was in place for studying even the largest macromolecular complexes by mass spectrometry. Practically, one obstacle remained: the dissociation of complexes during their transition from macromolecules in solution to gas phase. This phenomenon, exploited previously for examining protein ligands within large complexes, had to be overcome to record spectra of intact assemblies. Our initial attempts to record time-of-flight mass spectra of the 14 noncovalently bound subunits of the chaperonin GroEL invariably resulted in spectra of monomeric protein. To overcome this it was necessary to manipulate the pressure gradients within the mass spectrometer, introducing inert collision gas at various stages during the flight path to dampen the excess translational energy of the ions. The first spectrum of the intact $GroEL_{14}$ complex was a landmark for us since it demonstrated that species with a molecular mass close to 10^6 Da could be maintained intact during flight[12].

At the start of our research program the prospect of using mass spectrometry to study intact macromolecules of the size and complexity of ribosomes would have been unimaginable. Today the ability to maintain this macromolecular assembly and selectively dissociate groups of proteins that interact in dynamic complexes[13] is just one example of the phenomenal development of electrospray during the last century. For a technique that evolved from the spraying of paints and protective coatings on surfaces to early mass spectra of polymers through individual proteins to macromolecular particles, electrospray is now established as a major force in structural biology. I look forward to the next period of its development with high expectations.

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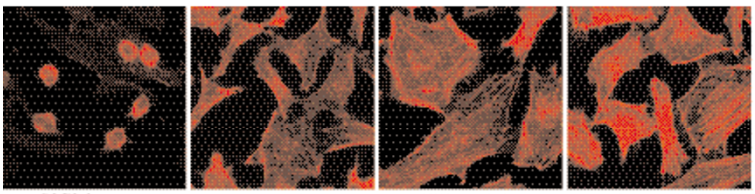
picture story

A pathogenic family business

Many invasive bacteria employ a specialized system, called type III secretion system, to inject toxins into the host cell. For example, the pathogenic species of *Yersinia*, including the causative agent of the bubonic plague, produce and secrete six toxins that function in concert to counteract the host's defense system; one of these toxins is YopT. At the cellular level, the toxicity of YopT is characterized by the disruption of the host's actin cytoskeleton and rounded cell morphology. However, the biochemical activity underlying these phenotypes has not been established. The study of Shao *et al.* (*Cell* **109**, 575–588; 2002) now provides insights into this activity.

The authors first identified that YopT is a prototypical member of a family of 19 proteins involved in bacterial pathogenesis. Sequence alignment of the YopT family suggests that three invariant residues — a Cys, a His and an Asp — may be important for YopT function. To verify the role of these invariant residues in YopT pathogenesis, the authors made single mutations at each of these sites. When expressed in HeLa cells, wild type YopT caused the previously observed effects — that is, rounded cell morphology and disrupted actin stress fiber (left panel). In



contrast, cells expressing mutant proteins had normal morphology and intact actin stress fiber (right panels); the expression level of the mutants was similar to that of wild type protein. These results demonstrate that all three residues are essential for the toxicity of YopT. Further experimentation established that YopT removes the membrane anchor of the Rho subfamily of G-proteins, which are known to regulate actin cytoskeleton. This biochemical activity therefore is likely responsible for the observed cytotoxic effect.

Shao *et al.* hypothesized that the invariant Cys/His/Asp residues may constitute the catalytic triad of cysteine proteases. In fact, the predicted secondary structure of YopT bears certain resemblance to that of a clan of cysteine proteases. The authors showed that a selective cysteine protease inhibitor blocked the biochemical activity of YopT — removal of the lipid anchor from RhoA and its subsequent membrane detachment. The authors further demonstrated that the C-terminal cysteine in RhoA bearing the lipid anchor was also removed by YopT. These observations provide experimental evidence that YopT is a *bona fide* cysteine protease.

To test whether the cysteine protease activity is a general property of the YopT family, the authors performed experiments on another YopT family member, AvrPphB, from bacteria that are pathogenic to plants. The results confirm that AvrPphB also has protease activity, which critically depends on the invariant Cys/His/Asp residues. This activity is essential for eliciting the hypersensitive response (a form of programmed cell death) in plants. Thus, the study of Shao *et al.* establishes that the cysteine protease activity of the YopT family functions in bacterial pathogenesis in both mammalian and plant cells.

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