Spermidine-induced Destabilization of Ornithine Decarboxylase (ODC) Is Mediated by Accumulation of Antizyme in ODC-overproducing Variant Cells*

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The mechanism of spermidine-induced destabilization of ornithine decarboxylase (ODC) was examined in newly isolated ODC-overproducing variant cells by use of an in vitro ODC degrading system. The cells accumulated ODC protein at a level of 2-4% of the total cytosolic protein in the presence of α-difluoromethylornithine. Addition of spermidine to the medium accelerated degradation of ODC protein concomitantly with induction of antizyme, a regulatory protein that binds to ODC, inhibiting its activity. Both the acceleration of ODC degradation and the induction of antizyme were inhibited by cycloheximide, but not by actinomycin D. ODC was degraded rapidly in extracts from spermidine-treated cells. The rate of ODC degradation correlated with the amount of antizyme in the extracts, and the degradation activity was abolished by treatment of the extracts with anti-antizyme antibody. Thus, antizyme induced by spermidine was essential for the accelerated degradation of ODC in the cells.

ODC was phosphorylated in the cells, probably at serine residue 303 in the first internal PEST region. ODC phosphorylation occurred even when its new synthesis was inhibited by cycloheximide. Antizyme accelerated the degradations of both dephosphorylated ODC and native ODC.

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fact, the half-life of ODC changes rapidly during the induction of the enzyme (4-6).

Polyamines stimulate the degradation of ODC as a type of negative feedback control (3). Results in one of our laboratories showed that ODC antizyme, a small ODC-binding protein that is induced by polyamines (7-9), mediates polyamine-induced acceleration of ODC degradation (10-15). ODC contains two PEST regions, amino acid sequences presumed to be involved in the degradation of proteins with rapid turnovers, at residues 298-333 and 423-449 (16, 17). There are reports that truncation of the C-terminal PEST region made the enzyme more stable (18) and that the first internal PEST region contains Ser-303, which is phosphorylated both in vivo (19) and by casein kinase II in vitro (20-23), although the role of this phosphorylation is not yet clear.

We recently isolated an ODC-overproducing cell line from mouse FM3A cells by using the method of stepwise increment of α-difluoromethylornithine (DFMO), an irreversible inhibitor of the enzyme. The cells, termed EXOD-1, produced ODC protein at a level of about 2-4% of their total cytosolic proteins when cultured in the presence of DFMO (24). Their ODC content, however, rapidly decreased after removal of the drug from the medium, mainly due to suppression of ODC synthesis and stimulation of ODC degradation, probably caused by an increase in the cellular level of polyamines (24).

The aim of the present study was to examine whether antizyme mediates the degradation of such a large amount of ODC and whether phosphorylation is involved in the regulation of ODC degradation. For these purposes, we first compared the degradations of ODC in cells in vivo and in a cell-free ODC degradation system in vitro (14, 15) and demonstrated that antizyme was essentially involved in spermidine-induced acceleration of ODC degradation in the ODC-overproducing cells. Then we examined the effects of dephosphorylation of ODC on its degradation rate in the in vitro system. The result indicated that dephosphorylated ODC, prepared by treating purified 35S-labeled ODC with alkaline phosphatase, was degraded as rapidly as native ODC in this system.

EXPERIMENTAL PROCEDURES

Materials—A mixture of L-[35S]methionine and L-[35S]cysteine (Expep[35S])S was purchased from Du Pont-New England Nuclear. 32P[Orthophosphoric acid was from American Radiolabeled Chemicals (St. Louis, MO). α-Difluoromethylornithine (DFMO) was kindly provided by Dr. P. P. McCann, Merrell Dow Research Institute, Cincinnati, OH. Nafamostat mesilate (Futhan) was a gift from Torii Yaktuin Co. (Tokyo). ES medium was from Nissui Pharmaceutical Co. (Tokyo), and fetal calf serum was from GIBCO/BRL. Alkaline phosphatase and reagents for immunoblotting were purchased from Sigma. Goat anti-rabbit IgG-alkaline phosphatase conjugate was a

Ornithine decarboxylase (ODC) catalyzes the conversion of ornithine to putrescine, the first step and a major site of regulation of polyamine biosynthesis (1). Large, rapid fluctuations of ODC activity are observed in growth and differentiation processes in which polyamines play essential roles (2). The level of ODC is known to be controlled at several sites, namely transcription, translation, and enzyme degradation (3). Much attention has recently been paid to the degradation of ODC, since ODC has the shortest half-life among known enzymes and therefore regulation of its decay rate may have prompt effects on its response to regulatory stimuli (3). In
product of Kirkegaard & Perry Laboratories (Gaithersburg, MD). Antizyme and rabbit anti-antizyme IgG were prepared as described (14, 24). A formalin-fixed Staphylococcus aureus Cowan I cell suspension (Pansorbin) was a product of Calbiochem.

**Cell Culture—EXOD-1 cells, an FMSA-derived, DFMO-resistant, ODC-overproducing cell line (24), were maintained in ES medium containing 2% (v/v) fetal-calf serum and 20 mM DMPO (basal medium). In this study, all media used for cell culture contained 20 mM DFMO. One day before experiments, cells were diluted to 3 × 10^5 cells/ml and incubated in basal medium. The cell concentration was about 6 × 10^5/ml at the start of experiments.**

**Metabolic Labeling of Cells and Immunoprecipitation of Labeled ODC—EXOD-1 cells were washed with ES medium, containing 2% (v/v) fetal-calf serum without either methionine or phosphate, and then labeled in the same medium with Expre^35S^S or [35S]Orthophosphate as indicated in the text. They were then washed with cold phosphate-buffered saline and collected by low speed centrifugation.

The cell pellet was freeze-thawed three times in 200 μl of extraction buffer (25 mM Tris-HCl, pH 7.5, 1 mM DTT, 1 mM EGTA, 0.1 mM EDTA, 10 mM NaF, 10 mM glycerophosphate, 2 mM pyridoxal 5'-phosphate, 10 μg/ml nafamostat mesilate) and cell extracts were prepared by centrifugation at 16,000 rpm for 20 min at 4°C in an Eppendorf-type microcentrifuge. Labeled ODC was immunoprecipitated from the extracts with anti-ODC antisera and formalin-fixed *S. aureus* Cowan I cells (Pansorbin) as described previously (26).

**Preparation of Labeled ODC and Dephosphorylated Labeled ODC—** For preparation of [35S]labeled ODC, 3 × 10^7 cells were labeled with [35S]Orthophosphate (1 mCi/ml containing 3 × 10^8 cells) harvested and lysed in 500 μl of extraction buffer as described above, and the lysate was centrifuged at 36,000 rpm (100,000 × g) for 1 h at 4°C. The extracts were directly applied to a 0.1-ml anti-ODC monoclonal antibody HO-101 Affi-Gel 10 column and the labeled ODC was eluted as described (27). The eluate was dialyzed against 50 mM Hepes buffer, pH 7.5, containing 1 mg MglCl, 0.1 mM EDTA, 0.01% Tween 20, 1 mM DTT, and 20% glycerol (dialysis buffer). The specific radioactivity of purified ODC was 427,000 cpm of [35S]μg of ODC protein. Part of the purified [35S]ODC (20 μg in 200 μl of dialysis buffer) was mixed with 4 μl of alkaline phosphatase (200 units) and incubated at 37°C for 40 min. For removal of alkaline phosphatase, the mixture was reappplied to an HO-101 Affi-Gel 10 affinity column and dephosphorylated labeled ODC was eluted as described above. Cells (6 × 10^6/ml) were incubated with [35S]Orthophosphate (1 mCi/ml) for 8 h, cell extracts were prepared, and [35P]-labeled ODC was purified as described above.

**Purification of ODC from EXOD-1 Cells and Production of ODC Antisera—** The amount of ODC in EXOD-1 cells was highest 1 day after removal of DFMO from the basal medium (24). Therefore, for cells/ml, cultured in basal medium lacking DFMO for 1 day, and then harvested and lysed in 500 pl of extraction buffer as described above, the resulting cell pellet (4.5 × 10^6 cells/ml) for 8 h, cell extracts was prepared, and 32P-labeled ODC was immunoprecipitated from the extracts with anti-ODC antisera and formalin-fixed *S. aureus* Cowan I cells (Pansorbin) as described previously (26). Therefore, we usually employed only the latter method.

**Determination of the Amount, and Rates of Synthesis, and Degradation of ODC Protein in EXOD-1 Cells—** The amount of ODC protein was determined by a sandwich enzyme immunoassay using anti-ODC monoclonal antibodies HO-101 and HO-202 as described (13). The cells were labeled with Expre[^35S]S as described above and the rate of synthesis and half-life of ODC protein were measured as indicated in the text. The labeled ODC protein was precipitated from cell extracts (containing 4 mg of protein) with 3 μl of anti-ODC antiserum or preimmune serum and 20 μl of Pansorbin. The precipitates were washed, and labeled ODC was extracted as described (26). The radioactivity in the extracts was measured directly in a liquid scintillation counter. The radioactivity precipitated with preimmune serum was subtracted as a background. The incorporation of [35S]into total soluble protein was determined as described previously (26).

**Other Methods—** SDS-PAGE and immunoblotting were carried out as described previously (25, 26). The band of labeled ODC on the dried gel was detected and quantitated using a Fujix Bas 2000 (imaging analyzer) (Fuji Photo Film Co., Tokyo).

**RESULTS**

**Effects of Spermidine on the Synthesis and Degradation of ODC in EXOD-1 Cells—** Both endogenously formed and exogenously added polyamines are known to suppress ODC synthesis and stimulate its degradation in a variety of cells (3, 26, 32, 33). Therefore, we first examined whether ODC-overproducing EXOD-1 cells could respond to exogenously

**FIG. 1.** Effects of spermidine, cycloheximide, and actinomycin D on decay of ODC protein in ODC-overproducing EXOD-1 cells. Cells were cultured as described under "Experimental Procedures." When the cell concentration was about 6 × 10^5/ml, the cultures were treated with 1 mM spermidine (●), 50 μg/ml cycloheximide (▲), spermidine plus 1 μg/ml actinomycin D (○), or spermidine plus cycloheximide (△). The cells were harvested at the indicated times thereafter, and cellular contents of ODC protein were determined by sandwich enzyme immunoassay. Data are for two independent experiments and ranges for triplicate assays. The contents of ODC at time zero of each experiment were 33 (●, ▲) and 20 (○, △) μg/mg cytosol protein, respectively.
results suggested that accelerated degradation of ODC caused by spermidine required new protein synthesis (3, 34).

**Antizyme Mediates Accelerated Decay of ODC in Spermidine-treated Cells**—Recently, an ODC degradation system with cell-free extracts was developed in one of our laboratories (14, 15). In this system, ODC degrading activity depends on both ATP and antizyme. We used this system to determine the mechanism of degradation of ODC in EXOD-1 cells. If the cell-free system really reflects ODC degradation in cells, extracts of spermidine-treated cells should show higher activity than extracts of control cells for ODC degradation. Therefore, cell-free extracts were prepared from the cells with or without spermidine treatment and their ODC-degrading activities were determined in the absence of exogenous antizyme by following the decay of 35S-labeled ODC which had been purified from cells labeled with Expre35S (Fig. 2a). The results demonstrated that the ODC-degrading activity in cell-free extracts increased with increase in time of treatment of the cells with spermidine (Fig. 2b). This increase was completely inhibited by simultaneous addition of cycloheximide, but not of actinomycin D (Fig. 2c), again suggesting that protein synthesis in the cells was required during spermidine treatment for the enhanced degradation of ODC observed in cell-free extracts. The degradation activity was ATP-dependent (Table II) as reported (14, 15, 35–37). Thus this system well reflected the accelerated degradation of ODC caused by polyamines in vivo (3, 26, 34).

Next we examined whether antizyme was involved in the accelerated degradation caused by spermidine in the EXOD-1 cells. Fig. 3 demonstrates the induction of antizyme in the EXOD-1 cells. The amount of antizyme in the cells increased time dependently after treatment with spermidine (Fig. 3a, lanes 2–5), and the increase was completely inhibited by cycloheximide, but not inhibited by actinomycin D (Fig. 3a, lanes 6 and 7). These treatments did not change the amount of antizyme mRNA (data not shown), suggesting that the induction of antizyme is controlled at the level of translation (25). Thus changes in the rate of ODC degradation in both

**Table I**

| Treatment | Rate of ODC synthesis | Half-life of 35S-ODC protein |
|-----------|-----------------------|-----------------------------|
| None      | 4.1 ± 0.9             | 180                         |
| Spermidine| 2.3 ± 0.3             | 70                          |

*Changes in the synthesis rate and half-life of ODC in EXOD-1 cells after treatment with spermidine*

Cells (6 x 10^6/ml) were incubated with or without spermidine (1 mM) for 8 h. Then, they were labeled with Expre35S (100 μCi/ml) for 5, 10, or 30 min to measure the synthesis rate of ODC, which was expressed relative to that of total soluble protein synthesis. Duplicate dishes were used for each time point, and linear increases in the incorporation of 35S into ODC and soluble protein were observed. Values are means ± S.D. for 6 determinations. For determination of the half-life, cells were labeled for 30 min with Expre35S (50 μCi/ml), and then the decay of 35S-labeled ODC was followed by incubating the cells in the same fresh medium containing 1 mM unlabeled L-methionine. The cells were harvested 0, 1, 2, and 3 h after the start of chasing. Duplicate dishes were used at each time point. Decay of labeled ODC showed first order kinetics during the chase period.

![Fig. 2. Degradation of purified 35S-labeled ODC protein in cell-free extracts from EXOD-1 cells.](image)

(a) Cells were labeled with Expre35S for 150 min. Labeled ODC was affinity-purified and analyzed by SDS-PAGE as described under "Experimental Procedures." Lane M was for molecular size markers with their molecular masses indicated on the right. (b) Crude extracts were prepared from cells that had been cultured in the presence of 1 mM spermidine for 4 h (△) or 8 h (●) or in the absence of spermidine for 8 h (○). ODC degradation activities in the extracts were determined by incubating the extracts for the indicated periods in the degradation assay mixture containing purified 35S-labeled ODC as described under "Experimental Procedures." Values were means for duplicate assays and are expressed as percentages of the initial amounts of ODC protein (∼500 nmol of 35S), which were determined at time zero. Thus, crude extracts were prepared from cells that had been incubated for 8 h with cycloheximide (50 μg/ml) (■), cycloheximide plus spermidine (1 mg/ml) (○), or actinomycin D (1 μg/ml) plus spermidine (△), or without any addition (○). The ODC degrading activities in the cell-free extracts were assayed, and results were expressed as described above. The apparent half-lives of 35S-labeled ODC in the extracts are indicated at the right of each plot.
cells and cell-free extracts correlated well with changes in the induced level of antizyme. Then we examined the effects of anti-antizyme IgG and antizyme on the ODC-degrading activity in cell-free extracts. Removal of antizyme from the extract of spermidine-treated cells by treatment with anti-antizyme IgG, resulted in loss of ODC-degrading activity (Fig. 4a).

Conversely, addition of antizyme to extracts of cycloheximide-treated cells, in which antizyme was not detectable (Fig. 3), resulted in dramatic degradation of ODC (Fig. 1 and Table I). Although antizyme was not detectable in crude extracts (Fig. 3a), a small amount of antizyme may be present and be involved in the degradation of ODC in these cells. Therefore, we partially purified antizyme together with ODC on an HO-101 anti-ODC monoclonal antibody affinity column. More than 90% of the ODC and antizyme were adsorbed to the column, as shown in Fig. 3a (compare lanes 2 and 6 with lanes 1 and 8, respectively). Then antizyme and ODC were eluted and the eluates were analyzed by immunoblotting. Fig. 3b shows that both antizyme and ODC were highly concentrated and that a faint band of antizyme was detectable 2 h after treatment with spermidine (Fig. 3b, lane 2), the time when the accelerated degradation started (Fig. 1). However, antizyme was not detected in the control and cycloheximide-treated cells (Fig. 3b, lanes 1 and 5). Thus, it is unlikely that antizyme contributes to the degradation of ODC in these cells. As the weak ODC-degrading activity observed in control and cycloheximide-treated cell extracts was ATP-dependent (Table II) and was not affected by treatment of anti-antizyme antibody (data not shown), it appeared to reflect basal ODC-degrading activity, as suggested previously (11, 14, 15).

**Degradation of Native and Dephosphorylated ODC in Cell-free Extracts**—Fig. 5 demonstrates the metabolic phosphorylation of ODC in EXOD-1 cells. For determination of the site of phosphorylation, 32P-labeled ODC was purified (Fig. 5b, lane 1), and phosphoamino acids were analyzed by two-dimensional electrophoresis after partial acid hydrolysis (39). The phosphopeptides were resolved by 11% SDS-PAGE after fragmentation with CNBr (21). The results indicated that 32P was exclusively incorporated into the serine residue in a single 10-kDa CNBr fragment (data not shown). These observations are consistent with previous reports that serine 303 is the phosphorylated residue of ODC in mammalian cells (19, 21).

The phosphorylation occurred even when new synthesis of ODC protein was inhibited by cycloheximide (Fig. 5, lanes 4 and 5). During incubation of cells with cycloheximide for 8 h, the cellular content of ODC decreased to 1/6 to 1/8 of that on incubation without cycloheximide. Roughly similar amounts of immunoprecipitated ODC were subjected to SDS-PAGE. As the weak ODC-degrading activity observed in control and cycloheximide-treated cells was ATP-dependent (Table II) and was not affected by treatment of anti-antizyme antibody (data not shown), it appeared to reflect basal ODC-degrading activity, as suggested previously (11, 14, 15).

We next examined whether ODC was degraded in a phosphorylated or dephosphorylated state when its degradation was stimulated by spermidine. For this purpose, we removed phosphate from ODC by treatment with alkaline phosphatase and compared the degradation rates of native and dephosphorylated ODC in the cell-free ODC-degrading system. Treatment with alkaline phosphatase for 10 min was sufficient to remove its phosphate as judged by disappearance of 32P from the ODC band (Fig. 6a). This treatment did not change the apparent molecular weight of ODC on SDS-PAGE (data not shown). As shown in Fig. 6b, native (upper panel) and dephosphorylated ODC (lower panel) were treated with spermidine for 0, 2, 4, and 8 h, respectively. Lane 6, cells were treated with spermidine and actinomycin D for 8 h. Lane 7, cells were treated with spermidine and cycloheximide for 8 h. Lane 8, cells were treated with spermidine and cycloheximide for 8 h.

**TABLE II**

| Source of cell extract | ATP | Incubation time (h) | % Remaining |
|------------------------|-----|---------------------|-------------|
| Control cells          | +   | 5380 ± 550         | 4530 ± 530  | 85  |
|                        | −   | 5500 ± 130         | 5380 ± 80   | 98  |
| Spermidine-treated cells| +   | 5540 ± 510         | 1660 ± 140  | 30  |
|                        | −   | 5780 ± 190         | 5960 ± 200  | 102 |

**Fig. 3. Induction of antizyme in EXOD-1 cells by treatment of spermidine.** a, cell extracts containing 30 µg of protein were fractionated by SDS-PAGE and transferred to a membrane filter. The membrane was cut between molecular size markers of 46 and 30 kDa. ODC and antizyme were detected by immunoblotting with anti-ODC antibody (upper panel) and anti-antizyme antibody (lower panel). Lanes 1, 2, 3, 4, 5, and 6, cells treated with spermidine for 0, 2, 4, and 8 h, respectively. Lane 7, cells were treated with spermidine and cycloheximide for 8 h. Lanes 5 and 8, fractions not adsorbed to an HO-101 affinity column of the same extracts of lanes 2 and 6, respectively. The same extracts as for a were applied to an HO-101 affinity column, and antizyme was eluted with ODC. Samples of 10 µg of protein in the eluate were precipitated with 5 µg of bovine serum albumin in 5% trichloroacetic acid and the precipitate was washed once with cold acetone and dissolved in 20 µl of sample buffer for SDS-PAGE. Antizyme and ODC were detected as described above. Lanes 1-4, cells treated with spermidine for 0, 2, 4, and 8 h, respectively. Lane 5, cells treated with spermidine and cycloheximide for 8 h.
Degradation of ODC in Vivo and in Vitro

with phosphate-buffered saline 3 times by centrifugation and the precipitate was used as immunoadsorbent.

and results are expressed as described above. To avoid dilution of cell extracts, from the cells that had been incubated with spermidine and cycloheximide for 8 h. ODC-degrading activity of the extracts were determined in the presence of cycloheximide.

Labeled ODC was immunoprecipitated from cells that had been incubated with spermidine for 8 h. The extracts (26 pl) were treated with 5 pl of 10 mg/ml anti-antizyme IgG (0), which was added to the medium 5 min before addition of [32P]orthophosphate. Cell extracts were prepared and the contents of ODC were determined by sandwich enzyme assay. Labeled ODC was immunoprecipitated from 5 pl (lanes 1–5) or 18 pl (lanes 6 and 7) of the cell extracts with preimmune serum (lane 1) or with anti-ODC antisera (lanes 2–7) and analyzed by SDS-PAGE.

DISCUSSION

In the present study we compared the degradations of ODC in cells in vivo and in cell-free extracts in vitro to determine the mechanism of polyamine-stimulated degradation of ODC in newly isolated ODC-overproducing cells (24). The results indicated that changes in ODC-degrading activity in the cell-free extracts well reflected accelerated decay of ODC in vivo caused by spermidine. We confirmed that antizyme protein was induced in the cells after treatment with spermidine and that increase in ODC-degrading activity in the cell-free extracts depended solely on the amount of antizyme in the extracts. In 1985, we proposed the working hypothesis that antizyme mediates polyamine-stimulated degradation of ODC, based on the observation that the rate of ODC degradation is correlated with the proportion of ODC that is complexed with antizyme (11). Two strong lines of evidence for this hypothesis were recently obtained in one of our laboratories. First, when antizyme cDNA is transfected into cultured cells in which the antizyme is programmed to be expressed by glucocorticoids, but not by polyamines, ODC degradation is stimulated by addition of dexamethasone (13). Second, in an ATP-dependent in vitro ODC degradation system established using extracts of cultured CHO cells, the degradation rate of ODC increases in proportion to the amount of antizyme added (14). The present results are consistent with these previous observations and demonstrate a direct relationship between increase in the cellular amount of antizyme and cell-free ODC-degrading activity upon treatment of the cells with spermidine. Therefore, we conclude that antizyme induced by polyamines mediates the accelerated degradation of ODC in EOXD-1 cells.

On the other hand, we could not detect antizyme in control cells or cycloheximide-treated cells, although ODC was degraded significantly in these cells. Extracts prepared from control cells with or without cycloheximide treatment showed weak but significant activity for ODC degradation, and this was not abolished by treatment with anti-antizyme antibody. This activity may reflect, at least in part, that for basol ODC degradation, as suggested in previous reports (11, 14). It is not yet clear whether this pathway is also involved in rapid fluctuation of ODC. We have reported that ODC induction by asparagine in primary cultured hepatocytes was in part due to stabilization of the enzyme (6). Removal of asparagine from the medium caused rapid degradation of ODC.2 There are several reports demonstrating that hypotonic stress stabilizes ODC protein and restoration of isotonicity causes its immediate destabilization (40–42). Antizyme is unlikely to be involved in induction in these processes, since isotonicity

2 R. Kanamoto and S. Hayashi, unpublished observation.
Degradation of ODC in Vivo and in Vitro

Fig. 6. Degradations of native and dephosphorylated ODC in cell-free extracts. a, purified 32P-labeled ODC (17,000 cpm) in 50 μl of dialysis buffer was mixed with 1 μl of alkaline phosphatase (50 units) and incubated at 37 °C. Aliquots were taken at 0 min (lane 1), 10 min (lane 2), and 40 min (lane 3), and analyzed by SDS-PAGE. The band of ODC was visualized with a Fujix Bas 2000 imaging analyzer. b, extracts were prepared from the cells treated with spermidine for 8 h or dephosphorylated (lower panel) 35S-labeled ODC were incubated in the degradation assay mixture for the indicated periods. Labeled ODC remaining in the mixture was determined as described. The apparent half-lives of 32P-labeled ODC in the extracts are indicated at the right of each plot.

causes prompt ODC destabilization without new protein synthesis and restoration of isotonicity and putrescine treatment together have synergistic stimulatory effects on enzyme degradation in cultured hepatocytes (42). Thus it is possible that the basal ODC degradation is accelerated under these conditions. The basal and antizyme-dependent ODC degradation systems are likely to share a common machinery, since a recent study in our laboratories has revealed that both basal and antizyme-dependent ODC degradation are catalyzed by purified 26S proteasomes in an ATP-dependent manner (15).

There are reports that ODC is phosphorylated (19–23, 38) and that Ser-303 in an internal PEST region is a site of phosphorylation by casein kinase II in vitro (19–21) and the sole apparent site of phosphorylation in vivo (19). We have also demonstrated that ODC is phosphorylated, possibly at Ser-303 in EXOD-1 cells. The involvement of phosphorylation in ODC degradation has been questioned from results on transfection of a mutant ODC cDNA in which Ser-303 was replaced by alanine (19, 43). We have demonstrated that antizyme phosphorylated Ser-303 in EXOD-1 cells. The involvement of phosphorylation in ODC degradation has been questioned from results on transfection of a mutant ODC cDNA in which Ser-303 was replaced by alanine (19, 43). From these results it was concluded that phosphorylation remains to be clarified.

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Degradation of ODC in Vivo and in Vitro

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