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Review

Dysregulation of Cell Signaling by SARS-CoV-2

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Pathogens usurp host pathways to generate a permissive environment for their propagation. The current spread of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection presents the urgent need to understand the complex pathogen–host interplay for effective control of the virus. SARS-CoV-2 reorganizes the host cytoskeleton for efficient cell entry and controls host transcriptional processes to support viral protein translation. The virus also dysregulates innate cellular defenses using various structural and nonstructural proteins. This results in substantial but delayed hyperinflammation alongside a weakened interferon (IFN) response. We provide an overview of SARS-CoV-2 and its uniquely aggressive life cycle and discuss the interactions of various viral proteins with host signaling pathways. We also address the functional changes in SARS-CoV-2 proteins, relative to SARS-CoV. Our comprehensive assessment of host signaling in SARS-CoV-2 pathogenesis provides some complex yet important strategic clues for the development of novel therapeutics against this rapidly emerging worldwide crisis.

Introduction to SARS-CoV-2

Coronaviruses are single-stranded RNA (ssRNA) viruses belonging to the family Coronaviridae. Viruses of this family have caused three major outbreaks worldwide: severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002, SARS-CoV-2 in 2019, and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 with smaller outbreaks in 2015 and 2018. In 2019, a major pandemic of SARS-CoV-2 emerged in Wuhan, China, and subsequently spread across the globe, with cases being confirmed in virtually all countries and infecting about 73 million people with over 1 627 046 deaths recorded as of November 30, 2020 (<https://www.worldometers.info/>). The spread, evolution, and the phylogenetic analysis of SARS-CoV-2 sequences are helping researchers to investigate the mutation patterns relative to differential pathogenicity and worldwide spread of the virus (Figure 1) [1]. Despite strong epidemiological evidence, the host–virus molecular interactions are poorly studied, which limits efficient pandemic control.

Both SARS-CoV and SARS-CoV-2 belong to the β subfamily of coronaviruses. SARS-CoV-2 consists of 29 different proteins, including 16 nonstructural proteins: proteases, RNA-dependent RNA polymerases, a helicase, nuclease, and methyltransferase encoded by 14 open reading frames (ORFs) (Table 1, Key Table) [2]. Structural proteins and nine putative accessory factors are encoded by 13 ORFs [2]. SARS-CoV-2 shares about 79% genome similarity with SARS-CoV and 50% with MERS-CoV [3]. Epidemiological data suggest that SARS-CoV-2 has the highest transmission rate but the smallest mortality rate (2.3%) when compared with SARS-CoV (9.6%) or MERS-CoV (35%). The higher transmission and pathogenicity of the SARS-CoV-2 could be attributed to the genetic alterations in different structural and nonstructural proteins. To identify potential molecular targets of SARS-CoV-2, and thus generate novel therapeutics against this devastating virus, it is important to understand the mechanisms by which the virus usurps host cell pathways and functions to cause disease. Here we discuss some of the genomic variations in viral proteins and contemplate their roles in the modulation of host cell signaling.

Highlights

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) proteins modulate host signaling to generate a permissive environment.

SARS-CoV-2 proteins upregulate or downregulate ~100 human kinases involved in cellular physiology, metabolism, and immune activation.

Targeting specific proviral cellular signaling could make the host microenvironment resistant to virus replication.

Most of the nonstructural proteins of SARS-CoV-2 participate in dysregulating the immune response.

Pertaining to the differences in sequence homology, NSP2 may be important in serodiagnosis.

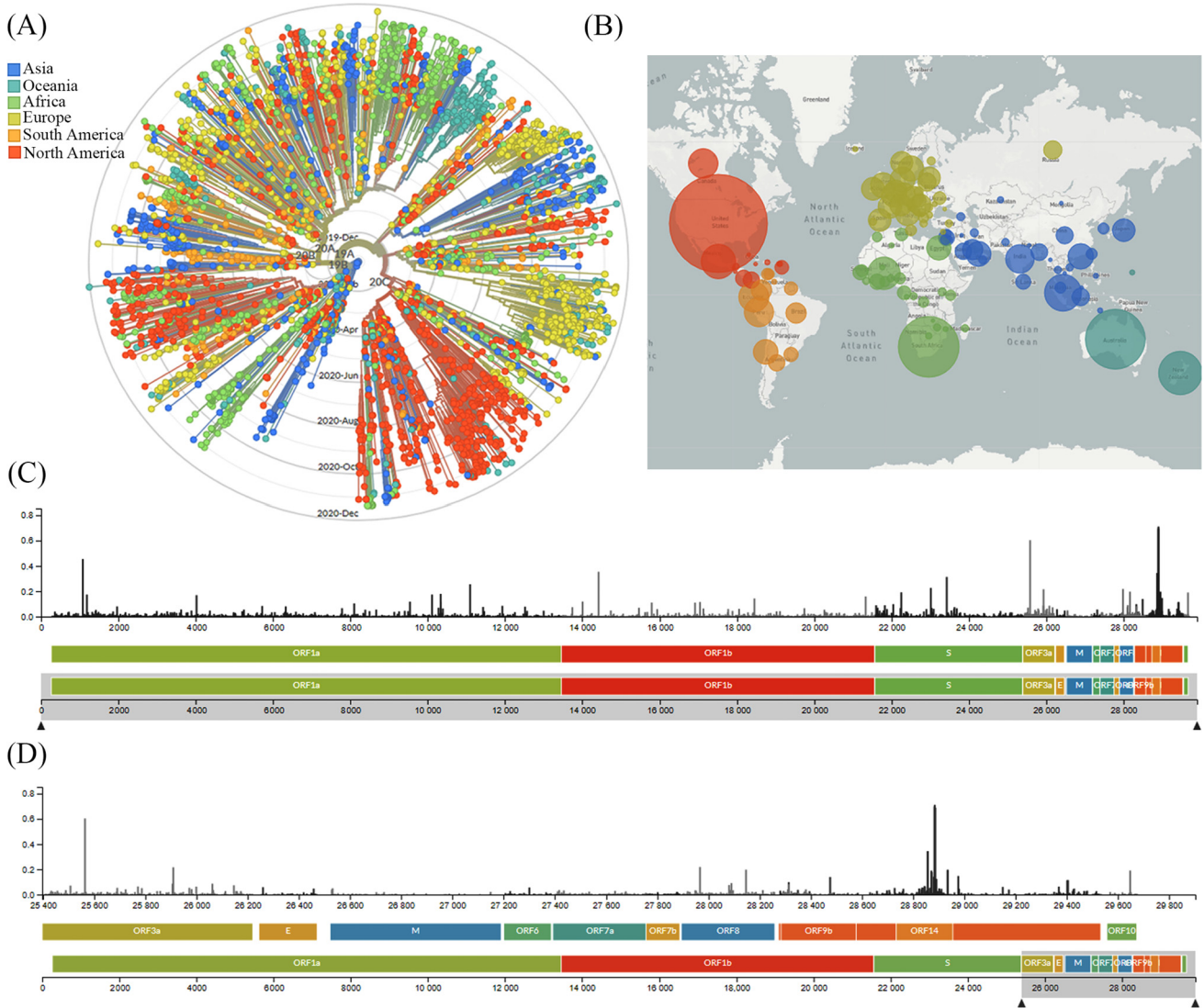
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Trends in Microbiology

Figure 1. Global Genomic Epidemiology of Novel Coronavirus Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). (A) Phylogenetic analysis of 3485 genomes of SARS-CoV-2 sequences globally from December 2019 to December 2020. The different genetic variants of circulating SARS-CoV-2 are grouped into five clades defined by specific signature mutations showing their global distribution on the time scale. The clades 19A and 19B dominated the early outbreak in Wuhan and represent a higher proportion in Asia. Clades 20A, 20B, and 20C dominate in Europe and North America. (B) Geographical distribution of genomes. Each circle is centered on an individual country. The color indicates the region, and the size (area) of the circle represents the number of genomes from that country. (C) A 'diversity' panel that shows the novel coronavirus genome, its genes, and sites of amino acid mutations. (D) Subsection of subfigure (C) highlighting the mutation pattern in the 25 400–29 800 bp range of the genome. Apart from the spike (S) region, the genomic regions of open reading frame (ORF)14, ORF9b, ORF8, and ORF3a appear to be highly variable between clinical isolates of SARS-CoV-2. Source: latest global SARS-CoV-2 updated daily at <https://nextstrain.org/sars-cov-2>. Abbreviations: E, envelope; M, membrane.

The Viral Life Cycle

The life cycle of SARS-CoV-2 begins with the priming of the viral spike (S) protein by host proteases or the transmembrane protease serine 2 (TMPRSS2) [4] to ensure fusion of the viral envelope with the host cell membrane and entry of the viral genome into the cell [5]. Alternatively, SARS-CoV-2 virions can be endocytosed by the cell after attaching to the angiotensin-converting

Key Table

Table 1. Overview of the Common Names and Currently Known Functions of the SARS-CoV-2 Viral Proteins^a

Structural protein	Function	Refs
Spike protein	Viral entry – binds to ACE2 receptor and heparan sulfate	[25,94]
Envelope protein	Virion structure	[3]
Membrane protein	Virion structure	[3]
Nucleocapsid	Houses genome, interferes with host translation and cell cycle, regulates cytoskeleton organization	[30,99]
Nonstructural protein		
NSP1	Inhibits host translation and the IFN response	[33,35]
NSP2	Disrupts cell cycle progression and apoptosis, possibly regulates actin assembly	[37,38]
NSP3	Facilitates transcription of the viral genome, arrests cell cycle in the G0/G1 phase, contains PLpro which antagonizes host immune responses and frees NSPs 1–3	[40,95,97]
NSP4	Participates in membrane rearrangement	[45]
NSP5	Contains the main protease, regulates epigenetic and gene expression, influences ER and mitochondrial functioning	[2,48,50]
NSP6	Restricts host autophagosome expansion	[52]
NSP7	Complexes with NSP8 to form RNA primase	[53]
NSP8	Complexes with NSP7 to form RNA primase	[53]
NSP9	RNA-binding phosphatase	[56]
NSP10	Activation cofactor for NSP14 and NSP16	[60,61]
NSP11	Unknown	[98]
NSP12	RdRp – complexes with NSP7 and NSP8 to replicate viral genome	[65]
NSP13	Helicase	[69,70]
NSP14	3'-5'-exoribonuclease, interacts with replication-transcription complex	[74]
NSP15	Endoribonuclease, IFN inhibitor, and modifies host cell cycle progression	[76,79]
ORF3a	Ion channel protein, influences cytokine responses	[81,82]
ORF6	Inhibits antiviral IFN response	[84]
ORF7a	Suppresses STAT1 phosphorylation, inhibits type 1 IFN response	[8]
ORF7b	Suppresses STAT1 and STAT2 phosphorylation, inhibits type 1 IFN response	[8]
ORF8	Inhibits antiviral IFN response, immune evasion virulence factor	[78]

^aThe proteins are divided between structural and nonstructural types. The functions and associated references are provided for each protein.

enzyme 2 (ACE2) receptor. Once internalized, host enzyme cathepsin L can cleave the S protein that results in a similar release of the viral genome to the cell [5].

Once inside the cell, the positive-sense ssRNA genome becomes translated using host ribosomes. The primary viral gene ORF1ab codes for the polyproteins pp1a and pp1ab which autocleaves the papain-like protease (PLpro) and 3C-like protease (3CL-pro) from itself [6]. PLpro further processes the viral polyprotein and releases nonstructural proteins (NSPs) 1–3, while 3CL-pro releases the remaining NSPs 4–16 [6]. Certain SARS-CoV-2 NSPs form an RNA replication complex, such as the RNA-dependent RNA polymerase (RdRp) NSP12 and the helicase NSP13 [7]. NSPs 10, 13, 14, and 16 participate in mRNA capping, and NSPs 10, 14,

and 15 proofread the nascent genome [7]. While the genome is replicating, multiple viral proteins – such as NSP1, NSP3, NSPs 12-14, ORF3, ORF6, and ORF7a/b – antagonize host immune responses by targeting the innate immune system, in particular the type 1 interferon (IFN) pathway [8,9]. However, the virus activates certain host Toll-like receptors (TLRs) during infection, which stimulates the release of numerous proinflammatory cytokines, including interleukin (IL)-1 β , IL-2, IL-6, IL-7, granulocyte-colony stimulating factor (G-CSF), IFN- γ , and tumor necrosis factor-alpha (TNF- α) [10–12]. Analysis of peripheral blood from severely infected patients revealed decreased CD4 and CD8 T cell counts, but the fractions of hyperactivated T cells increased [13]. Furthermore, the concentrations of T helper (Th)17 cells and perforin and granzyme-expressing CD8 T cells were enhanced, both of which contribute to hyperinflammation [13]. Host Th17 responses are major contributors to cytokine storms, a hallmark of severe coronavirus disease 2019 (COVID-19) infections [14].

Subsequently, the viral structural proteins and the viral genome assemble into the virion, which travels to the host cell surface using vesicles and is released via lysosomal, Arl8b-dependent exocytosis [3,15]. The host endoplasmic reticulum (ER) chaperone GRP78/BiP is cotrafficked with the virion and released during this process as well [15]. Alternatively, immature virions use the nucleocapsid (N) protein to localize to a glycosylated envelope (E) protein at the cell surface [16]. This interaction allows for a reorientation of the virion, followed by budding from the host cell [16]. Finally, the destruction of host organelles can lead to the release of lysosomal contents, triggering cell death and the release of viral progeny [17].

Host Signaling during SARS-CoV-2 Infection

During infection, viruses tend to exploit host machinery to create a permissive host micro-environment. A phosphoproteomic analysis of SARS-CoV-2-infected Vero cells revealed regulation of 97 cellular kinases [18]. The activated kinases include some of those involved in p38-mediated signaling and guanosine monophosphate-dependent protein kinases. Downregulated kinases include those involved in cell growth, the cell cycle, and cytoskeleton regulators. The most highly activated transcription factors, such as nuclear factor- κ B (NF- κ B), monocyte-specific enhancer factor 2C, and c-Jun, are downstream of the p38/MAPK (mitogen activated protein kinase) pathway which is known to mediate stimulation of proinflammatory cytokines, including IL-6, TNF- α , CCL2, CCL20, and CXCL8 [18]. Additionally, cell cycle arrest at the S/G2 transition is highly correlated with productive SARS-CoV-2 infection, whereas M phase progression is negatively correlated. S/G2 arrest during infection may be due to a dysregulation of cyclin-dependent kinase 2 (CDK2) activity during viral infection [18]. Studies have found atypical, delayed induction of host immune response to SARS-CoV-2 infection which favors virus replication in the absence of type I and III IFN responses and simultaneously induces high levels of chemokines [19,20]. An integrated immune analysis on a cohort of 50 COVID-19 patients with various levels of disease severity has shown that NF- κ B partially drives the inflammation. Such inflammatory responses are the outcome of increased TNF- α and IL-6 signaling [19]. Recent work shows that TNF- α and IFN- γ induce inflammatory cell death and tissue damage through extensive crosstalk among different modes of cell death, including pyroptosis, apoptosis, and necroptosis, together termed PANoptosis [21]. Treatment with a combination of TNF- α and IFN- γ was found to activate the Janus kinase (JAK)/STAT1 (signal transducer and activator of transcription 1)/IRF1 (interferon regulatory factor 1) axis, leading to nitric oxide production that drives caspase-8/FADD-mediated PANoptosis. Interestingly, TNF- α and IFN- γ together induce a SARS-CoV-2-like cytokine storm in mice, which can be reversed using PANoptosis inhibitors. Furthermore, treatment of SARS-CoV-2-infected mice with neutralizing antibodies against TNF- α and IFN- γ protects mice from death. This emphasizes the clinical significance of cytokine-mediated inflammatory cell death signaling pathways.

Structural Proteins

SARS-CoV-2 encodes five structural proteins: (i) spike, (ii) envelope, (iii) membrane, and (iv) nucleocapsid.

Spike Protein

The SARS-CoV-2 S protein mainly functions in viral entry. Mechanistically, the S protein binds to human heparan sulfate (HS) and ACE2 using its receptor-binding domain (RBD) and is activated by human proteases, but, unlike SARS-CoV, cell entry of SARS-CoV-2 is less dependent on host proteases as it is preactivated by the pro-protein convertase furin [22]. The rate of transmission of SARS-CoV-2 could be attributed to structural differences in the highly variable RBD of its S proteins (Box 1). Unlike many of its other proteins, the S protein of SARS-CoV-2 amplifies the activation of IFN- β [9]. A preprint by Hsu *et al.* suggests that the SARS-CoV-2 S protein contributes to host hyperinflammatory responses via its binding to the ACE2 receptor [23]. This interaction stimulates the MAPK–NF- κ B axis and results in the release of proinflammatory cytokines such as TNF- α and CCL2. MyD88 and TRIF are critical adaptor molecules for this signaling pathway to function. Furthermore, downstream signaling from the S–ACE2 interaction increases ER stress, causing an unfolded protein response and activation of NF- κ B. While these results are from a preprint, and should be viewed with some caution, an older study found that the SARS-CoV S protein induced the activation of NF- κ B signaling as well [24]. The induction of inflammatory cytokines by the S protein may contribute to the cytokine storm and the associated epithelial damage and organ injuries observed in COVID-19 patients.

In addition to ACE2, an increasing body of evidence points to the importance of cell surface HS in the initial cellular attachment and viral entry [25]. The higher binding affinity of S protein (Kd 55 nM) to HS compared with RBD (Kd 1 μ M) suggests that heparin, an HS mimetic, or soluble HS, can be used to block viral entry in the host cell [26]. HS is a complex glycosaminoglycan found ubiquitously on human cells, with documented involvement in key cellular processes of inflammation, cellular communication, and microbial invasion [27,28]. Recent evidence for the robust proviral actions of the HS-degrading enzyme heparanase (HPSE) across viral families make HS a promising system for the study of SARS-CoV-2 viral entry [29].

Envelope and Membrane Proteins

Envelope (E) and Membrane (M) proteins constitute important components of the SARS-CoV-2 virus envelope. Sequence comparison shows that these SARS-CoV-2 proteins are highly similar to those of SARS-CoV [9]. Interestingly, E and M proteins are nearly identical among bat and pangolin coronaviruses. The M protein also independently inhibits the activation of IFN- β , while the E protein stimulates the transcription of interferon-stimulated genes (ISGs) [9].

Nucleocapsid (ORF9/9a) Protein

The nucleocapsid (N) competes with host protein synthesis by dephosphorylating the translational regulator ribonucleoprotein LARP1, which lets it bind to untranslated regions of mRNA. Interestingly, overexpression of the SARS-CoV N protein upregulates casein kinase 2 activity, which induces actin polymerization and thereby regulates cytoskeleton organization, a process commonly associated with viral infection [30]. The SARS-CoV N protein also inhibits S phase

Box 1. Spike Protein Mutations in SARS-CoV-2

Structurally, in addition to 12 nucleotide insertions, amino acid mutations of the RBD of SARS-CoV-2 include L455, F486, Q493, S494, N501, and Y505. These modifications result in an increase in 3-O-linked glycans around the binding site and thus higher binding affinity for the human receptor ACE2. A variant carrying amino acid change D614G has been recently implicated to increase infectivity of SARS-CoV-2 [95].

progression by inhibiting CDK2, either by direct binding of N protein or by downregulating protein levels of CDK2, cyclin E, and cyclin A [31]. Further information on ORF9 isoforms is given in [Box 2](#).

Nonstructural Proteins

SARS-CoV-2 encodes 16 NSPs, which cooperate and form complexes to execute their functions. Here, we provide an overview of the role of these NSPs in the viral life cycle along with certain accessory ORF proteins expressed by the virus. Given the relative scarcity of peer-reviewed studies on SARS-CoV-2, we offer an overview of these proteins in both SARS-CoV and SARS-CoV-2 as their sequence similarities suggest that they could play analogous functions. However, information on SARS-CoV must be recognized as such, and caution must be exercised when it is applied to SARS-CoV-2.

NSP1

SARS-CoV-2 NSP1 shares 85% sequence identity with SARS-CoV and helps to neutralize the host response to infection. When HEK293T cells are stimulated with IFN- α , SARS-CoV-2 NSP1 inhibits phosphorylation of STAT1, thereby suppressing downstream IFN- α -mediated signaling. In addition, NSP1 also abolishes IFN- β production in HEK293T cells [9,32]. The mechanism by which this occurs is less clear as NSP1 likely inhibits proteins both upstream and downstream of IRF3. The mutations in NSP1 found in SARS-CoV and MERS-CoV appear to improve its ability to inhibit type 1 IFN responses [8].

Multiple roles have been discovered for NSP1 of SARS-CoV which may apply to its variant in SARS-CoV-2. SARS-CoV NSP1 blocks the host translation process by associating with the 40S subunit of the ribosome [33]. NSP1 also recruits a host endonuclease to create breaks in the 5'-UTR (untranslated region) region of host mRNA sequences, which are then degraded in an Xrn1-mediated manner [34]. Interestingly, viral mRNAs have a leader sequence in the 5'-region that confers protection against endonucleolytic cleavage and promotes the selective translation of viral proteins [35]. Mutations in SARS-CoV NSP1 jeopardize its ability to block the host IFN response, suggesting that NSP1 interacts with interferon response factor (IRF)-3, IRF7, and c-Jun signaling [36]. Both SARS-CoV and SARS-CoV-2 NSP1 function to negatively regulate the host immune response.

NSP2

NSP2 consists of 638 amino acids and shares only 68% sequence homology with its counterpart in SARS-CoV. One mutation in the endosome-associated-protein-like domain has been suggested to contribute to the increased contagiousness of SARS-CoV-2 [37]. NSP2 is one of the few SARS-CoV-2 proteins which significantly accelerates the activation of IFN- β [9]. While not confirmed in SARS-CoV-2, SARS-CoV NSP2 interacts with host proteins prohibitin 1 and 2 to disrupt cell cycle progression and apoptosis [38]. NSP2 has been shown to interact with the

Box 2. Functions of ORF9b and ORF9c

Functions of these two proteins remain elusive, with little information available in the literature to date. In SARS-CoV, ORF9b suppresses type I IFN signaling by severing a link between mitochondrial antiviral-signaling protein (MAVS) and downstream mediators TRAF3 and TRAF6 [96]. Protein expression of dynamin-related protein (DRP1), which is involved in physiological mitochondrial fission, was found to be diminished with overexpression of ORF9b, resulting in mitochondrial elongation [96]. While recent investigation on this protein has been limited, initial work directed at understanding host protein interactions of viral proteins suggests that SARS-CoV-2 ORF9b may influence cellular proliferation by binding to multiple MAP kinases, while ORF9c binds to several proteins involved in electron transport [2]. Interestingly, SARS-CoV-2 ORF9c may not be a true open reading frame or may not be required for viral replication, as multiple clinical isolates analyzed contained premature stop codons in the ORF sequence [2].

Wiskott–Aldrich syndrome protein and scar homology (WASH) complex component strumpellin [2]. Strumpellin is known to regulate actin assembly on intracellular vesicles, which may help in viral egress [2,39]. Considering the differences in sequence homology of NSP2 in SARS-CoV-2 compared with SARS-CoV, the protein merits additional research.

NSP3

NSP3 is a large, multidomain protein that plays versatile roles in the SARS-CoV-2 life cycle. SARS-CoV-2 NSP3 delays the activation of IFN- β , allowing more time for the virus to replicate safely [9]. However, the SARS-CoV NSP3 was a much more potent inhibitor of the IFN response and could deubiquitinate proteins more efficiently as well [40]. NSP3 contains a papain-like protease (PLpro) domain that cleaves the replicase polyprotein and frees NSP1, NSP2, and NSP3 [6]. The SARS-CoV PLpro was demonstrated to inhibit many components of the innate immune response, particularly by blocking type I IFN signaling through interaction with the stimulator of interferon genes (STING)–TRAF3 (TNF receptor associated factor 3)–TBK1 (Tank-binding kinase 1) complex and degradation of p53 [41,42]. SARS-CoV PLpro also impedes TLR7-mediated cytokine induction via the removal of Lys63-linked ubiquitin bound to TRAF3 and TRAF6 [43]. A macrodomain of NSP3, termed the X domain, forms an essential component of the coronavirus replication complex. The resulting protein from the X domain also inhibits type I IFN, ISGs, and cytokine responses by removing ADP-ribose from target proteins [44]. ADP-ribosylated proteins can stimulate a variety of immune responses, and many viruses have been found to encode proteins capable of removing ADP-ribose from host proteins and evading host immunity [44]. Given these results, SARS-CoV-2 PLpro may interact with the STING and TLR7 signaling pathways to dysregulate the host immune response.

NSP4

NSP4 is one of the viral proteins released during the cleavage of the viral polyproteins pp1a and pp1ab [45]. While there is a scarcity of information of SARS-CoV-2 NSP4, it may have similar roles as its SARS-CoV variant as they share nearly 80% sequence similarity [9]. Structural modeling of the SARS-CoV protein suggests that it has multiple transmembrane domains [46]. Early after its release, it becomes N-glycosylated upon interaction with the ER membrane. Two domains located in a luminal loop of SARS-CoV NSP4 interact with NSP3 to translocate from the ER to the perinuclear region [47]. SARS-CoV NSP4 (amino acid residues H120 and F120) participates in membrane rearrangement to facilitate the viral replication and transcription complex (RTC) formation.

NSP5

NSP5 is the main protease of SARS-CoV-2, with 3CL-pro being its putative functional domain [2]. The protein shows 99% and 96% similarity with BAT-SL-CovZXC21 and SARS-CoV respectively [48]. NSP5 acts as an epigenetic and gene expression regulator and can also influence the functions of the ER and mitochondria. In a protein interactome study, SARS-CoV-2 NSP5 interacted with histone deacetylase 2 (HDAC2), likely restricting its nuclear translocation to inhibit innate antiviral immunity and resulting in enhanced ACE2-mediated cell entry [2,49,50]. Studies also show that catalytically inactive NSP5 interacts with tRNA methyltransferase, which dimethylates guanosine on nuclear and mitochondrial tRNAs [2]. SARS-CoV-2 NSP5 may mediate the cleavage of TRMT1, making it lose its zinc-finger domain and nuclear localization sequence [2,51]. In addition, gene ontology data predict involvement of NSP5_C145A in regulation of the cell membrane matrix, cell death, response to oxidative stress, and sensory perception of mechanical stimuli.

NSP6

SARS-CoV-2 NSP6 is a potent inhibitor of the host type 1 IFN response [8]. NSP6 interacts with TBK1 to suppress the phosphorylation of IRF3, which is a prerequisite step for the stimulation of

IFN- β [8]. Interestingly, SARS-CoV NSP6 does not inhibit type I IFN responses [8]. Evolutionary analysis of NSP6 points to a mutation which is important for maintaining the stability of the SARS-CoV-2 protein and, in turn, contributes to its acquired role in immune inhibition [52]. However, one study has reported that SARS-CoV-2 NSP6 stimulated the promoters of the interferon-stimulated response element (ISRE) and ISG56 [9]. The NSP6 of SARS-CoV instead was reported to play an important role in the inhibition of host autophagy. It restricted the expansion of host autophagosomes by reducing the diameter of autophagosomes in infected cells [53]. NSP6 of the coronavirus infectious bronchitis virus (IBV) has been known to limit expansion of omegasomes, sites to which proteins forming the autophagosome complex are recruited [53]. Furthermore, IBV NSP6 disrupts the association of mammalian target of rapamycin (mTOR) with lysosomes [53]. Given these findings, SARS-CoV-2 NSP6 may have an uncharacterized role in impeding host autophagy as well.

NSP7 and NSP8

Both NSP7 and NSP8 are highly conserved between SARS-CoV-2 and SARS-CoV. SARS-CoV-2 NSP7 shares 98.8% sequence similarity with SARS-CoV NSP7, while SARS-CoV-2 NSP8 is 97.5% conserved, with 98.8% and 97.5% similarity, respectively. Therefore, NSP7 and NSP8 in SARS-CoV-2 likely serve similar roles, as in SARS-CoV. The SARS-CoV NSP8 is a 22-kD protein that forms a complex with NSP7 to act as an RNA primase [54]. In SARS-CoV, the NSP7+8 complex can efficiently bind to RNA and extend primers to template length [54]. Magnesium ions (as cofactors) and basic pH are required for the optimum activity of this complex [54]. The complex also indirectly facilitates replication of the viral genome. To initiate RNA replication, the RdRp NSP12 must first associate with the primase complex followed by NSP14. The SARS-CoV NSP14 is a key enzyme involved in proofreading of newly synthesized viral RNA [55]. NSP8 interacts with ribonucleoprotein 7 (LARP7), M-phase phosphoprotein 10, and cytokine-induced protein. LARP7 is an important part of the transcription assembly shown to control transcription of viral genes [18,56]. SARS-CoV-2 NSP7's interaction with Ras homology family member A (RHOA) may contribute to downregulation of Rho GTPases and the phosphorylation of the vimentin and stathmin proteins by PAK1/2 kinase [18]. Both vimentin and stathmin are known to have a role in cytoskeletal organization. Furthermore, SARS-CoV-2 NSP7 was demonstrated to impede IFN- α signaling independently of the other NSPs [8].

NSP9

NSP9 of SARS-CoV-2 acts as an RNA-binding phosphatase and participates in the RTC [57]. It shares 97.3% sequence homology with SARS-CoV NSP9. The structure of SARS-CoV NSP9 suggests that it may stabilize viral RNA during replication and assist in RNA processing [58]. During replication, SARS-CoV NSP9 dimerizes via the binding of two α -helices with GXXXG motifs, which is a necessary step for coronavirus replication [59]. Interestingly, SARS-CoV-2 NSP9 is a stimulator of the host type 1 IFN response [9].

NSP10 and NSP16

SARS-CoV-2 NSP10 is highly conserved with its counterpart in SARS-CoV [9]. SARS-CoV NSP10 forms a complex with NSP16 during the coronavirus life cycle [60]. NSP10 binds to and activates the 2-O-methyltransferase NSP16 [60]. The complex methylates the 5' guanosine cap of the viral RNA, which helps to avoid detection from molecules that recognize host cytosolic RNA, such as RIG-I [61]. This immune evasion ability of the SARS-CoV NSP10+16 complex interferes with activation of the IFN response [60]. Loss of the NSP10+16 interaction resulted in inhibition of viral replication, suggesting that formation of the complex is an essential step in the SARS-CoV life cycle [62]. Additionally, SARS-CoV NSP10 acts as an activation cofactor in its complex with NSP14. It enables NSP14 to proofread and edit newly synthesized RNA [63].

The NSP7+8+12 complex and NSP10 may each serve regulatory roles allowing the exoribonuclease to function optimally.

NSP11

SARS-CoV-2 NSP11 is a small cleavage product resulting from the processing of pp1a by 3CL-pro [64]. Its full 13 amino acid sequence is listed as follows: SADAQSFLNGFAV [65]. In the ribosomal frameshift of ORF1b, which is required for the translation of NSP11-16, it becomes the N terminus of NSP12. No independent function has been currently described for NSP11 [64].

NSP12

NSP12 is the RdRp of SARS-CoV-2 and is essential for viral replication. It must form a polymerase complex with its cofactors NSP7 and NSP8 prior to activation [66]. NSP7+8 functions as a primase and improves the RNA-binding capacity of NSP12, which facilitates its subsequent polymerase activity to replicate the SARS-CoV-2 genome [66]. Transfection of HEK293T cells with a NSP12 plasmid prior to infection with Sendai virus significantly inhibited IFN- β activation [9]. However, NSP12 also stimulates ISRE and ISG56, suggesting that it may have a nuanced relationship with the host immune response [9]. SARS-CoV-2 NSP12 displays decreased enzymatic activity and thermostability when compared with SARS-CoV NSP12 [67]. The experimental drug remdesivir, which has gained popularity in clinical use against SARS-CoV-2, is thought to inhibit SARS-CoV-2 by binding to NSP12 [62,68]. Furthermore, Ruan *et al.* found eight additional compounds that demonstrate strong binding affinity to the NSP7+8+12 complex [69]. NSP12 is known to interact with ribonucleoprotein 4B, CREB regulated transcription coactivator 3, E3 ubiquitin protein ligase, ubiquitin-associated protein 2, and PLAKHA5. Many SARS-CoV-2 NSP12-interacting proteins show decreased phosphorylation upon viral protein overexpression [18].

NSP13

NSP 13 is the SARS-CoV-2 helicase and is identical to its counterpart in SARS-CoV [48]. Therefore, information on SARS-CoV NSP13 is highly applicable to SARS-CoV-2. SARS-CoV NSP13 unwinds duplex DNA and RNA with a 5' single-stranded tail in the 5'-3' direction using ATP hydrolysis, and thereby plays a crucial role in viral replication [70,71]. In general, the duplex RNA-unwinding activity of SARS-CoV NSP13 is less efficient, when compared with duplex DNA unwinding [71]. However, with increased ATP concentrations, duplex RNA becomes a preferred substrate for helicase activity of SARS-CoV-2 NSP13, resulting in higher processivity in unwinding of duplex RNA compared with DNA [72]. Here, ATP causes a conformational change in helicase, diverting its affinity towards RNA. With this work, the authors conclude that ATP availability may influence the unwinding, translocation, and dissociation of SARS-CoV-2 NSP13 from its substrate (duplex DNA or RNA). Adedeji *et al.* further suggested that RdRp (NSP12 from SARS-CoV) enhances the catalytic efficacy of SARS-CoV NSP13 by twofold [73]. Similar to NSP6, the SARS-CoV-2 NSP13 also inhibits the activation of IFN- β in HEK293T cells by inhibiting the phosphorylation of both IRF3 and TBK1 [8,9,40]. NSP13 also inhibits IFN- α -mediated signaling in HEK293T cells [8]. Thus, SARS-CoV-2 NSP13 plays dual roles as a helicase and an inhibitor of the type 1 IFN response.

NSP14

SARS-CoV NSP14 has been shown to interact with other NSPs, like 7, 8, 10, and 12 [46,74]. Its primary role is to serve as a 3'-5'-exoribonuclease upon initial interaction with NSP10. Once active, it proofreads the viral genome and excises any errant nucleotides that it encounters. In the coronavirus murine hepatitis virus, mutations in NSP14 resulted in a 15-fold increase in genomic mutations, highlighting the essential role it plays in replication fidelity [75]. Similarly, the mutated NSP14 of SARS-CoV exhibited a 21-fold increase in genomic mutations [75]. Additionally,

SARS-CoV NSP14 serves as a (guanine-N7)-methyltransferase, which can protect newly synthesized viral RNA from degradation. Its capping function requires the presence of the exoribonuclease domain, although the enzymatic activity of the domain is dispensable [76]. Finally, SARS-CoV-2 NSP14 has been shown to inhibit IFN- β production and signaling by preventing the nuclear localization of IRF3 [9,40].

SARS-CoV NSP14 can also bind to the NSP7+8+12 complex, but it lacks exoribonuclease activity when associated [55]. However, upon the addition of NSP10 to the replication complex, SARS-CoV NSP14 regains its activity [55]. Alongside NSP7, 8, and 12, NSP14 may act as another unit of a super-complex of proteins with primase, polymerase, and exoribonuclease functions.

NSP15

SARS-CoV NSP15 acts as an endoribonuclease and cleaves RNA nucleotides in the 3' direction of uridylylates [77]. The conformation of NSP15 is highly sensitive to the presence of manganese cations [78]. Mutations in SARS-CoV NSP15, including those outside of the active site, inhibit viral infection [79]. Like NSP13 and 14, SARS-CoV-2 NSP15 can also interfere with the host IFN response during infection by blocking the localization of IRF3 to the nucleus [40]. Interestingly, SARS-CoV NSP15 contains a small domain capable of binding to the retinoblastoma protein pRb [80]. NSP15 alters the distribution of pRb in Huh-7 cells, increasing the ratio of cytoplasmic to nuclear pRb [80]. The reduction of Rb expression results in changes in cell cycle progression of NSP15-transfected cells [80]. Thus, NSP15 exhibits multifaceted roles as an endoribonuclease, an IFN inhibitor, and a binding partner for pRb.

ORF3a

ORF3a, alongside ORF8a and the E protein, is an ion channel protein encoded by SARS-CoV [81]. In SARS-CoV-2, ORF3a has been demonstrated to inhibit IFN- α signaling by suppressing the phosphorylation of STAT1 [8]. Another study found that ORF3 could impede IFN- β signaling as well, making it a potent inhibitor of the type 1 IFN response [9]. Recently, ORF3a has been implicated in lysosomal deacidification [15]. Raising the pH of the lysosome allows the virus to use lysosomal organelles as vehicles to reach the plasma membrane and egress from the host cell [15]. SARS-CoV ORF3a possesses three transmembrane domains and interacts with the M and E proteins during viral assembly [82]. ORF3a also contains a PDZ-binding motif, which conceivably allows it to bind to hundreds of cellular proteins and regulate signaling [82]. SARS-CoV virions lacking ORF3a exhibit defects in replication both *in vitro* and *in vivo* [82]. Viruses which lacked the PDZ-binding motif of ORF3a replicated normally, suggesting that other domains of ORF3a may be essential for replication [82]. However, in the absence of the E protein, the PDZ-binding motif becomes essential for replication [82]. Interplay between the E protein and ORF3a exerts significant influence over the viral replication cycle. ORF3a participates in cytokine responses independently of its interactions with the E protein (Box 3).

Box 3. ORF3a Participates in Cytokine Storm Induction

Independently of its interactions with the E protein, SARS-CoV ORF3a also participates in induction of the cytokine storm. In this pathway, TRAF3 acts as its key binding partner. The interaction of ORF3a and TRAF3 results in the activation of NF- κ B, which upregulates IL-1 β expression and secretion [97]. Additionally, SARS-CoV ORF3a expression leads to the ubiquitination of apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), a process mediated by TRAF3 once again [98]. Ubiquitination of ASC in NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3)-expressing cells activates caspase-1 and IL-1 β maturation [98]. Due to its role in the upregulation and maturation of IL-1 β , ORF3a stimulates formation of the NLRP3 inflammasome and the induction of a cytokine storm. Given these findings in SARS-CoV, the ORF3a of SARS-CoV-2 may induce cytokine storms during infection as well.

ORF6

ORF6 is the least conserved protein between SARS-CoV and SARS-CoV-2, with only 66.6% sequence similarity [9]. SARS-CoV NSP8 colocalizes with ORF6 in cytoplasmic punctate structures [83]. Although ORF6 associates with NSP8, mutants lacking ORF6 demonstrate similar replication kinetics as wild-type viruses [84]. SARS-CoV ORF6 binds to karyopherin $\alpha 2$ and $\beta 1$ to the ER/Golgi membrane, preventing them from forming a nuclear import complex [85]. Additionally, SARS-CoV ORF6 is known to interact with the NUP98/RAE complex and is correlated with increased phosphorylation of NUP98 at the S888 site. This prevents host mRNA nuclear export, resulting in inhibition of host protein synthesis [86]. The C terminus appears to be the essential region of the protein for the tethering to occur [85]. When IFN signaling is active, the loss of nuclear importation prevents STAT1 from entering the nucleus and blocks downstream STAT1 signaling [85]. In conjunction with these findings, SARS-CoV infection results in rapid production of high viral titers in STAT1 knockout mice [87]. Given the essential role of STAT1 signaling in the clearance of coronavirus infections, ORF6 plays a key role as an inhibitor of antiviral IFN signaling.

In support of these findings, recent studies have implicated SARS-CoV-2 ORF6 in the suppression of IFN- β -mediated IFN responses [8,9,40]. ORF6 binds to karyopherin α , a nuclear import factor, to prevent the translocation of IRF3 to the nucleus, which halts the subsequent production of IFN- β [8]. In addition, amino acids 53–61 of the ORF6 C terminus are sufficient for ORF6 to block the nuclear translocation of STAT1, thereby reducing the activity of the ISRE and ISG56 promoters [9].

ORF7a/b

ORF7a and 7b are accessory proteins of SARS-CoV-2. Both ORF7a and ORF7b inhibit the type 1 IFN response, but they do so through slightly different mechanisms. ORF7a inhibits the phosphorylation of STAT2, while ORF7b inhibits the phosphorylation of both STAT1 and STAT2 [8]. SARS-CoV-2 ORF7a and 7b prevent the STAT proteins from translocating to the nucleus and subsequent transcription of ISGs. Given the myriad of SARS-CoV-2 proteins which inhibit the type 1 IFN response, exogenous addition of certain IFNs may repress viral production. Prophylactic treatment of IFN- β proved sufficient to restrict viral replication in SARS-CoV-2-infected Calu-3 cells [9].

ORF8

ORF8 contains several hypervariable regions and constitutes one of the proteins that shares the smallest degree of homology with SARS-CoV [78]. ORF8 also serves as an important inhibitor of the type I IFN system. SARS-CoV-2 ORF8 overexpression results in dose-dependent inhibition of IFN- β promoter activation [88]. Its IFN-inhibitory activity could be due to downregulation of MHC-I [89]. Zhang *et al.* observed downregulation of human leukocyte antigen (HLA)-A2 with overexpression of ORF8 in 293T cells. SARS-CoV-2 ORF8 may serve as an immune evasion virulence factor through rapid mutation and evolution [90]. Divergent clinical isolates from various geographic regions have been identified with highly variable ORF8 and ORF 14 sequences [91–93]. Kakhki *et al.* proposed that ORF8 might serve as an excellent detection target as its sequence contains stretches of nucleotides that are wholly unique to SARS-CoV-2 [94].

Concluding Remarks

Two decades following its initial outbreak, no standardized therapies for SARS-CoV are available. Therefore, a great deal of in-depth research is needed to develop effective treatments for β -coronavirus infections such as SARS-CoV-2. In this review we have provided an overview of the

Outstanding Questions

Does the ability of the to interact with HS more strongly have a role in the high transmission rate for SARS-CoV-2? Should HS be considered an essential or accessory coreceptor for the S protein? What types of HS modification are essential for viral entry? Which viral proteins activate heparanase?

What are the structure-specific functional alterations of ORF8 and 14 which help the virus to adapt to different geographic locations? Moreover, how can these genetic variations be used to design specific immunotherapy against SARS-CoV-2?

What roles do mammalian target of rapamycin complex (mTORC)1/2 complexes play in virus protein translation and activation of immunity, and which viral proteins regulate them?

SARS-CoV-2 life cycle, specific details on its key proteins, and an account of the polymorphisms in the SARS-CoV-2 genome along with updated information on its global spread. The information may provide important clues about factors regulating the differential spread, high transmission, and pathogenesis of SARS-CoV-2. We have also discussed the relative differences in viral protein functions between the two SARS-CoV types. Both structural and nonstructural SARS-CoV-2 proteins play important roles in usurping cellular machinery, insights into which may be helpful to target the cellular signaling pathways to make the host microenvironment resistant to virus replication. Many SARS-CoV-2 proteins delay immune activation, which prolongs infection and allows further viral replication. This is followed by substantial IFN production, resulting in a cytokine storm that contributes to acute tissue injury. Proinflammatory viral factors are poorly studied and need to be further investigated (see [Outstanding Questions](#)). Elucidation of the crystal structures of these SARS-CoV-2 factors would likely contribute to development of safe and effective anti-COVID-19 therapies or vaccines. Collectively, it is clear that individual factors of SARS coronaviruses have diverse roles in disruption of cellular programs, and additional research focus directed in the areas outlined here may help in delineating the underlying mechanism of pathogenesis and spread in humans and will be instrumental in our quest for curative therapeutics.

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