

## The BC component of ABC toxins is an RHS-repeat-containing protein encapsulation device.

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ABC toxin complexes are a class of large, multi-subunit protein complexes produced by bacteria. They are widespread in pathogens of insects, and are also present in several mammalian pathogens<sup>1</sup>. These complexes typically contain three major protein components<sup>2</sup>, TcA, TcB, and TcC. The TcA component forms a pentameric assembly that is responsible for binding to the target cell and stimulating endocytosis. The TcC protein contains two distinct regions, a conserved N-terminal region (TcC<sup>NTR</sup>) and a variable C-terminal toxin region (TcC<sup>CTR</sup>). When co-expressed with TcB, the TcC protein is cleaved at the junction between these two regions, and all three polypeptides remain tightly associated.

I have studied the ABC toxin complex from a native New Zealand soil bacterium, *Yersinia entomophaga*<sup>3</sup>. I have determined the structure of several of the proteins that make up the complex, including two chitinase enzymes<sup>4</sup> and the complex formed by the TcB and TcC<sup>NTR</sup> proteins. The TcB/TcC<sup>NTR</sup> structure revealed an unprecedented, large, hollow shell with a previously unknown protein fold<sup>5</sup>. This shell is formed from a long strip of  $\beta$ -sheet that spirals around a central cavity,  $\sim 59,000 \text{ \AA}^3$  in volume. This hollow shell is believed to encapsulate the cytotoxic TcC<sup>CTR</sup> and deliver it into the cell cytoplasm.

The TcC proteins contain a conserved “RHS-repeat-associated core domain.” We show that this domain is an aspartic protease that cleaves the TcC protein into its two component regions, with TcC<sup>CTR</sup> encapsulated inside the shell.

TcC proteins also contain RHS (rearrangement hot-spot) repeats, which can be found throughout bacterial species, and also in eukaryotes as the related YD repeats. This is the first structure of a protein containing RHS repeats, and we are able to describe their three-dimensional structure. Each individual RHS repeat corresponds to an individual strand-turn-strand, and we predict that multiple RHS repeats will form a long strip of  $\beta$ -sheet that spirals around to form a hollow shell, as seen in our structure. RHS repeat proteins are therefore likely to be involved in the encapsulation and delivery of C-terminal peptides.

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