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.

References

1. A. Ciechanover, *Cell* **79**, 13 (1994); S. Jentsch, *Annu. Rev. Genet.* **26**,177 (1992); A. Hersko and A. Ciechanover, *Annu. Rev. Biochem.* **61**, 761 (1992).
2. J. M. Peters, *Trends Biochem. Sci.* **19**, 377 (1994); A. L. Goldberg, *Eur. J. Biochem.* **203**, 9 (1992); A. J. Rivett, *Biochem. J.* **291**, 1 (1993); K. Tanaka *et al.*, *New Biol.* **4**, 1 (1992); S. Wilk, *Enzyme & Protein* **47**, 306 (1993); W. Heinemeyer *et al., Biochemistry* **33**, 1229 (1994).
3. E. Eytan *et al., J. Biol. Chem.* **268**, 4668 (1993); R. W. King, P. K. Jackson, M. W. Kirschner, *Cell* **79**, 563 (1994); W. Seufert, B. Futcher, S. Jentsch, *Nature* **373**, 78 (1995); A. Hershko *et al., J. Biol. Chem.* **269**, 4940 (1994); R. W. King *et al., Cell,* in press (1995).
4. M. Treir, L. M. Stoszewski, D. Bohmann, *Cell* **78**, 787 (1994).
5. M. Scheffner *et al., ibid.* **63**, 1129 (1990).
6. V. J. Palombella *et al., ibid.* **78**, 773 (1994); V. J. Palombella *et al.*, unpublished observations.
7. K. L. Rock *et al., Cell* **78**, 761 (1994).
8. S. S. Wing and A. L. Goldberg, *Am. J. Physiol.* **64**, E668 (1993); R. Medina, S. S. Wing, A. L. Goldberg, *Biochem. J.* **307**, 639 (1995); S. S. Wing, A. L. Haas, A. L. Goldberg, *ibid.* **307**, 631 (1995); V. E. Baracos *et al., Am. J. Physiol.*, in press.
9. M. Gaczynsksa *et al., Proc. Natl. Acad. Sci. U.S.A.* **91**, 9213 (1994); M. Gaczynsksa, K. L. Rock, A. L. Goldberg, *Enzyme & Protein* **47**, 354 (1993); *Nature* **365**, 264 (1993); A. L. Goldberg and K. L. Rock, *ibid.* **357**, 375 (1992); J. Driscoll *et al., ibid.* **365**, 262 (1993); N. Shimbara *et al., J. Biochem.* **115**, 257 (1994).
10. E. Eytan *et al., Proc. Natl. Acad. Sci. U.S.A.* **86**, 7751 (1989); J. Driscoll and A. L. Goldberg, *J. Biol. Chem.* **265**, 4789 (1990).
11. M. Rechsteiner, L. Hoffman, W. Dubiel, *J. Biol. Chem.* **268**, 6065 (1993); G. N. Demartino *et al., ibid.* **269**, 20878 (1994); M. Chu-Ping *et al., ibid.* **269**, 3539 (1994); J. M. Peters *et al., J. Mol. Biol.* **234**, 932 (1993); T. Yoshimura *et al., J. Struct. Biol.* **111**, 200 (1993).
12. J. Löwe *et al., Science* **268**, 533 (1995).
13. E. Seemüller *et al., ibid.*, p. 579.
14. P. Zwickl, J. Kleizn, W. Baumeister, *Nature Struc. Biol.* **1**, 765 (1994); T. Wenzel and W. Baumeister, *ibid.* **2**, 199 (1995); E. Seemüller *et al., FEBS Lett.* **359**, 173 (1995).
15. G. Fenteany *et al., Science*, in press.
16. H. J. Fehling *et al., ibid.* **265**, 1234 (1994); L. Van Kaer *et al., Immunity* **1**, 533 (1994).

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

Jonathan S. Weissman, Paul B. Sigler, Arthur L. Horwich

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-

J. S. Weissman and A. L. Horwich are at the Howard Hughes Medical Institute and in the Department of Genetics, Yale University School of Medicine, New Haven, CT 06510, USA. P. B. Sigler is at the Howard Hughes Medical Institute and in the Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, CT 06510, USA.

lar, the extent to which a protein folds while residing in the GroEL cavity, recent studies indicate that GroEL undergoes cycling between states of high and low affinity for peptide, which are driven by the hydrolysis of adenosine triphosphate (ATP) and the binding and release of the heptameric co-chaperonin molecule, GroES (*7*, *8*). These cycles result in cycles of binding and release of substrate proteins with a portion of the molecules folding during each round of release (*7*, *9*).

Both GroEL and the proteasome exploit the ring architecture to create an internal protected environment that houses the functional sites and protects them from interacting with ambient proteins, as well as each other. In the proteasome, these functional domains are the catalytic sites, and placing them inside the ring prevents the indiscriminate hydrolysis of folded cellular proteins. In GroEL, the functional domains are the hydrophobic patches that form the polypeptide binding site (*10*). Their location inside the ring prevents the patches from interacting with each other and aggregating GroEL.

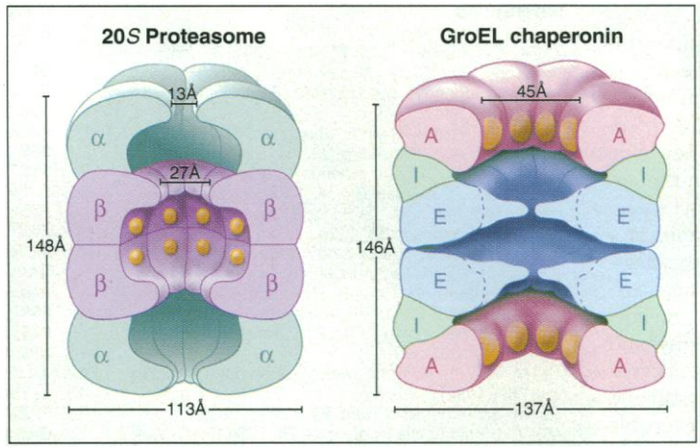

ILLUSTRATION: K. SUTLIFF

Ring complexes: The 20*S* proteasome and GroEL. (**Left**) The 20*S* proteasome is composed of four stacked, seven-membered rings. In the 20*S* proteasome from *T. acidophilum* (*7*) , the two outer rings comprise seven copies of a 25.9-kilodalton α subunit, and the two inner rings comprise seven copies of a 22.3-kilodalton β subunit. These rings form a central channel with three chambers: Two antechambers flank an inside chamber that houses the catalytic sites (yellow spheres). (**Right**) The GroEL chaperonin is a homo-oligomer formed by two seven-membered rings of 57-kilodalton subunits stacked back to back. The GroEL rings form a wide channel open at either end of the cylinder but likely to be blocked at the equator (*2*). The GroEL subunit is composed of an apical (A), intermediate (I), and equatorial (E) region. The putative polypeptide binding sites (yellow ovals) are hydrophobic patches on the inside face of each apical domain.

The ring motif is also exploited by the proteasome and GroEL to produce an array of functional sites that can act cooperatively on different regions of a single substrate. For example, although the proteasome has broad proteolytic specificity, the resulting peptides fall into a narrow size range, seven to eight amino acids (*11*). The length spanned by a peptide of this size in an extended conformation is ~28 Å, which corresponds to the distance separating catalytic sites in adjacent monomers in the crystal structure. This "molecular ruler" model is also supported by biochemical studies indicating that polypeptide substrates can be channeled between different catalytic sites within a single 20S complex (*12*). This channeling would be facilitated if the rate of proteolysis were slow relative to binding. Interestingly, classic studies (*13*) suggest that the same feature that allows the proteasome to act on the protein with broad specificity may also result in a relatively low degradation rate.

A fundamental difference between the GroEL and proteasome rings is that the entrance to the GroEL cavity is unobstructed. This large opening (~45 Å) allows ready access to the peptide-binding regions and presumably reflects the need for a large number of substrates to bind to GroEL shortly after synthesis. By contrast, most proteins must be protected from entering the proteasome cavity, since this would lead to irreversible degradation. Accordingly, the structure of the proteasome reveals an elaborate gating mechanism consisting of four narrow axial constrictions lined with hydrophobic residues. These constrictions create three distinct cavities (see the figure)—two antechambers located on opposite sides of a central chamber that houses the catalytic sites. Thus a polypeptide must pass through two narrow constrictions (*14*) before it can be hydrolyzed, explaining the preference of the 20S particle for unfolded substrates.

In the 26S proteasome, the 20S particle functions together with the 19S complex in the ATP-dependent degradation of ubiquitinated proteins. This requirement for ATP hydrolysis suggests that the 19S partner of the proteasome actively unfolds appropriate substrates in a chaperone-like manner.

Although unfolding would give the protein enough flexibility to pass through the narrow constrictions into the central cavity, it is not clear how the complete degradation of a polypeptide chain is achieved. Given the limited space in the catalytic chamber, it is likely that the substrate must be fed continually into the inner chamber and processively proteolyzed. The antechamber is well situated to store a portion of the substrate, maintaining it in an unfolded state as it awaits entry into the catalytic inner chamber. Threading into the inner chamber could be driven by binding to multiple catalytic sites. The effective force generated by this binding might be quite great, even if the affinities of the individual sites are relatively low, because the reactants confined to a box with 45 Å sides will have an apparent concentration of ~ 100 mM.

Despite the diametric differences in the reactions carried out by the proteasome and GroEL, these complexes both use ring structures to create a cavity that houses the active sites, allowing them to act on unfolded substrates in a protected environment. The differences in function so apparent in the structures reflects the need for the proteasome to sequester these sites behind an elaborate gating system, while for GroEL this cavity must be readily accessible to trap nonnative peptides before they aggregate. The gating in the proteasome, however, presents certain logistical problems. For example, how are hydrolyzed peptides removed from the central cavity? The only apparent egress is through the ends of the cylinder since, unlike in GroEL, there are no significant side windows. Also, how is commitment to the complete degradation of a polypeptide chain achieved when ubiquitination is confined to a limited number of positions along the substrate? Conversely, what is the mechanism by which this commitment is terminated at a specific residue, for example, in the proteasome-mediated partial degradation of the p105 subunit of NFκB to p50 (*15*)? With the rapid pace of biochemical analysis of the proteasome aided now by the existence of a crystal structure, the answers to these and other questions are likely to be forthcoming.

References

1. J. Löwe *et al.*, *Science* **268**, 533 (1995).
2. K. Braig *et al.*, *Nature* **371**, 578 (1994).
3. A. L. Goldberg, *Eur. J. Biochem.* **203**, 9 (1992). A. Hershko, A. Ciechanover, *Annu. Rev. Biochem.* **61**, 761 (1992); M. Rechsteiner, L. Hoffman, W. Dubiel, *J. Biol. Chem.* **268**, 60 (1993); J.-M. Peters, *Trends Biochem. Sci.* **19**, 377 (1994).
4. E. Seemüller *et al.*, *Science* **268**, 579 (1995).
5. G. Fenteany *et al.*, *ibid.*, p. 726.
6. F. U. Hartl and J. Martin, *Curr. Opin. Struct. Biol.* **5**, 92 (1995); G. H. Lorimer, *Structure* **2**, 1125 (1994).
7. M. J. Todd, P. V. Viitanen, G. H. Lorimer, *Science* **265**, 659 (1994).
8. J. Martin, M. Mayhew, T. Langer, F. U. Hartl, *Nature* **366**, 228 (1993).
9. J. S. Weissman, Y. Kashi, W. A. Fenton, A. L. Horwich, *Cell* **78**, 693 (1994).
10. W. A. Fenton, Y. Kashi K. Furtuk, A. L. Horwich, *Nature* **371**, 614 (1994).
11. T. Wenzel, C. Eckerskorn, F. Lottspeich, W. Baumeister, *FEBS Lett.* **349**, 205 (1994).
12. L. R. Dick, C. R. Moomaw, G. N. Demartino, C. A. Slaughter, *Biochemistry* **30**, 2725 (1991).
13. J. R. Knowles, *J. Theor. Biol.* **9**, 213 (1965).
14. T. Wenzel and W. Baumeister, *Nature Struct. Biol.* **2**, 199 (1995).
15. V. J. Palombella, O. J. Rando, A. L. Goldberg, T. Maniatis, *Cell* **78**, 773 (1994).