ABSTRACT

VapBCs, virulence-associated proteins, are the most abundant type II toxin-antitoxin (TA) systems in prokaryotes. Under normal conditions, toxin and antitoxin interact to form a heterooctameric complex, which upon binding to operator site, inhibits its own expression. Under stress conditions, the VapB antitoxin is degraded by cellular proteases to release a free VapC toxin, which in turn inhibits cell growth mainly by targeting protein translation. However, the detailed events involved in the formation of heterooctameric complex are not clear.

Here, we report crystal structures of *Mycobacterium tuberculosis* VapC20 toxin and VapBC11 TA complex, at 1.75 Å and 1.67 Å resolutions, respectively. Using analytical ultracentrifugation (AUC) studies, we show that VapB11 and VapC20 exists as homodimers in solution. The structural analysis of VapC homologs further suggests that VapCs form homodimers. We demonstrate that VapC20 is an obligate homodimer, and its self-association is critical for its folding and activity. AUC studies reveal that VapB11 and VapB20 exist as homodimers in solution. We report that homodimeric VapB20 associates with VapC20 dimers to form heterotetramers or heterooctamers in a concentration dependent manner. Our data suggests that both VapBC11 and VapBC20 TA modules form stable heterooctameric complexes. Surface plasmon resonance experiments suggest that VapC11 and VapC20 interact with their cognate VapB11 and VapB20 antitoxins to form a stable complex, with nanomolar range dissociation constant. A high association rate coupled with a very slow dissociation rate ensures minimal toxicity under normal growth conditions. Furthermore, we demonstrate that the VapBC11 TA module is essential for *Mtb* to establish infection in guinea pigs. RNA-sequencing revealed that overexpression of VapC11 toxin results in metabolic slowdown, suggesting that modulation of the growth rate is an essential strategy for *in vivo* survival. Interestingly, overexpression of VapC11 resulted in the upregulation of several chromosomal TA genes, suggesting the existence of highly coordinated crosstalk among TA systems.

Till date, there is no structure available for VapC toxin bound to its tRNA substrate. So, we used a combination of structural studies, toxin/tRNA binding kinetics, molecular docking, mutational analysis, and *in vitro* ribonuclease assays to gain insights to the mode of substrate recognition by the VapC toxin. We also identified a conserved surface Arg residue crucial for substrate binding. Using these

studies, we proposed a model for VapC11 bound to its tRNA substrate. Using VapBC11 TA structure as a template, we successfully designed VapB11 derived peptide-based inhibitors to target VapC11 ribonuclease activity. Taken together, we propose that the structure-guided design of inhibitors against *in vivo* essentia ribonucleases might be a novel strategy to hasten clearance of intracellular *Mtb*.