SENSORS AND PROBES

Degrading proteins with intracellular antibodies

Researchers develop a genetic method for degradation of GFP protein fusions in cells and living flies.

Markus Affolter from the University of Basel thinks that intracellular antibodies are going to revolutionize the way basic cell biology experiments are performed.

An intrabody is an antibody that works inside the cell to bind an intracellular protein. One can either introduce a whole purified antibody into a cell or engineer antibodies to be expressed and retained in the intracellular milieu.

Single-domain antibodies are a class of engineered antibodies that make great intrabodies. These smallest of antibodies—sometimes also called nanobodies given their nanoscale dimensions—have a similar affinity to antigens as whole antibodies but are more stable and about ten times smaller.

Single-domain antibodies have received much attention for their putative pharmaceutical value, and research has already shown that they have promise for the targeting of viral infection, cancer or even neurodegenerative diseases.

Affolter, however, is interested in their potential as tools for basic science. His group has shown recently how one can engineer a single-domain antibody to exert a particular function inside the cell—specifically, the degradation of intracellular proteins. "As developmental biologists," Affolter explains, "what we need in the next ten years are methods that allow modulating protein function in the living organism at the single-cell level." His postdoc, Emmanuel Caussinus, decided to use single-domain antibodies to develop such a method.

The group fused a GFP-specific nanobody to an F-box domain, a peptide responsible for binding proteins and targeting them for proteasomal degradation in eukaryotes. With this construct one could target proteins fused to GFP for degradation, so they called the construct 'deGradFP' for 'degrade GFP'.

They tested deGradFP in mammalian cells in culture and also in fruit flies. Among other experiments, they used the method to target a histone-GFP fusion in the fly epidermis and estimated that deGradFP can be used to eliminate the GFP fusion protein in about three hours. They then validated the use of deGradFP to phenocopy loss-of-function mutations in whole organisms. For this, they expressed deGradFP in flies, in which the only source of a given protein was its GFP fusion, and showed that the degradation of the protein fusion using deGradFP produced the same effects as those seen in the loss-of-function mutant. The researchers expressed the deGradFP in the fly under the control of the upstream activating sequence–GAL4 system. The GFP protein trap can be obtained from large collections that are available for the fruit fly.

An attractive property of deGradFP is that one can follow the degradation of the target fusion protein using fluorescence and monitor the resulting phenotypes as they occur over time. This method also does not require the normal turnover of the targeted protein to see the effect of a knockdown, as RNAi-based methods do. At the same time, one of deGradFP's limitations is that it is restricted to the targeting of GFP fusion proteins, at least for now. Affolter is actively collaborating with other research groups to implement the system using a variety of single-domain antibodies to endogenous proteins, which would extend the value of this approach enormously.

Inducibility and reversibility of the system are also important aspects that will need to be incorporated into deGradFP. Affolter and Caussinus plan to improve the system along these lines using optogenetic tools to make the system light-inducible.

Beyond improving this new 'degron' system, what really excites Affolter is to think about the potential of nanobodies in more general terms. Several projects in his laboratory are aimed at functionalizing these tiny antibodies in different ways, using them not only to get rid of proteins but also to play around with them, for example, by modifying their localization or their phosphorylation, "I think [this technology] has no limits," he adds with enthusiasm.

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RESEARCH PAPERS

Caussinus, E. *et al.* Fluorescent fusion protein knockout mediated by anti-GFP nanobody. *Nat. Struct. Mol. Biol.* **19**, 117–121 (2011).