Characterizing the bactericidal mechanism of F-type pyocins

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Abstract

Rising antibiotic resistance in bacterial pathogens such as *Pseudomonas aeruginosa* highlights the need for alternative antimicrobial strategies. One promising alternative is tailocins—protein-only bactericidal complexes that are evolutionarily related to the tails of contractile and non-contractile phages. Tailocins kill by puncturing the cell envelope and are promising candidates as targeted therapeutics due to their high specificity. *Pseudomonas aeruginosa* naturally produces two types of tailocins, known as pyocins. Although R-type pyocins are well characterized, the mechanisms underlying receptor recognition and membrane puncture by non-contractile F-type pyocins remain poorly understood.

F-type pyocins possess two tail fibers that have been implicated in target specificity: a central fiber that extends distally from the bottom of the tail and side fibers that attach directly to it. I demonstrated that central fibers bind specific side fibers, and this interaction is determined by a variable region within the central fiber. Swapping this region was sufficient to change which side fibers a given central fiber can bind. Furthermore, I found that F-type pyocin bactericidal specificity arises from the pairing of the central and side fibers, rather than either fiber independently.

Upon binding cognate receptors, the central fiber undergoes a dramatic conformational change. High-resolution structures of the F-type pyocin solved using cryogenic electron microscopy revealed that over 160 residues of the central fiber protein undergo a fold switch from a trimeric coiled-coil to a trimeric β-prism structure. I demonstrated that this transition is

essential for bactericidal activity—amino acid mutations that blocked the fold switch prevented the release of the tape measure protein, a step that is required for membrane puncture. I also identified diverse non-contractile phage tail fibers with similar sequence features, suggesting that fold-switching may be a conserved mechanism for membrane puncture.

This work advances our understanding of how non-contractile tails target bacteria and identifies fold-switching as a potentially widespread mechanism of membrane puncture, which will be an important consideration for engineering phage-based therapeutics.