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Mammalian membrane trafficking as seen through the lens of bacterial toxins

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Abstract

A fundamental question of eukaryotic cell biology is how membrane organelles are organised and interact with each other. Cell biologists address these questions by characterising the structural features of membrane compartments and the mechanisms that coordinate their exchange. To do so, they must rely on variety of cargo molecules and treatments that enable targeted perturbation, localisation, and labelling of specific compartments. In this context, bacterial toxins emerged in cell biology as paradigm shifting molecules that enabled scientists to not only study them from the side of bacterial infection but also from the side of the mammalian host. Their selectivity, potency, and versatility made them exquisite tools for uncovering much of our current understanding of membrane trafficking mechanisms. Here, we will follow the steps that lead toxins until their intracellular targets, highlighting how specific events helped us comprehend membrane trafficking and establish the fundamentals of various cellular organelles and processes. Bacterial toxins will continue to guide us in answering crucial questions in cellular biology while also acting as probes for new technologies and applications.

KEYWORDS
cell membrane, mechanism of action, toxins, trafficking

1 | INTRODUCTION

Bacteria and their hosts have co-evolved to produce a plethora of intricate host-pathogen interactions that have shaped their respective biological diversities (Masri et al., 2015). From such long-standing interactions, secreted bacterial exotoxins (hereby toxins) have emerged to be exquisitely precise and target very specific biological processes. For example, these toxins can target host functions to acquire nutrients, control host defences, and optimise their replicative niches.

The great advances that have been made in the characterisation of such toxins in the past 50 years have had a direct impact on infection biology but have also been instrumental in uncovering fundamental cellular mechanisms (Schiavo & van der Goot, 2001). These types of studies can be especially useful because toxins are generally extremely potent, that is, act at very low concentrations such that toxin action can be often monitored with a very clear and specific readout. Additionally, because toxins are secreted by bacteria, they can be purified and studied in isolation of the producing organism. Finally, their addition to the outside of cells allows for optimal temporal control.

As toxins are added outside but act inside cells, studying their modes of action has been particularly beneficial to understanding host cell membrane compartmentalization and vesicular trafficking. This review will follow the variety of routes undertaken by toxins to reach their cellular targets, highlighting the specific processes where toxins

have enriched our view of eukaryotic vesicular trafficking—from the cell-penetrating activities of toxins at the cell surface through to the endomembrane system and actions in the cytosol.

2 | TOXINS HIJACK HOST-CELL SURFACE MACHINERIES

Bacterial toxins can hijack diverse host-cell receptors: proteins, carbohydrates, lipids, or glycolipids. For example, anthrax toxin binds to two protein receptors, capillary morphogenesis gene 2 and tumour endothelial marker 8 (Bradley, Mogridge, Mourez, Collier, & Young, 2001; Scobie, Rainey, Bradley, & Young, 2003), cholera toxin targets monosialotetrahexosylganglioside (Fishman, 1982; Holmgren, Lönnroth, & Svennerholm, 1973), and Shiga toxin binds globotriaosylceramide (Lindberg et al., 1987; Sandvig, Olsnes, Brown, Petersen, & van Deurs, 1989). Even pore-forming toxins bind to diverse cell-surface molecules: Aerolysin binds the sugar moieties of glycophosphatidylinositol-anchored proteins (Abrami, Fivaz, Glauser, Parton, & van der Goot, 1998b) and cholesterol-dependent cytolysins, such as listeriolysin O or streptolysin O, target surface cholesterol (Duncan & Schlegel, 1975; Vazquez-Boland, Dominguez, Rodriguez-Ferri, Fernandez-Garayzabal, & Suarez, 1989). Because toxins are highly opportunistic molecules that make use of the cellular properties of their receptors, the study of the toxin-receptor behaviour provides highly relevant cellular information. For example, studies on the binding and internalisation of Shiga toxin have paved the way to the discovery of the galectin 3-mediated endocytic pathway (Lakshminarayan et al., 2014). Similarly, understanding how capillary morphogenesis gene 2 controls the level of collagen VI in the extracellular matrix was dictated by studies on anthrax toxin endocytosis (Bürgi et al., 2017).

Although bacterial toxins bind to extremely different receptors, a remarkable common feature is that these receptors tend to concentrate in specialised membrane domains. Because of this aspect, both cholera (Révész & Greaves, 1975) and tetanus (Montesano, Roth, Robert, & Orci, 1982) were instrumental in early observations that the cell surface is actually non-uniform, in contrast to the then proposed fluid mosaic model. These plasma membrane domains, often referred to as microdomains or lipid rafts, were found to be enriched in cholesterol, glycosphingolipids, and lipid-anchored proteins such as glycophosphatidylinositol-anchored proteins (Brown & Rose, 1992; Simons & Ikonen, 1997). Many other toxins, including Shiga toxin (Falguières et al., 2001; Kovbasnjuk, Edidin, & Donowitz, 2001) and pore-forming toxins (Abrami et al., 1998b; Waheed et al., 2001), were also shown to preferentially attach to microdomains, highlighting a shared mechanism for cellular activity/entry. Decades later, toxins continue to be exploited to probe for membrane compartmentalization (Dumitru et al., 2018; Maekawa, Yang, & Fairn, 2016; Russo et al., 2018). Their binding domains, conjugates, or derivatives have been used to not only monitor lipid distribution and trafficking but as probes that directly reorganise surface lipids, therefore driving the formation of membrane domains.

Compartmentalization in the two-dimensional membrane environment enables the local concentration of molecules. For toxins, this has several important consequences. One is that receptor clustering affects ligand binding avidity, thus allowing binding at lower toxin concentrations and also potentially facilitating downstream signalling (Bray, Levin, & Morton-Firth, 1998). This is particularly relevant for multivalent toxins, such as cholera and Shiga toxins whose receptor-binding subunits are pentameric (Chinnapen, Chinnapen, Saslowsky, & Lencer, 2007; Šachl et al., 2015). Other toxins are monomeric upon binding, but require oligomerization for their activity, as is the case for many pore-forming toxins and anthrax toxin (Abrami, Fivaz, & van der Goot, 2000; Abrami, Liu, Cosson, Leppla, & van der Goot, 2003). This oligomerization process will be favoured by receptor concentration, allowing the toxin to be active at lower overall concentrations (Abrami & van der Goot, 1999). In contrast, to satisfy the multivalency of the toxin or oligomerization process, toxins may also trigger the recruitment of additional receptors leading to increased, or even de novo, formation of membrane domains (Chinnapen et al., 2007; Šachl et al., 2015). This, in turn, may favour signalling events that are unrelated to the activity of the toxin itself, but merely triggered due to the toxin-induced receptor clustering, as shown for listeriolysin O (Gekara, Jacobs, Chakraborty, & Weiss, 2005). Receptor clustering is not a particularity of toxins and is now well established for eukaryotic cellular signalling (Alonso & Millán, 2001; Varshney, Yadav, & Saini, 2016). For example, after epidermal growth factor binds to its receptor, epidermal growth factor receptor, clustering allows distinct signalling entities at the plasma membrane (Needham et al., 2016).

Besides clustering within lipid microdomains, several toxins are activated by a limited proteolysis step at the cell surface. Studies on this activation were crucial in the discovery that membrane-anchored furin family members can be present and active at the cell surface of mammalian cells (Abrami et al., 1998a; Gordon, Klimpel, Arora, Henderson, & Leppla, 1995; Klimpel, Molloy, Thomas, & Leppla, 1992). These proprotein convertases were originally proposed to be located and active in the Golgi (Bresnahan et al., 1990), though the latest evidence using non-toxin-derived biosensors demonstrates that some proprotein convertases are active in non-Golgi compartments (Ginefra, Filippi, Donovan, Bessonnard, & Constam, 2018). A recent study on anthrax and aerolysin toxins demonstrated that not only should furin be present at the cell surface to process these toxins but the toxin-protease encounter needs to be forced through their colocalization in surface microdomains (Sergeeva & van der Goot, 2019). Beyond the intrinsic properties of most toxin receptors to be found in lipid microdomains, targeting furin to these locations was found to depend on palmitoylation of its cytosolic domain (Sergeeva & van der Goot, 2019). These toxin studies highlight how cells can ensure that interactions between low abundant molecules can occur in a highly efficient manner.

As demonstrated above, the general cell-surface dynamics of toxins involve binding to specific receptors that reside in or subsequently associate with membrane domains leading to some form of clustering, wherein cleavage by proprotein convertases can either contribute to or result from clustering. These steps have helped cell

biologists understand host-cell plasma membrane compartmentalization, activation, and receptor signalling, and the study of toxins is likely to continue revealing lipid-domain properties and receptor dynamics.

3 | TOXINS EXPLOIT HOST-CELL ENDOCYTIC AND RETROGRADE TRAFFICKING PATHWAYS

Apart from pore-forming toxins, most toxins require access to the cytosol to reach their targets. Their translocation mechanisms, however, are quite diverse and can occur in different cellular compartments. Rapid translocation upon toxin binding at the plasma membrane occurs for the Bordetella adenylyl cyclase toxin (Ladant & Ullmann, 1999), which utilises its intrinsic phospholipase activity to form proteolipid membrane pores that allow crossing of its large adenylyl cyclase domain (González-Bullón, Uribe, Martín, & Ostolaza, 2017). So far, all other toxins have been shown to enter the cell by endocytosis and only subsequently crossing some membrane of the endomembrane system.

Toxins such as botulinum and tetanus neurotoxins cross the endosomal membrane. They respond to endosome acidification by inserting a pore into the membrane of the early endosome, mediating the direct passage of active subunits (Pirazzini et al., 2016). pH-dependent translocation can also occur later in the endocytic pathway within multivesicular bodies. This is the case for the receptor-binding subunit of anthrax toxin, which translocates its enzymatic subunit, lethal factor, from the lumen of endosomes into the lumen of intraluminal vesicles (ILVs; Collier & Young, 2003; Friebe, van der Goot, & Bürgi, 2016). Access to the cytosol subsequently occurs by back fusion of ILVs with the limiting membrane of the endosome (Abrami, Lindsay, Parton, Leppla, & van der Goot, 2004). This trafficking route demonstrated that cargo within ILVs could remain in multivesicular endosomes without being delivered to lysosomes and could be stochastically released from ILVs to the cytosol (Luzio, Gray, & Bright, 2010; Stahl & Barbieri, 2002). Anthrax toxin was also shown to be released extracellularly through lethal factor-loaded exosomes upon fusion of multivesicular bodies with the plasma membrane (Abrami et al., 2013). These exosomes can then be taken up by neighbouring cells, leading to their intoxication (Abrami et al., 2013). Due to its unique ability to translocate its enzymatic subunit into the lumen of ILVs, anthrax toxin provides a unique tool for investigating how certain cargo within ILVs may escape lysosomal degradation and for following exosome biogenesis and uptake. These mechanisms are particularly relevant to viral cell biology given that multiple viruses have been also shown to utilise ILVs to traffic throughout the cell and spread to uninfected neighbouring cells (Nour & Modis, 2014).

Although the aforementioned toxins carry a subunit or domain with membrane translocation properties, not all toxins do. They instead rely on cellular translocation mechanisms and must travel from endosomes through the Golgi to the ER, where they can exploit the retro-translocation machinery. Early studies employing ricin (a plant toxin), cholera, and Shiga toxins, yielded the first clues of the existence of a retrograde pathway from the plasma membrane to the Golgi apparatus and then to the ER, which could be hijacked, but was not generated, by toxins (Moya, Dautry-Varsat, Goud, Louvard, & Boquet, 1985; Sandvig et al., 1989). Subsequent work helped characterise both clathrin-dependent and independent internalisation routes and demonstrated that toxin retrograde trafficking could occur through multiple pathways (Lauvrak, Torgersen, & Sandvig, 2004; Nichols et al., 2001; Sandvig & Brown, 1987). Trafficking occurs via early and recycling, Rab11-dependent, endosomes for Shiga and cholera toxins (Fuchs et al., 2007; Mallard et al., 1998), whereas Pseudomonas exotoxin A travels deeper in to the endocytic pathway through Rab9-dependent routes. At the trans-Golgi network (TGN), toxins also follow multiple routes to reach the ER. The classical route travelled by KDEL receptors involves transport through the cis-Golgi to the ER via COP-1 dependent trafficking (Spang, 2013). This is the path followed by exotoxin A, which contains the C-terminal KDEL ER-retrieval signal and binds the KDEL-receptor following exit from endocytic compartments and arrival to the TGN (Chaudhary, Jinno, FitzGerald, & Pastan, 1990). In contrast, Shiga and Shiga-like toxins do not harbour a KDEL sequence, which enabled the identification of COP-I-independent Golgi-to-ER trafficking (Girod et al., 1999). In both cases, transport depends on Rab6-mediated sorting and requires the integrity of the entire Golgi complex (Sandvig, Skotland, van Deurs, & Klokk, 2013). Cholera toxin, on the other hand, travels directly from the TGN to the ER, without crossing the entire Golgi apparatus. This helped uncover another unique retrograde pathway that does not require an intact Golgi apparatus (Feng et al., 2004) despite being dependent on its KDEL ER-retrieval signal (Fujinaga et al., 2003).

At the ER, toxins exploit ER-associated degradation pathways to translocate to the cytosol, which was actually suggested before the characterisation of the ERAD translocation machinery. Toxins were proposed to mimic misfolded proteins in the ER to reach the cytosol where they avoid subsequent ubiquitin-mediated targeting to the proteasome due to the low abundance of lysines in their sequences (Deeks et al., 2002; Hazes & Read, 1997). Since then, the ER translocation of toxins has helped to confirm the role of numerous ER chaperones and translocators in ER quality control mechanisms and has revealed the complex and flexible nature of ERAD degradation pathways (Morito & Nagata, 2015; Nowakowska-Gołacka, Sominka, Sowa-Rogozińska, & Słomińska-Wojewódzka, 2019).

Thanks to toxins, retrograde trafficking is now established as a combination of constitutive transport routes that deliver cargo (proteins and lipids) from early, recycling, or late endosomes back to the TGN, Golgi apparatus, or the ER. Physiological roles of this retrograde transport include the equilibration of membrane flow within the endomembrane system, retrieval of ER-resident proteins from the Golgi apparatus, receptor recycling, transport of proteins to the ER for retro translocation, and delivery of soluble extracellular antigens to the cytosol for antigen cross presentation (Bonifacino & Rojas, 2006; Johannes & Popoff, 2008). Thus, retrograde transport helps maintain cell homeostasis, coordinates signalling pathways, and controls antigen presentation (Johannes & Popoff, 2008; Spang, 2013).
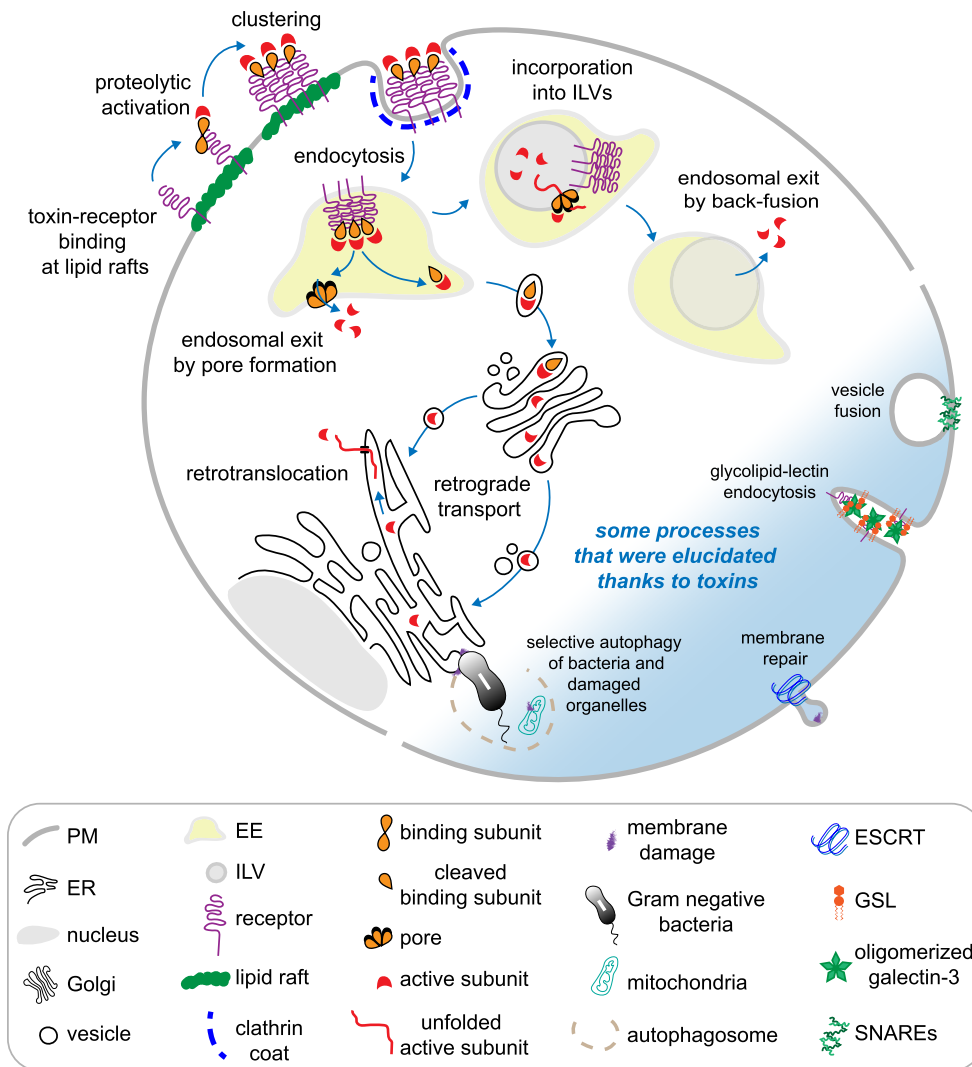
FIGURE 1 Trafficking routes influenced by bacterial toxins as shown for a hypothetical toxin with an active and binding subunit. The plasma membrane, endosome, and retrograde (Golgi to ER) pathways are depicted with their multiple mechanisms to access the cytosol. In addition, processes that were elucidated thanks to toxins are shown in the blue shading. EE, early endosome; ER, endoplasmic reticulum; ESCRT, endosomal sorting complexes required for transport; GSL, glycosphingolipids; ILV, intraluminal vesicle; PM, plasma membrane; SNAREs, soluble NSF-attachment protein receptors (We thank Giorgia Brambilla Pisoni for her illustration)

Additionally, toxin studies enabled the identification of various small molecule inhibitors and contributed to the characterisation of the molecular determinants that regulate sorting from different cellular membrane compartments, including clathrin adaptors, the retromer complex, Rab GTPases, and molecular motors (Bonifacino & Rojas, 2006; Johannes & Popoff, 2008).

4 | TOXINS HAMPER MEMBRANE TRAFFICKING MACHINERIES

Bacterial toxins have played an oft-forgotten though essential role in the understanding of vesicular trafficking. Indeed, the metalloprotease activity of tetanus and botulinum toxins against the vesicle-associated membrane protein (VAMP) synaptobravin-2 provided the first evidence that soluble NSF-attachment protein receptors (SNAREs) controlled vesicle fusion and neurotransmitters secretion from neuronal synapses (Schiavo et al., 1992). SNAREs are now recognised as key regulators of the sequential steps that lead to vesicle fusion during transport between different membrane compartments. The characterisation of key components of neurotransmitter release, such as VAMP/synaptobrevins, SNAP-25, and syntaxin 1, further helped cell biologists dissect SNARE function in neuronal exocytosis, trafficking, and survival (Lalli, Bohnert, Deinhardt, Verastegui, & Schiavo, 2003; Peng et al., 2013).

Similarly, lysosome secretion, visualised by the presence of lysosomal markers at the cell surface or the release of lysosomal enzymes, was first demonstrated to be induced by a rise in cytosolic calcium using both ionophores and plasma permeabilization by the pore-forming toxin streptolysin O (Rodríguez, Webster, Ortego, & Andrews, 1997). Subsequent studies using pore-forming toxins as triggers identified central regulators of lysosome exocytosis, including cytosolic calcium sensors (synaptotagmin VII), SNARE proteins (SNAP-23, syntaxin 4, VAMP7), and numerous effector and regulatory molecules (Andrews & Corrotte, 2018). Today, the exocytosis of lysosomal-related organelles has a recognised role in the repair of plasma membrane wounds and broader impact in various cell processes, such as polarity, immune granule secretion, and melanosome secretion (Andrews & Corrotte, 2018).

The damage to the plasma membrane caused by bacterial pore-forming toxins also enabled the understanding of the diversity of roles

of the endosomal sorting complexes required for transport (ESCRT) machinery, highlighting their involvement in membrane repair. The ESCRT machinery has established roles in the formation of vesicles that bud "out of the cytosol" such as ILV formation, viral budding at the cell surface, and cytokinesis. More recent studies using the pore-forming toxins, streptolysin O and listeriolysin O, and other sources of plasma membrane damage found that ESCRT complexes mediated budding of pore containing membrane patches and membrane remodelling of plasma membrane wounds (Jimenez et al., 2014). These findings paved the way for subsequent discoveries that the ESCRT machinery is a central mediator of nuclear envelope and organelle repair (Raab et al., 2016; Skowyra, Schlesinger, Naismith, & Hanson, 2018).

Membrane damage can also be caused by the translocon pore-forming proteins at the tip of Type III secretion systems of gram-negative bacteria. Groundwork on membrane damage caused by internalised bacteria was critical in identifying cytosolic membrane-damaging sensors (p62, NDP52, and optineurin) that promote selective autophagy of bacteria released into the cytosol as well as damaged organelles (Birmingham, Smith, Bakowski, Yoshimori, & Brumell, 2006; Thurston, Ryzhakov, Bloor, von Muhlinen, & Randow, 2009). Overall, these processes have not only showed us the clever ways toxins can harm host cells but the intricacies of mammalian cell trafficking.

5 | FUTURE PERSPECTIVES

Bacterial toxins have been truly exceptional tools for revealing the complexities of host cell mechanisms, and in this perspective, we have provided an overview of how they have improved our view of eukaryotic membrane trafficking. The selected examples underscore the detailed mechanistic insights and the broad conceptual changes that their study has made to cell biology (Figure 1). Toxins have also equally impacted other cell biology systems/processes, such as the eukaryotic cytoskeleton, cell cycle, and post-translational modifications of proteins. The story is not over, however—it is clear that we still have much to learn from the finely tuned, highly efficient (unmatched!) manner by which toxins make their way into cells. Also, the choice of their targets and the way they modify them is amazingly optimised and, therefore, every new mode of action of a bacterial toxin has also brought novel findings about cellular function and homeostasis. For example, what will we learn when we understand how toxins without a pore-forming domain make it from the endosome to cytosol (Alami, Taupiac, Reggio, Bienvenüe, & Beaumelle, 1998; Beaumelle, Alami, & Hopkins, 1993; Garcia-Castillo et al., 2015)? What is the relevance of multiple phenotypes targeted by pore-forming toxins, namely, how do pore-forming toxins lead to the fission or vacuolation of the ER (Brito, Cabanes, Sarmento Mesquita, & Sousa, 2019; Gonzalez et al., 2018; Mesquita et al., 2017)? Or how further can we exploit toxins to screen for regulators of organelle function and biosynthetic pathways (Tian et al., 2018)? Combined with model organisms, tailored pharmacological treatments, and emerging fine-tuned genetic manipulations, bacterial toxins will continue to reveal clever strategies that help us to dissect and identify fundamental, and exceedingly precise, properties of membrane trafficking.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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