Biology
of the Cell

REVIEW

A snapshot of protein trafficking in SARS-CoV-2 infection

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Abstract

SARS-CoV-2 is a human pathogenic virus responsible for the COVID-19 (coronavirus disease 2019) pandemic. The infection cycle of SARS-CoV-2 involves several related steps, including virus entry, gene expression, RNA replication, assembly of infectious virions and their egress. For all of these steps, the virus relies on and exploits host cell factors, cellular organelles, and processes such as endocytosis, nuclear transport, protein secretion, metabolite transport at membrane contact sites (MSC) and exocytotic pathways. To do this, SARS-CoV-2 has evolved multifunctional viral proteins that hijack cellular factors and modulate their function by unique strategies. In this Review, we highlight cellular trafficking factors, processes, and organelles of relevance to the SARS-CoV-2 infection cycle and how viral proteins make use of and perturb cellular transport during the viral infection cycle.

INTRODUCTION

Cellular homeostasis and cell survival require continuous cycling and trafficking of bio-molecules, which includes cell entry of proteins, carbohydrates, lipids and nucleic acids via endocytosis, exchange of molecules between the cytosol and the nucleus by nuclear transport and communication to the neighbouring cells by exocytosis. This flow of biomolecules ensures equilibrium between biosynthesis and degradation making the dynamics of bio-molecular trafficking vital to the survival of cells and organisms.

Viruses utilise the dynamics of bio-molecular trafficking in host cells to enter the cells, produce viral proteins, amplify and package progeny genomes, egress newly formed particles, and repeat this cycle in a neighbouring cell. Human coronaviruses (HCoVs) such as HCoV-229E, HCoV-OC43, and more recently identified

HCoV-NL63 and HCoV-HKU1, are known to cause common cold (V'Kovski et al., 2021). However, during the past 2 decades, highly pathogenic coronaviruses like severe acquired respiratory syndrome coronavirus (SARS-CoV), middle east respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 emerged having higher lethality in populations by causing acute lung injury and respiratory disstress (Weinstein, 2004; Zaki et al., 2012). Compared to SARS-CoV and MERS-CoV, SARS-CoV-2 has caused a major pandemic and in its course, evolved into several lineages and sub-lineages (Abdelrahman et al., 2020).

The infection cycle of SARS-CoV-2 initiates with the binding of the viral surface protein Spike (S) to the cellular receptor ACE2 (angiotensin-converting enzyme 2) (Lan et al., 2020; Shang et al., 2020; Wang et al., 2020). This interaction results in the internalisation of virions in endosomes where cellular proteases cleave S that mediates the fusion of the viral envelope with the endosomal membrane (Belouzard et al., 2012). Additionally, serine proteases like TMPRSS2 (Transmembrane protease, serine 2) promote the fusion of the viral envelope with the plasma membrane or endosomal membranes in close proximity to the plasma membrane (Hoffmann et al., 2020). Following membrane fusion, or concomitant with it, the viral RNA genome is separated from the nucleocapsid (uncoating) and upon binding of ribosomes, the two large open-reading frames (ORF)-1a and 1b are translated, giving rise to two polyproteins designated pp1a and pp1ab. These are cleaved by two viral proteases into 16 non-structural proteins (nsps). Massive ER membrane rearrangement due to the action of several nsps leads to the biogenesis of viral replication organelles that are composed of accumulations of double-membrane vesicles (DMVs) in which viral RNA replication and sub-genomic RNA (sgRNAs) synthesis occurs. Several sgRNAs encode structural proteins, some of them being inserted into the ER membrane and transported to the ERGIC (ER-Golgi intermediate compartment). Nucleocapsids that probably form close to DMVs, acquire the virion envelope by budding into the ERGIC lumen. Following vesicular transport through the Golgi, virions contained in secretory vesicles are released from the cell via exocytosis. Further details of the individual steps have been discussed in detail in previous reviews and therefore, will not be described here (Holmes et al., 2021; Hu et al., 2021; Jackson et al., 2022; Merad et al., 2022; V'Kovski et al., 2021).

At each step of the infection cycle, viral proteins and virus-induced organelles exploit cellular factors, organelles, and processes to achieve robust replication. This exploitation in turn, gives rise to profound perturbation of infected cells as exemplified by strongly altered protein and membrane trafficking in infected cells (Gordon et al., 2020; Klemm et al., 2021; Stukalov et al., 2021). In this Review, we will discuss cellular trafficking factors, processes, and organelles of relevance to the SARS-CoV-2 infection cycle and how viral proteins perturb cellular transport. We will structure this discussion by focusing on distinct transport pathways, that is, endosomal trafficking, nuclear transport, secretory processes and trafficking at membrane contact sites (MCS).

ENDOSOMAL TRAFFICKING AND SARS-COV-2 ENTRY

Endosomal trafficking is a key process involved in virus entry into host cells (Yamauchi & Greber, 2016). The majority of enveloped viruses gets internalised into host cells via endocytic or non-endocytic routes after binding to distinct cell surface receptors (Dimitrov, 2004). For SARS-CoV-2, the binding of the S glycoprotein to ACE2 triggers this first step of the infection cycle. Spike is composed of two domains, S1 and S2, comprising the receptor binding domain (RBD) and the membrane fusion domain, respectively, and was found to be cleaved at the S1/S2 site likely by furin proteases prior to release from infected cells (Hoffmann et al., 2020; Ou et al., 2020; Walls et al., 2020a). Once in endosome, further cleavage of S by endosome-localised protease cathepsin L at the S2' site exposes a membrane fusion peptide mediating fusion of the viral envelope with the membrane of a late endosome (Ou et al., 2020). In addition to this endocytic entry pathway, especially in respiratory cell lines plasma membrane-resident TMPRSS2 can also process S1/S2 at the S2' site (Bestle et al., 2020; Hoffmann et al., 2020) (Figure 1), thus accounting for the non-endocytic SARS-CoV-2 entry route. S1/S2 cleavage was shown to be essential for TMPRSS2, but not for cathepsin L processing of S at the S2' site (Tang et al., 2021).

Most of the recent research identified cellular factors and processes vital for the endocytic route of SARS-CoV-2 entry, including the endosomal retromer complex that together with the sorting nexins (SNXs) forms the trafficking complex mediating protein sorting at the endosomes (Seaman, 2021; Zhang et al., 2018a). One of the components of this complex, VPS29 (vacuolar protein sorting 29) was identified as a dependency factor for SARS-CoV-2 entry (Poston et al., 2022). VPS29 function was selective for SARS-CoV-2 entry and not required for the entry of other viruses entering via endosomes, such as influenza virus. Mechanistically, loss of VPS29 altered the morphology and acidity of endosomes, which in turn perturbed the activity of endosomal protease cathepsin L required for SARS-CoV-2 entry. Loss of VPS29 also alters the endosomal integrity leading to the enrichment of enlarged PI(3)P$^+$ vesicles (Poston et al., 2022) and possibly providing an explanation of how the absence of VPS29 leads to the loss of endosomal activity and hence, reduced viral entry.
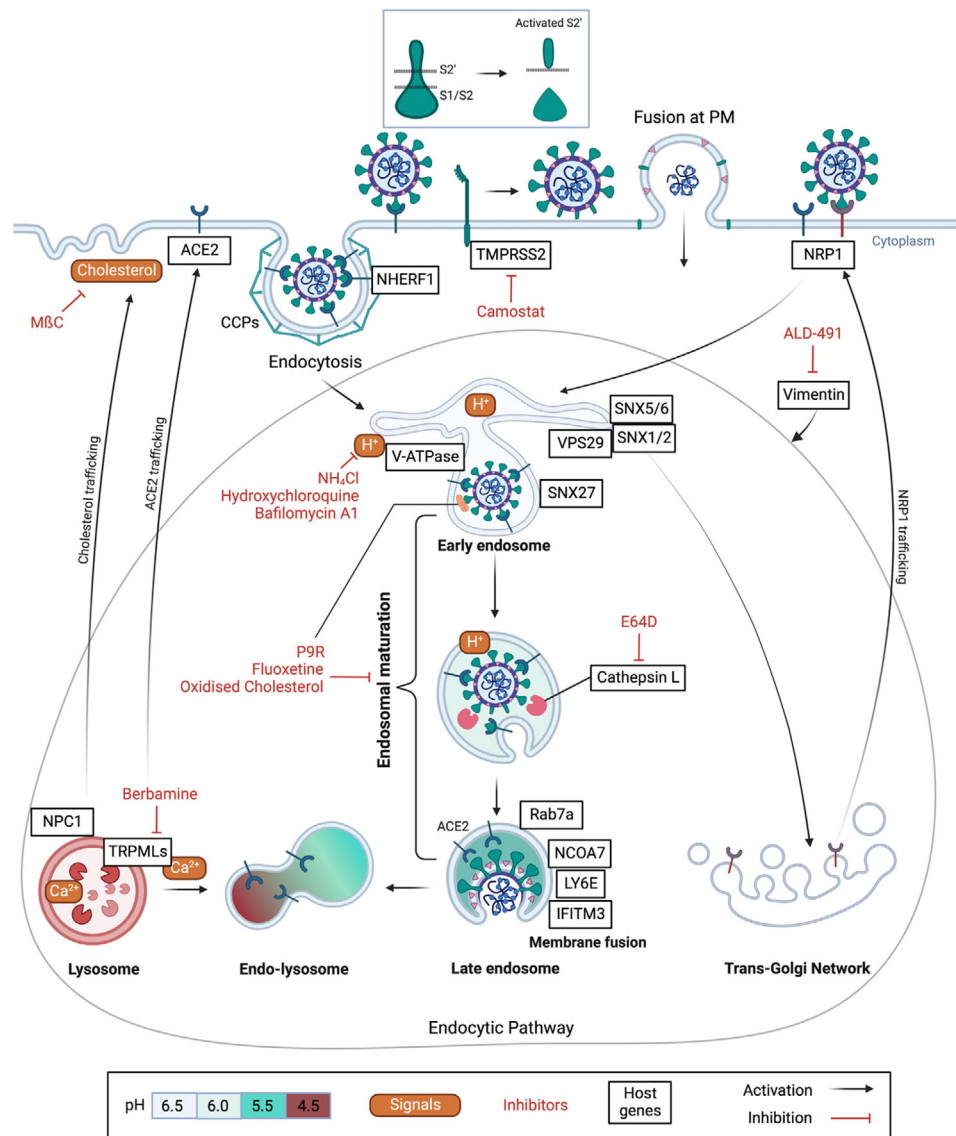
FIGURE 1 SARS-CoV-2 entry and endocytic trafficking. SARS-CoV-2 binds to cell surface receptor ACE2 and gets internalised in endosomes. Low pH endosomal environment and host proteases mediate viral fusion in the endocytic route (top left). Signals like endosomal pH, ions, and host cell factors (proteins and the lipid cholesterol) promote membrane fusion and uncoating of the SARS-CoV-2 genome. Non-endocytic entry of SARS-CoV-2 (top right) takes place by the action of the TMPRSS2 protease, exposing membrane fusion peptide S2' in S1/S2 cleaved spike. Binding of NRP1 also promotes the entry of SARS-CoV-2. Several pharmacological compounds inhibit individual steps in endocytic and non-endocytic route of SARS-CoV-2 entry.

Endosomes not only provide the route for virus entry, but also necessary cues, that is, chemical signals (ions, lipids) and topological environment (membrane curvature promoting interaction with membrane-bound receptors) that together facilitate conformational changes necessary for viral fusion (Yamauchi & Greber, 2016; Yamauchi & Helenius, 2013).

The importance of endosomal morphology and function for SARS-CoV-2 entry was demonstrated in a study reporting alteration of intermediate filaments (IF) to affect both entry and egress steps of SARS-CoV-2 (Li et al., 2021c) (Figure 1). Using the pharmacological inhibitor ALD-491 targeting the class III IF vimentin, the authors reported reduced SARS-CoV-2 entry and exosomal release in cell culture-based assays as well as mitigated disease severity and lung damage in aged mice. Changes in the cytoskeletal network of an infected cell also affect other steps of the infection cycle and will be discussed in later sections.

Apart from SARS-CoV-2 entry, cellular signals and cues at the endosomes are also required for virus trafficking. These signals may include endosomal acidity governed by H^+ and K^+ ions, and bio-molecular signalling molecules like proteins, lipids, and sugars.

Endosomal acidification is an important prerequisite for cargo trafficking and sorting at the endo-lysosomal network (Hu et al., 2015). Trafficking of SARS-CoV-2 also depends on endosomal acidity as binding of ACE2 to S is affected by low pH endosomal environment. S comprises receptor binding domains (RBDs) and cryo-electron microscopy (cryo-EM) showed that S trimers can assume prefusion confirmations, described as up or down confirmation (Walls et al., 2020b; Wrapp et al., 2020; Wrobel et al., 2020). It has been proposed that the 'up' conformations, referred to as pre-fusion spike, can freely interact with ACE2. At pH 5.5, S at the surface of virions freely adopts a single conformation where all RBDs take an up-conformation for the interaction with ACE2, but uniformly change to all-down conformation at lower pH (Zhou et al., 2020). This outlines the importance of endosomal pH in the entry of SARS-CoV-2 via the endosomal network.

Further evidence on the importance of pH for SARS-CoV-2 envelope fusion was provided by studies employing endosomal acidification inhibitors (Shang et al., 2021). such as hydroxychloroquine (HCQ), NH_4Cl, and Bafilomycin A1 that were shown to reduce SARS-CoV-2 yields. However, HCQ was also found to inhibit viral replication in cell culture experiments and innate immune hyperactivation iCOVID-19 patients, arguing for an additional role beyond entry (Hu et al., 2020; Savarino et al., 2003). In addition, since HCQ-mediated restriction primarily affects the endocytic route, over-expression of TMPRSS2 attenuates the inhibitory effect (Ou et al., 2021). Along similar lines, a broad-spectrum defensin-like peptide was shown to inhibit endosomal acidification and inhibit SARS-CoV-2 entry (Zhao et al., 2020). The peptide termed P9R interacted with virions and was designed on the assumption that virus binding alkaline peptides should broadly inhibit pH-dependent viruses. Consistent with the dependence of the endocytic entry route on pH, it was recently shown that the plasma membrane fusion of SARS-CoV-2 via TMPRSS2 protease is affected by extracellular pH (Kreutzberger et al., 2022).

Apart from the dependence on pH as signal (cue) to induce conformational changes triggering membrane fusion and uncoating of SARS-CoV-2, other biomolecular signals play an equally important role. Specifically, cholesterol and cholesterol level-altering factors such as cholesterol-rich lipid rafts and cholesterol-25-hydroxylase have been shown to affect SARS-CoV-2 entry into cells. Depletion of cell membrane-associated cholesterol with methyl-beta-cyclodextrin (MβC) reduces SARS-CoV-2 pseudovirus entry, which was rescued by cholesterol supplementation (Li et al., 2021b). Cholesterol export at the endosomes is another import factor that controls the membrane fusion of SARS-CoV-2. Cholesterol-25-hydroxylase is an interferon-stimulated gene participating in the endosomal cholesterol export (Liu et al., 2013). It is a broadly active antiviral factor

for a large range of enveloped viruses by synthesizing 25-hydroxycholesterol that prevents the fusion between host cell membranes and viral envelope mediated, for example, by SARS-CoV-2 S (Zang et al., 2020).

The importance of cholesterol in SARS-CoV-2 entry becomes evident from three additional observations. First, it was shown that an inhibitor of acid sphingomyelinase, fluoxetine, blocks viral entry by impairing endo-lysosomal acidification and promoting the accumulation of cholesterol within endosomes (Schloer et al., 2020). Second, oxidised cholesterol was shown to increase the endosomal pH, thus reducing the efficiency of SARS-CoV-2 entry (Wang et al., 2022). Third, SARS-CoV-2 entry depends on the Niemann-Pick C1 receptor (NPC1). NPC1 is a lysosomal membrane-localised cholesterol transporter that plays a role in transporting LDL-derived cholesterol to different subcellular sites, including the plasma membrane (Pfeffer, 2019). The absence or inhibition of NPC1 in cells lowers cholesterol level at the plasma membrane, reducing membrane fluidity and SARS-CoV-2 entry via clathrin-coated pits (CCPs) (Li et al., 2022) (Figure 1).

Endosomes also play an important role in the recycling of cellular receptors that promote virus binding and uptake. In the case of ACE2, several host factors have been reported to modulate recycling of this SARS-CoV-2 entry factor. ACE2 is a type I transmembrane metalloprotease and functions as a regulator of the renin-angiotensin system (RAS) (Gheblawi et al., 2020). ACE2 is expressed in the ER and traffics to the cell surface via the conventional secretory pathway (Badawi and Ali, 2021). Additionally, to achieve a regulatory role in the RAS system, ACE2 is cleaved by proteases such as ADAM17 (disintegrin and metalloproteinase 17) to release soluble ACE2 (sACE2) (Jia et al., 2009; Lambert et al., 2005). Maintenance of cell surface-resident ACE2 is tightly regulated, which includes ubiquitination, endosomal recycling and endolysosomal degradation (Shen et al., 2020; Zhang et al., 2018b). ACE2 contains type-I PDZ binding motif (PBM), by which it could interact with PDZ-domain containing proteins such as sorting nexin 27 (SNX27) of the retromer complex (Gallon et al., 2014). Indeed, it was reported that ACE2 interacts with SNX27 to regulate cell surface abundance of ACE2 (Yang et al., 2022). Moreover, it was shown that this interaction prevents the ACE2 - SARS-CoV-2 complexes from entering the late endosomes, hence preventing entry into cells of those variants where the endocytic pathway dominates.

The C-terminal PDZ-recognition motif of ACE2 has been shown to interact with PDZ-containing protein NHERF1 (Na$^+$/H$^+$ exchanger regulatory factor-1) tethering ACE2 to the cell surface and promoting SARS-CoV-2 entry (Zhang et al., 2021b). Another host factor, Rab7a, was also found to be important for SARS-CoV-2 entry by affecting the cell surface abundance of ACE2 (Daniloski et al., 2021). Rab7a is a member of Rab family of

GTPases and plays an essential role in vesicular membrane trafficking to late endosomes and lysosomes. The absence of Rab7a reduced surface ACE2 levels by promoting its accumulation in intracellular lysosomes. Berbamine (BBM) is a pharmacological compound that prevents the release of Ca^{2+} ions from lysosomes hence regulating lysosomal function (Fu et al., 2018). A study reported that treatment of cells with BBM reduced viral entry by impairing ACE2 trafficking resulting in lower cell surface ACE2 and increased ACE2 secreted in extracellular vesicles (EVs) (Huang et al., 2021). Mechanistically, BBM inhibited lysosomally localised TRPMLs (transient receptor potential mucolipin channels), hence reducing the release of Ca^{2+} from lysosomes. This led to a generalised decrease in cell surface receptors including ACE2 and MERS-CoV receptor DPP4 (dipeptidyl peptidase 4), and enhanced the exosomal release of these proteins (discussed in section 'SECRETION AND EXOSOMAL TRAFFICKING IN SARS-COV-2 INFECTION').

Trafficking of not only ACE2, but also other host factors important for SARS-CoV-2 entry is linked to endosomal sorting. Recently, another PDZ-binding domain containing host factor, NRP1 (neuropillin-1), was identified to be important for SARS-CoV-2 entry (Cantuti-Castelvetri et al., 2020; Daly et al., 2020). It was shown that virus internalisation was promoted when the S1 domain of S bound to cell surface-resident NRP1 (Figure 1). The binding of S1 to NRP1 was initially identified by applying the C-end rule according to which a R/KXXR/K motif is required at the carboxy-(C)-terminus of the ligand to bind to neuropilin C-end rule peptides. This interaction mediates neuropilin-1-dependent cell, vascular, and tissue penetration (Teesalu et al., 2009), and was shown to be important for SARS-CoV-2 entry. Endosomal sorting of NRP1 to the trans-golgi network (TGN) occurs via its direct interaction with ESCPE-1 (endosomal SNX-BAR sorting complex promoting exit 1), which is an endosomal coat complex important for retrograde trafficking of transmembrane proteins. ESCPE-1 consists of a heterodimer of functionally redundant sorting nexin 1 (SNX1) or SNX2, with SNX5 or SNX6 (Simonetti et al., 2022). In turn, ESCPE-1 was shown to interact with SARS-CoV-2 S and mediate the trafficking of S-coated nanoparticles to the TGN. The absence of ESCPE-1 complex proteins reduced the infection by SARS-CoV-2.

Endosomal trafficking and virus uptake can be affected by antiviral cytokines, most notably interferons (IFNs). These are small signalling proteins released by cells in response to viral infections. IFNs block viruses by inducing the expression of multiple genes with antiviral activities (Schoggins et al., 2011). IFN-stimulated genes (ISGs) are known to affect the trafficking of viruses (Kuroda et al., 2020). For SARS-CoV-2 and other coronaviruses, the ISG LY6E (Lymphocyte antigen 6 complex, locus E) was shown to inhibit viral entry into cells by interfering with S-mediated membrane fusion (Pfaender et al., 2020). Although the exact mechanism of this restriction is unknown, this observation is surprising because LY6E was earlier reported to promote the entry of multiple viruses such as influenza virus, human immunodeficiency virus and flaviviruses (Hackett & Cherry, 2018; Mar et al., 2018; Yu et al., 2017). Additional ISGs to be mentioned here are the IFN-induced transmembrane proteins (IFITMs). These are broadly active antiviral host factors inhibiting virus envelope - membrane fusion at the acidic endosomes by perturbing membrane curvature and lipid composition via their amphipathic helix (Chesarino et al., 2017; Guo et al., 2021; Li et al., 2013). Modification of IFITM3 by S-palmitoylation has been shown to be important for its antiviral activity (Yount et al., 2010). For SARS-CoV-2, IFITM 1, 2 and 3 were shown to exert antiviral activity. However, while the amphipathic helix of IFITM3 was important for its antiviral activity, S-palmitoylation was dispensable arguing for a novel mechanism of SARS-CoV-2 restriction by IFITM3 (Shi et al., 2021). A final ISG to be mentioned here is the IFN-inducible nuclear receptor coactivator 7 (NCOA7), shown to inhibit endocytic entry for a range of viruses, including SARS-CoV-2, HCoV-NL63, SARS-CoV and MERS-CoV (Khan et al., 2021). NCOA7 was reported to interact with vacuolar ATPase and affect endo-lysosomal vesicle acidification as well as lysosomal protease activity.

ROLE OF NUCLEAR TRANSPORT IN SARS-COV-2 INFECTION

Although most, if not all, of SARS-CoV-2 replication events occur in the cytoplasm of host cells, nuclear function plays a critical role during the viral infection cycle. For instance, the antiviral response to viral infection is controlled by activation of a transcriptional program, requiring mRNA splicing and export of mature mRNAs to allow cytoplasmic synthesis of innate immune factors. Nuclear accumulation of viral proteins is known to modulate the transcriptome and gene expression profile of infected cells. Indeed, the interactome of SARS-CoV-2 proteins revealed that the C-terminal region of envelope protein (E) mimics the N-terminal bromodomain binding region of histone H3, and interacts with bromodomains 2 and 4 via acetylated Lys63 (Gordon et al., 2020). Mimicking of histones by viral proteins and their interaction with bromodomains will likely suppress the innate immune response in an infected cell (Nicodeme et al., 2010).

An alternative way to suppress this antiviral defence response is the inhibition of the nuclear export of cellular mRNAs encoding ISGs. SARS-CoV-2 nsp1 inhibits this process by directly interacting with nuclear export receptor heterodimer NXF1-NXT1 (nuclear RNA export factor1-nuclear transport factor 2-related export protein
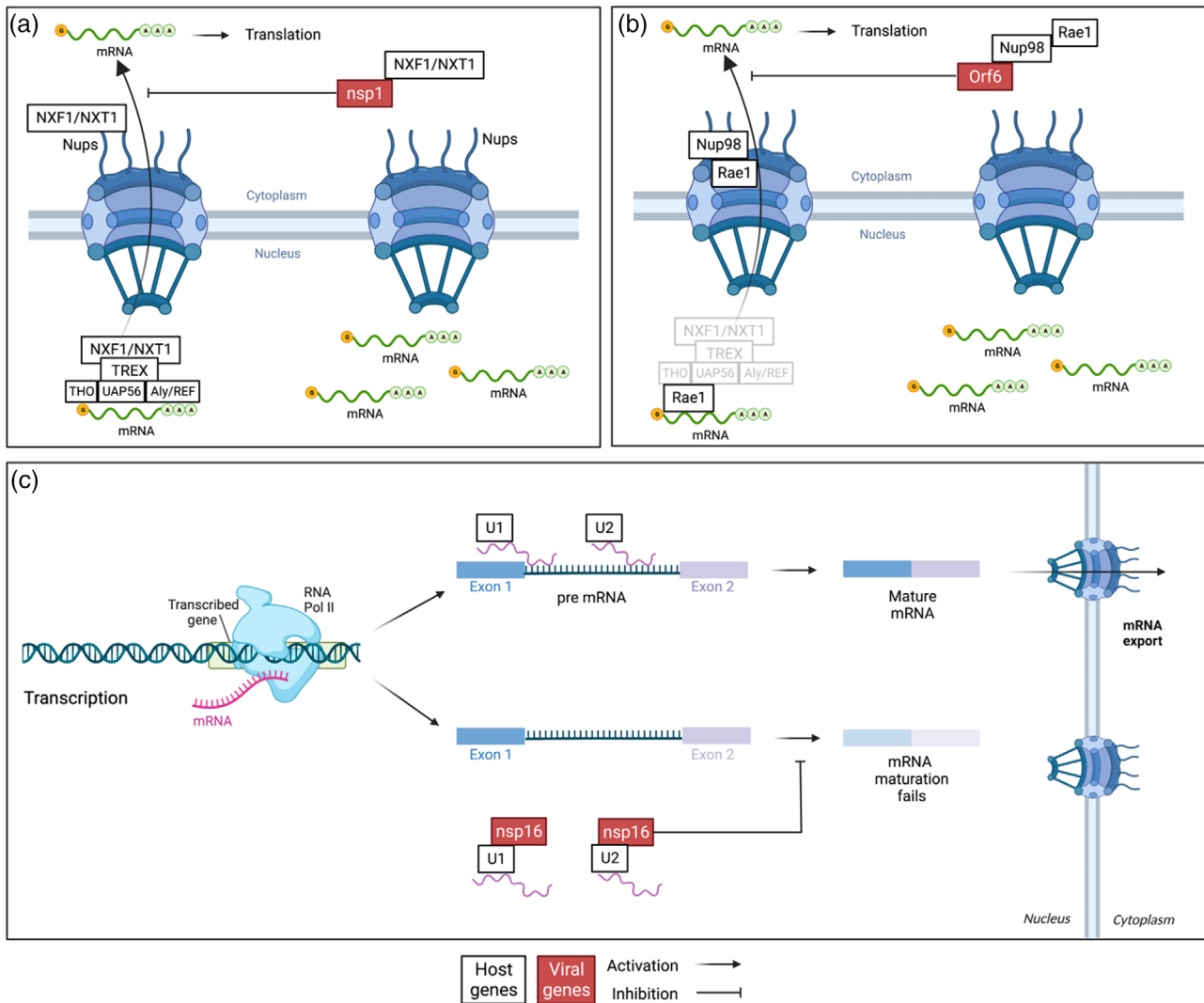
FIGURE 2 Inhibition of nucleo-cytoplasmic trafficking of host mRNA in SARS-CoV-2 infection. (a) SARS-CoV-2 nsp1 binds to the nuclear export receptor heterodimer NXF1-NXT1 preventing its association with nucleoporins and perturbing its docking to the nuclear pore complex. (b) ORF6 protein of SARS-CoV-2 interacts with nuclear pore complex components Rae1-Nup98 causing their dislocation from the nuclear pore complex. ORF6 bound Rae1-Nup98 was found predominantly in the cytoplasm leading to accumulation of cellular mRNAs in the nucleus. (c) SARS-CoV-2 nsp16 binds to the U1 and U2 snRNAs (small nuclear RNAs) preventing their association with the pre mRNA, there by inhibiting the maturation of cellular mRNA in the nucleus.

1) (Figure 2). NXF1-NXT1 is the key component of nuclear export receptor complex and mediates the docking and translocation of mRNAs through the nuclear pores by interacting with nucleoporins and nuclear export factors (Carmody & Wente, 2009; Stewart, 2010). Among these is the nuclear export factor complex TREX (transcription and export complex) comprising the multi-subunit THO complex, RNA helicases UAP56/DDX39B, and the RNA export adapter protein Aly/REF (Strasser et al., 2002). In the presence of nsp1, NXF1 was found to interact less with nucleoporins Nup358, Nup214, Nup153 and Nup62, thus perturbing its docking to the nuclear pore complex. In addition, nsp1 was also shown to interfere with the binding of NXF1 to mRNA export adaptor Aly/REF in the TREX complex, thereby preventing export of poly(A)-containing mRNA (Zhang et al., 2021a) (Figure 2a).

Another strategy for inhibiting the bidirectional nucleo-cytoplasmic trafficking/transport has been reported for the ORF6 protein of SARS-CoV-2. In infected cells, nuclear import via the karyopherin-dependent pathway is inhibited (Frieman et al., 2007; Lei et al., 2020; Xia et al., 2020). This included the MHC class-I transactivator NLRC5 (NLR family CARD domain containing 5) for which no interaction with nuclear import receptor importin alpha (KPNA) is required (Yoo et al., 2021). It was later revealed that ORF6 directly interacts with the nuclear pore complex components Rae1-Nup98 leading

to (a) their dislocation from the nuclear pore complex, (b) increased cytoplasmic localisation and (c) inhibition of nuclear export especially of newly transcribed mRNAs (Addetia et al., 2021; Kato et al., 2021; Li et al., 2021a) (Figure 2b). ORF6 interacts with the mRNA-binding site of the Rae1-Nup98 complex and hence competes with the binding of single-stranded RNA (Li et al., 2021a).

SARS-CoV-2 also inhibits global host cell gene expression by interfering with mRNA splicing. Nascent pre-mRNAs are spliced by the binding of U1 and U2 snRNAs (small nuclear RNAs) at the exon-intron junction and the branch point in the intron, respectively, thus facilitating removal of the introns (Seraphin et al., 1988). SARS-CoV-2 nsp16 localises to the nucleus of infected cells and binds to the 5' splice site recognition sequence of U1 snRNA as well as the branch point recognition site of U2 snRNAs (Banerjee et al., 2020) (Figure 2c). This disrupts the global mRNA splicing and dampens IFN response to SARS-CoV-2 infection.

SARS-CoV-2 nsp9 was found to interact with the nuclear pore complex component Nup62, reducing its expression and decreasing nuclear translocation of cellular proteins, including NF-kB subunit p65 under TNF-alpha stimulation (Makiyama et al., 2022). However, the role of this block for the SARS-CoV-2 infection cycle has not been clarified.

SECRETION AND EXOSOMAL TRAFFICKING IN SARS-COV-2 INFECTION

After uncoating of the SARS-CoV-2 genome, it is translated giving rise to the 1ab polyprotein. Amongst the cleavage products, nsp1 was not only reported to block nuclear export of cellular mRNAs (section 'ROLE OF NUCLEAR TRANSPORT IN SARS-COV-2 INFECTION', Figure 2a), but also to inhibit global mRNA translation by binding to the 18S ribosomal RNA, which is a structural component of the 40S ribosomal subunit (Banerjee et al., 2020).

Co-translational translocation of nascent peptides into the ER lumen for protein folding is initiated by the binding of signal recognition particle (SRP) to signal sequence (SS). SRP is a ribonucleoprotein complex containing SRP54, which is the protein responsible for signal peptide recognition, SRP-receptor binding, and translocation through ribosomes (Akopian et al., 2013), and 7SL RNA, which is the RNA component of SRP. The binding of SRP to SS on a nascent peptide halts translation elongation on ribosomes and the SRP complex-bound peptide enters the ER lumen through translocon, followed by the removal of SRP complex. Signal peptidases then cleave SS and the nascent peptides in the ER lumen undergo protein folding and enter the secretory pathway destined for secretion or targeting to different location in cells (Figure 3a). SARS-CoV-2

nsp8 and nsp9 proteins were found to bind to the 7SL RNA component of the SRP blocking the recognition of SS (Banerjee et al., 2020). This leads to the failure of nascent peptide translocation to the ER lumen, causing protein mislocalisation and degradation in the cytoplasm, and eventually protein secretion (Figure 3a).

One of the host proteins recycled during SARS-CoV-2 infection is its cell surface receptor ACE2 that is tightly regulated in a highly dynamic manner, involving both intracellular degradation and exosomal release to maintain a fine balance of ACE2 cell surface levels. As described in section 'ENDOSOMAL TRAFFICKING AND SARS-COV-2 ENTRY', BBM-mediated inhibition of lysosomal membrane TRPMLs prevents Ca^{2+} release and hence impairs lysosomal function. This reduces cell surface ACE2 levels and increases secretion of sACE2 in extracellular vesicles. Since sACE2 contributes to SARS-CoV-2 infectivity (Yeung et al., 2021), endolysosomal trafficking inhibitors reported to affect the exosomal release of proteins (Ortega et al., 2019) may lead to enhanced sACE2 (Figure 3b).

ACE2-containing extracellular vesicles (evACE2) have been detected in the plasma of COVID-19 patients (El-Shennawy et al., 2022). The authors also showed that evACE2 has up to 135-fold higher binding to the RBD of S compared to the vesicle-free and membrane-free form of sACE2, hence acting as an antiviral defence mechanism and possibly suitable as therapeutic tool as shown recently with the SARS-CoV-2 pseudovirus system (Cocozza et al., 2020). EvACE2 has similar membrane topology as cell surface-resident ACE2 with the RBD facing outside the cell. Secretion of evACE2 occurs via multi-vesicular bodies (MVBs) requiring palmitoylation of Cys141 and 498 residues of ACE2 (Xie et al., 2021) (Figure 3b).

SARS-CoV-2 induces profound remodelling of infected cells, one hallmark being the establishment of ER-derived membranous replication organelles composed of double membrane vesicles (DMVs). Infection alters the architecture of the ER and the network of DMVs was found to be surrounded by a 'cage-like' vimentin network and pharmacological inhibition of intermediate filaments with Withaferin A reduced SARS-CoV-2 replication (Cortese et al., 2020). Of note, vimentin appears to have a dual role in the SARS-CoV-2 infection cycle, by contributing to viral entry (see section 'ENDOSOMAL TRAFFICKING AND SARS-COV-2 ENTRY', Figure 1) and egress of infectious virus particles (Li et al., 2021c). It has been shown that newly assembled ß-coronaviruses exit the cells using lysosomal exocytosis with lysosomes being deacidified and lysosomal enzymes inactivated (Ghosh et al., 2020). Hence, inhibition of lysosomal degradation in SARS-CoV-2 infection is vital for virion egress. One of the mechanisms by which SARS-CoV-2 blocks lysosomal acidification requires viral protein Orf3a
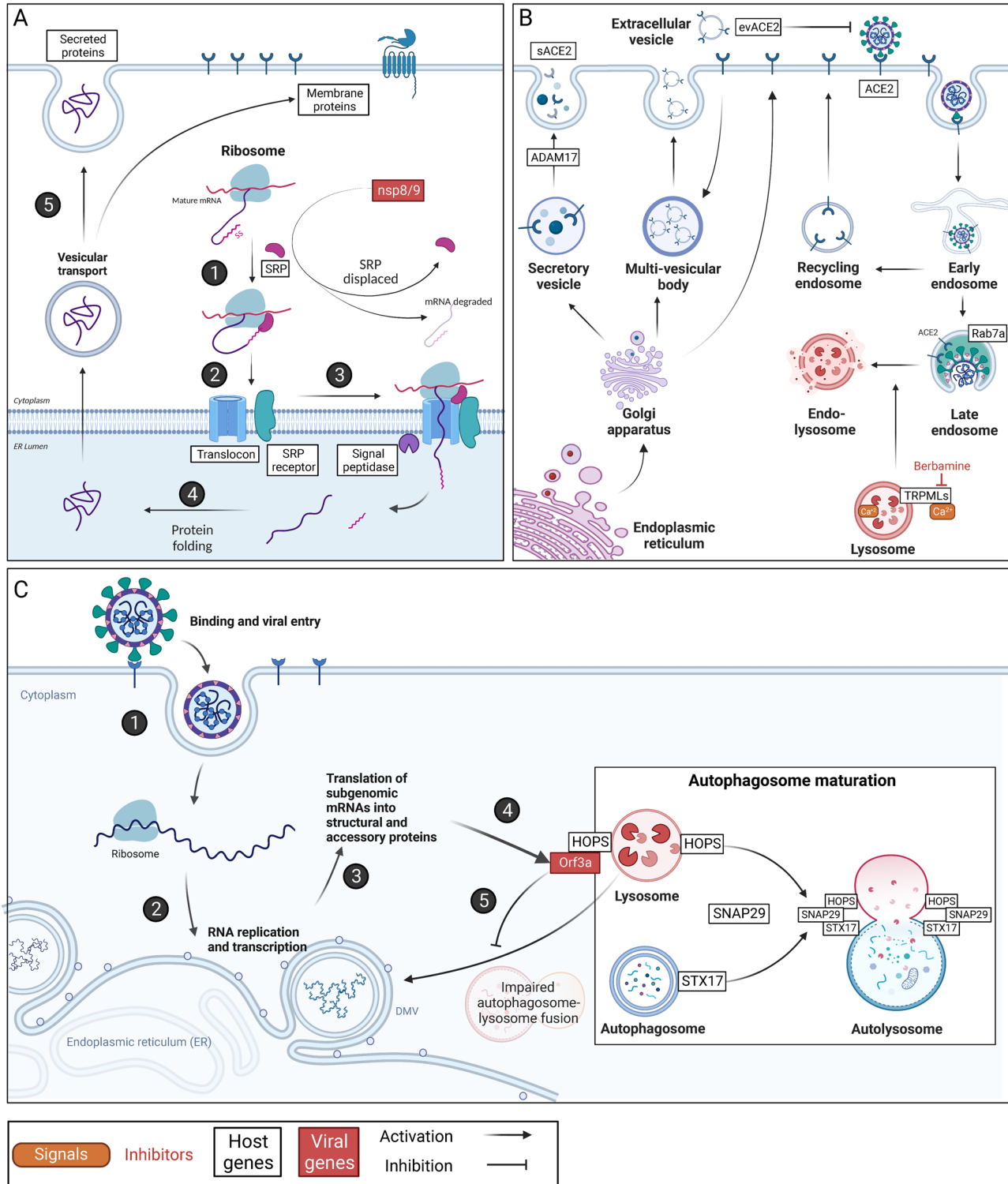
FIGURE 3 Secretion and exosomal trafficking in SARS-CoV-2 infection. (a) SARS-CoV-2 nsp8 and nsp9 block secretion by binding to the 7SL RNA of the SRP. Displacement of SRP by nsp8/9 leads to failure of nascent peptide-to-ER translocation, protein folding and secretion. Numbers indicate the progression of events from (1) identification of signal sequence (SS) on the mRNA by SRP complex; (2) assembly of the SRP-ribosome complex on the translocon; (3) cleaving of the SS and co-translational translocation; (4) protein folding in the ER lumen; and (5) vesicular transport. (b) Intracellular trafficking regulating ACE2 secretion pathways (left and right half of the figure, respectively) and ACE2 cell surface abundance (right panel). ACE2 is internalised after SARS-CoV-2 binding and is recycled to the cell surface via the endocytic route. Berbamine blocks TRPML-Ca^{2+} channels at the lysosomal membrane, leading to inhibition of endolysosomal trafficking of ACE2 and resulting in lower ACE2 cell surface abundance and increased secretion via the exosomal pathway. Cell surface ACE2 can be taken up in the multi-vesicular body and secreted as extra-vesicular ACE2 (evACE2), which can potentially bind to and block the extracellular SARS-CoV-2 particles. Newly synthesised ACE2 in the ER traveling to the cell surface in secretory vesicles can be cleaved by ADAM17 and secreted from

that blocks autophagosome-lysosome fusion (Miao et al., 2021; Zhang et al., 2021c). This fusion is regulated by the activity of STX17 (syntaxin 17), SNAP29 (synaptosomal-associated protein 29), and the HOPS complex (Viret & Faure, 2019). Orf3a was reported to interact with the HOPS component VPS39 on lysosomes and prevent HOPS-Rab7 interaction thereby inhibiting autophagosome-lysosome fusion. Since bulk autophagy is dispensable for SARS-CoV-2 infection whereas individual components of the autophagy machinery contribute to viral replication (Twu et al., 2021), the inhibition of lysosomal function by Orf3a might prevent the degradation of viral DMVs by blocking their fusion with lysosomes (Figure 3c).

TRAFFICKING AT MEMBRANE CONTACT SITES IN SARS-COV-2 INFECTION

MCS are regions of close apposition (~15–30 nm apart) between two different organelles (Phillips & Voeltz, 2016; Prinz et al., 2020), or two regions of the same organelle like, for example, intra-contact sites of the tubular ER network (Wang et al., 2016). In general, MCS allow mutual exchange of signals between involved organelles, thus modulating their function. This includes the regulation of organelle membrane dynamics, the transport of lipids between organelles, and the channelling of metabolite transfer.

Viruses are also known to utilise MCS to their benefit (Nagy et al., 2016). To enable the transfer of metabolites, lipids, and proteins for efficient viral replication, RNA viruses that induce cytoplasmic replication organelles can establish direct or indirect MCS. These virus-derived MCS (vMCS) may contain viral and cellular components and allow asymmetric lipid distribution, to enrich or deplete selectively cellular proteins in organelles and shape the morphology of replication organelles (Paul et al., 2014; Roulin et al., 2014; van der Schaar et al., 2016). In the following sections, we will discuss recent examples of how cellular MCS affect SARS-CoV-2 infection and how SARS-CoV-2 proteins induce the formation of vMCS to support viral replication.

ER - PLASMA MEMBRANE CONTACT SITES

Modulation of MCS between ER and the plasma membrane (PM) was found to impact SARS-CoV-2 infection.

Ca^{2+} signalling at the ER and the PM is regulated by the PM-resident calcium channel protein ORAI1 (calcium-release activated calcium channel protein 1) and the ER-localised Ca^{2+} sensor STIM1 (stromal interaction molecule 1). Apart from regulating the Ca^{2+} levels in cytoplasm and ER, ORAI1 and STIM1 also modulate the type I IFN (IFN-I) response by promoting Ca^{2+} signalling and calmodulin-dependent transcriptional response (Debant et al., 2015) (Figure 4). A study reported increased resistance to SARS-CoV-2 in STIM1 knockout (KO) cells because of higher baseline IFN-I signalling, and decreased baseline IFN-I signalling in ORAI1-KO cells increasing susceptibility to SARS-CoV-2 infection (Wu et al., 2022). Perturbation of this tonic IFN-I response after ORAI1- and STIM1-KO was attributed to the Ca^{2+} dependent transcriptional changes in cells due to activation of NF-AT (nuclear factor of activated T-cells) and NF-kB (nuclear factor-kappa B) responses (Debant et al., 2015).

MEMBRANE CONTACT SITES BETWEEN ER AND OTHER ORGANELLES

The ER localised transmembrane proteins VAPs (vesicle-associated membrane protein-associated proteins) are key components of the MCSs established between ER and other organelles. VAPs are small proteins on the cytoplasmic face of the ER and act as receptors for a range of proteins. Through their MSP (major sperm protein)-domain, VAPs associate with proteins containing FFAT-like (two phenylalanine in an acidic tract-like) motifs (Murphy & Levine, 2016). VAP interactors usually associate with other cellular membranes, hence creating molecular bridges and contact sites between ER and these organelles. A recent NMR-based study determined the interaction of six peptides containing the FFAT-like motifs of known VAP interactors (Furuita et al., 2021). Using the identified characteristics of these interactions, the authors searched for potential FFAT-like motifs in SARS-CoV-2 proteins and detected an EFYEA sequence at the C-terminus of nsp12 (the RNA-dependent RNA polymerase) as potential VAP interactor. NMR analysis with a peptide containing the nsp12 EFYEA sequence confirmed its interaction with VAP with an affinity comparable to the one of known interacting peptides (Furuita et al., 2021), raising the possibility of a role of nsp12-VAP interaction at ER-derived membranes during viral replication. Since nsp12 is the viral RNA-dependent RNA polymerase

cells as soluble ACE2 (sACE2). (c) SARS-CoV-2 Orf3a inhibits the maturation of autophagosomes by blocking autophagosome-lysosome fusion. Orf3a binds to the HOPS complex and inhibits HOPS-SNAP29-STX17 trimeric complex formation and autophagosome-lysosome fusion. Numbers show the progression of events in virus infection cycle from (1) virus binding and internalisation; (2) RNA transcription and replication; (3) translation of sub-genomic mRNAs to produce structural and accessory proteins; (4) inhibition of lysosome autophagosome fusion by Orf3a via interaction with HOPS; (5) inhibition of autophagosmal maturation and viral replication in the DMVs.
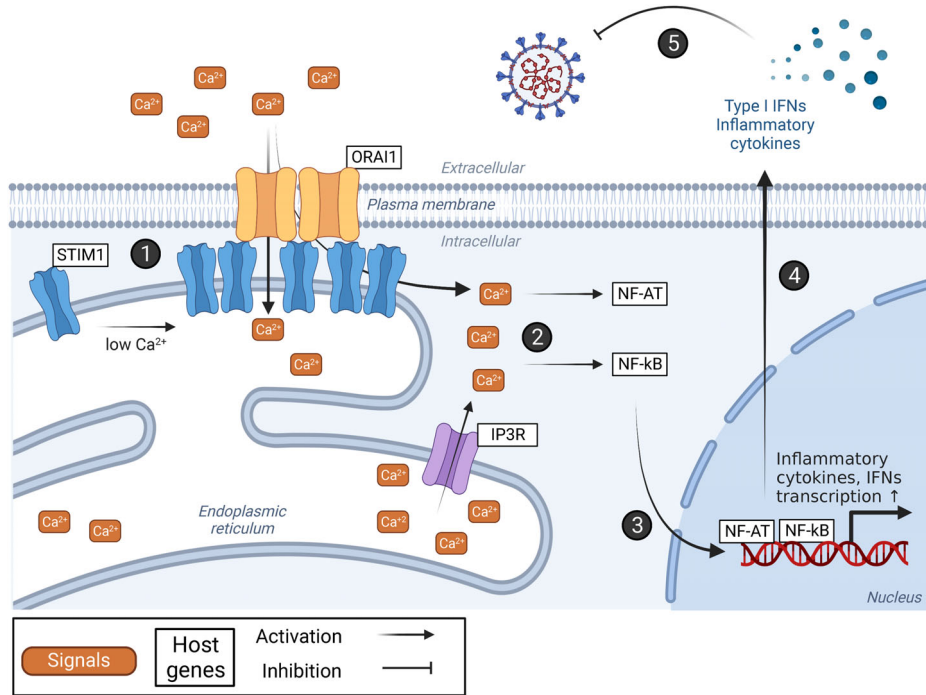
FIGURE 4 Membrane contact sites (MCS) at the PM - ER interface in SARS-CoV-2 infection. ORAI1-STIM1 regulated MCS at the PM - ER interface regulate cytoplasmic Ca^{2+} dependent IFN-I signalling response. (1) internalisation of Ca^{2+} ions from extracellular space to ER lumen via ORAI1-STIM1 establishes MCSs between PM and the ER; (2) movement of Ca^{2+} ions from ER to cytoplasm via IP3R Ca^{2+} channels; (3) Ca^{2+}-dependent activation of NF-AT and NF-kB and transcriptional upregulation of interferon and inflammatory cytokine encoding genes; (4) expression and secretion of IFNs and inflammatory cytokines; (5) inhibition of SARS-CoV-2 infection by released cytokines.

replicating viral RNA in the ER-derived DMVs, nsp12-VAP interaction might enrich viral and cellular co-factors required for viral replication.

MEMBRANE CONTACT SITES BETWEEN CIS-GOLGI AND ENDOSOMES

Recently, it was shown that the precursor of autophagosomes or phagophores is generated from a new hybrid organelle designate HyPAS (hybrid pre-autophagosomal structure), which is formed via close contacts between *cis*-Golgi and endosomes to support their fusion (Kumar et al., 2021). This compartment is created by close apposition of FIP200-positive vesicles derived from ER or Golgi and ATG16L1-positive endosomal vesicles (Lystad et al., 2019; Moreau et al., 2011; Ravikumar et al., 2010) (Figure 5a). The formation of HyPAS is dependent on Ca^{2+} and SNARE-dependent fusion via STX17, the Ca^{2+} regulator SIGMAR1 (sigma non-opioid intracellular receptor 1), E-SYT2 (extended synaptotagmins 2), and SERCA2 (calcium-ATPase type 2 in the sarco/-endoplasmic reticulum) (Hu & Reggiori, 2022). Importantly, it was shown that SARS-CoV-2 nsp6 resides in close proximity to E-SYT2, SIGMAR1, and other components of autophagy regulators and inhibits the formation of HyPAS (Kumar et al., 2021).

In addition, nsp6 was found to directly interact with SERCA2 thereby possibly disrupting Ca^{2+} dependent signalling during SARS-CoV-2 infection (see section 'ER - PLASMA MEMBRANE CONTACT SITES'). However, bulk autophagy was found to be dispensable for SARS-CoV-2 replication which occurs in morphologically similar DMVs and only individual components of the autophagy machinery are hijacked by SARS-CoV-2 for the replication organelle biogenesis (Twu et al., 2021).

MEMBRANE CONTACT SITES BETWEEN ER AND VIRUS-INDUCED REPLICATION ORGANELLES

SARS-CoV-2 induced DMVs serve as viral replication organelles and are derived from the ER (Cortese et al., 2020). These DMVs can be induced by the sole expression of nsp3 and nsp4 (Oudshoorn et al., 2017; Wolff et al., 2020) and require enrichment of ER-resident proteins (Tabata et al., 2021). Whereas host factors important for the function of DMVs are redistributed to the membranous organelle, other ER resident proteins like CNX (calnexin) and PDI (protein-disulfide isomerase) remain unaffected. It was recently shown that the DMVs are connected to the ER via
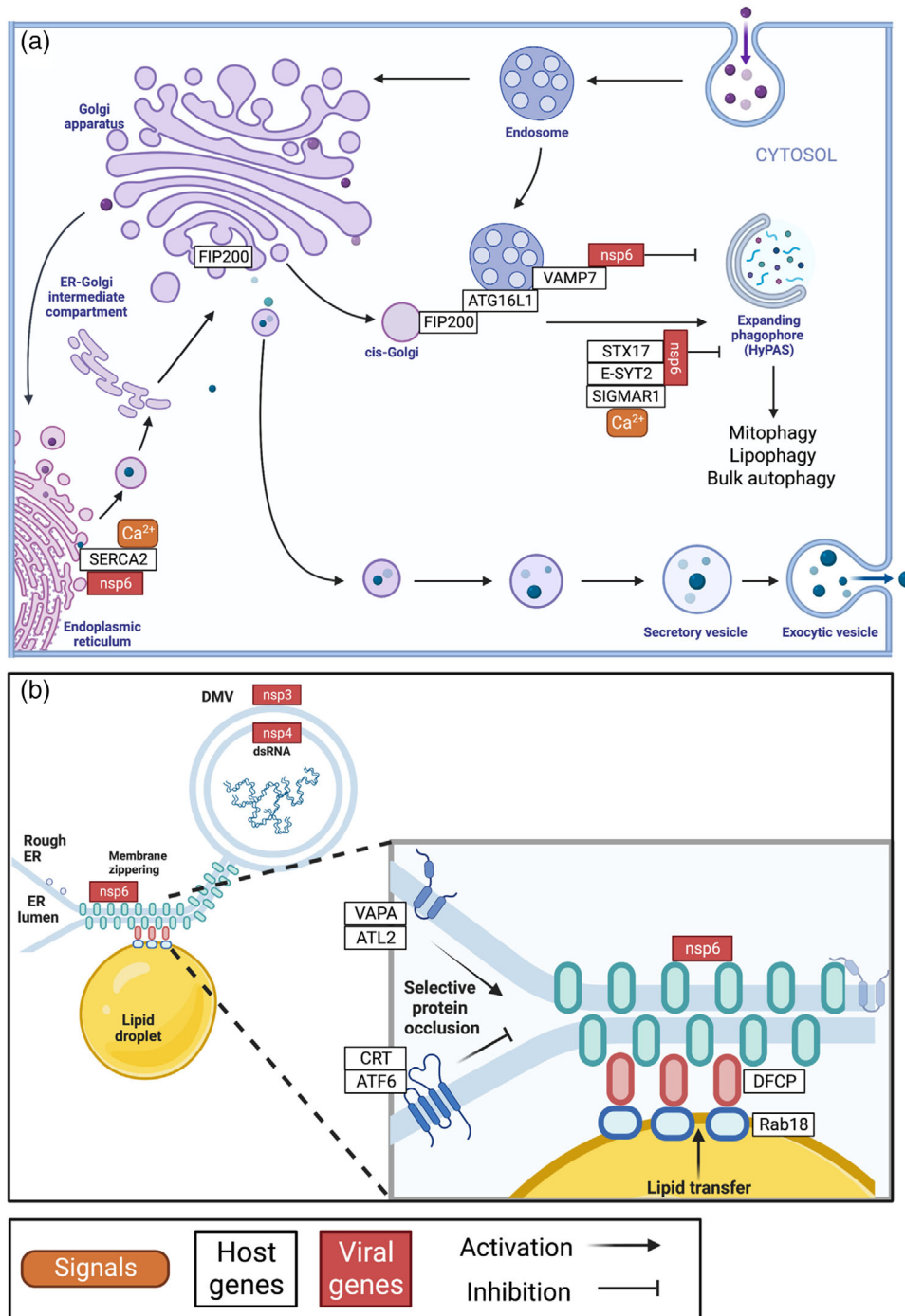
FIGURE 5   Membrane contact sites (MCS) at Golgi - endosome interface and ER-derived replication organelle in SARS-CoV-2 infection. (a) Hybrid pre-autophagosomal structure (HyPAS) formed by fusion of closely apposed FIP200-positive *cis*-Golgi-derived vesicles and ATG16L1-positive endosomes. HyPAS generated autophagosomes contribute to mito-, lipo- and bulk-autophagy. Formation of HyPAS depends on the SNARE STX17 and its interaction partners SIGMAR1, SERCA2, and E-SYT2. HyPAS formation is affected by Ca^{2+} and SARS-CoV-2 nsp6 inhibits HyPAS by interacting with E-SYT2, VAMP7, SIGMAR1 and SERCA2, hence disrupting Ca^{2+} regulation. (b) MCS between ER, SARS-CoV-2 induced DMVs, and lipid droplets generated by nsp6-mediated ER membrane zippering. Zoomed in region shows selective occlusion of the ER lumen and membrane proteins as well as regulated lipid transfer via recruitment of the DFCP1-Rab18 complex at the nsp6-zippered ER membrane region.

thin connectors, which are generated by viral protein nsp6 (Cortese et al., 2020; Ricciardi et al., 2022; Snijder et al., 2020). Through the action of a unique C-terminal localised amphipathic helix, nsp6 is thought to zipper the ER membranes, causing a collapse of the ER lumen and establishing a selective protein enrichment channel (Figure 5b). Thus, host proteins with large ER luminal domains are excluded whereas smaller proteins are retained in the connectors or DMVs. Moreover, nsp6 connectors were reported to regulate lipid distribution in the DMVs by establishing a membrane tethering complex involving host proteins DFCP1 and Rab18, the latter residing on the surface of lipid droplets. In this way, nsp6-induced ER zippers provide a unique vMCS allowing metabolite filtering to support efficient viral replication.

CONCLUSIONS AND OUTLOOK

The still ongoing SARS-CoV-2 pandemic has triggered global research efforts in numerous fields of life sciences. This enabled the discovery of novel principles in cell biology, immunology and coronavirus biology. Here, we have summarised some aspects related to the host cell factors and principles supporting the trafficking of SARS-CoV-2 virions and proteins in various cellular transport pathways and how viral infection induces or perturbs MCS. Some parallels have been discovered to non-coronaviruses such as the hepatitis C virus that replicates in DMV-like organelles as well, arguing for the convergent use of host cell factors and pathways by phylogenetically distinct viruses (Tabata et al., 2021; Twu et al., 2021). These results suggest that observations made with SARS-CoV-2 might help identifying targets suitable for broadly active antiviral therapies. In addition, discoveries related to SARS-CoV-2 host cell interaction should increase our understanding why infections with this virus cause such a perplexing plethora of COVID-19 disease manifestations.

CONFLICT OF INTEREST
None declared.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article as no new data were created or analysed in this study.

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