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Proteasomes: Machines for All Reasons

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Emerging data reveal that besides degrading proteins tagged with ubiquitin, the proteasome plays a more varied and decisive role in cellular regulation than previously imagined. In this issue, Hanna et al. (2007) expand our view of the proteasome by showing that under certain conditions, proteasome composition can be altered to control ubiquitin homeostasis.

In the strictest canonical model of proteasome function, the 26S proteasome selectively degrades proteins whose fates have been sealed by polyubiquitylation. The 26S proteasome is a 2.4 MDa complex composed of two multisubunit subcomplexes: a core protease, termed the 20S proteasome, and a regulatory element, termed PA700 or the 19S regulatory particle (Pickart and Cohen, 2004). The 20S proteasome is a 700 kDa complex composed, in eukaryotes, of two copies of 14 different gene products (α1–α7 and β1–β7) arranged in four axially stacked heptameric rings (α1–7, β1–7, β1–7, α1–7). The cylindrical structure contains two copies of each of three distinct catalytic subunits (β1, β2, and β5) whose active sites line a central lumen. Substrates reach this proteolytic chamber via 13 Å pores formed by the α subunit rings at either end of the cylinder (Baumeister et al., 1998). These pores, however, can be occluded by peptides from the amino termini of α subunits. PA700 mediates proteasome function, in part, by removing this occlusion and destabilizing the tertiary structure of protein substrates necessary for their passage through the narrow pores. PA700 is a 20-subunit complex that binds to either or both ends of the 20S cylinder, thereby positioning PA700 as a gatekeeper for substrate entry to the 20S proteasome. Although there is no crystal structure of PA700, its general architecture has been established by biochemical and imaging experiments (Ferrell et al., 2000). PA700 includes six distinct AAA-family ATPases (Rpt1–Rpt6) arranged in a hexameric ring that abuts axially to the outer α rings of the 20S proteasome (Smith and Goldberg, 2006). This ATP-dependent interaction promotes opening of pores and provides an access portal for substrates. Three non-ATPase subunits (Rpn1, 2, and 13) associate with the ATPase ring to form a subcomplex termed the "base." The remaining Rpn subunits constitute a separate subcomplex termed the "lid." Although the functions of most lid subunits are unknown, some display deubiquitylating activity, and one (Rpn10/S5a) features ubiquitin interaction motif (UIM) domains capable of binding to polyubiquitin. PA700 also displays chaperone-like activities for substrate destabilization and delivery to the proteolytic chamber. The overall process of 26S proteasome-catalyzed proteolysis depends on ATP hydrolysis. The exact energy-consuming steps in proteolysis remain unclear but are required when coupling deubiquitylation with degradation and plausibly could be linked to substrate unfolding, translocation, and deubiquitylation (Pickart and Cohen, 2004).

Proteasomes Exist in Multiple Structural Forms

The 26S proteasome, although commonly considered a single entity of invariant structure and dedicated function, exists as a heterogeneous group of structures with different functional features. Moreover, cells can regulate proteasome function in response to changing physiological demands both by altering the total number of proteasomes (Lecker et al., 2006) and by altering the subunit composition of proteasomes (Glickman and Raveh, 2005).

The 20S Proteasome Exists in at Least Two Forms

The 20S proteasome exists in at least two distinct forms that differ in their catalytic subunits. Higher eukaryotes contain two genes for each of the three catalytic subunits. Two of these genes (β1i and β5i) are encoded in the major histocompatibility locus and, with the third gene (β2i), are conditionally expressed and selectively incorporated into newly synthesized proteasomes instead of their constitutive counterparts under certain physiological states such as enhanced immune function (Baumeister et al., 1998). Proteasomes containing inducible catalytic subunits are termed "immunoproteasomes" as they participate in the production of some MHC class I antigenic peptides. Although antigen production also involves nonproteasomal events, immunoproteasomes display altered catalytic properties that favor production of certain class I peptides (Goldberg et al., 2002). Animals lacking genes for inducible catalytic subunits cannot produce these peptides, whereas overexpression of inducible genes enhances antigen production. Because class I peptides are derived from proteins degraded by a ubiquitin-dependent process, such results demonstrate that catalytic features of the 26S immunoproteasome represent an important regulatory determinant of the antigen production pathway.

Regulatory Complexes Bind to 20S Proteasomes

Most eukaryotic cells contain multiple proteins that bind directly to the outer α rings of 20S proteasomes as alternatives to PA700, thereby generating structurally different proteasome-regulatory complexes (Figure 1) (Schmidt et al., 2005). Although most of these proteins have defined biochemical effects on proteolytic properties of the proteasome, the precise physiological roles of the proteasome

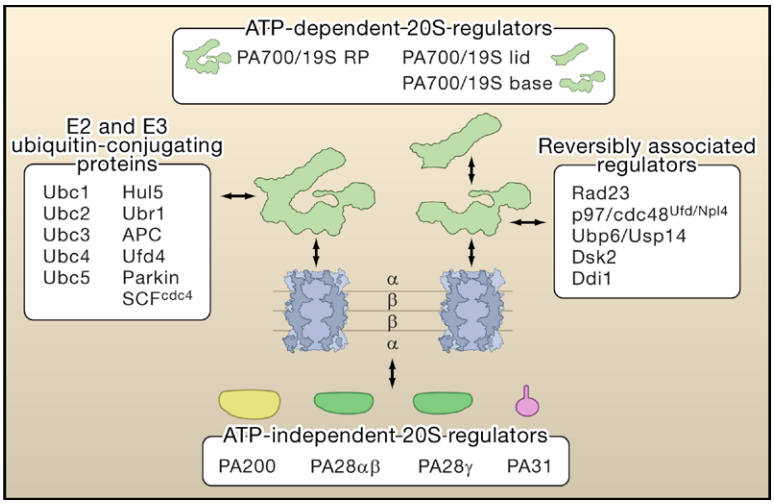

Figure 1. The Dynamic Proteasome
The proteasome is composed of a 20S core and a 19S regulatory subunit (PA700). The 20S core exists in at least two forms (constitutive and immuno), which differ in the composition of the catalytic subunits located on β rings. Many regulatory proteins (ATP-independent and ATP-dependent) that bind to 20S α rings affect the functions of the 20S proteasome and determine substrate specificity. PA700 or subcomplexes of it (base or lid) may function independently of the 20S proteasome in nonproteolytic roles. Other proteins—including ubiquitin-conjugating proteins, ubiquitin-chain-binding proteins, and deubiquitylating proteins—interact with the proteasome reversibly through interactions with proteasome regulators.

complexes they form remain largely unknown (Rechsteiner and Hill, 2005). Notably, unlike PA700, these alternative regulators are not ATPases and do not bind to polyubiquitin chains, suggesting that they may direct the proteasome in ubiquitin-independent proteolytic functions. The PA28 family of proteasome regulators illustrates these features particularly well. Mammals (and some other species, but not yeast) contain three homologous PA28 genes. PA28α and PA28β, are found in the MHC locus adjacent to the inducible 20S proteasome genes and are upregulated in response to cytokines such as interferon-γ (Rechsteiner et al., 2000). PA28α and PA28β proteins assemble into a heteromeric complex, whereas PA28γ, whose cellular regulation is unknown, forms a homoheptamer. Each of these ring-shaped complexes binds axially to the outer rings of the 20S proteasome and enhances proteasome activity by removing the occlusion at the proteasome pores. The molecular details of the PA28-proteasome interaction and the concomitant activation mechanism have been established by extensive biochemical studies and an informative cocrystal structure of the yeast 20S proteasome with a PA28 variant from trypanosomes (Glickman and Raveh, 2005). The exact relationship of this binding and activation mechanism to the corresponding mechanisms for other proteasome activators such as PA700 remains to be determined. Unlike PA700, PA28 enhances only the hydrolysis of short peptides and cannot specify the degradation of ubiquitylated proteins. Mice with disrupted PA28α and β genes are normal in most respects but have defective production of certain MHC class I antigens (Goldberg et al., 2002). The exact basis for this effect is unclear but may relate to altered catalytic specificity promoted by PA28 such that features of class I peptides are favored. Nonimmunological roles for PA28α and β also seem likely because of their wide expression and regulation under many physiological conditions, but they are still poorly understood. Mice with a disrupted PA28γ gene are smaller in size, and their cells display slower proliferation and increased susceptibility to apoptosis. The mechanistic basis of these effects is unknown, but the regulated degradation of at least one protein, steroid receptor coactivator-3, is blocked after reduction of PA28γ by RNAi (Li et al., 2006). These results support the general view that the type of regulatory protein associated with proteasomes can dictate biological outcomes of proteasome action.

Eukaryotes contain other proteins that bind to the outer rings of 20S proteasomes instead of PA700. PA200 and its yeast homolog, Blm10, contain HEAT repeats and activate proteasome hydrolysis of peptide substrates by relieving the occlusions at the proteasome's outer rings (Glickman and Raveh, 2005). Many physiological roles for PA200 have been reported, including DNA repair and response to stress, but most have been questioned subsequently. In addition, two proline-rich proteins, PI31 and Pr39, inhibit proteasome function in vitro by directly blocking 20S proteasome activity and attenuating binding of proteasome activators (Rechsteiner and Hill, 2005). The physiological functions of these proteins are unclear.

Hybrid Proteasomes Consist of Dissimilar Regulators

Because 20S proteasomes contain two identical outer α rings, a given molecule can accept two different regulators on opposite rings. Such combinatorial assembly could produce a large repertoire of proteasome structures featuring diverse catalytic properties to meet specific physiological demands. Although the cellular existence of proteasomes with all permutations of regulator combinations has not been established, some of these "hybrid" proteasomes display catalytic features that differ from those of their counterparts with only one type of regulator. For example, proteasomes containing PA700 and PA28 generate a qualitatively unique set of peptide products with characteristics of class I antigens. This form of proteasome could explain how the production of class I antigens can depend on both ubiquitin-dependent proteolysis and PA28. The mechanistic and physiological forces that govern the relative composition of various proteasome-regulator structures remain poorly understood but are likely to be highly regulated.

Reversibly Associated Proteins Regulate Proteasome Function

Regardless of their exact modular composition, proteasome-regulator complexes are usually depicted as defined structures containing stoichiometric levels of component subunits. However, proteasomes reversibly associate with many proteins whose proteasomal content is variable and

often substoichiometric (Schmidt et al., 2005). Several well-documented examples illustrate how such proteins are changing views of proteasome function and regulation.

A broad class of proteasome-associated proteins includes E2 ubiquitin-conjugating enzymes and E3 ubiquitin ligases that select substrates for ubiquitylation, indicating that substrate ubiquitylation and degradation can be physically and functionally coupled. Although molecular details of such coupling are largely lacking, it is easy to imagine how this arrangement could improve efficiency and specificity of degradation. In yeast, disrupted proteasomal binding of the ubiquitin ligase Ufd4 impairs proteolysis, showing that this interaction is functionally important. The ubiquitin ligase activity of Hul5 appears to be stimulated when it is bound to the proteasome, and ligase activity is closely tied to degradation of its substrates, further highlighting a tight relationship between proteasome-dependent ubiquitylation and degradation of substrates.

Many proteasome-associated proteins have functions intrinsic to the 26S proteasome, including polyubiquitin chain binding, deubiquitylation, and protein unfolding. Thus, functions canonically ascribed directly to the 26S proteasome might be supplemented by or subcontracted to reversibly associated proteins. For example, in yeast, deletion of Rpn10, the only well-characterized polyubiquitin-chain-binding subunit of the 26S proteasome, has little effect on global cellular protein degradation. Although other constituent proteasomal subunits might assume this role, proteasomes reversibly associate with multiple polyubiquitin-chain-binding proteins such as Rad23, Dsk2, Ddi1, the p97/cdc48$^{Ufp1/Npl4}$ complex, and p62. These proteins contain one or more of the domains capable of binding polyubiquitin (e.g., UBA domains) and N-terminal Ubl (*ub*iquitin-*l*ike) domains that reversibly bind to Rpn subunits of the PA700 base. Such proteins likely serve as "shuttles" for delivery of polyubiquitylated proteins to the proteasome. Cells lacking Rad23 display impaired degradation of some, but not all proteins, and Rad23 is required for 26S proteasome-catalyzed degradation of certain polyubiquitylated proteins in biochemically defined in vitro systems. In contrast, genetic deletion of both Rpn10 and Rad23 greatly impairs global protein degradation. These results indicate that cellular proteins are delivered to the proteasome by multiple routes, and that individual proteins may reach their proteasomal destinations by either dedicated or variable paths (Madura, 2004). The rules that govern such distinctions are not understood but seem likely to represent an important and complex element of proteasomal regulation. For example, some polyubiquitylated proteins may be transferred between several shuttle proteins before arriving at their proteasomal destination. The p97/cdc48$^{Ufp1/Npl4}$ complex, which is required for proteolysis of many polyubiquitylated proteins including those extracted from the endoplasmic reticulum, appears to transfer some of these proteins to Rad23, which then delivers them to the proteasome (Richly et al., 2005).

Deubiquitylation is a second important proteasome function supplemented by reversibly associated proteins. Removal of polyubiquitin chains from substrates is essential for proteolysis of polyubiquitylated proteins, and PA700 contains intrinsic deubiquitylation activities for this purpose (e.g., Rpn11) (Gutterman and Glickman, 2004). However, proteasomal deubiquitylation may be directed toward other means. Early work suggested that substrate deubiquitylation by Uch37, an intrinsic subunit of mammalian PA700, could also confer an editing function to the proteasome and spare certain proteins from degradation by prematurely removing the destruction tag. This mechanism further highlights the general notion that the proteasome actively participates in determining the fate of substrates that reach it. Ubp6 and its mammalian homolog Usp14 are deubiquitylating enzymes that bind reversibly to the base of the proteasome via their Ubl domains. The deubiquitylation activity of Ubp6/Usp14 is greatly enhanced upon proteasome binding and deletion of its Ubl domain mimics all features of the null mutant in yeast, showing that Ubp6 function is likely limited to the proteasome. Recent work shows that Ubp6 disassembles Hul5-generated polyubiquitin chains, providing further evidence that proteasome-associated substrate modifications determine the fate of proteins. Remarkably, Ubp6 also appears to regulate general aspects of both proteasome and ubiquitin homeostasis. Reduced catalytic function of Ubp6 due to an active site mutation or genetic deletion decreases total cellular ubiquitin levels as a consequence of increased ubiquitin degradation (as opposed to ubiquitin recycling) by the proteasome. In this issue of *Cell*, Finley and colleagues significantly expand our view of the functional and regulatory relationships among ubiquitin, the proteasome, and Ubp6 (Hanna et al., 2007). They show that yeast express catalytically inactive Ubp6 at higher levels than wild-type Ubp6. Genetic restoration of the otherwise reduced ubiquitin levels in these mutant strains reverts Ubp6 expression to normal levels. In contrast, genetic reduction of ubiquitin expression increases transcription of Ubp6 and increases the fraction of proteasomes associated with the protein, perhaps as a compensatory response to increase the supply of ubiquitin recovered from proteasomal processing. Cells that express catalytically inactive Ubp6 also have higher total levels of proteasome compared to wild-type strains. This effect appears to be mediated by the transcription factor Rpn4 and may reflect a response to the surprising inhibitory action of Ubp6 on proteasome activity. Rpn4 was originally identified as a putative proteasome subunit (hence its nomenclature) but subsequently was shown to be a rapidly degraded substrate. Inhibition of proteasome function increases Rpn4 content by sparing it from degradation; Rpn4 then acts as general transcription factor for proteasomal subunits via interaction with PACE (*P*roteasome-*A*ssociated *C*ontrol *E*lement) sequences common to proteasome genes (Xie and Varshavsky, 2001). Although a close structural homolog of Rpn4 has not been identified in higher eukaryotes, increased expression of proteasome subunits is a compensatory response to general proteasome inhibition in most cells. Remarkably, Hanna et al. (2007) show that Rpn4 is not necessary for increased

Ubp6 levels in response to decreased cellular ubiquitin content, suggesting an alternative transcriptional pathway for the regulation of ubiquitin levels. Thus, these results distinguish the general regulation of proteasome levels, "proteasome stress," from "ubiquitin stress," which selectively alters the proteasome composition; in the latter instance the selective assembly of proteasomes for selective tasks points to the high level of regulation that can be achieved through this adaptable complex. Many unresolved questions raised by the findings ensure future revelations about the important regulatory roles of the proteasome.

Nonproteolytic Functions of the Proteasome

Aside from its role as a protease, the proteasome also functions nonproteolytically in a variety of cellular processes including transcription, DNA repair, and chromatin remodeling. Any of the various subfunctions of the proteasome might be used for nonproteolytic processes, but the recurring identification of the ATPase subunits of PA700 as mediators of nonproteolytic functions suggests that chaperone-like activities form a general basis for these actions. Distinguishing nonproteolytic roles from closely intertwined mechanisms of proteasome-dependent proteolysis embedded in the same processes has been challenging.

Early studies in yeast identified specific Rpt mutant alleles as suppressors of a Gal4 transactivator mutant. Subsequent studies demonstrated at least two direct and genetically distinct nonproteolytic activities of PA700 in transcriptional initiation and elongation. The transcription-dependent localization of PA700 ATPases to Gal promoters and the demonstration that stable association of a transactivator with the histone acetyltransferase complex SAGA is driven by the ATPase activity of PA700 has further supported a cotransactivator role (Collins and Tansey, 2006).

PA700 also acts nonproteolytically in nucleotide excision repair (NER) (Reed and Gillette, 2007). Inhibition of proteasome-dependent proteolysis has no effect on NER activity in vitro, whereas inhibition of the ATPase activity of PA700 inhibits this activity in a Rad23-dependent manner. The Ubl domain of Rad23 is required for normal survival of yeast after exposure to UV light. The in vivo NER defect resulting from the loss of the Ubl domain of Rad23 is suppressed by specific mutations of ATPase subunits of PA700 independently of proteolysis. The same mutant alleles were originally identified as regulators of Gal gene transcription, suggesting a common mechanism of action for the two pathways. These results also indicate that reversibly associated proteasomal proteins such as Rad23 can mediate both proteolytic and nonproteolytic roles of the proteasome.

Chromatin remodeling is another nonproteolytic role of PA700, with implications for both transcription and DNA repair. PA700 ATPases mediate gene silencing at telomeres by a mechanism involving regulation of nucleosomal histone modification (Collins and Tansey, 2006). Specifically, ATPase subunits of PA700 control histone H3 methylation in response to histone H2B ubiquitylation. A global role for proteasomal ATPases in modulating chromatin structure is consistent with several reports showing widespread distribution of proteasomal components associated with chromatin. Whereas many of these interactions might result in proteolytic functions for the 26S proteasome, differential occupancy of PA700 and 20S proteasomal subcomplexes on DNA indicate that others represent nonproteolytic functions (Sikder et al., 2006). Moreover, transcription from a number of genes is modulated differentially and sometimes in opposing fashion by mutations in PA700 and 20S proteasome subunits. Perhaps proteolytic and nonproteolytic processes can be spatially and temporally separated by the regulated assembly and disassembly of the proteasome at specific sites during transcription and DNA repair.

The proteasome, once considered a static garbage disposal unit for cellular waste, now is recognized as a multifaceted mediator of many essential cellular processes via proteolytic and nonproteolytic mechanisms. The modular and dynamic composition of the proteasome and its multiple regulators allows proteasome subtypes to be adapted to a wide array of physiological roles.

REFERENCES

Baumeister, W., Walz, J., Zühl, F., and Seemüller, E. (1998). Cell *92*, 367–380.

Collins, G.A., and Tansey, W.P. (2006). Curr. Opin. Genet. Dev. *16*, 197–202.

Ferrell, K., Wilkinson, C.R.M., Dubiel, W., and Gordon, C. (2000). Trends Biochem. Sci. *25*, 83–88.

Glickman, M.H., and Raveh, D. (2005). FEBS Lett. *579*, 3214–3223.

Goldberg, A.L., Cascio, P., Saric, T., and Rock, K.L. (2002). Mol. Immunol. *39*, 147–164.

Guterman, A., and Glickman, M.H. (2004). Curr. Protein Pept. Sci. *5*, 201–211.

Hanna, J., Meides, A., Zhang, D.P., and Finley, D. (2007). Cell, this issue.

Lecker, S.H., Goldberg, A.L., and Mitch, W.E. (2006). J. Am. Soc. Nephrol. *17*, 1807–1819.

Li, X., Lonard, D.M., Jung, S.Y., Malovannaya, A., Feng, Q., Qin, J., Tsai, S.Y., Tsai, M.J., and O'Malley, B.W. (2006). Cell *124*, 381–392.

Madura, K. (2004). Trends Biochem. Sci. *29*, 637–640.

Pickart, C.M., and Cohen, R.E. (2004). Nat. Rev. Mol. Cell Biol. *5*, 177–187.

Rechsteiner, M., and Hill, C.P. (2005). Trends Cell Biol. *15*, 27–33.

Rechsteiner, M., Realini, C., and Ustrell, V. (2000). Biochem. J. *345*, 1–15.

Reed, S.H., and Gillette, T.G. (2007). DNA Repair (Amst.) *6*, 149–156.

Richly, H., Rape, M., Braun, S., Rumpf, S., Hoege, C., and Jentsch, S. (2005). Cell *120*, 73–84.

Schmidt, M., Hanna, J., Elsasser, S., and Finley, D. (2005). Biol. Chem. *386*, 725–737.

Sikder, D., Johnston, S.A., and Kodadek, T. (2006). J. Biol. Chem. *281*, 27346–27355.

Smith, D.M., and Goldberg, A.L. (2006). J. Struct. Biol. *156*, 72–83.

Xie, Y., and Varshavsky, A. (2001). Proc. Natl. Acad. Sci. USA *98*, 3056–3061.