RAB11-mediated trafficking in host–pathogen interactions

Annabel Guichard[1], Victor Nizet[2,3] and Ethan Bier[1]

Abstract | Many bacterial and viral pathogens block or subvert host cellular processes to promote successful infection. One host protein that is targeted by invading pathogens is the small GTPase RAB11, which functions in vesicular trafficking. RAB11 functions in conjunction with a protein complex known as the exocyst to mediate terminal steps in cargo transport via the recycling endosome to cell–cell junctions, phagosomes and cellular protrusions. These processes contribute to host innate immunity by promoting epithelial and endothelial barrier integrity, sensing and immobilizing pathogens and repairing pathogen-induced cellular damage. In this Review, we discuss the various mechanisms that pathogens have evolved to disrupt or subvert RAB11-dependent pathways as part of their infection strategy.

Recycling endosomes
Late vesicular compartments that are involved in recycling membrane proteins and *de novo* synthesized cargo from the Golgi complex to the cell surface.

To establish infection, pathogenic microorganisms have evolved many strategies to circumvent host defences and exploit the host cellular machinery. Specific virulence factors disable or subvert vesicular trafficking pathways to and from the host cell surface, which promotes pathogen entry, replication or escape.

RAB GTPases are key regulators of various membrane trafficking events (reviewed in REF. 1) (FIG. 1); they belong to the large protein family of small GTPases and comprise 30–70 members in different organisms. Other small GTPase subfamilies include RAS, RHO–RAC, ARF and RAN, as well as the more recently characterized RHEB, RAD and RIT subfamilies. Binding of GTP to small GTPases stimulates their effector functions, whereas association with GDP inactivates them (FIG. 1a). Guanine nucleotide exchange factors (GEFs) stimulate GDP release and are positive regulators, whereas GTPase-activating proteins (GAPs) inhibit GTPase activity by promoting the hydrolysis of GTP to GDP. In addition, guanine nucleotide dissociation inhibitory (GDI) proteins bind to GDP-bound RAB proteins to sequester them in the cytoplasm, and the opposing GDI displacement factors (GDFs) re-deliver GDP-bound RAB proteins to the membrane (FIG. 1a).

RAB11 mediates several cellular processes that involve intracellular vesicle trafficking, including the delivery of plasma membrane proteins to specialized sites (for example, bud sites in yeast or cell–cell junctions in mammalian cells), secretion of various factors (such as growth factors and other peptides, including interferons (IFNs), cytokines, bone morphogenetic protein (BMP) and transferrin receptor), establishment of cell polarity and targeting of proteins to organelles and cell compartments, which leads to the formation of edges of migrating cells and the midbody during cytokinesis (reviewed in REFS 2,3). Of note, RAB11-mediated targeting of proteins to organelles such as cilia[4,5] or tubular structures[6] also requires the activation of the downstream RAB GTPase RAB8, which may reflect the ancestral pathway, as this organization is also found in yeast[7]. Furthermore, RAB11-mediated trafficking events have a role in several aspects of innate immunity, including promoting epithelial and endothelial barrier integrity, targeting of proteins such as Toll-like receptor 4 (TLR4) to phagosomes for receptor-mediated phagocytosis of pathogens[8] and expulsion of pore-forming toxins (PFTs) from the cell surface[9]. Also, the exocyst, which is an eight-protein complex (see below) that mediates many functions of RAB11, has a key role in initiating IFN signalling in response to double-stranded DNA (dsDNA)[10] and regulating autophagy versus IFN signalling[11] (BOX 1).

One prominent trafficking pathway that pathogens modulate or exploit by various mechanisms is the final step of endocytic recycling, during which cargo-containing vesicles dock at the cell surface. Endocytic recycling of internalized extracellular molecules or membrane proteins is initiated by RAB5, which directs plasma membrane-derived vesicles to RAB4-positive early endosomes (FIG. 1b). Some cargo proteins can then recycle back to the plasma membrane from the early endosome via two pathways: a fast, direct route that depends on RAB4, or a slow route via RAB11-positive late recycling endosomes. RAB11-positive late recycling endosomes are delivered to the apical cell surface along

[1]Section of Cell and Developmental Biology, University of California.
[2]Department of Pediatrics, University of California.
[3]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093, USA.
Correspondence to V.N., E.B.
e-mails: vnizet@ucsd.edu; ebier@ucsd.edu
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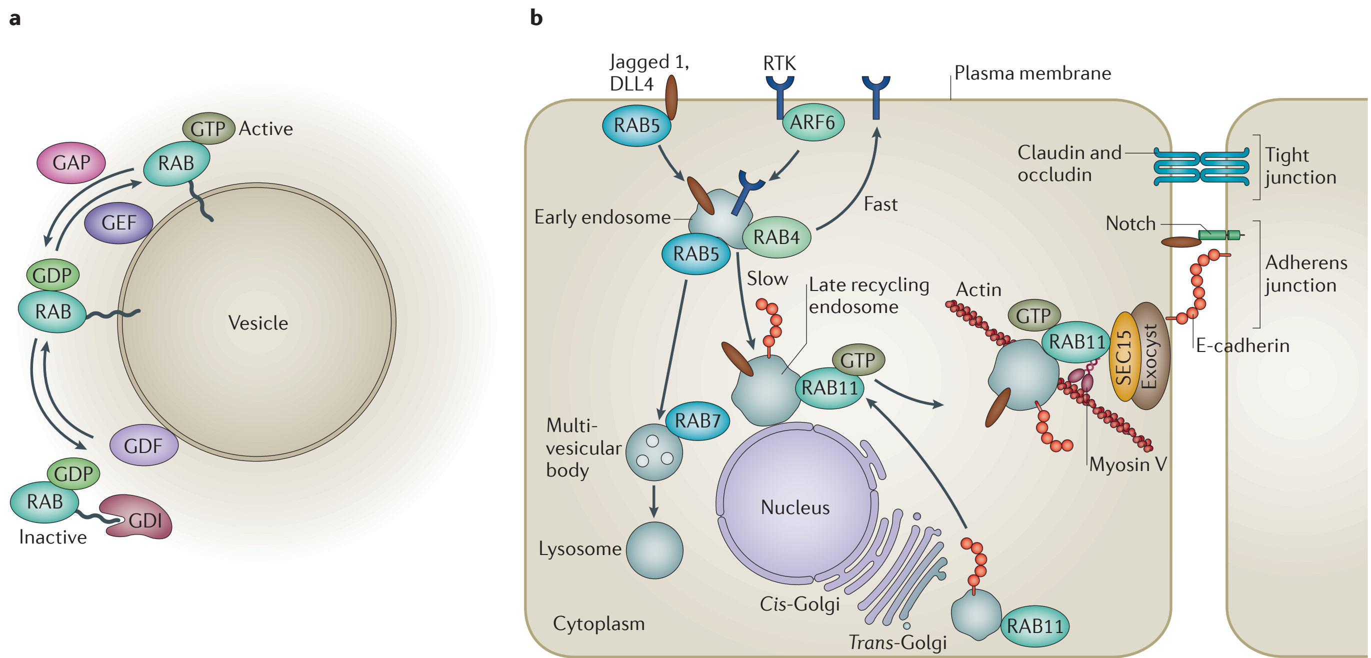

Figure 1 | **RAB-mediated endocytic recycling.** **a** | The figure shows RAB regulatory cycles. RAB GTPases are regulated by two coupled cycles: guanine nucleotide exchange factors (GEFs) exchange GDP for GTP to generate the active RAB•GTP form, whereas GTPase activating proteins (GAPs) generate the inactive RAB•GDP form. GEFs and GAPs can be highly selective for specific RABs. RABs are also regulated by extraction from vesicle membranes by cytoplasmic guanine nucleotide dissociation inhibitory (GDI) proteins that bind non-selectively to RAB•GDP and sequester it in the cytoplasm. GDI proteins have a hydrophobic pocket that binds to the prenylated moiety of RAB proteins (indicated by the line), which is otherwise inserted into the lipid bilayer of a vesicle. Membrane-bound GDI displacement factors (GDFs) extract RAB•GDP from complexes with GDI proteins and re-deliver them to the membrane compartments. **b** | The figure shows RAB11-mediated junctional transport. Retrieval of membrane proteins into early endosomes occurs via endocytosis, which is mediated by RAB5 or ARF6. Cargo from the early endosome can be recycled back to the cell surface by a fast (that is, within minutes) RAB4-mediated pathway and a slow (that is, within hours) RAB11-mediated process via late recycling endosomes. Early endosomes can also traffic cargo via RAB5 to the multivesicular body (MVB) and then via RAB7 to the lysosome. Late recycling endosomes can also acquire cargo (such as cadherins) via RAB11 from the Golgi complex. RAB11-positive late recycling endosomes are delivered to the cell surface along actin filaments via myosin V motors, where these vesicles are tethered to the plasma membrane by RAB11 binding to the exocyst component SEC15, which forms a tripartite docking complex. The late recycling endosomes are also decorated with vesicle SNARE (v-SNARE) and the SNARE component SNAP25 (not shown), which binds to the exocyst. Following docking, v-SNARE forms a trimeric complex with SNAP25 and the membrane-tethered t-SNARE to initiate fusion of the late recycling endosome with the plasma membrane and delivery of cargo. The Notch ligands DLL4 or Jagged 1, as well as epithelial cadherin (E-cadherin)–E-cadherin complexes, localize to adherens junctions as indicated. The tight junction proteins claudin and occludin are shown. RTK, receptor tyrosine kinase.

radial actin filaments by the motor protein myosin V (reviewed in REF. 2) and are then tethered at the plasma membrane by the binding of RAB11 and myosin V to the exocyst component SEC15 to form a tripartite docking complex. The exocyst is an octameric protein complex (which comprises SEC3, SEC5, SEC6, SEC8, SEC10, SEC15, EXO70 and EXO84) that interacts with SNARE complexes and subsequently mediates vesicle fusion and the delivery of cargo proteins to the plasma membrane.

Functioning in opposition to RAB11-mediated delivery of proteins to cell junctions, the small GTPase ARF6 promotes junctional disassembly in response to various acute inflammatory signals or vascular permeabilizing factors. In addition, ARF6 binds to the exocyst components SEC10 (REF. 12) and EXO70 (REF. 13), although the function of these direct links between ARF6 and the exocyst remain to be explored.

As epithelial and endothelial barriers provide a first line of defence against invasion by microbial pathogens, it is perhaps not surprising that several prominent pathogens secrete virulence factors that block RAB11-mediated trafficking to cell junctions. These activities compromise the integrity of membrane barriers and help pathogens to gain access to the host and/or cause tissue breakdown (for example, vascular leakage[14] and general tissue disintegration in anthrax infection and intestinal fluid loss in cholera infection[15]).

Certain pathogens also exploit or subvert RAB11-mediated trafficking; for example, *Salmonella enterica* subsp. *enterica* serovar Typhimurium uses the exocyst to recruit new membrane material to sites of bacterial attachment (known as invadosomes) to increase invasion efficiency[16], whereas some viruses, such as hantavirus, hijack recycling endosomes to bud or be released from

SNARE complexes
(Soluble NSF attachment protein receptor complexes). A family of related proteins that mediate the fusion of surface-bound vesicles with the plasma membrane (for example, SNAP25, a plasma membrane tethered SNARE and a vesicular SNARE form a trimeric complex to initiate membrane fusion).

Invadosomes
Subcellular structures that derive from the plasma membrane and mediate bacterial invasion of the host cell.

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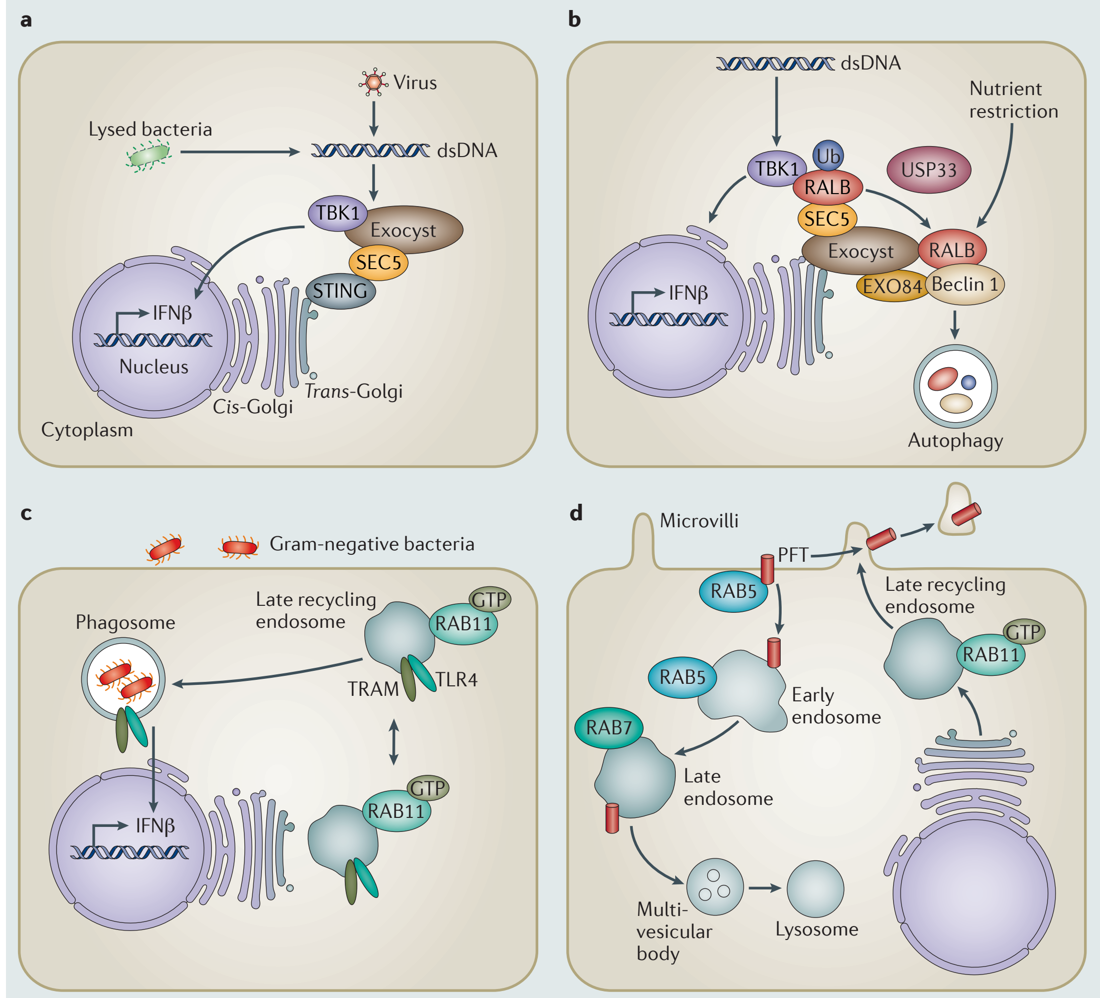
Box 1 | **Role of RAB11 and the exocyst in innate immunity**

The small GTPase RAB11 and the exocyst complex are involved in several aspects of innate immunity (see the figure). For example, the transmembrane protein STING (stimulator of interferon genes) senses foreign non-CpG double-stranded DNA (dsDNA), which is inadvertently liberated by pathogens, such as lysed bacteria or viruses[60] (see the figure, part **a**). In response to exogenous DNA, STING colocalizes with the exocyst component SEC5 and TANK-binding kinase-1 (TBK1)[10], which leads to the induction of interferon-β (IFNβ) gene expression[61]. The exocyst functions as a hub for the switch-like small GTPase RALB, which, depending on its ubiquitylation state, alternatively engages distinct exocyst components to trigger innate immune signalling (binding of SEC5 when ubiquitylated at lysine 47) or autophagy (when deubiquitylated by USP33, which triggers assembly of a RALB–EXO84–beclin 1 complex)[11] (see the figure, part **b**). The label 'exocyst' in parts **a** and **b** refers to exocyst components that are not specifically labelled. Similarly, in addition to contributing to the general process of phagocytic engulfment (reviewed in REF. 62), RAB11 is required for trafficking Toll-like receptor 4 (TLR4) from late recycling endosomes to phagosomes to initiate interferon signalling[8] (see the figure, part **c**) (note that a fraction of TLR4 in phagosomes derives from the plasma membrane in a RAB11-independent manner). Following binding to lipopolysaccharide on the surface of Gram-negative bacteria, TLR4 colocalizes with RAB11 in late recycling endosomes and translocates together with its transducer TRAM to the phagosome that is engulfing the pathogen, which leads to the induction of IFNβ expression. Last, RAB11-dependent trafficking has an important role in clearing pore-forming toxins (PFTs) from the cell surface and repair of the plasma membrane (see the figure, part **d**). For example, in *Caenorhabditis elegans*, RAB11 is essential against infection by bacteria that secrete PFTs, such as nemocidal *Bacillus thuringiensis* (which secretes Cry5 toxin) or *Vibrio cholerae* (which secretes *V. cholerae* cytolysin VCC). Endocytic recycling clears PFTs from the cell surface both by internalization — presumably followed by degradation in the lysosome by a RAB5- and RAB7-dependent process — and by RAB11-mediated expulsion of apical microvilli[9].



cells[17,18]. Other pathogens, including *Shigella flexneri*, disrupt the trafficking of cadherins and potentially of secreted factors such as antimicrobial peptides from the Golgi complex to recycling endosomes[19], whereas *Chlamydia* spp. exploit Golgi-derived membranes to create a specialized compartment that is suitable for their development[20].

In this Review, we first discuss the insights that have been gained into the pathogenic roles of anthrax toxin and cholera toxin in the inhibition of RAB11-dependent

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junctional trafficking. Next, we consider how several additional pathogens exploit RAB11-mediated trafficking to promote bacterial invasion and development. Last, we examine the subversion of exocyst-mediated trafficking as a viral exit strategy.

Inhibition of junctional transport

Bacillus anthracis and *Vibrio cholerae* have important places in the history of infectious disease. Robert Koch showed that *B. anthracis* is the causative agent of anthrax in 1876 (REF. 21), and he identified cholera toxin as a 'poisonous factor' that is produced by *V. cholerae* in 1884 (REF. 22). Louis Pasteur elucidated many aspects of anthrax pathogenesis and transmission[23] and ultimately developed a crude but effective vaccine[24] that ushered in the modern era of medical microbiology. Both of these pathogens secrete exotoxins that lead to the overproduction of intracellular cyclic AMP and thereby provoke the pathognomonic features of anthrax and cholera.

Anthrax toxins disrupt RAB11-mediated trafficking. Anthrax toxins have a bipartite structure in which a shared B subunit, known as protective antigen (PA), mediates the entry of the catalytic subunits, oedema factor (EF) and lethal factor (LF), into host cells (FIG. 2a). EF is a highly active calmodulin (CAM)-dependent adenylyl cyclase, and LF is a metalloproteinase that cleaves and inactivates host MEKs (reviewed in REFS 25,26). EF and LF are essential for the pathogenesis of *B. anthracis*, as highlighted in classic experiments by Smith and Keppie, which demonstrated that guinea pigs that were inoculated with *B. anthracis* were refractory to antibiotics after a crucial point during infection[81]. Importantly, they also showed that this lethality could be attributed to the accumulation of anthrax toxins, as immunization before infection with the anthrax toxin B subunit was protective, and this has also been shown to be effective in humans[82].

Anthrax toxins have several important roles during *B. anthracis* infection (reviewed in REF. 25). During the early, nearly asymptomatic prodromal stages of *B. anthracis* infection, toxins are essential for 'disarming' immune cells and promoting the transport of spores from the lungs to the lymph nodes. A recent study reported that mice that lack the key anthrax toxin receptor CMG2 in myeloid cells are resistant to infection by the attenuated non-capsulated Sterne strain of *B. anthracis*[27]. However, these mice remain fully sensitive to the lethality that is caused by the injection of oedema toxin (comprising EF and PA) or lethal toxin (comprising LF and PA), which indicates that the toxins exert their lethal effects on other cell types (see below). During the fulminant stages of *B. anthracis* infection, flu-like symptoms develop and the disease progresses rapidly, as the microbial toxins facilitate bacterial dissemination throughout the body and disrupt the function of almost every organ system. EF has a central role in causing oedema, hypotension and the loss of vascular endothelial barrier integrity, which can lead to shock-like death and which accounts for a substantial fraction of anthrax mortality in humans (reviewed in REF. 25). Anthrax toxins function cooperatively to induce lethality and oedema during infection of mice with *B. anthracis*[28]. Cell type-specific expression or deletion of the CMG2 receptor in mice showed that the cardiovascular system (that is, heart and smooth muscle cells) is the primary target of LF, whereas the primary target of EF is the liver[29]. The exact mechanisms that lead to the diverse effects of anthrax toxins that have been observed in different cell types remain elusive, and future studies are required to determine whether the inhibition of host endocytic recycling contributes to these various effects.

Although the structures and biochemical activities of anthrax toxins are well known, their cellular effects have been more difficult to elucidate. Expression of the enzymatic subunits of these toxins in the developing *Drosophila melanogaster* wing using the GAL4–UAS system[30], which bypasses the need for receptor-mediated toxin entry, yielded the expected effects of anthrax toxins (for example, growth inhibition by LF and cAMP-dependent activation of protein kinase A (PKA) by EF)[31]. In addition, these experiments revealed a novel cooperative activity of the two toxins in blocking Notch signalling[14]: both toxins reduce the cell surface expression of the Notch ligand Delta by inhibiting its endocytic recycling to adherens junctions[14], which is a process that is necessary for Notch receptor activation on adjacent cells[32]. In EF-expressing cells, the levels of RAB11 are decreased and endocytic recycling is inhibited, which suggests that EF targets RAB11 to block endocytic recycling (FIG. 2a). Consistent with this finding, a screen of dominant-negative forms of all 31 *D. melanogaster* RAB proteins showed that only expression of dominant-negative RAB11 mimics the effect of EF. Conversely, in flies and in human cells, overexpression of RAB11 rescues the junction-disrupting effects of EF[14]. Although LF does not reduce levels of RAB11, it does reduce the apical levels and function of the RAB11 mediator and binding partner SEC15 (REF. 14), which is an effect that cannot be rescued by overexpression of RAB11. EF also reduces apical SEC15 levels; however, in this case, the effect seems to be mediated indirectly by its reduction of RAB11 activity, as expression of dominant-negative RAB11 causes a similar reduction in SEC15 levels. Thus, EF functions by downregulating RAB11 levels and blocking vesicular transport (thereby also reducing apical SEC15 levels), whereas LF reduces apical levels of SEC15 in a RAB11-independent manner. Inhibition of endocytic recycling by either toxin also results in reduced levels of the cell–cell adhesion protein epithelial cadherin (E-cadherin) at adherens junctions. Similar effects of both EF and LF have been observed in human brain microvascular endothelial cells, in which the toxins block RAB11- and exocyst-mediated trafficking, reduce cadherin levels at junctions, inhibit Notch signalling and compromise barrier integrity (in transwell assays and *in vivo* in mice), with EF having a predominant role. Future experiments are required to determine the intersecting mechanisms by which EF, via increased cAMP, and LF, via inhibition of MEKs, disrupt RAB11- and exocyst-mediated junctional trafficking.

Notch signalling
A signalling pathway that controls a range of cell fate and growth decisions. It is activated at adherens junctions by cell surface-tethered ligands (for example, Delta) on one cell, which stimulate Notch receptors on adjacent cells.

Adherens junctions
Subapically localized cell–cell junctions that consist of transmembrane epithelial cadherin adhesion molecules, which interact with the cytoskeleton via α-catenins and β-catenins and link epithelial and endothelial cells, enabling them to form contiguous sheets.

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Cholera toxin disrupts intestinal barrier integrity. Cholera toxin causes the severe watery diarrhoea that is associated with cholera[33], and its mechanisms of action have been elucidated. Following translocation into the cytoplasm (which is mediated by the cholera toxin B subunit (CtxB)), the enzymatic moiety CtxA (which is an ADP ribosylase), binds to the host cofactor ARF6•GTP (which interestingly also functions in opposition to RAB11 to promote junction disassembly (FIG. 2b)). CtxA transfers ADP-ribose from NAD to the α-subunit of the stimulating G protein ($G_{s\alpha}$), which causes a pathological increase in cellular cAMP levels via the induction of host adenylyl cyclases at the plasma membrane (reviewed in REFS 33,34). Increased cAMP levels function via PKA to induce the ion channel cystic fibrosis transmembrane receptor (CFTR), which leads to Cl^- ion secretion into the intestinal lumen (reviewed in REF. 35). This transcellular secretion of Cl^- ions is accompanied by the paracellular efflux of Na^+ ions and water to preserve electroneutrality and osmotic balance, which results in a large volume of fluid loss (up to 10–20 litres per day). This model of CtxA action is supported by a range of experimental evidence, a definitive example being the failure of purified cholera toxin to induce fluid secretion in the small intestines of CFTR-null mice[36]. However, until recently, the mechanisms by which the paracellular efflux of Na^+ ions and water occurs had remained unclear.

As both CtxA and EF induce pathological levels of cAMP, the question arose whether CtxA might also disrupt cell junctions by inhibiting RAB11-mediated trafficking. Such an effect would facilitate the paracellular efflux of Na^+ ions and water that accompanies transcellular Cl^- secretion to generate the profuse diarrhoea that is caused by cholera infection. In agreement with this hypothesis, junctional levels of RAB11, SEC15 and E-cadherin are greatly reduced by treatment of human intestinal epithelial cell lines with CtxA[15] (FIG. 2b). CtxA also alters tight junctions, which disrupts their normal strict alignment with the more basal adherens junctions, and inhibits Notch signalling. Similar effects are also observed *in vivo* in mouse ligated ileal loop preparations, where CtxA induces fluid secretion, downregulates RAB11 and SEC15 levels and creates apical gaps between cells (FIG. 2c). In the fly gut, CtxA also causes dye leakage and large lacunal gaps between cells, which are visible at the ultrastructural level (FIG. 2c), as well as weight loss and lethality, all of which can be rescued by the overexpression of RAB11. Conversely, expression of a dominant-negative form of RAB11 mimics CtxA effects and causes the breakdown of intestinal barrier integrity, as judged by the ability of ingested dye to leak into the body cavity[15]. Junctional defects that are caused by CtxA in the fly gut and in human cells are remarkably similar to those that were previously reported in small-intestine biopsies from human patients with cholera[37] (FIG. 2c). As with purified toxin, the CtxA-dependent lethality and junctional defects that were observed following *V. cholerae* infection of adult flies can be mitigated by overexpressing RAB11 in the midgut (which is the fly equivalent of the small intestine). In flies and in human cells, evidence suggests that the effects of CtxA are mediated by a combination of the two primary known cAMP effectors, PKA and EPAC (a GEF for the small GTPase RAP1, which has several known links to the exocyst). Activation of either effector pathway by genetic means in flies or using pathway-specific cAMP analogues in mammalian cells also disrupts cell junctions, whereas chemical inhibition of either PKA or EPAC reduces the effects of CtxA[15]. These observations indicate that RAB11-dependent endocytic recycling helps to maintain normal intestinal barrier integrity and that boosting this process provides protection against the junctional disruptive effects of CtxA and infection with *V. cholerae*.

How pathological levels of cAMP, via PKA and EPAC, lead to the downregulation and inhibition of RAB11 in

Figure 2 | **The modes of action of anthrax toxins and cholera toxin. a** | *Bacillus anthracis* oedema toxin (which comprises oedema factor (EF) and protective antigen (PA)) and lethal toxin (which comprises lethal factor (LF) and PA) bind to the host receptors CMG2 or TEM8, which leads to their endocytic uptake and translocation to the cytoplasm. EF, which is a calmodulin-dependent adenylyl cyclase, remains perinuclear, where it produces cyclic AMP to activate protein kinase A (PKA) and EPAC–RAP1. The metalloproteinase LF disperses into the cytoplasm to cleave and inactivate MEKs. These pathways then reduce levels of RAB11 (for EF) on late recycling endosomes and of apical SEC15 (for EF and LF), which thereby blocks exocyst-mediated trafficking of junctional proteins (such as epithelial cadherins (E-cadherins) and Notch ligands Jagged 1 or DLL4)) to adherens junctions. The alignment of tight junctions with adherens junctions might also be altered. These effects of anthrax toxins on membrane junctions result in reduced endothelial barrier function and vascular leakage. **b** | The *Vibrio cholerae* toxin holoenzyme consists of the catalytic A subunit (CtxA) and five B subunits (CtxB). The toxin binds to the ganglioside GM1 on the plasma membrane, where it is endocytosed. CtxA is translocated into the cytoplasm, where it binds to ARF6 and activates host adenylyl cyclases (via the activation of G protein-coupled receptor (GPCR)) to increase cAMP levels and induce PKA and EPAC–RAP1. This results in the reduction of junctional levels of RAB11 and the exocyst component SEC15, which inhibits trafficking of cargo to adherens junctions and tight junctions. The junction-disrupting effects of CtxA may increase the paracellular efflux of Na^+ and water, thereby facilitating the transcellular secretion of Cl^- ions caused by PKA-dependent phosphorylation of the cystic fibrosis transmembrane receptor (CFTR). **c** | Cholera toxin produces apical gaps (arrows) and creates lacuna (asterisks) between intestinal epithelial cells and shortens microvilli in patients infected with *V. cholerae*, in cholera toxin-treated CACO2 cells and in the *Drosophila melanogaster* gut. Electron micrographs of human cholera biopsies in part **c** reprinted from *Gastroenterology*, **109**, Mathan, M. M., Chandy, G. & Mathan, V. I., Ultrastructural changes in the upper small intestinal mucosa in patients with cholera, 422–430, © (1995), with permission from Elsevier. Electron micrographs of human CACO2 cells and *Drosophila melanogaster* intestine in part **c** reprinted from *Cell Host Microbe*, **14**, Guichard, A. *et al*, Cholera toxin disrupts barrier function by inhibiting exocyst-mediated trafficking of host proteins to intestinal cell junctions, 481, © (2013), with permission from Elsevier.

Tight junctions
The most apically localized cell–cell junctions; they consist of adhesive claudins and occludin transmembrane proteins, which function as a diffusion barrier to ions, water and other small molecules.

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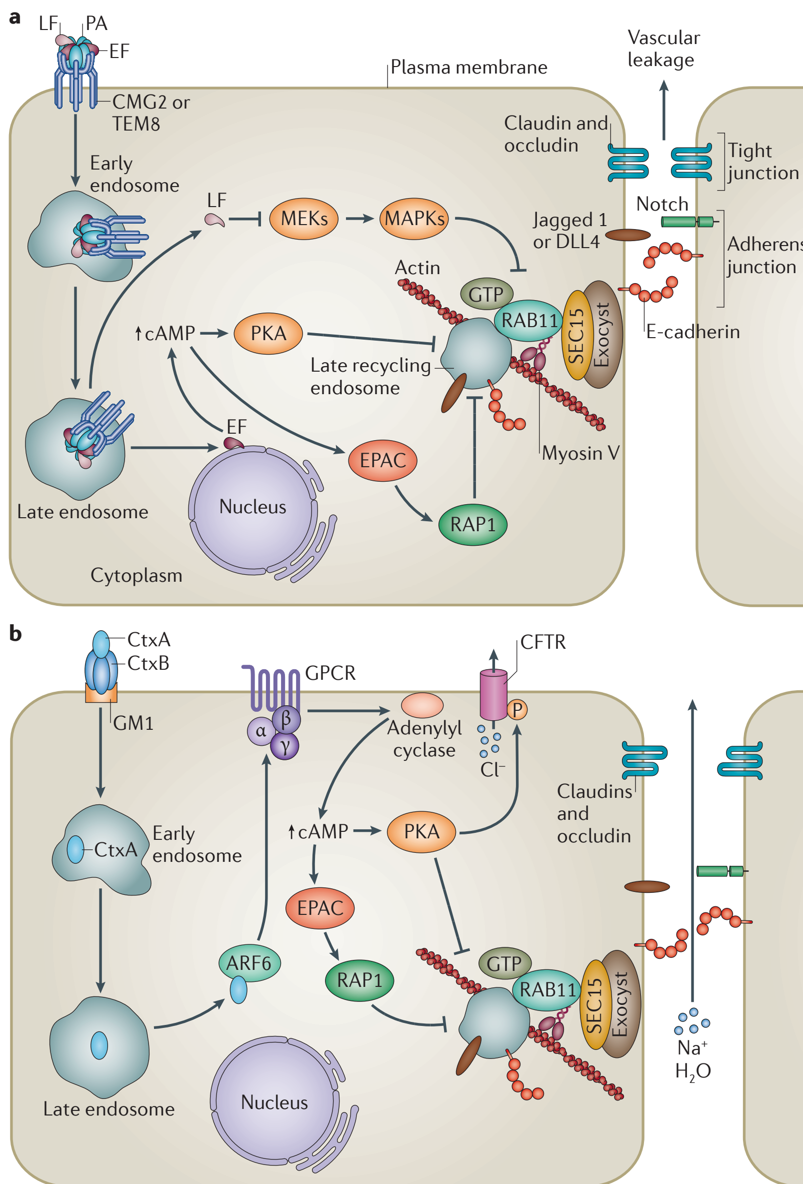

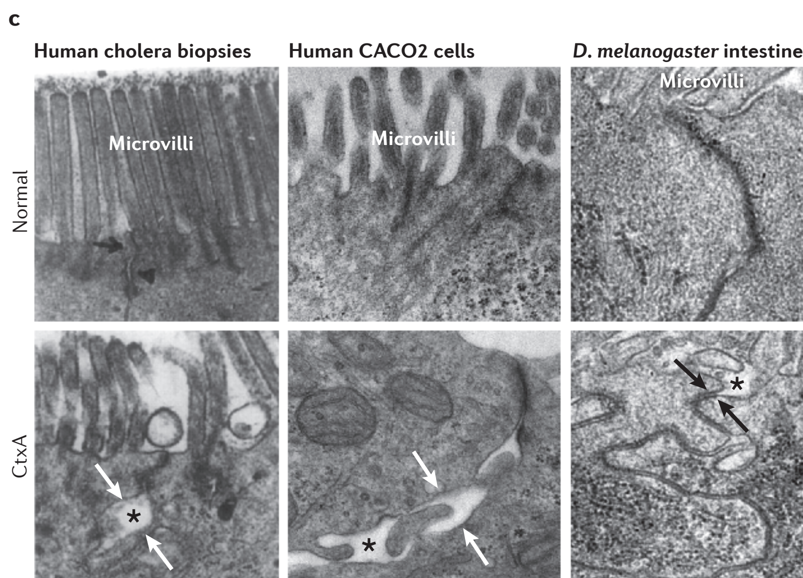

the vascular endothelium (that is, for EF) or gut (that is, for CtxA) is a central question for future research. It is conceivable that the observed decrease in RAB11 protein levels is caused by increased protein turnover (for example, proteasome-dependent degradation of RAB11), alterations in membrane compartment identities (for example, elimination of the late recycling endosome compartment), reduced production of proteins (for example, RAB11 mRNA instability or inhibition of RAB11 mRNA transcription) or a combination of these factors. Other pathogens liberating toxins that increase intracellular cAMP levels (cAMP toxins) (reviewed in REF. 38) may also possibly inhibit RAB11 activity to disrupt host cell barriers. Prominent examples of such pathogens include: toxigenic *Escherichia coli* (via heat-labile toxin (Ltx), which is highly related to CtxA, and heat-stable toxin (STa), which stimulates host guanylyl cyclases to indirectly increase cAMP levels and activates CFTR by cGMP-dependent protein kinase II; reviewed in REFS 39,40,48); *Bordetella pertussis* (via CyaA, which is a CAM-dependent adenylyl cyclase that is related to EF, and pertussis toxin (Ptx), which inhibits endogenous G_i subunits and thereby increases the activity of adenylyl cyclases); and *Pseudomonas aeruginosa* (via ExoY, which is an adenylyl cyclase). Finally, there are surprising parallels between the junction-disrupting actions of cAMP toxins and microbial PFTs that require further scrutiny (BOX 2).

Pathogens that exploit RAB11 trafficking

Various intracellular pathogens evade destruction following phagocytosis by subverting RAB-mediated trafficking events and reprogramming membrane compartment identities to create environments that support survival and replication within host cells (reviewed in REFS 41,42) (FIG. 3). In addition, several pathogens exploit or inhibit the endocytic recycling pathway to invade or escape host cells.

Commandeering the exocyst during bacterial invasion. *S.* Typhimurium is an intracellular pathogen that uses a type III secretion system (T3SS) to inject effector proteins into host cells. The tip of this injection complex comprises two proteins that penetrate the host cytoplasm. These proteins (SipB and SipC) not only participate in transferring virulence factors into the host cell but also interact with host factors, including the exocyst, to redirect resources to sites of bacterial invasion. SipC interacts with the plasma membrane-associated component EXO70 to recruit the exocyst complex to sites of *S.* Typhimurium attachment (FIG. 3). This might lead to the increased delivery of membrane proteins, which promotes membrane expansion and facilitates bacterial invasion[16]. At the same time, SopE and SopE2, which are two injected *S.* Typhimurium effectors, function as GEFs to activate the small GTPase RALA, which can bind in a mutually exclusive manner to either of the exocyst components SEC5 or EXO84. Recruitment of the exocyst and activated RALA is essential for bacterial entry, as RNAi of the exocyst components EXO70 or SEC5 or knockdown of RALA leads to reduced bacterial

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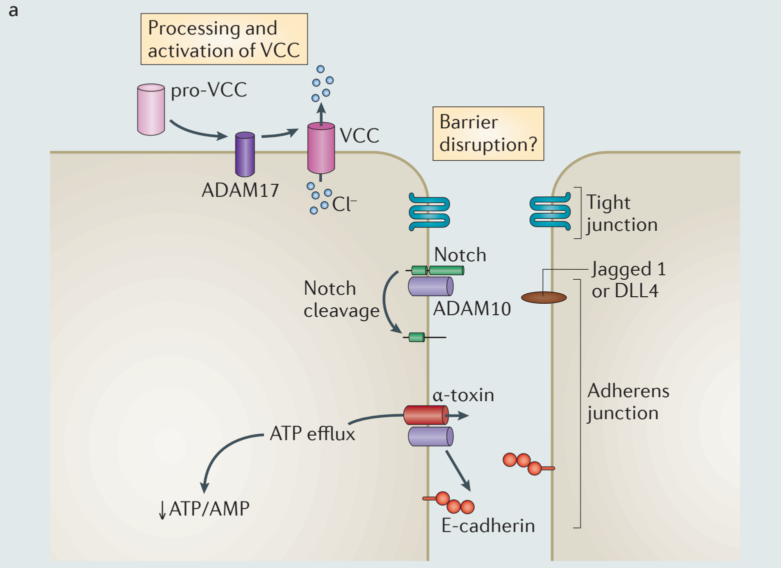
Box 2 | **Similarities between pore-forming toxins and cAMP toxins**

Many microbial pathogens can breach epithelial barriers during infection, and various virulence factors, including proteases and actin-modifying enzymes that aid in this process have been identified[63]. One particularly intriguing example is *Staphylococcus aureus* α-haemolysin (also known as α-toxin), which is a pore-forming toxin (PFT) that binds to the ADAM10 metalloproteinase as its host receptor[64] (see the figure). Binding of α-toxin activates the membrane-associated host metalloproteinase ADAM10 (REF. 64), which cleaves epithelial cadherin (E-cadherin) to disrupt junctional integrity[64–68]. ADAM10 also cleaves Notch receptors in mammals[69–72] (or its orthologue Kuzbanian in flies and nematode worms[73,74]) and thus we speculate that ADAM10-mediated Notch cleavage might have a role in the barrier-disruptive effects of α-toxin.

There are potentially important parallels between PFTs and toxins that increase intracellular cyclic AMP levels. For example, in the case of cholera, cholera toxin catalytic A subunit (CtxA) increases intracellular cAMP levels (cAMP toxins), which leads to Cl^- ion secretion via protein kinase A (PKA)-mediated activation of the cystic fibrosis transmembrane receptor (CFTR) ion transporter, whereas the PFT *Vibrio cholerae* cytolysin (VCC) is itself a Cl^- ion-conducting pore[75] that is processed and activated by the ADAM10-related protease ADAM17 (REF. 76) (see the figure). Also, as RAB11-dependent endocytic trafficking has a key role in eliminating PFTs from the cell surface (by endocytosis and expulsion of microvilli)[9], inhibition of this process by cAMP toxins may augment the effect of the PFTs. This strategy of expelling PFT-containing membrane has also been observed for *S. aureus* α-toxin[77], and the larger group A *Streptococcus* PFT streptolysin O[78] can similarly be shed via toxisome vesicles from several infected cell types.

Determining whether ADAM17, like ADAM10, cleaves E-cadherins or Notch receptors and exploring potential links between α-toxin, Notch cleavage and endocytic recycling will be interesting areas for further investigation. It may also be informative to study other potential parallels between α-toxin and cAMP toxins; for example, it is likely that both types of toxins reduce the cellular ATP/AMP ratio, as α-toxin results in ATP efflux through the pore, which is an effect that contributes to its toxicity[79], and high levels of cAMP production also deplete ATP reserves. α-toxin-mediated efflux of ATP (probably evoking host cellular responses via a reduction in the ATP/AMP ratio) is possibly mediated by effectors such as AMP-activated protein kinase (AMPK). Intriguingly, the anthrax toxin lethal factor (LF) also induces the depletion of ATP from macrophages (via a



uptake[16]. Association of RALA with the exocyst is important in this process, as the effect of RNAi-mediated depletion of SEC5 can be reversed by overexpressing wild-type SEC5 but not using a SEC5 point mutant that is defective in binding to RALA. SipC also binds to actin and remodels the apical cytoskeleton[43], which again provides a link to the exocyst, as this complex affects the architecture of the apical cytoskeletal. Thus, *S.* Typhimurium uses a coordinated set of effectors that induce localized membrane expansion and cytoskeleton remodelling to promote its engulfment by host cells.

Toxisome
A vesicle that contains toxic factors and that is expelled from the cell surface (for example, shed microvilli).

S. flexneri also uses the tip proteins (IpaB and IpaC) of a T3SS to penetrate into the host cytoplasm. One of these proteins (that is, IpaB) regulates lipid trafficking. IpaB redirects cholesterol to the site of bacterial entry[19] (FIG. 3) by altering the architecture and function of the recycling endosome, which causes tubulation of the RAB11-positive membrane compartment, although RAB11 is not the direct target of IpaB. Anterograde trafficking of E-cadherin via the Golgi complex and recycling endosome to cell junctions is greatly reduced by *S. flexneri* infection. This major disruption of the RAB11-positive compartment suggests that the trafficking of various secreted factors (for example, cytokines and antimicrobial peptides) and cell surface receptors that are required for defence may also be impeded during *S. flexneri* infection. IpaB binds to cholesterol and thereby depletes this lipid from the Golgi complex, which causes its fragmentation — an effect that can be reversed by co-treatment of cells with IpaB protein and exogenous cholesterol.

The intracellular pathogen *Chlamydia trachomatis*, which forms inclusion bodies in host cells, also leads to RAB11-dependent fragmentation of the Golgi complex, which is required for bacterial development and reproduction[20]. RNAi screening for host factors that are required for *C. trachomatis* replication identified RAB11 and RAB6 as essential elements, which is consistent with a previous study that reported the association of both GTPases with this bacterium during infection[44]. RAB11, which promotes anterograde trafficking, and RAB6, which promotes retrograde trafficking, are also essential for Golgi complex fragmentation during infection[20], as RNAi knockdown of either of these GTPases restores compact Golgi complex morphology and inhibits bacterial replication (FIG. 3). The importance of inducing Golgi complex fragmentation for *C. trachomatis* replication is further substantiated by the observation that RNAi of the resident Golgi protein p155, which alone causes fragmentation of this organelle, can overcome the rescuing effect of RNAi-mediated knockdown of RAB11 and restore the development of *C. trachomatis*. Interestingly, RNAi of another Golgi protein, golgin 84, which can also induce Golgi complex fragmentation, is unable to reverse either the restoration of Golgi complex structure or bacterial reproduction that is induced by RNAi-mediated knockdown of RAB11, which again links RAB11-mediated Golgi complex fragmentation and bacterial replication. These genetic epistasis experiments further suggest that RAB11 functions at a step following that of golgin 84 and before p155 in anterograde Golgi trafficking, which defines a pathway that needs to be investigated in further studies.

Subversion of vesicular trafficking by viruses. An important step in the viral life cycle is exit from the host cell. Some viruses assemble complete particles at the cell surface, whereas in other cases, particles are assembled intracellularly and then trafficked intact to the cell surface. As an example of a virus that traffics intact to the

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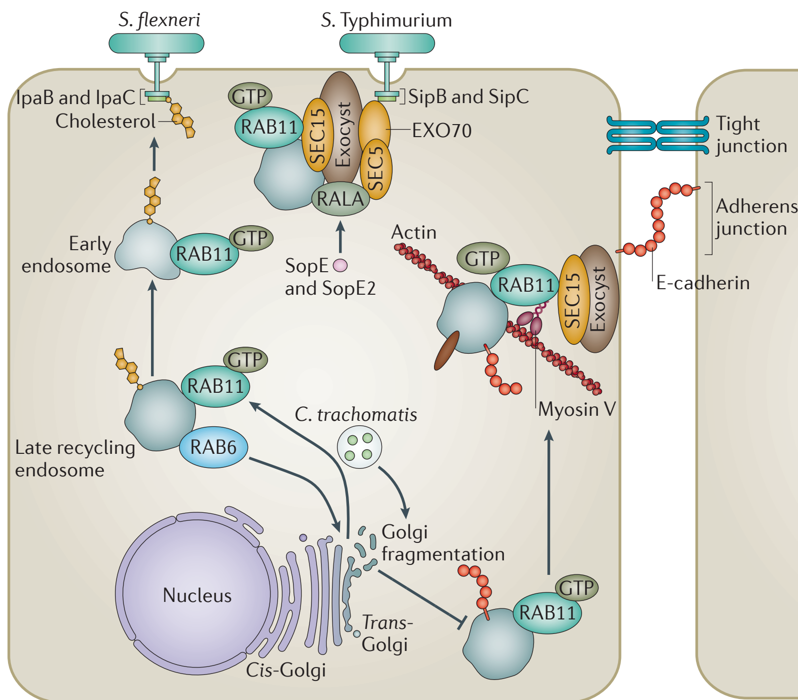

Figure 3 | **Pathogen subversion of RAB11 and exocyst functions.** The intracellular bacterial pathogen *Salmonella enterica* subsp. *enterica serovar* Typhimurium uses a type III secretion system (T3SS) to inject virulence factors into the host cell. The tips of the T3SS comprise two proteins that penetrate into the host cytoplasm, namely SipB and SipC. These proteins also interact with host proteins to manipulate membrane trafficking. SipC interacts with the exocyst component EXO70 to recruit membrane to sites of bacterial invasion (known as invadosomes). The *S.* Typhimurium T3SS also injects the effectors SopE and SopE2. These proteins are RALA guanine nucleotide exchange factors (GEFs) that promote an interaction between RALA and SEC5, which is essential for bacterial invasion. *Shigella flexneri* uses the T3SS proteins IpaB and IpaC to penetrate host cells. IpaB binds to and redirects cholesterol to the site of bacterial entry from the Golgi complex, which leads to Golgi complex fragmentation. Golgi complex fragmentation leads to tubulation of the RAB11 compartment and disruption of epithelial cadherin (E-cadherin) transport to adherens junctions and thus causes junctional disruption. *Chlamydia trachomatis* , which is another intracellular pathogen, also induces Golgi complex fragmentation via interactions with RAB11 and RAB6 to disrupt anterograde (that is, RAB11-mediated) and retrograde (that is, RAB6-mediated) transport between the Golgi complex and late recycling endosomes. This disrupted trafficking promotes *C. trachomatis* development and reproduction. Golgi complex fragmentation via RAB11 also requires the resident Golgi protein p155 (not shown).

cell surface, Andes virus (ANDV), which is a New World hantavirus, exploits RAB11-mediated trafficking for delivery to the apical cell surface (FIG. 4). This virus forms cytoplasmic inclusions that localize to both *trans*-Golgi and RAB8-positive and/or RAB11-positive vesicular compartments[18]. ANDV preferentially colocalizes with the inactive GDP-bound form of RAB11 (as well as both GDP- and GTP-bound forms of RAB8) in the perinuclear *trans*-Golgi compartment, where viral assembly is thought to take place. By contrast, active RAB11•GTP, which labels recycling endosomes en route to the cell surface, shows only partial overlap with ANDV. However, RAB8- and RAB11-mediated transport to the cell surface is essential for the egress of ANDV from cells, as inhibition of these RAB proteins resulted in more than a tenfold decrease in viral secretion under conditions in which viral entry was undisturbed.

Anterograde Golgi trafficking
A pathway via which vesicles that are derived from Golgi membranes are directed to the cell surface via anterograde trafficking. In addition, vesicles that are derived from the cell surface or other intracellular membrane compartments can be directed back to the Golgi via retrograde trafficking.

RAB11 is also essential for trafficking components of respiratory syncytial virus (RSV)[17], HIV-1 (REF. 45) and another retrovirus, Mason–Pfizer monkey virus[46], to the cell surface, where mature viral particles assemble; for example, RSV, which is a leading cause of lower respiratory tract illness in children and the elderly, co-opts the RAB11-dependent apical recycling machinery for exit from host cells[17] (FIG. 4). RSV release is strongly apically polarized, even when cultured cells are infected basally. Inhibition of apical endocytic recycling by the expression of dominant-negative forms of either the myosin V motor protein or the RAB11 family interacting protein (RAB11-FIP1) severely reduces RSV titres but does not affect propagation of either vaccinia virus or influenza virus. This disruption of viral replication does not alter the ability of RSV to infect cells but rather restricts its spread to other cells, presumably by preventing RAB11-dependent transport of viral components to the cell surface.

Conclusions and outlook

RAB11 and the exocyst have crucial host protective roles that contribute to several aspects of innate immunity, including the establishment and maintenance of endothelial and epithelial barrier integrity, trafficking of IFN signalling complexes to the phagosomes and the formation of scaffolds for building alternative protein complexes to promote innate immune signalling versus autophagy. However, these functions of RAB11- and exocyst-mediated trafficking are a 'double-edged sword', as they can be blocked or subverted by various pathogens.

The cAMP toxins anthrax EF and cholera CtxA (reviewed in REF. 38) are key virulence factors that disrupt RAB11- and exocyst-mediated trafficking of cargo, such as adhesion molecules and signalling complexes (for example, Notch ligands), to cell junctions. EF disrupts endothelial junctions, which leads to vascular leakage in mice, whereas CtxA disrupts intestinal epithelial barrier integrity and thereby potentially contributes to the massive fluid efflux that drives the severe life-threatening diarrhoea that is associated with cholera infection. Whether the reported barrier-disruptive effects that are caused by *P. aeruginosa* ExoY toxin[47] or enterotoxigenic *E. coli* Ltx and STa toxins (reviewed in REFS 39,40,48) also function by inhibiting the exocyst and whether cAMP production by CyaA and Ptx during *B. pertussis* infection[49] disrupt airway epithelial barrier function are important future questions to address. Similarly, the roles of cAMP-mediated inhibition of RAB11, which is potentially caused by the intracellular pathogen *Mycobacterium tuberculosis*, which secretes cAMP into the host cytoplasm[50], or by *Porphyromonas gingivalis*, which exploits host TLR2 signalling to induce endogenous cAMP production[51], require further investigation.

Although EF and CtxA can, on their own, recapitulate key pathologies of anthrax and cholera, respectively, these cAMP toxins are not the only potentially relevant virulence factors that are produced during infection. Indeed, in the case of anthrax, EF functions synergistically with the metalloproteinase LF to inhibit RAB11- and/or exocyst-mediated trafficking[14]. Similarly,

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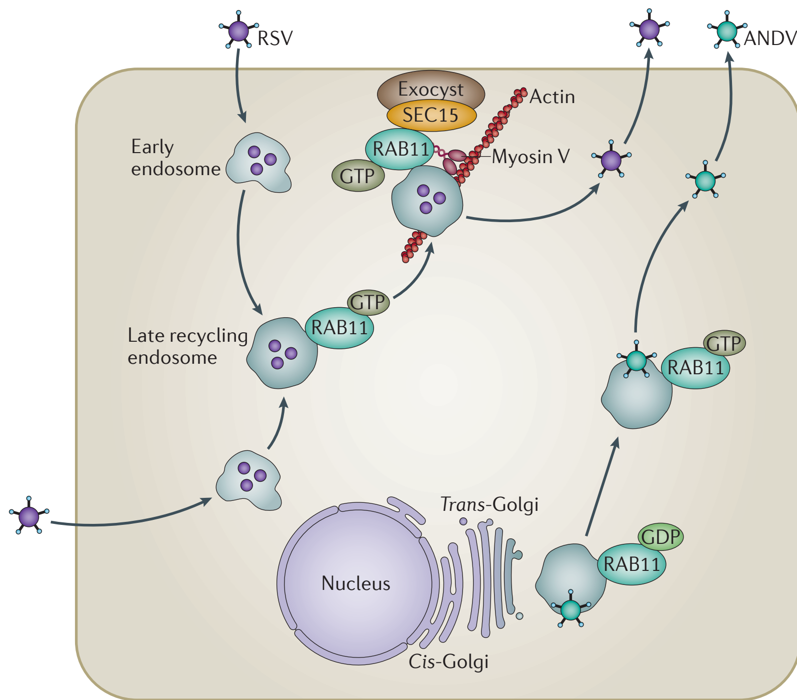

Figure 4 | **Viral exit via the recycling endosome.** Andes virus (ANDV), which is a New World hantavirus, forms cytoplasmic inclusions and undergoes assembly in the *trans*-Golgi (that is, RAB11•GDP-containing) and recycling endosomal (that is, RAB11•GTP-containing) compartments. Mature viral particles are then trafficked via the recycling pathway in a RAB11-dependent manner to the cell surface, where they are released. Respiratory syncytial virus (RSV) enters host cells either apically or basally and viral components are then transported to the apical cell surface via RAB11 and myosin V-dependent trafficking.

Yersinia pestis produces the adenylyl cyclase YpAC[38,52] and several factors that inhibit MEK signalling (for example, YopJ[53]). It remains to be determined whether synergy between these toxins contributes to the pathogenesis of plague. The cAMP toxins might also function collaboratively with other toxic factors, such as the PFTs of *B. anthracis* (that is, anthrolysin O) and *V. cholerae* (that is, *V. cholerae* cytolysin (VCC)), during infection. Finally, in the case of anthrax, as mitogen-activated protein kinase (MAPK) p38 (REF. 54) and TLR4 signalling[55] are key host defence responses to PFTs[54], their inhibition by LF and EF, respectively, may result in important neutralizing effects. Examination of these key nodes should provide fresh insights into cooperative interactions between virulence factors.

Another area for future investigation is whether other known features of disease that are caused by cAMP toxins may result from the inhibition of RAB11- and exocyst-mediated functions; for example, during the early and nearly asymptomatic phase of infection, anthrax toxins inhibit various aspects of the immune response, which enables the bacteria to proliferate and disseminate into the circulation (reviewed in REFS 56,57). As myeloid cells are crucial targets of anthrax toxins during this phase[27], it would be interesting to examine whether inhibition of RAB11 function by anthrax toxins alters cytokine secretion during the establishment of infection. Conversely, overexpression of RAB11 might mitigate aspects of toxin-mediated lethality. Similarly, as CtxA has been implicated in disrupting innate immune functions during the establishment of infection[58], how inhibition of RAB11- and exocyst-mediated functions contribute to this role (or roles) of CtxA should be studied. EF effects on the liver are also of interest, as a recent report found that hepatocytes are a key target of EF-mediated lethality in mice[29]. Experiments could probe how inhibition of RAB11- and exocyst-mediated trafficking by EF disrupts cell–cell junctions in bile duct cholangiocytes, whether it prevents hyperpolarization of hepatocytes to form canaliculi or whether this toxin impedes the secretion of key hepatic factors. If such effects are observed, a central question will be how they may contribute to the lethal effects of EF and whether selective overexpression of RAB11 in the liver can reverse these effects and restore viability. As liver pathology does not seem to be a notable feature of lethal anthrax cases in humans[59], one might ask whether more fine-scale analysis in mice can elucidate specific cellular phenotypes to examine more closely in human samples. Similarly, the cell type (or cell types) that is essential for generating oedema in response to EF (for example, lung oedema or footpad swelling) must be identified to examine whether such swelling can be counteracted by restoring endocytic recycling.

Another area for future study is to determine whether pathogens exploit or inhibit additional RAB11- and exocyst-mediated processes. Toxins that increase cAMP levels or other pathogenic factors may contribute to disease pathogenesis by inhibiting the establishment of apical–basal polarity (for example, in the liver), trafficking to cilia (for example, in the kidneys or in the regulation of vascular flow), cell migration (for example, of macrophages) or cell division (for example, of intestinal crypt cells). Pathogens may also manipulate the switch-like regulatory function of the exocyst in promoting the activation of innate immune signalling (for example, via ubiquitylated RALB–SEC5–TBK1 complexes) or autophagy (for example, via RALB–EXO84–beclin 1 complexes). Studies that probe these questions should lead to exciting discoveries and define new molecular targets for pharmacological intervention.

RALB
A small GTPase that can bind in a mutually exclusive manner to either of the exocyst components SEC5 or EXO84 to function as a molecular switch between immune signalling and autophagy.

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Competing interests statement
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