



Understanding Individual SARS-CoV-2 Proteins for Targeted Drug Development against COVID-19

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ABSTRACT Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the COVID-19 pandemic, responsible for millions of deaths globally. Even with effective vaccines, SARS-CoV-2 will likely maintain a hold in the human population through gaps in efficacy, percent vaccinated, and arising new strains. Therefore, understanding how SARS-CoV-2 causes widespread tissue damage and the development of targeted pharmacological treatments will be critical in fighting this virus and preparing for future outbreaks. Herein, we summarize the progress made thus far by using *in vitro* or *in vivo* models to investigate individual SARS-CoV-2 proteins and their pathogenic mechanisms. We have grouped the SARS-CoV-2 proteins into three categories: host entry, self-acting, and host interacting. This review focuses on the self-acting and host-interacting SARS-CoV-2 proteins and summarizes current knowledge on how these proteins promote virus replication and disrupt host systems, as well as drugs that target the virus and virus interacting host proteins. Encouragingly, many of these drugs are currently in clinical trials for the treatment of COVID-19. Future coronavirus outbreaks will most likely be caused by new virus strains that evade vaccine protection through mutations in entry proteins. Therefore, study of individual self-acting and host-interacting SARS-CoV-2 proteins for targeted therapeutic interventions is not only essential for fighting COVID-19 but also valuable against future coronavirus outbreaks.

KEYWORDS SARS-CoV-2, COVID-19, drug development, individual virus proteins, virulence factors, virus-host interactions, Nsp1, Nsp6, Orf3a, Orf6, Orf7a

Coronaviruses (CoVs) have been circulating among the human population for centuries and cause a portion of the common colds (human CoV HKU1 [hCoV-HKU1], hCoV-OC43, hCoV-229E, and hCoV-NL63). However, more recently, three CoVs have caused serious threats to global human health: severe acute respiratory syndrome coronavirus (SARS-CoV; 2002 to 2003) and Middle East respiratory syndrome coronavirus (MERS-CoV; 2012 to present) outbreaks and the ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; 2019 to present) pandemic. These coronaviruses share many features; however, marked differences in sequence, structure, and function have been identified. In order to understand how these viruses cause disease and to identify the most effective targets for therapeutic intervention, we need to identify the sites of virus-host interaction and their impact on host systems. Studies of individual virus proteins will be crucial to accomplish this.

Virus-encoded proteins, similar to those from higher-order organisms, carry out specialized functions, with shared and unique features found across virus families. Because viruses are dependent on host mechanisms for replication, these specializations are visible in virus-host interactions as well, including various tactics to manipulate host systems for immune evasion. For human immunodeficiency virus (HIV) (Vpr, Tat, and Nef proteins), influenza virus (M2 and NS1 proteins), and Zika virus (ZIKV) (NS4A protein), to name just a

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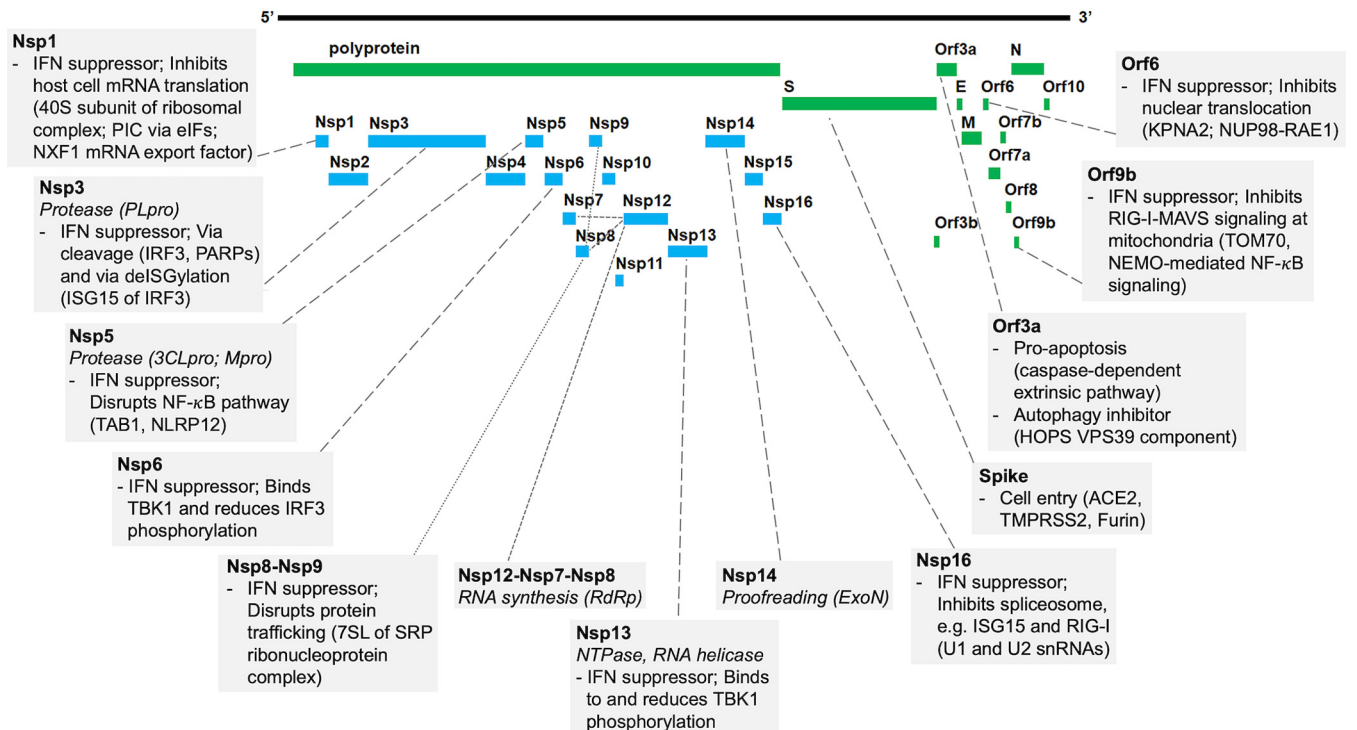


FIG 1 SARS-CoV-2 protein functions and host interactions. Shown is a graphic representation of the SARS-CoV-2 proteome. Boxes connected to proteins indicate the virus transcription and translation functions (italics) (summary of Table 1) and known human protein interaction with their effect on human pathways (summary of Table 2). Abbreviations: 3CLpro, 3-chymotrypsin-like protease; 7SL, RNA component of SRP ribonucleoprotein complex; ACE2, angiotensin I-converting enzyme 2; eIFs, eukaryotic translation initiation factors; ExoN, exoribonuclease; HOPS, homotypic fusion and protein sorting complex; IFN, interferon; IRF3, interferon regulatory factor 3; ISG, interferon-stimulated gene; KPNA2, karyopherin alpha 2; MAVS, mitochondrial antiviral-signaling protein; Mpro, main protease; NEMO, NF-κB essential modulator; NLRP12, NLR family pyrin domain containing 12; NUP98, nuclear pore complex protein 98; NXF1, nuclear RNA export factor 1; PARPs, poly(ADP-ribosyl) polymerases; PIC, ribosomal preinitiation complex; PLpro, papain-like protease; RAE1, RNA export 1; RdRp, RNA-dependent RNA polymerase; RIG-I, retinoic acid-inducible gene I; SRP, signal recognition particle; TAB1, TGF-β-activated kinase 1 (MAP3K7) binding protein 1; TBK1, TANK binding kinase 1; TMPRSS2, transmembrane serine protease 2; TOM70, translocase of outer membrane 70 (subunit of mitochondrial import receptor); VPS39, vacuolar protein sorting 39 (subunit of HOPS complex).

few human viruses, studies of individual virus proteins have revealed a tremendous amount about specific virus-host interactions, effects on host pathways, and resulting detrimental functional consequences in tissue-specific pathology (1–7). These studies have revealed the importance of individual virus proteins to pathogenicity by implicating host pathways enlisted to facilitate the virus replication cycle and to promote virus gene expression, hijacking of the host proteasome function, evasion of the host immune response (NF-κB; JAK/signal transducer and activator of transcription [STAT] signaling), and the role of host endocytic and secretory trafficking pathways, as well as the contribution of host-modifying factors. More importantly, these findings have provided a foundation for the development of new therapeutics driven by pharmacological inhibitors of specific virus-host interactions. The first of these compounds are entering clinical trials (for example, GS-6207, which targets the HIV-1 capsid protein).

COVID-19, the disease caused by SARS-CoV-2 infection, is notable for its detrimental effect on multiple organs and tissues. In-depth studies of how individual virus proteins disrupt host pathways will be crucial to identify the targets most effective for countering tissue damage and to develop inhibitors of these specific virus-host interactions for therapeutic intervention. In addition, these studies would provide insight into the differences between viral strains, e.g., why SARS-CoV-2 is so virulent, and aid in preparing for a future outbreak. This review covers studies available through PubMed prior to mid-February 2021.

SARS-CoV-2 PROTEOME

Genomic organization. The SARS-CoV-2 genome encodes 28 confirmed proteins (Fig. 1). Open reading frame 1ab (Orf1ab), by far the largest gene, is located at the 5'

end and encodes polyproteins PP1ab and PP1a. These polyproteins are cleaved into 16 nonstructural proteins (Nsp1 to Nsp16). The remaining 3' sequence contains genes encoding the four structural proteins (spike [S], envelope [E], membrane [M], and nucleocapsid [N]) and eight accessory proteins (Orf3a, Orf3b, Orf6, Orf7a, Orf7b, Orf8, Orf9b, [Orf9c lies outside verified SARS-CoV-2 open reading frame] and Orf10). Notably, sequence conservation among the three most pathogenic human CoVs is not uniform across the genome. Overall conservation is highest for the polyprotein Nsp proteins, while the genome sequence encoding the accessory factors (Orfs) diverges decidedly (8). MERS-CoV (C lineage beta-CoV) shows most differences and even has fewer Orfs than SARS-CoV and SARS-CoV-2 (B lineage beta-CoV). These accessory factors typically lack well-defined domain structures, which leaves their functions largely unresolved and suggests that they might hold the key to the properties unique to each virus. Based on the literature on SARS-CoV-2 protein functions and interaction with host cells, we grouped the virus proteins into three categories: host entry, self-acting, and host interacting. We review each group and focus on individual SARS-CoV-2 proteins that can be targeted either directly by drugs or through inhibiting their host interactions.

Host entry: virus spike protein and host factors to gain cell entry. The interaction of the SARS-CoV-2 spike with the host cell receptor at the host membrane has been a topic of great interest, especially in the context of transmission and infectivity. Due to its wide coverage in the literature, we keep it brief here and instead focus in more detail on the intracellular virus-host interactions and their pathogenic effects on host systems. The virus employs interaction of its spike protein with host plasma membrane receptors and proteases to facilitate membrane fusion and gain host cell entry (Fig. 1). The spike proteins of both SARS-CoV and SARS-CoV-2 use the host angiotensin I-converting enzyme 2 (ACE2) receptor, while the MERS-CoV spike interacts with the host dipeptidyl peptidase (DPP4; also known as CD26) receptor to do this (9). The spike protein is generally considered well preserved; however, SARS-CoV-2 spike protein binding affinity for the ACE2 receptor is significantly stronger than that of its SARS-CoV counterpart, which has been attributed to a structural rearrangement (10). Moreover, numerous mutations have been identified in the SARS-CoV-2 spike protein since the start of the pandemic, just over a year ago (11, 12). Both SARS-CoV-2 and SARS-CoV make use of the host transmembrane protease serine 2 (TMPRSS2) for cleavage of the spike protein trimer at the S1/S2 site upon virus-host cell contact to engage the necessary conformational change prior to cell entry (13). In addition, SARS-CoV-2 contains a furin cleavage site. This site is new to the B-lineage hCoVs, which include SARS-CoV, but has been known from other highly virulent human viruses (such as influenza, measles, mumps, and Ebola viruses and HIV-1) (14, 15). In fact, it has been shown that loss of this furin cleavage site reduces SARS-CoV-2 pathogenesis *in vitro* (16). Furthermore, it was shown this furin-cleaved site provides a substrate for neuropilin-1 (NRP1), which acts as a cofactor for ACE2-mediated fusion (17). NRP1 is highly expressed in pulmonary and olfactory tissues, and infected olfactory epithelial cells in COVID-19 patients were shown to be NRP1 positive (17). These data suggest that NRP1 could act as a crucial cofactor for virus entry in cells with low ACE2 expression, like olfactory epithelium, thereby enhancing SARS-CoV-2 tropism (i.e., ability to infect different cell types and tissues).

Self-acting: SARS-CoV-2 proteins dedicated to virus translation and replication. Viruses greatly depend on host systems for translation and replication; therefore, many of their proteins have evolved to interact with host proteins. These interactions target specific host proteins in order to hijack host pathways and create a favorable cellular environment. However, the virus genome also encodes several proteins specialized in virus replication. These carry out the virus-focused tasks of mRNA transcription, translation, and encapsulation (Table 1; Fig. 1). Among these replication-focused proteins are papain-like cysteine protease (PLpro; encoded within the Nsp3 gene) and 3-chymotrypsin-like protease (3CLpro; also known as main protease [Mpro]; encoded by the Nsp5 gene). PLpro cleaves Nsp1, Nsp2, and Nsp3 on Orf1a, while 3CLpro hydrolyzes the remaining Nsp4 to -16 units on the 1a and 1ab SARS-CoV-2 polyproteins. This step

TABLE 1 SARS-CoV-2 proteins for virus transcription and translation^a

SARS-CoV-2 protein(s)	Protein/complex role	Function	Compared to SARS-CoV	Model system(s) ^b	Reference(s)
Nsp3 (PLpro)	Papain-like cysteine protease (PLpro)	Proteolytic cleavage of Nsp1 to -3 of Orf1a for functional maturation	Inhibitors specific for SARS-CoV PLpro activity, similarly, showed efficacy against SARS-CoV-2 PLpro	<i>In vitro</i> ; cell lines, HEK293T and Vero CCL-81; cell-free system, <i>Escherichia coli</i>	20
Nsp5 (3CLpro)	3-Chymotrypsin-like protease (3CLpro), also known as main protease (Mpro)	Proteolytic cleavage of Orf1a and Orf1ab polyproteins for functional maturation of virus Nsp proteins (Nsp4 to -16), including itself	Compound (GRL-0496) effectively inhibits both SARS-CoV and SARS-CoV-2 3CLpro activity; anti-SARS-CoV 3CLpro antiserum cross-reactive with SARS-CoV-2 3CLpro	<i>In vitro</i> ; cell lines, HEK293T(17), VeroE6, and Caco-2 (ACE2/TMPRSS2)	19, 21
Nsp12-Nsp7-Nsp8 (polymerase complex)	RdRp complex	Nsp12 is the catalytic subunit with RdRp (RNA synthesis) activity. Nsp7 and Nsp8 cofactors are required to boost efficiency.	Reduced RNA synthesis activity and lower thermostability than with the SARS-CoV core polymerase complex	Cryo-EM	23, 54, 55
Nsp13	NTPase and RNA helicase activities	Viral RNA processing at many steps (replication, transcription, translation, and encapsidation)	Highly similar sequences; both have NTPase and RNA helicase activities; activity of both can be inhibited by bismuth salts in a dose-dependent manner	Cell-free system, <i>Escherichia coli</i>	22
Nsp14	Proofreading exoribonuclease (ExoN) + RNA recombination	RNA proofreading during virus replication (resistance to nucleoside analogs) and RNA recombination, which drives immune—and vaccine—evasion (generation of subgenomic mRNAs, DVG, and novel strain emergence)	Similar recombination networks (pattern of recombination junctions across the genomes and flanking nucleotide sequences, including DVG junctions, as well as types of recombined RNAs generated)	<i>In vitro</i> ; cell lines, DBT-9, Vero CCL-81, 7 and VeroE6	24

^aAbbreviations: RdRp, RNA-dependent RNA polymerase; cryo-EM, cryo-electron microscopy; DVG, defective viral genome.

^bDBT-9, delayed brain tumor, murine astrocytoma clone 9 cell line; HEK293T, human embryonic kidney cell line; VeroE6/Vero CCL-81, African green monkey (*Cercopithecus aethiops*) kidney cell lines.

is essential for maturation of the nonstructural proteins into functional proteins for virus replication and transcription, as well as to suppress the host immune system. Furthermore, this process enables the virus to carry a larger genome (18–21). Nsp13 possesses NTPase and RNA helicase activities; consequently, it facilitates many steps of viral RNA processing, including replication, transcription, translation, and encapsidation (22). Another key virus-encoded factor for replication is the RNA-dependent RNA polymerase (RdRp) complex, which is responsible for the synthesis of viral RNA. The SARS-CoV-2 complex consists of Nsp12, which forms the catalytic power core (although it is highly inefficient when by itself), and the Nsp7 and Nsp8 cofactors, which greatly boost its polymerase activity (23). Nsp14 provides proofreading services during RNA replication. It detects and then removes any mismatched nucleotides using its exonuclease (ExoN) function, which possibly also contributes to virus RNA recombination (24). The latter is a common strategy among viruses to generate new strains that are better at evading the host immune response, less susceptible to vaccines, or might cross over to new host species.

Host interacting: SARS-CoV-2 proteins targeting host proteins and the effects on host systems. Viruses carry relatively small genomes and largely depend on host cell machinery for their survival. Therefore, the vast majority of virus proteins, including some of the ones dedicated to virus transcription and translation described above, have evolved to interact with specific host proteins (Table 2; Fig. 1). Unlike the spike-ACE2/TMPRSS2/furin interaction to gain host cell entry, the majority of virus-host interactions take place intracellularly and aim to gain control over host cell systems. Host processes are employed for virus replication (transcription/translation and related protein trafficking), to create a virus-favorable environment (shutting down host systems to divert cellular resources to virus replication) and evasion of the host immune response.

(i) Nsp1. One of the first virus genes translated is the *Nsp1* gene. SARS-CoV Nsp1 is known to be a potent inhibitor of the interferon (IFN)-mediated innate immune response, to disrupt the cell cycle and to suppress host gene expression. Therefore, it has been a protein of great interest for SARS-CoV-2 studies. The Nsp1 protein also inhibits host gene expression; it does this by binding the host 40S subunit in ribosomal complexes, thereby blocking the mRNA entry channel, which inhibits the host mRNA translation machinery, which consequently disrupts the retinoic acid-inducible gene I-dependent innate immune responses (reduced IFN- β and interleukin 8 [IL-8] gene expression) (25–27). In addition, several eukaryotic translation initiation factors (eIFs) have been shown to allosterically modulate SARS-CoV-2 Nsp1 interaction with ribosomal preinitiation complexes (PICs) in the absence of mRNA, which provides another means to arrest host protein synthesis (28). And it has been shown that Nsp1 prevents physiological conformation of the 48S PIC by restricting rotation of the ribosome head domain (29). Further, it has been recently reported that SARS-CoV-2 Nsp1 directly binds the mRNA export factor NXF1 (30). This interferes with NXF1 interactions with mRNA export adaptors and the nuclear pore complex, effectively blocking host mRNA export. Thus, it seems that SARS-CoV-2 Nsp1 exerts multiple complementary strategies to take control of host cell systems.

(ii) Nsp3. Like its MERS-CoV and SARS-CoV counterparts, SARS-CoV-2 Nsp3 possesses deubiquitinating/deISGylating protease activity in addition to its PLpro (31–33). These provide another virus tactic to repress the host innate immune response, marked by reduced cytokine and chemokine production. SARS-CoV-2 Nsp3 has been shown to cleave ISG15 from interferon-regulatory factor 3 (IRF3), thereby inhibiting the host type I IFN (IFN-I) immune response (34, 35). Another strategy by which Nsp3 suppresses the host immune response is through countering poly(ADP-ribose) polymerases (PARPs). PARPs are among the first actors of the immune pathway, and several human PARPs are known to induce the IFN innate immune response. They carry out ADP-ribosylation of proteins and nucleic acids in order to establish an antiviral environment. In turn, viruses gain (ADP-ribose) hydrolase activity, which cleaves the host protein ADPr bond, to oppose this host protective mechanism. The macrodomain

TABLE 2 SARS-CoV-2 proteins interacting with intracellular host pathways^a

SARS-CoV-2 protein	Virus-host interaction	Effects on host ^b	Model system(s)	Reference(s)
Nsp1	40S subunit of ribosomal complex; ribosomal PIC via eIFs; blocking mRNA entry channel	mRNA translation machinery; inhibition of host cell mRNA translation; disruption of retinoic acid-inducible gene I-dependent innate immune responses (reduced IFN- β and IL-8 gene expression) mRNA translation machinery; inhibition of host cell mRNA translation	<i>In vitro</i> ; cell lines, HEK293T, HeLa, HEK293-EBNA, VeroE6, HEK-Blue-IFG, A549, Calu-3, HAP1, and H1299; cell-free system, <i>Escherichia coli</i>	25-29
Nsp3 (PLpro)	mRNA export factor NXF1; disrupts NXF1 interaction with mRNA export adaptors and nuclear pore complex IRF3; reduced deISGylation (ISG15) of IRF3; also, cleavage of IRF3	Innate immune response (IFN); indirect (deISGylation) and direct (cleavage) effects on the IRF3 pathways; strongly inhibits IRF3 activity and results in complete suppression of the type I IFN response	<i>In vitro</i> ; cell lines, HEK293T and VeroE6	30
	PARP5; macrodomain part of Nsp3 is an (ADP-ribosyl) hydrolase	Innate immune response (IFN); cleaves protein-ADP; bond to counteract host antiviral PARPs, which are induced during the IFN immune response	<i>In vitro</i> , cell lines, HeLa, A549, Caco-2, and 293T-ACE2; cell-free system, <i>Leishmania tarentolae</i>	34, 35
Nsp5 (3CLpro)	TAB1; cleavage of TAB1 results in loss of C terminus NLRP12; cleavage of NLRP12 (two cleavage sites)	Innate immune response (proinflammatory cytokines); cleaved TAB1 leads to reduced TAK1 activation, decreased NF- κ B signaling, and decreased cytokine production Innate immune response (proinflammatory cytokines); NLRP12 inhibits NF- κ B; cleaved NLRP12 leads to increased NF- κ B signaling and thus increased cytokine production and disrupts assembly of the NLRP12/NLRP3 inflammasome, increasing pro-caspase-1 cleavage and thus IL-1 β release; together, they might underly hypercytokinemia	Cell-free system, <i>Escherichia coli</i>	36, 37
	TBK1	Innate immune response (IFN); TBK1 binding suppresses IRF3 phosphorylation, which leads to reduced IFN production	<i>In vitro</i> ; cell line, 293T-ACE2; cell-free system, <i>Leishmania tarentolae</i>	34
Nsp6	Related host interactor undetermined	Related host pathway undetermined. Nsp6 induced cytotoxicity <i>in vitro</i> , and in flies induced early lethality, reduced trachea (lung equivalent) branching, muscle defects and reduced muscular mitochondria	<i>In vitro</i> ; cell line, 293T-ACE2; cell-free system, <i>Leishmania tarentolae</i>	34
	7SL RNA component of the signal recognition particle (SRP); directly binds 7SL RNA of the SRP	Innate immune response (IFN); disrupts global membrane protein trafficking and protein integration into the cell membrane; SRP-dependent transport is key to the IFN immune response (reduced SRP function resulted in a reduced IFN response)	<i>In vitro</i> ; cell lines, HEK293T, Vero CCL-81, BHK-21, Huh-7, and A549	38
Nsp8-Nsp9	TBK1	Innate immune response (IFN); TBK1 binding and suppression of TBK1 phosphorylation, which leads to reduced IFN production	<i>In vivo</i> (<i>Drosophila</i>); <i>in vitro</i> ; cell line, HEK293T	42, 46
Nsp13	TBK1	Innate immune response (IFN); TBK1 binding and suppression of TBK1 phosphorylation, which leads to reduced IFN production	<i>In vitro</i> ; cell lines, HEK293T, Calu-3 (SARS-CoV-2), and VeroE6 (SARS-CoV-2)	25
			<i>In vitro</i> ; cell lines, HEK293T, Vero CCL-81, BHK-21, Huh-7, and A549	38

(Continued on next page)

TABLE 2 (Continued)

SARS-CoV-2 protein	Virus-host interaction	Effects on host ^b	Model system(s)	Reference(s)
Nsp16	U1 and U2 snRNA components of the spliceosome; directly binds U1 and U2 snRNAs	Innate immune response (IFN); inhibits global mRNA splicing; reduces host innate immune response via increased intron retention in IFN-responsive genes (e.g., ISG15 and RIG-I genes)	<i>In vitro</i> ; cell lines, HEK293T and Calu-3 (SARS-CoV-2)	25
Orf3a	Extrinsic apoptotic pathway	Apoptosis (immune response?); induces cleavage/activation of caspase-8 (Bcl-2 of intrinsic pathway not affected); activated caspase-8 truncates Bid to tBid (increased), which induces release cytochrome c; this leads to apoptosome formation and caspase-9 cleavage/activation and ultimately apoptosis	<i>In vitro</i> ; cell lines, HEK293T, VeroE6, and HepG2	39
Orf7a	Components VPS39 of HOPS complex; directly binds HOPS VPS39 component	Immune response (autophagy); binding HOPS VPS39 prevents formation of the autophagosomal STX17-SNAP29-VAMP8 SNARE complex, thus blocking autophagy and enabling virus to evade lysosomal degradation	<i>In vitro</i> ; cell lines, HeLa with human ACE2 (SARS-CoV-2) and HEK293T	40
Orf6	KPNA2	Innate immune response (IFN); binds KPNA2 and binds C-terminal domain of NUP98-RAE1 nuclear pore complex, which blocks docking of karyopherin/importin cargo-receptor complex and prevents STAT1 and STAT2 nuclear translocation, thereby inhibiting the IFN immune response; in flies, Orf6 induced early lethality, reduced trachea (lung equivalent) branching, muscle defects, and reduced muscular mitochondria	<i>In vivo</i> (<i>Drosophila</i>)	38, 41
Orf7a	NUP98-RAE1 nuclear pore complex; directly interacts with NUP98-RAE1 nuclear pore complex ^c	Related host pathway undetermined. Nsp6 induced cytotoxicity <i>in vitro</i> , and in flies induced early lethality, reduced trachea (lung equivalent) branching, muscle defects, and reduced muscular mitochondria	<i>In vitro</i> ; primary cells, HBEC; cell lines, VeroE6, Calu-3, HEK293T, liver-derived (Huh7 and Hep3B); lung-derived (NCI-H210 A549, NCI-H1975), HRT-18G, and BHK-21J	42, 46
Orf7a	Related host interactor undetermined	Related host pathway undetermined. Nsp6 induced cytotoxicity <i>in vitro</i> , and in flies induced early lethality, reduced trachea (lung equivalent) branching, muscle defects, and reduced muscular mitochondria	<i>In vivo</i> (<i>Drosophila</i>)	42, 46
Orf9b	TOM70; interacts with TOM70	Innate immune response (IFN); mitochondrial, TOM70, and IFN immune response	<i>In vitro</i> ; cell line, HEK293T	43, 47
	NEMO; targets NEMO, an essential modulator of NF- κ B, and inhibits its activation by I κ B-linked polyubiquitination in response to viral infection	Innate immune response (IFN); disrupts NEMO-mediated NF- κ B signaling-induced IFN immune response	<i>In vitro</i> ; primary cells, HPAEpic; cell lines, VeroE660, HEK293T, Caco-2, BEAS-2B, Calu-3, and NCI-H1299	48

^aResults in bold are results of *in vivo* study. 3CLpro, 3-chymotrypsin-like protease; IFN, interferon; PI3K, phosphatidylinositol 3-kinase; RAB, rab GTPase; RPL, ribosomal protein L; RPS, ribosomal protein S; RPLP0, ribosomal protein L1; RPLP2, ribosomal protein L2; RPLP3, ribosomal protein L3; RPLP4, ribosomal protein L4; RPLP5, ribosomal protein L5; RPLP6, ribosomal protein L6; RPLP7, ribosomal protein L7; RPLP8, ribosomal protein L8; RPLP9, ribosomal protein L9; RPLP10, ribosomal protein L10; RPLP11, ribosomal protein L11; RPLP12, ribosomal protein L12; RPLP13, ribosomal protein L13; RPLP14, ribosomal protein L14; RPLP15, ribosomal protein L15; RPLP16, ribosomal protein L16; RPLP17, ribosomal protein L17; RPLP18, ribosomal protein L18; RPLP19, ribosomal protein L19; RPLP20, ribosomal protein L20; RPLP21, ribosomal protein L21; RPLP22, ribosomal protein L22; RPLP23, ribosomal protein L23; RPLP24, ribosomal protein L24; RPLP25, ribosomal protein L25; RPLP26, ribosomal protein L26; RPLP27, ribosomal protein L27; RPLP28, ribosomal protein L28; RPLP29, ribosomal protein L29; RPLP30, ribosomal protein L30; RPLP31, ribosomal protein L31; RPLP32, ribosomal protein L32; RPLP33, ribosomal protein L33; RPLP34, ribosomal protein L34; RPLP35, ribosomal protein L35; RPLP36, ribosomal protein L36; RPLP37, ribosomal protein L37; RPLP38, ribosomal protein L38; RPLP39, ribosomal protein L39; RPLP40, ribosomal protein L40; RPLP41, ribosomal protein L41; RPLP42, ribosomal protein L42; RPLP43, ribosomal protein L43; RPLP44, ribosomal protein L44; RPLP45, ribosomal protein L45; RPLP46, ribosomal protein L46; RPLP47, ribosomal protein L47; RPLP48, ribosomal protein L48; RPLP49, ribosomal protein L49; RPLP50, ribosomal protein L50; RPLP51, ribosomal protein L51; RPLP52, ribosomal protein L52; RPLP53, ribosomal protein L53; RPLP54, ribosomal protein L54; RPLP55, ribosomal protein L55; RPLP56, ribosomal protein L56; RPLP57, ribosomal protein L57; RPLP58, ribosomal protein L58; RPLP59, ribosomal protein L59; RPLP60, ribosomal protein L60; RPLP61, ribosomal protein L61; RPLP62, ribosomal protein L62; RPLP63, ribosomal protein L63; RPLP64, ribosomal protein L64; RPLP65, ribosomal protein L65; RPLP66, ribosomal protein L66; RPLP67, ribosomal protein L67; RPLP68, ribosomal protein L68; RPLP69, ribosomal protein L69; RPLP70, ribosomal protein L70; RPLP71, ribosomal protein L71; RPLP72, ribosomal protein L72; RPLP73, ribosomal protein L73; RPLP74, ribosomal protein L74; RPLP75, ribosomal protein L75; RPLP76, ribosomal protein L76; RPLP77, ribosomal protein L77; RPLP78, ribosomal protein L78; RPLP79, ribosomal protein L79; RPLP80, ribosomal protein L80; RPLP81, ribosomal protein L81; RPLP82, ribosomal protein L82; RPLP83, ribosomal protein L83; RPLP84, ribosomal protein L84; RPLP85, ribosomal protein L85; RPLP86, ribosomal protein L86; RPLP87, ribosomal protein L87; RPLP88, ribosomal protein L88; RPLP89, ribosomal protein L89; RPLP90, ribosomal protein L90; RPLP91, ribosomal protein L91; RPLP92, ribosomal protein L92; RPLP93, ribosomal protein L93; RPLP94, ribosomal protein L94; RPLP95, ribosomal protein L95; RPLP96, ribosomal protein L96; RPLP97, ribosomal protein L97; RPLP98, ribosomal protein L98; RPLP99, ribosomal protein L99; RPLP100, ribosomal protein L100.

^bBoldfacing refers to the *Drosophila in vivo* studies described in reference 46.

^cThe information for KPNA2 in "Effects on host," "Model system(s)," and "Reference(s)" also applies to NUP98-RAE1.

encoded by the SARS-CoV and SARS-CoV-2 *Nsp3* gene is responsible for this antagonistic cleavage against PARPs (36, 37).

(iii) Nsp5. The SARS-CoV-2 *Nsp5* gene encodes a 3C-like protease (3CLpro) which is crucial for virus replication by cleaving the Orf1a/ab polyprotein into functional Nsp units. And, similar to Nsp3 (PLpro), it has been shown to also cleave highly select host proteins: transforming growth factor β (TGF- β)-activated kinase 1 (MAP3K7) binding protein 1 (TAB1) and NLR family pyrin domain containing 12 (NLRP12) (34). TGF- β , IL-1, and tumor necrosis factor alpha (TNF- α) respond to viral infection and activate the TAB1/2/3/TAK1 complex. 3CLpro cleavage of TAB1 results in loss of its C terminus and disrupts complex formation. This results in reduced TGF- β -activated kinase 1 (TAK1) and subsequent diminished NF- κ B and MAP kinase signaling, ultimately reducing cytokine release (34). NLRP12 is an inhibitor of NF- κ B; cleavage by SARS-CoV-2 3CLpro releases this brake, leading to increased NF- κ B signaling and increased production of proinflammatory cytokines (34). In addition, NLRP12 cleavage disrupts inflammasome NLRP3/12 formation and might induce cleavage of pro-caspase-1, thereby enhancing release of IL-1 β (34).

(iv) Nsp6 and Nsp13. Evading the IFN-mediated early immune signaling pathway is a common strategy among viruses. Multiple studies reported screens to identify the individual SARS-CoV-2 proteins that antagonize IFN pathway signaling. These have implicated multiple virus proteins but typically have not investigated the virus-host interactions or pathways through which this antagonistic effect is conveyed. That being said, one study recognized two distinct mechanisms aimed at evading the IFN immune response: antagonism of IFN-I production and suppression of IFN-I signaling (38). The study identified several SARS-CoV-2 proteins that have IFN antagonistic capability, including Nsp1, Nsp6, Nsp12, Orf3a, Orf6, Orf7a, Orf7b, and M. Further, it was demonstrated that Nsp6 binds TANK binding kinase 1 (TBK1) and inhibits IRF3 phosphorylation (no effect on TBK1 phosphorylation), and Nsp13 was shown to bind TBK1 and inhibit TBK1 phosphorylation; both actions result in diminished IFN-I production. In addition, Nsp6 and Nsp13 were shown to inhibit IFN signaling by suppressing signal transducer and activator of transcription 1 and 2 (STAT1 and STAT2) phosphorylation, although the mechanism through which this occurs is not yet known (38).

(v) Nsp8 and Nsp9. Nsp8 and Nsp9 each bind specific regions of the 7SL component of the signal recognition particle (SRP) complex. SRP binds the 80S ribosome and is responsible for signal peptide recognition, SRP-receptor binding, and ribosome translocation. Nsp8 interacts with the binding site for SRP54 and with 28S rRNA, a component of the 60S subunit of the ribosome (25). Nsp9 binds to the SRP19 binding site, which is responsible for proper folding and assembly of the SRP; this includes adequate loading of SRP54 (25). Together, SARS-CoV-2 Nsp8 and Nsp9 suppress the IFN immune response to viral infection through disrupting SRP-mediated trafficking, upon which it is dependent (25).

(vi) Nsp16. SARS-CoV-2 Nsp16 binds host U1 and U2 small nuclear RNA (snRNA) components of the spliceosome (25). This was shown to disrupt spliceosome functioning and to result in global reduction of mRNA splicing (25). Several viruses are known to target the spliceosome as a tactic to evade the host immune response. Indeed, Nsp16-mediated intron retention in several IFN-responsive genes, including the ISG15 and RIG-I genes, was found to reduce IFN signaling in response to SARS-CoV-2 infection (25), yet another means by which SARS-CoV-2 hampers the host early innate immune response.

(vii) Orf3a. SARS-CoV Orf3a has been known to induce apoptosis in cells, and it was shown that SARS-CoV-2 Orf3a could similarly induce caspase-dependent apoptosis (39). SARS-CoV-2 Orf3a does this through the extrinsic apoptotic pathway, and its apoptotic activity could be significantly suppressed by caspase inhibitors. Interestingly, Orf3a plasma membrane localization was required for its apoptotic activity for SARS-CoV-2- but not SARS-CoV-encoded protein, which indicates that the viruses use different mechanisms (39). The authors suggest that the reduced Orf3a apoptotic activity in SARS-CoV-2, compared to that in SARS-CoV, and therewith the likely reduced

apoptosis-mediated immune response following infection, might contribute to the mild symptoms early on and asymptomatic transmissions observed in SARS-CoV-2.

SNARE complexes are essential to intracellular vesicular transport by mediating vesicle fusion to organelle or plasma membranes. Various viruses are known to disrupt SNARE complex formation, leading to accumulated autophagosomes/amphisomes and possibly facilitation of lysosome-mediated extracellular release of virus particles following replication. Interestingly, the SNARE proteins targeted to gain control of this process vary by virus. SARS-CoV-2 Orf3a disrupts homotypic fusion and protein sorting (HOPS)-mediated formation of the host STX17-SNAP29-VAMP8 SNARE complex (40). Orf3a directly interacts with the VPS39 component of HOPS; this sends it to the late endosomes, where VPS39 accumulates. And indirectly, Orf3a dysregulates the interactions of VPS39 with VPS11 and VPS18 (increased) and with VPS16-VPS33a (decreased) (40). Together, these Orf3a interferences result in diminished host autophagy. Similarly, SARS-CoV-2 M, Orf7a, and Nsp6 proteins reduced autophagy, the last doing so by limiting autophagosome size (40). Interestingly, in the same study, SARS-CoV Orf3a was not able to bind HOPS or block autophagy activity, suggesting that this feature is distinct to the SARS-CoV-2 Orf3a protein (40).

(viii) Orf6. SARS-CoV-2 Orf6 is a potent antagonist of the IFN immune response. It antagonizes IFN production through selective binding to karyopherin alpha 2 (KPNA2) to block IRF3 nuclear transport and by suppressing IFN signaling through inhibition of STAT1 nuclear translocation (38). Another report showed that Orf6 binding to the nuclear pore complex protein 98 (NUP98)-RAE1 complex prevents STAT nuclear import. This interaction further disrupts docking of the cargo KPNA1-KPNB1 complexes at the nuclear pore (41). Together, these actions inhibit the IFN-mediated innate immune response. We have shown that SARS-CoV-2 Orf6 expression is cytotoxic in an *in vitro* model (42). Further, using affinity purification and mass spectrometry, we identified a network of host proteins interacting with Orf6. Gene Ontology (GO) analysis revealed that these proteins were involved in a variety of processes, including translation initiation, viral gene expression, antigen processing and presentation, and IL-1-mediated signaling, and in the spliceosome. Further, they were enriched for nuclear transport-related proteins (nucleoporins, importins, and exportins), including RAE1, several NUPs, and XPO1, thus strengthening previous findings of interaction of SARS-CoV-2 Orf6 with the host nuclear pore complex (41–44). Moreover, we found that the SARS-CoV-2 virus-human host protein interaction network is highly conserved all the way from flies (45, 46). This enabled us to study SARS-CoV-2 Orf6 *in vivo*, using a *Drosophila* model, which showed that Orf6 causes early lethality, similar to the *in vitro* findings. Examination of tissue-specific pathogenic effects caused by Orf6 revealed reduced tracheal (fly equivalent of lung) branching, as well as muscle weakness with decreased presence of mitochondria (46).

(ix) Orf9b. RIG-I like receptors regulate a signaling complex on the mitochondrial outer membrane (including the adaptor proteins MAVS, TRAF3, TRAF6, and TOM70) responsible for activating the antiviral innate immune response and starting IFN production. SARS-CoV-2 Orf9b is another virus-encoded IFN antagonist. It antagonizes IFN in two ways, via TOM70 at the mitochondria (47) and via NEMO (48). Regarding the first, Orf9b localizes at the mitochondrial membrane, where it interacts with TOM70, which significantly reduces IFN activation (47). SARS-CoV and SARS-CoV-2 Orf9b proteins have similar binding affinities for TOM70, suggesting that this might be a shared mechanism among coronaviruses. Regarding the second (NEMO), immediately following virus infection, Orf9b rapidly accumulates and inhibits RIG-I-MAVS signaling-mediated IFN production. It does so by promoting MAVS degradation and subverting its downstream TBK1-IRF3 and IKK- $\alpha/\beta/\gamma$ -NF- κ B signaling pathways (48). Note that SARS-CoV-2 Orf9b targets the IKK regulator subunit NEMO, which is essential for NF- κ B but not IRF3 activation. As described above, SARS-CoV-2 Nsp3 protein disrupts the IRF3-regulated immune response (34, 35), and both Nsp6 and Nsp13 act upon TBK1-IRF3 as well (38). In fact, SARS-CoV-2 employs multiple strategies simultaneously (Nsp3, Nsp6 plus Nsp13, Nsp8-Nsp9, Nsp16, Orf6, and Orf9b) to ensure a nearly complete ablation of IFN signaling. This has been observed even during the later-stage cytokine storm.

TARGETED DRUG DEVELOPMENT FOR SARS-CoV-2 PROTEINS OR THEIR HOST INTERACTIONS

Drug host entry: vaccines and inhibitors targeting virus spike or the interaction with host factors to prevent cell entry. Not surprisingly, a huge amount of effort has been put into developing an effective vaccine in order to stem the SARS-CoV-2 pandemic. An alternative strategy to prevent infection is to block interaction between the virus spike protein with the host receptor. Drugs targeting the SARS-CoV-2 spike interaction with the host ACE2 entry receptor or the host factors, like TMPRSS2 and furin that facilitate the entry process, have been of great interest. Therefore, camostat (mesylate) and nafamostat (mesylate), both established TMPRSS2 inhibitors, were immediately identified as promising candidates (43). Indeed, early on it was shown that camostat mesylate attenuated SARS-CoV-2 cell entry *in vitro* (49). Another study proposed captopril, an ACE inhibitor, based on the spike-host protein interaction network (43). All three compounds are currently in clinical trials for the treatment of COVID-19. A different study showed that ipomoeassin F, a small molecule, inhibits Sec61-mediated endoplasmic reticulum membrane translocation, thereby prohibiting interaction between the virus spike and Orf8 proteins with the host ACE2 receptor (50). It is undeniable that vaccines and other means of prevention will be invaluable in the arms race with human viruses. Unfortunately, vaccines cannot provide complete immunity and viruses, especially their spike proteins, continue to mutate and develop new strains better equipped at evading host protective immune systems. Many new SARS-CoV-2 variants have been detected globally throughout this pandemic, including variants of concern (VOCs). Notably, several spike mutations have derived multiple times independently (12). Multiple reports in high-profile journals have been published recently on how effective current vaccines are at providing protection against these new variants. One study looked virus resistance to neutralization by the Pfizer-BioNTech (BNT162b2) or Moderna (mRNA-1273) vaccine following one or two doses (11). Encouragingly, the study found that neutralization was largely preserved against the newly emerging variant. However, the B.1.351 (South African origin) and B.1.1.28 (also known as 501Y.V2) lineage-derived variant P.1 (Brazilian origin) showed significantly reduced neutralization, even in the fully vaccinated group. Both manufacturers are currently developing boosters to expand protection against these new variants. In addition, to prepare for breaches of vaccine protection and new outbreaks in the human population, we need drugs that can minimize virus-caused damage in the host. SARS-CoV-2 is notorious for affecting so many different host tissues, which can culminate in system-wide shutdown as the disease (COVID-19) progresses. To be most effective, these drugs would be designed to target specific virus proteins, those self-acting and essential for virus replication processes and host-interacting ones that are most pathogenic through disrupting host pathways. Based on the studies of individual virus proteins described above, as well as virus proteome-wide host interaction networks, many such therapeutics have been proposed, multiple have been tested in the same *in vitro* systems used to identify them, and several are currently in clinical trials for the treatment of COVID-19 (Tables 3 and 4).

Drug self-acting: inhibitors targeting the viral proteins dedicated to virus replication processes. Due to their essential nature, the virus proteins specialized in virus replication (mRNA transcription, translation, and encapsulation) tend to be highly conserved, whereas, for example, proteins tasked with immune evasion have to constantly adapt to counteract new host tactics. In addition, inhibiting virus-specific proteins is less likely to lead to inadvertent side effects since the host systems would not carry homologous proteins. Together, these characteristics have made these virus replication-dedicated proteins of great interest as potential therapeutic targets (Table 3).

Many PLpro (Nsp3) inhibitors have been developed over the years; however, one study found that these were, in general, unsuccessful at inhibiting SARS-CoV-2 PLpro activity (20), except for the compounds known to be effective inhibitors of SARS-CoV PLpro, which showed similar efficacy against SARS-CoV-2 PLpro (20), as did noncovalent SARS-CoV PLpro-specific inhibitor GRL-0617 (35, 51). In addition to these existing inhibitors, two novel compounds (VIR250 and VIR251) have been shown to specifically inhibit protease

TABLE 3 Inhibitors of SARS-CoV-2 transcription and translation

Drug group	Examples ^a	Virus protein	Host protein	Pathway	Reference(s)
PLpro inhibitors	rac5c (less effective, rac3j and rac3k), GRL-0617, novel inhibitors VIR250 and VIR251	PLpro (Nsp3)	NA ^b	Protease; virus mRNA maturation	20, 33, 35, 51
3CLpro inhibitors	GRL-0496 (inhibitor SARS-CoV 3CLpro), GC376 (inhibitor SARS-CoV 3CLpro), lopinavir/ritonavir , ASC09F , GC813, and SK80	3CLpro (Nsp5)	NA	Protease; virus mRNA maturation	19, 21, 52
Nucleotide/nucleoside analog inhibitors	Remdesivir , faripiravir , galidesivir , and penciclovir Remdesivir , ribavirin , 5-fluorouracil, and β -D-N ⁴ -hydroxycytidine (NHC, EIDD 1931/2801)	Polymerase complex (Nsp12-Nsp7-Nsp8) Exoribonuclease (Nsp14)	NA	Polymerase; virus RNA synthesis Exoribonuclease; virus RNA synthesis (proofreading and recombination)	23, 54 24
Bismuth salts	Bismuth-peptic complex and ranitidine bismuth citrate	NTPase and RNA helicase (Nsp13)	NA	RNA processing; virus replication, transcription, translation, and encapsidation	22

^aBold indicates that the compound is in a clinical trial for treatment of COVID-19.

^bNA, not applicable.

activity of SARS-CoV and SARS-CoV-2 but not MERS-CoV (33). The *Nsp5* gene encodes the main protease (Mpro; 3CLpro); due to its importance in viral protein maturation for SARS-CoV-2 and many other viruses, it has been a therapeutic target of great interest for a long time. These have included a wide variety of potential SARS-CoV and MERS-CoV 3CLpro inhibitors (52). Urged on by the pandemic, efforts to develop inhibitors for SARS-CoV-2 3CLpro have included drug repurposing. GRL-0496 and GC376 are known inhibitors of SARS-CoV 3CLpro and were shown to be effective *in vitro* (dose dependent) against 3CLpro activity following SARS-CoV-2 infection (19, 21). In addition, several existing 3CLpro inhibitors are currently in (pre)clinical trials for the treatment of COVID-19, including pyri-thiobac derivatives, unsymmetrical aromatic disulfides and SK80 (SARS), GC813 (MERS), and GC376, a peptidomimetic inhibitor and a neuraminidase inhibitor analog (SARS and MERS), as well as anti-HIV protease inhibitors (lopinavir/ritonavir and ASC09F, a fixed-dose combination of ASC09 and ritonavir, combined with oseltamivir) (52). Structural studies, molecular docking, virtual screens, and artificial intelligence are employed to predict

TABLE 4 Inhibitors of SARS-CoV-2 host protein-protein interactions

Drug ^a	Drug class	Virus protein	Host protein	Pathway	Reference(s)
Amiodarone		Nsp6	SIGMAR1		43
Amodiaquine	Antimalarial	Nsp6	SIGMAR1		43
(Hydroxy)chloroquine	Antimalarial	Nsp6	SIGMAR1		8, 43
Chlorpromazine	Typical antipsychotic	Nsp6	SIGMAR1		43
Propranolol		Nsp6	SIGMAR1		43
Tamoxifen		Nsp6	SIGMAR1		43
GRL-0617		Nsp3 (PLpro)		Immune response (IFN; NF- κ B)	35
Indomethacin	Anti-inflammatory (NSAID)	Nsp7	PTGES-2		8, 43
Metformin		Nsp7/Orf9c	NDUF5		8
Fostamatinib	Tyrosine kinase inhibitor	Nsp13	MARK2		58
Ribavirin		Nsp14	IMPDH2		8
Ruxolitinib		Orf9b	MARK2/3		
Selinexor		Orf6 (Nsp4–Nsp9)	XPO1; NUP98-RAE1	Immune response (IFN); nuclear export inhibitor	8, 42, 46
Tocilizumab		Nsp10	NF- κ B-repressing factor	Immune response; IL-6 inhibitor	44

^aAll the compounds listed are in clinical trials for treatment of COVID-19.

binding affinity and potential for inhibitory effect in order to guide selection of candidates and design of new compounds (53).

It has been shown in a cell-free system that bismuth salts could inhibit both the NTPase and RNA helicase activities of SARS-CoV-2 Nsp13 in a dose-dependent manner (22). These compounds are in clinical use for the treatment of diarrhea (bacterial infection) and related stomach ulcers; clinical trials to test their efficacy in treating COVID-19 are currently not listed (<https://clinicaltrials.gov>). Given its essential function to virus survival, disrupting the virus-encoded RdRp has been a strategy for drug design against many viruses. Indeed, many nucleotide/nucleoside analog inhibitors have been designed for this purpose: their incorporation into viral RNA stops further synthesis. Several of these compounds are currently in clinical trials for COVID-19 (23, 54), including remdesivir (55), the only approved therapeutic for the treatment of SARS-CoV-2 infection. In addition, these drugs prohibit SARS-CoV-2 Nsp14 from carrying out its RNA proofreading duties (24). Remdesivir is a broad-spectrum antiviral, and while initial results looked promising for improving outcomes for COVID-19 patients (56), more recent data from a larger study showed little or no effect on disease progression and survival (57). A better molecular understanding of SARS-CoV-2 polymerase (Nsp12-Nsp7-Nsp8) and proofreading exoribonuclease (Nsp14) activities, through studying the individual virus proteins, could inform virus-specific design and thereby the efficacy of nucleotide/nucleoside analog inhibitors for the treatment of COVID-19.

Drug host interacting: inhibitors targeting the virus proteins, or their host factors, in specific virus-host interactions. Comprehensive proteomics studies that generated extensive virus-host protein-protein interaction networks have contributed greatly to identification of targets of interest and in screening of existing drug libraries for candidate inhibitors (43, 44). One such study found that around 40% of SARS-CoV-2-interacting proteins were associated with host endomembrane compartments or vesicle trafficking pathways (43). The protein networks further revealed SARS-CoV-2 interaction with host proteins representing innate immune pathways, host translation machinery, ubiquitination complex (commonly targeted by viruses for replication and pathogenesis) through interaction with a cullin ubiquitin ligase, and bromodomain proteins (this could interfere with transcriptional process in support of an antiviral response) (43). By overlaying the interaction networks with pharmacological profiles for a library of pharmacological agents, the authors identified 69 compounds targeting 62 of the identified virus-host protein interactions. *In vitro* testing of these host-directed compounds for antiviral activity showed compounds that inhibit virus-targeted interaction with translation pathways and sigma-1 and sigma-2 receptors to be most promising (43). At this time, several of the candidate compounds are in clinical trials for COVID-19 (<https://clinicaltrials.gov>): chloroquine targets Nsp6-SIGMAR1, indomethacin targets Nsp7-PTGES2, metformin targets Nsp7/Orf9c-NDUFs, ribavirin targets Nsp14-IMPDPH2, ruxolitinib targets Orf9b-MARK2/3, and selinexor targets Nsp4/9/Orf6-NUPs RAE1) (Table 4). In a follow-up study, the same group used a similar approach but overlaid the virus-host protein interaction networks of MERS-CoV, SARS-CoV, and SARS-CoV-2 (8). Of the promising (FDA-approved) candidates following *in vitro* validation, several are currently in clinical trials for COVID-19 (<https://clinicaltrials.gov>): indomethacin, an anti-inflammatory (nonsteroidal anti-inflammatory drug [NSAID]) (targets Nsp7-PGES-2), and chlorpromazine (typical antipsychotic), antimalarials (amodiaquine, hydroxychloroquine, and chloroquine), amiodarone, tamoxifen, and propranolol (all target Nsp6-SIGMAR1) (Table 4).

A different group used a similar approach to generate a SARS-CoV-2 human protein-protein network; they further determined global proteome profiles obtained from peripheral blood mononuclear cells (PBMCs) of patients with mild and severe COVID-19 (44). Analysis of the latter revealed that over 350 host proteins had been dysregulated. Proteins involved in neutrophil activation and blood coagulation were upregulated, while downregulated proteins were enriched for mediators of T cell receptor signaling. The authors confirmed the SARS-CoV-2 Nsp10 interaction with NF- κ B-repressing factor (NKRF) in HEK293T cells, which was not present in the previously published network (43).

This interaction facilitates an IL-8-mediated response and might contribute to the chemotaxis of neutrophils and cytokine storm (hypercytokinemia) observed in COVID-19 patients. Based on these findings, they proposed several drugs—targeting IL-8, IL-6, or the downstream JAK kinases—for pharmacological intervention in COVID-19 (44). Of these, tocilizumab, an IL-6 inhibitor, is currently in a clinical trial for COVID-19 (<https://clinicaltrials.gov>) (Table 4).

Another group sought to identify the sections of the virus-host protein network most affected by virus interaction, which they termed master regulators (58). Their analysis was based on interactions between SARS-CoV-2 and human host proteins predicted by structural genomics (59). Among the host systems most affected were apoptotic and mitochondrial pathways and downregulation of the ACE2 entry receptor. Based on these findings, the proposed targets of pharmacological intervention included, more broadly, antivirals and anti-inflammatories, recombinant ACE2, TMPRSS2 inhibitors (e.g., camostat mesylate), reviving innate immunity by targeting monocytes/macrophages, and boosting the downregulated apoptotic mechanisms, as well as, more specifically, interrupting the SARS-CoV-2 Nsp13-host MARK2 interaction by using fostamatinib (used to treat rheumatoid arthritis and immune thrombocytopenic purpura) (58). This tyrosine kinase inhibitor is currently in clinical trials for the treatment of COVID-19 (<https://clinicaltrials.gov>) (Table 4).

Several of the studies that investigated the pathological consequences for host cell pathways caused by individual SARS-CoV-2 proteins (see “Host interacting: SARS-CoV-2 proteins targeting host proteins and the effects on host systems” above) have also proposed and sometimes tested therapeutic inhibitors for the virus-host interactions. Selected ones are currently in clinical trial for the treatment of COVID-19 (Table 4). For the SARS-CoV-2 Nsp3 gene, which encodes PLpro and also possess deubiquitination/delSGylation activity, treatment with GRL-0617 (Table 4), a known SARS-CoV PLpro inhibitor, attenuated both its protease and nonhydrolysis functions, thus reducing Nsp3-mediated cytopathogenic suppression of the host IFN immune response via the IRF3 pathways and by countering PARPs. Furthermore, treatment with GRL-0617 reduced virus replication in infected cells (35). These findings demonstrate the potentially broad range of PLpro inhibitors in targeting Nsp3 functions. GRL-0617 is in COVID-19 clinical trials. The Nsp5 gene encodes the main protease (Mpro; 3CLpro), which is a target of major interest for pharmacological intervention (see “Drug self-acting: inhibitors targeting the viral proteins dedicated to virus replication processes” above and Table 3). Since its direct effect on host TAB1 and NLRP12 proteins is through protease-mediated cleavage, potent SARS-CoV-2 3CLpro inhibitors might similarly block cleavage of host proteins. It appears that no inhibitors have yet been designed or tested to do so; however, given that this Nsp5-NLRP12 interaction might contribute to the devastating cytokine storm (hypercytokinemia) observed in severe COVID-19 patients, this potential intervention warrants further investigation. SARS-CoV-2 Orf6 targets the host nuclear pore complex. Selinexor is an FDA-approved drug that targets XPO1 and inhibits nuclear export at the NUP98-RAE1 complex (43) (Table 4). We have previously shown that treatment with selinexor attenuates Orf6-mediated pathogenic effects (dose dependent) in both *in vitro* and *in vivo* systems (42, 46). Interestingly, SARS-CoV-2 Nsp6 and Orf7a induced phenotypes similar to Orf6 in fly; however, these did not improve following treatment with selinexor. These findings suggest that the underlying pathogenic pathways are a unique feature of each individual virus protein. This notion is supported by the virus protein-specific host interaction networks (43, 44).

SARS-CoV-2 is exceptional in its ability to suppress the IFN-mediated innate immune response. As we have seen above (see “Host interacting: SARS-CoV-2 proteins targeting host proteins and the effects on host systems”), it applies multiple strategies simultaneously to nearly abolish the response even at late stages of disease (COVID-19). The extent of this virus effort is also evident from the two SARS-CoV-2 proteomics studies (43, 44). Even though there is limited (~16%) overlap between the virus-host protein interaction networks reported by each study, the host proteins involved in the innate immune response to viral infections were preserved (44). Several clinical trials

on treatment with IFN in COVID-19 patients are under way, and some encouraging preliminary findings have been reported (60). However, it might be more effective to directly target the virus-host interactions that drive this immune suppression, for example, blocking the Nsp8-Nsp9 interaction with the SRP complex or inhibiting both Orf9b (NEMO-NF- κ B pathway) and Nsp3 (IRF3 pathway) simultaneously since they act on different branches of a shared host pathway regulating the IFN response. By studying the individual virus proteins and the host pathways favored to carry out their mission, we could identify more efficient drugs to boost the patient immune system.

CONCLUSION

It has been just over a year since SARS-CoV-2 entered the world stage; in that time, the research community has developed multiple vaccines that are currently being rapidly administered to stem this pandemic. This has been accomplished through Herculean effort and unprecedented global data sharing, yet a year is a long time to wait during a raging pandemic with life effectively on hold. Future outbreaks are a matter of when rather than if. Already, mutations in the SARS-CoV-2 spike protein have been linked to increased resistance to some of the vaccines. We need to prepare: on the one hand by developing pan-coronavirus vaccines and on the other by providing clinical staff with the knowledge and medications needed to treat patients effectively as a first response to novel outbreaks. Therefore, in addition to prevention (vaccines), which focuses on virus-host entry proteins, we need to study the role of virus self-acting (dedicated to virus replication processes) and host-interacting proteins. Research into the role of individual SARS-CoV-2 proteins with their host interactors and the pathways driving the multitude of pathogenic effects has already provided a tremendous amount of information. These form the foundation for drug target identification and design. In fact, several lead compounds have already made it into clinical trials for the treatment of COVID-19. This is only the beginning, as these studies will be elemental to giving patients a fighting chance during the next outbreak.

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