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The proteasome makes sense of mixed signals

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Abstract

Protein ubiquitination is an important degradative signal, yet not all ubiquitinated proteins are degraded. Recent results reveal insights into the proteasome's strategy for integrating biophysical signals when choosing substrates for degradation.

Many of our 401(k) accounts have recently become 201(k)s, so let's talk about money. Imagine that money flows into your bank account but never out, so your balance grows and grows. Lucky you! Now let us imagine a more realistic scenario in which the rates of incoming and outgoing funds are nearly equal. Your balance may fluctuate up or down in the short term but will remain relatively constant over time. Substitute a mammalian cell for the bank account and proteins for funds, and we have a rough analogy for what a typical cell must accomplish to maintain appropriate levels of thousands of different proteins. The rates of protein synthesis and degradation must be nearly equal to keep protein levels relatively constant. Although human nature leads us to focus on the 'supply side' (that is, transcription and translation), our appreciation for the regulatory strategies and mechanisms governing protein degradation is increasing. A report from Prakash *et al.* in this issue of *Nature Chemical Biology*¹ reveals insights into how proteasomes integrate a variety of biophysical signals when choosing proteins for degradation.

Ubiquitin is a 76-residue protein that is covalently attached to lysine side chains of target proteins, and ubiquitin itself has seven lysine residues that often serve as sites for additional ubiquitination. Mammalian genomes encode hundreds of different proteins involved in ubiquitination, so the protein machinery responsible for regulating this post-translational modification is quite complex². Deubiquitinating enzymes are active players in this process, and small domains that are similar to ubiquitin (for example, SUMO) can compete with ubiquitin for acylation of substrates³. The engagement of these myriad enzymatic activities on protein substrates creates the possibility for a dynamic equilibrium in which one or more lysine side chains of a potential substrate can be tagged with a variety of modifications. Given the many possibilities for modification, it is not entirely clear how the cellular machinery integrates these varied post-translational modifications to maintain homeostasis.

Proteasome-mediated degradation is one of the most thoroughly studied fates of ubiquitinated proteins, and current thinking holds that four or more ubiquitin domains are required to efficiently target substrates for degradation⁴. Additionally, it appears that polyubiquitin linkages through the ubiquitin Lys48 side chain are preferred by the degradative machinery⁵. There is, however, much more to learn. Which ubiquitin conjugating enzymes regulate which substrates? How significant are the deubiquitination enzymes in this dynamic equilibrium? Levels of many cyclin proteins are tightly regulated by proteasome-mediated degradation, yet their CDK partners routinely escape degradation⁶. How does the proteasome choose its substrates, especially when confronted with a variety of ubiquitinated proteins?

The hypothesis underlying the study reported in this issue of *Nature Chemical Biology* is that protein substrates have two structural features relevant to the proteasome. The first is

ubiquitination, which targets proteins to the proteasome. The second feature is the presence of some form of unstructured region (USR) that facilitates efficient proteasome-mediated degradation⁷. Prakash *et al.* pursued a biochemical strategy to address this question of substrate selectivity. They used preparations of functional proteasomes from reticulocyte lysates as well as purified yeast proteasomes. Substrate proteins were expressed in bacteria, which allowed the investigators to tag their substrates with ubiquitin or a USR as desired. In short, the use of pure substrates and enzymes enabled a tightly controlled structure/function study of the proteasome.

There was an additional experimental twist that revealed interesting behavior on the part of the proteasome. Instead of using a single polypeptide as a proteasome substrate, they used barnase and barstar, two polypeptides that form a well-characterized bimolecular complex⁸. This strategy allowed the investigators to tag either barnase or barstar with a USR and/or ubiquitin and monitor the fates of the individual proteins, revealing insights into how these two structural features are handled by the proteasome (Fig. 1). Proteins that had a USR but lacked ubiquitin were stable. Ubiquitinated proteins that lacked a USR were also stable. Predictably, a protein that had both structural features was degraded. The USR marks a protein for degradation but only if the substrate is delivered to the proteasome through ubiquitination. The investigators further demonstrated that the ubiquitin modification can be present on either the polypeptide that contains the USR or the other member of the complex. For example, ubiquitinated barstar caused the degradation of a USR-containing barnase. Strikingly, the ubiquitinated barstar was not degraded, which raises the possibility that a ubiquitinated member of a complex can act both 'in *trans*' and in a catalytic fashion, thereby leading multiple copies of a USR-containing partner down the path to degradation. This mechanism, if general, expands the range of proteins whose stabilities may be regulated by a particular ubiquitin ligase.

By experimental necessity, the full range of possible ubiquitin linkages could not be represented in this study. The authors had no way to control the fates of their N-end rule substrates in reticulocyte lysates. Once the N terminus was liberated, the protein substrate could be modified by ubiquitin ligases, possibly yielding a heterogeneous pool of substrates. In the experiments using purified proteasomes, the investigators used a linear repeat of four ubiquitin genes followed by the substrate protein. The fused ubiquitins were made resistant to deubiquitinating enzymes, which allowed the repeating ubiquitins to target substrates to proteasomes. In essence, the linear tetraubiquitin fusion served as a mimic for the typical polyubiquitination of a lysine side chain. However, it is possible that more physiologically relevant linkages would result in alternative fates for the ubiquitinated proteins. On the enzyme side, all proteasomes are not created equal⁹. These multiprotein molecular machines are comprised of many different polypeptide chains, some of which are interchangeable, and distinct proteasomes may display different substrate preferences.

Yen *et al.* recently described a high-throughput platform to monitor the turnover of thousands of proteins in mammalian cells, and they found no correlation between the presence of an unstructured region and protein stability¹⁰. The findings of Prakash *et al.* are consistent with this observation and remind us once again that much of cell biology is molecular. Understanding the structures of biological molecules and the stoichiometries with which they interact with each other can be critical for making sense of their various functions. The biochemical study described in this issue of *Nature Chemical Biology* opens our eyes to a possible role for ubiquitinated proteins as *trans*-acting catalytic adaptors that regulate the degradation of partner substrates, which is certainly something to keep in mind when evaluating screens for proteins having hallmarks of past ubiquitination¹¹.

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Figure 1.

Candidate substrates are localized to the proteasome through conjugated ubiquitin domains, and the presence of a USR is also important to initiate proteolytic degradation. **(a)** Barnase lacking a USR is stable when presented to the proteasome by ubiquitinated barstar. **(b)** Barnase displaying a USR is not degraded if barstar is not ubiquitinated. **(c)** Ubiquitinated barstar displaying a USR is degraded by the proteasome, but barnase is stable. **(d,e)** When ubiquitinated barstar delivers a USR-displaying barnase to the proteasome, the barnase is proteolyzed but the ubiquitinated barstar is spared **(d)**. This observation suggests that ubiquitinated barstar would be capable of ‘turning over’ multiple copies of a USR-displaying barnase for proteasome-mediated degradation **(e)**.