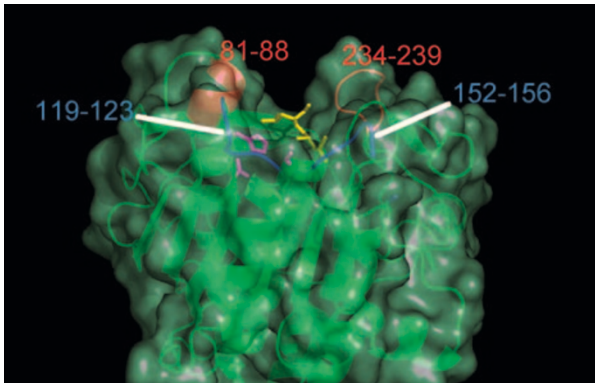
TOXINS

SubAB — a specifically deadly toxin

> Disrupting this crucially important chaperone is inevitably fatal for eukaryotic cells.

The armaments of many bacterial pathogens include toxins, which often contribute significantly to the pathology associated with infection. Adrienne Paton and colleagues now report that the subtilase cytotoxin (SubAB), a recently discovered potent AB_5 toxin, kills eukaryotic cells by cleaving the essential endoplasmic reticulum (ER) chaperone BiP — a new way to trigger cell death.

The AB_5 toxin family includes Shiga toxin, which was named after Kiyoshi Shiga, who identified *Shigella dysenteriae* as the causative agent of dysentery in 1897. This family also includes the pertussis toxin and the cholera toxin. Annual deaths resulting from infection with AB_5 toxin-producing bacteria total more than one million. These toxins share a similar arrangement of subunits, with a B subunit that forms a pentamer that facilitates uptake into susceptible cells, and an enzymatic A subunit that disables key cell functions.



Cartoon representation of SubA viewing from the S′ end of the active site. The catalytic triad is in magenta stick and the position of a modelled substrate is shown in yellow stick. Highlighted in red are the 234–239 loop and the N terminus of the helix flanking the active site (81–88). The elements of the secondary structure lining the channel leading out of the S′ side of the active site are highlighted in dark blue (119–123 and 152–156). Reproduced with permission from *Nature* 5 Oct 2006 (doi: 10/1038/nature05124) © (2006) Macmillan Publishers Ltd.

In 2004 the Paton group identified the plasmid-borne SubAB toxin, which is produced by a Shiga toxigenic *Escherichia coli* O113:H21 strain 98NK2 that causes haemolytic uraemic syndrome in humans. SubAB is the prototype member of a new class of AB_5 toxins, and has a serine protease activity that is essential for cytotoxicity. Paton and co-workers used a proteomics approach to search for SubAB targets. After challenging cells with either functional SubAB or a mutant that lacked serine protease activity, they looked for proteins that were specifically cleaved by functional toxin. The only SubAB target identified was BiP, an ER chaperone. Among other functions, BiP ensures correct folding of secretory proteins, targets misfolded proteins to the proteasome, is the ER master regulator of the unfolded protein response and, through opposing the functions of caspase, can modulate apoptosis. Disrupting this crucially important chaperone is inevitably fatal for eukaryotic cells.

Co-localization studies revealed that SubAB and BiP are both found in the ER lumen. Importantly, SubAB cleaved BiP in mouse livers after injection with the purified toxin, with no cleavage of BiP when mice were challenged with the mutant form of SubAB lacking serine protease activity, showing that the toxin is physiologically functional. Using purified SubAB, Paton *et al.* revealed that unlike other subtilisin-like enzymes this toxin is exquisitely specific, and only cleaves BiP, leaving even related proteins intact. They also pinpointed the cleavage site in BiP using purified proteins.

Inspection of the crystal structure of SubA localized the trio of amino acids necessary for the serine protease activity inside a deep cleft that is partly occluded by protein loops, thereby accounting for the precise substrate specificity of SubA. Finally, Paton and co-workers engineered a toxin-resistant BiP mutant that was able to protect cells from SubAB, proving that BiP is the physiological target of the toxin and that the effects of SubAB on BiP directly contribute to the pathology of toxin-producing bacterial infection.

SubAB is the first toxin identified that targets a chaperone, so this research will be a boon to cell biologists interested in diseases that are thought to be related to the malfunction of ER chaperones, including Parkinson's and Alzheimer's disease.

Susan Jones

ORIGINAL RESEARCH PAPER Paton, A.W. *et al.* AB_5 subtilase cytotoxin inactivates the endoplasmic reticulum chaperone BiP. *Nature* 5 Oct 2006 (doi: 10/1038/nature05124)