Functions of the Proteasome: The Lysis at the End of the Tunnel

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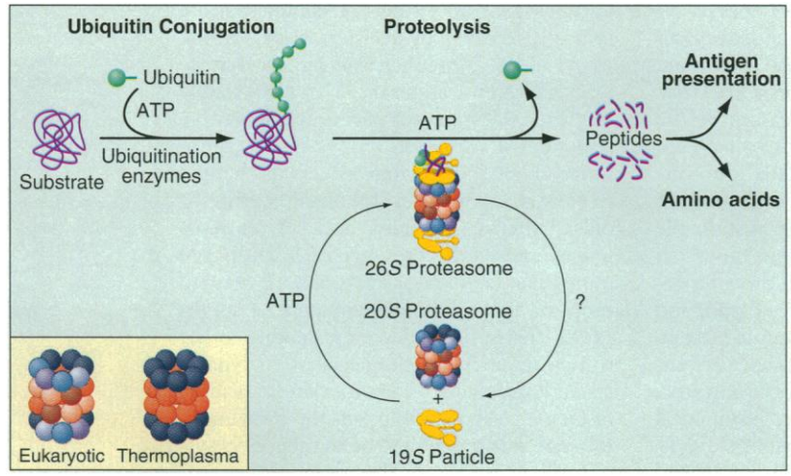
Most proteins in eukaryotic cells are degraded by a soluble, adenosine triphosphate (ATP)–dependent pathway present in both the nucleus and cytosol (*1*, *7*). Before proteolysis, proteins are usually first covalently conjugated to multiple molecules of the polypeptide ubiquitin (*1*). This modification marks them for rapid hydrolysis by a 26*S* (2000-kilodalton) complex, which contains as a key proteolytic component the 20*S* proteasome (*2*)—the x-ray structure of which is now reported by Löwe and co-workers in this issue (*12*).

This non-lysosomal pathway was first discovered almost 20 years ago as mediating the rapid elimination of highly abnormal proteins that arise by mutation or postsynthetic damage (*1*). This pathway is now known to degrade many critical regulatory proteins that must be rapidly destroyed for normal growth and metabolism (*1*). For example, progression through the cell cycle requires the programmed ubiquitination and destruction of the mitotic cyclins (*3*). Moreover, alterations in the stability of regulatory proteins can have major consequences: Mutations that prevent proteasome degradation of the Jun oncoprotein lead to tumors (*4*), and papilloma virus induces tumors by stimulating ubiquitin-mediated degradation of the tumor suppressor p53 (*5*). In several instances, phosphorylation of a protein triggers its ubiquitination and subsequent degradation. For example, the production of many inflammatory mediators is initiated by the phosphorylation and rapid breakdown of the critical inhibitory factor IκB (*6*). Its elimination allows the generation of the active form of the transcription factor NF-κB by limited proteolysis of a large inactive precursor. The process also requires ubiquitin and the 26*S* proteasome. Thus, this pathway can catalyze also the signal-induced destruction of specific portions of cell proteins.

The slower degradation of most cell proteins is also by the proteasome (*7*) and is precisely regulated (*8*). During fasting, infection, or cancer, mammals mobilize amino acids from muscle protein by hormone-induced stimulation of the ubiquitin-proteasome pathway (*8*). The immune system uses this pathway to screen the cytosol for abnormal (viral) antigens (*7*, *9*). Generally, proteasomes degrade proteins to small peptides, most of which are rapidly hydrolyzed to amino acids by cytosolic exopeptidases. However, some of the oligopeptides generated by proteasomes, especially in response to interferon, are presented to the immune system on major histocompatibility complex (MHC) class I molecules (*7*, *9*).



Regulatory functions of the proteasome.

The 20*S* particles, which comprise about 1 percent of the protein in mammalian cells (*3*), were discovered about 1980 in several contexts (*3*). In 1989 these various particles were shown to be identical and to function as the proteolytic core of the 26*S* complex (*10*). This larger structure includes a 19*S* particle, which contains five different ATPases and a binding site for ubiquitin chains (*11*). The 19*S* complex serves as the "mouth" that engulfs substrates, stimulates the peptidase activity of the 20*S* proteasome (*11*), and probably functions as a chaperone to unfold the polypeptides and inject them into the degradative tunnel.

Two articles in this issue (*12*, *13*) from the laboratories of Huber and Baumeister describe major advances in our understanding of this degradative apparatus. They report the x-ray crystallographic analysis of the 20*S* proteasome from the archaebacterium *Thermoplasma acidophilum* (*2*). Like the eukaryotic proteasome, it contains four stacked rings, each composed of seven subunits (*3*, *14*). Its marked symmetry and the extreme stability of enzymes from thermophilic organisms have allowed its crystallization, which has proven impossible for the more complex eukaryotic proteasomes. In the archaebacterium, the outer rings contain a single type of α subunit and the central rings a single β subunit, whereas in eukaryotes the seven α and seven β subunits are distinct (but related) gene products (*3*) (see inset).

For intracellular proteolysis to be extremely selective, safeguards must exist that prevent nonspecific digestion of cell constituents. Löwe and coworkers (*12*) demonstrated that the archaebacterial proteasome has 14 active sites in the inner channel, one on each β subunit. Thus, the hydrolytic sites are geographically isolated from the intracellular milieu (just as lysosomal proteases are segregated within membrane vacuoles). Because only unfolded proteins can enter the small opening in its central channel (*14*), the α rings form a barrier that helps prevent nonspecific proteolysis. Presumably, this entry point is a site of regulation, and the 19*S* complex and other factors that stimulate the 20*S* complex (*3*, *11*) may temporarily enlarge this opening. Also, the requirement for ubiquitination probably evolved in eukaryotes to ensure that polypeptide entry into the 20*S* proteasome is highly selective. However, alternative modes of regulation of this entry step appear likely, because not all substrates require ubiquitination and because 20*S* proteasomes clearly exist in archaebacteria and have recently been discovered in eubacteria (*13*), where neither 26*S* complexes nor ubiquitin are seen.

The hydrolytic mechanism of the 20*S* proteasome has long been a mystery. Although all eukaryotic and prokaryotic α and β subunits are homologous (*12*, *13*), they show no sequence similarities to those of other proteases. Also, the proteasome's sensitivity to inhibitors differs from those of the classical families of proteases. Traditionally, enzymologists have divided such enzymes into four classes: the serine and cysteine proteases, whose hydroxyl or thiol groups serve as the active-site nucleophile that attacks peptide bonds, and the metalloproteases and acidic proteases, where the critical nucleophile is a bound

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water molecule. Proteasomes had been suggested to be an unusual type of serine protease; however, systematic mutagenesis (*13*) indicated that no serine was essential for their activity (*14*). The data (*12, 13*), reported in this issue, demonstrate a new type of proteolytic mechanism in which the active-site nucleophile is the hydroxyl group on the threonine at the amino-terminus of the β subunit. Competitive inhibitors associate with this threonine (*2*), and mutagenesis of this residue to an alanine prevents activity (*13*). Furthermore, Fenteany and co-workers (*15*) have made the unexpected discovery that the antibiotic lactacystin and related lactones are specific, irreversible proteasome inhibitors that covalently bind to the terminal threonine on the β subunit of mammalian proteasomes. Thus, the proteolytic mechanisms of the mammalian and *Thermoplasma* particles appear similar, as would be expected from the strong conservation of primary and quarternary structures (*2, 13*). In the archaebacterial proteasome, replacement of the terminal threonine by a serine allows full proteolytic activity (*13*), so it is unclear why threonines (and not serines) have been conserved in the active sites of proteasomes from bacteria to man.

Unlike bacteria, eukaryotic proteasomes contain diverse subunits that allow specialized catalytic functions and important regulatory opportunities. Mammalian proteasomes exhibit up to five different peptidase activities, including sites that preferentially cleave after basic, hydrophobic, or acidic residues (*3*). Which β subunits catalyze these different activities is still unclear, and because three or four mammalian β subunits lack terminal threonine residues (*13*), some probably serve structural rather than hydrolytic functions. The particular β subunits comprising a mammalian proteasome determine its functional properties and are regulated by cytokines (*9, 16*). Interferon-γ, which enhances antigen presentation, induces the expression of three β subunits, including the MHC-encoded proteins LMP-2 and -7 (*10*). These subunits are incorporated into proteasomes in place of homologous, normal subunits. The resulting proteasomes cleave preferentially after hydrophobic and basic residues. Thus, interferon favors the production of oligopeptides with hydrophobic or basic carboxyl-termini—exactly those peptides that are preferentially transported into the endoplasmic reticulum and that bind tightly to MHC class I molecules. Thus, during the evolution of the immune system, the phylogenetically ancient proteasome has undergone adaptations that favor antigen presentation (*9, 16*) and probably additional adaptations that are important in other physiological situations.

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From the Cradle to the Grave: Ring Complexes in the Life of a Protein

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It is an irony of nature that the chaperonin GroEL, which promotes protein folding, and the proteasome, which catalyzes protein degradation, have strikingly similar architectures. Viewed at the modest resolution of the electron microscope, it takes a skilled eye to distinguish between the two complexes. Both are cylindrical ring structures measuring 110 to 150 Å in length and diameter. Along the cylindrical axis, the two complexes exhibit sevenfold rotational symmetry around a large central cavity, and from the side both appear to have four stacked layers. However, when revealed at near-atomic resolution by the crystal structure of the 20S proteasome from the archeon *Thermoplasma acidophilum*, reported in this week's issue of *Science* (*1*), and by the recently reported crystal structure of GroEL (*2*), fundamental differences come into focus. As suggested by the lack of primary sequence homology, there is no similarity in the secondary or tertiary structures of the two complexes. These differences reflect the particular functional requirements of the distinct processes these assemblies carry out.

The 20S proteasome is composed of 28 subunits that form four stacked heptameric rings. The rings of eukaryotic proteosome are composed of up to 14 different species of subunits, while those of the archeon *T. acidophilum* are homo-oligomers of either α- or β-type subunits, arranged with two outer rings of seven α subunits each and two inner rings of seven β subunits (see figure). Löwe and co-workers took advantage of the simplicity of this 20S complex in their structural studies. Lest one not be left wondering whether the interesting cavity structure formed by these rings (see figure) is in fact the site of action, they also examined the structure of the proteasome with a bound peptide-aldehyde inhibitor. These studies revealed 14 catalytic sites deep within the central cavity and suggested an unusual proteolytic mechanism in which the hydroxyl group of the β subunit's amino-terminal threonine acts as the nucleophile in peptide hydrolysis. This mechanism is supported by mutagenesis experiments reported in an accompanying paper (*4*) and by the observation that a natural inhibitor of the proteasome, lactacystin (*5*), covalently modifies the amino-terminal threonine.

The action that occurs inside GroEL is of a different sort—regulated binding instead of cleavage. Unfolded proteins are held, at least in part, within the central cavity formed by the two seven-membered rings. Although many questions remain about the mechanism of GroEL action (*6*), in particu-

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