CHEMICAL BIOLOGY

SNIPer pulls the trigger

A split protease under small-molecule control selectively activates the executioner caspases involved in apoptosis.

Through apoptosis, cells die a programmed death whereby their components are systematically dismantled and recycled. The executioner caspases are the final proteases to be activated, triggering the apoptotic cascade by cleaving more than a thousand proteins. These caspases themselves are translated as inactive zymogens that become active only upon being cleaved. How these proteases are regulated and their precise roles in orchestrating apoptosis are questions that James Wells of the University of California, San Francisco has long been interested in pursuing. Recently Wells' group reported an approach for activating individual procaspases with high selectivity in human cells (Gray *et al.*, 2010). To ensure selective activation, Wells and graduate student Daniel Gray engineered a split protease that could be controlled with a small molecule. Tobacco etch virus (TEV) protease seemed like a good choice, as no natural TEV protease cleavage sites exist in the human proteome. Only the procaspase into which a TEV protease cleavage site had been engineered would be cleaved, and thus activated, upon addition of the small molecule rapamycin. Though a split TEV system had been previously described (Wehr *et al.*, 2006), explains Wells, "the pieces can come together on their own, so that when you overexpress them they'll just come together and start working, which is a real problem for studying apoptosis." Gray reengineered the split TEV protease interface to avoid this, ensuring that the aptly named SNIPer construct—for 'single nick in proteome'—would be under the tight control of rapamycin.

By using SNIPer to activate each executioner caspase in turn, the researchers found that activating caspase-3 or caspase-7 induced apoptosis, whereas caspase-6 activation alone did not. By analyzing proteomic changes they also discovered that the caspases attacked the proteasome during apoptosis, suggesting that a negative reciprocal relationship exists between the caspases and the proteasome. "This shows how important post-translational control of caspase function is," notes Gray.

SNIPer could be applied to investigate many types of proteolysis events. "We're picking our targets carefully; …a number of those targets will be really exciting," says Wells.

Allison Doerr

RESEARCH PAPERS

Gray, D.C. *et al.* Activation of specific apoptotic caspases with an engineered small-molecule-activated protease. *Cell* **142**, 637–646 (2010).

Wehr, M.C. *et al.* Monitoring regulated protein-protein interactions using split TEV. *Nat. Methods* **3**, 985–993 (2006).