SARS-CoV-2
Classification, Zoonosis, Viral Cycle

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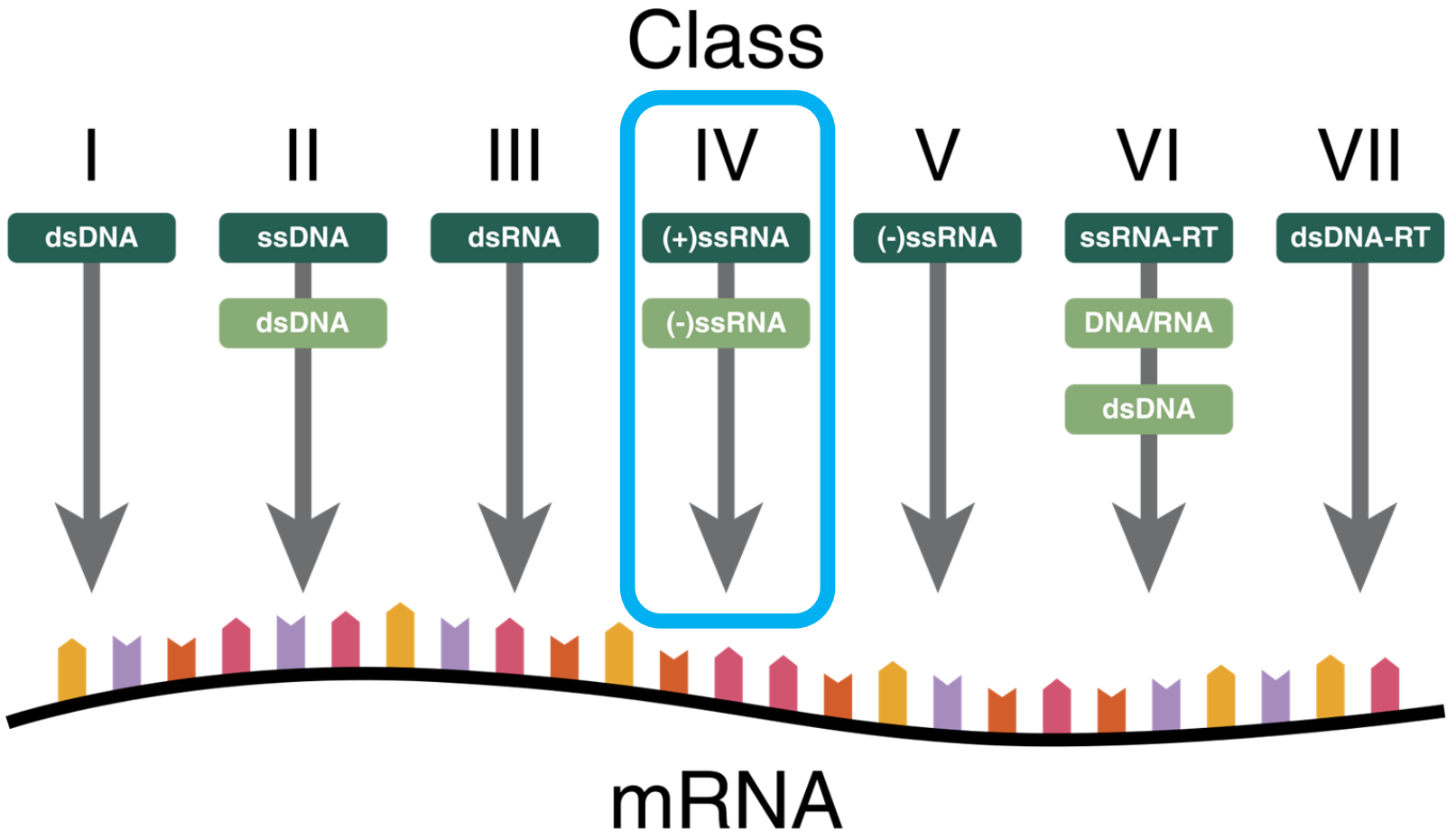
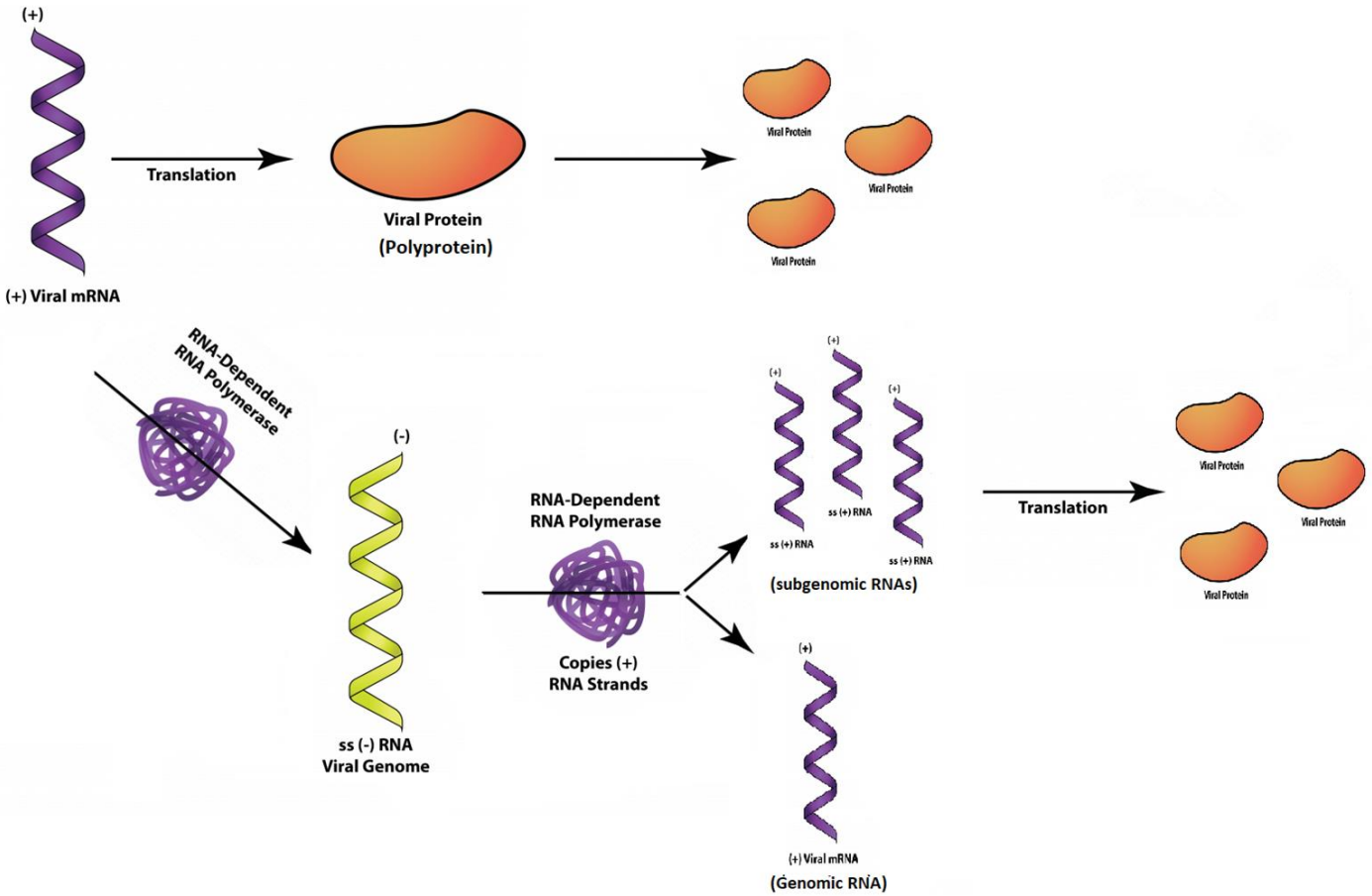
Baltimore classification (Baltimore, 1971)

- Baltimore classification is based on viral genome and mode of transcription /replication

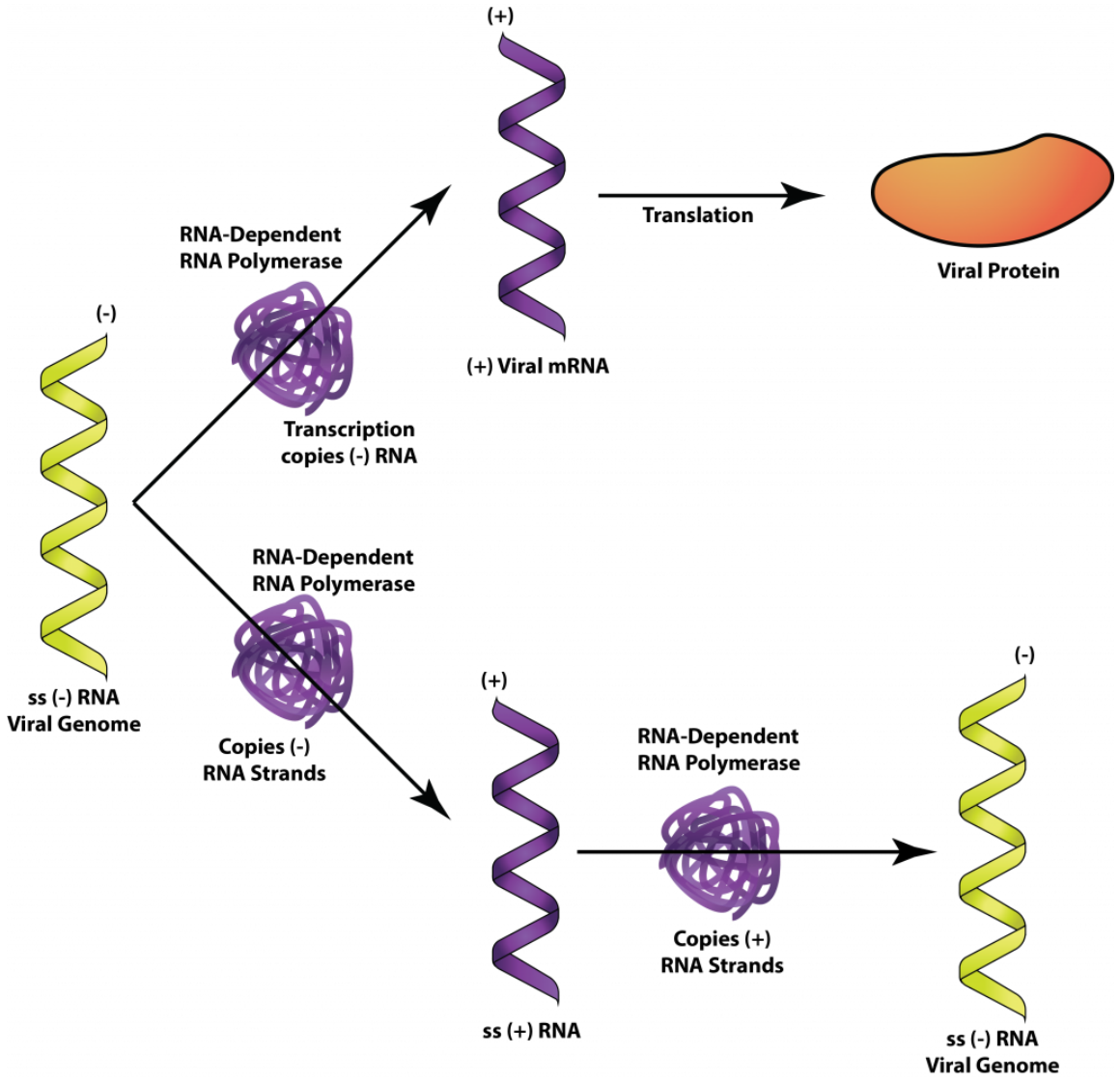


Class IV: positive sense ssRNA viruses



- Viruses provide RdRP
- Translation:
  - Polyprotein from polysistronic RNA (and/or frame shifting)
  - Proteins from subgenomic transcripts
- Picornaviruses (e.g. polio), **Coronaviruses**, Togaviruses (e.g. encephalitis/arthritis related diseases)

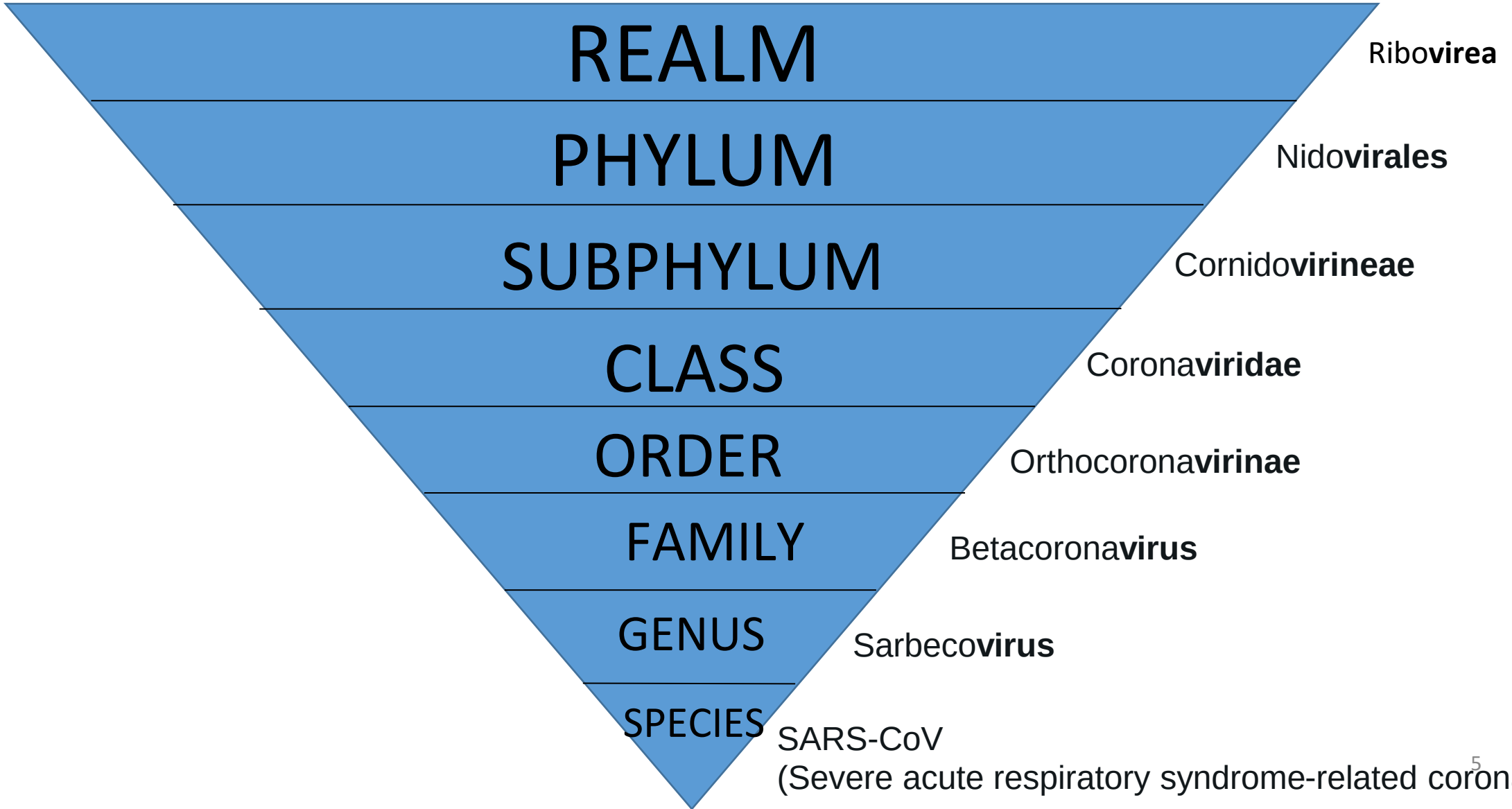
Class V: negative sense ssRNA viruses



- Sequence is complementary to mRNA
- RdRP provided by the virus
- Influenza Viruses, Rabies Virus, Ebola virus

image referance: https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(Bruslind)/22%3A_The_Viruses

ICTV (International Committee on Taxonomy of Viruses) classification



Coronaviruses in Humans

Coronavirus Name	Year	Location	Mortality Rate
229E	1965	England*	
OC43	1967	United States*	
SARS-CoV	2003	China	~15% (WHO, 2003)
NL63	2004	Netherlands	
HKU-1	2005	Hong Kong	
MERS-CoV	2012	Saudi Arabia	~35% (WHO, 2020)
SARS-CoV-2	2019	China (Wuhan)	~2.3% (Chen and Li, 2020)

*Research institute locations

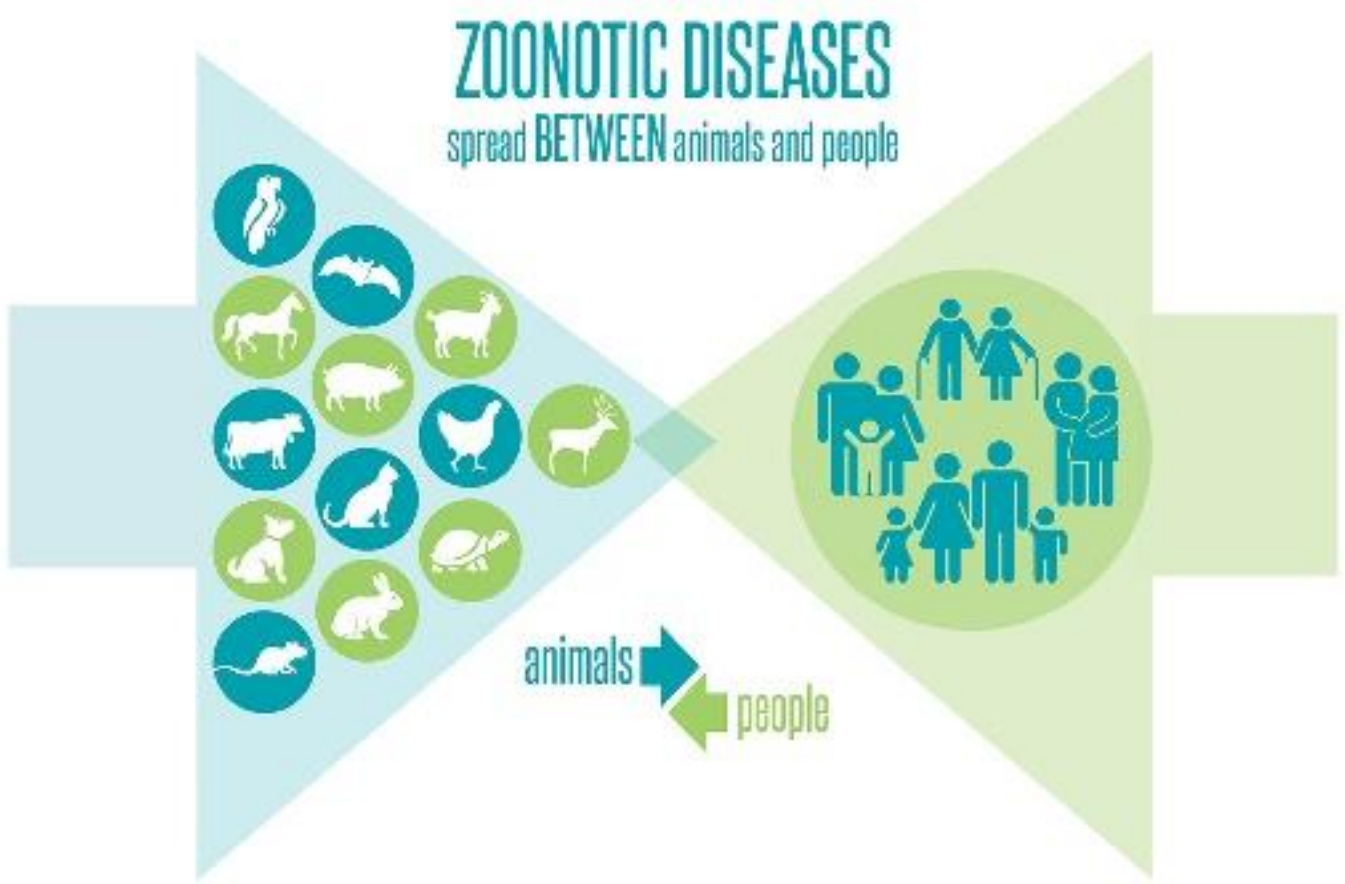
Jefrey and Kenneth, 2005

One Health One World

As stated by the World Organization of Animal Health (OIE), 60% of the known infectious diseases in humans are caused by animals, and 75% of newly emerged infectious diseases are triggered by animal-borne pathogens (OIE, 2020).

https://medium.com/@shwetachelluboina_74686/how-one-health-structures-human-healthcare-863af6aa14d1

Zoonosis

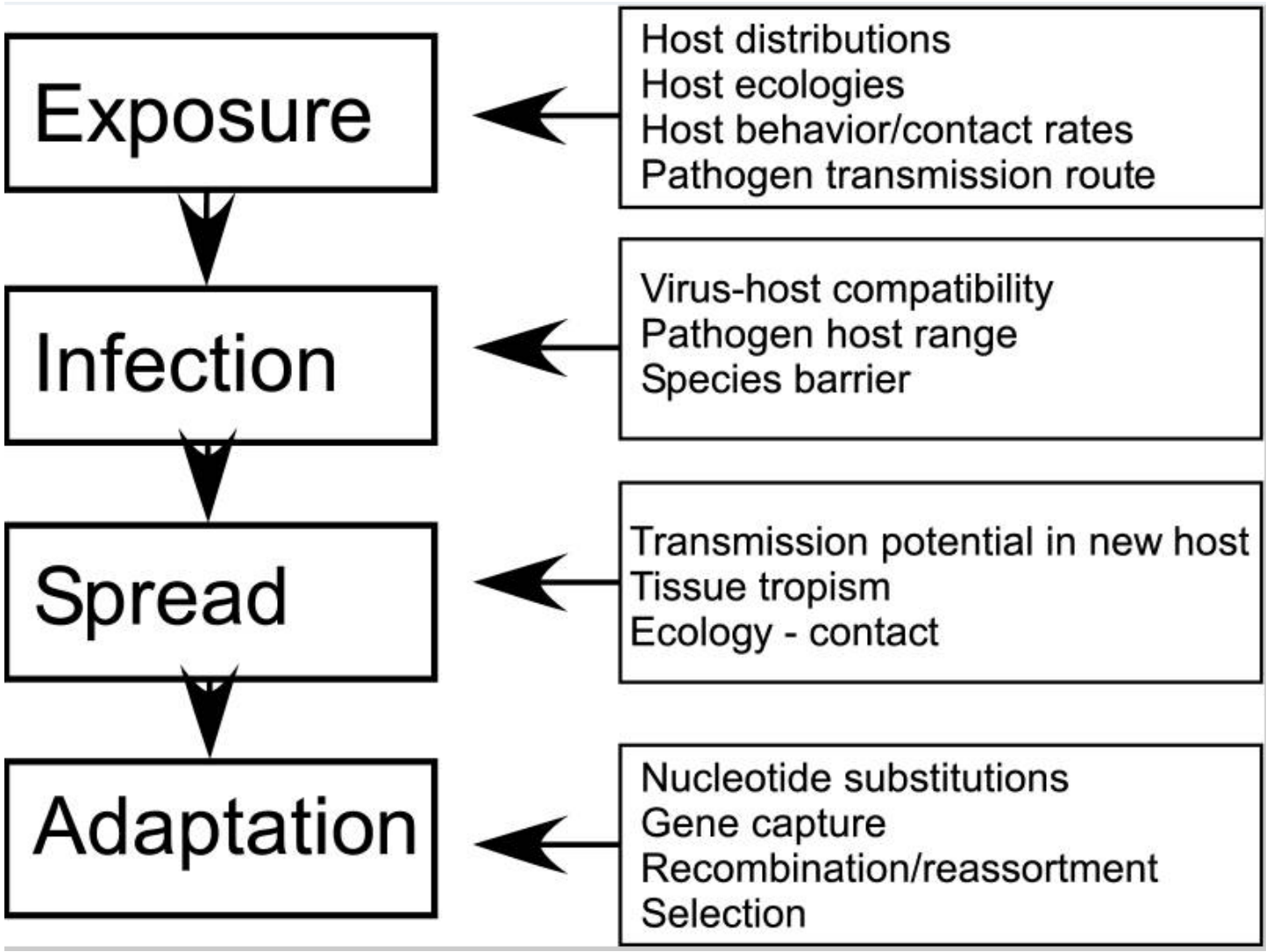


Zoonosis is the event of disease/pathogen transmission from animals to to human.

19th century by Dr. Rudolf Virchow

Reverse zoonotic disease transmission (Human to Animal) is called as Zooanthroponosis.

Host Switch Steps



Parrish et al., 2008

Barriers to Host Switching

- Host External Barriers (Mucus, Skin etc.)
- Receptor Binding
- Internal Barriers (Interferons, Absence of required enzymes etc.)

Zoonotic Transmission Examples

Viruses	Original Host	Intermediate Host	New Host
Influenza A Virus	Aquatic Birds	Swine/poultry/quail	Human
HIV-1 (SIV?)	Old World Primates		Human
SARS-CoV	Bats	Masked Palm Civets	Human
Dengue Virus	Old World Primates	Mosquito	Human
Ebola Virus	Bats	Monkey	Human
West Nile Virus	Birds	Mosquito	Human
Hendra Virus	Fruit Bats	Horse	Human

Parrish et al., 2008

Coronaviruses

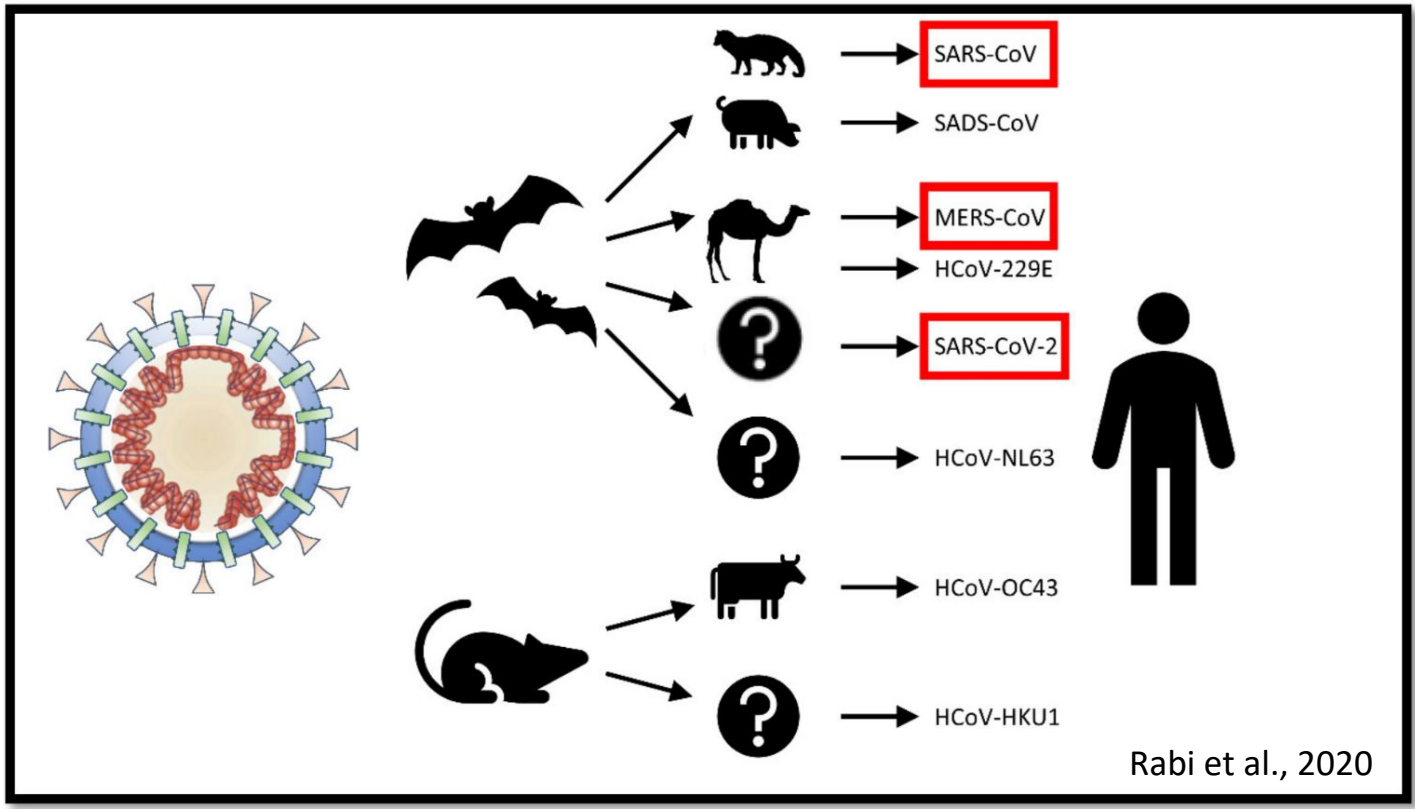
Alphacoronavirus	• Alphacoronavirus 1 comprising: Feline Coronavirus (FCoV) serotype 2 Canine Coronavirus (CCoV) serotype 2 Transmissible gastroenteritis virus (TGEV) • Human coronavirus 229E • Human coronavirus NL63 • Porcine Epidemic Diarrhea Coronavirus (PEDV) • Rhinolophus bat coronavirus HKU2 • Scotophilus bat coronavirus 512/05 • Miniopterus bat coronavirus 1 • Miniopterus bat coronavirus HKU8

Betacoronavirus	• Betacoronavirus 1 comprising: Bovine coronavirus (BCoV) Human coronavirus OC43 (HCoV-OC43) Equine coronavirus (ECoV) Human enteric coronavirus (HECoV) Porcine haemagglutinating encephalomyelitis virus (PHEV) Canine respiratory coronavirus (CrCoV) • Murine coronavirus • Human coronavirus HKU9 • Rousettus bat coronavirus HKU4 • Tylonycteris bat coronvirus HKU5 • SARSr-CoV (SARS related Coronavirus) comprising Human SARS-CoV Rhinolophus bat viruses

SARS-CoV-2

Gamma-coronavirus	• Avian coronavirus comprising: IBV Various coronaviruses infecting turkey, pheasant, duck, goose and pigeon • Beluga Whale coronavirus SW1
Delta-coronavirus	• Bulbul coronavirus HKU11 • Thrush coronavirus HKU12 • Munia coronavirus HKU13

Host Range



- ss(+)RNA virus
- Enveloped (spherical)
- 60-140nm in diameter
- ~30kb genome (the largest)
- Encoding
  - 4 structural proteins (Spike, Membrane, Envelope, Nucleocapsid)
  - 16 non-structural proteins (nsp1-nsp16)

Genetic material of ss(+) RNA viruses can be infectious (Peleg, 1969; Burroughs et al., 1978; Roner et al., 1990)

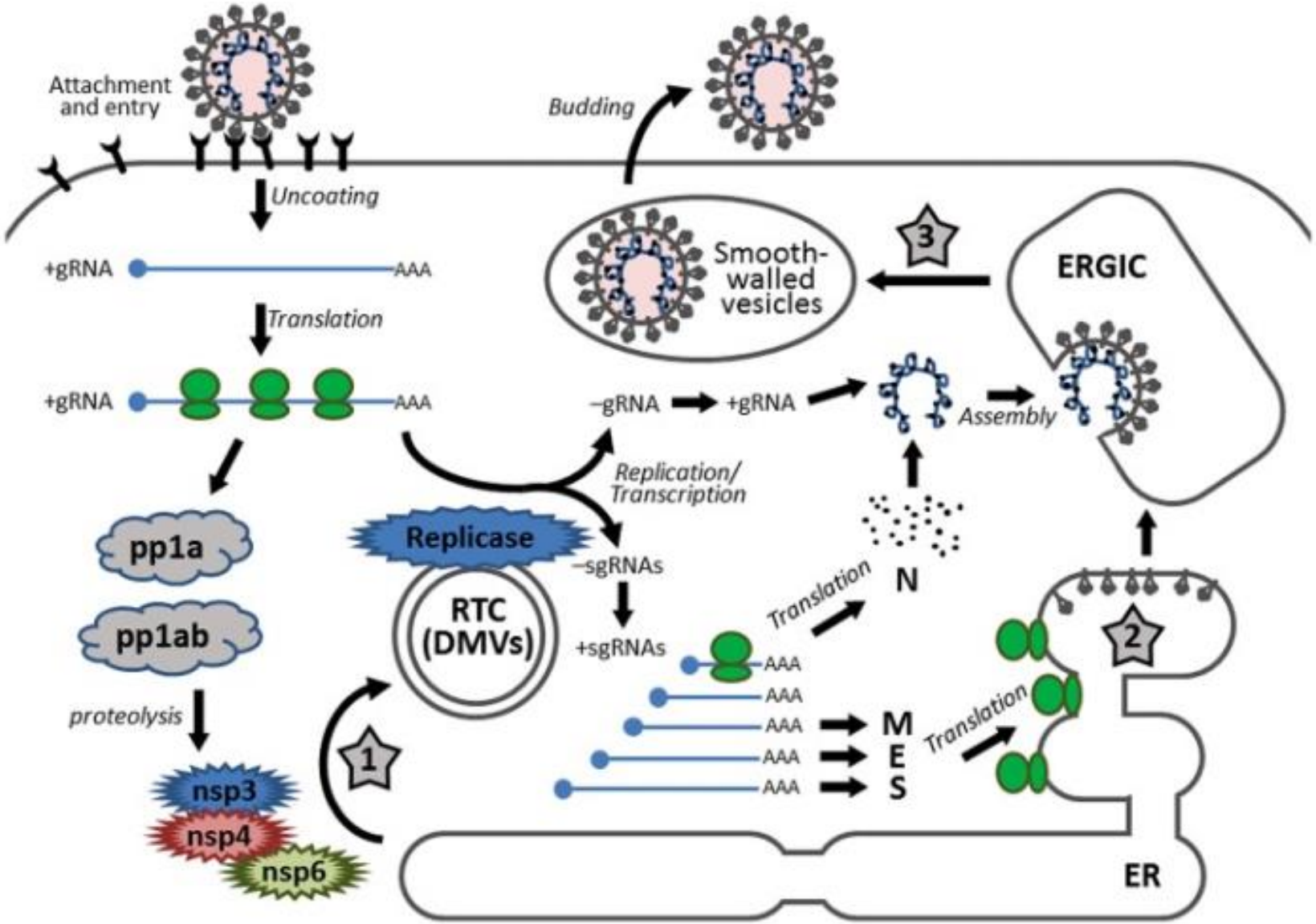
Is Intermediate Host Necessary?

NO

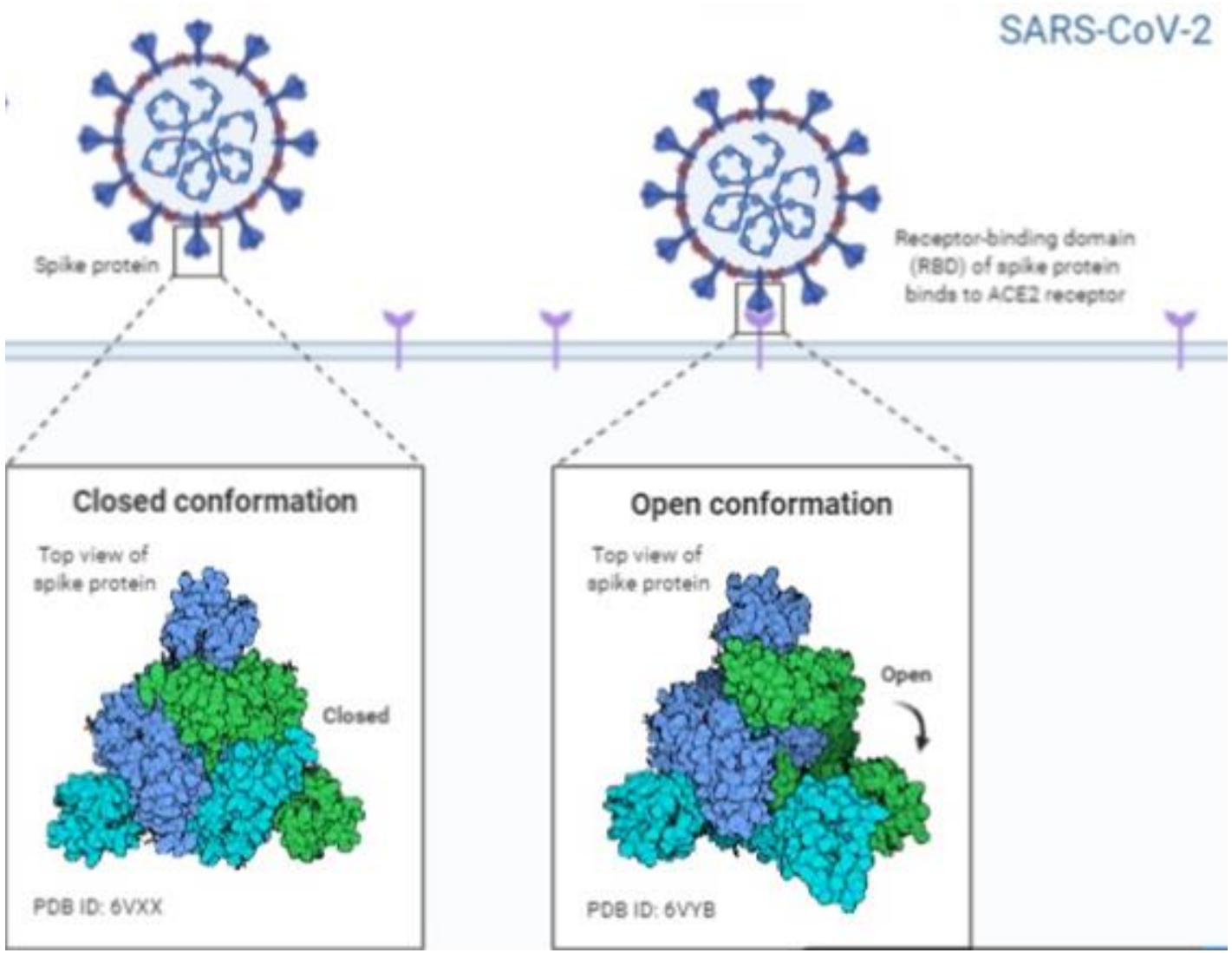
- A live SARS-like coronavirus was isolated from fecal samples of Chinese horseshoe bats , which could use the SARS-CoV cellular receptor – huACE2 for cell entry (Ge et al., 2013).

- Direct human infection is possible by some bat coronaviruses, avian influenza, Crimean-Congo etc.

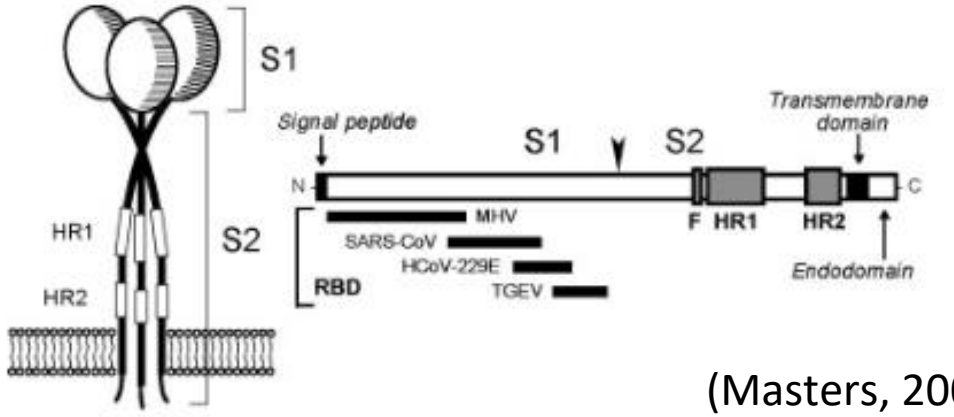
SARS-CoV-2 Viral Cycle



Cell Attachment

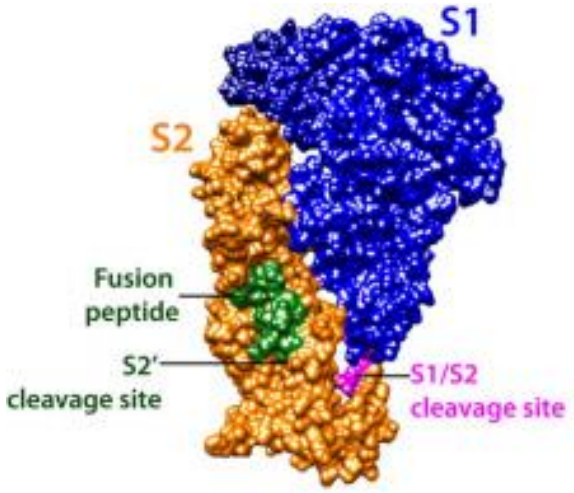


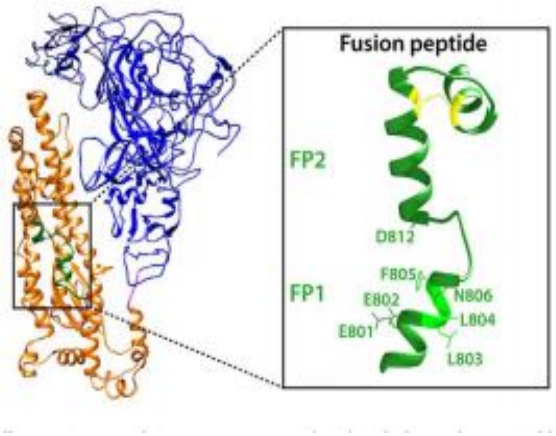
- Receptor binding site of Spike protein attaches on the receptor.
- Attachment cause conformational change on the Spike protein.
  - Reveals the cleavage S1/S2 site



(Masters, 2006)

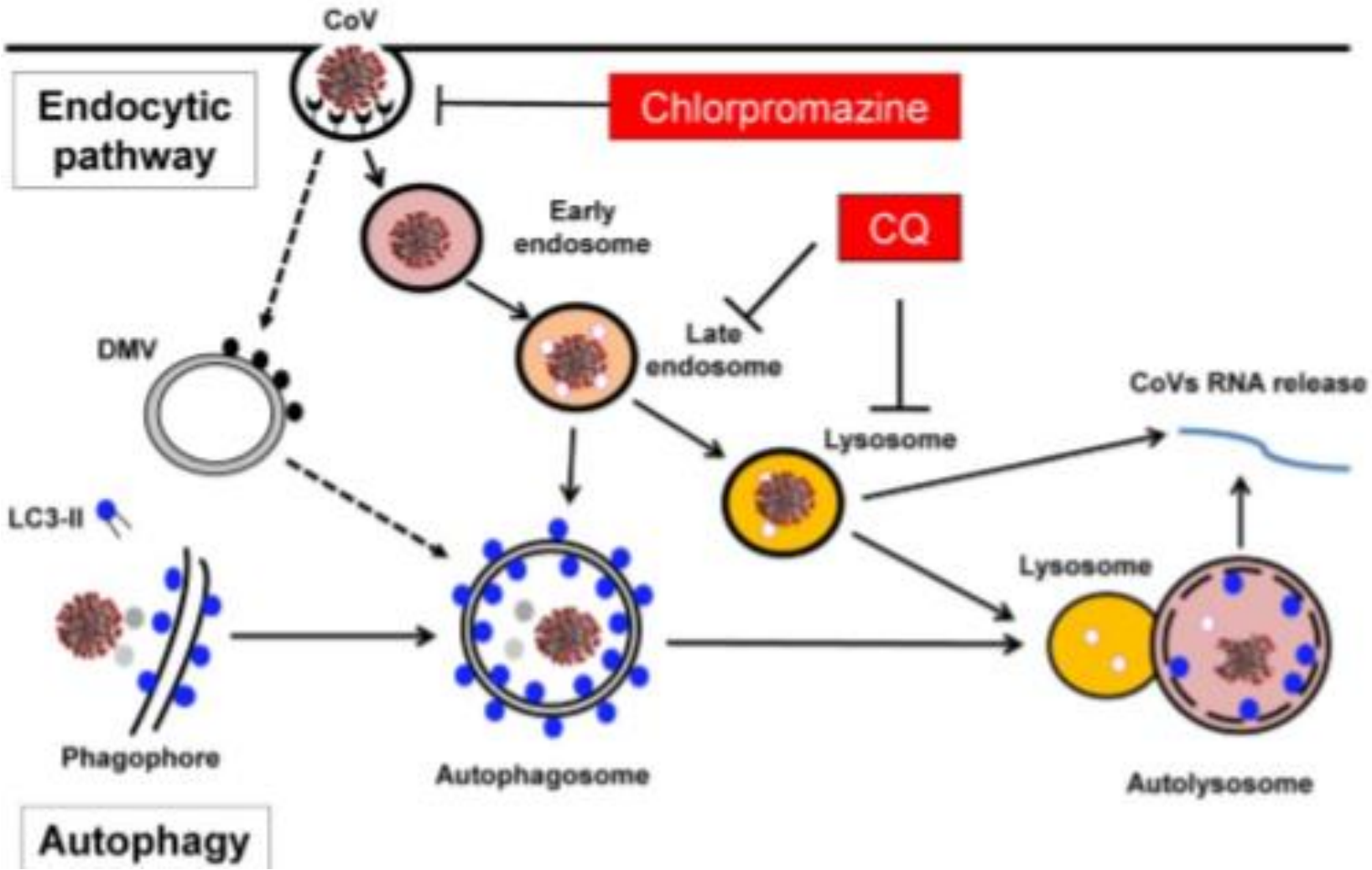
Viral Entry





- After attachment, S protein cleavage occurs at two sites.
  - Cleavage at S1/S2 site is essential for separating the RBD and fusion domains of the S protein.
  - Cleavage at S2' site is important for exposing the fusion peptide.
- Fusion peptide interacts with the host cellular membrane.
- Two heptad repeats and fusion peptide forms six-helix "trimer-of-dimers" bundle.
- Juxtaposition of the viral and cellular membranes.
- Biphasic cell entry: Early-entry or Late-entry
- Receptor-dependent, pH and Ca sensitive endocytosis

Viral Entry



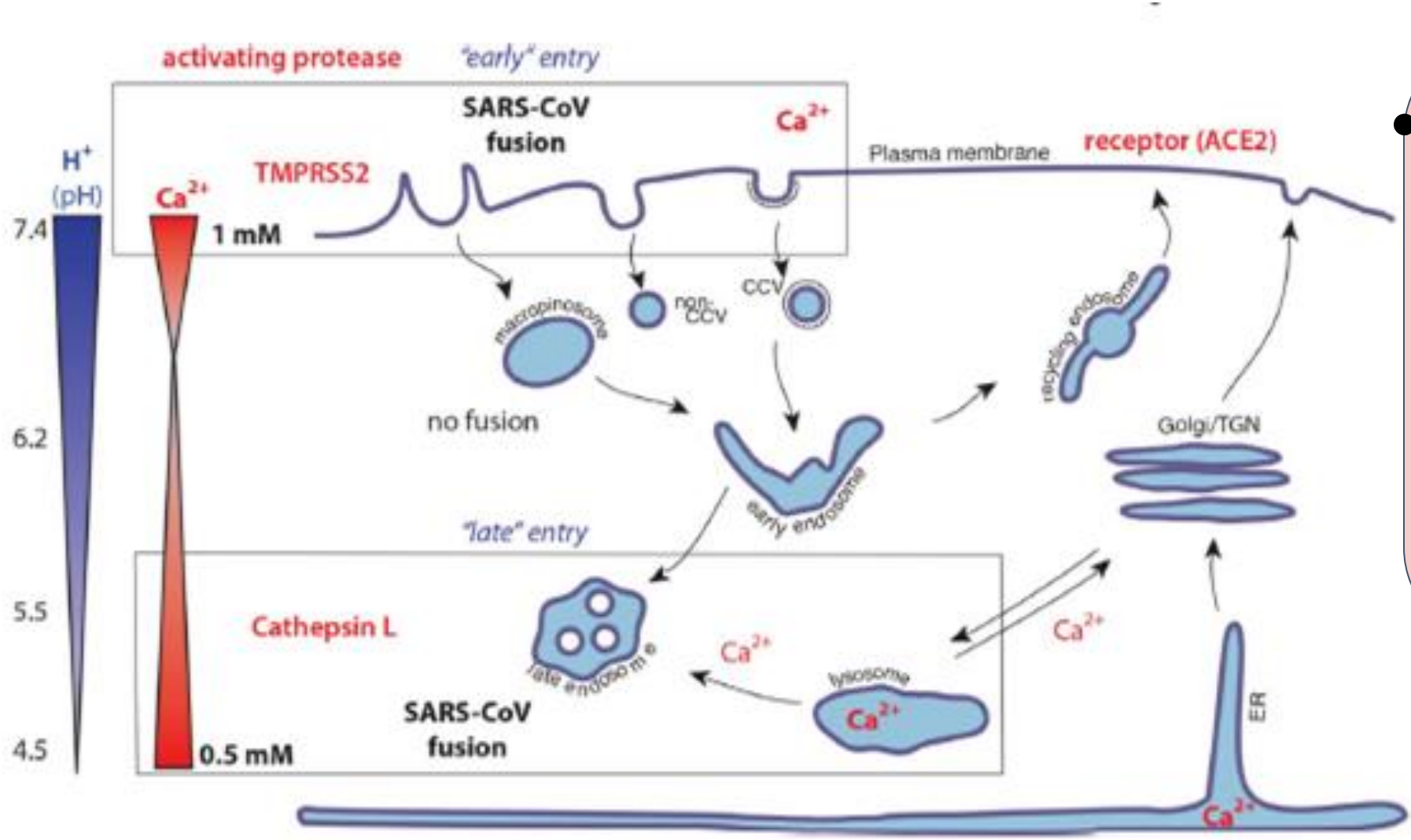
- Coronaviruses enter the host cells via two routes
  - Endocytic pathway
  - Non-endosomal pathway
- The autophagy has also been implicated in the viral replication in the cells, a process partly related to the formation of DMV in the host cells.

Viral Entry

Virus and cells tested	Part of endocytic pathway studied	Main findings
SARS-CoV/Vero E6 cells	S protein-mediated entry	SARS-CoV entry requires acidification of endosomes
SARS-CoV S glycoprotein/Vero cells	S-protein mediated entry	S-protein mediated entry is pH-dependent
SARS-CoV /Vero E6 cells	Endo-lysosomal pH/cysteine protease	SARS-CoV entry requires acidification of endosomes
SARS-CoV/Vero cells, 293T cells	Endo-lysosomal cysteine protease Cathepsin L	Cathepsin L is required for infection of cells with ACE2 expression
SARS-CoV/HepG2 cells	Clathrin-dependent endocytosis	Virus entry is mediated by clathrin-dependent endocytosis
SARS-CoV/Vero cells	Late endosome	Amiodarone inhibits late endosome to suppress SARS-CoV infection
SARS-CoV/HEK293E cells	Clathrin- and caveolae-mediated endocytic pathway	Virus entry is mediated by a clathrin- and caveolae-independent endocytic pathway
SARS-CoV 2/ Vero E6	Endosomal pH	SARS-CoV-2 entry requires acidification of endosomes

(Yang, et al. 2020)

Viral Entry

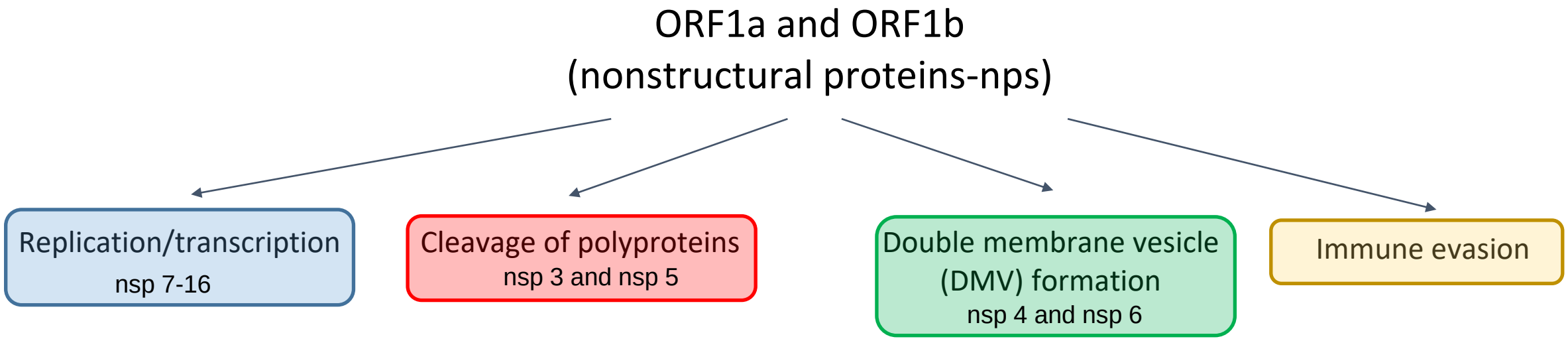


- After endosomal uptake, entry of SARS-CoV is inhibited by blocking the endosomal/lysosomal calcium channels.

(Millet, et al. 2017)

Encoded proteins

- Sub-genomic RNAs ⟹ Structural Proteins - spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins



-nsp1: host shot off by degradation

-nsp2: UNKNOWN

-nps3: cleave pp1a and pp1a/b, interfere with type I IFN production by deubiquitination of components of IFN production pathways.

-nsp4: formation of double membrane vesicles (DMVs) that contains replication/transcription complex (RTC)

-nsp5: cleave pp1a and pp1a/b; inhibiting IFN signaling via cleavage of NEMO and STAT6.

-nsp6: limits autophagosome expansion and prevent release of viral proteins to be released from DMVs that can be recognized as antigens.

-nsp7/8: together produce short RNA stretches to be used as primers.

-nsp8: Primes RNA synthesis. Interacts with nsp7 and nsp12.

-nsp9: RNA binding of RTC

-nsp10: interacts with nsp14 and nsp16, regulation of replication

-nsp11: UNKNOWN

-nsp12: RNA dependent RNA polymerase

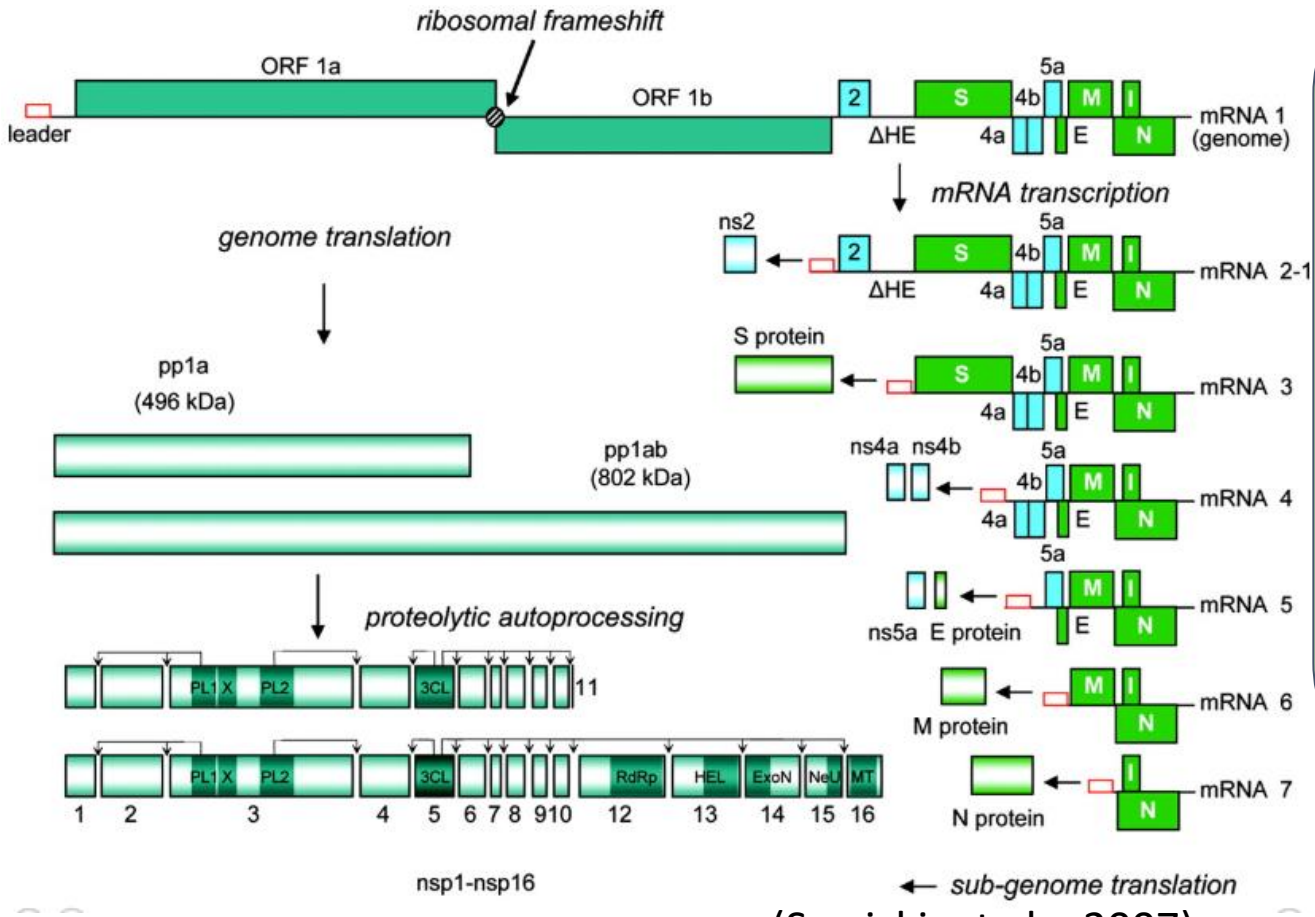
-nsp13: Helicase, 5'triphosphatase (5'cap formation)

-nsp14: 3' to 5' exoribonuclease (genome maintenance), N7-MTase (5' cap formation)

-nsp15: endoribonuclease (for dsRNA), evasion from innate immune system

-nsp16: 2′-O-methyltransferase (5'cap formation), decrease activation of IFN production by inhibiting activation of poly I:C-, RIG-I- and MDA5

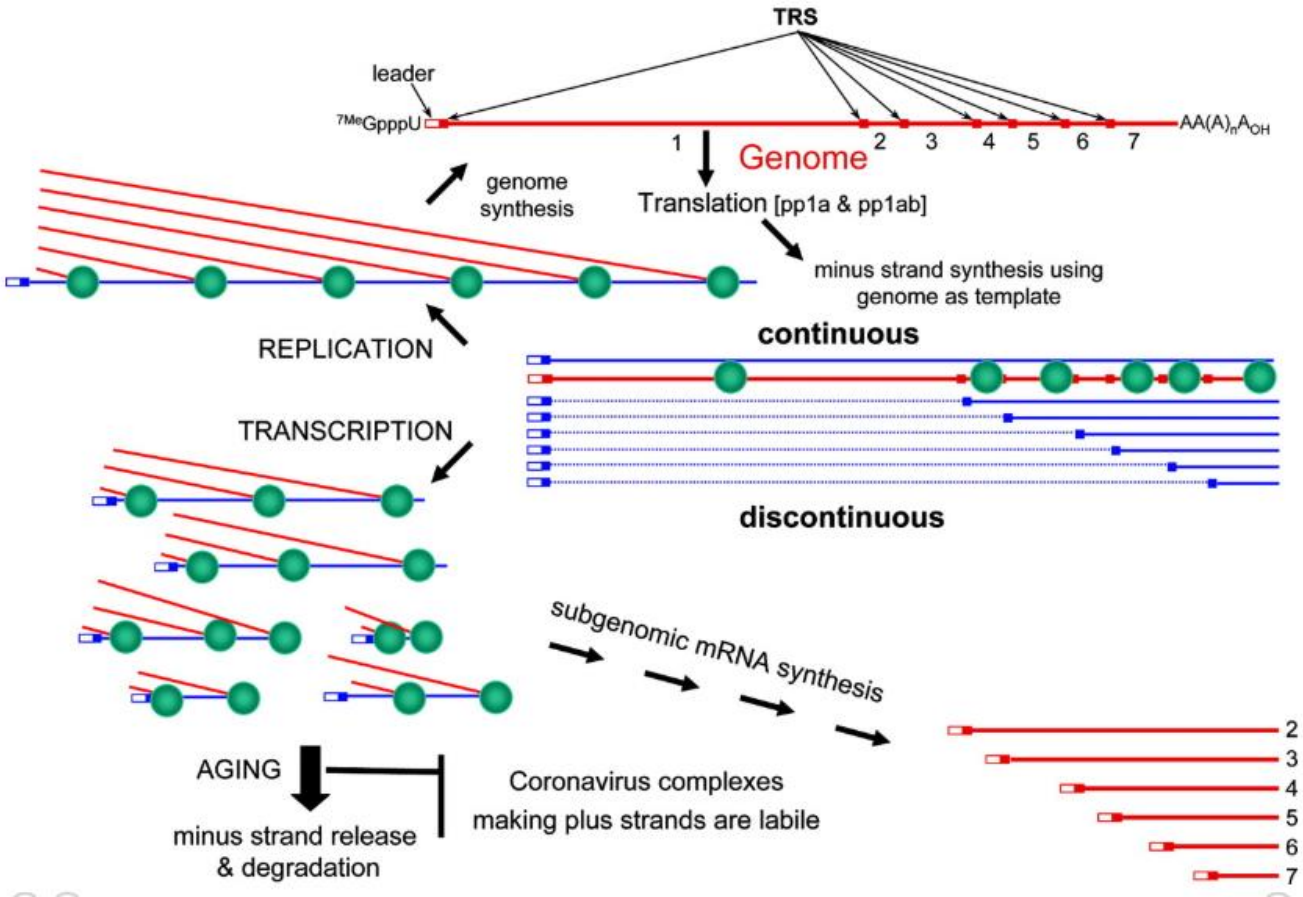
Translation



(Sawicki, et al. , 2007)

- Replicase gene encodes two open reading frames.
  - ORF1a and ORF1b are translated into pp1a and pp1b
- Pp1a and pp1b contain non-structural proteins 1-11 and 1-16 respectively.
- Pp1a and pp1b can cleaved by virus encoded proteases (nsp3 ve nsp5).
  - Papain-Like proteases (PLpro)
  - Serine type protease
  - Main protease (Mpro)

Replication and Transcription



(Sawicki, et al. , 2007)

- Many non-structural proteins assemble and form replicase-transcriptase complex (RTC) (nsp7-nsp16)
- These non-structural proteins use the genome as a template to generate full-length negative sense RNAs (-gRNA)
- -gRNA serve as templates in generating additional full-length genomes (+gRNA)
- Subgenomic RNAs then serve as templates for viral mRNA production.

Assembly and Release

- After viral structural proteins Spike (S), Membrane (M), Envelope (E), and Nucleocapsid (N) proteins are translated, they moved into the ER.
- S, E and M proteins move to the endoplasmic reticulum–Golgi intermediate compartment (ERGIC) by secretory pathway
- Viral genomes encapsidated by N protein bud into membranes of the ERGIC containing viral structural proteins, forming mature virions.
- Following assembly, virions are transported to the cell surface in vesicles and released by exocytosis.

- Infection causes ER stress on the cells and induce the Unfolded Protein Response (UPR) that is simply global translation shutdown to regain the ER homeostasis.
-
- Prolonged ER stress might result in apoptotic cell death.

- Thus, ER Stress and UPR activation plays a significant role in viral replication & pathogenesis.

Spike Protein

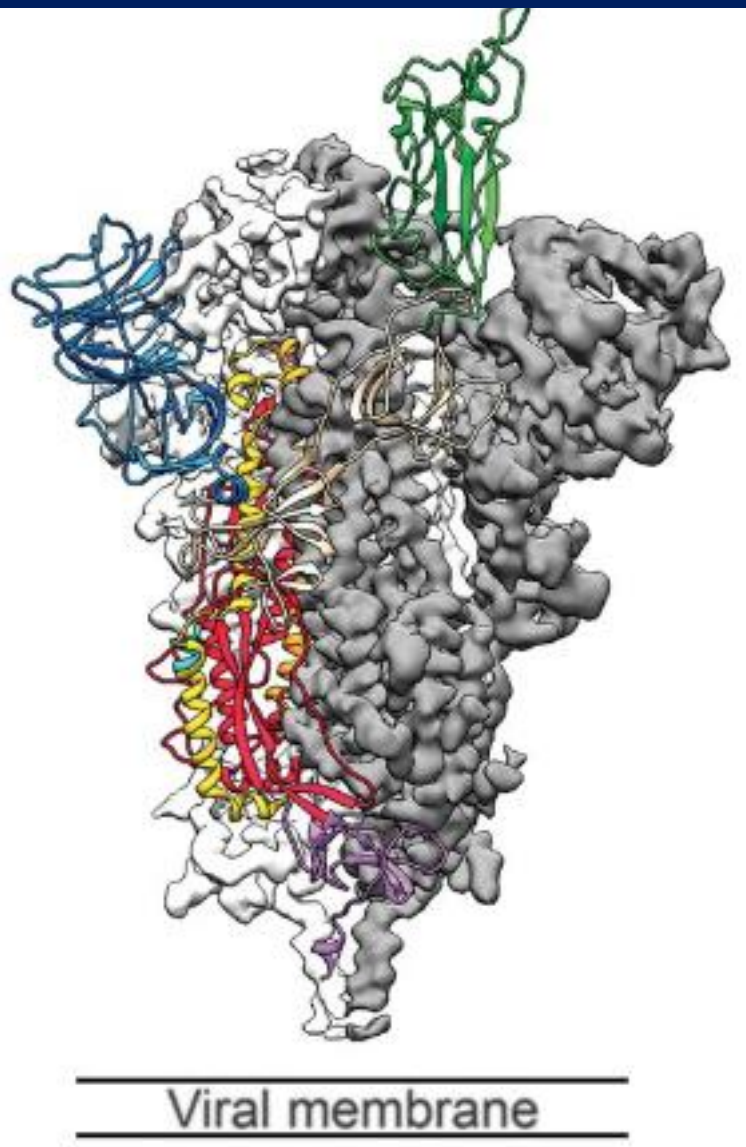
Coronavirus entry into host cells is mediated by the transmembrane spike (S) glycoprotein

- ✔ Trimeric class I fusion protein
- ✔ Main host-determining factor

The entry receptor for SARS-CoV-2 has been identified as the human angiotensin-converting enzyme 2 (hACE2).

S comprises two functional subunits:

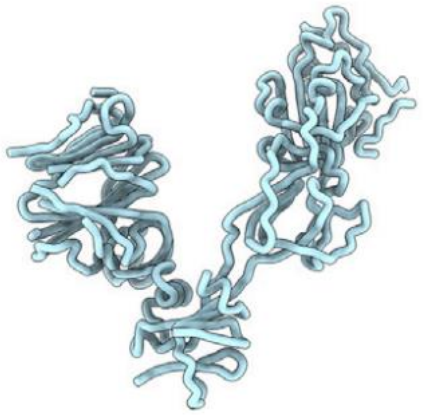
- ✔ S1 subunit
- ✔ S2 subunit



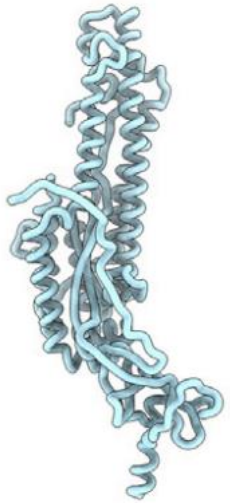
Wrapp et al., 2020

Spike Protein Subunits

Prior to membrane fusion, the S protein should be cleaved and activated to allow for the fusion peptide releasing onto host cell membranes



S_1 subunit



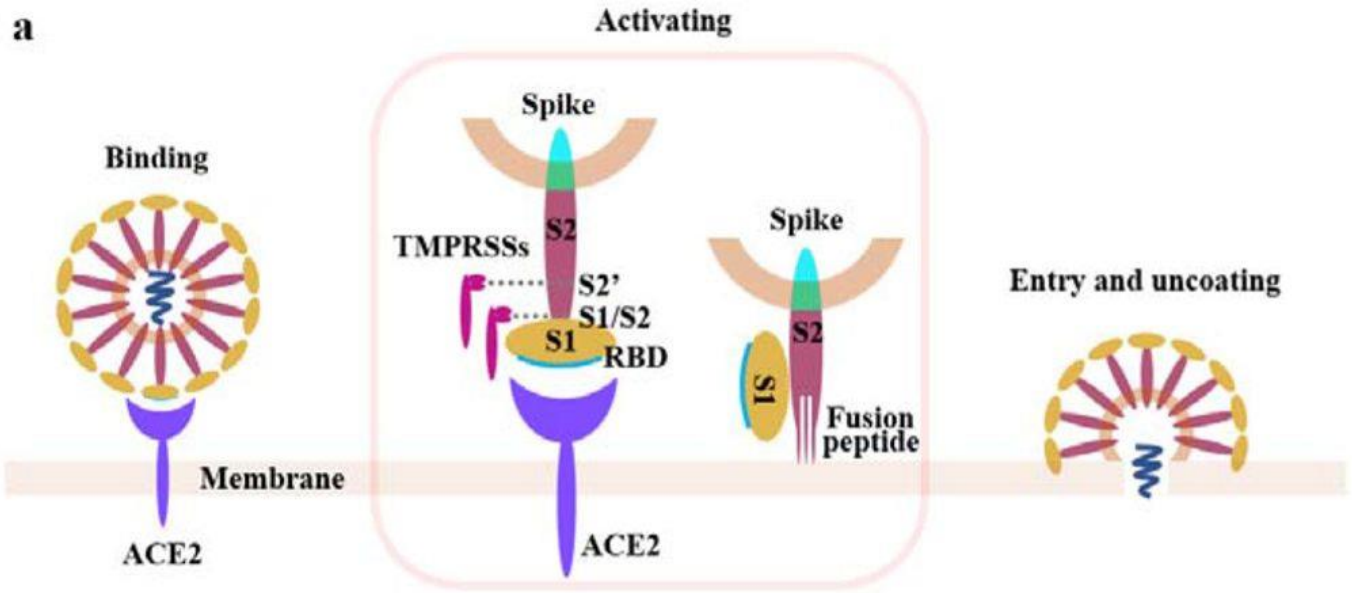
S_2 subunit

- ✔ S1 mediates the entry into host cells by binding to cell receptor
- ✔ S2 subunit regulates the fusion of viral and cellular membranes

Walls et al., 2020

Cleavage of S Protein

The entry of SARS-CoV-2 into host cells depends on the cell receptor recognition and cleavage by cell proteases.

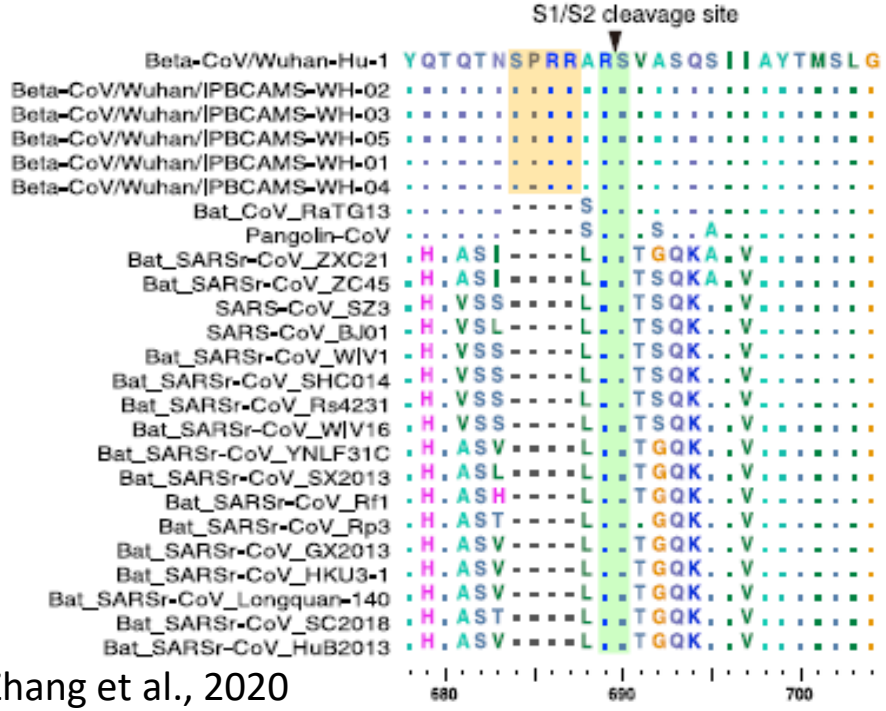


CoV S proteins may be cleaved by one or several host proteases. Such as;

Trypsin, Cathepsins, Transmembrane protease serine protease-2 (TMPRSS-2), TMPRSS-4, Human airway trypsin-like protease (HAT), Furin
(Ou et al., 2020)

Meng et al., 2020

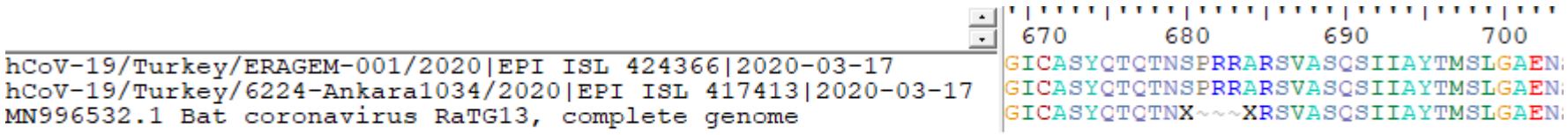
Furin-site



Zhang et al., 2020

SARS-CoV-2 S protein:
74% and 90.1% homologous to SARS-CoV and RaTG13 (Ou et al., 2020)

The furin sequence motif at the S1/S2 site (amino acids 682-685, RRAR) is missing in the S protein of **Pangolin-CoV** and all other **SARS-CoVs** (Zhang et al., 2020)

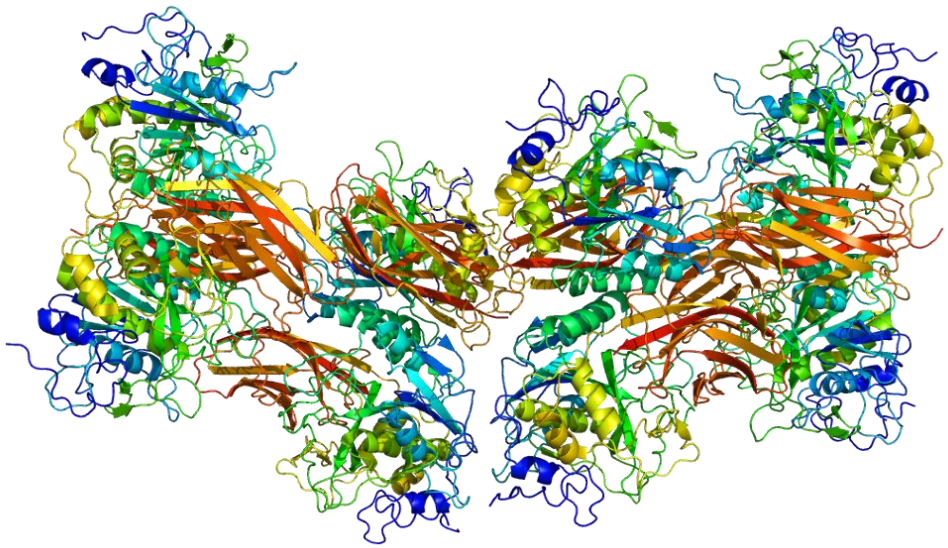


Data were obtained from GISAID/EpiCov database

Two sequences that were isolated from Turkey have furin-site.

Whether the presence of this furin site has any effect on viral pathogenesis and virus spreading among humans remains to be investigated.

Furin



✔ Furin is an enzyme in the proprotein convertase family which cleaves precursor proteins and facilitates their conversion to a biologically active state.

✔ Furin is expressed in significant concentrations in many organs such as brain, lungs, salivary gland, gastrointestinal tract, liver, pancreas, kidney, urinary bladder.

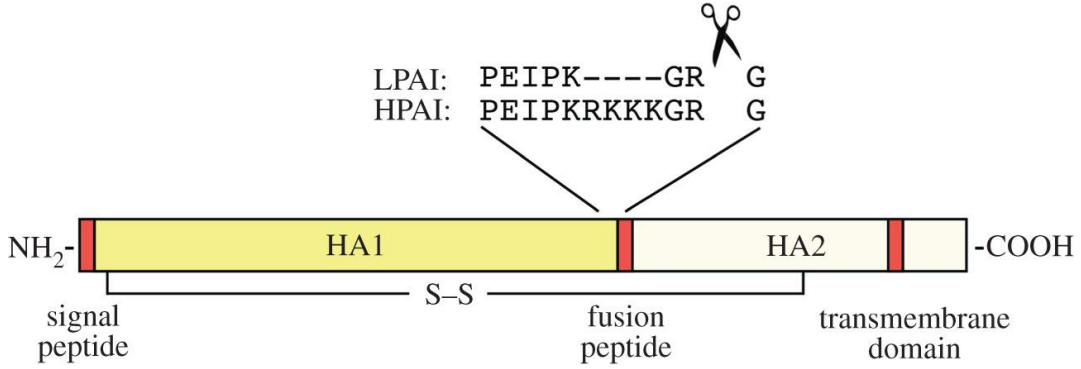
https://www.proteinatlas.org/ENSG00000140564-FURIN/tissue

Multiple Amino Acid Insertion

Similar to SARS-CoV-2:

Low-pathogenic avian influenza viruses contain a single basic amino acid at the cleavage site in the HA glycoprotein which is cleaved by proteases that are found in limited organs.

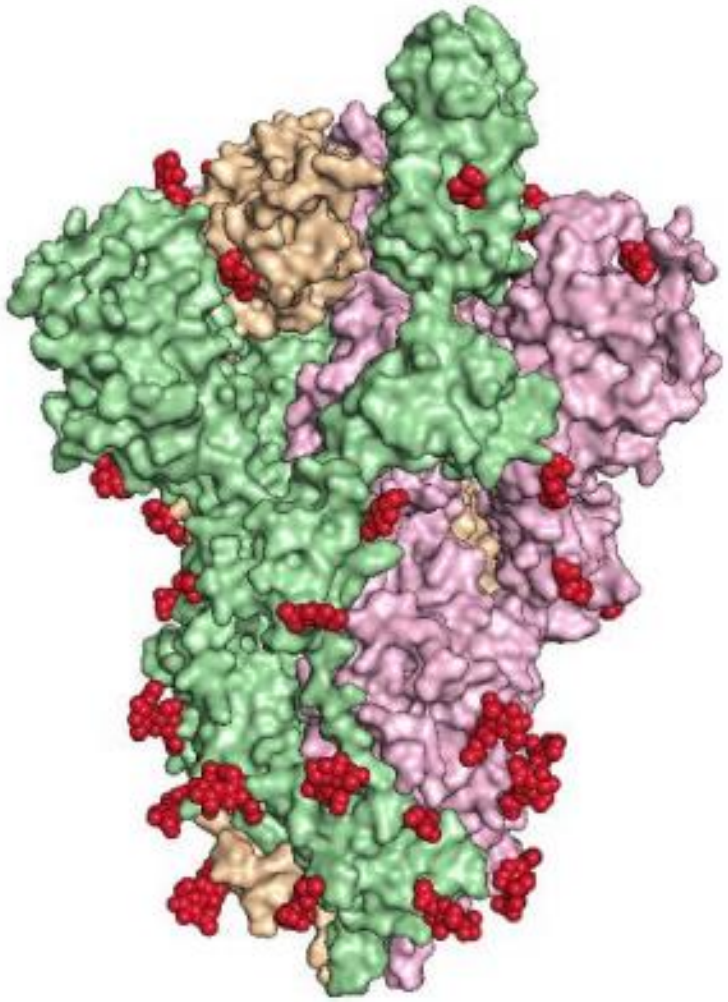
Insertion of a polybasic cleavage sites in the HA glycoprotein of highly pathogenic avian influenza viruses leads to replication in multiple tissues and higher pathogenicity.



Lycett, 2019

Glycosylation of S Protein

- S glycoproteins are densely decorated by heterogeneous N-linked glycans (Walls et al., 2020).
- 22 N-linked glycosylation sites were predicted on SARS-CoV-2 Spike protein (Watanabe et al., 2020; Zhou et al., 2020)
- In a trimeric structure, the spike glycoprotein of SARS-CoV-2 contains 66 glycosylation sites (Wrapp et al., 2020)



Wrapp et al., 2020

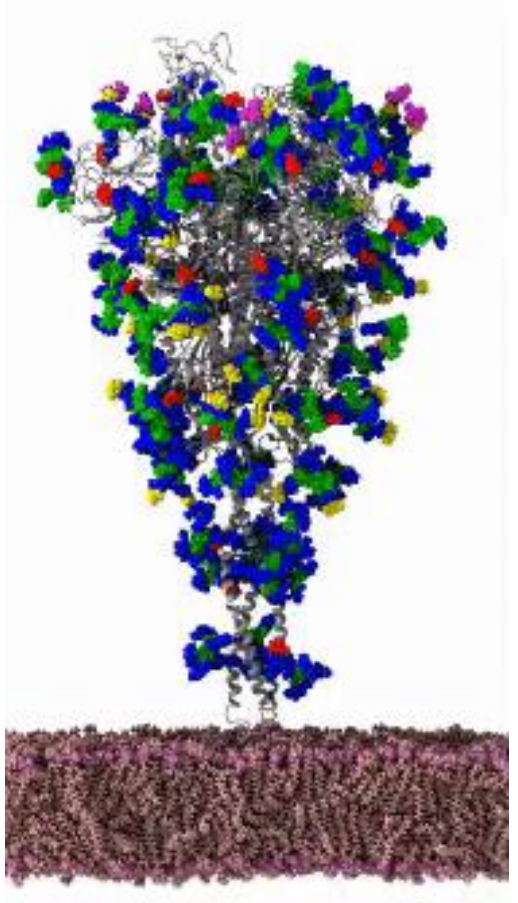
Glycosylation of S Protein

- Glycan diversity is used by both host to evade recognition by pathogens and the pathogens to escape the immune system response.

  Chloroquine could inhibit SARS-CoV entry through changing the glycosylation of ACE2 receptor and spike protein (Vincent et al., 2005)

- The N-glycans on S protein play important roles in proper protein folding and priming by host proteases.

  3 amino acid differences on S protein between HKU and MERS-CoV cause introducing N-linked glycosylation site and blocks the access of human endosomal cysteine protease (Yang et al., 2015)

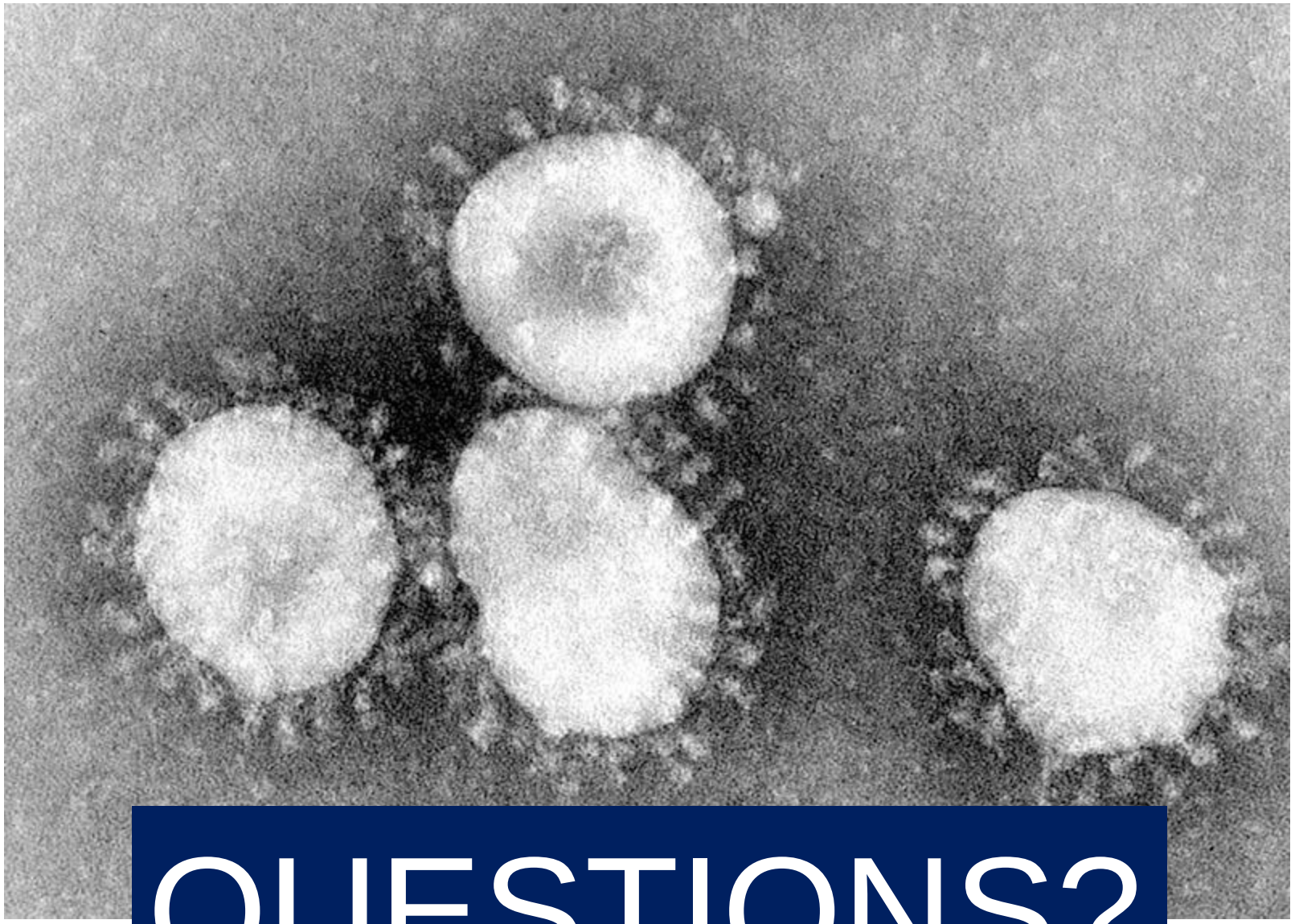


The colorful balls representing glycans
Video: Lorenzo Casalino, Zied Gaieb, and Rommie Amaro, UC San Diego

Glycosylation of HA Protein in IAV

Glycosylation of HA glycoprotein of IAV may affect virulence and pathogenicity of the virus by shielding the HA from antibodies and/or changing receptor binding affinity.

- The introduction of 2 glycosylation sites on HA resulted in the enhancement of virulence and pathogenicity of pH1N1 in mice (Zhang et al., 2013).
- In another study, naturally occurring 2 glycosylation sites on HA protein of H5N1 enhanced viral productivity in mice (Zhao et al., 2017).
- Besides, the loss of one glycosylation site in HA resulted in decreased receptor binding affinity in mice (Tan et al., 2019).
- In contrast, the loss of one glycosylation site in HA caused an increase up to 80% mortality in domestic poultry (Deshpande et al., 1987).



QUESTIONS?