MODULATION OF RIBONUCLEASE P EXPRESSION IN
ESCHERICHIA COLI BY POLYAMINES

CHRISTOS A. PANAGIOTIDIS,†* DENIS DRAINAS‡ and SHU-CHING HUANG†

Departments of †Pharmacology and ‡Molecular Biophysics and Biochemistry, Yale University,
New Haven, CT 06511, U.S.A.

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Abstract—1. The presence of polyamines in the growth medium of Escherichia coli can modulate the
activity of the RNA-processing enzyme, ribonuclease protein ribonuclease P (RNase P), by altering the
expression of the nprA and nprB genes, which encode its C5 protein and M1 RNA subunits, respectively.
2. Following growth in the presence of 1 mM spermidine the levels of C5 protein mRNA and catalytic
M1 RNA were significantly elevated in the wild type E. coli K-12 strain MG1655.
3. The nprA mRNA, together with the ribosomal protein L34 (rpmH) mRNA, was found to constitute a
dicistronic rpmH–nprA message whose half-life did not change upon Escherichia coli growth in the
presence of spermidine.
4. This suggests that the spermidine effect is on the transcriptional level.
5. Increased expression of the nprA and nprB genes was reflected in the activity of RNase P, which
almost doubled.
6. These results identify yet another component of the protein synthetic machinery which is specifically
affected by polyamines.

INTRODUCTION

The polyamines, putrescine, spermidine and spermine, are polycationic compounds found at high
concentrations (1–20 mM) in nearly all cells (Tabor and Tabor, 1985). The role(s) of polyamines in
cellular metabolism, however, is highly conjectural at present. The broad spectrum of their properties
renders the localization of their essential function(s) a difficult task. Polyamines are known to increase the
rate of protein synthesis in vitro and to participate in a large variety of other reactions, including DNA
replication, RNA synthesis, and protein phosphorylation (for review see Tabor and Tabor, 1984, 1985,
and references therein). As part of an effort to determine the roles of polyamines in vivo, genetic
blocks have been engineered for various polyamine-synthesizing enzymes of Escherichia coli
(Cunningham-Rundles and Maas, 1975; Hafner et al., 1979; Morris and Jorstad, 1970). Hafner et al.
(1979) found that the normal pathways (speA,B, speC, speD) for polyamine production could be
blocked by deletion mutations, and yet the bacteria carrying these mutations still grew, albeit at a slower
rate than normal. Subsequently it was found that these deletion mutants acquire a polyamine-dependent
phenotype upon introduction of a streptomycin-resistance mutation (rpsL9) (Tabor et al., 1981).
Since rpsL codes for a ribosomal protein (S12), it was concluded that the fidelity and/or efficiency of protein
synthesis may be intimately related to polyamine dependence.

Another line of investigation, quite unexpectedly, has led to a similar conclusion. In the process of
studying the regulation of the polyamine-biosynthetic enzymes, ornithine and arginine decarboxylase, in
E. coli, Canellakis and co-workers (Heller et al., 1983; Kyriakidis et al., 1978) discovered three polyamine-
ducible protein inhibitors of these enzymes; two of them were identified as the ribosomal proteins S20
and L34 (Panagiotidis and Canellakis, 1984). This rather surprising finding suggested that the regulation of
polyamine levels is closely related to ribosomal components, and thus, to protein synthesis.

The gene for ribosomal protein L34 (rpmH) was shown to be part of the same operon as the nprA gene of C5
protein, the protein subunit of the RNA-

*Address correspondence to: Dr Christos Panagiotidis,
Department of Microbiology, Columbia University,
College of Physicians and Surgeons, 701 W. 168th Street,
New York, NY 10032, U.S.A. [Tel. (212) 305-4137; Fax
(212) 305-1468].
†Present address: Department of Medicine, Laboratory of
Biological Chemistry, University of Patras, Patras
26110, Greece.
MATERIALS AND METHODS

Bacterial strains and growth conditions

E. coli K-12, strain MG1655 (ΔrpsH::FRT) (Guyer et al., 1981) was grown in minimal medium 56 (Monod et al., 1951; Huang et al., 1990), supplemented with 0.4% glucose and 1 μg·ml⁻¹ thiamine, at 37°C with vigorous shaking. Where indicated, spermidine was added to a final concentration of 1 mM.

Probes and mRNA analysis

Total E. coli RNA was isolated from logarithmically growing cells (A600 = 0.5), as previously described (Mackie, 1987). RNA was denatured and analyzed on 5% polyacrylamide gels containing 2.2M formamide and MOPS buffer pH 7.0 (40 mM MOPS, 10 mM sodium acetate, 1 mM EDTA); MOPS buffer was also used as electrode buffer. RNAs were transferred electrophoretically from the gels onto Zeta-Probe membranes (Bio-Rad), in 40 mM Tris-acetate buffer, pH 8.0 containing 2 mM EDTA, and the membranes were hybridized with the indicated cRNA or DNA probes following the manufacturers’ instructions.

The 32P base-pair long EcoRV-EcoRI fragment of plasmid pRBIA (Braun et al., 1985), which contains the rpmH gene and the beginning of the rnpA gene, was subcloned in plasmid pGEM-4 to yield plasmid pHPC-3 (Huang et al., 1990). Following linearization with EcoRV, plasmid pHPC-3 was used as the template in synthesizing a 32P-labelled RNA probe (Melton et al., 1984) complementary to L34 (rpmH) with the 5’-end of rnpA mRNA. Following linearization of pHPC-3 with Hpal an RNA probe complementary to the 5’-end of the rnpA mRNA sequence was synthesized as described. An oligodeoxynucleotide complementary to the last 33 bases of the rnpA mRNA coding sequence was custom synthesized (Genetic Designs, Inc., Houston, Tex.) and used as an 3’-end specific rnpA probe. Plasmid pNL3100 (Lawrence and Altman, 1986), containing the M1 RNA (rnpB) gene, was used as an M1 RNA probe after nick-translation with [α-32P]dCTP (Maniatis et al., 1982).

Plasmid pHPC-4 was used as agmatine ureohydrolase (AUH) specific probe. Plasmid phPC-4 was constructed by subcloning the 3.5-kb EcoRI–BamHI DNA fragment, containing the agmatine ureohydrolase gene, from plasmid pKAS (Boyle et al., 1984) into the vector pGEM-4. mRNA half-lives were measured as previously described (Huang et al., 1990).

RNase P assay

RNase P activity was assayed as previously described (Guerrier-Takada et al., 1983), except that the precursor tRNA used, pre-sups1, was an in vitro synthesized and labelled transcript of the Schizosaccharomyces pombe tRNAγ gene, sup51 (Krupp et al., 1986). The concentration of pre-sups1 in the reaction mixture was 1.95 mM. Reaction products were resolved on 8% denaturing polyacrylamide gels (Nichols et al., 1988) after phenol extraction and ethanol precipitation. Protein concentrations were determined according to Bradford (1976).

The X-ray films from the northern blots or the RNase P assays were scanned with a Zeinleh sol laser scanning densitometer (model SL-TRF) connected to an Apple IIE computer and analyzed using a program created by Mr. Robert Dreyer of this department.

All experiments were repeated at least 3 times with similar results and data from representative experiments are presented here.

RESULTS

When a cRNA spanning the 325 bp EcoRV–EcoRI fragment containing the L34 (rpmH) gene, and the beginning of the gene encoding the C5 protein (rnpA) (Hansen et al., 1982, 1985), was used to probe the RNA blots of E. coli grown in the presence or absence of spermidine, 3 RNA bands were detected (Fig. 1A). Two of these appeared to be L34 mRNAs initiating from each of the 2 major rpmH promoters (Hansen et al., 1982). Their sizes of approx 400 and 280 nucleotides, agreed well with the calculated distance between the 2 promoters based on DNA sequence data (Hansen et al., 1982). The third RNA band had an approximate size of 700 nucleotides, suggesting that it was a dicistronic message coding for both the L34 ribosomal protein (rpmH gene product) and the C5 protein (rnpA gene product). The size of the transcript was in good agreement with DNA sequence data (Hansen et al., 1985). Increased levels of all three RNA bands hybridizing with the L34 gene probe (Fig. 1A) were observed after growth in the presence of 1 mM spermidine. This treatment, however, did not affect the growth rate of the E. coli strain MG1655, which was growing with a doubling time of 85 min (Huang et al., 1990). Densitometric scanning of the autoradiograms showed that the quantity of rpmH2 promoter transcript increased by 50%, following growth in the presence of spermidine, and both the rpmH1 promoter transcript and the 700 nucleotide transcript increased by 80–100%. To ensure that the 700 nucleotide long RNA was indeed an rpmH–rnpA dicistronic message, an oligodeoxynucleotide complementary to the 33 bases at the 5’ end of the rnpA mRNA coding sequence (Hansen et al., 1985) was used to probe the Northern blots. Hybridization with the 700 nucleotide RNA, but not with the 400 or 280 nucleotide RNA transcripts, was detected (Fig. 1C). This indicates that the rnpA message is contained within the 700 nucleotide transcript. In contrast, a cRNA probe containing 80 nucleotides from the 5’ end of the rnpA gene but none of the rpmH sequence (Hpal–EcoRI restriction fragment) (Hansen et al., 1982), hybridized with all 3 RNA bands (Fig. 1B). The above data suggest that transcription of the monocistronic rpmH transcripts terminates within the rnpA coding sequence. The possibility that the dicistronic rpmH–rnpA RNA transcript is synthesized but processed within that region post-transcriptionally, however, cannot be excluded.

As in the case of the monocistronic rpmH mRNAs (Huang et al., 1990), the effect of polyamines on the levels of the dicistronic rpmH–rnpA mRNA appeared to occur at the transcriptional level. Thus, the half-life of this transcript was found to be identical (70 sec) in cells growing either in the absence or in the presence of 1 mM spermidine (Fig. 2).

It has been proposed (Hansen et al., 1985) that expression of the rnpA gene is coupled to expression
Fig. 1. Polyamine effect on the levels of monocistronic rpmH and dicistronic rpmH-rnpA mRNAs. Total RNA was isolated from E. coli grown in the absence (−) or presence (+) of 1 mM spermidine and equal amounts (4 μg) were electrophoresed, and blotted as described in Materials and Methods. The blots were hybridized with cRNA probes complementary to rpmH-5' rnpA (A) or the 5' end of rnpA mRNA only (B), or with a synthetic oligodeoxynucleotide complementary to the 3' end of the rnpA mRNA (C). The positions of relevant RNA size markers (BRL) are indicated. Growth in the presence of spermidine did not affect the growth rate.

Fig. 3. Spermidine increases the levels of the M1 RNA without affecting the levels of the agmatine ureohydrolase (AUH) mRNA. Total E. coli RNA was isolated from cells grown in the absence (−) or in the presence (+) of 1 mM spermidine and equal amounts (4 μg) were electrophoresed, and blotted as described in Materials and Methods. The 2 blots came from separate gels; the RNA for the M1 RNA detection was electrophoresed on a 5% polyacrylamide gel, whereas a 0.7% agarose gel was used for the analysis of AUH mRNA. The blots were hybridized either with nick-translated plasmid pNL3100 containing the M1 RNA gene or with an agmatine ureohydrolase (AUH) gene probe.
Fig. 4. Influence of polyamines on RNase P activity. (A) RNase P activity was assayed in *E. coli* extracts (10 and 20 ng of protein) prepared from cells which had grown in the absence (−) or presence (+) of 1 mM spermidine. The positions of the substrate precursor tRNA pre-supS1, processed supS1, and the excised 5′ flank are indicated by arrows. (B) The RNase P activities shown in (A) were quantitated, as described in the text. (●) No spermidine, (○) 1 mM spermidine.
of the rpmB gene coding for M1 RNA, the catalytic RNA subunit of RNase P. It was of interest, therefore, to determine whether polyamines exert an influence on the expression of M1 RNA or if their effect is localized to the expression of the rpmH–rpmA operon. Plasmid pNL3100, which contains the M1 RNA gene (rpmB) (Lawrence and Altman, 1986), was nick-translated (Maniatis et al., 1982) and used as a probe in Northern blot analysis of total E. coli RNA. As shown in Fig. 3, the amount of M1 RNA significantly increased in E. coli MG1655 grown in medium containing 1 mM spermidine. The effect of spermidine here is also most likely to take place at the transcriptional level since the M1 RNA is one of the stable E. coli RNAs (King and Schlessinger, 1987) and its in vivo regulation by any further prolongation of its half-life seems quite unlikely. To demonstrate the specificity of the observed effects we also measured the levels of the mRNA of the gene encoding for the enzyme agmatine ureohydrolase (AUH) and we found that they remained unaltered when the cells had grown in the presence of spermidine (Fig. 3).

Subsequently, we investigated whether growth in the presence of spermidine also caused an increase in the level of functional RNase P enzyme. This was necessary since antibodies against the C5 protein were not available to us and the 2-fold increase in the amounts of its mRNA did not guarantee an equivalent increase in the functional C5 protein levels. RNase P activity was measured in extracts of E. coli MG1655 grown in the presence or absence of 1 mM spermidine. Figure 4 shows that extracts of E. coli MG1655 grown in the presence of 1 mM spermidine possess higher RNase P activity than extracts prepared from cells grown in its absence. Quantitation of the activities from three separate experiments, by densitometric scanning and/or counting the radioactivity of individual bands, revealed that the increase (75 ± 5%) in RNase P activity was in good agreement with the observed increases in the levels of its components, i.e. the products of the rpmA and rpmB genes. In addition we observed that spermidine did not influence the RNase P-catalyzed reaction when added to our in vitro assay mixtures (data not shown). Therefore it is highly unlikely that the spermidine effect on RNase P activity stems from its direct interaction with the enzyme or the substrate.

**DISCUSSION**

In the present study we have presented evidence that RNase P activity can be modulated in vivo by the levels of spermidine in the growth medium of Escherichia coli cultures. The stimulative effect of spermidine on RNase P activity results from equivalent increases in the expression levels of the 2 genes (rpmA and rpmB), encoding the 2 subunits of RNase P. This occurs in spite of the fact that these 2 genes are 13 min apart in the E. coli linkage map (Bachmann, 1987). To our knowledge, this is the first report of a condition, other than alterations in the growth rate, which elicits changes in the transcription levels of the genes encoding the 2 RNase P subunits, and in the activity of RNase P in E. coli. The inclusion of 1 mM spermidine in the growth medium of the wild type E. coli K-12 strain MG1655 did not influence its growth rate (Huang et al., 1990), nor did it affect the expression of ribosomal protein genes other than those of S20 and L34 (Huang et al., 1990), whose products are involved in the negative regulation of the polyamine-biosynthetic enzymes (Heller et al., 1983; Panagiotidis and Canellakis, 1984). In addition, the inclusion of spermidine did not affect the levels of agmatine ureohydrolase mRNA which was included as a control in the present study. Therefore our findings indicate that the observed spermidine effect is specific for the genes studied and also that it is not due to differences in growth rates.

Since the rpmH and rpmA genes constitute an operon and most likely use the same promoters (Hansen et al., 1985), it could be argued that the increased expression of the rpmA gene, in the form of the dicistronic rpmH–rpmA mRNA, by spermidine is merely the result of increased transcriptional activity from the rpmH promoters (Huang et al., 1990). Such an interpretation, though, would ignore the facts (i) that the expression of the rpmA gene product is considerably less efficient than that of the rpmH gene product (Hansen et al., 1985) and (ii) that even when the 2 genes were placed under the control of a highly inducible promoter the product of the rpmA gene increased only 6-fold in fully induced cells while the rpmH gene product was massively over-produced (Vioque and Altman, 1986). Apparently there is a second level of negative control in the expression of the rpmA gene which is very efficient even under
conditions of extremely high transcriptional activity; yet specifically allows increased synthesis of di-cistronic rpmH—rnapA mRNAs when cells grow in the presence of spermidine.

Our findings, taken together with previous studies on E. coli RNase P showing that the presence of spermidine is necessary to maintain the activity of the catalytic M1 RNA under certain conditions (Guerrier-Takada et al., 1983), may suggest that spermidine can modulate RNase P by affecting both its expression and its enzymatic activity. In a similar manner spermidine has been reported also to both activate and increase the specificity of another RNA processing enzyme, an endonuclease involved in the splicing reaction during maturation of yeast tRNAs (Peebles et al., 1983). It should be noted, however, that when spermidine was included in our reaction mixtures it had no effect on the in vitro activity of the RNase P holoenzyme (data not shown).

The present study provides additional support for the idea that polyamines are intimately involved in the regulation of protein synthesis. As the number of genes whose expression is affected by polyamines increases, one can not help wondering whether polyamines can elicit a global response, through a set of transcriptional activator(s) and inhibitor(s). The identification of the members of such a polyamine regulon could shed light on the more specific functions of these important biogenic amines.

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REFERENCES


Hafner E. W., Tabor C. W. and Tabor H. (1979) Mutants of Escherichia coli that do not contain 1,4-diaminobutane (putrescine) or spermidine. J. biol. Chem. 254, 12,419–12,426.


