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Regulated Degradation of Ornithine Decarboxylase Requires Interaction with the Polyamine-Inducible Protein Antizyme

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Intracellular degradation of vertebrate ornithine decarboxylase (ODC) is accelerated by polyamines, the products of the pathway controlled by ODC. Antizyme, a reversible, tightly binding protein inhibitor of ODC activity, is believed to be involved in this process. Mouse and *Trypanosoma brucei* ODCs are structurally similar, but the trypanosome enzyme, unlike that of the mouse, is not regulated by intracellular polyamines when expressed in hamster cells (L. Ghoda, D. Sidney, M. Macrae, and P. Coffino, Mol. Cell. Biol. 12:2178–2185, 1992). We found that mouse ODC interacts with antizyme in vitro but trypanosome ODC does not. To localize the region necessary for binding, we made a series of enzymatically active chimeric mouse-trypanosome ODCs and tested them for antizyme interaction. Replacing residues 117 to 140 within the 461-amino-acid mouse ODC sequence with the equivalent region of trypanosome ODC disrupted both antizyme binding and in vivo regulation. Formation of an antizyme-ODC complex is therefore required for regulated degradation.

Proteins that turn over rapidly are of special interest because they can quickly adjust their abundance in response to changes in synthesis or degradation. Some labile proteins interact with a second protein that promotes their degradation, e.g., lysosome-degraded proteins interact with a 70-kDa heat shock protein (3), and tumor suppressor p53 interacts with a viral oncoprotein (4). Mammalian ornithine decarboxylase (ODC), a key enzyme in the biosynthesis of polyamines, is one of the most short lived of proteins. Its turnover can proceed along two different pathways: constitutive and polyamine dependent. Polyamine-promoted degradation of ODC seems also to need the involvement of another protein.

The negative effect of polyamines on ODC activity is phenomenologically well characterized (2). Elevation of intracellular polyamines causes a marked reduction of ODC activity, commonly 10- to 100-fold, within a few hours. Loss of activity reflects a reduction in ODC protein (26). This, in turn, results from both accelerated destruction of the ODC present before polyamine augmentation and a reduced rate of production. The level of ODC mRNA is not, however, altered by perturbation of cellular polyamine pools. Polyamine-induced degradation of ODC is correlated with the appearance of a protein termed antizyme (5, 10). Induction of the 26,500-Da protein is sensitive to cycloheximide but not to actinomycin D, indicating that induction is posttranscriptional (5, 16, 18). Antizyme forms a high-affinity, enzymatically inactive, and reversible complex with ODC (17). An association has been found between the proportion of ODC within cells complexed to antizyme and the rate of its degradation (18). Forced intracellular expression of recombinant antizyme reduces the level of ODC and accelerates degradation of the enzyme (19). Such evidence suggests but does not demonstrate that antizyme participates in regulated degradation and leaves its mechanism of action undetermined.

Studies of genetically engineered ODCs have shown that an element required for constitutive degradation lies within the C-terminal 37 amino acids (7). However, polyamine-dependent degradation can take place without this region. Some mutations within the C-terminal sequence increase the half-life of the protein but leave ODC responsive to exogenous polyamine treatment, although the speed or extent of the response may be attenuated (6). These studies exploited the properties of ODC from the parasite *Trypanosoma brucei*. The latter is structurally very similar to mouse ODC, but when expressed in animal cells it is stable and indifferent to the regulatory influence of polyamines. Both ODCs are homodimers. The mouse ODC monomer contains 461 amino acids; trypanosome ODC lacks a region that corresponds to the 36 C-terminal amino acids of the mouse protein. Truncated and deleted forms of mouse ODC and enzymatically active chimeras composed of complementary elements from both species were studied. A mouse ODC with the last 37 amino acids removed was fully active when expressed in cells but showed no evidence of decay over a period of 4 h, in contrast to the full-size protein which, when similarly expressed, decayed with a half-life of 1 h. However, the ODC activity of cells expressing the shortened protein fell in response to elevation of intracellular polyamine levels (6). Because mouse ODC, whether truncated or not, can be made unstable by raising polyamine levels within the cell, there must be a second domain, outside the last 37 residues and not present in trypanosome ODC, that is required for control by polyamines. This conclusion was confirmed by the regulatory behavior of a chimera, a trypanosome ODC with the last 86 residues from mouse ODC. The chimera was constitutively rapidly degraded (8) but did not respond to polyamines.

The reversible nature of the inhibition of ODC by antizyme in vitro indicates that antizyme does not itself degrade ODC. We set out to determine where antizyme binds within ODC and whether intracellular degradation requires binding. A critical test of whether antizyme is involved in polyamine-mediated degradation of ODC would be identification and disruption of its binding site within ODC and

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determination of whether this interferes with regulated degradation. Recently, rat antizyme cDNA has been cloned from a λ gt11 cDNA library (14). The recombinant protein, like antizyme from the native source, reversibly inhibits ODC activity. The clone, by facilitating the production and structural manipulation of functional antizyme, has allowed us to assess the interaction of ODC and antizyme more readily. We found that antizyme binding is essential to polyamine-mediated degradation. This was substantiated by showing that mouse ODC binds antizyme but trypanosome ODC does not, by identifying the region of mouse ODC required for antizyme binding, and by examining the polyamine responsiveness of a series of mouse-trypanosome ODC chimeras. By replacing the relevant region of mouse ODC with the equivalent region of trypanosome ODC, we converted mouse ODC from a polyamine-dependent to a polyamine-independent enzyme.

MATERIALS AND METHODS

ODC activity and antizyme inhibition assays of cell lysates. About 10^6 cells were plated on each 100-cm-diameter dish and grown in Dulbecco's modified Eagle's H21 medium containing 5% fetal calf serum and 1% nonessential amino acids overnight at 37°C. To induce antizyme, intracellular polyamines were elevated by addition of putrescine (final concentration, 500 μM) for 4 h. The cell layer was then rinsed with 0.9% sodium chloride, and cells were collected by scraping and centrifugation and suspended in 100 μl of PDE buffer (10 mM potassium phosphate [pH 7.5], 2 mM dithiothreitol, 0.1 mM EDTA). Cells were lysed by sonication, and 30 μl was used for the ODC assay. The enzymatic reaction was initiated by addition of 8 μl of [^{14}C]ornithine (0.1 μCi/μl), 4 μl of 1% bovine serum albumin, and 3 μl of 25× reaction buffer (1 M potassium phosphate [pH 7.5], 50 mM dithiothreitol, 2.5 mM pyridoxal phosphate) and carried out for 2 h at 37°C. The reaction product, [^{14}C]-labelled CO_2, was collected and counted as previously described (7). To measure inhibition by antizyme, lysates of treated and control cells were mixed and incubated on ice for about 10 min before initiation of the reaction.

In vitro transcription and translation. Mouse or trypanosome ODC cDNA was amplified by the polymerase chain reaction (PCR) using as the 5′ oligonucleotide a primer with a T7 polymerase promoter coupled directly upstream of the first 19 nucleotides of the mouse or trypanosome ODC coding region and using as the 3′ oligonucleotide a primer positioned downstream of the natural stop codon. The PCR products were purified by Centricon centrifugation and transcribed in vitro with T7 RNA polymerase. These RNAs were then translated in a rabbit reticulocyte lysate in the presence of [^{35}S]methionine for the antizyme-binding assay or identically but with nonradioactive methionine for ODC activity and inhibition assays.

Preparation and use of recombinant antizyme and its insoluble matrix. A rat antizyme λ gt11 clone that encodes a β-galactosidase (β-gal)–antizyme fusion protein (14) was kindly provided by S.-I. Hayashi. Antizyme fusion protein or control β-gal protein was prepared by infecting Y1090 cells with the bacteriophage and incubating it for 4 h at 37°C. The antizyme fusion protein or β-gal protein was induced by addition of 10 mM isopropyl-β-D-thiogalactopyranoside and incubation for another 12 h. Crude extracts containing recombinant protein were eluted after incubation with PDE buffer, and the eluate was collected. Monoclonal antibody against β-gal was added to the eluate and incubated with shaking at room temperature for 40 min. The immunological complex was then coupled to Pansorbin by incubation for another 40 min. Antizyme–β-gal–Pansorbin or β-gal–Pansorbin was precipitated by centrifugation and suspended in PE buffer (PDE buffer without dithiothreitol). Antizyme–β-gal–Pansorbin, diluted as indicated with control β-gal–Pansorbin, was used for either the binding or the inhibition assay. For the binding assay, in vitro-translated ^{35}S-labeled ODC (20 μl) was mixed with 10 μl of antizyme affinity Pansorbin and 10 μl of PE buffer and incubated at room temperature for 30 min. The complex was centrifuged, and the pellet was washed three times with 100 μl of PE buffer. ODC was made soluble, fractionated, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. For antizyme inhibition, in vitro-translated ODC was mixed with antizyme affinity Pansorbin on ice for 10 min and the ODC activity of the mixture was measured as described above.

Construction of chimeric ODCs. M285T, M314T, and T314M were made by exchanging restriction fragments between mouse ODC and trypanosome ODC cDNA after introducing restriction sites (*Hpa*I at residue 288 and *Sna*BI at residue 317) by site-directed mutagenesis as described in reference 6. Construct Δ1–41 was made by appending a T7 promoter 5′ of codon 42 by PCR. M141 and M169 were obtained by PCR by introducing a stop codon into primers at residue 141 or 169. T61M was made by replacing mouse ODC residues 1 to 61 with trypanosome ODC cDNA at a *Bst*UI site. T110M was constructed through overlap extension PCR (12) at a homology region of mouse ODC and trypanosome ODC. To make Δ78–145, mouse ODC cDNA was deleted through *Hgi*AI digestion, blunt ended, and religated. T145M was constructed by introducing an *Hgi*AI site at residue 145 of trypanosome ODC and exchanging *Hgi*AI fragments between mouse and trypanosome ODCs. M110T145M was made from T145M by exchange of the region between residues 1 and 110 of mouse ODC at the residue 110 to 116 homology region between mouse and trypanosome ODCs through overlap extension PCR (12). The structures of all constructs were verified by restriction mapping or sequence analysis.

In vivo expression of constructs. The constructs with ODC activity in vitro were transfected into ODC-deficient mutant CHO cells (28) as described by Ghoda et al. (7). Expression vectors utilized the simian virus 40 early promoter (27) and hepatitis B virus polyadenylation region (25) and were constructed by starting with pODC461 (7), which encodes full-length mouse ODC, by appropriate substitution of chimera-coding sequences.

RESULTS

Mouse ODC is inhibited by antizyme, but trypanosome ODC is not. ODC from the parasite *T. brucei* is structurally very similar to mouse ODC (22). When expressed in animal cells, the trypanosome ODC is both stable and indifferent to the regulatory influence of polyamines (6). Mouse ODC must therefore have an element required for control by polyamines that is not present in trypanosome ODC. If antizyme promotes degradation by direct interaction, its binding site should not be present in trypanosome ODC and disruption of its binding site within mouse ODC should interfere with regulated degradation.

We first determined whether mouse and trypanosome ODCs interact differently with antizyme. Mutant CHO cells devoid of endogenous ODC activity (28) and transfected

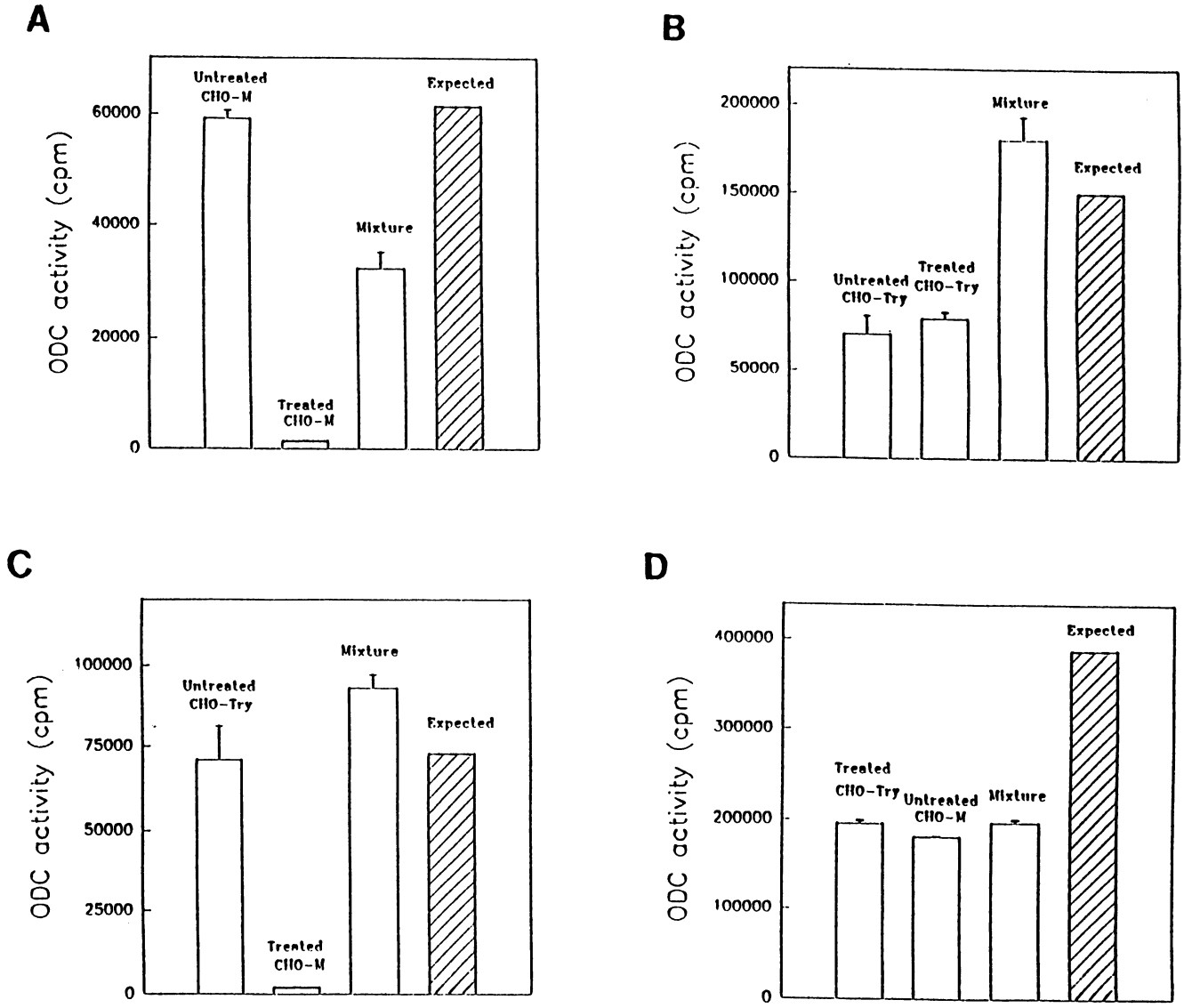

FIG. 1. Inhibitory effect of polyamine-induced antizyme on mouse ODC versus trypanosome ODC. ODC-inhibitory activity was induced by putrescine treatment of cells (500 μM putrescine for 4 h), and an extract was prepared and mixed with an extract from untreated cells. The resulting activity (Mixture) was compared with the summed activity (Expected) of each extract assayed separately. Cells expressed transfected mouse ODC (CHO-M) or trypanosome ODC (CHO-Try), as indicated. Panels: A, treated plus control CHO-mouse cells; B, Treated plus control CHO-tryp cells; C, Treated CHO-mouse cells plus control CHO-tryp cells; D, treated CHO-tryp cells plus control CHO-mouse cells.

with genes that encode mouse or trypanosome ODC (6), here termed, respectively, CHO-mouse and CHO-tryp cells, were used to provide the target enzyme. The same cells were treated to augment polyamines; these served as a source of crude antizyme activity. Polyamine-treated and control cell extracts were prepared and mixed, and ODC activity was measured. When extracts were prepared from untreated and putrescine-treated CHO-mouse cells and assayed, the latter had much less ODC activity, as reported previously. A mixture of the two extracts had less-than-additive activity (Fig. 1A), suggesting the presence of an excess of free inhibitor in the treated cells. When CHO-tryp cells were similarly examined, putrescine treatment had no effect on ODC activity and the activity of mixed extracts was additive (Fig. 1B). Two possible explanations for this result are that (i) in CHO-tryp cells, antizyme was not induced or (ii) trypanosome ODC could not respond to antizyme. To distinguish between these possibilities, we mixed lysates from untreated CHO-tryp and putrescine-treated CHO-mouse cells, which had been found to contain the inhibitor. Activities were again additive (Fig. 1C), suggesting that the inhibitor cannot act on the trypanosome enzyme. The converse experiment, in which treated CHO-tryp extracts were mixed with untreated CHO-mouse extracts, exhibited less-than-additive activity, indicating that the inhibitor could be induced in CHO-tryp cells and could act on mouse ODC (Fig. 1D). Our results suggested that mouse ODC, but not trypanosome ODC, was inhibited by extracts having antizyme activity. These results are consistent with the conclusion that the failure of trypanosome ODC to be regulated by intracellular polyamines is a consequence of its inability to respond to the inhibitor antizyme.

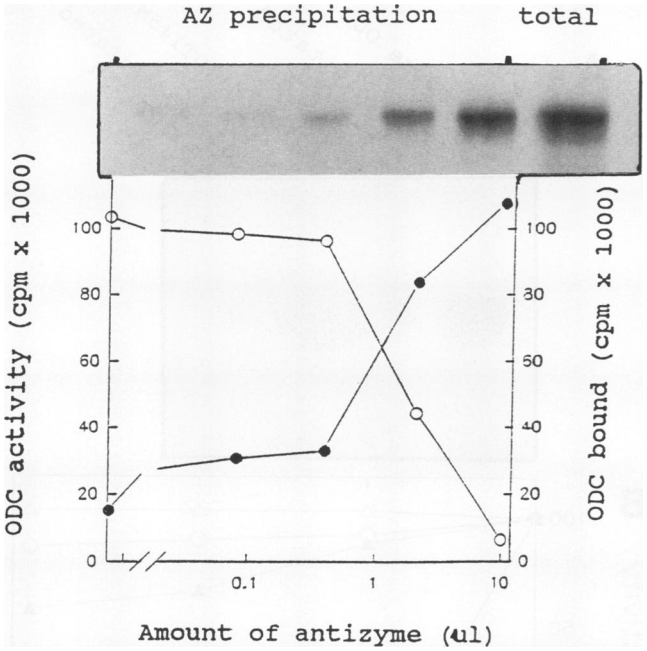

FIG. 2. Binding of antizyme to ODC is associated with inhibition of enzymatic activity. For the antizyme (AZ)-ODC binding assay, ^{35}S-labelled mouse ODC was incubated with different amounts of an antizyme–β-gal–antibody–Pansorbin complex (0, 0.08, 0.4, 2, and 10 µl) and the bound radiolabelled ODC was recovered by centrifugation. Bound ODC was assayed either by scintillation counting of radioactivity in the precipitate (closed symbols) or by SDS-PAGE and autoradiography (inset; lanes are aligned to indicate the amounts of antizyme used). The total ^{35}S-labelled ODC present before binding was also determined by SDS-PAGE to assess the efficiency of precipitation (total). To measure inhibition, unlabelled mouse ODC was incubated with the antizyme complex as for the binding assay and then assayed for ODC activity (open symbols). β-Gal–antibody–Pansorbin, as a nonspecific control, was used to maintain a constant amount of the antigen-antibody-Pansorbin complex in all assays.

Antizyme inhibition and binding are associated. A better-defined source of antizyme and ODC is required to study the nature of the interaction between antizyme and its target and to establish the basis of its inhibitory activity. We wanted to know in particular whether or not trypanosome ODC was unresponsive because it did not associate with antizyme. A recombinant antizyme in the form of a λ gt11 clone of rat antizyme fused to bacterial β-gal (14) was made available to us by S. Hayashi and colleagues. The fusion protein, like native antizyme, associates with mouse ODC with high affinity and inhibits its activity (14). The antizyme fusion protein was coupled to an insoluble matrix (Pansorbin) through a monoclonal antibody against β-gal. The antizyme-Pansorbin complex was used for both inhibition and binding assays. To produce mouse, trypanosome, and chimeric ODCs, mRNAs were produced by in vitro transcription and translated in vitro. Mouse ODC was inhibited by the recombinant antizyme in a dose-dependent manner (Fig. 2, open symbols). Precipitated radiolabelled mouse ODC was assayed either through direct counting of the radiolabel (Fig. 2, closed symbols) or by SDS-PAGE and autoradiography (inset). Mouse ODC was specifically precipitated by antizyme, and the extent of precipitation was dependent on the amount of antizyme added. Inhibition of activity was closely related to precipitation of [^{35}S]methionine-labelled ODC.

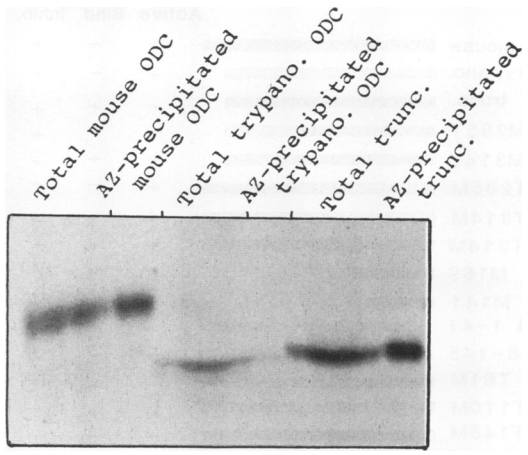

FIG. 3. Comparison of antizyme (AZ) binding and inhibition properties among mouse ODC, trypanosome ODC, and truncated mouse ODC425. Mouse ODC, trypanosome ODC, and truncated mouse ODC (M425) were amplified, transcribed, and translated as previously described (6). Translated ODCs were analyzed for binding to antizyme as described in the legend to Fig. 2. Equal aliquots of each labelled ODC were analyzed by SDS-PAGE before precipitation (Total) or after precipitation (AZ-precipitated). Overloading of unlabeled proteins in lanes marked "Total" resulted in broadening compared with the other lanes.

Mouse ODC is bound by antizyme, but trypanosome ODC is not. The binding and activity assays were next applied to trypanosome ODC. Trypanosome ODC activity was neither inhibited (5×10^4 cpm with control β-gal–Pansorbin versus 4.7×10^4 cpm with antizyme-Pansorbin) nor bound (Fig. 3) by antizyme, in contrast to mouse ODC activity (4.9×10^4 cpm with control β-gal–Pansorbin versus 0.6×10^4 cpm with antizyme-Pansorbin). In addition, we assessed the effect of antizyme on a C-truncated form of mouse ODC which is stable but polyamine regulated (6). Like intact mouse ODC, the truncated form, devoid of the last 37 amino acids, both binds to (Fig. 3) and is regulated by antizyme (23.4×10^4 cpm with control β-gal–Pansorbin versus 4.6×10^4 cpm with antizyme-Pansorbin). This result implies two conclusions. (i) The C terminus, the most prominent single structural domain present in mouse but not trypanosome ODC, is not a prerequisite for antizyme binding. (ii) A major structural alteration of mouse ODC that does not abolish polyamine-dependent regulation also does not alter interaction with antizyme.

Chimeric proteins: antizyme binding and regulatory properties. To localize the binding site and to examine the relationship between binding, inhibition, and polyamine-dependent regulation, we constructed a series of chimeric proteins of mouse and trypanosome ODCs. Figure 4 indicates the structures of the ODC-derived proteins we studied and summarizes their functional properties. Each construct was composed of the ODC amino acid sequence from mice (solid bars) or trypanosomes (hatched bars). The enzymatic activities of constructs were assessed by assay of the in vitro translation products. In all of the cases examined, except T61M and T110M, full-size ODCs were enzymatically active. All constructs were tested for antizyme binding, and those with enzymatic activity were tested for inhibition by antizyme.

The properties of a pair of reciprocal chimeras, M285T and T285M, indicated that the site of antizyme binding lies in

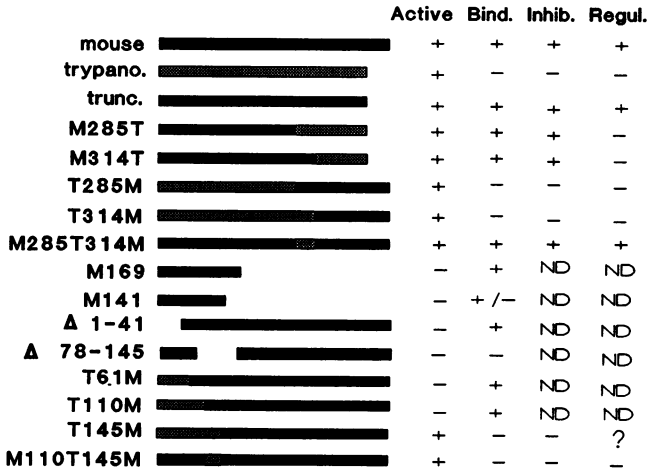

FIG. 4. Structures, antizyme binding, and inhibition properties of chimeric mouse-trypanosome ODCs and truncated mouse ODCs. The constructs indicated were transcribed in vitro, and translated proteins were generated for antizyme-binding and inhibition assays. The ODC activities, antizyme binding, and inhibition properties of the proteins were determined as described in the legends to Fig. 1 and 2; in vivo regulation by polyamines was determined after stable expression in ODC$^-$ CHO cells as described in reference 7. ND, not determined. ?, activity in vivo too low for assessment of regulation.

the first 285 amino acids of mouse ODC, because the former bound and the latter did not. A second reciprocal pair, M314T and T314M, supported that conclusion, for the former bound and the latter did not. T61M and T110M both bound antizyme, constraining the site of binding to a region on the C-terminal side of residue 110, a conclusion consistent with the finding that the N-terminal truncation, Δ1–41, also bound antizyme. N-terminal ODC fragments M141 and M169 bound (the former more weakly than the latter), placing the site between residues 110 and 141. This inference was further supported by failure of the internal deletion MΔ78–145 to bind. These results indicated that the locus of antizyme binding lies between residues 110 and 145. In every case, determinations of binding and inhibition concurred. All enzymatically active constructs were stably expressed in ODC$^-$ CHO cells and tested for regulation by polyamines. All constructs without binding activity were incapable of regulation in vivo.

As a critical test of these inferences, we constructed a chimera, M110T145M, in which the DNA that encodes the putative binding region of mouse ODC (110 to 145) was replaced by the cognate region of trypanosome ODC. Because amino acids 110 to 116 and 141 to 143 of the mouse and trypanosome ODCs are identical and because an *Hgi*AI site introduced at 145 converts Lys-144 and Thr-145 of trypanosome ODC to Arg-144 and Ala-145 of mouse ODC, only 24 amino acids, 117 to 140, were replaced in the chimera. M110T145M scarcely bound antizyme (Fig. 5A), and its activity was not inhibited by antizyme (2.8×10^4 cpm with β-gal–Pansorbin and 2.9×10^4 cpm with antizyme-Pansorbin). To determine whether disruption of the binding site altered polyamine-dependent regulation in vivo, the same chimera was stably expressed in mutant CHO cells lacking endogenous ODC activity. M110T145M-derived ODC activity was not markedly diminished by augmentation of intracellular polyamines (Fig. 5B), even when putrescine treatment was prolonged to 24 h (data not shown). Both in vitro

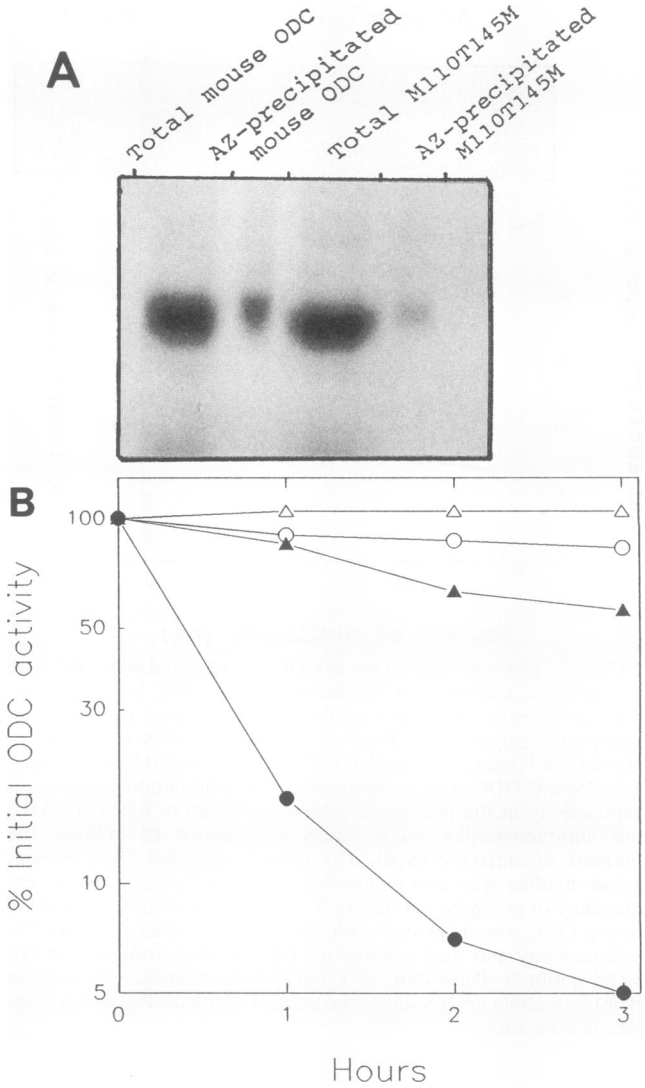

FIG. 5. In vitro antizyme binding and polyamine-dependent regulation of mouse ODC and M110T145M. Mouse and M110T145M ODC cDNAs were amplified, transcribed, and translated as described in reference 6 and Materials and Methods. Translated ODCs were analyzed for binding to antizyme, and equal aliquots of the labelled ODCs were analyzed by SDS-PAGE before precipitation (Total) or after precipitation (AZ-precipitated) (A). For the study of polyamine-dependent regulation, mutant ODC-deficient CHO cells were stably transfected with expression vectors (6) that encode M110T145M chimeric (triangles) or mouse (circles) ODC. Cells were untreated (open symbols) or treated (closed symbols) with 500 μM putrescine to expand polyamine pools, and cultures were harvested at the indicated times after treatment. Cell extracts were prepared, and ODC activities were determined. Percentages of ODC activity of untreated cells (initial time point) are shown. Initial activities for untreated and treated mouse cells were 24.6 and 30 pmol/min/mg of protein, respectively, and those for untreated and treated M110T145M were 1.9 and 2.7 pmol/min/mg of protein, respectively.

and in vivo, M110T145M behaved like trypanosome ODC. Mouse ODC, identical to M110T145M but for the 24-amino-acid substitution, in contrast, showed binding, inhibition, and full regulation.

Inhibition of activity was measured for all proteins that were enzymatically active. In every case, binding and in

vitro inhibition determinations concurred. Not so for in vivo regulation: no construct without binding activity was capable of regulation; however, M285T and M314T bound but did not regulate. Our recent findings suggest that antizyme binding is necessary but not sufficient for polyamine-dependent regulation (unpublished data). Because we did not measure the binding affinities of the various forms of ODC quantitatively and do not know whether the binding site consists of a single linear epitope, we can conclude only that amino acids 117 to 140 of mouse ODC contribute importantly to antizyme binding.

DISCUSSION

Mammalian ODC is selectively degraded by cells when polyamines rise. This specificity requires a degradation pathway that selectively recognizes its target. The polyamine-mediated decline of ODC activity is associated with the appearance of a polyamine-induced protein, antizyme (5, 10). Numerous in vitro studies have demonstrated that antizyme binds ODC tightly and reversibly inhibits enzymatic activity. More direct evidence for a regulatory role comes from forced cellular expression of recombinant antizyme, which accelerates ODC degradation (19). Antizyme may promote degradation by associating with ODC, but it cannot act directly as a proteinase. Understanding the molecular interaction between antizyme and ODC is thus an important step in elucidating the mechanism of polyamine-mediated degradation.

Three lines of evidence presented here strongly support the conclusion that antizyme promotes degradation by forming a complex with ODC. (i) Our studies demonstrated that the difference in polyamine regulation of mouse and trypanosome ODCs is associated with a difference in sensitivity to antizyme. (ii) We identified the region of mouse ODC between amino acids 117 and 140 as necessary for antizyme binding. The constructs containing this region were shown to be inhibited and precipitated by antizyme in vitro. (iii) We showed that replacing this region with the corresponding one from trypanosome ODC converted mouse ODC into a form resistant to antizyme in vitro and unresponsive to polyamines in vivo. These studies strongly support the conclusion that antizyme binding promotes degradation of ODC and imply that interaction between antizyme and ODC is a necessary step in polyamine-induced degradation.

A comparison of the amino acid sequences of mouse and trypanosome ODCs in the antizyme-binding region reveals that 12 of 24 residues are identical:

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                117                 140
Mouse:          VSQIKYAASNGVQMMTFDSEIELM
Trypanosome:    ISHIRYARDSGVDVMTFDCVDELE
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The specific residues responsible for interaction must be determined by site-directed mutagenesis.

Ubiquitin conjugation plays a well-documented role in protein degradation, but there is in vivo and in vitro evidence that ODC is not a substrate of the ubiquitin pathway (1, 9, 24). Labile proteins have been found to contain strings of amino acids rich in PEST residues (single-letter code) (23). Mouse ODC contains two such regions, one central and one C terminal, but the antizyme-binding region corresponds to neither. Truncation of the C-terminal PEST-rich region changes the constitutive stability of ODC (7) but not its ability to respond to polyamines (6). These results support the hypothesis that the C-terminal region of ODC is required for basal degradation and the antizyme-binding region is required for polyamine-mediated degradation. The presence within antizyme itself of a PEST region (9a) suggests that the PEST motif functions as a portable element that can confer lability upon proteins with which it associates.

Polyamines have been perceived to alter ODC levels in two mechanistically distinct ways: production and degradation. The findings that manipulation of polyamine pools does not alter ODC mRNA levels but does change both the rate of incorporation of labelled amino acids into ODC and the steady-state amount of the enzyme have been interpreted to indicate translational regulation by polyamines (11, 13, 15, 20, 21). However, recent results (29) are more consistent with another interpretation: ODC can be degraded during or immediately after synthesis, and the probability of destruction versus conservation depends on the polyamine status of the cell. This concept unites the apparently disparate effects of polyamines on production of newly synthesized ODC and destruction of pre-existing enzyme molecules but allows limited time for co- or posttranslational degradation. The presence of the antizyme-binding site close to the amino terminus of ODC may permit the recognition process to be initiated on the nascent protein. It is attractive to imagine that antizyme binding distorts the conformation of the ODC polypeptide, both preventing folding into an enzymatically active form and facilitating degradative processing. Thus, upon induction by polyamines, antizyme acts stoichiometrically to annul ODC enzymatic activity and recurrently to encumber production and enhance destruction of the ODC polypeptide.

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REFERENCES

1. **Bercovich, Z., Y. Rosenberg-Hasson, A. Ciechanover, and C. Kahana.** 1989. Degradation of ornithine decarboxylase in reticulocyte lysate is ATP-dependent but ubiquitin-independent. J. Biol. Chem. **264**:15949–15952.
2. **Canellakis, E. S., and S. Hayashi.** 1989. The role of antizyme in the regulation of ornithine decarboxylase activity in eukaryotic cells, p. 315–330. *In* U. Bachrach and Y. M. Heimer (ed.), The physiology of polyamines, vol. I. CRC Press, Inc., Boca Raton, Fla.
3. **Chiang, H. L., S. R. Terlecky, C. P. Plant, and J. F. Dice.** 1989. A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins. Science **246**:382–385.
4. **Crook, T., J. A. Tidy, and K. H. Vousden.** 1991. Degradation of p53 can be targeted by HPV E6 sequences distinct from those required for p53 binding and trans-activation. Cell **67**:547–556.
5. **Fong, W. F., J. S. Heller, and E. S. Canellakis.** 1976. The appearance of an ornithine decarboxylase inhibitory protein upon the addition of putrescine to cell culture. Biochim. Biophys. Acta **428**:456–465.
6. **Ghoda, L., D. Sidney, M. Macrae, and P. Coffino.** 1992. Structural elements of ornithine decarboxylase required for intracellular degradation and polyamine-dependent regulation. Mol. Cell. Biol. **12**:2178–2185.
7. **Ghoda, L., T. van Daalen Wetters, M. Macrae, D. Ascherman, and P. Coffino.** 1989. Prevention of rapid intracellular degradation of ODC by a carboxyl-terminal truncation. Science **243**: 1493–1495.
8. **Ghoda, L. Y., M. A. Phillips, K. E. Bass, C. C. Wang, and P. Coffino.** 1990. Trypanosome ornithine decarboxylase is stable

because it lacks sequences found in the carboxyl terminus of the mouse enzyme which target the latter for intracellular degradation. J. Biol. Chem. **265:**11823–11826.

9. **Glass, J. R., and E. W. Gerner.** 1987. Spermidine mediates degradation of ornithine decarboxylase by a non-lysosomal, ubiquitin-independent mechanism. J. Cell. Physiol. **130:**133–141.

9a. **Hayashi, S.** Personal communication.

10. **Heller, J. S., W. F. Fong, and E. S. Canellakis.** 1976. Induction of a protein inhibitor to ornithine decarboxylase by the end products of its reaction. Proc. Natl. Acad. Sci. USA **73:**1858–1862.

11. **Hölttä, E., and P. Pohjanpelto.** 1986. Control of ornithine decarboxylase in Chinese hamster ovary cells by polyamines. J. Biol. Chem. **261:**9502–9508.

12. **Horton, R. M., H. D. Hunt, S. N. Ho, J. K. Pullen, and L. R. Peasse.** 1989. Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlap extension. Gene **77:**61–68.

13. **Kahana, C., and D. Nathans.** 1985. Translational regulation of mammalian ornithine decarboxylase by polyamines. J. Biol. Chem. **260:**15390–15393.

14. **Matsufuji, S., Y. Miyazaki, R. Kanamoto, T. Kameji, Y. Murakami, T. G. Baby, K. Fugita, and S. Hayashi.** 1990. Analyses of ornithine decarboxylase antizyme mRNA with a cDNA clone from rat liver. J. Biochem. **108:**365–371.

15. **McConlogue, L., S. L. Dana, and P. Coffino.** 1986. Multiple mechanisms are responsible for altered expression of ornithine decarboxylase in overproducing variant cells. Mol. Cell. Biol. **6:**2865–2871.

16. **Mitchell, J. L. A., D. W. Mahan, P. P. McCann, and P. Qasba.** 1985. Dicyclohexylamine effects on HTC cell polyamine content and ornithine decarboxylase activity. Biochim. Biophys. Acta **840:**309–316.

17. **Murakami, Y., K. Fujita, T. Kameji, and S. Hayashi.** 1985. Accumulation of ornithine decarboxylase-antizyme complex in HMO_A cells. Biochem. J. **225:**689–697.

18. **Murakami, Y., and S. Hayashi.** 1985. Role of antizyme in degradation of ornithine decarboxylase in HTC cells. Biochem. J. **226:**893–896.

19. **Murakami, Y., S. Matsufuji, Y. Miyazaki, and S. Hayashi.** 1991. Destabilization of ornithine decarboxylase by transfected antizyme gene expression in hepatoma tissue culture cells. J. Biol. Chem., in press.

20. **Persson, L., I. Holm, and O. Heby.** 1988. Regulation of ornithine decarboxylase mRNA translation by polyamines. J. Biol. Chem. **263:**3528–3533.

21. **Persson, L., S. M. Oredsson, S. Anehus, and O. Heby.** 1985. Ornithine decarboxylase inhibitors increase the cellular content of the enzyme: implications for translational regulation. Biochem. Biophys. Res. Commun. **131:**239–245.

22. **Phillips, M. A., P. Coffino, and C. C. Wang.** 1987. Cloning and sequencing of the ornithine decarboxylase gene from Trypanosoma brucei. J. Biol. Chem. **262:**8721–8727.

23. **Rogers, S., R. Wells, and M. Rechsteiner.** 1986. Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science **234:**364–368.

24. **Rosenberg-Hasson, Y., Z. Bercovich, A. Ciechanover, and C. Kahana.** 1989. Degradation of ornithine decarboxylase in mammalian cells is ATP dependent but ubiquitin independent. Eur. J. Biochem. **185:**469–474.

25. **Russnak, R., and D. Ganem.** 1990. Sequences 5′ to the polyadenylation signal mediate differential poly(A) site use in hepatitis B viruses. Genes Dev. **4:**764–776.

26. **Seely, J. E., and A. E. Pegg.** 1983. Changes in mouse kidney ornithine decarboxylase activity are brought about by changes in the amount of enzyme protein as measured by radioimmunoassay. J. Biol. Chem. **258:**2496–2500.

27. **Simonsen, C. C., and A. D. Levinson.** 1983. Isolation and expression of an altered mouse dihydrofolate reductase cDNA. Proc. Natl. Acad. Sci. USA **80:**2495–2499.

28. **Steglich, C., and I. E. Scheffler.** 1982. An ornithine decarboxylase-deficient mutant of Chinese hamster ovary cells. J. Biol. Chem. **257:**4603–4609.

29. **van Daalen Wetters, T., M. Macrae, M. Brabant, A. Sittler, and P. Coffino.** 1989. Polyamine-mediated regulation of mouse ornithine decarboxylase is posttranslational. Mol. Cell. Biol. **9:**5484–5490.