MICROBIOLOGY

Translocation of Anthrax Toxin: Lord of the Rings

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Eukaryotic cells are endowed with exquisite machineries for moving proteins across their lipid membranes and out of the cell. But just as every coin has two sides, microorganisms have evolved equally ingenious machineries that allow them to ship toxic proteins into target cells. Although these machineries seem to have evolved largely independently of one another, they have converged onto a common basic principle: to thread proteins across membranes as linear, unfolded polypeptide chains rather than as more unwieldy, tightly folded globular structures.

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Even when unfolded, a polypeptide cannot by itself penetrate through a lipid membrane. It needs some kind of proteinaceous channel to provide a passageway. On page 777 of this issue, Krantz *et al*. (*1*) provide new insight into how such a channel—the anthrax toxin pore—works.

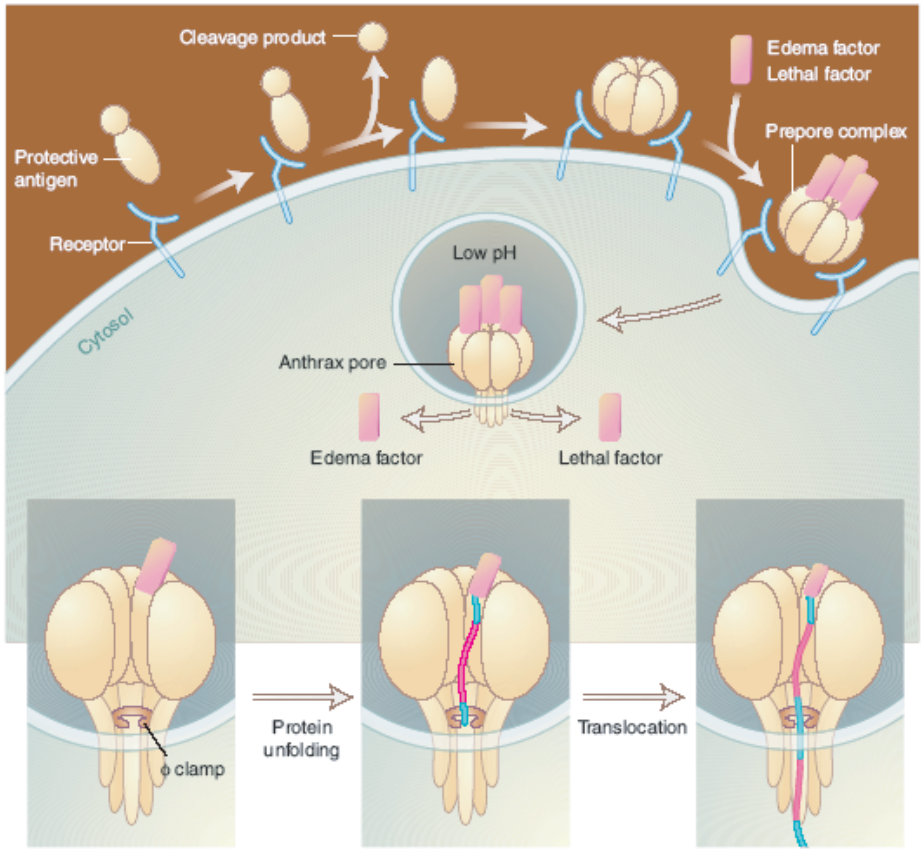
Anthrax is caused by the bacterium *Bacillus anthracis* and normally spreads to humans via infected animals or contaminated animal products. Inhalation of bacterial spores is particularly dangerous, with a mortality rate of nearly 100% if no treatment is given. Fortunately, anthrax is a rare disease, although it is regarded as a threat in the bioterrorism arena.

Anthrax toxin is secreted by the bacterium in the form of three distinct proteins (*2*) (see the figure). The pore-forming protective antigen protein first binds to a receptor on the surface of the target cell. It is activated by a proteolytic cleavage event and assembles into a heptameric prepore complex. The lethal factor and edema factor proteins then bind to the prepore complex, whereupon the whole assembly is taken up by the target cell and delivered into an acidic intracellular compartment. The low-pH environment triggers a conformational change in the prepore that leads to formation of the toxin pore proper. The low pH simultaneously causes a partial unfolding of the lethal and edema factor proteins, priming them for transport through the pore. Finally, each factor is threaded through the pore in a poorly understood process driven by the electrochemical potential across the membrane.

The pore itself has the overall shape of a mushroom with its stem penetrating the membrane of the target cell. The current model of the stem is that of a 14-stranded β barrel, 15 Å in diameter, with a water-filled pore running down its center (*3*). This is the correct diameter to allow the passage of an unfolded polypeptide, but how can the pore catalyze the complete unfolding of the toxin proteins to promote their transport into the cell?

To address this question, Krantz *et al*. mutated amino acids thought to line the pore to cysteine, and then modified these residues with a small organic reagent. Modification of two neighboring residues in the pore—Phe^{427} and Ser^{429}—blocked ion conductance, suggesting that they define the most narrow part of the pore. Further studies of Phe^{427} by electron paramagnetic resonance showed that the seven copies of this residue—one from each of the seven subunits—move close to each other during the prepore-to-pore transition and thus presumably form an aromatic ring or "ϕ-clamp" that constricts the pore.

The authors then made a very surprising observation. The pore's ion conductance increases, as expected, when Phe^{427} is mutated to smaller residues such as Ala or Ser. In contrast, the translocation rate through the pore of a fragment from the lethal factor protein decreases to an undetectable level when Phe^{427} is replaced by Ala. The Ala^{427} mutation consequently inactivates the toxin. Paradoxically, removal of the ϕ-clamp makes the pore a less efficient translocator, which strongly suggests that the ϕ-clamp is



How anthrax infects cells. Insets show how hydrophobic segments (blue) in the unfolded toxin protein (edema or letal factor) bind in succession to an aromatic ring, or ϕ clamp, promoting translocation into the host cell cytoplasm.

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not simply constricting the pore but has an active role in the translocation process.

What, then, might be the function of the ϕ-clamp? To probe its substrate-binding properties, the authors tested the inhibitory effects on ion conductance of a panoply of small blocking agents such as tetrabutylammonium or tetraphenylphosphonium. They concluded that the ϕ-clamp binds these agents by nonspecific hydrophobic as well as aromatic π-π and cation-π interactions. This led them to the idea that the role of the ϕ-clamp is to grab successive hydrophobic segments in the lethal and edema factor proteins as each chain is pulled through the pore. In essence, they propose a "Brownian ratchet" model where transient unfolding of the protein exposes hydrophobic segments that bind to the ϕ-clamp, preventing refolding and facilitating the conversion to a translocation-competent form.

There is a striking analogy between the ϕ-clamp structure proposed by Krantz *et al*. and the so-called hydrophobic gasket found in the Sec61 translocon that mediates protein translocation across the inner membrane of bacteria and the endoplasmic reticulum membrane of eukaryotes (*4*). In the Sec61 channel, a ring of hydrophobic Ile residues is thought to provide a flexible seal around the translocating polypeptide, preventing ion leakage through the membrane. At the same time, the Ile ring is expected to bind to hydrophobic segments in the polypeptide, perhaps shunting very hydrophobic transmembrane helices into the surrounding lipid membrane. Another comparable case is the GroEL/ES chaperonin in which hydrophobic residues project into a central cavity. This configuration is thought to unfold entrapped, misfolded proteins by pulling on hydrophobic residues exposed on their surface (*5*). And recently, it was proposed that movable loops protruding into the central channel of the ATP-driven ClpA protease push unfolded substrates toward their destruction (*6*).

These likely represent only a few examples of how cells manipulate unfolded proteins. By exploiting the most basic characteristic of the unfolded state—the exposure of hydrophobic residues—cells have microengineered sophisticated molecular machines to push and pull proteins, delivering them to their final destinations.

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