CHEMICAL BIOLOGY

Rapamycin to the rescue!

Researchers describe a strategy to control protein function by rescuing a protein from degradation with split ubiquitin and a three-hybrid technology.

To really understand a protein's function in the cell, often the best strategy is to get rid of it and see what the consequences are. Powerful genetic methods and chemical biology strategies can suppress the function of a protein of interest, but both factions come with their own strengths and weaknesses. With RNA interference, gene silencing can cause unwanted off-target effects, and it can be challenging to control protein levels. Small molecules act rapidly in a dose-dependent fashion, but the main limitation, of course, is that a specific inhibitor is needed for every protein of interest.

To develop more general strategies, several groups have turned to small molecules that act as chemical inducers of dimerization, to mediate the interaction of two proteins in a dose-dependent manner. These three-hybrid technologies have been used to control protein function in a number of different applications. A well-characterized system is the FK506-binding protein 12 and FKBP12-rapamycin-binding protein (FKBP12-FRB) interaction, mediated by the small molecule rapamycin. The groups of Gerald Crabtree and Tom Wandless of Stanford University have previously used degrons—a sequence that gets rapidly polyubiquitylated and shunted to the proteasome—attached to a three-hybrid system to rescue cargo proteins from the fate of degradation.

Tom Muir of the Rockefeller University has long been interested in developing laboratory tools to control protein function. Muir and his colleagues reasoned that it should be possible to adapt and combine existing technologies to release the protein of interest in its native form, thereby providing a 'traceless' method for controlling protein function. "One can conceive of situations where having the degron still there may affect [protein] function," explains Muir. Furthermore, "we can actually use our system to look at the half-life of the endogenous protein," he says.

Muir and his colleagues designed a system based on a genetically split ubiquitin, with one ubiquitin half attached to FRB and a degron at one terminus, and the protein of interest attached at the other end, with the other ubiquitin half attached to FKBP. When rapamycin arrives on the scene, it initiates dimerization of FKBP and FRB, reconstituting the split ubiquitin. Cellular ubiquitin hydrolases then cleave off the protein of interest, freeing it and restoring its function (**Fig. 1**). They named their system 'split ubiquitin for the rescue of function', or SURF for short.

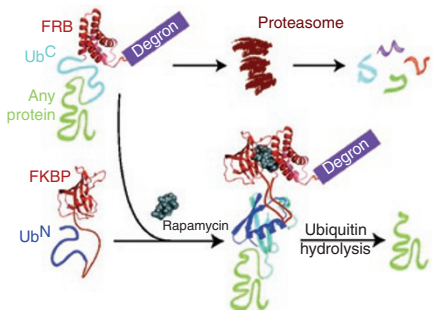

Figure 1 | The SURF technology. The protein of interest is rescued from degradation through the dose-dependent addition of the drug rapamycin. Reprinted with permission from the National Academy of Sciences, USA, copyright 2007.

The researchers showed that the method was general by testing a few different degrons as well as several different proteins of interest, including firefly luciferase, a protease, a kinase and a transcription factor. Notably, compared to the immense challenge of developing novel small-molecule inhibitors for every protein of interest, "there's a lot known about the pharmacology and cellular effects of rapamycin, so we know the playing field a little bit, which is not always the case for a new small molecule," says Muir.

Perhaps most importantly, however, such a system is not limited to simply rescuing proteins from degradation. "In work ongoing in the lab, we have applications of this technology where we replace the degron with other things, like localization sequences or other regulatory sequences that also affect the activity of the protein to which they are attached in *cis*, but not in *trans*," Muir says. "And at the end of the day it really only involves standard molecular cloning and transfection approaches, so there's really no fancy chemistry involved, only a little imagination in terms of design."

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RESEARCH PAPERS
Pratt, M.R. *et al.* Small-molecule-mediated rescue of protein function by an inducible proteolytic shunt. *Proc. Natl. Acad. Sci. USA* **104**, 11209–11214 (2007).