CHEMICAL BIOLOGY

Regulation through degradation

Two groups present small molecule–induced degradation systems for controlling protein function in living systems.

Researchers have long studied protein function by using various methods to 'remove' the protein and then observe the effects on the cell. Control at the protein level is attractive because dosage can be easily controlled and function can be knocked down rapidly. Small-molecule inhibitors specific to each protein in the cell would be ideal tools for controlling function, but this is far from reality as it is extremely difficult to identify or design such molecules. A more general technique involves using a fusion protein whose function is controlled by a small-molecule ligand. One successful approach has been to create a fusion with tunable stability such that the cell recognizes the protein as being misfolded and thus degrades it.

Two groups recently reported advances in this arena. Tom Wandless of Stanford University and colleagues used a small molecule to induce degradation of a fusion protein based on FK506- and rapamycin-binding protein (FKBP). And Craig Crews of Yale University and colleagues used a covalent hydrophobic tagging approach to induce degradation of a HaloTag protein fusion.

A few years ago, Wandless and his colleagues introduced a technique for regulating protein stability using a so-called destabilizing domain. They generated mutants of FKBP that could be stabilized by a ligand called Shield-1. In the absence of Shield-1, the FKBP fusion protein was degraded by the cell. "From the beginning, we thought: 'wouldn't it be interesting if we could make it work in the opposite direction, to get ligand binding to be destabilizing to the cell'," says Wandless. They hypothesized that a C-terminal peptide tag that bound the active site of FKBP in the absence of the Shield-1 ligand would stabilize the fusion protein. After extensive screening, they identified a 19–amino acid peptide with the desired property. The addition of Shield-1 interrupts the intermolecular interaction, destabilizes the fusion and results in degradation (Bonger *et al.*, 2011).

This ligand-induced degradation (LID) system has the advantage over Wandless's previous system in that constant dosing with a ligand to stabilize the fusion protein is not needed; instead, the ligand is only added when you want to turn off the protein's function. Although the researchers have not yet tested the LID system in animals, Wandless believes that the method should be particularly attractive to those using mouse models and long-term experimental windows who do not want to have to constantly dose their mice with a rather expensive ligand.

Crews recalls his group's inspiration for their hydrophobic tagging (named HyT) system (Neklesa *et al.*, 2011). "I thought, could there be some way we could append a hydrophobic patch to the surface of a protein to make the cell think that the internal, normally hidden hydrophobic residues are now exposed?" he says. "It's kind of hijacking the cell's own quality control mechanism." They considered the HaloTag fusion system, a variant of haloalkane dehalogenase, an enzyme which removes a halide from an aliphatic carbon chain substrate, forming a covalent bond with an aspartate residue of the enzyme in the process. They reasoned that by using a highly hydrophobic substrate to form a covalent bond with the HaloTag, the cell could be 'tricked' into thinking that the HaloTag fusion protein is misfolded and thus rapidly degrade the fusion.

Crews and his colleagues used the hydrophobic system to control the function of several proteins in cell lines, in zebrafish embryos and in mice, and found, notably, that it did not cause any toxic effects. Another advantage of the method, he notes, is the commercial availability of the entire human 'ORFeome' as fusions to the HaloTag. "Anyone can order up their protein of interest and control it using our technology," he says. He foresees the method as being particularly useful for studying protein function in genetically intractable systems such as parasites.

So which of these new small molecule–based degradation systems should the curious researcher try? "These systems just have to be tried out in the field to see how robust they're going to end up being," says Wandless.

Allison Doerr

RESEARCH PAPERS

Bonger, K.M. *et al.* Small-molecule displacement of a cryptic degron causes conditional protein degradation. *Nat. Chem. Biol.* **7**, 531–537 (2011).

Neklesa, T.K. *et al.* Small-molecule hydrophobic tagging-induced degradation of HaloTag fusion proteins. *Nat. Chem. Biol.* **7**, 538–543 (2011).