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Intracellular trafficking of bacterial toxins

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Bacterial toxins often translocate across a cellular membrane to gain access into the host cytosol, modifying cellular components in order to exert their toxic effects. To accomplish this feat, these toxins traffic to a membrane penetration site where they undergo conformational changes essential to eject the toxin's catalytic subunit into the cytosol. In this brief review, we highlight recent findings that elucidate both the trafficking pathways and membrane translocation mechanisms of toxins that cross the plasma, endosomal, or endoplasmic reticulum (ER) membrane. These findings not only illuminate the specific nature of the host–toxin interactions during entry, but should also provide additional therapeutic strategies to prevent or alleviate the bacterial toxin-induced diseases.

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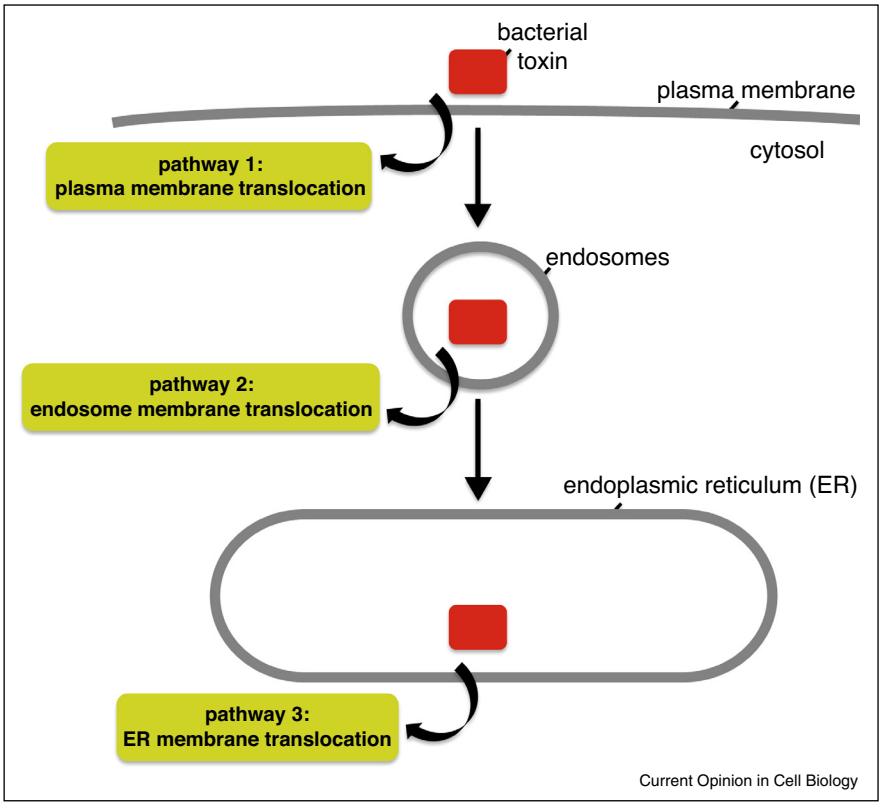
Introduction

To cause disease, many bacterial toxins must reach the cytosol of the target cell where they modify the activities of host components, leading to changes in cellular physiology that ultimately promote bacterial pathogenesis [1,2]. To do so, the toxins first bind to receptor(s) on the surface of the host cell. These engagements can initiate toxin translocation across the plasma membrane, enabling the toxin to gain access into the cytosol (Figure 1, pathway 1). Alternatively, the toxin–receptor interaction can stimulate endocytosis, bringing the toxin into endosomal compartments. Some toxins subsequently breach the endosomal membrane in order to reach the cytosol (Figure 1, pathway 2). Others traffic further along the retrograde pathway and are directed to the endoplasmic reticulum (ER). Here, translocation across the ER membrane allows the toxin to arrive into the cytosol (Figure 1, pathway 3). How a toxin selects a specific membrane translocation pathway depends partly on the nature of the toxin and the availability of host components that support all cellular events leading to cytosol translocation. In recent years, seminal reports have provided insights into the trafficking pathways and membrane translocation mechanisms of certain bacterial toxins that use the plasma, endosomal, or ER membrane for membrane penetration. In this review, our objective is to focus on one toxin for each membrane penetration site — these examples underscore the exquisite demand required to deliver an extracellular bacterial toxin into the interior of a host.

Translocation across the plasma membrane

The best case of a bacterial toxin that translocates across the plasma membrane is seen in the adenylate cyclase toxin (CyaA) (Figure 2a) secreted by *Bordetella pertussis*, the causative agent for pertussis or whooping cough [3]. Infection by this bacterium often compromises the host immune system. Structurally, CyaA contains an N-terminal adenylate cyclase (AC) domain followed by a C-terminal hydrophobic hemolysin domain important for pore formation and receptor-binding [4]. To cause disease, CyaA binds to the β2 integrin complement receptor (CR3) on the cell surface of phagocytes [5]. Although this step is not absolutely required for toxin entry, it enhances the process. Part of the hemolysin domain is then inserted into the plasma membrane, enabling the AC domain to subsequently cross the plasma membrane and reach the cytosol [6]. How the hemolysin domain assists in membrane translocation of AC is not well-understood, although there is evidence that an α-helical peptide located at the N-terminus of the hemolysin domain possesses a membrane-destabilizing activity [7] — this activity has been proposed to locally disrupt membrane bilayer integrity that in turn promotes AC translocation across the plasma membrane. Importantly, while cellular components involved in supporting this translocation event remained largely unclear [8–10], a recent report using an elegant *in vitro* translocation system was able to address this question [11••]. In this study, AC translocation across an artificial lipid bilayer (designed to mimic the plasma membrane) was clearly demonstrated to be dependent only on the presence of calcium ions and a negative membrane potential, without requiring additional host factors [11••]. Although calmoduin (CaM) was placed in the *trans* side of this *in vitro* membrane translocation system, it likely stimulates AC's catalytic activity [12] without necessarily playing any role in driving AC's membrane translocation [13]. Such an *in vitro* approach offers the advantage of evaluating a membrane translocation event under a highly controlled condition, a

Figure 1



Intracellular transport of bacterial toxins. Bacterial toxins can enter host cells by at least three different pathways. The toxins can bind to the host cell surface, initiating translocation across the plasma membrane to reach the cytosol (pathway 1). Alternatively, the toxins can undergo endocytosis, targeting to endosomal compartments where they penetrate the endosomal membrane to reach the cytosol (pathway 2). Other toxins traffic further along the retrograde pathway to reach the ER — here they translocate across the ER membrane and arrive in the cytosol (pathway 3).

strategy that should be used to further evaluate membrane translocation of other bacterial toxins.

Upon reaching the cytosol, the AC domain converts cytosolic ATP to the crucial second messenger cyclic AMP (cAMP) [14]. cAMP in turn stimulates a signaling cascade that disrupts the bactericidal functions of the phagocytes, and impairs other host cell innate immune responses against the bacteria [15]. Curiously, when a natural ligand binds to CR3, this interaction normally stimulates signal transduction events that lead to cellular responses important for innate immune defense [16], including CR3-dependent phagocytosis of bacteria [17]. How then does binding of CyaA to CR3 avoid activating this normal cascade? A partial clue to this enigma was revealed in a new study demonstrating that CyaA binds to an atypical binding site in the so-called 'bent and closed' CR3 conformation, in contrast to natural ligands that typically interact within a well-defined canonical binding site in the 'extended and open' CR3 conformation [18••]. Although this atypical binding still allows translocation of CyaA's AC domain across the plasma membrane, it fails to stimulate the normal ligand-activated CR3 signaling pathway. In fact, the AC-generated rise in cAMP can counter the usual CR3-dependent signaling induced by a natural ligand. In this manner, CyaA binding to its host receptor is thought to thwart the ability of a host to properly mount an immune response. This example emphasizes how subtle differences in receptor engagement can lead to profound differences in cellular physiology.

Translocation across the endosomal membrane

By contrast to CyaA, other toxins undergo receptor-mediated endocytosis to reach endosomal compartments where they translocate across the endosomal membrane to access the cytosol. In addition to anthrax and tetanus toxins that use this entry pathway to reach the cytosol [19], another well-characterized toxin that also uses this route is the botulinum neurotoxin (BoNT) (Figure 2b). BoNTs are secreted by various *Clostridia* species and are classified into seven serotypes (A–G) [20]. These toxins cause severe flaccid paralysis of muscles observed in