PROTEIN BIOCHEMISTRY

A protein alternative to RNAi

A general method of targeting tagged proteins for rapid degradation in the cell promises to provide a powerful alternative to RNA interference (RNAi) for studying the functions of proteins in living cells.

There is no disputing the fact that RNAi is an indispensable method for achieving targeted knockdown of proteins. The award of this year's Nobel Prize in Medicine to Fire and Mello, who first described the process, recently underscored this fact for anyone who has not been paying attention. No method is perfect, however, and RNAi still has limitations in specificity, speed and tunability. Specificity problems are being addressed, but other limitations are inherent to a method that targets precursors rather than final products.

Even before the advent of RNAi, researchers have been trying to design general methods to selectively inhibit protein function. Many strategies have been tried, but so far none have been widely adopted because of practical limitations. In the September issue of *Cell*, Tom Wandless and colleagues report a method of targeting specific proteins for rapid degradation in mammalian cells (Banaszynski *et al.*, 2006). Just in the first three weeks after publication, they already had received more than 40 requests for reagents, so it seems they may have hit on something that promises to be widely adopted.

The path to their method started as many scientific breakthroughs do, with a completely unexpected result in a 'failed' experiment. Wandless was collaborating with Jerry Crabtree at Stanford around 2001 on a method that used a derivative of rapamycin as a conditional switch to mislocalize, and thus turn off, proteins tagged with a rapamycin-binding domain and coexpressed in cells with a second protein that would direct the mislocalization of the rapamycin-bound tagged protein.

They made several strains of mice that expressed tagged versions of different proteins. Wandless exclaims, "Much to our unpleasant surprise the knockins phenocopied the traditional knockouts." It turned out that the protein tag triggered degradation of the fusion protein in the absence of the rapamycin derivative, but the protein was stable in the presence of the compound. They published this curious result in 2003 (Stankunas *et al.*, 2003).

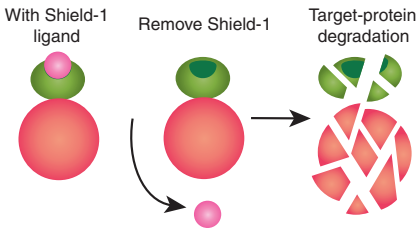

Figure 1 | Ligand-dependent targeted protein elimination using a destabilization domain. A small destabilization domain (green) is fused to a target protein of interest (red). The small molecule Shield-1 (pink) stabilizes the destabilization domain, protecting it from degradation. But when the molecule is removed, the entire fusion protein is rapidly degraded.

"It was immediately apparent to me and everyone on the project that this was not going to be a great long-term solution [for protein knockdown]," says Wandless. The system required two different protein domains and an expensive rapamycin derivative. "To this day nobody has ever asked me for that drug," adds Wandless jokingly. Right then they decided to try and fix the system by using a single protein domain system with a high-affinity small molecule that is easy to synthesize and is reasonably soluble.

They chose a single 107-residue protein called FKBP12 and synthesized a ligand (Shield-1) that exhibited the properties they wanted (**Fig. 1**). They generated a library of FKBP12 random mutants, cloned these upstream of a fluorescent protein, and selected for clones that were fluorescent in the presence of Shield-1 but lost fluorescence in its absence. They found one FKBP12 mutant that displayed only 1–2% of wild-type fluorescence in the absence of Shield-1.

They determined that this loss of fluorescence was caused by the rapid proteasome-dependent degradation of FKBP12 and whatever protein it was attached to. Wandless says, "We've tested 24–25 proteins now and have yet to see it not work." It appears that the intrinsic stability of the target protein affects the speed of degradation. Although the highly stable GFP takes 4 hours to degrade, Wandless says a less stable protein like luciferase takes only 30 minutes.

Rates of elimination of 4 hours or less are considerably faster than what is possible with RNAi. For example, RNAi knockdown of a stable protein like GFP takes 48–72 hours because the effect on protein level is delayed until previously synthesized protein is naturally degraded. In contrast, by altering the concentration of Shield-1, Wandless showed that they could dynamically control the levels of target protein in the cell. "The tunability is actually quite powerful," he notes.

Although it provides exquisite specificity, the need to tag a target protein with the destabilization domain is a practical limitation of this system—but one they are trying to exploit. His team is now developing a second destabilization domain using a completely different protein and small molecule. This would allow a researcher to tag two different proteins with different domains and independently control the level of each protein. Wandless says, "There are already a number of projects in the lab that are taking advantage of this." Although their 'DD system', as they call it, is not likely to lead to a Nobel Prize, it looks certain to provide researchers with a valuable and powerful complement to RNAi.

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RESEARCH PAPERS

Banaszynski, L.A. *et al.* A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules. *Cell* **126**, 995–1004 (2006).

Stankunas, K. *et al.* Conditional protein alleles using knockin mice and a chemical inducer of dimerization. *Mol. Cell* **12**, 1615–1624 (2003).