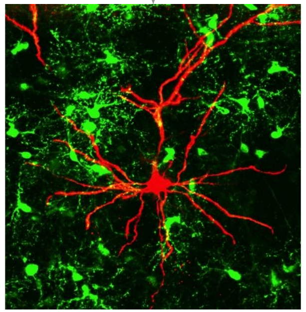




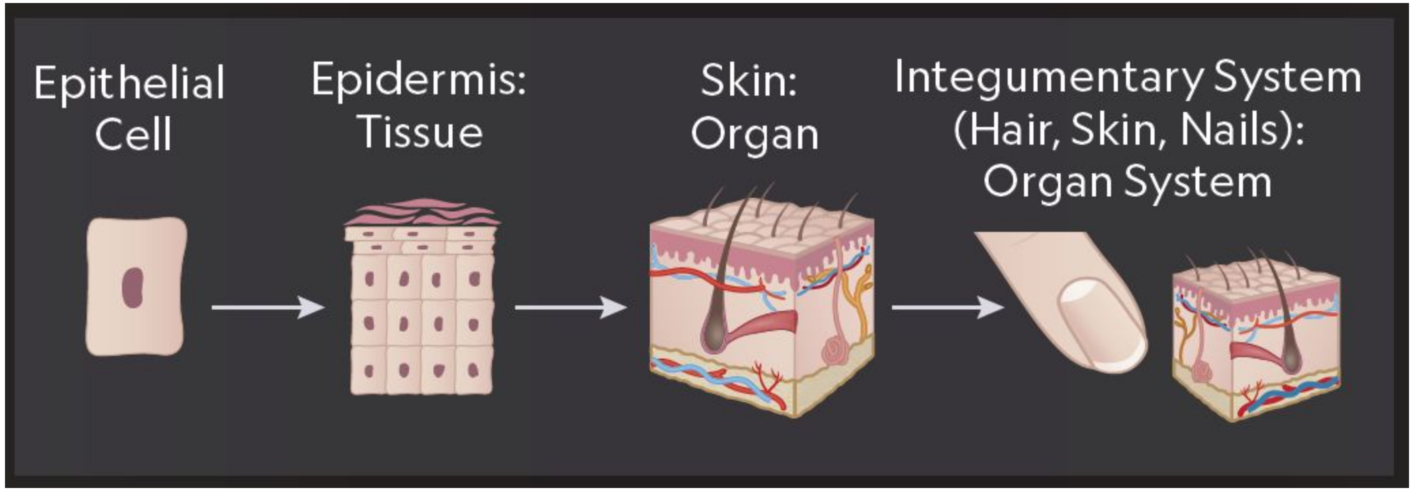








A hierarchical system of cells, tissue, organ, organ system and human body



Cells: basic building blocks, variable morphologies and functions

Tissues: groups of specialized cells that communicate and collaborate

Organ: group of tissues to perform specific functions

Organ systems: group of organs and tissues

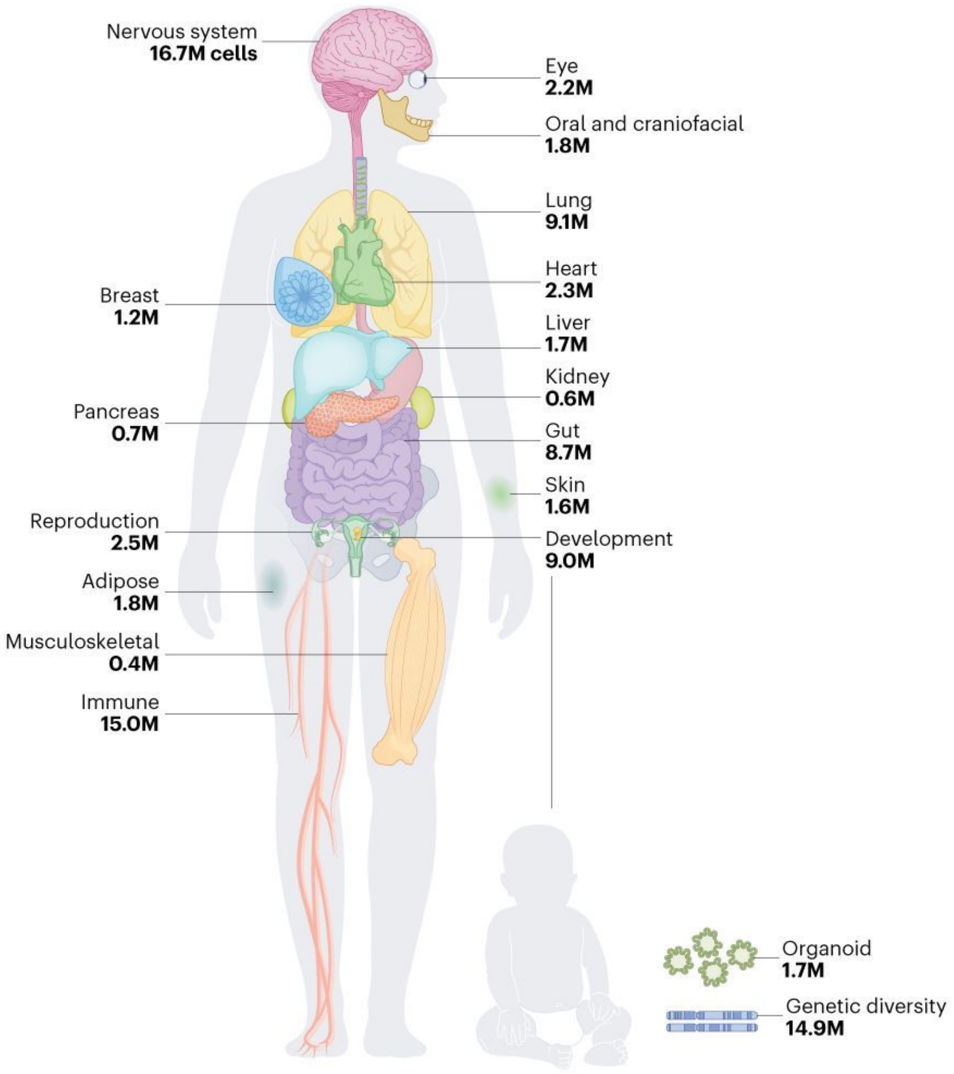
Human Cell Atlas: a consortium effort creating a 3D map of human cells

Established in 2016, the Human Cell Atlas (HCA) consortium set out to create a comprehensive biological map of cells within the human body.

See a collection of articles celebrating the first draft of the atlas, and find out more about the resource at its website, https://www.humancellatlas.org/.

The effort is a typical reductionist approach to study human biology.

An open question is the benefit and risk of an reductionist approach, and for what purposes and under which circumstances, a holistic approach would be more advantageous.



18 HCA Biological Network Atlases, as of end 2024. Numbers: cells profiled for which data have been generated.

Reductionist *versus* holistic approach

A Sunday Afternoon on the Island of La Grande Jatte, George Seurat
Example inspired by Derek Lowe, author of *In the Pipeline*

Extended reading on reductionist and holistic approaches: antibiotics research

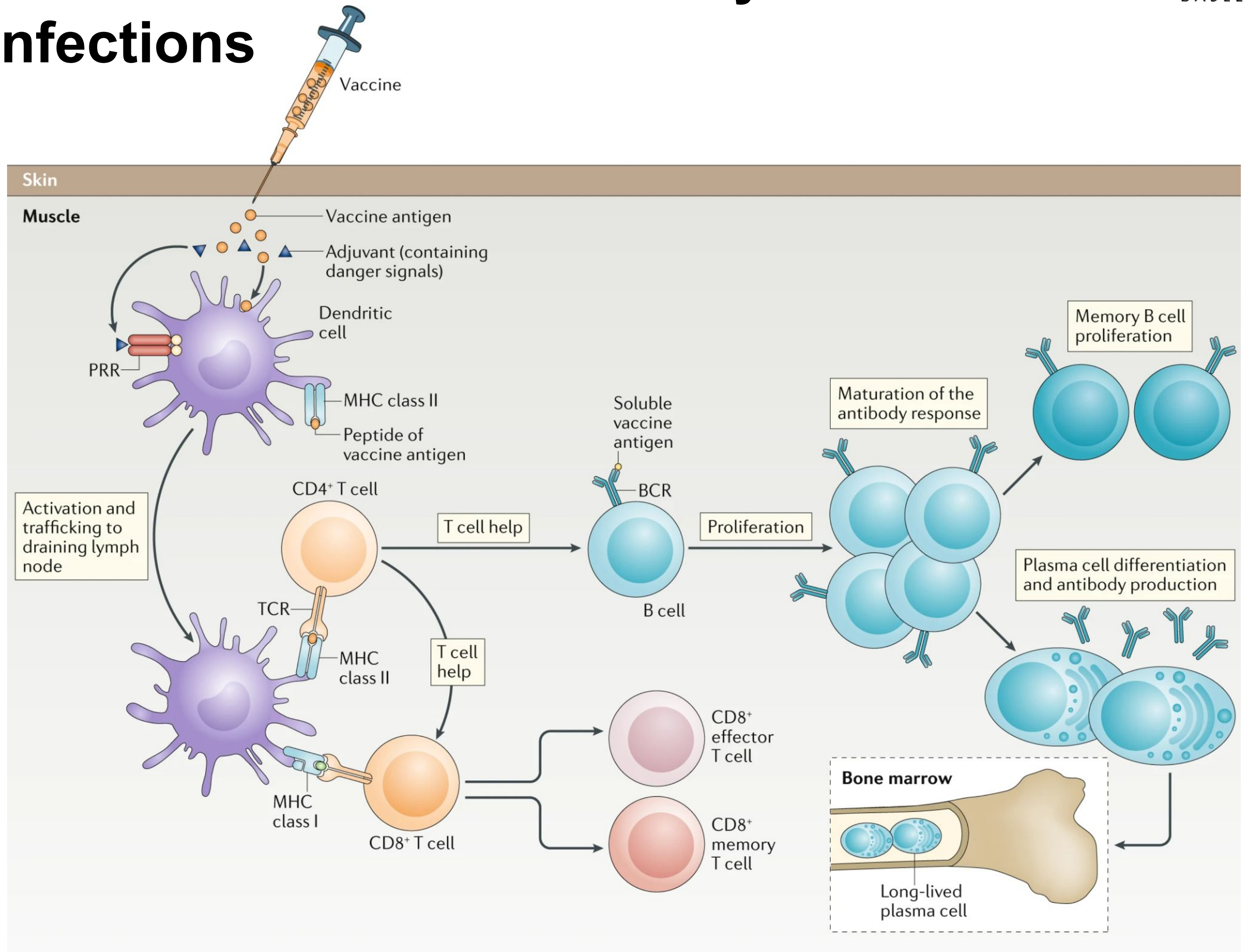
- *Drugs for bad bugs: confronting the challenges of antibacterial discovery*, Payne *et al.*, Nature Reviews Drug Discovery, 2006: https://www.nature.com/articles/nrd2201, an industrial perspective on learnings from failed reductionist approaches;
- *Recover the lost art of drug discovery,* Kim Lewis, Nature, 2012, https://www.nature.com/articles/485439a, recounting the Waksman platform discovering antibiotics from soil bacteria;
- *The Science of Antibiotic Discovery,* Kim Lewis, Cell, 2020: https://www.cell.com/cell/fulltext/S0092-8674(20)30233-6, highlighting recent trends and future directions of antibiotics discovery and development;

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.

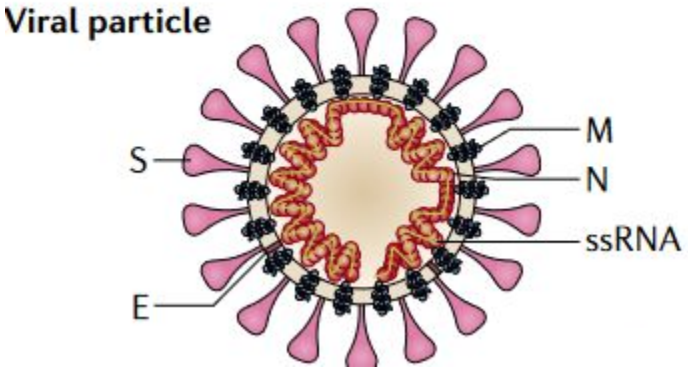
Key players in the game:

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).

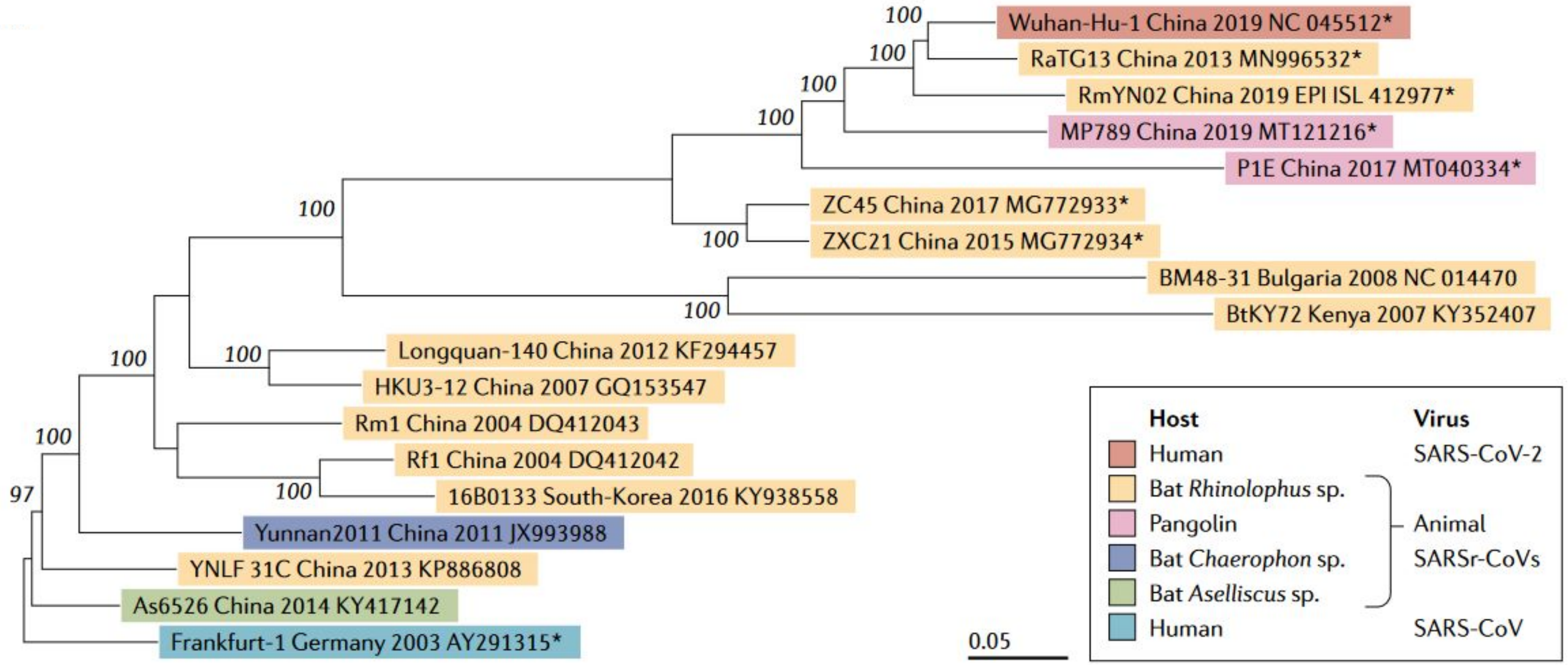


Pollard, A. J. & Bijker, E. M. A guide to vaccinology: from basic principles to new developments. Nature Reviews Immunology 21, 83–100 (2021).

Coronavirus is a RNA virus infecting human and other species



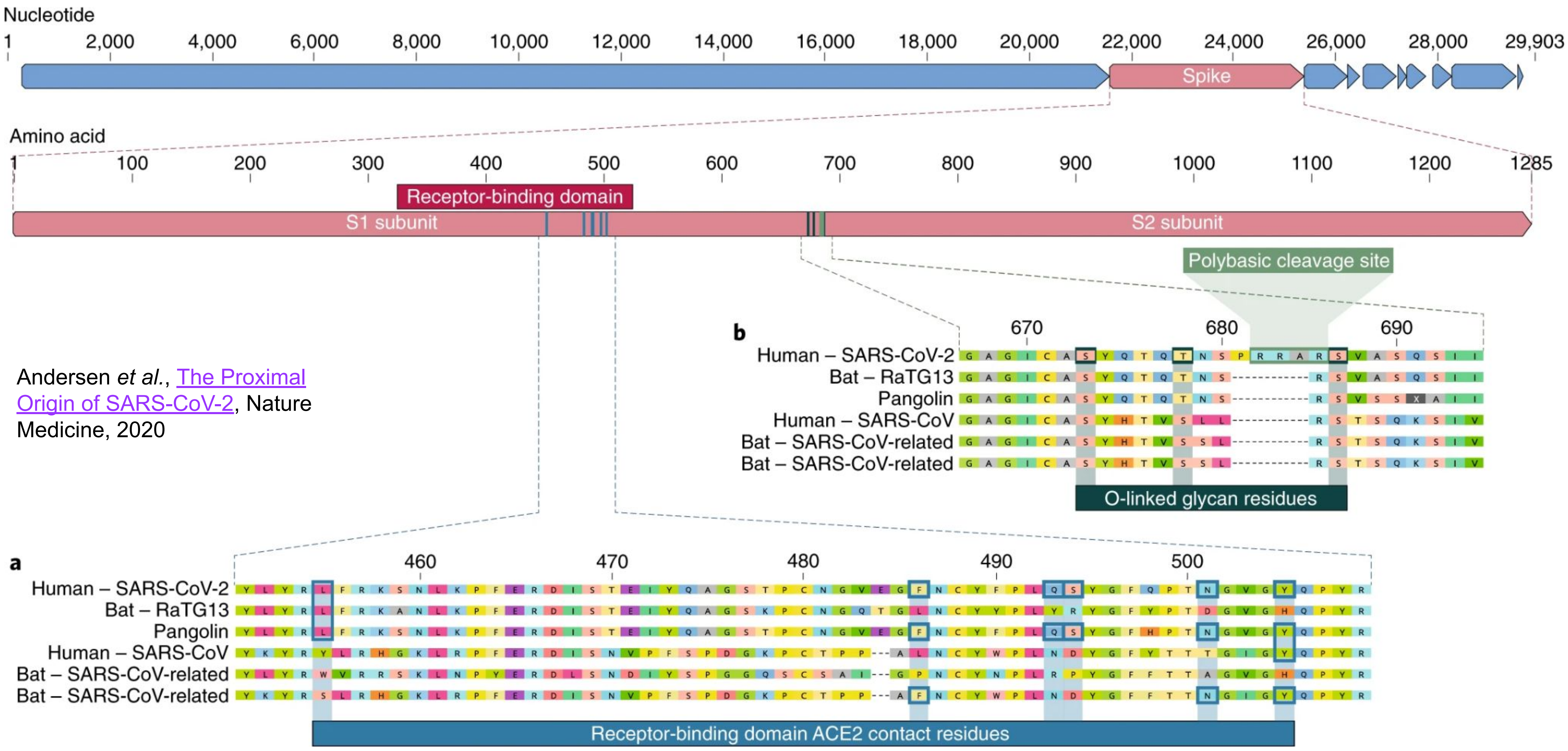
The coronavirus virion consists of structural proteins, namely spike (S), envelope (E), membrane (M), nucleocapsid (N)



Phylogenetic relationships of representative members of the species Severe Acute Respiratory Syndrome (SARS)-related coronavirus

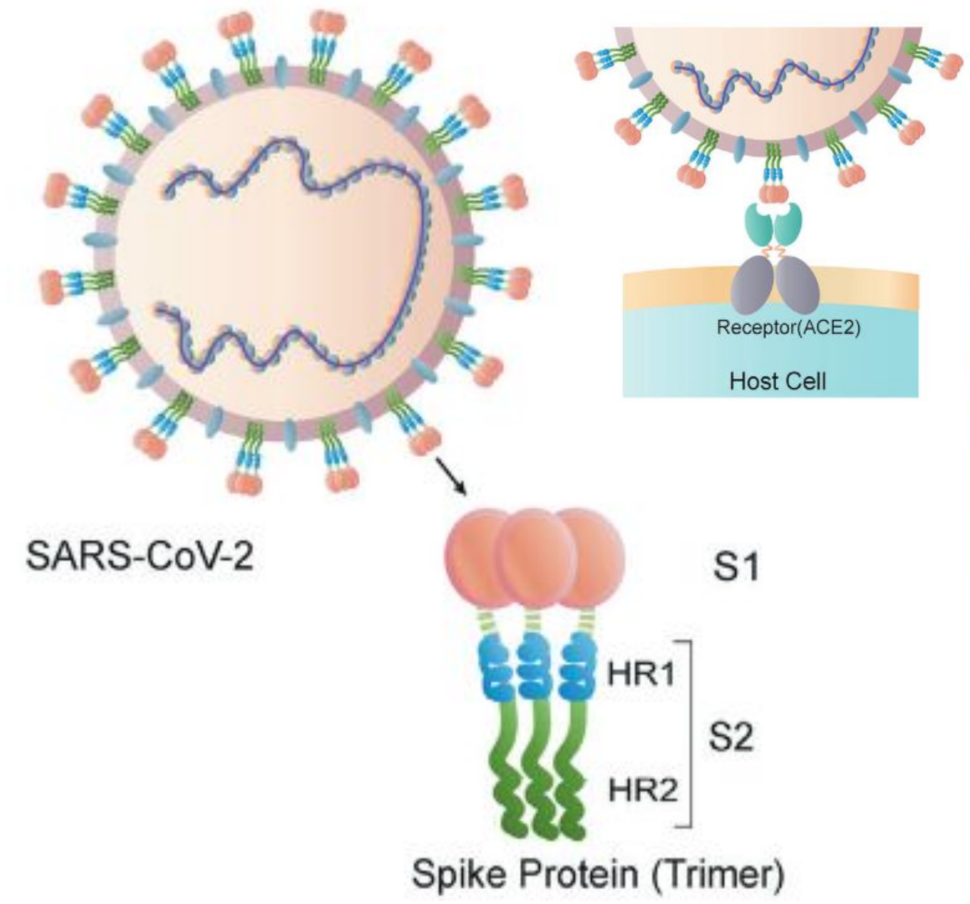
V'kovski, P., Kratzel, A., Steiner, S., Stalder, H. & Thiel, V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol 19, 155–170 (2021).

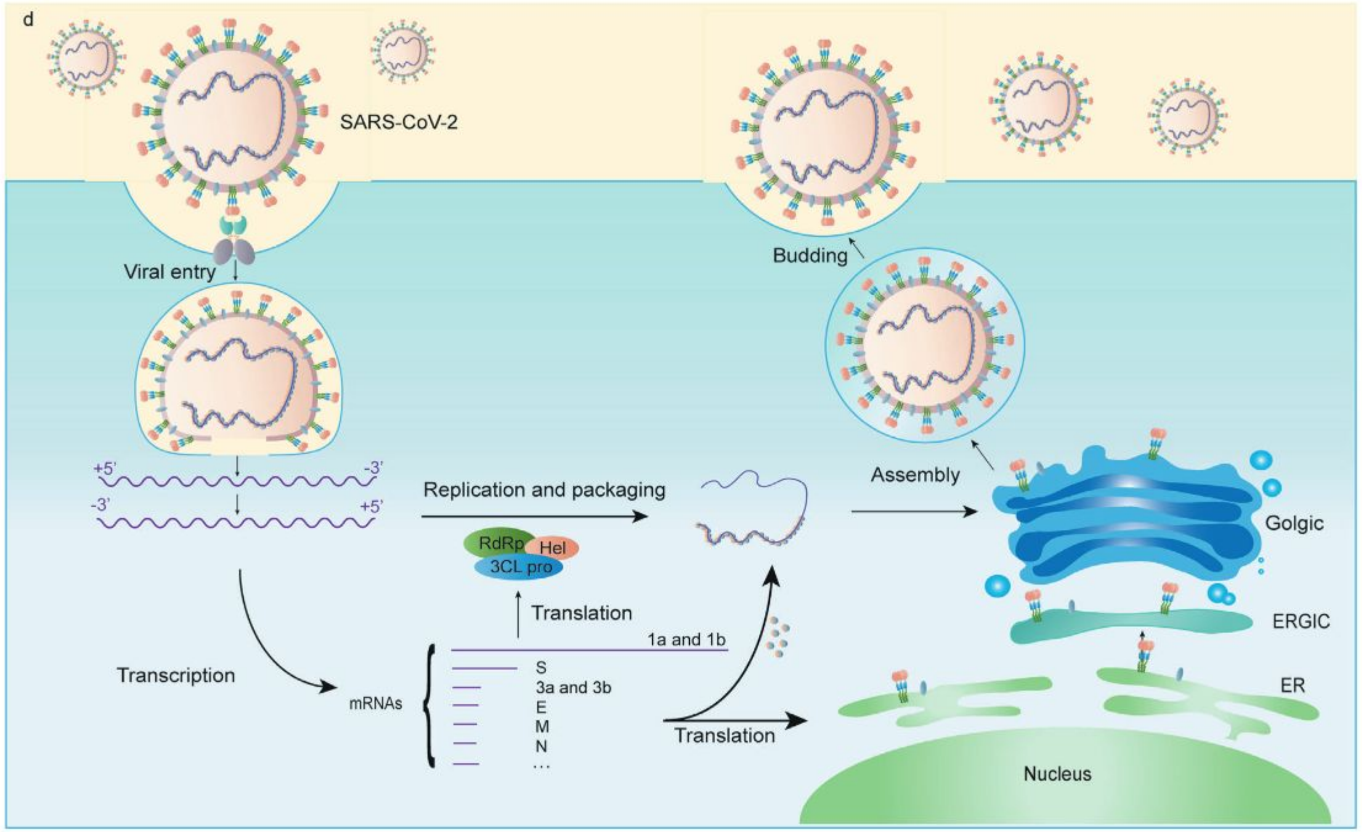
Sequence of the spike protein is largely conserved between SARS-CoV-2 and related viruses



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



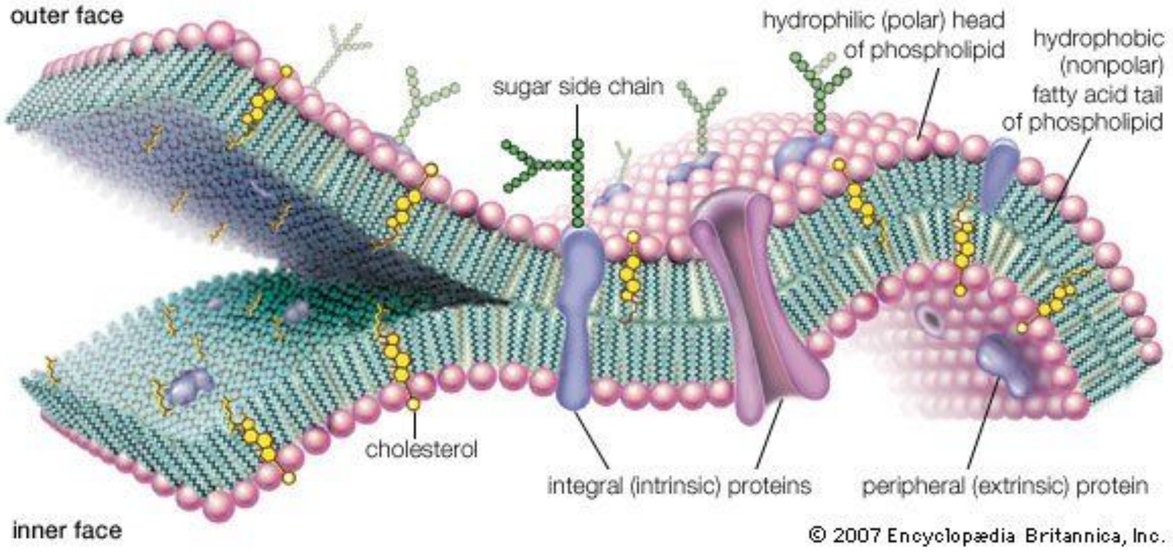


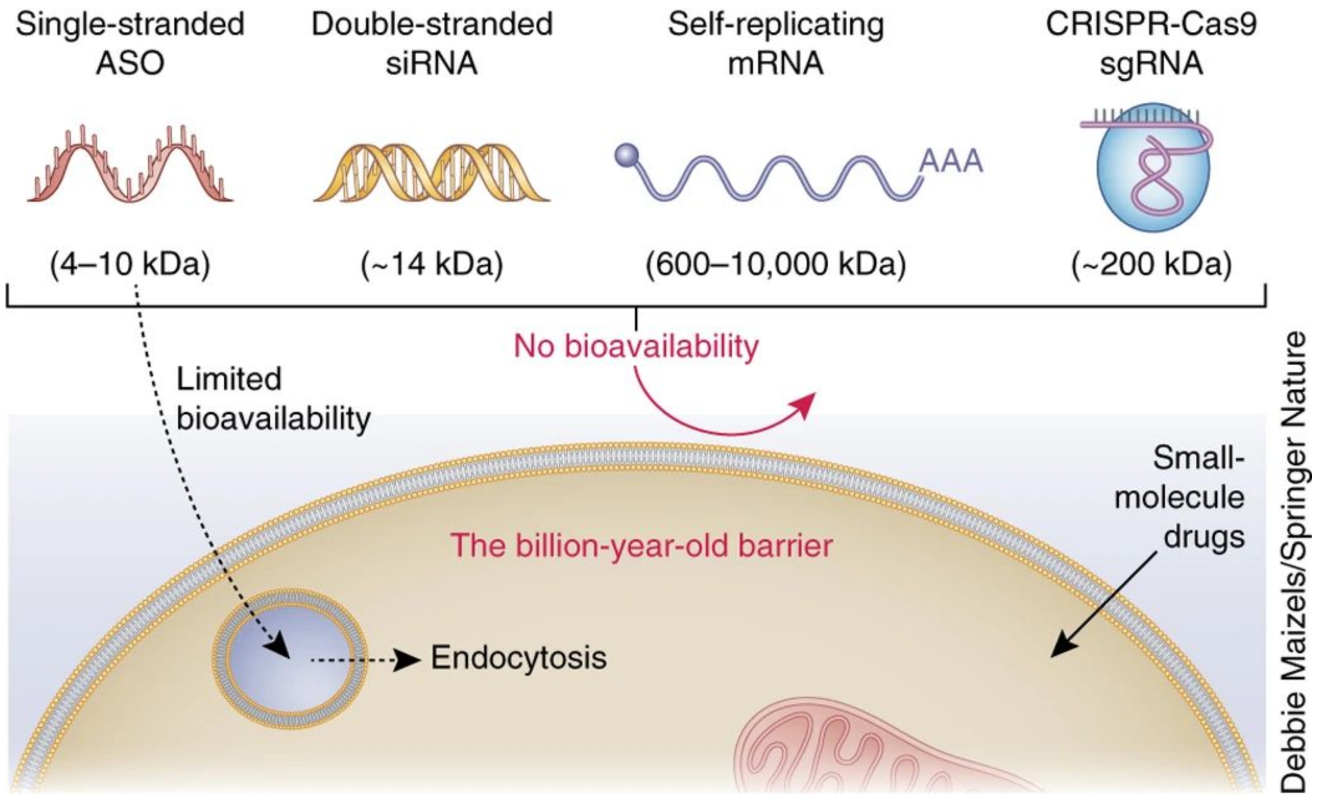
Huang, Y., Yang, C., Xu, X., Xu, W. & Liu, S. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin 41, 1141–1149 (2020).

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





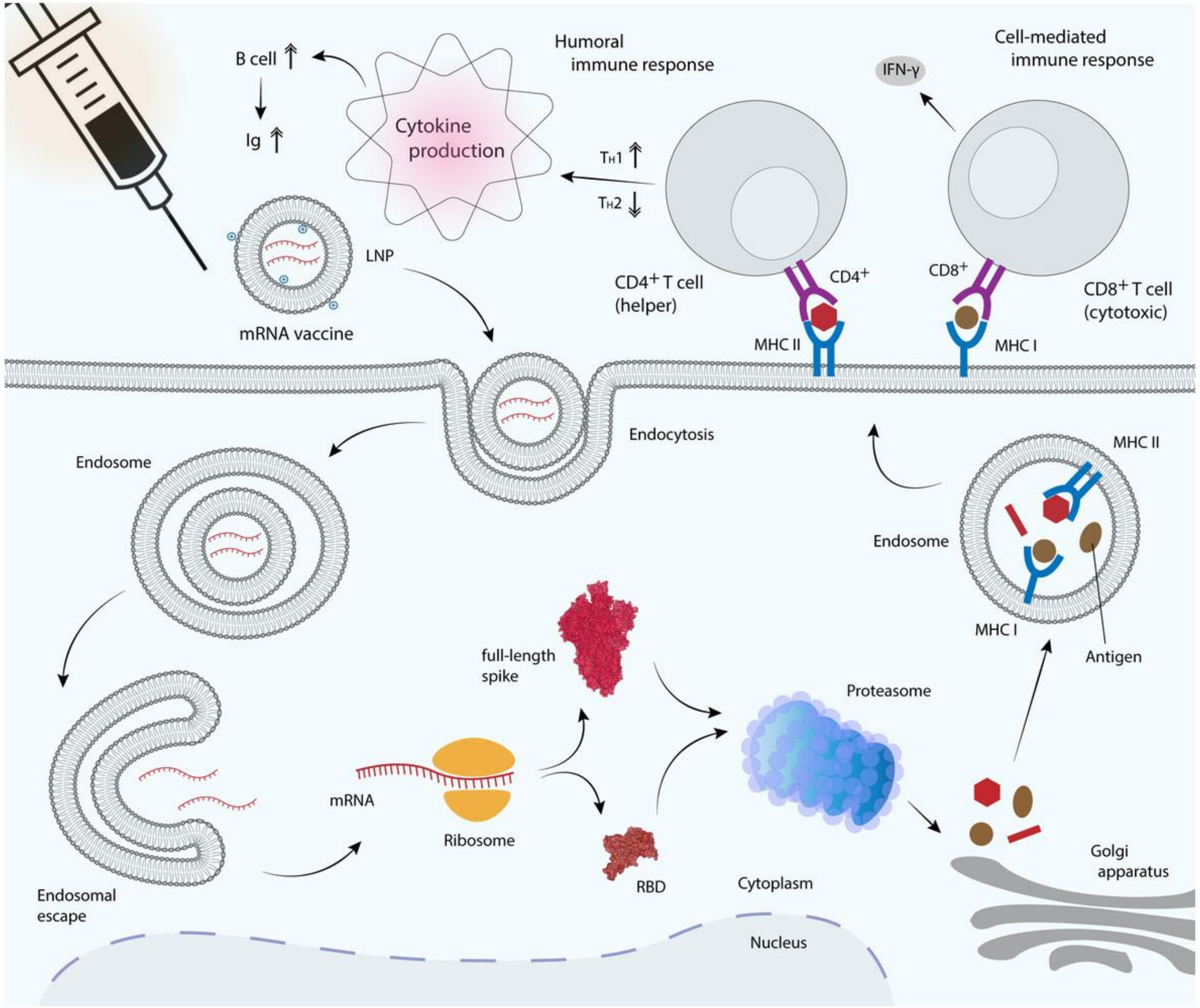
Key challenges:

- mRNAs are too large and charged to pass lipid bilayers.
- mRNAs are degraded, e.g. by ribonucleases.
- *Exogenous* mRNAs cause *immunogenicity*.

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

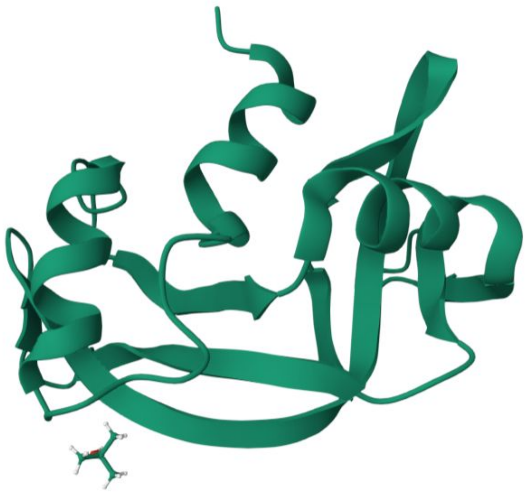
Lipid NanoParticles (LNP) helps delivering RNAs into cells

- 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.

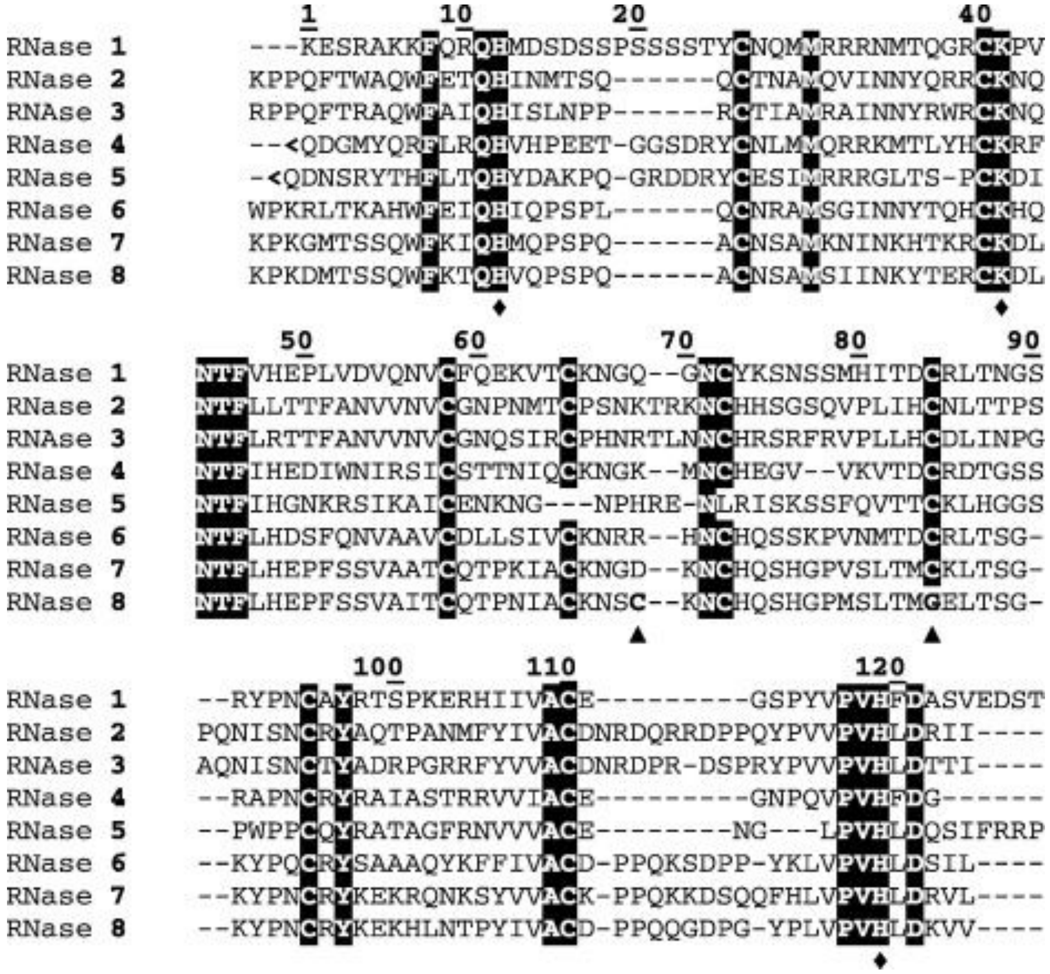


Salleh, Mohd Zulkifli *et al.* "[Immunogenicity Mechanism of mRNA Vaccines and Their Limitations in Promoting Adaptive Protection against SARS-CoV-2.]" PeerJ 10 (March 9, 2022)

RNAs are degraded by proteins known as ribonucleases (RNAases)



mRNAs are degradable by ribonucleases (RNases). RNases belong to *enzymes*, a class of proteins that catalyse chemical reactions.



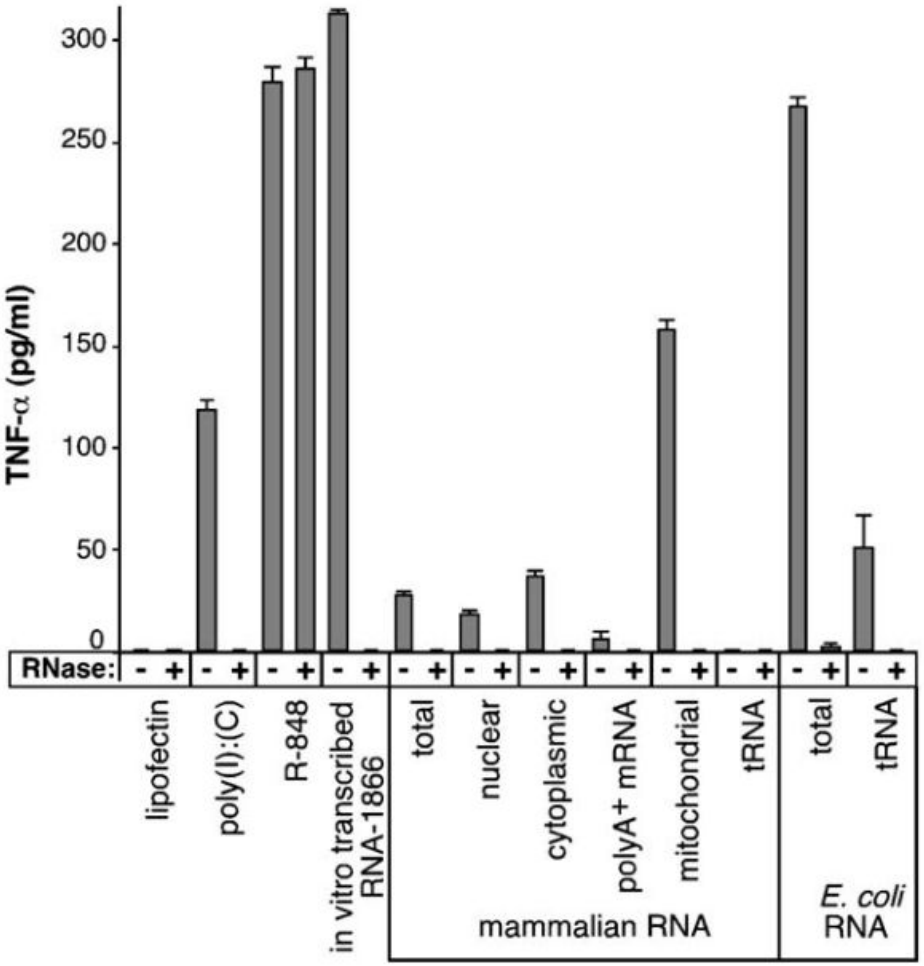
Left: Structure of PDB 7RSA. Right: alignment of protein sequences of 8 canonical human RNases (ribonuclease A family). Sorrentino FEBS Letters, 2010.

Unmodified RNA induces unwanted immune reactions: modifying RNA can reduce or remove them

Exogenous **RNAs induce immunogenicity.** RNAs are synthesized from four ribonucleotides: ATP (adenosine triphosphate), CTP (cytidine triphosphate), UTP (uridine triphosphate), and GTP (guanosine triphosphate).

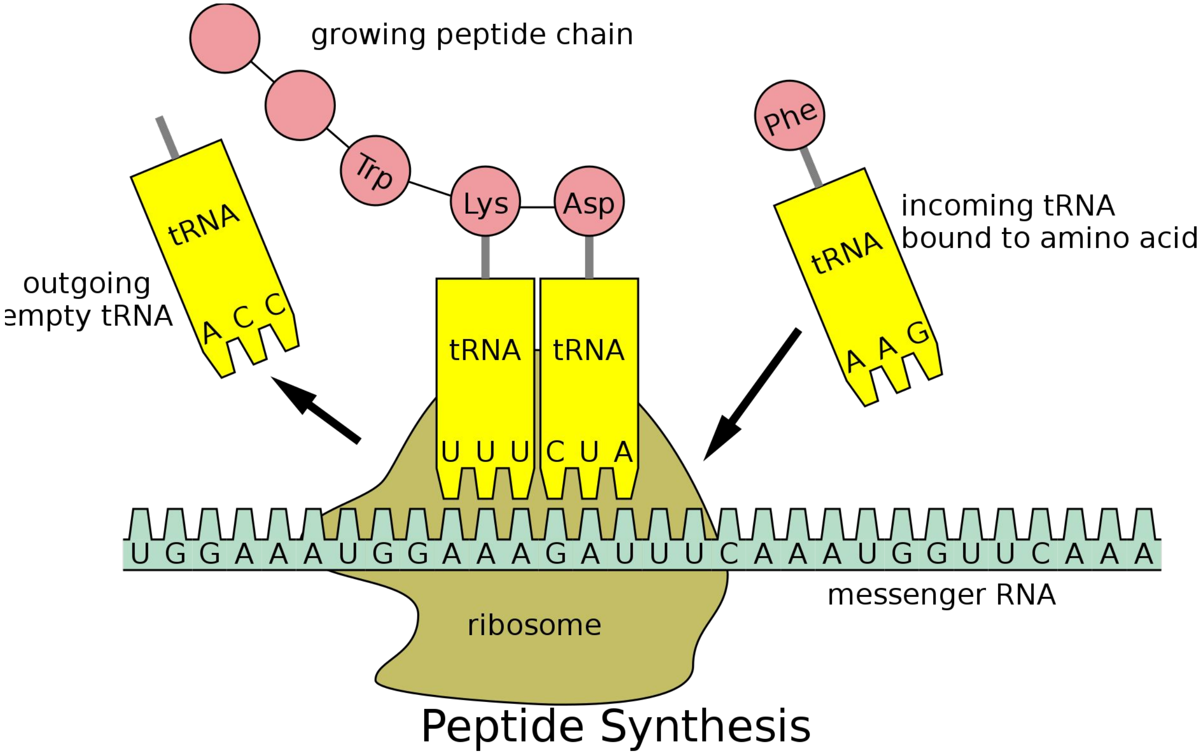
When unmodified RNAs are delivered into cells, they induce unwanted immune reaction. They activate the surface proteins known as Toll-like receptors (TLRs), which leads to the release of cytokines including the tumor necrosis factor alpha (TNF-alpha). TLRs and TNF-alpha are also activated by bacterial and viral infections and mediate their killing.

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

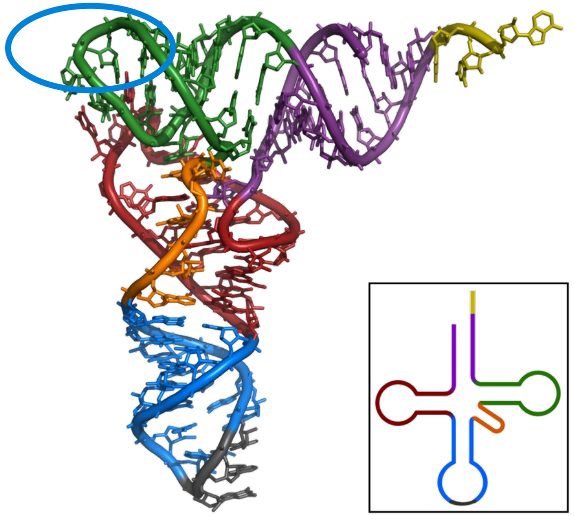


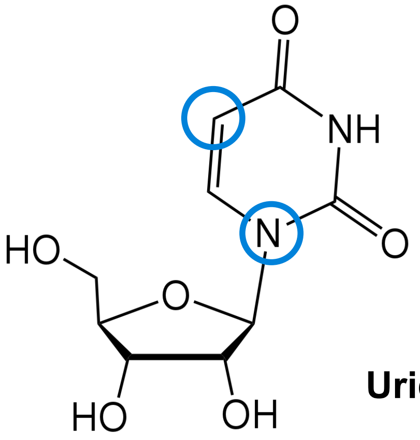
Karikó, K., Buckstein, M., Ni, H. & Weissman, D. Suppression of RNA Recognition by Toll-like Receptors: The Impact of Nucleoside Modification and the Evolutionary Origin of RNA. Immunity 23, 165–175 (2005).

Human tRNA contains *pseudouridine*, a modified uridine, which does not induce immunogenicity

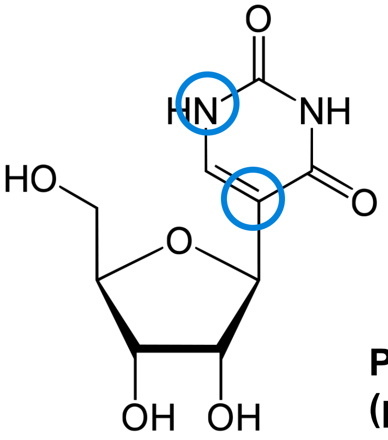


Left: tRNAs transfer amino acids to ribosome for protein translation. Top right: tRNA structure, with the TΨC loop highlighted in the blue ellipse. Below: structures of uridine and pseudouridine.



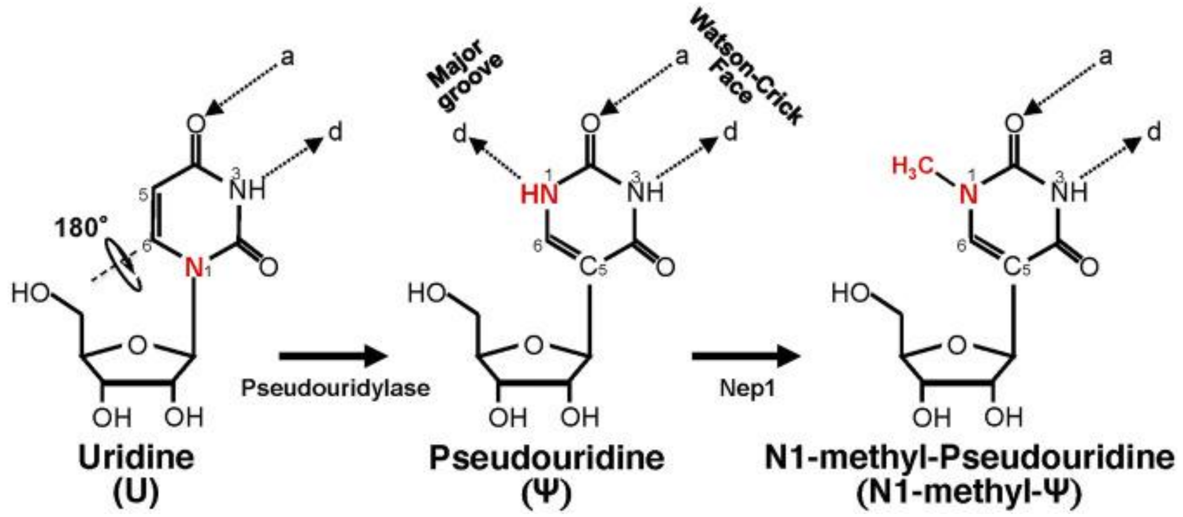


Uridine (U)

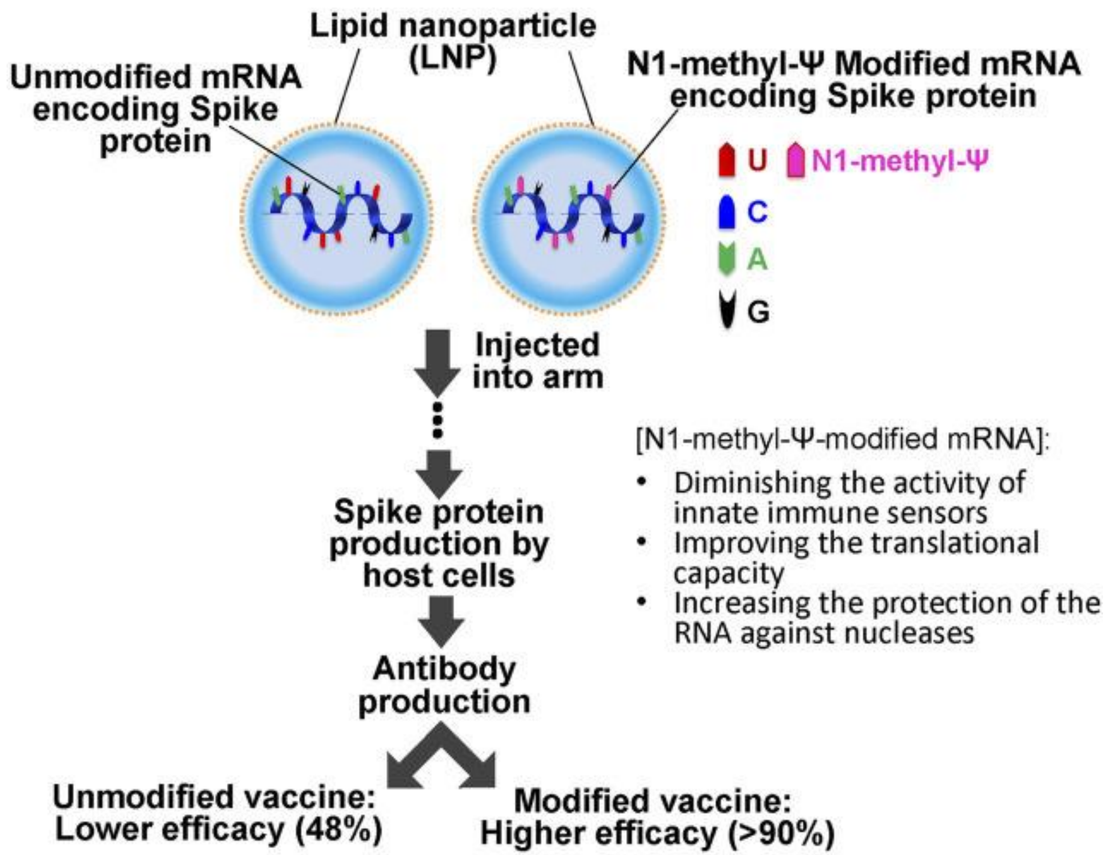


Pseudouridine (psi, Ψ)

Further modification (N1-methyl-Ψ) and LNP delivery are critical for the success of mRNA 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 benefited from 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).

Medical need: we need a large amount of vaccines

Challenge #1: No coronavirus vaccine is available
Challenge #2: Classical vaccines are least feasible due to capability and time
Challenge #3: Genetic sequence of the coronavirus may change over time.

Target & modality: Spike protein + mRNA vaccine

Challenge #4: mRNAs alone are too large and charged to pass lipid bilayers.
Challenge #5: mRNAs are readily degraded by ribonucleases.
Challenge #6: Exogenous mRNAs cause immunogenicity.

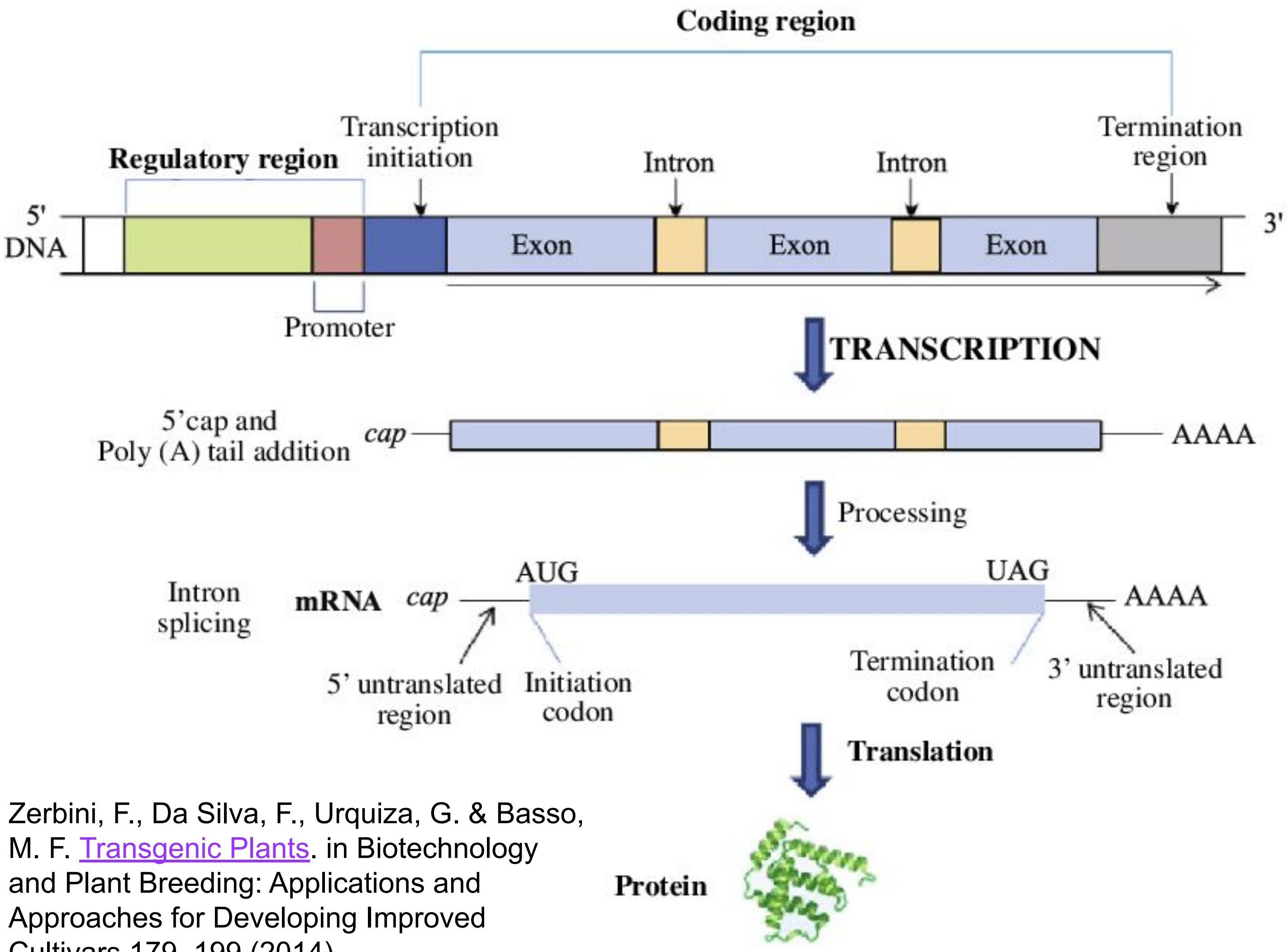
Modifications to improve **PK/PD profiles**: (1) LNPs delivering and protecting mRNA, (2) N1-methyl-Ψ modification, (3) hemoglobin 5’-UTR

Benefit/risk assessment: efficacy and toxicity profiles in animals, healthy volunteers and other populations, i.e. children, immunocompromised patients, …

Patient stratification: who benefit most? Who suffer from more severe adverse effects?

LNP: lipid nanoparticles

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

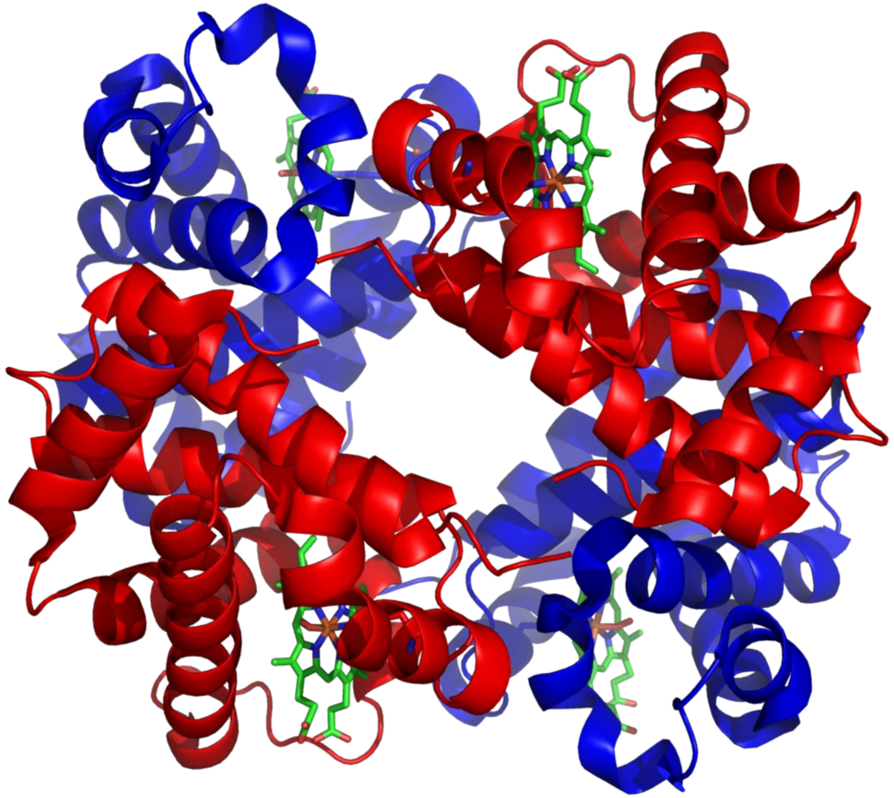


Zerbini, F., Da Silva, F., Urquiza, G. & Basso, M. F. Transgenic Plants. in Biotechnology and Plant Breeding: Applications and Approaches for Developing Improved Cultivars 179–199 (2014).

The process of gene expression in eukaryotes:

1. RNA polymerase, an enzyme, binds to the promoter region of the gene. It reads the DNA from the 5′ untranslated region (UTR) to the 3′ UTR to synthesize pre-mRNA.
2. Pre-mRNA receives a modified nucleotide (7-methylguanosine triphosphate) at the 5′ end as a cap, and a repeated adenine sequence (poly-A tail) at 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. Mature mRNA is 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 protein that transports oxygen.
- Hemoglobin consists of three subunits: alpha, beta, and delta. They are encoded by three highly similar genes known as HBA, HBB, and HBD (above).
- Hemoglobin is present in erythrocytes (red blood cells) of almost all vertebrates.
- The protein is essential, therefore the mRNA is relatively stable and highly translated.

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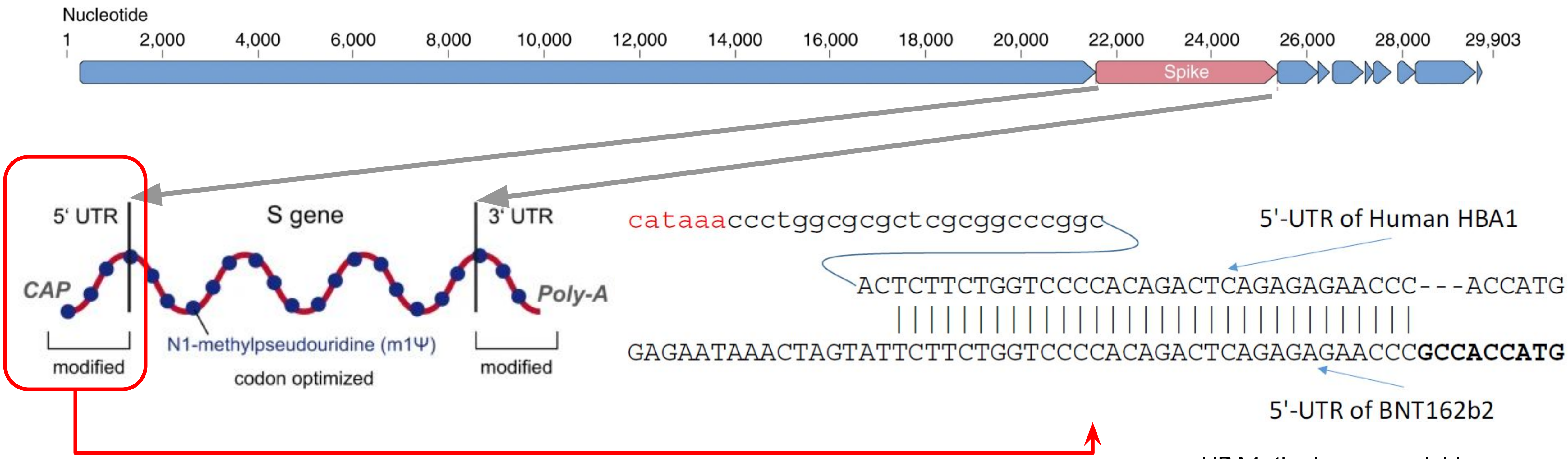
LNP, modified RNA, and 5’-UTR of HBA are all essential to make effective *and safe* vaccines against coronavirus



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

Interested in learning more? Read this report by WHO on potential benefits and limitations of mRNA vaccines.

Most drugs work by binding to and modulating protein targets

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Table 1 | **Molecular targets of FDA-approved drugs**

Drug target class	Targets			Drugs		
	Total targets	Small-molecule drug targets	Biologic drug targets	Total drugs	Small molecules	Biologics
Human protein	667	549	146	1,194	999	195
Pathogen protein	189	184	7	220	215	5
Other human biomolecules	28	9	22	98	63	35
Other pathogen biomolecules	9	7	4	79	71	8

The list also includes antimalarial drugs approved elsewhere in the world.

Summary

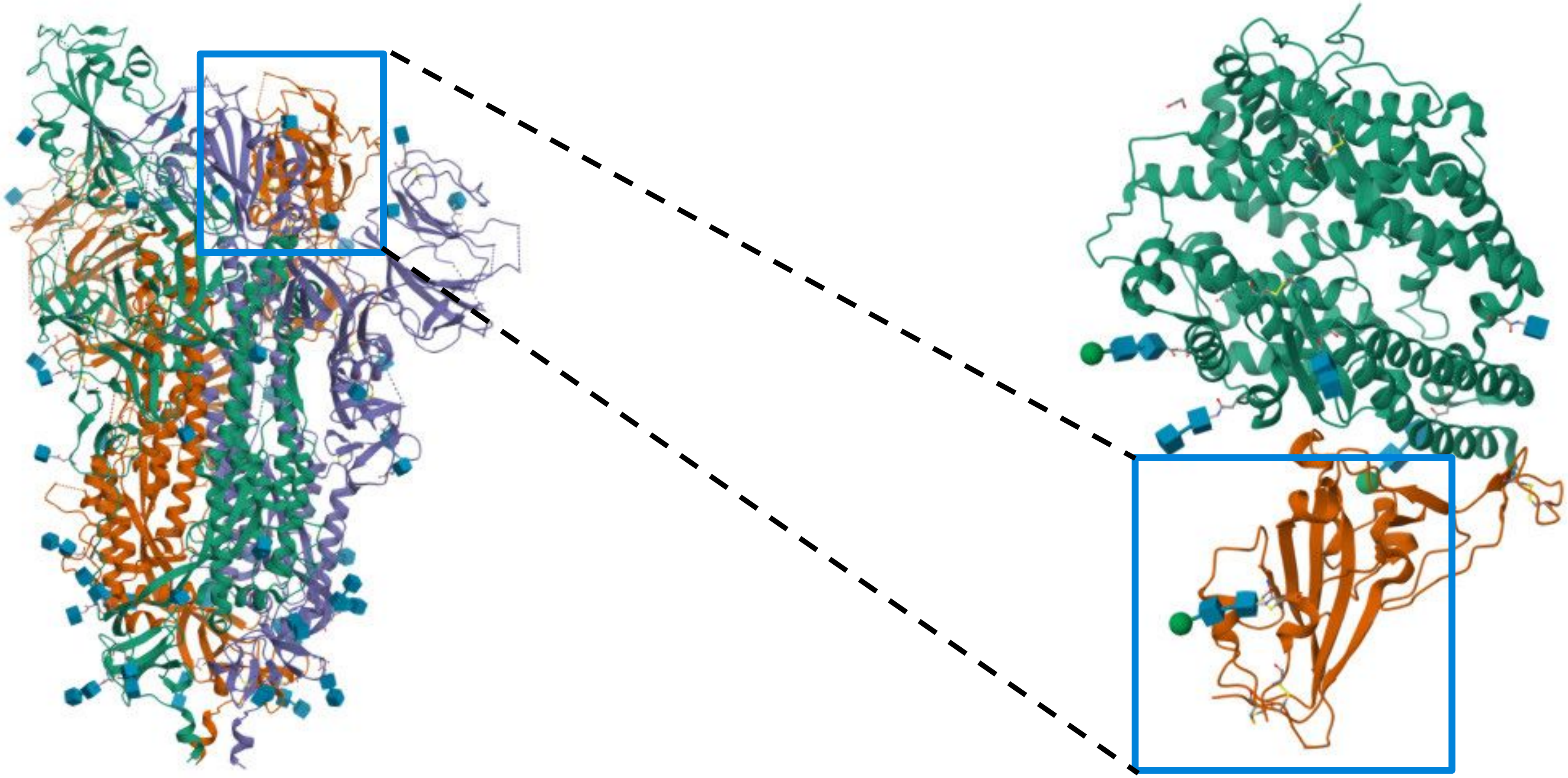
1. The central dogma dictates information flow in biological systems. Its components - DNA, RNA, proteins, metabolites - and processes - transcription, translation, and enzymic reactions - serve as drug targets.
2. Human bodies consist of cells that show different patterns of ‘active’ DNAs, expressed RNAs and proteins. The efficacy and safety of drugs need to be considered in the context of different cells. This is one of the reasons why animal studies are indispensable.
3. The example of SARS-CoV-2 vaccine discovery highlights the key questions in drug discovery, and its interdisciplinary nature.

Offline activities

1. Read the *Popular Information* of Nobel Prize 2025 in Physiology or Medicine 2025, https://www.nobelprize.org/prizes/medicine/2025/popular-information/. What was the most interesting learning for you?
2. Read the article *Principles of Early Drug Discovery* by Hughes *et al.* (2011) twice. The first time, read the whole paper however as you wish. The second time, use one sentence to summarize each paragraph of the sections 'target identification' and 'target validation'. Submit your summary sentences (no formatting/polishing needed).

Backup slides

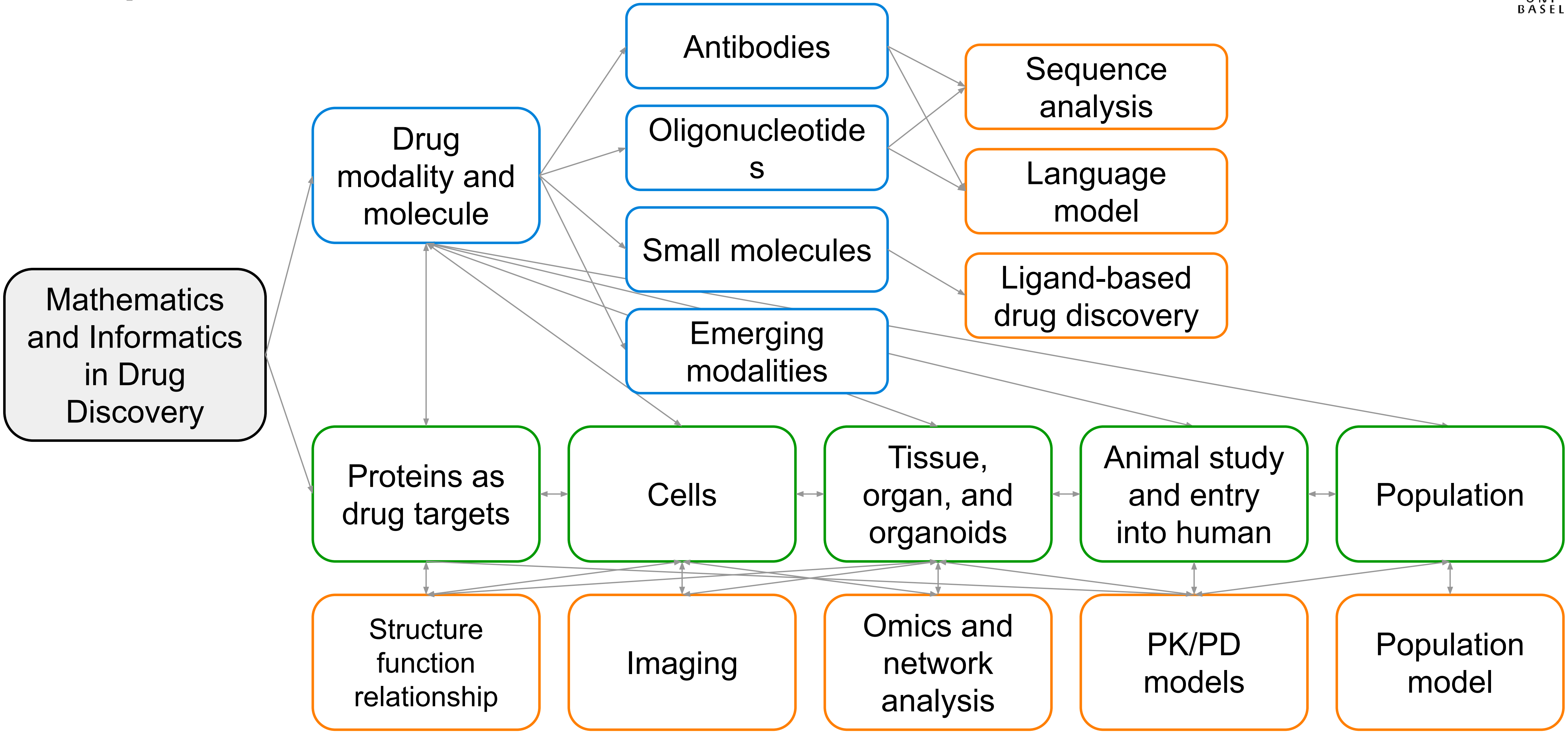
Structure of SARS-CoV-2 spike protein, and the receptor binding domain-ACE2 complex



SARS-CoV-2 spike glycoprotein with a single receptor-binding domain up. PDB 6VSB

Receptor binding domain (PDB) of spike protein (in orange) and human ACE2 (blue). PDB 6VW1

The path of the course



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

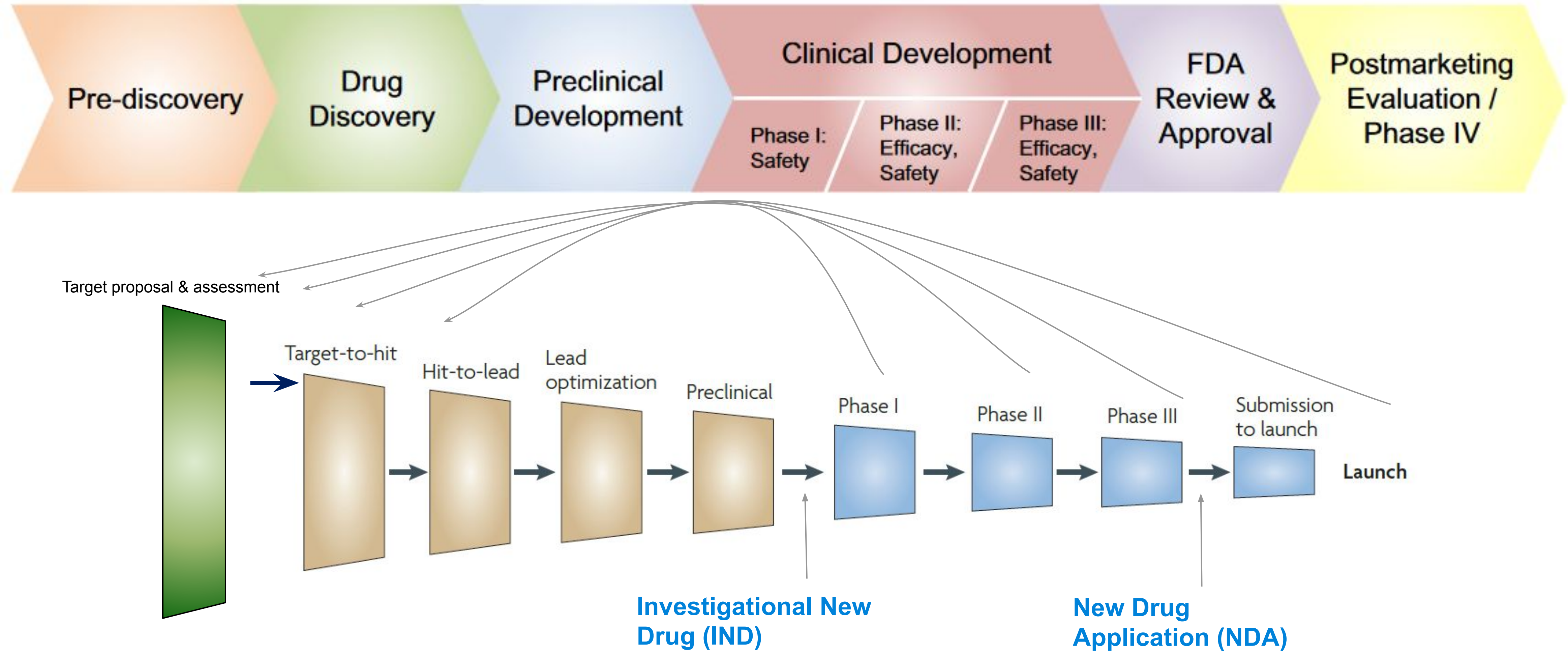
Interests

- 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

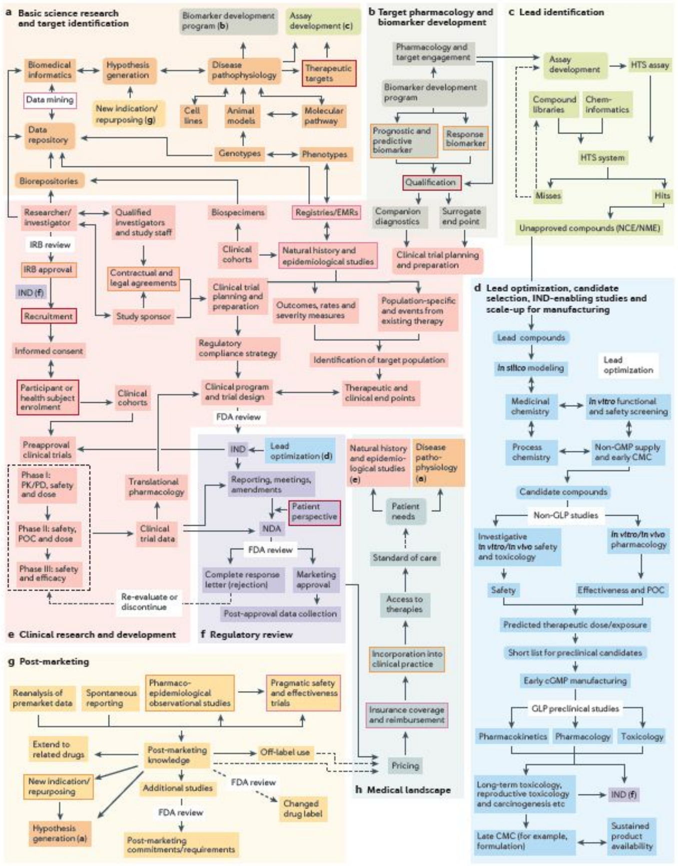
Chevron diagrams as a pipeline view of drug discovery and development



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.

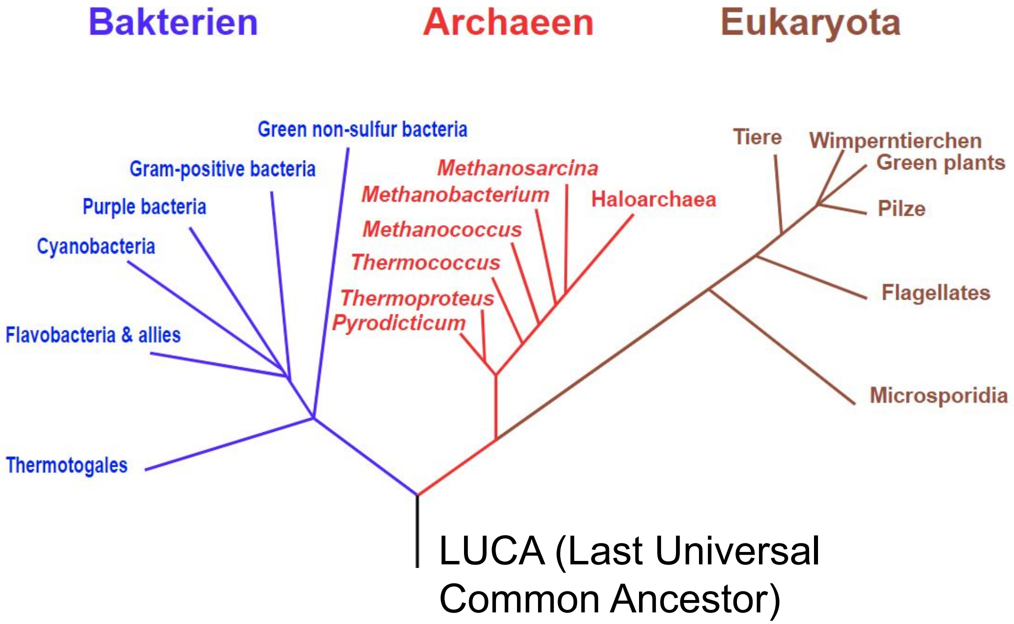
A dynamic map for drug discovery, development, and deployment

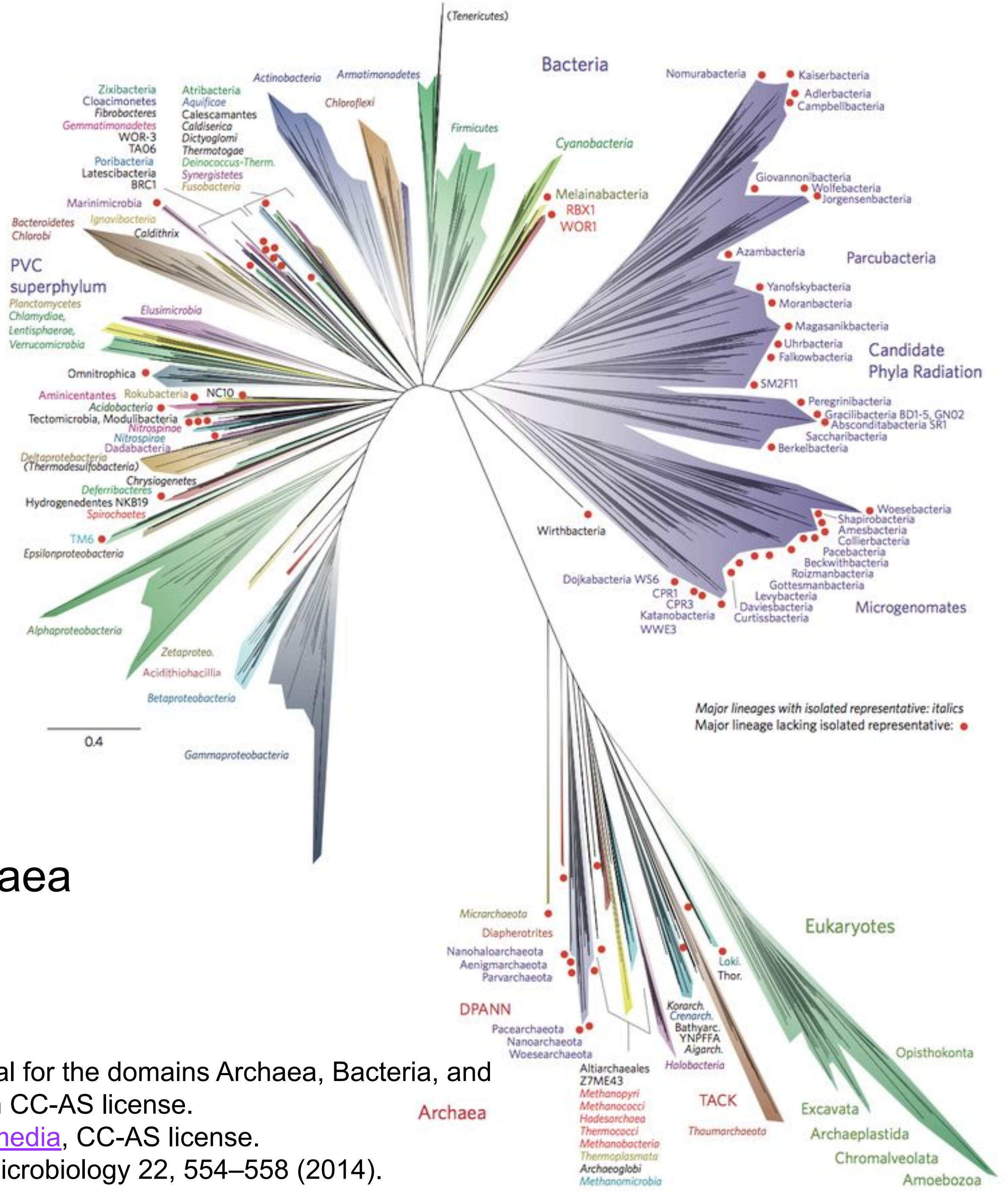
1. **Basic science research and target identification.** *What causes the disease? What do we want to achieve? Which protein can I target with which modality?*
2. **Target pharmacology and biomarker development.** *What is the effect of targeting the protein? What we can measure to confirm that the protein 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).

Virus is evolutionarily special

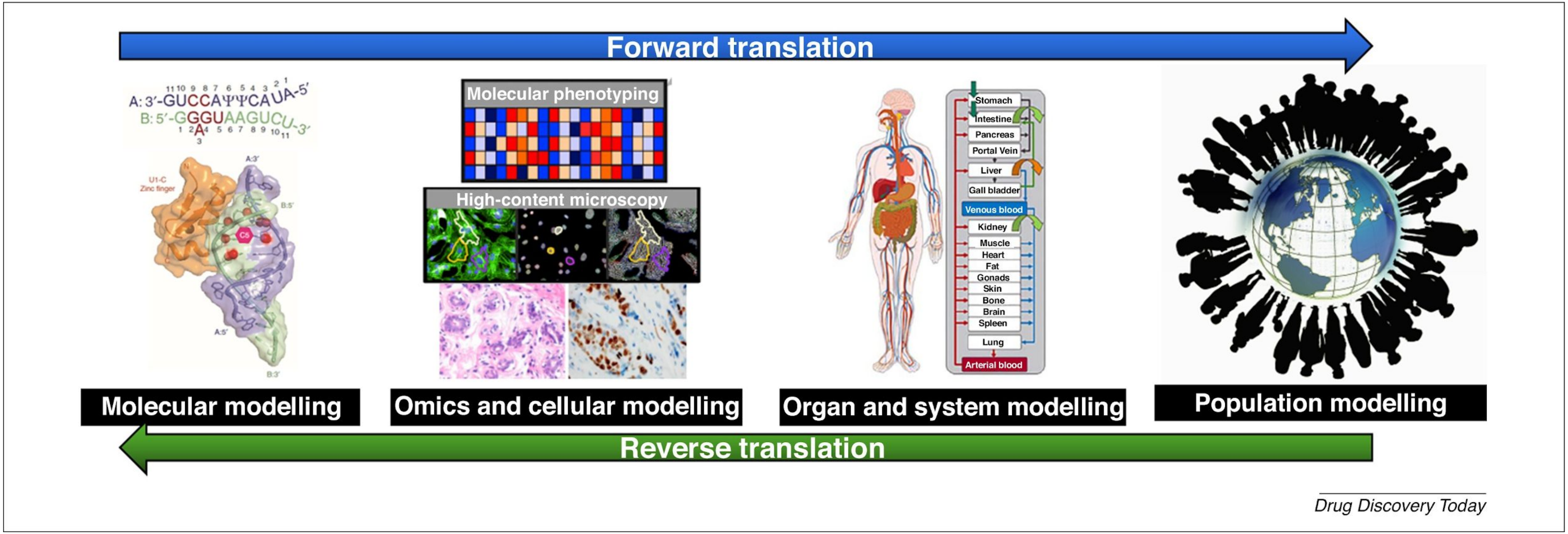




- The three-domain model of *cellular* life: (eu-)bacteria, archaebacteria, and eukaryotes.
- The two-domain model: bacteria as one branch, archaea and eukaryotes as the other.
- Virus fits in no domain of neither models.

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).

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.

Five key questions in drug discovery

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?

Right target
- Strong link between target and disease
- Differentiated efficacy
- Available and predictive biomarkers

Right tissue
- Adequate bioavailability and tissue exposure
- Definition of PD biomarkers
- Clear understanding of preclinical and clinical PK/PD
- Understanding of drug–drug interactions

Right safety
- Differentiated and clear safety margins
- Understanding of secondary pharmacology risk
- Understanding of reactive metabolites, genotoxicity and drug–drug interactions
- Understanding of target liability

Right patient
- Identification of the most responsive patient population
- Definition of risk–benefit for a given population

Right commercial potential
- Differentiated value proposition versus future standard of care
- Focus on market access, payer and provider
- Personalized health-care strategy, including diagnostics and biomarkers

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