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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

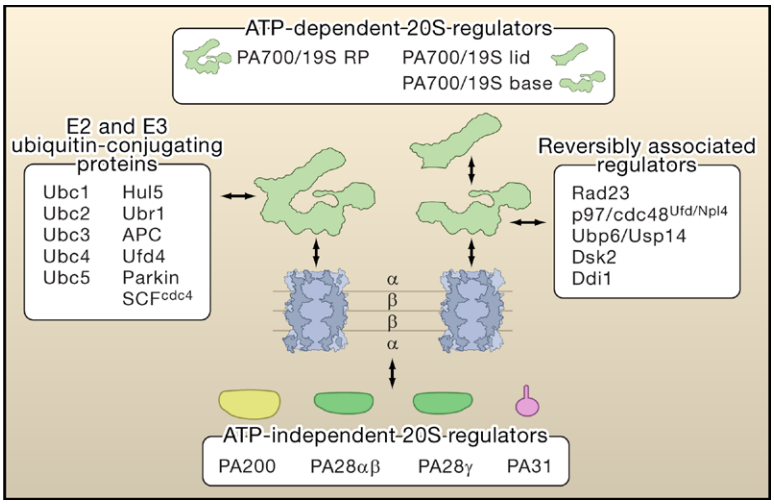

Figure 1. The Dynamic Proteasome
The proteasome is composed of a 20S core and a 19S regulatory subunit (PA700). The 20S core exists in at least two forms (constitutive and immuno), which differ in the composition of the catalytic subunits located on β rings. Many regulatory proteins (ATP-independent 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