MOLECULAR AND CELLULAR BIOLOGY, Dec. 1989, p. 5484–5490
0270-7306/89/125484-07$02.00/0

Vol. 9, No. 12

Polyamine-Mediated Regulation of Mouse Ornithine Decarboxylase Is Posttranslational

TED VAN DAALEN WETTERS,[1] MADHU MACRAE,[1] MARC BRABANT,[1] ANNIE SITTLER,[1] AND PHILIP COFFINO,[1,2]*

Departments of Microbiology and Immunology[1] and Medicine,[2] University of California, San Francisco, San Francisco, California 94143

Received 28 June 1989/Accepted 28 August 1989

The activity of ornithine decarboxylase (ODC) is negatively regulated by intracellular polyamines, which thereby mediate a form of feedback inhibition of the initial enzyme in the pathway of their synthesis. This phenomenon has been believed to result, at least in part, from translational regulation. To investigate this further, we performed four series of experiments. First, we found that a chimeric protein encoded by an mRNA containing the ODC 5′ leader sequence did not exhibit polyamine-dependent regulation. Second, we showed that transcripts containing the protein-coding sequence of ODC, but no other ODC-derived sequence information, exhibited regulation. Third, we found that the association of ODC mRNA with ribosomes was not altered when intracellular polyamine levels were modulated under conditions previously deemed to cause translational regulation. Last, we carried out experiments to measure the incorporation of [^{35}S]methionine into ODC in polyamine-starved and polyamine-replete cells. Differential incorporation diminished progressively as pulse-label times were shortened; at the shortest labeling time used (4 min), the difference in favor of ODC in polyamine-starved cells was less than twofold. These findings suggest that it is necessary to reevaluate the question of whether polyamines cause alterations of translation of ODC mRNA.

Translational regulation in eucaryotic cells is well documented (19). The enzyme ornithine decarboxylase (ODC) has been widely regarded as subject to that form of regulation. ODC is the first enzyme in the pathway committed to polyamine synthesis (24, 40). It converts ornithine to putrescine, the precursor to the polyamines spermidine and spermine. Elevation of intracellular levels of polyamines results in a prompt reduction in enzymatic activity, thereby mediating a form of feedback inhibition. Because ODC is rapidly degraded in cells (3, 12, 13, 20, 21, 31, 33, 34), alterations in the rate of synthesis should lead promptly to new steady-state levels of activity (32).

Indications of translational regulation of ODC mRNA by polyamines are as follows. Experimental manipulations of the levels of polyamines within cells result in rapid changes in ODC activity, but such treatments have no discernible effect on the amount of ODC mRNA (5, 12, 16, 18, 27–29, 35). It has been reported that the rate of synthesis of the enzyme, measured by metabolic labeling with radioactive amino acids, is reduced when intracellular polyamines are augmented, and the opposite result is found when cells are depleted of polyamines (11, 12, 16, 20, 21, 25, 27). ODC mRNA has been found to exhibit translational regulation in response to mitogenic stimulation of lymphocytes (41). Lastly, ODC mRNA has an uncommonly long GC-rich 5′ untranslated leader that can be anticipated to have extensive secondary structure (1, 17). It is plausible that polyamines could directly or indirectly alter the structure of the untranslated leader (14, 30), thereby modifying the translational efficiency of the mRNA.

The magnitude of the effect of manipulating polyamine levels on ODC activity is cell type dependent but has never been reported to be less than 10-fold. We have previously carried out studies on this phenomenon in S49 mouse lymphoma cells (20, 21). Some of the studies reported here also utilize these cells. In S49 cells (as in other cells), a portion of the regulatory effect of polyamines on ODC activity is due to a change in ODC stability (21). These investigations demonstrated that pharmacological manipulations of polyamine levels resulted in approximately a 10-fold change in ODC activity and that approximately half of that change could be accounted for by alteration of enzyme stability. Metabolic labeling studies with [^{35}S]methionine indicated, conversely, about a threefold change in incorporation (20, 21). Those results suggested that the response has two components of approximately equal importance, one related to stability and the other related to synthesis. Because ODC mRNA levels did not change (21), we attributed the second regulatory mode to changes in translational efficiency. The present paper constitutes a reevaluation of this phenomenon.

MATERIALS AND METHODS

Construction of recombinant DNAs used conventional methods. Experimental details will be provided on request. ODC$^-$ CHO C55.7 cells were transfected and selected as previously described (7). DNA coprecipitates included 15 μg of ODC-bearing plasmids and 15 μg of pSV2neo plasmids. ODC enzyme activity and β-galactosidase activity were determined as described in references 20 and 10, respectively. β-Galactosidase activity is expressed as (optical density at 420 nm × 100) per minute per milligram of protein.

For polysome gradient analysis, S49 cells in exponential growth were treated with either 100 μM putrescine or 2 mM difluoromethyl ornithine (DFMO) or were untreated. After 22 h, cells were centrifuged and washed in ice-cold Dulbecco modified Eagle medium, and the pellets were suspended in 1.5 ml of buffer (10 mM Tris chloride [pH 7.4], 10 mM NaCl, 1.5 mM $MgCl_2$) (15) containing 100 μg of cycloheximide and 20 U of RNasin per ml. A portion of the cells (EDTA control) was suspended in a buffer solution which was the same but contained 20 mM EDTA as well. Cells were incubated on ice

* Corresponding author.

for 7 min and then lysed by the addition of Nonidet P-40 to 0.5% for 3 min. Nuclei were removed by centrifugation (HB4 rotor, 8,000 rpm for 10 min), and the supernatants were layered on 34-ml 10 to 40% sucrose gradients prepared in lysis buffer containing 100 μg of cycloheximide per ml. The EDTA gradient was supplemented with 20 mM EDTA. The gradients were spun in an SW27 rotor at 9,000 rpm for 16 h, and 24 1.5-ml fractions were collected. The optical density at 260 nm of each fraction was determined. The sucrose concentration of every second fraction was measured by refractometry. The 24 fractions from each gradient were reduced to eight pools by combining trios of contiguous fractions. RNA was isolated (23). After electrophoresis on a 1% agarose formaldehyde gel and transfer to a Nytran membrane (Schleicher & Schuell, Inc.), blots were probed with ^{32}P-labeled single-stranded ODC cDNA probe (21) and then reprobed with the randomly primed (6) β-actin probe pAct-1 (37).

For metabolic labeling and immunoprecipitation, 5-ml suspension cultures of D2.88 cells (21) were grown for 22 h in low-methionine (6 μg/ml) medium containing 100 μM putrescine or 2 mM DFMO. [^{35}S]methionine (Dupont, NEN Research Products) was then added to a final concentration of 0.5 mCi/ml. Incorporation of label was terminated by adding 1-ml samples of the cultures to 10 ml of ice-cold phosphate-buffered saline containing 30 μg of unlabeled methionine per ml. After low-speed centrifugation, washed cell pellets were lysed in 200 μl of 25 mM Tris hydrochloride (pH 7.5)–1% Triton X-100–0.5% sodium deoxycholate–5 mM EDTA–150 mM NaCl. Incorporation into acid-precipitable material was determined for duplicate 5-μl samples of the microfuged extracts. Samples containing 10^6 cpm of acid-precipitable material were immunoprecipitated with rabbit antisera directed against recombinant mouse ODC (8) or with normal rabbit serum, and sodium dodecyl sulfate gel electrophoresis was done as previously described (20). Autoradiograms were scanned by a Zeineh soft laser scanning densitometer (model SL-504; Biomed Instruments, Inc.).

RESULTS

Role of the 5′ untranslated leader. Because of its large size and rich potential for secondary structure (1, 17), the 313-nucleotide GC-rich 5′ leader of mouse ODC mRNA invites consideration as a potential site of translational regulation. To test whether the presence of the mouse ODC 5′ leader sequence sufficed to confer polyamine-dependent translational regulation on an mRNA, we created a chimeric gene, pT9OGALA. Within the polylinker of pUC18, the pT9OGALA construct contains the following (successively from the 5′ direction): (i) an approximately 1.7-kilobase *Pst*I-*Pst*I genomic DNA fragment from pOD100 (1, 4) containing the ODC promoter and upstream regions; (ii) the contiguous region of ODC cDNA (8), contained within a 385-nucleotide *Pst*I-*Ava*II fragment that encompasses the 5′ untranslated leader and first (amino-terminal) 25 codons; (iii) two nucleotides (GG) from the *Sma*I site in the polylinker of pUC18; (iv) a 2.5-kilobase *Bam*HI-*Cla*I fragment from pMC1871 (2) containing the *lacZ* gene of *Escherichia coli*, encoding β-galactosidase; and (v) a 2-kilobase *Cla*I-*Bam*HI fragment from pCH110 (9) containing a polyadenylation site from simian virus 40. The gene was constructed so as to produce, when the gene was transfected into cells, a transcript composed of the full length of the ODC 5′ leader, the first 25 codons of mouse ODC fused in frame with the *E. coli* β-galactosidase-coding region, followed, lastly, by a 3′ untranslated sequence containing a simian virus 40 polyadenylation site. To ensure the fidelity of the site of transcriptional initiation, the promoter-proximal portion of the chimeric gene was constructed to include approximately 2 kilobases of 5′ untranscribed sequences of the mouse ODC gene.

TABLE 1. β-Galactosidase activity in NIH 3T3 cells transfected with pT9OGALA[a]

Lysate background	ODC		β-Galactosidase activity
	cpm	Activity	
Without putrescine	4,607 ± 1,279	0.680	517 ± 57
With putrescine[b]	661 ± 207	0.065	482 ± 53
None	247 ± 5		

[a] Cells were harvested 48 h after transfection from independent triplicate plates, and lysates were analyzed for ODC and β-galactosidase activity. ODC activity is expressed as nmoles of CO_2 per 100-mm culture dish per hour. The background of β-galactosidase activity in mock-transfected cells was less than 1% of the values reported.

[b] Immediately after transfection, a portion of the dishes were treated with 100 μM putrescine.

If the ODC 5′ leader sequence (and the amino-terminal 25 amino acid codons) suffice for polyamine-mediated translational regulation, the rate of synthesis of the fusion protein, reflected in β-galactosidase activity, should decline when cells transfected with the gene are treated with polyamines. Furthermore, ODC activity could be measured in samples of the same cellular extracts, thereby providing an internal control for integrity of the response. Mouse NIH 3T3 cells were transfected with the chimeric gene. Simultaneously with transfection, putrescine (100 μM) was added to some of the culture media. (Cells promptly metabolize putrescine to spermidine; it is the latter that likely mediates the regulatory effects on ODC [18].) At 48 h after transfection, cellular extracts were prepared and assayed for β-galactosidase activity and for ODC activity (Table 1). Putrescine treatment resulted in approximately a 10-fold reduction in ODC activity but produced no significant change in β-galactosidase activity. The same chimeric gene was used to establish stably transfected pools of NIH 3T3 cells. In these cells, as with the transiently transfected recipients, polyamine treatment promptly abolished ODC activity but failed to perturb β-galactosidase activity (results not shown).

Expression of recombinant ODC-coding sequence. The inability of the ODC leader sequence to confer polyamine-dependent regulation on a heterologous gene implies that this function resides elsewhere within the ODC gene. We tested this hypothesis with an ODC minigene construct that encodes the entire ODC mRNA minus the leader sequence, pOD-ODC3′. This construct includes 280 base pairs of the ODC promoter region, ODC cDNA with both natural poly(A) addition sites, and about 1 kilobase of 3′ untranscribed genomic DNA.

ODC$^-$ CHO cells were used as recipients for minigene DNA. These cells express no detectable ODC activity and are auxotrophic for putrescine (38, 39). A pool of cells transfected with a plasmid bearing the ODC minigene was tested for regulation by polyamines. As a control, we used a pool of cells which were similarly transfected but with an ODC genomic clone, pOD12.7 (1), that encodes the entire natural mouse ODC mRNA. Putrescine caused ODC activity to decay with similar extent and kinetics (half-life of ~25 min) in the genomic and minigene transformants (Fig. 1A). Therefore, ODC mRNA lacking the 5′ leader sequence is sufficient to evoke a full response to putrescine.

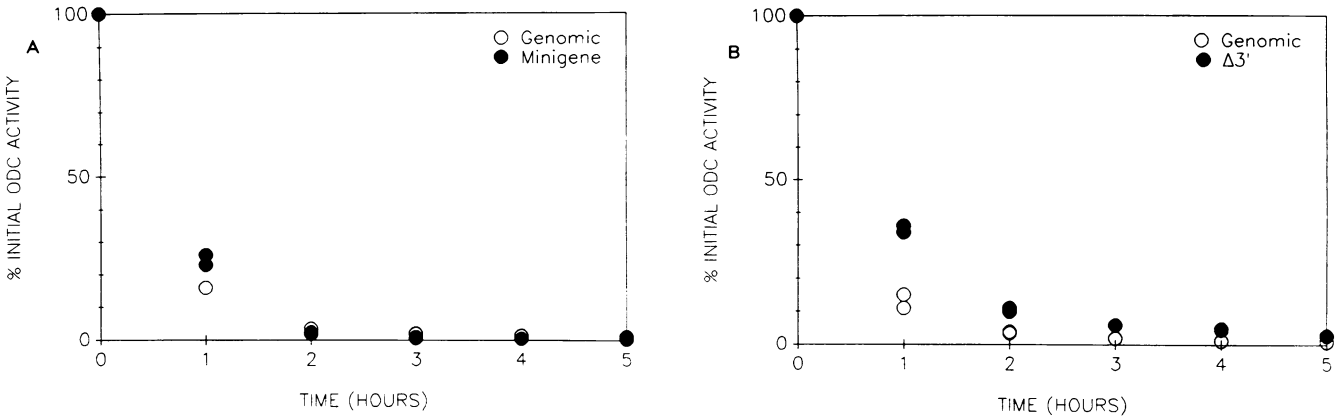


FIG. 1. Regulation by putrescine of ODC activity in transfected cells. Exponentially growing cells were treated with 0.5 mM putrescine and harvested at the indicated times, and the ODC activities in the microfuged supernatants were measured. Each point represents a duplicate assay of a single plate. In each graph, the transfected cells, the ODC mRNA they express, and the ODC activities in untreated control lysates are as follows. (A) pOD12.7, complete ODC mRNA (178 pmol/min per mg of protein) (○); pOD-ODC3', ODC mRNA lacking 5' leader (70 pmol/min per mg of protein) (●). (B) pOD12.7, complete ODC mRNA (121 pmol/min per mg of protein) (○); pODM3Δ3', ODC protein-coding region only (213 pmol/min per mg of protein) (●).

We sought to delimit further the region of ODC mRNA that could encode putrescine-mediated regulation. The plasmid expression vector pODM3Δ3' contains ODC cDNA encompassing only the protein-coding region; i.e., it contains neither the 5' leader nor the 3' untranslated sequences of ODC. In this construct, ODC mRNA expression is driven by the simian virus early region promoter, and signals for polyadenylation and transcription termination are provided by sequences from the 3' end of the hepatitis B virus surface antigen gene (36). Putrescine-mediated regulation of ODC activity expressed in pooled transfectants of the pODM3Δ3' mutant appears similar to that in cells expressing the natural ODC mRNA (Fig. 1B). The small difference in apparent initial half-lives for decay of ODC activity was not reproduced in subsequent experiments. It appears, therefore, that the ODC-coding region itself is sufficient for regulation by polyamines.

Association of ODC mRNA with polysomes. If an increase in the intracellular polyamine pools reduces the efficiency of translation of ODC mRNA, it could in principle do so in two ways: by reducing the frequency of initiation or by prolonging the process of elongation. If the initiation rate is reduced, the number of ribosomes found on each molecule of ODC mRNA should become smaller; if elongation slows, that number should become larger. It is also possible that both processes, i.e., those of initiation and elongation, are affected to a similar degree, in which case the distribution of ribosomes would not change.

To determine whether an effect on polysome size occurred, cells were treated to augment or reduce their polyamine pools. Cell lysates were prepared and fractionated on sucrose density gradients to separate polysomes according to size. S49 mouse lymphoma cells were treated overnight with putrescine to increase polyamines or with DFMO, an inhibitor of ODC, to reduce polyamine levels (22). Each of these treatments has been shown to alter the activity of ODC in S49 cells. Both affect the half-life (determined by incubation of cells with cycloheximide [21]) and the apparent rate of synthesis (determined by pulse-label experiments [20, 21]) of the enzyme. Neither treatment changes the steady-state level of ODC mRNA in S49 cells (21). The three conditions (putrescine treatment, no treatment, and DFMO treatment) should expose the cells to the full gamut of intracellular polyamine levels attainable by physiological or pharmacological manipulation. Polysomes from cells subjected to each of these conditions were fractionated as described above, and the distribution of ODC-specific RNA was determined by Northern (RNA) blot analysis (Fig. 2). The position of sedimentation of β-actin mRNA was determined as a control. As a further control, extracts were incubated and centrifuged with EDTA, which dissociates ribosomal subunits from mRNA (26). EDTA treatment caused ODC mRNA to sediment with fractions lighter than monosomes, which suggests that ODC mRNA that cosediments with polysomes in the presence of free divalent cations is in fact bound to polysomes.

The results (Fig. 2) indicated the following. First, neither putrescine nor DFMO treatment caused an apparent change in the distribution of ODC-specific mRNA. Similar results with Ehrlich ascites cells have recently been reported (11). Second, about half of the ODC-specific RNA is found in fractions lighter than polysomes and presumably represents untranslated mRNA. Third, the portion of ODC-specific RNA found within the polysome region of the gradient is seen almost exclusively on the smallest polysomes. In contrast, almost all of the signal associated with β-actin mRNA was found within the region of the gradient associated with the largest polysomes.

Metabolic pulse-labeling of ODC. Observations that more [^{35}S]methionine is incorporated into ODC in cells deprived of polyamines have contributed to the conclusion that ODC synthesis is subject to translational regulation. Incorporation of label in such experiments can reflect not only the true rate of synthesis but also posttranslational events such as degradation. The extent of incorporation should more faithfully reflect the true rate of synthesis as the labeling period becomes shorter, because posttranslational events have more opportunity to manifest themselves during longer labeling periods. To test whether differential incorporation reflected differential synthesis, we measured the incorporation of [^{35}S]methionine into ODC with labeling periods that varied from 4 min to half an hour. The cells used (clone D2.88) are a variant of S49 lymphoma cells that overproduce ODC about 10-fold compared with wild-type parental cells. D2.88 cells have been isolated in a single-step selection, and they overproduce ODC mRNA to the same degree as ODC

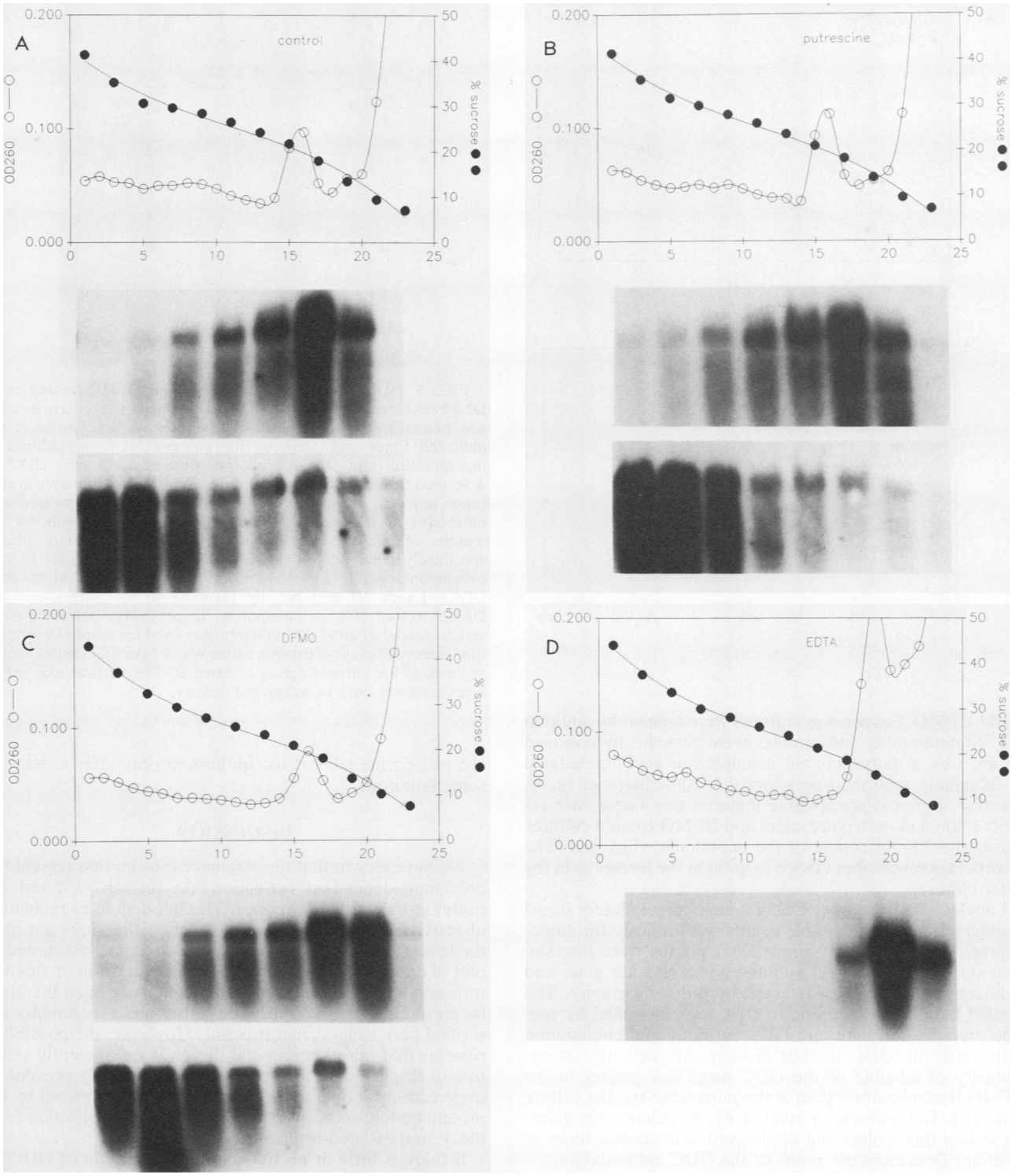
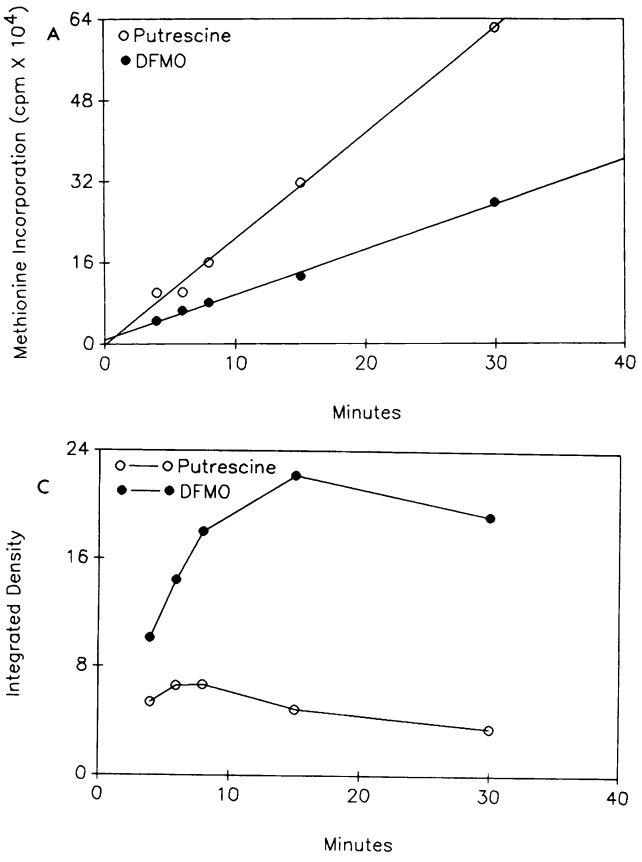


FIG. 2. Distribution of ODC mRNA and β-actin mRNA on polyribosome gradients. S49 cells were untreated (A) or were treated with 100 μM putrescine (B) or with 2 mM DFMO (C). After 22 h, cells were lysed and fractionated on sucrose density gradients. A portion of the control cell lysate was fractionated in gradients containing 20 mM EDTA (D). Fractions were collected, pooled, and analyzed for ODC (upper blot) and β-actin (lower blot) mRNA. Lanes are aligned with the sucrose gradient fractions to which they correspond. OD260, Optical density at 260 nm.

polypeptide and display the same magnitude of regulation of ODC activity in response to polyamine repletion and depletion as do their wild-type parent cells (21). These overproducing cells were used to facilitate experiments utilizing very short pulse-label times for detection of labeled ODC; results similar to those reported here have been obtained with wild-type S49 cells (results not shown).

Cells were treated for 22 h with 100 μM putrescine or with



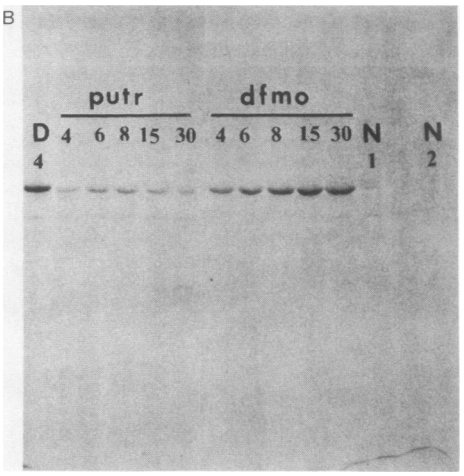


FIG. 3. ODC labeling in putrescine- and DFMO-treated cells. D2.88 cells were labeled with [^{35}S]methionine in the presence of 100 μM putrescine or 2 mM DFMO. Samples were removed at the indicated times and prepared for analysis for total radioactive incorporation and specific incorporation of label into ODC as described in Materials and Methods. (A) [^{35}S]methionine incorporation into acid-precipitable material. (B) ODC in cells treated with putrescine or DFMO. Cells incorporated [^{35}S]methionine for the number of minutes indicated. Lane D4, [^{35}S]methionine-labeled specifically immunoprecipitated cytoplasmic lysate from D4.1 ODC-overproducing cells (20) (the band marks the position of migration of ODC). Lanes N1 and N2 are identical to the putrescine- and DFMO-treated 30-min time points, respectively, but preimmune serum instead of anti-ODC antisera was used for immunoprecipitation. Autoradiographic exposure time was 4 days. (C) Densitometric analysis of the autoradiogram in panel B. The vertical axis represents arbitrary units of integrated density.

2 mM DFMO. Suspension cultures were labeled by addition of [^{35}S]methionine, and samples were periodically removed for analysis. A portion of each sample was acid precipitated to determine total incorporation of [^{35}S]methionine. Incorporation into acid-precipitable material was linear with respect to time in both putrescine- and DFMO-treated cultures during the 30-min course of the experiment (Fig. 3A). The incorporation was about twice as great in the former as in the latter culture.

Labeled ODC was specifically immunoprecipitated. Equal numbers of acid-precipitable counts were utilized for immunoprecipitation of each sample. Precipitates were fractionated on sodium dodecyl sulfate-polyacrylamide gels, and radioactive protein was detected by autoradiography. The labeled band corresponding to ODC was identified by specific precipitation with anti-ODC antibody and comigration with authentic ODC marker protein. At each time point, intensity of labeling of the ODC band was greater in the DFMO-treated culture than in the putrescine-treated culture (Fig. 3B). The differences in intensity, however, were greatest at late time points and diminished with shorter times of labeling. Densitometric scans of the ODC autoradiographic bands (Fig. 3C) showed that incorporation was about 5.5-fold greater in the DFMO-treated cells at 30 min but only about 1.9-fold greater at 4 min of labeling. This result suggests that the true rate of synthesis of ODC under the two treatment conditions differs at most by a factor of two. With each culture condition, the fraction of acid-precipitable counts represented by full-size immunoprecipitable ODC rose during initial times of labeling. This likely reflects the fact that acid-precipitable counts increase linearly, virtually from zero time, but that ODC (and other protein) nascent chains become labeled to equilibrium only after a delay of some minutes.

DISCUSSION

We have shown that the sequence information relevant to polyamine-dependent regulation lies primarily, if not entirely, in the coding sequence. The full degree of regulation of activity is exhibited by a transfectant that does not have the leader sequence, and the leader sequence, when made a part of another gene, does nothing. The question of whether translational regulation is indeed taking place must therefore be carefully reconsidered. The polysome experiments described here suggest that it is not. However, the possibility remains that our experimental design is insufficiently sensitive to display such a form of regulation if it is present. If there is translational regulation, it is being carried out by the protein-coding sequence, possibly with a contribution from the 3′ untranslated sequences.

If there is little or no translational regulation of ODC by polyamines, how have we and others come to believe otherwise? The most convincing data in favor of the hypothesis come from observations that when cells are treated with putrescine or polyamines, mRNA levels do not change but there is a reduction of incorporation of radioactive amino acids into ODC polypeptide. In none of these previously reported experiments has the duration of labeling been less than 7 min (21). We speculate that newly synthesized ODC is subject to two kinetic components of degradation. The slower of these acts on ODC molecules that have assumed a

native and enzymatically active tertiary and quaternary form. It is this component of degradation, typically measured as a half-life of 20 to 60 min (3, 12, 13, 20, 21, 31, 33, 34), that is measured in experiments that utilize inhibitors of protein synthesis to examine the rate of disappearance of enzymatic activity and in pulse-chase experiments with labeling times in excess of several minutes. We hypothesize that there is a second component of degradation that acts on newly synthesized protein. The rate or extent of this process could be controlled by the level of intracellular polyamines. This alternate hypothesis leads to several predictions. One is that the polyamine-dependent differences in incorporation of radioactive amino acids into ODC should diminish as labeling times approach a few minutes, the time required to synthesize a polypeptide. A second prediction of this hypothesis is that because all regulation is carried out at the level of the ODC protein, only protein-coding sequences should be required to display the full gamut of regulation. Both of these predictions are confirmed by the results reported here.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants CA29048 and CA47721 from the National Institutes of Health and by grant DCB 8707375 from the National Science Foundation to P.C.

LITERATURE CITED

1. **Brabant, M., L. McConlogue, T. van Daalen Wetters, and P. Coffino.** 1988. Mouse ornithine decarboxylase gene: cloning, structure, and expression. Proc. Natl. Acad. Sci. USA **85:** 2200–2204.
2. **Casadaban, M. J., A. Martinez-Arias, S. K. Shapira, and J. Chou.** 1983. Beta galactosidase gene fusions for analyzing gene expression in *Escherichia coli* and yeast. Methods Enzymol. **100:**293–308.
3. **Clark, J. L.** 1974. Specific induction of ornithine decarboxylase in 3T3 mouse fibroblasts by pituitary growth factors: cell density-dependent biphasic response and alteration of half-life. Biochemistry **13:**4668–4674.
4. **Coffino, P., and E. L. Chen.** 1988. Nucleotide sequence of the mouse ornithine decarboxylase gene. Nucleic Acids Res. **16:** 2731–2732.
5. **Dircks, L., A. Grens, T. C. Slezynger, and I. E. Scheffler.** 1986. Posttranscriptional regulation of ornithine decarboxylase activity. J. Cell. Physiol. **126:**371–378.
6. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:**6–13.
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. **Gupta, M., and P. Coffino.** 1985. Mouse ornithine decarboxylase: complete amino acid sequence deduced from cDNA. J. Biol. Chem. **260:**2941–2944.
9. **Hall, C. V., P. E. Jacob, G. M. Ringold, and F. Lee.** 1983. Expression and regulation of *Escherichia coli lacZ* gene fusions in mammalian cells. J. Mol. Appl. Genet. **2:**101–109.
10. **Herbomel, P., B. Bourachot, and M. Yaniv.** 1984. Two distinct enhancers with different cell specificities coexist in the regulatory region of polyoma. Cell **39:**653–662.
11. **Holm, I., L. Persson, L. Stjernborg, L. Thorsson, and O. Heby.** 1989. Feedback control of ornithine decarboxylase expression by polyamines. Biochem. J. **258:**343–350.
12. **Hölttä, E., and P. Pohjanpelto.** 1986. Control of ornithine decarboxylase in Chinese hamster ovary cells by polyamines. J. Biol. Chem. **261:**9502–9508.
13. **Isomaa, V. V., A. E. I. Pajunen, C. W. Bardin, and O. A. Jänne.** 1983. Ornithine decarboxylase in mouse kidney: purification, characterization and radioimmunological determination of the enzyme protein. J. Biol. Chem. **258:**6735–6740.
14. **Jacobson, A. B., H. Kumar, and M. Zuker.** 1985. Effect of spermidine on the conformation of bacteriophage MS2 RNA electron microscopy and computer modeling. J. Mol. Biol. **181:**517–531.
15. **Jones, C. L., and E. Ehrenfeld.** 1983. The effect of poliovirus infection on the translation in vitro of VSV messenger ribonucleoprotein particles. Virology **129:**415–430.
16. **Kahana, C., and D. Nathans.** 1985. Translational regulation of mammalian ornithine decarboxylase by polyamines. J. Biol. Chem. **260:**15390–15393.
17. **Katz, A., and C. Kahana.** 1988. Isolation and characterization of the mouse ornithine decarboxylase gene. J. Biol. Chem. **263:** 7604–7609.
18. **Madhubala, R., J. A. Secrist III, and A. E. Pegg.** 1988. Effect of inhibitors of S-adenosylmethionine decarboxylase on the contents of ornithine decarboxylase and S-adenosylmethionine decarboxylase in L1210 cells. Biochem. J. **254:**45–50.
19. **Mathews, M. B. (ed.).** 1986. Translational control. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **McConlogue, L., and P. Coffino.** 1983. Ornithine decarboxylase in difluoromethylornithine-resistant mouse lymphoma cells. J. Biol. Chem. **258:**8384–8388.
21. **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.
22. **McConlogue, L. C., L. J. Marton, and P. Coffino.** 1983. Growth regulatory effects of cyclic AMP and polyamine depletion are dissociable in cultured mouse lymphoma cells. J. Cell. Biol. **96:**762–767.
23. **Pachter, J. S., T. J. Yen, and D. W. Cleveland.** 1987. Autoregulation of tubulin expression is achieved through specific degradation of polysomal tubulin mRNAs. Cell **51:**283–292.
24. **Pegg, A. E.** 1986. Recent advances in the biochemistry of polyamines in eukaryotes. Biochem. J. **234:**249–262.
25. **Pegg, A. E., R. Madhubala, T. Kameji, and R. J. Bergereon.** 1988. Control of ornithine decarboxylase activity in alpha-difluoromethylornithine-resistant L1210 cells by polyamines and synthetic analogues. J. Biol. Chem. **263:**11008–11014.
26. **Penman, S., C. Vesco, and M. Penman.** 1968. Localization and kinetics of formation of nuclear heterodisperse RNA, cytoplasmic heterodisperse RNA and polyribosome-associated messenger RNA in HeLa cells. J. Mol. Biol. **34:**49–69.
27. **Persson, L., I. Holm, and O. Heby.** 1986. Translational regulation of ornithine decarboxylase by polyamines. FEBS Lett. **205:**175–178.
28. **Persson, L., I. Holm, and O. Heby.** 1988. Regulation of ornithine decarboxylase mRNA translation by polyamines. J. Biol. Chem. **263:**3528–3533.
29. **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.
30. **Quigley, G. J., M. M. Teeter, and A. Rich.** 1978. Structural analysis of spermine and magnesium ion binding to yeast phenylalanine transfer RNA. Proc. Natl. Acad. Sci. USA **75:**64–68.
31. **Russell, D. H., and S. H. Snyder.** 1969. Amine synthesis in regenerating rat liver: extremely rapid turnover of ornithine decarboxylase. Mol. Pharmacol. **5:**253–262.
32. **Schimke, R. T.** 1973. Control of enzyme levels in mammalian tissues. Adv. Enzymol. **37:**135–187.
33. **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.
34. **Seely, S. E., H. Pösö, and A. E. Pegg.** 1982. Effect of androgens on turnover of ornithine decarboxylase in mouse kidney. J. Biol. Chem. **257:**7549–7553.
35. **Sertich, G. J. and A. E. Pegg.** 1987. Polyamine administration

reduces ornithine decarboxylase activity without affecting its mRNA content. Biochem. Biophys. Res. Commun. **143**:424–430.

36. **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.
37. **Spiegelman, B. M., M. Frank, and H. Green.** 1983. Molecular cloning of mRNA from 3T3 adipocytes. J. Biol. Chem. **258**: 10083–10089.
38. **Steglich, C., A. Grens, and I. E. Scheffler.** 1985. Chinese hamster cells deficient in ornithine decarboxylase activity: reversion by gene amplification and by azacytidine treatment. Somatic Cell Mol. Genet. **11**:11–23.
39. **Steglich, C., and I. E. Scheffler.** 1982. An ornithine decarboxylase-deficient mutant of Chinese hamster ovary cells. J. Biol. Chem. **257**:4603–4609.
40. **Tabor, C. W., and H. Tabor.** 1984. Polyamines. Annu. Rev. Biochem. **53**:749–790.
41. **White, M. W., T. Kameji, A. E. Pegg, and D. R. Morris.** 1987. Increased efficiency of translation of ornithine decarboxylase mRNA in mitogen-activated lymphocytes. FEBS Lett. **170**: 87–92.