antibiotic-induced iNOS induction is already sufficient to foster *Enterobacteriaceae* overgrowth.

In summary, recent work has provided unprecedented insights into the complex mechanisms of infections by antibiotic-dependent pathogens (Ng et al., 2013; Spees et al., 2013). On the one hand, antibiotics insult the delicate crosstalk between the microbiota and the mucosal immune system, thereby leading to alteration of immune homeostasis, which ultimately increases susceptibility to pathogens. On the other hand, antibiotic-dependent disruption of microbial ecology generates free nutrient niches, which can be exploited by pathogens for infiltrating this complex and stable ecosystem to initiate disease. Given the increased thread of antibiotic-dependent pathogens in hospital settings, the work of Ng et al. (2013) provides an important basis for the future development of therapeutic intervention against these infections.

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Cholera Toxin Notches Epithelial Junctions

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Cholera toxin (CT) is the factor responsible for watery diarrhea associated with *Vibrio cholerae* infection. In this issue, Guichard et al. (2013) report that CT compromises intestinal epithelium barrier function via cyclic AMP (cAMP)-induced disruption of Rab11- and exocyst-dependent delivery of endocytic recycling cargo to cell-cell junctions.

Infections by *Vibrio cholerae* remain a global public health burden. Cholera toxin (CT) is the toxic factor responsible for the induction of a unique, profuse, watery diarrhea of typically 10 to 20 l a day in adult patients infected by toxigenic strains of *Vibrio cholerae*. CT is closely homologous to the heat-labile toxin produced by toxigenic strains of *Escherichia coli*, the causative agent of traveler's diarrhea. As with other enteropathogens triggering watery diarrhea, the mechanism by which water flows across and out of the epithelium is not fully understood. Now, Guichard et al. (2013) reveal that dysfunction of Rab11 and exocyst machinery, which is triggered by CT via forced induction of cyclic AMP (cAMP) signaling, compromises epithelium barrier integrity and contributes to efflux of water and solutes associated with *V. cholera* infection.

CT belongs to the AB_5 family of toxins. It is composed of an enzyme (A subunit, CtxA) associated with a pentameric crown of B polypeptides for binding to GM1 gangliosides at the surface of host cells. CT enters into cellular vesicles and undergoes a retrograde traffic to the endoplasmic reticulum, where the A subunit translocates into the cytosol. CtxA is a mono-ADP-ribosyltransferase that works in combination with the ADP-ribosylation factor 6 (ARF6) cellular cofactor. CtxA catalyzes the transfer of an ADP-ribose from nicotinamide adenine dinucleotide (NAD) onto a key arginine residue in the α-stimulating subunits of heterotrimeric G proteins (Gsα). This leads to the activation of cellular adenylate cyclases at the plasma membrane provoking a pathological rise of cAMP. cAMP is a broad signaling molecule that activates different cellular factors, such as the cAMP-dependent protein kinase A (PKA), thereby inducing the phosphorylation of several ion channels. Notably, this activates the conductance of the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride ion channel (Gabriel et al., 1994). CFTR drives the efflux of Cl^- ions and the resulting compensatory, and partly paracellular, secretion of Na^+ (Gabriel et al., 1994). The net secretion of sodium chloride (NaCl) generates an osmotic gradient that is compensated by water efflux. Both basolaterally located K^+ channels and an

ATP-dependent $Na^+/K^+/Cl^-$ cotransporter cooperate to establish the osmotic gradient.

The study of infectious diseases in genetically tractable model organisms, such as the fruit fly *Drosophila melanogaster*, is a valuable strategy to pinpoint major actors in host pathogen interactions. Flies infected with the bacterium *V. cholerae* display weight loss and increased mortality (Blow et al., 2005). By direct cytosolic expression of the catalytic subunit of cholera toxin in *Drosophila* cells in vivo, combined with genetic approaches, Guichard et al. (2013) reveal that activation of both Gsα and the *Drosophila* adenylate cyclase *rutabaga* triggers a phenotype of the wings reminiscent of the Notch mutant phenotype. Epistasis experiments demonstrate that CtxA acts downstream of the Notch ligand Delta (Dl) and upstream of the Notch receptor. Stimulation of Notch requires trafficking of Dl from the apical face of the epithelia to the basolateral side via the endocytic recycling compartments (EC) (Figure 1). Transfer of cargo from EC to the basolateral side is under the control of the small guanosine triphosphatase (GTPase) Rab11 and its effector Sec15, a component of the exocyst complex (Guichard et al., 2010; Heider and Munson, 2012). Guichard et al. (2013) reveal that CtxA induces a decrease of cellular levels of Rab11 and Sec15-GFP at the apical side of epithelial cells. This affects the Rab11-driven transfer of endocytic recycling cargo, thus restricting levels of adherens junction (AJ) molecules to the basolateral face of the epithelium. Moreover, the expression of CtxA in mature enterocytes of the midgut has a profound impact on the level and distribution of cadherin and beta-catenin at AJs, with major consequences on intercellular membrane apposition, as evidenced by transmission electron microscopy. By feeding the flies with a colored food dye and assessing dye diffusion in tissues, the authors clearly demonstrate that expression of CtxA or a dominant-negative form of Rab11 induces loss of epithelial barrier integrity. Overexpression of the wild-type Rab11 rescues CtxA-induced disruption of AJ integrity and epithelium barrier function. Moreover, the overexpression of Rab11 has a protective effect in a model of CtxA-driven *V. cholera* infection in flies.

The authors further examine the cytotoxic effects of CT on human intestinal epithelial cell monolayers. Intoxication results in a dramatic reduction of E-cadherin staining associated with its redistribution along the entire apical-basal axis, while the staining of the tight junction (TJ) molecules ZO1 and Claudin-2 remains restricted to apical regions. Nevertheless, the TJ adopts a convoluted organization with a loss of apposition with AJs. A few gaps in ZO1 staining at TJ are also evidenced. The use of pharmacological inhibitors and analogs implicates the cAMP effectors Epac and PKA in these cytotoxic effects. Overexpression of Rab11 restores cadherin signal at AJs and reduces the extent of intercellular gaps. Analysis of CT effects on a model of ligated murine ileal loop also reveals cell-cell disjunctions associated with an accumulation of albumin in the lumen.

This study thus ascribes to cholera toxin a new cytotoxic feature, namely the disruption of the intestinal epithelium barrier by targeting Rab11, a critical component of endocytic recycling compartments. This represents a major step forward in our understanding of the molecular basis for the massive water efflux induced by CT. An exciting challenge for future studies will be to establish the molecular link between the cAMP-driven Epac/PKA signaling axis and Rab11. Moreover, it will be important to decipher why the amplitude and duration of cAMP signaling induced by several toxins trigger a rupture of epithelial and endothelial barriers, whereas physiological stimuli rather tighten these barriers.

Interestingly, a series of recent findings points to the importance of Rab11 signaling in host pathogen interactions (Figure 1). The adenylate cyclase toxin (edema toxin) from *Bacillus anthracis* disrupts endothelial cell adherens junction cohesion by corrupting the Rab11 and exocyst machinery (Guichard et al., 2010). *Shigella flexneri* compromises the organization and trafficking of cargo through Rab11-dependent Golgi and endocytic recycling endosomes by corrupting cholesterol distribution (Mounier et al., 2012). As reported for CT, this phenomenon likely also contributes to disrupting the trafficking of epithelial cell AJ molecules. Rab11 is also instrumental for the assembly, trafficking, and budding of some enveloped viruses (Bruce et al., 2012). *Chlamydia trachomatis* usurps Rab11 signaling in order to fragment the Golgi apparatus and

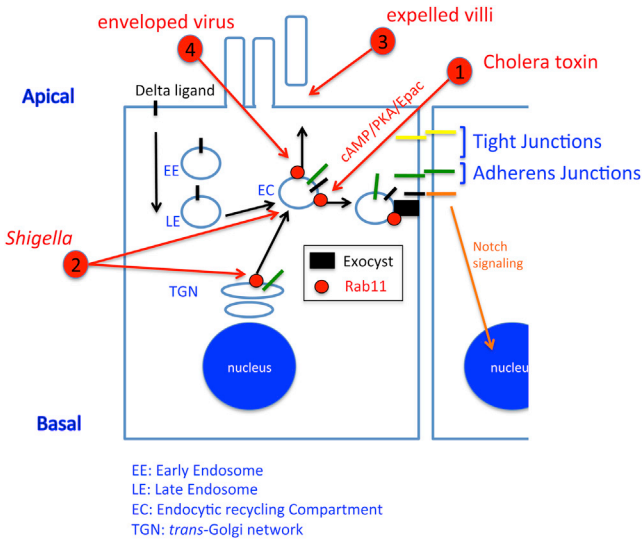

Figure 1. Central Role of Rab11-Driven Vesicular Trafficking in Pathogen Interactions with Intestinal Epithelium

Adherens junction (AJ) molecules (green), such as E-cadherin and beta-catenin, maintain epithelia cell-cell cohesion. In vertebrates, tight junction (TJ) molecules (yellow), such as claudin-2 and ZO1 (shown above AJs), limit the paracellular permeability. TJ molecules are also essential for the establishment of epithelial cell polarity, owing to their properties to restrict the apicobasal diffusion of molecules such as the Notch receptor ligand Delta. Rab11 drives the vesicular trafficking of cargo from endocytic recycling compartments (EC) and *trans*-Golgi network (TGN) to specific areas at the plasma membrane. (1) Guichard et al. (2013) show the impact of CT-driven forced induction of cAMP signaling on Rab11-driven Sec15-exocyst-dependent trafficking of E-cadherin at AJs. (2) *Shigella* triggers a tubulation of Rab11-positive compartment, also impairing E-cadherin targeting to plasma membrane. (3) The noxious effects of small pore-forming toxins are limited by Rab11-driven expulsion of microvilli lined with toxin molecules. (4) Rab11 is critical for the assembly, trafficking, and budding of negative-strand RNA viruses.

establish a replicative niche (Rejman Lipinski et al., 2009). Conversely, Rab11 actively limits the noxious permeabilization effect of small pore-forming toxins (Los et al., 2011). Cells achieve this notably by expelling microvilli by a Rab11-dependent mechanism, likely requiring vesicle fusion. Rab11 also controls the trafficking of the Toll-like receptor 4 (TLR4) from endocytic recycling compartments to phagosomes containing *Escherichia coli*. This allows a local stimulation of TLR4 together with interferon regulatory factor 3 (IRF3) for robust production of interferon-γ (Husebye et al., 2010). Collectively, these studies point to a critical function of Rab11 signaling in host pathogen interactions.

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Host Restriction Factor Screening: Let the Virus Do the Work

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In this issue of *Cell Host & Microbe*, Varble et al. (2013) engineer a library of RNA viruses to express small interfering RNAs and couple this with the power of virus evolution and selection to screen for host genes that when silenced resulted in greater viral infection in vivo.

Over the last five years, a variety of screening methods have been used to identify host factors that restrict viral infection. Some investigators have used genome-wide screening approaches, whereas others have targeted specific antiviral pathways, including the type I interferon (IFN) response. Experimentally, these screens have relied on short hairpin RNA-based gene silencing or ectopic gene expression largely in transformed cell culture models of virus infection. In gene silencing approaches, virus infectivity is enhanced when expression of restriction factors is diminished. In the context of treating cells with exogenous IFN, gene silencing can define the relative contribution of individual interferon-stimulated genes (ISGs) to the host antiviral responses. In ectopic expression screens, particularly of genes in the IFN signaling and effector pathway, host factors that are sufficient to protect cells from virus infection have been revealed.

Each screening method has strengths and limitations. In genome-wide siRNA screening approaches, an underlying assumption is that a restriction factor will be expressed basally at levels that are sufficient to control an incoming virus. The identification of the antiviral activity of IFITM gene family members is a successful example of this strategy (Brass et al., 2009). Other genome-wide siRNA screens have been performed in the context of IFN treatment and uncovered numerous putative host restriction factors, many of which regulate cellular antiviral responses even though they are not induced by IFN (Fusco et al., 2013; Zhao et al., 2012). Two screens that specifically targeted the IFN pathway by silencing a comprehensive panel of ISGs identified novel host factors that had direct effector functions or regulated IFN response pathways (Li et al., 2013; Metz et al., 2012). In addition to gene silencing strategies, ectopic expression screens also have identified ISGs that inhibit virus infection. When hundreds of ISGs were tested for their ability to suppress virus