power of the high-resolution phenotyping afforded by zebrafish and the value of the large-scale databases linking phenotype and genotype in this organism. As these databases progress toward completeness, the validity of the approach will continue to increase. Although this strategy is unlikely to be universally effective, the fumagillin success makes a strong case for considering the approach as a potent, albeit "noncanonical," path to deciphering small-molecule mechanisms of action.

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A Stability Switch for Proteins

A paper published in the September 8 issue of *Cell* [1] describes a generally applicable approach for chemical control of protein stability, with potential for broad use in chemical genetics.

An oft-stated goal for chemical biology is to provide new tools for analyzing gene and protein function that complement classical genetics. This aspiration, encapsulated in the phrase "chemical genetics," is increasingly being fulfilled as chemical probes are used to inhibit proteins of interest and generate chemically-induced alleles of protein function [2].

What chemistry has to offer genetics above all else is the dimension of time [2, 3]. Essentially, small molecules can be used to effect "instant mutagenesis" by specifically inhibiting (or activating) a protein of interest in real time. By contrast, generation of knockout mice to look at the functional consequences of protein loss is a slow process, often stymied by embryonic lethality or confounding compensatory changes in gene expression. RNAi [4] provides a new way to knock down protein function, but is still slower to take effect as it does not directly target the protein. Thus, chemical approaches have the potential to illuminate otherwise invisible areas of biology.

Unfortunately, as a field we are not yet at a point where we can routinely generate suitable chemical inhibitors for every protein of interest. This is particularly true if in vivo studies are contemplated, meaning that the additional hurdle of pharmacology needs to be cleared. An alternative strategy is to "tag" a protein of interest in such a way that it can be targeted by a generic ligand—for example by fusing it to a heterologous protein [3]. This powerful approach in principle allows any protein of interest to be chemically manipulated in real time without the need for a specific inhibitor. "Tagging" strategies have both drawbacks and advantages. They assume that protein function is unaffected by the tag, and they also require genetic steps to express the modified protein in cells. In addition, because endogenous, unmodified protein molecules will be unaffected by the generic ligand, either the protein of interest needs to have a dominant effect, or the study needs to be performed in the background of a knockout of the endogenous protein. On the other hand, since the effects of the small molecule are restricted to cells expressing the tagged protein, these approaches can often be more precise than the use of a direct chemical inhibitor.

Many successful "tagging" strategies have been described [3]. For example, dimeric small molecules can be used to inducibly dimerize tagged proteins, allowing study of proteins and pathways controlled by protein-protein associations [5, 6]. Expression of protein kinases that are modified to bind certain inhibitors uniquely has allowed very precise delineation of the roles of individual kinases and substrates in cell biology [7]. Several of these technologies have "crossed over" from the field of chemical biology to become established mainstream biological research tools. However, they are generally restricted to subsets of proteins that share a certain mechanism of action. A truly versatile technology for chemically regulating protein function, generally applicable to any protein, is a highly desirable goal.

Such an approach was described a few years ago by Stankunas et al. [8], who developed a system for controlling protein stability—a shared property essential to all proteins (see Figure 1). These authors exploited the chance observation that a certain mutated protein

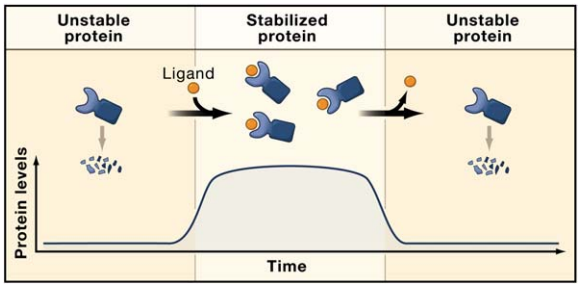

Figure 1. Control of Protein Stability Using Small Molecules and a Destabilizing Protein Tag

A protein of interest (rectangle) is fused to a destabilizing protein tag (horseshoe), leading to proteolytic elimination of the fusion protein. Addition of a tag-specific ligand stabilizes the tagged protein, leading to its accumulation, which can be reversed by removal or breakdown of the ligand.

domain (termed FRB*) is unstable and confers instability to any protein to which it is fused. This instability can be rescued by addition of rapamycin or a specific analog called Ma-rap, which bind to FRB* in complex with endogenous protein FKBP. The investigators showed that fusing FRB* to a protein of interest targets it to the proteasome, whereas the ligand-bound FRB* is spared. Thus, this "stability switch" allows the induction of a brief, defined period of protein function and is in principle applicable to any protein.

Unfortunately this prototype system is limited by issues associated with the ligands. Rapamycin is a highly bioactive molecule whose anti-proliferative effects will often confound studies of cellular function; Ma-rap lacks these effects but cannot be used in mice due to poor pharmacology [8, 9]. In principle one could engineer the system to work with different rapamycin analogs [6, 9]. Instead, in their *Cell* paper, Banaszynski et al. [1] have devised a more versatile and broadly applicable stability switch based on a different ligand-protein pair. They turned to FKBP, a small chaperone-like protein that has proved to be fertile ground for both protein engineers and chemical biologists. Particularly useful are ligands that have been engineered to bind only to a specific mutant, F36V, rather than the wild-type protein, as these ligands are physiologically inert and have been widely used in vivo [10].

Using F36V as a starting point, Banaszynski et al. [1] pummeled the normally stable protein by random mutagenesis and used a clever selection scheme involving N-terminal fusion to the fluorescent protein YFP and rounds of flow cytometry to identify destabilizing mutants. A broad panel of such mutants was obtained, each of which confer different levels of destabilization and therefore rates of degradation. All were rescuable by Shld1, a specific monomeric F36V ligand [11] designed to be cell permeant—addition of Shld1 induced protein accumulation and prevented degradation by the proteasome. In an impressive demonstration of the "tuneability" of the new system, protein function (in this case YFP fluorescence) was iteratively adjusted through several levels between zero and 100% and back again over the course of a week by exposing cells to different concentrations of Shld1.

Banaszynski et al. went to considerable lengths to establish the generality of their approach. They identified separate mutants optimized to function as C-terminal fusions, for cases where N-terminal fusion would interfere with protein function. They showed that the system was able to control the stability of 14 different proteins of disparate classes, including membrane proteins, and to function in multiple cell lines. They also validated that it could be used to bring cellular phenotypes under chemical control by inducing reversible changes in cell morphology through Shld1-mediated control of three different cell cycle regulatory proteins.

An important additional experiment reported by the authors is a comparison of their approach with RNAi (although not, unfortunately, with the same target protein). Control with the stability system achieved a faster (maximal at 4 versus 48 hr) and more complete knockdown of protein expression than transfection with synthetic siRNA. These comparisons are rarely done with new chemical genetic tools, but are important if these new approaches are to be understood and embraced by the broader cell biology research community.

A crucial next step will be a demonstration of true functionality and versatility in the in vivo setting. Here the omens are good, since F36V ligands have been successfully used in dozens of studies in mice, rats, and nonhuman primates [6], and even humans [12]. It should be noted that most of these studies used dimeric ligands, which would likely be inappropriate for use in controlling stability. Shld1-like monomeric ligands have been used in some mouse studies [13], but tweaking may be required to identify a compound (and formulation) with optimal pharmacokinetics, metabolism, and tissue distribution for use with the stability switch. However, it is likely that this new "tagging" system will prove broadly applicable to the analysis of protein function both in vitro and in experimental animals.

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