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Cell entry and egress are essential steps in the viral life cycle that govern pathogenesis and spread. Mammalian orthoreoviruses (reoviruses) are nonenveloped viruses implicated in human disease that serve as tractable models for studies of pathogen–host interactions. In this review we discuss the function of intracellular vesicular transport systems in reovirus entry, trafficking, and egress and comment on shared themes for diverse viruses. Designing strategic therapeutic interventions that impede these steps in viral replication requires a detailed understanding of mechanisms by which viruses coopt vesicular trafficking. We illuminate such targets, which may foster development of antiviral agents.

Mammalian Orthoreoviruses

The family Reoviridae encompasses prototypical double-stranded (ds)RNA viruses that include important pathogens of plants, animals, and humans. Mammalian orthoreoviruses (herein referred to as reoviruses) spread between humans by respiratory or fecal–oral transmission with initial exposure often occurring in childhood [1]. Serologic evidence indicates that at least half the human population has experienced reovirus infection [1,2]. However, most active infections remain undetected due to the absence of symptoms or a mild clinical presentation, usually of respiratory or gastrointestinal illness [3]. In rare cases, infection of children has been associated with encephalitis or meningitis [4,5]. Reoviruses isolated from humans cause significant disease in mice, infecting multiple organs including the intestine, liver, spleen, heart, and brain. Associated pathophysiology includes cholestatic liver disease, myocarditis, encephalitis, and hydrocephalus [3]. Infection with some reovirus strains abrogates oral immunological tolerance in mice and may be linked to celiac disease in humans [6,7]. Studies of reovirus pathogenesis have informed general mechanisms by which viruses infect specific tissues to cause disease. Critical steps governing viral invasion of a host include how viruses enter and spread between cells. Several primary and immortalized cell lines support reovirus replication and are tractable for mechanistic studies of steps in reovirus infection, including how the virus enters, synthesizes RNA and protein, and is released from cells [3]. Recent advances expand our understanding of reovirus entry and egress to include multiple distinct pathways for each.

Entry

Viruses must enter host cells to initiate productive infection since they depend on intracellular machinery for replication. Specific host factors are responsible for the attachment and uptake of different viruses, but shared themes in the exploitation of cellular biology can be found across diverse viruses. Many viruses first make contact with the cell surface by binding oligosaccharides [8]. This initial attachment often is followed by specific interactions between virions and proteinaceous receptors that coordinate uptake into the cell [9]. Virus entry into cells commonly relies on host endocytic and vesicular trafficking machinery.
Viruses Contact Cell-Surface Molecules to Initiate Infection

The initial event in reovirus infection is attachment to terminal sialic acid moieties on cell-surface polysaccharides of susceptible cells [10]. Sialic acid binding is common to viral pathogens [11], and species-specificity and tropism often are determined by preferential use of specific sialylated glycans (see Glossary) by individual viruses. Although glycans are found on the surface of all cells, glycosylation patterns and saccharide monomer linkages differ between cell types and species based on host genetics, enzymatic activity, and secretory processing [12]. Reoviruses bind to α-2,3-, α-2,6-, or α-2,8-linked sialylated glycans, depending on the viral strain [13–15]. The reovirus outer-capsid protein, σ1, functions in glycan binding (described in Box 1). While not strictly required for productive infection, glycan attachment promotes reovirus replication by enhancing virion interactions with entry receptors and contributes to binding avidity at the cell surface [10,16].

Bona fide entry receptors are responsible for virion attachment and internalization. Immunoglobulin superfamily (IgSF) proteins, integrins, and phosphatidyl serine (PS) receptors are transmembrane proteins that often are targeted by viruses to facilitate uptake [8]. Two known receptors facilitate reovirus infection: junctional adhesion molecule A (JAM-A), an IgSF receptor that localizes to epithelial and endothelial tight junctions, leukocytes, and platelets, and Nogo receptor 1 (NgR1), a neuronal cell-surface protein involved in regulating axonal plasticity and growth [17,18]. Reovirus attachment protein σ1 binds to JAM-A as well as glycans (Box 1); the viral ligand for NgR1 is likely to be outer-capsid protein σ3. These receptors do not fully explain reovirus tropism, as reovirus strains that differ in tropism can use JAM-A and NgR1 as entry receptors. This tropism difference is particularly notable in the brain, where type 1 reoviruses infect ependymal cells and type 3 reoviruses infect neurons [19]. Thus, there are likely additional reovirus receptors yet to be discovered, and multiple receptors may act in concert or individually to mediate cell type- or pathway-specific internalization.

Viral Uptake Is Mediated by Host Endocytic Machinery

Viruses coopt cellular internalization processes, including clathrin-mediated endocytosis, caveolar endocytosis, phagocytosis, and pinocytosis, to reach the cytosol. Clathrin-mediated endocytosis is the predominant form of reovirus entry in nonpolarized cell lines following JAM-A binding, although alternative entry modes exist (Figure 1, Key Figure) [20–23]. The capacity to

Box 1. Reovirus Structure and Replication

The reovirus genome consists of ten dsRNA segments packed in two concentric protein shells. Reovirus encodes eight proteins that form the complex capsid; σ1, σ3, and μ1 comprise the outer capsid, while σ2, λ1, λ2, μ2, and λ3 comprise the inner capsid [3] (Figure I). The protein σ1 functions in receptor binding to initiate cell entry. Specific σ1 sequences interacting with attachment and entry receptors have been identified by mutational analyses and structural studies [14,15,91,92]. The σ1 protein determines the serotype of reovirus strains. Alterations in σ1 sequence between reovirus serotypes likely control differential receptor binding interactions that dictate preferences for target cell selection and pathogenesis [93].

During the infectious cycle, virions are disassembled by acid-dependent proteases to form defined intermediates. Acidification of late endosomes activates cathepsin B and cathepsin L proteases, which digest major outer-capsid protein σ3 and cleave μ1 forming infectious subviron particles (ISVPs) from virions [33]. In ISVPs, the λ2 protein is further exposed, and the trimeric attachment protein σ1 adopts an extended conformation (Figure I). These conformational changes likely alter capsid-receptor interactions for extracellularly produced ISVPs. Further capsid modifications to ISVPs occur in the endosome through loss of σ1 and exposure of hydrophobic residues and release of the N-terminal fragment from μ1 (μ1N) to form ISVPS [64]. The μ1N fragment aids in penetration of endosomal membranes by ISVPs and release of a transcriptionally active viral core into the cytoplasm (Figure I). Viral cores containing the dsRNA genome catalyze synthesis and release of positive-sense, single-stranded RNA transcripts that are translated by the host machinery [3]. Viral nonstructural proteins act in the formation of viral factories (VFs). Reovirus μNS is required to initiate VF formation, while μ2 interacts with the cytoskeleton to anchor VFs and influences their shape [67,95–97]. Host and viral components of VFs promote assembly of progeny virions prior to their release by lytic or nonlytic pathways.

Glossary

Endoplasmic reticulum (ER): an intracellular membranous network that functions in protein synthesis and transport.

Endosome: a single-membrane-bound organelle that transports cargo from endocytic vesicles to other membranous compartments for recycling, sorting, or degradation.

Glycan: a polymeric sugar compound, also referred to as a polysaccharide, often conjugated to a protein or lipid.

Immunoglobulin superfamily (IgSF): a group of cell-surface proteins with a shared immunoglobulin-like domain structure that function in binding and adhesion.

Infectious subviron particle (ISVP): a reovirus disassembly intermediate with a modified capsid formed by protease treatment of virions.

Lysosome: a single-membrane-bound organelle that participates in the degradation of biomolecules.

Membranous carrier (MC): a small membrane-bound organelle that buds from sorting organelles and functions to transport mature virions to the cell surface.

Plasma membrane (PM): a semipermeable lipid bilayer delimiting cellular contents from the external environment.

Sorting organelle (SO): an organelle formed from modified lysosomes during reovirus infection that functions to collect mature progeny virions.

Trans-Golgi network (TGN): a membranous organelle system that directs vesicular sorting of cargo for transport, secretion, or degradation.

Viral factory (VF): a cluster of actively replicating viruses organized by infection-induced changes to intracellular structures.

Viroporin: a viral protein that interacts with host cell compartments to form ion channels.
use multiple entry pathways might promote viral replication or pathogenesis in diverse cellular environments. In addition to clathrin-mediated endocytosis, caveolar endocytosis provides a cell entry route for virions and infectious subvirion particles (ISVPs), which are reovirus disassembly intermediates that are produced in the intestinal lumen (Figure 1) [21]. ISVP biogenesis is described in Box 1. In neurons, a highly polarized cell type, entry occurs by a mechanism consistent with macropinocytosis (Figure 1) [24]. In macropinocytosis, membrane ruffling driven by cytoskeletal rearrangement and phosphoinositide 3 kinase (PI3K) activity enables cells to take up large volumes of extracellular material that is then trafficked through the same endocytic
Figure 1. Reoviruses enter cells via uptake of virions or infectious subvirion particles (ISVPs) into vesicular compartments after macropinocytosis (i), clathrin-mediated endocytosis (ii), or caveolar endocytosis (iii). Reovirus entry by a particular uptake route may be dictated by receptor binding to initiate distinct signaling pathways or cellular preference for a particular uptake mechanism. The formation and transport of virus-containing vesicles after internalization depends on coordination by cellular components. Vesicles can be transported long distances before acidification results in virion-to-

(Figure legend continued at the bottom of the next page.)
pathways as cargo endocytosed by clathrin- or caveolin-mediated pathways. Although neurons are currently the only cell type confirmed to internalize reovirus by macropinocytosis, reovirus also may use this pathway to enter other cell types that are specialized to intake large volumes. Distinctions in cell entry likely contribute to reovirus pathogenesis, although cellular and viral factors that determine the specific entry pathway remain to be elucidated.

Many viral receptors function directly in intracellular signaling following ligand binding. Despite possessing a cytoplasmic domain, JAM-A signal transduction is not required for reovirus uptake. Instead, β1 integrin is required to recruit the cellular endocytic machinery to coordinate reovirus internalization [20,25]. Integrins function to relay signals between extracellular and intracellular environments, resulting in cytoskeletal rearrangements that allow uptake of a variety of viruses, including Ebola virus [26], human cytomegalovirus [27], reovirus, and vaccinia virus [28], among others. The reovirus capsid protein λ2 contains two conserved integrin-binding motifs (RGD and KGE) [25,29], although it remains to be confirmed whether λ2 directly contacts the β1 integrin extracellular domain to activate outside-in signaling to internalize or influence endocytic sorting. Precedent for engagement of integrins by viruses of the family Reoviridae is set by rotavirus, which directly engages αvβ3 integrin via capsid protein VP7 to mediate entry [30,31]. It is unclear whether JAM-A, NgR1, or integrin engagement is involved in coordinating macropinocytic uptake of reovirus. Similarly, it is unknown whether NgR1 relies on a partner molecule for signal transduction, as is the situation with integrins and JAM-A. It is formally possible that distinct receptor engagement dictates the entry mode and subsequent trafficking pathways used by reovirus.

Disruption of viral entry is an attractive therapeutic option. Preventing viral replication early in the infectious course diminishes the need to manage symptoms and has great public health benefit in pathogen eradication. While pharmacologic inhibitors of clathrin-mediated endocytosis, caveolar endocytosis, macropinocytosis, and phagocytosis aid as research tools to study the cell biology of viral entry, their widespread clinical use is precluded by significant adverse effects on cellular physiology, which would pose harm. A preferred strategy to block viral entry is to disrupt virus–host interactions in a manner that does not alter cellular function but specifically targets a viral component. Continued research to identify common viral motifs involved in host interactions raises the possibility of developing mimetics for common attachment factors, such as glycans or integrins, to impede host-binding capsid proteins as a broad-spectrum prophylaxis measure.

Reovirus Transport and Signaling through the Endocytic Pathway Determines the Outcome of Infection

Following receptor-mediated endocytosis, both enveloped and nonenveloped viruses transit through endosomes. Viruses use cues within the endocytic pathway to gain cytosolic entry at appropriate sites, barring which they risk degradation or recycling out of the cell. In the case of reovirus, JAM-A binding promotes clathrin-mediated internalization mediated by β1 integrins for transit through distinct endosomal compartments marked by Rab GTPases: early (Rab5), late (Rab7 and Rab9), and recycling endosomes (Rab4 and Rab11) (Figure 1) [25,32]. While sorting into recycling endosomes appears to be a nonproductive entry route, distribution to late endosomes is required
for cytosolic entry and subsequent replication [32]. Proteolytic processing of reovirus virions by cathepsin proteases in acidified endosomal compartments is described in Box 1. Blocking either cathepsin activity or endosomal acidification inhibits infection by virions [33]. Endosomal pH regulation is essential for productive infection by many nonenveloped and enveloped viruses; acid-dependent proteolytic processing or conformational changes allow membrane penetration, dissolution, or fusion to release the viral genome or genome-containing subassembly [34].

Interactions of viral and host factors along the endocytic pathway can be productive or antagonistic, thereby influencing the success of viral infection. After internalization, NPXY motifs in the cytoplasmic tail of β1 integrin [20,25] and src kinase activity mediate productive sorting of reovirus into late endosomes [35]. Mutation of NPXY motifs or inhibition of src kinase does not affect reovirus internalization. Instead, these alterations misdirect reovirus virions to lysosome-like organelles during entry, resulting in diminished infectivity. By contrast, lysosome-like organelles function productively in the reovirus life cycle during nonlytic egress following replication (Figure 1) [36]. Whether reovirus directly engages β1 integrin, and how src signaling is triggered to influence virus sorting after uptake, is not understood. One possibility is that activated src kinase phosphorylates integrin NPXY motifs to enable recruitment of adaptor proteins for cargo sorting. Multiple viruses activate src family kinases to remodel host cells for viral replication [37], which makes these enzymes potential targets for therapeutic intervention.

Viruses also encounter innate immune defenses within the endosomal pathway that antagonize infection. Members of the interferon-induced transmembrane family (IFITM) have antiviral activity against several viruses, including dengue virus, Ebola virus, influenza A virus, severe acute respiratory syndrome (SARS) coronavirus, and West Nile virus [34]. IFITM3 localizes to late endosomes and antagonizes viral infection by restricting fusion of enveloped viruses and shuttling these viruses to lysosomes [34,38]. IFITM3 impedes reovirus infection by either modulating endosome acidification or diminishing virus uncoating [39]. Proteolytic processing of reovirus in the endocytic pathway triggers NF-κB-dependent apoptotic signaling that results in cell death and eventual egress of viral progeny (Figure 1) [40]. Reovirus-induced NF-κB signaling has tissue-specific effects. NF-κB-mediated host gene expression protects the heart yet is linked to neural injury in the brain (Box 2) [41]. Interestingly, the capacity of reovirus strains to induce apoptosis

**Box 2. Cellular Entry and Egress Are Pathogenesis Determinants for Reoviruses**

The interplay of entry and egress mechanisms is required to coordinate transmission of reovirus between cells and defines the course of infection. Reoviruses establish initial infection in the intestine following fecal–oral transmission. Following primary replication, reovirus spreads to multiple organs and causes disease in a serotype-specific manner. Reovirus uses the receptor JAM-A to enter endothelial cells lining blood vessels, from which progeny virions are released into the bloodstream to disseminate hematogenously [56,98]. Reovirus also spreads by a JAM-A–independent neural route [98]. Enteric nerve termini may be the main point of access to the nervous system for reoviruses.

Viruses must exit infected cells to spread to new cells and hosts. The mechanisms by which reoviruses exit the bloodstream to initiate infection in organs of secondary replication remain to be identified, but the release process may be mediated by nonlytic egress from infected endothelial cells [36]. Likewise, much remains to be discovered about mechanisms of reovirus neuronal egress and spread through neural networks. A directional, nonlytic egress mechanism may direct spread through neural networks. However, reovirus-induced apoptotic cell death is a critical disease correlate in mice [44,99]. Reovirus infection is associated with physiologic dysfunction in multiple target tissues. Intestinal infection of adult mice results in aberrant T cell differentiation and a loss of tolerance to newly introduced food antigens [83]. Reovirus strains that produce this effect trigger apoptosis in intestinal epithelial cells less efficiently than strains that do not [42]. However, caspase-mediated apoptosis induced by reovirus infection results in cardiac and neural injury [99,100]. Initiation of apoptotic signaling occurs during viral entry. Conversion of virions to infectious subviral particles (ISVPs) in acidified endosomes is required for apoptotic death of cultured cells [101]. It is possible that apoptotic cell death may enhance release of progeny virus from infected cells, thus further linking reovirus entry and egress.
in the intestine inversely correlates with the capacity to block immunological tolerance to a newly introduced food antigen. It appears that virus-induced apoptosis limits the duration of reovirus replication in the intestine and thus dampens inflammatory immune responses linked to tolerance loss \([6,7,42]\). Thus, apoptotic responses must be fine-tuned by the host to limit viral replication without leading to tissue damage or aberrant inflammation.

The form of virus entering a cell influences the requirements and results of infection. ISVPs, which are thought to be the predominant reovirus particle form infecting intestinal cells, bypass requirements for proteolytic disassembly in late endosomes and establish infection more efficiently than virions (Box 1). ISVPs escape endosomes soon after endocytosis before reaching late endosomes (Figure 1) and, therefore, ISVPs are not restricted by IFITM3 [39]. Since ISVPs can be produced by proteolytic treatment of virions in the intestinal lumen, they do not require functions of integrin NPXY motifs [20], src kinase [35], endosome acidification, or cathepsin proteases [3] to establish productive infection. The early endosomal escape by ISVPs results in a dampened innate immune response relative to virions, which activate a strong innate immune response by activation of Toll-like receptors and RIG-I-like receptor pathways [43]. ISVPs also induce a pro-survival state by transforming growth factor (TGF)-\(\beta\) production during infection of intestinal epithelial cells [43]. It is not clear whether ISVPs are produced extracellularly at other sites during natural infection in vivo or whether ISVPs formed in this way influence pathogenesis.

Reovirus infects a variety of polarized cells during infection, including intestinal and airway epithelial cells, vascular endothelial cells, and neurons. Neurons in the cortex, thalamus, and hippocampus are preferentially infected by neurotropic reoviruses, although distinguishing factors allowing neurons at these sites to support reovirus replication remain to be elucidated [44]. Infection of polarized cells is complicated by the necessity for directional transit to access sites of replication. Dynein mediates microtubule-dependent, long-distance retrograde transport of reovirus, which is necessary to establish neuronal infection [24,45]. Reovirus also colocalizes with dynein and requires microtubule-based transport for delivery to late endosomes in nonpolarized cells [46]. During retrograde neuronal transport, reovirus remains within nonacidified vesicles until reaching the neuronal soma, where acidification and core release ensue [24]. Such a mechanism of long-distance transit in nonacidified vesicles also is employed by other neurotropic viruses such as adenovirus and rabies virus, neuronal cargo, and misfolded proteins implicated in neuropathology [47–49]. Influenza virus and some rotavirus strains trigger signaling through Akt (protein kinase B), extracellular signal-regulated kinase, and PI3K during entry to modulate vacuolar proton pump function and endosomal pH [50,51]. It is unknown whether reovirus employs similar mechanisms to actively regulate endosomal pH, nor is it clear whether \(\beta1\) integrin and src kinase signaling are used for active sorting into specific endosomal compartments in neurons. Delayed endosome acidification effected by IFITM3 may be beneficial in this context by allowing time for reovirus transport to neuronal soma before proteolytic processing.

Egress

After replication and assembly in cytoplasmic factories, progeny virions must escape the host cell. Many viruses use cellular vesicular trafficking routes for egress and cell-to-cell transmission [52,53]. Enveloped viruses can be released by budding at the plasma membrane (PM) or the bounding membrane of an internal compartment such as the endoplasmic reticulum (ER), thereby acquiring their envelopes [54]. In the latter case, viruses gathered in the ER lumen exit the cell via the conventional secretory pathway. By contrast, nonenveloped viruses, such as reovirus, lack an external membrane. These viruses are usually thought to disrupt the integrity of the lipid bilayer, leading to cell egress by lysis. However, mature virions of several nonenveloped

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**Box 1**

ISVPs: Intracellular Single-Particle Vesicles that escape endosomes and establish infection more efficiently than virions.
viruses can exit infected cells using nonlytic mechanisms of egress without compromising cell viability [52]. A summary of viral egress mechanisms is shown in Figure 2.

Figure 2. Summary of Known Egress Pathways Used by Viruses to Exit Infected Cells. Multiple modes of egress are used for viral release. Enveloped viruses acquire membranes during egress by budding at the plasma membrane (PM) (i), while transiting intracellular membranes in the endoplasmic reticulum and Golgi network (ii), or directly budding at the nuclear membrane (iii). The egress pathways of enveloped and nonenveloped viruses converge at intracellular membranous networks that traffic cargo to the PM, allowing nonenveloped viruses to exit cells without lysis. Viral particles are recruited from viral factories (VFs) into vesicular trafficking pathways bound for extracellular release. Multiple virions can be packaged in multivesicular bodies (MVBs) and released at the PM (iv). Autophagosome-like vesicles can enclose progeny virions for extracellular release without degradation (v). Modified lysosomal pathways may be similarly exploited by viral pathogens for exocytic release (vi). Both enveloped and nonenveloped viruses can induce cell death, resulting in release of viral contents (vii). Reovirus egress occurs by a nonlytic lysosomal pathway or lysis, depending on the cell type.

Abbreviation: TGN, trans-Golgi network. Figure prepared using BioRender.

Nonlytic Reovirus Egress Uses Cellular Organelle Trafficking Pathways

Reovirus infection is lytic in some types of cultured cells, in which viral disassembly leads to activation of transcription factor NF-kB, inducing apoptotic signaling (Figure 1). NF-kB modulates gene expression and multiple effectors downstream to execute reovirus-induced cell death by apoptosis [55] and potentially more inflammatory means. However, reovirus can undergo nonlytic egress from other types of cell, such as human brain microvascular endothelial cells (HBMECs). Studies using polarized HBMECs showed that reovirus release occurs predominantly from the apical surface, allowing access to the bloodstream for systemic dissemination in the host [56]. However, in nonpolarized HBMECs, progeny virions exit cells at discrete zones at the basal surface.

We discovered that the reovirus egress machinery is composed of two different membranous elements called sorting organelles (SOs) and membranous carriers (MCs) (Figure 1) [36].
SOs are recruited to the periphery of viral factories (VFs) during late phases of infection and appear to be modified lysosomes. In fact, reovirus infection promotes the modification of lysosome dynamics, morphology, and function. The number and size of lysosomes are increased in reovirus-infected cells, and these organelles frequently form aggregates [36]. It is possible that these modifications alter lysosome function and activity for subsequent use in reovirus egress. In reovirus-infected cells, SOs selectively collect mature virions from VFs for transport to the PM. VFs are formed by the accumulation of ER-derived tubulovesicular membranes [57,58]. Therefore, VF-associated membranes might fuse with SO-bounding membranes to facilitate movement of virions from VFs to SOs. Interestingly, mature, genome-containing virions are attached to filaments, whereas empty particles are not. Although the identity of these filaments remains to be determined, their morphology suggests that they are formed from actin. Actin is a cytoskeletal component that functions in reovirus transport. The presence of filaments in all compartments of the reovirus egress machinery suggests that actin or other cytoskeletal components participate in reovirus sorting at the VF periphery before egress. In addition, specific points of membrane fusion connecting VFs with SOs might rely on cytoskeletal filaments to mediate the selective uptake of mature virions into the egress machinery. After SOs are filled with mature virions, the smaller MC organelles bud from these structures to transport the new progeny particles to the cell periphery for egress (Figure 3) [36].

Several nonenveloped viruses, including members of the families Parvoviridae [59], Picornaviridae [60–63], and Reoviridae [56,64–66], use nonlytic mechanisms for egress, depending on the virus and cell type. Directional release of rotavirus from the apical surface of intestinal epithelial cells is reminiscent of reovirus release from endothelial cells, although the rotavirus egress mechanism does not involve lysosomes [65]. Nonlytic exit may prolong the viability of infected cells, enhancing yields of progeny virus and allowing continuous release. Furthermore, in most viral infections, nonlytic egress consists of a nonconventional secretion process that could mediate the directional release of virus. The contribution of directional entry and egress in reovirus pathogenesis
is discussed in Box 2. Additional advantages of nonlytic egress may include avoiding inflammatory signaling associated with lytic cell death and enhancing transmission potential by including multiple virions in a single transmissible unit [67,68].

Nonlytic egress mechanisms frequently involve the formation of new compartments derived from cellular organelles, whose membranous characteristics allow the exit of virions without disrupting cellular integrity. The origin of these compartments is frequently associated with autophagic and multivesicular sorting pathways. Indeed, infection by some reoviruses is facilitated by the autophagy machinery. Plant reoviruses induce the formation of autophagosomes to carry virions, mediating virus spread between cells using nonconventional secretion in the insect vector [69]. The intracellular transport of new virions from viral factories to the cell surface is likely facilitated by microtubules and the actin cytoskeleton. In addition, plant reoviruses assemble tubules formed by viral proteins and actin, which dictate the intercellular movement of new particles [69,70]. While there is little homology shared between plant and mammalian reoviruses, both use the cytoskeleton and infection-induced membranous compartments for their egress mechanisms. Therefore, a similar means of viral transport may be mediated by distinct viral proteins. The precise transport mechanisms used by reovirus-containing organelles from viral assembly sites to the PM for egress remain under study.

The Role of Environmental pH in Viral Progeny Protection and Nonlytic Viral Egress

Most viruses require pH regulation of cellular compartments in one or more stages of their life cycle [71], although the effect of cellular pH on nonlytic viral egress is poorly understood. Plant and animal reoviruses use two distinct nonlytic mechanisms for egress: the autophagosomal machinery [69,72] and SOs [36], respectively. In both pathways, plant and animal reoviruses must avoid degradation by acidic lysosomal pH to exit successfully, but mechanisms by which this protective effect is accomplished are unknown.

Other viruses exploiting the autophagic pathway use different strategies to avoid acidic lysosomal pH during egress [73]. Rotaviruses block autophagy maturation, redirecting autophagic membranous trafficking to the ER [74]. Influenza and parainfluenza viruses prevent fusion of autophagosomes with lysosomes using the matrix M2 protein and the phosphoprotein, respectively [75,76], while members of the family Herpesviridae inhibit the recruitment of Atg6/Beclin-1 to the PI3K complex, blocking autophagy and its pernicious effects on virus progeny [77,78]. In the case of mammalian reovirus, infection raises the lumenal pH of the SOs from 4.5–5 to 6.1 [36], thereby potentially blocking the activity of pH-dependent cathepsins and preventing premature virion disassembly. Mechanisms by which reovirus alters the lysosomal pH are not clear.

Viruses have developed two main strategies to modify the pH of cellular compartments to facilitate viral propagation: viroporins and alteration of vacuolar ATPase (V-ATPase) distribution and function. Examples of viroporins include hepatitis C virus (HCV) p7, influenza A virus (IAV) M2, and picornavirus 2B proteins [71]. Coxackievirus B (CVB), HCV, and IAV change the pH of several cellular compartments using viroporin proteins to establish pores for egress [79]. Positively charged protons flow from these compartments into the cytosol, producing an alkaline environment inside the vesicle. In this way, IAV alkalinizes the lumen of the Golgi complex and the trans-Golgi network (TGN), preventing premature conformational changes in the viral hemagglutinin during exit of viral progeny [80] while directing trafficking of the hemagglutinin along the secretory pathway to the PM [81]. The CVB viroporin releases charged calcium ions and protons from the Golgi complex and ER into the cytosol [82], reducing cellular protein trafficking and promoting persistent CVB infection [82,83]. This process additionally inhibits glycosylation of proteins in the Golgi complex, resulting in dampened host cell immune responses [84]. Similarly,
HCV p7 protein alkalizes cellular secretory compartments, which protects nascent virions from inactivation [85].

Other viruses modify the pH of cellular compartments by interacting with the vacuolar-ATPase proton pumps that regulate acidification of vesicles and organelles. HIV-1 prevents the recruitment of a functional V-ATPase to endosomes where it buds and accumulates in macrophages [86]. Dengue virus prM protein binds to endosomal V-ATPases, increasing the pH in that compartment to promote efficient viral release [87]. The increase in intravesicular pH also prevents pH-dependent conformational changes of dengue virus E glycoprotein during viral secretion [88].

Due to the contribution of pH in viral replication, inhibitors of viroporins and cellular V-ATPase proton pumps [69,89,90] are an important focus of study for development of antiviral countermeasures [71]. Research on mechanisms by which reoviruses regulate or avoid acidic lysosomes in the egress pathway may lead to new targets for antiviral drugs.

Concluding Remarks and Future Perspectives
Antiviral therapeutics must selectively target viral replication without significantly altering normal cell function. Since viral replication is tightly linked to host cell physiology, research to uncover viral factors that interact with the host to coordinate infection, replication, and spread is essential to designing appropriately targeted interventions (see Outstanding Questions). Multiple points in the reovirus life cycle rely on interactions with host machinery. The use of cellular architecture by reoviruses at two distinct steps in the viral life cycle, namely entry and egress, converge on viral manipulation or exploitation of intracelluar vesicular and cytoskeletal trafficking systems. Many viral pathogens use these same cellular components to replicate, although different viral mechanisms may be used to coordinate the function of this cellular machinery during infection. Ongoing work to elucidate interactions between reovirus and host cells during entry and egress will lead to discoveries of the specific viral factors that mediate the pathways discussed and potentially illuminate new targets for antiviral drug development.

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Outstanding Questions
Do additional membrane proteins function as reovirus receptors?
Are reovirus entry mechanisms dictated by engagement of specific receptors?
What host factors mediate macropinocytic reovirus entry?
Are reovirus entry and egress mechanisms cell-type-specific or shared broadly?
What viral mechanisms regulate endosomal pH during entry, long-range transport, and egress?
What aspects of host machinery mediate formation and transport of reovirus-containing macropinocytic vesicles?
How are lysosomes recruited to viral factories during infection?
What viral factors function to modify lysosomes into sorting organelles?
Do viral factors interact with lysosomal proton pumps?
Does reovirus encode a viroporin?
How are only mature, genome-containing reovirus particles recruited to sorting organelles?
What cellular factors guide membranous carriers to the cell surface for reovirus release?
Does reovirus dissemination or transmission benefit from nonlytic egress?
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