Aiming to kill: Rhs effector delivery by the Type VI Secretion System.

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The type VI secretion system (T6SS) is a molecular machine utilised many Gram-negative pathogens during bacterial warfare. The T6SS delivers toxins directly into adjacent target cells, often providing a competitive advantage. T6SS toxins delivered by non-covalent interactions with T6SS machinery are termed cargo effectors. A class of T6SS effectors, called rearrangement hotspot (Rhs) proteins are known cargo effectors, but the precise delivery and activation of these toxins is poorly defined. We present the cryoEM structure of a novel T6SS Rhs effector (Tse15) from the multidrug resistant hospital-acquired pathogen *Acinetobacter baumannii*. Tse15 forms a triple layered β-cocoon Rhs domain with an N-terminal α-helical clade domain and an unfolded C-terminal toxin domain located entirely inside the Rhs cage. We show recombinant Tse15 is auto-cleaved into three domains, through two independent auto-cleavage events involving a nucleophilic glutamic acid for cleavage of the N-terminal clade domain and aspartyl protease activity for C-terminal toxin cleavage. Proteomic analyses showed that the N-terminal clade and toxin domains, but not the Rhs cage, are delivered outside of the host cell, suggesting a novel mechanism for Rhs toxin delivery and activation. Our findings suggest that this delivery mechanism requires an interaction between the N-terminal clade and toxin domains, with the clade domain acting as an internal chaperone to mediate tethering of the toxin to the T6SS machinery. Conservation of the clade domain in other Gram-negative bacteria suggest this may be a common mechanism for T6SS Rhs toxin delivery. Underpinning T6SS toxin delivery may allow for anti-T6SS therapeutics, and for artificial payloads to be delivered by this nanomachine.