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Regulated Degradation of Yeast Ornithine Decarboxylase*

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Ornithine decarboxylase (ODC) declines in cells that accumulate an excess of polyamines, the downstream products of the enzyme. Superfluous production of polyamines is thus prevented. In animal cells, polyamines reduce ODC activity by accelerating its degradation. Similar down-regulation of ODC activity has been observed in the budding yeast *Saccharomyces cerevisiae*, but induced degradation has not been documented. Here we show using pulse-chase analysis that the loss of enzyme activity is the result of increased degradation of ODC. Polyamines reduce the half-life of the newly synthesized protein from 3 h to approximately 10 min. Degradation of bulk ODC pools is also accelerated by polyamines, but the absolute rate of turnover is slower, with a half-life of 5 h in untreated and 1 h in treated cells. Newly synthesized ODC polypeptide thus undergoes a process of maturation that renders it relatively resistant to both basal and polyamine-induced degradation. Proteasome mutants have a blunted or absent regulatory response, implicating both the core protease and the regulatory cap of the proteasome in induced degradation of yeast ODC.

The enzymes that control polyamine metabolism share a common characteristic: the regulation of ornithine decarboxylase (ODC)[1] (1, 2), *S*-adenosylmethionine decarboxylase (3), and spermidine/spermine acetyltransferase (4) depends in large part on post-translational mechanisms. This property has perhaps been documented most fully for ODC of animal cells (1, 2). ODC catalyzes the initial step in the biosynthesis of polyamines, small, ubiquitous, abundant, and essential cellular polycations (5). When these rise to excess levels, the half-life of the enzyme becomes shorter, and its steady-state level falls. This sequence of events provides a form of feedback regulation whereby the end products of the biosynthetic pathway limit the activity of the initial enzyme of the pathway. A second protein, termed antizyme, controls the process. Production of antizyme requires a translational frameshift to align a small upstream ORF with a second ORF that encodes all known functions of the protein. Polyamines greatly enhance frameshifting, and thus control the level of antizyme (6). Antizyme associates with ODC, disrupting the homodimeric enzyme and rendering it inactive. The ODC:antizyme heterodimer thus formed is degraded by the proteasome (7). ODC represents an unusual substrate for the proteolytic action of the proteasome. Proteasomes act predominantly on proteins that have been modified by covalent association with multiple copies of the protein ubiquitin (8), but accelerated ODC degradation depends instead on 1:1 stoichiometric noncovalent association with antizyme (9, 10).

In the budding yeast *Saccharomyces cerevisiae*, the mechanism of regulation of ODC (yODC) is less well understood. However, many key attributes are similar to those observed in animal cells (11, 12). Augmenting polyamines reduces yODC activity and protein, without changing the amount of yODC mRNA; changes in transcription or yODC mRNA stability are therefore implausible as mechanisms of control. Polyamines do not change the distribution of yODC mRNA on the polyribosomes, implying that translation is unaltered. Expression of the yODC open reading frame without flanking regions of the mRNA confers activity that remains under the control of polyamines, suggesting that the protein itself is subject to control (11). Degradation of yODC in response to polyamines subsists as the most probable explanation both because it is not excluded by the data and because animal cells utilize this mechanism. Direct evidence for this conclusion has, however, proven elusive. In yeast, genetic evidence has demonstrated that the proteasome digests yODC (13, 14), but polyamine excess has not been shown to influence this process. Here we provide evidence that polyamines accelerate the degradation of yODC by the proteasome in yeast.

EXPERIMENTAL PROCEDURES

Culture of Yeast—Cells were grown in liquid culture with aeration by shaking at 30 or at 25 °C for proteasome mutant strains. Synthetic minimal medium (SM) consisted of 0.67% yeast nitrogen base (Difco), 2% glucose, and amino acid omission mixtures (BIO 101, Inc.), deficient in the appropriate amino acids required for selection. Transformation of yeast was done by the lithium acetate method (15). Polyamine treatment was with 1 mM spermidine, 1 mM spermine. Yeast manipulations were carried out as described (16). General methods for DNA manipulation made use of standard procedures (17).

Strains and Plasmids—The wild type background strain used in these studies was PSY93 (*matα,leu2,ura3,trp1,his3*) from A. Johnson, University of California, San Francisco. In the PSY93-derived strains described here, unless otherwise stated, the *SPE2* gene encoding *S*-adenosylmethionine decarboxylase was replaced by a *spe2::LEU2* disruption using a plasmid obtained from C. Tabor and H. Tabor, National Institutes of Health (18). The effect of this mutation was to prevent conversion of putrescine to the polyamines spermidine and spermine. Proteasome mutant strains were *pre1–1, pre2–2* in a WCG4 strain background (19), or *cim3–1* (20) backcrossed 4x to the W303 strain. Activity of yODC in each of these proteasome mutant strains was compared with that in their respective isogenic or congenic wild type control strain. The yODC (*SPE1*) genomic clone (21) was obtained from W. Fonzi, University of California, Irvine. A strain with three yODC gene copies (3x strain) was constructed by cloning *SPE1* expressed from its native promoter in the *CEN-ARS* plasmids (22) pRS313 (*HIS3*) and pRS314 (*TRP1*), transforming *SPE1* cells with both constructs and subsequently maintaining continuous selection for both markers. Cells with a single chromosomal copy of *SPE1* are termed 1x and isogenic cells with three gene copies 3x.

yODC Enzymatic Activity—Cells were washed in H_2O and resus-

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[1] The abbreviations used are: ODC, ornithine decarboxylase; ORF, open translational reading frame; yODC, yeast ornithine decarboxylase; PAGE, polyacrylamide gel electrophoresis; TBS-T, Tris-buffered saline with Tween 20.

pended in 450 μl of lysis buffer (0.02% Brij, 25 mM Tris pH 8.0, 0.1 mM EDTA, 2 mM dithiothreitol, 0.1 mM pyridoxal phosphate, 0.1% Triton X-100, 1 mM Mg Cl_2), disrupted by agitation with glass beads (BioSpec Bead-Beater), and the 14,000 × *g* supernatant recovered for determination of protein concentration (Bradford, Bio-Rad, bovine serum albumin standard) and yODC enzymatic activity, using an assay that measures release of $^{14}CO_2$ from ^{14}C-carboxy-labeled ornithine (12). Enzymatic activity units are expressed as pmol of CO_2 released/min/mg of protein. A protease inhibitor mix containing phenylmethylsulfonyl fluoride, leupeptin, aprotinin, and pepstatin A (Roche Molecular Biochemicals) was included in the lysis buffer.

Metabolic Labeling of Cells and yODC Immunoprecipitation—Cultures in mid-logarithmic phase (OD_{600} 0.2 to 0.5) in SM medium at 30° were washed once in labeling medium (identical to SM, but without methionine and equimolar NH_4Cl replacing $(NH_4)_2SO_4$), resuspended in the same medium to a final cell density of 2 ODU_{600}, cultured for 30–40 min, and then labeled for 3 min by addition of S^{35} Express label (ICN) containing [^{35}S]methionine and ^{35}S-cysteine. When cells were labeled for longer periods, cold methionine and cysteine were added with S^{35} Express label to prolong incorporation; acid-precipitable incorporation of ^{35}S was found to be linear for at least 3 h under these conditions. Chase was initiated by centrifuging the cells and resuspending them in pre-warmed SM containing unlabeled 1 mM cysteine and 1 mM methionine. Aliquots were collected periodically and diluted into ice-cold tubes containing NaN_3, 30 mM final concentration. Cells were centrifuged, washed and stored at −80° for subsequent analysis by immunoprecipitation. For immunoprecipitation of labeled yODC, all manipulations were done at 4°, and all solutions contained 1× protease inhibitors (Roche Molecular Biochemicals) and 1 mM phenylmethylsulfonyl fluoride in addition to the indicated constituents. Cells were broken by glass bead agitation (BioSpec Bead-Beater) in 200 μl of phosphate-buffered saline + 1% Triton X-100 (PBS/TX-100). After lysis, 800 μl of PBS/TX-100 + 0.2% SDS (wash buffer) was added, and the glass beads were briefly vortexed. The solution was cleared by centrifugation at 14,000 × *g*. A portion of the high speed supernatant was used to determine acid precipitable ^{35}S, and the volumes used for immunoprecipitation were adjusted to contain equal counts. Extracts were pre-cleared by incubation with pre-immune serum and protein A-Sepharose (Amersham Pharmacia Biotech), and yODC was then immunoprecipitated with polyclonal rabbit antiserum prepared (Animal Pharm Services) by immunizing rabbits with purified recombinant yODC, produced in *Escherichia coli*. The anti-yODC antibody was rendered monospecific before use by adsorption with an acetone powder extract of yeast (23) in which the *SPE1* gene was disrupted. Antibody-yODC complexes were collected by addition of protein A-Sepharose and washed sequentially in wash buffer, urea/NaCl buffer (2 M urea, 10 mM Tris, pH 7.5, 1% Triton X-100, 2 M NaCl), wash buffer, and low salt buffer (10 mM Tris, pH 7.5, 10 mM NaCl). The precipitates were analyzed by SDS-PAGE, and radiolabeled bands were visualized by autoradiography or by using a phosphoimager (Molecular Dynamics). The density of bands corresponding to labeled yODC was measured by ImageQuant (MD).

Colony and Western Immunoblot Assay of yODC—An assay developed to measure yeast carboxypeptidase Y (24) was adapted to assess the level of yODC in cells grown on nitrocellulose filter overlays in SM agar plates. Yeast strains were streaked onto nitrocellulose filters (Hybond-C, Amersham Pharmacia Biotech) placed upon agar plates containing the SM medium. Polyamines, where indicated, were added to the medium subsequent to plate preparation and before application of filters. The cells were allowed to grow for 3 days at 25 °C and then lysed upon the filters (24). Filters were developed using the Amersham ECL detection kit and protocol. Filters were blocked in TBS-T including 5% powdered low-fat milk and 5% bovine serum albumin. Primary anti-yODC antibody was identical to that used for immunoprecipitation (1:10,000 dilution) in TBS-T plus 0.3% bovine serum albumin. Secondary antibody was goat anti-rabbit conjugated with horseradish peroxidase (Sigma, 1:15,000 dilution) in TBS-T. For Western blot analysis, cells were treated with polyamines or were untreated, extracts prepared via glass bead lysis, subjected to SDS-PAGE, and immunoreactive yODC visualized by ECL as above. Equality of sample loading was verified by using monoclonal antibody YL1/2 (25) directed against yeast tubulin.

RESULTS

Response of yODC Activity and Protein to Polyamines—Polyamine excess causes a reduction of ODC activity in yeast and other eukaryotes. We created a yeast strain with two kinds of genetic changes intended to make investigation of this form of regulation more tractable. First, a null mutation was introduced in *SPE2*. Its gene product, *S*-adenosylmethionine decarboxylase, is required for conversion of putrescine, the direct product of yODC, to polyamines (18). By thus preventing endogenous synthesis of polyamines, cellular pools could be made to depend solely on the amounts of polyamines provided in the medium. Because polyamines are essential, spermidine and spermine were added to the medium at a concentration of 10^{-7} each for routine cell propagation and added at a level 10,000-fold higher to down-regulate yODC. Second, because yODC is a low abundance protein, biochemical and molecular investigation of the mechanism of the response would be facilitated by expressing it to a higher level than that found in wild type cells. However, previous investigations (11) have shown and we have confirmed that high level expression of yODC from a multicopy plasmid greatly blunts the regulatory response. Therefore, the extent of overexpression must be limited if the desired strain is to maintain regulation. To this end, we established a strain with three copies of the yODC gene (3x strain), one chromosomal and two on plasmids, and measured the response. The cells were treated with polyamines to expand intracellular pools, and time-dependent changes in yODC activity were measured. As has been previously reported for cells with a single gene copy, yODC activity fell with a half-life of about 1 h, declining more than 50-fold within 8 h (Fig. 1*A*). The decline in activity displayed similar kinetics to that observed in isogenic cells with a single chromosomal copy of the yODC gene (1x strain).

To determine whether polyamines reduce yODC protein as well as activity, we used a colony-based immunologic assay (24). Cells were transferred to nitrocellulose filters, and grown as an overlay on synthetic complete medium plates supplemented or not with polyamines. After cell lysis, the amount of immunoreactive yODC present on the filters was assessed. We tested the 3x strain, as well as a strain in which the yODC gene had been disrupted, the latter to determine the intensity of assay background. Incubation with polyamines for 3 days, a period required to establish grossly visible colonies, significantly reduced the amount of yODC detected in the 3x strain (Fig. 1*B*). To confirm this inference and to determine whether regulation of protein level takes place more promptly, we used Western immunoblot analysis of extracts of cells exposed to polyamines in suspension culture for only 4 h. Immunoreactive yODC declined markedly as a result of this treatment (Fig. 1*C*). No immunoreactive yODC was detected in the *spe1* disruption strain. Because polyamines induced a comparable reduction of yODC activity and yODC protein within hours, we conclude that the loss of activity results from a diminution of protein level.

Degradation of yODC—To test directly whether polyamine treatment of cells accelerates yODC degradation, we metabolically labeled cells and measured how fast labeled yODC diminished in cells that were pretreated with polyamines or were untreated. The labeling time was 3 min (pulse), followed by subsequent incubation under conditions that prevented further labeling of newly synthesized proteins (chase). Extracts were immunoprecipitated with antibody specific for yODC, and labeled immunoprecipitated protein was visualized by SDS-PAGE and phosphoimager processing. The specificity of precipitation was verified using yODC gene disruption and overexpressor strains (Fig. 2*A*). yODC in 3x strain cells not pretreated with polyamines had a half-life of about 3 h (Fig. 2*B*). Polyamine pretreatment reduced the half-life to about 10 min. Although signal intensity declined much faster in the treated cells, the intensity of yODC labeling at the earliest time

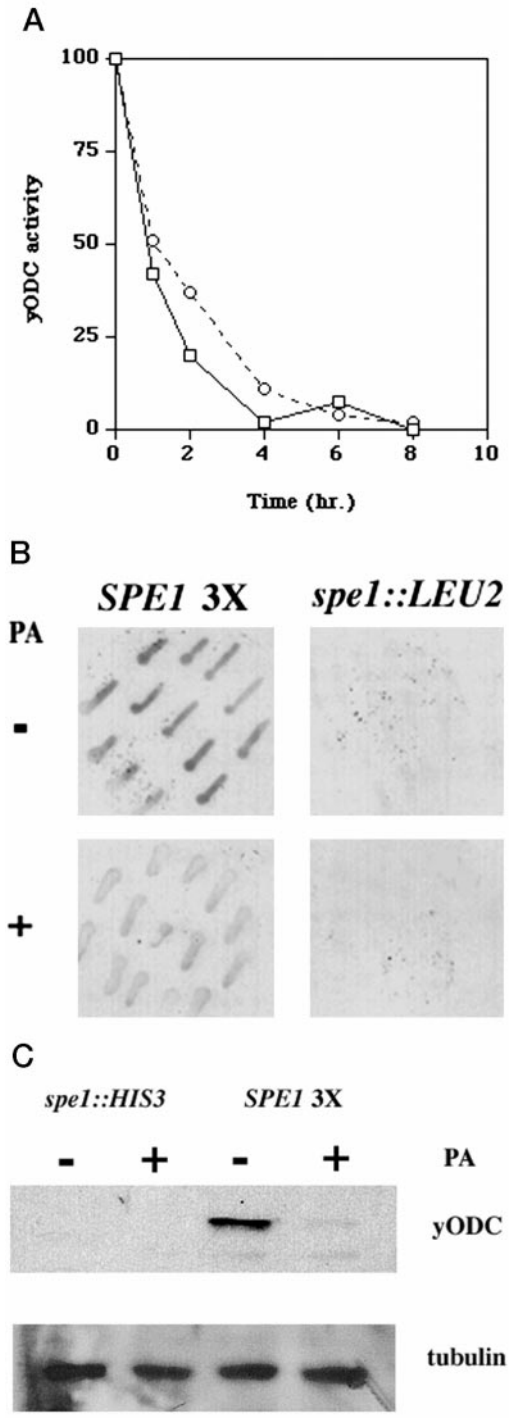

FIG. 1. **Loss of yeast ODC upon polyamine treatment in cells with one or three gene copies.** *A*, time-dependent change in yODC activity. At the initial time point, spermidine and spermine at concentrations of 1 mM each were added to the medium of 1x (□)and 3x (○) strains. Portions were removed periodically for measurement of yODC activity. The data are plotted as a percent of initial activity, which was 27.8 and 60.8 pmol/min/mg of protein in the 1x and in the 3x strains, respectively. *B*, immunoreactive yeast ODC in cell colonies after polyamine treatment. Cells with three copies of the yODC gene (*SPE1 3x*) were patched to filters, treated with polyamines or left untreated, and processed to display immunoreactive yODC. Cells with the yODC gene disrupted (*spe1*::*His3*) were processed in parallel and serve as a control for nonspecific immunoreactivity. *C*, Western immunoblot analysis of yODC in cell extracts after polyamine treatment. Cells with the yODC gene disrupted (*spe1*::*His3*) or with three copies of the yODC gene (*SPE1 3x*) were untreated or treated with polyamines, as indicated, and yODC in extracts were visualized by Western blotting. Immunoreactive bands were visualized with antibody to yODC or to yeast tubulin, which serves as a loading control, as indicated.

point observed was not strongly or consistently influenced by polyamine treatment. These results imply that polyamine treatment accelerates degradation more than ten-fold, but has little or no effect on synthesis. Pulse-chase experiments with 1x cells produced results that were qualitatively similar to those seen with the 3x strain (results not shown), but signal strength was diminished, making data analysis problematic. Consistent with the observation that activity is not regulated in a strain expressing yODC from a high copy plasmid (11),[2] pulse-chase experiments showed that yODC has about the same 3-h half-life as in the 3x strain and that polyamines do not stimulate turnover (Fig. 2*C*).

Degradation of Steady-state yODC Pools—The pulse-chase experiments described above demonstrate that polyamine treatment labilizes newly synthesized yODC protein, those molecules metabolically labeled during the brief 3-min pulse employed. Pools of yODC that exist prior to treatment may be made similarly labile. However, it is possible instead that yODC undergoes a process of folding or other form of maturation that renders it more stable. In that case, polyamines might induce cells to accelerate the degradation only of newly synthesized yODC, whereas pre-existing pools of the molecule decay with the 3 h half-life characteristic of newly synthesized yODC in untreated cells or even remain entirely stable, decaying only through a dilution process dependent on cell growth. To test this, cells were labeled for 2.5 h before imposing polyamine treatment. Chase was initiated simultaneously with polyamine treatment, and the amount of labeled yODC was assessed during a subsequent 4-h chase period (Fig. 3). As was true for yODC populations labeled for 3 min, polyamine treatment also accelerated the degradation of long labeled yODC. However, the absolute rate of degradation after polyamine treatment is much slower in the second case. In contrast to short-labeled yODC, for which polyamines changed the half-life from 3 h to 10 min, polyamines altered the half-life of long-labeled yODC from 5 h to 60 min. In summary, in cells with either a basal or augmented level of polyamines, yODC undergoes a process of maturation that reduces its susceptibility to degradation.

Effect of Proteasome Mutations—Both mammalian ODC and yeast ODC are degraded *in vitro* by the yeast proteasome. *In vivo*, mutations in the yeast proteasome impede degradation of yODC, but these experiments have been carried out in cells not subject to treatment with polyamines. To determine whether the accelerated form of degradation induced by polyamines also depends on the proteasome, we utilized two temperature-sensitive mutants: the first, a double mutant (*pre1–1,pre2–2*) which impairs four of the six proteolytic sites of the 20 S proteasome core (19); the second (*cim3–1*), a temperature-sensitive mutant of the Rpt6 ATPase subunit of the regulatory cap of the 26 S proteasome (20). In *pre1–1,pre2–2* cells, polyamines produced a 2-fold reduction, compared with a 28-fold change in the corresponding wild type cells (Table I). In *cim3–1* cells, the polyamine-induced change was 0.5-fold (a small increase rather than reduction), compared with 8-fold for the wild type. Mutations of either the core proteolytic chamber or regulatory complex interfere with polyamine-induced reduction of yODC activity, presumably by interfering with polyamine-induced accelerated degradation.

DISCUSSION

As described in "Introduction," previous findings suggested that cellular polyamines reduce yODC activity and protein by accelerating the degradation of the enzyme. However, the low level of expression of yODC has impeded direct measurements of stability. We constructed well regulated cells with aug-

[2] C. Toth and P. Coffino, unpublished results.

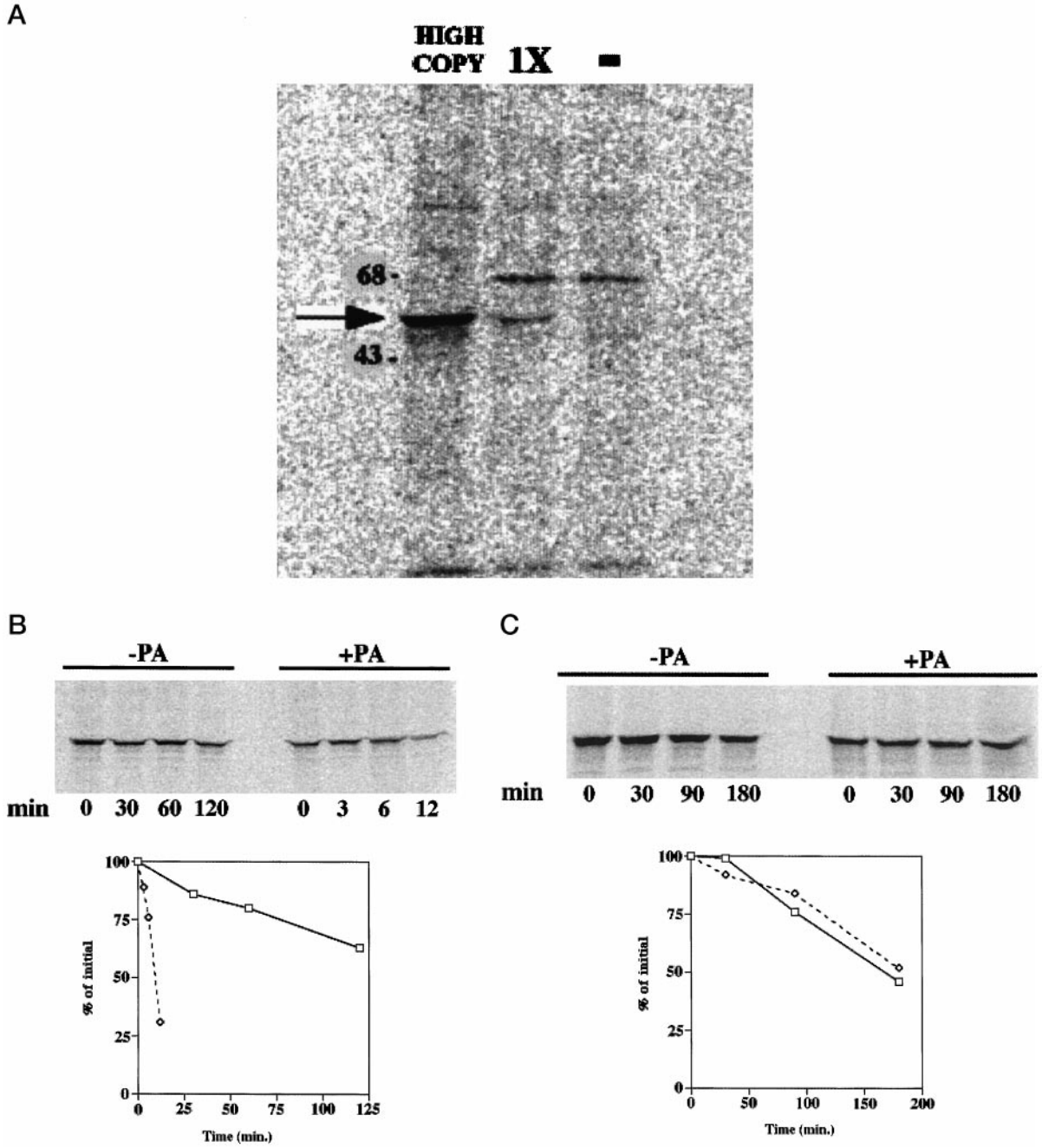

FIG. 2. **Pulse-chase experiment to determine yODC turnover.** *A*, the specificity of yODC immunoprecipitation from metabolically labeled cell extracts was determined by comparing the pattern of labeled bands obtained using cells with no wild type yODC gene copy (−), with one copy (*1X*), and with multiple copies carried on a 2 μM plasmid (*HIGH COPY*). The *arrow* indicates the position of migration of yODC (52.3 kDa expected molecular mass (21)) on the SDS-PAGE gel. *B*, cells with three yODC gene (*SPE1*) copies and a *spe2* disruption were treated for 4 h with spermidine and spermine at concentrations of 1 mM each or were untreated. Each culture was then pulse-labeled with [^{35}S]methionine and ^{35}S-cysteine for 3 min. Cells were transferred to medium without label, and samples were removed periodically and processed for determination of radiolabeled yODC. Chase times were 0, 3, 6, and 12 min for treated cells and 0, 30, 60, or 120 min for untreated cells. The time-dependent changes in intensity of the radiolabeled yODC protein of untreated (□) or treated (○) cells are plotted as a percent of initial value. *C*, cells with multiple yODC gene (*SPE1*) copies carried on a 2 μM plasmid and wild type for *SPE2* were treated, labeled, and analyzed as in *panel B*. □, untreated cells; ○, treated cells. Chase times were 0, 30, 90, and 180 min.

mented yODC expression and produced highly specific antiserum ("Experimental Procedures"). These technical tools made it possible to measure changes in the level of immunoreactive yODC and to perform pulse-chase analysis to measure its rate of turnover. We found that polyamine treatment greatly reduces the amount of yODC and diminishes the half-life of newly synthesized yODC, reducing it from about 3 h to 10 min. These changes in degradation were not associated with altered synthesis; using a short pulse labeling time of 3 min, polyamines were found to cause no consistent alteration in metabolic labeling of yODC. This result is consistent with that previously found in animal cells (26). Although it is difficult to exclude small changes in translation rate, it is clear that the bulk and perhaps all of the polyamine-induced change in yODC activity results from a post-translational process. This is in contrast to the fungus *Neurospora crassa*, in which polyamines exert a repressive influence on ODC by reducing the abundance of ODC mRNA (27).

Previous investigations of yODC stability employed inhibitors of synthesis, predominantly cycloheximide, to assess the

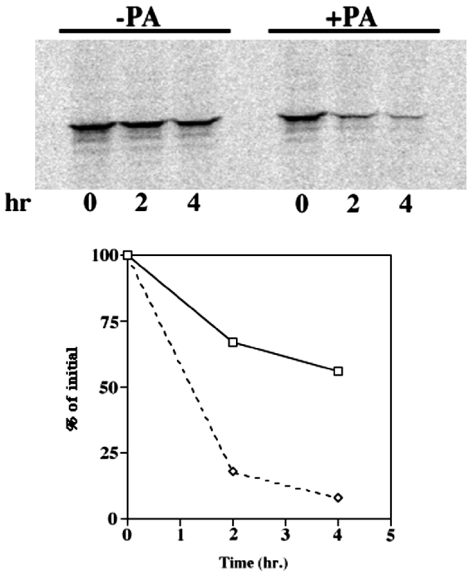

FIG. 3. **Chase experiment to determine turnover of radiolabeled yODC in cells subjected to a prolonged label period.** A 3x strain culture was metabolically labeled for 2.5 h. One portion of the culture was chased in medium containing spermidine and spermine at concentrations of 1 mM each and a second portion in medium without polyamines. Chase samples were removed after 0, 2, and 4 h and processed and analyzed as in Fig. 2*B*. □, untreated cells; ○, treated cells.

TABLE I
Comparison of yODC activities

Cultures were treated with polyamines or were untreated, incubated for 16 h at 25 °C, incubated at 34 °C for a further 4 h, and harvested for determination of yODC activity. The yODC activities of strains with mutations in proteasome genes were compared with those in isogenic or congenic strains wild type for the corresponding genes.

Proteasome allele	Polyamine treat	yODC activity	Polyamine-induced reduction
		pmol/min/mg of protein	
PRE1/PRE2	−	3.9	
	+	0.14	28x
pre1–1, pre2–2	−	6.9	
	+	3.5	2x
CIM3	−	2.8	
	+	0.35	8x
cim3–1	−	9.5	
	+	20	0.5x

rate at which activity falls when synthesis of proteins is halted (11, 12). These studies showed that by this measure of stability, yODC has a half-life of more than an hour, regardless of whether or not polyamines are augmented. These results are only apparently discrepant with those reported here. Two models could reconcile these results with ours. Model 1 asserts that yODC is subject to accelerated degradation only or predominantly during a brief period after translation, perhaps before the newly synthesized polypeptide folds into an enzymatically active conformation. In that case, steady-state pools of enzymatically active yODC, those that are determined in cycloheximide chase experiments, would be predominantly in a matured form no longer susceptible to rapid degradation. Experiments using inhibitors of synthesis would reveal little or no effect of polyamines on degradation. To directly test this, we carried out prolonged labeling to uniformly label yODC pools. Cells were labeled for 2.5 h before initiating a chase. At the end of the label period, most of the labeled yODC cohort present will have been synthesized more than an hour ago, a calculation based on an 3-h yODC half-life and a 2-h cell division time. These labeled yODC pools were then chased; at the time of initiation of the chase, cells were either treated with polyamines or left untreated. In long labeled cells not treated with polyamines, labeled yODC pools declined with a 5-h half-life. In polyamine-treated cells, the half-life was reduced to 1 h. Polyamines therefore accelerate degradation of long-labeled yODC 5-fold. These results show that model 1 is untenable.

Model 2 offers a offers a different resolution of the apparent conflict between cycloheximide chase *versus* pulse-chase experiments: simultaneous treatment with polyamines and inhibitors of protein synthesis may prevent the production of a polyamine-induced protein required for accelerated degradation. This is the case in animal cells. There the more marked effect on ODC activity of treatment with polyamines alone, compared with cycloheximide or cycloheximide plus polyamines (28), is because of the polyamine-induced synthesis of the protein antizyme, which binds to ODC and accelerates its degradation. Cycloheximide prevents antizyme synthesis but allows preformed ODC to be degraded at a rate characteristic of an antizyme-independent process or one dependent on the lesser amount of antizyme present in cells with basal levels of polyamines. In yeast as well, cycloheximide plus polyamines produce changes in yODC activity identical to those seen with cycloheximide and slower than those seen with polyamines alone (12). This suggests that, in yeast too, excess polyamines stimulate the synthesis of a protein responsible for degradation. In yeast, polyamine treatment produces similar kinetics of decline in yODC activity, regardless of whether or not cycloheximide treatment is superimposed an hour after polyamines are added (12). This too is consistent with the possibility that polyamines cause a protein to appear which accelerates degradation of yODC and which can persist if synthesis is later impeded. Model 2 is fully compatible with our data and that of others.

Our attempts to obtain direct biochemical evidence for induction in yeast of a hypothetical functional analog of antizyme have, however, been unsuccessful. Such an analog is likely to act catalytically on its substrate, as does mammalian antizyme, and hence be present in cells in a still smaller amount than the already scarce yODC. This suggests that genetic approaches to its identification will likely prove more useful than attempts at biochemical isolation. Whatever the mechanism of yODC regulation, amounts greatly in excess of that normally present in the cell exceed its capacity (Ref. 11 and Fig. 2*C*). As shown here, a modest degree of over-expression, about 3-fold normal, is compatible with full or near-full regulation. The nature of the component limiting regulatory capacity remains to be determined, as does its relationship with the hypothetical yeast antizyme analog.

Proteasome mutations in either the catalytic 20 S core (29) or 19 S regulatory cap (13) interfere with yODC degradation in cells with basal polyamine levels. The same mutations are here shown to strongly impede polyamine-induced reduction in yODC. Induced degradation as well as basal degradation therefore depends on the integrity of both these functional elements of the proteasome, the core, and regulatory cap. As both elements of the yeast proteasome are required *in vitro* for proteolysis of mouse and yeast ODC (14), it is very likely that the proteasome itself is the agent of induced yODC degradation.

The observed difference in polyamine-induced lability of "new" *versus* "aged" yODC is not because of the use of a 4-h polyamine pre-treatment before pulse-chase for the short label experiment, *versus* addition of polyamines at initiation of the chase in the long label experiment. A treatment period of 1 h is in fact sufficient to fully establish yODC lability. That 1 h suffices to establish lability is consistent with our data and that of others (Ref. 12, and Fig. 1*A*) and directly supported by our

observation that the short label experiment yields an identical result if a 1-h rather than 4-h pretreatment is used (data not shown). Hence the cellular conditions required to produce extreme lability of newly synthesized yODC had been established within one h. of inception of the chase in the long label experiment. At what point after synthesis does yODC become less susceptible to degradation? Compared with newly synthesized yODC, "mature" molecules degrade about 2-fold more slowly in untreated cells (half-life 3 h *versus* 5 h) and about 6-fold more slowly in polyamine-treated cells (half-life 10 min *versus* 60 min). We know little about the time scale over which maturation takes place because we compared cohorts of molecules labeled for very dissimilar times, 3 min or 2.5 h. The timing and mechanism of the transition of yODC from more to less degradation-susceptible, therefore, remain highly conjectural. The relevant transition could be associated with one or more of several steps: completion of polypeptide translation, folding to a native monomeric configuration, self-association of monomers to form the enzymatically active homodimer, or association with some other cellular component. Two decades ago, Wheatley *et al.* (30) demonstrated using pulse-chase labeling of cultured mammalian cells that newly synthesized proteins are surprisingly labile. After a 5-min pulse with ^{3}H-leucine, about one third of incorporated (acid precipitable) counts were hydrolyzed by cells within 1 h, a fraction that fell progressively as the labeling time was increased. This result was interpreted to indicate that a large class of nascent proteins are initially at high risk for degradation and then undergo time-dependent stabilization. It is possible that yODC represents but a special case of this general phenomenon.

Is accelerated degradation sufficient to account for the reduction in yODC activity observed in cells exposed to high polyamine concentrations? This treatment causes activity to fall with a 1-h half-life (Ref. 12, and Fig. 1*A*). Some of the reduction in activity is because of very fast degradation of newly synthesized yODC, functionally equivalent to a near cessation of synthesis. The remaining reduction in activity is the result of a change in the half-life of the bulk steady-state yODC pool from 5 h to 1 h, a new rate consistent with the rate of loss of enzymatic activity. Significantly, polyamines cause similar kinetics of decline in enzymatic activity and in long labeled yODC, implying that these are measuring different properties of a common molecular population. Degradation therefore accounts for loss of activity.

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