ANNUAL REVIEWS

*Annual Review of Microbiology*

# Human Coronavirus: Host-Pathogen Interaction

To Sing Fung and Ding Xiang Liu

Guangdong Province Key Laboratory of Microbial Signals and Disease Control and Integrative Microbiology Research Centre, South China Agricultural University, Guangzhou 510642, Guangdong, People's Republic of China; email: dxliu0001@163.com

Annu. Rev. Microbiol. 2019. 73:529–57

First published as a Review in Advance on June 21, 2019

The *Annual Review of Microbiology* is online at micro.annualreviews.org

https://doi.org/10.1146/annurev-micro-020518-115759





## Keywords

coronavirus, host-virus interaction, ER stress, MAPK, apoptosis, innate immunity

## Abstract

Human coronavirus (HCoV) infection causes respiratory diseases with mild to severe outcomes. In the last 15 years, we have witnessed the emergence of two zoonotic, highly pathogenic HCoVs: severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). Replication of HCoV is regulated by a diversity of host factors and induces drastic alterations in cellular structure and physiology. Activation of critical signaling pathways during HCoV infection modulates the induction of antiviral immune response and contributes to the pathogenesis of HCoV. Recent studies have begun to reveal some fundamental aspects of the intricate HCoV-host interaction in mechanistic detail. In this review, we summarize the current knowledge of host factors co-opted and signaling pathways activated during HCoV infection, with an emphasis on HCoV-infection-induced stress response, autophagy, apoptosis, and innate immunity. The cross talk among these pathways, as well as the modulatory strategies utilized by HCoV, is also discussed.

## Contents



## INTRODUCTION

Coronaviruses are a group of enveloped viruses with nonsegmented, single-stranded, and positive-sense RNA genomes. Apart from infecting a variety of economically important vertebrates (such as pigs and chickens), six coronaviruses have been known to infect human hosts and cause respiratory diseases. Among them, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) are zoonotic and highly pathogenic coronaviruses that have resulted in regional and global outbreaks.

According to the International Committee on Taxonomy of Viruses, coronaviruses are classified under the order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae*. Based on early serological and later genomic evidence, *Coronavirinae* is divided into four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus* (126). Four distinct lineages (A, B, C, and D) have been assigned within the genus *Betacoronavirus*. Among the six known human coronaviruses (HCoVs), HCoV-229E and HCoV-NL63 belong to *Alphacoronavirus*, whereas HCoV-OC43 and HCoV-HKU1 belong to lineage A, SARS-CoV to lineage B, and MERS-CoV to lineage C *Betacoronavirus* (**Figure 1**).

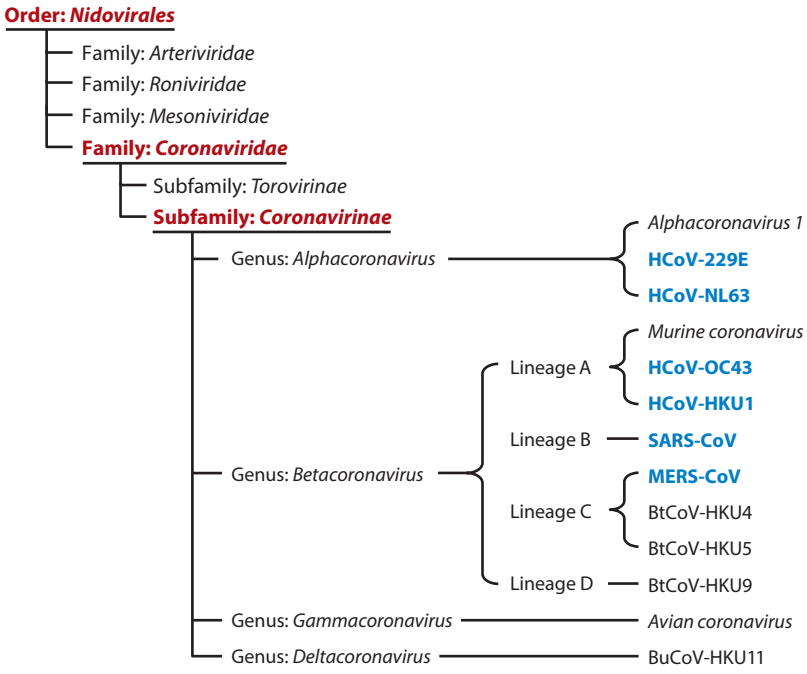


**Figure 1**

Taxonomy of HCoVs: the updated classification scheme of HCoV and other coronaviruses. The six known HCoVs are in blue. Abbreviations: BtCoV, bat coronavirus; BuCoV, bulbul coronavirus; HCoV, human coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus.

In November 2002, a viral respiratory disease first appeared in southern China and quickly spread to other countries, leading to over 8,000 confirmed cases at the end of the epidemic in June 2003, with a mortality rate of ~9.6% (98). The etiologic agent was identified as SARS-CoV, a zoonotic betacoronavirus originated in horseshoe bats that later adapted to infect the intermediate host palm civet and ultimately humans (64). After an incubation period of 4–6 days, SARS patients develop flu-like symptoms and pneumonia, which in severe cases lead to fatal respiratory failure and acute respiratory distress syndrome (96). Although SARS-CoV infects multiple organs and causes systemic disease, symptoms indeed worsen as the virus is cleared, suggesting that aberrant immune response may underlie the pathogenesis of SARS-CoV (98). While no cases of SARS have been reported since 2004, a rich gene pool of bat SARS-related coronaviruses was discovered in a cave in Yunnan, China, highlighting the necessity to prepare for future reemergence (50).

In June 2012, MERS-CoV emerged in Saudi Arabia as the causative agent of a SARS-like respiratory disease (25). Although human-to-human transmission is considered limited, MERS-CoV has caused two major outbreaks in Saudi Arabia (2012) and South Korea (2015), with the global confirmed cases exceeding 2,000 and a mortality rate of ~35% (10). Elderly people infected with MERS-CoV, particularly those with comorbidities, usually develop more severe and sometimes fatal disease (42). Similar to SARS-CoV, MERS-CoV originated in bats, but it later adapted to dromedary camels as intermediate hosts (17). Currently, no vaccine or specific antiviral drug has been approved for either SARS-CoV or MERS-CoV.

Prior to the emergence of SARS-CoV, only two HCoVs (HCoV-229E and HCoV-OC43) were known, both causing mild upper respiratory symptoms when inoculated to healthy adult

volunteers (45). Two more HCoVs, HCoV-NL63 and HCoV-HKU1, were identified in 2004 and 2005, respectively (31, 127). Together, these four globally distributed HCoVs presumably contribute to 15–30% of cases of common cold in humans (69). Although diseases are generally self-limiting, these mild HCoVs can sometimes cause severe lower respiratory infections in infants, elderly people, or immunocompromised patients (41, 97). Similar to SARS-CoV and MERS-CoV, HCoV-NL63 and HCoV-229E originated in bats, whereas HCoV-OC43 and HCoV-HKU1 likely originated in rodents (22). Importantly, a majority of alphacoronaviruses and betacoronaviruses were identified only in bats, and many coronaviruses phylogenetically related to SARS-CoV and MERS-CoV were discovered in diverse bat species (22). Therefore, emerging zoonotic HCoVs such as SARS-CoV and MERS-CoV likely originated in bats through sequential mutation and recombination of bat coronaviruses, underwent further mutations during the spillover to intermediate hosts, and finally acquired the ability to infect human hosts (22).

In this review, we first revisit the replication cycle of HCoV, with a particular focus on the host factors co-opted during individual stages of HCoV replication. Next, we summarize the current knowledge of important signaling pathways activated during HCoV infection, including stress response, autophagy, apoptosis, and innate immunity. The cross talk among these pathways and the modulatory strategies utilized by HCoV are also discussed.



## HCoV REPLICATION AND THE INVOLVEMENT OF HOST FACTORS

### Morphology and Genomic Structure of HCoV

Coronaviruses are spherical or pleomorphic, with a diameter of 80–120 nm. Under the electron microscope, the virion surface is decorated with club-like projections constituted by the trimeric spike (S) glycoprotein (79). Shorter projections made up of the dimeric hemagglutinin-esterase (HE) protein are observed in some betacoronaviruses (such as HCoV-OC43 and HCoV-HKU1) (24). Both S and HE are type I transmembrane proteins with a large ectodomain and a short endodomain. The viral envelope is supported by the membrane (M) glycoprotein, the most abundant structural protein that embeds in the envelope via three transmembrane domains (79). Additionally, a small transmembrane protein known as the envelope (E) protein is also present in a low amount in the envelope (71). Finally, the nucleocapsid (N) protein binds to the RNA genome in a beads-on-a-string fashion, forming the helically symmetric nucleocapsid (79).

The coronavirus genome is a positive-sense, nonsegmented, single-stranded RNA, with an astoundingly large size ranging from 27 to 32 kilobases. The genomic RNA is 5′-capped and 3′-polyadenylated and contains multiple open reading frames (ORFs). The invariant gene order is 5′-replicase-S-E-M-N-3′, with numerous small ORFs (encoding accessory proteins) scattered among the structural genes (**Figure 2**). The coronavirus replicase is encoded by two large overlapping ORFs (ORF1a and ORF1b) occupying about two-thirds of the genome and is directly translated from the genomic RNA. The structural and accessory genes, however, are translated from subgenomic RNAs (sgRNAs) generated during genome transcription/replication as described below.

The coronavirus replication cycle is divided into several steps: attachment and entry, translation of viral replicase, genome transcription and replication, translation of structural proteins, and virion assembly and release (**Figure 3**). In this section, we briefly review each step and summarize host factors involved in coronavirus replication (**Table 1**).

### Attachment and Entry

Coronavirus replication is initiated by the binding of S protein to the cell surface receptor(s). The S protein is composed of two functional subunits, S1 (bulb) for receptor binding and S2 (stalk) for membrane fusion. Specific interaction between S1 and the cognate receptor triggers a

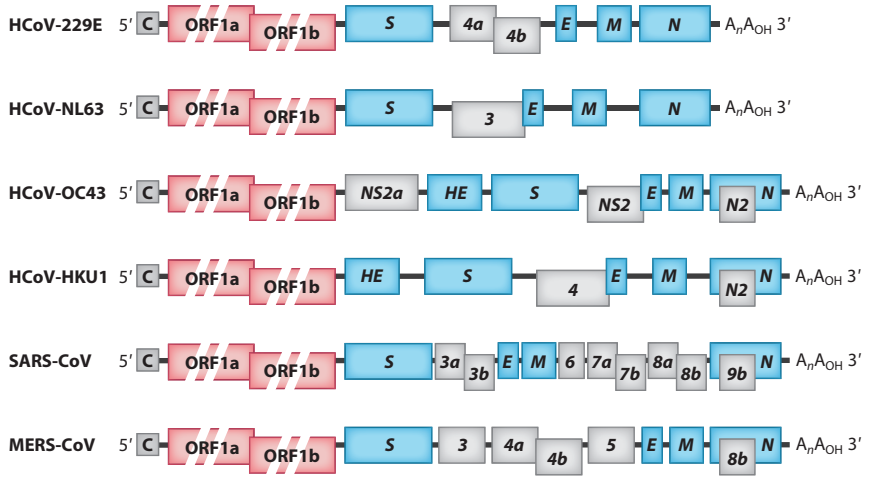


**Figure 2**

Genome structure of human coronaviruses (HCoVs). Schematic diagram showing the genome structure of six known HCoVs (not to scale). The 5′-cap structure (5′-C) and 3′-polyadenylation ($A_nA_{OH}$-3′) are indicated. The open reading frame 1a (ORF1a) and ORF1b are represented as shortened red boxes. The genes encoding structural proteins spike (S), envelope (E), membrane (M), nucleocapsid (N), and hemagglutinin-esterase (HE) are shown as blue boxes. The genes encoding accessory proteins are shown as gray boxes.

drastic conformational change in the S2 subunit, leading to the fusion between the virus envelope and the cellular membrane and release of the nucleocapsid into the cytoplasm (79). Receptor binding is the major determinant of host range and tissue tropism for a coronavirus. Some HCoVs have adopted cell surface enzymes as receptors, such as aminopeptidase N (APN) for HCoV-229E, angiotensin converting enzyme 2 (ACE2) for HCoV-NL63 and SARS-CoV, and dipeptidyl peptidase 4 (DPP4) for MERS-CoV, while HCoV-OC43 and HCoV-HKU1 use 9-*O*-acetylated sialic acid as a receptor (69).

The S1/S2 cleavage of coronavirus S protein is mediated by one or more host proteases. For instance, activation of SARS-CoV S protein requires sequential cleavage by the endosomal cysteine protease cathepsin L (7, 105) and another trypsin-like serine protease (4). On the other hand, the S protein of MERS-CoV contains two cleavage sites for a ubiquitously expressed protease called furin (84). Interestingly, whereas the S1/S2 site was cleaved during the synthesis of MERS-CoV S protein, the other site (S2′) was cleaved during viral entry (84). A similar cleavage event was also observed in infectious bronchitis virus (IBV), a prototypic gammacoronavirus that infects chickens, in an earlier study (132). Additionally, type II transmembrane serine proteases TMPRSS2 and TMPRSS11D have also been implicated in the activation of S protein of SARS-CoV (6) and HCoV-229E (5). Apart from S activation, host factors might also be involved in subsequent stages of virus entry. For example, valosin-containing protein (VCP) contributed to the release of coronavirus from early endosomes, as knockdown of VCP led to decreased replication of both HCoV-229E and IBV (125).

Host factors could also restrict the attachment and entry of HCoV. For example, interferon-inducible transmembrane proteins (IFITMs) exhibited broad-spectrum antiviral functions against various RNA viruses (2). The entry of SARS-CoV, MERS-CoV, HCoV-229E, and HCoV-NL63 was restricted by IFITMs (51). In sharp contrast, however, HCoV-OC43 used IFITM2 or IFITM3 as an entry factor to facilitate its infection (144). A recent study identified several amino acid residues in IFITMs that control the restriction versus enhancing activities on HCoV entry (145).

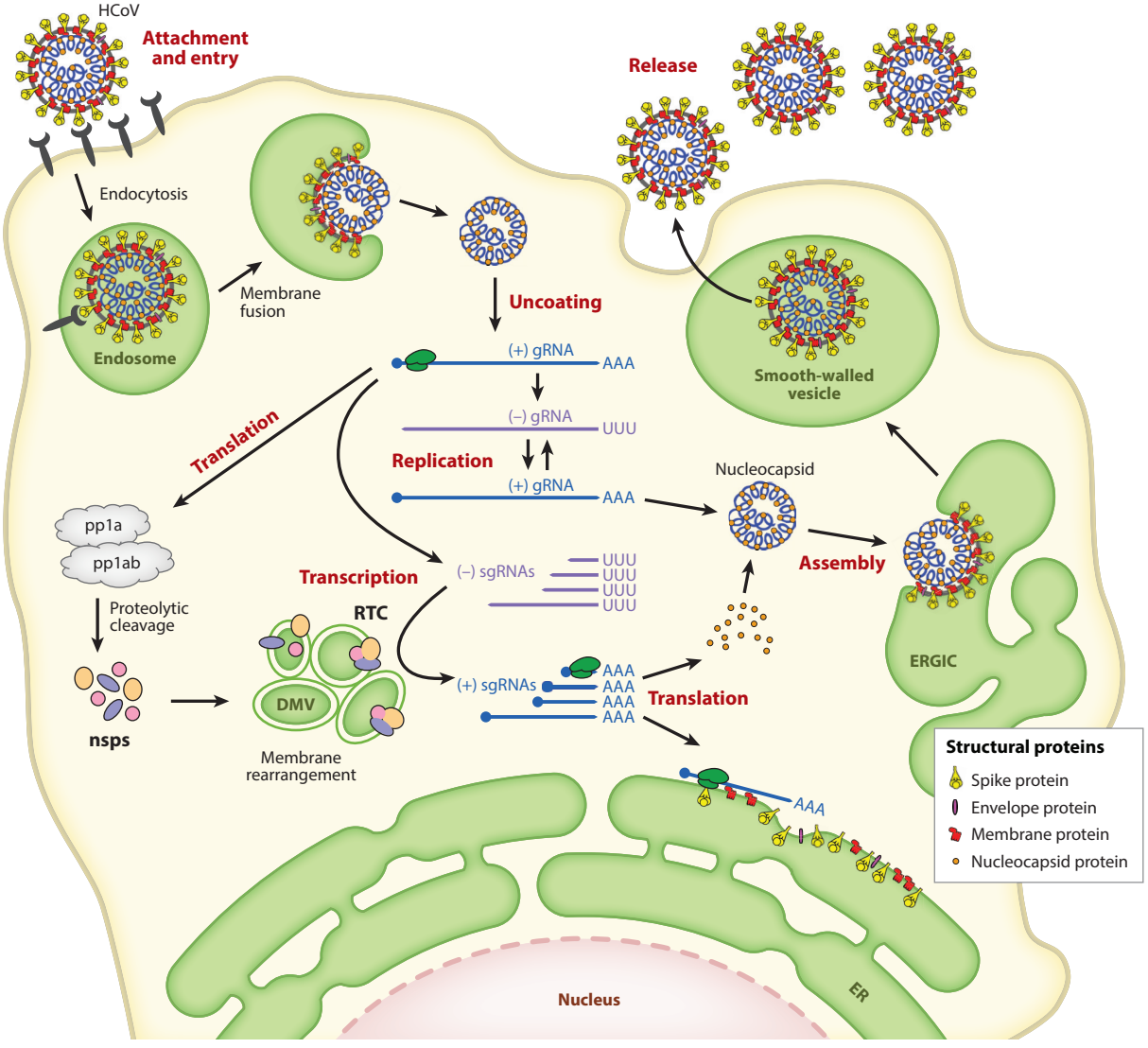


**Figure 3**

Replication cycle of human coronaviruses (HCoVs). Schematic diagram showing the general replication cycle of HCoVs. Infection starts with the attachment of HCoVs to the cognate cellular receptor, which induces endocytosis. Membrane fusion typically occurs in the endosomes, releasing the viral nucleocapsid to the cytoplasm. The genomic RNA (gRNA) serves as the template for translation of polyproteins pp1a and pp1ab, which are cleaved to form nonstructural proteins (nsps). nsps induce the rearrangement of cellular membrane to form double-membrane vesicles (DMVs), where the viral replication transcription complexes (RTCs) are anchored. Full-length gRNA is replicated via a negative-sense intermediate, and a nested set of subgenomic RNA (sgRNA) species are synthesized by discontinuous transcription. These sgRNAs encode viral structural and accessory proteins. Particle assembly occurs in the ER-Golgi intermediate complex (ERGIC), and mature virions are released in smooth-walled vesicles via the secretory pathway.

## Translation of Replicase and Assembly of the Replication Transcription Complex

After entry and uncoating, the genomic RNA serves as a transcript to allow cap-dependent translation of ORF1a to produce polyprotein pp1a. Additionally, a slippery sequence and an RNA

**Table 1 Host factors involved in HCoV replication**

| Replication stage | Host factor(s) | HCoV (other CoV) | Function |
|---|---|---|---|
| Attachment and entry | APN | HCoV-229E | Cellular receptor |
| | ACE2 | SARS-CoV, HCoV-NL63 | Cellular receptor |
| | DPP4 | MERS-CoV | Cellular receptor |
| | 9-*O*-acetylated sialic acid | HCoV-OC43, HCoV-HKU1 | Cellular receptor |
| | Cathepsin L | SARS-CoV | Cleave and activate S protein |
| | Furin | MERS-CoV, (IBV) | Cleave and activate S protein |
| | TMPRSS11D | SARS-CoV, HCoV-229E | Cleave and activate S protein |
| | VCP | HCoV-229E, (IBV) | Facilitate virus release from early endosomes during entry |
| | IFITM | SARS-CoV, MERS-CoV, HCoV-229E, HCoV-NL63 | Restrict virus entry |
| | IFITM2/IFITM3 | HCoV-OC43 | Facilitate virus entry |
| Translation of replicase and RTC assembly | Annexin A2 | (IBV) | Bind to RNA pseudoknot and regulate ribosomal frameshifting |
| | GBF1 and ARF1 | (MHV) | Facilitate the formation of double-membrane vesicle |
| Genome replication and transcription | GSK3 | SARS-CoV, (MHV-JHM) | Phosphorylate N protein and facilitate viral replication |
| | DDX1 | (MHV-JHM) | Facilitate template switching and synthesis of genomic RNA and long sgRNAs |
| | hnRNPA1 | SARS-CoV | Regulate viral RNA synthesis |
| | ZCRB1 | (IBV) | Bind to 5′ UTR of the viral genome |
| | Mitochondrial aconitase | (MHV) | Bind to 3′ UTR of the viral genome |
| | PABP | (Bovine CoV) | Bind to poly(A) tail of the viral genome |
| Translation of structural proteins | *N*-linked glycosylation enzymes | SARS-CoV | Modify S and M protein; *N*-linked glycosylation of the S protein facilitates lectin-mediated virion attachment and constitutes some neutralizing epitopes |
| | *O*-linked glycosylation enzymes | (MHV) | Modify M protein; *O*-linked glycosylation of the M protein affects interferon induction and virus replication in vivo |
| | ER chaperones | SARS-CoV | Proper folding and maturation of S protein |
| Virion assembly and release | Tubulin | HCoV-229E, HCoV-NL63, (TGEV) | Bind to cytosolic domain of S protein; facilitate particle assembly and release |
| | β-Actin | (IBV) | Bind to M protein; facilitate particle assembly and release |
| | Vimentin | (TGEV) | Bind to N protein; facilitate particle assembly and release |
| | Filamin A | (TGEV) | Bind to S protein; facilitate particle assembly and release |

Abbreviations: RTC, replication transcription complex; sgRNA, subgenomic RNA.

pseudoknot near the end of ORF1a enable 25–30% of the ribosomes to undergo −1 frameshifting, thereby continuing translation on ORF1b to produce a longer polyprotein pp1ab (79). The autoproteolytic cleavage of pp1a and pp1ab generates 15–16 nonstructural proteins (nsps) with various functions. Importantly, the RNA-dependent RNA polymerase (RdRP) activity is encoded in nsp12 (130), whereas papain-like protease (PLPro) and main protease (Mpro) activities are encoded in nsp3 and nsp5, respectively (149). nsp3, 4, and 6 also induce rearrangement of the cellular membrane to form double-membrane vesicles (DMVs) or spherules (1, 77), where the coronavirus replication transcription complex (RTC) is assembled and anchored.

Apart from the RNA secondary structures, programmed ribosomal frameshifting (PRF) might also be regulated by viral and/or host factors. For example, PRF in the related arterivirus porcine reproductive and respiratory syndrome virus (PRRSV) was transactivated by the viral protein nsp1β, which interacts with the PRF signal via a putative RNA-binding motif (65). A host RNA-binding protein called annexin A2 (ANXA2) was also shown to bind the pseudoknot structure in the IBV genome (62).

In terms of DMV formation and RTC assembly, host factors in the early secretory pathway seemed to be involved. Golgi-specific brefeldin A–resistance guanine nucleotide exchange factor 1 (GBF1) and its effector ADP ribosylation factor 1 (ARF1) are both required for normal DMV formation and efficient RNA replication of mouse hepatitis virus (MHV), a prototypic betacoronavirus that infects mice (119).

## Genome Replication and Transcription

Using the genomic RNA as a template, the coronavirus replicase synthesizes full-length negative-sense antigenome, which in turn serves as a template for the synthesis of new genomic RNA (79). The polymerase can also switch template during discontinuous transcription of the genome at specific sites called transcription-regulated sequences, thereby producing a 5′-nested set of negative-sense sgRNAs, which are used as templates for the synthesis of a 3′-nested set of positive-sense sgRNAs (79).

Although genome replication/transcription is mainly mediated by the viral replicase and confines in the RTC, the involvement of various host factors has been implicated. For instance, coronavirus N protein is known to serve as an RNA chaperone and facilitate template switching (150, 151). Importantly, the N protein of SARS-CoV and MHV-JHM was also phosphorylated by glycogen synthase kinase 3 (GSK3), and inhibition of GSK3 was shown to inhibit viral replication in Vero E6 cells infected with SARS-CoV (129). Additionally, GSK3-mediated phosphorylation of the MHV-JHM N protein recruited an RNA-binding protein DEAD-box helicase 1 (DDX1), which facilitates template read-through, favoring the synthesis of genomic RNA and longer sgRNAs (128). Another RNA-binding protein called heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) can also bind tightly to SARS-CoV N protein and potentially regulate viral RNA synthesis (74).

Host RNA-binding proteins could also bind directly to untranslated regions (UTRs) of the coronavirus genome to modulate replication/transcription, such as zinc finger CCHC-type and RNA-binding motif 1 (ZCRB1) binding to the 5′-UTR of IBV (111), mitochondrial aconitase binding to the 3′-UTR of MHV (90), and poly(A)-binding protein (PABP) to the poly(A) tail of bovine coronavirus (108).

## Translation of Structural Proteins

Most of the coronavirus sgRNAs are functionally monocistronic, and thus only the 5′-most ORF is translated in a cap-dependent manner (79). However, some sgRNAs can also employ other

mechanisms, such as ribosome leaky scanning and ribosome internal entry, to translate additional ORFs (71). Transmembrane structural proteins (S, HE, M, and E) and some membrane-associated accessory proteins are translated in the ER, whereas the N protein is translated by cytosolic free ribosomes (79). Recent studies using ribosome profiling have identified ribosome pause sites and revealed several short ORFs upstream of, or embedded within, known viral protein-encoding regions (52).

Most coronavirus structural proteins are subjected to posttranslational modifications that modulate their functions (40). For example, both S and M proteins were modified by glycosylation (147). Although N-linked glycosylation of SARS-CoV S protein does not contribute to receptor binding (109), it might be involved in lectin-mediated virion attachment (46) and might constitute some neutralizing epitopes (107). Also, *O*-linked glycosylation of M protein affects the ability of MHV to induce type I interferon and its replication in mice (26). Proper folding and maturation of viral transmembrane proteins (in particular S) also rely heavily on ER protein chaperones such as calnexin (33).



### Virion Assembly and Release

Particle assembly occurs in the ER-Golgi intermediate compartment (ERGIC) and is orchestrated by the M protein (57, 79). Homotypic interaction of M protein provides the scaffold for virion morphogenesis, whereas M-S and M-N interactions facilitate the recruitment of structural components to the assembly site (48). The E protein also contributes to particle assembly by interacting with M and inducing membrane curvature (68). Finally, coronavirus particles budded into the ERGIC are transported in smooth-wall vesicles and trafficked via the secretory pathway for release by exocytosis.

Various host factors have been implicated in the assembly and release of coronavirus. In particular, interactions between the cytoskeleton and structural proteins seem to be essential. Interactions between tubulins and the cytosolic domain of S protein of HCoV-229E, HCoV-NL63, and TGEV are required for successful assembly and release of infectious viral particles (103). Similarly, interactions between IBV M protein and β-actin, between TGEV N protein and vimentin (an intermediate filament protein), and between TGEV S protein and filamin A (an actin-binding protein) have been shown to facilitate coronavirus particle assembly and/or release (121, 143).

## ACTIVATION OF AUTOPHAGY DURING HCoV INFECTION

Macroautophagy (hereafter referred to as autophagy) is a conserved cellular process involving self (auto) eating (phagy). Specifically, cells under stress conditions (such as starvation, growth factor deprivation, or infection by pathogens) initiate autophagy in nucleation sites at the ER, where part of the cytoplasm and/or organelles are sequestered in autophagosomes and degraded by fusing with lysosomes (135). Autophagy is tightly regulated by highly conserved autophagy-related genes (ATGs) (**Figure 4**).

Autophagy activation is yet to be characterized for human alphacoronavirus infection. In the related porcine alphacoronavirus PEDV, autophagy was activated in Vero cells infected with PEDV strain CH/YNKM-8/2013, and autophagy inhibition suppressed viral replication and reduced the production of proinflammatory cytokines (44). Similarly, activation of autophagy and mitophagy in porcine epithelial cells (IPEC-J2) infected with TGEV (strain SHXB) benefited viral replication and protected infected cells from oxidative stress and apoptosis (148). In contrast, in two separate studies using swine testicular cells infected with TGEV (strain H165) or IPEC-J2 cells infected with PEDV (strain SM98), activation of autophagy indeed suppressed viral replication

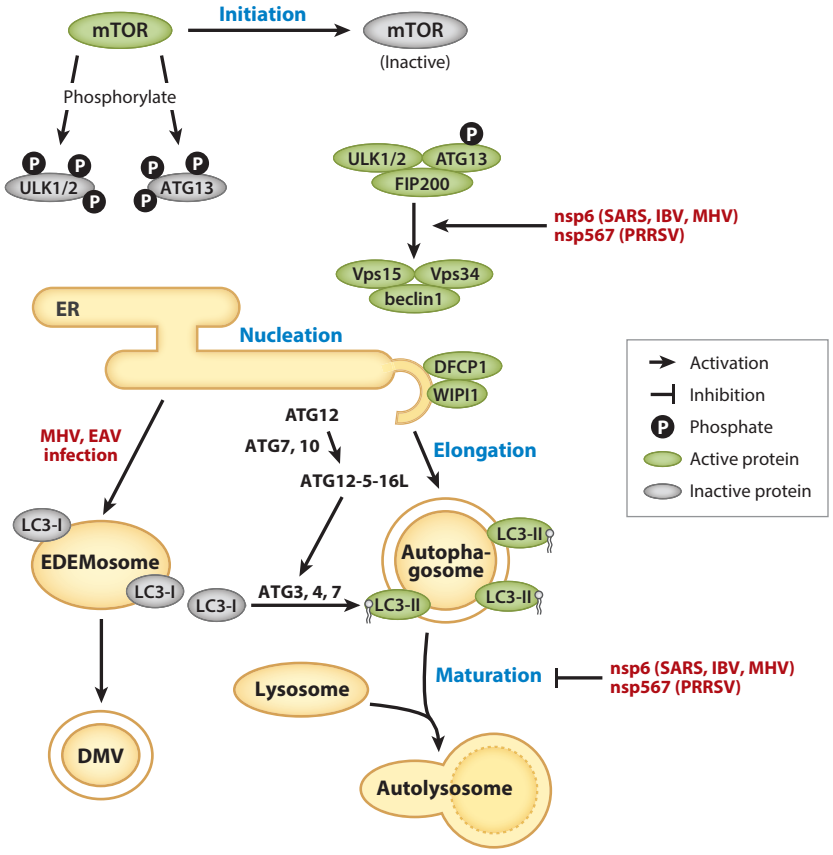


**Figure 4**

Induction and modulation of autophagy by HCoV infection. Schematic diagram showing the signaling pathway of autophagy and the modulatory mechanisms utilized by HCoV. Viruses and viral components modulating the pathway are bolded in red. Abbreviations: ATG, autophagy-related gene; beclin1, coiled-coil myosin-like Bcl2-interacting protein; DFCP1, double-FYVE-containing protein 1; DMV, double-membrane vesicle; EAV, equine arteritis virus; FIP200, FAK family kinase–interacting protein of 200 kDa; IBV, infectious bronchitis virus; LC3, microtubule-associated protein 1 light chain 3; MHV, mouse hepatitis virus; mTOR, mammalian target of rapamycin; PRRSV, porcine reproductive and respiratory syndrome virus; SARS, severe acute respiratory syndrome; ULK, Unc-51-like autophagy-activating kinase; Vps15, vacuolar protein sorting; WIPI1, WD repeat domain, phosphoinositide interacting 1.

(43, 58). Such discrepancies might arise from differences in cell lines and virus strains, calling for more comprehensive in vivo studies.

As for betacoronavirus, initial studies observed colocalization of autophagy protein LC3 and Atg12 with MHV replicase protein nsp8, hinting that DMV formation might utilize components of cellular autophagy (99). However, MHV replication was not affected in ATG5$^{-/-}$ mouse embryonic fibroblasts (MEFs) (146). Also, replication of SARS-CoV was comparable in wild-type or ATG5$^{-/-}$ MEFs overexpressing ACE2, suggesting that intact autophagy is not required for betacoronavirus replication (104). Later, it was shown that MHV co-opted the host machinery for COPII-independent vesicular ER export to derive membranes for DMV formation. This process required the activity of nonlipidated LC3 but was independent of host autophagy (101). Such autophagy-independent activity of LC3 was also implicated in the replication of equine arteritis

virus (EAV) of the family *Arteriviridae* (89). Therefore, it is quite likely that other viruses in the *Nidovirales* order share this LC3-hijacking strategy for replication.

Coronavirus nsp6 is a multipass transmembrane protein implicated in the formation of DMVs during SARS-CoV infection (1). Overexpression of nsp6 of IBV, MHV, or SARS-CoV activated the formation of autophagosomes from the ER via an omegasome intermediate (18). However, autophagosomes induced by IBV infection or overexpression of coronavirus nsp6 had smaller diameters compared with those induced by starvation, indicating that nsp6 might also restrict the expansion of autophagosomes (19).

## INDUCTION OF APOPTOSIS DURING HCoV INFECTION

Apoptosis is one form of programmed cell death characterized by the highly controlled dismantling of cellular structures, which are released in membrane-bound vesicles (known as apoptotic bodies) that are engulfed by neighboring cells or phagocytes (114). Due to its self-limited nature, apoptosis is not immunogenic, thereby distinguishing it from necrotic cell death, where uncontrolled leakage of cellular contents activates an inflammatory response.

Apoptosis can be activated by two pathways (**Figure 5**). The intrinsic pathway is orchestrated by the B cell lymphoma 2 (Bcl2) family proteins (114). Among them, BAX and BAK are proapoptotic, channel-forming proteins that increase the mitochondrial outer membrane permeability (MOMP), whereas Bcl2-like proteins (such as Bcl2, Bcl-xL, and Mcl-1) are antiapoptotic factors that inhibit this process. Under stressful conditions (DNA damage, growth factor deprivation, etc.) BH3-only proteins are activated to overcome the inhibitory effect of Bcl2-like proteins. The resulting increase in MOMP leads to release of cytochrome *c* and formation of an apoptosome, thereby activating effector caspase 3/7. In the extrinsic pathway, binding of the death ligands [such as FasL and tumor necrosis factor-α (TNF-α)] to the cell surface death receptors (such as Fas and TNF receptor 1) leads to the formation of death-inducing signaling complex and activation of caspase 8, which either directly activates effector caspases or engages in cross talk with the intrinsic pathway by activating the BH3-only protein Bid (114).

Apoptosis induced by HCoV infection has been extensively investigated. In autopsy studies, hallmarks of apoptosis were observed in SARS-CoV-infected lung, spleen, and thyroid tissues (61). Also, apoptosis induced by infection of SARS-CoV, MERS-CoV, or other HCoVs was described in various in vitro systems and animal models (113, 136). Apart from respiratory epithelial cells, HCoVs also infect and induce apoptosis in a variety of other cell types. For example, HCoV-OC43 induced apoptosis in neuronal cells (30), while MERS-CoV induced apoptosis in primary T lymphocytes (15). HCoV-229E infection also causes massive cell death in dendritic cells, albeit independent of apoptosis induction (82). Collectively, induction of cell death in these immune cells explains the lymphopenia observed in some HCoV diseases (such as SARS) and may contribute to the suppression of host immune response.

Apoptosis can be induced by multiple mechanisms in HCoV-infected cells. SARS-CoV was shown to induce caspase-dependent apoptosis, which is dependent on but not essential for viral replication, as treatment of pan-caspase inhibitor z-VAD-FMK or overexpression of Bcl2 did not significantly affect SARS-CoV replication (36). In contrast, although MERS-CoV infection of human primary T lymphocytes was abortive, apoptosis was induced via activation of both intrinsic and extrinsic pathways (15). Apoptosis in neuronal cells infected with HCoV-OC43 involved mitochondrial translocation of BAX but was independent of caspase activation (30).

Apoptosis was also induced in cells overexpressing SARS-CoV proteins, including S, E, M, N, and accessory protein 3a, 3b, 6, 7a, 8a, and 9b (70). Among them, SARS-CoV E and 7a protein activated the intrinsic pathway by sequestering antiapoptotic Bcl-$X_L$ to the ER (112). Other

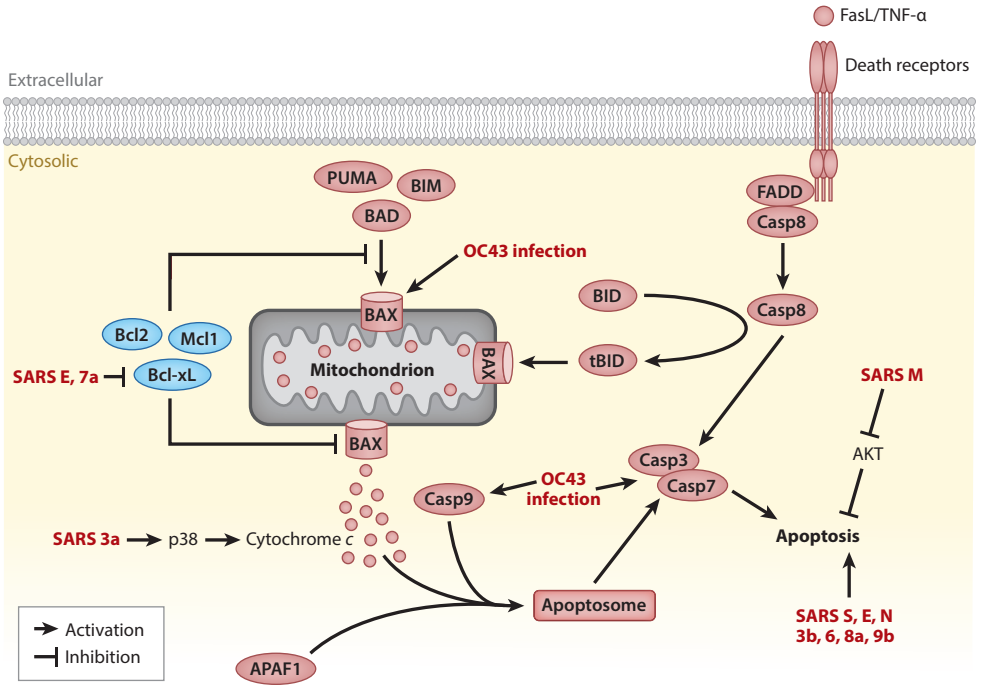


**Figure 5**

Apoptosis induced by HCoV infection and modulatory mechanisms. Schematic diagram showing the signaling pathway of intrinsic and extrinsic apoptosis induction and the modulatory mechanisms utilized by HCoV. Blue ovals are antiapoptotic proteins, whereas pink ovals are proapoptotic proteins. Viruses and viral components modulating the pathway are bolded in red. Abbreviations: AKT, RAC-alpha serine/threonine-protein kinase; APAF1, apoptotic peptidase-activating factor 1; BAD, Bcl2-associated agonist of cell death; BAX, Bcl2-associated X; Bcl-xL, Bcl-2-like protein 1; Bcl2, B cell lymphoma 2; BID, BH3-interacting domain death agonist; BIM, Bcl2-interacting mediator of cell death; Casp, caspase; FADD, Fas associated via death domain; FasL, Fas ligand; HCoV, human coronavirus; Mcl1, myeloid cell leukemia 1; PUMA, p53-upregulated modulator of apoptosis; SARS, severe acute respiratory syndrome; TNF-α, tumor necrosis factor alpha.

proapoptotic mechanisms by SARS-CoV included interfering with prosurvival signaling by M protein and the ion channel activity of E and 3a (70). HCoV infection also modulated apoptosis by activating ER stress response and mitogen-activated protein kinase (MAPK) pathway, as discussed in detail in the following sections.

## ACTIVATION OF ENDOPLASMIC RETICULUM STRESS DURING HCoV INFECTION

ER is a membranous organelle and the main site for synthesis, folding, and modification of secreted and transmembrane proteins. Affected by the extracellular environment and physiological status, the amount of protein synthesized in the ER can fluctuate substantially. When the ER folding capacity is saturated, unfolded proteins accumulate in the ER and lead to ER stress. During HCoV infection, viral structural proteins are produced in massive amounts. In particular, the S glycoprotein relies heavily on the ER protein chaperones and modifying enzymes for its folding and maturation (33). Indeed, overexpression of SARS-CoV S alone was sufficient to

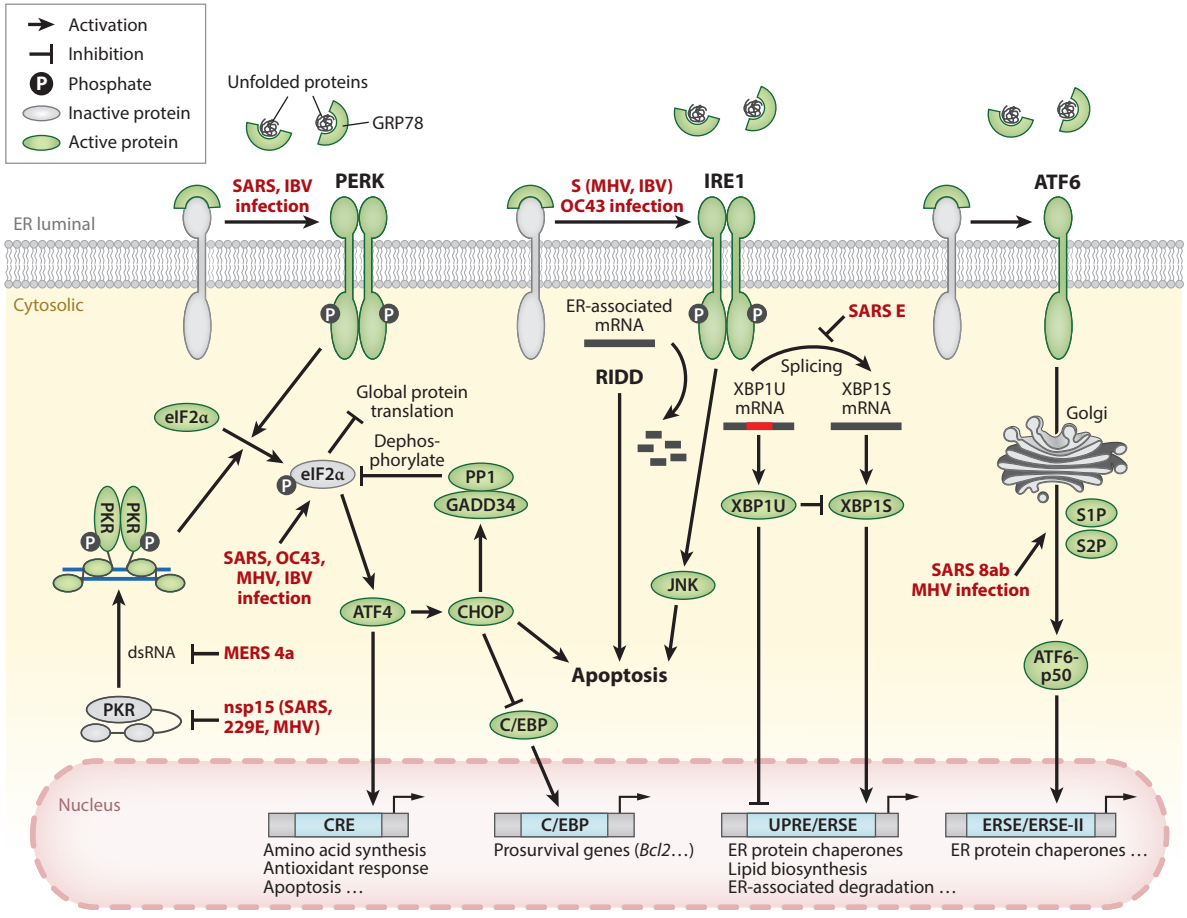


**Figure 6**

Induction and modulation of unfolded protein response by HCoV infection. Schematic diagram showing the three branches of UPR signaling pathway activated and regulated by HCoV infection. Viruses and viral components modulating the pathway are bolded in red. Abbreviations: ATF6, activating transcription factor 6; C/EBP, CCAAT enhancer binding protein; CHOP, C/EBP-homologous protein; CRE, cAMP response element; eIF2α, eukaryotic initiation factor 2 subunit α; ERSE, ER stress response element; GADD34, growth arrest and DNA damage–inducible 34; GRP78, glucose-regulated protein, 78 kDa; HCoV, human coronavirus; IBV, infectious bronchitis virus; IRE1, inositol-requiring enzyme 1; c-Jun N-terminal kinase; MERS, Middle East respiratory syndrome; MHV, mouse hepatitis virus; PERK, PKR-like ER protein kinase; PKR, protein kinase RNA-activated; PP1, protein phosphatase 1; RIDD, IRE1-dependent mRNA decay; SARS, severe acute respiratory syndrome; UPR, unfolded protein response; UPRE, unfolded protein response element; XBP, X-box-binding protein.

induce a potent ER stress response (11). In addition, membrane reorganization for DMV formation and membrane depletion for virion assembly may also contribute to ER stress during HCoV infection (38).

To restore ER homeostasis, signaling pathways known as unfolded protein response (UPR) will be activated. UPR consists of three interrelated pathways, named after the transmembrane sensors: protein kinase RNA-activated (PKR)-like ER protein kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6) (**Figure 6**). In the following section, activation of the three UPR branches by HCoV infection is discussed.

## PERK Pathway and Integrated Stress Response

The PERK pathway is the first to be activated among the three UPR branches. In the stressed ER, protein chaperone GRP78 binds to unfolded proteins and dissociates from the luminal domain of PERK, leading to oligomerization and activation of PERK by autophosphorylation. Activated PERK phosphorylates the α subunit of eukaryotic initiation factor 2 (eIF2α), which inhibits the conversion of inactive GDP-bound eIF2α back to the active GTP-bound form, thereby suppressing translation initiation. The resulting global attenuation of protein synthesis reduces the ER protein influx and allows the ER to reprogram for preferential expression of UPR genes. Besides PERK, eIF2α can also be phosphorylated by three other kinases: heme-regulated inhibitor kinase (HRI), general control nonderepressible 2 (GCN2), and PKR. PKR is an interferon-stimulated gene (ISG) activated by binding of double-stranded RNA (dsRNA), a common intermediate during the replication of DNA and RNA viruses. Together, these four eIF2α kinases and their convergent downstream signaling pathways are known as the integrated stress response (ISR) (102).

Although global protein synthesis is attenuated under ISR, a subset of genes is preferentially translated (102). One of them is activating transcription factor 4 (ATF4), a basic leucine zipper (bZIP) transcription factor that switches on UPR effector genes. ATF4 also induces another bZIP protein C/EBP-homologous protein (CHOP), which is responsible for triggering apoptosis in cells under prolonged ER stress. ATF4 and CHOP further induce growth arrest and DNA damage–inducible protein 34 (GADD34), a regulatory subunit of protein phosphatase 1 (PP1) that dephosphorylates eIF2α. This negative feedback mechanism enables protein synthesis to resume after resolution of ER stress.

In one early study, phosphorylation of PKR, PERK, and eIF2α was observed in 293/ACE2 cells infected with SARS-CoV (61). Surprisingly, knockdown of PKR had no effect on SARS-CoV replication or virus-induced eIF2α phosphorylation, although SARS-CoV-induced apoptosis was significantly reduced. These data suggested that SARS-CoV-induced PKR activation might trigger apoptosis independent of eIF2α phosphorylation (61). As detailed in the section titled Innate Immunity and Proinflammatory Response, recent studies showed that the endoribonuclease activity of coronavirus nsp15 and dsRNA-binding activity of MERS-CoV protein 4a could also suppress PKR activation (28, 56, 100). Activation of ISR by other HCoVs is not fully understood. In neurons infected with HCoV-OC43, only transient eIF2α phosphorylation was observed at early infection, with no induction of ATF4 and CHOP (30).

As for animal coronaviruses, MHV-A59 infection induced significant eIF2α phosphorylation and ATF4 upregulation, but the CHOP/GADD34/PP1 negative-feedback loop was not activated, leading to a sustained translation attenuation (3). TGEV infection also induced eIF2α phosphorylation, and TGEV accessory protein 7 interacted with PP1 and alleviated translation attenuation by promoting eIF2α dephosphorylation (21). Finally, IBV infection triggered transient PKR, PERK, and eIF2α phosphorylation at early infection, which was rapidly inactivated by GADD34/PP1-mediated negative feedback (66, 123). Nonetheless, accumulation of CHOP promoted IBV-induced apoptosis, presumably by inducing proapoptotic protein tribbles homolog 3 (TRIB3) and suppressing the prosurvival extracellular regulated kinase 1/2 (ERK1/2) (66).

## IRE1 Pathway

Besides being activated like PERK via dissociation of GRP78, IRE1 is also activated by direct binding of the unfolded protein to its N-terminal luminal domain (20). Upon activation by oligomerization and autophosphorylation, the cytosolic RNase domain of IRE1 mediates an unconventional splicing of the mRNA of X-box-binding protein 1 (XBP1) (138). The spliced and frameshifted transcript encodes XBP1S, a bZIP transcription factor inducing the expression of

numerous UPR effector genes that enhance ER folding capacity (134). On the other hand, the unspliced transcript encodes XBP1U, a highly unstable protein that negatively regulates XBP1S activity (116). Under prolonged ER stress, the RNase domain of IRE1 can also degrade ER-associated mRNAs in a process called IRE1-dependent mRNA decay (RIDD) (49). Although RIDD facilitates ER homeostasis by reducing ER-associated mRNA, degradation of mRNAs encoding prosurvival proteins contributes to ER-stress-induced cell death (81). Finally, the kinase activity of IRE1 also activates a signaling cascade that ultimately activates c-Jun N-terminal kinase (JNK) (118). Activation of the IRE1-JNK pathway is required for induction of autophagy and apoptosis in cells under ER stress (93).

In one early study, overexpression of MHV S protein was found to induce XBP1 mRNA splicing (120). Also, infection with MHV-A59 induced XBP1 mRNA splicing, although XBP1S protein was not produced, presumably due to translation suppression by the PERK/PKR-eIF2$\alpha$ pathway (3). In sharp contrast, neither SARS-CoV infection nor overexpression of SARS-CoV S protein could induce XBP1 mRNA splicing (27, 120). However, when the SARS-CoV E gene was deleted by reverse genetics, the recombinant virus efficiently induced XBP1 mRNA splicing and upregulated stress-induced genes, leading to a more pronounced apoptosis compared with wild-type control (27). Thus, SARS-CoV E protein might serve as a virulent factor that suppressed activation of the IRE1 pathway and SARS-CoV-induced apoptosis. Infection with another *Betacoronavirus* HCoV-OC43 induced XBP1 mRNA splicing and upregulation of downstream UPR effector genes (30). Notably, two point mutations in the S protein were reproducibly observed during persistent infection of HCoV-OC43 in human neural cell lines. Compared with wild-type control, recombinant HCoV-OC43 harboring these two mutations induced a higher degree of XBP1 mRNA splicing and apoptosis (30). Taken together, activation of the IRE1 pathway seems to promote apoptosis during HCoV infection.

Efficient XBP1 mRNA splicing and upregulation of UPR effector genes were also observed in cells infected with IBV (37). In contrast with its role during HCoV infection, IRE1 indeed suppressed apoptosis in IBV-infected cells, presumably by converting proapoptotic XBP1U to antiapoptotic XBP1S, and by modulating phosphorylation of key kinases such as JNK and AKT (37).

## ATF6 Pathway

Similar to PERK and IRE1, ATF6 is activated by ER stress-induced dissociation from GRP78. Alternatively, underglycosylation or reduction of disulfide bonds in its ER luminal domain can also activate ATF6 (69). Upon activation, ATF6 is translocated to the Golgi apparatus, where protease cleavage releases its N-terminal cytosolic domain (ATF6-p50). ATF6-p50 is a bZIP transcription factor that translocates to the nucleus and induces the expression of UPR effector genes harboring ER stress response element (ERSE) or ERSE-II in the promoters (139). Apart from ER protein chaperones, ATF6 also induces the expression of CHOP and XBP1, thereby connecting the three UPR branches into an integrated signaling network (102).

Activation of the ATF6 pathway by HCoV infection is less studied, and most studies have relied on indirect methods, such as luciferase reporter, due to the lack of a specific antibody. No ATF6 cleavage was detected in cells infected with SARS-CoV (27), and overexpression of SARS-CoV S protein failed to activate ATF6 luciferase reporter (11). However, ATF6 cleavage and nuclear translocation were observed in cells transfected with SARS-CoV accessory protein 8ab, and physical interaction between 8ab and the luminal domain of ATF6 was also determined (110). The SARS-CoV 8ab protein was only detected in early isolates during the pandemic, while two separated proteins 8a and 8b were encoded in later isolates resulting from a 29-nucleotide genome deletion (94).

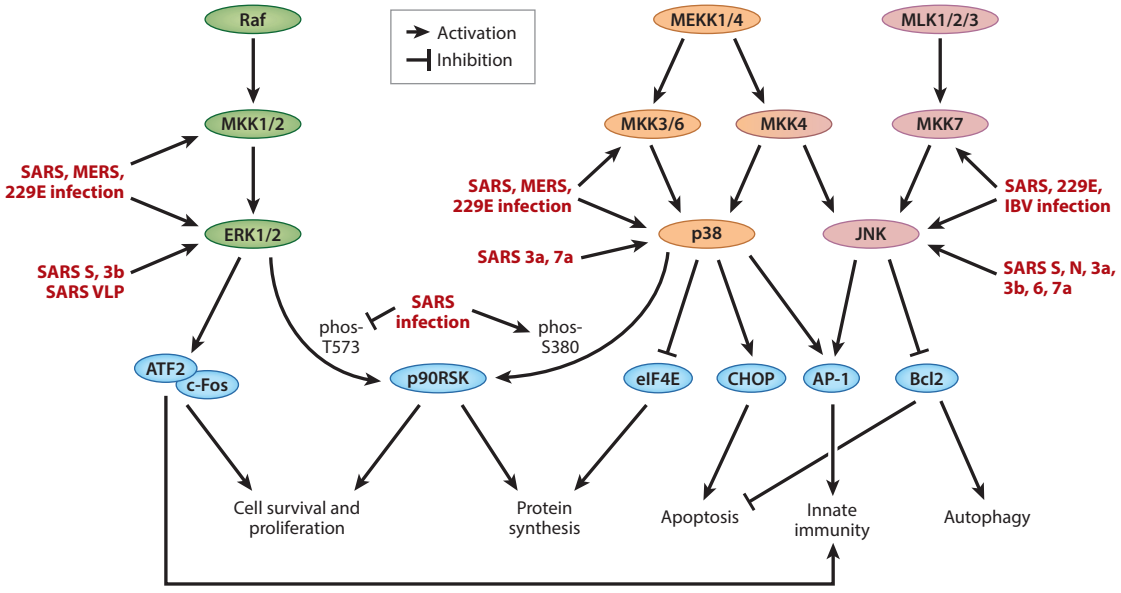


**Figure 7**

Activation and modulation of MAPK signaling pathways by HCoV infection. Schematic diagram showing the activation and modulation of MAPK signaling pathway by HCoV infection. Viruses and viral components modulating the pathway are bolded in red. Abbreviations: AP-1, activator protein 1; ATF2, activating transcription factor 2; Bcl2, B cell lymphoma 2; c-Fos, Fos proto-oncogene; CHOP, C/EBP-homologous protein; eIF4E, eukaryotic translation initiation factor 4E; ERK, extracellular signal–regulated kinase; MAPK, mitogen-activated protein kinase; MEKK, MAPK/ERK kinase kinase; MKK, MAPK kinase; MLK, mixed lineage kinase; p90RSK, 90-kDa ribosomal protein S6 kinase 1; Raf, Raf-1 proto-oncogene.

## ACTIVATION OF MAPK PATHWAYS DURING HCoV INFECTION

MAPKs are evolutionarily conserved serine/threonine protein kinases, which are activated in response to a variety of environmental stimuli, such as heat shock, DNA damage, and the treatment with mitogens or proinflammatory cytokines (55). MAPKs are currently classified into four groups, namely ERK1/2, ERK5, p38, and JNK. To become activated, MAPKs require dual phosphorylation of threonine and tyrosine by upstream MAPK kinases (MKKs) within a conserved TxY motif. MKKs are in turn activated by MKK kinases (MKKKs, also known as MAP3Ks). MAP3Ks are usually activated in multiple steps and regulated by complex mechanisms, such as allosteric inhibition and/or activation by yet other kinases (MAP4Ks) (55). Because MKKs have high substrate specificity toward the cognate MAPKs, classical MAPK signaling pathways are typically multi-tiered and linear. However, some levels of signaling cross talk do occur, and some atypical MAPKs can be directly activated by MAP3K. By phosphorylating their protein substrates (in many cases transcription factors), activated MAPKs regulate numerous critical cellular processes such as proliferation, differentiation, apoptosis, and immune response (55). The activation of p38, ERK, and JNK pathways during HCoV infection is discussed below (**Figure 7**).

### p38 Pathway

Activated p38 translocates to the nucleus and directly or indirectly phosphorylates a broad range of substrate proteins, including important transcription factors such as cAMP response element-binding protein (CREB), ATF1, signal transducer and activator of transcription 1 (STAT1), and STAT3 (140). By mediating the phosphorylation of eIF4E, activated p38 can suppress the

initiation of protein translation. The p38 pathway may also regulate apoptosis by phosphorylating of p53 or other proapoptotic proteins such as CHOP (8, 124).

In early studies, phosphorylation of p38, its upstream kinase MKK3/6, and its downstream substrates was detected in Vero E6 cells infected with SARS-CoV (85, 86). Specifically, p38-dependent phosphorylation of eIF4E might contribute to the suppression of cellular protein synthesis during SARS-CoV infection. However, SARS-CoV genome replication and viral protein synthesis were not affected by the treatment with p38 inhibitor, suggesting that p38 phosphorylation was not essential during SARS-CoV infection in cell culture (86). Notably, overexpression of SARS-CoV accessory protein 7a alone could induce p38 phosphorylation and inhibit cellular protein synthesis (60). Moreover, activation of the p38 pathway was also implicated in apoptosis induced by overexpression of SARS-CoV protein 3a or 7a (60, 95). Phosphorylation of p38 was also observed in human fetal lung cells L132 infected with HCoV-229E, and p38 inhibition was found to inhibit HCoV-229E replication (59). Activation of the p38 pathway was also observed in cells infected with feline coronavirus (FCoV), TGEV, MHV, or IBV (34).



## ERK Pathway

Similar to p38, activated ERK also exerts its function by phosphorylating numerous transcription factors, such as ATF2, c-Fos, and Bcl6 (137). Unlike p38, activated ERK mediates the phosphorylation eIF4E binding protein 1 (eIF4EBP1), causing its dissociation from eIF4E and thereby promoting protein synthesis. ERK also directly phosphorylates 90-kDa ribosomal protein S6 kinases (p90RSKs), which are important kinases regulating protein translation and cell proliferation (32). ERK also regulates Bcl2 family proteins such as BAD, thereby suppressing apoptosis and promoting cell survival (137).

In an early study, phosphorylation of ERK and upstream kinases MKK1/2 was observed in Vero E6 cells infected with SARS-CoV (85). In fact, incubation of A549 cells with SARS-CoV S protein or SARS-CoV virus-like particles was sufficient to induce ERK phosphorylation (14). However, activation of p90RSK, one of the key substrates of ERK, was complicated in SARS-CoV-infected cells (88). Upon mitogen stimulation, p90RSK is first phosphorylated by ERK at Thr573 at the C terminus, which leads to autophosphorylation at Ser380. This then allows for the binding of another kinase that phosphorylates p90RSK at Ser221 in the N terminus, leading to its full activation (23). Interestingly, a basal level of Thr573 phosphorylation in p90RSK was abolished in SARS-CoV-infected Vero E6 cells (88). On the other hand, phosphorylation of p90RSK at Ser380 was significantly induced by SARS-CoV infection, which was dependent on the activation of the p38 pathway (88). Therefore, activation of p90RSK might adopt a completely different mechanism in SARS-CoV-infected cells, involving potential cross talk between the ERK and p38 pathways. The same study also observed that treatment with MKK1/2 inhibitor had no effect on SARS-CoV-induced apoptosis, suggesting that activation of the ERK pathway was not sufficient to antagonize apoptosis during SARS-CoV infection (88). This is different from infection with IBV, where ERK apparently served as an antiapoptotic factor (66). Finally, activation of the ERK pathway was also observed in cells infected with MERS-CoV and HCoV-229E (69).

## JNK Pathway

Similar to p38 and ERK, active JNK translocates to the nucleus to phosphorylate a number of transcription factors such as c-Jun and ATF2 (106). Phosphorylated c-Jun then dimerizes with other proteins to form the activator protein 1 (AP-1) complex, which binds to promoters with 12-*O*-tetradecanoylphobol-13-acetate response element (TRE) and activates gene expression (47). Besides inducing the transcription of proapoptotic genes such as *Bak* and *FasL* in the nucleus, JNK

also translocates to the mitochondria and directly phosphorylates Bcl2 family proteins, thereby promoting stress-induced apoptosis (133).

Phosphorylation of JNK and its upstream kinases MKK4 and MKK7 was observed in Vero E6 cells infected with SARS-CoV (87). Additionally, JNK phosphorylation was detected in 293T cells overexpressing SARS-CoV S protein, mediated by protein kinase C epsilon in a calcium-independent pathway (72). Interestingly, treatment with JNK inhibitor abolished persistent infection of SARS-CoV in Vero E6 cells, suggesting a prosurvival function of the JNK pathway (87). This is quite unexpected because apoptosis induced by overexpression of SARS-CoV N or accessory protein 6 or 7a was JNK dependent (69), and activation of JNK also promoted IBV-induced apoptosis (37, 39). Presumably JNK might be proapoptotic during initial SARS-CoV infection but later switched to a prosurvival role in persistently infected cells.

## INNATE IMMUNITY AND PROINFLAMMATORY RESPONSE

The innate immune system is a conserved defense strategy critical for the initial detection and restriction of pathogens and later activation of the adaptive immune response. Effective activation of innate immunity relies on the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and RIG-I-like receptors (RLRs) (69). Upon activation by PAMPs, PRRs recruit adaptor proteins, which initiate complicated signaling pathways involving multiple kinases. This ultimately leads to the activation of crucial transcription factors including interferon regulatory factor 3 (IRF3), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and AP-1. Synergistically, these factors promote the production of type I interferons (IFN-I), which are released and act on neighboring cells by binding to IFN-α/β receptor (IFNAR) (69). The antiviral activity of IFN-I is mediated by the induction of numerous interferon-stimulated genes (ISGs), which antagonize viral replication by various mechanisms (**Figure 8**). Meanwhile, cytokines and chemokines are also induced to activate an inflammatory response, which is also sometimes responsible for extensive tissue damage and other immunopathies associated with HCoV infection (98).

While mild HCoVs such as HCoV-229E typically induced a high level of IFN-I production (82), SARS-CoV and MERS-CoV were shown to utilize numerous mechanisms to suppress the activation of host innate immune response. Several structural proteins (M and N), nonstructural proteins (nsp1 and nsp3), and accessory proteins of SARS-CoV and/or MERS-CoV were identified as interferon antagonists (40, 69, 70). In the following section, the involvement of UPR/ISR and MAPK in HCoV-induced innate immunity is discussed, followed by two important strategies utilized by HCoV to modulate the innate immune response.

### Involvement of ER Stress and ISR

UPR pathways may modulate innate immune and cytokine signaling by multiple mechanisms, including activation of NF-κB and cross talk with MAPK pathways (38). Also, PKR/eIF2α/ATF4-dependent upregulation of GADD34 was essential for the production of interferon beta (IFN-β) and interleukin 6 (IL-6) induced by polyI:C or chikungunya virus infection (16). Moreover, UPR transcription factors such as XBP1 may directly bind to the promoter/enhancer of IFN-β and IL-6 to activate transcription (78). Recently, it was found that while the PERK branch of UPR suppressed TGEV replication by activating NF-κB-dependent IFN-I production (131), the IRE1 branch indeed facilitated IFN-I evasion by downregulating the expression level of miRNA miR-30a-5p (75). Whether similar mechanisms apply during HCoV infection will require further investigation.

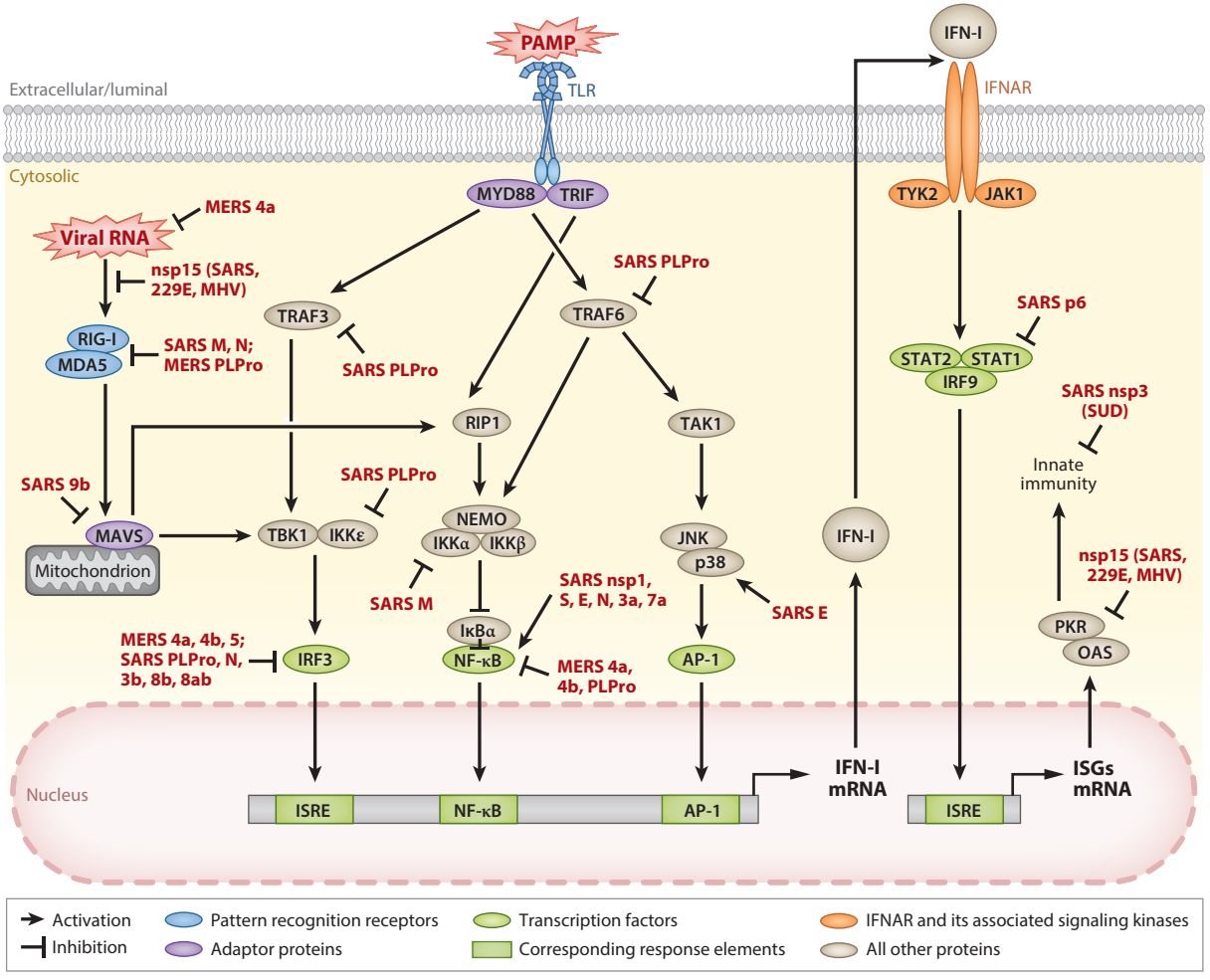


**Figure 8**

Type I interferon induction and signaling during HCoV infection and modulatory mechanisms. Schematic diagram showing the induction and signaling pathways of type I interferon during HCoV infection, and known modulatory mechanisms. Viruses and viral components modulating the pathway are bolded in red. Abbreviations: AP-1, activator protein 1; HCoV, human coronavirus; IκBα, NF-κB inhibitor alpha; IFN-I, type I interferon; IFNAR, IFN-α/β receptor; IKKα, IκB kinase α; IRF3, interferon regulatory factor 3; ISG, interferon-stimulated gene; ISRE, interferon-stimulated response element; JAK1, Janus kinase 1; JNK, c-Jun N-terminal kinase; MAVS, mitochondrial antiviral signaling protein; MDA5, melanoma differentiation-associated protein 5; MERS, Middle East respiratory syndrome; MHV, mouse hepatitis virus; MYD88, myeloid differentiation primary response 88; NEMO, NF-κB essential modulator; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; nsp, nonstructural protein; OAS, 2′-5′-oligoadenylate synthetase; PAMP, pathogen-associated molecular pattern; PKR, protein kinase RNA-activated; PLPro, papain-like protease; RIG-I, retinoic acid–inducible gene I; RIP1, receptor-interacting serine/threonine kinase 1; SARS, severe acute respiratory syndrome; STAT1, signal transducer and activator of transcription 1; TAK1, TGF-β-activated kinase 1; TBK1, TANK-binding kinase 1; TLR, Toll-like receptor; TRAF3, TNF receptor–associated factor 3; TRIF, TIR domain–containing adaptor inducing interferon-beta; TYK2, tyrosine kinase 2.

Another important antiviral protein in innate immunity is PKR, which requires dsRNA binding for full activation. In a recent study, endoribonuclease (EndoU) activity encoded by coronavirus nsp15 was found to efficiently suppress the activation of host dsRNA sensors including PKR (56). Replication of EndoU-deficient MHV was greatly attenuated and restricted in vivo even during the early phase of infection. It also triggered an elevated interferon response and

induced PKR-dependent apoptosis (28, 56). Moreover, EndoU-deficient coronavirus also effectively activated MDA5 and OAS/RNase L, caused attenuated disease in vivo, and stimulated a protective immune response (28). Interestingly, protein 4a (p4a) of MERS-CoV was also identified as a dsRNA-binding protein (100). By sequestering dsRNA, MERS-CoV p4a suppressed PKR-dependent translational inhibition, formation of stress granules, and the activation of interferon signaling (100).

## Involvement of MAPK

The MAPK pathways contribute to innate immunity mainly by activating AP-1 and other transcription factors regulating the expression of proinflammatory cytokines. For instance, activation of p38 was essential for cytokine production and immunopathology in mice infected with SARS-CoV (53). Also, upregulation and release of CCL2 and IL-8 induced by the binding of SARS-CoV S protein was dependent on the activation of ERK (12, 14). Similarly, the JNK pathway was required for the induction of cyclooxygenase 2 (COX-2) and IL-8 in cells overexpressing SARS-CoV S protein (12, 72). Similar involvement of MAPK pathway in the induction of proinflammatory cytokines (such as IL-6, IL-8, and TNF-α) was determined for numerous animal coronaviruses as well (34). In addition, MAPK may also regulate cytokine signaling. For example, SARS-CoV infection caused dephosphorylation of STAT3 at Tyr705 in VeroE6 cells, leading to its nuclear exclusion (85). Inhibition of p38 partially inhibited this process, suggesting a suppressive role of p38 in STAT3 signaling during SARS-CoV infection (85).

## Deubiquitinating and deISGylating Activity of HCoV PLPro

Coronaviruses typically encode one or two PLPros in nsp3. Besides the polyprotein-cleaving protease activity, deubiquitinating activity was also identified for PLPro of SARS-CoV, MERS-CoV, and IBV, as well as PLP2 of HCoV-NL63 and MHV-A59 (40). Additionally, PLPro of SARS-CoV and MERS-CoV also recognized proteins modified by ISG15 and catalyzed its removal (deISGylation) (83). Expectedly, deubiquitination and deISGylation of critical factors in the innate immune signaling were utilized by HCoV to antagonize host antiviral response. For instance, overexpressing PLPro of SARS-CoV or MERS-CoV significantly reduced the expression of IFN-β and proinflammatory cytokines in MDA5-stimulated 293T cells (83). Also, SARS-CoV PLPro catalyzed deubiquitination of TNF-receptor-associated factor 3 (TRAF3) and TRAF6, thereby suppressing IFN-I and proinflammatory cytokines induced by TLR7 agonist (63). The deubiquitinating activity of SARS-CoV PLPro also suppressed a constitutively active phosphomimetic IRF3, suggesting its involvement in the postactivation signaling of IRF3 (80). Nonetheless, HCoV PLPro could also antagonize innate immunity by mechanisms independent of its deubiquitinating/deISGylating activity (29).

## Ion Channel Activity and PDZ-Binding Motif of Viroporins Encoded by HCoV

Viroporins are small hydrophobic viral proteins that oligomerize to form ion channels on cellular membrane and/or virus envelope. They are encoded by a wide range of viruses from different families (35). For coronaviruses, ion channel activity has been described for the E protein of MHV (76), SARS-CoV (67), and IBV (117); 3a (73) and 8a (13) of SARS-CoV; ORF3 of PEDV (122); ORF4a of HCoV-229E (141); and ns12.9 of HCoV-OC43 (142).

Ion channel activity is essential for viral replication for some coronaviruses. For instance, recombinant IBV harboring ion channel–defective mutation T16A or A26F in the E gene produced

similar intracellular viral titers but released a significantly lower level of infectious virions to the supernatant, suggesting that ion channel activity might specifically contribute to IBV particle release (117). Similarly, compared with wild-type HCoV-OC43, recombinant virus lacking ns12.9 suffered a tenfold reduction of virus titer in vivo and in vitro (142). Unlike IBV, however, intracellular titers of HCoV-OC43-Δns12.9 were markedly reduced, and electron microscopy suggested defective virion morphogenesis (142). Experiments using small interfering RNA (siRNA) also showed that silencing SARS-CoV 3a (73), HCoV-229E ORF4a (141), or PEDV ORF3 (122) resulted in reduced virion production or release of the correspondent virus. Although ion channel activity of SARS-CoV E protein is not essential for viral replication, it contributes to viral fitness as revealed in a competition assay (91).

Ion channel activity also contributes to HCoV virulence and pathogenesis, particularly induction of stress response and proinflammatory response. In one early study using recombinant virus lacking the E gene, SARS-CoV E protein was shown to downregulate the IRE1 pathway of UPR, reduce virus-induced apoptosis, and stimulate the expression of proinflammatory cytokines (27). Later, using SARS-CoV mutants lacking the E protein ion channel activity ($EIC^-$), it was shown that although viral replication was not affected, in vivo virulence in a mouse model was markedly reduced for $EIC^-$ mutants (91). Remarkably, compared with wild-type control, lung edema accumulation was significantly reduced in mice infected with the $EIC^-$ mutants, accompanied by reduced production of proinflammatory cytokines IL-1β, TNF-α, and IL-6 (91). Specifically, the ion channel activity of SARS-CoV E protein increased the permeability of ERGIC/Golgi membrane and caused the cytosolic release of calcium ion, thereby activating the NLRP3 inflammasome to induce IL-1β production (92). Similarly, compared with wild-type control, BALB/c mice intranasally infected with HCoV-OC43-Δns12.9 showed significant reduction in viral titers and the production of proinflammatory cytokines IL-1β and IL-6 (142).

Apart from the ion channel activity, some coronavirus viroporins also harbor PDZ-binding motifs (PBMs) at their C terminus, which are recognized by cellular PDZ proteins. For example, the last four amino acids of SARS-CoV E protein (DLLV) formed a PBM that interacted with protein associated with Lin seven 1 (PALS1) and modified its subcellular localization. This further led to altered tight junction formation and epithelial morphogenesis, which might contribute to the disruption of lung epithelium in SARS patients (115). Importantly, compared with wild-type control, recombinant SARS-CoV with E protein PBM deleted or mutated was attenuated in vivo and caused reduced immune response (53). SARS-CoV E protein PBM was found to interact with host PDZ protein syntenin and led to its relocation to the cytoplasm, where it activated p38 and induced the expression of proinflammatory cytokines (53). Interestingly, when recombinant SARS-CoV with defective E protein PBM was passaged in cell culture or in vivo, virulence-associated reverting mutations accumulated that either restored the E protein PBM or incorporated a novel PBM sequence to the M or 8a gene (54). This suggests at least one PBM on a transmembrane protein is required for the virulence of SARS-CoV. Accessory protein 3a, another viroporin encoded by SARS-CoV, also harbors a C-terminal PBM. Interestingly, while recombinant SARS-CoV lacking both E and 3a gene was not viable, the presence of either protein with a functional PBM could restore viability (9). Except for HCoV-HKU1, all HCoV E proteins contain PBMs, but their functional significance requires further investigation.

## CONCLUSION

As obligate intracellular parasites restricted by limited genomic capacities, all viruses have evolved to hijack host factors to facilitate their replication. Meanwhile, host cells have also developed intricate signaling networks to detect, control, and eradicate intruding viruses, although these antiviral

pathways are often evaded, inhibited, or subverted by various viral countermechanisms. Virus-host interaction therefore represents an ongoing evolutionary arms race perfected at the molecular and cellular levels. In this review, we have summarized recent progress in studies of HCoV-host interaction, with an emphasis on co-opted host factors and critical signaling pathways. Evidently, every step of the HCoV replication cycle engages certain host factors, and dramatic alterations in cellular structure and physiology activate host stress response, autophagy, apoptosis, and innate immunity. With the recent advance in multi-omics analysis and genome editing (such as CRISPR), it is very likely that more and more host factors and pathways implicated in HCoV infection will be uncovered and characterized in the future. Supplemented with the several well-established HCoV animal models and reverse genetics systems, these studies will hopefully unravel previously unknown mechanisms underlying the molecular biology of HCoVs and how they interact with the host.

From a practical perspective, the study on HCoV-host interaction is also critical in the face of potential future emergence and/or reemergence of highly pathogenic HCoV. In the last 15 years, we have witnessed outbreaks of two zoonotic and highly pathogenic HCoVs. Severe symptoms observed in SARS and MERS patients are indeed largely contributed by immunopathies due to the aberrant activation of the immune system. In sharp contrast, other mild HCoVs cause self-limiting upper respiratory tract infections, which only rarely develop into life-threatening diseases in immune-compromised individuals. How can these related viruses manifest so differently in terms of pathogenesis? To a certain extent, this may be explained by the different patterns of HCoV interaction with the host cells. One example is that mild HCoVs generally induce a high level of IFN-I production, whereas SARS-CoV and MERS-CoV are known to antagonize interferon induction and signaling via numerous mechanisms. A better understanding of HCoV-host interaction will enable us to pinpoint critical viral and host factors that control the pathogenesis of HCoV and to develop therapeutic approaches more effective against HCoV infection. For instance, drugs targeting essential host factors are less likely to select for drug-resistant HCoV variants. Also, while overactive immune response must be suppressed in severe HCoV diseases, enhancing the activation of the immune system would be beneficial during vaccine administration. Finally, findings on HCoV-host interaction may also be extrapolated to other animal and zoonotic coronaviruses, shedding new light on the prevention and control of these economically important and veterinary pathogens as well as emergence of novel zoonotic coronaviral pathogens.



## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

## ACKNOWLEDGMENTS

This work was partially supported by Guangdong Natural Science Foundation grant 2018A030313472, and Guangdong Province Key Laboratory of Microbial Signals and Disease Control grants MSDC-2017-05 and MSDC-2017-06, Guangdong, People's Republic of China.

## LITERATURE CITED

1. Angelini MM, Akhlaghpour M, Neuman BW, Buchmeier MJ. 2013. Severe acute respiratory syndrome coronavirus nonstructural proteins 3, 4, and 6 induce double-membrane vesicles. *mBio* 4(4):e00524-13
2. Bailey CC, Zhong G, Huang I-C, Farzan M. 2014. IFITM-family proteins: the cell's first line of antiviral defense. *Annu. Rev. Virol.* 1:261–83

3. Bechill J, Chen Z, Brewer JW, Baker SC. 2008. Coronavirus infection modulates the unfolded protein response and mediates sustained translational repression. *J. Virol.* 82(9):4492–501
4. Belouzard S, Chu VC, Whittaker GR. 2009. Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. *PNAS* 106(14):5871–76
5. Bertram S, Dijkman R, Habjan M, Heurich A, Gierer S, et al. 2013. TMPRSS2 activates the human coronavirus 229E for cathepsin-independent host cell entry and is expressed in viral target cells in the respiratory epithelium. *J. Virol.* 87(11):6150–60
6. Bertram S, Glowacka I, Müller MA, Lavender H, Gnirss K, et al. 2011. Cleavage and activation of the severe acute respiratory syndrome coronavirus spike protein by human airway trypsin-like protease. *J. Virol.* 85(24):13363–72
7. Bosch BJ, Bartelink W, Rottier PJM. 2008. Cathepsin L functionally cleaves the severe acute respiratory syndrome coronavirus class I fusion protein upstream of rather than adjacent to the fusion peptide. *J. Virol.* 82(17):8887–90
8. Bulavin DV, Saito S, Hollander MC, Sakaguchi K, Anderson CW, et al. 1999. Phosphorylation of human p53 by p38 kinase coordinates N-terminal phosphorylation and apoptosis in response to UV radiation. *EMBO J.* 18(23):6845–54
9. Castaño-Rodriguez C, Honrubia JM, Gutiérrez-Álvarez J, DeDiego ML, Nieto-Torres JL, et al. 2018. Role of severe acute respiratory syndrome coronavirus viroporins E, 3a, and 8a in replication and pathogenesis. *mBio* 9(3):e02325-17
10. Chafekar A, Fielding BC. 2018. MERS-CoV: understanding the latest human coronavirus threat. *Viruses* 10(2):E93
11. Chan CP, Siu KL, Chin KT, Yuen KY, Zheng B, Jin DY. 2006. Modulation of the unfolded protein response by the severe acute respiratory syndrome coronavirus spike protein. *J. Virol.* 80(18):9279–87
12. Chang Y-J, Liu CY-Y, Chiang B-L, Chao Y-C, Chen C-C. 2004. Induction of IL-8 release in lung cells via activator protein-1 by recombinant baculovirus displaying severe acute respiratory syndrome-coronavirus spike proteins: identification of two functional regions. *J. Immunol.* 173(12):7602–14
13. Chen C-C, Krüger J, Sramala I, Hsu H-J, Henklein P, et al. 2011. ORF8a of SARS-CoV forms an ion channel: experiments and molecular dynamics simulations. *Biochim. Biophys. Acta* 1808(2):572–79
14. Chen I-Y, Chang SC, Wu H-Y, Yu T-C, Wei W-C, et al. 2010. Upregulation of the chemokine (C-C motif) ligand 2 via a severe acute respiratory syndrome coronavirus spike-ACE2 signaling pathway. *J. Virol.* 84(15):7703–12
15. Chu H, Zhou J, Wong BH-Y, Li C, Chan JF-W, et al. 2016. Middle East respiratory syndrome coronavirus efficiently infects human primary T lymphocytes and activates the extrinsic and intrinsic apoptosis pathways. *J. Infect. Dis.* 213(6):904–14
16. Clavarino G, Cláudio N, Dalet A, Terawaki S, Couderc T, et al. 2012. Protein phosphatase 1 subunit Ppp1r15a/GADD34 regulates cytokine production in polyinosinic:polycytidylic acid-stimulated dendritic cells. *PNAS* 109(8):3006–11
17. Corman VM, Ithete NL, Richards LR, Schoeman MC, Preiser W, et al. 2014. Rooting the phylogenetic tree of Middle East respiratory syndrome coronavirus by characterization of a conspecific virus from an African bat. *J. Virol.* 88(19):11297–303
18. Cottam EM, Maier HJ, Manifava M, Vaux LC, Chandra-Schoenfelder P, et al. 2011. Coronavirus nsp6 proteins generate autophagosomes from the endoplasmic reticulum via an omegasome intermediate. *Autophagy* 7(11):1335–47
19. Cottam EM, Whelband MC, Wileman T. 2014. Coronavirus NSP6 restricts autophagosome expansion. *Autophagy* 10(8):1426–41
20. Credle JJ, Finer-Moore JS, Papa FR, Stroud RM, Walter P. 2005. On the mechanism of sensing unfolded protein in the endoplasmic reticulum. *PNAS* 102(52):18773–84
21. Cruz JL, Sola I, Becares M, Alberca B, Plana J, et al. 2011. Coronavirus gene 7 counteracts host defenses and modulates virus virulence. *PLOS Pathog.* 7(6):e1002090
22. Cui J, Li F, Shi Z-L. 2019. Origin and evolution of pathogenic coronaviruses. *Nat. Rev. Microbiol.* 17(3):181–92

23. Dalby KN, Morrice N, Caudwell FB, Avruch J, Cohen P. 1998. Identification of regulatory phosphorylation sites in mitogen-activated protein kinase (MAPK)-activated protein kinase-1a/p90rsk that are inducible by MAPK. *J. Biol. Chem.* 273(3):1496–505
24. de Groot RJ. 2006. Structure, function and evolution of the hemagglutinin-esterase proteins of corona- and toroviruses. *Glycoconj. J.* 23(1–2):59–72
25. de Groot RJ, Baker SC, Baric RS, Brown CS, Drosten C, et al. 2013. Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group. *J. Virol.* 87(14):7790–92
26. de Haan CAM, de Wit M, Kuo L, Montalto-Morrison C, Haagmans BL, et al. 2003. The glycosylation status of the murine hepatitis coronavirus M protein affects the interferogenic capacity of the virus in vitro and its ability to replicate in the liver but not the brain. *Virology* 312(2):395–406
27. DeDiego ML, Nieto-Torres JL, Jiménez-Guardeño JM, Regla-Nava JA, Álvarez E, et al. 2011. Severe acute respiratory syndrome coronavirus envelope protein regulates cell stress response and apoptosis. *PLOS Pathog.* 7(10):e1002315

**28. This paper demonstrates how HCoV nsp15 mediates evasion of dsRNA sensing and suppresses apoptosis.**

**28. Deng X, Hackbart M, Mettelman RC, O'Brien A, Mielech AM, et al. 2017. Coronavirus nonstructural protein 15 mediates evasion of dsRNA sensors and limits apoptosis in macrophages. *PNAS* 114(21):E4251–60**

29. Devaraj SG, Wang N, Chen Z, Chen Z, Tseng M, et al. 2007. Regulation of IRF-3-dependent innate immunity by the papain-like protease domain of the severe acute respiratory syndrome coronavirus. *J. Biol. Chem.* 282(44):32208–21
30. Favreau DJ, Meessen-Pinard M, Desforges M, Talbot PJ. 2012. Human coronavirus-induced neuronal programmed cell death is cyclophilin D dependent and potentially caspase dispensable. *J. Virol.* 86(1):81–93
31. Fouchier RAM, Hartwig NG, Bestebroer TM, Niemeyer B, de Jong JC, et al. 2004. A previously undescribed coronavirus associated with respiratory disease in humans. *PNAS* 101(16):6212–16
32. Frödin M, Gammeltoft S. 1999. Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. *Mol. Cell Endocrinol.* 151(1–2):65–77
33. Fukushi M, Yoshinaka Y, Matsuoka Y, Hatakeyama S, Ishizaka Y, et al. 2012. Monitoring S protein maturation in the endoplasmic reticulum by calnexin is important for the infectivity of severe acute respiratory syndrome coronavirus. *J. Virol.* 86(21):11745–53
34. Fung T, Liao Y, Liu DX. 2016. Regulation of stress responses and translational control by coronavirus. *Viruses* 8(7):184
35. Fung T, Torres J, Liu D. 2015. The emerging roles of viroporins in ER stress response and autophagy induction during virus infection. *Viruses* 7(6):2834–57
36. Fung TS, Huang M, Liu DX. 2014. Coronavirus-induced ER stress response and its involvement in regulation of coronavirus-host interactions. *Virus Res.* 194:110–23

**37. This paper demonstrates how the IRE1 branch of UPR promotes cell survival during CoV infection.**

**37. Fung TS, Liao Y, Liu DX. 2014. The ER stress sensor IRE1$\alpha$ protects cells from apoptosis induced by coronavirus infectious bronchitis virus. *J. Virol.* 88(21):12752–64**

38. Fung TS, Liu DX. 2014. Coronavirus infection, ER stress, apoptosis and innate immunity. *Front. Microbiol.* 5:296

**39. This paper demonstrates how CoV infection activates JNK to modulate apoptosis induction.**

**39. Fung TS, Liu DX. 2017. Activation of the c-Jun $NH_2$-terminal kinase pathway by coronavirus infectious bronchitis virus promotes apoptosis independently of c-Jun. *Cell Death Dis.* 8(12):3215**

40. Fung TS, Liu DX. 2018. Post-translational modifications of coronavirus proteins: roles and function. *Future Virol.* 13(6):405–30
41. Gorse GJ, O'Connor TZ, Hall SL, Vitale JN, Nichol KL. 2009. Human coronavirus and acute respiratory illness in older adults with chronic obstructive pulmonary disease. *J. Infect. Dis.* 199(6):847–57
42. Graham RL, Donaldson EF, Baric RS. 2013. A decade after SARS: strategies for controlling emerging coronaviruses. *Nat. Rev. Microbiol.* 11(12):836–48
43. Guo L, Yu H, Gu W, Luo X, Li R, et al. 2016. Autophagy negatively regulates transmissible gastroenteritis virus replication. *Sci. Rep.* 6:23864
44. Guo X, Zhang M, Zhang X, Tan X, Guo H, et al. 2017. Porcine epidemic diarrhea virus induces autophagy to benefit its replication. *Viruses* 9(3):E53

45. Hamre D, Procknow JJ. 1966. A new virus isolated from the human respiratory tract. *Exp. Biol. Med.* 121(1):190–93
46. Han DP, Lohani M, Cho MW. 2007. Specific asparagine-linked glycosylation sites are critical for DC-SIGN- and L-SIGN-mediated severe acute respiratory syndrome coronavirus entry. *J. Virol.* 81(21):12029–39
47. Hess J, Angel P, Schorpp-Kistner M. 2004. AP-1 subunits: quarrel and harmony among siblings. *J. Cell Sci.* 117(25):5965–73
48. Hogue BG, Machamer CE. 2008. Coronavirus structural proteins and virus assembly. In *Nidoviruses*, ed. S Perlman, T Gallagher, EJ Snijder, pp. 179–200. Washington, DC: ASM
49. Hollien J, Weissman JS. 2006. Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. *Science* 313(5783):104–7
50. Hu B, Zeng L-P, Yang X-L, Ge X-Y, Zhang W, et al. 2017. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLOS Pathog.* 13(11):e1006698
51. Huang I-C, Bailey CC, Weyer JL, Radoshitzky SR, Becker MM, et al. 2011. Distinct patterns of IFITM-mediated restriction of filoviruses, SARS coronavirus, and influenza A virus. *PLOS Pathog.* 7(1):e1001258
52. Irigoyen N, Firth AE, Jones JD, Chung BY-W, Siddell SG, Brierley I. 2016. High-resolution analysis of coronavirus gene expression by RNA sequencing and ribosome profiling. *PLOS Pathog.* 12(2):e1005473
**53. Jimenez-Guardeño JM, Nieto-Torres JL, DeDiego ML, Regla-Nava JA, Fernandez-Delgado R, et al. 2014. The PDZ-binding motif of severe acute respiratory syndrome coronavirus envelope protein is a determinant of viral pathogenesis. *PLOS Pathog.* 10(8):e1004320**
54. Jimenez-Guardeño JM, Regla-Nava JA, Nieto-Torres JL, DeDiego ML, Castaño-Rodriguez C, et al. 2015. Identification of the mechanisms causing reversion to virulence in an attenuated SARS-CoV for the design of a genetically stable vaccine. *PLOS Pathog.* 11(10):e1005215
55. Keshet Y, Seger R. 2010. The MAP kinase signaling cascades: a system of hundreds of components regulates a diverse array of physiological functions. *Methods Mol. Biol.* 661:3–38
56. Kindler E, Gil-Cruz C, Spanier J, Li Y, Wilhelm J, et al. 2017. Early endonuclease-mediated evasion of RNA sensing ensures efficient coronavirus replication. *PLOS Pathog.* 13(2):e1006195
57. Klumperman J, Locker JK, Meijer A, Horzinek MC, Geuze HJ, Rottier P. 1994. Coronavirus M proteins accumulate in the Golgi complex beyond the site of virion budding. *J. Virol.* 68(10):6523–34
58. Ko S, Gu MJ, Kim CG, Kye YC, Lim Y, et al. 2017. Rapamycin-induced autophagy restricts porcine epidemic diarrhea virus infectivity in porcine intestinal epithelial cells. *Antiviral Res.* 146:86–95
59. Kono M, Tatsumi K, Imai AM, Saito K, Kuriyama T, Shirasawa H. 2008. Inhibition of human coronavirus 229E infection in human epithelial lung cells (L132) by chloroquine: involvement of p38 MAPK and ERK. *Antiviral Res.* 77(2):150–52
60. Kopecky-Bromberg SA, Martinez-Sobrido L, Palese P. 2006. 7a protein of severe acute respiratory syndrome coronavirus inhibits cellular protein synthesis and activates p38 mitogen-activated protein kinase. *J. Virol.* 80(2):785–93
61. Krähling V, Stein DA, Spiegel M, Weber F, Mühlberger E. 2009. Severe acute respiratory syndrome coronavirus triggers apoptosis via protein kinase R but is resistant to its antiviral activity. *J. Virol.* 83(5):2298–309
62. Kwak H, Park MW, Jeong S. 2011. Annexin A2 binds RNA and reduces the frameshifting efficiency of infectious bronchitis virus. *PLOS ONE* 6(8):e24067
63. Li S-W, Wang C-Y, Jou Y-J, Huang S-H, Hsiao L-H, et al. 2016. SARS coronavirus papain-like protease inhibits the TLR7 signaling pathway through removing Lys63-linked polyubiquitination of TRAF3 and TRAF6. *Int. J. Mol. Sci.* 17(5):E678
64. Li W, Shi Z, Yu M, Ren W, Smith C, et al. 2005. Bats are natural reservoirs of SARS-like coronaviruses. *Science* 310(5748):676–79
65. Li Y, Treffers EE, Napthine S, Tas A, Zhu L, et al. 2014. Transactivation of programmed ribosomal frameshifting by a viral protein. *PNAS* 111(21):E2172–81
66. Liao Y, Fung TS, Huang M, Fang SG, Zhong Y, Liu DX. 2013. Upregulation of CHOP/GADD153 during coronavirus infectious bronchitis virus infection modulates apoptosis by restricting activation of the extracellular signal-regulated kinase pathway. *J. Virol.* 87(14):8124–34

**53. This paper demonstrates how the PDZ-binding motif of the SARS-CoV E protein contributes to viral pathogenesis.**

67. Liao Y, Yuan Q, Torres J, Tam JP, Liu DX. 2006. Biochemical and functional characterization of the membrane association and membrane permeabilizing activity of the severe acute respiratory syndrome coronavirus envelope protein. *Virology* 349(2):264–75
68. Lim KP, Liu DX. 2001. The missing link in coronavirus assembly retention of the avian coronavirus infectious bronchitis virus envelope protein in the pre-Golgi compartments and physical interaction between the envelope and membrane proteins. *J. Biol. Chem.* 276(20):17515–23
69. Lim Y, Ng Y, Tam J, Liu D. 2016. Human coronaviruses: a review of virus-host interactions. *Diseases* 4(4):26
70. Liu DX, Fung TS, Chong KK-L, Shukla A, Hilgenfeld R. 2014. Accessory proteins of SARS-CoV and other coronaviruses. *Antiviral Res.* 109:97–109
71. Liu DX, Inglis SC. 1991. Association of the infectious bronchitis virus 3c protein with the virion envelope. *Virology* 185(2):911–17
72. Liu M, Yang Y, Gu C, Yue Y, Wu KK, et al. 2007. Spike protein of SARS-CoV stimulates cyclooxygenase-2 expression via both calcium-dependent and calcium-independent protein kinase C pathways. *FASEB J.* 21(7):1586–96
73. Lu W, Zheng BJ, Xu K, Schwarz W, Du L, et al. 2006. Severe acute respiratory syndrome-associated coronavirus 3a protein forms an ion channel and modulates virus release. *PNAS* 103(33):12540–45
74. Luo H, Chen Q, Chen J, Chen K, Shen X, Jiang H. 2005. The nucleocapsid protein of SARS coronavirus has a high binding affinity to the human cellular heterogeneous nuclear ribonucleoprotein A1. *FEBS Lett.* 579(12):2623–28
75. Ma Y, Wang C, Xue M, Fu F, Zhang X, et al. 2018. Coronavirus TGEV evades the type I interferon response through IRE1α-mediated manipulation of the miR-30a-5p/SOCS1/3 Axis. *J. Virol.* 92:e00728-18
76. Madan V, García M de J, Sanz MA, Carrasco L. 2005. Viroporin activity of murine hepatitis virus E protein. *FEBS Lett.* 579(17):3607–12
77. Maier HJ, Hawes PC, Cottam EM, Mantell J, Verkade P, et al. 2013. Infectious bronchitis virus generates spherules from zippered endoplasmic reticulum membranes. *mBio* 4(5):e00801-13
78. Martinon F, Chen X, Lee A-H, Glimcher LH. 2010. TLR activation of the transcription factor XBP1 regulates innate immune responses in macrophages. *Nat. Immunol.* 11(5):411–18
79. Masters PS. 2006. The molecular biology of coronaviruses. *Adv. Virus Res.* 66:193–292
80. Matthews K, Schäfer A, Pham A, Frieman M. 2014. The SARS coronavirus papain like protease can inhibit IRF3 at a post activation step that requires deubiquitination activity. *Virol. J.* 11:209
81. Maurel M, Chevet E, Tavernier J, Gerlo S. 2014. Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem. Sci.* 39(5):245–54
82. Mesel-Lemoine M, Millet J, Vidalain P-O, Law H, Vabret A, et al. 2012. A human coronavirus responsible for the common cold massively kills dendritic cells but not monocytes. *J. Virol.* 86(14):7577–87
83. Mielech AM, Kilianski A, Baez-Santos YM, Mesecar AD, Baker SC. 2014. MERS-CoV papain-like protease has deISGylating and deubiquitinating activities. *Virology* 450–451:64–70
**84. Millet JK, Whittaker GR. 2014. Host cell entry of Middle East respiratory syndrome coronavirus after two-step, furin-mediated activation of the spike protein. *PNAS* 111(42):15214–19**
85. Mizutani T, Fukushi S, Murakami M, Hirano T, Saijo M, et al. 2004. Tyrosine dephosphorylation of STAT3 in SARS coronavirus-infected Vero E6 cells. *FEBS Lett.* 577(1–2):187–92
86. Mizutani T, Fukushi S, Saijo M, Kurane I, Morikawa S. 2004. Phosphorylation of p38 MAPK and its downstream targets in SARS coronavirus-infected cells. *Biochem. Biophys. Res. Commun.* 319(4):1228–34
87. Mizutani T, Fukushi S, Saijo M, Kurane I, Morikawa S. 2005. JNK and PI3k/Akt signaling pathways are required for establishing persistent SARS-CoV infection in Vero E6 cells. *Biochim. Biophys. Acta Mol. Basis Dis.* 1741(1):4–10
88. Mizutani T, Fukushi S, Saijo M, Kurane I, Morikawa S. 2006. Regulation of p90RSK phosphorylation by SARS-CoV infection in Vero E6 cells. *FEBS Lett.* 580(5):1417–24
89. Monastyrska I, Ulasli M, Rottier PJM, Guan J-L, Reggiori F, de Haan CAM. 2013. An autophagy-independent role for LC3 in equine arteritis virus replication. *Autophagy* 9(2):164–74

**84. This study shows how host protease furin activates the spike protein of MERS-CoV.**

90. Nanda SK, Leibowitz JL. 2001. Mitochondrial aconitase binds to the 3′ untranslated region of the mouse hepatitis virus genome. *J. Virol.* 75(7):3352–62
**91. Nieto-Torres JL, DeDiego ML, Verdiá-Báguena C, Jimenez-Guardeño JM, Regla-Nava JA, et al. 2014. Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. *PLOS Pathog.* 10(5):e1004077**
92. Nieto-Torres JL, Verdiá-Báguena C, Jimenez-Guardeño JM, Regla-Nava JA, Castaño-Rodriguez C, et al. 2015. Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. *Virology* 485:330–39
93. Ogata M, Hino S, Saito A, Morikawa K, Kondo S, et al. 2006. Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol. Cell. Biol.* 26(24):9220–31
94. Oostra M, de Haan CAM, Rottier PJM. 2007. The 29-nucleotide deletion present in human but not in animal severe acute respiratory syndrome coronaviruses disrupts the functional expression of open reading frame 8. *J. Virol.* 81(24):13876–88
95. Padhan K, Minakshi R, Towheed MAB, Jameel S. 2008. Severe acute respiratory syndrome coronavirus 3a protein activates the mitochondrial death pathway through p38 MAP kinase activation. *J. Gen. Virol.* 89(Part 8):1960–69
96. Peiris J, Lai S, Poon L, Guan Y, Yam L, et al. 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* 361(9366):1319–25
97. Pene F, Merlat A, Vabret A, Rozenberg F, Buzyn A, et al. 2003. Coronavirus 229E-related pneumonia in immunocompromised patients. *Clin. Infect. Dis.* 37(7):929–32
98. Perlman S, Netland J. 2009. Coronaviruses post-SARS: update on replication and pathogenesis. *Nat. Rev. Microbiol.* 7(6):439–50
99. Prentice E, Jerome WG, Yoshimori T, Mizushima N, Denison MR. 2004. Coronavirus replication complex formation utilizes components of cellular autophagy. *J. Biol. Chem.* 279(11):10136–41
100. Rabouw HH, Langereis MA, Knaap RCM, Dalebout TJ, Canton J, et al. 2016. Middle East respiratory coronavirus accessory protein 4a inhibits PKR-mediated antiviral stress responses. *PLOS Pathog.* 12(10):e1005982
101. Reggiori F, Monastyrska I, Verheije MH, Cali T, Ulasli M, et al. 2010. Coronaviruses hijack the LC3-I-positive EDEMosomes, ER-derived vesicles exporting short-lived ERAD regulators, for replication. *Cell Host Microbe* 7(6):500–8
102. Ron D, Walter P. 2007. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.* 8(7):519–29
103. Rüdiger A-T, Mayrhofer P, Ma-Lauer Y, Pohlentz G, Müthing J, et al. 2016. Tubulins interact with porcine and human S proteins of the genus *Alphacoronavirus* and support successful assembly and release of infectious viral particles. *Virology* 497:185–97
104. Schneider M, Ackermann K, Stuart M, Wex C, Protzer U, et al. 2012. Severe acute respiratory syndrome coronavirus replication is severely impaired by MG132 due to proteasome-independent inhibition of M-calpain. *J. Virol.* 86(18):10112–22
105. Simmons G, Gosalia DN, Rennekamp AJ, Reeves JD, Diamond SL, Bates P. 2005. Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. *PNAS* 102(33):11876–81
106. Smeal T, Binetruy B, Mercola DA, Birrer M, Karin M. 1991. Oncogenic and transcriptional cooperation with Ha-Ras requires phosphorylation of c-Jun on serines 63 and 73. *Nature* 354(6353):494–96
107. Song HC, Seo M-Y, Stadler K, Yoo BJ, Choo Q-L, et al. 2004. Synthesis and characterization of a native, oligomeric form of recombinant severe acute respiratory syndrome coronavirus spike glycoprotein. *J. Virol.* 78(19):10328–35
108. Spagnolo JF, Hogue BG. 2000. Host protein interactions with the 3′ end of bovine coronavirus RNA and the requirement of the poly(A) tail for coronavirus defective genome replication. *J. Virol.* 74(11):5053–65
109. Sui J, Li W, Murakami A, Tamin A, Matthews LJ, et al. 2004. Potent neutralization of severe acute respiratory syndrome (SARS) coronavirus by a human mAb to S1 protein that blocks receptor association. *PNAS* 101(8):2536–41
110. Sung SC, Chao CY, Jeng KS, Yang JY, Lai M. 2009. The 8ab protein of SARS-CoV is a luminal ER membrane-associated protein and induces the activation of ATF6. *Virology* 387(2):402–13

**91. This paper demonstrates how ion channel activity of SARS-CoV E contributes to viral pathogenesis.**

**111. This paper demonstrates how CoV 5′-UTR recruits host factors that regulate viral replication and transcription.**

**111. Tan YW, Hong W, Liu DX. 2012. Binding of the 5′-untranslated region of coronavirus RNA to zinc finger CCHC-type and RNA-binding motif 1 enhances viral replication and transcription. *Nucleic Acids Res*. 40(11):5065–77**

112. Tan Y-X, Tan THP, Lee MJ-R, Tham P-Y, Gunalan V, et al. 2007. Induction of apoptosis by the severe acute respiratory syndrome coronavirus 7a protein is dependent on its interaction with the Bcl-XL protein. *J. Virol.* 81(12):6346–55

113. Tao X, Hill TE, Morimoto C, Peters CJ, Ksiazek TG, Tseng C-TK. 2013. Bilateral entry and release of Middle East respiratory syndrome coronavirus induces profound apoptosis of human bronchial epithelial cells. *J. Virol.* 87(17):9953–58

114. Taylor RC, Cullen SP, Martin SJ. 2008. Apoptosis: controlled demolition at the cellular level. *Nat. Rev. Mol. Cell Biol.* 9(3):231–41

115. Teoh K-T, Siu Y-L, Chan W-L, Schlüter MA, Liu C-J, et al. 2010. The SARS coronavirus E protein interacts with PALS1 and alters tight junction formation and epithelial morphogenesis. *Mol. Biol. Cell* 21(22):3838–52

116. Tirosh B, Iwakoshi NN, Glimcher LH, Ploegh HL. 2006. Rapid turnover of unspliced Xbp-1 as a factor that modulates the unfolded protein response. *J. Biol. Chem.* 281(9):5852–60

**117. This paper demonstrates that CoV E ion channel activity is required for efficient CoV release.**

**117. To J, Surya W, Fung TS, Li Y, Verdià-Bàguena C, et al. 2017. Channel-inactivating mutations and their revertant mutants in the envelope protein of infectious bronchitis virus. *J. Virol.* 91(5):e02158-16**

118. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, et al. 2000. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 287(5453):664–66

119. Verheije MH, Raaben M, Mari M, Te Lintelo EG, Reggiori F, et al. 2008. Mouse hepatitis coronavirus RNA replication depends on GBF1-mediated ARF1 activation. *PLOS Pathog.* 4(6):e1000088

120. Versteeg GA, Van De Nes PS, Bredenbeek PJ, Spaan WJM. 2007. The coronavirus spike protein induces endoplasmic reticulum stress and upregulation of intracellular chemokine mRNA concentrations. *J. Virol.* 81(20):10981–90

121. Wang J, Fang S, Xiao H, Chen B, Tam JP, Liu DX. 2009. Interaction of the coronavirus infectious bronchitis virus membrane protein with β-actin and its implication in virion assembly and budding. *PLOS ONE* 4(3):e4908

122. Wang K, Lu W, Chen J, Xie S, Shi H, et al. 2012. PEDV ORF3 encodes an ion channel protein and regulates virus production. *FEBS Lett.* 586(4):384–91

123. Wang X, Liao Y, Yap PL, Png KJ, Tam JP, Liu DX. 2009. Inhibition of protein kinase R activation and upregulation of GADD34 expression play a synergistic role in facilitating coronavirus replication by maintaining de novo protein synthesis in virus-infected cells. *J. Virol.* 83(23):12462–72

124. Wang X, Ron D. 1996. Stress-induced phosphorylation and activation of the transcription factor CHOP (GADD153) by p38 MAP kinase. *Science* 272(5266):1347–49

125. Wong HH, Kumar P, Tay FPL, Moreau D, Liu DX, Bard F. 2015. Genome-wide screen reveals valosin-containing protein requirement for coronavirus exit from endosomes. *J. Virol.* 89(21):11116–28

126. Woo PC, Lau SK, Lam CS, Lau CC, Tsang AK, et al. 2012. Discovery of seven novel mammalian and avian coronaviruses in *Deltacoronavirus* supports bat coronaviruses as the gene source of *Alphacoronavirus* and *Betacoronavirus* and avian coronaviruses as the gene source of *Gammacoronavirus* and *Deltacoronavirus*. *J. Virol.* 86:3995–4008

127. Woo PCY, Lau SKP, Chu C, Chan K, Tsoi H, et al. 2005. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J. Virol.* 79(2):884–95

**128. This paper demonstrates how a host protein regulates template read-through during HCoV genome transcription.**

**128. Wu C-H, Chen P-J, Yeh S-H. 2014. Nucleocapsid phosphorylation and RNA helicase DDX1 recruitment enables coronavirus transition from discontinuous to continuous transcription. *Cell Host Microbe* 16(4):462–72**

129. Wu C-H, Yeh S-H, Tsay Y-G, Shieh Y-H, Kao C-L, et al. 2009. Glycogen synthase kinase-3 regulates the phosphorylation of severe acute respiratory syndrome coronavirus nucleocapsid protein and viral replication. *J. Biol. Chem.* 284(8):5229–39

130. Xu X, Liu Y, Weiss S, Arnold E, Sarafianos SG, Ding J. 2003. Molecular model of SARS coronavirus polymerase: implications for biochemical functions and drug design. *Nucleic Acids Res.* 31(24):7117–30
131. Xue M, Fu F, Ma Y, Zhang X, Li L, et al. 2018. The PERK arm of the unfolded protein response negatively regulates transmissible gastroenteritis virus replication by suppressing protein translation and promoting type I interferon production. *J. Virol.* 92(15):e00431-18
132. Yamada Y, Liu DX. 2009. Proteolytic activation of the spike protein at a novel RRRR/S motif is implicated in furin-dependent entry, syncytium formation, and infectivity of coronavirus infectious bronchitis virus in cultured cells. *J. Virol.* 83(17):8744–58
133. Yamamoto K, Ichijo H, Korsmeyer SJ. 1999. BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G2/M. *Mol. Cell. Biol.* 19(12):8469–78
134. Yamamoto K, Yoshida H, Kokame K, Kaufman RJ, Mori K. 2004. Differential contributions of ATF6 and XBP1 to the activation of endoplasmic reticulum stress-responsive *cis*-acting elements ERSE, UPRE and ERSE-II. *J. Biochem.* 136(3):343–50
135. Yang Z, Klionsky DJ. 2010. Eaten alive: a history of macroautophagy. *Nat. Cell Biol.* 12(9):814–22
136. Yeung M-L, Yao Y, Jia L, Chan JFW, Chan K-H, et al. 2016. MERS coronavirus induces apoptosis in kidney and lung by upregulating Smad7 and FGF2. *Nat. Microbiol.* 1(3):16004
137. Yoon S, Seger R. 2006. The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. *Growth Factors* 24(1):21–44
138. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. 2001. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 107(7):881–91
139. Yoshida H, Okada T, Haze K, Yanagi H, Yura T, et al. 2001. Endoplasmic reticulum stress-induced formation of transcription factor complex ERSF including NF-Y (CBF) and activating transcription factors 6α and 6β that activates the mammalian unfolded protein response. *Mol. Cell. Biol.* 21(4):1239–48
140. Zarubin T, Han J. 2005. Activation and signaling of the p38 MAP kinase pathway. *Cell Res.* 15(1):11–18
141. Zhang R, Wang K, Lv W, Yu W, Xie S, et al. 2014. The ORF4a protein of human coronavirus 229E functions as a viroporin that regulates viral production. *Biochim. Biophys. Acta* 1838(4):1088–95
142. Zhang R, Wang K, Ping X, Yu W, Qian Z, et al. 2015. The ns12.9 accessory protein of human coronavirus OC43 is a viroporin involved in virion morphogenesis and pathogenesis. *J. Virol.* 89(22):11383–95
143. Zhang X, Shi H, Chen J, Shi D, Dong H, Feng L. 2015. Identification of the interaction between vimentin and nucleocapsid protein of transmissible gastroenteritis virus. *Virus Res.* 200:56–63
144. Zhao X, Guo F, Liu F, Cuconati A, Chang J, et al. 2014. Interferon induction of IFITM proteins promotes infection by human coronavirus OC43. *PNAS* 111(18):6756–61
145. Zhao X, Sehgal M, Hou Z, Cheng J, Shu S, et al. 2018. Identification of residues controlling restriction versus enhancing activities of IFITM proteins on entry of human coronaviruses. *J. Virol.* 92(6):e01535-17
146. Zhao Z, Thackray LB, Miller BC, Lynn TM, Becker MM, et al. 2007. Coronavirus replication does not require the autophagy gene *ATG5*. *Autophagy* 3(6):581–85
147. Zheng J, Yamada Y, Fung TS, Huang M, Chia R, Liu DX. 2018. Identification of N-linked glycosylation sites in the spike protein and their functional impact on the replication and infectivity of coronavirus infectious bronchitis virus in cell culture. *Virology* 513:65–74
148. Zhu L, Mou C, Yang X, Lin J, Yang Q. 2016. Mitophagy in TGEV infection counteracts oxidative stress and apoptosis. *Oncotarget* 7(19):27122–41
149. Ziebuhr J, Snijder EJ, Gorbalenya AE. 2000. Virus-encoded proteinases and proteolytic processing in the *Nidovirales*. *J. Gen. Virol.* 81(4):853–79
150. Zúñiga S, Cruz JLG, Sola I, Mateos-Gómez PA, Palacio L, Enjuanes L. 2010. Coronavirus nucleocapsid protein facilitates template switching and is required for efficient transcription. *J. Virol.* 84(4):2169–75
151. Zúñiga S, Sola I, Moreno JL, Sabella P, Plana-Durán J, Enjuanes L. 2007. Coronavirus nucleocapsid protein is an RNA chaperone. *Virology* 357(2):215–27

Annual Review of Microbiology

Volume 73, 2019

# Contents

**Indexes**



**Errata**

An online log of corrections to *Annual Review of Microbiology* articles may be found at http://www.annualreviews.org/errata/micro