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**Title:** Pre-pore oligomer formation by *Vibrio cholerae* cytotoxin: Insights from a truncated variant lacking the pore-forming pre-stem loop

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Pore-forming toxin  
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**Abstract:** *Vibrio cholerae* cytotoxin (VCC), a  $\beta$ -barrel pore-forming toxin ( $\beta$ -PFT), induces killing of the target eukaryotic cells by forming heptameric transmembrane  $\beta$ -barrel pores. Consistent with the  $\beta$ -PFT mode of action, binding of the VCC toxin monomers with the target cell membrane triggers formation of pre-pore oligomeric intermediates, followed by membrane insertion of the  $\beta$ -strands contributed by the pre-stem motif within the central cytotoxin domain of each protomer. It has been shown previously that blocking of membrane insertion of the VCC pre-stem motif arrests conversion of the pre-pore state to the functional transmembrane pore. Consistent with the generalized  $\beta$ -PFT mechanism, it therefore appears that the VCC pre-stem motif plays a critical role toward forming the structural scaffold of the transmembrane  $\beta$ -barrel pore. It is, however, still not known whether the pre-stem motif plays any role in the membrane interaction process, and subsequent pre-pore structure formation by VCC. In this direction, we have constructed a recombinant variant of VCC deleting the pre-stem region, and have characterized the effect(s) of physical absence of the pre-stem motif on the distinct steps of the membrane pore-formation process. Our results show that the deletion of the pre-stem segment does not affect membrane binding and pre-pore oligomer formation by the toxin, but it critically abrogates the functional pore-forming activity of VCC. Present study extends our insights regarding the structure-function mechanism associated with the membrane pore formation by VCC, in the context of the  $\beta$ -PFT mode of action.

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