[S5-1]

The Multifunctional-Autoprocessing RTX Toxin of *Vibrio cholerae*

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The life-threatening diarrheal disease cholera is caused by toxigenic strains of the Gram-negative organism *Vibrio cholerae*. In addition to the well-characterized ADP-ribosylating cholera toxin (CT), *V. cholerae* secretes a novel cytotoxin that is the founding member of a new family of the RTX (repeats-in-toxin) family. This toxin of *V. cholerae* has recently been demonstrated to contribute to virulence in mice and has been shown to be important for the bacterium to establish prolonged colonization of the small intestine. Hence, we now propose that this toxin is important for initiation of disease during the earliest stages of bacterial exposure in cholera patients when bacteria are in limited numbers in the intestine.

Beyond its potential importance for pathogenesis, the recently named Multifunctional-Autoprocessing RTX toxin of *V. cholerae* ($MARTX_{Vc}$) is of intrinsic interest due to its novel biochemical properties and mode of action. At 4545 aa and a predicted size of >480 kDa, $MARTX_{Vc}$ is one of the largest single polypeptide toxins. However, unlike other RTX toxins, $MARTX_{Vc}$ is not a pore-forming toxin, but rather induces actin depolymerization and cell rounding. Our current knowledge about the process of cell rounding by $MARTX_{Vc}$ is diagrammed in Fig. 1.

Based on the mechanism of translocation of RTX toxin *Bordetella pertussis* adenylate cyclase, we predict that $MARTX_{Vc}$ self-inserts into the eukaryotic cytoplasmic membrane and then transfers activity domains to the cytoplasm. Thus far, we have described three activities of this toxin conferred by three discrete activity domains.

First, we have demonstrated that this toxin covalently crosslinks actin into oligomers. This activity is associated with the actin crosslinking domain (ACD) that is shared with a type 6 secretion effector of *V. cholerae* and with the putative MARTX toxin of *Aeromonas hydrophila* ($MARTX_{Ah}$). We have shown that the substrate for crosslinking is free monomeric G-actin and crosslinking occurs dependent upon the hydrolysis of ATP. We hypothesize that cell rounding then occurs by depletion of the free G-actin pool leading to an equilibrium shift that drives the depolymerization of assembled actin fibers.

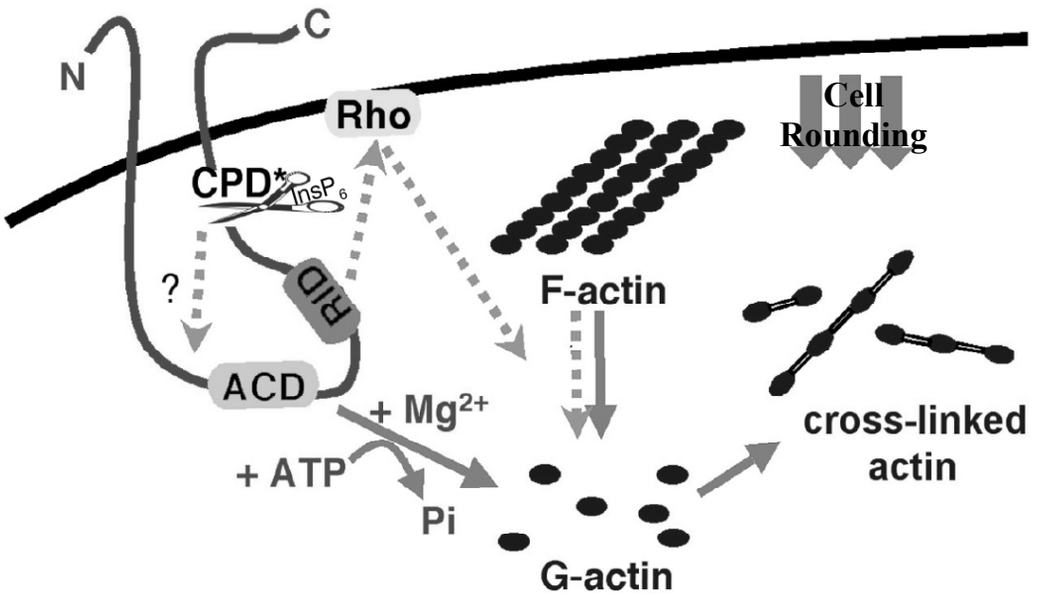

Fig. 1. Model for mechanism of $MARTX_{Vc}$-mediated cell rounding. Figure adapted from original drawn by Christina Cordero.

Second, we have demonstrated that this toxin inactivates RhoGTPases, the master regulators of actin cytoskeletal assembly, by a mechanism that is distinct from all other known Rho-modulating toxins. This activity is associated with a Rho-inactivation domain (RID) that is shared with the MARTX toxin from *V. vulnificus* ($MARTX_{Vv}$) and the putative MARTX toxins from *Xenorhabdus* sp..

Finally, $MARTX_{Vc}$ and all other members of the newly recognized MARTX family of toxins have a cysteine protease domain (CPD) with autoprocessing activity that cleaves the protein after binding cytosolic stimulatory factor inositol hexakisphosphate (InsP6), a molecule found exclusively in the eukaryotic cell cytosol. Thus, processing would be induced only after translocation to the eukaryotic cell cytosol to release the ACD and RID to access the substrates. This mode of toxin delivery is novel among the bacterial proteins toxins.

Overall, we propose a model for MARTX function wherein N- and C-terminal repeat regions of the toxins form a membrane pore for translocation of the ACD, RID, and CPD to the eukaryotic cytosol. Upon translocation, the CPD is activated, releasing the ACD and RID to the cytosol where they are free to access substrates actin and Rho. The inactivation of Rho would signal for actin depolymerization increasing the pool of G-actin for crosslinking, ultimately leading to permanent destruction of the cytoskeleton.