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Previews

To Translocate or Not: That Is the Problem

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The botulinum toxins (BoNTs) enter the cytosol of host cells by translocation across the limiting membrane of acidic endosomes. In this issue, Sun et al. (2011) show that BoNT binding to one of its cell surface receptors renders it susceptible to pH-dependent conformational changes required for translocation and cellular toxicity.

Without exception, all living cells exhibit a fundamental requirement to translocate some proteins into or across membranes. Most proteins are translocated through aqueous protein-conducting channels, perhaps best typified by the Sec61 and SecY protein complexes of the endoplasmic reticulum and prokaryotic cell membrane. Some proteins, such as the A-B subunit bacterial toxins, translocate enzymatic domains into the cell cytoplasm by crossing a membrane on their own. This is no small feat, as the toxins are secreted as fully folded water-soluble proteins that undergo a series of structural changes that allow them to integrate into lipid membranes and inject the enzymatic domain (the A subunit) in unfolded conformations across the membrane to the cytosol of host cells. Once in the cytosol, the A subunit refolds into a functional enzyme that attacks specific cellular systems. Typically, these toxins are taken up into endosomes where the acidification of the endosome serves to trigger the conformational changes in the B subunit necessary for translocation of the catalytic A subunit into the cytosol. How this occurs has been the topic of investigation for many years and for good reason: the problem is biologically important, and for the A-B toxins many facets of the translocaton process have yet to be understood.

In this issue, Sun et al. (Sun et al., 2011) present new studies that address the membrane translocation mechanism of the Botulinum toxin B (BoNT/B). The Botulinum toxin serotypes A, B, E, and F are Zn^{2+}-dependent proteases that cause paralytic disease (and death) by entering the cytosol of cholinergic neurons to block release of stimulatory neurotransmitters. BoNT/B typifies the structure and function of BoNT/A and E. They all have a trimodular structure, with an enzymatic, membrane-translocation, and receptor-binding domain (Figure 1) (Montal, 2010; Swaminathan and Eswaramoorthy, 2000), suggesting that they share a common cellular intoxication mechanism. Since these toxins are the most potent toxins currently known and are potential biological weapons, it is of interest to understand how they translocate their catalytic fragment into neurons to effect the disruption of neurotransmitter release.

It has long been known that BoNT must bind to both a cell-surface ganglioside and protein for efficient receptor-mediated endocytosis into neurons and functional toxicity (Montecucco, 1986). In the case of BoNT/B, the receptors are ganglioside GT1b and synaptotagmin I or II (syt I/II). Recently, the structural basis for the dual receptor model has been elucidated (Chai et al., 2006; Jin et al., 2006). Of note, receptor binding did not alter the structure of BoNT, at least when assessed at neutral pH. Abundant evidence also shows that within the acidic endosome the BoNT/B translocation domain forms an aqueous channel capable of conducting ions and translocating the enzymatic domain (Montal, 2010). For these reasons, and because the receptor-binding domain appeared to be fully dispensable for pore formation and toxin translocation (Fischer et al., 2008), BoNT/B binding to GT1b and syt I/II has been considered a mechanism for cell tropism and efficient endocytosis.

Herein, Sun et al. (Sun et al., 2011) presents data showing that receptor binding is also essential to the formation of the translocation channel at low pH. They show that when bound to GT1b, and at acidic pH, BoNT becomes more hydrophobic and looses α-helical structure as measured by circular dichroism. Neither low pH nor binding to GT1b alone induces such conformational changes, which is fully consistent with previous studies as described above and is correlated with toxin function. Hence, the toxin's receptor-binding domain is found to inhibit function of the translocation domain at low pH, rendering it unable to form channels in cell membranes unless the toxin is also bound to GT1b (as implied by earlier studies, see Fischer et al., 2008). Importantly, GT1b is justifiably identified as a "coincidence receptor" by Sun et al.—the toxin's GT1b binding motif must be conformationally coupled to, or form a part of, the pH sensing apparatus of BoNT that regulates the formation of the translocation system, since neither alone is sufficient to initiate the requisite conformational changes.

Why some neurotoxins have evolved to couple receptor interaction with the control of the subsequent translocation of its catalytic domain is currently unknown, but suggests that there are deeper relationships between cellular targeting and translocation of the neurotoxins, perhaps linked to its transit from the intestinal tract, where BoNT is initially absorbed, to the neuron. Why a ganglioside is targeted for BoNT binding is another question still unanswered. The gangliosides are a family of glycosphingolipids enriched in the plasma membrane and endosomal compartments of host cells, suggesting a role in trafficking BoNT to the acidic endosome. But this cannot be the only explanation as there is no obvious reason why syt I or II alone could not provide the same function. Perhaps

the sphingolipid functions to associate BoNT with membrane nanodomains more amenable to the insertion of toxin channels.

The paper also raises the interesting idea that homo-oligomerization may be required for BoNT assembly into protein-conducting channels and thus for toxicity. Here, the evidence is correlative, based on studies conducted in the presence and absence of the small molecule inhibitor Toosendanin, which is shown to partially block translocation of BoNT and toxin function in vitro and in vivo (Montal, 2010). In the Sun et al. study, Toosendanin is found to block GT1b- and pH-dependent homo-oligomerization of BoNT/B when bound to artificial lipid membranes containing GT1b, as assessed by atomic force microscopy (AFM), and Toosendanin also blocks pH-induced oligomerization of the GT1b-BoNT complex in solution, as assessed by migration of toxin monomers and oligomers on blue-native gels (Sun et al., 2011). The evidence is consistent (by inference) with a requirement for homo-oligomerization in channel formation, but whether either assay measures a physiologic state of toxin bound to membranes of host cells remains to be confirmed.

The idea for homo-oligomerization in BoNT function has been suggested before, but this model is provocative, as it competes with substantial evidence supporting the view that a single BoNT molecule can form aqueous channels in host cell membranes—that it is fully capable of conducting ions and the BoNT enzymatic domain (Fischer and Montal, 2007; Koriazova and Montal, 2003). The two models for BoNT action (oligomer and single protein channels) are not necessarily mutually exclusive. Sun et al. (Sun et al., 2011) point out similarities with other protein-conducting channels endogenous to mammalian cells, such as the Sec61, Tom40, and Tim22 complexes of the ER and mitochondrial membranes. Homo-oligomerization for these proteins has also been observed, but strong evidence, at least for the Sec61 and SecY complexes, indicates that protein translocation occurs through aqueous channels formed by single proteins (reviewed in Rapoport, 2007). Oligomerization of Sec61 and SecY may contribute to protein translocation in other ways, and the emerging view is that only one copy of a translocation channel in a larger complex may be active at any given time (Rapoport, 2007). The same may be true for BoNT and related toxins when homo-oligomerized, although it is not immediately obvious whether formation of these complexes is necessary for translocation or result from a posttranslocation structure of membrane monomers that promotes their interaction.

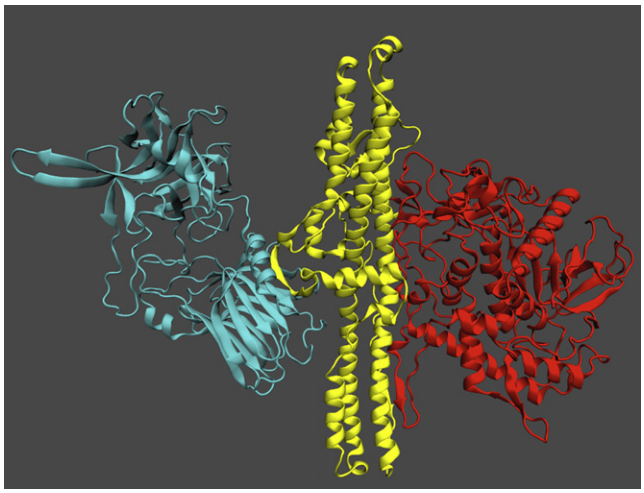

Figure 1. Structure of BoNT
Receptor binding domain in cyan, translocation domain in yellow, enzymatic domain in red (modified from Swaminathan and Eswaramoorthy, 2000).

While many aspects of BoNT channel assembly remain to be discovered, the new paper by Sun et al. identifies GT1b as a coincidence receptor, directing channel formation and the efficient intoxication of host cells. As noted by the authors, the result implies that host cell membranes "play a crucial role in shaping the behavior of bacterial toxins" (Sun et al., 2011). This is an important idea with broad impact on our understanding of host-pathogen interactions in general.

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