Keeping proteasomes under control—a role for phosphorylation in the nucleus

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The 26S proteasome is the primary site for protein breakdown in the nucleus and cytosol of eukaryotic cells. This 60-subunit proteolytic machine selectively degrades ubiquitinated proteins. These substrates initially bind to its 19S regulatory complex, which is located at the ends of the barrel-shaped 20S core proteasome, within which proteins are hydrolyzed to small peptides. This ATP-driven process involves several different enzymatic steps including substrate binding, deubiquitination, unfolding, and translocation into the 20S particle (Fig. 1). However, only recently have clear insights emerged about how these multiple processes are coordinated and how proteasomes become localized to different cellular compartments. For example, recent studies show that binding of ubiquitinated proteins activates the proteasome's degradative machinery (1). Nevertheless, many ubiquitinated proteins bind to the 26S but escape proteolysis, only to be deubiquitinated and released (2). These observations of inherent regulation make it likely that there are also exogenous cellular mechanisms that regulate proteasome activity.

A number of studies have reported that proteasome subunits can be modified by phosphorylation, glycosylation, ubiquitination, or proteolysis by caspases. However, the physiological significance of these posttranslational modifications and their effects on proteasome function has been unclear. Unfortunately, most of these intriguing findings have not yet been reproduced by other investigators. During the past two decades, proteasome phosphorylation has often been reported, but it remains unclear whether such modifications actually perturb rates or selectivity of protein degradation. Various subunits have been shown to be phosphorylated *in vivo* including four of the seven α -subunits that comprise the outer rings of the 20S particle, $\alpha 7$, $\alpha 3$, $\alpha 2$, and $\alpha 4$ (3–7), and two of the six ATPases, Rpt6 (8–11) and Rpt2 (12), that comprise a ring at the base of the 19S complex. Because these rings lie adjacent to each other, their interactions are very likely to be influenced by phosphorylation and dephosphorylation.

Given the importance of the proteasome and protein phosphorylation in regulating other cellular processes, it is surprising that so little is known about the role of phosphorylation in modulating

Fig. 1. Role of the 26S proteasomes in degrading ubiquitinated proteins. Polyubiquitinated substrates initially bind to subunits Rpn10 and Rpn13 and become deubiquitinated. This process activates proteolysis (24). Substrates are unfolded by the six ATPase subunits (Rpt1–6), which open the gated pore in the α -ring to allow translocation into the 20S core, where they are cleaved to peptides by the six active sites on β -subunits. This illustration is adapted from the EM images provided by Wolfgang Baumeister (Max Planck Institute, Martinsried, Germany) (25).

proteasome function. Various kinases have been reported to copurify with proteasomes including casein kinase II (7, 13), Polo-like kinase (4), and calmodulin-dependent protein kinase II (CAMKII) (14), although it is unclear how these kinases bind. Several groups have found that dephosphorylation *in vitro* inhibits the hydrolysis of small peptides by 20S proteasomes (3, 15) and 19S ATPase activity (16). However, very few studies analyzed whether phosphorylation affects degradation of the proteasome's natural substrates, ubiquitinated proteins. Recently, Zhang et al. found in cells that cAMP-dependent protein kinase (PKA) phosphorylates Rpt6 and thus enhances degradation of the fusion protein, GFP-CL1 (8), whereas Djakovic et al. reported that CAMKII phosphorylates Rpt6 and enhances degradation of both GFP-CL1 and GFP-ODC (11), whose degradation does not require ubiquitination.

In PNAS, Guo et al. (17) present further evidence for a key regulatory role for phosphorylation of a proteasome subunit and identify a phosphatase, UBLCP1, that binds to the 26S proteasome. This phosphatase UBLCP1 contains a ubiquitin-like (Ubl) domain that binds to the 19S subunit Rpn1. Surprisingly, the

knockdown of UBLCP1 was found to promote the degradation of a model substrate in the nucleus, but not in the cytoplasm. Thus, subunit phosphorylation can facilitate the degradation of specific substrates by altering proteasome function in only one compartment. Future studies of the turnover of different substrates and total cell protein degradation are clearly necessary to clarify the importance of phosphorylation–dephosphorylation in regulating proteasome activity.

Another significant finding in this study is that subunit phosphorylation enhances proteolysis by promoting the association of the 19S regulatory particle with the 20S proteasome to form the 26S complex, which catalyzes the degradation of most cell proteins. There were prior indications that phosphorylation promotes 26S assembly (10, 18) and that dephosphorylation of Rpt6 in purified proteasomes promoted their dissociation into 20S and

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19S components by disrupting the interaction between Rpt6 and $\alpha 2$ (10). Conversely, phosphorylation by PKA appears to promote 26S in vitro and in vivo (18). The present study provides compelling evidence that phosphorylation promotes 26S assembly in vivo and that this critical step is under physiological regulation. Because the most frequent sites of phosphorylation are on the α -subunits and ATPases, which associate to form the 26S complex, it is conceivable that their phosphorylation modulates total 26S content and thereby controls overall rates of proteolysis. However, further evidence is needed to show that phosphorylation indeed regulates the degradation of multiple polyubiquitinated substrates and affects proteolysis generally.

In addition to enhancing 26S assembly, phosphorylation of α - and Rpt-subunits might also affect 26S function by altering ATPase activity, which regulates gate opening in the 20S complex as well as substrate unfolding and translocation. During protein degradation, the ATPases' C termini dock into pockets between the α -subunits and trigger opening of the gated channel for substrate entry (19). This gate is formed by the N termini of the α -subunits, and possibly phosphorylation influences gate opening and thus may contribute to the enhanced peptide hydrolysis noted earlier (20). Although the phosphorylation sites on $\alpha 7$ were identified as serine residues close to the C terminus (6, 7) and thus are not within the gating residues, their phosphorylation might alter $\alpha 7$ conformation (and possibly

that of adjacent subunits) to influence substrate entry.

During proteolysis, the ATPases function in an ordered, cyclical manner, and the resulting conformational changes drive gate opening, substrate unfolding, and translocation (21). Consequently, factors that influence the proteasome's ATPase activity are of particular interest. O-GlcNAcylation of Rpt2 has been reported to reduce 19S ATPase activity (22) and to be dominant over Rpt6 phosphorylation in affecting degradation. Dephosphorylation has also been reported to decrease ATP hydrolysis by purified 19S complexes (16), which further implies that phosphorylation of 19S subunits can enhance proteolysis by the 26S complex.

In addition to phosphorylation, other proteasome modifications have been shown to modulate different steps in substrate degradation. For instance, degradation of a polyubiquitinated protein begins with its binding to the 19S subunits, Rpn10 and Rpn13. This "receptor" protein, Rpn10, in yeast is itself subject to monoubiquitination, which reduces its affinity for ubiquitin conjugates (23) and thus decreases proteasome function. Although all these potential mechanisms for regulating protein breakdown merit in-depth investigation, there have been very few physiological conditions where global changes in rates of proteolysis have been demonstrated (except yeast in stationary phase or atrophying muscle), and none has been shown as yet to be due to altered proteasomes.

The study by Guo et al. shows a clear difference in proteasome function in different cellular compartments. The specific interaction between the UBLCP1 phosphatase and the 26S proteasome was demonstrated only in the nucleus and this selective association suggests that nuclear protein degradation can be specifically modulated by UBLCP1, without affecting this process elsewhere. In the nucleus, proteasomes have important roles, for example in the degradation of transcriptional regulators, in recovery from DNA damage, and perhaps also directly in gene transcription. In other cell compartments, proteasome function may be modulated by analogous mechanisms through interaction with specific kinases or phosphatases. In fact, recent studies in neurons indicate that CAMKII influences synaptic plasticity by modulating proteasome function through phosphorylation of Rpt6 and the recruitment of proteasomes to dendritic spines (11, 14).

Thus, protein turnover in cells is regulated not just through the activity of ubiquitin ligases, but also through control of the proteolytic machinery in different cellular compartments. The existence of mechanisms regulating proteasome function, especially through phosphorylation, also suggests that this process can be a target of pharmacological manipulation—not just to inhibit proteasome function, as has been used successfully to treat hematological cancers, but also perhaps to enhance the proteasome's degradative capacity to help clear the toxic proteins that cause many human diseases.

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