A mammalian sequence-dependent upstream open reading frame mediates polyamine-regulated translation in yeast

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ABSTRACT

In mammals, control of S-adenosylmethionine decarboxylase (AdoMetDC) translation is one component of a feedback network that regulates intracellular levels of the polyamines, spermidine, and spermine. AdoMetDC mRNA from mammals contains a highly conserved upstream open reading frame (uORF) within its leader sequence that confers polyamine-regulated suppression of translation on the associated downstream cistron. This regulation is mediated through an interaction that depends on the amino acid sequence of the uORF-encoded hexapeptide. It remains to be shown whether polyamines participate directly in this interaction or indirectly through a specialized signal transduction pathway. We show that *Saccharomyces cerevisiae* does not have a uORF associated with its AdoMetDC gene (*SPE2*) and that ribosome loading on the *SPE2* mRNA is not positively influenced by polyamine depletion, as it is in mammalian cells. Nevertheless, the mammalian AdoMetDC uORF, when introduced into a polyamine auxotroph of yeast, conferred polyamine regulation of both translational efficiency and ribosome loading on the associated mRNA. This regulatory activity depended on the amino acid sequence encoded by the fourth and fifth codons of the uORF, as in mammalian cells. The fact that the regulatory properties of this mammalian translational control element are quite similar in both mammalian and yeast cells suggests that a specialized signal transduction pathway is not required. Rather, it seems likely that polyamines may be directly participating in an interaction between the uORF-encoded peptide and a constitutive component of the translation machinery, which leads to inhibition of ribosome activity.

Keywords: mRNA; polyamines; S-adenosylmethionine decarboxylase; translation; upstream open reading frame

INTRODUCTION

The biosynthesis of the naturally occurring polyamines, spermidine and spermine, is tightly regulated by a variety of mechanisms (Heby & Persson, 1990; Morris, 1991; Grillo & Colombatto, 1994; Shantz & Pegg, 1999). Fine control of intracellular polyamine levels is necessary, because overproduction of these compounds can lead to cellular toxicity and oncogenic transformation (Heby & Persson, 1990; Morris, 1991), whereas polyamine depletion inhibits cell growth (Pegg, 1988; Marton & Pegg, 1995).

S-Adenosylmethionine decarboxylase (AdoMetDC) is one of the regulated enzymes in the pathway of polyamine biosynthesis. Translation of mammalian AdoMetDC is controlled by the intracellular level of polyamines (White et al., 1990) through a unique mechanism.

The mammalian AdoMetDC mRNA contains within its leader sequence a small open reading frame that encodes a peptide of sequence MAGDIS (Hill & Morris, 1992). This upstream open reading frame (uORF) suppresses translation of associated downstream cistrons through a mechanism that requires specific structural features in the carboxy-terminus of the encoded peptide (Hill & Morris, 1993; Mize et al., 1998). The stringent requirements of the peptide structure implies that it interacts with a specific cellular target and, furthermore, this target seems to be conserved across species from mammals to yeast (Mize et al., 1998). Intracellular polyamine levels in mammalian cells regulate the suppressive activity of the AdoMetDC uORF through a mechanism that involves modulation of the interaction of MAGDIS with its target (Ruan et al., 1996). Although the mammalian uORF suppresses translation in yeast (Mize et al., 1998), it has not been established whether this activity is regulated by polyamines.

In *Saccharomyces cerevisiae*, the level of the first enzyme of the polyamine pathway, ornithine decarboxy-

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lase (ODC) is feedback controlled by cellular polyamine content through a posttranslational mechanism (Fonzi, 1989) that involves regulated degradation by the proteasome (Toth & Coffino, 1999). Although the pattern of regulation of yeast ODC is similar to that found in mammalian cells, it is not known whether AdoMetDC is under translational control in yeast as it is in mammals. In the present study, we find that the yeast *SPE2* gene, which encodes AdoMetDC, does not have an associated uORF. Additionally, ribosome loading on *SPE2* mRNA is not enhanced by lowering cellular polyamine levels. The mammalian AdoMetDC uORF was placed upstream of a reporter gene and introduced into a polyamine auxotroph of yeast. Translation of mRNA generated from this construct was regulated by the uORF in a manner identical to that observed in mammalian cells. Polyamine depletion of the cells enhanced both translational efficiency and density of ribosome loading on the mRNA. This regulatory activity depended on the amino acid sequence in the fourth and fifth positions of the uORF-encoded peptide. The fact that the endogenous yeast *SPE2* gene is not regulated by polyamines through an associated uORF suggests that the mammalian AdoMetDC uORF is not controlled through a dedicated regulatory pathway, and is consistent with a model involving interaction of the uORF-encoded peptide and polyamines with a component of the constitutive translational machinery.

RESULTS

In mammalian cells, reduction of polyamine levels results in an overall inhibition of protein synthesis, but an enhancement of ribosome loading on the AdoMetDC mRNA (Ruan et al., 1996). To test the behavior of the endogenous *SPE2* mRNA in yeast, a polyamine auxotroph containing a disruption of the ornithine decarboxylase gene (*SPE1*) was starved of polyamines for 22 and 47 h and ribosome loading on mRNA was compared to an unstarved control culture by sucrose gradient centrifugation. The distribution of *SPE2* (Fig. 1A) and actin (Fig. 1B) mRNAs are compared across the sucrose gradients. With progressive time of starvation, both mRNAs move to positions in the gradient corresponding to a lower density of ribosome loading, consistent with slowed cell growth and a general depression of protein synthesis. However, there is no indication of preferential translation of the *SPE2* mRNA as occurs in mammalian cells. There was no additional change in the distribution of these mRNAs in polysomes after 70 h of starvation (not shown).

The structure of the leader of the *SPE2* mRNA was investigated by RNase protection. Four probes were generated from yeast genomic sequence covering a region -511 to +77 nt relative to the translational start codon of the yeast *SPE2* gene. RNase protection experiments with these probes, using RNA isolated from

FIGURE 1. Loading of ribosomes on mRNA in a polyamine auxotroph. Yeast strain Y532 (*MAT α* , *ura3-52*, *his6*, *leu2*, *spe1::LEU2*; a gift from H. Tabor) was grown as described in Materials and Methods in the presence of polyamines (closed triangles) or 22 and 47 h after removal of polyamines from the medium (open squares and circles, respectively). After sucrose gradient centrifugation, 12 fractions were collected from each tube and analyzed for *SPE2* and actin mRNA. Direction of sedimentation was from left to right and the positions of the fractions containing monosomes and polysomes with five ribosomes were determined from a simultaneous A_{254} trace. The mRNA levels in each fraction are presented as the percentage of the sum of the signals for each species in the 12 fractions. **A:** *SPE2* mRNA. **B:** Actin mRNA.

yeast with a wild-type *SPE2* gene, demonstrated a single cluster of transcription start sites located at about 75 nt upstream of the AdoMetDC open reading frame. The experiment shown in Figure 2 used an antisense RNA probe encompassing the region from -289 to +75 relative to the first nucleotide of the initiator AUG. A major series of fragments of approximately 150 nt was protected from RNase digestion, consistent with a leader of approximately 75 nt in length. Inspection of the sequence of this protected fragment revealed the presence of no uORFs associated with the *SPE2* mRNA.

FIGURE 2. Analysis of the 5' end of the *SPE2* mRNA by RNase protection assay. A 364-bp fragment, from the region of the yeast *SPE2* gene -289 to $+75$ relative to the first nucleotide of the initiator AUG codon, was amplified by PCR and cloned into pBluescript SK⁺. The identity of the fragment was confirmed by sequence analysis and this construct was used to generate a ³²P-labeled antisense RNA transcript. Using this transcript as probe, RNase protection assay was performed as described previously (Mize et al., 1998). Ten micrograms of total RNA from a strain with wild-type *SPE2* (strain CRY1; Kean et al., 1993) was used in lanes 2–4 and 10 μ g of tRNA was used as a control in lane 1. Hybridization to the probe was carried out at 26°C (lane 2), 37°C (lanes 1 and 3), and 45°C (lane 4). Fragment sizes were determined from an RNA ladder and “FL” indicates the expected position of the full-length, undigested probe (includes sequence from the multiple cloning site at both ends).

There is also a faint cluster of fragments approximately 190 nt in length, suggesting a minor product with a leader of 115 nt, which also does not contain a uORF. The minor, diffuse band at about 380 nt, which was observed at 26°C and 37°C, did not withstand hybridization at 45°C (Fig. 2, lane 4). Based on these results, taken together with the polysome experiment, we conclude that there is no evidence for regulation of expression of the endogenous yeast *SPE2* gene by a uORF-mediated mechanism.

The yeast strain used for the remaining studies in this article is a polyamine auxotroph that contains deletions in genes *SPE1* and *SPE2* (Toth & Coffino, 1999), which encode ODC and AdoMetDC, respectively. When polyamines were removed from the culture medium (Fig. 3A), this strain shows a gradually declining growth rate over several days due to depletion of intracellular spermidine and spermine. To test possible polyamine regulation through the mammalian AdoMetDC uORF in yeast, the auxotroph was transformed with expression constructs containing wild-type and altered uORFs inserted into the transcript leader (Mize et al., 1998). Expression of the β -galactosidase gene associated with

FIGURE 3. Influence of polyamine starvation on translational control by the AdoMetDC uORF in yeast. Auxotrophic strains, containing β -galactosidase expression vectors with wild-type and AUG/GUG uORFs in the 5' transcript leaders (Materials and Methods), were grown in uracil-deficient synthetic dextrose medium containing 100 μ M spermidine. At 0 h, cultures were washed twice in sterile water, inoculated into medium in the presence or absence of 100 μ M spermidine, and grown at 30°C. **A:** Growth was monitored by measuring absorbance at 600 nm (no polyamines: open triangles; spermidine-supplemented: filled triangles). Cultures were diluted into fresh media when necessary to prevent saturation. Although the growth curve shown is for wild type, an identical growth pattern was observed for cells containing the AUG/GUG construct. Identical growth curves were also seen when the strains were grown with spermine alone or with a combination of spermidine and spermine (data not shown). Samples were removed from the cultures at the indicated times and processed for measurement of β -galactosidase activity and mRNA level (Materials and Methods). **B:** The translational efficiencies were normalized to the values of the construct containing the wild-type uORF in the presence of spermidine at 24 h. Filled symbols indicate values obtained during growth in 100 μ M spermidine and open symbols are from polyamine-starved cultures. Circles represent data from cultures containing the wild-type uORF and squares represent data from cultures containing the AUG/GUG construct.

these leaders was monitored during polyamine starvation and normalized to mRNA levels, yielding a measure of translational efficiency (Fig. 3B). Translational efficiency of the construct with the wild-type uORF was elevated threefold over an unstarved culture by 48 h of starvation and over fivefold by 144 h, demonstrating enhanced translation of this construct in response to

polyamine starvation. A construct in which the initiator AUG of the uORF had been altered to GUG was expressed at a 20-fold higher level (note difference in y axis) as expected (Mize et al., 1998), but exhibited no change in expression during polyamine starvation, suggesting that the presence of the uORF was necessary to confer the translational control.

Expression of β -galactosidase relative to mRNA level suggested that polyamine starvation enhanced translation of mRNAs containing the intact AdoMetDC uORF. To test this conclusion, ribosome loading on mRNAs was measured. Extracts were prepared from unstarved cells and from cells that had been polyamine-starved for 120 h. The extracts were fractionated by sucrose gradient centrifugation and mRNA location was ascertained by either nuclease protection or northern blots. The absorbance profile from the polyamine-starved culture (Fig. 4A) revealed relative underloading of polysomes due to the slow growth that resulted from a combination of minimal medium and polyamine starvation. Consistent with a reduction in global protein synthesis, polyamine starvation reduced the ribosome loading on the actin (*ACT1*) mRNA (Fig. 4B). The ma-

jority of the β -galactosidase (*lacZ*) mRNA in the cells grown in the presence of spermidine (+PA) sedimented in the region of single ribosomes and ribosomal subunits, in agreement with previous results with the wild-type uORF in yeast prototrophic for polyamine biosynthesis (Mize et al., 1998). In obvious contrast with the behavior of *ACT1* mRNA, there was a clear increase in amount of the *lacZ* mRNA loaded with two ribosomes or more, consistent with the increase in translational efficiency after this length of starvation (see Fig. 3B, 120 h).

Removal of polyamines from the culture media of this auxotroph not only depletes intracellular polyamine levels, but also drastically reduces the growth rate (Fig. 3A). Therefore, one cannot distinguish between a direct regulatory influence of polyamine levels and an indirect effect mediated through altered growth rate. To test the influence of down-regulation of growth rate on expression of the uORF-containing construct, cells were transferred from dextrose as a carbon source to maltose, which caused an immediate and drastic reduction in growth rate (Fig. 5). No change in the translational efficiency of the uORF-containing mRNA was observed during this transition (Fig. 5, inset). Likewise, no change was seen in the expression of the construct in which the initiator AUG of the uORF had been altered to GUG. From this experiment, one concludes that the change in translational efficiency observed in the experiment of Figure 3 resulted from depleted polyamine levels and was not due to the altered growth rate of the cells.

Previous results have shown that the nature of codons 4, 5, and 6 of the AdoMetDC uORF are critical for regulation by polyamines in mammalian cells (Ruan et al., 1996). Missense alterations in the important codons were introduced and the resulting impact on translational efficiency in yeast was tested by the same approach as described in Figure 3. Samples were taken for β -galactosidase and mRNA analysis 18 h after transfer to polyamine-free medium ("control") and after an additional 102 h of culture time in this medium ("starved"). The data are presented in Table 1 as the ratios of translational efficiencies under the two conditions. Consistent with the results in Figure 3, the wild-type uORF showed a ratio of 4.5 between the two conditions, whereas there was no significant change in expression of the construct in which the initiator AUG of the uORF had been altered to GUG. Interestingly, the conservative replacement of valine for isoleucine at position 5 (construct I5V) resulted in, if anything, better regulation than wild type, whereas the similarly conservative substitution of leucine at the same position (construct I5L) abolished regulation by polyamines. A conservative substitution at codon 4 (D4E) showed an intermediate value of translational efficiency. The other missense alterations tested, as well as termination codons at positions 4, 5, and 6, destroyed the regulatory activity of the uORF. These results are consistent with

FIGURE 4. Ribosome loading in yeast containing the β -galactosidase expression construct with the wild-type uORF. Extracts were prepared from cultures exponentially growing in medium supplemented with 100 μ M spermidine (+ PA) or cultures that had been polyamine-depleted for 120 h (- PA). Sucrose gradient centrifugation and fractionation were performed. **A:** A₂₅₄ tracing from the "- PA" culture, with sedimentation being from left to right. **B:** RNA was isolated from each of 24 fractions indicated by the tick marks in **A**. mRNA from the β -galactosidase construct (*lacZ*) was detected by nuclease protection. Actin mRNA (*ACT1*) was monitored by northern blot analysis. The vertical dashed line separates the polysomal and subpolysomal regions of the gradient.

FIGURE 5. Influence of growth rate on translational efficiency of mRNA associated with the AdoMetDC uORF. Yeast containing wild-type and AUG/GUG expression vectors were grown in uracil-deficient synthetic dextrose medium containing 100 μ M spermidine. At 3 h on the growth curve, cultures were washed once in sterile water and inoculated into the same medium with either dextrose (filled circles) or maltose (open circles) as carbon sources, supplemented with 100 μ M spermidine. Cultures were grown at 30 °C and growth was monitored by absorbance at 600 nm. Growth curves are shown for the wild-type construct, but no difference in growth pattern was observed between cells containing the constructs with the wild-type and AUG/GUG uORFs (data not shown). Samples were removed from the cultures at the indicated times and processed for β -galactosidase activity and mRNA level as described in Materials and Methods. Lower case letters indicate points where samples were taken and the data are reported in the inset as translational efficiency (β -galactosidase activity/mRNA) normalized to the value with the wild-type uORF in sample a.

those obtained previously both in mammalian cells (Ruan et al., 1996) and in cell-free extracts (Raney et al., 2000).

A series of triamine homologs of spermidine (Jorstad et al., 1980) were tested for their ability to replace the natural polyamine in suppression of translation of the construct containing the wild-type AdoMetDC uORF. As expected, spermidine supported growth of the auxotroph (Fig. 6), whereas none of the homologs stimulated growth to a significant extent over the unsupplemented culture, with the possible exception of the AP-5, which is one methylene group longer than spermidine. All of the spermidine homologs are taken up by the cells and all suppress the translational efficiency of the β -galactosidase constructs containing the wild-type uORF as efficiently as spermidine (Table 2). In contrast, neither spermidine nor any of the homologs in-

TABLE 1. Sequence dependence of translational control by the AdoMetDC uORF. Yeast polyamine auxotrophs, containing β -galactosidase expression vectors with various uORFs described in Mize et al. (1998), were grown in uracil-deficient synthetic dextrose medium containing 100 μ M spermidine. At 0 h, cultures were washed twice in sterile water and inoculated into polyamine-free medium. Cultures were grown at 30 °C and were diluted into fresh medium when necessary to prevent saturation. No difference in growth rate was noted between the strains carrying the different constructs (data not shown). Samples were removed from the cultures at 18 and 120 h (see Fig. 3). β -Galactosidase activity was measured and corrected for mRNA level as described in Materials and Methods. Results from two independent experiments are shown and the data are reported as the ratio of the translational efficiencies between the 120-h ("starved") and 18-h ("control") time points. "Wild type" refers to the unaltered uORF inserted into the expression construct, "AUG/GUG" designates the construct in which the initiation codon was destroyed and the remaining constructs are either missense alterations or conversions into termination codons ("Ter").

uORF derivative	Starved/Control	
	Experiment 1	Experiment 2
Wild type	4.5	4.3
AUG/GUG	1.1	1.3
I5V	6.2	6.5
I5L	1.2	0.9
D4E	2.4	2.5
D4C	1.2	1.3
D4R	1.5	1.4
D4Ter	1.2	1.1
I5Ter	1.3	1.2
S6Ter	1.2	0.9

fluences the translation of the construct in which the initiator AUG codon has been changed to GUG. Therefore, although none of the spermidine analogs was able to support appreciable growth of the polyamine auxotroph, all were taken up to levels that regulated translation through the uORF similarly to spermidine.

DISCUSSION

Previous evidence has shown that the uORF from the mammalian AdoMetDC gene, when placed in a yeast transcript leader, suppressed translation of an associated reporter gene in wild-type yeast cells (Mize et al., 1998). The strict dependence of this activity on the amino acid sequence of the uORF-encoded peptide in the critical fourth and fifth positions was identical in yeast and mammalian cells. These results suggested that at least some mechanistic features of the suppressive interactions of the peptide are strongly conserved phylogenetically.

The suppressive activity of the uORF is regulated in mammalian cells by intracellular polyamine levels (Ruan et al., 1996). In this article, we document the striking observation that the mammalian AdoMetDC uORF is regulated by polyamines in yeast and this regulatory activity shares apparently identical properties across phylogenetic lines. The AdoMetDC uORF mediates reg-

FIGURE 6. Growth in the presence of spermidine analogs. The *spe1*, *spe2* double mutant containing the expression vector with the wild-type uORF was grown in uracil-deficient synthetic dextrose medium containing 100 μ M spermidine. At 0 h, cultures were washed twice with sterile water and inoculated into polyamine-free medium (closed circles) or supplemented with 100 μ M spermidine (filled triangles), 100 μ M AP-3 (open circles), 100 μ M AP-5 (open squares), or 100 μ M AP-6 (open triangles). Cultures were grown at 30 °C and monitored by measuring absorbance at 600 nm. Cultures were diluted into fresh media when necessary to prevent saturation. Identical growth patterns were observed with cells containing the AUG/GUG construct.

ulated ribosome loading in both mammals and yeast. At normal intracellular polyamine levels, the uORF-containing mRNA is located largely on monosomes and disomes, whereas depletion of intracellular polyamines increases the density of ribosome loading on the mRNA in both cell types. As in mammalian cells, the polyamine regulation in yeast is exquisitely dependent on the amino acid sequence of the uORF-encoded peptides. This extraordinary conservation of these regulatory interactions, despite the fact that yeast contains no uORF associated with its own *SPE2* gene, has clear implications with regard to the mechanism of regulation.

Two key observations regarding possible mechanisms of regulation of translation by the AdoMetDC uORF in mammalian cells are that the translationally repressed mRNA is associated primarily with single ribosomes (Hill & Morris, 1992) and that the uORF seems to act in *cis* on the ribosome that translated it (Hill & Morris, 1993). These findings and others led to a model for sequence-dependent uORFs (Geballe & Morris, 1994) in which the nascent peptide product of the uORF interacts with a component of the translation machinery to arrest the ribosome at or close to the termination codon of the uORF. This arrested ribosome would then create a blockade to entry of additional scanning ribosomes onto the mRNA and thereby suppress translation. This model predicts the presence of a cellular target with which the nascent peptide specifically interacts. Previous work (Mize et al., 1998) suggested that this target and the nature of its interaction with the uORF-encoded peptide are widely conserved across

TABLE 2. Influence of spermidine analogs on translational control by the AdoMetDC uORF. Yeast strains containing the β -galactosidase expression vector with the wild-type or AUG/GUG uORFs were transferred to culture medium lacking polyamines as described in Figure 6. After 24 h of polyamine starvation at 30 °C, growth was continued in the absence of added polyamines or with the indicated triamine added at 100 μ M. After 24 h of additional growth, samples were taken for measurements of β -galactosidase activity and cellular polyamine levels (Materials and Methods). β -Galactosidase activity was corrected for mRNA and normalized to the cultures grown in the presence of spermidine. "Wild type" refers to the unaltered uORF inserted into the expression construct, and "AUG/GUG" designates the construct in which the initiation codon was mutated.

uORF derivative	Triamine added	Translational efficiency ^a	Cellular triamine ^b
Wild Type	No addition	2.6	0.05
	Spermidine	1.0	0.13
	AP-3	0.6	0.18
	AP-5	0.9	0.35
	AP-6	1.0	0.31
AUG/GUG	No addition	1.0	0.06
	Spermidine	1.0	0.22
	AP-3	0.8	0.10
	AP-5	0.9	0.16
	AP-6	0.8	0.46

^aTranslation efficiency expressed as β -galactosidase/mRNA and normalized to the cultures grown in the presence of spermidine.

^bCellular levels of spermidine or the homologs are expressed as nmol/ μ g protein.

nature. From the results presented in this article, we conclude that not only is the target conserved, but the regulatory response to polyamines is conserved as well.

There are two general classes of models for the regulation of translation by polyamines through the AdoMetDC uORF. Polyamines could participate directly in the interaction between the nascent peptide product of the uORF and its target, perhaps as a component of a ternary complex. Alternatively, polyamine deficiency could act indirectly, perhaps by activating a specific signal transduction pathway, which in turn could regulate the interaction between the nascent peptide and translation machinery. The findings reported in this article are relevant in weighing the relative merits of these two models. The second model implies a refined and specialized regulatory pathway, whereas the first model could be as simple as an interaction of the uORF-encoded peptide with a component of the constitutive protein synthesis machinery, for example, a release factor or a ribosomal component. Although the mammalian AdoMetDC uORF mediates translational control by polyamines in yeast, ribosome loading on the endogenous yeast *SPE2* mRNA is not regulated by polyamines and this mRNA contains no associated uORF. The fact that yeast does not normally express a regulatory mechanism specific to this uORF would argue against complex, specialized regulatory mechanisms and favor the simpler interaction implicit in the first model. Another argument against more complicated reg-

ulatory pathways comes from a recent study in vitro (Raney et al., 2000), in which the rates of synthesis of peptides encoded by wild-type and altered AdoMetDC uORFs were examined. Consistent with the regulatory properties of the uORF, the rate of translation of the wild-type peptide was more sensitive to elevated levels of polyamines than was synthesis of altered peptides. The fact that this aspect of the regulatory mechanism could be recapitulated in vitro in cell extracts also argues for a simpler, more direct mechanism of regulation by polyamines. We therefore favor, at this point, a model in which polyamines directly enhance the interaction between the uORF-encoded peptide and its target, perhaps by participating in formation of a ternary complex.

MATERIALS AND METHODS

A polyamine auxotrophic strain of *S. cerevisiae* (*MAT α* , *leu2*, *ura3*, *trp1*, *his3*, *spe1::HISG*, *spe2::LEU2*) from P. Coffino, which contained deletions in the genes encoding ornithine decarboxylase (*SPE1*) and S-adenosylmethionine decarboxylase (*SPE2*; Toth & Coffino, 1999), was transformed with chimeric *GCN4/lacZ*, *URA3* constructs (Mize et al., 1998) using the lithium acetate procedure (Ausubel et al., 1994). For growth of these cells, special care was taken to maintain polyamine-free conditions. All glassware used for preparation of medium and cell culture was acid-washed as described in Balasundaram et al. (1991). Solutions and culture media were prepared using Nano-pure™ water. Unless otherwise indicated, yeast was grown in polyamine-free uracil-deficient synthetic dextrose medium. Spermidine, spermine, and the spermidine analogs N-(3-aminopropyl)1,3-propane-diamine (AP-3; Aldrich, Milwaukee, Wisconsin), N-(3-aminopropyl)1,5-pentane-diamine (AP-5), and N-(3-aminopropyl)1,6-hexane-diamine (AP-6) (Jorstad et al., 1980) were added to final concentrations of 100 μ M where indicated. Cultures were grown at 30 °C and cell growth was monitored by measurement of absorbance at 600 nm.

Samples were removed from yeast cultures for analysis of β -galactosidase activity and mRNA, polyamine, and protein levels. After harvesting by centrifugation, the cells were washed three times with phosphate-buffered saline (2 mM KCl, 1.5 mM KH_2PO_4 , 8 mM Na_2HPO_4 , and 140 mM NaCl), split into three tubes, and centrifuged. The pellets were stored at -70 °C until analysis. β -Galactosidase activity and *lacZ* mRNA levels were measured as described (Mize et al., 1998). Actin (*ACT1*) mRNA levels were measured by northern blot with a probe kindly provided by Ken Dombek (Dombek et al., 1993).

Polyamine analysis was performed by thin layer chromatography of the dansyl derivatives (Seiler, 1983) with the following modifications: Insoluble material from perchloric acid extraction was retained for protein measurements and thin layer plates were run three times in a mixture of cyclohexane/ethylacetate (3:2), and the dansyl derivatives were quantified using the Storm 840 (Molecular Dynamics, Sunnyvale, California). Protein was determined in the insoluble material by resuspending the pellets in 0.5 M NaOH, incubating overnight at 37 °C and analysis was carried out using the Bio-Rad detergent compatible protein assay kit (Bio-Rad, Hercules, California).

Polysomes were isolated from yeast cultures and analyzed as described (Mize et al., 1998), with the exception of the experiment of Figure 4, where the gradient was centrifuged for 2 h and separated into 24 fractions.

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