Regulated degradation of ornithine decarboxylase (ODC) is mediated by its association with the inducible protein antizyme. The N terminus of antizyme (NAZ), although unneeded for the interaction with ODC, must be present to induce degradation. We report here that covalently grafting NAZ to ODC confers lability that normally results from the non-covalent association of native antizyme and ODC. To determine whether NAZ could act similarly as a modular functional domain when grafted to other proteins, we fused it to a region of cyclin B (amino acids 13–90) capable of undergoing degradation or to cyclin B (amino acids 13–59), which is not subject to degradation. The association with NAZ made both NAZ-cyclin B13–90 and NAZ-cyclin B13–59 unstable. Furthermore, NAZ and cyclin B 13–59 were together able to induce in vitro degradation of Trypanosoma brucei ODC, a stable protein. The ODC-antizyme complex bound to the 26 S protease but not the 20 S proteasome, consistent with the observation that ODC degradation is mediated by the 26 S protease. The association was shown to be independent of NAZ, suggesting that NAZ does not act as a recognition signal.

Cellular polyamines are essential for cells to grow and proliferate. Ornithine decarboxylase (ODC),1 the key enzyme in the biosynthesis of the polyamines, is highly regulated. Its activity is dramatically increased by stimulating cell growth and decreased by excess polyamines. Feedback regulation of ODC activity by polyamines occurs via induction of the protein antizyme (1–5). Antizyme binds to ODC, inhibits its activity, and accelerates its degradation. The determinants within each protein needed for their association have been identified; one element is near the N terminus of ODC (6), and the other element is the C-terminal half of antizyme (7). Besides regions that bring the two proteins together, two additional elements are necessary for antizyme-dependent degradation to occur. Both the C-terminal degradation domain of ODC (8) and the N terminus of antizyme are required for proteolysis of ODC. The C terminus of ODC has been characterized as a degradation domain that is sufficient for polyamine-independent basal degradation (8, 9). Deletion forms of antizyme devoid of the N-terminal half still interact with ODC, but accelerated ODC degradation can be directed only by molecules in which an antizyme-N-terminal region is also present (7).

ODC, like other short-lived proteins, is degraded by the 26 S protease in an ATP-dependent manner. Ubiquitination is a modification that triggers proteolysis of many short-lived proteins (10–13), but ODC does not utilize this mechanism. Instead, regulated degradation of ODC requires association with antizyme (14–16). The means by which the N terminus of antizyme (NAZ) acts in place of ubiquitin for ODC degradation is unclear. It might function as a signal domain to promote recognition by the 26 S protease or as an activation domain to stimulate degradation. Here we report that AZ contains a module that can be grafted to other proteins to make them labile. We show also that the association of the ODC-AZ complex with the 26 S protease is independent of NAZ.

**MATERIALS AND METHODS**

Recombinant DNA Constructs—Recombinant DNAs used for the expression of fusion proteins by in vitro transcription and translation were made as follows. The DNA sequence encoding each constituent protein fragment to be expressed was copied by PCR, using oligonucleotides that incorporated a common restriction endonuclease recognition site at the point of fusion. The PCR fragments encoding the protein regions to be fused were digested with the common restriction enzyme, ligated, and reamplified using the distal 5′- and 3′-oligonucleotides. The 5′-oligonucleotide used to copy the N-terminal element of each fusion contained a T7 RNA polymerase recognition site placed upstream of the translation initiation AUG codon. The resultant PCR product after the second round of amplification thus contained at the 5′-end a T7 RNA polymerase recognition site, followed by an open reading frame encoding the fusion protein. Plasmids encoding sea urchin cyclin B were kindly provided by A. W. Murray (University of California, San Francisco). The rat antizyme λgt11 partial cDNA clone Z1 (4) was obtained from S. Hayashi (Ike University, Tokyo). Z1 has a 212-amino acid open reading frame (5). We use here a numbering convention that specifies the start of this reading frame to be amino acid 1 of AZ. The sources and sequences of mouse ODC (MODC) and Trypanosoma brucei ODC (TbODC) were as described previously (6–8). To fuse the 1–97 N-terminal amino acids of antizyme (NAZ) to various proteins, Z1 DNA was amplified by PCR using a 3′-oligonucleotide with a HindIII site. Similarly using PCR, a HindIII site was introduced at the 5′-ends of MODC, TbODC, Tb376M, Tb422M, and cyclin B amino acids 13–90. PCR-amplified fragments were digested or partially digested with HindIII and ligated to make NAZ-MODC, NAZ-TbODC, NAZ-Tb376M, NAZ-Tb422M, and NAZ-cyclin B 13–90. To make the C-terminal deletion NAZ-cyclin B 13–90, sequence changes were incorporated into the 3′-oligonucleotides, which created translation stop codons immediately after cyclin B amino acid 59. CAZ-MODC was made using a similar strategy by ligating AZ cDNA encoding amino acids 106–212 and mouse ODC cDNA with a HindIII linker. To make fusion proteins of TbODC coupled to the degradation domain of p53, DNA was also amplified by PCR using oligonucleotides that contained a BamHI site at the TbODC 3′-end and at the 5′-end of the p53 degradation domain (amino acids

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1 The abbreviations used are: ODC, ornithine decarboxylase; NAZ, N terminus of antizyme; AZ, antizyme; PCR, polymerase chain reaction; MODC, mouse ODC; TbODC, T. brucei ODC; CAZ, C-terminal half of antizyme; PAGE, polyacrylamide gel electrophoresis; ATP-5′-S, adenosine 5′-O-(thiotriphosphate); GST, glutathione S-transferase.

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2 The abbreviations used are: ODC, ornithine decarboxylase; NAZ, N terminus of antizyme; AZ, antizyme; PCR, polymerase chain reaction; MODC, mouse ODC; TbODC, T. brucei ODC; CAZ, C-terminal half of antizyme; PAGE, polyacrylamide gel electrophoresis; ATP-5′-S, adenosine 5′-O-(thiotriphosphate); GST, glutathione S-transferase.
Degradation of Mouse ODC Is Induced by NAZ—MODC, like other short-lived proteins, is relatively stable in an in vitro degradation system derived from rabbit reticulocytes (8). Its degradation is promoted by the addition of the regulatory protein antizyme (8). Although proteolysis of ODC is maximally stimulated by AZ at a 1:1 stoichiometric ratio, AZ appears to act catalytically, efficiently directing ODC degradation even when the target is present at 100-fold or greater excess (20, 21). Hence AZ acts catalytically to promote ODC degradation. AZ has two functionally distinguishable regions. The C-terminal half of the protein (CAZ) binds to ODC (7). The N-terminal half of AZ (NAZ-MODC) is largely de-
graded in 2 h (Fig. 1B). In contrast, the CAZ-MODC fusion protein was relatively stable. Therefore, NAZ can direct degradation of ODC if it is provided with a means to associate with its target, whether that association is non-covalent as with intact AZ or covalent as in AZ-MODC.

Two Functional Domains Can Jointly Direct Degradation of Trypanosome ODC—Ornithine decarboxylase from T. brucei (TbODC), although almost 70% identical to MODC in its core structure, is a stable protein (9, 22). It is also insensitive to regulation by polyamines in the native context of the parasite (23) or when expressed in mammalian cells (24). It lacks both the C-terminal degradation domain and the antizyme-binding domain of mouse ODC. The protein can be converted into one that is unstable in vivo (but still unresponsive to polyamines) by replacing its C terminus with the degradation domain of mouse ODC. This has been done by making chimeric proteins in which a junction between mouse and trypanosome ODC (without insertion or deletion) is created at amino acid 376 (to make Tb376M) (17) or at 422 (to make Tb422M) (8), as shown in Fig. 2A. Both chimeras were shown to be unstable in vivo by treating cells that expressed the chimeras with an inhibitor of protein synthesis and measuring the rate of decline of ODC activity. However, the Tb376M and Tb422M chimeras, like most other short-lived proteins, were relatively stable in the in vitro degradation assay.

To test whether NAZ was capable of inducing Tb376M degradation in vitro, we coupled NAZ in front of the protein, to make NAZ-Tb376M (Fig. 2A). In vitro degradation analysis showed that NAZ-Tb376M was degraded (Fig. 2B). Both NAZ and the C terminus of mouse ODC were needed to induce efficient degradation of TbODC; neither Tb376M nor NAZ-TbODC alone was capable of changing the stability of TbODC (Fig. 2B). Densitometric analysis of the data of Fig. 2B is displayed as Fig. 2C. NAZ also stimulated the degradation of Tb422M (Fig. 2D). NAZ-Tb422M contains the entire open reading frame of trypanosome ODC except for the last 2 amino acids, sandwiched between NAZ and the C terminus (amino acids 422–461) of mouse ODC. Therefore, these two functional domains are together necessary and sufficient to cause in vitro degradation of a stable protein, trypanosomal ODC.

We made control fusion proteins consisting of NAZ-TbODC extended at its C terminus by either full-length human papillomavirus 16 E6 (151 amino acids) or the N-terminal 90 amino acids of that protein to determine whether any extension, regardless of sequence specificity, can induce degradation of NAZ-TbODC. Both fusion proteins were subjected to the degradation assay. During the 2-h incubation, the proteins remained undegraded (data not shown). Therefore, the C terminus of mouse ODC must contain a specific functional sequence motif that is able to cooperate with NAZ.

Antizyme-mediated degradation of ODC by the 26 S proteasome is independent of ubiquitination (16). To examine
whether degradation induced by appending NAZ involves ubiquitin modification, we incubated NAZ-Tb376M and NAZ-Tb422M as for in vitro degradation but substituting ATPγS in place of ATP. ATPγS has been shown to block degradation but not ubiquitination (25), thereby leading to the accumulation of high molecular weight forms of a target protein decorated by multiple ubiquitin chains. Both NAZ-Tb376M and NAZ-Tb422M were degraded in the presence of ATP (Fig. 3A). Substitution of ATPγS for ATP blocked degradation, but high molecular weight conjugates of NAZ-Tb376M and NAZ-Tb422M did not appear. As a positive control, we used HPV16 E6-mediated degradation of p53, which is ubiquitin-dependent. In the presence of E6 and ATP, p53 was degraded (Fig. 3A). When ATPγS was used in place of ATP, degradation was blocked and high molecular weight forms of p53 accumulated. Immunoprecipitation with a monoclonal antibody to p53 (PAb421) followed by Western blot analysis using anti-ubiquitin antiserum confirmed that the high molecular weight proteins enhanced in the presence of ATPγS consisted of polyubiquitinated p53 (Fig. 3B). Therefore, NAZ-induced degradation, like antizyme-mediated degradation of ODC, is ubiquitin-independent.

The Destruction Box of Cyclin B Can Cooperate with NAZ to Confer Degradation—Sea urchin cyclin B is a well-characterized short-lived protein that accumulates at interphase and is degraded at metaphase (26). Degradation of the protein allows cells to exit metaphase and enter interphase. The protein is stable in frog oocyte extracts prepared from interphase cells, but it is rapidly degraded in metaphase extracts (27). The specific capacity of mitotic cell extracts to degrade cyclin B thus reproduces faithfully the property of the cell of origin. Degradation of cyclin B requires that it contain both ubiquitination sites and a destruction box (27). Its N terminus contains all the structural information needed for degradation; the amino acid 13–90 region fused to staphylococcal protein A is able to induce degradation in metaphase cell extracts. A smaller region, amino acids 13–59, does not contain the ubiquitination sites and does not undergo proteolysis, presumably because the lysines within amino acids 60–66 are not present to serve as ubiquitin modification sites. Likewise, a single mutation at the conserved arginine in the destruction box (amino acids 42–50), a conserved region in the N terminus, prevents cycle-specific degradation.

To test whether the cyclin B degradation domain within amino acids 13–90 can confer lability on the otherwise stable TbODC, we fused this region to the C terminus of TbODC (Fig. 4A). As shown in Fig. 4B, the TbODC-cyclin B13–90 fusion protein was stable in the reticulocyte lysate. The result agreed with the conclusion that this portion of cyclin alone cannot promote degradation of protein A (27). However, it became unstable after NAZ was appended to form NAZ-TbODC-cyclin B13–90. Furthermore, deleting the lysine residues that are putative sites of ubiquitination from NAZ-TbODC-cyclin B13–90 to form NAZ-TbODC-cyclin B13–59 did not alter its degradation. These results suggested that cyclin B amino acids 13–59 con-
tain a degradation domain that can cooperate with NAZ to promote trypanosome ODC degradation in vitro. Furthermore, the degradation is independent of the presence of lysine residues (contained within amino acids 60–90), which are the normal targets for ubiquitination, suggesting that NAZ can provide an alternate signal for degradation that bypasses the requirement for ubiquitin modification.

To test whether NAZ was able directly to induce degradation of the cyclin B degradation region 13–90 in a rabbit reticulocyte lysate, we coupled NAZ in front of either amino acids 13–90 or amino acids 13–59 (Fig. 5A). Both fusion proteins were degraded (Fig. 5B). As expected for a metaphase-specific substrate subjected to degradation in the reticulocyte-based degradation assay system, the cyclin B degradation region 13–90 alone used as a control was relatively stable. NAZ therefore can alter the degradation properties of cyclin B amino acids 13–90 in two ways: promoting its lability in the absence of cycle-specific signals and diverting it to a pathway that does not require ubiquitination.

Association of ODC<sub>z</sub>AZ Complex with the 26 S Protease Does Not Require NAZ—To determine whether NAZ is needed for the association of ODC<sub>z</sub>AZ with the 26 S protease, we prepared two forms of antizyme to complex with ODC, AZ amino acids 55–212 and AZ amino acids 106–212. We have shown that the larger of the two truncated proteins can induce ODC degradation in vitro, but the smaller one cannot (7). However, both efficiently form a complex with ODC. [35S]Methionine-labeled mouse ODC was made by in vitro translation and allowed to associate with purified GST recombinant fusion proteins GAZ 55–212 or GAZ 106–212. The ODC-AZ complexes were mixed with the 26 S protease or with 20 S proteasome or with 11 S activator (18, 19) and fractionated by non-denaturing gel electrophoresis. The positions on the gel of 20 S proteasome, 26 S protease, or 11 S activator were visualized by Coomassie Blue staining or by an overlay assay with a fluorogenic peptide (18, 19). Association of ODC-GAZ 55–212 with the 26 S protease was observed (Fig. 6). The complex did not bind to the 20 S proteasome, in agreement with a previous report that ODC-AZ was degraded by the 26 S protease, not the 20 S proteasome (16). There was no association of the complex with the 11 S activator. The complex of ODC-GAZ 106–212, which lacks NAZ, was able to bind the 26 S protease as well as ODC-GAZ 55–212. This result demonstrates that NAZ is not required, under the conditions of this assay, for the association between substrate and proteolytic machine.

To test whether each component of the ODC-AZ complex can separately associate with the 26 S proteasome, we produced [35S]methionine-labeled ODC and AZ as GST fusion proteins by in vitro translation. The fusion proteins, purified by affinity chromatography with glutathione-Sepharose 4B, were mixed with the 20 S proteasome or 26 S proteasome for the association assay. GST-ODC and GST-AZ fusion proteins were each able to associate with the 26 S protease but not the 20 S proteasome (Fig. 7A). GST alone was unable to associate with either of the proteasomes. Next, to test whether NAZ is necessary for the association of AZ with 26 S, we analyzed both forms of AZ, GST-AZ 55–212 and GST-AZ 106–212. We found that both truncated proteins were able to associate with the 26 S protease, again indicating that NAZ is not required for association (Fig. 7B). Therefore, NAZ must serve some other function in the destruction of ODC.

Finally, we examined the capacity of trypanosome ODC association of GST, GST-MODC, or GST-AZ with 20 S proteasome and 26 S protease. B, association of GAZ 55–212 or GAZ 106–212 with 26 S protease. C, association of GST, GST-MODC, GST-M314T, and GST-TbODC with 26 S protease.
(TbODC) to associate with proteasomes. Using a GST-TbODC fusion protein, we found that TbODC, unlike MODC, did not associate with the 26 S protease. To find out whether the C terminus of MODC, required for protein instability, is also necessary for MODC association with 26 S, we made a GST-M314T chimera, containing the N terminus of MODC and the C terminus of TbODC, with the junction at amino acid 314. This GST-M314T chimera was able to associate with the 26 S protease (Fig. 7C). This result suggests that the difference between TbODC and MODC in their ability to associate with the 26 S protease does not depend on the C terminus of mouse ODC but rather on sequence information contained within its first 314 amino acids.

**DISCUSSION**

Vertebrate ODC is a labile protein with a half-life in cells of less than an hour (28). C-terminal deletions or mutations can make it stable, and the C terminus appended to other proteins can confer on these a short half-life (17, 24). The C terminus is therefore both necessary and sufficient to provide a moderate degree of lability. ODC becomes still more labile in its N terminus. Mutations in that binding site destroy the regulatory degree of lability. ODC becomes still more labile in the presence of AZ (28). The AZ binding site within ODC is near its N terminus. Mutations in that binding site destroy the regulatory effect of AZ on ODC activity and abolish the regulatory effect of polyamines on ODC in cells. The AZODC complex is an efficient substrate for in vitro degradation. Normally, AZ binds to ODC and is not itself consumed as rapidly as ODC but acts catalytically to mediate ODC degradation (20, 21). We have shown here, by directly coupling it to ODC, that the N terminus of AZ is solely responsible for its degradative role. The fusion construct NAZMODC contains the two domains needed for degradation, the AZ N terminus and the mouse ODC C terminus. These are enough to destabilize the otherwise stable protein trypanosome ODC. This finding has encouraged us to use NAZ fusion proteins as a general means to identify and analyze degradation domains of other proteins uncoupled from earlier steps in the degradation process (29).

NAZ could act either as a bridge to bring together substrate ODC and 26 S protease or as a protease activator. Our results are inconsistent with the first possibility: the 26 S protease associates with ODC-AZ independently of the presence of NAZ. In fact, each protein alone can associate with the protease, and this recognition process is dependent on neither the N terminus of AZ nor the C terminus of mouse ODC. It is therefore improbable that these degradative elements serve as recognition signals.

Regulatory proteins, such as cyclins and oncoproteins, are usually short-lived in cells. Understanding the signals that direct their degradation is facilitated by mutagenesis and in vitro analysis of those signals. It may be difficult, however, to interpret the results of such experiments with proteins that require ubiquitination for turnover. Mutations of target proteins that interfere with degradation could do so by inhibiting ubiquitination or, alternatively, by impeding downstream steps. One can bypass the need for ubiquitination and thus simplify analysis by attaching a protein element that provides a functional alternative to ubiquitination to target proteins. NAZ has these properties. Its presence drives proteins down a proteasome-mediated degradation pathway with downstream elements common to ubiquitinated and non-ubiquitinated targets, thus bypassing the need for that modification. Furthermore, the NAZ domain is effective in promoting in vitro degradation. By conferring in vitro lability on natural substrates of in vivo degradation, it can be used to assist the analysis of cis-acting structural determinants of degradation. We have applied this form of analysis to establish that a region of cyclin B containing the destruction box acts as a degradation domain that can function independently of ubiquitin. This was further confirmed by demonstrating that the degradation domain of cyclin can effectively replace the C-terminal degradation domain of mouse ODC.

Most short-lived proteins require polyubiquitination to be degraded. The exact role of ubiquitination in proteolysis is not yet understood. It has been proposed that the modification is a direct recognition signal for the protease complex. Recently a specific protease subunit has been identified as the locus of interaction with polyubiquitin (30). A degradation domain could act as a proteolysis site, which is made available for digestion by ubiquitin modification, or a site of association with other proteins required for proteolysis, e.g. a chaperone. The work described here supports the hypothesis that NAZ shares with polyubiquitination the need for collaboration with a degradation domain. Because NAZ can function as an independent module when appended to diverse proteins, it can be used as an analytic reagent for probing the structure of degradation domains. This method of analysis is applied to the tumor suppressor p53 in the accompanying paper (29).

**REFERENCES**

1. Fong, W. F., Heller, J. S., and Canelakis, E. S. (1976) Biochim. Biophys. Acta 428, 456–465
2. Heller, J. S., Fang, W. F., and Canellakis, E. S. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 1850–1862
3. Kitani, T., and Fujisawa, H. (1984) J. Biol. Chem. 259, 10036–10040
4. Matsufuji, S., Miyazaki, Y., Kanamoto, K., Kameji, T., Murakami, Y., Baby, T. G., Fujita, Ohno, T. K., and Hayashi, S. (1990) Biochem. (Tokyo) 108, 365–371
5. Miyazaki, Y., Matsufuji, S., and Hayashi, S. (1992) Gene (Amst.) 113, 191–197
6. Li, X., and Coffino, P. (1992) Mol. Cell. Biol. 12, 3556–3562
7. Li, X., and Coffino, P. (1994) Mol. Cell. Biol. 14, 87–92
8. Li, X., and Coffino, P. (1993) Mol. Cell. Biol. 13, 2377–2383
9. Ghoda, L. V., van Daalen Wetters, T., Macrae, M., Aschnerman, D., and Coffino, P. (1989) Science 243, 1493–1495
10. Goldberg, A. L., and Rock, K. L. (1992) Nature 357, 375–379
11. Gottesman, S., and Maurizi, M. R. (1992) Microbiol. Rev. 56, 592–621
12. Hershko, A., and Ciechanover, A. (1992) Annu. Rev. Biochem. 61, 761–807
13. Rechsteiner, M., Hoffman, L., and Dubiel, W. (1993) J. Biol. Chem. 268, 6065–6068
14. Glass, J. R., and Gerber, E. W. (1987) J. Cell. Physiol. 130, 133–141
15. Berovich, Z., Rosenberg-Hasson, Y., Ciechanover, A., and Kahana, C. (1989) J. Biol. Chem. 264, 15949–15952
16. Murakami, Y., Matsufuji, S., Kameji, T., Hayashi, S., Igarashi, K., Tamura, T., Tanaka, K., and Ichihara, A. (1992) Nature 357, 597–599
17. Ghoda, L. V., Phillips, M. A., Bass, K. E., Wang, C. C., and Coffino, P. (1990) J. Biol. Chem. 265, 11823–11826
18. Hoffman, L., Pratt, G., and Rechsteiner, M. (1992) J. Biol. Chem. 267, 22362–22368
19. Dubiel, W., Pratt, G., Ferrel, K., and Rechsteiner, M. (1992) J. Biol. Chem. 267, 22369–22377
20. Murakami, Y., Tanaka, K., Matsufuji, S., Miyazaki, Y., and Hayashi, S. (1992) Biochem. J. 283, 661–664
21. Mannroid-Kidron, E., Omer-Itsacovich, M., Berovich, Z., Tobias, K. E., Rom, E., and Kahana, C. (1994) Eur. J. Biochem. 226, 547–554
22. Phillips, M. A., Coffino, P., and Wang, C. C. (1987) J. Biol. Chem. 262, 8721–8727
23. Bass, K. E., Sommer, J. M., Cheng, Q. L., and Wang, C. C. (1992) J. Biol. Chem. 267, 11034–11037
24. Ghoda, L. V., Sidney, D., Macrae, M., and Coffino, P. (1992) Mol. Cell. Biol. 12, 2178–2185
25. Schaffer, M., Werness, B. A., Hubregtse, J. M., Levine, A. J., and Howley, P. M. (1990) Cell 63, 1129–1136
26. Evans, T., Rosenthal, E. T., Youngblom, J., Distel, D., and Hunt, T. (1983) Cell 33, 389–396
27. Gliedter, M., Murray, A. M., and Kirschner, M. W. (1991) Nature 349, 132–138
28. Murakami, Y., and Hayashi, S. (1985) Biochem. J. 226, 893–896
29. Li, X., and Coffino, P. (1996) J. Biol. Chem. 271, 4447–4451
30. Deveraux, Q., Ustrell, V., Pickart, C., and Rechsteiner, M. (1994) J. Biol. Chem. 269, 7059–7061
The N Terminus of Antizyme Promotes Degradation of Heterologous Proteins
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