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Molecular Virology of SARS-CoV-2 and Related Coronaviruses

Yu-An Kung,[a] Kuo-Ming Lee,[a,b] Huan-Jung Chiang,[a] Sheng-Yu Huang,[a] Chung-Jung Wu,[a] Shin-Ru Shih[a,c,d,e,f,g]

[a]Research Center for Emerging Viral Infections, College of Medicine, Chang Gung University, Taoyuan, Taiwan
[b]Division of Infectious Diseases, Department of Pediatrics, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan
[c]Department of Laboratory Medicine, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan
[d]Department of Medical Biotechnology and Laboratory Science, College of Medicine, Chang Gung University, Taoyuan, Taiwan
[e]Research Center for Chinese Herbal Medicine, College of Human Ecology, Chang Gung University of Science and Technology, Taoyuan, Taiwan
[f]Research Center for Food and Cosmetic Safety, College of Human Ecology, Chang Gung University of Science and Technology, Taoyuan, Taiwan
[g]Graduate Institute of Health Industry Technology, College of Human Ecology, Chang Gung University of Science and Technology, Taoyuan, Taiwan

Yu-An Kung, Kuo-Ming Lee, and Huan-Jung Chiang contributed equally to this article. Author order was determined by drawing straws.

SUMMARY Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The global COVID-19 pandemic continues to threaten the lives of hundreds of millions of people, with a severe negative impact on the global economy. Although several COVID-19 vaccines are currently being administered, none of them is 100% effective. Moreover, SARS-CoV-2 variants remain an important worldwide public health issue. Hence, the accelerated development of efficacious antiviral agents is urgently needed. Coronavirus depends on various host cell factors for replication. An ongoing research objective is the identification of host factors that could be exploited as targets for drugs and compounds effective against SARS-CoV-2. In the present review, we discuss the molecular mechanisms of SARS-CoV-2 and related coronaviruses, focusing on the host factors or pathways involved in SARS-CoV-2 replication that have been identified by genome-wide CRISPR screening.

KEYWORDS SARS-CoV-2, coronavirus

INTRODUCTION

In late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged. It is the causative pathogen of coronavirus disease 2019 (COVID-19), which has become a global pandemic. It was thought that coronaviruses (CoVs) have only mild adverse effects on human health until the emergence of several coronavirus diseases, including severe acute respiratory syndrome (SARS or SARS-CoV-1), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2, all of which pose serious threats to public health. As of January 2022, the rapid spread of SARS-CoV-2 has

resulted in over 318 million confirmed cases and more than 5.5 million deaths (https://covid19.who.int/). These numbers are even higher when mild and asymptomatic cases are also considered. The devastating impact of the SARS-CoV-2 pandemic is extensive, and COVID-19 continues to affect every aspect of human life (1). Various measures, such as social distancing, hard-surface disinfection, and face masks, have been implemented in an effort to halt the spread of the disease. Nevertheless, the virus continues to thrive and evolve as various mutations and variants have emerged (2).

SARS-CoV-2 was so named because its clinical manifestations resembled those of its predecessor SARS-CoV-1. However, COVID-19 is highly contagious and is characterized by influenza-like symptoms such as fever, sore throat, cough, diarrhea, and breathing difficulties (3). Various risk factors are associated with disease severity, including age, sex, heredity, and preexisting health conditions; these include diabetes, cancers, cardiovascular diseases, obesity, and allergic diseases. The above-mentioned factors may contribute to the development of pneumonia, dyspnea, lymphopenia, acute respiratory distress syndrome (ARDS), multiple-organ failure, and even death in infected individuals (4–7). About 80% of all COVID-19 patients present with mild to moderate symptoms, while 5% end up in critical condition. The estimated worldwide mortality rate of COVID-19 is 1 to 2% (8–10). However, COVID-19 particularly targets older adults. It was estimated by the U.S. Centers for Disease Control and Prevention in January 2022 that >75% of all COVID-19-related deaths occurred in individuals aged ≥65 years (https://covid.cdc.gov/covid-data-tracker) due to dysregulated cytokine responses. Similar to other RNA viruses, the pathogen-associated molecular patterns of SARS-CoV-2, such as the spike (S) protein and double-stranded RNA (dsRNA), are recognized by pattern recognition receptors (e.g., Toll-like receptors and retinoic acid-inducible gene I [RIG-I]-like receptors), triggering a host innate immune response (11). But unlike many other respiratory pathogens, SARS-CoV-2 can induce an aberrant immune response. Interferon (IFN) responses of type I (IFN-I) and type III (IFN-III) were only slightly activated, whereas chemokines were highly upregulated in the sera and autopsied lungs of COVID-19 patients (12). In addition, severe COVID-19 cases often exhibit an IFN-I deficiency signature coupled with an exacerbated inflammatory response (13). Overall, the consequences of these dysregulated immune responses include a cytokine storm and death (14).

The scientific and medical communities have acted swiftly in the face of this unprecedented crisis brought on by the COVID-19 pandemic. In the present review, we summarize and review our current knowledge of the mechanisms of the coronavirus life cycle (Fig. 1). We highlight the molecular functions of viral proteins and host factors revealed by genome-wide CRISPR screening, discuss potential therapeutic agents, and endeavor to deepen our understanding of this rapidly moving and evolving virus.

EVOLUTION OF SARS-CoV-2 AND CELL ENTRY MECHANISMS

The family *Coronaviridae* is in the order *Nidovirales*. Members of this family are positive-sense single-stranded RNA [(+)ssRNA] viruses that are genetically categorized into the genera *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus* (Table 1) (15, 16). The coronavirus genome is ~30 kb in size and is the largest among all RNA viruses. Seven coronaviruses known to infect humans are designated HCoVs (human coronaviruses). They belong to the *Alphacoronavirus* (HCoV-229E and HCoV-NL63) and *Betacoronavirus* (HCoV-OC43, HCoV-HKU1, SARS-CoV-1, MERS-CoV, and SARS-CoV-2) genera. Both alphacoronaviruses and betacoronaviruses primarily infect mammals, whereas viruses in the *Gammacoronavirus* and *Deltacoronavirus* genera infect birds and mammals (17). The *Betacoronavirus* genus is further divided into several subgenera, including *Sarbecovirus*, *Hibecovirus*, *Nobecovirus*, *Merbecovirus*, and *Embecovirus* (16). Of the five HCoVs among the betacoronaviruses, MERS-CoV is a merbecovirus, whereas both HCoV-OC43 and HCoV-HKU1 are embecoviruses. Full-genome sequencing revealed that both SARS-CoV-1 and SARS-CoV-2 are in the *Sarbecovirus* subgenus (18) and share ~79% genetic similarity at the nucleotide level (19). The extremely high overall genetic
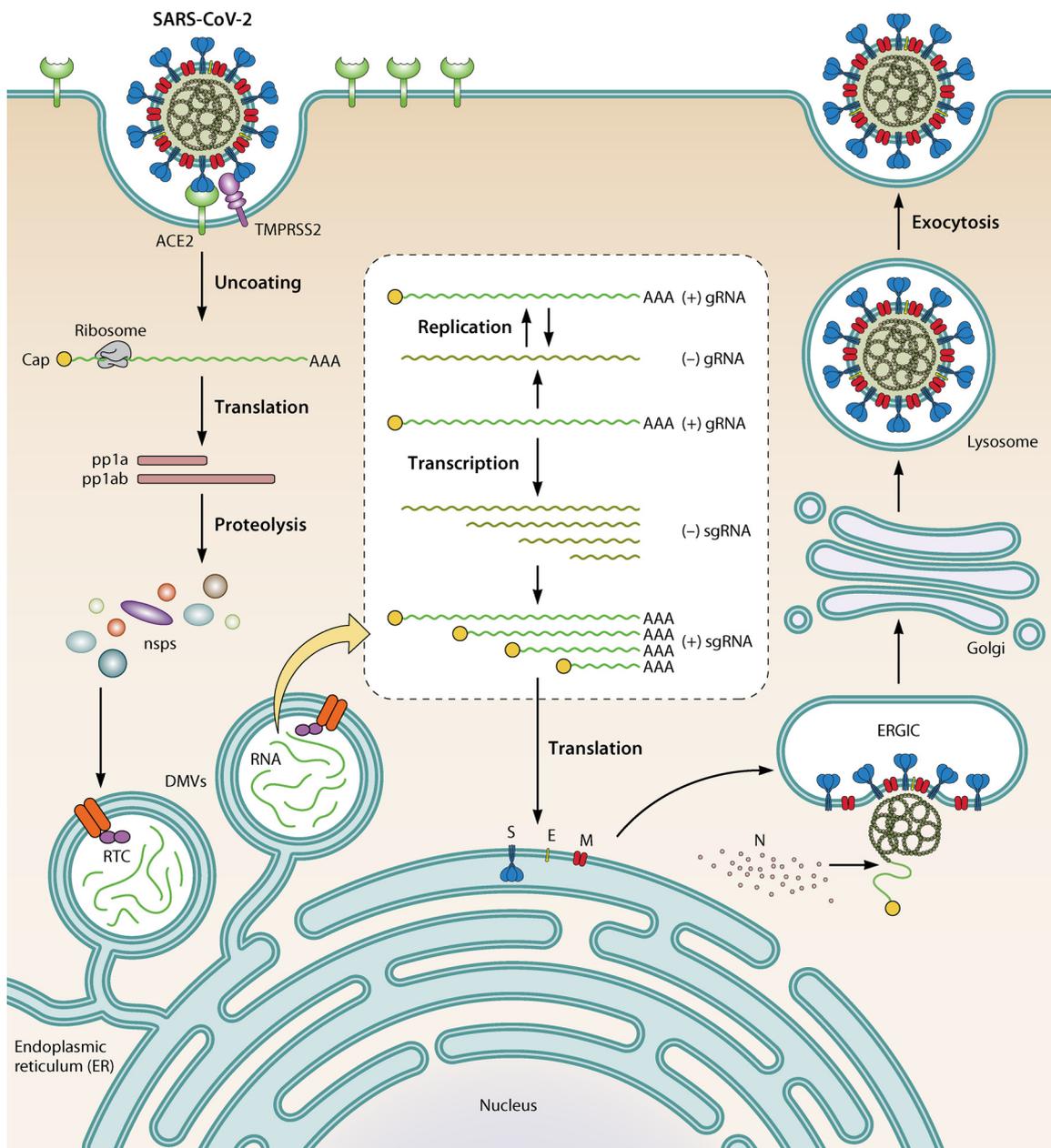
FIG 1 SARS-CoV-2 life cycle. SARS-CoV-2 interacts with the ACE2 receptor, and the spike (S) protein is cleaved by TMPRSS2, after which fusion between the viral and host membranes occurs. SARS-CoV-2 can also enter host cells by endocytosis, and the S protein is then activated by endosomal cathepsins. After the viral (+)ssRNA genome is released into the host cytoplasm, it is translated and produces the polyproteins pp1a and pp1ab, which are autoproteolytically processed into the nonstructural proteins nsp1 to -16. The nsps assemble the coronavirus replicase-transcriptase complex (RTC) and remodel the membranes to form organelles for viral RNA synthesis. Viral replication and transcription occur in double-membrane vesicles (DMVs) derived from the ER. Newly synthesized viral genomic RNA is exported from the DMV interior via the pore channel and is then encapsidated by the nucleocapsid (N) protein. The nested transcribed subgenomic RNAs (sgRNAs) are translated into the structural proteins S, envelope (E), membrane (M), and N and accessory proteins. S, E, and M are anchored to the ER membrane and migrate to the virion assembly site known as the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). The viral ribonucleoprotein (vRNP) complexes migrate to the ERGIC and bud into the lumen. The enveloped virion is then released from the cells via lysosomes.

similarity (>96%) between SARS-CoV-2 and a SARS-related bat coronavirus indicates that SARS-CoV-2 might have originated in bats (19–21). Interestingly, the overall sequence similarity between SARS-CoV-2 and the virus infecting Malayan pangolins is lower than that observed between SARS-CoV-2 and the SARS-related bat coronavirus (92% versus 96%, respectively). However, much higher similarity was found between the spike receptor binding domain (RBD) of SARS-CoV-2 and that of the virus infecting

TABLE 1 Classification of the human coronaviruses[a]

Genus	Natural host(s)	Subgenus	Species	Intermediate host	Presence of furin cleavage site
Alphacoronavirus	Mammals dominant		HCoV-229E	Camelids	−
			HCoV-NL63	?	+ (at S2′)
Betacoronavirus	Mammals dominant	*Embecovirus*	HCoV-OC43	Bovines	+
			HCoV-HKU1	?	+
		Hibecovirus			+
		Merbecovirus	MERS-CoV	Dromedary camels	+
		Nobecovirus			+
		Sarbecovirus	SARS-CoV-1	Palm civets	−
			SARS-CoV-2	Pangolins	+
Gammacoronavirus	Birds dominant; mammals				+
Deltacoronavirus	Birds dominant; mammals				−

[a]Coronaviruses belong to the order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae*. "+" indicates presence of furin cleavage site; "−" indicates absence of furin cleavage site; "?" indicates yet to be identified/clarified.

Malayan pangolins (97.4%) than that of the SARS-related bat coronavirus (89.2%). Moreover, SARS-CoV-2-related CoVs have been detected in pangolins in Southeast Asia. For these reasons, pangolins are considered potential intermediate hosts of SARS-CoV-2 (22, 23).

The spike protein, particularly the RBD region, plays a pivotal role in the host cell entry of CoV by binding to cell surface receptors. Spikes can be destabilized by long-term virus storage, which reduces their human angiotensin-converting enzyme II (ACE2) binding affinity (24). The SARS-CoV-2 RBD shares high sequence identity (~73.9%) with the SARS-CoV-1 RBD, and both develop highly similar structures, especially in terms of their receptor binding motifs (25–27). Thus, both SARS-CoV-2 and SARS-CoV-1 utilize ACE2 for host cell entry (25, 28, 29). ACE inhibitors (ACEis), such as perindopril, ramipril, enalapril, and lisinopril, alter the conformation of ACE2 and prevent SARS-CoV-2 from binding to host cells, thereby reducing SARS-CoV-2 infectivity (30). Other cell surface factors, such as soluble ACE2 (sACE2) (31) and heparan sulfate proteoglycans (HSPGs) (32), also contribute to virus entry. The trimeric form of the heavily glycosylated spike protein consists of S1 and S2 subunits, and it is dispersed on the surface of a mature virus (Fig. 2A). The spike protein enables the virus to attach to ACE2 and initiate viral entry into the host cell. The S1 subunit contains the N-terminal domain (NTD) and RBD that facilitate viral binding to the host cell. The S2 subunit comprises a fusion peptide (FP), heptapeptide repeat sequence 1 (HR1), heptapeptide repeat sequence 2 (HR2), a transmembrane (TM) domain, and a cytoplasmic (CP) domain (25, 33, 34). S2 mediates membrane fusion and is characterized as the stalk region (Fig. 2A). The RBD of S1 interacts with ACE2, is characterized as the head region, and is often considered a valuable therapeutic target for drug and vaccine development (35). The NTD of S1 is also immunogenic as neutralizing antibodies binding to this region can hamper viral attachment (36, 37); it therefore constitutes a promising therapeutic option for SARS-CoV-2 infection. Numerous efforts have been made to produce effective therapeutic antibodies against SARS-CoV-2. Anti-RBD antibodies isolated from the sera of infected patients impaired ACE2 binding (38, 39). As SARS-CoV-1 and SARS-CoV-2 have similar structures and receptor usages, antibodies targeting the SARS-CoV-1 spike protein have been tested as potential therapeutic agents against SARS-CoV-2. There was a certain degree of cross-reactivity between SARS-CoV-1 antibodies and the SARS-CoV-2 spike (40–42). However, one study conducted by Wrapp et al. (34) evaluating the cross-reactivity of SARS-CoV-1 antibodies against SARS-CoV-2 yielded less optimistic results; no binding could be detected for some SARS-CoV-1 RBD antibodies to SARS-CoV-2 spike (34). Yuan et al. (42) further demonstrated with a neutralizing antibody that the antibody binding epitope could be masked by the RBD conformation. Various *in vitro* binding assays demonstrated that the SARS-CoV-2 RBD exhibited a higher ACE2 binding affinity than the SARS-CoV-1 RBD; however, receptor binding is regulated by different degrees of RBD exposure in response to conformational changes
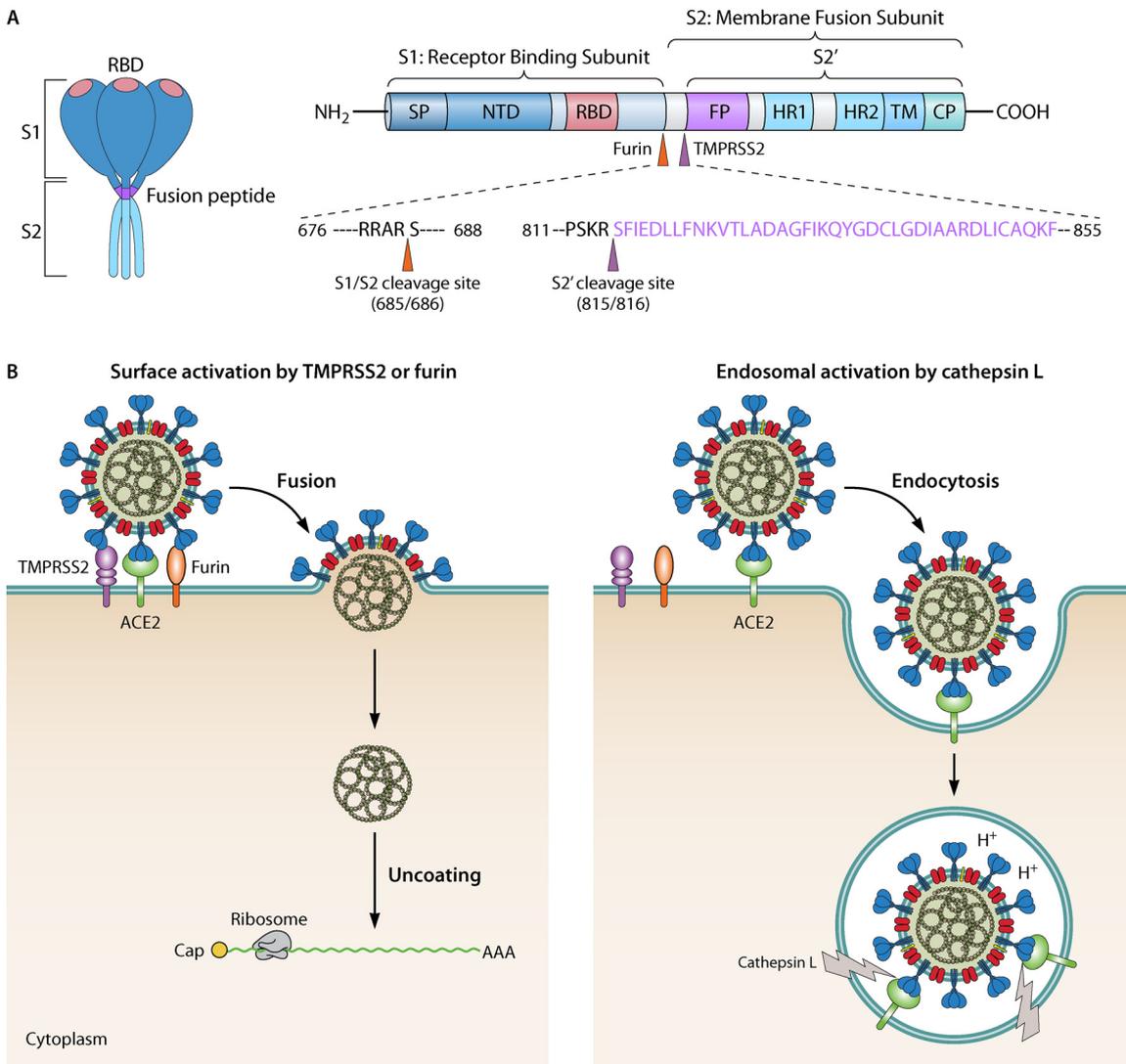
FIG 2 SARS-CoV-2 spike protein structure, activation, and host cell entry. (A) Schematic drawing of the coronavirus spike three-dimensional (3D) structure and domain structure, including the signal peptide (SP), N-terminal domain (NTD), receptor-binding domain (RBD), fusion peptide (FP), heptad repeat 1 (HR1), heptad repeat 2 (HR2), transmembrane (TM), cytoplasmic (CP), and proteolytic (S1/S2 and S2′) cleavage sites. Arrowheads indicate the cleavage sites of furin and TMPRSS2. (B) SARS-CoV-2 can enter the host cell by TMPRSS2 or furin activation of fusion of the viral and host cell membranes (surface activation). SARS-CoV-2 can also enter the host cell via binding of the viral spike (S) protein to a cellular receptor and virion endocytosis. In the endosome, the pH-dependent cysteine protease cathepsin L (CatL) activates the S protein, causing fusion within the endosomal membrane (endosomal activation). The viral genome is released by TMPRSS2-mediated host cell entry or from the endosome and is partially and completely replicated and translated in the ER to form new SARS-CoV-2 virions.

of the spike protein. Based on images of intact virions, Yao et al. (43) provided detailed molecular insights into the SARS-CoV-2 spike conformations before and after fusion. The SARS-CoV-2 spike can effectively bind ACE2 as the low distribution density of the spike enables it to rotate on the viral envelope with high plasticity. In addition, furin cleavage is believed to force the SARS-CoV-2 RBD into a lying-down state. Consequently, the SARS-CoV-2 RBD is less accessible to receptor binding than the SARS-CoV-1 RBD, even though the former has a strong ACE2 binding affinity. Wide host protease usage and strong RBD binding affinity compensate for the apparent disadvantage of a less accessible receptor binding status, while the hidden RBD contributes to immune evasion wherein the virus undergoes conformational masking to escape host immunity (26, 44).

Proteolytic spike protein cleavage is also necessary for coronavirus entry into host cells (45). SARS-CoV-2 acquires a polybasic furin cleavage site (amino acid residues

682-Arg-Arg-Ala-Arg-685 [RRAR]) at the junction of the S1 and S2 subunits (46) (Fig. 2A). The furin cleavage site is common to other *Betacoronavirus* subgenera but is seldom detected among sarbecoviruses and may promote infectivity and transmission (47–52). Cleavage at the polybasic site exposes the S2′ cleavage site within the S2 domain. The latter is cleaved by either serine protease or cathepsins to expose the S2 FP that subsequently inserts into the host cell membrane (Fig. 2A). Membrane fusion is then initiated, and the viral genome is released into the cytoplasm (53). TMPRSS2 is a serine protease that anchors at the host cell membrane and activates the SARS-CoV-2 spike, inducing membrane fusion (Fig. 2B) (28, 54). TMPRSS2 is widely expressed on the epithelial cells lining the respiratory, gastrointestinal, and urogenital tracts (55, 56). SARS-CoV-2 can also bypass TMPRSS2 and enter cells via endocytosis. Spike cleavage then occurs in the endosome in a pH-dependent manner, mediated by the cysteine protease cathepsin L (CatL) (57). Zhao et al. (58) demonstrated that the licensed influenza drug amantadine inhibits CatL and prevents SARS-CoV-2 infection *in vivo*. Cathepsin B was initially regarded as another pivotal protease since it cleaves the SARS-CoV-1 spike protein. However, its inhibition could not prevent SARS-CoV-2 infection *in vitro*. Hence, CatL cleavage might suffice for SARS-CoV-2 infection *in vitro* (59).

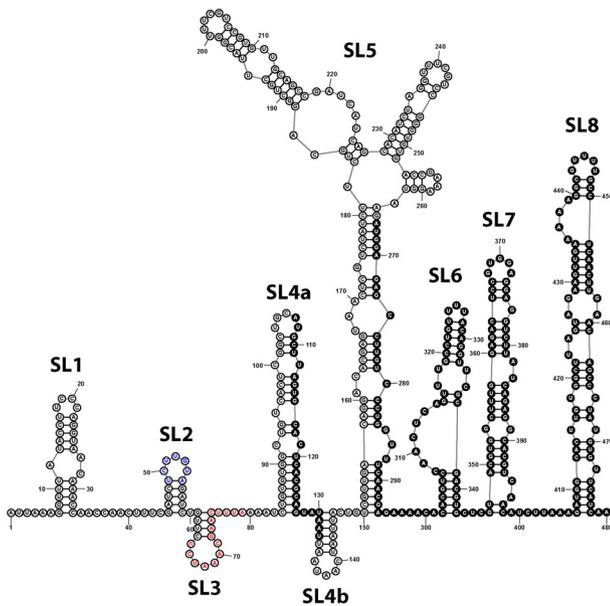## GENOME STRUCTURES OF SARS-CoV-2 IN TRANSLATIONAL REGULATION

S2′ cleavage triggers virus-host cell fusion, and the viral RNA (vRNA) is released into the cytoplasm where translation and genome replication occur (60). The SARS-CoV-2 (+)ssRNA genome is decorated with a 5′ cap and has a 3′ poly(A) tail. Hence, it functions as translation-ready mRNA. Two-thirds of the genome at the 5′ end contains open reading frame 1a (ORF1a) and ORF1b encoding the nonstructural proteins nsp1 to -16. The remaining one-third of the genome at the 3′ end encodes the structural proteins S, envelope (E), membrane (M), and nucleocapsid (N) and several accessory proteins, including ORF3a, -6, -7a/b, -8, and -10 (Fig. 3A). Chemical probing and nuclear magnetic resonance (NMR) *in vivo* and *in vitro* genome-wide RNA structure analyses revealed complex secondary structures, including those that are evolutionarily conserved at the 5′ and 3′ ends (61–65). The 5′ end is characterized by a series of stem-loops, SL1 to SL8, with various lengths and structures (Fig. 3B). SL1 and SL2 of the 5′ untranslated region (UTR) are highly conserved in coronaviruses, whereas SL4 is structurally conserved in betacoronaviruses (61, 64, 66). SL5 to SL8 show that folding extends into the coding sequence. The 3′-end structure folds from the ORF10 coding region toward the 3′ terminus. Important structures include an evolutionarily conserved bulged stem-loop (BSL), an overlapping hairpin (SL1)/pseudoknot (PK), and a unique three-helix junction formed by the retrograde folding of the downstream hypervariable region (HVR) (Fig. 3C). The structures at the 5′ and 3′ ends of the genome markedly influence translation and genome replication (61).

Upon cell entry, coronavirus utilizes cap-dependent translation, which is also utilized by cellular capped mRNAs. However, coronavirus infection is characterized by global mRNA degradation and robust inhibition of translation (33). Until recently, it was unknown how the translation machinery distinguished between host and viral mRNAs. A host shutoff mechanism depends on viral nsp1 binding to the 18S rRNA near the mRNA entry channel of the 40S ribosomal subunit, and ribosomal scanning is then blocked (67–69). This translational inhibition is abolished when the viral RNAs contain a 5′ leader sequence encompassing SL1 and SL2. The discrepancy in the translational regulation of the viral and host mRNAs is determined by the precise structure and location of SL1 within the leader sequence. Thus, the highly conserved SL1 and SL2 regulate viral translation in a structure-dependent manner (69).

Viral RNA translation produces polyprotein 1a (pp1a), which is then proteolytically processed into nsp1 to -11. A pseudoknot structure near the junction of ORF1a and ORF1b causes −1 programmed ribosomal frameshifting (PRF), which in turn leads to the production of another, longer polyprotein, pp1ab, wherein the short nsp11 is replaced by the RNA-dependent RNA polymerase (RdRp) nsp12 and is followed by
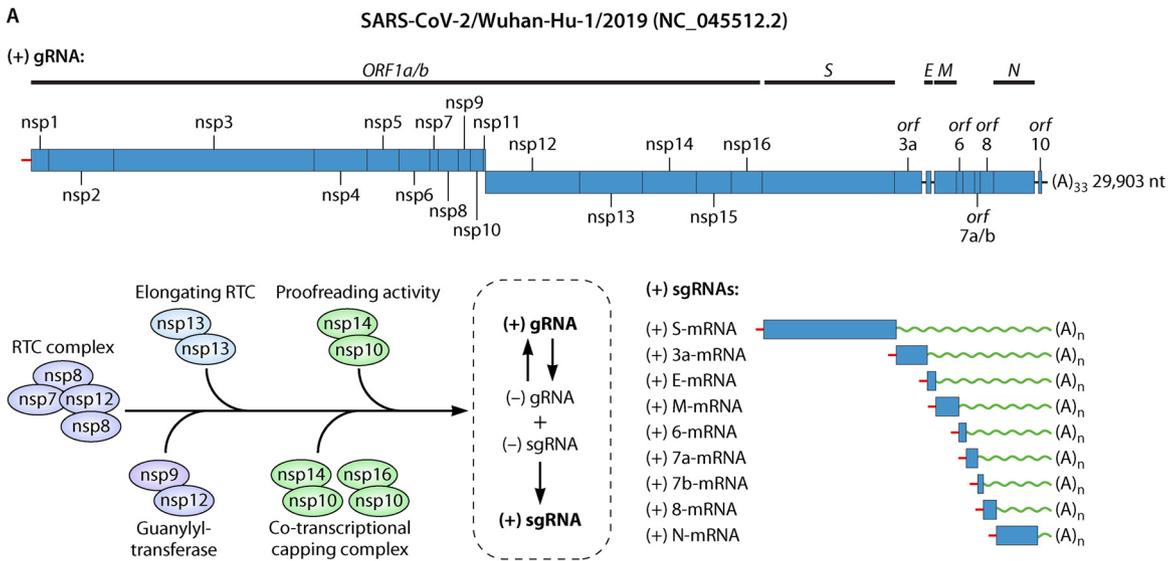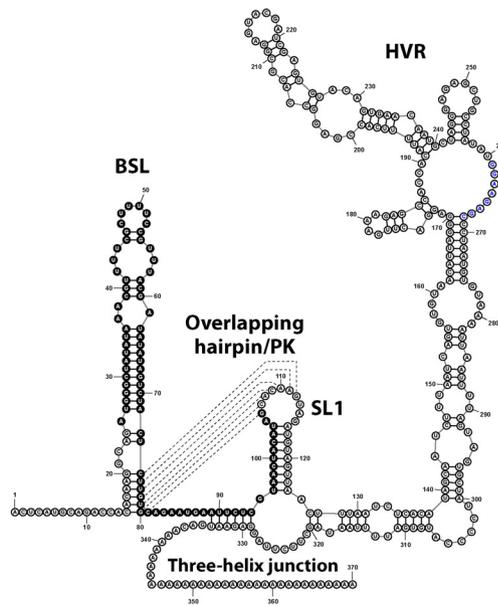
FIG 3 Genome structure and transcriptome architecture of SARS-CoV-2. (A) Schematic representation of the SARS-CoV-2 genome with annotations based on the reference sequence of the Wuhan-Hu-1 strain (GenBank accession number NC_045512.2). The distributions of open reading frames (ORFs) and coding regions of each nonstructural protein (nsp) across the genome are indicated. Both (+)gRNA and positive-stranded subgenomic RNA [(+)sgRNA] carry an identical leader sequence at the 5′ terminus (red). The (+)gRNA serves as the template for the synthesis of (−)gRNA as well as (−)sgRNAs that are subsequently used to synthesize (+)gRNA and (+)sgRNAs. The replication and transcription processes are carried out by nsps. Complexes with various functions are comprised of different nsps, as indicated in the schematic. (B) The 5′ end of the genomic sequence can fold into multiple stem-loops (SLs). The transcription/replication sequence (TRS) of SL3 is marked in red. The upstream region of the TRS encompassing SL1 and SL2 is defined as the leader sequence. Coding sequences located at the 5′ genomic region, including the upstream ORF in SL4, are represented by closed circles. (C) The secondary structures of the 3′ genomic sequences include an evolutionarily conserved bulged stem-loop (BSL) and a three-helix junction structure that is formed by base pairing between the hypervariable region (HVR) and the upstream/downstream sequences of SL1. The unwinding of the three-helix structure by the replicase-transcriptase complex (RTC) leads to a conformational change, allowing base pairing between the SL1 apical loop and the stem of the BSL to form a pseudoknot (PK).

nsp13 to -16 (Fig. 3A). Despite the low protein levels of these nsps compared with other nsps because of PRF processing, they furnish the enzymatic activity required for viral mRNA synthesis and processing. Both the viral RNA and nascent polyprotein chain contribute to the PRF by causing the ribosome to stall at the slippery sequence. Therefore, the PRF may be targeted for the development of novel antiviral agents (70–73). Proteolytic

cleavage of pp1a and pp1ab is executed by the virally encoded proteases nsp3 and nsp5, also known as the papain-like protease PLpro and the 3C-like protease 3CLpro, respectively. Nsp3 and nsp5 cleave the junctions from nsp1 to nsp4 and from nsp4 to nsp16, respectively (74). Because these proteases play vital roles in viral protein maturation, previous research has attempted to identify potent, broad-spectrum protease inhibitors (75–79).

SARS-CoV-2 REPLICATION-TRANSCRIPTION COMPLEX

The nsps liberated by proteolysis participate in the viral replication-transcription complex (RTC) within organelles coopted by nsp3, nsp4, and nsp6 (Fig. 3A) (74, 80, 81). Other than the nsp12 catalytic subunit, at least one nsp7 subunit and two nsp8 copies form a minimal RTC that efficiently incorporates nucleotides in vitro (82–85). Cryo-electron microscopy (EM) analyses revealed that the nsp7-nsp8 factor pair binds the finger domain of nsp12 and may serve as a primase in a manner similar to that of the SARS-CoV cognate (84, 86). The other nsp8 binds the thumb domain of nsp12. Hence, the N-terminal extensions of both nsp8 subunits sit in opposite sites and accommodate the exiting RNA. Investigations of the RTC structure in RNA molecule complexes revealed that the incorporation of the nucleotide analog remdesivir might cause delayed-chain termination, possibly explaining why remdesivir can escape the surveillance of the coronavirus proofreading activity (82, 84). Elongation of the RTC is contributed by two copies of the nsp13 helicase of the 1B helicase superfamily. They also possess RNA 5'-triphosphatase activity and join the RTC via interactions between its N-terminal Zn binding domains and the N-terminal extensions of each nsp8 unit. The position of the nsp13 nucleoside triphosphatase (NTPase) domain behind the RTC enables the latter to permit template switching, backtracking, and proofreading (87). Therefore, the recruitment of the RTC-nsp13 complex to the 3' triple-helix structure might destabilize the BSL structure, forming the PK structure, thus facilitating RNA unwinding and proceeding of nsp12 (70).

Unlike the replicases of most other RNA viruses, the coronavirus replicase is characterized by proofreading activity that invariably diminishes the efficacy of nucleotide-derived analog inhibitors. Proofreading is conducted by nsp14, which is a bifunctional protein bearing the N-terminal 3'-to-5' exoribonuclease (ExoN) domain and C-terminal N7-methyltransferase (N7-MTase) activity. ExoN activity is stimulated by binding with nsp10. In the active form of the nsp14-nsp10 complex with the RNA substrate, the active site of nsp14 ExoN cooperates with the N terminus of nsp10 and binds the 3' end of the RNA (88, 89). This active-site conformation resembles that of Lassa virus (Arenaviridae) nucleoprotein. The Arenaviridae are the only other RNA viruses known to possess ExoN activity (88). ExoN function depends on two metal cations irrespective of the N7-MTase domain (89). A free 3'-OH in the RNA substrate is required for ExoN processivity. This configuration may facilitate the design of nucleotide analogs for use in ExoN evasion (88). Nsp14 regulates viral RNA capping via the N7-MTase domain. nsp14-nsp10 joins the RTC to form a cotranscriptional capping complex (RTC-CCC) (90). A remarkable feature of SARS-CoV-2 RNA processing is the role of nsp12 in capping. Nsp12 possesses a recently characterized second catalytic domain termed nidovirus RdRp-associated nucleotidyltransferase (NiRAN) that can catalyze nucleotidyl monophosphorylation (NMPylation) using both RNA and protein as the substrates (91, 92). The N-terminal region of nsp9, which is a small RNA binding protein, binds the catalytic pocket of the NiRAN domain. Nsp9 might bring newly synthesized transcripts to the NiRAN domain and generate a GpppA cap in cooperation with the RNA 5'-triphosphatase activity of nsp13. Alternatively, nsp9 GMPylated by the NiRAN domain might be a primer for RNA synthesis in the RdRp active site of nsp12 (93). After GpppA core cap formation, N7-methylation and 2'-O-methylation are performed by nsp14 and nsp16, respectively (89, 94).

SUBGENOMIC RNA SYNTHESIS AND APPLICATION IN MOLECULAR DIAGNOSIS

Another phenotype unique to nidoviruses is the synthesis of multiple discontinuous transcripts known as subgenomic mRNAs (sgRNAs) (Fig. 3A). They encode structural and accessory proteins (95–99). The biogenesis of sgRNA involves long-range RNA-RNA pairing of the transcription/replication sequence (TRS) and mediation of replicase template switching (70, 100, 101). The consensus TRS is short ($\sim$15 nucleotides [nt]), and its core sequence (5′-ACGAAC-3′ in SARS-CoV-2) is localized to SL3 of the 5′ UTR (TRS leader) and the immediate upstream regions of most accessory and structural genes (TRS body) (Fig. 3B) (20, 97). During minus-strand genomic RNA [($-$)gRNA] synthesis, the antisense TRS body sequence in the nascent transcripts pairs with the complementary TRS leader of the positive-stranded genome template. This mechanism facilitates the replicase jump from the 3′ region to the 5′ end of the template and generates several discontinuous ($-$)sgRNAs with identical terminal sequences antisense to the leader sequence. These ($-$)sgRNAs serve as the templates generating protein-coding positive-stranded sgRNAs [($+$)sgRNAs] characterized by 5′ leader sequences identical to those of ($+$)gRNAs (Fig. 3A). This extraordinary template-switching process enables the ($+$)sgRNAs to acquire the 5′ leader sequence containing vital SL1 and SL2 structures, preventing nsp1-mediated translational shutoff (61).

The presence of both continuous gRNA and discontinuous sgRNA during viral replication confounds the interpretation of nucleic acid amplification test results for infectious COVID-19 patients. Host antibody, viral protein/antigen, and viral genome detection assays have been developed for SARS-CoV-2 diagnosis (102). As real-time reverse transcription-PCR (RT-PCR) has high sensitivity and specificity, it is considered the gold standard for SARS-CoV-2 detection. However, the correlation between the cycle threshold (C_T) ratio and the culture-based infectivity assay in COVID-19 patients remains controversial. The ranges of C_T ratios obtained for successful SARS-CoV-2 isolation vary among studies (103–107). Various amplicons of ORF1a/b and the structural E, N, and S genes have been widely used. Nevertheless, numerous primer sets recognizing structural genes fail to distinguish gRNAs from sgRNAs (108). Persistent, highly abundant E and N sgRNAs in COVID patients cast doubt as to whether viral RNA detection is truly representative of active viral replication and shedding (109). Moreover, low-C_T specimens that failed in virus isolation were found to carry disproportionate numbers of copies of structural and nonstructural genes. Hence, disrupted genomes or residual replicating intermediates may have been detected (105). In contrast, monitoring the RNA levels of various replicating intermediates and their associations with clinical specimen culturability revealed that culturable specimens were characterized by the detection of ($-$)gRNAs and sgRNAs soon before the onset of COVID-19 symptoms (110, 111). This sgRNA-associated infectiousness was also observed in rhesus macaques and African green monkeys infected with SARS-CoV-2 (112, 113). Thus, sgRNA detection could be used to improve therapeutic decision-making for COVID-19 (111). Nevertheless, RT-PCR results must be carefully interpreted and correlated with infectiousness, irrespective of gRNA and sgRNA detection (106, 114).

STRUCTURAL AND ACCESSORY PROTEINS ENCODED BY sgRNAs AND HOST IMMUNOMODULATION

Protein products of the sgRNAs participate in viral replication despite their absence from genome replication/transcription. Structural proteins, including S, E, M, and N, are essential for packaging mature virions. Glycosylated S proteins play pivotal roles in viral entry into host cells. The E protein is a viroporin that selectively transports cations. A homopentameric ion channel, characteristic of a five-helix bundle, surrounds a narrow pore and is integrated into lipid bilayers. Both the N- and C-terminal regions are exposed outside the membrane and can interact with viral and host proteins (115). The M protein is the most abundant glycoprotein in the coronavirus. It contains three transmembrane domains and functions as a scaffold protein by interacting with itself and the S and N proteins (116, 117). The N protein is the only structural protein that

binds RNA. As N bears several intrinsically disordered regions, it was characterized by liquid-liquid-phase separation with viral RNA; N tentatively bound the single-stranded sequence and especially the linker sequence embedded in the highly structured 5′ and 3′ regions (118). Under physiological conditions, the viral genome forms a string of shell-like structures consisting of 12 copies of N wrapped with ~800 nt of RNA (43, 119). Phase separation of N/genomic RNA in the membrane fraction revealed the accumulation of nsp12 and M in the viral ribonucleoprotein (vRNP) complex condensates. This discovery suggests that N facilitates viral replication and packaging (116, 120).

There are also accessory proteins encoded by the sgRNAs or overlapping genes of certain sgRNAs (Fig. 3A), which play auxiliary roles in viral replication. The compositions and coding capacities of the accessory genes vary among coronaviruses (15). The coding capacities and annotations of the accessory proteins of SARS-CoV-2 are inconclusive (96, 99, 121, 122). Certain accessory proteins are encoded by annotated ORFs, such as ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10. Other proteins include overlapping ORF3c and ORF3d within the ORF3a coding region. ORF9b and ORF14 are the products of leaky scanning of the *N* sgRNA (99, 122, 123). The functions of the SARS-CoV-2 accessory proteins have yet to be fully characterized. The high sequence identities of most accessory proteins, except ORF8, to the SARS-CoV-1 cognate suggest that they might create environments conducive to viral replication (124). ORF3a and ORF6 in SARS-CoV-2 have been thoroughly researched; the former has a transmembrane domain, forms a dimeric or tetrameric ion channel (125), and participates in apoptosis and autophagosome blockage and release (126, 127). ORF6 is a potent IFN antagonist (128–130); host cells infected with ORF6-deleted recombinant SARS-CoV-2 did not present with IFN inhibition (129). IFN inhibition is mediated by the interaction between the C-terminal domain of ORF6 and the Nup98-Rae1 nuclear pore complex proteins. In this way, the nuclear import of the STAT1 and STAT2 transcription factors is inhibited (130). In addition, reporter assays indicated that IFN inhibition might also be mediated by nsp1, nsp3, nsp12, nsp13, nsp14, nsp15, ORF3, and M (128, 130). The less conserved SARS-CoV-2 ORF8 regulates immune evasion by targeting major histocompatibility complex (MHC) class I for lysosomal degradation (131). Several strains were characterized by ORF8 deletions. Patients infected with the longest Δ382 variant of SARS-CoV-2 presented with comparatively mild symptoms (132–134). ORF9b suppresses the IFN-I response by localizing to the mitochondria and sequestering Tom70, which is an adaptor protein in RIG-I/MDA5 signaling (135). SARS-CoV-2 can also prevent RIG-I/MDA5 activation through sequence trimming mediated by nsp15, which is also known as nidovirus endoribonuclease specific for U (NendoU). Nsp15 functions in a hexameric form by the dimerization of the trimeric nsp15; thus, the C termini of the nsp15 hexamer assemble into a catalytic domain resembling those of EndoU family enzymes (136). Nsp15 preferentially cleaves the 3′-phosphate of uridines (U's), especially U's followed by purines. Therefore, nsp15 can efficiently cleave poly(U) tracts that are enriched in the 5′ ends of (−)gRNA and also U's along with the following purine throughout the genome (137). Through the interaction with the RTC, nsp15 specifically degrades the (−)gRNA of the dsRNAs, which are the ligands of RIG-I/MDA5 (138, 139). Altering the activity of these immunomodulatory proteins might be another approach toward developing antiviral drugs (140). Various other strategies are also utilized by SARS-CoV-2 to induce host shutoff. For example, nsp16 binds the mRNA recognition motif of U1/U2 snRNP and inhibits splicing. Nsp8 and nsp9 associate with signal recognition particles and selectively disrupt protein trafficking (69). Meanwhile, viral proteins might cooperate with host proteins to facilitate viral replication and translation (141–143). These mechanisms can be elucidated by genetic screening via small interfering RNA (siRNA)- and CRISPR-based techniques.

CORONAVIRUS MODIFIES HOST CELL MEMBRANES FOR VIRAL REPLICATION

In coronavirus-infected cells, viral replication organelles are formed by the membranous rearrangement of the endoplasmic reticulum (ER) for viral RNA synthesis (Fig. 1). Coronavirus induces convoluted membranes (CMs) or a zippered ER, small open double-membrane spherules (DMSs), and double-membrane vesicles (DMVs), all of which are

thought to be derived from the ER. Immunogold electron microscopy (IEM) revealed that CMs are major sites of nsp accumulation; moreover, dsRNA, the site of viral RNA synthesis, is mainly localized to the interior of SARS-CoV-1-induced DMVs (144). These phenomena were further confirmed by metabolic labeling of newly synthesized coronavirus RNA followed by quantitative EM autoradiography. This revealed that DMVs are the primary sites of RNA synthesis in MERS-CoV, SARS-CoV-1, and the gammacoronavirus infectious bronchitis virus (IBV). Viral RNA synthesis is not linked to CMs, the zippered ER, or DMSs (145). Additional evidence also suggests that DMVs are dedicated sites for coronavirus RNA replication. Klein et al. (119) observed RNA filaments with secondary structures inside the DMVs. Using cryo-EM, Wolff et al. (81) identified a molecular pore complex that exports newly synthesized viral RNA from the DMVs to the cytosol. Nsp3, nsp4, and nsp6 contain multiple hydrophobic, membrane-spanning domains and might participate in DMV formation (146, 147). MERS-CoV nsp3 and nsp4 coexpression suffices to induce DMVs in Huh7 cells; however, IBV nsp3, nsp4, and nsp6 coexpression did not induce DMVs. Several studies indicate that IBV nsp6, HCoV-NL63 nsp3, or mouse hepatitis virus (MHV) infection induces autophagosome formation associated with the viral replication complex (148). Therefore, different coronaviruses utilize different nsps in DMV formation. However, it remains to be determined whether coronavirus is involved in the autophagy pathway.

The RTC is thought to concentrate the viral RNA (vRNA) synthesis machinery, and it can protect viral dsRNA recognition by pattern recognition receptors of the innate immune system. van Hemert et al. (149) isolated the SARS-CoV-1 RTC in an *in vitro* system and proved that cytoplasmic host factors are required for RTC activity; moreover, the membrane structure of replication organelles protects the vRNA and RTC activity from protease and nuclease digestion. V'kovski et al. (150) engineered a recombinant MHV harboring a biotin ligase to the RTC and identified more than 500 RTC-proximal host proteins that were involved in vesicular ER-Golgi apparatus trafficking pathways, ubiquitin-dependent and autophagy-related processes, and translation initiation factors. Moreover, viral nsp2 to -10, nsp12 to -16, and the N protein were also enriched in the microenvironment of the coronavirus RTC. They also found that the silencing of eukaryotic initiation factor 3e (eIF3e), eIF3f, and eIF3i was significantly decreased in MHV replication and moderately affected cellular translation, suggesting that a number of the translation initiation factors have an important function in coronavirus replication.

VIRAL ASSEMBLY AND EGRESS

Coronavirus virion assembly and budding occur in the ER-Golgi intermediate compartment (ERGIC) (Fig. 1). S, M, E, and N are major coronavirus structural proteins. ER membranes are decorated by viral S, M, and E proteins, and the mature virions containing the viral genome are encapsidated by N protein. The vRNPs may be recruited to the membrane by the N protein and contribute to membrane curvature. Moreover, the S protein alone cannot initiate virus budding (81). The M proteins of SARS-CoV-1 and SARS-CoV-2 alone also cannot induce virion assembly (151). For virus-like particle production, the M protein of SARS-CoV-1 must be coexpressed with the E and N proteins. However, virus-like particle formation in MHV is N independent (152).

E is the smallest integral membrane protein in coronavirus. Nevertheless, it plays multiple roles in virion assembly and release. It may induce membrane curvature, and it has ion channel activity. E prevents M from aggregating into insoluble complexes in an E palmitoylation-dependent manner (153). M determines the shape of the virion envelope and is the most abundant structural protein in coronavirus; it might play a central role in virion assembly since most structural proteins interact with it. M forms homodimers, and two distinct conformations were observed by cryo-EM (154). One conformation is elongated, enters into contact with RNP, and participates in membrane rigidity, uniform curvature, and spike protein incorporation. The other conformation is compact and regulates spike protein flexibility and maintains its low density. Newly synthesized S protein is transported into the ER via the signal peptide at its N terminus. There, it is modified by *N*-glycosylation. The mature S protein is a trimer, and

terminal glycosylation occurs after trimerization (155). The S protein trimers are transported to the ERGIC where virion assembly occurs. S protein processing is also influenced by the other viral structural proteins. Boson et al. (117) reported that E and M of SARS-CoV-2 can induce S retention in the Golgi/ERGIC compartments as well as S protein maturation and *N*-glycosylation. Virion particles are trafficked to the Golgi apparatus and the *trans*-Golgi network (TGN) where S is separated into S1 and S2 subunits by furin-like host cell protease. After protease processing, virion particle egress proceeds via lysosomal trafficking instead of the biosynthetic secretory pathway (156).

HIGH-THROUGHPUT SCREENING IDENTIFICATION OF HOST CELL FACTORS REQUIRED FOR CORONAVIRUS REPLICATION

Elucidation of the roles of host factors in viral infection may help clarify virus-host interactions and identify potential antiviral targets. Several genome-wide CRISPR studies have been conducted to identify host factors involved in SARS-CoV-2 and other coronavirus infections (Table 2). Daniloski et al. (157) found that most genes were clustered into the vacuolar-ATPase proton pump, the endosomal protein-sorting retromer complex, the endosomal trafficking commander complex, or the phosphatidylinositol 3-kinase (PI3K) pathway, which are involved in viral membrane fusion and endosome recycling. Inhibitors targeting PIK3C3 can reduce SARS-CoV-2 by >1,000-fold. In *RAB7A* knockout cells, viral entry was inhibited as the ACE2 receptor was sequestered in endosomal vesicles. In addition, affinity purification mass spectrometry revealed that Rab7A interacted with SARS-CoV-2 nsp7 (142). Wang et al. (158) found that cholesterol is required for S-mediated SARS-CoV-2 entry. Compounds targeting the PI3K, MBTPS1, KEAP1-NRF2, and cholesterol pathways exhibited different degrees of inhibitory efficacy against SARS-CoV-2, HCoV-229E, and HCoV-OC43. Lipid homeostasis and the cholesterol biosynthesis pathway apparently affect SARS-CoV-2 (157–160). However, the precise mechanisms remain to be clarified. Wei et al. (161) reported that HMGB1 regulates *ACE2* expression and is critical for SARS-CoV-2, SARS-CoV-1, and HCoV-NL63 entry. Zhu et al. (162) used genome-wide CRISPR screening with a SARS-CoV-2 spike deletion (Sdel) virus to identify SARS-CoV-2 endosomal entry-specific factors. The Sdel virus has a 21-nucleotide deletion at the S1/S2 cleavage site and enters cells via the endosome pathway. Therefore, the S1/S2 boundary of the SARS-CoV-2 spike protein modulates viral entry via the endosomal or fusion pathway. In a golden Syrian hamster model, the Sdel virus markedly reduced viral transmission. Other host factors, such as COMMD3, VPS29, VPS35, CCDC53, and CCDC22, regulate *ACE2* expression in SARS-CoV-2 infection. The cholesterol transporter gene *NPC1* regulates intracellular cholesterol traffic and is important for Sdel virus infection. The NPC1 inhibitor U18666A blocks cholesterol movement out of the lysosomes, impairs endosome traffic, and mitigates Sdel virus infection. Daniloski et al. (157) also indicated that *NPC1* may be involved in SARS-CoV-2 infection. The ER-resident protein TMEM41B facilitates autophagosome formation and is an essential host protein for HCoV-229E, HCoV-OC43, HCoV-NL63, SARS-CoV-1, MERS-CoV, and SARS-CoV-2 (160, 163, 164). However, the reported effects of TMEM41B on SARS-CoV-2 are inconsistent among studies (163–166) and must be further investigated. Trimarco et al. (164) proposed that TMEM41B may participate in HCoV-229E replication complex formation by regulating lipid membrane composition and facilitating ER membrane expansion and curvature. The role of TMEM41B in coronavirus might resemble the model proposed for flavivirus (167). Baggen et al. (163) found that TMEM106B functions in lysosomal acidification and is a proviral host factor for the replication of SARS-CoV-2 but not HCoV-229E. The *TMEM106B* expression level was higher in the airway epithelia of COVID-19 patients than in noninfected donors. Thus, *TMEM106B* upregulation increases SARS-CoV-2 infection rates. Biering et al. (168) utilized Calu-3 cells in CRISPR screening and found that mucins restricted SARS-CoV-2 infection. They also discovered that mucins were significantly upregulated in the epithelial cell fractions of bronchoalveolar lavage fluid (BALF) from COVID-19 patients. Those authors identified several genes involved in the inflammatory response, the cell cycle, and epigenetic modifiers. Goujon et al. (169) found that mucins have antiviral action against SARS-CoV-2. They also revealed that AP1G1 and

TABLE 2 Overview of genome-wide CRISPR screens for coronavirus host factors[c]

Group (reference)[a]	Cell line(s)	Library (reference)	Virus strain(s)	Validated gene(s)[b]	Pathway(s)	Inhibitor(s) tested
Daniloski et al. (157)	A549-ACE2	GeCKOv2 library (171)	SARS-CoV-2 (USA/WA-1 isolate)	ACE2 CCDC22, NPC1, RAB7A ATP6AP1, ATP6V1A, CTSL, TMPRSS2 COMMD3, PIK3C3, SNX27, VPS35 SPEN BAX, CNOT4, CXCL5, FBXO27, FBXO33, HDAC9, HNRNPC, INO80E, PCID2, PRKCA, PSMB5, SIGMAR1, TMEM165, TMPRSS13, TOR1AIP1, TRIM4, ZC3H18	Viral receptor Endosomal entry Spike cleavage and membrane fusion Endosome recycling Transcription Other functions	Cathepsin L inhibitors (Odanacatib, SID26681509, ALLN), PIK3C3 inhibitors (Serabelisib, Idelalisib, Buparlisib, PIK-III, Compound 19, SAR405, Autophinib), PRKCA inhibitors (Sotrastaurin, Enzastaurin, Tamoxifen), MMP12 inhibitors (Doxycycline, Ilomastat), BRPF1 inhibitor (GSK6853), DRD2 inhibitor (Olanzapine), MAPK3 inhibitors (Binimetinib, Mirdametinib), CALR inhibitor (Gentamicin), HDAC9 inhibitors (BRD4354, TMP195, Vorinostat, Belinostat, Panobinostat, Pracinostat), calcium channel antagonist (Amlodipine)
Wang et al. (158)	Huh7.5.1, Huh7.5.1-ACE2-IRES-TMPRSS2	GeCKOv2 library (171)	SARS-CoV-2 (USA/WA-1 isolate), HCoV-OC43, HCoV-229E	ACE2 CCZ1B, RAB7A, VPS16 MBTPS1, MBTPS2, PIKFYVE, SCAP BECN1, PIK3R4, UVRAG, VAC14 EXOC2, EXOC6, EXOC8 TMEM106B	Viral receptor Endosome maturation Cholesterol homeostasis Phosphatidylinositol kinase complexes Exocyst complex Lysosome/autophagosome-related function Other functions	PI3K inhibitor (SAR405), PIKfyve inhibitor (YM201636), SREBP activation inhibitor (Fatostatin), MBTPS1 inhibitor (PF-429242), 25-hydroxycholesterol (25-HC), KEAP1-NRF2 activator (Bardoxolone)
Hoffmann et al. (159)	Huh7.5	332 human genes that were previously identified in SARS-CoV-2 protein interactome (142)	SARS-CoV-2 (USA/WA-1 isolate), HCoV-OC43, HCoV-229E, HCoV-NL63	ARF5 RAB2A, RAB7A, VPS11, VPS39 EMC1, SCAP, NPC2, SREBF1, SREBF2 RAB10, RAB14 GPAA1, PIGO, PIGS HS2ST1, INSIG1, MIB1, RAB18, RAB1A, RAB8A, RAB5C	Other functions Viral receptor Common Rabs and HOPS Cholesterol homeostasis SARS-CoV-2 Rabs GPI biosynthesis Other functions	NA
Schneider et al. (160)	Huh7.5	Brunello genome-wide CRISPR library (172)	SARS-CoV-2 (USA/WA-1 isolate), HCoV-OC43, HCoV-NL63, HCoV-229E	ACE2, ANPEP MBTPS1, MBTPS2, SREBF2 EXT2 PIK3C3, VAC14 COG3 BMPR1A, CDX2, DDX1, DHX36, EGLN1, EP300, NRIP1, OR1A1, RAB7A, RIC1, RTCB, SLC30A1, SLC3949, TM9SF3, TMEM106B, TMEM41B, VMP1, VRK1	Viral receptor SREBP control of lipid biosynthesis Glycosaminoglycan biosynthesis: heparin sulfate PIP biosynthesis at late endosome COG and exocyst complex Other functions	NA
Wei et al. (161)	Vero E6	African green monkey genome-wide CRISPR knockout library (CP0070) designed according to the Brunello human genome-wide library (173)	SARS-CoV-2 (USA/WA-1 isolate), rcVSV-SARS-CoV-2-S, HKU5-SARS-CoV-1-S, MERS-CoV (WT and T1015N)	ACE2 ARID1A, SMARCA4, SMARCE1, SMAD3, SMAD4 CTSL CABIN1, HIRA SMARCA5 ACVR1B, ATRX, DOLK, DPF2, DYRK1A, HMGB1, JMJD6, KDM6A, PCBD1, PHF6, PHIP, PIAS1, PIAS2, RAD54L2, SIAH1, UBXN7	Viral receptor SWI/SNF complex RUNX3 regulates CDKN1A transcription Cystatin and endolysosome lumen Regulator of histone variant H3.3 NURF complex Other functions	Cathepsin L inhibitor (Calpain inhibitor III), SMARCA4 and SMARCA2 inhibitor (PFI-3), SMAD3 inhibitor (SIS3)

(Continued on next page)

TABLE 2 (Continued)

Group (reference)[a]	Cell line(s)	Library (reference)	Virus strain(s)	Validated gene(s)[b]	Pathway(s)	Inhibitor(s) tested
Zhu et al. (162)	A549-ACE2	Brunello genome-wide CRISPR library (Addgene no. 73178) (172)	Patient-isolated SARS-CoV-2 SH01 strain	ACE2	Viral receptor	TMPRSS2 inhibitor (Camostat mesilate), Cathepsin B and L inhibitor (aloxistatin), NPC1 inhibitor (U18666A)
				CTSL	Membrane fusion	
				C18orf8, CCZ1, CCZ1B, RAB7A, VSP29, VSP35	Retromer-associated genes	
				C16orf62, CCDC22, CCDC93, COMMD10, COMMD2, COMMD3-BMI1, COMMD3, COMMD4, COMMD5, COMMD7, COMMD8	CCC complex	
				CCDC53, KIAA0196, KIAA1033, ACTR2, ACTR3, ARPC3, ARPC4	WASH complex / Arp2/3 complex	
				NPC1, NPC2	Cholesterol trafficking	
				ASCC3, TFE3, WDR81, WDR91	Other functions	
Baggen et al. (163)	Huh7	Brunello genome-wide CRISPR library (Addgene no. 73179) (172)	SARS-CoV-2 (SARS-CoV-2/Belgium/GHB-03021/2020), HCoV-229E	ACE2, ANPEP, EXT1, ITGB6	Viral receptor/heparan sulfate synthesis	PI3K type 3 inhibitors (VPS34-IN1, VPS34-IN2, SAR405, autophinib)
				CCZ1B, PTPN23, RSG1	Membrane/vesicle trafficking	
				PIK3C3, TMEM41B	Endocytic trafficking and autophagy	
				OSBPL9, PTDSS1, TMEM30A	Lipid homeostasis	
				TMEM106B	Lysosome/autophagosome-related function	

ATP8B1 are cofactors for SARS-CoV-2, MERS-CoV, HCoV-NL63, and HCoV-229E. Kratzel et al. (166) indicated that TMEM41B, FKBP8, and MINAR1 are cofactors for MERS-CoV, HCoV-229E, SARS-CoV-1, and SARS-CoV-2. However, they used genome-wide CRISPR screening for MERS-CoV and HCoV-229E in Huh7 cells. The three above-described genes may be implicated in vesicle formation during autophagy. Grodzki et al. (165) also identified *TMEM41B* and *RAB7A*. They demonstrated that CCZ1 and EDC4 were required for SARS-CoV-2 and HCoV-OC43 infection in *CCZ1* or *EDC4* knockdown cells. However, CTSL could not promote HCoV-OC43 infection. Those authors used several inhibitors targeting the cell cycle and the endocytosis pathway and showed that both are critical for coronavirus infection. Kung et al. (170) found that ACSL4-mediated ferroptosis is required for HCoV-229E infection. They identified host factors using CRISPR loss-of-function screening with coxsackievirus A6. Ferroptosis inhibitors suppressed HCoV-229E, HCoV-OC43, and SARS-CoV-2. Daniloski et al. (157) also reported the upregulation of the pathway affecting ferroptosis in selected target gene-perturbed cells, and the observations were summarized in a heat map. Vero E6, Huh7, Huh7.5, A549-*ACE2*, Calu-3, and other cell lines used to identify host proteins involved in coronavirus might have influenced the results. Several overlapping genes, including *RAB7A*, *PIK3C3*, *NPC1*, *COMMD3*, *TMEM106B*, *TMEM41B*, and others, were identified among different groups and coronaviruses. These factors are potential targets for antiviral drug development.

CONCLUDING REMARKS

The COVID-19 pandemic remains a global threat despite the growing numbers of fully vaccinated individuals. SARS-CoV-2 variants are still public health concerns. Moreover, neutralization antibody titers decline with time after vaccination; consequently, the number of breakthrough infections is increasing. Therefore, efficacious antiviral treatments against COVID-19 are urgently required. Clarification of the molecular mechanisms of SARS-CoV-2 and virus-host interactions will reveal how the virus manipulates the host cell into promoting viral replication. Antiviral drugs should target host factors or the virus itself. Nevertheless, drug resistance readily occurs as RNA viruses have high mutation rates. Therefore, antiviral drugs targeting host factors might be efficacious against viral infection. Genome-wide CRISPR screening can help identify host factors essential for coronavirus infection. Genes and pathways enriched during coronavirus infection were previously identified; however, their precise mechanisms in the viral replication cycle remain to be elucidated. CRISPR screening has also been used to identify inhibitors that efficiently decrease viral titers. Nevertheless, the lag time until the clinical application of these inhibitors could be very long. Therefore, accelerated clinical testing of these inhibitors is crucial for effective and timely responses to the SARS-CoV-2 pandemic. Future research should endeavor to find antiviral agents with substantial activity against SARS-CoV-2.

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