



# Coronaviruses 101: Focus on Molecular Virology

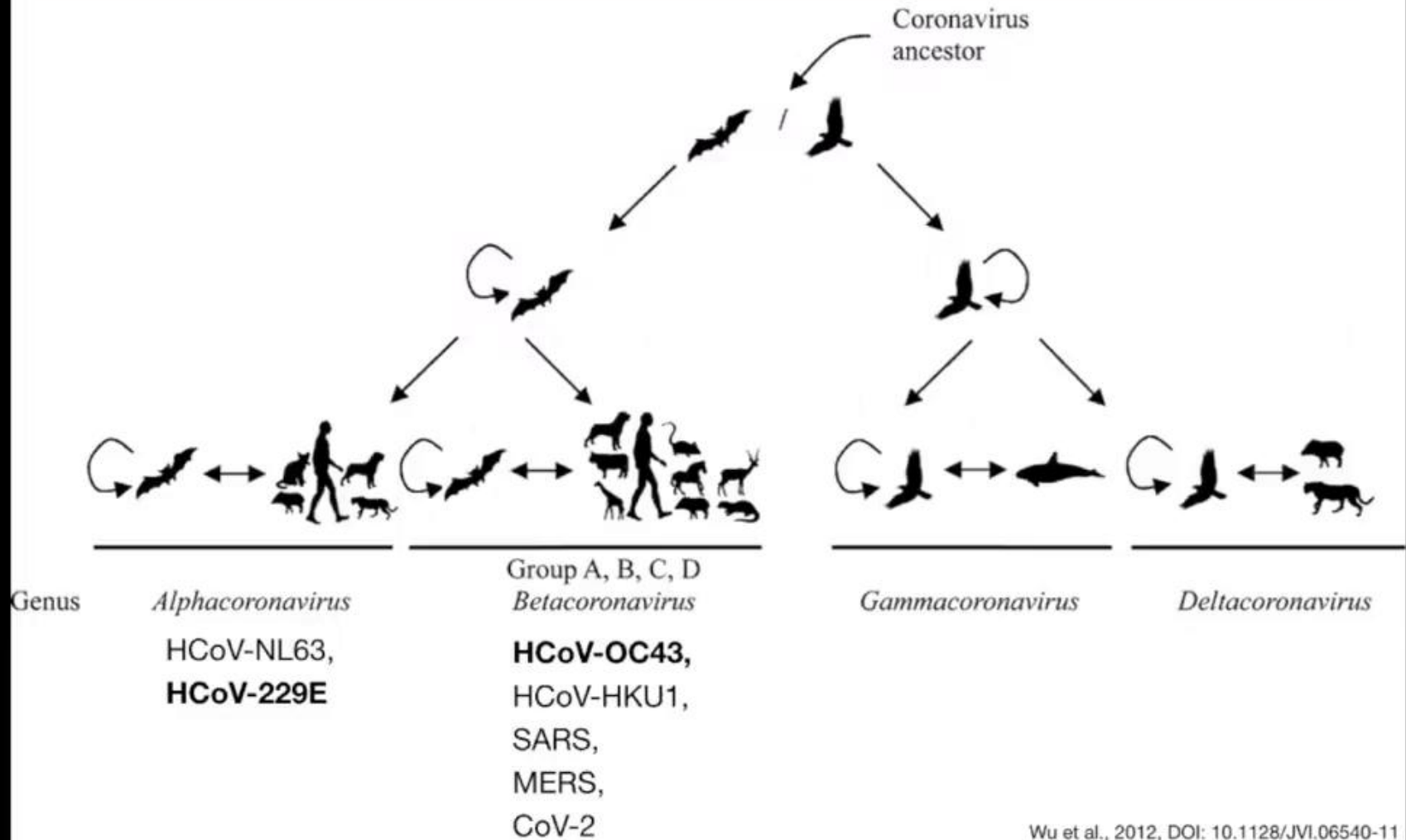
**Britt Glaunsinger, PhD**  
**Howard Hughes Medical Institute**  
**University of California Berkeley**  
**Departments of Plant & Microbial Biology and**  
**Molecular and Cell Biology**



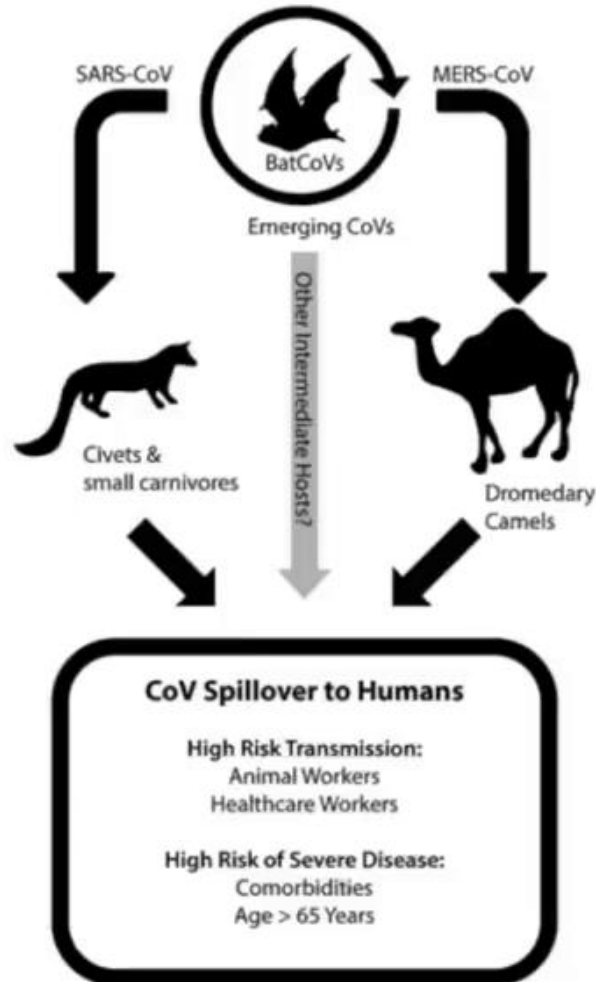
**March 24, 2020**



# There are 7 human CoVs, present in the alpha-and betacoronavirus genera



# SARS CoV-1, MERS, and SARS CoV-2 likely all emerged from bats and were transferred to humans via intermediate host



- More than 500 CoVs have been identified in bats in China, with estimates of unknown bat CoV diversity reaching >5,000.

SARS: 8,098 cases, 774 total deaths (epidemic ended)

MERS: 2,521 cases, 866 total deaths (infections still occurring)

## Why has CoV-2 been so much harder to control than SARS?

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CoV-1/SARS	CoV-2/COVID19
<p>'spillover' reservoir known: civit cats</p> <p><i>-cull to break the chain</i></p>	<p>'spillover' reservoir unknown</p>
<p>Most transmission occurred in hospital setting (hubs)</p> <p><i>-implemented barrier nursing</i></p>	<p>Widespread community transmission</p>
<p>No transmission until 24-36h after symptoms, lack of asymptomatic cases</p> <p><i>-contact tracing effective</i></p>	<p>Possible abundant asymptomatic/mild cases</p>



Total Confirmed

**396,249**

Confirmed Cases by  
Country/Region/Sovereignty

- 81,588 China
- 63,927 Italy
- 46,548 US
- 39,673 Spain
- 31,370 Germany
- 24,811 Iran
- 20,149 France
- 9,117 Switzerland
- 9,037 Korea, South
- 6,818 United Kingdom

Admin1

Last Updated at (M/D/YYYY)  
3/24/2020, 8:01:27 AM



Cumulative Confirmed Cases    Active Cases

169

Lancet Inf Dis Article: [Here](#). Mobile Version: [Here](#). Visualization: JHU CSSE. Automation Support: Esri Living Atlas team and JHU APL. Contact US. FAQ. Data sources: WHO, CDC, ECDC, NHC, DXY, 1point3acres.

Total Deaths

**17,252**

6,077 deaths  
Italy

3,160 deaths  
Hubei China

2,696 deaths  
Spain

1,934 deaths  
Iran

860 deaths  
France

337 deaths  
United Kingdom

Total Recovered

**103,334**

60,324 recovered  
Hubei China

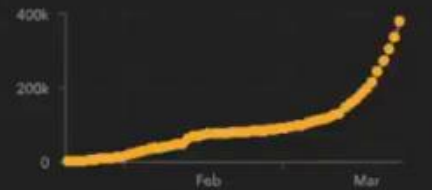
8,913 recovered  
Iran

7,432 recovered  
Italy

3,794 recovered  
Spain

3,507 recovered  
Korea, South

2,200 recovered  
France

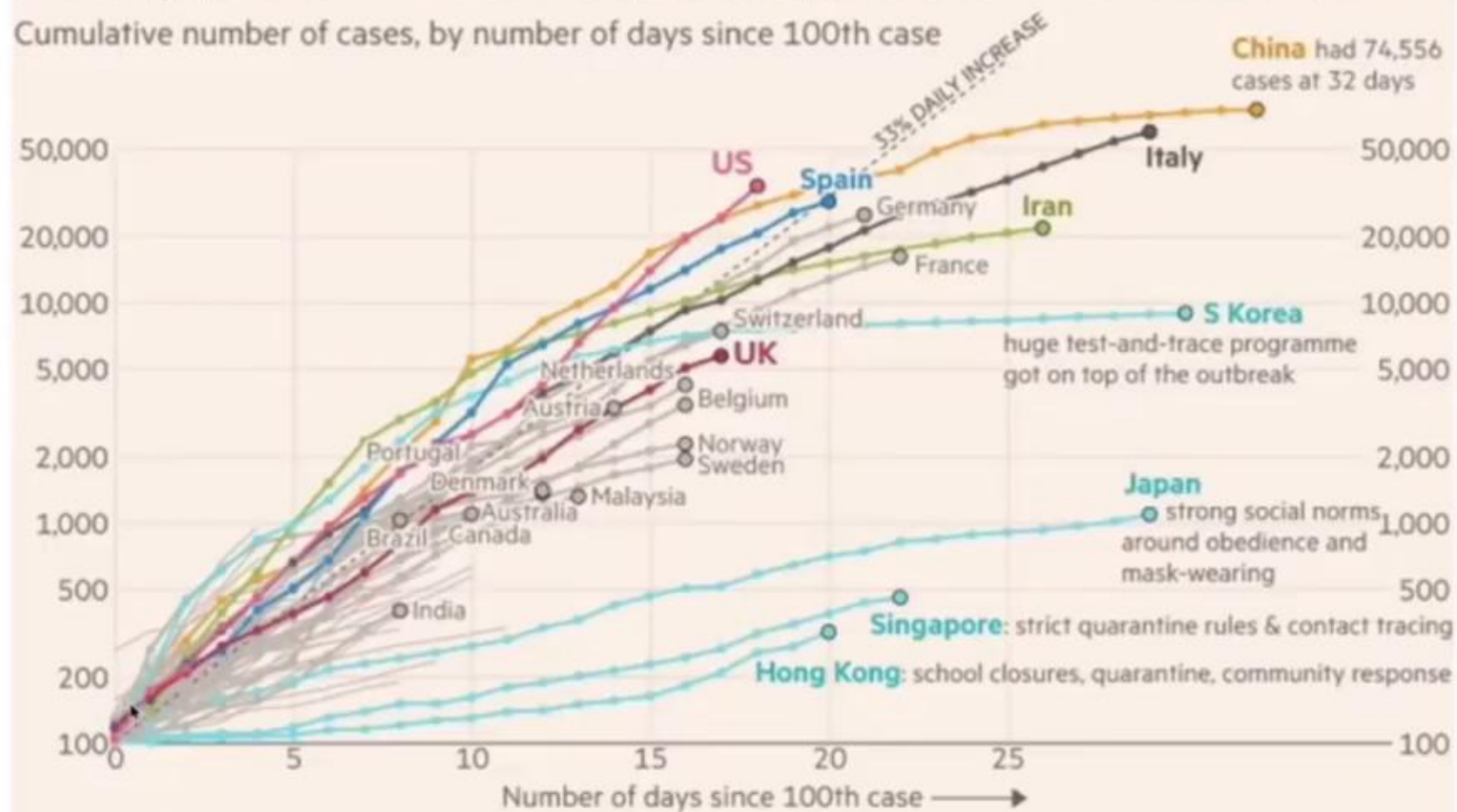


Confirmed    Daily Increase

**\*Actual number of cases presumed much higher, due to limitations in testing**

Most western countries are on the same coronavirus trajectory. Hong Kong and Singapore have limited the spread; Japan and S Korea have slowed it

Cumulative number of cases, by number of days since 100th case

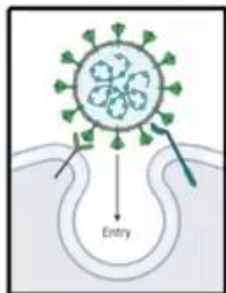


FT graphic: John Burn-Murdoch / @jburnmurdoch

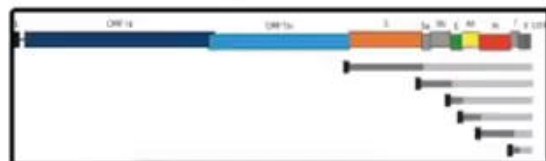
Source: FT analysis of Johns Hopkins University, CSSE; Worldometers. Data updated March 22, 19:00 GMT

© FT

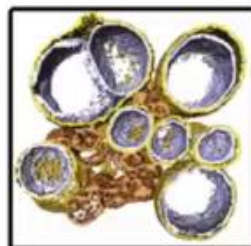
### 1. Entry



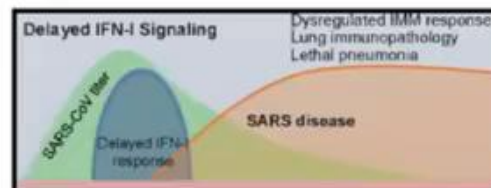
### 2. Replication & gene expression



### 3. RTCs

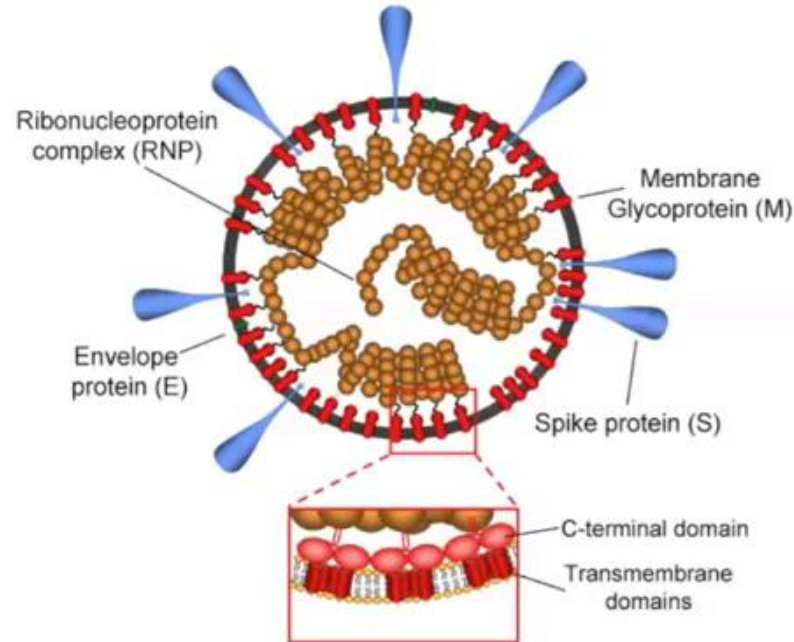


### 4. Immune interactions

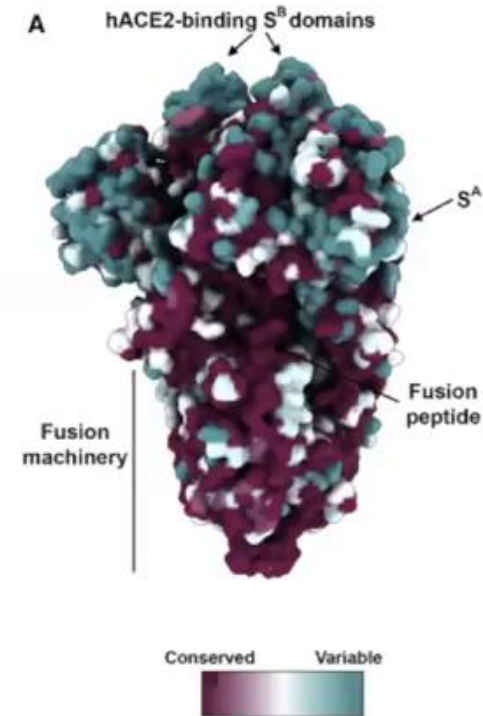


# CoV particles are pleomorphic with a helical nucleocapsid

~125 nm diameter, 30 Kb (+) RNA genome



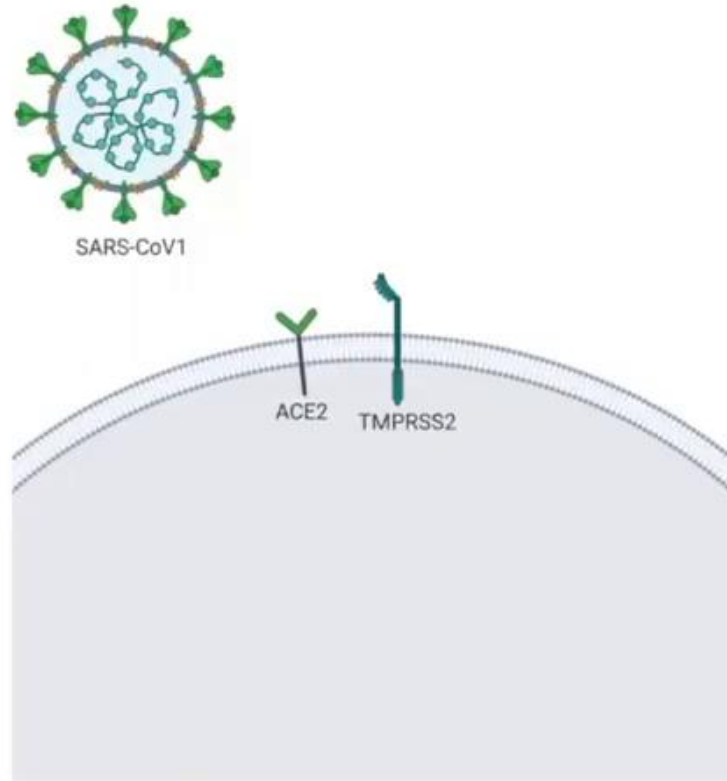
CoV Spike conservation





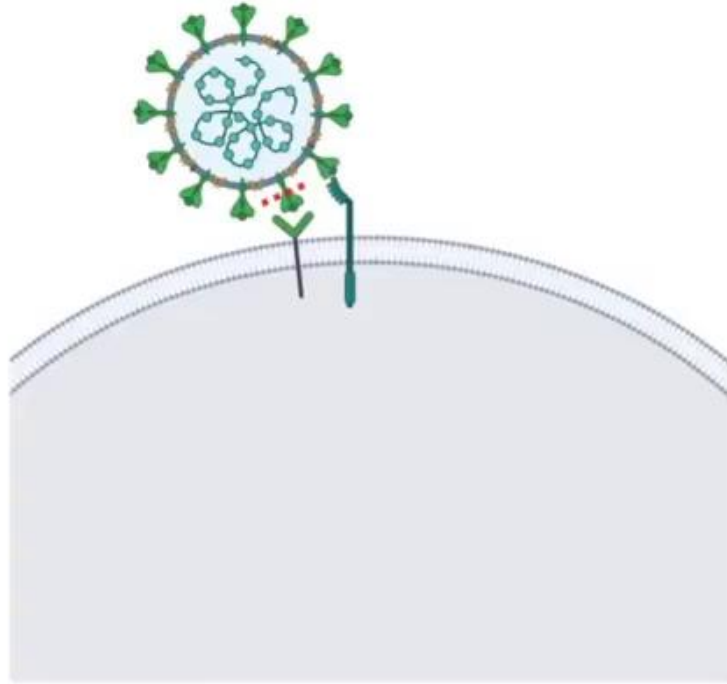
# CoV-2 entry is driven by interactions between Spike and angiotensin-converting enzyme 2 (ACE2); subsequent protease cleavage drives fusion

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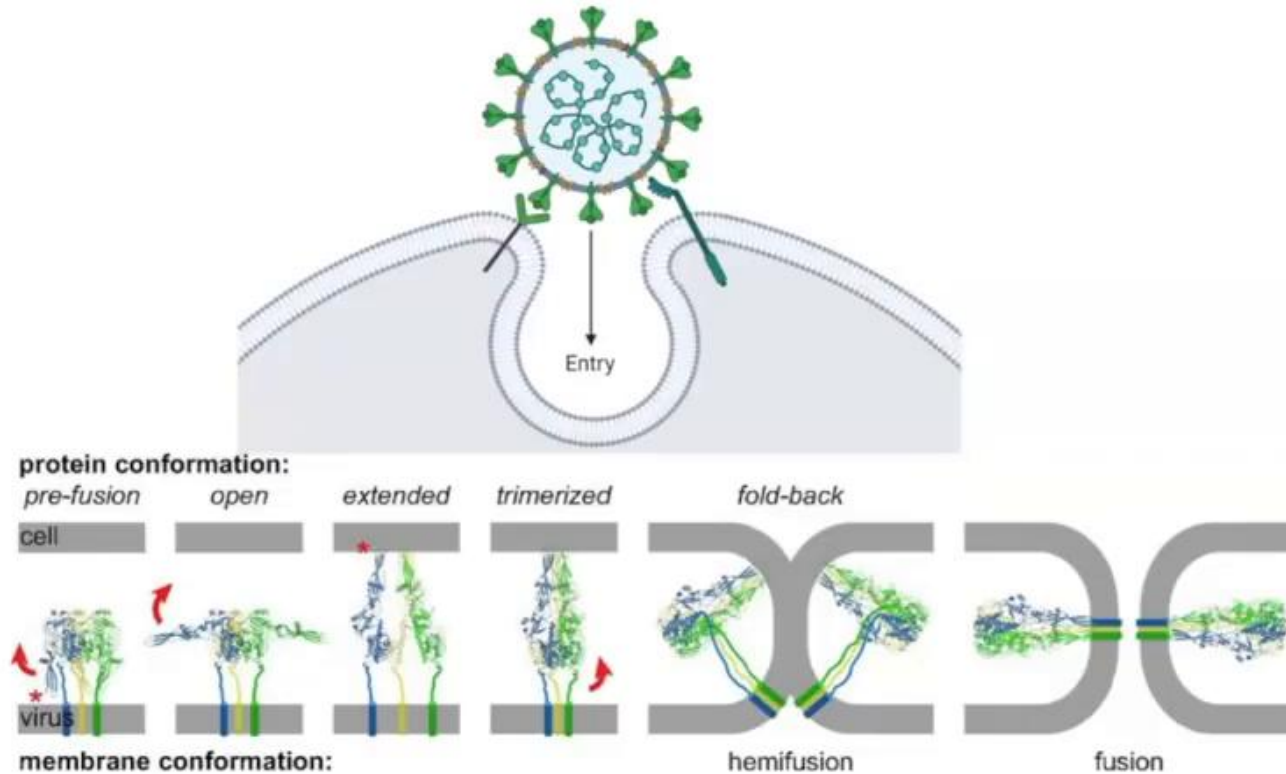


**CoV-2 entry is driven by interactions between Spike and angiotensin-converting enzyme 2 (ACE2); subsequent protease cleavage drives fusion**

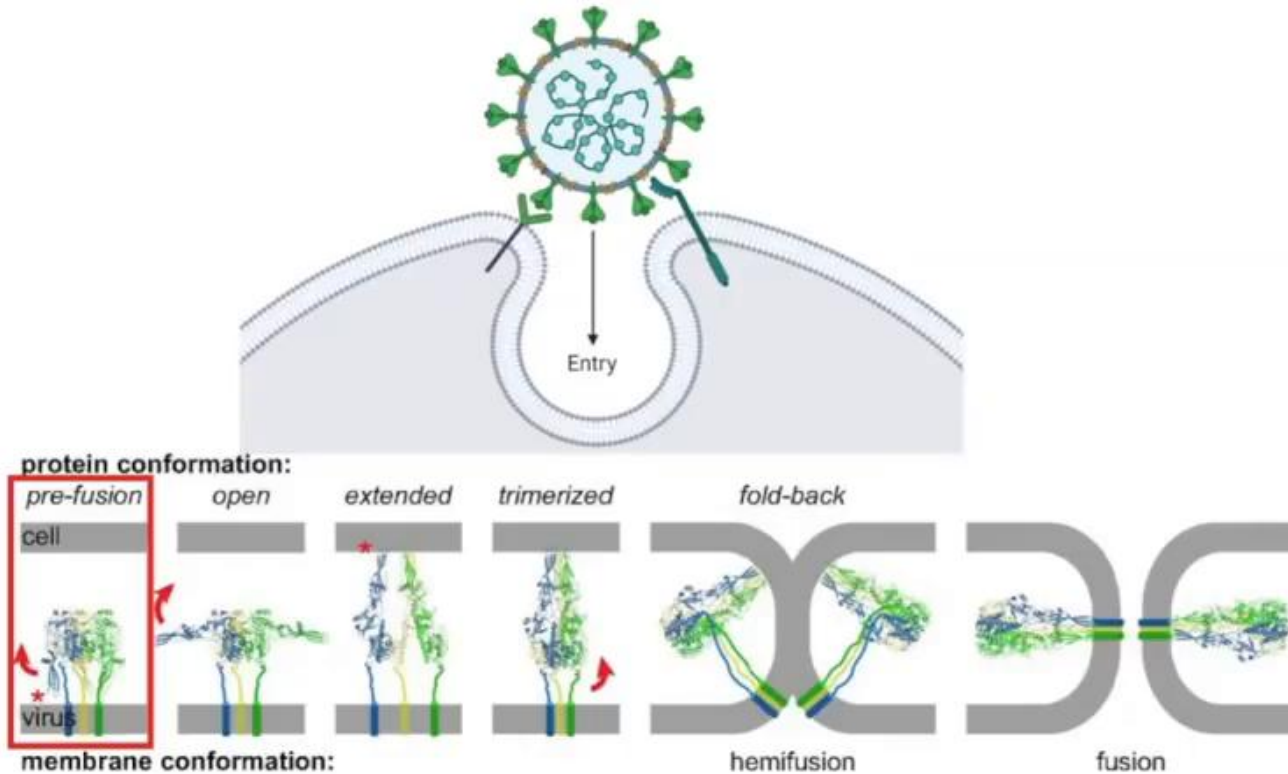
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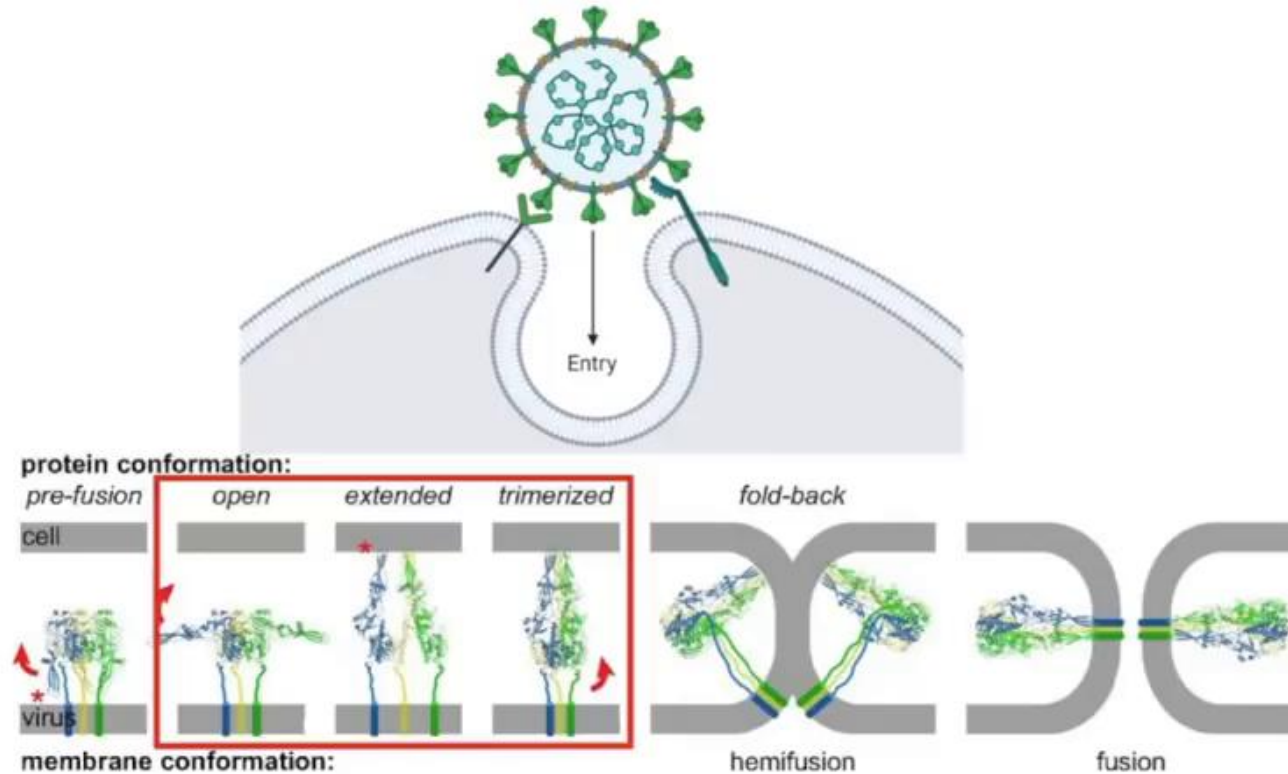
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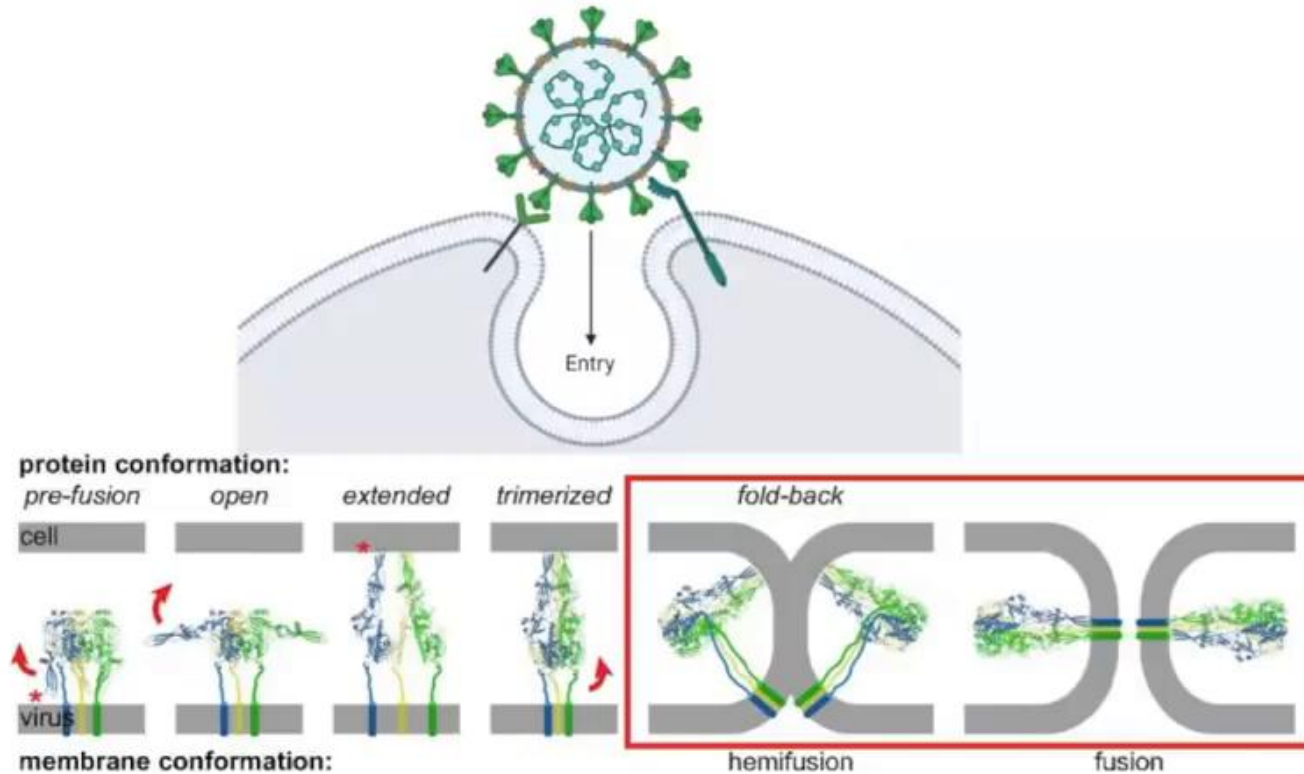
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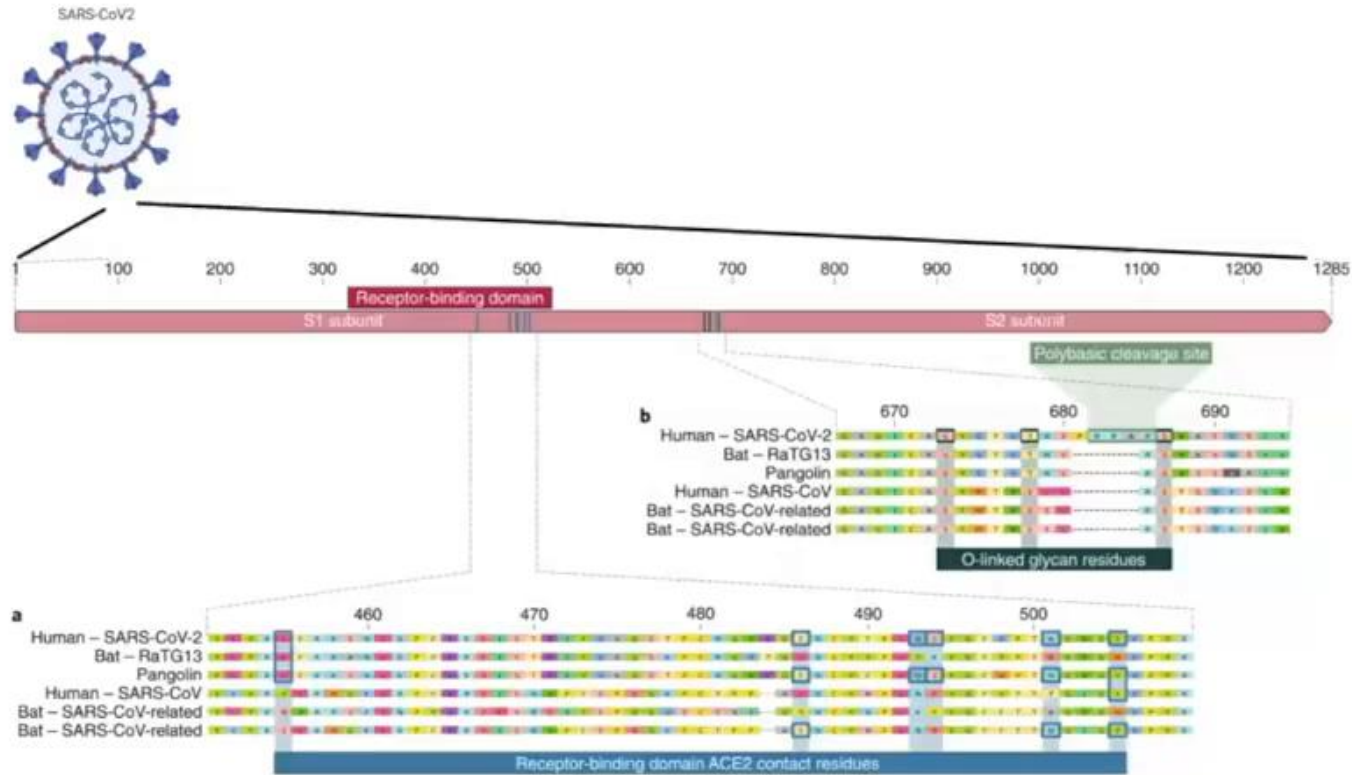
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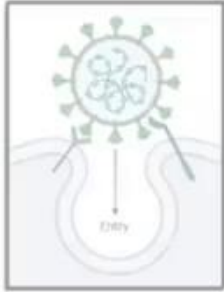
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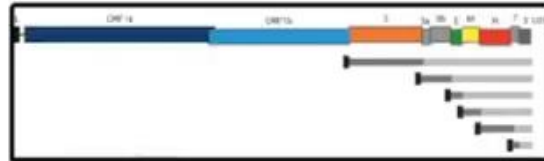
# Acquisition of polybasic cleavage site in CoV-2 spike may increase viral transmissibility



### 1. Entry



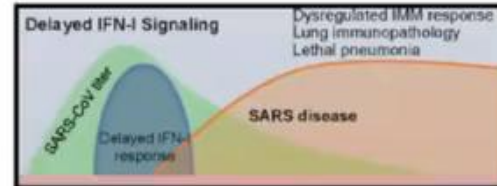
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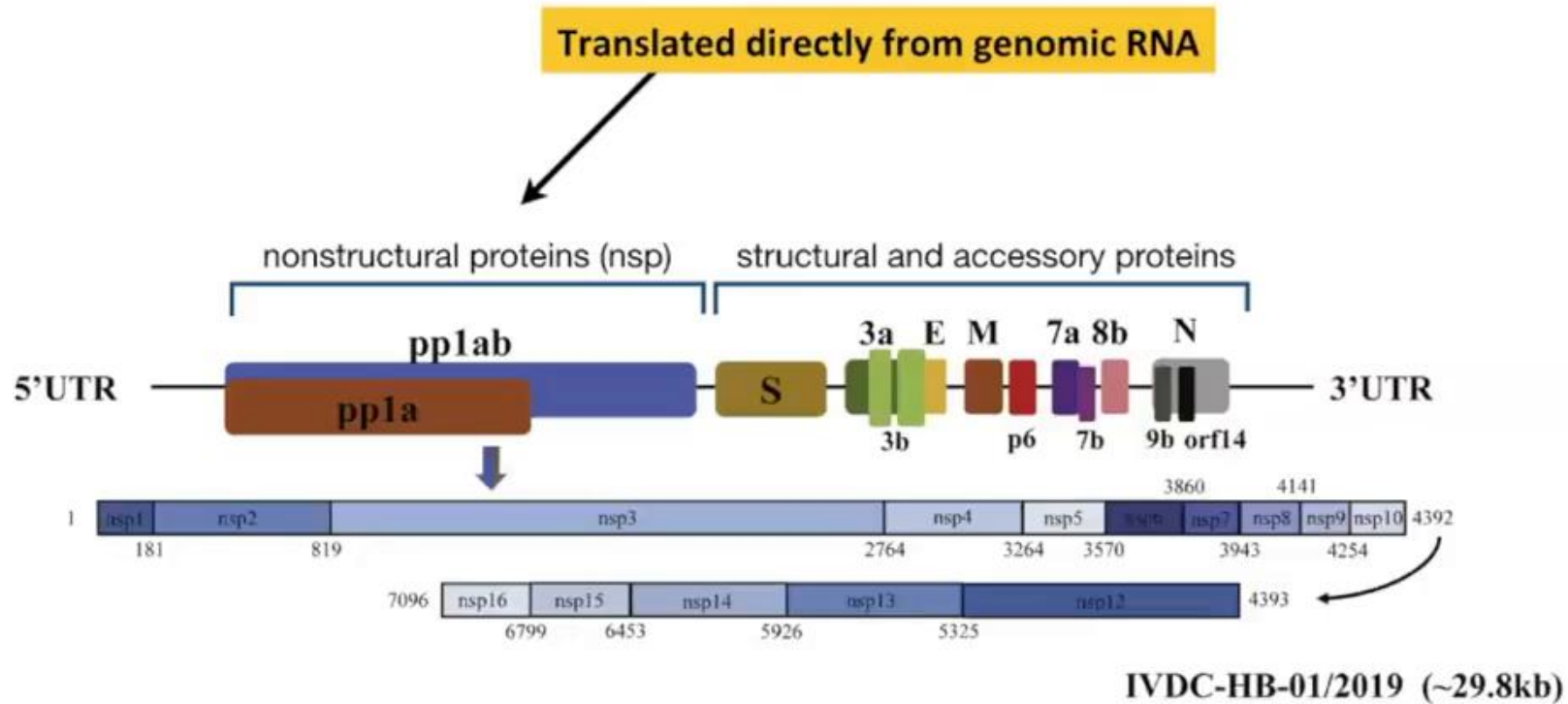


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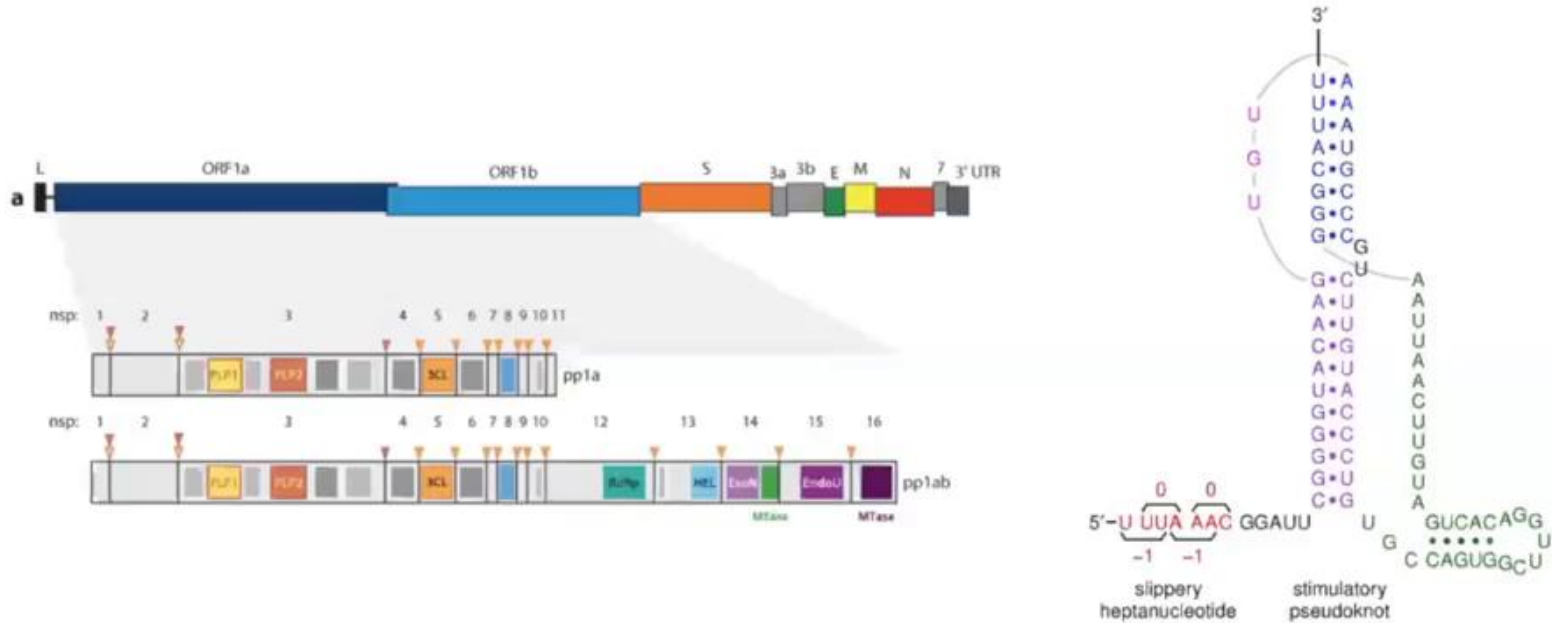




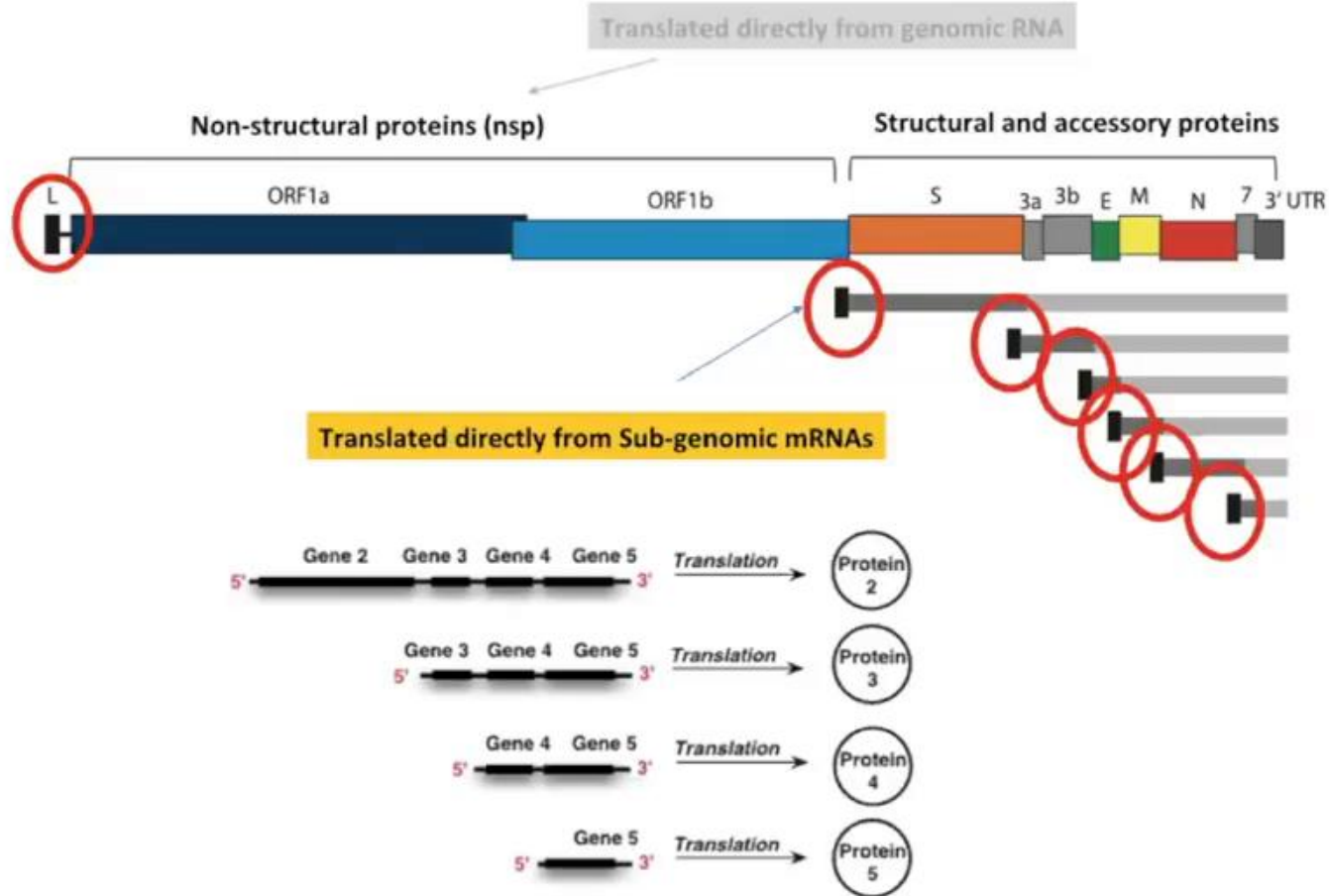
# The 2019-nCoV genome was annotated to possess ~14 ORFs encoding 27 proteins



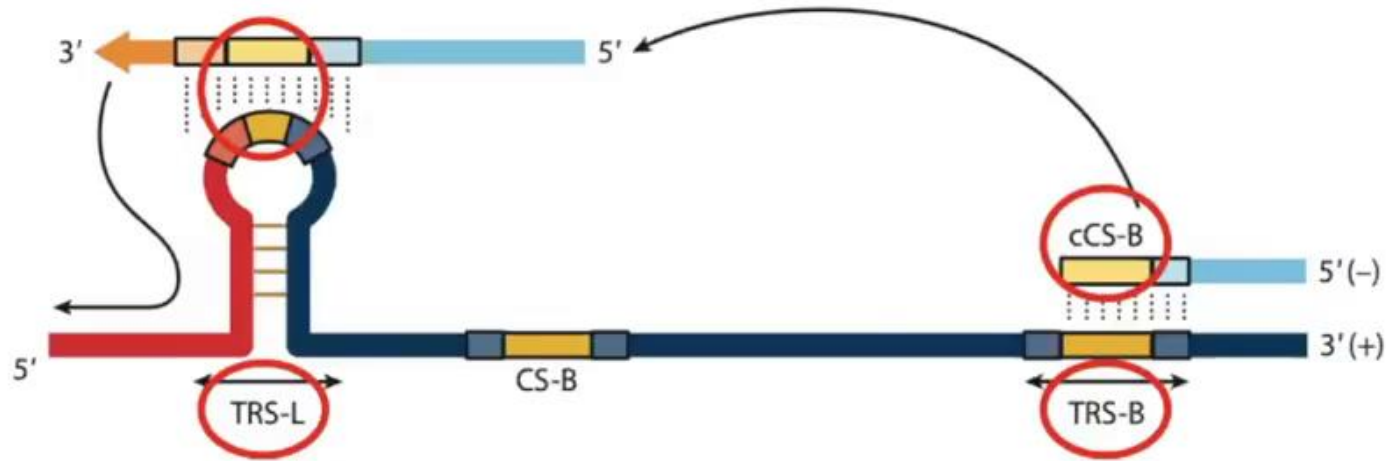
# Programmed ribosomal frameshifting generates two polyproteins encoding the replicase proteins



# Structural proteins are made from a nested set of sub-genomic mRNAs with shared 5' and 3' sequences

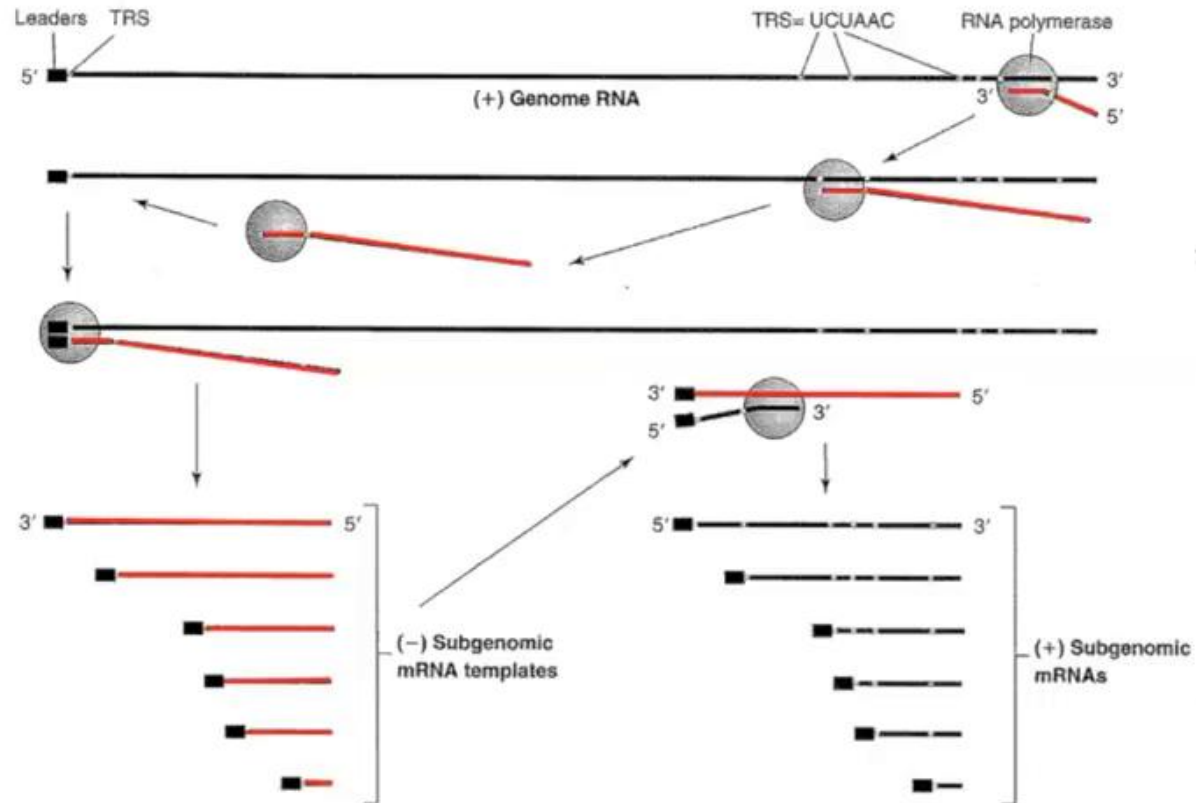


# Sub-genomic RNA transcription is discontinuous and is facilitated by shared transcription regulatory sequences



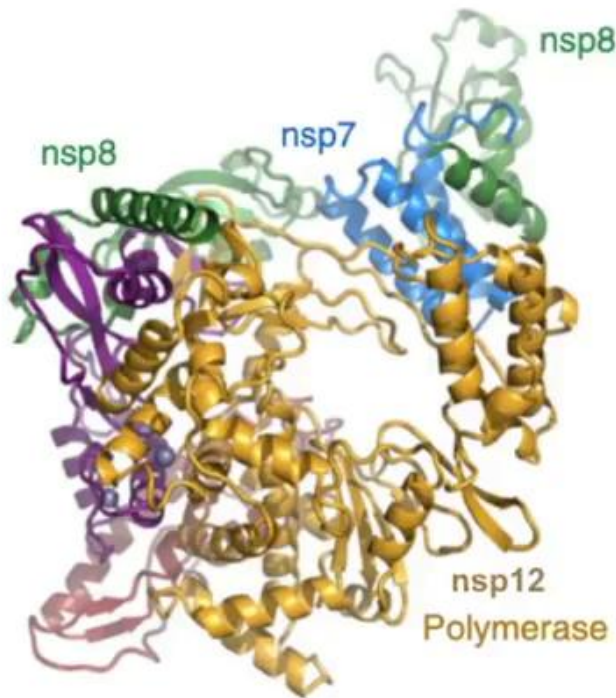
*CS= core conserved sequence within TRS*

# Sub-genomic RNA transcription is discontinuous and is facilitated by shared transcription regulatory sequences



# The CoV replicase requires functional integration of RNA polymerase, capping, and proofreading activities

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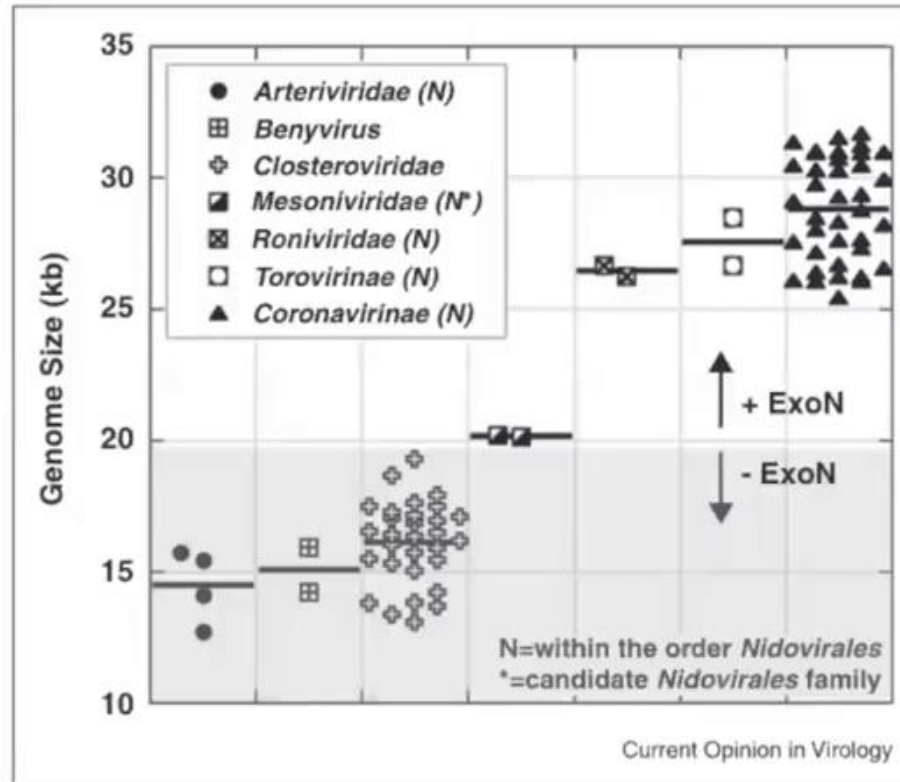


nsp7/nsp8/nsp12/nsp14  
capping,  
ExoN

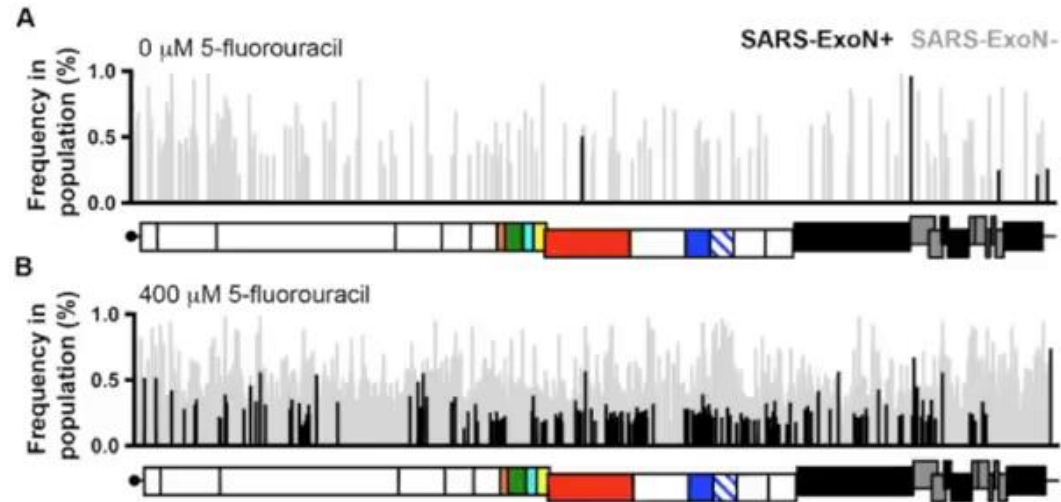
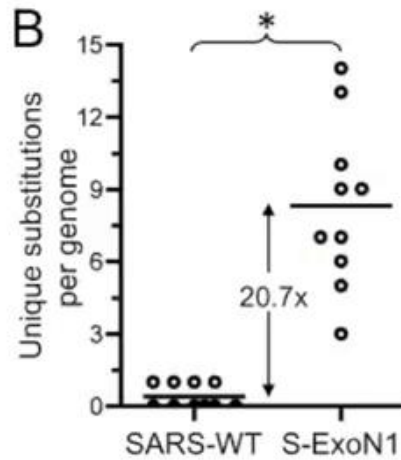
polymerase 'holoenzyme'  
initiates de novo RNA syn.

**Variety of other viral processing proteins/  
activities associated with replicase  
complex, cellular proteins**

# Exonuclease is present only in viruses with genomes > 20 kb



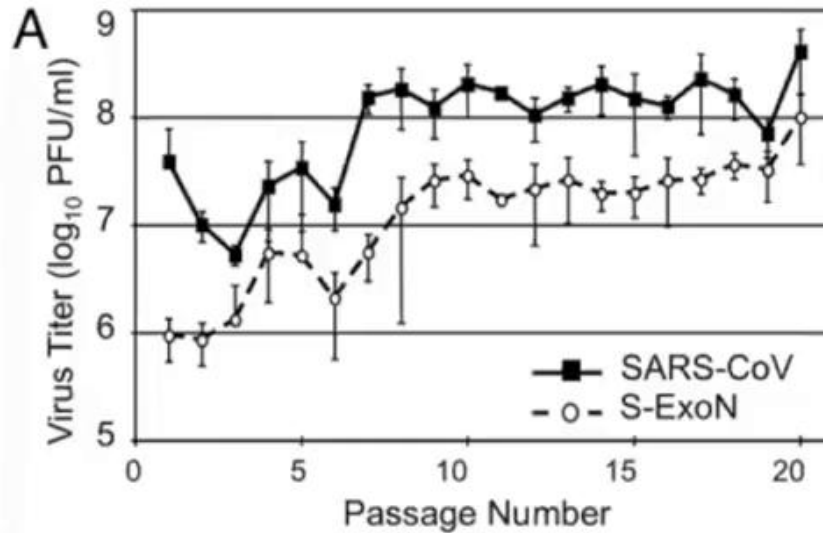
# Loss of ExoN activity dramatically increases the sensitivity of CoVs to RNA mutagens





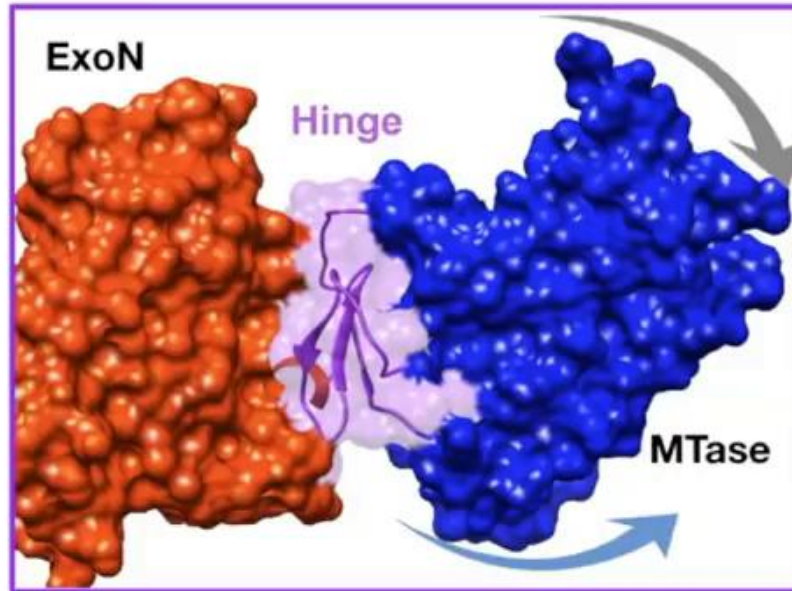
## However... the mutants adapt over multiple passages to stabilize populations and prevent lethal mutagenesis

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- MHV ExoN mutant showed promise as a vaccine strategy (Graham et al., 2012, Nat Med)

# nsp14 is a bimodular protein composed of ExoN and N7-MTase domains



Ogando et al. 2019; [doi.org/10.3389/fmicb.2019.01813](https://doi.org/10.3389/fmicb.2019.01813)

- SARS-CoV nsp10/ExoN operate as a heterodimer in a mismatch repair mechanism
- ExoN can efficiently excise ribavirin 5'-monophosphate (Snijder et al., 2003; Ferron et al., 2018).

- Replication of MHV ExoN1-knockout was inhibited more efficiently than WT virus by Remdesivir, suggesting ExoN may reduce its incorporation—>try simultaneous targeting of RdRp and ExoN with nucleoside analog + a specific exoribonuclease inhibitor? (Warren et al., 2016; Sheahan et al., 2017; Agostini et al., 2018)

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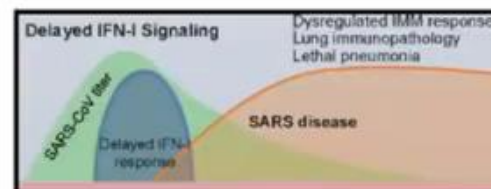
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### 4. Immune interactions

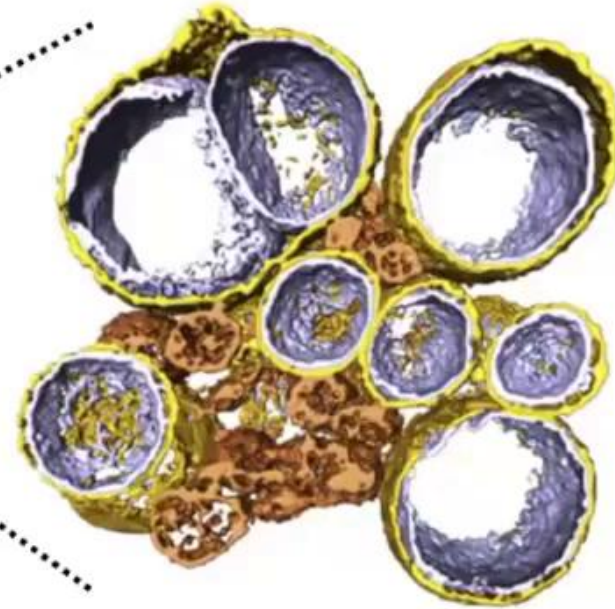
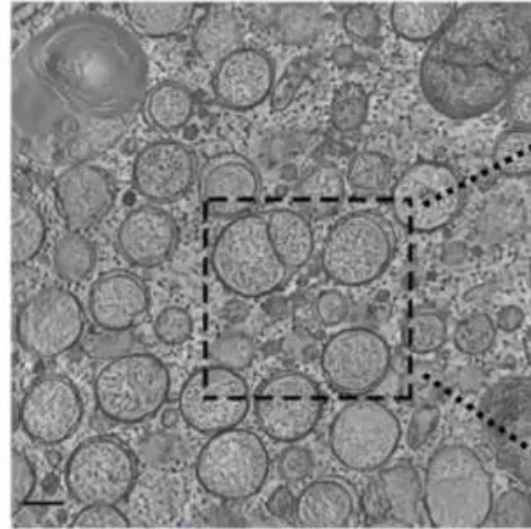
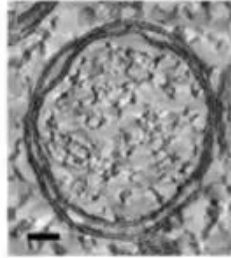


# CoVs form interconnected double membrane vesicles where viral replication and transcription occur

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SARS replication-transcription complexes (RTC)

Double membrane vesicle

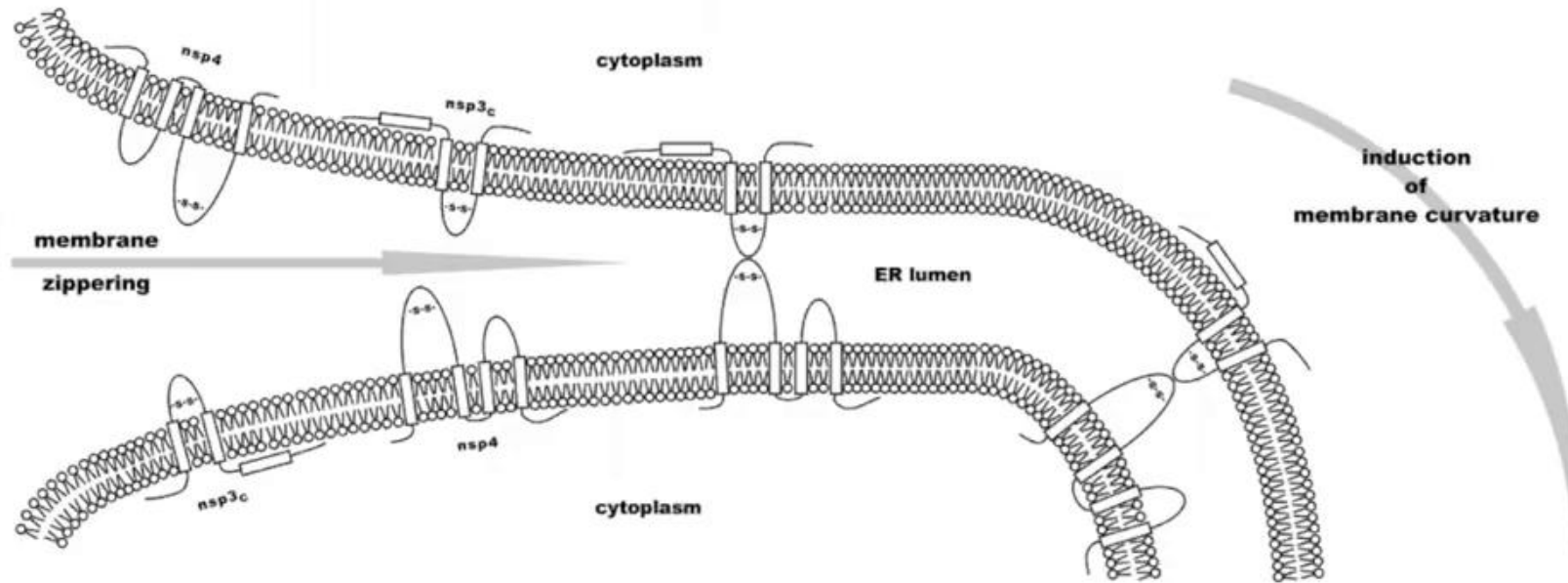


Knoops et al., 2008 <https://doi.org/10.1371/journal.pbio.0060226>

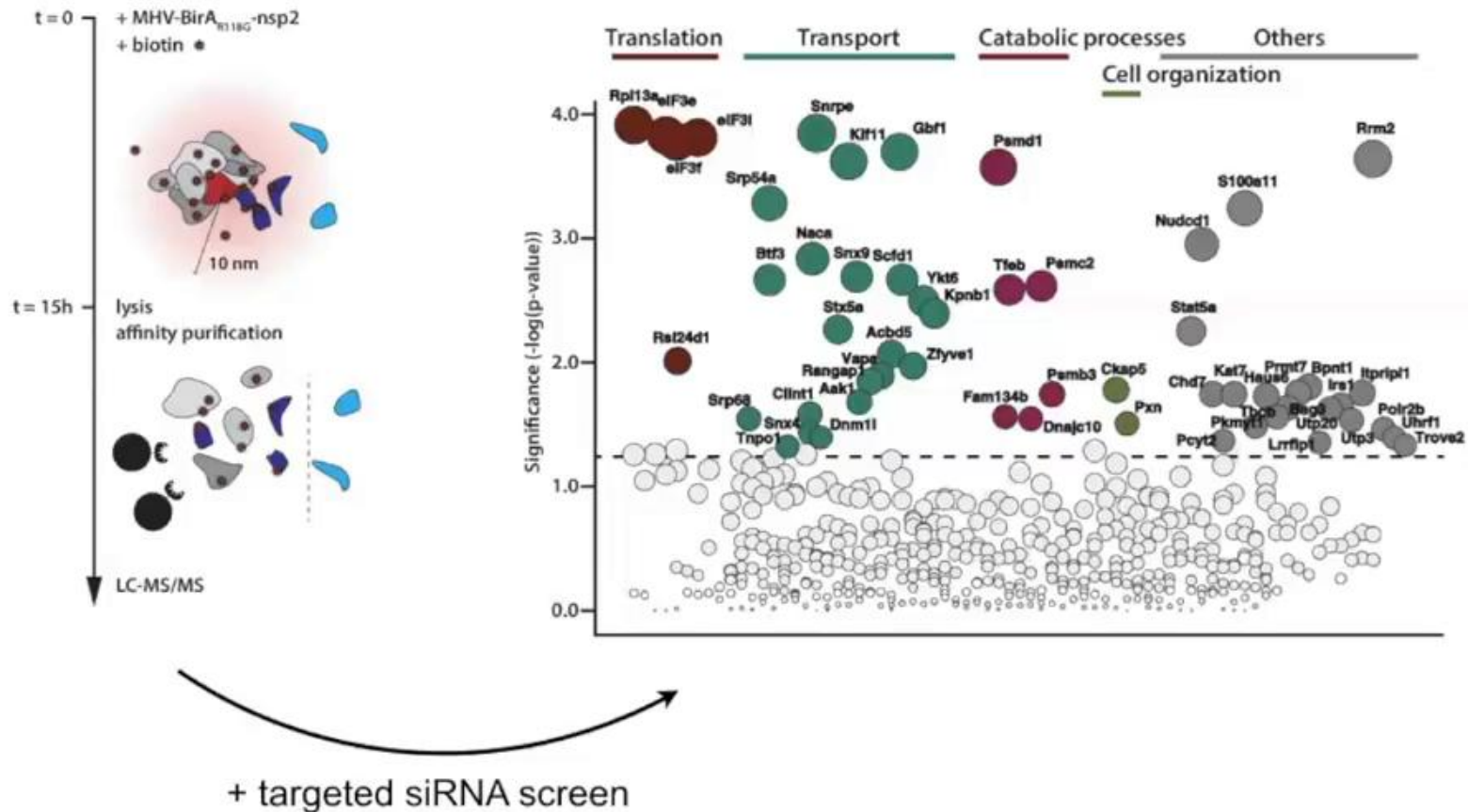
**-CoV RTCs are potential antiviral targets**

# Integral membrane replicase proteins function in vesicle biogenesis and recruitment of factors necessary for viral transcription and amplification

-nsp3, 4, 6 involved in vesicle formation



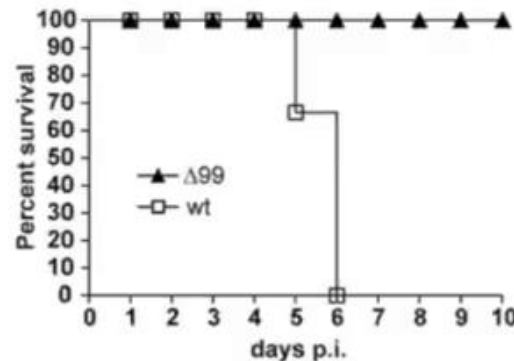
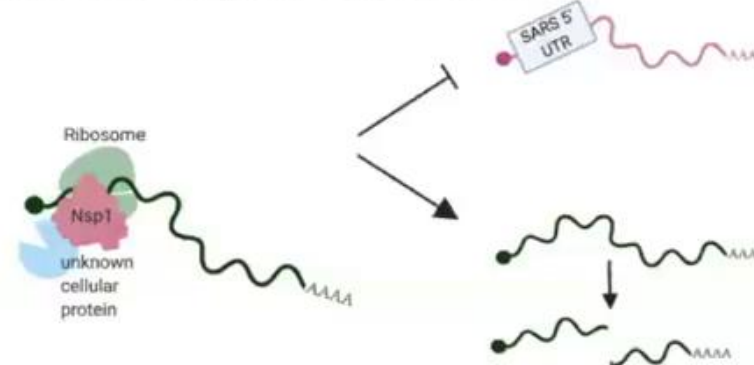
# Proximity labeling has been used to characterize the RTC-proximal proteome in the beta-coronavirus MHV



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MHV protein	log2FC	adj p-value
nsp1	2.18	0.1858
nsp2	10.70	0.0403
nsp3	9.32	0.0078
nsp4	29.92	0.0007
nsp5	27.28	0.0004
nsp6	25.66	0.0008
nsp7	23.89	0.0020
nsp8	28.37	0.0007
nsp9	27.41	0.0004
nsp10	27.51	0.0003
nsp11	n.d.	n.d.
nsp12	28.70	0.0007
nsp13	28.38	0.0007
nsp14	27.36	0.0007
nsp15	28.08	0.0007
nsp16	27.97	0.0007
2a	0.93	0.4742
HE	n.d.	n.d.
S	-1.29	0.0354
ORF4	n.d.	n.d.
5a	n.d.	n.d.
E	n.d.	n.d.
M	-0.99	0.1893
N	4.05	0.0280


nsp1 is a CoV pathogenicity factor that restricts host gene expression



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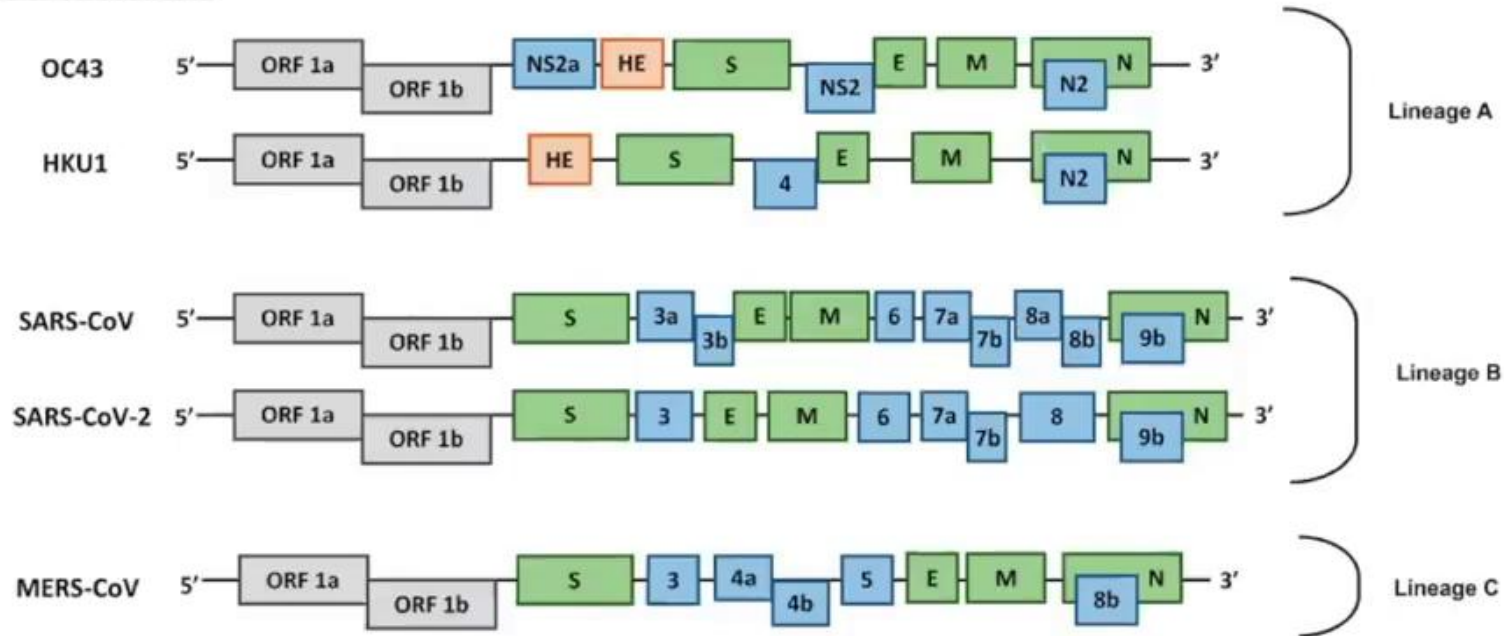
Several assembly/virion proteins, accessory proteins





# Accessory genes are genera/species specific and are usually dispensable for viral replication *in vitro* but required *in vivo*

## Betacoronavirus



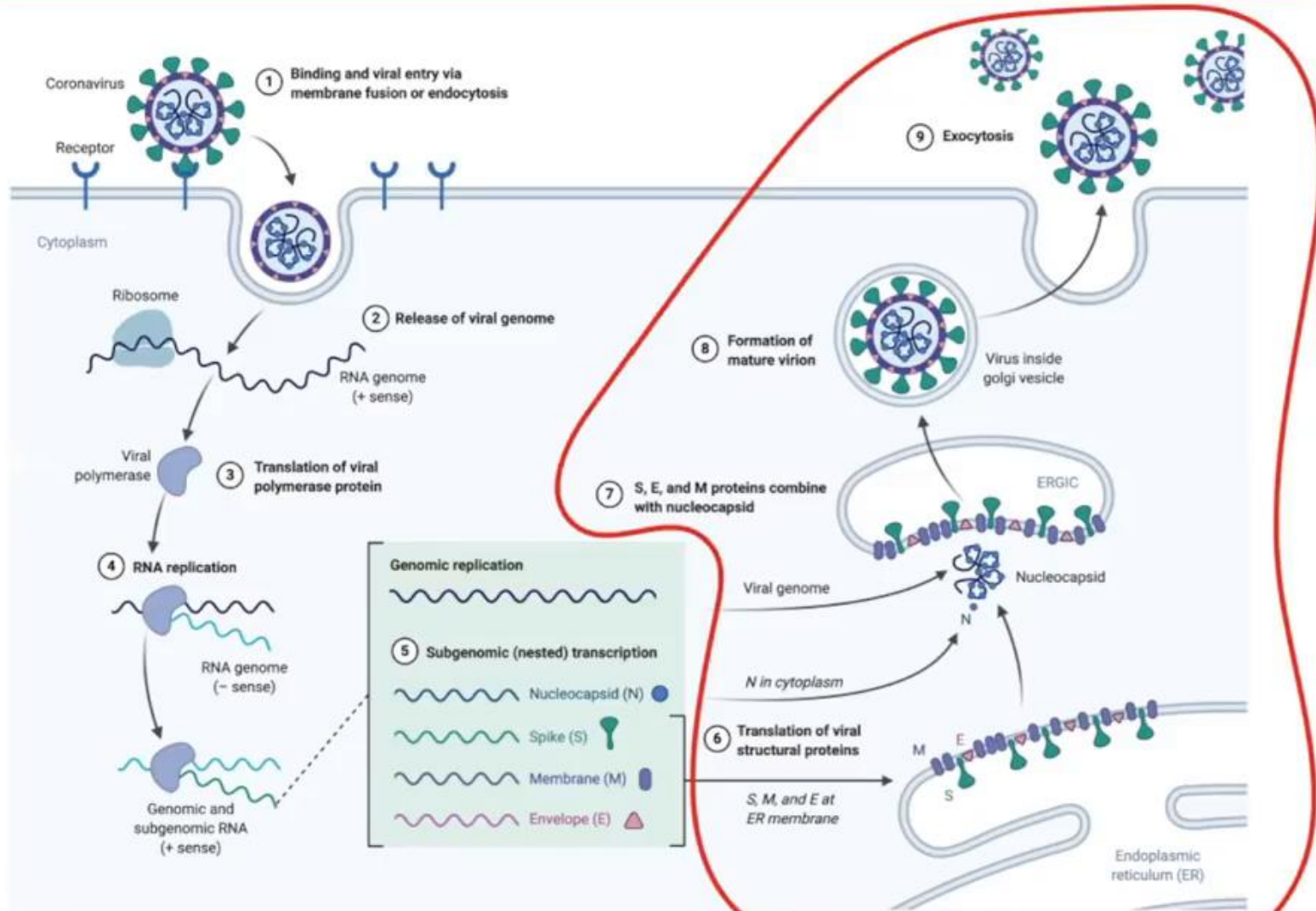
Accessory proteins labeled in blue

## CoV-2 and SARS may have a similar set of accessory genes, with some differences among the interferon antagonists

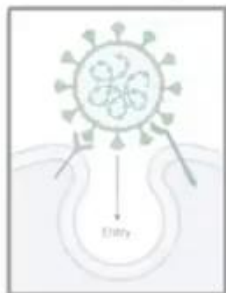
Accessory Proteins	Incorporation into Virions/VLPs	Function(s)	Notable SARS-CoV-2 variations (from Wu et al., 2020 genome annotation)
3a	Yes	NF- $\kappa$ B $\uparrow$ , JNK $\uparrow$ , IL-8 $\uparrow$ , RANTES $\uparrow$ , ion-channel activity, apoptosis induction, and cell cycle arrest	
3b	Unknown	Type I IFN production and signaling inhibition, JNK $\uparrow$ , ERK $\uparrow$ apoptosis and necrosis induction, and cell cycle arrest.	Truncated to 20aa (154aa in SARS-CoV)
6	Yes	Type I IFN production and signaling inhibition, and cellular DNA synthesis $\uparrow$	Only 69% aa identity with SARS-CoV
7a	Yes	NF- $\kappa$ B $\uparrow$ , JNK $\uparrow$ , p38 MAP kinase $\uparrow$ , host translation inhibition, apoptosis induction and cell cycle arrest.	
7b	Yes	No known function	
8a	Unknown	Apoptosis induction	
8b	Unknown	Cellular DNA synthesis $\uparrow$ and caspase-independent cell death $\uparrow$	Single ORF8 gene (121aa)
9b	Yes	Apoptosis induction	

( $\uparrow$ ) up-regulation/activation

# Assembly of nucleocapsids into virions occurs in ER/golgi



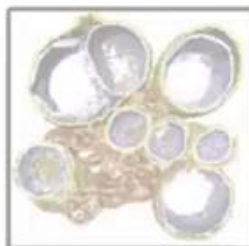
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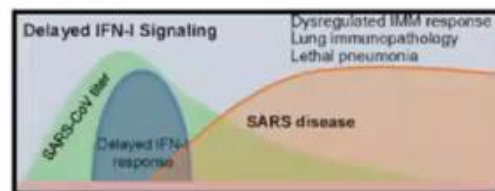
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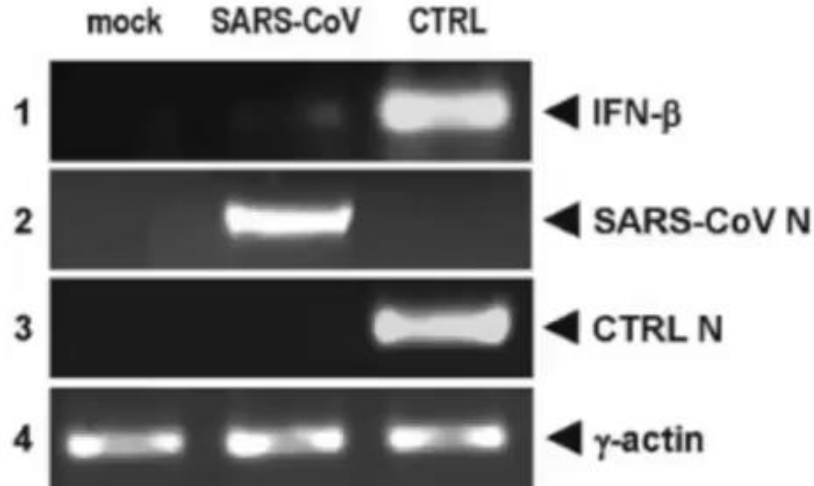
### 3. RTCs



### 4. Immune interactions



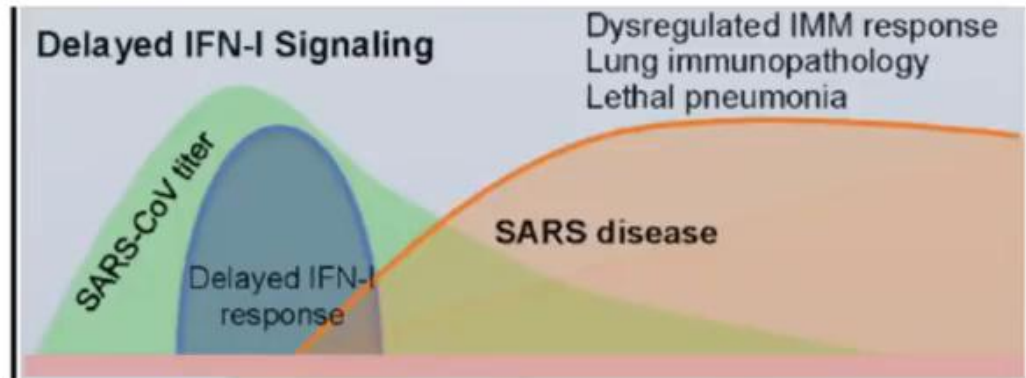
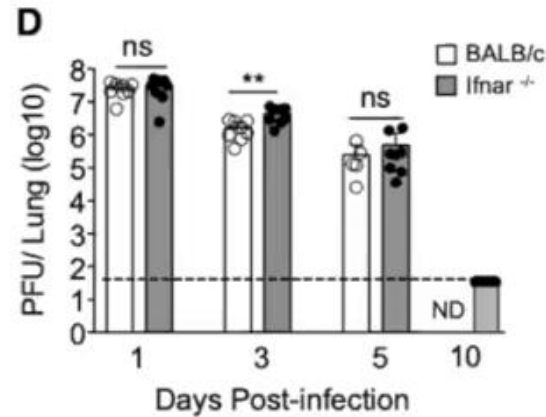
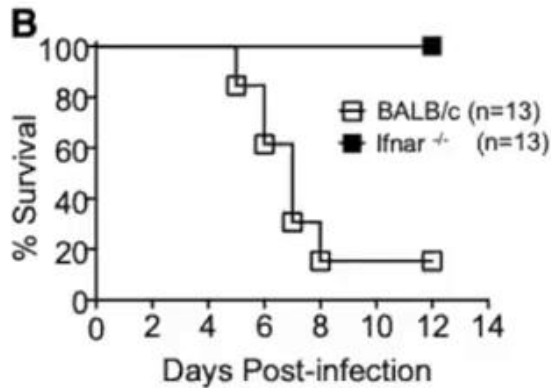
# SARS-CoV and MERS-CoV induce very little – if any – IFN in most cells



A number of putative IFN antagonists have been identified in the SARS-CoV genome:

- nsp1, 3, 7, 15, 16
- ORF 3a,3b, 6
- M and NP protein

# SARS pathogenesis is linked to delayed IFN-I signaling and subsequent immune toxicity

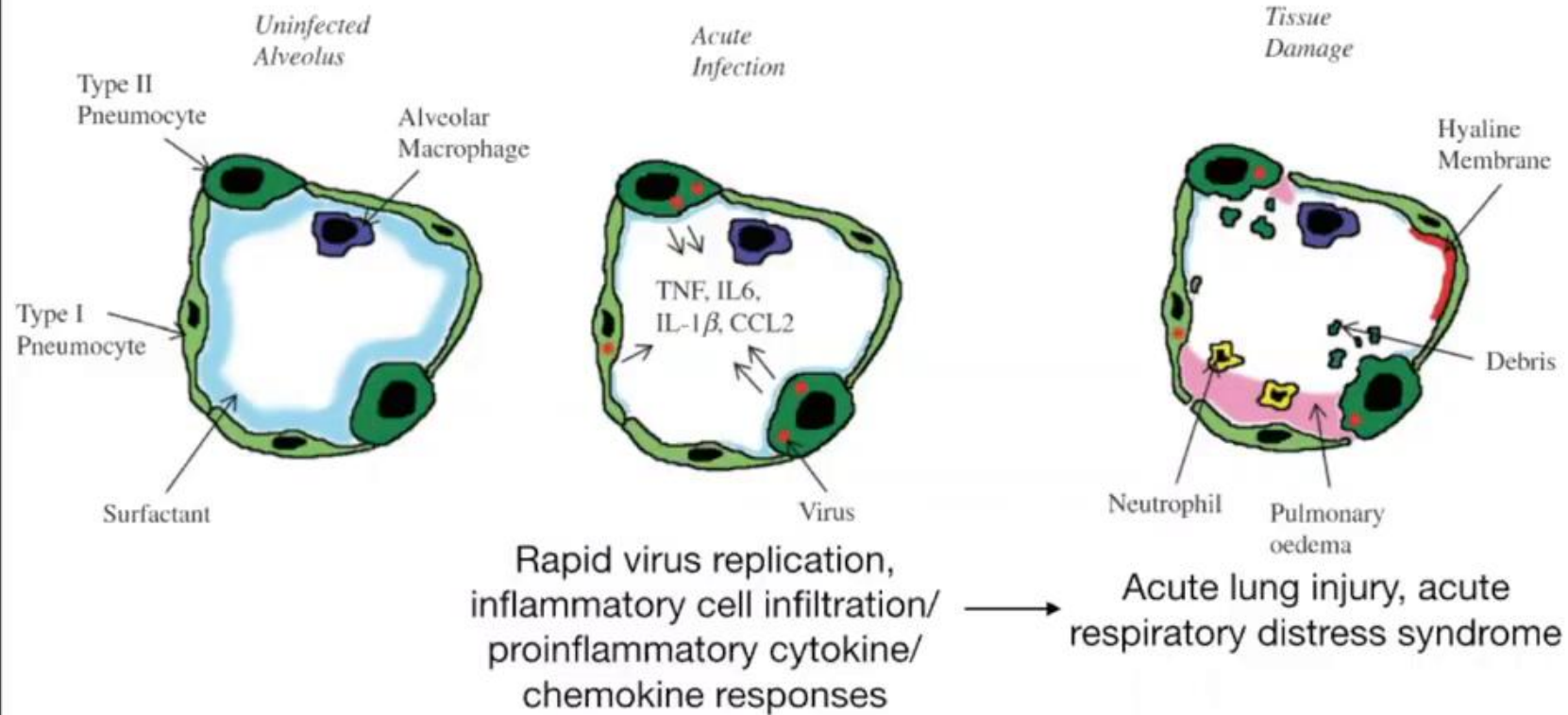


Cell Host & Microbe 19, 181–193, February 10, 2016

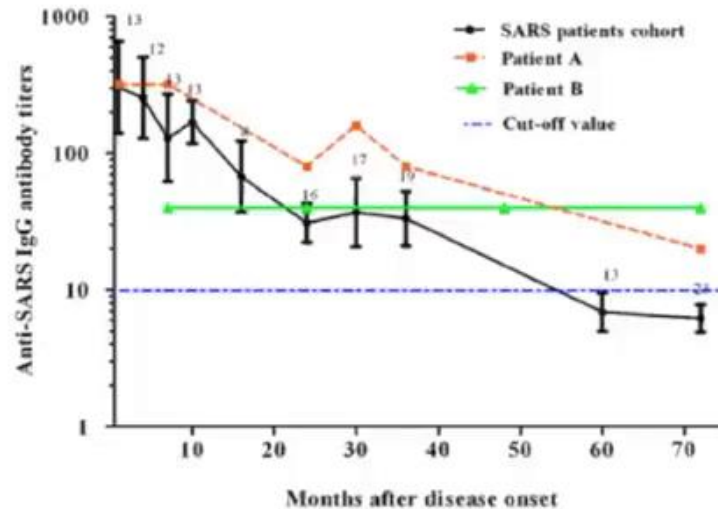
High initial virus titers due to a late interferon response

- > drives aberrant recruitment of pathogenic inflammatory monocyte-macrophages (IMMs), activation of innate immune response
- > cytotoxicity

# A dysregulated/exuberant innate response is a leading contributor to SARS-CoV-mediated pathology



# Neutralizing antibody titers and the memory B cell response are short lived in SARS-recovered patients



J Immunol 2011; 186:7264-7268

- How does seroconversion look for CoV-2?
- How long do recovered individuals stay immune, can they be reinfected?
- What type of immunity will we get from vaccines and how does it compare to infection response?
- Need more info about what's happening in older people: immunology, inflammation. What parallels should be looked for in animal models (need development)?



## **(Some) Key open basic science questions**

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- What is the role of the polybasic site in the spike protein in CoV-2 transmission?
- What are the pathways involved in CoV-induced membrane remodeling, and how do RTCs temporally and functionally coordinate various stages of the viral lifecycle?
- What are the biochemical activities and roles of the various proteins that form the replication-transcription complex, and how do they coordinate genome replication vs. transcription?
- How do CoVs maintain such a large genome and still have sufficient mutation rates for adaptation and trans-species movement?
- What are the functions of the CoV-2 accessory proteins, and how do they impact the in vivo growth and virulence of the virus?
- Will CoV-2 infected individuals (or vaccines) mount protective long-term immune responses?

# Thank you!

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## Slide assistance from:

Laurent Coscoy  
Divya Nandakumar  
Ella Hartenian  
Michael Ly  
Azra Lari  
Jessica Tucker  
Allison Didychuk

★ **The coronavirus researchers**

★ **All the scientists and medical personnel who are working tirelessly to fight the pandemic, thank you.**



<https://www.ibiology.org/microbiology/kshv/>