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Unlocking the secrets of chitinase secretion

Researchers discover a new mechanism of protein secretion in a pathogenic bacterium.

Focal Point

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(Left to right) Jaeger Hamilton, Sarah Coulthurst, Matthias Trost, Frank Sargent, Tracy Palmer, Nicola Stanley-Wall, and colleagues (not pictured) identify a novel secretion pathway that the pathogenic bacterium *S. marcescens* uses to export chitinolytic proteins. Chitinase secretion depends on the expression of an inner membrane holin-like protein called ChiW and an endopeptidase called ChiX. ChiW appears to transport ChiX to the periplasm, where it may clear a path through the peptidoglycan layer to facilitate chitinase release. The image on the right uses fluorescent reporters to show that a subpopulation of *S. marcescens* cells coordinately express ChiX (red) and the chitinase ChiA (green).

To secrete proteins into their extracellular environment, gram-negative bacteria must transport them across both the inner and outer cell membranes, as well as the intervening peptidoglycan-rich periplasm. To date, researchers have identified six different pathways for protein secretion (1). Some of them take secretory proteins directly from the cytoplasm to the cell exterior, whereas others involve a two-legged crossing, with a brief layover in the periplasm en route. Hamilton et al. now uncover a seventh pathway, which the pathogenic bacterium *Serratia marcescens* uses to secrete enzymes that break down the polysaccharide chitin (2).

S. marcescens, a major cause of healthcare-acquired infections, secretes large amounts of three chitinases (ChiA, ChiB, and ChiC) and a chitin-binding protein, Cbp21. These proteins allow *S. marcescens* to use chitin as a food source, but they may also facilitate the bacterium's attachment to host cell membranes (3, 4). Frank Sargent, a biotechnologist from the University of Dundee in the UK, was interested in using *S. marcescens* chitinases to transform chitin, which is highly abundant throughout nature, into biofuel. First, however, he told graduate student Jaeger Hamilton to look up how the enzymes are secreted so that the whole chitinolytic machinery could be transferred into a more convenient host, such as *E. coli*.

"He came back and said there was nothing known about how these chitinases were secreted," Sargent recalls. "*S. marcescens* doesn't even have half of the secretion systems written about in the literature." Hamilton et al. therefore carried out a genetic screen to identify *S. marcescens* mutants unable to break down chitin (2).

The screen revealed that bacteria lacking a gene that the researchers named *chiW* were unable to release the chitinolytic machinery from the cell. The chitinases ChiA and ChiC, for example, got trapped in the periplasm in ChiW's absence. *chiW* encodes a member of the holin family of membrane proteins, which bacteriophages often express at high levels to form membrane pores and induce host cell lysis. Indeed, *chiW* is located in an operon containing several other genes encoding homologues of viral lysis proteins. Hamilton et al. found that one of these proteins, an L-alanyl-D-glutamate endopeptidase called ChiX, was also required for chitinase secretion.

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Sargent and colleagues hypothesized that, as an inner membrane holin-like protein, ChiW might translocate ChiX to the periplasm, allowing the endopeptidase to clear a path through the peptidoglycan layer for the chitinolytic machinery to follow on its way to the cell exterior. The researchers therefore tagged ChiX with a signal peptide that allowed the endopeptidase to reach the periplasm even in the absence of ChiW. "It completely rescued the [Δ*chiW*] phenotype," Sargent says. "We got secretion of the chitinases again. So that told us that ChiX and ChiW work together and that the holin is a specific transporter for the endopeptidase. ChiX might then help the chitinases negotiate the thick peptidoglycan layer in the periplasm."

Given that ChiW and ChiX are homologues of bacteriophage lysis proteins, an alternative possibility was that they promote the release of chitinases by bursting a subpopulation of *S. marcescens* cells apart. Hamilton et al. therefore generated bacterial strains containing fluorescent reporters of both ChiX and chitinase expression. "We could follow them by live imaging and found that the cells didn't lyse," Sargent explains. "The cells that switched on the secretion system were perfectly fine and could go on to divide."

ChiX and ChiW are therefore components of a new bacterial secretion pathway. Many details of this pathway remain unclear, however. ChiA and Cbp21 contain signal peptides that could direct them to the periplasm through the Sec secretion pathway, but how ChiB and ChiC cross the inner cell membrane is uncertain. And how all four secretory proteins traverse the outer membrane once ChiX has escorted them through the peptidoglycan layer is equally unclear. "It's been really fun working with a new organism, and we want to keep doing it," Sargent says. "There are lots more things to find out."

1. Trost, M., et al. 2005. *Proteomics*. 5:1544–1557.
2. Hamilton, J.J., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201404127.
3. Tran, H.T., et al. 2011. *Histol. Histopathol.* 26:1453–1464.
4. Frederiksen, R.F., et al. 2013. *Microbiology*. 159:833–847.