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# Know your enemy and know yourself – the case of SARS-CoV-2 host factors

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the etiologic agent that causes Coronavirus Disease 2019 (COVID-19) pandemic, is a newly emerging respiratory RNA virus with exceptional transmissibility and pathogenicity. Numerous COVID-19 related studies have been fast-tracked, with the ultimate goal to end the pandemic. Here we review the major stages of SARS-CoV-2 infection cycle in cells, with specific emphasis on essential host factors. Insights into the cell biology of SARS-CoV-2 infection have accelerated the development of host-directed therapeutics, as shown by dozens of clinical trials evaluating COVID-19 treatments using host-targeting compounds.

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## Introduction

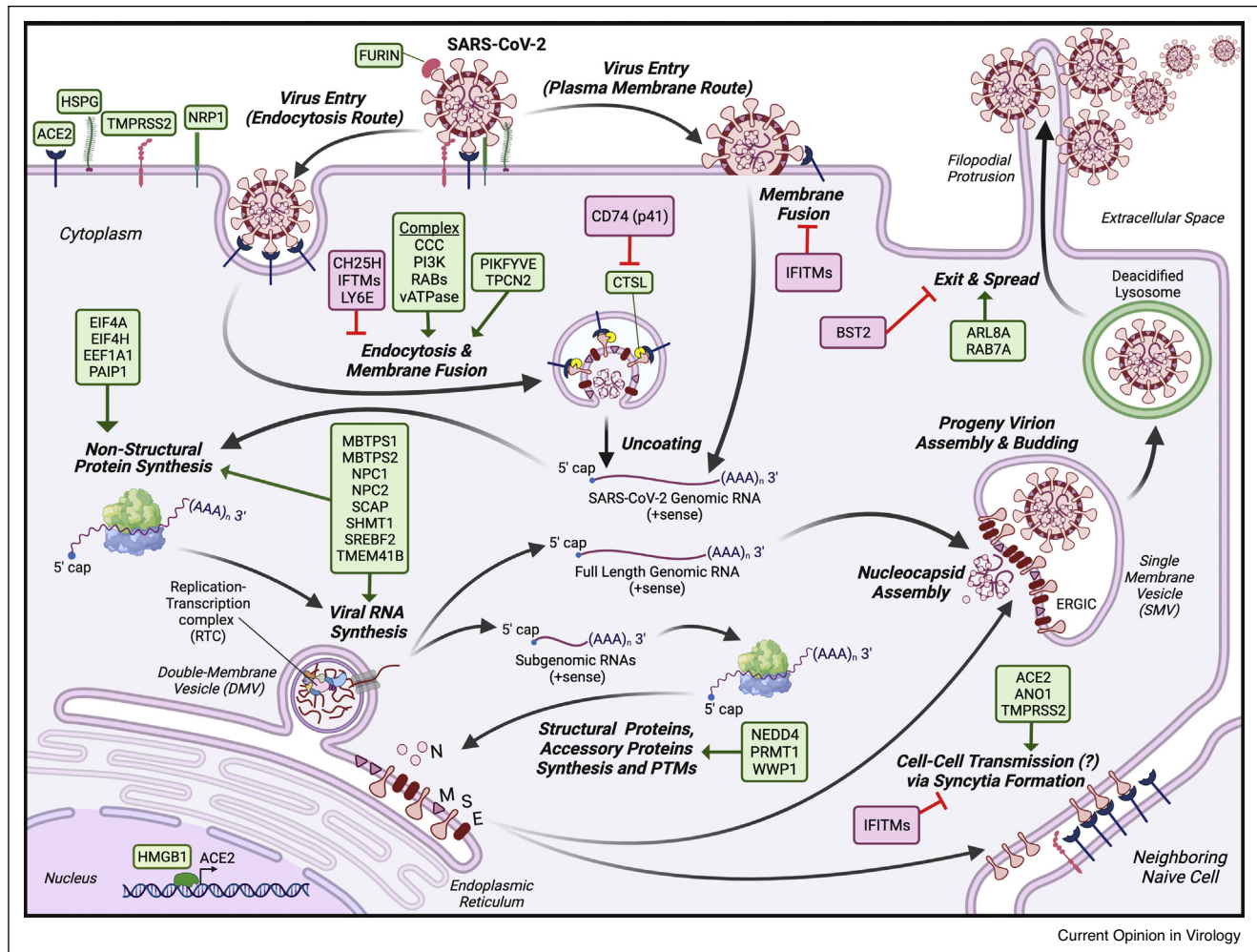
COVID-19 is one of the most grievous pandemics of modern human history. As of 20 months post-outbreak, SARS-CoV-2 has globally infected more than 200 million people causing over 4 million deaths (<https://coronavirus.jhu.edu/map.html>). A few vaccines have been authorized for emergency usage, but these efforts have yet to contain the pandemic due to limited productions and distributions [1–3]. COVID-19 research does not seem to be impacted by the stringent lockdowns and restrictions, instead, it has been conducted at an extraordinary pace to demystify the molecular biology of SARS-CoV-2 infection and pathogenesis. In this review, we summarize recently uncovered host factors critical for the various stages of the SARS-CoV-2 infection cycle (Figure 1). We further highlight promising druggable targets.

## SARS-CoV-2 entry

Similar to SARS-CoV-1 [4], SARS-CoV-2 binds to the cognate proteinaceous receptor, Angiotensin Converting Enzyme 2 (ACE2) at the plasma membrane of permissive cells using the Spike (S) protein [5<sup>\*</sup>,6<sup>\*\*</sup>,7<sup>\*\*</sup>,8]. ACE2 is a membrane-bound dipeptidyl carboxypeptidase that may play a physiological role in the regulation of cardiovascular and renal function [9,10]. The S protein comprises two major subunits, S1 and S2, and the former subunit interacts with ACE2 via a receptor-binding domain (RBD) to mediate the attachment process. ACE2 is the key receptor but not the sole factor facilitating SARS-CoV-2 entry (reviewed in Ref. [11]). A few other cell surface factors have been shown to promote the virus entry such as the heparan sulfate proteoglycans (HSPGs) — common attachment factors for many viruses [12<sup>\*\*</sup>]. The S protein’s RBD can directly interact with heparan sulfate to promote the viral attachment, also likely enhance interaction between the S protein and ACE2 [12<sup>\*\*</sup>]. Multiple genes involved in the heparan sulfate biosynthesis pathway were implicated in functional genomic screens for SARS-CoV-2, further strengthening the role of heparan sulfate in enhancing SARS-CoV-2 infection [13<sup>\*</sup>,14,15<sup>\*</sup>,16]. Additionally, a few other host proteins such as a secreted isoform of ACE2, AXL Receptor Tyrosine Kinase (AXL, a receptor tyrosine kinase), Basigin (Ok Blood Group) (BSG, a.k.a. CD147, a ubiquitously expressed multifunctional plasma membrane protein), Scavenger Receptor Class B Member 1 (SCARB1, a receptor for high-density lipoprotein cholesterol), and Transmembrane Protein 106B (TMEM106B), have been reported to promote SARS-CoV-2 entry [16–20]. Intriguingly, enhancement of virus entry could be accomplished without ACE2, suggesting ACE2 may not be the only host factor determining tissue tropism for SARS-CoV-2 [18]. Some cellular factors, such as High Mobility Group Box 1 (HMGB1), a multifunctional DNA-binding nuclear protein, have been shown to indirectly impact SARS-CoV-2 entry by regulating the cell surface expression of ACE2 [21<sup>\*</sup>].

Upon binding to ACE2, the S1–S2 subunits dissociate. The S1–S2 dissociation involves proteolytic cleavage by a ubiquitously expressed serine endoprotease furin (FURIN) at the S1–S2 junction within a multibasic motif, ‘Arg-Arg-Ala-Arg’ (RRAR) [22<sup>\*</sup>,23<sup>\*\*</sup>]. The multibasic cleavage motif is critical for SARS-CoV-2 to infect human airway cells, and it can be a contributing factor for SARS-CoV-2 tropism and transmissibility [24–29,30<sup>\*</sup>,31<sup>\*</sup>].

Figure 1



### The SARS-CoV-2 infection cycle.

Upon attachment to human cells, SARS-CoV-2 can fuse at the plasma membrane or internalize and later fuse in the endosome. Uncoated viral RNA genomes are rapidly translated into non-structural proteins to facilitate the full-length and subgenomic viral RNA syntheses. Structural and accessory proteins are translated from the subgenomic RNA transcripts to support the assembly and budding of progeny virions. During egress, SARS-CoV-2 progeny virions hijack a non-canonical lysosomal exocytosis pathway to exit cells. SARS-CoV-2 infected cells can fuse with neighboring naïve cells via syncytia formation.

Abbreviation: Angiotensin Converting Enzyme 2 (ACE2); Heparan sulfate proteoglycans (HSPGs); Transmembrane Serine Protease 2 (TMPRSS2); Neuropilin 1 (NRP1); Furin (FURIN); Cholesterol 25-Hydroxylase (CH25H); Interferon-induced Transmembrane proteins (IFITMs); Lymphocyte Antigen 6 Family Member E (LY6E); COMMD/CCDC22/CCDC93 Complex (CCC); Class III Phosphatidylinositol 3-kinase Complex (PI3K); RAB GTPases; vacuolar ATPase (vATPase) complex; Phosphoinositide Kinase, FYVE-Type Zinc Finger Containing (PIKFYVE); Two Pore Segment Channel 2 (TPCN2); Cathepsin L (CTSL); p41 isoform of the CD74 protein (CD74(p41)); Eukaryotic Translation Initiation Factor 4A1 (EIF4A); Eukaryotic Translation Initiation Factor 4H (EIF4H); Eukaryotic Translation Elongation Factor 1 Alpha 1 (EEF1A1); Poly(A) Binding Protein Interacting Protein 1 (PAIP1); Membrane Bound Transcription Factor Peptidase, Site 1 (MBTPS1); Membrane Bound Transcription Factor Peptidase, Site 2 (MBTPS2); NPC Intracellular Cholesterol Transporter 1 (NPC1); NPC Intracellular Cholesterol Transporter 2 (NPC2); SREBF Chaperone (SCAP); Serine Hydroxymethyltransferase 1 (SHMT1); Sterol Regulatory Element Binding Transcription Factor 2 (SREBF2); Transmembrane Protein 41B (TMEM41B); High Mobility Group Box 1 (HMGB1); NEDD4 E3 Ubiquitin Protein Ligase (NEDD4); WW Domain Containing E3 Ubiquitin Protein Ligase 1 (WWP1); Protein Arginine Methyltransferase 1 (PRMT1); Anoctamin 1 (ANO1); ADP Ribosylation Factor Like GTPase 8A (ARL8A); RAB7A, Member RAS Oncogene Family (RAB7A); Tetherin (BST2). Host dependency factors are boxed in green, and host restriction factors are boxed in red. Lipid bilayer membrane is indicated by double lines in purple. Deacidified lysosome is indicated by double lines in green.

However, it remains unclear whether furin is indispensable during the SARS-CoV-2 entry, as other alternative proteases may be able to cleave the S1–S2 junction in the absence of furin [27–29]. A few recent reports

demonstrated that the untied S1 subunit could bind to Neuropilin 1 (NRP1), a cell surface receptor that recognizes and binds CendR motif, ‘Arg/Lys-X-X-Arg/Lys’ (R/KXXR/K, where X indicates any amino acid),

containing ligands [32–34]. The interaction between NRP1 and the S1 subunit can promote SARS-CoV-2 infection, probably by enhancing the virus internalization process [32,33]. Similar to SARS-CoV-1, the S2 subunit is further processed by either a transmembrane serine protease, TMPRSS2, or lysosomal cysteine proteinases (cathepsins, such as CTSL) to trigger final conformational changes of the S2 subunit, exposing and inserting the viral fusion peptide into the cellular membrane. As a result, the viral membrane fuse with the host membrane to deposit the viral genome into the cytoplasm [29]. Currently, TMPRSS2 inhibitors, such as Camostat Mesylate and Nafamostat Mesylate, are being tested in over a dozen of clinical trials (Table 1).

Since proteolytic maturation of the S protein is a prerequisite for the virus entry, the entry site of SARS-CoV-2 is dictated by the subcellular localization of the host proteases. Thus, SARS-CoV-2 can enter cells through two main entry routes: fusing directly at the cell membrane after binding to ACE2, or entering cell through receptor-mediated endocytosis and then fusing to endosomal membrane. In cells with surface expression of ACE2

and TMPRSS2, such as human airway epithelial cells, SARS-CoV-2 readily binds and fuses at the plasma membrane [7<sup>\*\*</sup>,35,36]. Interestingly, other TMPRSS2-like proteases such as TMPRSS4 and TMPRSS13 may similarly promote the S protein processing [36,37]. Alternatively, the virus can first enter host cells via receptor-mediated endocytosis and subsequently fuses with the endosomal membrane. This entry route allows the virus to access cathepsins in the endosomes, especially when TMPRSS2 is absent from the cell surface [38].

Cathepsin L (CTSL) is the principal endosomal protease responsible for priming the S protein in endosomes. It was observed that a higher level of circulating CTSL in the blood is associated with severe disease in COVID-19 patients [39]. Intriguingly, pharmacological inhibition of CTSL, but not Cathepsin B (CTSB), can reduce SARS-CoV-2 infection *in vitro* [8,38]. Some other proteases such as Trypsin and Human Airway Trypsin-Like Protease (TMPRSS11D, a.k.a. HAT) have been proposed to play a similar role in the *in vitro* processing of the S protein [8,27,36]. Notably, the p41 isoform of the CD74 protein (a chaperone that regulates MHC class II

Table 1

Examples of host-directed therapeutics against COVID-19 undergoing clinical trials(<https://clinicaltrials.gov/ct2/home>)

Drug	Pharmacological actions	Targeting host factors	Stage of SARS-CoV-2 infection cycle	Clinical trials	Phase	Reference
Apilimod	Inhibitor	PIKFYVE	Entry	NCT04446377	2	[8,74,117*,118]
Camostat mesylate	Inhibitor	TMPRSS2	Entry	NCT04353284	2	[36,119]
				NCT04583592	2	
				NCT04608266	3	
				NCT04524663	2	
				NCT04657497	3	
				NCT04681430	2	
				NCT04470544	2	
Nafamostat mesylate	Inhibitor	TMPRSS2	Entry	NCT04625114	2	[120–122]
				NCT04352400	2, 3	
				NCT04390594	3	
				NCT04418128	2, 3	
				NCT04473053	2, 3	
				NCT04623021	2	
				NCT04628143	2	
Niclosamide	Inhibitor	ANO1	SARS-CoV-2 induced syncytia and spread (?)	NCT04399356	2	[114*,123]
				NCT04603924	2, 3	
				NCT04858425	2	
				NCT04753619	2	
				NCT04558021	3	
				NCT04870333	2, 3	
Plitidepsin	Inhibitor	EEF1A	Translation	NCT04750759	2	[59]
				NCT04382066	1	
Statin	Inhibitor	HMGCR	Multiple	NCT04784559	3	[124–127]
				NCT04486508	3	
				NCT04904536	3	
				NCT04472611	3	
Zotatifin	Inhibitor	EIF4A	Translation	NCT04380402	2	[58**,128,129]
				NCT04632381	1	



antigen processing) can hinder CTSL-mediated activation of the S protein and induce resistance in human cells by arresting viral fusion inside endosomes [40]. In addition to the host proteases, SARS-CoV-2 membrane fusion in endosomes requires a low pH, as neutralization of acidic endosomes inhibits SARS-CoV-2 infection in specific cell types [7\*\*,8,38,39,41]. The impact of the endosomal pH neutralization can be reversed by overexpressing TMPRSS2, supporting the model that SARS-CoV-2 can penetrate the cellular membrane barrier either at the plasma membrane or in the endosome [7\*\*,38]. Some host factors known to play a role in endocytosis and endosomal trafficking pathways, such as subcomponents of COMMD/CCDC22/CCDC93 Complex, Class III Phosphatidylinositol 3-kinase (PI3K) Complex, RAB GTPases, and vacuolar ATPase (vATPase) complex, have been reported to promote SARS-CoV-2 infection [13\*,20,42\*,43,44]. Chemical perturbations of Two Pore Segment Channel 2 (TPCN2, a nicotinic acid adenine dinucleotide phosphate receptor), and its upstream regulator PIKFYVE, a phosphoinositide kinase that recruits protein complexes to cell- and endosomal-membrane, have been shown to potently block virus entry [8]. PIKFYVE functions can be chemically perturbed using Apilimod — a candidate therapeutic for treating COVID-19 (Table 1).

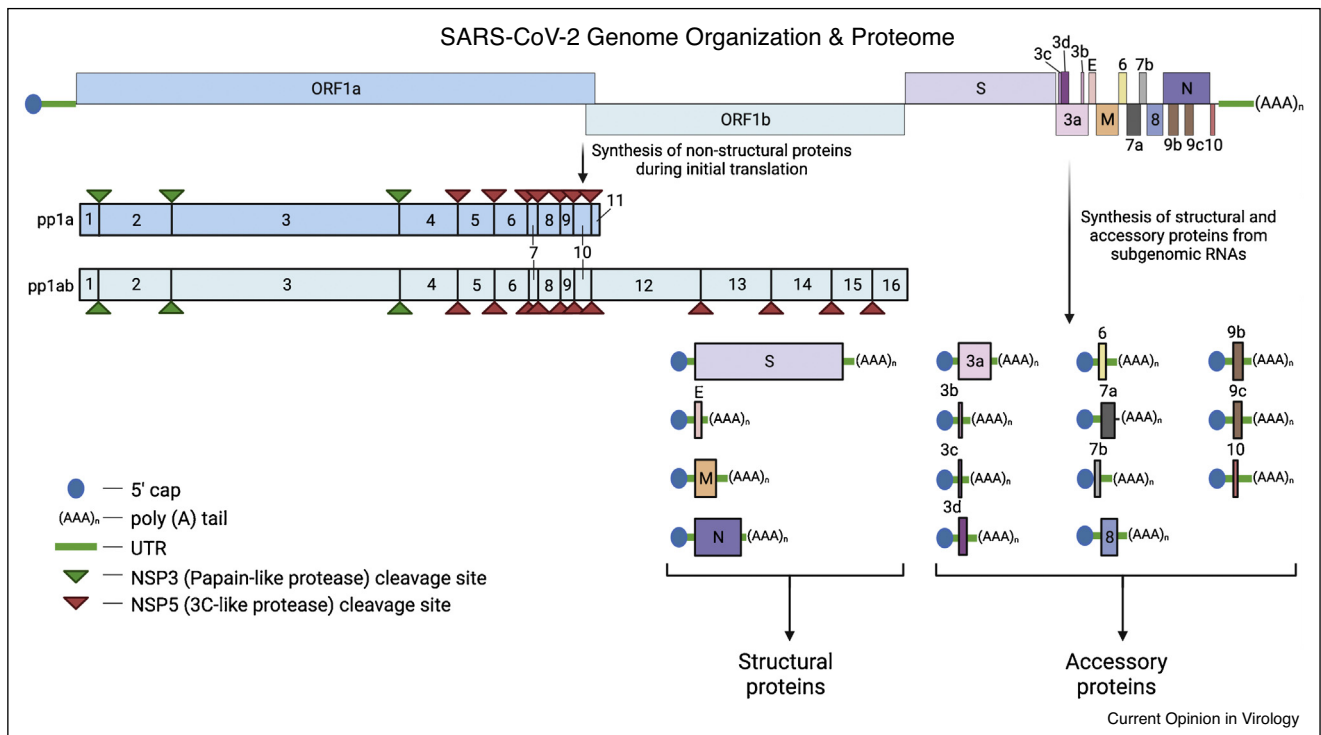
Regardless of where the S protein-mediated membrane fusion occurs, host cells can intercept the penetration using broad-acting restriction factors such as Cholesterol 25-Hydroxylase (CH25H), Lymphocyte Antigen 6 Family Member E (LY6E), and Interferon-induced Transmembrane proteins (IFITMs, e.g. IFITM1, IFITM2, IFITM3) [45–51]. CH25H catalyzes the conversion of cholesterol to 25-hydroxycholesterol (25HC). This activates Acetyl-CoA Acetyltransferase 1 at the endoplasmic reticulum (ER) resulting in depletion of plasma membrane cholesterol and accumulation of 25HC in the late endosomes, which blocks the viral membrane fusion [49,50]. Similarly, LY6E, an interferon-stimulated glycosylphosphatidylinositol-anchored protein, can block SARS-CoV-2 entry by impairing the membrane fusion regardless of the S protein maturation stages [45,46]. IFITMs are known to block the membrane fusion of many enveloped viruses, including SARS-CoV-1 [52]. Nonetheless, IFITMs may act as double-edged swords in responding to the SARS-CoV-2 entry. IFITMs can principally inhibit SARS-CoV-2 infection, but the subcellular localization of distinct IFITMs may impose different modulatory effects on the virus entry. For instance, ectopically expressed IFITM3 that traffics between the plasma membrane and endosomes can block the SARS-CoV-2 fusion in the endosome. Conversely, cell surface-immobilized IFITM3 mutant that harbors dysfunctional endocytosis YxxΦ motif can enhance SARS-CoV-2 fusion at the plasma membrane [47].

### SARS-CoV-2 translation and RNA replication

Upon deposition in the cytoplasm, the uncoated viral RNA genome can rapidly recruit cellular translation machinery to synthesize SARS-CoV-2 proteins [53\*\*,54–56]. SARS-CoV-2 genome (Figure 2) is a positive-sense, single-stranded RNA, approximately 30 kb in length, comprising multiple open reading frames (ORFs) that encodes for relatively large numbers of non-structural proteins (NSPs), structural proteins, and accessory proteins [57]. NSPs are directly translated from the full-length viral genome, whereas structural and accessory proteins are encoded by subgenomic RNA transcripts [84]. Each viral transcript mimics host mRNA, that is, decorated with a 5' cap structure and a 3' poly (A) tail, enabling the virus to synthesize its proteins by co-opting the cap-dependent mRNA translation machinery. Approximately two-thirds of the viral transcript from the 5' terminus encodes for two overlapping ORFs, ORF1a, and ORF1b, translated into two polypeptides, pp1a and pp1ab, respectively. Upon cleavages by its viral proteases (NSP3 and NSP5), these polypeptides mature into 16 non-structural proteins (NSPs, NSP1–NSP16). The remainder of the viral transcript towards the 3' terminus encodes for structural proteins, S protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein, and multiple accessory proteins.

Overall, in SARS-CoV-2 infected cells, the global host protein translation is reduced via rapid degradation of cytoplasmic mRNA and sequestration of nascent mRNA in the nucleus, allowing viral RNAs to dominate the access to ribosomes [54]. A few translational components, such as translation initiation factors EIF4A and EIF4H, as well as a translation elongation factor EEF1A1, have been demonstrated to be indispensable for the virus infection [58\*\*,59,60]. Excitingly, some of these pro-viral translation factors can be targeted using specific drugs such as Zotatfin and Plitidepsin to inhibit SARS-CoV-2 infection (Table 1). Interestingly, to enhance the translation of viral proteins, the NSP3 protein can interact with human Poly (A) Binding Protein Interacting Protein 1 (PAIP1), which can stimulate translation via interaction with translational initiation components [61]. SARS-CoV-2 NSP1 protein can bind and block the cellular mRNA entry tunnel of the 40S ribosomal subunit, resulting in disruption of the host mRNA translation and rewiring the translation machinery to accommodate biasedly viral protein synthesis [62–67]. In addition, the NSP1 protein can also occupy the host mRNA nuclear export complex, NXF1-NXT1, to disrupt the mRNA nuclear export [68]. Furthermore, SARS-CoV-2 ORF6 protein can alter the nuclear pore complex via interaction with Ribonucleic Acid Export 1 (RAE1) and Nucleoporin 98 (NUP98) to disrupt the bidirectional nuclear-cytoplasmic trafficking of cellular RNA [69,70]. Additionally, SARS-CoV-2 NSP16 can interact with U1/U2 snRNA, critical components of the major spliceosome, to inhibit global pre-mRNA maturation [67].

Figure 2



#### SARS-CoV-2 genome organization and proteome.

SARS-CoV-2 genome is a ~30 kb positive-sense, single-stranded RNA. The 5' terminal harbors two overlapping ORFs (ORF1a and ORF1b), encoding polyproteins pp1a and pp1ab. These polyproteins are proteolytically processed by viral proteases (NSP3 and NSP5) into 16 non-structural proteins (NSP1–NSP16). The 3' end of the viral genome encodes for structural and accessory proteins via multiple subgenomic RNAs. Sizes of SARS-CoV-2 genes and proteins are not drawn to scale.

Abbreviation: Spike (S); Envelope (E); Membrane (M); Nucleocapsid (N); accessory proteins (3a, 3b, 3c, 3d, 6, 7a, 7b, 8, 9b, 9c and 10); open reading frame (ORF); untranslated region (UTR).

Post-translational modifications (PTMs) of SARS-CoV-2 proteins play an essential role in promoting virus infection. The S protein is heavily N- and O-glycosylated, and these glycan decorations are crucial in promoting SARS-CoV-2 entry [71–73]. Multiple SARS-CoV-2 proteins (e.g. NSP3, NSP9, NSP14, S, M, N, and ORF9b) are phosphorylated, and chemical inhibitions of the PTMs can block virus infection, suggesting these phosphorylations may exhibit a pro-viral role [60,74]. The S protein is ubiquitinated by HECT-E3 ubiquitin ligases, for example, NEDD4 and WWP1, and chemical inhibitions of these E3 ubiquitin ligases can potently decrease SARS-CoV-2 infection and egress [75,76]. The ORF7a protein requires polyubiquitylation to confer the ability to antagonize host innate immunity by blocking STAT2 phosphorylation [77]. SARS-CoV-2 N protein is methylated by Protein Arginine Methyltransferase 1 (PRMT1), and the PTM is required for viral RNA packaging and interaction with cellular stress granules [78].

SARS-CoV-2 NSPs orchestrate the cascade of events during viral RNA synthesis including vast cellular

membrane remodeling. The viral Replication and Transcription Complex (RTC), comprising NSP2–16 proteins, is the core component of the viral RNA synthesis machinery [53\*\*]. RTCs are anchored on unique, interconnected compartments called double-membrane vesicles (DMVs) [79–81]. Like other virus-induced RNA replication compartments, these DMVs serve as hideouts to avoid host innate immune sensing and RNA decay machinery, allowing SARS-CoV-2 to clandestinely synthesize its subgenomic and full-length genomic RNA transcripts [82,83]. Specific host factors co-opted by SARS-CoV-2 to facilitate viral RNA synthesis are yet to be revealed but likely to be similar to those reported for other coronaviruses [85]. In addition, a plethora of SARS-CoV-2 RNA-interacting host proteins have been comprehensively uncovered, and some of these RNA-binding proteins are likely to play a role during virus infection in cells, including viral RNA synthesis [86\*,87,88\*,89\*].

Overall, substantial alterations of cellular organelles and compartments, such as fragmentation of Golgi apparatus, were observed in SARS-CoV-2 infected cells, indicating

cell membrane homeostasis and lipid metabolism can play an essential role in accommodating productive propagation of progeny virions [80]. Furthermore, accumulation of lipid droplets and reprogramming of lipid metabolism have been observed in SARS-CoV-2 infected cells and COVID-19 patients' samples, suggesting a potential causative link between dysregulation of lipid metabolism and viral pathogenesis [90–92]. Notably, the strategy of treating COVID-19 via pharmacological inhibition of host lipid metabolism using statin and derivatives, a group of commonly used cholesterol-lowering drugs, is currently being evaluated in clinical trials (Table 1). Some vital cellular pathways that regulate cellular lipid metabolism, such as the PI3K and autophagy pathways, have been implicated to play a role during coronavirus infections and pathogenesis [13\*,42\*,93]. Specific cellular factors that are known to regulate lipid and cholesterol homeostasis, such as Sterol Regulatory Element Binding Transcription Factor 2 (SREBF2, a.k.a. SREBP2), SREBF Chaperone (SCAP), Membrane Bound Transcription Factor Peptidases (MBTSP1, MBTSP2), and NPC Intracellular Cholesterol Transporters (NPC1, NPC2), have been identified as host dependency factors for SARS-CoV-2 via functional genomic screens [15\*,42\*,43,44]. SARS-CoV-2 ORF3a can directly interact with VPS39 subunits of the Homotypic Fusion and Protein Sorting (HOPS) Complex to inhibit fusion of autophagosomes with lysosomes, preventing the formation of late-stage autophagy organelles, autolysosomes [94,95]. The ORF3a-VPS39 interaction intercepts the subsequent interplay between HOPS-RAB7A, and HOPS-STX17-SNAP29-VAMP8-SNARE, which modulates the autolysosome maturation. Interestingly, depletion of VPS39 can protect human cells from SARS-CoV-2 mediated cytopathic effects, highlighting the role of VPS39 in promoting virus infection [44]. In addition, SARS-CoV-2 infection requires an ER-resident phospholipid scramblase, TMEM41B, that was first known to regulate autophagosome formation and lipid mobilization [15\*,16,96–98]. TMEM41B plays a physiological role in maintaining the ER integrity and cellular lipid homeostasis, especially the distribution of cholesterol and phosphatidylserine [99,100]. In addition to lipid metabolism, SARS-CoV-2 can rewire glycolysis and one-carbon metabolism via hijacking Serine Hydroxymethyltransferase 1 (SHMT1) to induce *de novo* purine synthesis for supporting mega-scale of viral RNA synthesis [101].

### SARS-CoV-2 assembly and exit

Generally, the assembly and exit of enveloped viruses involve a cascade of irreversible events that is well-orchestrated by both viral factors and cellular components [83]. For SARS-CoV-2, the process begins when newly synthesized SARS-CoV-2 RNA genomes are exported from the DMVs via a crown-like, cylindrical membrane pore complex comprising viral proteins (e.g. NSP3) and perhaps host proteins that are yet to be identified [81,102,103]. This channel may also play a role in

shuttling host factors and ingredients (e.g. metabolites and nucleotides) into the DMVs to fuel the viral RNA synthesis [80]. Subsequently, via an unknown mode of trafficking, perhaps involving the association of specific viral RNA sequences (e.g. cis-acting elements) with viral proteins and host factors such as cytoskeleton and RNA binding proteins (as suggested in Refs. [86\*,87,89\*,104]), the viral genomes shuttle to reach the N proteins near the budding site. SARS-CoV-2 RNA genomes can directly interact with the N proteins to form viral ribonucleoprotein complexes (vRNPs) via liquid–liquid phase separation that may involve association with G3BP-mediated stress granules [87,105,106]. vRNPs are frequently seen to accumulate and bud into curved cytosolic surfaces of single-membrane vesicles (SMVs), where viral structural proteins (S, N, E, and M) happen to be co-localized [79,80]. As a result, SARS-CoV-2 progeny virions are assembled in the SMVs that are likely derived from the endoplasmic reticulum-Golgi-intermediate compartment (ERGIC) [79]. The underlying mechanistic details about SARS-CoV-2 assembly and budding remain murky. The budding process is likely promoted by a membrane bending mechanism driven by unknown viral components and host factors [79].

Despite hitch-hiking the canonical exocytosis pathway to exit cells like other enveloped viruses [107], SARS-CoV-2 virions are likely to unconventionally utilize lysosomal exocytosis as an exit strategy [108\*\*]. During exit, SARS-CoV-2 progeny virions may first travel from the SMV to the Golgi intermediate compartments and subsequently re-routed to a deacidified lysosomal compartment before getting released from host cells. A few host factors that are known to regulate lysosomal trafficking, such as RAB7A (a RAS-related GTP-binding protein that regulates endolysosomal trafficking), ADP Ribosylation Factor Like GTPase 8A (ARL8A, a regulator of lysosome mobility), and TMEM106B (a regulator of lysosomal pH, morphology trafficking, and exocytosis), may play a role in modulating the lysosomal exocytosis process [13\*,16,108\*\*,109]. Intriguingly, colocalization of SARS-CoV-2 virions on cell surface filopodial protrusions was commonly observed on infected cells, suggesting a strategy for the virus to release or spread from cell to cell [80]. The formation of these protrusions involves a Casein kinase II (CK2)-dependent remodeling of the cytoskeleton that is activated by the N protein [58\*\*]. Nevertheless, the exit of viruses is not always smooth sailing; SARS-CoV-2 release from host cells can be blocked by Tetherin (BST2), an IFN-induced restriction factor targeting a broad spectrum of enveloped viruses [110,111\*]. Similar to HIV-1's counteraction of Tetherin using its Vpu accessory protein [112], SARS-CoV-2 has evolved to thwart BST2 inhibition using accessory protein ORF7a [111\*].

Although the spread of SARS-CoV-2 virions remains poorly understood, they are likely to transmit via both

cell-free and cell-to-cell routes. Syncytia formation is commonly observed in some SARS-CoV-2 infected cells and COVID-19 patients' tissues, and it has been proposed to play a critical role in cell-to-cell transmission and pathogenesis [113]. The underlying mechanism for the syncytia formation is surprisingly similar to the TMPRSS2-dependent SARS-CoV-2 entry — triggered by the interaction between the S protein (present on the surface of donor cells) and ACE2 (on the surface of neighboring cells) [48,114\*,115]. Mechanistically, syncytia formation is regulated by a plasma membrane protein, Anoctamin 1 (ANO1, a.k.a. TMEM16), but can be restricted by IFITMs, especially IFITM1, and can be chemically intervened using Niclosamide (Table 1) [48,114\*]. Puzzlingly, the impact of the IFITMs-mediated restriction can be reversed by high levels of TMPRSS2 expression, further clouding the differences between SARS-CoV-2 entry and spread [48].

### Host-directed therapeutics against COVID-19

As drug discovery and approval for use entail significant resources and time, drug repurposing represents the primary line of treatment for the COVID-19 pandemic. The strategy is especially advantageous for drugs with a pre-established clinical profile and manufacturing arrangements that could expedite treatment options [116,117\*]. A few SARS-CoV-2 host factors have served as promising therapeutic targets to fuel numerous pre-clinical screens and clinical trials (Table 1). Host-directed therapeutics enjoy the benefits of reduced viral resistance and their broad-spectrum antiviral potential. However, selecting a suitable host target essential for a virus but dispensable for a host remains challenging. In order to avoid undesirable host responses, rigorous validation and extensive monitoring are necessary for the optimal selection of drugs and host factor candidates.

### Concluding remarks

Rapid multi-omics studies on SARS-CoV-2 have painted a complex picture of its infection mechanism. This molecular information is crucial for the innovation of novel antiviral strategies including new vaccine designs and host-directed antiviral therapeutics [130,131]. It is perhaps worth noting a few caveats about these discoveries. Since the genuine SARS-CoV-2 must be studied in a contained BSL3 environment with restricted access and limited resources, a substantial number of SARS-CoV-2 studies were performed at BSL2 labs using surrogate systems, such as individual viral protein expression constructs, pseudotyped viruses, viral replicons, and other betacoronaviruses (e.g. HCoV-OC43 and MHV). In addition, non-human cell types (e.g. Vero cells and derivatives) and artificially engineered systems (e.g. human ACE2 overexpressing cell lines and lab animals) have been widely used. These systems have greatly expedited SARS-CoV-2 research, but the outcomes must be carefully interpreted and cross-validated before making

conclusions about the genuine virus. For example, African green monkey kidney epithelial cells, Vero cells, are widely used for COVID-19 research, including virus isolation and propagation. However, Vero cells express only ACE2 but not TMPRSS2 at the cell surface, forcing SARS-CoV-2 to enter these cells solely via the endocytosis route. Endosomal inhibitors such as hydroxychloroquine tend to inhibit SARS-CoV-2 infection in Vero cells potently, but not other cell types (e.g. Calu-3, human lung epithelial cells) that allow the virus to opt for fusion at the plasma membrane and in the endosomes [132\*\*]. Worryingly, repeated passaging of SARS-CoV-2 in Vero cells can result in the loss of the furin-cleavage site on the S protein, altering the authenticity and pathogenicity of the virus [25,31\*,133,134]. Therefore, in-depth studies using physiological relevant tools are needed to uncover the *bona fide* molecular mechanisms underlying the SARS-CoV-2 infection and pathogenesis.

In a nutshell, an unprecedented scale of coronavirus research has been rapidly conducted in response to the COVID-19 pandemic, and significant progress has been achieved to fill up knowledge gaps in some aspects of coronavirus biology. However, these findings may represent the tip of the iceberg. Humankind has witnessed the emergence of three novel coronaviruses in the past two decades, and SARS-CoV-2 is unlikely to be the last. To better prepare for the next pandemic, a comprehensive understanding of the SARS-CoV-2 biology is direly in need to stimulate the development of novel antiviral strategies, such as host-directed therapeutics that can potentially combat a broad spectrum of coronaviruses.

### Conflict of interest statement

Nothing declared.

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