Biochemical and Biophysical Research Communications **267,** 1–6 (2000)
doi:10.1006/bbrc.1999.1706, available online at http://www.idealibrary.com on IDEAL®

BREAKTHROUGHS AND VIEWS

Degradation of Ornithine Decarboxylase by the 26S Proteasome

Yasuko Murakami,* Senya Matsufuji,* Shin-ichi Hayashi,*
Nobuyuki Tanahashi,† and Keiji Tanaka†

**Department of Biochemistry 2, Jikei University School of Medicine, 3-25-8, Nishi-shinbashi, Minato-ku, Tokyo 105-8461, Japan; and †Tokyo Metropolitan Institute of Medical Science, and CREST, Japan Science and Technology Corporation (JST), 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8613, Japan*

Received October 12, 1999

Ornithine decarboxylase (ODC) is a key enzyme in polyamine biosynthesis. Turnover of ODC is extremely rapid and highly regulated, and is accelerated when polyamine levels increase. Polyamine-stimulated ODC degradation is mediated by association with antizyme (AZ), an ODC inhibitory protein induced by polyamines. ODC, in association with AZ, is degraded by the 26S proteasome in an ATP-dependent, but ubiquitin-independent, manner. The 26S proteasome irreversibly inactivates ODC prior to its degradation. The inactivation, possibly due to unfolding, is coupled to sequestration of ODC within the 26S proteasome. This process requires AZ and ATP, but not proteolytic activity of the 26S proteasome. The carboxyl-terminal region of ODC presumably exposed by interaction with AZ plays a critical role for being trapped by the 26S proteasome. Thus, the degradation pathway of ODC proceeds as a sequence of multiple distinct processes, including recognition, sequestration, unfolding, translocation, and ultimate degradation mediated by the 26S proteasome. © 2000 Academic Press

Ornithine decarboxylase (ODC) is the first and rate-limiting enzyme in a metabolic pathway for polyamine biosynthesis (1, 2). Polyamines are essential for normal cell growth but cytotoxic in excess, therefore, cells have developed several ways to increase the levels of polyamines rapidly and transiently. ODC is induced dramatically in response to various growth stimuli. It turns over with the shortest half-life of all known enzymes ranging from minutes to more than one hour. Instability of ODC is strongly augmented when cellular polyamine levels increase. Several unique features for molecular mechanisms underlying the polyamine-regulated ODC degradation in mammalian cells have been documented (3, 4). First, polyamine-enhanced degradation is mediated exclusively by antizyme (AZ), a 26.5 kDa protein induced by polyamines (5). Second, ODC associated with AZ is degraded by the 26S proteasome without ubiquitination. Third, polyamines promote biosynthesis of AZ by raising efficiency of programmed, ribosomal frameshifting (6). Fourth, AZ-mediated ODC degradation is canceled by AZ inhibitor, which is the product of an ODC-related gene without enzymatic activity, and bind to AZ more firmly than ODC does (7). AZ inhibitor apparently stabilizes newly synthesized ODC during the phase of ODC increase after growth stimuli, whereas AZ accelerates ODC degradation during the phase of ODC decline. In addition to the role in the polyamine-regulated ODC degradation, AZ also suppresses cellular uptake of polyamines (8, 9). These dual functions of AZ effectively prevent excessive accumulation of cellular polyamines. This article will review our current understanding of the mechanism by which ODC associated with AZ is targeted to the proteasome.

STRUCTURAL ELEMENTS OF ODC AND AZ REQUIRED FOR RAPID ODC DEGRADATION

Mouse ODC is composed of two identical subunits of 461 amino acids and two active sites are formed at their interface (10).The active homodimer is in equilibrium with the inactive monomer to which AZ preferentially binds (11). Coffino's group reported the structural elements of ODC responsible for its degradation (4, 12). Various deletion mutants and chimeras between stable trypanosome ODC and unstable mouse ODC reveal that the C-terminal region of ODC (amino acids 423–461) is essential for both constitutive and AZ-dependent degradation of ODC (Fig. 1). The region

0006-291X/00 $35.00
Copyright © 2000 by Academic Press
All rights of reproduction in any form reserved.

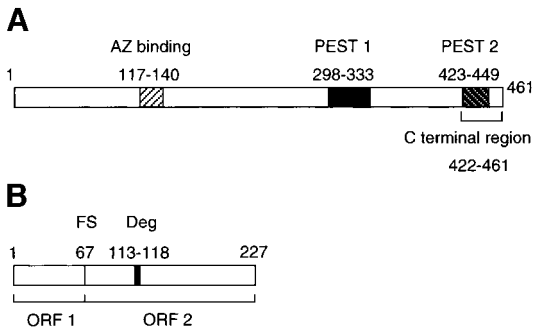

FIG. 1. Structural features of mouse ODC and rat AZ. (A) Mouse ODC. See text for the details of AZ binding, PEST 1/2, and the C-terminal region that functions as the putative "degradation signal." (B) Rat AZ. Deg, the region required for destabilization of ODC; FS, the amino acid residue corresponding to the translational frameshift site; ORF, open reading frame. Note that ORF1 of AZ mRNA is normally translated, but its ORF2 is consecutively translated to form the full-length AZ only in the presence of polyamines.

contains one of two PEST regions in ODC, but removal of the last five amino acids (outside the PEST region) or a single amino acid exchange in the PEST region (Cys441 to Trp) stabilizes ODC, suggesting that this PEST sequence is not a site for self-contained determination of ODC instability. Another internal PEST region (amino acids 298–333) also does not appear to be associated with ODC instability. In addition, an internal region of mouse ODC (amino acids 117–140) required for binding with AZ is needed for AZ-dependent degradation of ODC. This region is highly conserved in mammals, avians (chicken), and amphibians (frog) whose ODC are rapidly degraded by an AZ-dependent process (3). Recently, unique ODC was found in the trypanosomatid, *Crithidia fasciculata*. This ODC lacks both the C-terminal degradation domain and AZ binding region, but shows a rapid turnover even when expressed in mammalian cells, suggesting that another signal(s) mediates the rapid turnover of the ODC (13).

Mutational analyses of AZ indicated that, although the C-terminal half of AZ is sufficient for binding to ODC and inhibition of its activity, an adjacent region (amino acids 113–118) is also required for destabilization of ODC (3, 4) (Fig. 1). This finding suggests that binding of AZ maintains ODC in the inactive monomer state, but this dissociation is not sufficient for destabilization of ODC. Consistently, AZ accelerates degradation of a single amino acid mutant ODC that can not form dimers (14).

Very recently, new types of AZ molecular species were found in fish (15) and mammals (16, 17). They can inhibit ODC activity, but cannot accelerate ODC degradation by the 26S proteasome, at least *in vitro*, implying that the effect of AZ on the inhibitory activity and induction of destabilization against ODC can be clearly discriminated.

THE ENZYME RESPONSIBLE FOR ODC DEGRADATION

ODC is degraded rapidly in the crude extract of cultured cells or reticulocytes in the presence of AZ and ATP (see below). Immunoremoval of the proteasome from these extracts causes almost complete loss of ODC degradation. In living cells, lactacystin (proteasome inhibitor) almost completely inhibits the degradation of ODC. These findings strongly indicate that the proteasome is the main enzyme for ODC degradation.

The proteasome is a multisubunit complex, consisting of a central proteinase (called the 20S proteasome) and two terminal regulatory subcomplexes, termed PA700 (also called 19S complex) and PA28 (equivalent to 11S regulator), that are attached to both ends of the central portion in opposite orientations (18–21). The 20S proteasome is a 700 kDa, cylinder-shaped particle arranged as four axially stacked heptameric rings made up of two outer α-rings and two inner β-rings. The α- and β-rings are each made up of seven structurally similar α- and β-subunits, respectively. It has multiple catalytic centers located within a hollow cavity of the β-ring and exists as a latent form in cells. The structural observation of the yeast proteasome showed that entrances of both α-rings are almost closed by the N-terminal portions of the α-subunits (19, 21). Interaction between the 20S core particle and regulatory complexes such as PA700 and PA28 presumably opens the proteasome channel for entry of the protein substrate, thereby accessing the catalytic sites (19–20). Binding of PA28 activates degradation of peptides, while PA700 enhances degradation not only of peptides, but also of ubiquitinated proteins (see below). PA700 can be dissociated into two subcomplexes, named "base" and "lid" (22). The base complex contains six AAA-type ATPases and two non-ATPase subunits, and is directly linked to the α-ring of the 20S core particle. The base is sufficient to activate the peptidase activity of the proteolytic 20S core in the presence of ATP, possibly by opening the channel. The lid complex contains at least eight non-ATPase subunits and is attached to the base complex. The lid is required for the degradation of ubiquitinated proteins, suggesting that it has a role for trapping ubiquitinated proteins.

In cells at least four different forms of proteasomes exist: 26S proteasome (with PA700 at its both ends), 20S proteasome (without regulatory proteins), homo-PA28 proteasome (with PA28 at its both ends) and hybrid-type proteasome (a hetero-complex containing both PA28 and PA700) (23). ODC is degraded rapidly *in vitro* by purified 26S, but not 20S, proteasome in the presence of ATP and AZ (24). Recently, the hybrid-type proteasome also was found to be responsible for ODC degradation, although the homo-PA28 proteasome was

incapable of degrading ODC (Tanahashi *et al.*, submitted for publication).

ODC DEGRADATION REQUIRES ENERGY BUT NOT UBIQUITINATION

The 26S proteasome has been shown to act as the protein-destroying machinery for selective degradation of numerous cellular proteins tagged with the polyubiquitin (Ub) chain functioning as a degradation signal. Intriguingly, it degrades ODC without ubiquitination as mentioned above, and ATP hydrolysis is required for the degradation of ODC analogous to that of ubiquitinated proteins. The base complex of PA700 contains six ATPases which may account for energy dependency (22). Although the exact functions of the ATPases remain elusive, mutation analysis showed that they are functionally different (25). For degradation of mouse ODC in yeast cells, two ATPases, Cim3 and Cim5, are required, whereas only Cim3 is required for degradation of yeast ODC, suggesting their contribution to the direct recognition of target proteins (26). It has been proposed that these ATPases unfold substrate proteins and translocate them into the inner aspect of the β-ring of the 20S proteasome on which the proteolytically active sites are located. However, it has not yet been shown that a protein substrate is unfolded by the proteasome in an ATP-dependent manner. To examine the degradation mechanism of the proteasome, especially unfolding of a substrate protein, ODC appears to be an advantageous substrate, since ODC is a naturally-occurring sole substrate of the 26S proteasome without ubiquitination and changes in both ODC protein and its activity can be determined easily. A recombinant AZ is capable of providing the degradation signal of cell-free translated ODC *in vitro*. In addition, inactive ODC-AZ complex, the form of ODC recognized by the 26S proteasome, can be completely reactivated by AZ inhibitor as mentioned above. Following alterations of both the activity and protein levels of ODC, it becomes clear that the degradation pathway of ODC by the 26S proteasome can be dissected into multiple steps, which proceed in a sequential fashion, as discussed below (27).

ATP-DEPENDENT INACTIVATION OF ODC BY THE 26S PROTEASOME

When ODC was incubated with cell extracts in the presence of ATP and AZ, the extent of decrease in ODC activity was much greater than that of ODC protein. This observation indicates that inactivated ODC was formed and remained at least in part without degradation in the reaction mixture. This inactivated ODC was different from ODC inhibited by AZ which could be reactivated fully by AZ inhibitor. The apparently irreversible inactivation of ODC occurs in an energy- and AZ-dependent fashion like its degradation. A selective proteasome inhibitor *Clasto*-lactacystin β-lactone did not inhibit ODC inactivation caused by cell extracts, but resulted in considerable accumulation of inactivated ODC. Immunodepletion of proteasomes from cell extracts, however, almost completely prevented this inactivation. These findings indicate that inactivation of ODC is catalyzed by the proteasome, but the proteolytic activity is not required for the process. Purified 26S proteasome, but not the 20S proteasome, catalyzed AZ- and energy-dependent ODC inactivation. PA700 alone could not inactivate ODC, but preincubation with the 20S proteasome in the presence of ATP-Mg revealed inactivation activity, suggesting that the 26S proteasome reconstituted from PA700 and the 20S proteasome is responsible for ODC inactivation.

SEQUESTRATION OF ODC INTO THE 26S PROTEASOME

The apparent size of the inactivated ODC resembles that of the native ODC on SDS–PAGE, but its mobility on the nondenaturing PAGE was much slower than that of the native ODC. Subsequently, it was found that the inactivated ODC was immunoprecipitated with anti-proteasome antibody, but not with anti-ODC antibody, suggesting that ODC is trapped within the proteasome. Indeed, glycerol density gradient centrifugation analysis revealed co-sedimentation of ODC and the 26S proteasome, indicating that most of the ODC binds preferentially to the 26S proteasome, but not to free PA700. However, it is not shown whether inactivated ODC is incorporated into the interior of the core or trapped by PA700 of the 26S proteasome. Sequestration of the inactivated ODC within the proteasome was demonstrated by its resistance to proteinase K digestion: immunoprecipitatable ODC with anti-proteasome antibody resisted proteinase K digestion while unprecipitatable ODC (free ODC) was rapidly degraded by proteinase K.

INACTIVATION AND SEQUESTRATION OF ODC IS PREREQUISITE FOR DEGRADATION

Time courses of ODC inactivation, sequestration and degradation indicated that ODC sequestration and inactivation occurred almost simultaneously and preceded ODC degradation. Inactivation coupled to sequestration required AZ and ATP hydrolysis, but not proteolytic activity of the 26S proteasome. Thus, the energy and AZ-dependent pathway for ODC degradation by the 26S proteasome can be dissociated into two distinct steps: first irreversible inactivation and subsequent degradation, which may occur sequentially at a very rapid rate in living cells, because the inactivated

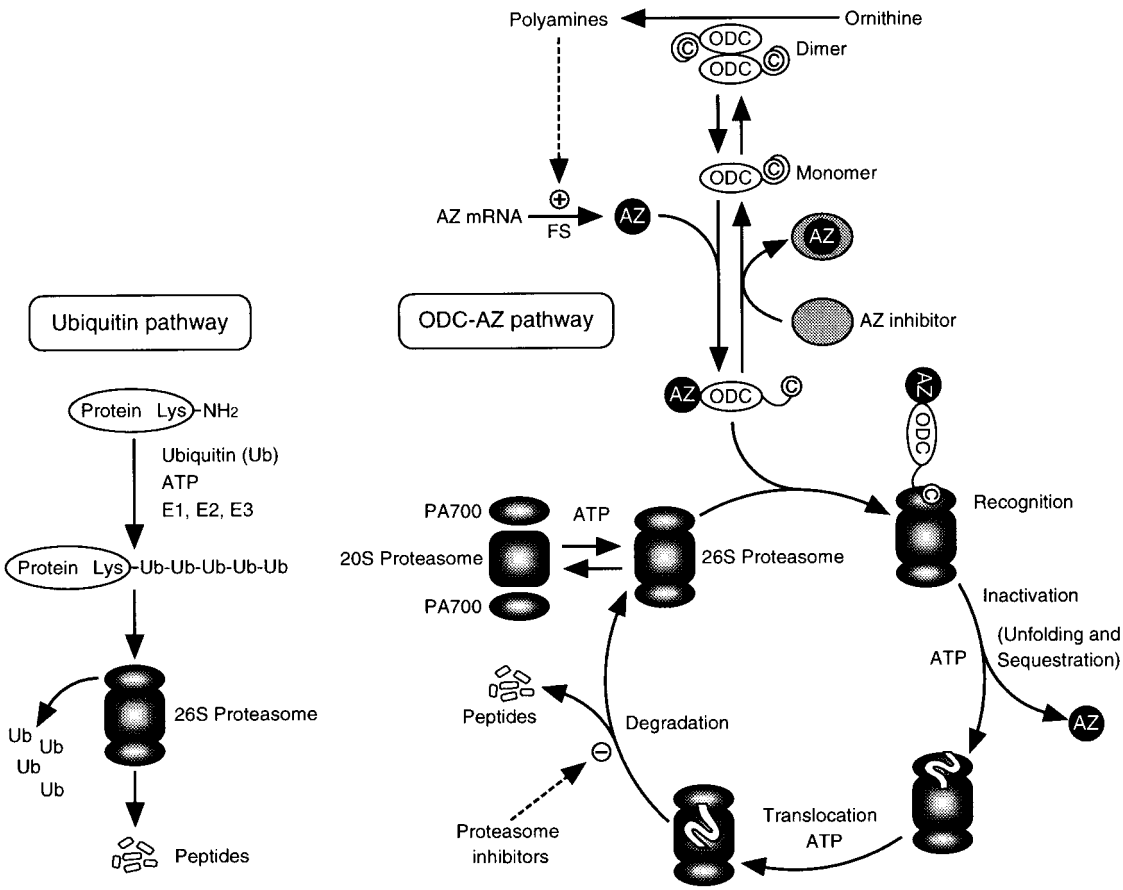

FIG. 2. Model of AZ-dependent ODC degradation pathway. The enzymatically active dimeric form of ODC is in rapid equilibrium with the inactive monomeric form. AZ, which is induced by polyamine-dependent translational frameshifting (see Fig. 1B), binds to the monomeric ODC to form an ODC–antizyme complex and is thought to expose the carboxy-terminal region of ODC to attack from the 26S proteasome. Thus, ODC is targeted for degradation through a unique negative-feedback regulation. The 26S proteasome degrades not only a number of proteins tagged with a poly-Ub chain (left) but also ODC moiety in the ODC-AZ complex (right) in an ATP-dependent manner, and both Ub and AZ are recycled. The degradation pathway of ODC proceeds as a sequence of multiple distinct processes, including recognition, sequestration, unfolding, translocation, and ultimate degradation. FS, translational frameshifting; E1, Ub-activating enzyme; E2, Ub-conjugating enzyme; E3, Ub-ligating enzyme.

intermediate form of ODC is barely detected under normal conditions (Fig. 2).

HOW DOES THE 26S PROTEASOME RECOGNIZE ODC FOR DEGRADATION?

ODC mutants lacking the intact C-terminal region were not subject to the AZ-dependent inactivation by the 26S proteasome. Thus, although both inactivation and degradation are triggered by AZ, they also depend on the cis-acting element on the substrate. A most likely interpretation of these observation is that the putative "degradation signal" of ODC is located at the C-terminal region which is normally masked, but exposed by attachment of AZ and finally recognized in some way by the 26S proteasome (Fig. 2). An alternative possibility that AZ bound to ODC together with the signal on ODC molecule provides a recognition site for the 26S proteasome cannot be ruled out completely. However, it seems unlikely that AZ, presumably dissociated from ODC before sequestration of ODC into the 26S proteasome, is directly recognized by the 26S proteasome, because a few AZ molecules could promote the degradation of a large number of ODC molecules (3) and because AZ was not appreciably degraded under the conditions of exhaustive degradation of ODC to oligopeptides (28). The recycling of AZ seems to be similar to that of ubiquitin (Ub), but their mechanisms of action are different. Ub is directly recognized by the poly-Ub chain binding receptor of the 26S proteasome and is removed from the protein substrate tagged with a poly-Ub chain by isopeptidase associated with the 26S proteasome for its reutilization. Furthermore, the degradation of polyubiquitinated protein is inhibited by excessive amounts of either poly-Ub receptor or poly-Ub chain, whereas ODC degradation is inhibited

by C-terminal peptides of ODC (unpublished results), but not excessive AZ. Finally, ODC can be degraded slowly by the purified 26S proteasome even in the absence of AZ. These findings strongly suggest that ODC rather than AZ is directly recognized by the 26S proteasome.

To date, Ub-independent degradation by the 26S proteasome has been rare (29) and a specific regulatory protein-dependent degradation by the proteasome is not known except for ODC. Interestingly, however, an example resembling the regulated ODC degradation is found in *Escherichia coli* lacking the Ub system. Rpos, the stationary-phase sigma factor, is degraded during exponential growth by ClpXP protease, an ATP-dependent protease. The degradation is dependent on RssB, a protein with homology to the family of response regulator (30). Action of RssB resembles that of AZ in various aspects: (1) Specific interaction with substrate. (2) The interaction causes modification of the substrate activity. (3) Stimulation of substrate degradation in a recycling manner.

HOW IS ODC SEQUESTRATED INTO THE 26S PROTEASOME?

It is plausible that the 26S proteasome degrades both ODC and polyubiquitinated proteins by the same mechanism requiring ATP, except for the manner of substrate recognition. Energy and AZ dependency of ODC inactivation and sequestration into the 26S proteasome indicates that ATP-energy is required for the process before proteolysis. The energy of ATP hydrolysis may be required to induce a conformational change of ODC (equivalent to unfolding) and/or to translocate the unfolded ODC into the 20S proteasomal inner cavity centralized within the 26S proteasome complex. Sequestration and irreversible inactivation of ODC caused by unfolding may be coupled to substrate translocation. Strictly speaking, unfolding has not been demonstrated directly so far, but has been suggested based on the findings that ODC is ATP-dependently sequestrated into the proteasome (27) and that the α-rings of the 20S proteasome discriminate between unfolded and folded proteins (31).

Recently, Rechsteiner proposed two possible mechanisms of proteolysis catalyzed by the 26S proteasome: the “ribosome model” and the “solid-state model” (32). According to the “ribosome model,” free PA700 can trap target proteins, mostly ubiquitinated, and recruit them to the 20S proteasome, yielding the 26S proteasome, for ultimate degradation. In the “solid-state model,” the 26S proteasome can directly trap target proteins for their degradation. Our observations that inactivated ODC cosedimented with the 26S proteasome, but not PA700, appears to support the second “solid-state model.” However, the “ribosome model” cannot be excluded completely, because of the possibility that the small number of PA700 molecules is sufficient to recruit the target protein to the 20S catalytic proteasome. Alternatively, inactivated ODC may be reactivated by PA700 in the absence of 20S core particle. Indeed, Braun *et al.* (33) showed that human and yeast 26S proteasome can bind denatured protein and can refold the protein in an ATP-dependent reaction. They mapped the chaperone-like activity to the base complex of PA700.

Prokaryotes and certain eukaryotic organelles contain several ATP-dependent proteases resembling the 26S proteasome, although their functions are independent of Ub. For example, the ClpAP protease of *Escherichia coli* is composed of a protease (ClpP) and two ATPase particles (ClpA). ClpA can exert chaperone-like activity by itself or even in the proteolytic ClpAP complex without dissociation of ClpA from the complex (34). Recently, Weber-Ban *et al.* (35) showed global unfolding of a substrate protein by ClpA. The unfolded proteins are released into solution or, in the presence of a mutant form of molecular chaperone GroEL that cannot release substrate protein, they are captured by it. In the presence of ClpP the substrate proteins are translocated from ClpA into the ClpP proteolytic cavity. Similarly, a proteolytically inactive Lon mutant remains substrate protein sequestrated without release, suggesting that ATP-dependent unfolding may be coupled to translocation to the proteolytic chamber (36). Lon protease is another bacterial ATP-dependent protease with an ATPase domain and a proteolytic domain within a single polypeptide chain. Thus, the concurrent chaperone and proteolytic activities may be a common feature of various ATP-dependent proteases including the 26S proteasome (34). Baumeister and his colleagues propose the term a self-compartmentalizing protease for these proteases with ATP-dependent chaperone functions cooperating with proteolysis (19).

PERSPECTIVES

ODC is a good target for studies on the selective proteolysis mechanism. Degradation of ODC shows both unique and common features to those of other polyubiquitinated proteins. The molecular mechanism underlying the polyamine-regulated ODC degradation in mammalian cells has been largely clarified. The regulatory proteins, AZ and AZ inhibitor, also have short half-lives comparable to ODC, but their degradation pathways are almost unknown. It is plausible that binding of ODC or AZ inhibitor may affect the stability of AZ. Clarification of their degradation mechanism is necessary for better understanding of regulation of polyamine metabolism.

Finally, one major unanswered aspect is how the 26S proteasome recognizes ODC attached to AZ at the molecular level. For this, specification of the proteasome

subunit that interacts directly with the ODC molecule should be required. Tertiary structural analysis of ODC (37) and ODC-AZ complex will also provide valuable information to explore the mechanism by which the 26S proteasome can recognize ODC. More detailed knowledge about the structure-function relationship of the 26S proteasome is also needed. The X-ray structural information of the 20S proteasome alone is available to date, and three-dimensional structures for the lid, base, PA700, and finally the 26S proteasome will enable us to scrutinize the physical interaction between the 26S proteasome and ODC.

ACKNOWLEDGMENTS

This work was supported in part by Grants-in-Aid for Scientific Research on Priority Areas (Intracellular Proteolysis) from the Ministry of Education, Sports, and Culture of Japan, a grant from the Uehara Foundation, and the Human Frontier Science Promotion Organization.

REFERENCES

1. Pegg, A. E. (1986) *Biochem. J.* **234,** 249–262.
2. Hayashi, S. (1989) *in* Ornithine Decarboxylase: Biology, Enzymology, and Molecular Genetics (Hayashi, S., Ed.), pp. 35–45, Pergamon Press, New York.
3. Hayashi, S., Murakami, Y., and Matsufuji, S. (1996) *Trends Biochem. Sci.* **21,** 27–30.
4. Coffino, P. (1998) *in* Ubiquitin and the Biology of the Cell (Peters, J.-M., Harris, J. D., and Finley, D., Eds.), pp. 411–428, Plenum Press, New York.
5. Heller, J. S., Fong, W. F., and Canellakis, E. S. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1858–1862.
6. Matsufuji, S., Matsufuji, T., Miyazaki, Y., Murakami, Y., Atkins, J. F., Gesteland, R. F., and Hayashi, S. (1995) *Cell* **80,** 51–60.
7. Murakami, Y., Ichiba, T., Matsufuji, S., and Hayashi, S. (1996) *J. Biol. Chem.* **271,** 3340–3342.
8. Mitchell, J. L. A., Judd, G. G., Bareyal-Leyser, A., and Ling, S. Y. (1994) *Biochem. J.* **299,** 19–22.
9. Suzuki, T., He, Y., Kashiwagi, K., Murakami, Y., Hayashi, S., and Igarashi, K. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8930–8934.
10. Coleman, C. S., Stanley, B. A., Viswanath, R., and Pegg, A. E. (1994) *J. Biol. Chem.* **269,** 3155–3158.
11. Mitchell, J. L., and Chen, H. J. (1990) *Biochim. Biophys. Acta* **1037,** 115–121.
12. Li, X., and Coffino, P. (1993) *Mol. Cell. Biol.***13,** 2377–2383.
13. Svensson, F., Ceriani, C., Wallström, E. L., Kockum, I., Algranati, I. D., Heby, O., and Persson, L. (1997) *Proc. Natl. Acad. Sci. USA*, **94,** 397–402.
14. Mamroud-Kidron, E., Omer-Itsicovich, M., Bercovich, Z., Tobias, K. E., Rom, E., and Kahana, C. (1994) *Eur. J. Biochem.* **226,** 547–554.
15. Saito, T., Hascilowicz, T., Ohkido, I., Kikuchi, Y., Okamoto, H., Hayashi, S., Murakami, Y., and Matsufuji, S. (1999) *Biochem. J.*, in press.
16. Ivanov, I. P., Gesteland, R. F., and Atkins, J. F. (1998) *Genomics* **52,** 119–129.
17. Zhu, C., Lang, D. W., and Coffino, P. (1999) *J. Biol. Chem.* **274,** 26425–26430.
18. Coux, O., Tanaka, K., and Goldberg, A. L. (1996) *Annu. Rev. Biochem.* **65,** 801–847.
19. Baumeister, W., Walz, J., Zühl, F., and Seemüller, E. (1998) *Cell* **92,** 367–380.
20. DeMartino, G. N., and Slaughter, C. A. (1999) *J. Biol. Chem.* **274,** 22123–22126.
21. Bochtler, M., Ditzel, L., Groll, M., Hartmann, C., and Huber, R. (1999) *Annu. Rev. Biophys. Biomol. Struct.* **28,** 295–317.
22. Glickman, M. H., Rubin, D. M., Coux, O., Wefes, I., Pfeifer, G., Cjeka, Z., Baumeister, W., Fried, V. A., and Finley, D. (1998) *Cell* **94,** 615–623.
23. Hendil, K. B., Khan, S., and Tanaka, K. (1998) *Biochem. J.* **332,** 749–754.
24. Murakami, Y., Matsufuji, S., Kameji, T., Hayashi, S., Igarashi, K., Tamura, T., Tanaka, K., and Ichihara, A. (1992) *Nature* **360,** 597–599.
25. Rubin, D. M., Glickman, M. H., Larsen, C. N., Dhruvakumar, S., and Finley, D. (1998) *EMBO J.* **17,** 4909–4919.
26. Mamroud-Kidron, E., and Kahana, C. (1994) *FEBS Lett.* **356,** 162–164.
27. Murakami, Y., Matsufuji, S., Hayashi, S., Tanahashi, N., and Tanaka, K. (1999) *Mol. Cell. Biol.*, in press.
28. Tokunaga, F., Goto, T., Koide, T., Murakami, Y., Hayashi, S., Tamura, T., Tanaka, K., and Ichihara, A. (1994) *J. Biol. Chem.* **269,** 17382–17385.
29. Pickart, C. M. (1997) *FASEB J.* **11,** 1055–1066.
30. Zhou, Y.-N., and Gottesman, S. (1998) *J. Bacteriol.* **180,** 1154–1158.
31. Wenzel, T., and Baumeister, W. (1995) *Nature Struct. Biol.* **2,** 199–204.
32. Rechsteiner, M. (1998) *in* Ubiquitin and the Biology of the Cell (Peters, J. -M., Harris, J. D., and Finley, D., Eds.), pp. 147–189, Plenum Press, New York.
33. Braun, B. C., Glickman, M., Kraft, R., Dahlmann, B., Kloetzl, P.-M., Finley, D., and Schmidt, M. (1999) *Nature Cell Biol.* **1,** 221–226.
34. Pak, M., Hoskins, J. R., Singh, S. K., Maurizi, M. R., and Wickner, S. (1999) *J. Biol. Chem.* **274,** 19316–19322.
35. Weber-Ban, E. U., Reid, B. G., Miranker, A. D., and Horwich, A. L. (1999) *Nature* **401,** 90–93.
36. Melderen, L. V., and Gottesman, S. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 6064–6071.
37. Kern, A. D., Oliveira, M. A., Coffino, P., and Hackert, M. L. (1999) *Structure* **7,** 567–581.