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A small-molecule SMASh hit

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There are many techniques that can be employed to control gene expression at the post-translational level. However, a novel system called SMASh (small molecule–assisted shutoff), which allows for chemically-induced degradation of target proteins, presents some distinct advantages.

Technological advances produce breakthroughs in biomedical research, and vice versa. As scientists' understanding of biological systems becomes more sophisticated, so do their methods and research tools. To accurately decipher how genes function within the cell, tight control of their intracellular abundance is essential. In this issue, Chung *et al.*¹ report the SMASh system, in which gene products are fused to a drug-sensitive viral protease and a protein destabilization element (degron), which allows for a simple way to turn off expression of the protein of interest¹. This approach, which is simple, straightforward and conducive to high-throughput technology, has clear applications as a research tool and may be potentially useful for therapeutic systems.

Many different techniques have been developed to alter the function, localization and degradation of gene products at the post-translational level (recently reviewed in ref. 2). In the ubiquitin proteasome system (UPS), E3 ubiquitin ligases confer specificity in the process of substrate ubiquitination and subsequent degradation by the proteasome. It therefore is possible to either attach a degron (a domain which is required for degradation) to an exogenous protein of interest or to engineer a chimeric E3 ligase that binds to an endogenous protein and promotes its ubiquitination. In the former approach, the degron can either confer binding to a known E3 ligase or promote degradation via different means, including the unfolded protein response, the N-end rule (in which certain N-terminal residues promote ubiquitination) or direct recruitment to proteasomes or autophagosomes. These approaches can also be coupled with pretranslational approaches such as RNA interference to target gene products from multiple angles and achieve a maximal level of gene knockdown³.

Inducible post-translational modes of protein knockout utilize small molecules, which often confer a greater degree of control to the system. Some techniques, such as the plant-derived auxin-inducible ubiquitination system, allow for chemical regulation of E3 ligase activity⁴. Alternatively, proteases (and protease inhibitors) can be used to either separate degrons from the protein of interest or reveal masked degrons. Independent of engineered E3 ligases,

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synthetic molecules called ‘protacs’ have been developed to facilitate interactions between endogenous proteins and E3 ligases and promote target protein degradation⁵. Aside from small molecules, temperature-sensitive proteins can also be engineered, and these undergo conformational changes to either expose degrons or destabilize target proteins.

Each of these systems has advantages and disadvantages. Extensive protein engineering can be laborious and cause unintended phenotypic effects. Small molecules generally confer greater control but either rely on engineered proteins or must be carefully designed. In addition, there are risks of unintended effects from off-target binding and/or intrinsic toxicity. This is also a concern for temperature-sensitive systems or chemicals with global cellular effects such as translational or proteasome inhibitors. Conversely, over-reliance on the endogenous UPS and other pathways may also disrupt existing cellular functions.

The system reported in this issue by Chung *et al.*¹ is minimally disruptive, effective and inducible. The protein of interest is tagged with the hepatitis C virus protease NS3 as well as a destabilizing degron (Fig. 1). After translation, the tagged protease cleaves off the degron as well as NS3 itself, yielding unmodified gene product. To halt expression of nascent target proteins, the protease inhibitor asunaprevir can be administered, resulting in retention of the degron and the degradation of all future NS3-degron–fused gene products. The authors have dubbed this technology small molecule–assisted shutoff, or SMASh.

In their report, Chung *et al.*¹ demonstrate that the SMASh system requires minimal genetic modification. The degron-protease tag works on multiple target genes and is equally effective whether attached to the N or C terminus of the protein of interest. Also, once cleavage occurs, the protein is devoid of structural modifications, so the gene product itself is assumed to function normally. With SMASh, target protein degradation is drug inducible, which allows for tight control and rapid recovery of normal protein production after drug removal. One concern is that the NS3 protease can cleave proteins involved in the innate immune response, but this could be easily overcome with minor modifications of either the NS3 protease or its host substrates⁶. Unlike tools such as chimeric E3 ligases, inducible degrons and protacs, the SMASh system does not require extensive engineering. Although the SMASh system cannot be used for direct targeting of endogenous proteins, as certain chimeric E3 ligases can, the SMASh tag can be added to endogenous genes^{7,8}. A recent report used the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 gene-editing system to add inducible degrons to the *TCOF1* locus in human cells, and Chung *et al.*¹ demonstrate in their report that SMASh tag modification of endogenous yeast genes (*YSH1* and *SEC14*) is feasible^{1,9}.

The SMASh system is a simple and controllable means of regulating protein expression that offers advantages over existing protein knockout techniques. Furthermore, SMASh may also be conducive to therapeutic applications such as terminating the activity of therapeutic proteins at will and controlling oncolytic viral replication¹.

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Figure 1.

Schematic of the SMASh system. After translation of a SMASh-tagged protein, intramolecular cleavage by NS3 releases the protein of interest in its native form and the NS3 protease and degron are degraded by intracellular machinery. Addition of asunaprevir inhibits the NS3 protease and, as a result, the degron promotes proteasomal and/or autophagosomal-lysosomal degradation of the SMASh-tagged protein. In this system, adding protease inhibitor halts expression of any protein with a SMASh tag and effectively shuts down its activity. Inset, schematic of the SMASh tag, comprising a destabilizing degron (D) and the NS3 protease, inserted 5' to the gene of interest.