CHEMICAL BIOLOGY

Caught in action

Screening reveals a chemical activator that triggers apoptosis by locking inactive but dynamic proenzymes into a more active state, suggesting a promising strategy for targeting proteases.

Many proteases start off as inactive proenzymes, kept in check by portions of their structure that essentially act as 'safeties'. Removal of these domains—either through the action of external factors or via autoproteolytic self-cleavage—leads to enzyme maturation, freeing the active proteases to go about their business.

Caspases, those enzymatic harbingers of apoptotic death, are a prime example of this, and each of the so-called 'executioner' caspases (3, 6 and 7) undergoes a catalytic transition from inactive zymogen to active enzyme. Jim Wells and his colleagues at the University of California at San Francisco have been investigating modes of caspase-3 activation for some time, but mostly at a relative distance—targeting activators and inhibitors upstream of the protease. "We've had a lot of interest in moving down that chain of command," he says.

In a recent study, his team directly targeted the enzyme, using a high-throughput screening approach to identify small molecules that could stimulate self-cleavage by procaspase-3. They identified a dozen candidate compounds and selected the robust activator 'compound 1541' for further investigation. This molecule showed clear target selectivity, with strong activation of procaspase-3 and -6 cleavage but minimal effect on procaspase-7 activation. It also had an unusual kinetic profile. "If we add our compound to the proenzyme, we see a lag in the activation process," says Wells. "In that lag, what's happening is that it's 'cocking' the enzyme partway into an active conformer."

Caspase-3 dimers are known to be rather dynamic, shifting between fully active 'on' and proenzyme-like 'off' states even after proteolytic maturation, but it was unclear whether the procaspase-3 dimers were equally dynamic. Indeed, the kinetic data obtained with compound 1541 provide strong support for such behavior and suggest that this drug helps bias the proenzyme into a partially active conformation. "It binds to one side of the protease—and can actually act as a partial inhibitor at that site—but since the protease is a dimer, it actually stabilizes the active form so that the other, unoccupied active site is now really in position to process itself," says Wells. Importantly, active caspase-3 molecules can efficiently process procaspase-3, and this early phase of slow 1541-mediated activation soon leads to a rapid burst of enzyme maturation.

Accordingly, Wells's team demonstrated that 1541 can efficiently induce apoptosis in a wide variety of cell lines. One notable exception was the MCF-7 breast cancer cell line, which lacks functional caspase-3 and proved highly resistant to the effects of 1541, suggesting that proenzyme activation is this compound's primary mode of action. This conclusion was further supported by experiments indicating that 1541 successfully kills cells with crippling upstream defects in either the 'intrinsic' (induced by mitochondrial release of cytochrome *c*) or 'extrinsic' (induced by DNA damage and p53 activation) apoptotic pathways.

Many other proteases undergo the zymogen-to-enzyme transition, and although the mechanisms may differ radically, Wells suggests that the findings from this study could help guide approaches that exploit structural characteristics of proenzymes as a mode of activation. "People generally don't look for these," he says, "and so I think that just the fact that we found them will inspire people to begin looking." His team is continuing to explore caspase activation, through both new screening experiments and efforts to fine-tune the activity of 1541, but they are also hoping to develop similar chemical modulators for other important cellular targets such as protein kinases. "Many of them sit in dormant states which are then either activated by phosphorylation or binding to a partner," says Wells, "so the same principle should hold."

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RESEARCH PAPERS
Wolan, D.W. *et al.* Small-molecule activators of a proenzyme. *Science* **326**, 853–858 (2009).