The Upstream Open Reading Frame of the mRNA Encoding S-Adenosylmethionine Decarboxylase Is a Polyamine-responsive Translational Control Element*

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S-Adenosylmethionine decarboxylase (AdoMetDC) is a key enzyme in the pathway of polyamine biosynthesis. The cellular levels of the polyamines specifically regulate AdoMetDC translation through the 5′-leader of the mRNA, which contains a small upstream open reading frame (uORF) 14 nucleotides from the cap. Mutating the initiation codon of the uORF, which encodes a peptide product with the sequence MAGDIS, abolished regulation. In addition, the uORF is sufficient by itself, to provide polyamine regulation when inserted into the 5′-leader of the human growth hormone mRNA. Changing the amino acid sequence at the carboxyl terminus of the peptide product of the uORF abolished polyamine regulation. In contrast, altering the nucleotide sequence of the uORF at degenerate positions, without changing the amino acid sequence of the peptide, did not affect regulation. Extending the distance between cap and uORF, thereby changing the rate of initiation at the initiator AUG of the uORF, did not alter polyamine regulation. When the uORF was extended so as to overlap, out of frame, the downstream major cistron, polyamine regulation was abolished. We propose that polyamines do not modulate the rate of recognition of the uORF but rather regulate interaction of the peptide product of the uORF with its target.

Polyamines (putrescine, spermidine, and spermine) are a group of low molecular weight compounds necessary for the optimal growth of all cells, eukaryotic and prokaryotic (for reviews, see Marton and Morris (1987) and Jänne et al. (1991)). Depletion of cellular polyamine levels has been shown to lead to decreased cell growth and alterations in cell differentiation (Pegg and McCann, 1982; Pegg, 1988; Marton and Pegg, 1995). On the other hand, excessive levels of polyamines may have toxic effects (reviewed in Heby and Persson (1990); Morris (1991)). Derepression of polyamine synthesis has been shown to cause neoplastic transformation of cultured cells and a propensity toward tumor development in transgenic animals (Auvinen et al., 1992; Moshier et al., 1993; Shantz and Pegg, 1994; Megosh et al., 1995). Probably because of the ramifications of derepression, polyamine levels are tightly regulated by a variety of mechanisms, including feedback regulation of expression, activity, and stability of key enzymes as well as polyamine degradation and excretion from cells (reviewed in Heby and Persson (1990); Morris (1991); Large (1992); Grillo and Colombatto (1994)).

There are two key regulated enzymes in the pathway of polyamine biosynthesis, ornithine decarboxylase and S-adenosylmethionine decarboxylase (AdoMetDC).¹ The levels of these enzymes are regulated not only by exogenous signals (such as growth factors, hormones, and tumor promoters), but also by the endogenous level of the polyamines. AdoMetDC catalyzes the formation of an intermediate necessary for the conversion of putrescine to spermidine and spermine and is located at an important branch point in the metabolism of S-adenosylmethionine. The intracellular levels of polyamines influence AdoMetDC expression at multiple steps, including mRNA level, translation, and protein half-life (Shirahata and Pegg, 1985, 1986; Pajunen et al., 1988; White et al., 1990), forming feedback loops to maintain the normal concentration of polyamines in cells.

The translation of AdoMetDC mRNA in reticulocyte lysates has been reported to be more strongly inhibited by increasing concentrations of polyamines than general protein synthesis (Kameji and Pegg, 1987). In intact cells, several studies have focused on translational control of AdoMetDC by polyamines, using polyamine-depleting drugs, such as α-difluoromethylornithine (DFMO), a potent irreversible inhibitor of the first enzyme in polyamine biosynthesis, ornithine decarboxylase (for review, see Marton and Pegg (1995)). Intra cellular polyamine levels influence the translation of AdoMetDC mRNA in vivo, and the major effect seems to be at the initiation step (White et al., 1990). More recently, it has been demonstrated that the 5′-leader of AdoMetDC mRNA mediates this polyamine regulation, pointing to the presence of a polyamine-responsive element in this region of the mRNA (Shantz et al., 1994).

The AdoMetDC 5′-leader is unusually long (330 nucleotides) with a small upstream open reading frame (uORF) located 14 nucleotides from the cap (Hill and Morris, 1992; Pulkka et al., 1993; Waris et al., 1993). This uORF plays a dominant role in repressing AdoMetDC translation in T cells (Hill and Morris, 1992) through a mechanism that is critically dependent on the unique peptide sequence (MAGDIS) coded by the uORF. Missense mutations in the 3′-terminal three codons of the uORF relieve translational suppression, while synonymous changes, having no effect on the coding status, retain suppressive activ-
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ity (Hill and Morris, 1993). The uORF is largely ignored in nonlymphoid cells because of its proximity to the 5'-end of the mRNA, resulting in efficient translation of the downstream cistron. In T cells, the uORF is efficiently recognized and translated, resulting in strong suppression of downstream translation. This cell-specific translational control involves regulation of recognition and initiation at the uORF but not modulation of the interaction of the peptide product of the uORF with its target and is independent of the position of the uORF relative to the mRNA 5'-cap. This mode of regulation is notably different from that utilized in cell-specific translational control.

EXPERIMENTAL PROCEDURES

Chimeric Constructs—Chimeras between AdoMetDC and human growth hormone (hGH) were described in previous publications (Hill and Morris, 1992, 1993; Ruan et al., 1994). Briefly, the various modified 5'-leaders of AdoMetDC were synthesized by polymerase chain reactions (Saiki et al., 1985) using pRS227 (Hill and Morris, 1992) as template. We inserted the modified 5'-leaders (with BamHI sites added on each end) into the BamHI site of pRbGHΔ5TL (Hill and Morris, 1992) by standard methods (Sambrook et al., 1990). The sequence of each construct was verified from the middle of the promoter through the hGH translational initiation site using the dideoxy chain termination method (Sanger et al., 1977) with a sequencing kit from U.S. Biochemical Corp.

Cell Culture—Chinese hamster ovary (CHO) cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 200 μM t-proline, 100 units/ml penicillin and 50 μg/ml streptomycin. In experiments in which polyamines were depleted, a final concentration of 5 mM DFMO was added to the media.

Polyamine Levels—CHO cells were seeded at 50,000 cells/plate in 35-mm plates in the presence or absence of 5 mM DFMO. For some samples, 10 μM spermidine and 1 mM aminoguanidine (to inhibit the action of oxidase present in serum) were added 24 h after. When 72 h in culture, the cells were washed with ice-cold 1 × Tris-buffered saline (25 mM Tris-HCl, pH 7.4, 137 mM NaCl, 0.7 mM CaCl₂, 0.5 mM MgCl₂, and 0.6 mM Na₂HPO₄) twice, scraped into 1.5 ml tubes, and harvested by centrifugation. Cell pellets were extracted with 10% (w/v) trichloroacetic acid. Polyamine content was determined as described previously (Pegg et al., 1989).

[35S]Methionine Pulse Labeling of CHO Cells—Cells were cultured as described for polyamine determination. Triplicate cultures at each time point were washed twice with culture medium lacking methionine, and medium containing [35S]methionine was added (600 μl of medium containing 10% fetal bovine serum with 2 μCi of [35S]Met per 35-mm plate). 5 mM DFMO was added when indicated. After incubation at 37°C for 90 min, cells were washed twice with ice-cold 1 × TBST, precipitated with 10% trichloroacetic acid, and scraped into 1.5 ml tubes. After harvesting by centrifugation, the cell pellet was washed with 10% trichloroacetic acid twice, once with an ethanol/ether mixture (1:1), and once with ether. The dry pellet was dissolved in 3 ml KOH, and the protein concentration of the cell pellet was measured using the standard Bradford method (Bio-Rad). The radioactivity in each sample was counted using a liquid scintillation counter.

DNA Transfection—CHO cells were transfected using lipofectamine (Life Technologies, Inc.) according to the manufacturer’s instructions. Approximately 120,000 cells were seeded per 35-mm plate and allowed to grow for 48 h. For cells depleted of polyamines, a final concentration of 5 mM DFMO was added at plating and was present throughout the transfection. Cells were transfected with 5 μg of the indicated construct in 1 ml serum-free medium containing 1 μl of lipofectamine. After incubation for 5 h at 37°C, the solution was replaced with 2 ml of fresh culture medium, and the cultures were incubated for 48 h before harvesting for analysis. In experiments where polyamines were restored to depleted cells, a final concentration of 10 μM spermidine (with 1 mM aminoguanidine) was added at the time of transfection.

Polyosome Analysis—For analysis of polyosomes, we isolated and pu

| Treatment | Spermidine | Spermine |
|-----------|------------|----------|
| None      | 9.0 ± 0.6  | 11.2 ± 0.5 |
| 5 mM DFMO | <0.5       | 5.2 ± 0.6  |
| 5 mM DFMO + 10 μM spermidine | 16.3 ± 1.0 | 11.0 ± 1.5 |

TABLE I

Effect of DFMO on polyamines in CHO cells

Cells were grown for 24 h as described under “Experimental Procedures.” The medium was then changed, and the cells were allowed to grow for 48 h, when they were harvested and assayed for polyamine content. DFMO was added at the time the cells were plated, and spermidine was added when medium was changed.

RESULTS

Influence of DFMO Treatment on Cellular Polyamines and Protein Synthesis—DFMO, which inhibits ornithine decarboxylase activity (Pegg, 1986), has been shown previously to deplete polyamine levels and decrease the cellular rate of protein synthesis (Pegg and McCann, 1982; Pegg, 1986, 1988). Treatment of CHO cells with DFMO for 72 h results in depletion of polyamines, especially spermidine (Table I). The cellular levels of spermidine and spermine could be maintained in cells

= 11.2 ± 0.5 |

nmol/mg protein

= 11.0 ± 1.5 |
treated with DFMO by adding 10 mM spermidine to the culture medium (Table I).

At various times after initiating treatment of CHO cells with DFMO, the rate of protein synthesis was assessed by labeling with [35S]methionine (Fig. 1A). There was significant inhibition of methionine incorporation at 24 and 48 h after the start of DFMO treatment, and this inhibition reached approximately 10-fold by 72 h. This inhibition of protein synthesis by DFMO treatment is also reflected in reduced accumulation of cellular protein in the cultures (Fig. 1B).

Influence of Polyamine Level on AdoMetDC Translation—Cytoplasmic extracts were prepared from DFMO-treated and control CHO cells to analyze the distribution of AdoMetDC mRNA in polysomes. The extracts were fractionated by centrifugation in sucrose gradients, and AdoMetDC mRNA was analyzed on Northern blots (Fig. 2). In untreated cells, AdoMetDC mRNA is broadly distributed from monosomes to polysomes of small size. When cells were treated with DFMO, AdoMetDC mRNA distribution is more narrow and centered in the region of five ribosomes per molecule. The increase in size of polysomes containing AdoMetDC mRNA in polyamine-depleted cells suggests that polyamines regulate the number of ribosomes associated with AdoMetDC mRNA and hence regulate the relative efficiency of translation of this mRNA. Consistent with previous results (White et al., 1990), there is no influence of polyamine depletion on the distribution of actin mRNA in polysomes (Fig. 2) or on the absorbance profile of the sucrose gradients at 254 nm (not shown).

Previous results (Shantz et al., 1994) demonstrated that translational control of AdoMetDC was mediated through the 5′-leader of its mRNA. As shown in Fig. 2, distribution of chimeric mRNA containing the 5′-leader of AdoMetDC mRNA (from construct pRS327) mimics that of endogenous AdoMetDC mRNA in both untreated and DFMO-treated CHO cells. This chimeric mRNA moved from small and medium polysomes onto large polysomes after 72 h of DFMO treatment. By comparison, a control mRNA containing hGH 5′-leader (from construct pRNN) remained essentially at the same position in polysomes after cells were treated with DFMO, similar to the behavior of endogenous actin mRNA. Hence, the AdoMetDC 5′-leader governs the regulation by polyamines of ribosome loading onto the AdoMetDC mRNA.

Cells were transfected with constructs pRS327 and pRNN under different conditions of polyamine depletion (see Table II). We measured the concentrations of hGH in the culture media 48 h after transfection (Hill and Morris, 1992). The hGH concentrations were then normalized to the levels of mRNAs from the respective constructs to obtain a measure of relative translation. As shown in Table II, depletion of polyamines causes 86% decrease (ratio of DFMO to none is 0.14) in the translation of the mRNA containing the hGH 5′-leader. This was expected, based on the reduction in overall cellular protein synthesis demonstrated above. However, when hGH mRNA is instead downstream of the AdoMetDC 5′-leader, expression was reduced by only 19% (ratio is 0.81). Therefore, the AdoMetDC/hGH chimera is roughly 6-fold more resistant to translational inhibition caused by polyamine depletion than is general protein synthesis represented by construct pRNN. From experiment to experiment the ratio (DFMO/non) was found to vary from 0.5 to 0.8, which perhaps reflects a variation in the degree
of polyamine depletion. However, in all instances, the AdoMetDC 5'-leader was 4–6-fold more resistant to DFMO treatment than the hGH 5'-leader. The addition of spermidine to the cultures prevented the DFMO-induced decrease of translation efficiency for both constructs (Table II), demonstrating that the effect on translation is specifically caused by polyamine depletion.

**Sequence Elements in the AdoMetDC 5'-Leader Involved in Polyamine Regulation**—Constructs containing mutations in several potentially important areas of AdoMetDC 5'-leader were tested. For experimental purposes, we divided the 5'-leader into three regions; region A is the 14 nucleotides between 5'-cap and the uORF, region B is the uORF itself, and region C includes the long intercistronic region between the uORF and the downstream cistron (hGH in these constructs). In construct pRS362 (Fig. 3), region A was replaced with 47 nucleotides from hGH 5'-leader. When this construct was transfected into CHO cells, polyamine depletion only slightly reduces its expression (12% reduction), while expression of control construct pRNN (hGH leader) showed a 90% reduction, showing that altering region A does not abolish polyamine regulation. Next, we tested constructs containing large deletions in region C. Internal deletions in constructs pRS103 and pRS66 collectively covered the entire region C. As shown in Fig. 3, expression of constructs pRS103 and pRS66 after polyamine depletion were, respectively, 64 and 43% of those under normal culture conditions; in this experiment, those values were comparable with that with wild type AdoMetDC 5'-leader. Therefore, we conclude that sequences in region C are not involved in polyamine regulation.

To test the uORF region, the initiator AUG for the uORF was mutated into GUG in construct pRSG316327, thereby eliminating the uORF. When this construct was transfected into CHO cells, its expression dropped by 86% upon polyamine depletion, similar to the result with pRNN. To further test the involvement of the uORF in polyamine regulation, we transferred the uORF into the hGH 5'-leader, 14 nucleotides from the cap, generating construct pRS(uORF)hGH. This construct was much more resistant to polyamine depletion, which reduced expression by only 13%, as compared with 89% for the parent pRNN construct. These results suggest that the uORF is the single important element in the AdoMetDC 5'-leader that mediates polyamine regulation and that the uORF needs to be translated in order to function as the mediator of polyamine regulation.

**Influence of the uORF on Translational Regulation by Polyamines**—The coding capacity of the three 3'-terminal codons of the AdoMetDC uORF is critical for suppressing translation (Hill and Morris, 1989). To test the role of the coding sequence of uORF in mediating polyamine regulation, we altered these three codons (Table III, top) and tested the constructs in CHO cells. Changing the fourth codon, which encodes aspartic acid, to arginine abolished polyamine regulation (see construct pRS/D/R327 in Table III (middle)). Similarly, alteration of the fifth codon from isoleucine to alanine or the sixth codon from serine to alanine interfered with polyamine regulation (constructs pRS/I/A327 and pRS/S/A327 in Table III (middle)).

To test whether the nucleotide sequence or the coding capacity of the last three codons is required to be conserved for polyamine regulation, we changed six different nucleotides within the uORF while retaining the amino acid sequence of the putative peptide (construct pRS(WuORF)327 in Table III (top)). The translation efficiency of this construct is at least as high as the wild type construct after polyamine depletion (Table III, bottom). These results suggest that the regulation mediated by polyamines requires preservation of the amino acid sequence of the putative product but not the nucleotide sequence of the uORF itself.

We have previously shown there is no influence on translation by extending the uORF of the AdoMetDC leader so that it overlaps, out of frame, the downstream cistron, regardless of the cell type tested (Ruan et al., 1994). This construct (pRSSTTG, Fig. 4) was tested for polyamine regulation and compared with the wild type pRS327 as well as pRS66. As Fig. 4 shows, extending uORF into the downstream cistron had little or no effect on expression in the absence of DFMO but abolished polyamine regulation. This result clearly distinguishes polyamine regulation from the cell-specific regulation described previously (Ruan et al., 1994). The result is also consistent with the conclusion that the sequence of the peptide product from uORF needs to be preserved to achieve efficient polyamine regulation.

**DISCUSSION**

Polyamines are required for normal cell growth and protein synthesis (Lökwist et al., 1987; Bitonti et al., 1988; Mihm et al., 1989). In the present study, treatment of CHO cells with the inhibitor of ornithine decarboxylase, DFMO, resulted in polyamine depletion and caused a dramatic reduction in the rate of protein synthesis. Against this background of general inhibition of protein synthesis, the translation of AdoMetDC mRNA is sustained (Persson et al., 1989; Kameji and Pegg, 1987; White et al., 1990; Strømberg et al., 1993). Here, we define in detail those aspects of mRNA structure that are required for translational control of AdoMetDC by polyamines.

The maintenance of the rate of AdoMetDC translation during polyamine deprivation comes about through a specific elevation of the rate of translational initiation on this mRNA relative to the rate of polypeptide chain elongation (White et al., 1990). This regulation is mediated, at least in part, through the 5'-leader of the mRNA (Shantz et al., 1994). These conclusions are supported in the present study through the use of chimeric constructs containing hGH as a reporter gene. Translation from the intact wild type hGH mRNA was inhibited by

### Table II

**Effect of DFMO treatment on translation efficiency**

| Chimeric construct | Treatment | hGH protein | hGH mRNA | Relative translation | Ratio (DFMO/none) |
|--------------------|-----------|-------------|-----------|----------------------|-------------------|
| pRS327             | None      | 25.3 ± 4.1  | 2.5 ± 0.6 | 32.4 ± 6.6           | 0.81              |
| pRS327             | DFMO      | 18.6 ± 4.6  | 2.4 ± 0.5 | 26.3 ± 2.8           |                   |
| pRS327             | DFMO + spermidine | 42.5 ± 0.2 | 4.2 ± 0.6 | 29.6 ± 5.0           |                   |
| pRS327             | None      | 110 ± 12.8  | 3.5 ± 0.08| 100 ± 6.6            | 1.00              |
| pRS327             | DFMO      | 13.3 ± 3.4  | 2.9 ± 0.30| 14.0 ± 3.2           |                   |
| pRS327             | DFMO + spermidine | 118 ± 7.6  | 3.2 ± 0.5 | 115 ± 16.7           |                   |

* a mRNA level corrected for recovery.

b Relative translation is defined as the rate of accumulation of hGH protein divided by the level of the chimeric hGH mRNA. The value for construct pRNN in nontreated CHO cells was arbitrarily designated as 100.
DFMO treatment to an extent similar to total protein synthesis, while the chimera containing the AdoMetDC 5'-leader was more resistant to polyamine depletion. In agreement with the increase in relative translatability of the AdoMetDC/hGH chimera, its mRNA moved into larger polysomes upon polyamine depletion, paralleling the behavior of endogenous AdoMetDC mRNA (White et al., 1990). In contrast, the efficiencies of ribosome loading onto the mRNAs from the wild type hGH construct and the endogenous actin mRNA were unchanged.

The results from the current studies clearly show that the structural features necessary for polyamine regulation of AdoMetDC translation reside solely in the uORF of the 5'-leader. The clearest demonstration of this comes from the construct where polyamine regulation was conferred on the unregulated hGH leader through insertion of the AdoMetDC uORF with no additional sequence. Previous work has shown that the AdoMetDC uORF suppresses translation of downstream cis-trans through a mechanism that depends not only on its own translation but also on the sequence of the encoded peptide (Hill and Morris, 1993). These properties place the AdoMetDC uORF among the "sequence-specific uORFs," which have been proposed to suppress translation by a "ribosome-stalling" mechanism (Hill and Morris, 1993; Geballe and Morris, 1994; Cao and Geballe, 1996). In this mechanism, the nascent peptide encoded by the uORF interacts with a component of the translation machinery, and this interaction causes the ribosome to be stalled at the termination codon of the uORF. The stalled ribosome serves as a blockade, which prevents addi-
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| Constructs | Treatment | Relative translation | Ratio (DFMO/None) |
|------------|-----------|----------------------|-------------------|
| pRS327     | +DFMO     | 19.2                 | 0.19              |
|            | None      | 100.0                |                   |
| pRS66      | +DFMO     | 26.1                 | 0.74              |
|            | None      | 35.5                 |                   |
| pRSSTTG    | +DFMO     | 30.8                 | 0.67              |
|            | None      | 46.1                 |                   |
| pRNN       | +DFMO     | 11.6                 | 0.24              |
|            | None      | 48.5                 |                   |

*Fig. 4. Influence on polyamine regulation of an extended uORF that overlaps the hGH cistron.* Details of construct pRSSTTG are described under "Results." Relative translation was determined as described in the legend to Fig. 3, and the value for construct pRNN in untreated CHO cells was arbitrarily designated as 100.

Polyamine regulation was abolished by missense substitutions in the carboxyl half of the uORF sequence but not by synonymous mutations that extensively altered the nucleotide sequence but not the coding capacity of the uORF. These results demonstrate clearly that the specific peptide sequence encoded by the uORF, and not the nucleotide sequence, mediates polyamine regulation. The other important aspect of uORF-mediated polyamine regulation is that altering the distance between 5′-cap and uORF from 14 to 47 nucleotides did not change regulation by polyamines. This suggests that changing the efficiency of recognition of the uORF by the scanning ribosomes has little or no effect on the response to polyamine depletion. This is in clear contrast to the previously reported cell-specific regulation of AdoMetDC translation, which is mediated through recognition of the uORF and sensitive to the distance from the 5′-cap (Ruan et al., 1994).

The requirement in polyamine regulation for both translation of the AdoMetDC uORF and a unique peptide sequence is identical to the reported requirement for translational suppression by this uORF in T cells (Hill and Morris, 1993). As noted above, since polyamine control is independent of the distance of the initiator codon of the uORF from the cap, regulation by polyamines does not seem to be mediated by altering the efficiency of recognition of the uORF. To account for these results, we suggest that physiological levels of the polyamines stabilize specific interactions between the peptide encoded by the uORF and its target. According to this model, the strength of these interactions would be weakened in the polyamine-depleted state, leading to a reduction in the amount of ribosome stalling. By this mechanism, the inhibitory effect of the uORF would be relieved by polyamine depletion, with a consequent increase in downstream translation. When the peptide sequence encoded by the uORF is changed, the altered peptide no longer interacts with its target, and therefore, polyamines would no longer specifically influence downstream translation of the associated mRNA.

According to the above model, two factors contribute to regulation of ribosome stalling on the AdoMetDC uORF: the sequence of the peptide encoded by the uORF and the cellular level of polyamines. Changing either of these two factors would abolish both ribosome stalling and uORF-mediated regulation. One can envision three ways in which polyamines could stabilize the interaction between the nascent peptide and its target. First, polyamines might participate directly in this interaction, forming a stable ternary complex with peptide and target. Second, appropriate polyamine levels might provide a critical intracellular pH value required for this interaction. This possibility is suggested by the recent finding that polyamines may exert their physiological function through maintaining intracellular pH (Poulin and Pegg, 1995). Last, the interaction could be stabilized by a trans-acting factor, whose function is influenced in turn by polyamines. Regardless of which, if any, of these hypotheses is correct, the implication is that the specific structure of the nascent peptide encoded by the AdoMetDC uORF plays a critical role in polyamine-mediated regulation. The fact that the peptide sequence encoded by the AdoMetDC uORF (MAGDIS) is identical among the reported mammalian cDNAs is consistent with this model. Also consistent with a unique role for the MAGDIS peptide in regulation by polyamines is the observation that, in the currently available compendium of uORFs (Kozak, 1987), there is none that encodes this or a related sequence.

There are only a limited number of uORFs for which the mechanism of regulation is understood. There are several examples of uORFs that suppress downstream translation constitutively, and their recognition is regulated (for reviews, see Geballe and Morris (1994); Morris (1995)). The present example is of particular interest, since it appears that the suppressive influence of the uORF is regulated independently of the efficiency of its recognition. Another possible example where regulation by a uORF might be mediated through interactions with its peptide product is the CPA1 gene of yeast (Werner et al., 1987; Delbecq et al., 1994), in which feedback regulation by arginine is mediated at the translational level through a sequence-specific uORF. These two examples, AdoMetDC and CPA1, provide prototypes for regulated interactions between uORF-encoded peptides and their cellular targets.

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