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; McConilogue 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-[3,4,5H]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 μg of streptomycin/ml). The cells were seeded at a density of 1.0 × 10⁶ cells/ml in the absence or presence of 5 mM-DFMO and various concentrations (1 μM–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 mM-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 × 10⁶ cells/ml) in methionine-free Eagle's minimum essential medium supplemented with [35S]methionine (10 μCi/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 μg), isolated by the guanidinium/ CsCl method (Glin 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-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 mM-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 μg) in the presence of 0.1 mM-KCl, 1.3 mM-MgCl₂, 1.0 mM-ATP, 0.2 mM-GTP, 10 mM-phosphocreatine, 1.0 mM-glucose, 50 μM of an amino acid mixture lacking methionine, and 20 μCi 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-phthalaldehyde 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 ± S.E.M. (n = 3): N.D., not detectable.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Treatment</th>
<th>Activity (nmol of CO₂ formed/h per 10⁶ cells)</th>
<th>Protein (ng of ODC/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>0.51 ± 0.05</td>
<td>0.41 ± 0.23</td>
</tr>
<tr>
<td>1</td>
<td>DFMO</td>
<td>0.15 ± 0.01</td>
<td>6.32 ± 1.67</td>
</tr>
</tbody>
</table>

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 (O) or spermidine (△). The amount of ODC protein was determined after 1 day. Untreated controls contained 0.41 ± 0.23 ng of ODC/10⁶ cells at this time (Table 1). Results are means ± S.E.M. (n = 3).

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 ± S.E.M. (n = 3): *P < 0.05, **P < 0.01, ***P < 0.001 as compared with controls (day 1).

<table>
<thead>
<tr>
<th></th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (day 0)</td>
<td>0.11 ± 0.01***</td>
<td>0.80 ± 0.06***</td>
<td>0.39 ± 0.04</td>
</tr>
<tr>
<td>Control (day 1)</td>
<td>0.77 ± 0.04</td>
<td>2.16 ± 0.11</td>
<td>0.54 ± 0.06</td>
</tr>
<tr>
<td>DFMO</td>
<td>0.02 ± 0.00***</td>
<td>0.80 ± 0.06***</td>
<td>1.01 ± 0.04**</td>
</tr>
<tr>
<td>DFMO + 1 μM-putrescine</td>
<td>0.07 ± 0.01***</td>
<td>1.47 ± 0.13*</td>
<td>0.97 ± 0.07*</td>
</tr>
<tr>
<td>DFMO + 10 μM-putrescine</td>
<td>0.78 ± 0.08</td>
<td>2.56 ± 0.22</td>
<td>0.77 ± 0.04*</td>
</tr>
<tr>
<td>DFMO + 100 μM-putrescine</td>
<td>1.37 ± 0.12**</td>
<td>2.52 ± 0.17</td>
<td>0.71 ± 0.06</td>
</tr>
<tr>
<td>DFMO + 1 μM-spermidine</td>
<td>0.21 ± 0.07**</td>
<td>1.67 ± 0.03*</td>
<td>0.89 ± 0.08*</td>
</tr>
<tr>
<td>DFMO + 10 μM-spermidine</td>
<td>0.10 ± 0.01***</td>
<td>2.54 ± 0.44</td>
<td>0.43 ± 0.13</td>
</tr>
<tr>
<td>DFMO + 100 μM-spermidine</td>
<td>0.09 ± 0.02***</td>
<td>2.93 ± 0.12*</td>
<td>0.63 ± 0.07</td>
</tr>
</tbody>
</table>
This stimulation effective DFMO the polyamines. concentration a putrescine. Immunoprecipitation and 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 μM. 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.

[35S]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 μM. 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).

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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 [35S]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, 10² and 10³ μM 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, 10², 10³ and 10⁴ μM respectively). Arrow indicates the migration of [3H]DFMO-labelled purified mouse kidney ODC (53 kDa).

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.
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 μg/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
ODC synthesis was obtained even by exposure to micro-
molar polyamine concentrations, although the intra-
cellular 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 supple-
mented 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 syn-
thesis 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 ob-
served in two other cell lines (Kahana & Nathans, 1985;
Hölttä & Pohjanpello, 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 ampli-
fication.

The present results with DFMO show that ODC
synthesis is suppressed by the actual cellular concen-
trations of putrescine and spermidine in exponentially
growing cells. However, this suppression is not maximal
since addition of more polyamines inhibited ODC syn-
thesis even further. Hence the cells are equipped with an
excellent machinery for regulating the cellular concen-
trations 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

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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 immuno-
precipitated [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.
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, 1983; 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.

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