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REVIEW

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Coronavirus interactions with the cellular autophagy machinery

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ABSTRACT

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, is the most recent example of an emergent coronavirus that poses a significant threat to human health. Virus-host interactions play a major role in the viral life cycle and disease pathogenesis, and cellular pathways such as macroautophagy/autophagy prove to be either detrimental or beneficial to viral replication and maturation. Here, we describe the literature over the past twenty years describing autophagy-coronavirus interactions. There is evidence that many coronaviruses induce autophagy, although some of these viruses halt the progression of the pathway prior to autophagic degradation. In contrast, other coronaviruses usurp components of the autophagy pathway in a non-canonical fashion. Cataloging these virus-host interactions is crucial for understanding disease pathogenesis, especially with the global challenge of SARS-CoV-2 and COVID-19. With the recognition of autophagy inhibitors, including the controversial drug chloroquine, as possible treatments for COVID-19, understanding how autophagy affects the virus will be critical going forward.

Abbreviations: 3-MA: 3-methyladenine (autophagy inhibitor); AKT/protein kinase B: AKT serine/threonine kinase; ATG: autophagy related; ATPase: adenosine triphosphatase; BMM: bone marrow macrophage; CGAS: cyclic GMP-AMP synthase; CHO: Chinese hamster ovary/cell line; CoV: coronaviruses; COVID-19: Coronavirus disease 2019; DMV: double-membrane vesicle; EAV: equine arteritis virus; EDEM1: ER degradation enhancing alpha-mannosidase like protein 1; ER: endoplasmic reticulum; ERAD: ER-associated degradation; GFP: green fluorescent protein; HCoV: human coronavirus; HIV: human immunodeficiency virus; HSV: herpes simplex virus; IBV: infectious bronchitis virus; IFN: interferon; LAMP1: lysosomal associated membrane protein 1; MAP1LC3/LC3: microtubule associated protein 1 light chain 3; MCoV: mouse coronavirus; MERS-CoV: Middle East respiratory syndrome coronavirus; MHV: mouse hepatitis virus; NBR1: NBR1 autophagy cargo receptor; CALCOCO2/NDP52: calcium binding and coiled-coil domain 2 (autophagy receptor that directs cargo to phagophores); nsp: non-structural protein; OS9: OS9 endoplasmic reticulum lectin; PEDV: porcine epidemic diarrhea virus; PtdIns3K: class III phosphatidylinositol 3-kinase; PLP: papain-like protease; pMEF: primary mouse embryonic fibroblasts; SARS-CoV: severe acute respiratory syndrome coronavirus; SKP2: S-phase kinase associated protein 2; SQSTM1: sequestosome 1; STING1: stimulator of interferon response cGAMP interactor 1; ULK1: unc-51 like autophagy activating kinase 1; Vps: vacuolar protein sorting

Introduction to coronaviruses

The pandemic of COVID-19, arising from the newest member of the coronavirus family SARS-CoV-2, has transitioned this relatively understudied group of viruses to a worldwide public health priority in a matter of months. In this review, we intend to summarize the large amount of work done over the past twenty years to understand how this class of viruses interacts with the autophagy pathway.

Coronaviruses (CoVs) are a group of enveloped viruses with a positive-sense, single-stranded RNA genome [1]. Much like other viral families, coronaviruses replicate by hijacking their host cell's machinery. The infection cycle begins as the viral receptors bind to the host cell and fuse with the membrane. Once the virus enters the host, its genomic RNA is translated from a number of open reading frames encoding structural (capsid) and non-structural proteins.

Negative-sense RNA is produced, which serves as a template for synthesizing progeny genomes [1]. Rearranged host cell membranes serve as physical substrates for the assembly of viral replication complexes, improving the efficiency of RNA synthesis [2].

Prior to 2002, only two human coronavirus serotypes were known, HCoV-229E and HCoV-OC43 [3]. Both are responsible for mild respiratory diseases, which resolve relatively quickly in healthy patients. In 2004 and 2005, two novel coronaviruses were identified, HCoV-NL63 and HCoV-HKU1, which also cause relatively mild flu-like illnesses [4,5]. These four human coronaviruses are likely responsible for somewhere between 15–30% of common colds annually in the world, although they cause more severe disease in infants, the immunocompromised, and the elderly [6]. In general, the four endemic strains of coronaviruses are not considered

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major threats to public health, and coronaviruses were not a major research focus for years.

That changed in November 2002 with the emergence of Severe acute respiratory syndrome coronavirus (SARS-CoV) in southern China [7–9]. This viral respiratory disease quickly spread to other countries, leading to over 8,000 confirmed cases by June 2003 [3]. Following a 4- to 6-day incubation period, SARS patients seemed to exhibit both flu-like symptoms as well as pneumonia. SARS-CoV infects multiple organs, causing systemic disease, and symptoms tend to worsen as the virus is cleared. Severe cases lead to fatal respiratory failure and acute respiratory distress. The causative agent was determined to be a Betacoronavirus originating in horseshoe bats, later evolving the ability to infect palm civets before it spread to humans [10–12]. Years later, a fertile gene pool containing bat SARS-related coronaviruses was found in a cave in Yunnan, China, foreshadowing the possibility of a future resurgence [13].

A second major coronavirus emerged in June 2012, when Middle East respiratory syndrome coronavirus (MERS-CoV) first appeared in Saudi Arabia and led to major outbreaks in Saudi Arabia and, in 2015, South Korea [14]. Even with limited human-to-human transmission, the global confirmed cases seemed to exceed 2,000, with a 35% mortality rate, mostly comprised of elderly patients [15–17]. Much as was learned for SARS-CoV, MERS-CoV viral origins were also traced to bats, with the virus passing through dromedary camels as intermediate hosts before infecting humans [18–21]. To date, there is no approved vaccine for either SARS-CoV or MERS-CoV [3].

Seventeen years after SARS, and seven years after MERS, a new human coronavirus triggered the largest pandemic since 1918. Coronavirus disease 2019 (COVID-19) is a novel viral respiratory illness caused by infection with SARS-CoV-2 [22–24]. Following its emergence at the very end of 2019, the number of infected patients, as well as deaths, has escalated dangerously and the World Health Organization has classified COVID-19 as a global public health emergency [25]. The virus has been found in bronchoalveolar-lavage, sputum, saliva, throat, and nasopharyngeal swabs [26]. Analyses and characterizations of the SARS-CoV-2 genome depict mutation and recombination events, and we are just beginning to gain insight into SARS-CoV-2 origins, emergence, and genome evolution [27–29].

Autophagy

As with several other RNA viruses, coronaviruses have long been known to interact with the cellular macroautophagy/autophagy (hereafter autophagy) pathway to promote their replication [30,31]. Autophagy is a conserved cellular process involving the formation of autophagosomes which enclose cytoplasmic cargo, including long-lived proteins, protein aggregates and organelles, and deliver this cargo to lysosomes for degradation. Although autophagy is a constitutive pathway, it is upregulated when cells are under stressful conditions, such as starvation or infection by pathogens [32]. The autophagy pathway involves multiple steps. First, the sequestering compartment of autophagy, known as the phagophore, nucleates and expands. When the phagophore closes to form the autophagosome, it traps cargo in its double-membraned

structure. The autophagosome then fuses with the endosome to form the acidic amphisome. Finally, the amphisome fuses with the lysosome, allowing for the degradation of vesicular contents in what is termed the autolysosome (Figure 1).

The origin of the nucleating phagophore in eukaryotes is still a matter of debate [33,34]. A leading hypothesis is that the initial phagophore is an endoplasmic reticulum (ER)-associated structure, termed the omegasome [35]. The omegasome structure resides separately from the ER and forms as an autophagy-specific phagophore through the action of the class III phosphatidylinositol 3-kinase (PtdIns3K) complex containing the human/yeast proteins PIK3C3/Vps34, PIK3R4/Vps15, NRBF2/Atg38, ATG14/Atg14 and BECN1/Vps30/Atg6 [36,37]. Next, the expansion of the phagophore occurs by means of the ULK complex including ULK/Atg1, ATG13/Atg13, RB1CC1/FIP200/Atg17 and ATG101 [38]. In addition, mammalian Atg8-family proteins (commonly known as LC3 and GABARAP subfamilies) play a key role in the maturation of the phagophore. LC3 exists as a soluble cytoplasmic protein (LC3-I). LC3-I undergoes phosphatidylethanolamine (PE)-modifications to become LC3-II prior to its insertion into the phagophore membrane. The mammalian ATG12–ATG5-ATG16L1 complex, ATG3, and ATG7 participate in the conversion of LC3-I to LC3-II [39]. Phagophores then recruit cytoplasmic cargo through promiscuous autophagic cargo receptors, including mammalian SQSTM1, NBR1, and CALCOCO2/NDP52, before self-fusing to form double-membraned vesicles [40,41]. These vesicles fuse with endosomes, which deliver vacuolar ATPases, inducing acidification of the vesicle and generating the amphisome [42]. In the last step, fusion of the amphisome and the lysosome forms the so-called autolysosome, which contains the cargo designated for degradation [43].

Individual components of autophagy play many roles in the cell. The strict definition of *autophagy* is the degradation of components via the autophagosome and lysosome. Coronaviruses, similar to other viruses, likely utilizes certain components of the pathway to possibly inhibit the degradative process itself, though these components may not always be required. We begin our analysis of this complex relationship with a component of the LC3 lipidation machinery, ATG5.

Mouse coronavirus and ATG5

Some of the earliest work on coronaviruses and autophagy was performed using mouse hepatitis virus (MHV), also known as mouse coronavirus (MCoV). MHV is a widely used model to study basic coronavirus replication, pathogenesis, and host-immune response, due to its ability to be used in BSL-2 environments, and the permissiveness of some variants in multiple cell types and host-species [44–48]. Much as for other RNA viruses, MHV infection induces cellular autophagy, resulting in the development of double-membrane vesicles (DMVs) [49,50]. These structures mimic autophagosomes in several ways, but are distinct in size, and are the sites of RNA replication during MHV infection. Although some viruses benefit from interactions with the cellular autophagy machinery, it remains unclear if all coronaviruses require autophagy for viral replication or pathogenesis [31,49,51]. A wide variety of host factors, including autophagy and
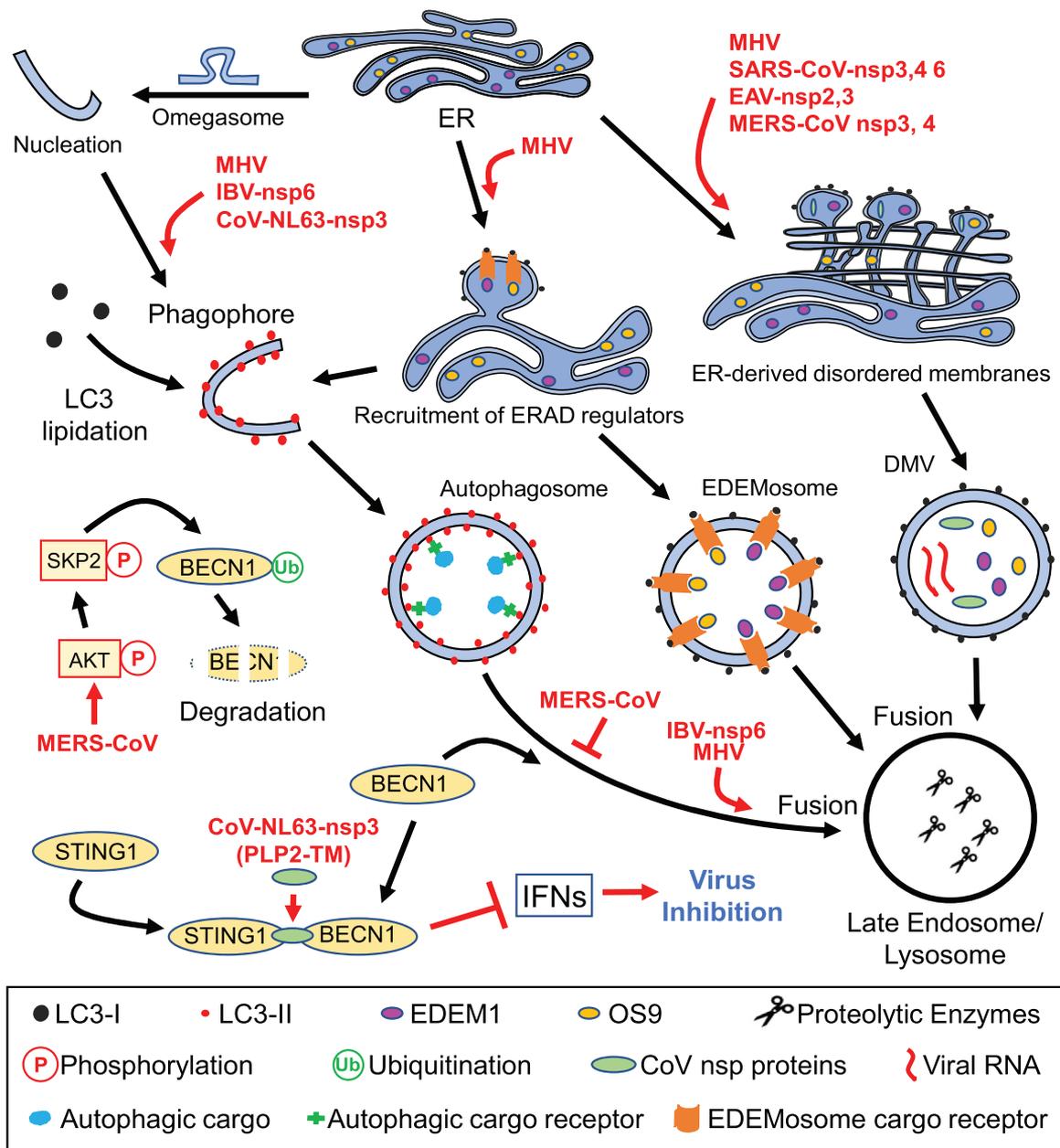
Figure 1. Coronavirus interference in the autophagic pathway. Upon induction of the canonical autophagy pathway, ER membranes rearrange to form membranous structures known as omegasomes. These omegasomes then self-fuse into double-membrane vesicles (DMVs), which are termed autophagosomes. Infection by MHV, nsp6 of IBV, and nsp3 of CoV-NL63 promote the formation of these autophagosomes, and viral replication complexes often associate with these structures. However, coronavirus proteins may induce the formation of DMVs directly from the ER independent of the canonical autophagosome machinery, as seen for SARS-CoV nsp3, 4, and 6, EAV nsp2 and 3, and MERS-CoV nsp3 and 4 (see text.) MHV infection induces ER-derived DMVs independent of the autophagic pathway through hijacking of the host cell ERAD machinery. As the autophagic pathway progresses, the autophagosome fuses with the late endosome, then the lysosome, which results in degradation of the autophagosomal cargo. Nascent RNA of MHV colocalizes with late endosomal markers, suggesting that MHV may allow or promote the fusion of the autophagosome with the late endosome. In other cases, coronaviruses inhibit fusion of the autophagosome with the lysosome. One mechanism of blocking fusion is through direct or indirect inhibition of BECN1, a host protein known to promote this fusion. Specifically, the PLP-domain of nsp3 of CoV-NL63 binds BECN1 and STING1, which prevents BECN1 from promoting autophagosome and lysosome fusion and inhibits production of interferon. MERS-CoV inhibits BECN1-mediated fusion through a separate mechanism, by activation of SKP2, which promotes degradation of BECN1. All of these pathways converge in the late endosome or lysosome, although some coronaviruses inhibit fusion with these compartments.

transport proteins, were recently found to be associated with MHV replication organelles, indicating a commonality for these mechanisms if not necessarily for autophagy itself [52]. The first studies investigating the relationship between autophagy and coronaviruses focused on MHV, which induces cellular autophagy and requires ATG5 for normal levels of virus replication [53]. Lipidation and membrane association

of LC3 is dependent on ATG5, and these events are crucial for formation of autophagic vesicles. The study evaluated MHV replication and growth under autophagic and autophagy-inhibited conditions in murine embryonic stem cells and delayed brain tumor cells.

A few years later, a second study determined that ATG5 and intact autophagy are not required for coronavirus replication in

bone marrow macrophages (BMMs) and primary mouse embryonic fibroblasts (pMEFs) [54]. BMMs are biologically relevant cells for coronavirus infection and pathogenesis, whereas pMEFs are a low-passage primary cell line permissive to coronavirus infection. These two studies used different genetic systems and studied different cell types, providing some possible explanations for the conflicting results. Another explanation could be non-canonical roles for autophagy proteins during coronavirus infections, including a role for LC3 in forming ER-associated degradation (ERAD) organelles during MHV infection. Although the second study showed that ATG5 is not required for MHV replication in BMMs and pMEFs, it did not rule out that other autophagy proteins may still play roles in coronavirus replication. One such example may be a protein that ATG5 plays a role in lipidating, the LC3 protein itself.

Non-canonical roles of LC3 in coronavirus replication

MHV replication does not appear to be dependent on the canonical autophagic pathway, as demonstrated by normal virus replication in cells lacking ATG5 and ATG7 [54,55]. However, this does not preclude the involvement of individual components of the autophagic pathway in MHV replication and packaging. For example, one group demonstrated that the LC3/Atg8 protein is present on MHV-induced DMVs and colocalizes with the MHV nucleocapsid protein [53]. However, other data suggest that nsps from the RNA replication complex do not colocalize with LC3 [56,57]. One explanation for these different results may involve whether endogenous or overexpressed LC3 are examined. In a study directly comparing the two, endogenous LC3 localized with MHV nsp2 and nsp3 while exogenously expressed GFP-LC3 did not [55]. It is important to note and specify that coronavirus nsps may not localize only to DMVs, but potentially other components of viral replication organelles.

In the canonical autophagy pathway, modification of LC3 with PE is critical for LC3 membrane association and vesicle formation. Interestingly, non-lipidated LC3-I associates with the MHV-induced DMVs [55]. These and other data suggest that the MHV-induced, ER-derived DMVs arise from the hijacking of the ERAD machinery.

ERAD, which eliminates misfolded or incompletely translated proteins from the ER, involves three steps: recognition of improperly folded or formless proteins in the ER; retro-translocation into the cytosol; and ubiquitin-dependent degradation by the proteasome [58]. Short-lived ER proteins, including human EDEM1 and OS9, are essential for the transport of ERAD substrates from the ER to the cytosol [59,60]. Under normal conditions, the intra-lumenal levels of these regulators are very low in order to prevent interruption of correct folding programs [61]. The manipulation of ERAD levels by these regulators is called ERAD tuning, and involves recruitment of ERAD regulators by an unknown receptor; generation of structures known as EDEMosomes, which are distinct, ER-derived vesicles; and finally transport of EDEMosomes to endosomes or to lysosomes for degradation. LC3-I is associated with the outer membrane of these EDEMosomes. The ERAD E3 ubiquitin ligase SEL1L may be a receptor for these ERAD regulators, serving as a physical bridge between EDEM1 or OS9 and LC3-I [62]. The interactions

between the regulation of ERAD tuning and autophagy are still unknown.

Accumulation of EDEM1, a chaperone that is normally degraded during ERAD, is visible in cells infected with MHV. EDEM1 is maintained on MHV-induced DMVs, supporting the authors' hypothesis that MHV infection interferes with ERAD to produce the ER-derived DMVs that go on to become sites of viral replication. Although the mechanism through which MHV hijacks the ERAD pathway is not sufficiently understood, the current working hypothesis is that nsps of MHV interact with an unknown EDEMosome cargo receptor that induces EDEM1 export from the ER for ERAD [55]. However, EDEM1 knockdowns do not affect MHV replication, whereas knockdown of LC3 does [55]. Therefore, the autophagic pathway and the replication of MHV on DMVs may be two distinct processes, although both utilize the LC3 protein. However, it is unknown how LC3 associates with EDEMosomes in the absence of PE modification.

Previously, there was no direct evidence for the involvement of ERAD tuning in the generation of DMVs for coronaviruses other than MHV. ERAD tuning may be involved in the generation of DMVs for other coronaviruses, particularly SARS-CoV for which the ER is hypothesized to be the source of the virus-induced DMVs. It is thought that all studied coronaviruses have conserved replication organelle elements strongly indicating they have a broadly conserved mechanism for formation [63]. This story is a caution to researchers, that "autophagy," defined as degradation, does not necessarily follow just because a single autophagy-related protein, such as LC3, is involved.

Coronavirus-induced double-membraned vesicles and vesicle acidification

For all positive-strand RNA viruses studied to date, the viral replication complex associates with host intracellular membranes, although the origin and nature of these membranes vary [64–67]. Some alphavirus replication complexes associate with endosomal and lysosomal membranes, while there is also evidence for the ER as the source of poliovirus-induced viral vesicles [64,67]. Many viruses induce complex rearrangements of cellular membranes prior to formation of DMVs in the cytoplasm of the host cell [68]. For some viruses, including poliovirus and hepatitis C virus, these DMVs can serve as sites for viral replication, though they may not be the primary replication sites [67,69]. For coronaviruses, the replication complex of MHV associates with DMVs, as does the replication complex of the comparable equine arteritis virus (EAV) [70,71]. MERS-CoV and SARS-CoV proteins similarly have been associated with DMVs and other replication organelles [72]. Although not fully understood, DMVs may arise from viral hijacking of the host autophagy pathway, and there is evidence for the involvement of autophagy proteins in DMV generation in rhinovirus- and poliovirus-infected cells [49].

Viral DMVs, much like autophagosomes, may fuse with late endosomes or lysosomes, suggesting that vesicle acidification may play a role in viral replication or maturation of the virion. For example, vesicle acidification promotes cleavage

maturation of a poliovirus capsid protein to result in infectious viral particles [73]. For some coronaviruses, vesicle acidification is important for release of the viral genome into the cytoplasm of the host cell during virus entry [74]. Previous studies show little evidence to suggest a possible role for acidification of DMVs in coronavirus replication or maturation. It was thought that the colocalization of nascent MHV RNA with late endosomal markers suggested that acidification may play a role in MHV replication or maturation [75]. This colocalization suggested fusion of DMVs, which are known sites of MHV replication, with endosomes. However, more recent work using improved imaging technology clearly depicts nascent viral RNA localizing to virus DMVs confirming their role as the primary if not only, site of viral RNA synthesis within replication organelles. It is possible that endosomal markers are re-localized to viral replication organelles [63].

This DMV-endosome fusion is an important step in the canonical autophagy pathway, supporting the proposed interaction between MHV and the host cell autophagy machinery. Interactions between the canonical autophagy machinery and DMVs may take place during the life cycles of other coronaviruses, though this remains to be investigated [53]. As is true for viruses such as arteriviruses and poliovirus, viral DMVs are smaller than autophagosomes and are not necessarily functional for degradation [76,77]. Coronavirus DMVs likely do not to function as degradative vesicles because DMVs act as the primary site of RNA synthesis for coronaviruses [63]. These differences may reduce the number of proteins shared by viral replication and the host autophagy pathway, with those proteins involved in membrane curvature and "pinching" of vesicles most likely to be in common.

Roles of individual coronavirus non-structural proteins

In many cases, non-structural proteins of RNA viruses induce autophagy, though some may only induce certain steps in the pathway. Specifically, autophagy-related membrane rearrangements induced and directed by individual coronavirus proteins have been studied in infectious bronchitis virus (IBV). In comparison, SARS-CoV and MERS-CoV proteins induce and direct the formation of replication organelles such as DMVs. A brief summary of these studies is listed in Table 1. For SARS-CoV, individual proteins of the RNA replication complex associate with DMVs localized to the ER [57]. Using uninfected HEK293T cells, concurrent expression of nsp3, nsp4, and nsp6, three of the sixteen non-structural proteins (nsps) of SARS-CoV, is sufficient to induce DMV formation similar to that observed in SARS-CoV-infected cells. When expressed alone, full-length nsp3 or the isolated C-terminal domain of nsp3 results in disordered and proliferating membrane structures. Expression of nsp4 alone produces no distinct phenotype, but co-expression of nsp3 with nsp4 is sufficient to promote the formation of multi-lamellar bodies described as "maze-like." Nsp6 alone results in the production of single-membrane spherical vesicles localized to the microtubule organizing center, though this phenotype disappears when nsp6 and nsp4 are co-expressed. Only when all three constructs are expressed concurrently do they produce DMVs similar in structure and organization to those formed in SARS-CoV-infected cells [78]. Electron tomography also showed these maze-like bodies with areas of zippered ERs and later confirmed SARS-CoV and MERS-CoV expression of nsp3 and nsp4 induce the formation of DMVs [72].

For MERS-CoV, nsp3 and nsp4 are involved in the formation of DMVs. Co-expression of nsp3 and nsp4, either individually or within the same plasmid as a self-cleaving product, is sufficient to induce the formation of DMVs in HuH-7 cells. Similarly for SARS-CoV, where the formation of DMVs resembling those produced during SARS-CoV infection can occur during co-expression of nsp3 and nsp4 or expression of nsp3, 4, and 6, the MERS-CoV nsp6 is not a requirement for formation of DMVs and, when co-expressed with nsp3 and nsp4, does not alter DMV morphology [72]. For the related equine arteritis virus (EAV), nsp2 and nsp3 co-expression in BHK-21 cells is sufficient for DMV formation [79].

Expression of nsp6 of the avian coronavirus IBV is sufficient for generation of LC3-puncta [80–82]. However, the complex disordered membrane structures formed do not

Table 1. Coronavirus non-structural proteins and the autophagy pathway.

Virus Name	Viral protein	Effects	Cells
HCoV-NL63	PLP domain of nsp3	Autophagosomes, but inhibited autophagic flux[91]	HeLa, HEK293T, and MCF-7
SARS-CoV	nsp3, nsp4, and nsp6	DMV formation[78]	HEK293T
MERS-CoV	nsp3 and nsp4	DMV formation[72]	HuH-7
EAV	nsp2 and nsp3	DMV formation[79]	BHK-21
IBV	nsp6/infection	Autophagosome formation[80]·[82]	CHO and Vero
	nsp6	Autophagosome formation[81]	Avian
MHV	Infection	DMV formation[55]	MEF and HeLa
		ERAD/EDEMosome formation[55]	HeLa

A summary of what is known about expression of coronavirus proteins, responses of the cellular autophagy machinery, and the cell types used in each experiment.

strongly resemble autophagosomes [83]. The LC3 puncta, however, also colocalize with LAMP1, indicating possible endosome/lysosome fusion with these disordered membranes [80]. These data have been confirmed in relevant avian cells, using avian LC3 [81]. IBV nsp6 induces LC3 puncta formation, but colocalizes with ER markers, whereas SARS-CoV nsp6 colocalizes tightly with LC3, suggesting that SARS-CoV nsp6 may travel to the lysosome, if the canonical autophagy pathway proceeds normally [80]. IBV nsp6 constricts the expansion of the LC3 puncta, which is interpreted as limiting the size of autophagosomes [82]. Interestingly, IBV itself does not induce the autophagic pathway in avian cells, although IBV nsp6 expression induces autophagic signaling in both avian and Vero cells [81]. Furthermore, IBV nsp4 has been confirmed to be the driving protein for membrane pairing where IBV nsp3 co-expression does not alter membrane rearrangement [83]. This same study also found that co-expression of IBV nsp3 and nsp4 and co-expression of nsp3, nsp4, and nsp6 do not result in the formation of DMVs but may require other viral proteins to induce DMVs. Taken together, these results from SARS-CoV, MERS-CoV, and IBV demonstrate a variety of roles for coronavirus nsps in the generation of DMVs and progression in the autophagic pathway.

Coronaviruses and BECN1

Critical steps in the autophagy pathway rely on the action of the BECN1 protein, part of the class III PtdIns3K complex that participates in early membrane rearrangements leading to the formation of the autophagosome [84]. Additionally, BECN1 promotes fusion of the autophagosome with the lysosome for content degradation [85]. Proteins of negative-sense RNA viruses and DNA viruses, including Influenza A M2, HIV nef, and HSV-1 ICP34.5, target BECN1 to inhibit either nucleation of the autophagosome or fusion of the autophagosome with the lysosome to increase viral replication [86–89]. BECN1 negatively regulates the innate immune response with CGAS by preventing excessive IFN (interferon) production while promoting autophagic degradation of intracellular pathogens such as HSV-1 (Figure 1) [90]. It appears that the interaction of viral proteins with BECN1 thus serves to increase viral replication and influence the innate immune response.

Coronavirus proteins downregulate BECN1 in multiple ways. Coronaviruses have different strategies to modulate BECN1 activity. A transmembrane-containing portion of the papain-like protease domain of nsp3, PLP-TM, of HCoV-NL63, which is known to induce autophagosome formation in HeLa, HEK293T, and MCF-7 cells, binds to BECN1 to inhibit the fusion of autophagosomes with lysosomes (Figure 1) [91]. Specifically, the nsp3 PLP-TM domain is necessary to complex BECN1 to STING1 (stimulator of interferon response cGAMP interactor 1), which prevents the stimulation of IFN production (Figure 1) [91]. Though the interaction between BECN1 and the PLP2-TM domain of HCoV-NL63 is best characterized, it is possible that BECN1 may also associate with the PLP domains of the nsp3 proteins of other coronaviruses; for example, the nsp3 proteins of SARS-CoV and the porcine epidemic

diarrhea virus (PEDV), because these domains are known to act as IFN antagonists, and a knockdown of BECN1 with siRNA decreases PEDV replication [91–93].

Other coronaviruses may downregulate BECN1 by indirectly downregulating its protein levels. The cellular E3 ubiquitin ligase SKP2 ubiquitinates BECN1, resulting in its degradation. SKP2 therefore acts as a negative regulator of autophagy. The kinase AKT1 activates SKP2 by phosphorylation. MERS-CoV was found to increase phosphorylation of AKT1, increasing its kinase activity [94]. This increase in activation of AKT1 leads to phosphorylation and activation of SKP2, stalling progression of the autophagic pathway [95]. This hypothesis supports the increase in SKP2 phosphorylation and the comparable increase in BECN1 degradation upon infection of VeroB4 cells with MERS-CoV as a mechanism for increasing viral replication. As expected, the number of autophagosomes seems to increase upon infection, despite the indication of so few autolysosomes, which is likely due to a decrease in BECN1 levels [95]. Therefore, MERS-CoV interferes with host cell autophagy by promoting BECN1 degradation. This is consistent with the increase in BECN1 levels and decrease in MERS-CoV replication when infected cells are treated with a SKP2 inhibitor [95]. In this model, MERS-CoV infection activates AKT1 by phosphorylation, which in turn activates SKP2, resulting in the degradation of BECN1 (Figure 1). Loss of BECN1 inhibits the fusion of autophagosomes with lysosomes, potentially protecting viral replication complexes located on cellular double-membraned structures [72].

It is important to contextualize these data; while coronaviruses often inhibit BECN1 function, it does not necessarily mean that the autophagy pathway is inhibited or not involved. Other viruses inhibit upstream autophagy signaling while triggering LC3 lipidation, double-membraned vesicle formation, or utilizing other parts of the pathway. For example, picornaviruses do not require ULK1, which is upstream of BECN1, but they do require LC3 lipidation [96]. Presumably some advantage is afforded to the viruses by non-canonically signaling to induce the membrane-reshaping machinery of autophagy.

Conclusion

As for many other RNA viruses, evidence suggests that coronaviruses interact with the cellular autophagy pathway to enhance virus replication. The development of ER-derived double-membraned vesicles in the host cytoplasm is so similar to autophagosome development that it suggests that coronaviruses are mimicking the cellular autophagy pathway. For many RNA viruses, these double-membrane vesicles serve as genome replication sites, and there is conclusive evidence that virus-induced DMVs are the site of coronavirus RNA synthesis [63].

However, the link between formation of DMVs, true autophagy, and coronavirus replication remains unclear. An example is MHV: one study determined that MHV replication requires the LC3 modification protein ATG5, whereas a later study using biologically relevant knockout cells concluded that ATG5, and thus autophagy, is not required for MHV replication. Another set of studies posited that MHV does not use canonical autophagy at all, but rather subverts the LC3 protein for a non-

canonical role in formation of ERAD-associated structures. The roles of the autophagic pathway and its components appear to vary widely across coronaviruses.

Going forward, what lessons can we take from the history of coronavirus-autophagy studies in understanding the cell biology of SARS-CoV-2 infection? One lesson from the history of the field is that each coronavirus is likely to interact with this pathway in its own unique fashion. Therapeutics such as chloroquine and its derivatives, which inhibit the autophagic pathway, have been suggested as treatments for COVID-19. Therefore, understanding how this and other intracellular pathways affect SARS-CoV-2, and if it is similar to other coronaviruses in these interactions, is an important factor in confronting this and future coronavirus outbreaks.

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