COVID-19 Grand Rounds, April 1, 2020

SARS-CoV-2: Virology and Clinical Implications for COVID-19

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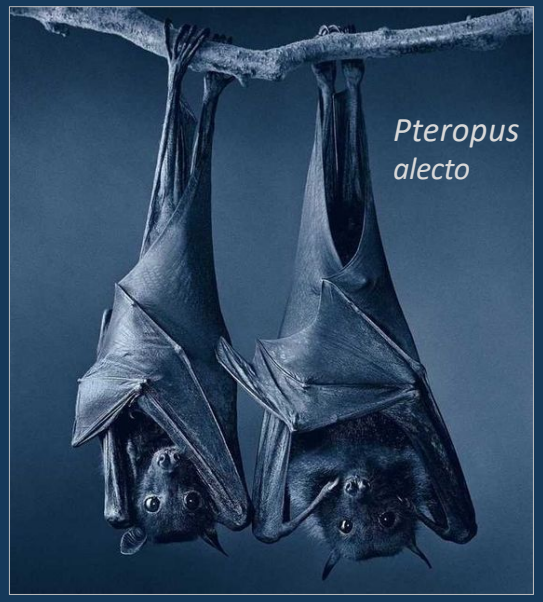
Bats (Mammalian Order Chiroptera)

Megachiroptera



Large, frugivorous, sight-locating

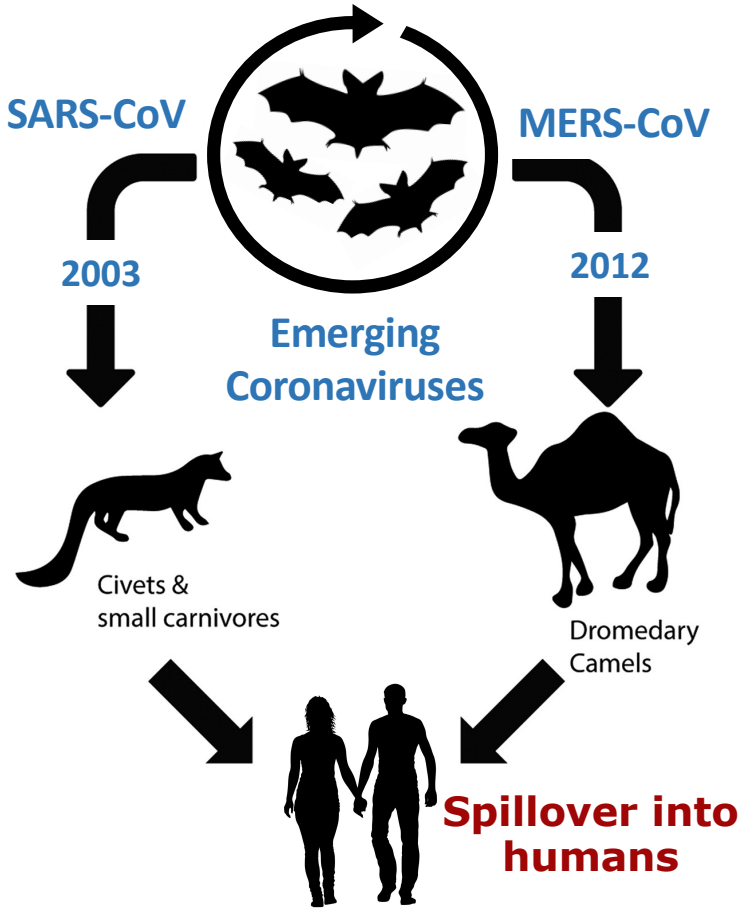
Microchiroptera

Small, insectivorous, echolocating

Alternative molecular phylogeny-based suborders:

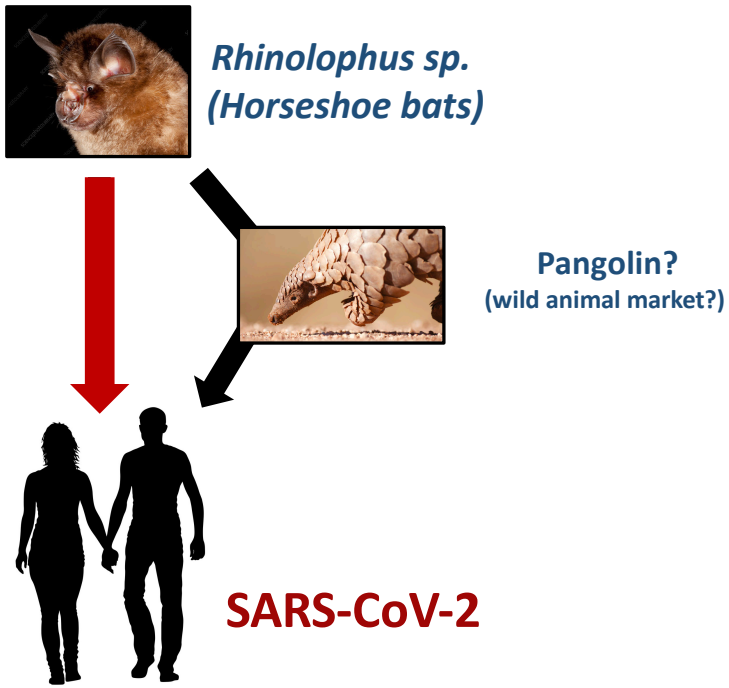
- Yinpterochiroptera (Pteropodiformes)
- Yangochiroptera (Vespertilioniformes)

Viral phylogenetic data: the first SARS Virus (SARS-CoV) & MERS virus emerged from bats and transferred to humans, probably via intermediate mammalian hosts.



shorthand: "SARS2" "SARS1"

For the recently emergent SARS-CoV-2 (like SARS-CoV), bats in the genus *Rhinolophus* are the likely reservoir

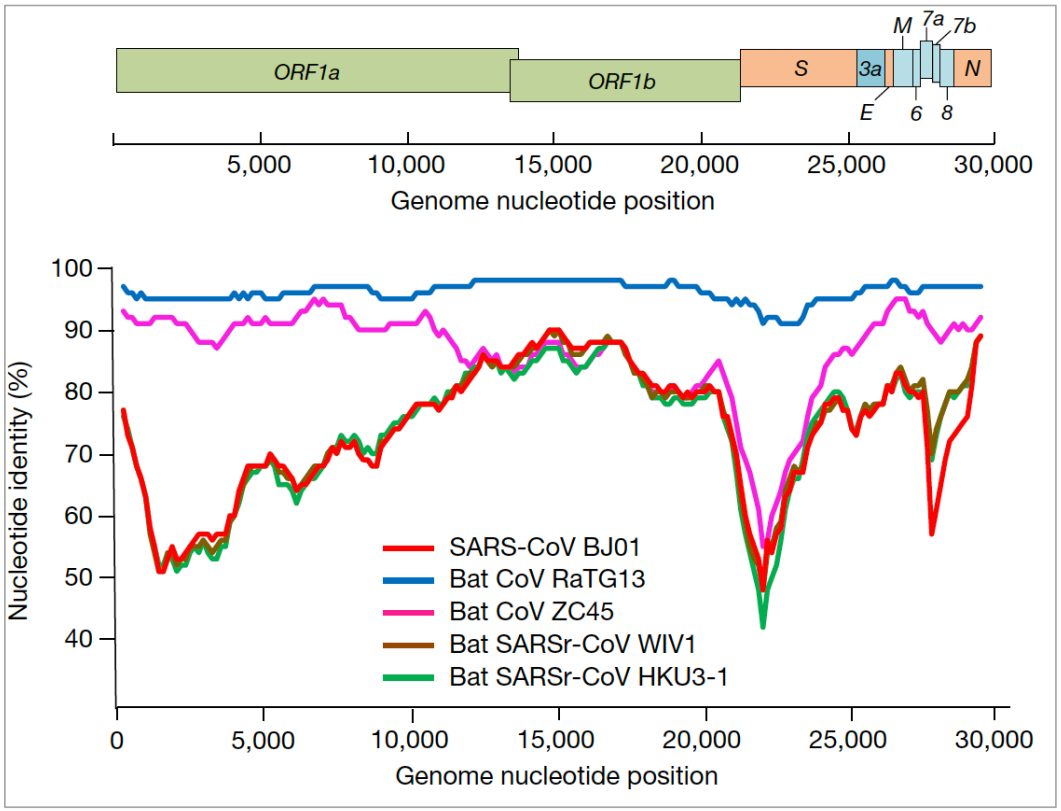


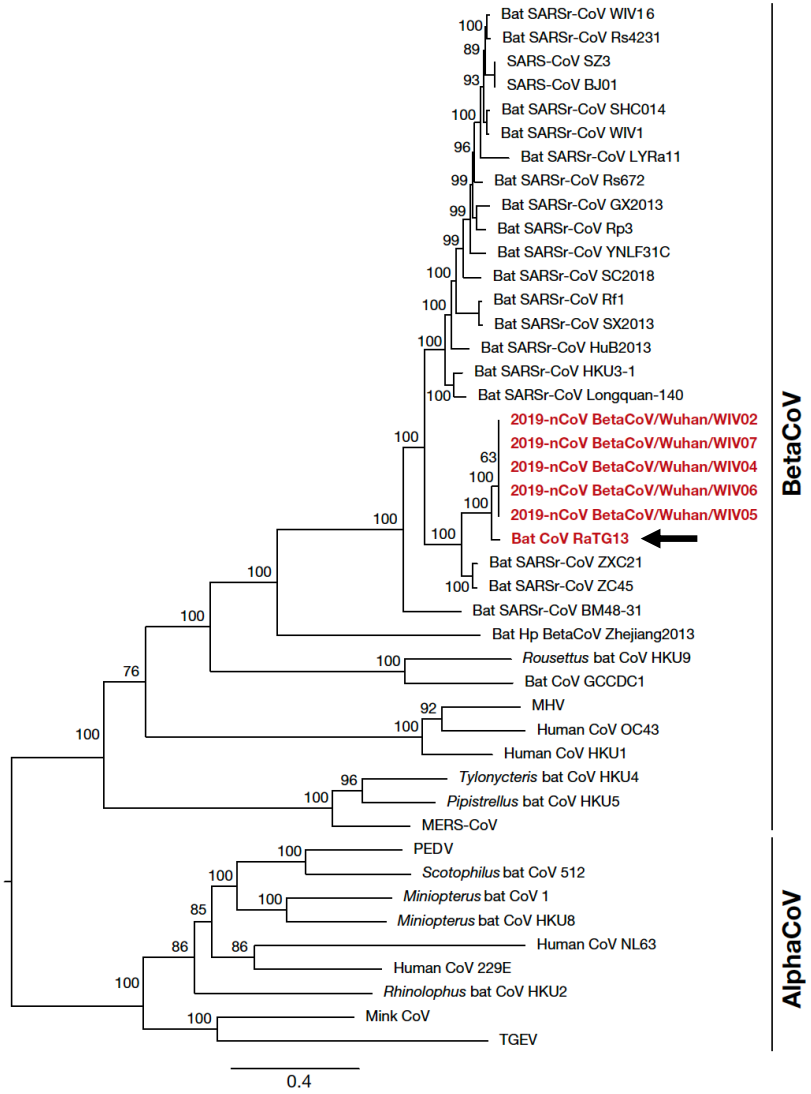
Poeschla 4-1-20

Figure adapted from Totura et al. [https://www.tandfonline.com/doi/full/10.1080/17460441.2019.1581171]

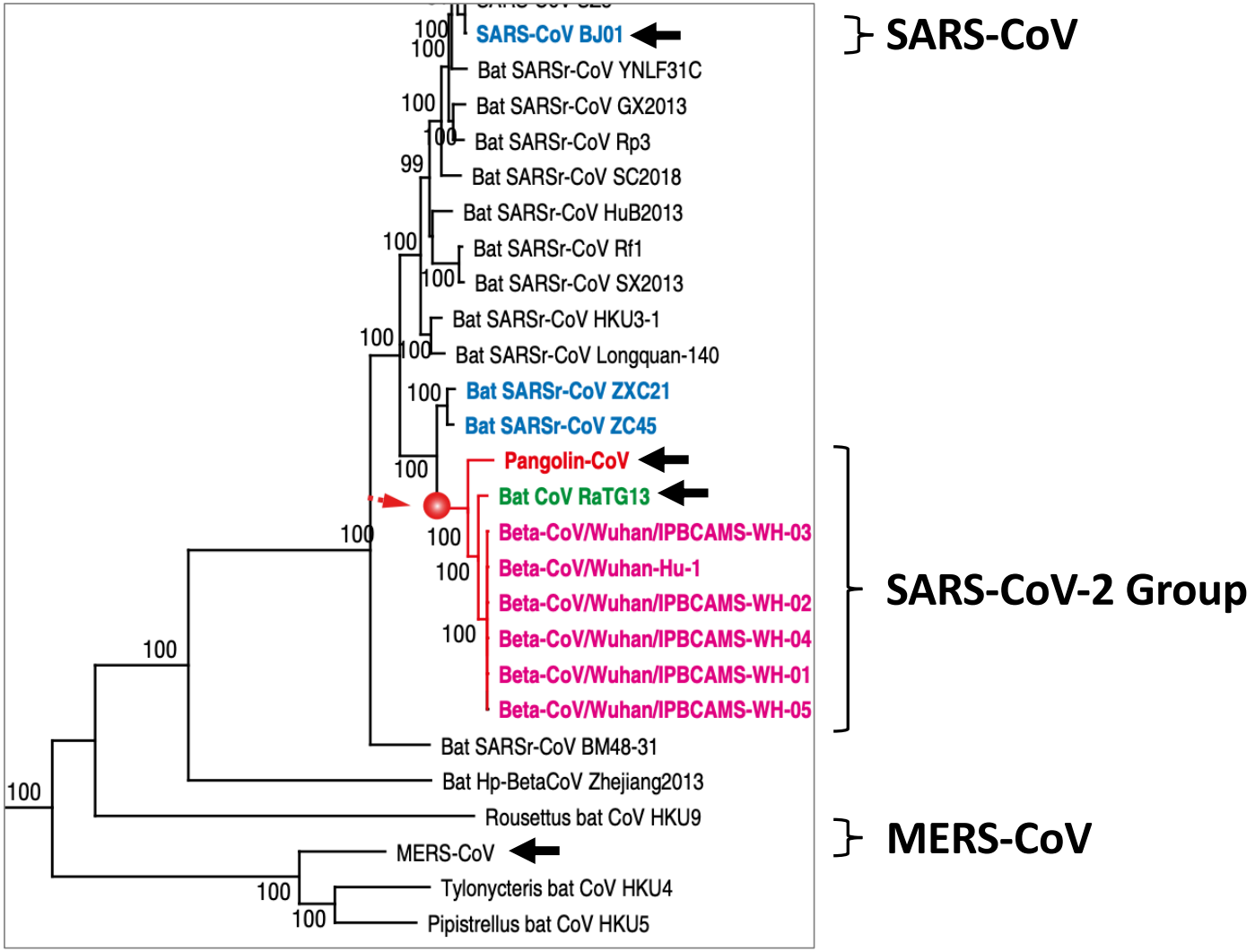
See: Andersen K et al. The Proximal Origin of SARSS-CoV-22. Nature Medicine, March 17

Viral phylogenetic data





Zhou, P et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579: 270-273 (2020).


SARS-CoV BJ01
Bat SARSr-CoV YNLF31C
Bat SARSr-CoV GX2013
Bat SARSr-CoV Rp3
Bat SARSr-CoV SC2018
Bat SARSr-CoV HuB2013
Bat SARSr-CoV Rf1
Bat SARSr-CoV SX2013
Bat SARSr-CoV HKU3-1
Bat SARSr-CoV Longquan-140
Bat SARSr-CoV ZXC21
Bat SARSr-CoV ZC45
Pangolin-CoV
Bat CoV RaTG13
Beta-CoV/Wuhan/IPBCAMS-WH-03
Beta-CoV/Wuhan-Hu-1
Beta-CoV/Wuhan/IPBCAMS-WH-02
Beta-CoV/Wuhan/IPBCAMS-WH-04
Beta-CoV/Wuhan/IPBCAMS-WH-01
Beta-CoV/Wuhan/IPBCAMS-WH-05
Bat SARSr-CoV BM48-31
Bat Hp-BetaCoV Zhejiang2013
Rousettus bat CoV HKU9
MERS-CoV
Tylonycteris bat CoV HKU4
Pipistrellus bat CoV HKU5
SARS-CoV
SARS-CoV-2 Group
MERS-CoV

Why Bats?

Ancient Mammalian Order (80-90 Mya)

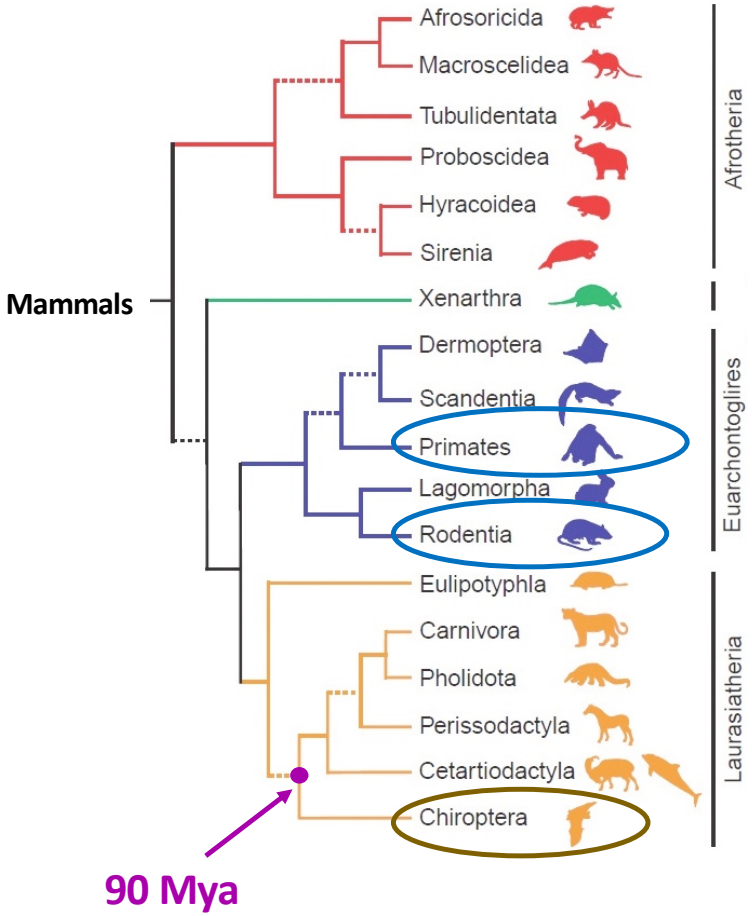
- Closer phylogenetically to horses, whales, carnivores

Recurrent reservoirs for severe, lethal human viral pathogens

- Lyssaviruses (Rabies)
- Paramyxoviruses (Nipah/Hendra)
- Filoviruses (Ebola/Marburg)
- Coronaviruses (SARS1, MERS, **SARS2**)

Why are these animals such prolific sources of viruses?

- Only mammals with powered flight (mobility/dispersal)
- Immensely abundant/diverse (>1,200 species; 20% of all mammalian species)
- Huge, dense colonies (millions in single roosts; up to thousands/m^2)
- Extreme longevity, often > 25 years, and accumulate viruses
- Dampened innate immunity (metabolic demands of flight, torpor)
  - Contracted interferon gene repertoire
  - Dampened NLRP3 inflammasome
  - Dampened viral DNA sensing (cGAS-STING)
- A picture of chronic virus tolerance, viremia, asymptomatic shedding



nature

Ge et al. **Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor.** Nature, 503:536-8 (2013).

2013

nature medicine

Menachery et al., **A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence.** Nature Medicine, 21:1508-13 (2015).

2015

PNAS

Menachery et al. **SARS-like WIV1-CoV poised for human emergence.** PNAS, 113:3048-53 (2016).

2016

Coronaviruses: Taxonomy

ssRNA genome

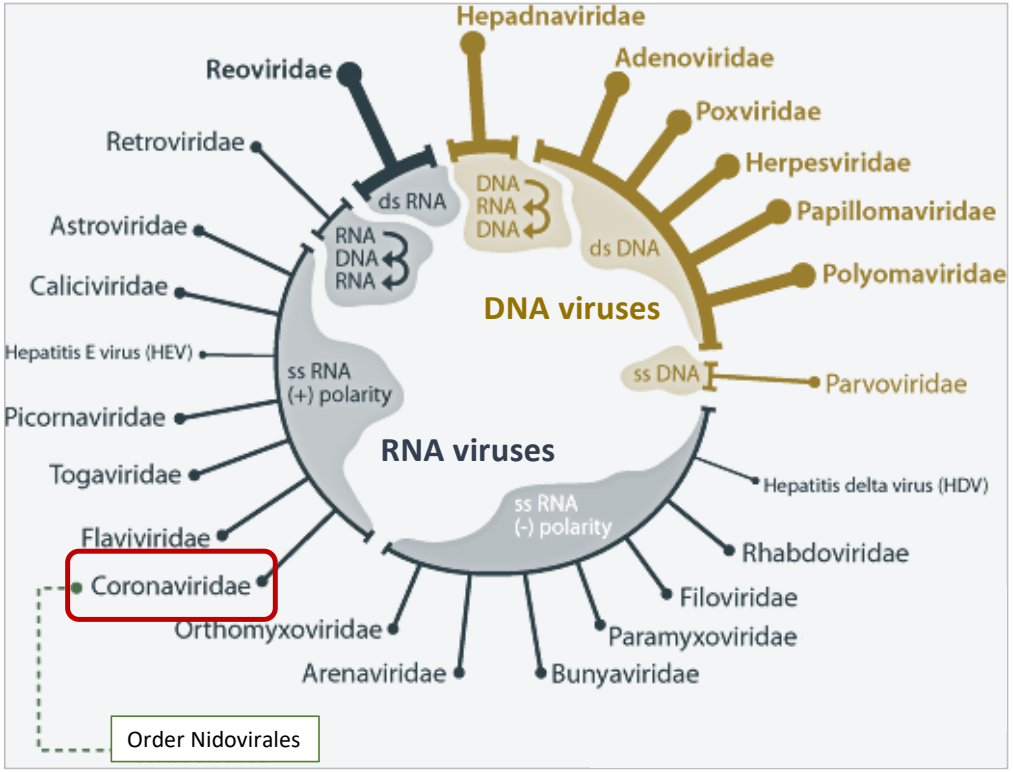
Largest genomes of all RNA viruses (~ 30 kilobases)

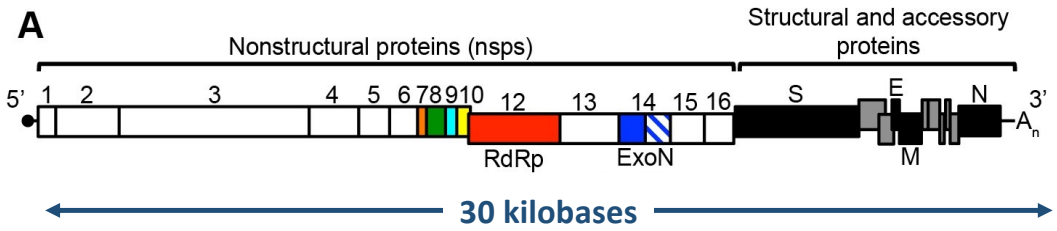
Order Nidovirales

Family Coronaviridae

SARS1/2 & MERS are beta-coronaviruses

SARS2: 27 proteins





SARS2 is the seventh coronavirus known to infect humans

Virus		Emergence	
1. CoV HKU1 2. CoV NL63 3. CoV OC43 4. CoV 229E	Seasonal viruses. About 8-15% of URIs, typically mild symptoms	Hundreds of years ago	
5. SARS1 6. MERS 7. SARS2	Can cause severe disease	17 years ago 7 years ago 0.25 years ago	Increase in the animal-human interface, accelerating cross-species movement in 21st century

→ SARS1 considered extinct in human populations, at least for now.
(Community transmission did not eventuate)

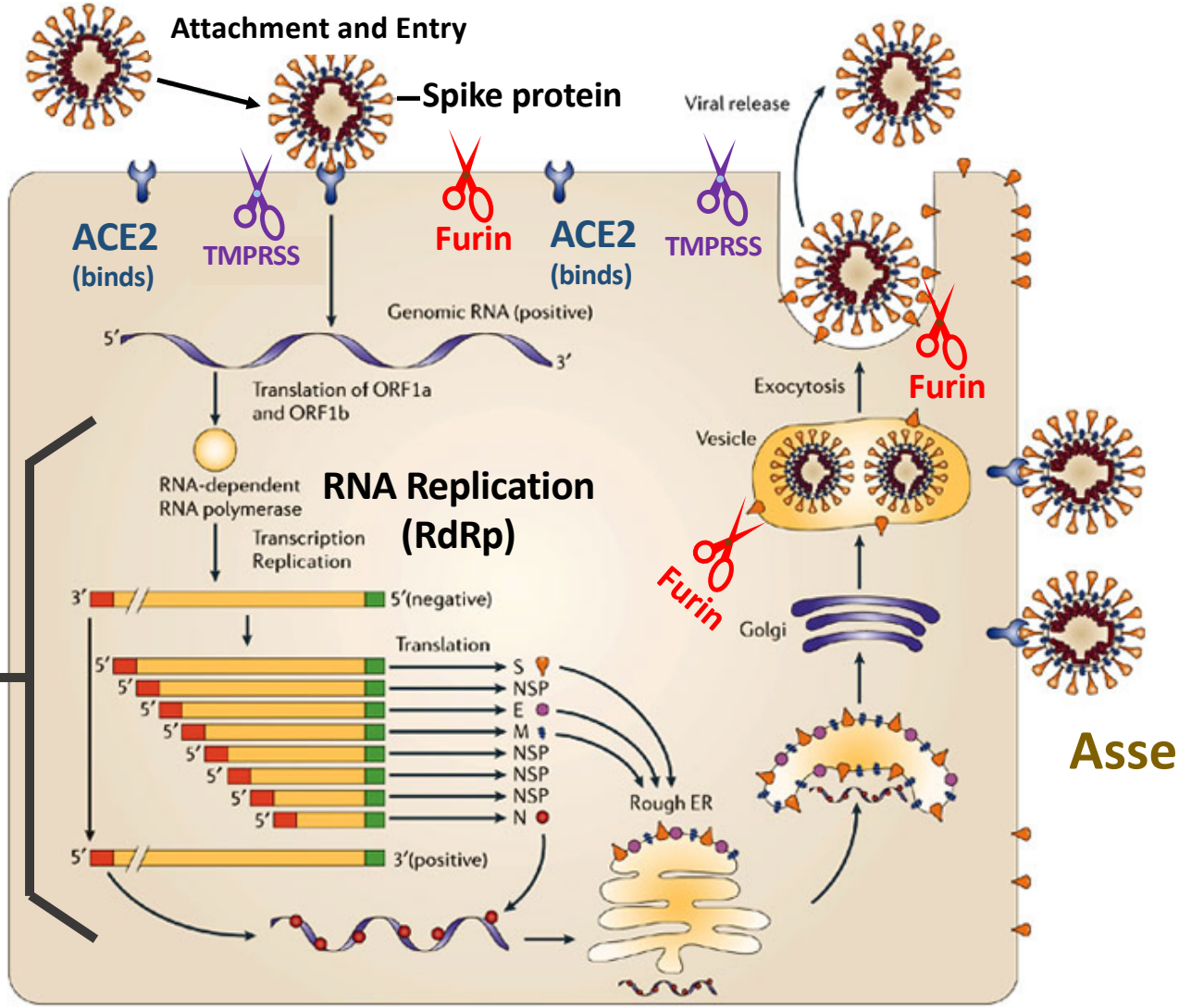
SARS1(2002-4)	SARS2 (2019-20)
• Most transmission in hospitals (hubs) • Transmits 24-36 hr after symptoms • Few asymptomatic cases	• Widespread community transmission • Many asymptomatic/mild cases. • More oropharyngeal virus than SARS1

See Wölfel et al. Virological assessment of hospitalized patients with COVID-2019. Nature, 4-1-2019.

SARS-CoV-2 Life Cycle

Entry process

Replication Factories



Assembly

Adapted from MHV replication cycle shown in Bergmann et al.. Nat Rev Microbiol, 4 (2), 121-32 (2006)

Poeschla 4-1-20

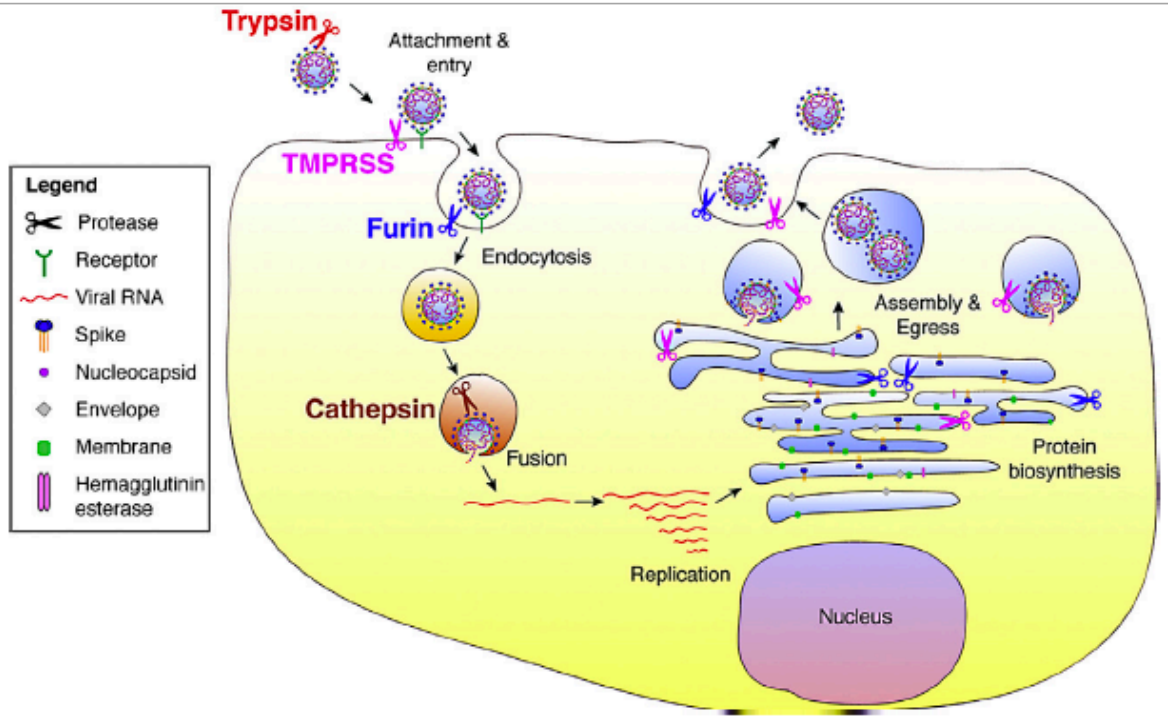
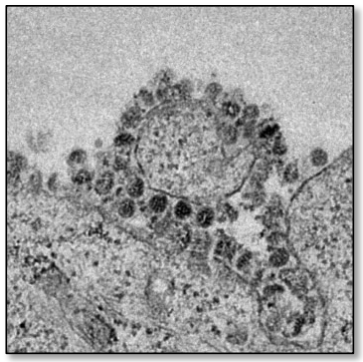
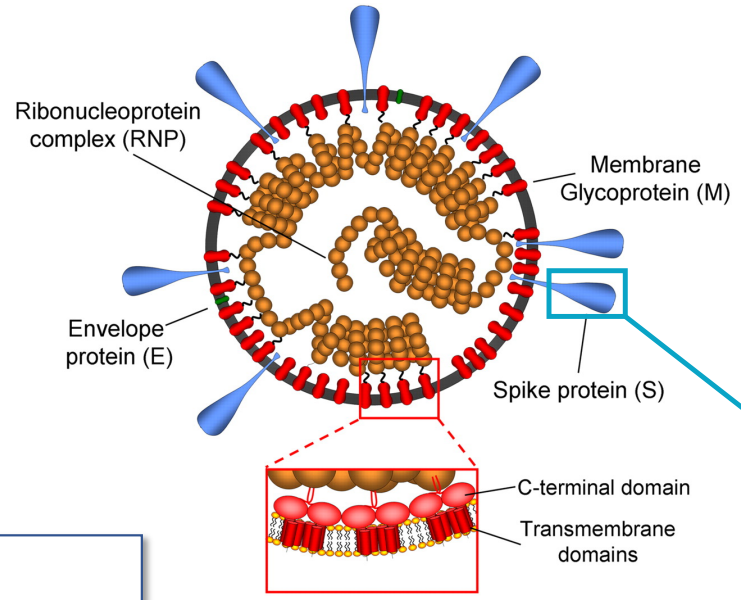


Fig. 2. Host cell proteases involved in activating the coronavirus spike (S) protein. (A) Schematic of a protease cleavage site and substrate binding pocket. The sites within the protease that accommodate substrate residues are designated with the letter S. The residues of the substrate protein involved in recognition and proteolytic processing are denoted with the letter P. The scissile bond is cleaved by the protease and the residues involved in this bond are denoted P1–P1′. (B) Structures of three common host cell proteases known to activate coronavirus S: crystal structures of trypsin (PDB: 2PTN), furin (PDB: 1P8J), and the pro-form of cathepsin L (PDB: 1CJL). (C) Diagram of a coronavirus life cycle and the various host cell proteases known to cleave and activate some coronavirus S proteins. Note that for certain coronaviruses, fusion can occur directly at the plasma membrane.

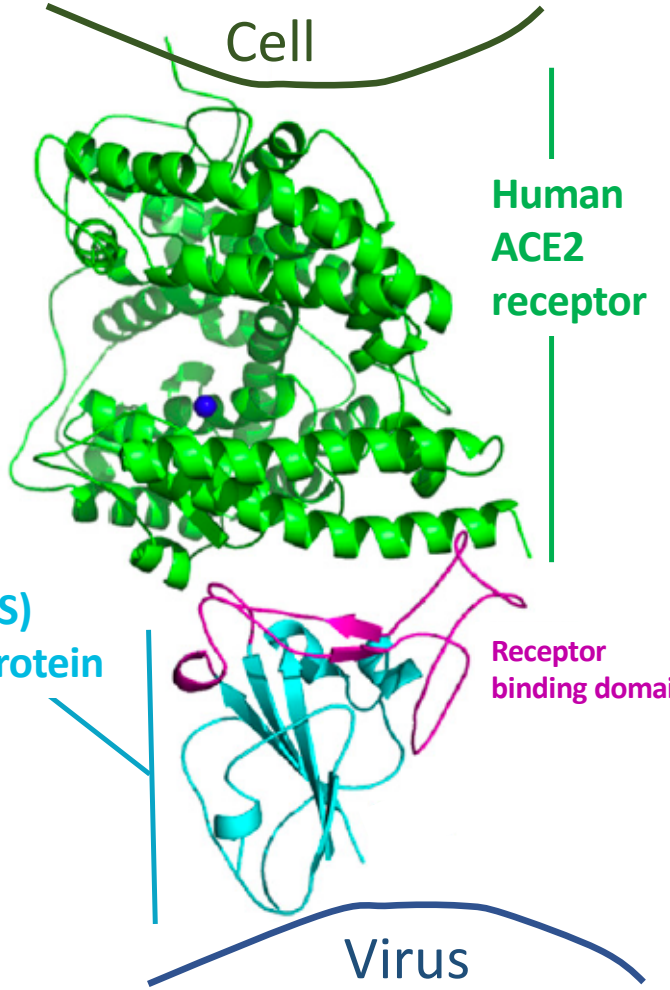
Millet and Whittaker, Virus Research, 2015. https://pubmed.ncbi.nlm.nih.gov/25445340/

The Virion and the Receptor





Barcena et al https://www.pnas.org/content/106/2/582.long



Physical properties

RNA virus; Enveloped (lipid shell)

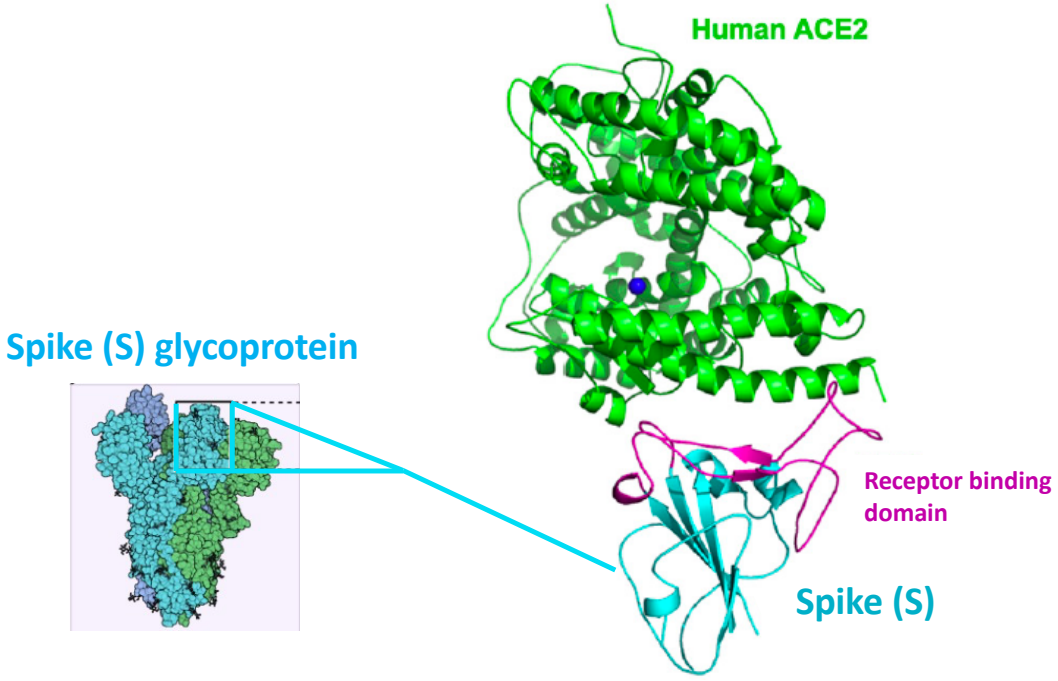
- Soaps kill
- Alcohols kill
- May survive on surfaces for days (esp., hard, smooth ones):

Shang et al. Structural basis of receptor recognition by SARS2. Nature, 30 March 2020.

Entry into the Target Cell -- two key processes (Spike attachment, Spike cleavage-activation):

1. Viral envelope spike protein **attaches** to the human protein **ACE2 (angiotensin converting enzyme 2)**

1. Coronavirus spike proteins are cleaved and then activated during biogenesis *or* entry by cellular enzymes (e.g., **TMPRSS**) to orchestrate their fusion capacity (S1/S2 and S' sites respectively). SARS2 has acquired a polybasic motif for cleavage at S1/S2 by **Furin.** SARS1 lacked this.



Clinical implications:

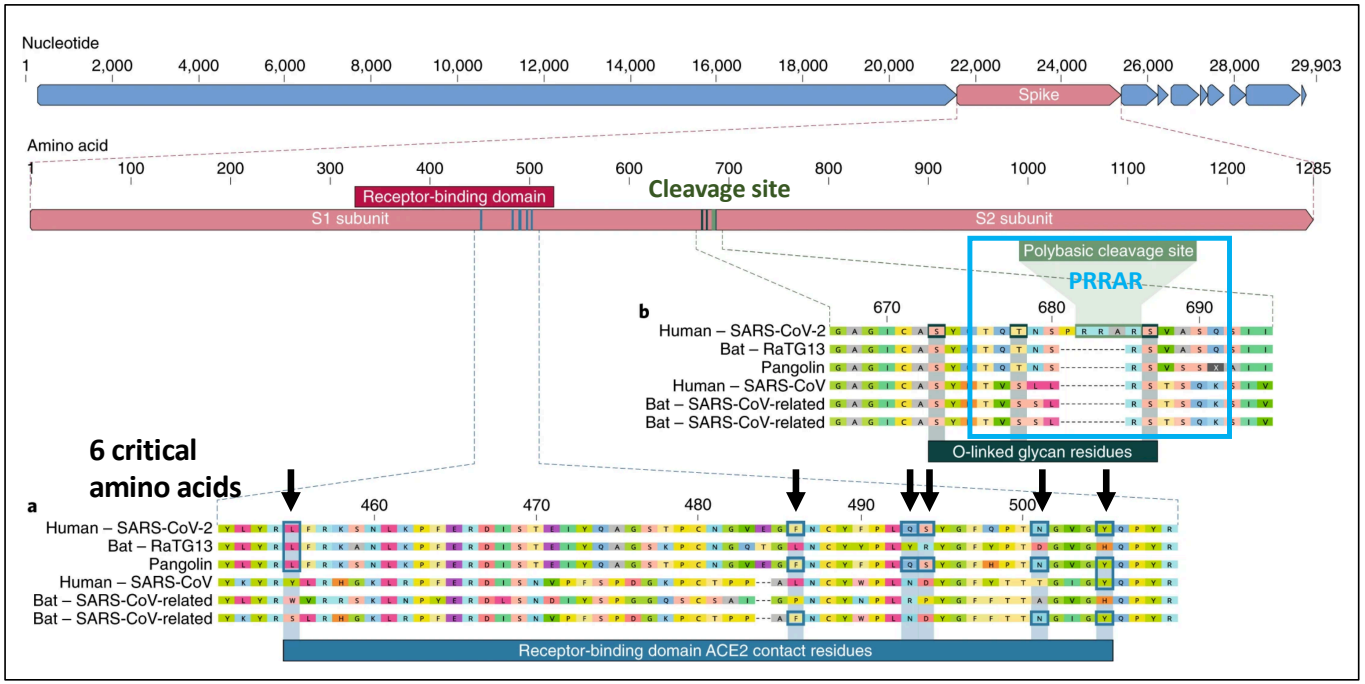
- Spike protein is a leading **vaccine target.**
- The ACE2 receptor is present on endovascular cells and cardiac myocytes in addition to lung cells. And a main clinical issue arising now is viral myocarditis.
- **ACE inhibitors** & **Angiotensin Receptor Blockers** elevate levels of **ACE2.**
  → Could be bad, good, or neutral.

  Renin-angiotensin-aldosterone Inhibitors in patients with COVID-9. *New Eng J Med*, 3-31-2020)
- Furin present in Golgi and at cell surface of cells in many tissues.

Two very interesting things happened on the way to SARS2

1. The affinity for ACE2 of the SARS2 Spike protein is **10-fold higher** than the SARS1 spike. **Six critical amino acids** mediate these virus's ACE2 receptor attachment, and **five of them** are different from SARS1. But **all 6 are identical** in the pangolin virus and SARS2.

2. On the way from bats, the SARS2 Spike also acquired an insertion of a new **polybasic cleavage site** for the cellular protease (convertase) **furin**, which other related beta-coronaviruses, such as the RaTG13, pangolin ones, etc., do not have:



Andersen K et al. The Proximal Origin of SARS-CoV-2. Nature Medicine, March 17

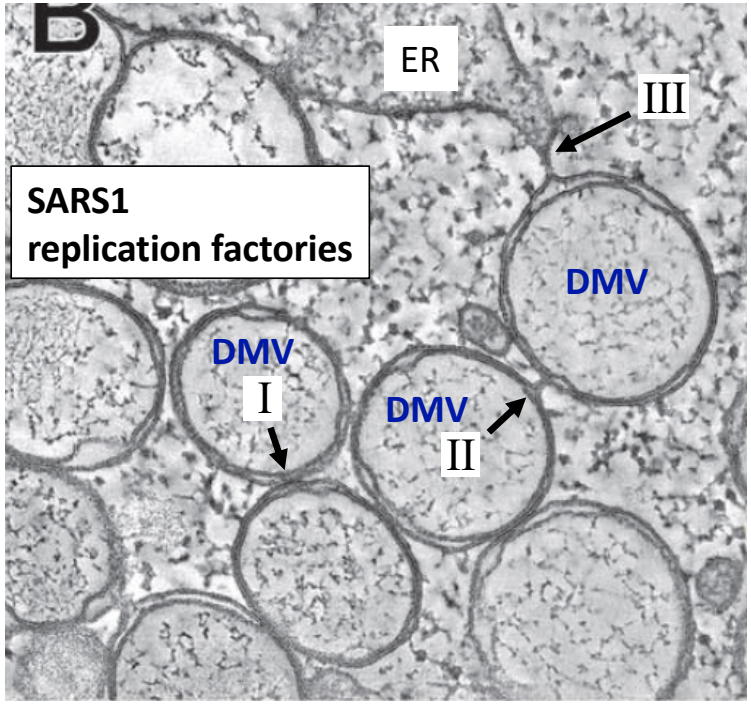
Importance/Hypotheses Data Raise:

- Both things may have facilitated human emergence and spread.
- The RBD six amino acids likely arose prior to move to humans; the furin site perhaps after it, during human-human transmission. [Tentative but reasonable inferences at present].
- Insertion of this kind of **furin activation site** has been shown to increase transmissibility of other respiratory viruses, particularly highly pathogenic influenza viruses. Function needs exp. testing however.
- Predicted are 3 O-linked glycosylation sites. Speculative: could contribute to immuno-evading glycan shield.
- Furin is very abundant in lungs, & other organs, so might could perhaps influence tissue tropism/invasion.

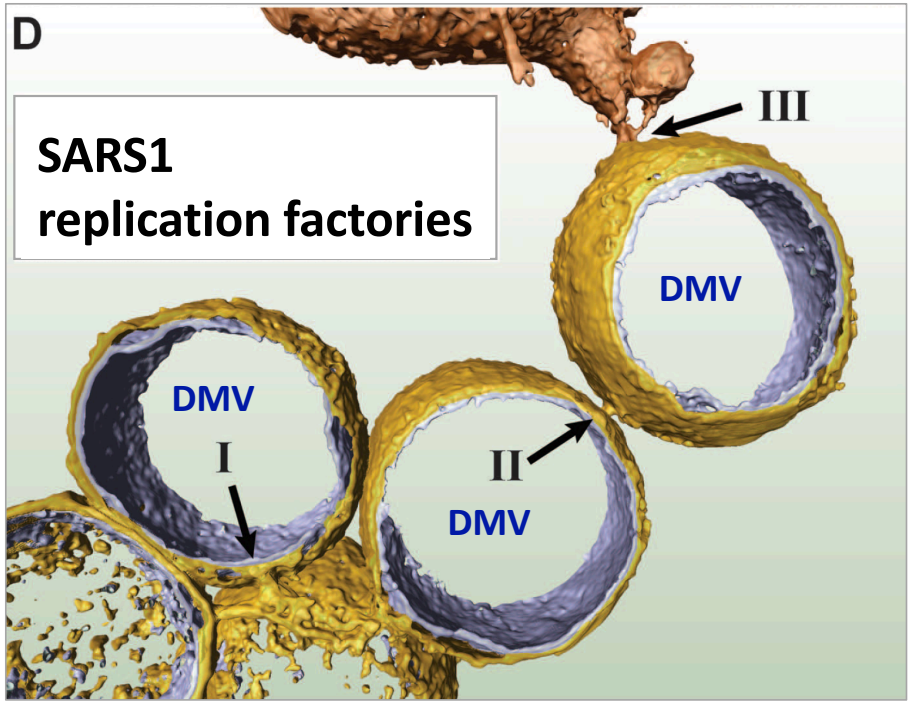
See also: Coutard et al. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Research (2020).

Stealth: How the virus hides while replicating furiously inside a patient's cells

- It sequesters RNA genome amplification inside **"replication factories"** it derives from intracellular membranes.
- This can limit detection by key **cellular warning systems** that detect viral dsRNA intermediates (e.g., MDA5).



DMVs: double membrane vesicles



Electron tomographic 3-D surface model

Knoops et al. PLoS Biology 2008

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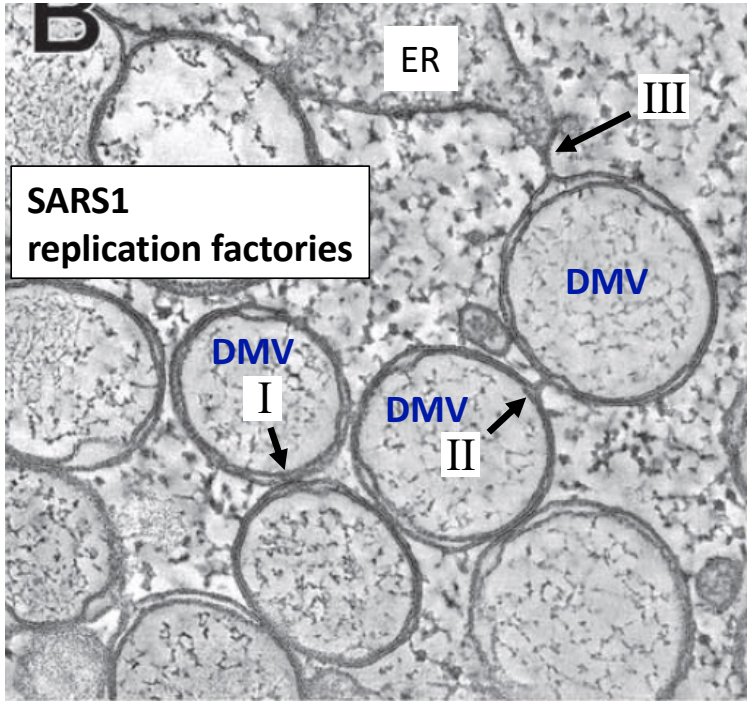

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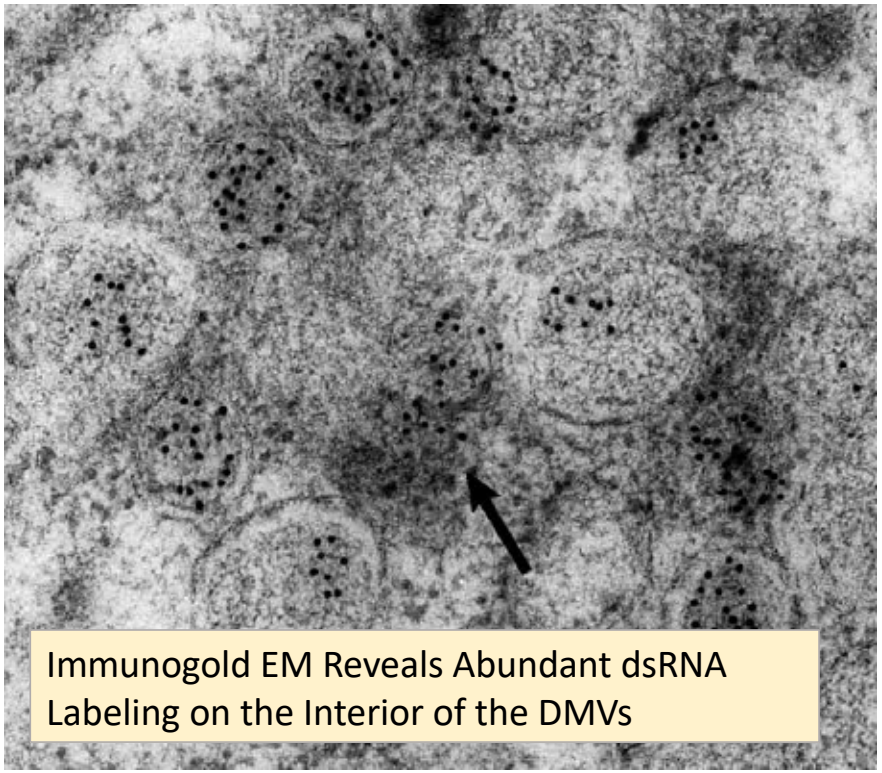
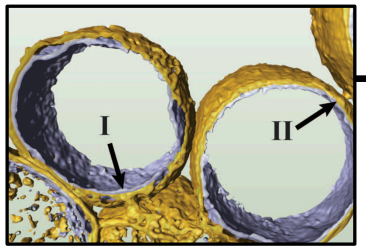
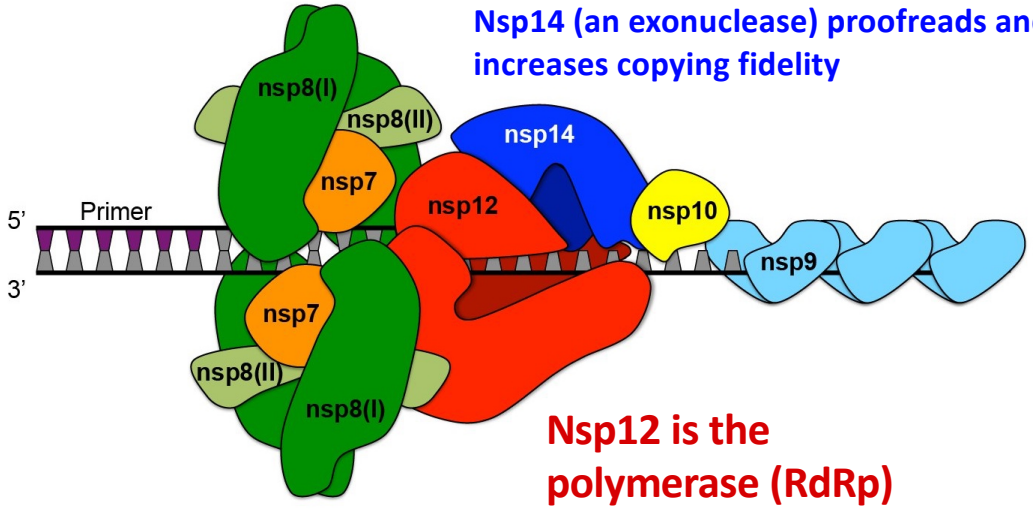


Knoops et al. PLoS Biology 2008



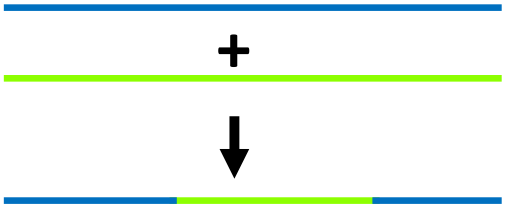
Inside those replication factories, the RNA genome is replicated by a viral 'holoenzyme' (Nsp7, Nsp 8, Nsp12, Nsp14 work together):

- **Polymerase inhibited by remdesivir** (chain-terminator; IC_{50} 0.7 µM in primary cells).
- **Remdesivir more potent when the proofreading exonuclease Nsp14 is inactive. Drug target for combination therapy?**

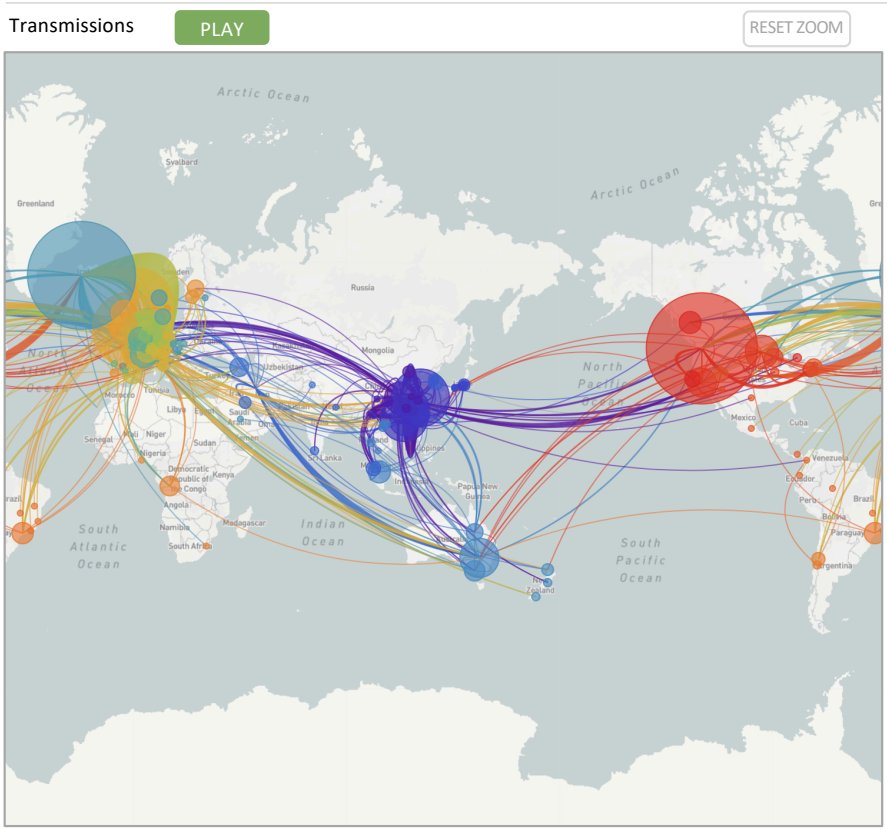


When two different coronaviruses co-infect the same animal or person and hence get produced inside the same cell, there are high rates of <u>recombination</u> (RNA genome segment swapping)

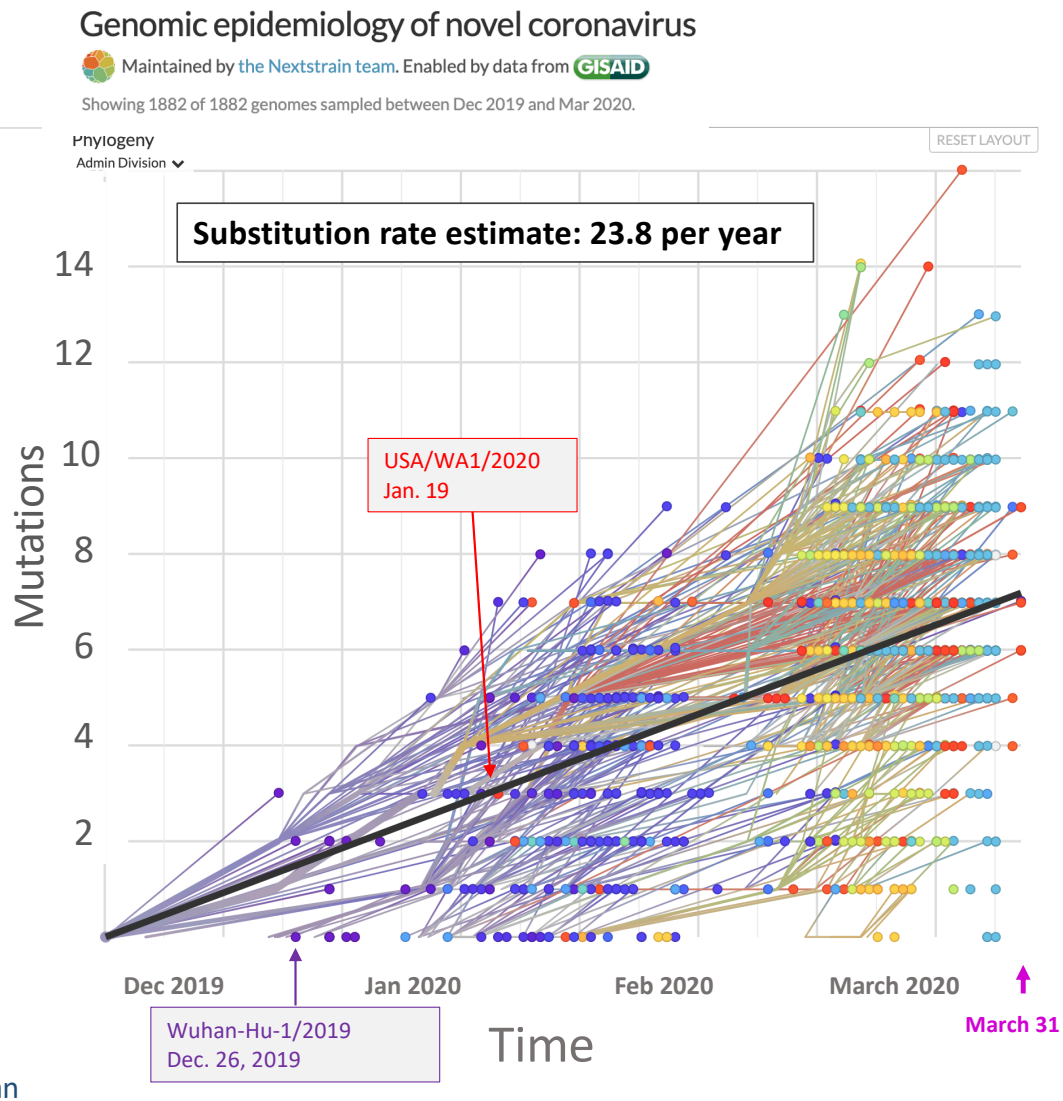
- **25% during mixed infections**
- **Possibly operative in origin of SARS-CoV-2**



Is SARS-CoV-2 changing?



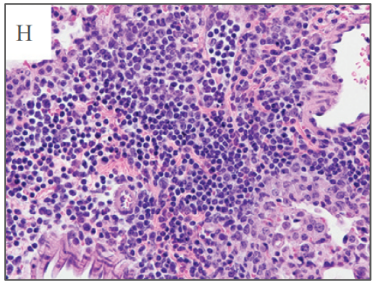
- RNA viruses generate quasispecies (diverse swarms of viruses).
- At present, there are no data to indicate evolution to greater or lesser virulence is particularly likely.
- SARS2 is already highly adapted & spreading efficiently by using the human respiratory tract as an aerosolization device.



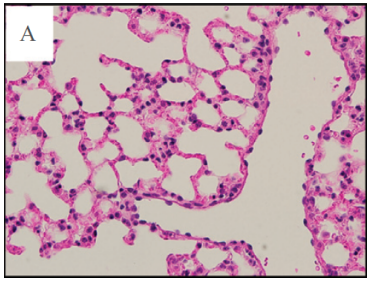
Emerging concept in our hospital & across the world: A dysregulated, over-exuberant immune response contributes to COVID-19 pathology

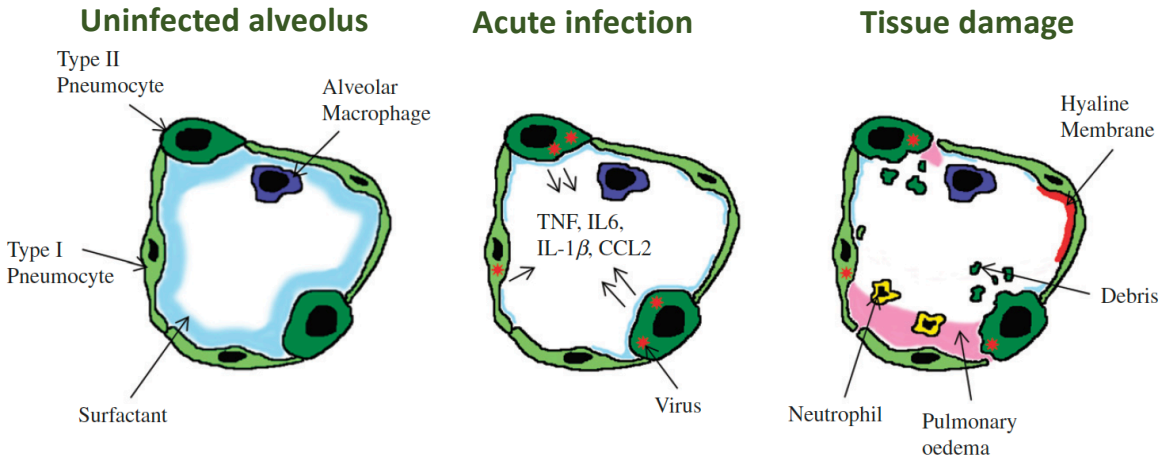
(IL6, TNF, IL-1beta, CCL2, etc.)

Mouse lung inflammation 7 days after **SARS1** infection



Mock-infected animal's lung



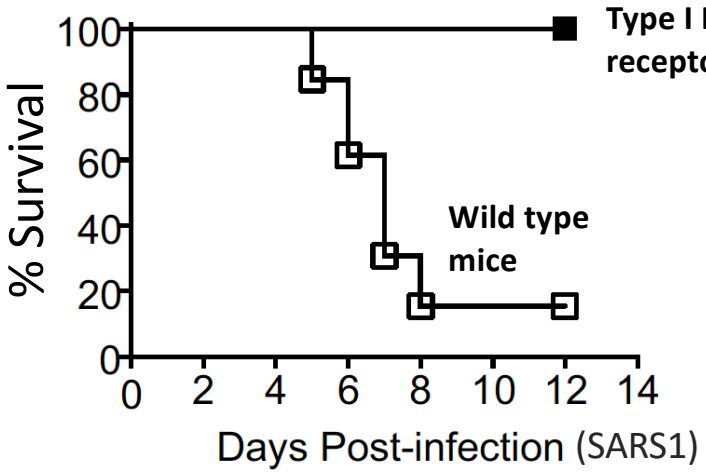


Journal of Pathology, 235: 185–195 (2015)

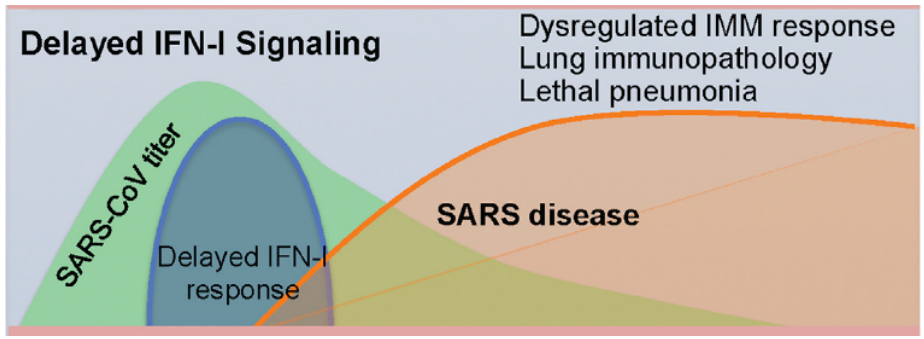
Candidate immunosuppressive agents: Hydroxychloroquine, IL6 Receptor mAbs (tocizilumab, sarilumab), corticosteroids, ...

Immunopathogenesis

Interferon (IFN) receptor knockout mice survive SARS1 infection better than WT mice.



Channappanavar et al., *Cell Host & Microbe* (2016)

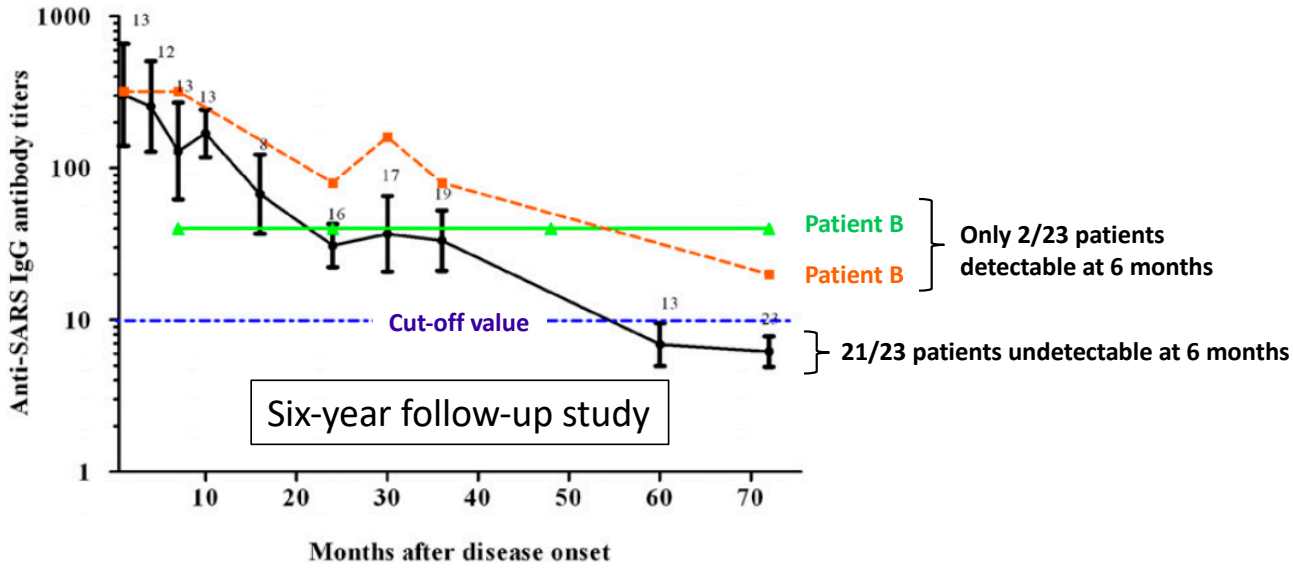


***And, importantly, WT & KO mouse virus titers in lung tissue were the same.**

IFN might possibly help very early on, but when late or sustained, it promotes accumulation of pathogenic monocyte-macrophages → lung immunopathology, vascular leak, and suboptimal T cell responses

Adaptive immune system: issues for antibody responses and durable individual/herd immunity (data from SARS1 patients)

Waning of Peripheral Memory B Cell Responses in Recovered Human SARS1 Patients



- **Peripheral memory B cell responses were undetectable in 21/23 recovered SARS patients**
- **IgG may eventually vanish**
- **In contrast, T cell memory responses maintained for at least 6 years**
- **Implications for vaccines, and for convalescent plasma therapy.**

Tang F, et al. Lack of Peripheral Memory B Cell Responses in Recovered Patients with Severe Acute Respiratory Syndrome: A Six-Year Follow-Up Study. *Journal of Immunology*, 2011.

Clinical Virology of Transmission

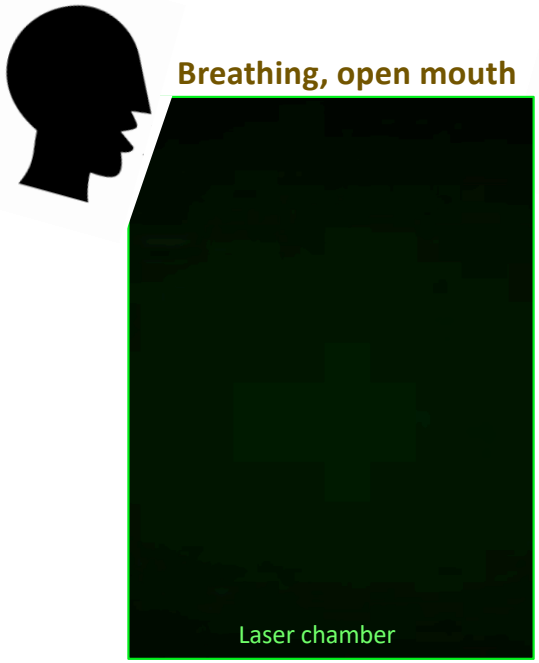
Just Speaking = Exchanging Saliva

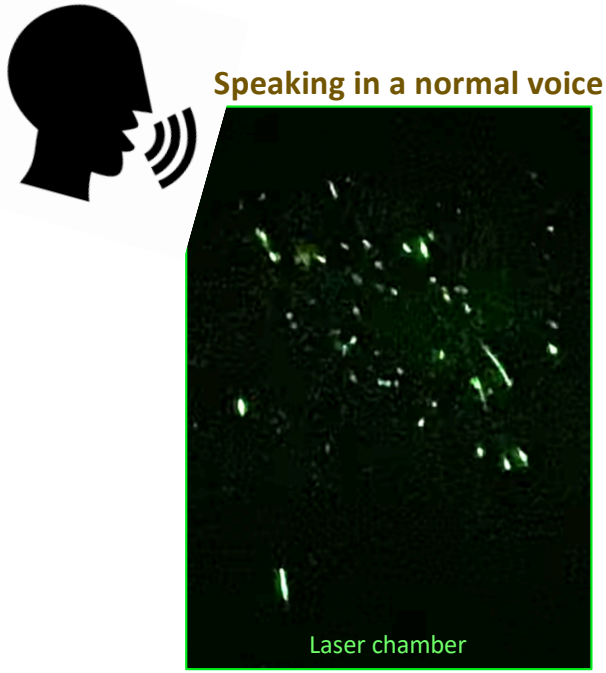
Main value of paper masks is to prevent outward transmission to others.

https://www.youtube.com/watch?v=qzARpgx8cvE&feature=youtu.be

"You've seen the evidence. Now we know. Let's talk virtually and not face to face, and we'll put this crisis behind us."

-- Harold Varmus (Nobel, 1989, Virology)





I acknowledge:

- My amazing colleagues in our Infectious Disease Division, who are going above and beyond in countless ways.
- Everyone else across the city who is contributing all that they are.
- A special tribute to our nurses.
- My lab members, who know something about viruses (and bats).
- Many researchers who provided fast data/info on preprint servers and elsewhere:
  - Glaunsinger B. https://www.youtube.com/watch?time_continue=97&v=8_bOhZd6ieM&feature=emb_logo
  - Baric R. https://special.croi.capitalreach.com/)