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Retrograde transport of cholera toxin into the ER of host cells

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Abstract

Cholera toxin moves from the plasma membrane to the ER of host cells to cause disease. Here we discuss recent studies on the mechanism of transport from plasma membrane to the ER and on the reactions that unfold and retrotranslocate a portion of the toxin to the cytosol where toxicity is induced.

Key words: cholera toxin – lipid rafts – membrane transport – endoplasmic reticulum – Golgi

Introduction

Cholera toxin (CT) is an A/B_5-subunit protein responsible for the massive secretory diarrhea caused by infection with *Vibrio cholerae*. The toxin is secreted into the intestinal lumen without damage to the intestinal epithelium, and it enters host cells by co-opting a membrane glycolipid to breech the intestinal barrier and cause disease.

CT binds to the epithelial cell apical membrane using the lectin-like activity of its B-subunit. The B-subunit consists of a highly stable ring-like assembly of five identical polypeptide chains, and contains five binding sites for the oligosaccharide domain of the monosialoganglioside GM_1 (Merritt et al., 1994). Binding GM_1 tethers the toxin to the membrane and results in the association of CT with lipid rafts. GM_1-mediated localization of CT in lipid rafts is required for toxin function (Orlandi and Fishman, 1998; Wolf et al., 1998, 2002).

CT can also enter host cells by clathrin-dependent mechanisms, but there are conflicting reports on whether this can lead to Golgi transport and a functional response (Nichols et al., 2001; Shogomori and Futerman, 2001). When internalized by both clathrin-dependent and -independent mechanisms, the toxin can be found in early and recycling endosomes, the Golgi apparatus, and the ER (Henley et al., 1998; Richards et al., 2002). Movement into the Golgi can be inhibited by blockade of COPI- and COPII-mediated vesicular transport, and this affects toxin function implicating trafficking through Golgi as a necessary step in toxin action (Richards et al., 2002).

There is very good evidence that the toxic A-subunit must travel retrograde from the apical plasma membrane into the ER of host intestinal cells. In the ER, a portion of the A-subunit, the A1 chain, unfolds and retrotranslocates to the cytosol to cause disease (Tsai et al., 2001; Tsai and Rapoport, 2002; Fujinaga et al., 2003). Exactly how the A-subunit moves from the cell surface to the ER is not clear. It is claimed that the A- and B-subunits of CT dissociate in the Golgi apparatus, and that the A-subunit, which contains an ER-targeting KDEL motif at its C-terminus, may then traffic retrograde into the ER by binding the KDEL receptor ERD2 (Bastiaens et al., 1996; Majoul et al., 1998, 2001).

The structure of CT typifies the structure of other A/B_5-enterotoxins, including Shiga and the *E.*

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coli heat labile type I and II toxins. Like CT, these enterotoxins also bind membrane lipids, enter the cell by clathrin-dependent and -independent mechanisms, and move retrograde into the ER to induce disease (Falguieres et al., 2001; Wolf et al., 1998). Shiga toxin, however, does not contain an ER-targeting KDEL motif, and there is evidence that the B-subunit of Shiga toxin travels from Golgi to ER independently of COP-1-mediated mechanisms (Girod et al., 1999; White et al., 1999). The efficiency of trafficking retrograde into the ER may be affected by the ceramide structure of the Shiga toxin glycolipid receptor Gb3 (Arab and Lingwood, 1998; Sandvig et al., 1994), and the idea that Gb3 sorts Shiga toxin into a COP-1-independent pathway has been proposed (Girod et al., 1999; White et al., 1999).

This short communication summarizes recent advances in our understanding of the cellular mechanisms co-opted by CT to move from the plasma membrane to the ER. We also discuss recent studies that explain some of the reactions that occur in the ER and account for the unfolding and retrotranslocation of the CT A1 chain.

Mechanisms of transport from plasma membrane to ER

Dependency on membrane cholesterol for toxin function and endocytosis

Several studies suggested that CT enters host cells by binding GM1 in lipid rafts (Orlandi and Fishman, 1998; Wolf et al., 1998). Since certain lipid rafts depend on membrane cholesterol for function, we examined whether cholesterol affected CT action. Methyl β-cyclodextrin (mβ-CD) was used to deplete human intestinal T84 cells of membrane cholesterol. This reversibly inhibited CT-induced Cl^- secretion and prolonged the lag phase of the toxin: i.e. the time between toxin binding at the cell surface and the induction of toxicity. In our hands, cholesterol depletion does not affect anthrax edema toxin (EdTx)-induced Cl^- secretion. EdTx does not bind to raft domains at the cell surface and enters the intestinal cell by a different mechanism. Thus, there is specificity for the effects of cholesterol depletion in human intestinal cells. There is a report, however, that EdTx must utilize lipid rafts to enter other cell types (Abrami et al., 2003). The discrepancy between these studies may reflect differences in cell phenotypes or perhaps in the extent of cholesterol depletion induced by the two experimental conditions.

In intestinal cells, the inhibition in toxin function after cholesterol depletion correlates with inhibition of toxin endocytosis and with a change in raft structure. Nearly 50% of proteins normally associated with lipid rafts are lost from this fraction after cholesterol depletion. The CT-GM1 complex, however, is not displaced from the raft fraction. Thus, toxin trafficking and function in intestinal cells depend on membrane cholesterol, but toxin localization in apical plasma membrane rafts does not. These results suggested to us that cholesterol may function to couple the CT-GM1 complex with membrane components involved in raft function and/or downstream signal transduction that is required for toxicity in polarized intestinal T84 cells.

The B-subunit carries the A-subunit to the ER by binding GM1 in lipid rafts

We have recently completed studies that explain how CT moves from the plasma membrane to the ER of host cells (Fujinaga et al., 2003). Here, we find that the B-subunit of CT serves as a carrier for the A-subunit to the ER where disassembly occurs. The B-subunit binds to gangliosides in lipid rafts and travels with the ganglioside to the ER. Non-raft glycolipids do not traffic toxin into the Golgi or ER. These results suggest that retrograde transport of CT from plasma membrane to ER is mediated by its association with gangliosides with affinity for lipid rafts. We believe this represents a general pathway from plasma membrane to ER (Fig. 1).

Mechanisms of retrotranslocation across the ER membrane

Our studies on B-subunit trafficking into the ER also show that CT enters the ER as a fully folded protein (Fujinaga et al., 2003). In the ER, the luminal chaperone protein disulfide isomerase (PDI) recognizes the A1-chain (Fig. 2). In its reduced form, PDI binds to the A1 chain, dissociates it from the B-subunit, and unfolds it. PDI will recognize the A1 chain only when the peptide loop that connects the A1 and A2 chains is proteolytically cleaved. This site of cleavage has long been recognized to represent a critical post-translational modification required for toxicity. Thus, cleavage of the peptide loop between the A1 and A2 chains acts as a molecular switch. When the loop is intact, CT is folded properly in the periplasm of *V. cholerae*. When the loop is proteolytically cleaved, CT is unfolded in the ER of host cells. Both the periplasm and ER are similar oxidiz-

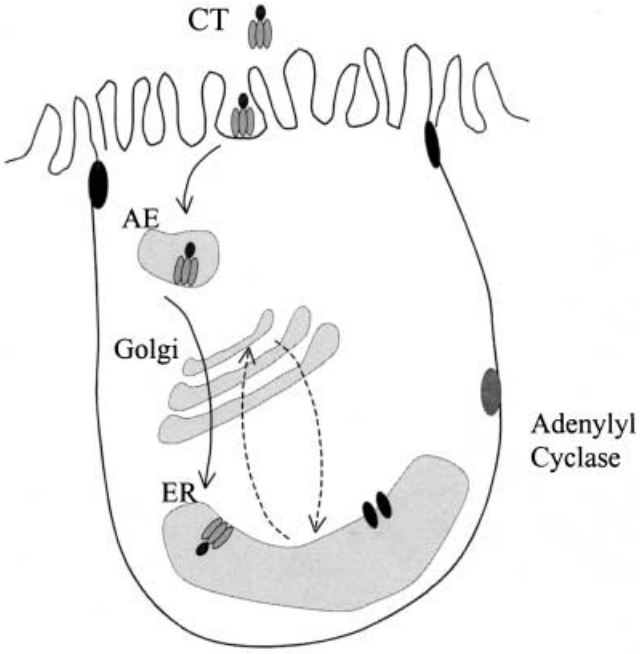

Fig. 1. Transport of CT from plasma membrane to ER by association with lipid raft glycolipids. CT enters the cell by binding ganglioside GM1 in lipid rafts and travels with the ganglioside to the Golgi and then retrograde to the ER. The toxin enters the ER as a fully assembled protein.

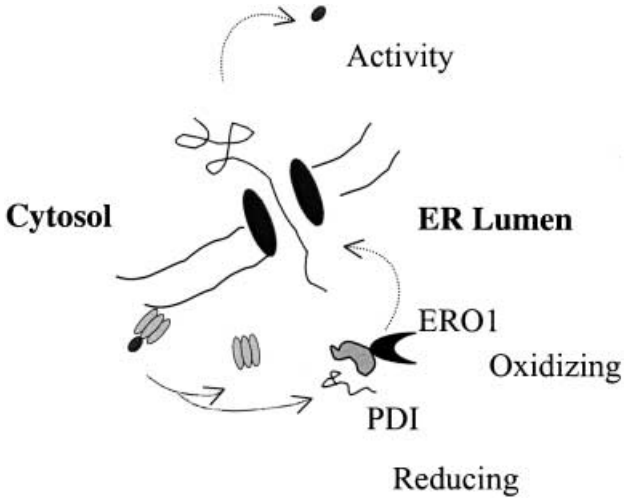

Fig. 2. Unfolding of the CT A1 chain and dissociation from the B-subunit in the ER of host cells. In the ER, protein disulfide isomerase in its reduced form recognizes the proteolytically nicked form of the CT A subunit and unfolds and dissociates the A1 chain from the B-subunit. The PDI-A1 chain complex may be targeted to the ER luminal membrane where the oxidoreductase ERO1 catalyzes the oxidation of PDI causing the release of the unfolded A1 chain. Retrotranslocation may occur by passage of the A1 chain through the sec61 channel. The driving force for movement of the A1 chain out of the ER into the cytosol does not depend on polyubiquitination of the A1 chain.

ing environments containing chaperones to assist in protein folding and unfolding.

When PDI is oxidized, it releases the A1 chain; and the A1 chain rapidly and spontaneously refolds. Thus, PDI acts as a chaperone that is driven by a redox- rather than an ATP-cycle. The ER luminal thioreductase ERO1 oxidizes PDI and catalyzes the release of the A1 chain (Tsai and Rapoport, 2002). PDI may target the unfolded A1 chain to the ER luminal membrane, perhaps directly to the protein translocation channel that may be sec61 (Schmitz et al., 2000).

Since most proteins that are dislocated from the ER are polyubiquitinated and targeted for degradation by the proteasome, we recently tested whether polyubiquitination is required for the retrotranslocation reaction. Here, we prepared a mutant toxin lacking all lysines in the A1 chain and with the N-terminus blocked by carbamylation. When applied to human intestinal cells, this mutant toxin lacking all sites for polyubiquination still induced a robust Cl^- secretory response (Rodighiero et al., 2002). Thus, polyubiquitination is not required for retrotranslocation of the A1 chain to the cytosol.

Why are the two lysines in the wild-type toxin not ubiquitinated after retrotranslocation to the cytosol? Since the A1 chain must be unfolded initially after entry into the cytosol, we hypothesized that the toxin may refold rapidly after emerging from the translocation channel. To test this idea, we modeled the refolding reaction in vitro, and found that the A1 chain refolds in as little as 5 seconds after release from PDI. Such rapid refolding renders the A1 chain resistant to polyubiquitination and may explain the driving force for the retrotranslocation reaction itself (Rodighiero et al., 2002).

Conclusions

Recent studies are discussed that show CT moves from the plasma membrane to the ER by binding to GM1 gangliosides in lipid rafts. We propose that raft glycolipids are a general vehicle for transport in this pathway. The toxin enters the ER as a fully folded protein and it is unfolded in the ER by the ER luminal chaperone PDI. The dislocation reaction that moves the A1 chain to the cytosol does not depend on polyubiquitination and may be driven by the intrinsic ability of the A1 chain to refold spontaneously.

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