CHEMICAL BIOLOGY

Rapid allosteric activation

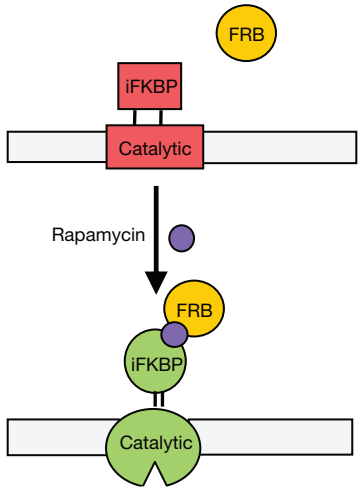
Engineered kinases remain off in cells until turned on by small molecules.

The Nobel prize may have been awarded for the creation of knockout mice, but there are many questions that cannot be answered by deleting a gene and thus its protein product. Controlling protein function rather than protein production has several advantages, including a potentially quicker response to stimuli and the ability to study an enzyme's catalytic function without disrupting its role in protein complexes.

Techniques to control kinases in living cells include methods to induce dimerization and proteolytic degradation. Another method relies on small molecules that restore function to proteins with an inactivating mutation. Now, researchers led by Klaus Hahn and Nikolay Dokholyan at the University of North Carolina, Chapel Hill added another component to this toolbox that does not require modification of a protein's active site or prevent it from forming protein complexes. Instead, they modify a kinase such that its catalytic domain is specifically activated by the addition of a small molecule.

The researchers designed their tool around a small protein known as FKBP12 that binds FKBP12-rapamycin binding domain (FRB) in the presence of the small molecule rapamycin. First author Andrei Karginov began by inserting modified FKBP12 into sites of focal adhesion kinase (FAK) that seemed important for protein-protein interactions. The researchers expected the inserted version of FKBP12 would disrupt FAK-containing protein complexes in the presence of the drug. Instead, says Hahn, "we started to notice curious behavior. Some of those engineered sites would very surgically knock out the catalytic activity but not the binding. Then we tried the drug, and it turned the kinase back on." Hahn and Karginov decided to optimize this activity. Karginov designed a very small version of FKBP12 dubbed insertable FKBP (iFKBP) to avoid disrupting the kinase domain. This rendered FAK inactive in cells, but its activity could be restored by adding rapamycin or a rapamycin analog.

The researchers also turned to Dokholyan to model protein movement and explain what was happening. Though iFKBP was placed outside FAK's active site, it still affects the site's stability: unbound iFKBP is highly mobile and destabilizes the catalytic site. In the presence of rapamycin, however, iFKBP binds FRP and becomes more rigid. Then, explains Hahn, "it tightens up the catalytic domain, and the whole thing works."



A small molecule activates an engineered kinase. Image reprinted from *Nature Biotechnology*.

The portion of the catalytic domain that is destabilized is a protein loop that is highly conserved in both serine/threonine and tyrosine kinases. "That mechanism indicates that this will be broadly generalizable," says Hahn. Hahn and colleagues already have evidence that similar modifications to p38, Src and some half-dozen other kinases also allow inducible activation of those enzymes' catalytic activities without disrupting the formation of protein complexes.

Meanwhile, Hahn is hoping for even more specific control. Inducing activity with small molecules allows the researchers to activate FAK in cells within two minutes, but Hahn is looking for systems that would allow spatial as well as temporal control. The next step is to modify activating mechanisms to work only when a kinase is in a particular cellular location or in complex with certain other proteins. "Now we have to turn them on all at once," Hahn says, "but we want to look at specific subsets."

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RESEARCH PAPERS
Karginov, A.V. *et al.* Engineered allosteric activation of kinases in living cells. *Nat. Biotechnol.* **28**, 743–747 (2010).