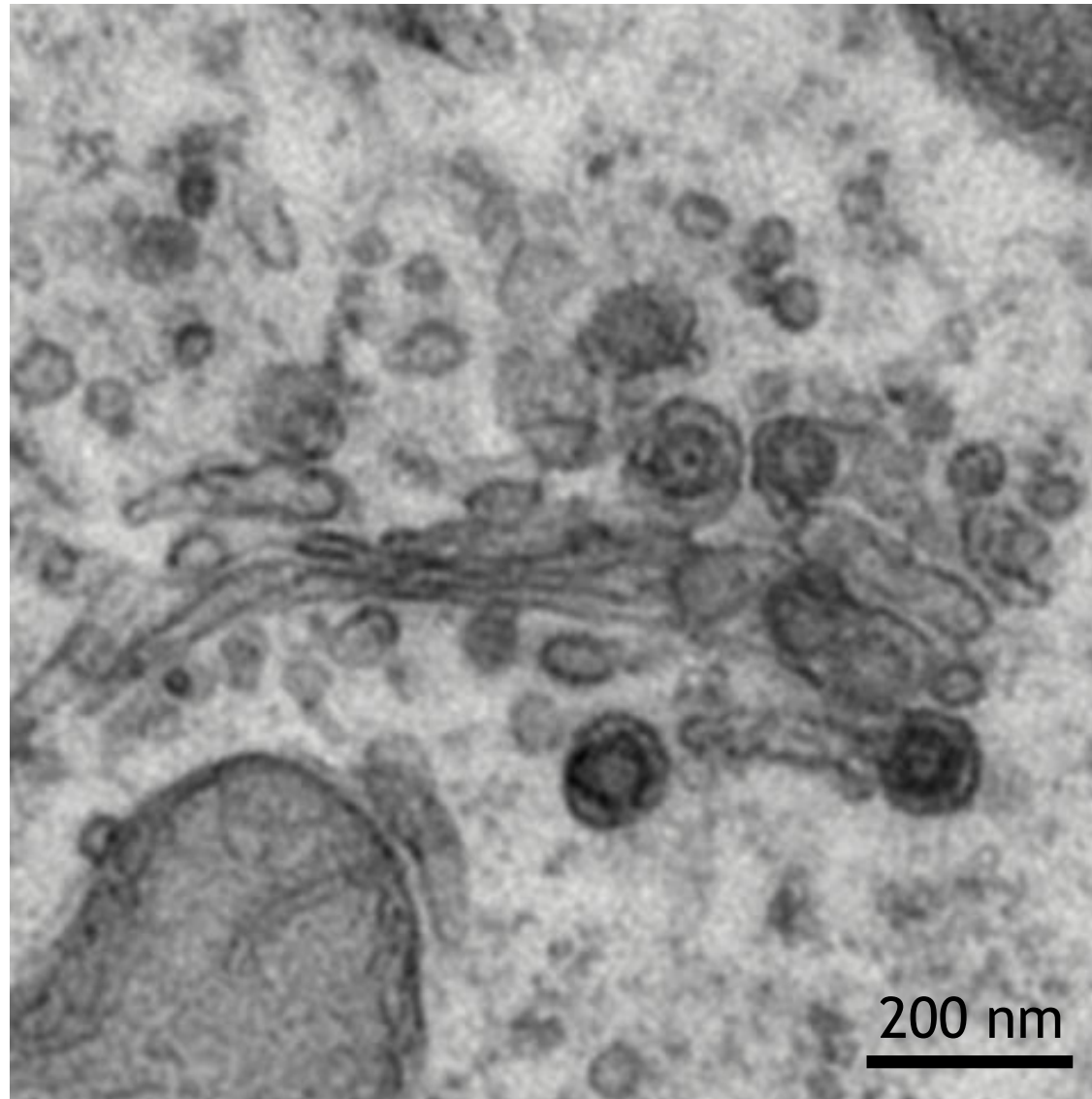


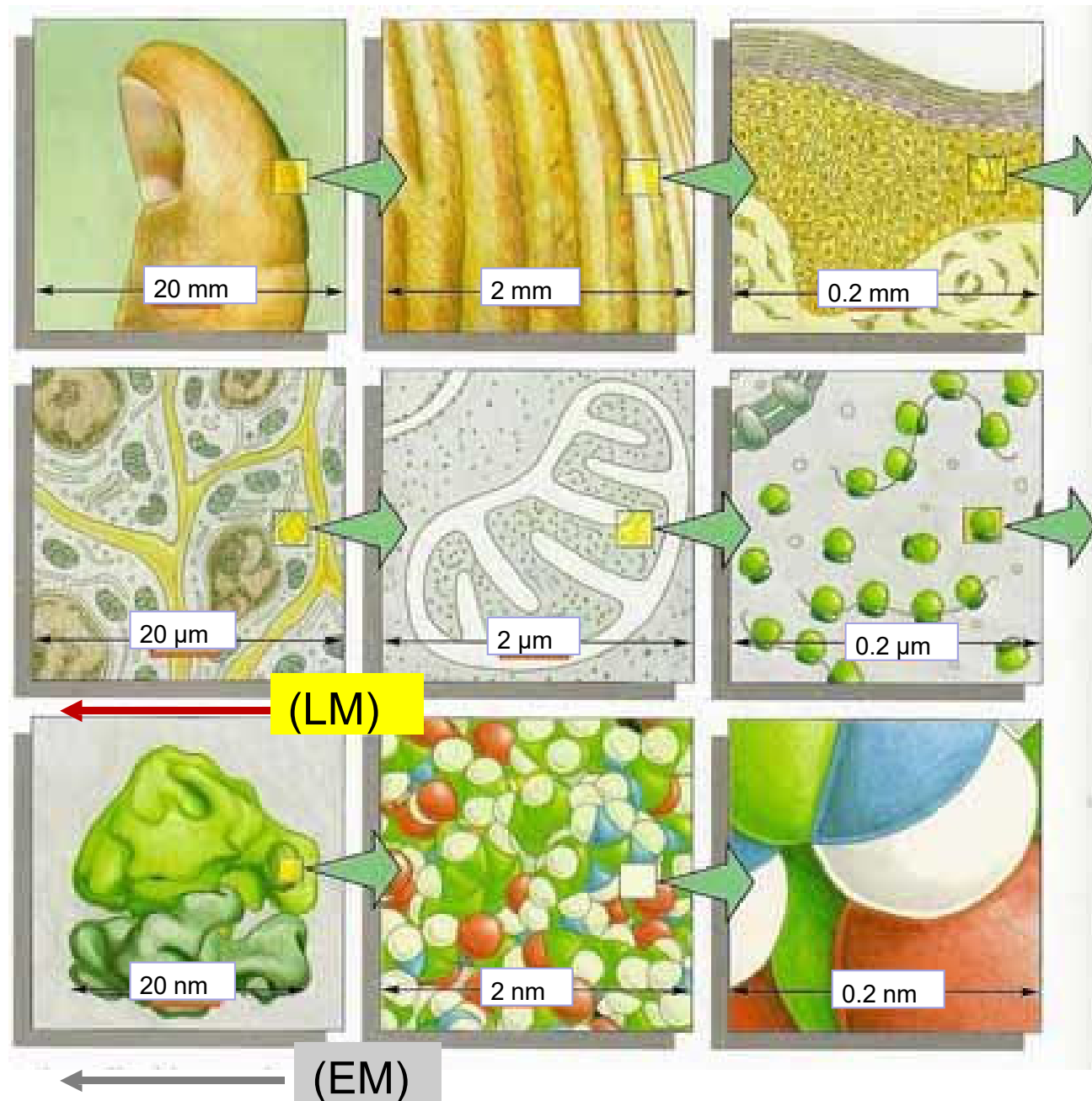
Tutorial 1

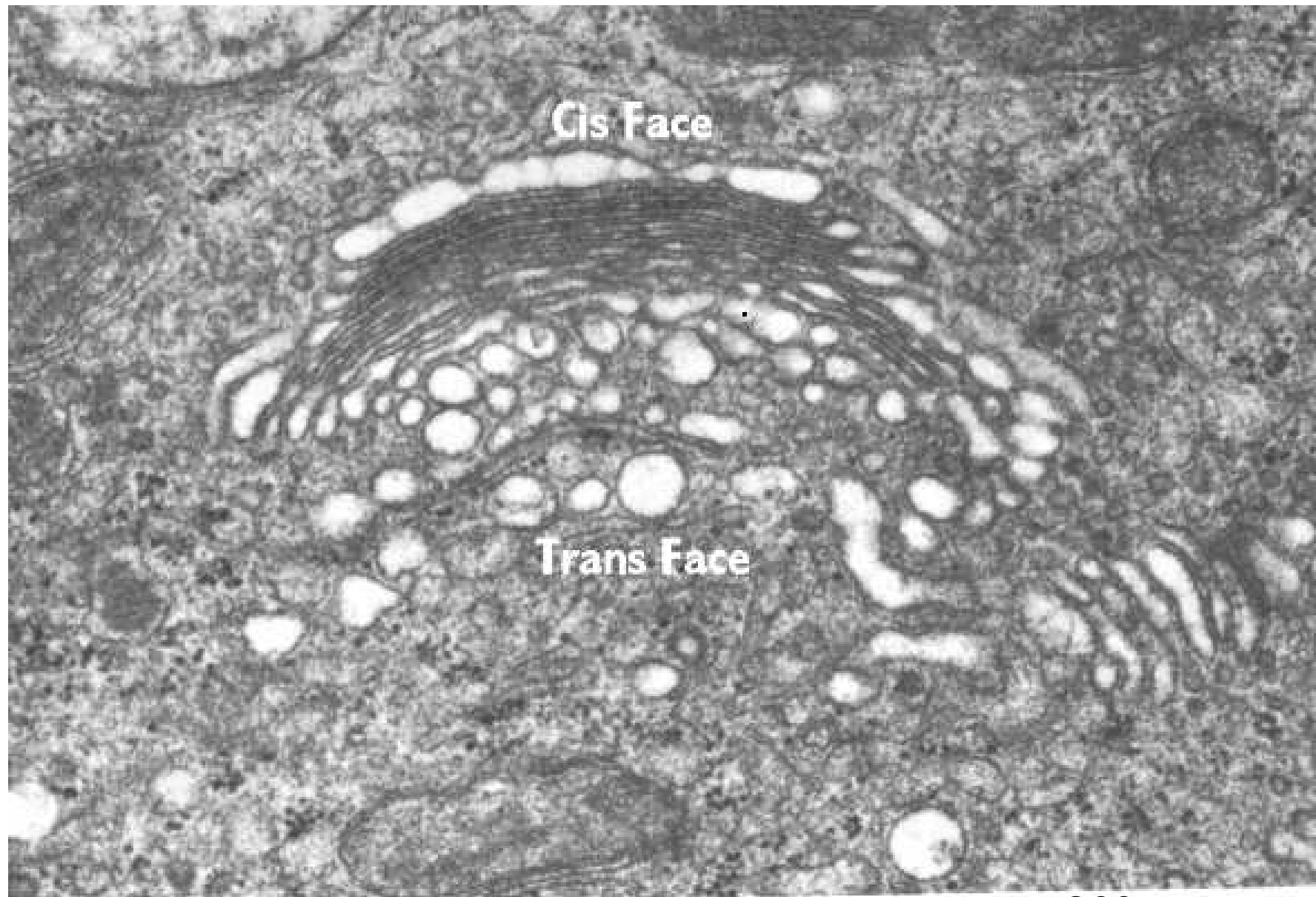


Transmission electron microscopy (TEM)

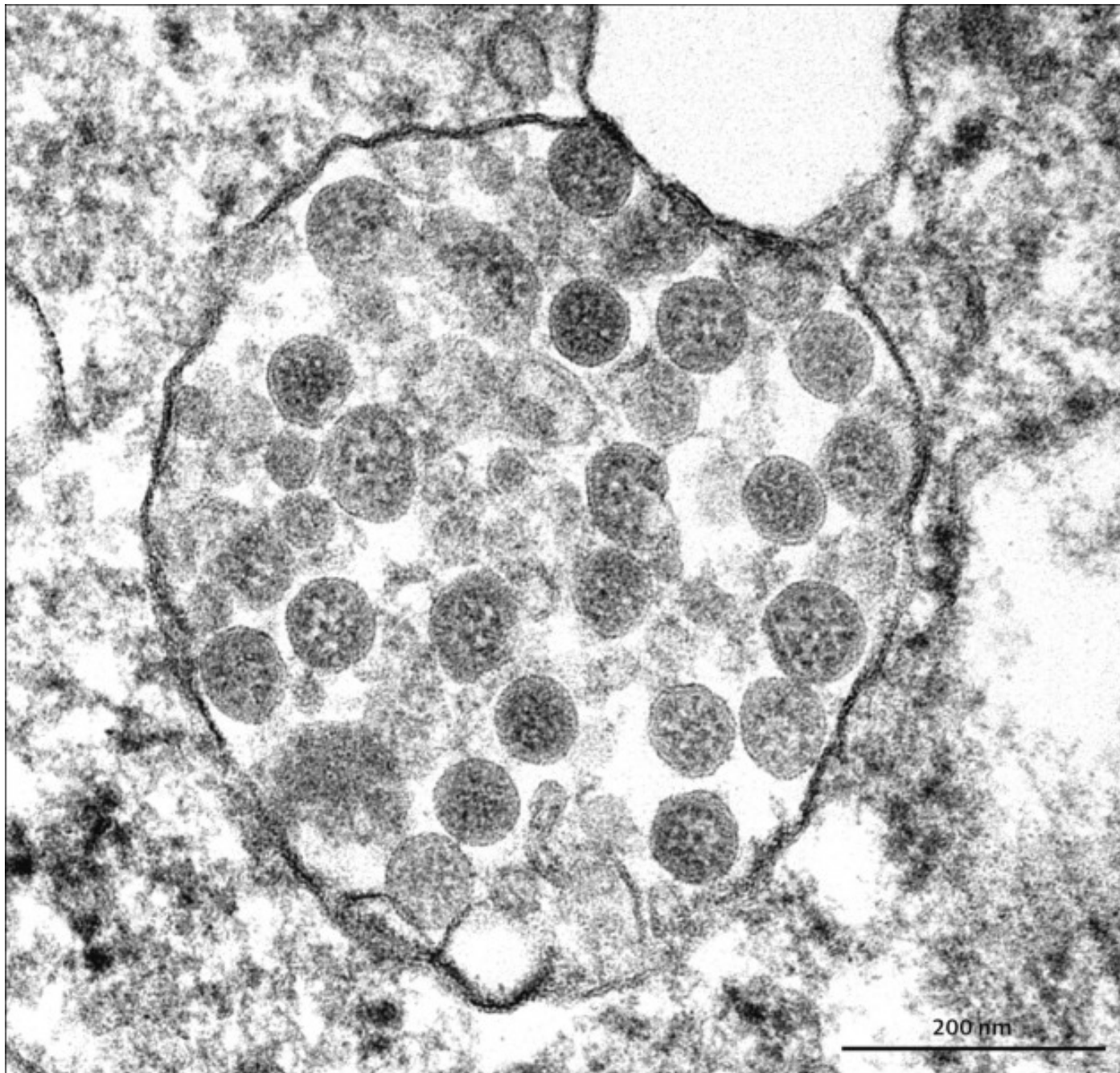
Z. Lygerou, Patras Medical School

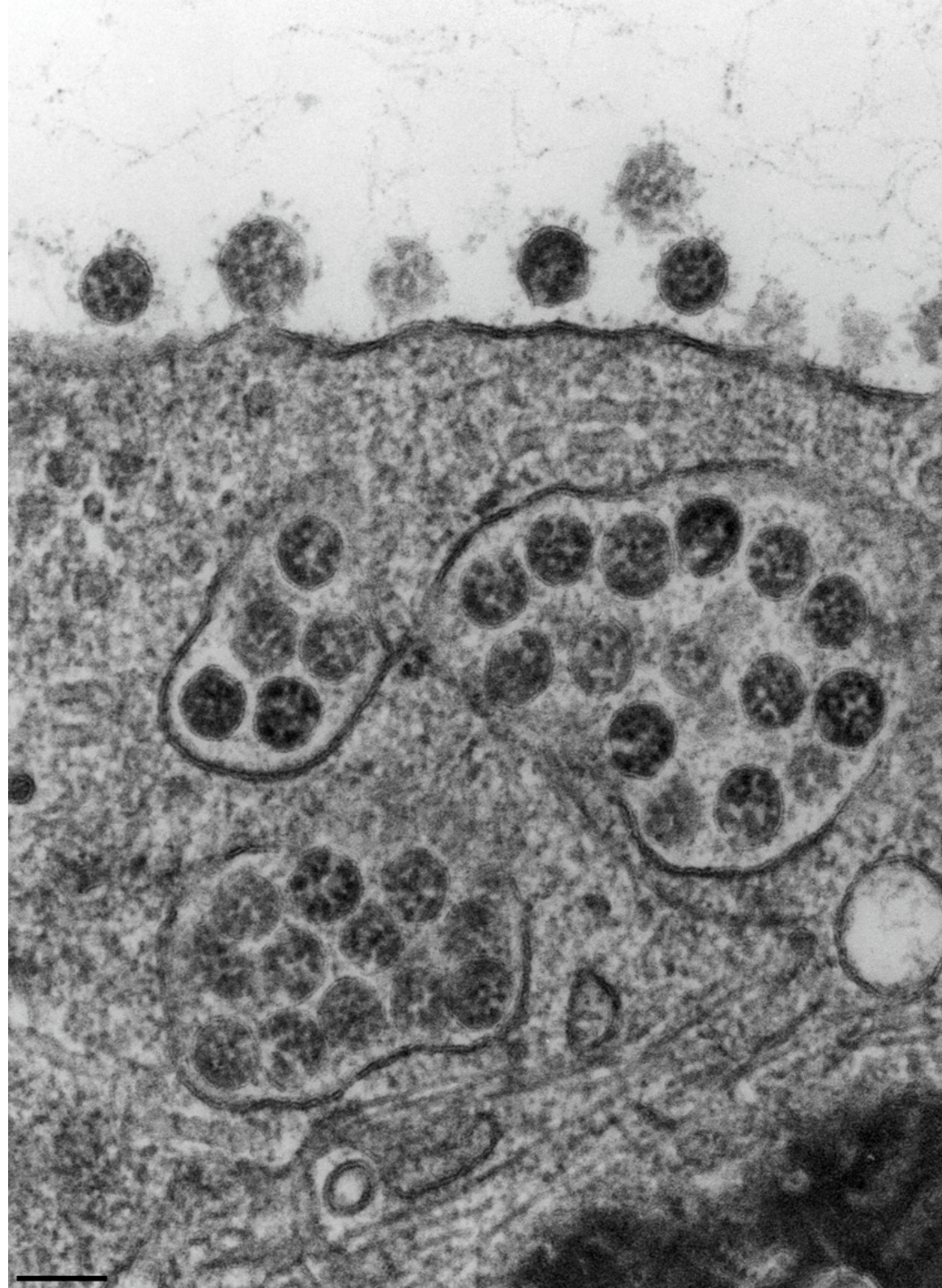
A conventional light microscope cannot resolve objects less than 200 nm apart (resolution).





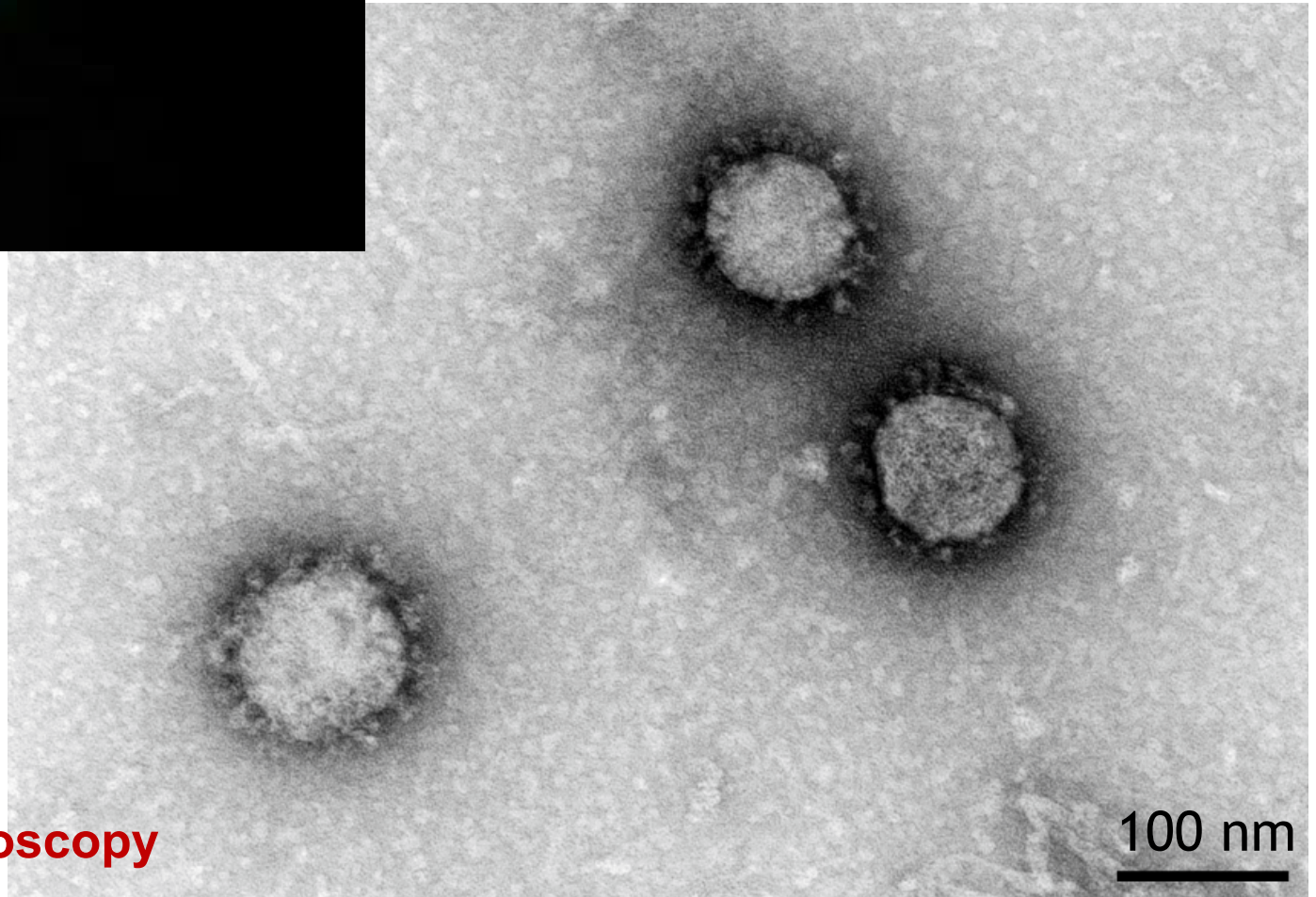
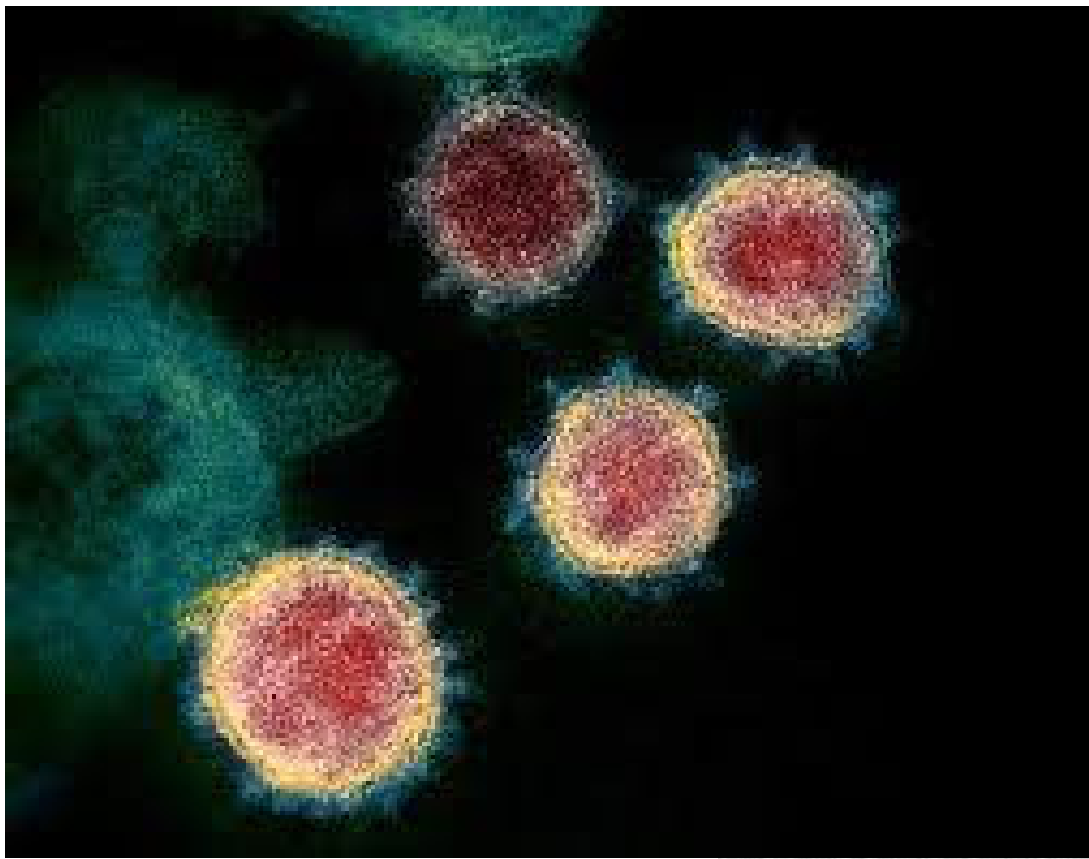
200 nm





100 nm

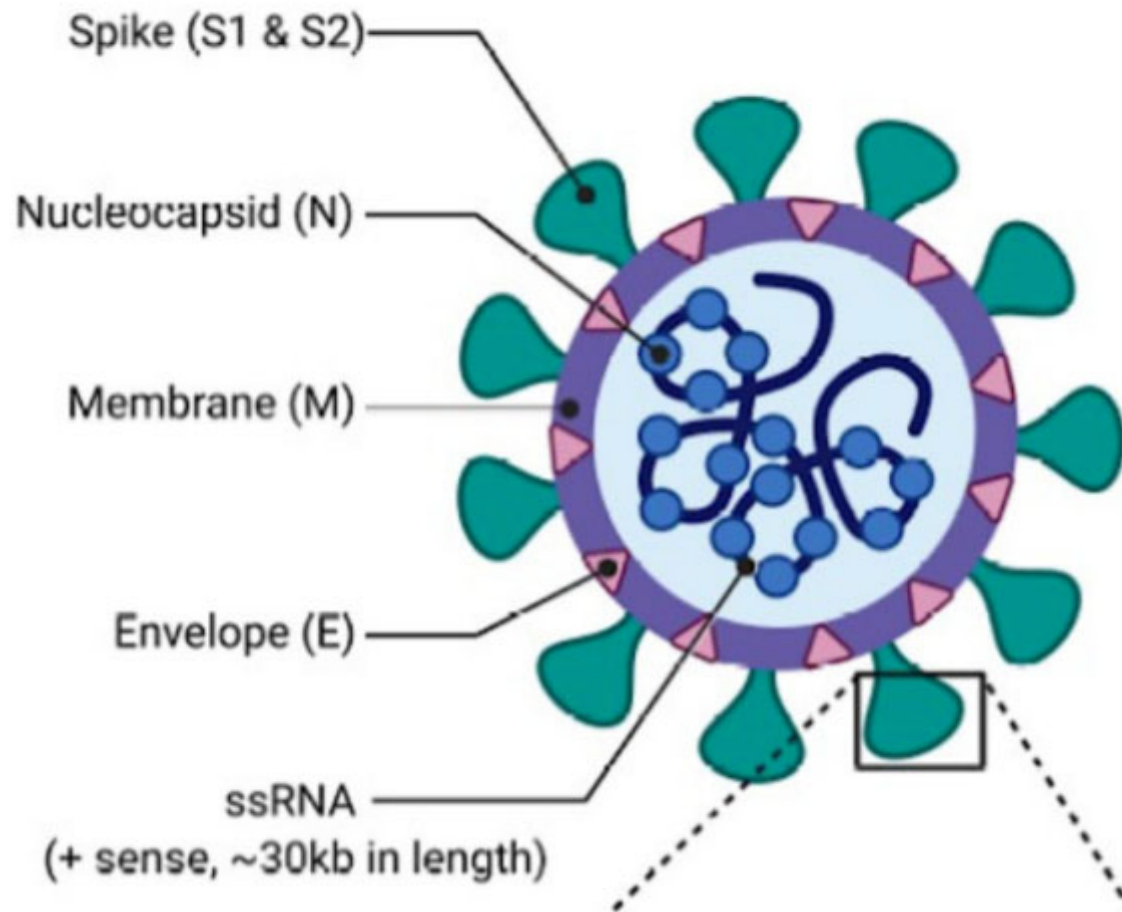




Transmission electron microscopy

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2): the virus

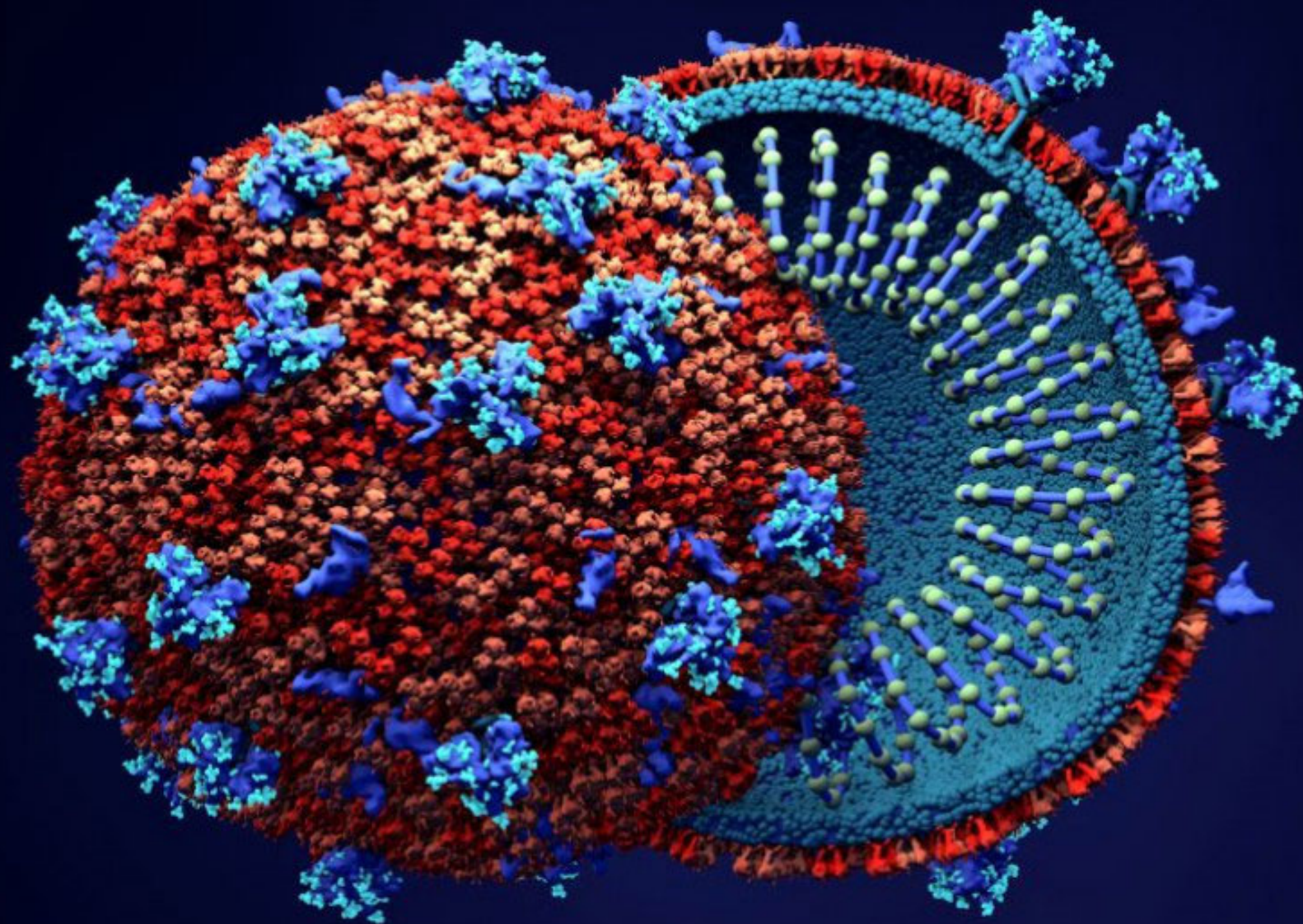
COVID-19 (Coronavirus Disease 2019): the disease



<https://www.youtube.com/watch?v=5DGwOJXSxqg>

<https://www.youtube.com/watch?v=RN81h85V6D4>

<https://www.youtube.com/watch?v=GQUCCkHNjN8>



SARS-CoV-2

Escherichia coli

Homo sapiens

Size

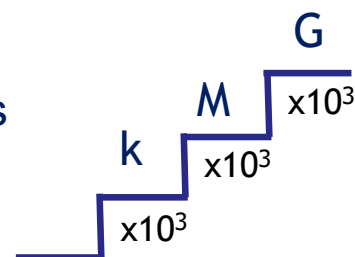
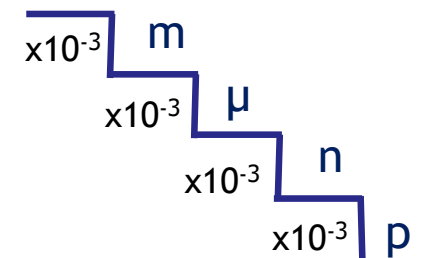
60 -120 nm*

0.5 x 1-2 μm *

100 μm

* m= meters, mm= 10^{-3} m, $\mu\text{m}=10^{-6}$ m, nm = 10^{-9} m
(Angstrom = 10^{-10} m)

** b= bases, bp=base pairs
kb= 10^3 bases, Mb = 10^6 bases, Gb= 10^9 bases



SARS-CoV-2

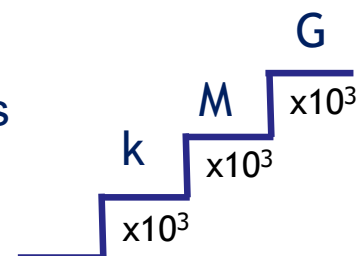
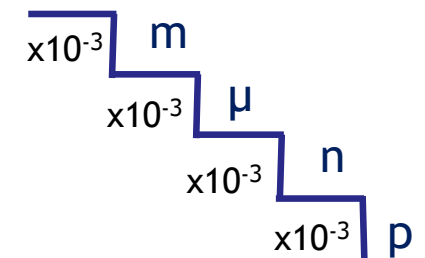
Escherichia coli

Homo sapiens

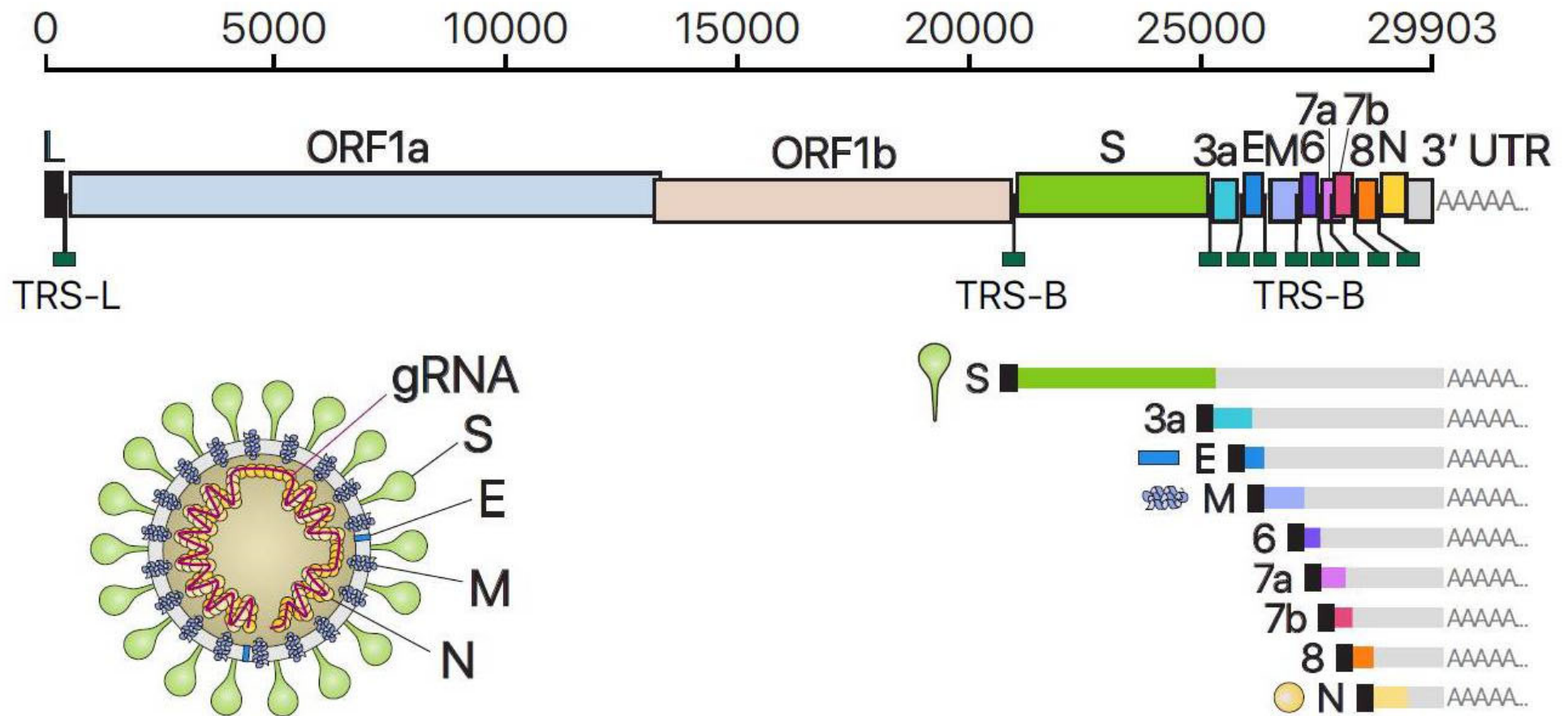
Size	60 -120 nm*	0.5 x 1-2 μm^*	100 μm
Genetic material	RNA (+) Single-stranded 30 kb** 1 linear molecule	DNA (double-stranded) 4.6 Mbp** 1 circular molecule	DNA (double-stranded) 3 Gbp (haploid)** 23 linear molecules (mitochondrion = 16 kb)
Proteins (coding sequences – CDS)	29 4 structural		

* m= meters, mm= 10^{-3} m, $\mu\text{m}=10^{-6}$ m, nm = 10^{-9} m
(Angstrom = 10^{-10} m)

** b= bases, bp=base pairs
kb= 10^3 bases, Mb = 10^6 bases, Gb= 10^9 bases



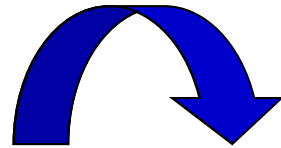
The SARS-CoV-2 genome



ORF = Open Reading Frame

A region of genetic material which starts with a start codon (AUG) and has no stop codons that can therefore be translated into a protein

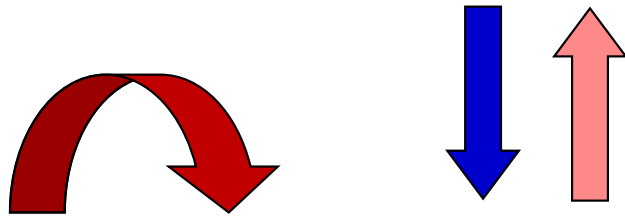
The central dogma of molecular biology



Replication



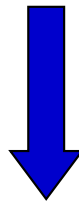
DNA



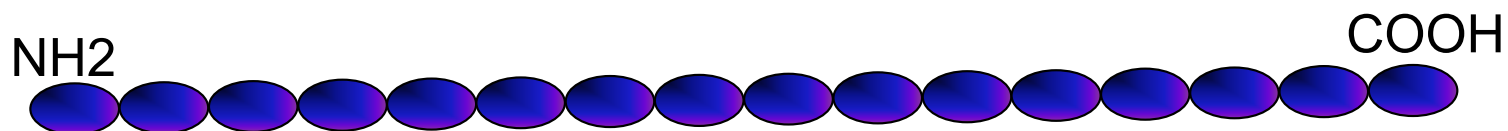
Transcription



RNA

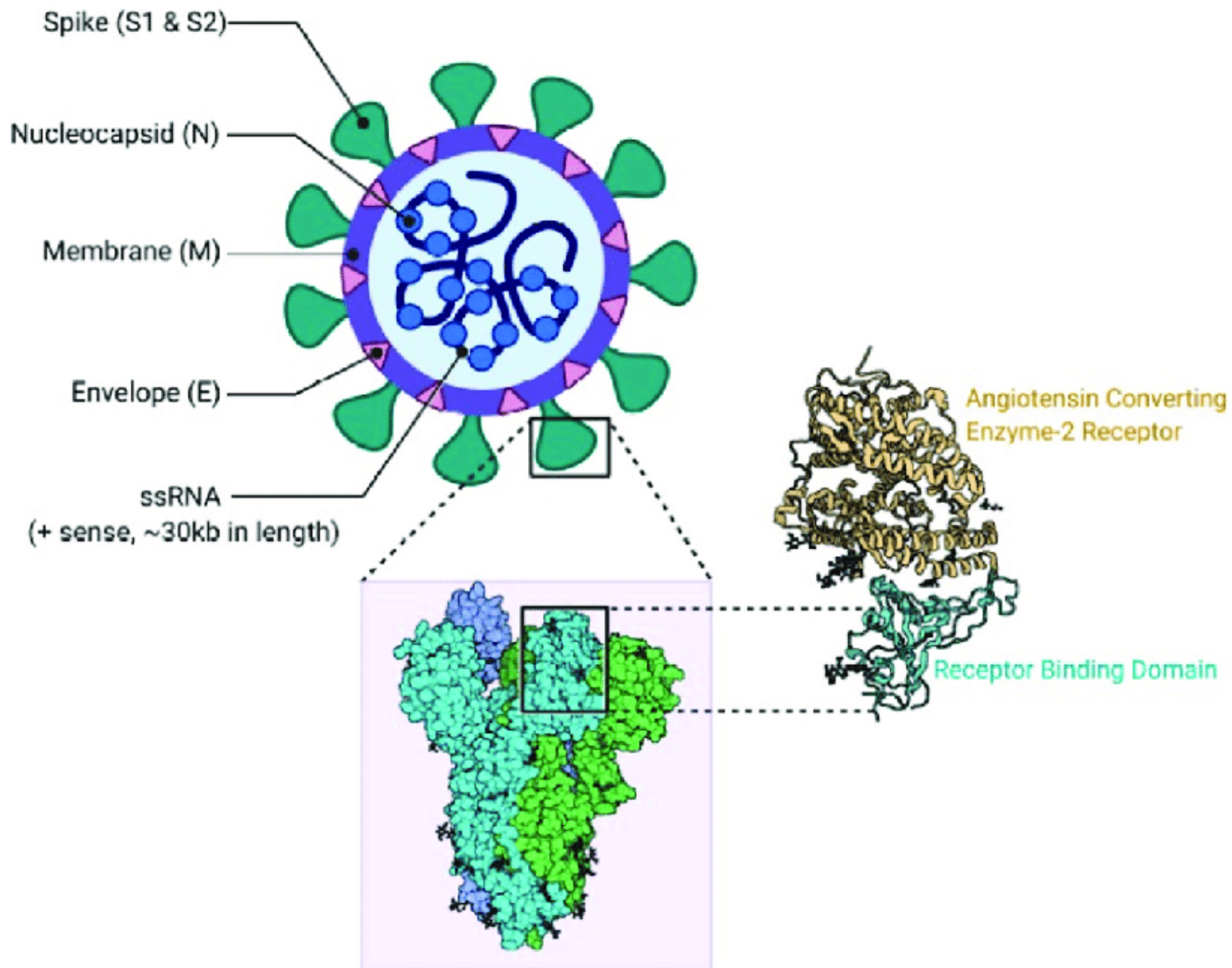


Translation



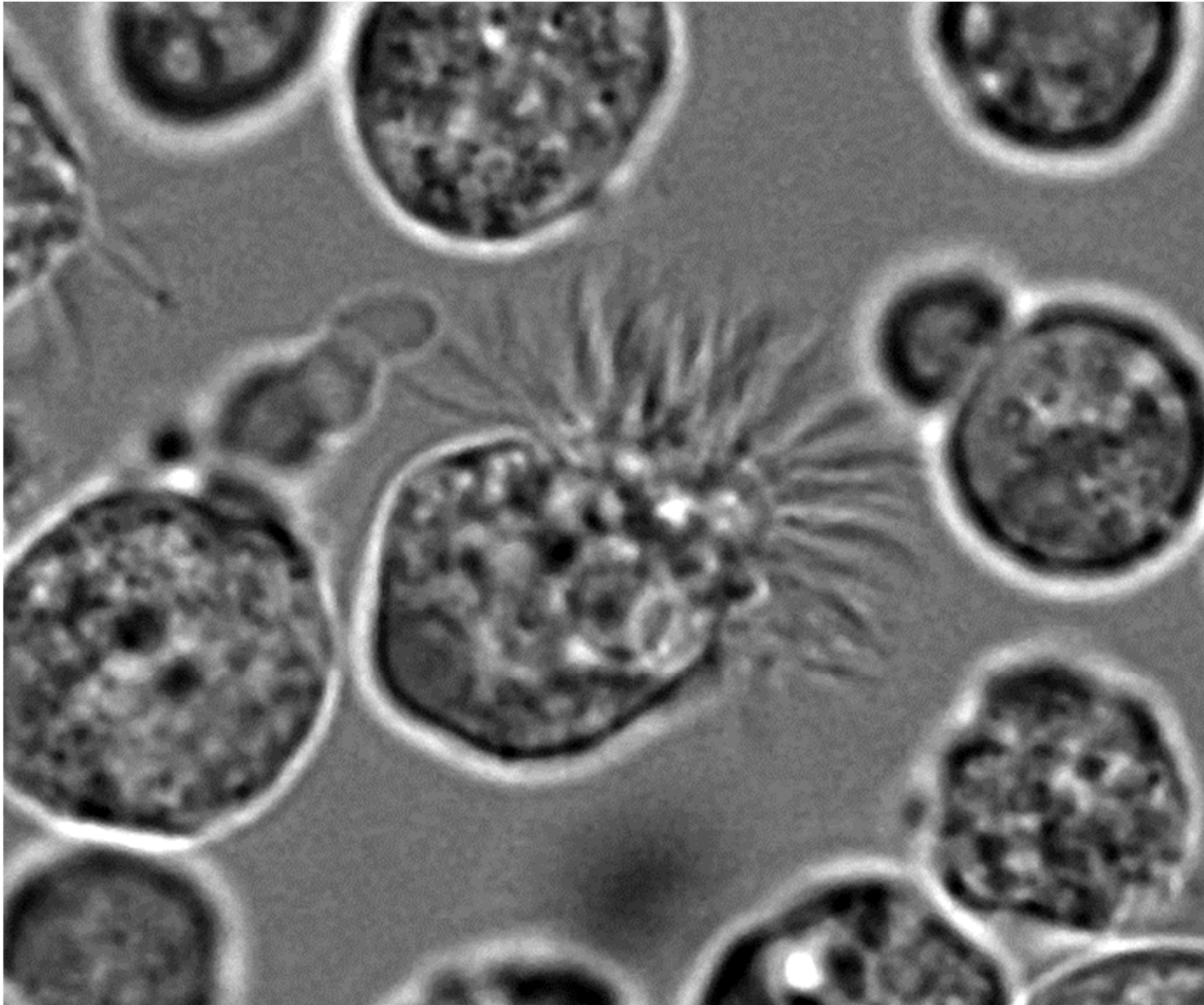
Protein

	SARS-CoV-2	<i>Escherichia coli</i>	<i>Homo sapiens</i>
Size	60 -120 nm	0.5 x 1-2 µm	100 µm
Genetic material	RNA (+) Single-stranded 30 kb 1 linear molecule	DNA (double-stranded) 4.6 Mbp	DNA 3 Gbp (haploid)
Proteins (CDS)	29 4 structural RNA-dependent RNA polymerase (RdRp)	5,000	20,000–30,000 (mitochondrion: 13...)
What fraction of the genetic material codes for proteins?	All – overlapping ORFs	90%	2%
Membrane	Yes	Yes (cell membrane)	Yes (+ internal membranes)
Ribosomes	No	Yes (70 S)	Yes (80 S)



Protein structure – Angström resolution ($=0,1\text{nm}$)
(X-ray crystallography, NMR, cryo-electron microscopy)

**Ciliated cells of the respiratory epithelium
express the ACE2 receptor**

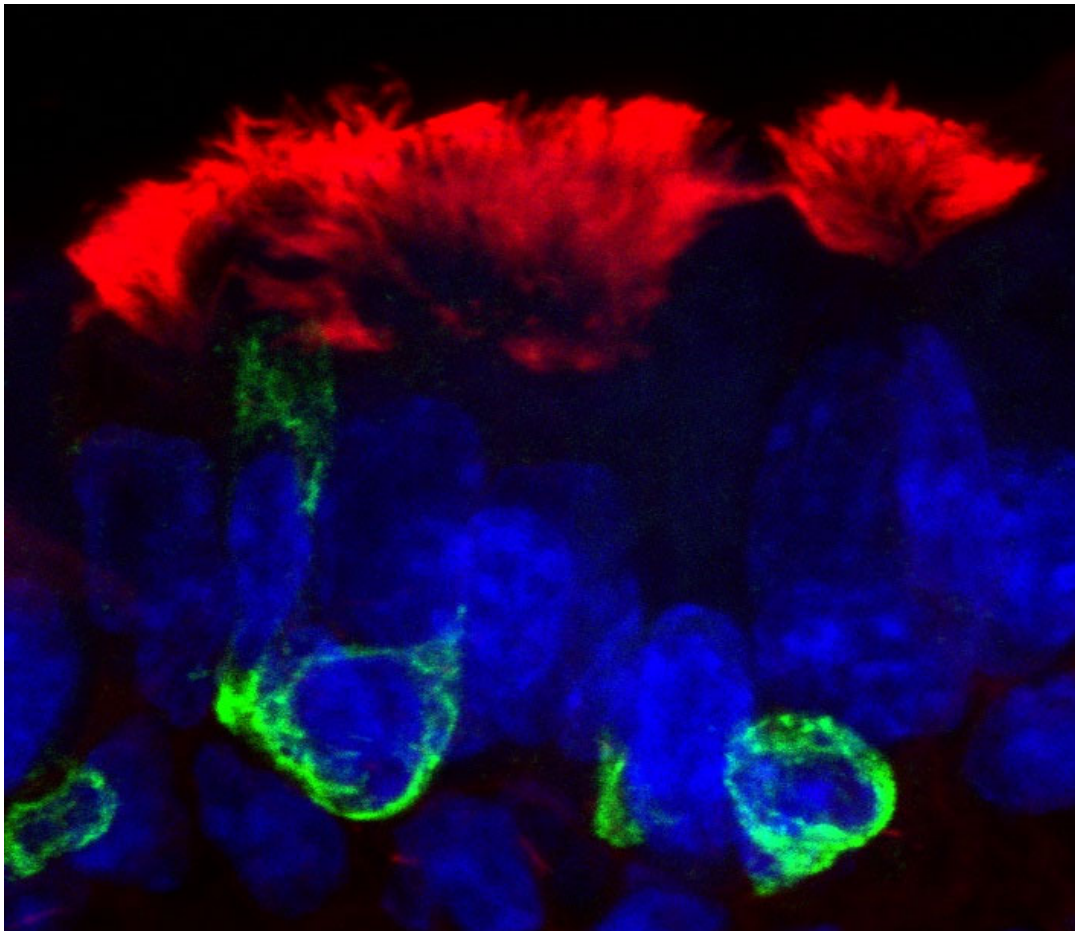


Light microscopy – phase contrast

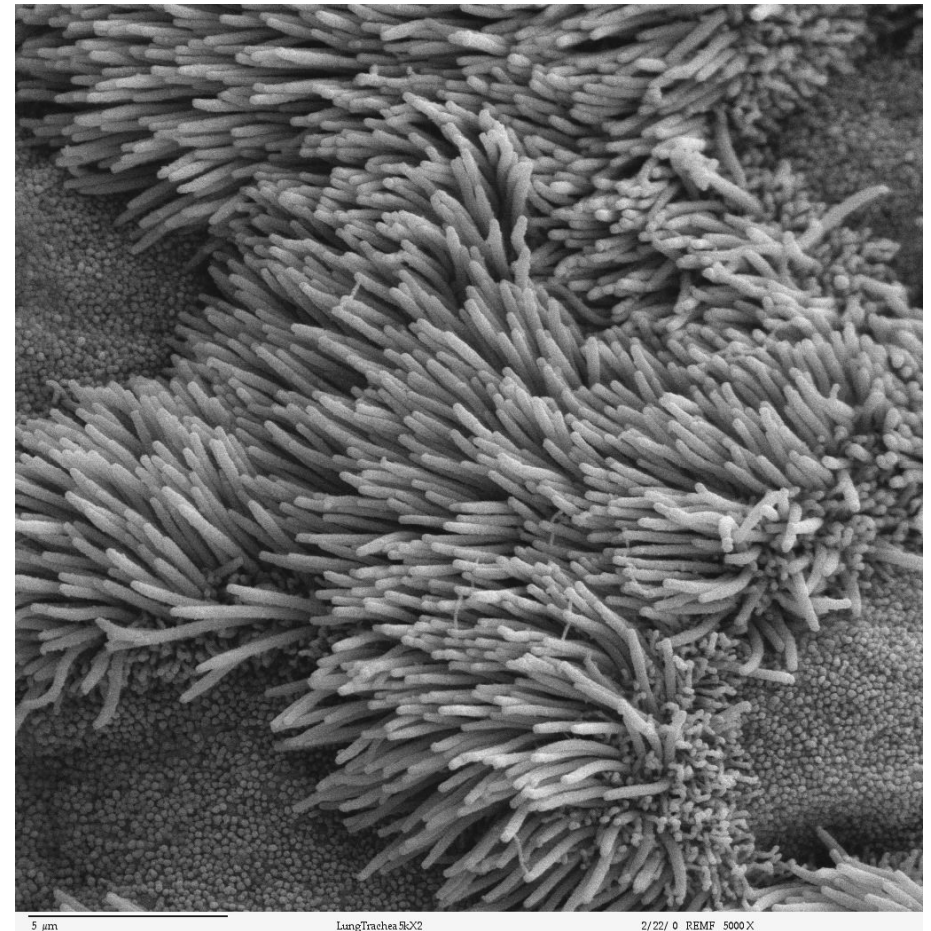


Light microscopy

Fluorescence – confocal



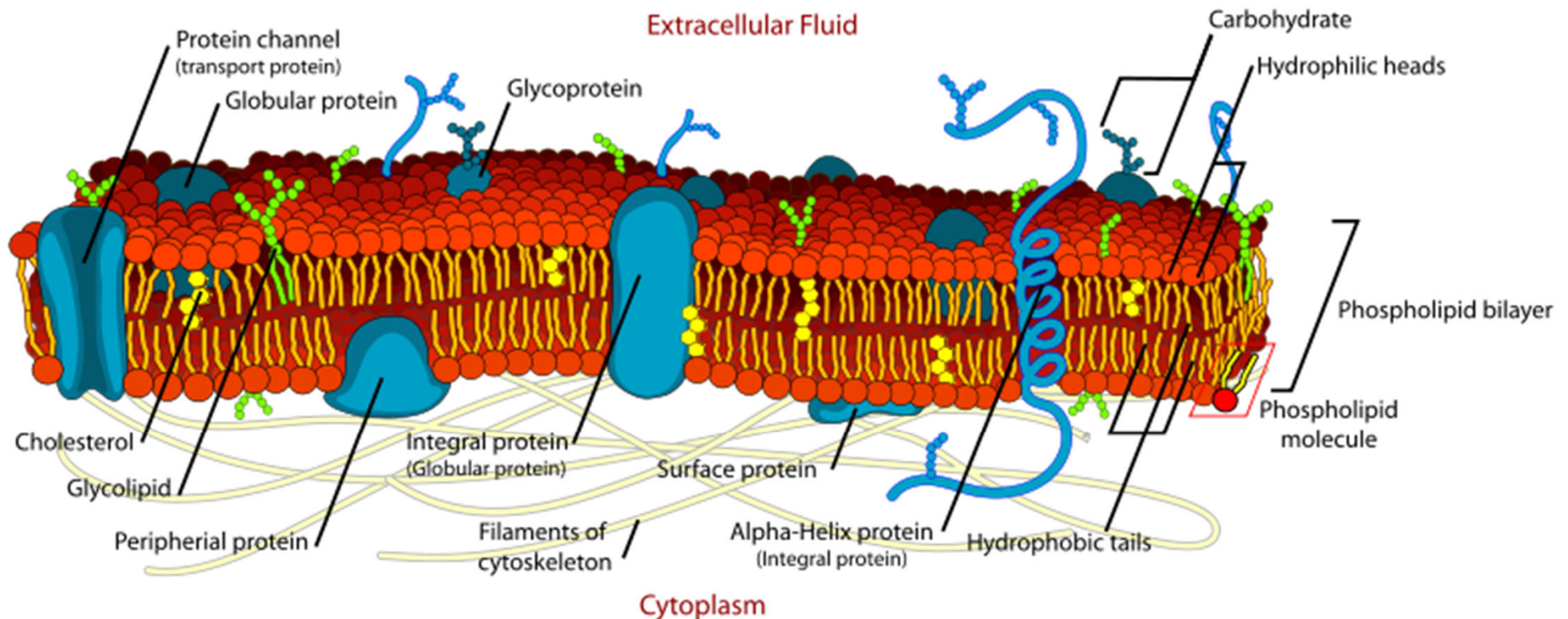
Scanning electron microscopy

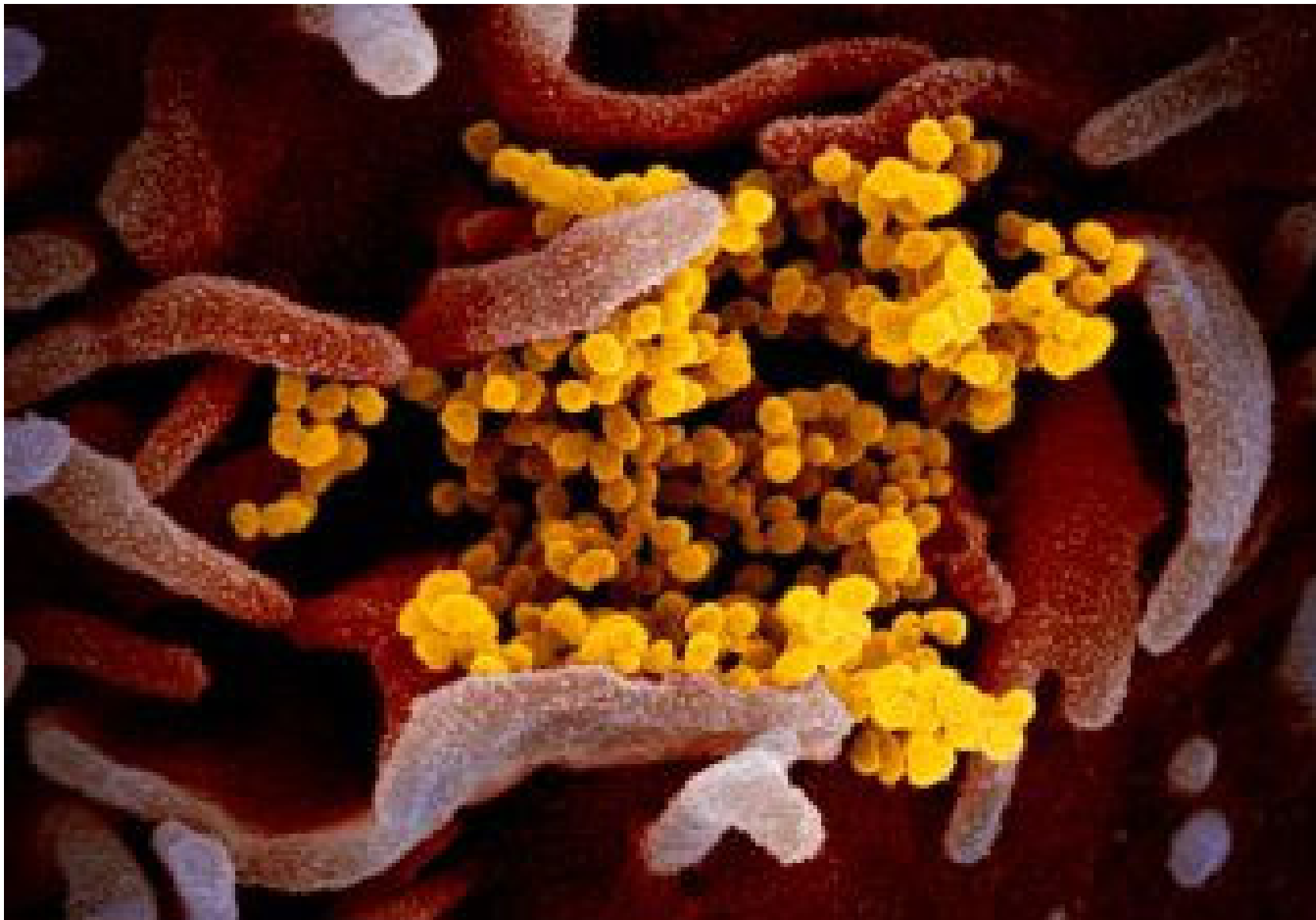


All cells are surrounded by a cell membrane

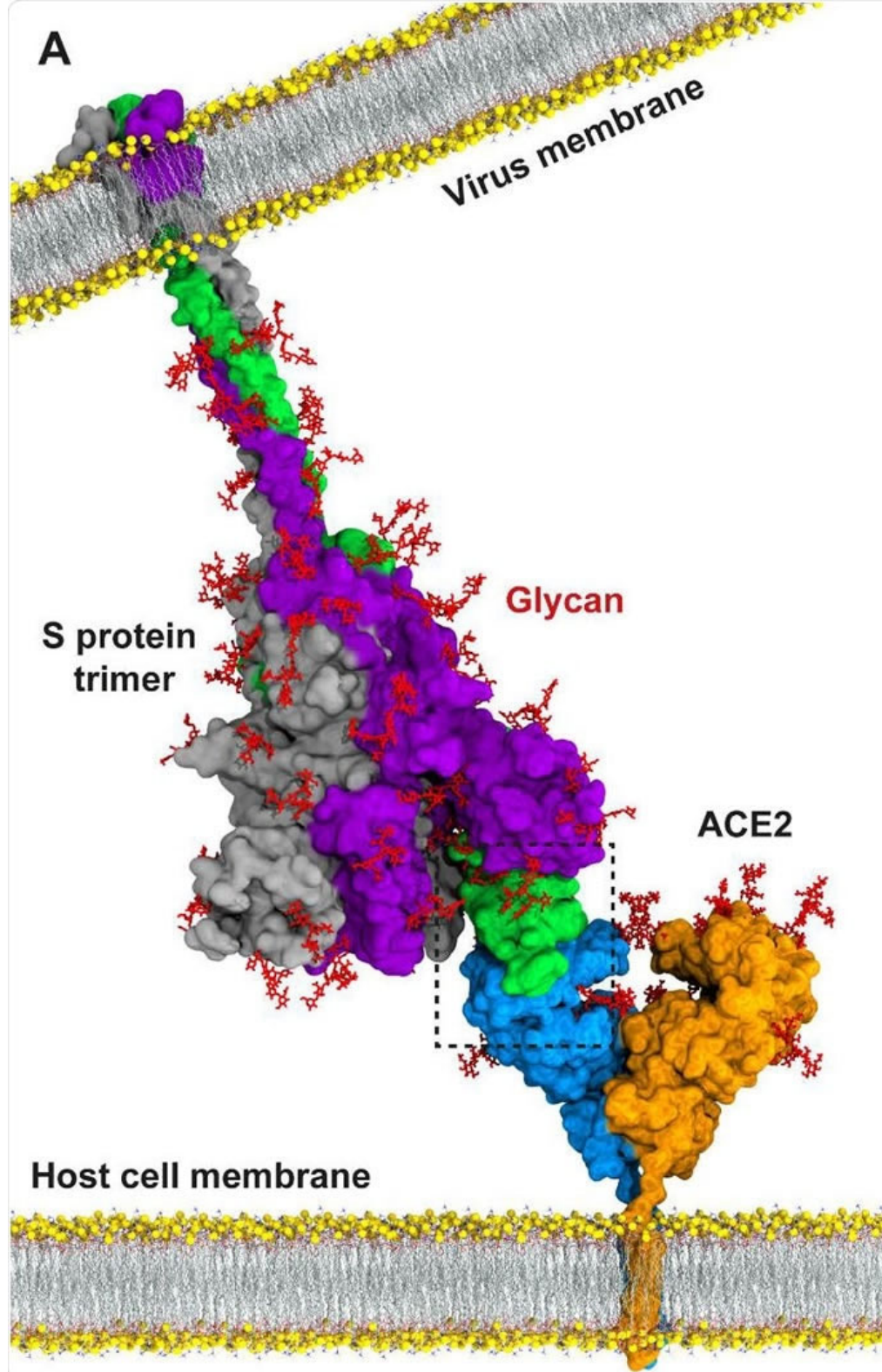
Different cell types express different transmembrane proteins

ACE2 is expressed on the membrane of respiratory epithelial cells but also in lungs, intestinal tract, kidneys, cardiomyocytes, endothelial cells, nervous system





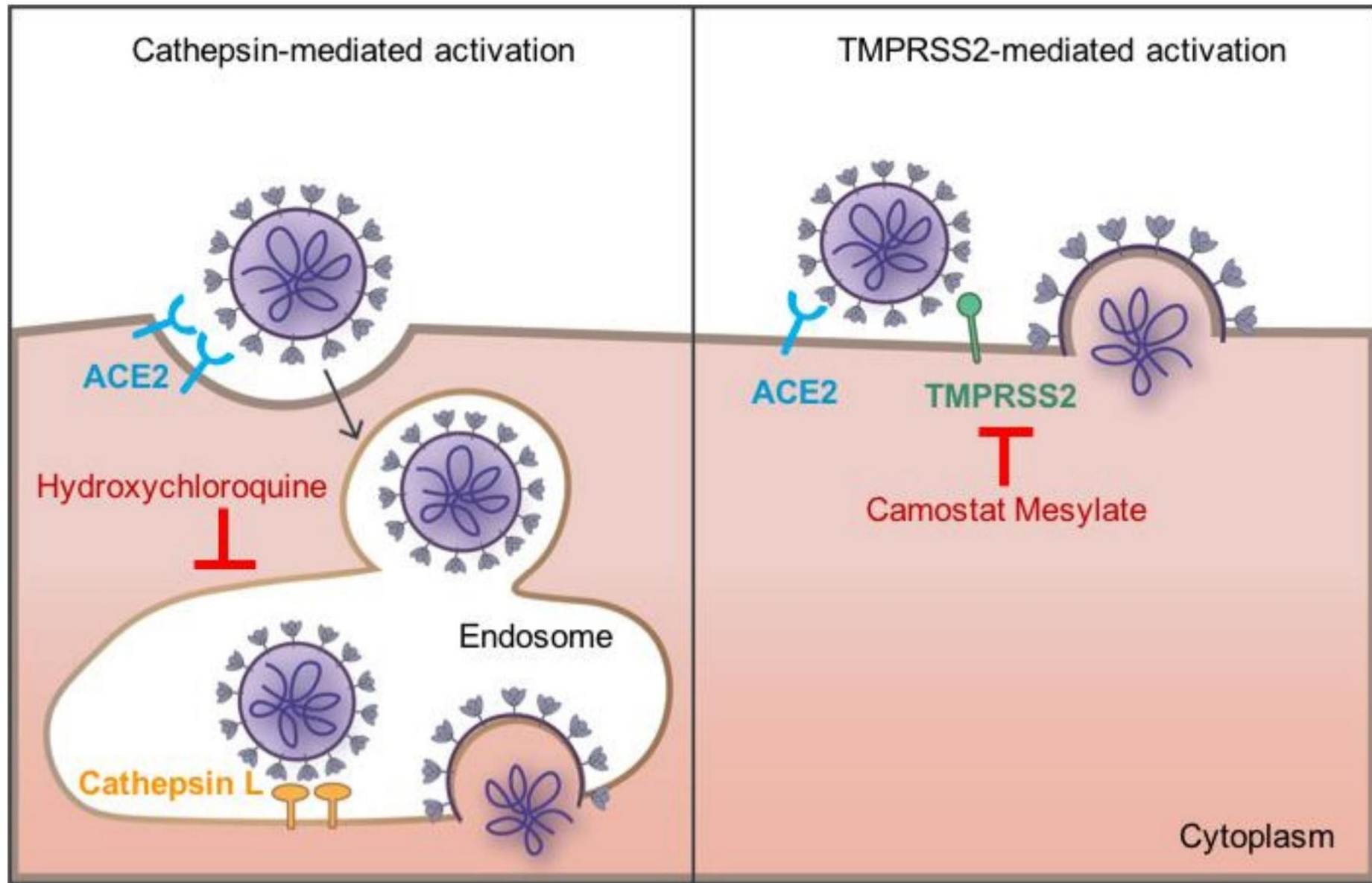
Scanning electron microscopy



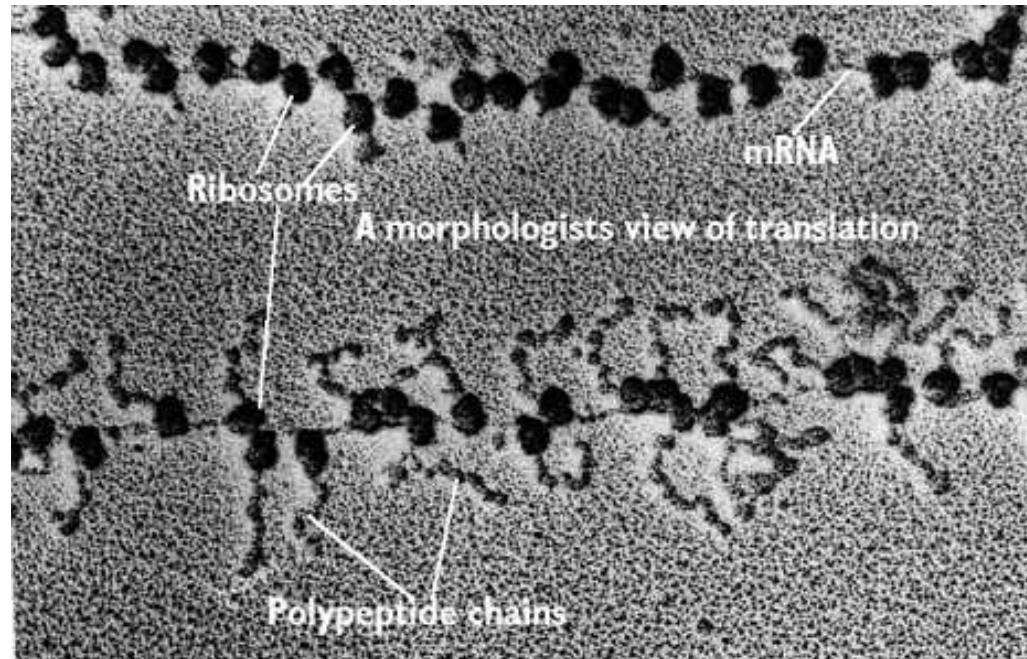
The virus enters cells

- through endosomes: endocytosis
- through fusion with the cell membrane

Viral RNA is released into the cytoplasm



Viral RNA is initially translated by free ribosomes in the cytosol, producing the viral RNA-dependent RNA polymerase (RdRp).



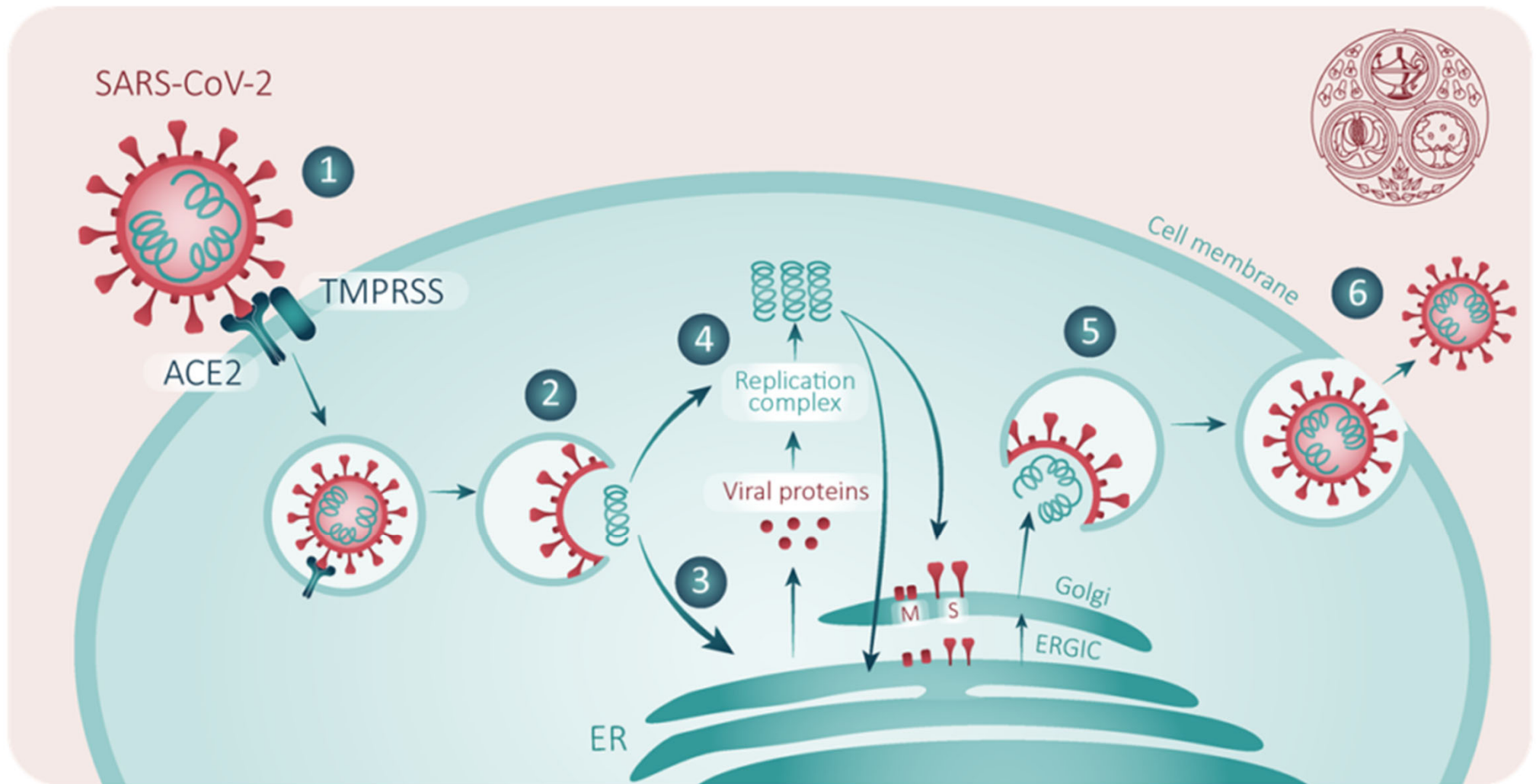
Using the viral polymerase...

Viral RNA is replicated to produce many copies of genomic RNA

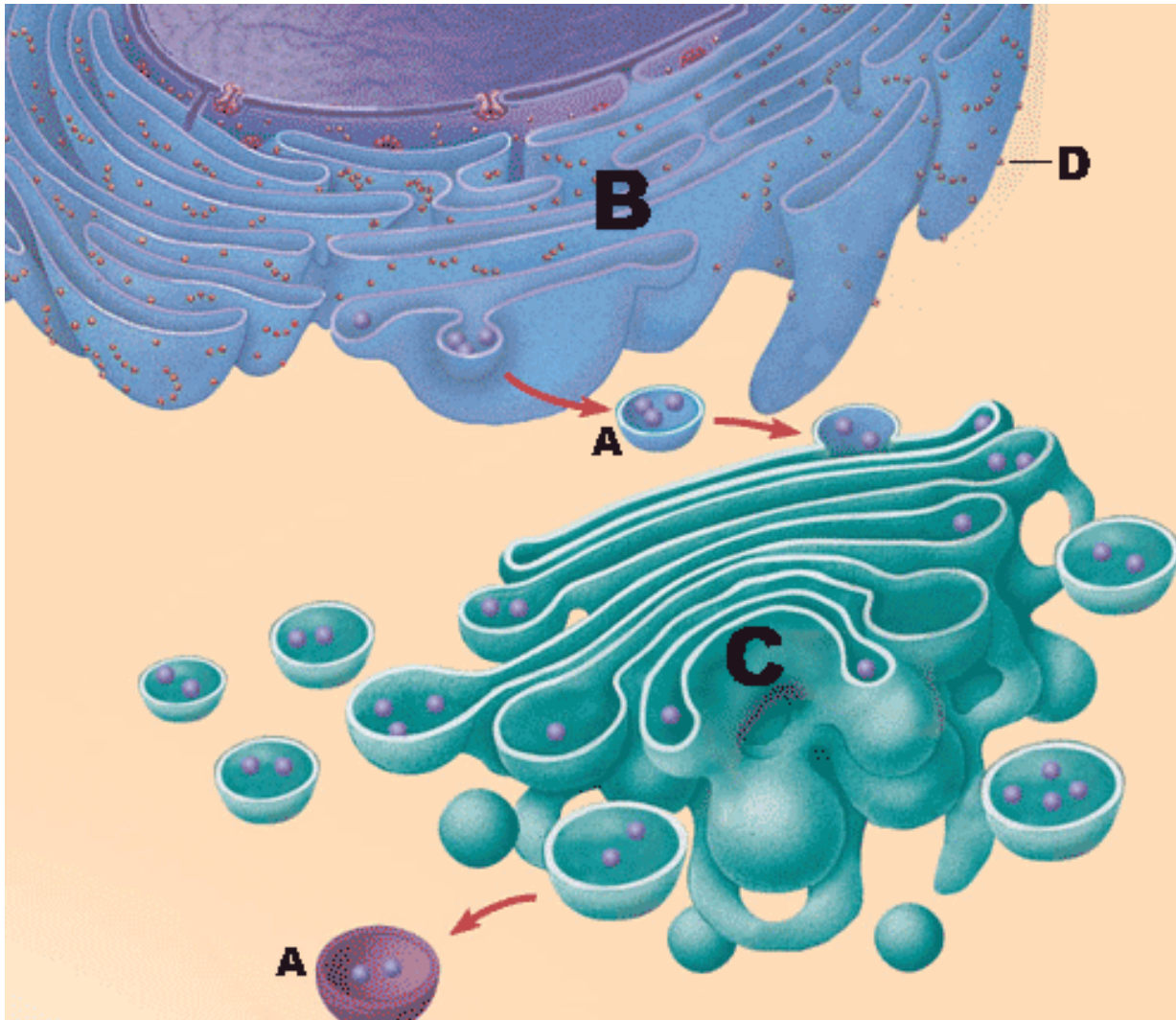
and transcribed to produce mRNAs

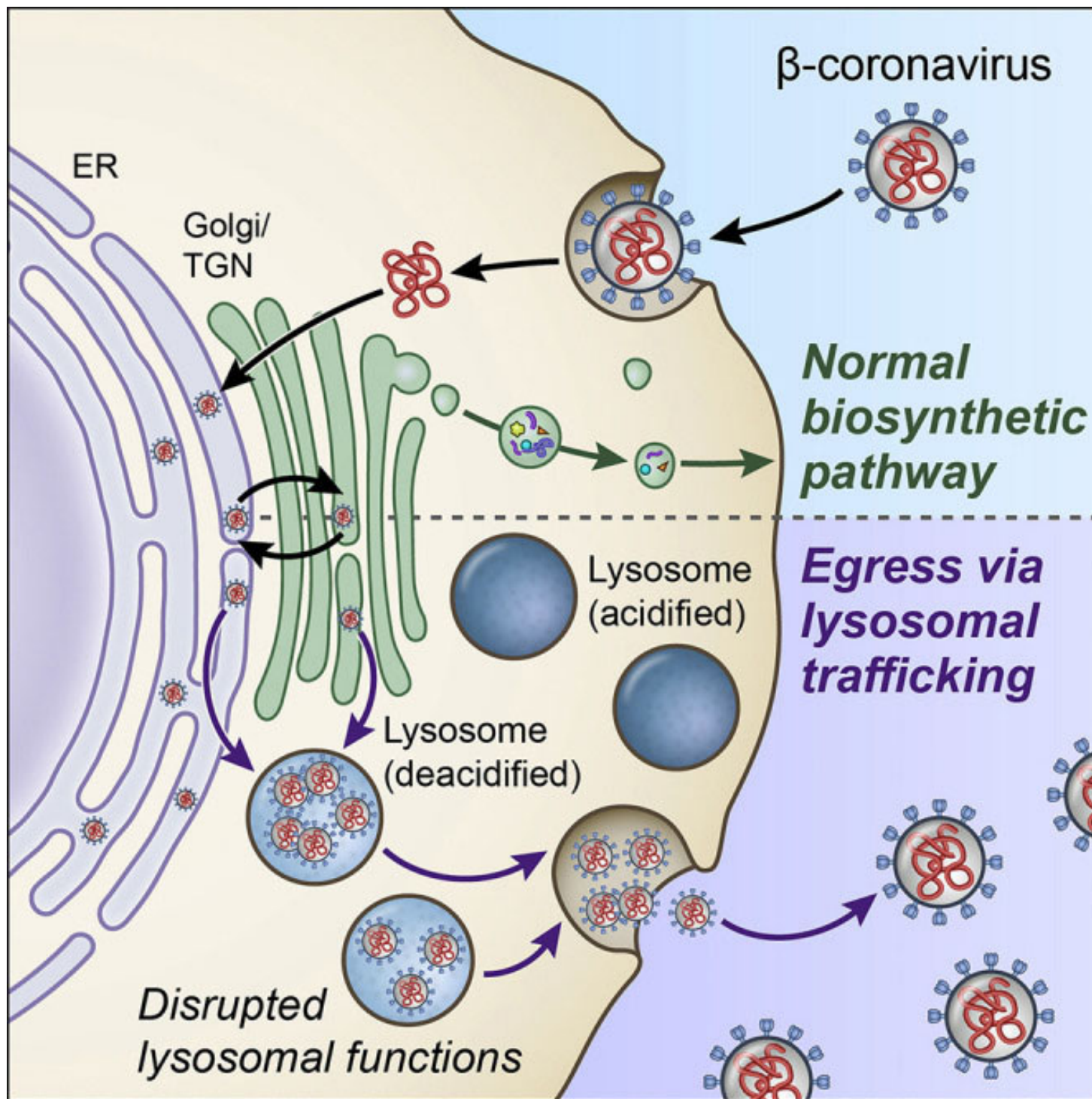
for the synthesis of viral capsid proteins

The SARS-CoV-2 life cycle



Viral envelope proteins are translated by ribosomes on the **endoplasmic reticulum**. Viral particles assemble, move to the **Golgi apparatus** and are finally released by **exocytosis**.

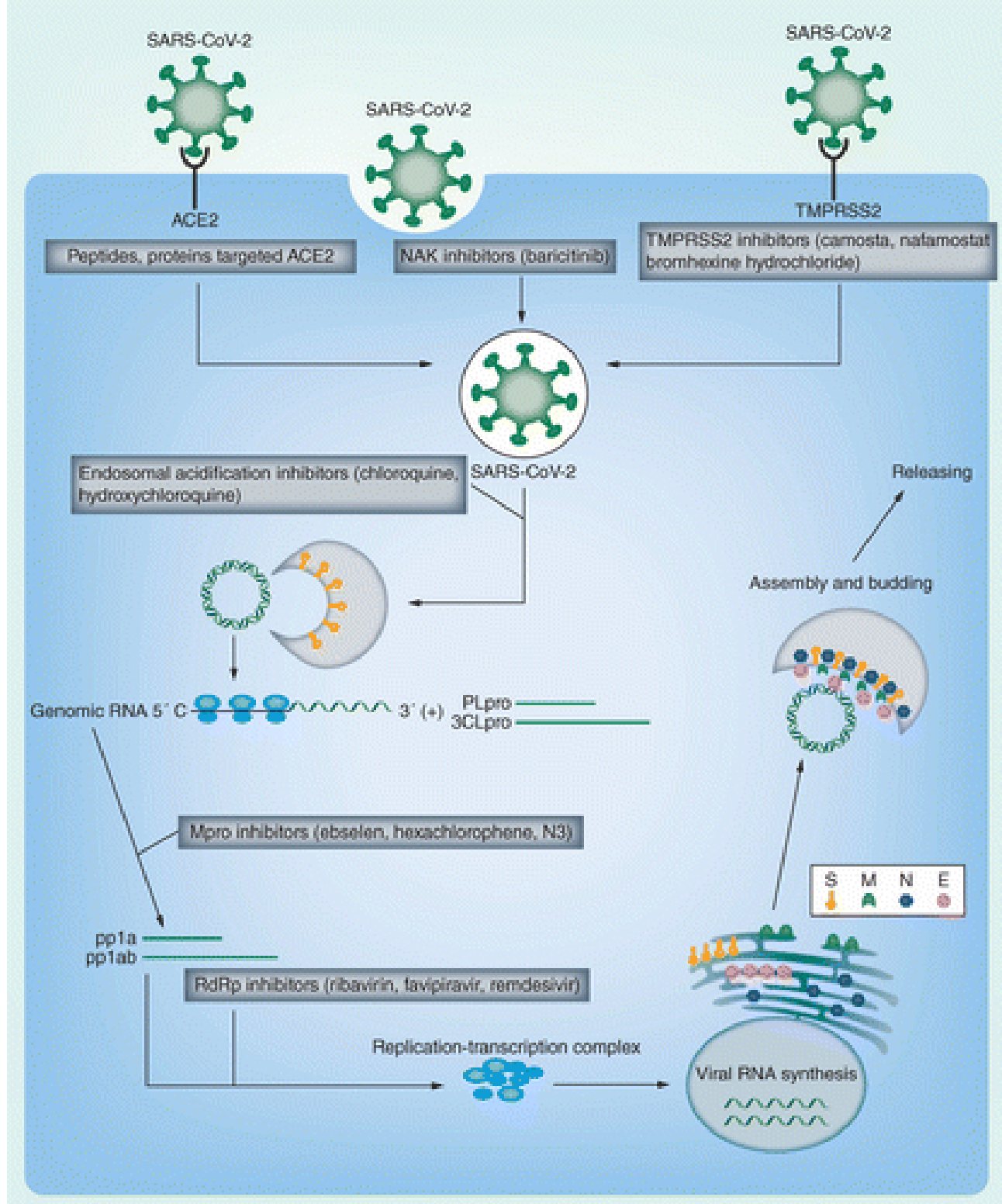


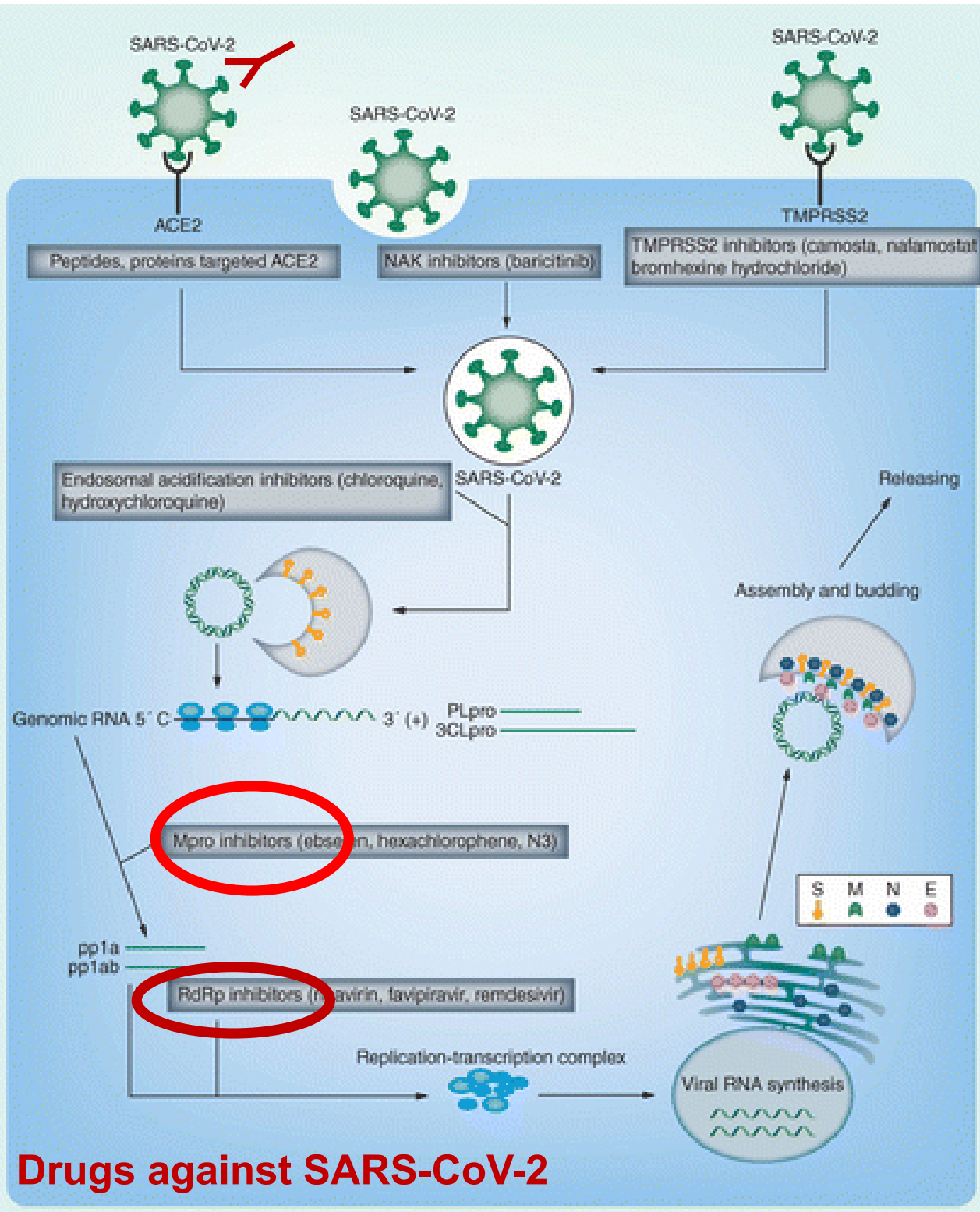


After fusion
with lysosomes...

Gosh et al, Cell,
December 2020

Drugs against SARS-CoV-2



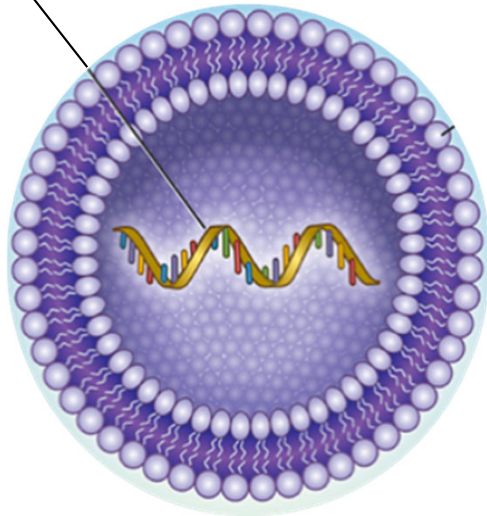


Drugs against SARS-CoV-2



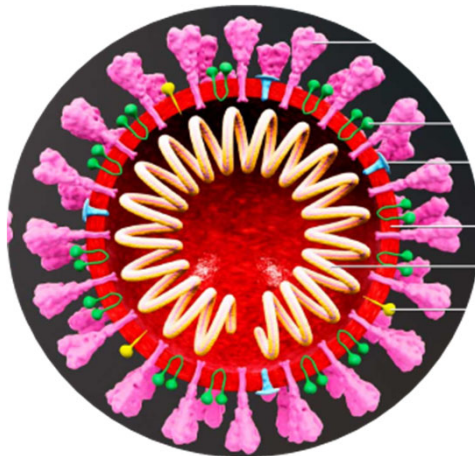
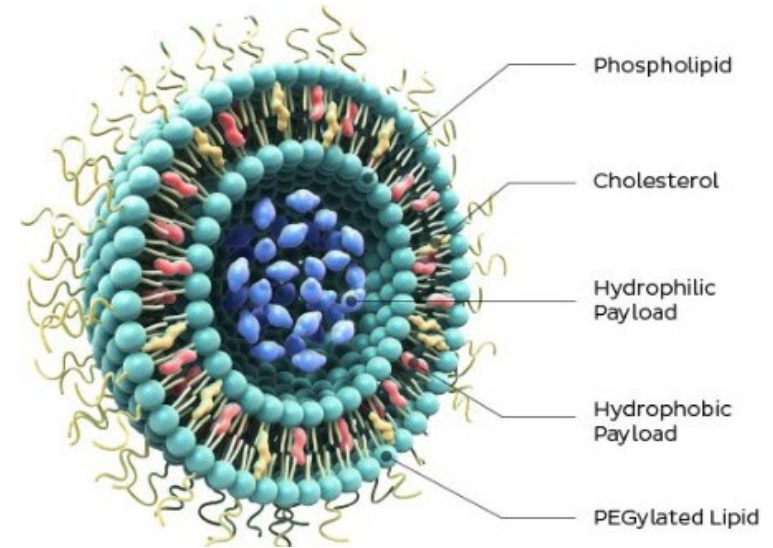
mRNA vaccines

mRNA (+ sense, encoding the S protein)



Lipid nanoparticle
LNP

(nanometre scale)



Resembles SARS-CoV-2

(similar size, positive-sense ssRNA, cap, poly(A), lipid bilayer)

But simpler (no proteins, shorter RNA, no ability to replicate, different lipid composition)

Only the S protein is produced: immunity without disease

The Nobel Prize in Physiology or Medicine 2023



Ill. Niklas Elmehed © Nobel Prize Outreach

Katalin Karikó

Prize share: 1/2



Ill. Niklas Elmehed © Nobel Prize Outreach

Drew Weissman

Prize share: 1/2

"for their discoveries concerning base modifications that enabled the development of effective mRNA vaccines against COVID-19"

Some videos about vaccines

<https://www.youtube.com/watch?v=mvA9gs5gxNY>

<https://www.youtube.com/watch?v=v-NEr3KCug8>

<https://www.youtube.com/watch?v=-92HQA0Gcl8>

Exams and question types

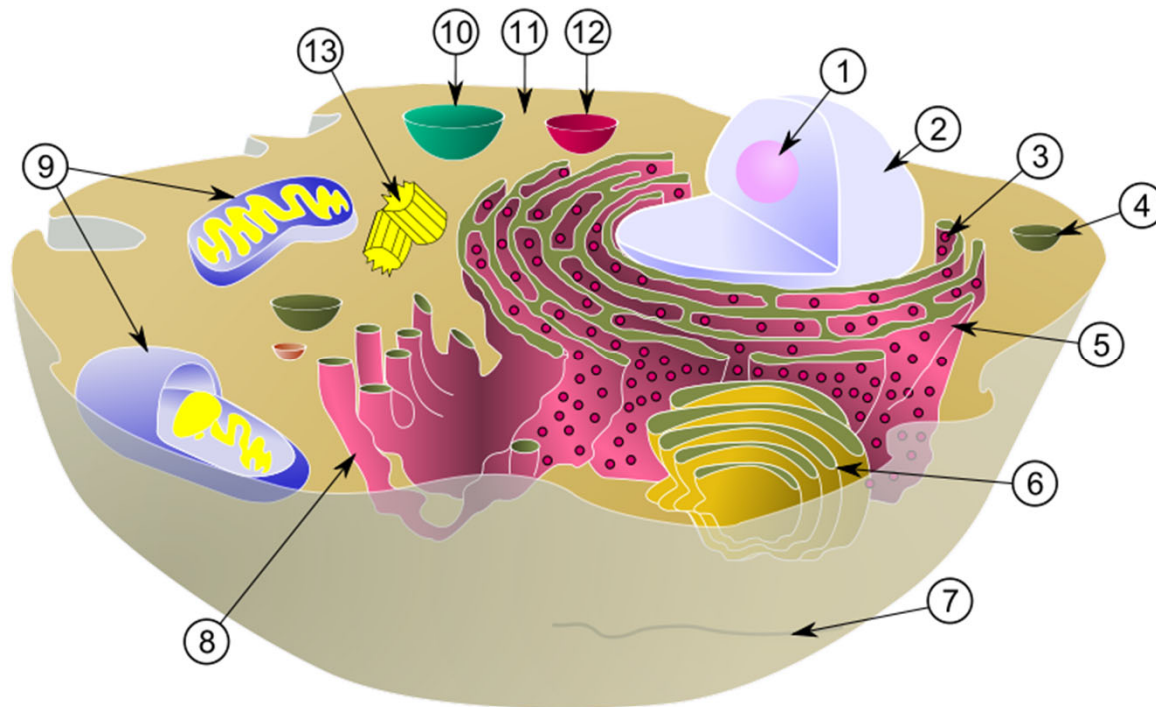
Exams cover core knowledge: main concepts and basic terminology.
Avoid getting lost in detail.

Exams assess understanding, rather than rote memorisation.

Question types

Multiple matching (with or without an image, with or without justification)

Match the following cellular components and functions to the correct numbers.



True or false with justification

Which of the following statements are true and which are false? Briefly justify your answers.

The nucleolus is surrounded by a double membrane.

In the cytoplasm, ribosomal proteins bind to ribosomal RNAs to form ribosomes.

Nuclear proteins are synthesised in the nucleus.

Lysosomes break down cellular components.

Ribosomes are present on the membrane of the Golgi apparatus.

Ribosomes in the cytosol, on the rough endoplasmic reticulum and in mitochondria are identical.

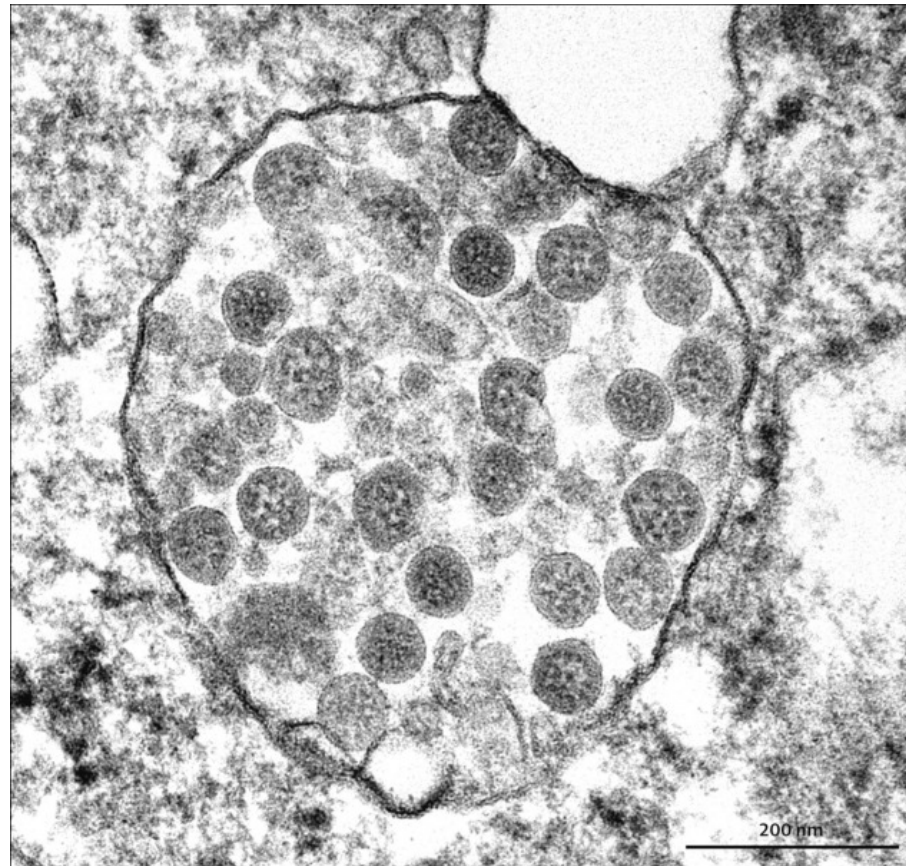
The cytoskeleton (like the human skeleton) is a stable structure that does not change during the life of the cell.

Image

The image below shows ... (SARS-CoV-2 particles leaving a human cell)

Write a caption

Or answer a question about the image



Logical pairs

Form logical pairs using the following terms and justify your answers.

1. Ribosome production
2. Low pH
3. Mitochondrion
4. Catalase
5. ATP production
6. Nucleolus
7. Peroxisome
8. Lysosome

Multiple-matching questions

Match the organelles/cellular compartments (a–e) to the functions that take place in them (1–8).

Note: more than one function may occur in a compartment, and a function may occur in more than one compartment.

- a. Nucleus
 - b. Mitochondrion
 - c. Golgi apparatus
 - d. Rough endoplasmic reticulum
 - e. Cytosol
-
- 1. Synthesis of nuclear proteins
 - 2. Synthesis of secreted proteins
 - 3. Synthesis of mitochondrial proteins
 - 4. Protein glycosylation
 - 5. DNA replication
 - 6. Transcription
 - 7. Translation
 - 8. Intron removal