Feedback control of ornithine decarboxylase expression by polyamines

Analysis of ornithine decarboxylase mRNA distribution in polysome profiles and of translation of this mRNA *in vitro*

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Cell growth and differentiation require the presence of optimal concentrations of polyamines. Ornithine decarboxylase (ODC) catalyses the first and rate-controlling step in polyamine synthesis. In studies using cultures of Ehrlich ascites-tumour cells, we have shown that the expression of ODC is subject to feedback regulation by the polyamines. A decrease in the cellular polyamine concentration results in a compensatory increase in the synthesis of ODC, whereas an increase in polyamine concentration results in suppression of ODC synthesis. These changes in ODC synthesis were attributed to changes in the efficiency of ODC mRNA translation, because the steady-state amount of ODC mRNA remained constant. We now show that the number of ribosomes associated with ODC mRNA is low, and that the increase in ODC mRNA translation takes place without a shift in the distribution of ODC mRNA towards larger polysomes. This finding indicates that the polyamines regulate the efficiency of ODC mRNA translation by co-ordinately affecting the rates of initiation and elongation. By analysing ODC mRNA translation *in vitro*, using a rabbit reticulocyte lysate, polyadenylated RNA from a cell line with an amplified ODC gene, and a monospecific anti-ODC antibody, we also show that spermidine, but not putrescine, exerts a direct regulatory effect on ODC synthesis.

INTRODUCTION

It is well established that the polyamines putrescine, spermidine and spermine are essential for cell growth and differentiation (Heby, 1981; Tabor & Tabor, 1984; Pegg, 1986). Therefore, it is not surprising that their biosynthesis is highly regulated. The first and rate-limiting step, i.e. the synthesis of putrescine, is catalysed by ornithine decarboxylase (ODC). This enzyme is very important from a regulatory point of view. Accordingly, the turnover rate of ODC is extremely rapid, and the half-life can be as short as 5–10 min (Russell & Snyder, 1969; Hogan & Murden, 1974). There is no evidence that the ODC activity is regulated by post-translational modification of the enzyme molecule. Instead it appears to be regulated by changes in rate of synthesis and/or degradation.

Cells that have been treated with reversible inhibitors of ODC, to deplete their polyamine content, usually exhibit a compensatory increase in ODC activity as measured in diluted or dialysed extracts (Inoue *et al.*, 1975; McCann *et al.*, 1977; Mamont *et al.*, 1978; Oredsson *et al.*, 1980). Conversely, cells that have been treated with polyamines, to increase their intracellular polyamine content, are deprived of their ODC activity (Kay & Lindsay, 1973; Clark, 1974; Jänne & Hölttä, 1974). Part of these changes can be explained by stabilization/destabilization of the enzyme. However, changes

in the rate of ODC synthesis may also contribute to altered enzyme activity (Persson *et al.*, 1985, 1986).

ODC synthesis has been little investigated because of difficulties related to the small quantities of the enzyme present in most cells (Pegg, 1986). However, the preparation of monospecific anti-ODC antibodies (Kameji *et al.*, 1982; Persson, 1982; Isomaa *et al.*, 1983; Seely & Pegg, 1983) as well as ODC cDNAs (Berger *et al.*, 1984; Kahana & Nathans, 1984; Kontula *et al.*, 1984; McConlogue *et al.*, 1984) has provided adequate tools for such studies. In previous reports we have demonstrated that the polyamines exert a feedback control of ODC synthesis (Persson *et al.*, 1985, 1986). When cells are treated with the irreversible inhibitor of ODC, 2-difluoromethylornithine (DFMO) (Metcalf *et al.*, 1978), to decrease their polyamine content, there is a compensatory increase in the cellular amount of ODC. This increase is not due to a stabilization of the enzyme, but is mainly caused by a change in the rate of synthesis (Persson *et al.*, 1985, 1986). Addition of polyamines, on the other hand, causes a suppression of ODC synthesis (Persson *et al.*, 1986). Since there are no changes in the cellular amount of ODC mRNA, the polyamines are suggested to exert their effects at the level of translation rather than transcription. In the present work we have further examined the regulatory effect of the polyamines on ODC synthesis by analysing the distribution of ODC mRNA in polysome profiles and by studying the translation of ODC mRNA in a cell-free system.

Abbreviations used: ODC, ornithine decarboxylase; DFMO, 2-difluoromethylornithine; poly(A)⁺ RNA, polyadenylated RNA.

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MATERIALS AND METHODS

Materials

[³⁵S]Methionine and [³²P]dCTP were obtained from Amersham. L-[1-¹⁴C]Ornithine and DL- α -[3,4-³H]DFMO were purchased from New England Nuclear. DFMO was generously provided by Merrell Dow Research Institute (Strasbourg, France) and cDNA (pODC 934) encoding mouse kidney ODC was kindly given by Dr. Franklin G. Berger (Berger *et al.*, 1984).

Cells

Ehrlich ascites-tumour cells were grown in a 1:1 (v/v) mixture of Eagle's minimum essential medium and Ham's F12 medium (lacking putrescine) supplemented with 0.2% bovine serum albumin and antibiotics (50 units of penicillin/ml and 50 μ g of streptomycin/ml). The cells were seeded at a density of 1.0×10^5 cells/ml in the absence or presence of 5 mM-DFMO and various concentrations (1 μ M–10 mM) of putrescine or spermidine. Cells were harvested 24 h after seeding.

Assay of ODC activity

The cells were sonicated in a small volume of ice-cold 0.1 M-Tris/HCl, pH 7.5, containing 0.1 mM-EDTA and 0.5 mM-dithiothreitol. After centrifugation at 20000 g for 20 min, ODC activity was determined in samples of the supernatants by measuring the release of ¹⁴CO₂ from [*carboxy*-¹⁴C]ornithine in the presence of saturating concentrations of pyridoxal 5'-phosphate (0.1 mM) and L-ornithine (0.5 mM) (Jänne & Williams-Ashman, 1971).

Assay of ODC protein content

The amount of ODC protein was measured essentially as described by Seely & Pegg (1983). Portions of the supernatants were incubated at room temperature for 30 min with a monospecific antibody against mouse kidney ODC (Persson, 1982) diluted 1:256000. Purified mouse kidney ODC (Persson, 1981) labelled with [³H]DFMO was then added, and the samples were incubated for a further 30 min. Antibody-bound radioactivity was determined after precipitation with bacterial protein A adsorbent (60 min) and centrifugation at 12000 g for 2 min. Purified mouse kidney ODC was used as standard.

Determination of ODC synthesis

ODC synthesis was determined by measuring the incorporation of [³⁵S]methionine into the enzyme. The cells were reseeded (1.0×10^6 cells/ml) in methionine-free Eagle's minimum essential medium supplemented with [³⁵S]methionine (10 μ Ci/ml). After incubation at 37 °C for 25 min (the half-life of ODC in the cells used is about 1 h; Persson *et al.*, 1985), the cells were collected by centrifugation and sonicated as described above. Portions of the supernatants (containing equal amounts of acid-insoluble radioactivity) were incubated with an excess of anti-ODC antiserum (Persson, 1982) at room temperature for 30 min. ODC bound to the antibody was precipitated by incubation with protein A adsorbent for a further 30 min. After thorough washing in 10 mM-Tris/HCl, pH 7.5, containing 0.1 mM-EDTA, 0.05 mM-dithiothreitol, 0.1% bovine serum albumin, 0.1% Triton X-100 and 0.1% SDS, the precipitate was fractionated by SDS/polyacrylamide-gel electrophoresis essentially as described by Persson *et al.* (1984). Autoradiography

was carried out after incubating the gels in Amplify (Amersham).

Determination of ODC mRNA

ODC mRNA was determined by Northern-blot analysis. Total RNA (20 μ g), isolated by the guanidinium/CsCl method (Glisin *et al.*, 1974) was fractionated in formaldehyde-containing 1%-agarose gels (Elliot & Berger, 1983). After transfer to Gene-Screen (New England Nuclear), the RNA was hybridized to pODC 934 labelled with [³²P]dCTP (Feinberg & Vogelstein, 1983).

Polysome isolation and fractionation

The cells were swollen in 2 vol. of 10 mM-KCl/1.5 mM-magnesium acetate/7 mM-2-mercaptoethanol/10 mM-Hepes (pH 7.5) and lysed by the addition of 0.25% Nonidet P-40. After centrifugation at 10000 g for 10 min, the supernatants were layered on 15–50% (w/v) linear sucrose gradients prepared in 0.1 M-KCl/3 mM-magnesium acetate/20 mM-Tris/HCl (pH 7.6) and centrifuged at 40000 rev./min (2 h, 4 °C) in a Beckman SW 41 rotor. After the gradients were pumped through the flow cell of a recording spectrophotometer (254 nm), fractions were collected. Each fraction was treated with Proteinase K (0.1 mg/ml) and 0.5% SDS for 30 min at 37 °C. Thereafter they were extracted once with 1 vol. of phenol/chloroform/3-methylbutan-1-ol (50:49:1, by vol.) and once with 1 vol. of chloroform. RNA from equal volumes of each fraction was analysed for ODC mRNA content as described above.

Translation *in vitro* of poly(A)⁺ RNA

The polyamine content of rabbit reticulocyte lysates was depleted by gel filtration on Bio-Gel P-6DG (Bio-Rad) by using 10 mM-Hepes buffer (pH 7.2), containing 1.2 mM-MgCl₂, 25 mM-KCl, 10 mM-NaCl, 0.05 mM-EDTA and 0.1 mM-EGTA. Poly(A)⁺ RNA was isolated from ODC-overproducing mutant L1210 cells (Persson *et al.*, 1988) and translated (0.5 μ g) in the presence of 0.1 M-KCl, 1.3 mM-MgCl₂, 1.0 mM-ATP, 0.2 mM-GTP, 10 mM-phosphocreatine, 1.0 mM-glucose, 50 μ M of an amino acid mixture lacking methionine, and 20 μ Ci of [³⁵S]methionine (1 h; 30 °C). Incorporation of radioactivity into ODC was determined as described above. Total protein synthesis was measured after precipitation with 5% (w/v) trichloroacetic acid.

Determination of polyamines

Chromatographic separation and quantitative determination of the polyamines in the cell extracts were carried out with an automatic amino acid analyser (Biotronik LC 5001), with *o*-phthaldialdehyde as the reagent.

RESULTS

Effects of DFMO and polyamine treatment on the cellular ODC activity and content

As shown in Table 1, dilution of Ehrlich ascites-tumour cells in fresh medium caused a dramatic increase in ODC activity. As expected, this rise in ODC activity was partly restrained when cells were seeded in a medium containing DFMO, a highly specific enzyme-activated irreversible inhibitor of ODC. Nevertheless, the radio-

Table 1. Effects of DFMO treatment on ODC induction

Cells were seeded in the absence or presence of DFMO (5 mM). They were harvested 1 day later (during exponential growth) and analysed for ODC activity and ODC protein content. Results are means $\pm$ S.E.M. ($n = 3$): N.D., not detectable.

Time (days)	Treatment	ODC	
		Activity (nmol of CO ₂ formed/h per 10 ⁶ cells)	Protein (ng of ODC/10 ⁶ cells)
0	—	N.D.	N.D.
1	—	0.51 $\pm$ 0.05	0.41 $\pm$ 0.23
1	DFMO	0.15 $\pm$ 0.01	6.32 $\pm$ 1.67

immunoassay revealed a large increase in ODC content. The ODC content was more than 10-fold greater in DFMO-treated cells than in cells grown in the absence of DFMO (Table 1).

Addition of putrescine or spermidine to the medium dramatically decreased the DFMO-induced increase in ODC content of the cells (Fig. 1). Spermidine gave rise to a marked inhibitory effect at a concentration as low as 1 μ M and seemed a little more effective than putrescine. At the highest polyamine concentration (10 mM) there was no detectable ODC protein in the cells.

Effects of DFMO and polyamine treatment on the cellular polyamine content

The increase in ODC activity, induced by dilution of the cells in fresh medium, resulted in elevated polyamine content (Table 2). The increase in putrescine and spermidine content was effectively restrained by the addition of DFMO to the medium, but that of spermine was not. Instead there was a further increase in the spermine content during DFMO treatment as a result of conversion of putrescine and spermidine.

The DFMO-mediated depletion of cellular putrescine and spermidine was effectively counteracted by supplementing the medium with these amines (Table 2). When putrescine was added at a concentration of 1 μ M, the

Fig. 1. Effects of polyamines on DFMO-induced increase in cellular ODC content

Cells were seeded in the presence of DFMO (5 mM) and various concentrations of putrescine (○) or spermidine (△). The amount of ODC protein was determined after 1 day. Untreated controls contained 0.41 $\pm$ 0.23 ng of ODC/10⁶ cells at this time (Table 1). Results are means $\pm$ S.E.M. ($n = 3$).

major intracellular change was an increase in spermidine content, demonstrating that most of the putrescine taken up by the cells was converted into spermidine. The cellular putrescine and spermidine contents increased, even above normal, when higher concentrations of putrescine were used. Treatment with spermidine, at a concentration of 1 μ M, nearly sufficed to produce a normal cellular spermidine content in the presence of DFMO. At 10–100 μ M-spermidine concentrations, the cellular spermidine pool increased above normal, yet only a very small fraction appeared to be converted into putrescine.

Effects of DFMO and polyamine treatment on ODC synthesis

To examine whether the regulatory effects of polyamines on the cellular ODC content were due to changes in ODC synthesis rate, cells were pulse-labelled with

Table 2. Effects of DFMO and polyamines on the cellular polyamine content

Cells were seeded in the absence or presence of DFMO (5 mM) and various concentrations of putrescine or spermidine. The cellular polyamine contents (nmol/10⁶ cells) were determined 1 day after seeding. Results are means $\pm$ S.E.M. ($n = 3$): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared with controls (day 1).

	Putrescine	Spermidine	Spermine
Control (day 0)	0.11 $\pm$ 0.01***	0.80 $\pm$ 0.06***	0.39 $\pm$ 0.04
Control (day 1)	0.77 $\pm$ 0.04	2.16 $\pm$ 0.11	0.54 $\pm$ 0.06
DFMO	0.02 $\pm$ 0.00***	0.80 $\pm$ 0.06***	1.01 $\pm$ 0.04**
DFMO + 1 μ M-putrescine	0.07 $\pm$ 0.01***	1.47 $\pm$ 0.13*	0.97 $\pm$ 0.07*
DFMO + 10 μ M-putrescine	0.78 $\pm$ 0.08	2.56 $\pm$ 0.22	0.77 $\pm$ 0.04*
DFMO + 100 μ M-putrescine	1.37 $\pm$ 0.12**	2.52 $\pm$ 0.17	0.71 $\pm$ 0.06
DFMO + 1 μ M-spermidine	0.21 $\pm$ 0.07**	1.67 $\pm$ 0.03*	0.89 $\pm$ 0.08*
DFMO + 10 μ M-spermidine	0.10 $\pm$ 0.01***	2.54 $\pm$ 0.44	0.43 $\pm$ 0.13
DFMO + 100 μ M-spermidine	0.09 $\pm$ 0.02***	2.93 $\pm$ 0.12*	0.63 $\pm$ 0.07

Fig. 2. Effects of DFMO and polyamine treatment on ODC synthesis

Cells were seeded in the absence or presence of DFMO (5 mM) and various concentrations of spermidine (a) or putrescine (b). ODC synthesis was determined 1 day after seeding by measuring the incorporation of [35 S]methionine into ODC protein [precipitated with an excess of anti-ODC-antibody (Persson, 1982) after a 25 min labelling pulse]. (a) Lane 1, untreated control cells; lane 2, DFMO-treated cells (non-immune serum); lane 3, DFMO-treated cells; lanes 4–7, cells treated with DFMO + spermidine (1, 10, 10^2 and 10^3 μ M respectively). (b) Lane 1, DFMO-treated cells (non-immune serum); lane 2, DFMO-treated cells; lanes 3–7, cells treated with DFMO + putrescine (1, 10, 10^2 , 10^3 and 10^4 μ M respectively). Arrow indicates the migration of [3 H]DFMO-labelled purified mouse kidney ODC (53 kDa).

[35 S]methionine, and the relative amount of radioactivity incorporated into the enzyme was determined after immunoprecipitation and SDS/polyacrylamide-gel electrophoresis (Figs. 2a and 2b). In the presence of DFMO the cells increased their rate of ODC synthesis. This stimulation was inhibited by simultaneous addition of polyamines. Spermidine again appeared to be more effective than putrescine, with a marked effect already at a concentration as low as 1 μ M. At the same concentration, putrescine exerted only a slight inhibitory effect on ODC synthesis. Higher concentrations of putrescine or spermidine effectively restrained the rate of ODC synthesis to values below that of untreated control cells. Notably, ODC synthesis was never totally abolished, but proceeded even at a polyamine concentration (10 mM) which sufficed to eradicate the ODC activity (Persson *et al.*, 1986).

Effect of DFMO and polyamine treatment on the cellular ODC mRNA content

Steady-state contents of ODC mRNA were analysed by Northern-blot hybridization using a cDNA encoding mouse kidney ODC. As seen from Fig. 3, the only significant change in cellular ODC mRNA content occurred after growth stimulation. Thus dilution of the cells in fresh medium resulted in a markedly elevated ODC mRNA content. This increase in ODC mRNA was neither enhanced by DFMO nor suppressed by putrescine or spermidine, indicating that the polyamine-mediated regulation of ODC expression is not operating at the transcriptional level.

Effects of DFMO treatment on the distribution of ODC mRNA in polysome profiles

Northern-blot analysis was used to determine the distribution of ODC mRNA in polysomes obtained from

cells grown in the absence or presence of DFMO. The fractions containing the largest amounts of ODC mRNA were fractions 2 and 3 (Fig. 4). These represent mainly untranslated mRNA (not associated with ribosomes). Notably, the DFMO-induced increase in ODC mRNA translation was not associated with a shift in the distribution of ODC mRNA towards larger polysomes.

Fig. 3. Effects of DFMO and polyamine treatment on the cellular ODC mRNA content

Lane 1, untreated 0 h control cells; lane 2, untreated 1-day control cells; lane 3, cells treated for 1 day with 5 mM-DFMO; lane 4, cells treated for 1 day with 5 mM-DFMO + 1 mM-putrescine; lane 5, cells treated for 1 day with 5 mM-DFMO + 1 mM-spermidine. The relative amounts of ODC mRNA, as determined by densitometric scanning, were 1:3.8:3.6:4.5:3.7.

Fig. 4. Distribution of ODC mRNA in polysome profiles from control and DFMO-treated cells

Cytosolic extracts from cells grown for 1 day in the absence or presence of 5 mM-DFMO were subjected to sucrose-gradient centrifugation and analysed for absorbance at 254 nm (top panels). Serial fractions were analysed for their ODC mRNA content by hybridization to a cDNA probe (bottom panels).

Thus the increase was not due to an elevated number of ribosomes associated with the mRNA.

That the ODC mRNA was in fact translatable was demonstrated by pre-treating the cells for 30 min with a low dose (0.75 $\mu\text{g}/\text{ml}$) of cycloheximide (which preferentially slows elongation relative to initiation). Thus most of the ODC mRNA was found to be associated with larger polysomes in cycloheximide-treated cells (results not shown).

Effects of polyamines on the translation of ODC mRNA in rabbit reticulocyte lysates

Effects of polyamines on the translation of ODC mRNA *in vitro* were studied by using rabbit reticulocyte lysates. The lysates were gel-filtered to remove the endogenous polyamines. This procedure seriously decreased the translation capacity of the lysate. Addition of spermidine, however, reversed this effect and caused maximum stimulation of ODC synthesis and total protein synthesis at concentrations of 0.2 and 0.4 mM respectively (Fig. 5a). At higher spermidine concentrations ODC synthesis decreased to a non-detectable value, whereas total protein synthesis exhibited a relatively small decrease.

At variance with spermidine, putrescine did not stimulate protein synthesis in the lysate. To determine whether

putrescine might still exert an inhibitory effect on ODC mRNA translation at high concentrations, the maximal rate of translation was produced by adding 0.2 mM-spermidine to the gel-filtered lysate. Then putrescine was added at various concentrations. The high rates of ODC mRNA translation and total protein synthesis achieved by spermidine addition were largely unaffected by putrescine concentrations up to at least 1.0 mM (Fig. 5b).

In addition to the band corresponding to the 53 kDa subunit molecular mass of ODC, several bands with lower molecular masses were seen (Fig. 5). These are probably the result of internal initiation or premature termination. By and large, the polyamines affected the synthesis of the smaller species of ODC-immunoreactive proteins in a similar way to that of full-length ODC.

DISCUSSION

The present work provides evidence for direct feedback regulation of ODC expression by the polyamines. Depletion of the cellular putrescine and spermidine contents, by using DFMO, resulted in a compensatory increase in ODC synthesis. This DFMO-mediated stimulation of ODC synthesis was counteracted by addition of putrescine or spermidine to the medium. Marked inhibition of

Fig. 5. Effects of polyamines on the translation of ODC mRNA *in vitro*

Poly(A)⁺ RNA isolated from ODC-overproducing cells was translated in gel-filtered reticulocyte lysate in the presence of spermidine alone (a) or spermidine plus putrescine (b). Top panels: SDS/polyacrylamide-gel electrophoresis of immunoprecipitated [³⁵S]methionine-labelled ODC. (a) Lanes 1–8: 0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 or 1.0 mM-spermidine. (b) Lanes 1–6: 0.2 mM-spermidine plus 0, 0.2, 0.4, 0.6, 0.8 or 1.0 mM-putrescine. Arrows indicate the migration of pure ODC (53 kDa). Bottom panels: Effects of putrescine and spermidine on total protein synthesis (●) and on ODC mRNA translation (○). The latter was quantified by densitometric scanning of the 53 kDa band in the corresponding autoradiograms.

ODC synthesis was obtained even by exposure to micromolar polyamine concentrations, although the intracellular concentrations normally are in the millimolar range. This may be explained by the fact that mammalian cells have a very effective polyamine transport system, which can operate against a steep concentration gradient (Kano & Oka, 1976). When the medium was supplemented with high concentrations of the polyamines, together with DFMO, ODC synthesis decreased to values which were even lower than those observed in untreated control cells. This is in agreement with our previous report, demonstrating a decrease in ODC synthesis rate when cells were treated with an excess of polyamines (Persson *et al.*, 1986).

Since no correlation was observed between the change in ODC synthesis (caused by addition of DFMO or polyamines) and the cellular ODC mRNA content, it appears that the negative control of ODC expression exerted by the polyamines is carried out at the translational rather than the transcriptional level. The only change in ODC mRNA content was observed during the initiation of growth. Thus a marked increase in ODC mRNA content was found to accompany the rise in ODC activity that occurred when the cells were diluted in fresh medium. This increase occurred even in the presence of DFMO and/or polyamines, and was thus independent of the cellular polyamine content. The elevated ODC mRNA content may be due to increased transcription (Katz & Kahana, 1987) and/or increased stability of the message (Rose-John *et al.*, 1987).

Translational regulation of ODC has also been observed in two other cell lines (Kahana & Nathans, 1985; Hölttä & Pohjanpelto, 1986). However, these cell lines were selected for ODC overproduction, and thus do not necessarily reflect the normal situation. Moreover, since the treatment periods with polyamines were relatively short, ranging from 15 min to 4 h, a long-term effect on ODC-gene transcription could not be excluded. The present results, together with our previous observations on Ehrlich ascites-tumour cells (Persson *et al.*, 1985, 1986), clearly demonstrate that the polyamines regulate ODC expression mainly at the translational level. This is shown to be true even under normal conditions, i.e. when ODC is not overproduced as a result of gene amplification.

The present results with DFMO show that ODC synthesis is suppressed by the actual cellular concentrations of putrescine and spermidine in exponentially growing cells. However, this suppression is not maximal since addition of more polyamines inhibited ODC synthesis even further. Hence the cells are equipped with an excellent machinery for regulating the cellular concentrations of polyamines. If, for any reason, the polyamine pools decrease, ODC synthesis and thus also polyamine synthesis will increase. An excess of polyamines, on the other hand, will immediately suppress the synthesis of ODC, resulting in a rapid decrease in ODC activity owing to the fast turnover of this enzyme (Russell & Snyder, 1969; Hogan & Murden, 1974). In view of the large number of cell types in which the polyamines have

been shown to suppress the ODC activity (Kay & Lindsay, 1973; Clark, 1974; Jänne & Hölttä, 1974), it may be suggested that the polyamine-mediated translational regulation of ODC expression observed here is of general importance.

Molecular analyses have revealed that ODC mRNA contains a long non-coding 5' leader (Gupta & Coffino, 1985; Katz & Kahana, 1988). Since the polyamines have a high affinity for nucleic acids, it is conceivable that these amines, by changing the secondary structure of the leader, affect the rate of initiation. By analysing the distribution of ODC mRNA in polysome profiles we demonstrate that the increase in ODC mRNA translation, resulting from polyamine depletion, is not merely due to stimulation of initiation. In addition, there has to be a corresponding increase in the rate of elongation, since there was no significant shift of the ODC mRNA from the untranslated to the translated compartment. Notably, only a few ribosomes are associated with each ODC mRNA. This is true even when ODC translation is maximal.

To analyse further the polyamine-mediated feedback control of ODC synthesis, a rabbit reticulocyte-lysate system was used. Such lysates contain large amounts of polyamines, which are essential for mRNA translation (Jackson *et al.*, 1983). Thus removal of the polyamines by gel filtration markedly decreased the translation of ODC mRNA (and other mRNAs) in the lysate. Addition of spermidine, but not putrescine, was found to stimulate translation. At higher, yet physiological, concentrations spermidine exerted a strong inhibitory effect on ODC mRNA translation. The translation of total mRNA was only slightly inhibited by spermidine, resulting in a decrease in the fraction of protein synthesis accounted for by ODC. This fact may explain previous difficulties in translating ODC mRNA in rabbit reticulocyte lysates (McConlogue & Coffino, 1983; Kahana & Nathans, 1984) which contain high concentrations of spermidine (Jackson *et al.* 1983). A similar inhibitory effect of polyamines on ODC synthesis *in vitro* has been found using poly(A)⁺ RNA from mouse kidney (Kameji & Pegg, 1987).

At variance with spermidine, putrescine exerted no significant stimulatory or inhibitory effect on the translation of ODC mRNA or total mRNA in the lysate. Therefore putrescine appears to have no major regulatory role in ODC synthesis. In apparent contrast, addition of putrescine to cells in culture was found to inhibit ODC synthesis markedly. However, this finding may be explained by the fact that the putrescine added was rapidly converted into spermidine. The addition of spermidine did not markedly affect the cellular content of the other polyamines, except for a small decrease in spermine content.

Protein synthesis is normally limited by the rate of initiation, and translational control mechanisms are likely to operate at this level (Hershey *et al.*, 1986). Thus several cases have been described in which selective control of mRNA translation appears to occur at the level of polypeptide-chain initiation. However, the present study strongly suggests that chain elongation is an additional site for regulation of protein synthesis. Translational control at this level is also suggested by studies of some other proteins, e.g. secretory proteins (Hershey *et al.*, 1986). Further studies on the polyamine-mediated regulation of ODC mRNA translation are

likely to add more information concerning the role of elongation in controlling protein synthesis.

This study was supported by grants from the Swedish Natural Science (B-BU 4086-115) and Medical (04X-02212) Research Councils, the Medical Faculty (University of Lund) and the John and Augusta Persson, the Harald and Greta Jeansson, the Berta Kamprad and the Magnus Bergvall Foundations.

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Received 4 July 1988/17 October 1988; accepted 20 October 1988