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The translation *in vitro* of rat ornithine decarboxylase mRNA is blocked by its 5′ untranslated region in a polyamine-independent way

Harry VAN STEEG,* Conny T. M. VAN OOSTROM,* Hennie M. HODEMAEKERS,* Lily PETERS† and Adri A. M. THOMAS†

*National Institute of Public Health and Environmental Protection, P.O. Box 1, 3720 BA Bilthoven, The Netherlands, and †University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands

The enzyme ornithine decarboxylase (ODC, EC 4.1.1.17) is believed to play an essential role in the growth and differentiation of cells by regulating the biosynthesis of polyamines. The 5′ untranslated region (5′ UTR) of the ODC mRNA of different species is rather unusual in length and GC content, and may therefore be involved in translational control of ODC protein synthesis. We cloned the rat ODC cDNA downstream of the phage T7 promoter in order to perform transcription/translation studies *in vitro*. Our results show that the intact 5′ UTR of rat ODC mRNA, which is 303 nt in length, is a potent inhibitor of translation. Efficient synthesis *in vitro* of ODC protein is obtained when either 172 nt from the 5′-end or 236 nt from the 3′-end of the 5′ UTR are removed. A truncated 5′ UTR with a calculated free energy of less than −272 kJ (−65 kcal/mol) is unable to support the synthesis *in vitro* of ODC protein. The short open reading frame (ORF) present in the 5′ UTR of rat ODC mRNA does not contribute to the observed inhibitory effect on translation efficiency *in vitro*. At low polyamine concentration the efficiency of translation *in vitro* of intact ODC mRNA is not relatively increased compared with that of an ODC mRNA having a truncated 5′ UTR or with that of control globin mRNA. From this we conclude that the well-documented negative feedback control of intracellular polyamines on ODC expression is not regulated by effects of polyamines on the secondary structure of the 5′ UTR of ODC mRNA.

INTRODUCTION

Polyamines are essential for optimal growth of mammalian cells (Tabor & Tabor, 1984; Pegg, 1986). The polyamine concentration in the cell is accurately regulated by the enzyme ornithine decarboxylase (ODC; EC 4.1.1.17), which catalyses the first and rate-limiting step in polyamine biosynthesis. ODC enzyme activity, and thus the concentration of polyamines, is increased in rapidly growing cells as compared with slowly proliferating or quiescent cells (Pegg & McCann, 1982; Tabor & Tabor, 1984). Furthermore, transient induction of ODC enzyme activity can be observed in response to several proliferative stimuli such as growth factors, tumour promoters, cyclic AMP-elevating agents and hormones (Berger *et al.*, 1984; Hovis *et al.*, 1986; Olson & Spizz, 1986; Gilmour & O'Brien, 1989). Although a considerable number of studies have dealt with the elucidation of the mechanism of ODC enzyme induction, an overall model is still missing. Regulation of (i) transcription (Katz & Kahana, 1987), (ii) translation (Kahana & Nathans, 1985; Kameji & Pegg, 1987; Pegg *et al.*, 1988; Holm *et al.*, 1989), (iii) mRNA stability (Berger *et al.*, 1986; Hölttä *et al.*, 1988) and (iv) protein stability (Pegg *et al.*, 1988; Ghoda *et al.*, 1989; Van Daalen Wetters *et al.*, 1989*a*,*b*) has been reported.

An intriguing observation concerning the regulation of ODC enzyme activity by polyamines is the negative feedback mechanism at the translational level (Kahana & Nathans, 1985; Hölttä & Pohjanpelto, 1986). It has been suggested (Katz & Kahana, 1988; Van Steeg *et al.*, 1990) that the highly stable secondary structure postulated to be present in the 5′ untranslated region (5′ UTR) of mouse and rat ODC mRNA may play a role in this phenomenon. Since we isolated a full-length rat ODC cDNA clone (Van Kranen *et al.*, 1987), we were able to test the effect of the 5′ UTR on ODC protein synthesis *in vitro*. For this purpose intact rat ODC cDNA as well as cDNA constructs having deletions in the 5′ UTR were cloned downstream of the phage T7 promoter. Transcripts synthesized and capped *in vitro* were translated in a standard reticulocyte lysate. The effect of polyamine concentration on ODC protein synthesis *in vitro* was determined in cell-free systems with purified translational components. Our results demonstrate that the intact 5′ UTR (303 nt in length) of rat ODC mRNA is a potent inhibitor of its own translation and that the regulatory inhibition of ODC protein synthesis by polyamines is not caused by stabilizing potential secondary structures in the 5′ UTR.

MATERIALS AND METHODS

Materials

L-[^{35}S]Methionine (1300 Ci/mmol) was purchased from Amersham International. T7 RNA polymerase and exonuclease *Bal*31 were obtained from Pharmacia and Biolabs respectively. Avian-myeloblastosis-virus (AMV) reverse transcriptase and RNasin were purchased from Promega. DNA restriction endonucleases and other nucleic-acid-modifying enzymes were obtained from Boehringer.

cDNA constructs

A 34 bp double-stranded DNA fragment, encoding the phage T7 promoter (21 bp) plus the extreme 5′ 13 bp of the rat ODC cDNA sequence:

5′-AGCTTAATACGACTCACTATAGTCAGTCCCTGCA-3′

was cloned as a *Hin*dIII/*Pst*I fragment into pEMBL9 (Dente *et al.*, 1983) resulting in clone p9T7ODC1. A 5′ 208 bp *Pst*I/

Abbreviations used: AMV, avian myeloblastosis virus; ODC, ornithine decarboxylase; ORF, open reading frame; nt, nucleotide(s); UTR, untranslated region.

*Hin*dII fragment and a 2101 bp *Hin*dII/*Eco*RI fragment (coding region) of rat ODC cDNA clone pODC.E10 (Van Kranen *et al.*, 1987) were cloned (in two steps) in p9T7ODC1. The final clone (p9T7ODC303) comprises the nt sequences of the phage T7 promoter, the intact 5′ untranslated region (5′ UTR) of 303 bp, the coding region of rat ODC (1383 bp) and 633 bp of the 3′ trailer sequence.

In order to obtain 5′-end deletions in the 5′ UTR, plasmid pODC.E10 was linearized with *Bam*HI and digested with exonuclease *Bal*31 for various times at room temperature. Truncated *Bal*31/*Eco*RI fragments were cloned into p9T7ODC1 (digested with *Pst*I/Klenow and *Eco*RI). The size of the deletions in the 5′ UTR was determined by sequence analysis (Sanger *et al.*, 1980). The upstream AUG codon in clones p9T7ODC303 and p9T7ODC184 was changed to UUG by site-directed mutagenesis (Amersham site-directed mutagenesis system RPN.1523) and the mutations were confirmed by sequence analysis.

For the 3′-end deletions in the 5′ UTR a unique *Bam*HI site was introduced (by site-directed mutagenesis) 9 bp upstream of the AUG start codon of clone p9T7ODC303. This clone (designated as p9T7ODC303B; see Fig. 1 below) was linearized with *Bam*HI and digested with exonuclease *Bal*31 as described above. The truncated ORF of ODC was removed by digestion with *Eco*RI and replaced by the 2025 bp *Eco*RI/*Bam*HI/Klenow fragment of p9T7ODC303B. The size of the 3′-end deletions in the 5′ UTR was determined by sequence analysis.

Transcription *in vitro*

Plasmids were linearized with *Eco*RI and transcribed with T7 polymerase in the presence of a 5-fold molar excess of 7mGpppG over GTP as described (Pelletier *et al.*, 1988). The transcripts were purified by Sephadex G-50 spun-column chromatography (Maniatis *et al.*, 1982). The integrity of the mRNAs synthesized *in vitro* was checked by non-denaturing agarose-gel electrophoresis. Yield was estimated by comparison with known amounts of ribosomal RNA, run on the same gel. RNA-containing bands were excised from the gel and eluted overnight in 0.4 ml of 0.1% SDS. After precipitation the mRNAs were used for primer-extension studies.

Primer extension

Oligonucleotides complementary to nt 125–144 and nt 312–333 of rat ODC cDNA (see the underlined sequences in Fig. 1*a* below) were 5′ end-labelled (Maniatis *et al.*, 1982) and hybridized [in 2 μl of 200 mM-KCl/10 mM-Tris (pH 7.8)/RNasin (250 units/ml)] for 1 h at 45 °C with 15 ng of synthesized ODC mRNA *in vitro*. For transcripts synthesized with p9T7ODC303 as a template, the oligonucleotide complementary to nt 125–144 was used, whereas for all other transcripts the oligonucleotide complementary to nt 312–333 was taken for hybridization. After hybridization 2.4 μl of reverse-transcriptase reaction mixtures [25 mM Tris(pH 7.8)/16 mM-$MgCl_2$/8 mM-dithiothreitol/0.4 mM-dATP+dCTP+dTTP/0.8 mM-dGTP/RNasin (3000 units)/AMV reverse transcriptase (250 units/ml)] were added. Reactions were done at 42 °C for 2 h, whereafter the extension products were analysed by electrophoresis on a 6% denaturing polyacrylamide gel, followed by autoradiography (for 16 h). To determine the exact length of the extended products, a DNA sequence analysis was performed using the same 5′-end-labelled oligonucleotides and p9T7ODC303 DNA as a template.

Translation *in vitro*

Translation was performed (*a*) in rabbit reticulocyte lysates and (*b*) with purified components (pH 5 system).

(*a*) Translation in reticulocyte lysates. This was performed in 5 μl as described (Pelham & Jackson, 1976) with 200 μCi of [^{35}S]methionine/ml assay. Polyamines were not added. Rabbit globin mRNA was included in each assay as an internal standard.

(*b*) Translation in pH 5 systems. This was performed as described (Thomas *et al.*, 1980). In short, 10 μl systems contained 20 mM-Hepes/KOH, pH 7.6, 1 mM-ATP, 0.4 mM-GTP, 5 mM-creatine phosphate, 0.05 unit of creatine kinase, 2 mM-dithiothreitol, 120 mM-potassium acetate, 2 mM-magnesium acetate, 100 μM-spermine (or as indicated), 50 μM amino acids minus methionine, 2 μCi of [^{35}S]methionine, 0.4 μg of tRNA, 2.5 μg of 'pH 5 enzymes', 10 μg of ribosomal salt wash, 3 pmol of ribosomal subunits and 0.1 μg (or amounts as indicated) of template.

After translation for 60 min, 1 μl aliquots were taken to determine hot-trichloroacetic-acid-precipitable radioactivity. The remaining material was analysed on a 12.5%-(w/v)-polyacrylamide gel as described by Laemmli (1970). ODC protein-containing gel slices were cut from the dried gel, rehydrated and counted. Alternatively, different exposures of the fluorogram were scanned to determine ODC protein content.

Computer analysis

The free energies of the 5′ UTRs of the different ODC transcripts were calculated as described (Zuker & Stiegler, 1981).

RESULTS

Transcription of ODC mRNA *in vitro*

In order to study translation of rat ODC mRNA *in vitro* we cloned the intact ODC cDNA downstream of the bacteriophage T7 promoter (clone p9T7ODC303; see Fig 1). Furthermore, to examine the influence of the 5′ UTR on the efficiency of translation of ODC mRNA, deletions ranging from 119 bp to 272 bp were made in this part of rat ODC cDNA (see Fig. 1*b*). After transcription of the linearized plasmids *in vitro*, using T7 RNA polymerase, only single discrete RNA species were discernible on a non-denaturing 1%-(w/v)-agarose gel (result not shown). To ascertain that transcription initiation had occurred at the same site used *in vivo* (Wen *et al.*, 1989; Van Steeg *et al.*, 1990), we performed primer-extension studies with the ODC mRNAs synthesized *in vitro*. A typical result with plasmids p9T7-ODC303, -ODC131, -ODC71 and -ODC31 is depicted in Fig. 2. As determined from the length of the extended products, we conclude that all ODC mRNAs (including those synthesized with plasmids p9T7-ODC184, -ODC136 and -ODC67; results not shown) start at the G residue at nt 1 (indicated by an asterisk in Fig. 1). This implies that the ODC mRNA303 synthesized *in vitro* has an identical 5′ UTR compared with the rat ODC mRNA found *in vivo*.

Translation *in vitro*

The transcripts of wild-type ODC303 and the 5′-truncated derivatives ODC31, ODC71, ODC131 and ODC184 were translated in the two types of assay systems. The fluorogram of the reticulocyte system is shown (Fig. 3*a*). A protein with an M_r corresponding to ODC (about 55 kDa) is produced with all transcripts, although in strongly variable amounts. Wild-type ODC mRNA (ODC303) was poorly translated, giving rise to only marginal amounts of full-length ODC protein. Deletion of increasingly more nts from the 5′ UTR resulted in a marked increase in the amount of the 55 kDa ODC protein, highest synthesis being obtained with the 131-nt and 71-nt 5′ UTR transcripts of clones p9T7ODC131 and p9T7ODC71 (see Figs. 3*a* and 3*b*). A further 40 nt deletion of the 5′ UTR decreased ODC synthesis about 50%. The reason for this observation

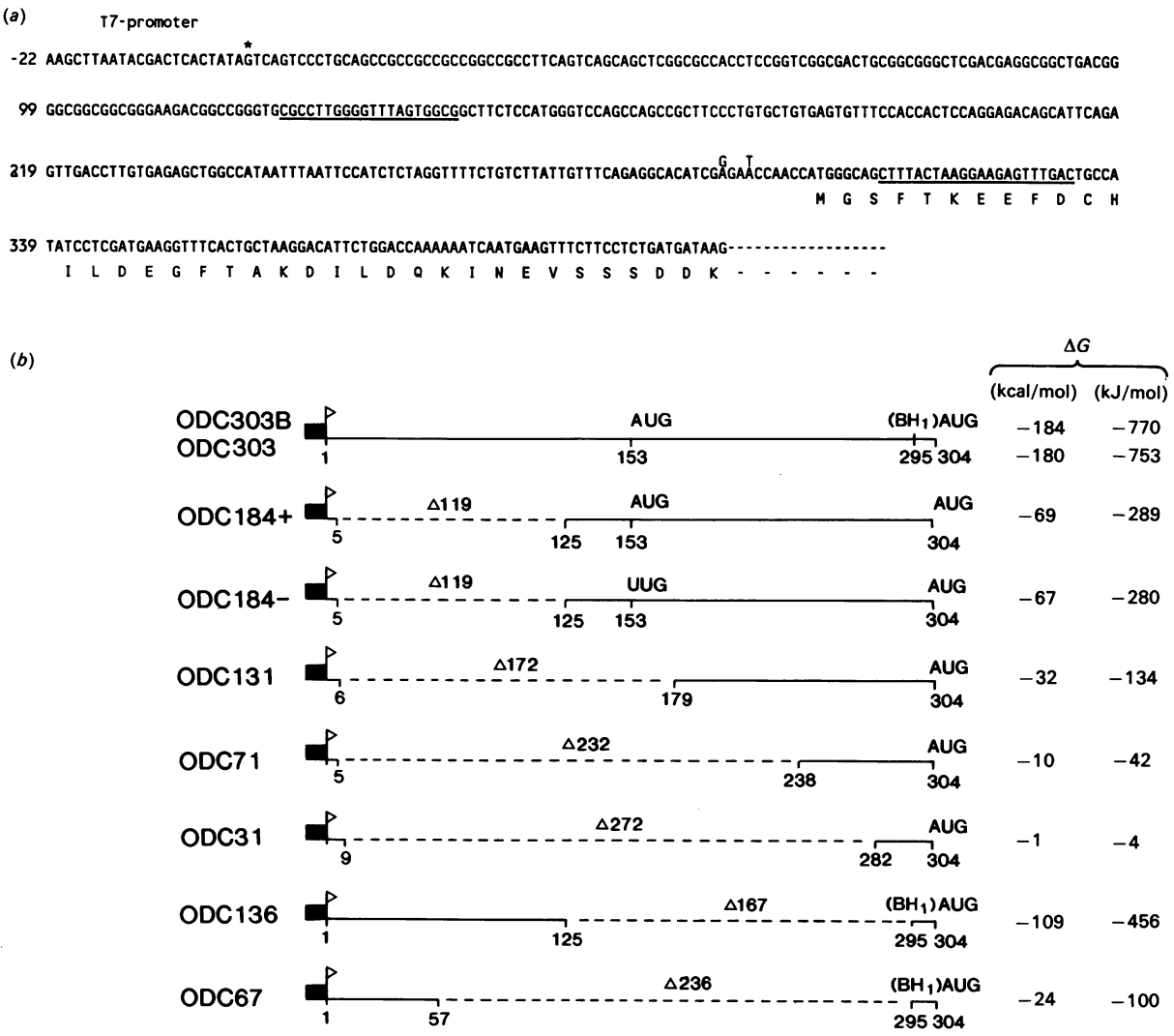

Fig. 1. Nucleotide sequence of the 5′ UTR region of cDNA clone p9T7ODC303 and related clones

(*a*) Nucleotides are numbered starting from the G residue at the '*in vivo*' and '*in vitro*' transcription start point (indicated by an asterisk). Sequences complementary to the synthetic oligonucleotides used for primer-extension analysis (see also Fig. 2) are underlined. By means of site-directed mutagenesis, a *Bam*HI site was introduced in clone p9T7ODC303 (i.e. $A^{294} \rightarrow G^{294}$ and $A^{297} \rightarrow T^{297}$), and the clone was designated p9T7ODC303B. Starting from A^{304}, part of the ODC ORF is depicted. (*b*) By means of *Bal*31 digestion, parts of the 5′ UTR (as indicated) of clones p9T7ODC303 and p9T7ODC303B were removed. The size of the deletions was determined by sequence analysis and is indicted by Δ. In clone p9T7ODC184− the upstream AUG (nt 153) has been replaced by a UUG sequence. The filled box (■) represents the phage T7 promoter and the pennant (▷) the transcription start point. The calculated free energy (Δ*G*; Zuker & Stiegler, 1981) of each individual 5′ UTR is depicted at the right.

remains unclear, but it was also observed in the cell-free pH5 system (results not shown). Finally, non-capped transcripts appeared to be inactive in both assay systems (results not shown).

As depicted in Fig. 1, the 5′ UTR of rat ODC mRNA contains an upstream AUG codon (at nt 153), which is followed nine triplets further downstream by an in-frame stop codon. Upstream AUGs have, in general, an inhibitory effect on the translation of a downstream ORF (Kozak, 1989*a*). However, from Fig. 4(*a*) lanes 3 and 4, it is clear that replacement of this AUG sequence by UUG in the 5′ UTR of wild-type ODC303 mRNA did not result in a stimulatory effect on the synthesis of the 55 kDa ODC protein. Since ODC303 mRNA is a poor template, the same 5′ UTR $A^{153} \rightarrow U^{153}$ mutation was made in the leader of ODC184 mRNA. Again, removal of the upstream AUG codon had no effect on the efficiency of ODC protein synthesis (Fig. 4*b*, lanes 1 and 2).

The results with the mRNAs with deletions in the 3′-end of the 5′ UTR support the conclusion that the upstream AUG does not have a major effect on translation of ODC-mRNA (Fig. 5). Although the 5′ UTRs of ODC67 and ODC136 both lack an upstream AUG, their ability to support translation is poor. ODC67 transcripts gave rise to only 15% of the ODC protein obtained with ODC71 transcripts and are 14-fold more active than the transcripts derived from clone p9T7ODC136.

As has been shown (Glass *et al.*, 1987) the difference in translational activity of ODC transcripts can be caused by differences in the amount of ODC template added to an '*in vitro*' system of protein synthesis. However, even at a concentration of 60 μg/ml, ODC303 mRNA is still a poor template compared with ODC31 mRNA and globin mRNA (results not shown).

The observed inefficient translation of ODC mRNA and some of its truncated derivatives can be explained by the presence of highly stable stem–loop structures in the 5′ UTR (Wen *et al.*,

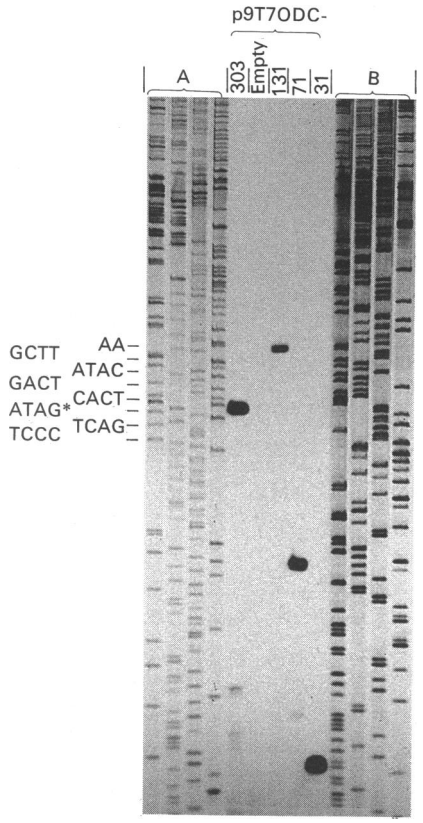

Fig. 2. Determination of the transcription start point of synthesized ODC mRNAs *in vitro* by primer extension analysis

RNA transcripts derived from clones p9T7ODC-303, -131, -71 and -31 were subjected to primer-extension analysis (see the Materials and methods section). An oligonucleotide complementary to nts 125–144 (see Fig. 1*a*) was used to determine the 5′ end of the ODC303 transcript. Lanes A show the DNA sequence of p9T7ODC303 using the same labelled oligonucleotide. The sequence around the transcription start point of the ODC303 transcript (indicated by an asterisk) is depicted at the left. The '*in vitro*' transcription start point of the remaining transcripts was mapped using an oligonucleotide complementary to nts 312–333 (see Fig. 1*a*). The DNA sequence of p9T7ODC303 using this latter oligonucleotide is depicted in lanes B.

1989; Van Steeg *et al.*, 1990). Such structures are known to block the translational apparatus (Kozak, 1989*b*; Pelletier & Sonenberg, 1985). The free energy [ΔG in kJ (kcal)/mol] of the different leaders was calculated (Fig. 1*b*). 5′ UTRs with a calculated ΔG of less than -272 kJ (-65 kcal)/mol are almost unable to support translation of the downstream ODC ORF. ODC transcripts with 5′ UTRs having less secondary structure allow ribosome recognition of their initiation codon, which is in agreement with literature data (Kozak, 1989*b*; Pelletier & Sonenberg, 1985). The 5′ UTR of ODC 31 mRNA, although $\pm$100-fold more efficient than the 5′ UTR of wild-type ODC303 mRNA (Fig. 3), is apparently too short for maximal support of ODC protein synthesis (see also Kozak, 1983).

Examination of the predicted secondary structure (not shown) revealed that transcripts having one of the two stable stem–loop structures present in the wild-type 5′ UTR (see Van Steeg *et al.*, 1990) were inactive in translation. Deletions entering the 'hairpins' destroyed the secondary structure and thereby facilitated initiation.

Polyamines do not have differential effect on the translatability of ODC mRNAs having a different 5′ UTR

The results obtained so far indicate that the structures present in the 5′ UTR of the rat ODC mRNA have an inhibitory effect on ODC protein synthesis. Either the translational apparatus fails to initiate on this type of 5′ UTR or components present in '*in vitro*' systems for protein synthesis interact with these structures, resulting in a block of initiation. Polyamines are likely candidates for such interactions, since they negatively affect ODC mRNA translation (Kahana & Nathans, 1985; Kameji & Pegg, 1987; Pegg *et al.*, 1988; Persson *et al.*, 1988; Holm *et al.*, 1989). The plausible assumption is that polyamines exert their effect on ODC mRNA translation by stabilizing its 5′ UTR and that, under conditions of polyamine depletion, full-length ODC mRNA is relatively better translated than non-polyamine-regulated mRNAs. In order to test this hypothesis, we compared, in a pH 5 system, the relative translatability of wild-type rat ODC mRNA (ODC303) with that of an ODC mRNA having less secondary structure in its 5′ UTR (ODC71) and with control globin mRNA (not shown), as a function of the spermine and spermidine concentration. Cell-free pH 5 system are particularly useful in such studies, since translation completely depends on added polyamines. The results in Fig. 6 show, however, that optimal translation of ODC303 and ODC71 mRNA was obtained at identical spermine and spermidine concentrations (i.e. 90 μM and 400 μM respectively). The same polyamine-dependence was found for the translation of globin mRNA in these pH 5 systems (results not shown). These results indicate that polyamine depletion cannot convert an inactive ODC mRNA into a relatively more active one.

DISCUSSION

We have shown that intact rat ODC mRNA synthesized *in vitro* is a very poor template for synthesis *in vitro* of ODC protein (Figs. 3–5). This result is in line with translation studies using hybrid-selected mRNA and ODC-specific antibodies (Kahana & Nathans, 1984). Efficient translation of ODC mRNA ($\pm$200-fold increase) is achieved when 172 nt of the 5′ end of the 5′ UTR are removed, leaving the remaining part of the messenger intact (Figs. 1–3).

It has been demonstrated that the level of secondary structure in the 5′ UTR is an important element in the translational ability of an mRNA (Kozak, 1989*b*; Pelletier & Sonenberg, 1985). On the basis of the calculated free energy (ΔG) of the 5′ UTR of the different ODC templates tested (Fig. 1), we conclude that the efficiency of ODC synthesis is related to the predicted secondary structure present in the leader. Leaders with a calculated ΔG of less than -272 kJ (-65 kcal)/mol are almost unable to support translation.

The template activity of the transcripts derived from clones p9T7ODC303 and p9T7ODC184, with and without an upstream AUG, was very similar. Therefore, it is not likely that the upstream AUG at nt 153 is involved in the inhibitory action of the leader in protein synthesis *in vitro*. A similar finding was recently reported for the 5′ UTR of the BCR/ABL transcript (Muller & Witte, 1989); this leader is also rich in G-C residues, contains a short upstream ORF and is strongly inhibitory in translation *in vitro*. Also, in this case, the short ORF does not contribute significantly to the inhibition observed in translation *in vitro*.

It was postulated (Katz & Kahana, 1988; Van Steeg *et al.*, 1990) that the negative feedback control of polyamines on ODC translation is brought about by interactions of polyamines with the unusually stable secondary structures present in the 5′ UTR of ODC mRNA. We therefore determined the optimal polyamine

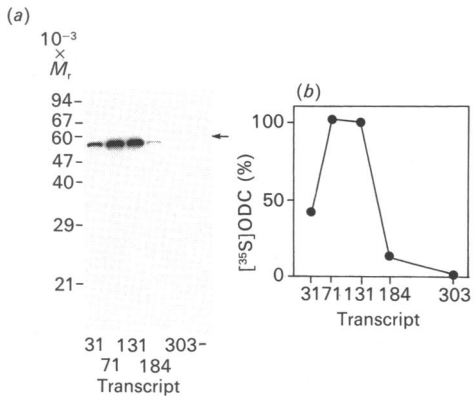

Fig. 3. Translation in reticulocyte lysates of ODC mRNAs with 5′-end deletions in the 5′ UTR

Reticulocyte lysates were incubated for 1 h at 30 °C with 50 ng of '*in vitro*'-synthesized transcripts as described in the Materials and methods section. The numbers on the horizontal scale refer to the added transcripts (p9T7ODC31 etc.) derived from the clones depicted in Fig. 1(*b*). (*a*) '*In vitro*'-synthesized ODC protein was analysed on an SDS/12.5%-(w/v)-polyacrylamide gel (Laemmli, 1970). The Figure shows a 16 h fluorogram of the dried gel. The arrow indicates the position to which the intact ODC protein migrates. Positions of marker proteins in the gel are indicated at the left. (*b*) ODC-protein-containing bands were cut from the gel and the radioactivity counted. Incorporation of [^{35}S]methionine into ODC protein using ODC71 transcript as a mRNA template was arbitrarily adjusted to 100% (i.e. 44000 c.p.m.). [^{35}S]Methionine incorporation into globin protein (200 ng of mRNA added) was 18105 c.p.m./μl of translation mixture.

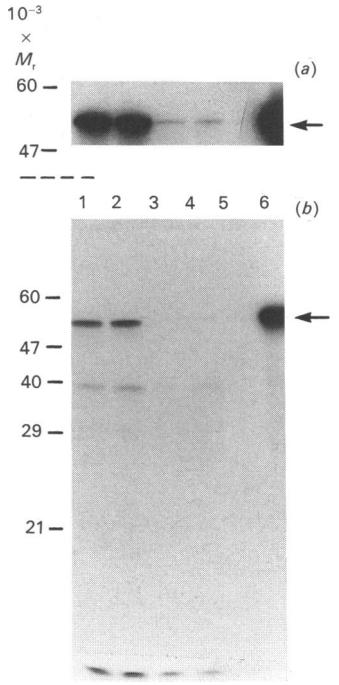

Fig. 4. The upstream AUG in the 5′ UTR does not contribute to the observed inhibition of *in vitro* translation

The reticulocyte assays were performed as described in the legend to Fig. 3. Transcripts (100 ng) added are: lane 1, ODC184− (UUG-mutant); lane 2, ODC184+; lane 3, ODC303− (UUG-mutant); lane 4, ODC303+ (wild-type); lane 5, no mRNA added; lane 6, ODC71; 4 μl of each translation mixture was analysed on the gel, except for ODC71, for which 0.5 μl was applied. (*a*) shows a 4-day overexposure of the ODC protein region of the same gel. The incorporation into globin protein was 10363 c.p.m. (see the legend to Fig. 3).

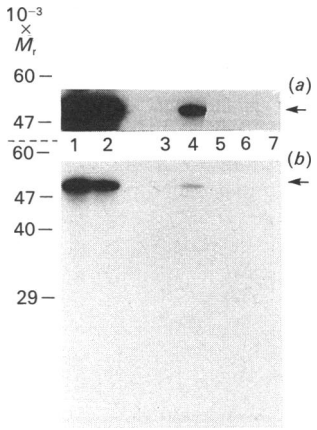

Fig. 5. Comparison of the translational activity of ODC transcripts having a 5′- or 3′-end deletion in the 5′ UTR

The reticulocyte assays were performed as described in the legend to Fig. 3. Transcripts (50 ng) added were: lane 1: ODC71; lane 2: ODC131; lane 3: ODC303; lane 4: ODC67; lane 5: ODC136; lane 6: ODC303B; lane 7: no mRNA added. (*a*) is a longer exposure of (*b*) to show that all transcripts are able to support ODC synthesis. See, for further details, the legend to Fig. 3. The incorporation into globin protein was 19084 c.p.m. (see the legend to Fig. 3).

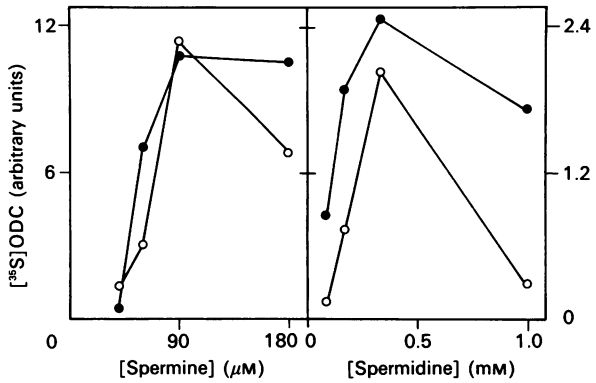

Fig. 6. Polyamine titration in a cell-free pH 5 translation system

Assays were performed as described in the Materials and methods section. After fluorography the relative amount of ODC protein present in each lane was determined by scanning the fluorogram. The left scale refers to the results obtained with the translation of ODC71 (●) transcripts, whereas the right scale refers to those obtained with the ODC303 (○) transcripts.

concentration for ODC synthesis *in vitro* using templates having a high (ODC303) or low (ODC71) degree of secondary structure in the 5′ UTR (Fig. 6). Optimal translation with both templates is obtained at the same polyamine concentration, which appeared to be identical with that for control globin mRNA translation. More important, low polyamine concentrations were not favourable for translation of ODC303 mRNA. We conclude, therefore, that the negative feedback control of polyamines on ODC expression is not exerted at the level of the initiation of translation. We cannot exclude, however, that '*in vitro*' pH 5 translation mixtures are devoid of an (until now) unknown mediator of polyamine regulation of ODC protein synthesis. Our results are, however, in line with recent observations by Van Daalen Wetters *et al.* (1989*a*) that polyamine feedback, at least for mouse ODC protein synthesis, is exhibited by the protein-encoding part of ODC mRNA only. These, as well as our present

results, point towards the assumption that the polyamine-mediated regulation of ODC expression is post-translational.

The remaining question is: 'how do ribosomes *in vivo* circumvent the unwinding of an extremely stable 5′ UTR in order to reach the nearly perfect consensus sequence CAACCAUGG at the initiation site (Kozak, 1989*a*)?' It was suggested that the NIH 3T3 cells used in the study by Muller & Witte (1989) possess the ability to relieve the translational block of the inhibitory 5′ UTR of the BCR/ABL-oncogene transcript. The same suggestion can be made for the expression of ODC protein. Indeed, in many cell systems studied (Gilmour *et al.*, 1985; Katz & Kahana, 1987; Van Daalen Wetters *et al.*, 1989*b*) the induction of ODC activity after treatment with a variety of mitogenic stimuli is accompanied by only a moderate induction of ODC mRNA. This suggests that modulation of ODC activity is to a great extent regulated at the post-transcriptional level. Furthermore, recent results (Van Daalen Wetters *et al.*, 1989*b*) indicate that regulation of ODC activity in NIH 3T3 cells in response to serum factors and phorbol 12-myristate 13-acetate ('TPA') is at the level of modulation of ODC enzyme degradation/stabilization. Another important regulatory device might be the relief of the translational block set by the 5′ UTR of ODC mRNA.

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