# COVID-19(武漢肺炎)之病毒學解析

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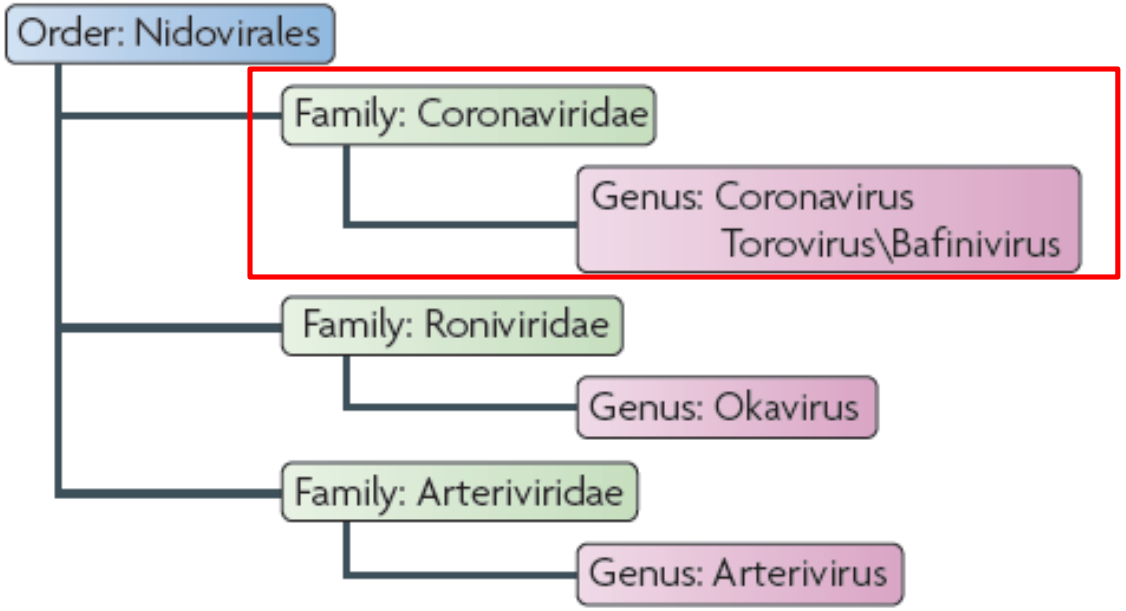
# Outline of this lecture

- I. Classification and nomenclature
- II. Genome structure
- III. Life cycle and replication
- IV. Laboratory diagnosis
- V. Ecology and interspecies transmission

What we are going to talk is **the coronavirus genus of coronaviridae family**

They belong to the Order of Nidovirales



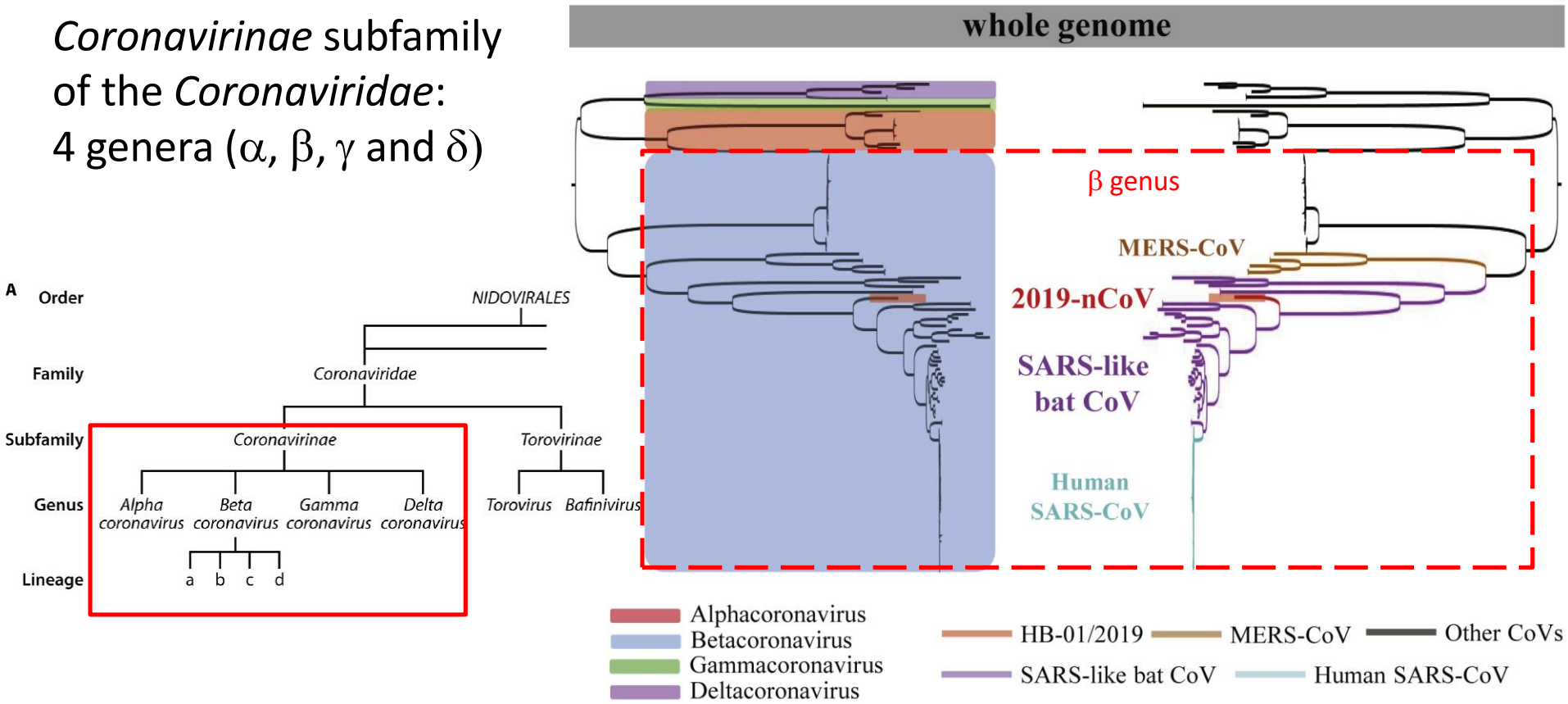

**Updated: Four genera:**
**1. Alphacoronavirus**:
**2. Betacoronavirus**:
**3. Gammacoronavirus**:
**4. Deltacoronavirus:**

Figure 1 | **The Nidoviruses.** Phylogenetic relationship of viruses in the order Nidoviruses.

Pertman and Netland, 2009

# Classification of coronavirus

*Coronavirinae* subfamily of the *Coronaviridae*: 4 genera (α, β, γ and δ)



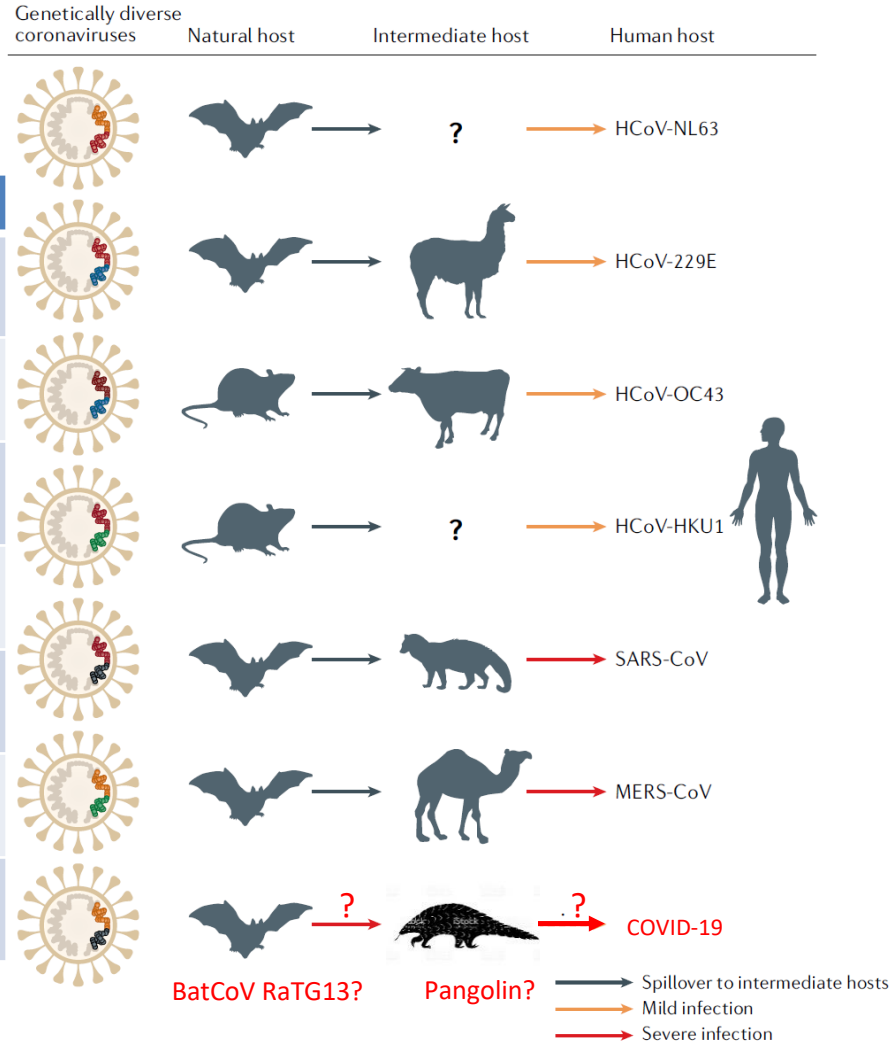

Chan et al., *Clin. Microbiol. Rev.* (2015), 28:465;
Wu et al., *Cell Host Microbe* (2020), doi: 10.1016/j.chom.2020.02.001.

# Human coronaviruses

List of human pathogenic coronaviruses

| Virus | Genus | Symptoms |
|---|---|---|
| Human CoV-229E | α | Mild respiratory tract infections |
| Human CoV-NL63 | α | Mild respiratory tract infections |
| Human CoV-OC43 | β | Mild respiratory tract infections |
| Human CoV-HKU1 | β | Pneumonia |
| SARS-CoV | β | Severe acute respiratory syndrome, 10% mortality rate |
| MERS-CoV | β | Severe acute respiratory syndrome, 37% mortality rate |
| COVID-19 | β | Severe acute respiratory syndrome, ?% mortality rate |

Chen et al., *J. Med. Virol.* (2020), doi: 10.1002/jmv.25681;
Cui et al., *Nat. Rev. Microbiol.* (2019), 17:181



# New coronavirus

- Disease: coronavirus disease (COVID-19)

From WHO

- Virus: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

此病毒剛開始命名為2019 novel coronavirus (2019-nCoV)，
後來國際病毒命名委員會(ICTV) 將之命名為SARS-CoV-2

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- I. Classification and nomenclature
- II. Viral morphology and genome structure
- III. Life cycle and replication
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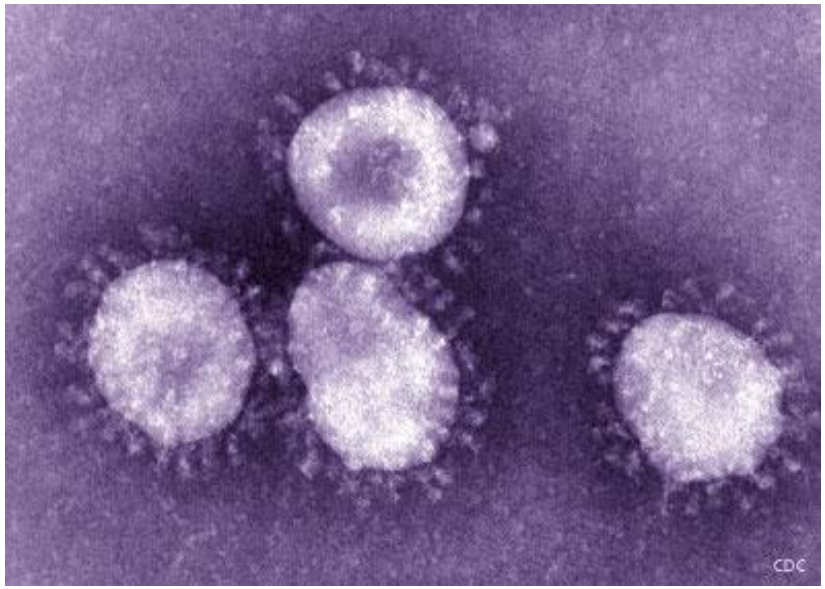
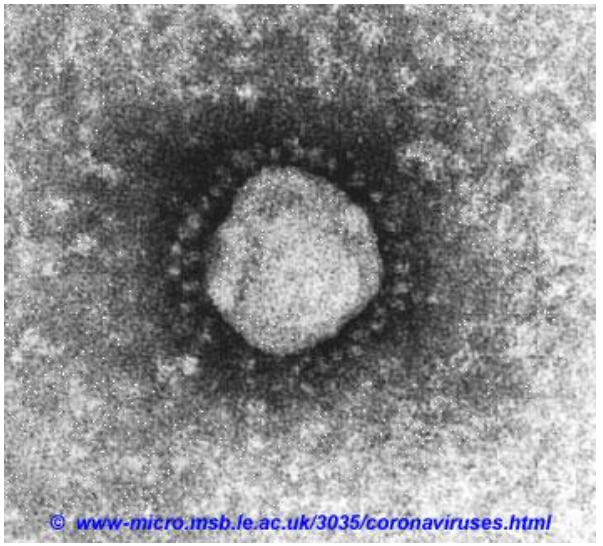
# Why is it called “corona”?

From Latin, “**corona**” means crown

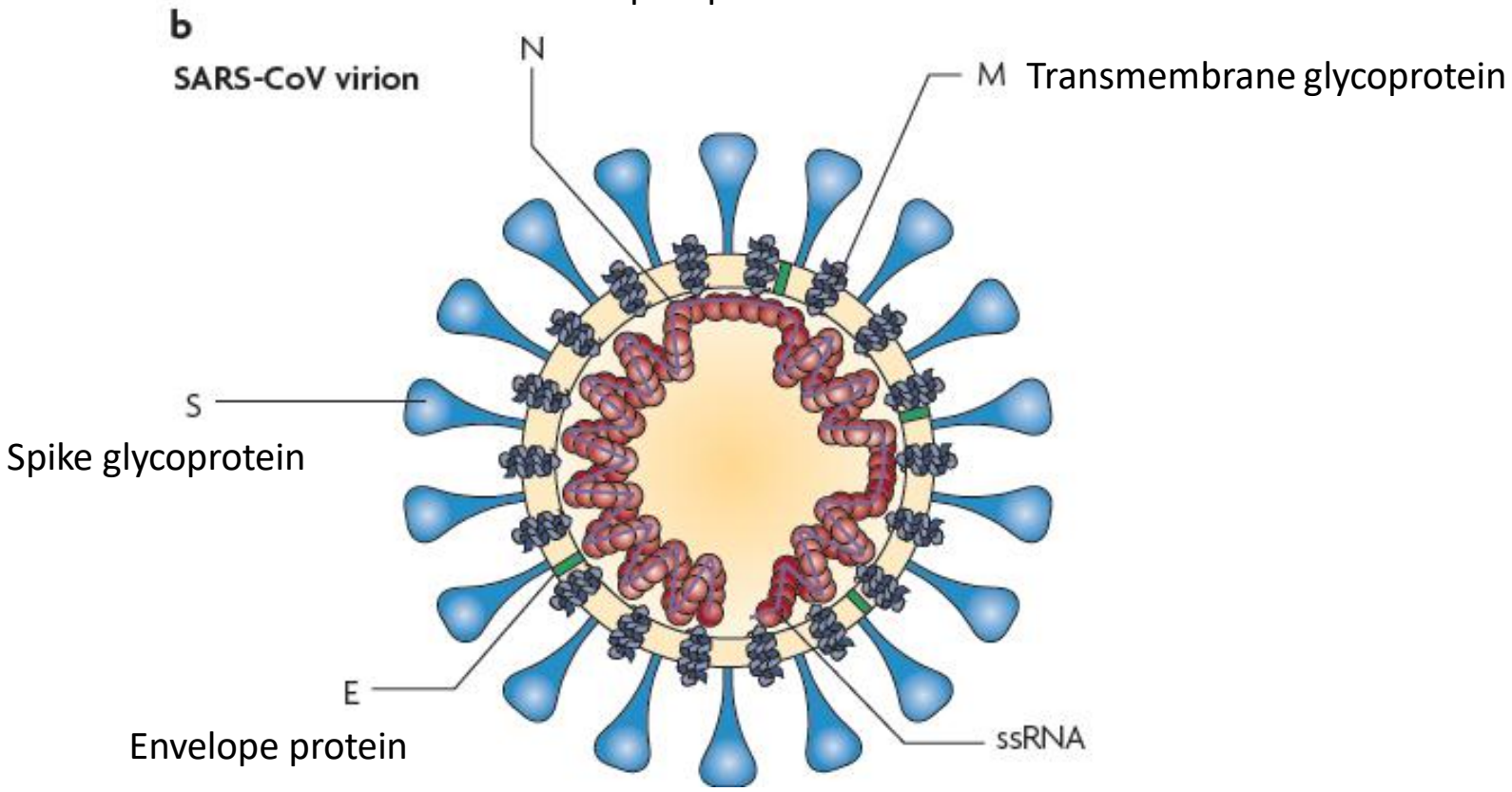
**Virion structure**: there are spikes projecting from envelope, looks like crown




CDC, USA; Stanford University

Nucleocapsid protein




Transmembrane glycoprotein

Spike glycoprotein

Envelope protein

# The envelope proteins (structural proteins):

Example: group II coronavirus

| | |
|---|---|
| *Hemagglutinin-esterase (HE)* | Envelope protein in some but not all coronaviruses. Virus entry, pathogenesis, virus release. |
| *Spike (S)* | Envelope protein. Receptor binding, fusion, tropism. |
| *Envelope (E)* | Minor envelope protein. Required for envelope formation. |
| *Membrane (M)* | Major envelope protein that contacts both spike and nucleocapsid protein. Required for envelope formation. |
| *Nucleocapsid (N)* | Forms helical nucleocapsid with genome RNA. Interacts with M protein; may form icosahedral shell. |
| *Accessory or group-specific proteins* | Three to five proteins, dispensible for replication *in vitro*. Functions unknown. |

# The nonstructural proteins:

Example: group II coronavirus (**nonstructural proteins**)

TABLE 19.2 **Group II coronavirus proteins and functions**

| Protein | Functions |
|---|---|
| *nsp1, 2, 7-10, 11* | Unknown functions in replication; nsp1 may mediate cell-cycle arrest during overexpression in culture. |
| *nsp3* | One or two papain-like proteinase domains (PLP1, PLP2) responsible for cleavage of nsp1, nsp2, and nsp3; zinc ribbon motifs with predicted transcription factors; transmembrane sequences with membrane integration. |
| *nsp4, nsp 6* | Membrane-spanning proteins, may localize replication complexes to membranes. |
| *nsp5* | Picornavirus 3C-like proteinase (3CLpro or Mpro) responsible for cleavage of nsp4 through nsp16. |
| *nsp12* | Predicted RNA-dependent RNA polymerase responsible for genome replication and transcription. |
| *nsp13* | RNA helicase, nucleoside triphosphatase activity *in vitro*. Likely involved in genome unwinding, separation, and packaging; may be virulence factor. |
| *nsp14, 15, 16* | Predicted RNA modifying enzymes: 3′ to 5′ exonuclease (14), endoribonuclease (15), and O-methyl transferase (16). |

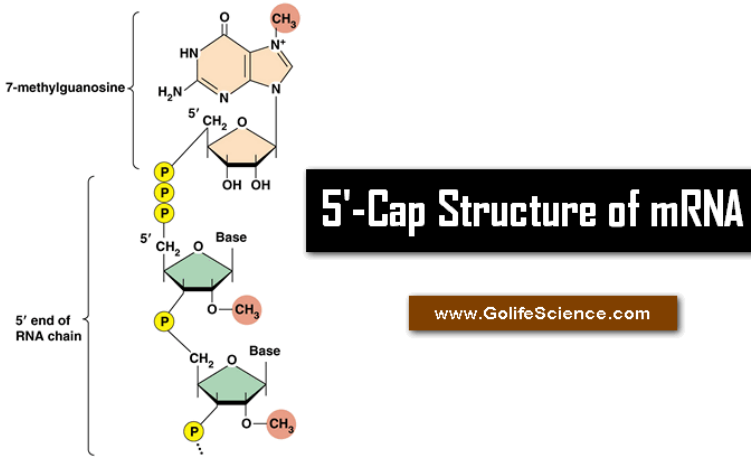
## The RNA genome structure:

1. Large (27-32Kb), single-stranded, positive-sense RNA genome

2. 5’ methyl-guanosine cap and 3’ poly(A) tail

3. It contains 6-10 genes

4. The order of genes is highly conserved:
   2/3: gene 1, for replication
   1/3: gene 2-7, for structure proteins



# Example: Murine hepatitis virus (MHV):

**Gene 1 (genomic RNA as mRNA)**: **2 ORFs, 1a and 1b**, are translated into polyproteins then cleaved by viral proteinases into 16 proteins

**Gene 2-7** are expressed by **"subgenomic mRNA"**

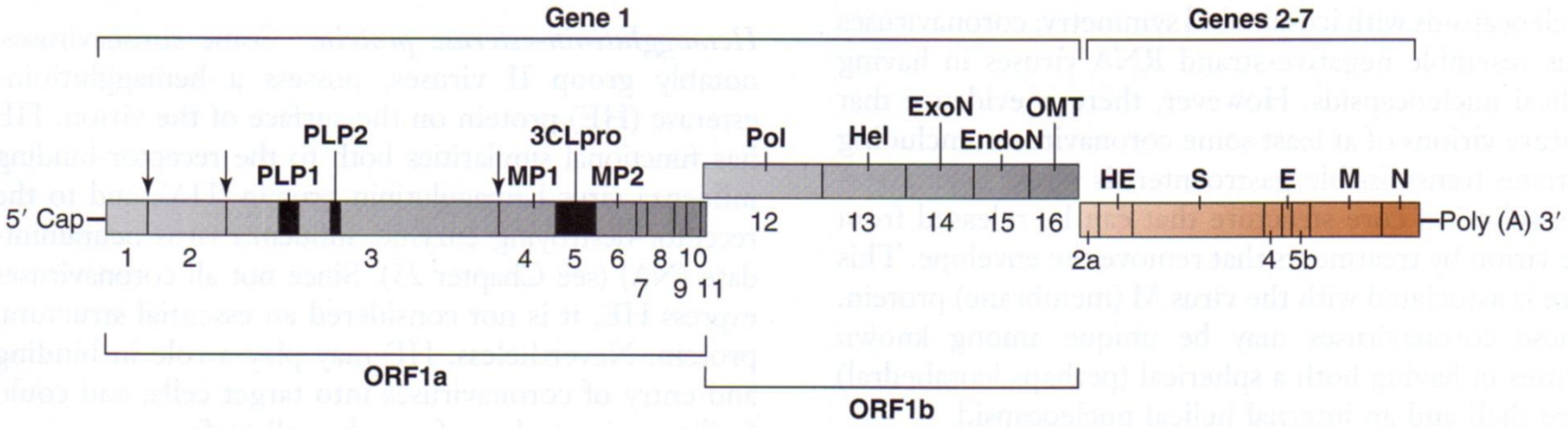
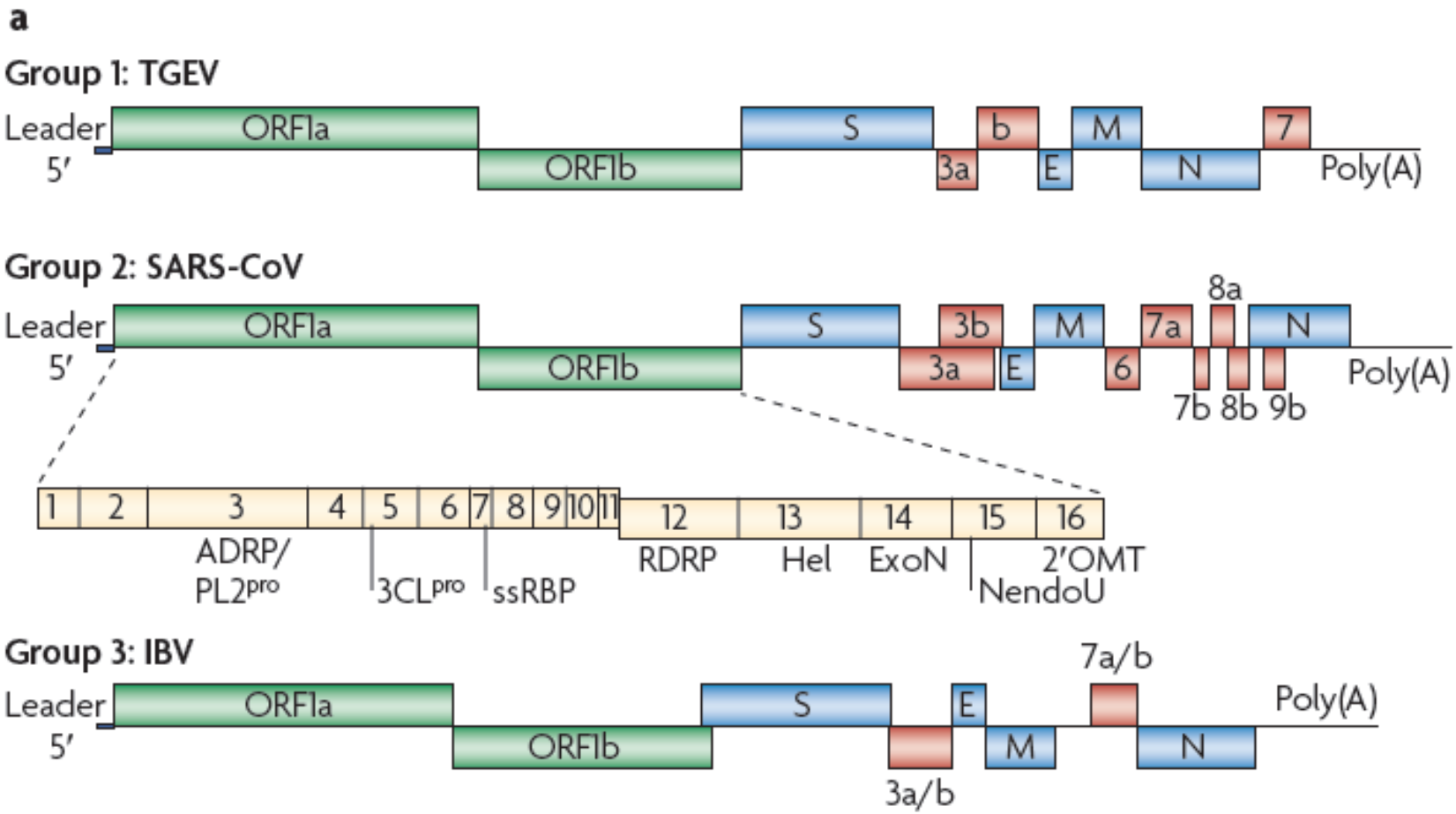


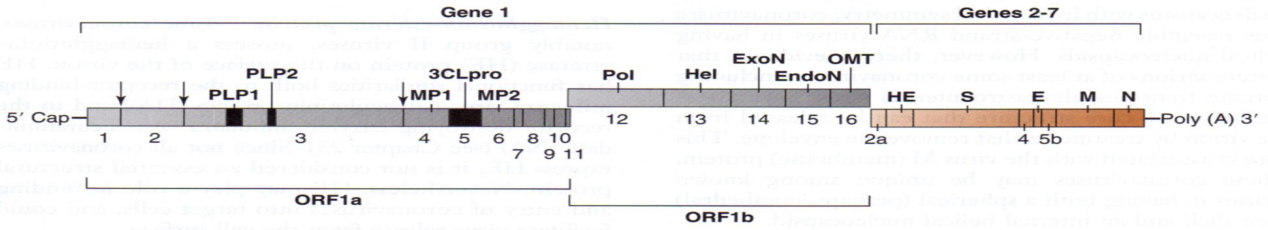

FIGURE 19.1 **Organization of the coronavirus genome.** The 31.5 Kb genome of murine hepatitis virus genome is shown. A short 5′ nontranslated leader region (gray) is followed by gene 1, which consists of two overlapping reading frames (ORF1a and 1b) that are translated as polyproteins from genome RNA. Genes 2–7 (orange) are expressed from subgenomic messenger RNAs. Where known, the names or function of gene products are shown above the genome map. The nonstructural proteins nsp1–16 (shown below the map) are generated by cleavage from the 1a/1b fusion protein by viral proteinases. Cleavage by proteinases PLP1 and PLP2 (black, cleavage sites shown as vertical arrows), yields nsp1–3, and cleavage by 3CLpro (black) yields nsp4–16.

# The comparison of the RNA genome structure:




Pertman and Netland, 2009

# The translation of gene I (ORF1a and ORF1b)




## ORF1a

1. Translation by cellular ribosomes begins at an AUG shortly beyond the 60-100 nt untranslated leader sequence
2. A polyprotein for ORF1a is produced
3. The polyprotein is then cleaved by viral proteinases to generate 11 nonstructural proteins

## ORF1b

1. Some of the ribosomes translating **ORF1a** pause on a complex RNA structure (**pseudoknot**) in the overlap between ORF1a and 1b.
2. **Ribosomes shift** to ORF1b and generate a larger 1a/1b polyprotein.
3. The polyprotein is then cleaved to 16 nonstructural proteins

# The expression of structural proteins:

**By a nested set of mRNAs, subgenomic mRNA**

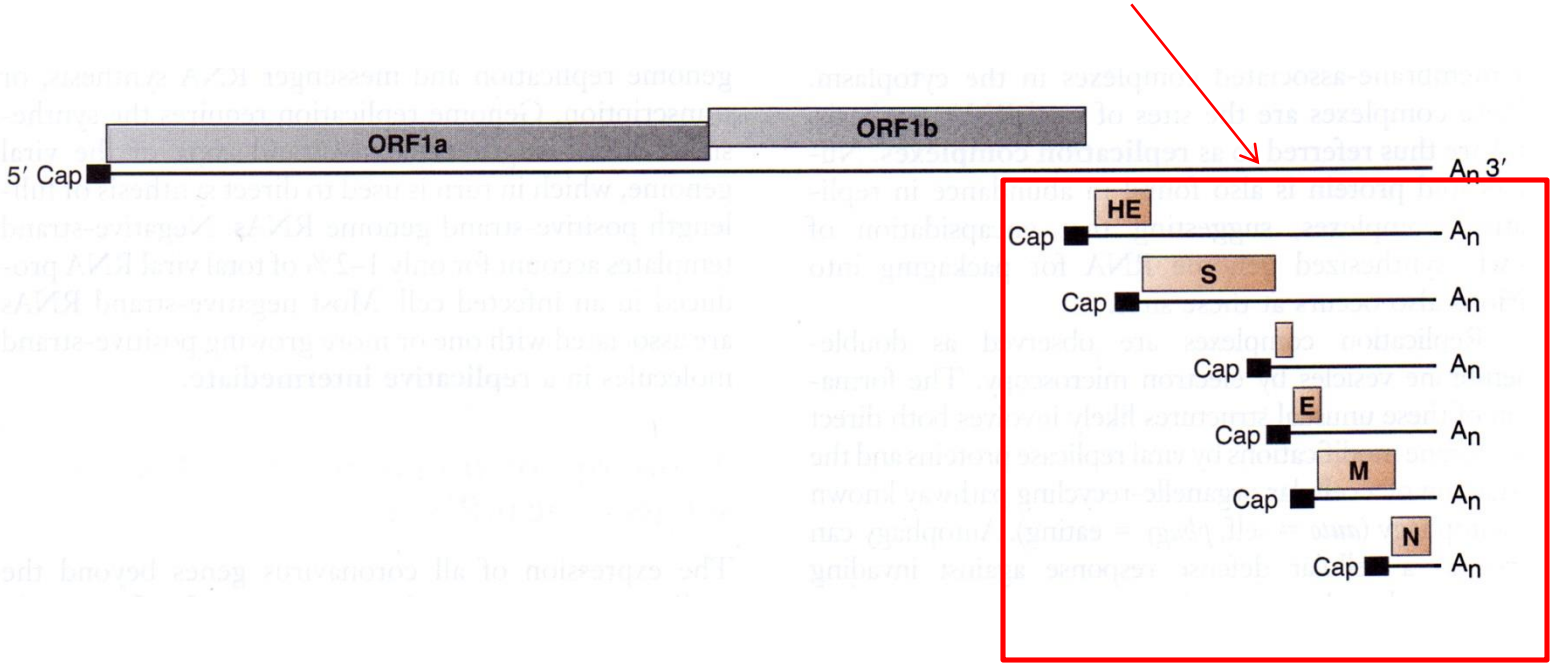
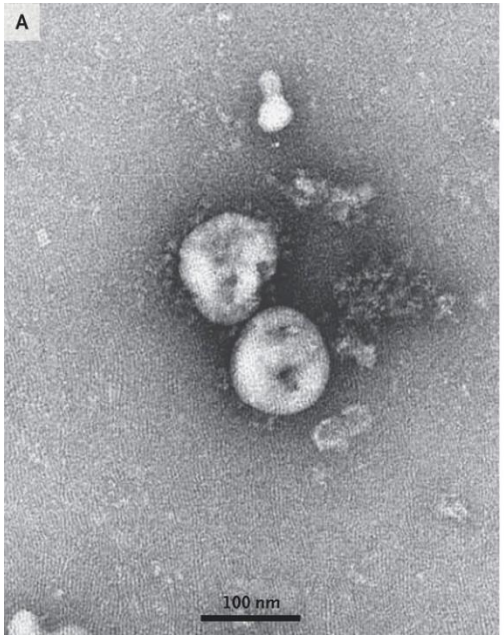
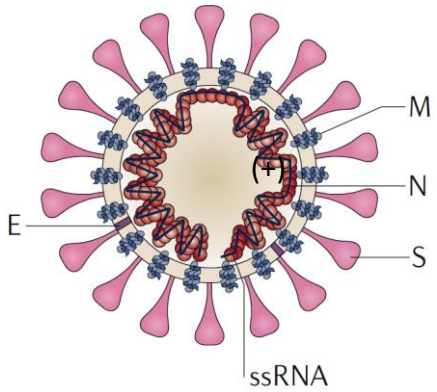
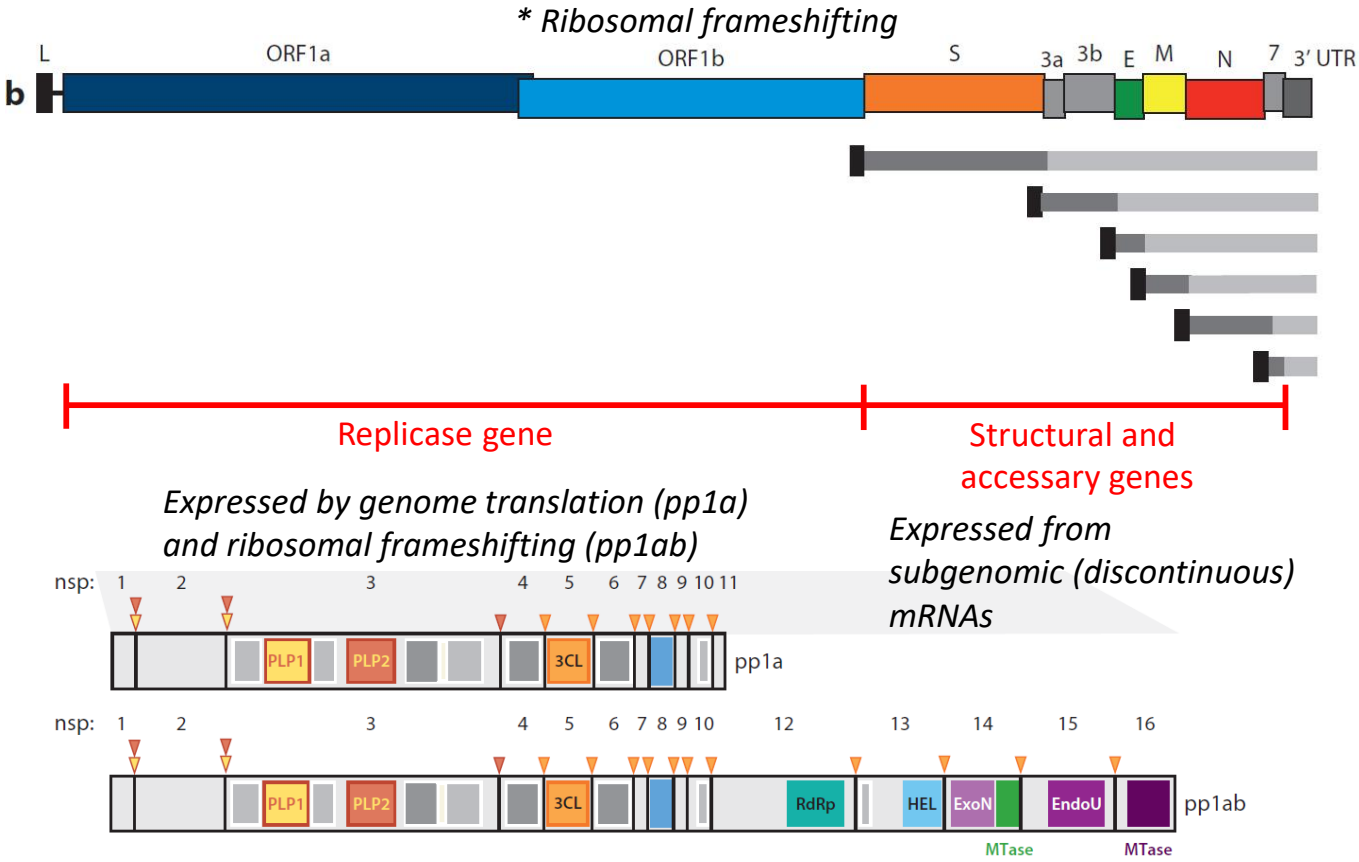



FIGURE 19.3 **Coronavirus messenger RNAs: a nested set.** The seven messenger RNAs of murine hepatitis virus are shown, along with the open reading frames they use to synthesize viral proteins. All share the same 5′ leader sequence and all contain overlapping sequences at their 3′ ends.

# Morphology of SARS-Cov-2 and its gene expression




Cui et al., *Nat. Rev. Microbiol.* (2019), 17:181; Sola et al., *Annu. Rev. Virol.* (2015), 2:265; Zhu et al., *N. Engl. J. Med.* (2020), doi: 10.1056/NEJMoa2001017

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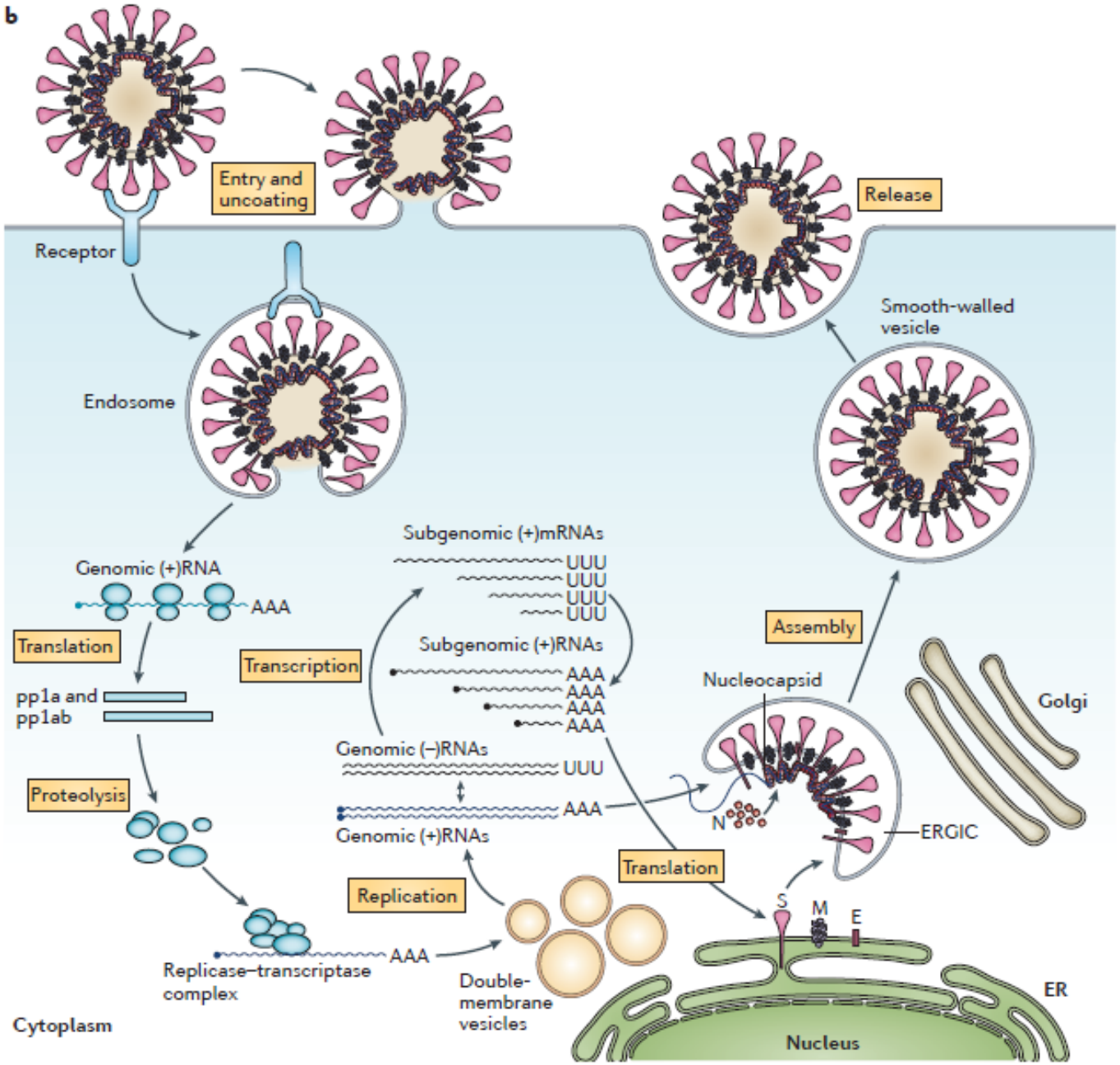



Wit et al., Nat Rev Microbiol, 2016

# Receptors of coronaviruses

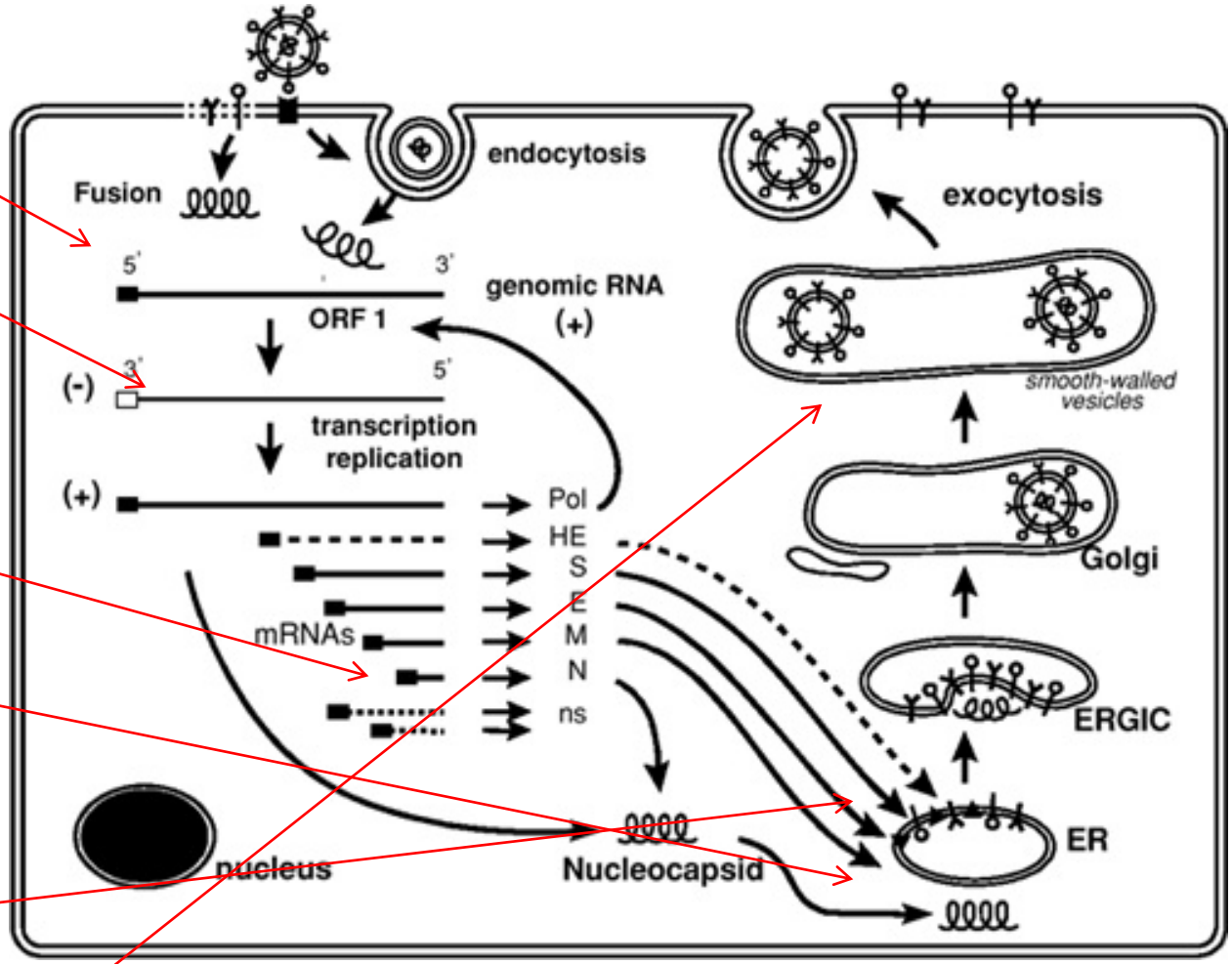
Table 1 | **Representative coronavirus species and their receptors**

| Group | Host | Virus | Cellular receptor |
|---|---|---|---|
| Group 1a | Bat‡ | BtCoV | Unknown |
| | Cat | FCoV | APN |
| | Cat | FIPV | APN |
| | Dog | CCoV | APN |
| | Pig | TGEV | APN |
| Group 1b | Human | HCoV-229E | APN |
| | Human | HCoV-NL63 | Angiotensin-converting enzyme 2 (ACE2) |
| | Pig | PEDV | Unknown |
| Group 1* | Rabbit | RbCoV | Unknown |
| Group 2a | Cattle, ruminants, alpaca | BCoV and related viruses | 9-*O*-acetylated sialic acid |
| | Dog | CRCoV | Unknown |
| | Human | HCoV-HKU1 | Unknown |
| | Human | HCoV-OC43 | 9-*O*-acetylated sialic acid |
| | Mouse | MHV | Carcinoembryonic antigen adhesion molecule 1 |
| | Pig | PHEV | Unknown |
| Group 2b | Bat‡ | BtCoV (multiple species) | Unknown |
| | Human | SARS-CoV | ACE2 |
| Group 2* | Manx shearwaters | PCoV | Unknown |
| | Rat | RtCoV | Unknown |
| | Rat | SDAV | Unknown |
| Group 3a | Chicken | IBV | Unknown |
| | Pheasant | PhCoV | Unknown |
| | Turkey | TCoV | Unknown |
| Group 3b | Beluga whale | SW1 | Unknown |
| Group 3c | Bulbul | BuCoV-HKU11 | Unknown |
| | Thrush | ThCoV-HKU12 | Unknown |
| | Munia | MuCoV-HKU13 | Unknown |
| | Asian leopard cat, Chinese ferret badger | ALCCoV | Unknown |

Pertman and Netland, 2009

# Replication cycle of coronavirus

1. Products of Gene 1 replicate the genomic RNA and synthesize the subgenomic RNAs
2. The mRNA and subgenomic mRNAs are transcribed
3. The structural proteins are synthesized. The **nucleocapsid** protein and newly synthesized genomic RNA assemble to form **helical nucelocapsids**
4. **M protein** is inserted in ER and anchored in Golgi. **Nucleocapsid** interacts with M .
5. E and M interaction triggers the budding process
6. S and HE are translated on membrane-bound polysomes, inserted into RER , and transported to Golgi
7. **Virions are released by exocytosis-like fusion of smooth-walled vesicles**




**Lai** et al., Fields Virology, 2007.

# RNA replication proteins

**RNA-dependent RNA polymerase:** nsp12, 100 kDa, ORF1b

**ATPase and RNA helicase:** nsp13

**RNA modifying activity:** exonuclease (nsp14); methyltransferase (nsp16); endoribonuclease (nsp15)

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# WHO建議之病毒檢驗測試

| 檢驗 | 檢體種類 | 備註 |
| --- | --- | --- |
| 可用的核酸增幅試驗（可參照WHO所提供各國家用於檢測可能病例的引子序列） | 呼吸道檢體<br>(病毒核酸萃取) | 若有病例出現則進行檢體採集。<br>一旦試驗驗證完成則由專業實驗室進行。 |
| 全基因組測序 | 呼吸道檢體<br>(病毒核酸萃取) | 若有病例出現則進行檢體採集。<br>由專業實驗室進行。 |
| 血清抗體檢測，針對病人配對血清檢體的血清抗體檢測。 | 血清 | 針對配對血清檢體的確認，第一次血清採集於發病第一週，第二次則採集於發病後3-4週。<br>若收集到單一血清檢體，則至少在發病後三週再次進行收集血清檢體。<br>由專業實驗室進行，直至獲得更多有關可用試驗的效能的資訊。 |

資料來源：Laboratory testing for SARS-Co-V2 in suspected human cases

# 檢測執行現況-檢測法

疾病管制署提供 primer, probe
-針對三個基因片段設計real-time RT-PCR
-篩檢敏感度3.7~9.6 RNA copies/rxn

**First line screening assay: E gene assay**
**Confirmatory assay: RdRp gene assay**
**Additional confirmatory assay: N gene assay**

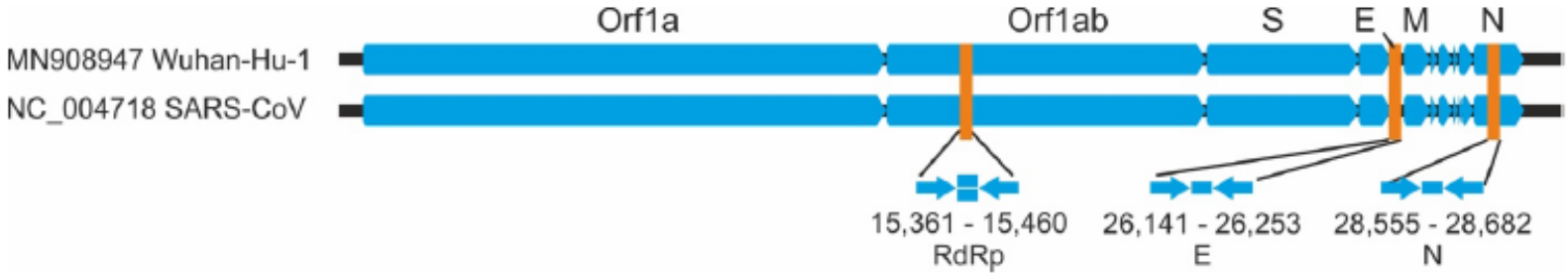


**Figure 1 relative positions of amplicon targets on SARS-CoV ad Wuhan-CoV genome.** N: nucleocapsid; ORF: open reading frame; RdRp: RNA-dependent RNA polymerase. Numbers below amplicon are genome positions according to SARS-CoV, NC_004718.

Euro Surveill. 2020;25(3):pii=2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

# 檢測執行現況- 檢驗流程

收件核對
(0.5 hr)

前處理
(1hr, 20撿體)

萃取核酸
(2 hr, 20檢體)

配製試藥
(0.5 hr)

檢測反應
(2.5 hr)

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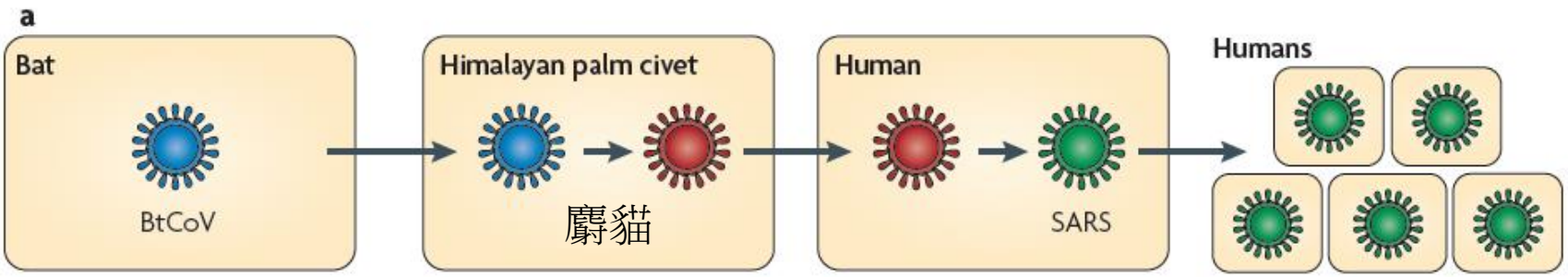
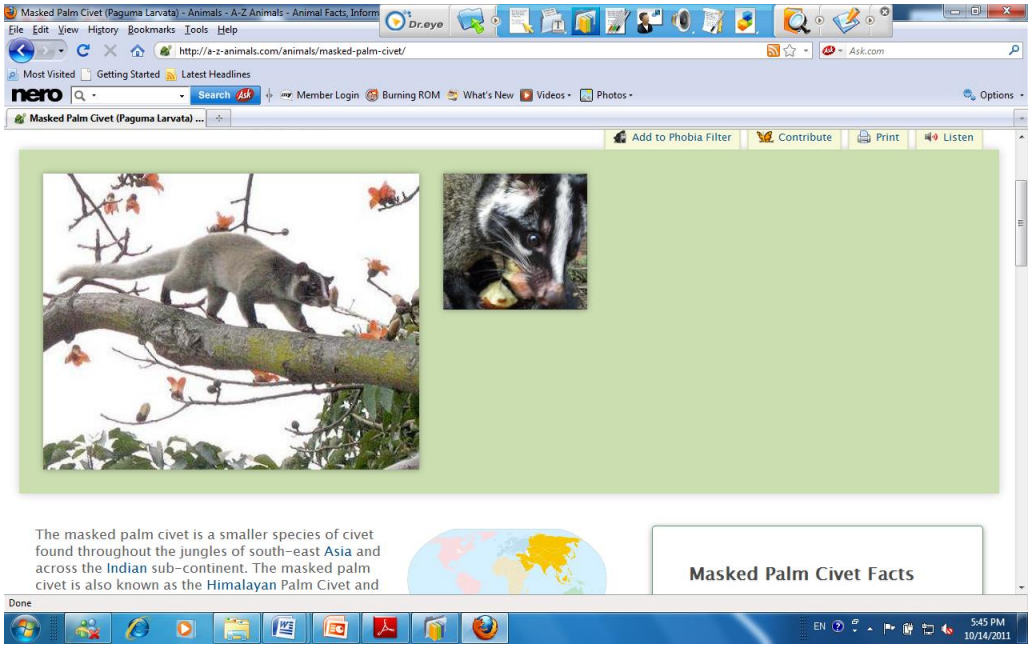
# Cross-species transmission of coronavirus

## a. SARS-CoV



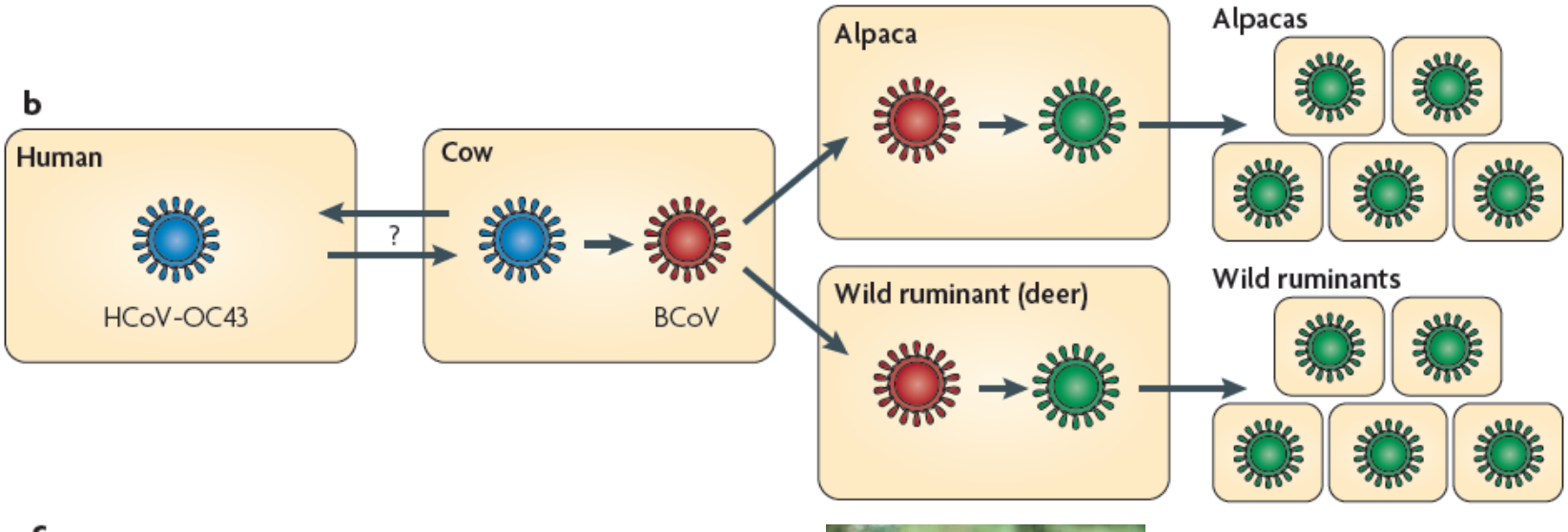

Pertman and Netland, 2009



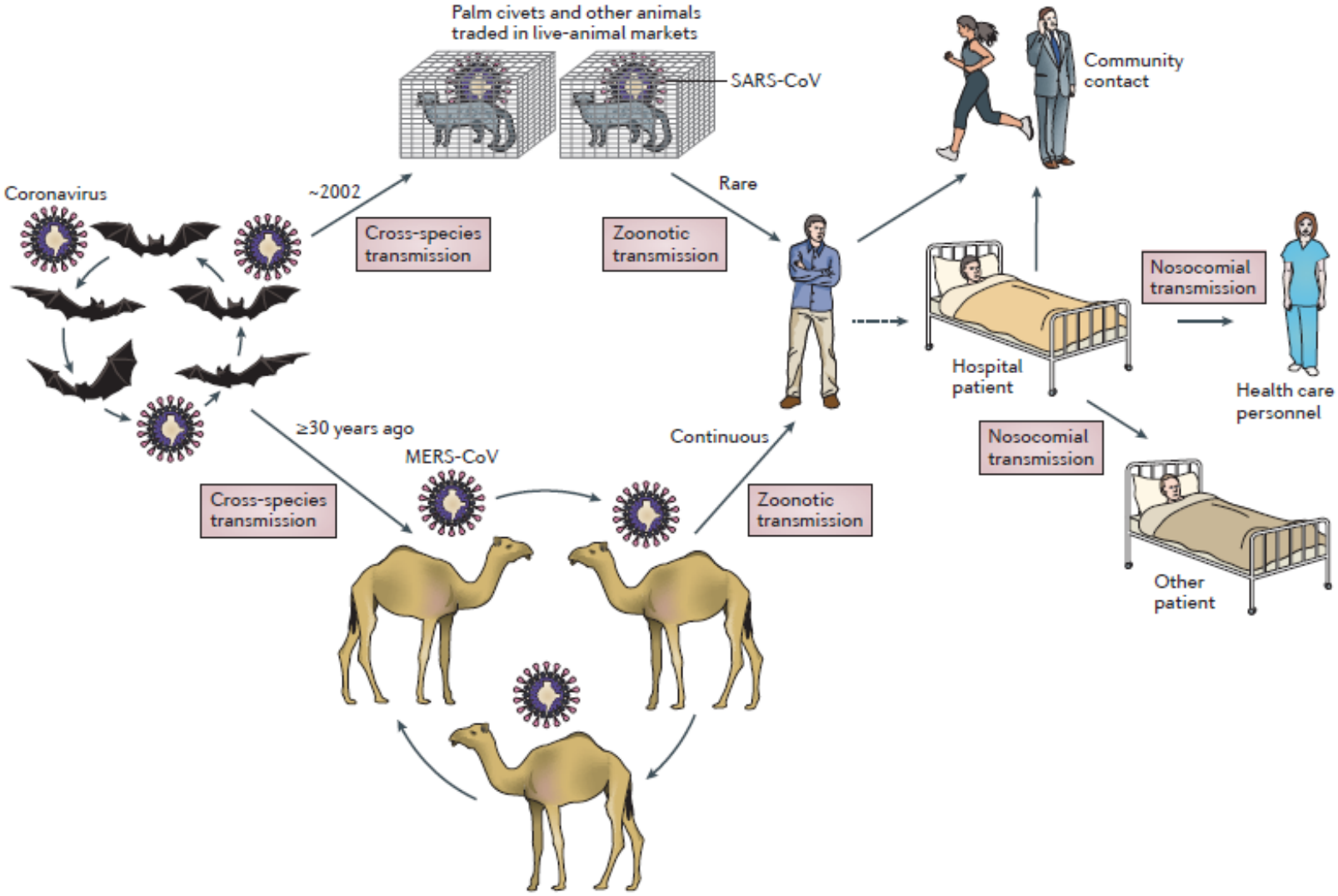
## b. Human HCoV-OC43

羊駝

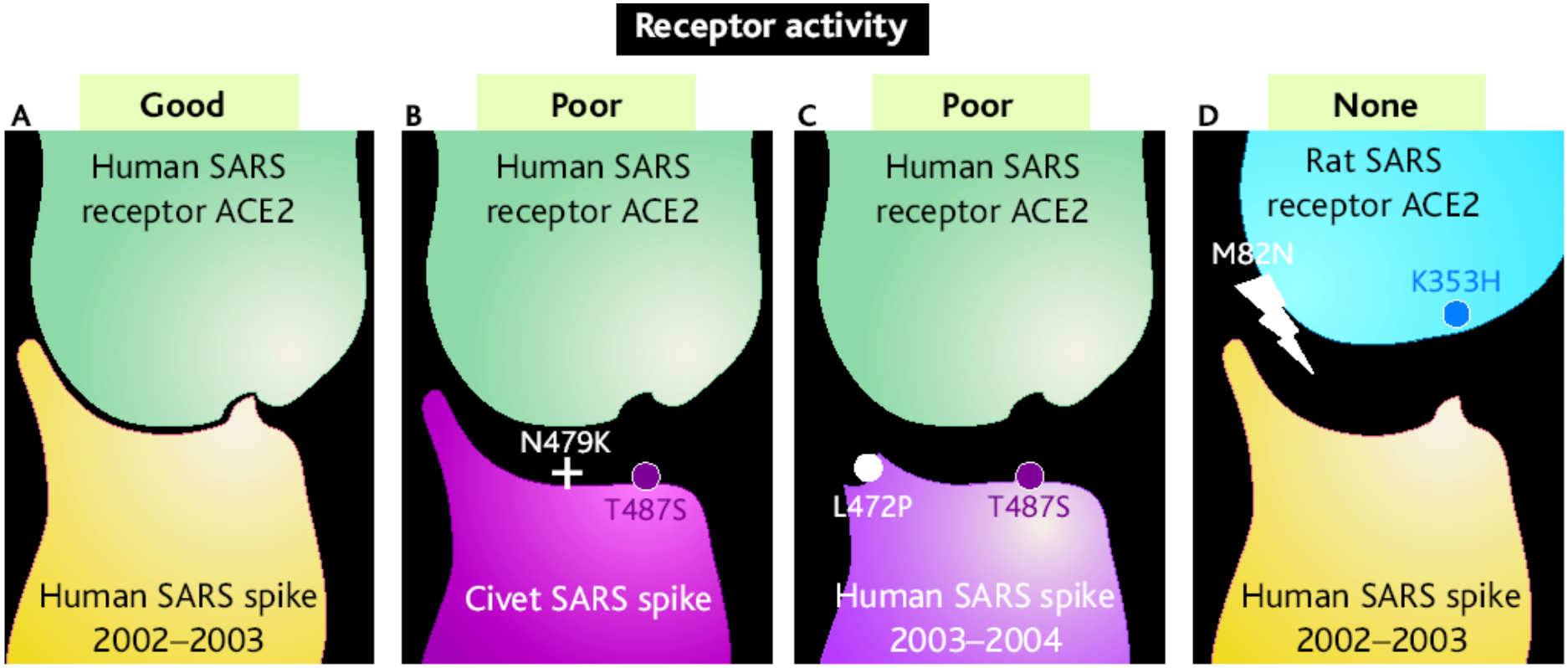
http://www.visitcumbria.com/visit-cumbria.htm



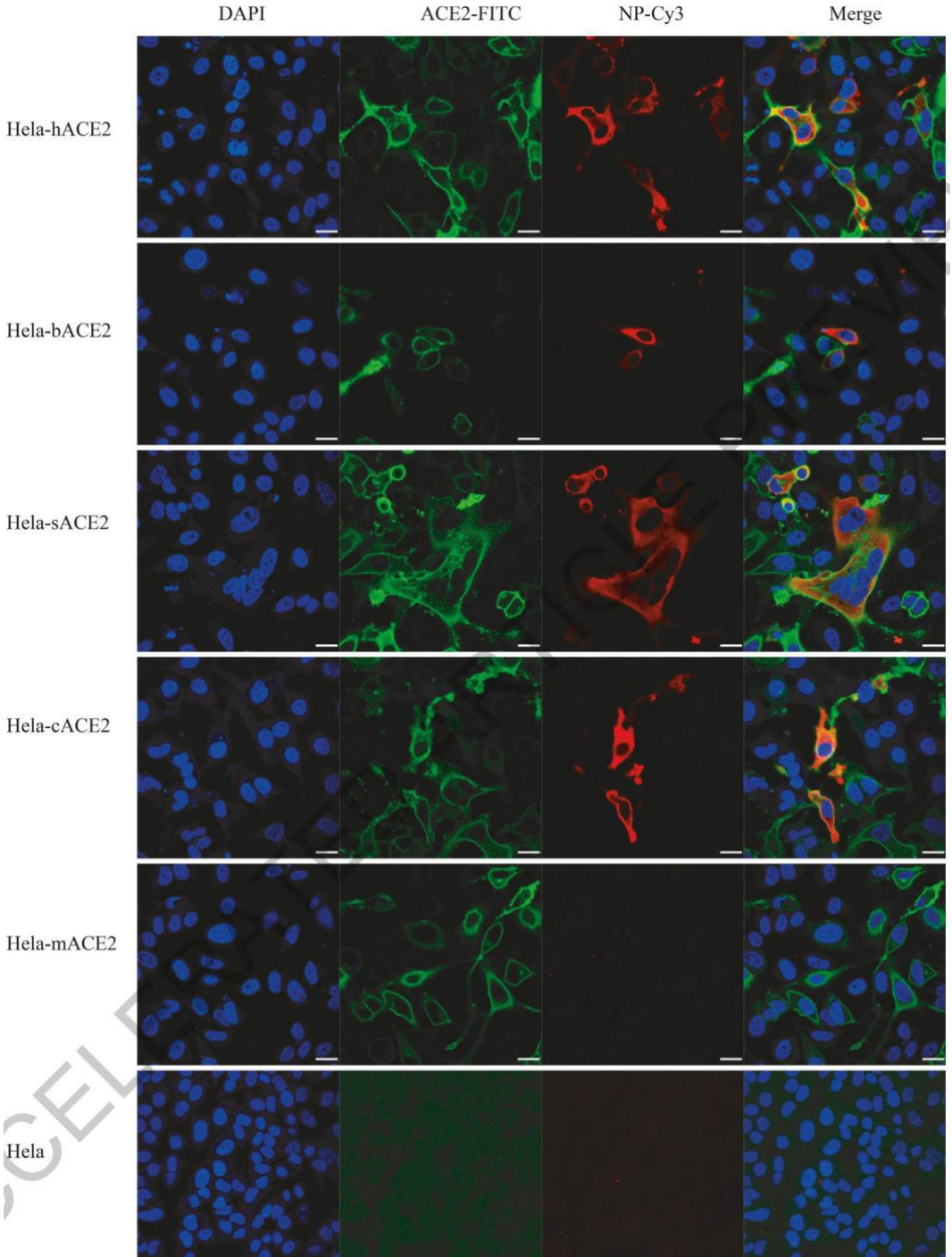

http://www.tpwd.state.tx.us/huntwild/hunt/planning/rut_whitetailed_deer/


Palm civets and other animals traded in live-animal markets
SARS-CoV
Community contact
Coronavirus
~2002
Cross-species transmission
Rare
Zoonotic transmission
Nosocomial transmission
Hospital patient
Health care personnel
≥30 years ago
MERS-CoV
Continuous
Cross-species transmission
Zoonotic transmission
Nosocomial transmission
Other patient

# Adaptation of SARS-CoV to human cells (1) mutations at receptor binding sites:



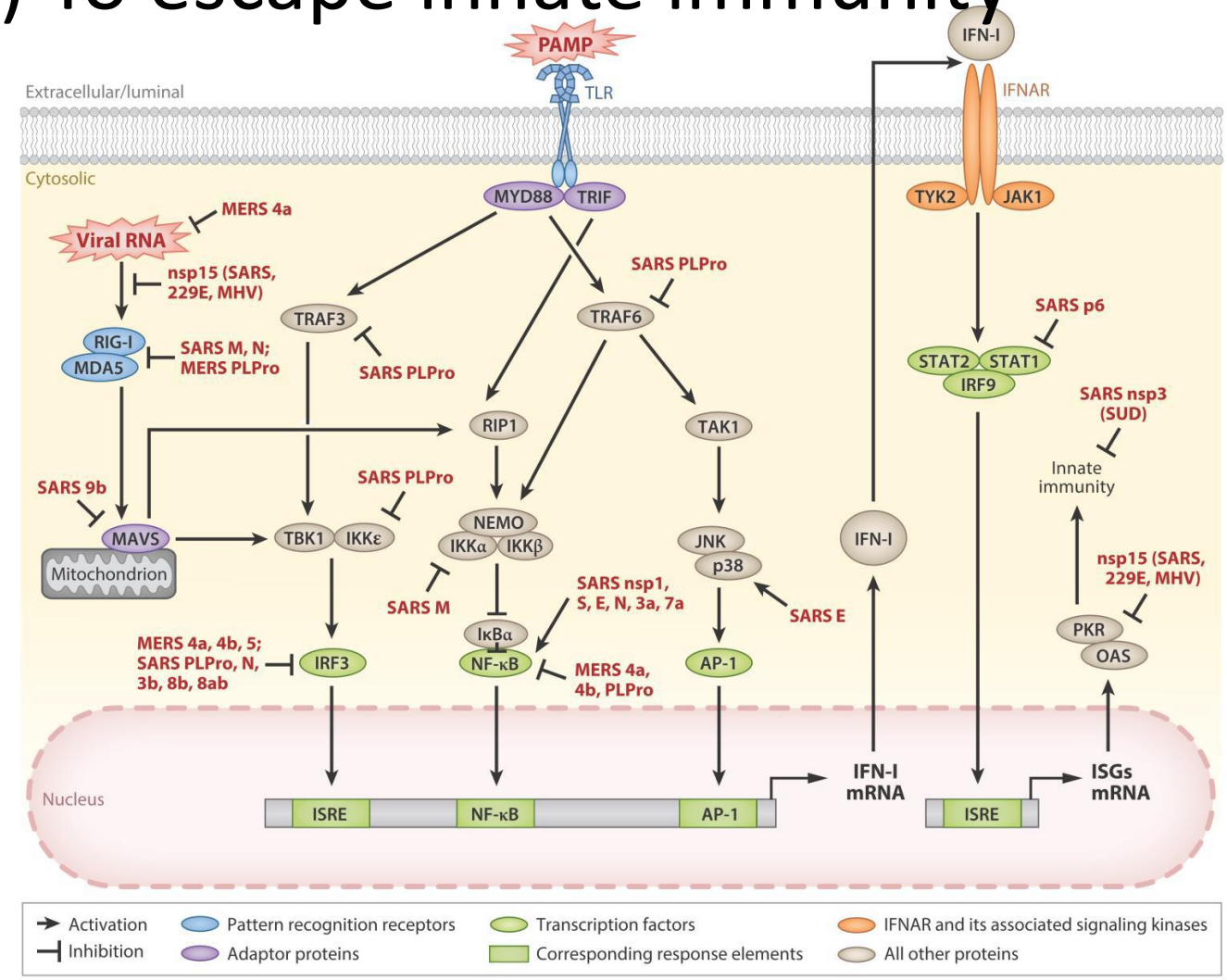

Holmes KV et al., Science, 2005




SARS-CoV-2 binds to human ACE2

Zhou P et al., Nature 2020

# Adaptation of SARS-CoV to human cells
# (2) To escape innate immunity




Type I interferon induction and signaling during CoV infection

Fung TS and Liu DX, Annu Rev Microbiol, 2019

# Summary of this lecture

- I. SARS-CoV-2 is a newly discovered virus in the family of coronaviridae (beta)
- II. Its virion structure and genome are similar to other coronaviruses
- III. Life cycle and replication (ACE2 is its receptor)
- IV. Laboratory diagnosis
- V. Ecology and interspecies transmission (the origin is from bat coronavirus)