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The life cycle and enigmatic egress of coronaviruses

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Abstract

There has been considerable recent interest in the life cycle of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), the causative agent of the Covid-19 pandemic. Practically every step in CoV replication – from cell attachment and uptake via genome replication and expression to virion assembly has been considered as a specific event that potentially could be targeted by existing or novel drugs. Interference with cellular egress of progeny viruses could also be adopted as a possible therapeutic strategy; however, the situation is complicated by the fact that there is no broad consensus on how CoVs find their way out of their host cells. The viral nucleocapsid, consisting of the genomic RNA complexed with nucleocapsid proteins obtains a membrane envelope during virus budding into the lumen of the intermediate compartment (IC) at the endoplasmic reticulum (ER)-Golgi interface. From here, several alternative routes for CoV extracellular release have been proposed. Strikingly, recent studies have shown that CoV infection leads to disassembly of the Golgi ribbon and the mobilization of host cell compartments and protein machineries that are known to promote Golgi-independent trafficking to the cell surface. Here we discuss the life cycle of CoVs with special focus on different possible pathways for virus egress.

Introduction

Effective egress of mature virus particles from host cells is one of the important determinants of virus infectivity. While some viruses are released after mediating lysis of the infected cells, others acquire a membrane envelope by budding at the host cell plasma membrane (PM). Yet other viruses, like coronaviruses (CoVs), obtain their envelope as they bud into a membrane compartment inside the cell, and must therefore be transported from their site of assembly to the cell surface to be able to reach the extracellular milieu via exocytosis (Sturman & Holmes, 1983; Hernandez-Gonzalez et al., 2021). The progeny CoVs assemble by budding into the lumen of the intermediate compartment (IC) (Tooze et al., 1984; Klumperman et al., 1994; Stertz et al., 2007), functionally situated between the endoplasmic reticulum (ER) and the Golgi apparatus in the early secretory pathway (Saraste & Kuismanen, 1984; Saraste & Marie, 2018). However, the IC also turns out to communicate directly with endocytic compartments (Saraste & Prydz, 2019), opening for unconventional modes of egress that bypass the Golgi stacks participating in the conventional secretory process (Ghosh et al., 2020; Saraste & Prydz, 2021). Assembly at the IC membranes is a common property of CoVs belonging to different genera (α-, β-, and γ-CoVs), including the Severe Acute Respiratory Syndrome (SARS)-CoV causing the serious outbreak in 2002 (SARS-CoV) and the recent pandemic (SARS-CoV-2) in humans (Stertz et al., 2007; Bracquemond & Muriaux, 2021). In the assembly process, the virus nucleocapsid – consisting of the positive-stranded genomic RNA complexed with nucleocapsid (N) proteins – is enclosed in a lipid bilayer derived from a subdomain of the IC membrane, that incorporates the viral membrane glycoproteins designated as S (spike), M (membrane), and E (envelope) into the forming virions (**Fig.1**) (Scherer et al., 2022).

For a long time, viral envelope glycoproteins have provided important tools for studies of intracellular transport of membrane proteins between the ER and the PM (Bergmann et al., 1981; Saraste & Kuismanen, 1984). Figuring out the transport routes for virus particles from their intracellular site of assembly to the site of release via exocytosis can be more challenging than studying conventional glycoprotein transport to the cell surface. Namely, the large size of intracellularly budding virus particles – for example, CoVs range from 80 to 120 nm in diameter

– means that they have to be packaged into specialized transport carriers, which currently remain poorly characterized. Another reason is that virus infection typically leads to alterations in the structure and function of endomembrane compartments, resulting in the redistribution of traditional organelle markers. Indeed, viruses seem to have developed the ability to inhibit or reorganize secretory transport routes in a manner that is in their best interest.

In this Micro Review, we focus on the structural and functional changes occurring in the host cells during CoV infection and discuss how these changes may influence the mode of egress of newly assembled virus particles. Since the different stages of the virus life cycle are closely interconnected, we also briefly address earlier steps of virus infection.

Virus entry and egress: glycan binding at the right place?

To be able to enter their host cells and release their genome to the cytoplasm, CoVs must first bind to transmembrane protein receptors at the cell surface. Subsequently, the viral membrane can either fuse directly with the host cell PM, or the virus is endocytosed and releases its genome from an endosomal compartment (**Fig. 1**) (Fung & Liu, 2019; Jackson et al., 2022). The attachment and entry steps of the virus particles are mediated by the trimeric S glycoprotein. Direct fusion with the PM requires that the spike protein has been primed by the proprotein convertase furin during virus egress from a producer cell, and that the new host cell expresses the TMPRSS2 protease at its surface that introduces a second proteolytic cleavage. Alternatively, the two successive cleavages of the S protein may be created by proteases (cathepsins) after the virus has entered the lumen of the endosome (Millet & Whittaker, 2015; Hoffmann et al., 2020; Zhang & Zhang, 2021). For SARS-CoV and SARS-CoV-2, angiotensin-converting enzyme 2 (ACE2) has been shown to act as an obligate receptor for host cell entry, while for the Middle East Respiratory Syndrome (MERS)-CoV the reported receptor is dipeptidyl peptidase IV (DDP4). In addition, several CoVs bind to the glycosaminoglycan (GAG) chains of heparan sulfate (HS) proteoglycans (de Haan et al., 2005; Milewska et al., 2014). SARS-CoV-2 depends both on ACE2 and HS for efficient S protein interaction with the host cell (Clausen et al., 2020), while HCoV-NL63 – another human CoV – seems to interact with HS via the M

protein (Naskalska et al., 2019). It is important to note that the pattern of HS sulfation, which determines the biological specificity of the GAG chains, changes with age, which could significantly affect the susceptibility of different age groups to CoV infection (Feyzi et al., 1998; Kreuger et al., 1999). Diversity of cell surface glycan structures in a population is beneficial in an evolutionary perspective to ensure that certain individuals survive severe threats from disease-causing microorganisms (Varki 2011). The extent of variation in HS structure among individuals is not known in detail, and will also vary in different tissues. Studies of the receptor-binding domain (RBD) of the S protein using glycan arrays and ACE2-positive HEK cells demonstrated its additional affinity for sialic acid, preferentially in the context of mono-sialylated gangliosides. The affinity was similar to that observed for binding to HS, and reduced levels of cell surface sialic acid were inhibitory to virus attachment and cell entry (Nguyen et al. 2022).

Many viruses depend on glycans as receptors or co-receptors for efficient binding to and entry into their host cells (Russell et al., 2006; Aquino & Park, 2016). Since the same glycans are synthesized and modified in the secretory pathway, progeny viruses undergoing egress must either avoid binding to the glycan receptors, or be able to detach from the bound glycan at the cell surface by an appropriate enzymatic activity. Influenza viruses bind to variants of sialic acid both during entry into and egress from their host cells, and utilize the activity of viral neuraminidase to promote the release of newly synthesized virions from cell surface glycans (McAuley et al., 2019). Enzymatic release of SARS-CoV-2 from host cell HS GAGs has not been demonstrated, but has been described for instance in the case of Herpes simplex virus 1, which is released from the cell surface by heparanase degradation of HS GAGs attached to syndecan-1 (Hadigal et al., 2020; de Pasquale et al., 2021). As discussed below, CoV infection causes disassembly of the Golgi apparatus (Lavi et al., 1996; Ulasli et al., 2011; Ruch & Machamer, 2012; Cortese et al., 2020; Hackstadt et al., 2021), where HS synthesis normally takes place (Prydz and Dalen, 2000). How CoV-mediated Golgi disassembly influences the biosynthesis and transport of HS is not known in detail, but Golgi fragmentation caused by the depletion of two Golgi-associated peripheral membrane proteins, GRASP55 and GRASP65, leads to a reduction in HS synthesis (Ahat et al. 2022). In the early phase of infection, HS chains are most likely still

normally synthesized, but the SARS-CoV-2 virions may prefer egress route(s) where the S protein avoids encountering and binding to the newly synthesized glycosaminoglycans.

Virus RNA replication and protein synthesis

Virus-infected cells rearrange their endomembranes to establish viral factories, where the viral genome is replicated and transcribed (Miller & Krijnse-Locker, 2008; Blanchard & Roingeard, 2016; Sachse et al., 2019; Hernandez-Gonzalez et al., 2021; Wong et al., 2021). CoV infection leads to the formation of double membrane vesicles (DMVs) that are continuous with ER-derived convoluted membranes (**Fig.1**) (Stertz et al., 2007; Knoops et al., 2008; Fehr & Perlman, 2015; Cortese et al., 2020; Klein et al., 2020; Eymieux et al., 2021a; Mendonça et al., 2021; Wong et al., 2021) and may develop into structures called vesicle packages. These membrane enclosed environments are thought to protect the viral RNA from recognition by host cell innate immunity mechanisms, thus providing safe havens for viral RNA replication (Sachse et al., 2019; Malone et al., 2022). Interestingly, there appears to be a close connection between the DMVs and the IC subdomains where CoV assembly takes place (Cortese et al., 2020; Mendonça et al., 2021; Scherer et al., 2022); however, how the genomic RNAs actually reach the sites of assembly remains poorly understood.

Interestingly, the biogenesis of the DMVs engages the machineries operating in autophagy (Blanchard & Roingeard, 2015; Twu et al., 2021), with possible additional contribution from peroxisomes (Cortese et al., 2020). The autophagic pathway has also been implicated as an egress route for SARS-CoV-2 virus particles in light of ubiquitination of the M protein (Yuan et al., 2022). Following replication and transcription of viral RNAs, the genomic and sub-genomic mRNAs leave the DMVs, most likely through special pores (Wolff et al., 2020). After entering the cytoplasm they are ready to be translated in ER-associated ribosomes to yield the viral envelope proteins E, M, and S, which contain N-terminal signal sequences for ER translocation. By contrast, the nucleocapsid protein N is synthesized on free ribosomes **(Fig.1)**. The non-structural proteins (nsps) and accessory proteins (ORFs) of CoVs are not included in the virus particles, but by interacting with specific host proteins (Gordon et al., 2020; Stukalov et al.,

2020) can these proteins participate not only in viral RNA replication (**Fig.1**), but also in the virus-induced organelle rearrangements, such as Golgi disassembly (see below). Consequently, these proteins may also be linked to the mechanisms of virus egress.

Virus assembly

CoV assembly is initiated by coating of the RNA genome by N proteins, leading to the formation of phase-separated condensates in association with the M protein – the major viral membrane protein present in the IC membranes – and virus budding (Lu et al., 2021). Co-expression of the structural proteins of CoVs has demonstrated that the formation of virus-like particles (VLPs) also requires the E protein (Vennema et al., 1996; Fischer et al., 1998; Xu et al., 2020). The S protein is not required for VLP formation, but is essential for virus infectivity; that is, all three proteins must co-localize in the IC to ensure the formation of fully functional CoV particles. The three proteins – the E protein (Corse et al., 2000; Cohen et al., 2011; Li et al., 2014), the M protein (Machamer & Rose, 1987; Swift & Machamer, 1991; Krijnse-Locker et al., 1992; Klumperman et al., 1994) and the S protein (Lontok et al., 2004; McBride et al., 2007) – are all transmembrane proteins that following their insertion into the ER membrane are transported to the IC (**Fig.1**). Efficient incorporation of these proteins into the virus envelope requires that they harbor signals for retention or retrieval to the perinuclear Golgi region of the host cell, which has been demonstrated by studies of individually expressed proteins. Generally, receptor-mediated retrieval of endogenous ER proteins via C-terminal KDEL signals functions throughout the Golgi apparatus (Miesenböck & Rothman, 1995), and likewise, membrane proteins with a terminal double lysine motif (KKXX) in their cytoplasmic tails are retrieved retrogradely from the PM and distal regions of the Golgi apparatus to the IC and the ER (Nilsson et al., 1989; Jackson et al., 1990; Townsley & Pelham, 1994; Itin et al., 1995).

During the early stages of CoV infection, before the Golgi apparatus is severely affected, viral proteins can be returned to the IC from more distal compartments by well-known mechanisms (Bracquemond & Muriaux, 2021). Both retention and retrieval signals operate to maintain their concentration in the perinuclear Golgi region (reviewed by Ujike & Taguchi, 2015). For instance, the M proteins of certain CoVs localize to secretory compartments that lie beyond the sites of

CoV assembly at the IC (Klumperman et al., 1994; Perrier et al., 2019). The S protein forms trimers in the ER, which are incorporated into virions at the IC through their interactions with the highly abundant M protein (Godeke et al., 2000). When expressed in BHK cells, the S protein displays a more widespread distribution in the secretory compartments, and is also detected at the PM (Nal et al., 2005). The S protein also contains a di-basic signal in its cyto-plasmic tail that mediates its COPI mediated retrieval to the IC (McBride et al., 2007), where the protein can be retained through its interaction with the M protein (Opstelten et al., 1995). Furthermore, the E protein contains intrinsic information that retains it in the perinuclear IC/Golgi region (Corse & Machamer 2000; 2002), where the E and M proteins interact via their cytoplasmic tails (Corse & Machamer, 2003).

The E and the S proteins of CoVs are both S-acylated/palmitoylated at cysteines in their cytoplasmic domains (Lopez et al., 2008; McBride & Machamer 2010). Inhibition of acylation of the S protein reduced its interaction with the M protein (Thorp et al., 2006) and inhibited fusion between viral and cellular membranes (Petit et al., 2007; Li et al, 2022), suggesting that its association with particular lipid domains is important at different stages of the virus life cycle. Interestingly, it has been recently reported that the cytoplasmic domain of the S protein is acylated at a total of 10 cysteines. The hyper-acylation process starts in the ER with palmitate addition to cysteines close to the transmembrane domain, and continues at additional cysteines after ER exit, with each S protein trimer arriving at the IC being decorated by up to 30 acyl chains (Mesquita et al., 2021). Based on its extensive acylation the S protein triggers the formation of cholesterol-rich membrane nanodomains in the IC membranes, thereby facilitating virus budding (Mesquita et al., 2021) possibly by promoting membrane curvature (Ernst et al., 2019). The formation of "lipid rafts" at the IC during SARS-CoV-2 infection may also play a role in the formation of specialized transport carriers mediating the egress of progeny viruses.

Endomembrane alterations support CoV replication, assembly, and egress

As mentioned above, the first observable change occurring intracellularly in CoV-infected cells is the formation of DMVs – the sites of RNA replication – which appear already at 3 hours post infection (Stertz et al., 2007; Cortese et al., 2020; Eymieux et al., 2021a; Mendonça et al., 2021). Other early membrane rearrangements in CoV-infected cells include alterations in the appearance of mitochondria and the recruitment of peroxisomes to the vicinity of the DMVs (Cortese et al., 2020).

It has been recognized for some time that virus infection impacts autophagy, a key process that regulates cellular homeostasis by directing dysfunctional organelles and proteins towards degradation, thereby providing building blocks for biosynthesis during starvation. The initiation of autophagy involves the formation of a double membrane structure called the phagophore (Seglen et al., 1990), which grows to form the autophagosome, enclosing in a selective or non-selective manner cytoplasmic material for delivery to lysosomes for degradation. Autophagy can be activated in response to virus infection, to shield the invading virus and to deliver it to pre-lysosomes or lysosomes for proteolytic degradation and presentation of peptide fragments to the adaptive immune system (Liang et al., 2021). However, many viruses encode proteins that inhibit autophagy, redirecting membrane sources normally used for this process to alternative purposes for their benefit (Blanchard & Roingeard, 2015; Roth et al., 2020). The IC/*cis*-Golgi membranes that are known to provide such a membrane source (Ge et al., 2015) were recently shown to co-operate with the endosomal system in the formation of a precursor membrane structure designated as the pro-phagophore. Moreover, the formation of this hybrid compartment that initiates autophagy was reported to be inhibited by the nsp6 of SARS-CoV-2 (Kumar et al., 2021).

Another link between autophagy and SARS-CoV-2 infection is provided by phosphatidylinositol-3-kinase (PI3K), which besides being involved in autophagosome biogenesis, is required for the formation of DMVs (Twu et al., 2021; Williams et al., 2021). In addition, the ORF3a protein of SARS-CoV-2 has been shown to block autophagy by inhibiting the machinery – including the tethering complex (HOPS) and the SNARE (syntaxin 17) - that mediates the fusion between autophagosomes and lysosomes (Miao et al., 2021). Besides escaping engulfment by the autophagic pathway, a potential benefit for the virus could be ensuring that the IC membranes

are preferentially used for virus assembly, instead of being depleted by autophagosome formation. The egress of β-CoVs was shown to be enhanced by ORF3a, which also contributes to Golgi fragmentation (see below), while the progeny CoVs were suggested to follow an exit route that passes via late endosomes and/or lysosomes (**Fig.1**; Chen et al., 2021).

CoV infection, like infection of cells by a variety of other viruses, has been shown to induce fragmentation of the Golgi apparatus (Lavi et al., 1996; Ulasli et al., 2011; Ruch & Machamer, 2012; Cortese et al., 2020; Hackstadt et al., 2021; Glingston et al., 2019). This striking organelle alteration can be observed already at 6 hours post-infection, when the first progeny viruses are released to the medium of cultured cells (Lavi et al., 1996; Ulasli et al., 2010; Cortese et al., 2020; Hackstadt et al., 2021). Expression of individual CoV proteins has also been shown to induce Golgi fragmentation, with both the E protein and ORF3a having this ability. Notably, both proteins have been reported to function as ion channels (Freundt et al., 2010; Ruch & Machamer, 2011; Hackstadt et al., 2021), indicating that the induction of Golgi fragmentation is an intrinsic property of viral proteins. However, it may involve additional host factors (Ruch and Machamer, 2011; Westerbeck & Machamer, 2019).

Strikingly, instead of interfering with virus release, the dramatic Golgi alterations seem to facilitate this process (Ruch and Machamer, 2012). Indeed, Golgi-independent egress via lysosomes (**Fig. 1**), as shown by its resistance to Brefeldin A (BFA) treatment, has been suggested to dominate during β-CoV infection (Ghosh et al., 2020. This mode of egress appears to be stimulated by the ORF3a protein of SARS-CoV-2 which redirects the lysosomal membrane proteins LAMP1 and LAMP2 to the PM (Chen et al., 2021), in analogy to observations made with reovirus-infected cells (Fernandez de Castro et al., 2020). SARS-CoV ORF3a does not seem to promote the same mechanism, but is still stimulatory to virus egress, possibly by forming ion channels in the cell membrane (Lu et al., 2006). What has been designated as lysosomal egress is reported to involve a pathway traversing late endosomes and/or lysosomes (Ducatelle & Hoorens, 1984; Freundt et al., 2010; Ghosh et al., 2020; Chen et al., 2021); however, the detailed intracellular route remains to be characterized. The same holds also for virus exit at the cell surface, where two modes of release to the cell exterior have been reported, one via membrane tunnels providing openings for large virus-containing carriers (Mendonça et al.

2021) and another involving secretory vesicles that generally contain a single virus particle (Eymieux et al., 2021b).

We have previously drawn the attention to the direct connection between the IC and the endocytic recycling system – consisting of the widely distributed recycling endosomes (REs) and the pericentrosomal endocytic recycling compartment (ERC) – in providing a pathway for the delivery of CoVs from the IC to the extracellular space (**Fig. 1**; Saraste & Prydz, 2021). These compartments – the IC and REs, defined by the GTPases Rab1 and Rab11, respectively – also appear to co-exist at the non-compact zones of the Golgi ribbon, adjacent to the dilated rims of the stacked Golgi *cisternae,* with which they share structural similarity (**Fig. 1**; Saraste & Prydz, 2019). Interestingly, besides its role in ER-Golgi trafficking, the IC has been implicated in Golgi-independent transport routes, that can be defined, for instance, by their resistance to BFA (Sannerud et al., 2006; Prydz et al., 2008; Tveit et al., 2009; Marie et al., 2009; Prydz et al., 2013; Zhang et al., 2020). A secretory Golgi-bypass route via RE has also been demonstrated in neuronal dendrites (Kennedy and Hanus, 2019).

Interestingly, an increasing number of viruses turn out to exploit the endocytic recycling apparatus defined by Rab11 for their assembly and/or release, regardless of whether they bud intracellularly or at the PM (Rowe et al., 2008; Bruce et al., 2012; Coller et al., 2012; Pereira et al., 2014; Vale-Costa & Amorim, 2016; Lučin et al. 2018). For example, infection of cells with influenza virus results in the accumulation of Rab11-positive REs at the pericentrosomal ERC, creating cholesterol-rich membrane domains for the binding of viral genome-containing ribo-nucleoproteins (vRNPs) (Kawaguchi et al., 2015). Subsequently, the vRNPs are delivered in a Rab11- and microtubule-dependent fashion to the PM where virus assembly by budding is completed (Bruce et al., 2012). The functional association of IC membranes with the REs/ERC (Marie et al., 2009; Saraste and Marie, 2018) raises the possibility that this pericentrosomal membrane system at the crossroads of the endo- and exocytic transport routes also plays a role in the release of CoVs (Saraste & Prydz, 2021).

Summary and outlook

Taking into consideration the recently expanding literature, we summarize here the available information on the pathways and mechanisms that the intracellularly budding CoVs employ as they are exported from their host cells (**Fig. 1)**. As is often the case with recently emerged "hot topics", the situation is quite puzzling and further research is required to obtain a better picture on how these viruses take advantage of the more or less conventional cellular machineries to promote their release. Nevertheless, it is clear that the long-prevailing concept on the role of the classical secretory pathway in CoV egress has been seriously questioned. Indeed, the lack of inhibition of β-CoV release by BFA set the stage for the search and characterization of an alternative Golgi-independent exit route via the endo-lysosomal system (Ghosh et al., 2021). One potential problem with this pathway is that the progeny viruses would undergo proteolytic degradation as they pass through lysosomes and become non-infectious. This could probably be partially avoided if CoV infection leads to the reported neutralization of the acidic lumen of endosomes and lysosomes over time (Ghosh et al., 2021). However, recent cryo-EM studies indicated that the spikes of SARS-CoV-2 particles residing in lysosome-like organelles have undergone proteolysis, although it remained unclear whether they represent newly made or re-internalized virus particles (Mendonça et al., 2021). Another problem with this pathway is to understand how viruses are delivered from the IC to lysosomes, since they evidently cannot follow the BFA-sensitive pathway via the Golgi stacks and the *trans*-Golgi/TGN (**Fig.1**). Indeed, the effects of BFA and other transport inhibitors on the release of the various CoVs from different host cells, including epithelial cells, deserve further analysis.

In line with data obtained with other viruses, the role of secretory autophagy in CoV egress has also been considered (Yuan et al., 2022). However, the detailed mechanisms of this process, which until now has been predominantly described in the case of cytosolic proteins, remain poorly understood. Indeed, one may ask how it can be responsible for the efficient secretion of large-sized intralumenal virus particles. Finally, the collective results showing that a number of viruses highjack the endocytic recycling apparatus and the master GTPase Rab11 during the late stages of their replication makes the direct IC-RE pathway a particularly attractive candidate to consider in the case of CoVs (**Fig.1**). Moreover, this pathway can readily explain the puzzling findings regarding efficient CoV release after the Golgi apparatus has been subjected to

extensive reorganization due to virus infection (Saraste and Prydz, 2021). In fact, it may even be beneficial for the virus to be able to down-regulate alternative paths or remove obstacles as it embarks on its "unconventional journey" from the IC via REs to the extracellular space.

Figure legends

Figure 1. A cartoon illustrating early and late stages of the CoV life cycle. *Upper panel*: Following attachment to specific receptor(s) – such as ACE2 – CoV enters cells either by fusing directly with the cell surface, or following its uptake into endosomes, where the viral envelope fuses with the endosomal membrane. In both cases, the viral nucleocapsid enters the cytoplasm and undergoes uncoating, resulting in the release of the viral RNA genome. The positive-sense RNA associates with host cell ribosomes directing the synthesis of non-structural proteins (nsps), which provide subunits of the viral RNA replicase or act in the biogenesis of an ER-derived convoluted membrane compartment, which includes double membrane vesicles (DMVs) – the sites for viral RNA replication and transcription. *Lower panel*: Sub-genomic mRNAs produced in the DMVs function in the synthesis of the viral structural proteins – the cytoplasmic nucleocapsid (N) protein and three membrane proteins (E, M and S) – in free- or ER membrane-bound ribosomes, respectively. Vesicle-mediated transport and accumulation of the membrane proteins at the IC membranes sets the stage for virus assembly by budding into the IC lumen. Three alternative pathways for CoV delivery from the IC to the extracellular space are depicted: *Route 1*) The progeny viruses highjack the constitutive secretory pathway as they segregate into the dilated rims of Golgi *cisternae* and pass across the Golgi stacks (*cis*-to-*trans*) based on cisternal progression. At *trans*-Golgi the viruses are sorted into post-Golgi carriers which move to the PM and undergo exocytosis. *Route 2*) This pathway bypassing the Golgi stacks is based on a direct connection between the IC elements and REs, defined by Rab1 and Rab11, respectively. Prior to Golgi fragmentation these compartments reside at the non-compact zones of the Golgi ribbon, connecting the different Golgi stacks. In this case the endocytic recycling system provides the carriers for the final delivery of the virus for exocytosis. *Route 3*) The progeny viruses are released from cells via lysosomal exocytosis. They may reach the lysosomes via *trans*-Golgi; for example, following *Route 1*, or employ a direct IC-to-lysosome pathway, which

remains to be identified. For simplicity, only one CoV particle in the lumen of the carriers is shown, although many of them contain numerous viruses.

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Abbreviated summary

A number of egress routes have been suggested for corona virions from the pre-Golgi intermediate compartment (IC), where the virus capsids obtain their membrane envelope, in direction of the cell surface. We here discuss coronavirus egress routes in relation to the observed changes that occur to host cell intracellular compartments during coronavirus infection.