Polyamines Enhance Synthesis of the RNA Polymerase α^{38} Subunit by Suppression of an Amber Termination Codon in the Open Reading Frame*

The mechanisms by which polyamines stimulate synthesis of the RNA polymerase α^{38} subunit in Escherichia coli were studied. Polyamine stimulation was observed only in strains in which the 33rd codon of RpoS mRNA is a UAG termination codon instead of a CAG codon for glutamine in wild-type E. coli. Readthrough of the termination codon by Gln-tRNA^{supE} was stimulated by polyamines. This stimulation was found to be caused by an increase in both the level of suppressor tRNA^{supE} and the binding affinity of Gln-tRNA^{supE} for ribosomes. The stimulatory effect was observed with a UAG termination codon but not with UGA and UAA codons. Readthrough of the UAG termination codon at the 270th amino acid position of RpoS mRNA was also stimulated by polyamines, indicating that polyamines stimulate read-through of a UAG codon regardless of its location within the RpoS mRNA. When cell viability of an E. coli strain having a termination codon in the 33rd position of RpoS mRNA was compared using cells cultured with or without putrescine, it was higher in cells cultured with putrescine than in cells cultured without putrescine. The level of α^{38} subunit in the cells cultured with putrescine was higher than that in cells cultured without putrescine on days 2, 4, and 8, but the level of α^{39} subunit was almost the same in cells cultured with or without putrescine. These results confirm that elevated expression of the rpoS gene is important for cell viability at late stationary phase.

Polyamines, aliphatic cations present in almost all living organisms, are necessary for normal cell growth (1, 2). Because polyamines interact with nucleic acids and mostly exist as polyamine-RNA complexes in cells (3, 4), their proliferative effects are presumed to be caused by stimulation of nucleic acid and protein synthesis. In fact, it has been reported that polyamines stimulate the synthesis of some protein species in vitro (5–7) and in vivo (8, 9), induce the in vivo assembly of 30 S ribosomal subunits (10–12), and increase the fidelity of protein synthesis (13–15), altogether suggesting that polyamines regulate protein synthesis at several different steps.

In Escherichia coli, the synthesis of OppA protein, a periplasmic substrate-binding protein of the oligopeptide uptake system, is strongly stimulated by the addition of putrescine to a polyamine-requiring mutant, MA261 (9). We found that (i) the stimulation of OppA synthesis takes place at the level of translation; (ii) the position and secondary structure of the Shine-Dalgarno (SD) sequence (16) on OppA mRNA are important for this stimulation (17); and (iii) polyamines cause a structural change of the SD sequence and the initiation codon AUG of OppA mRNA, facilitating formation of the initiation complex (18). We also found that polyamines increase the translation of adenylate cyclase (Cya) mRNA by facilitating UUG codon-dependent initiation (19). Analysis of RNA secondary structure suggests that exposure of the SD sequence of mRNA is a prerequisite for polyamine stimulation of UUG codon-dependent initiation (19).

In the present study we found a novel mechanism of the polyamine stimulation, which is involved in the enhanced synthesis of RNA polymerase α^{38} subunit by polyamines (19). This type of the polyamine stimulation was observed only in E. coli strains in which the 33rd codon of RpoS mRNA is a UAG amber termination codon instead of a CAG codon for glutamine in wild-type E. coli strains. Readthrough of the termination codon by Gln-tRNA^{supE} was stimulated by polyamines.

**Experimental Procedures**

**Bacterial Strains and Culture Conditions—**Polyamine-requiring mutants, E. coli MA261 (speB speC gly leu thr thi) (20), HT263 (speA speB speC speED thr pro thi) (21), and DR112 (speA speB) (22) were kindly provided by Dr. W. K. Maas (New York University School of Medicine), Dr. H. Tabor (National Institute of Health), and Dr. D. R. Morris (University of Washington), respectively. A α^{38}-deficient mutant, E. coli KT1100 rpoS::tet (23), was kindly supplied by Dr. K. Tanaka (University of Tokyo). E. coli MA261 rpoS::tet, HT263 rpoS::tet, and DR112 rpoS::tet were isolated by transduction with P1 phage (24) using E. coli KT1100 as the donor. E. coli W3110 type A, B, and D were used in this study. TheluxA::tet strain was used as a new (type F) strain derived from type A. E. coli C600 (supE44 hsdR thi-1 thr-1 leuB6 lacY1 tolA21) was from our laboratory stock.

E. coli MA261 and MA261 rpoS::tet were grown at 37 °C in medium A supplemented with 5 amino acids (100 μg/ml each of Gly, Leu, Met, Ser, and Thr) in the presence (100 μg/ml) of absence of putrescine (9). HT263 and DR112 strains were cultured according to the method of Hafer et al. (21) and Linderoth and Morris (22), respectively, in the presence (100 μg/ml) or absence of putrescine. Antibiotics used were 100 μg/ml ampicillin, 50 μg/ml kanamycin, 30 μg/ml chloramphenicol, and 15 μg/ml tetracycline. Cell growth was monitored by measuring the absorbance at 540 nm.

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1. The abbreviations used are: SD, Shine-Dalgarno; Cya, adenylate cyclase; ORF, open reading frame.
Fig. 1. Effect of polyamines on the synthesis of ρ30 subunit of RNA polymerase in polyamine-requiring mutants of E. coli. A, Western blotting of σ subunits was performed using 10 μg of cell lysate protein for ρ30 or 5 μg of cell lysate protein for ρ31. Cell lysates were prepared from cells cultured with or without 100 μg/ml putrescine (PUT) and harvested at A600 = 0.2. B, dot blotting of RpoS (ρ30) mRNA was performed using 1, 3, 10, and 30 μg of total RNA. Total RNA was prepared as described under “Experimental Procedures” from E. coli MA261 cells cultured with or without 100 μg/ml putrescine and harvested at A600 = 0.2.

FIG. 2. Nucleotide sequence of rpoS and its encoded amino acid residues in various E. coli strains. A, nucleotide sequence of rpoS and its encoded amino acid residues in various E. coli strains are shown. B, nucleotide sequences for 31–35 amino acid residues of the ORF of rpoS gene are shown.

Plasmids—Plasmids pMRTC (pMW33TCC) and pMRTAG (pMW33TAG) (26) were kind gifts from Dr. Y. Kami (Tohoku University). Total chromosomal DNA from E. coli was prepared according to the method of Ausubel et al. (27). For construction of pMWpoS(33CAG) (pMW33CAG) or pMWpoS(270TAG) (pMW270TAG), PCR was performed using 5′-GAAGGATCCGACGTGTCAA-3′ (P1) and 5′-GACCTTGCTCAGCGCA-3′ as primers and total chromosomal DNA of W3110 type A and B, respectively, as templates. After cutting with EcoRI and HindIII, the 1.9-kb fragment was inserted into the same restriction sites of pMW119 (Nippon Gene, Japan) and pACYC184 (PerkinElmer Life Sciences).

For preparation of ρ30(33TAA)-His6, PCR was performed using MA261 chromosomal DNA as templates and 5′-AGGGAATTCGGGTA-3′ (P2) and 5′-GCGGGATCCCTCGA-3′ (P3) as primers. The 0.9-kb fragment prepared similarly to the probe described above (19). A plasmid in which the rpoS(33TAA)-His6 gene is under the control of T7 promoter was selected.

PCR and DNA Sequencing of rpoS Gene and metT Operon—PCR for rpoS gene or metT operon was performed using chromosomal DNA of various E. coli strains as templates and the primer pair of 5′-GCGAGGATTCTCGGAAGATCAGTCAACCAAACATCGAC-3′ (P5) and 5′-GAAGGATCCGACGTGTCAA-3′ (P4) as primers. After cutting with BamHI, the 0.9-kb fragment was inserted into the same restriction sites of pCMV119 (Takara Bio Inc., Japan) and pACYC184 (PerkinElmer Life Sciences).

For preparation of ρ30(33TAA)-His6, PCR was performed using MA261 chromosomal DNA as templates and 5′-AGGGAATTCGGGTA-3′ (P2) and 5′-GCGGGATCCCTCGA-3′ (P3) as primers. The 0.9-kb fragment prepared similarly to the probe described above (19). A plasmid in which the rpoS(33TAA)-His6 gene is under the control of T7 promoter was selected.

PCR and DNA Sequencing of rpoS Gene and metT Operon—PCR for rpoS gene or metT operon was performed using chromosomal DNA of various E. coli strains as templates and the primer pair of 5′-GCGAGGATTCTCGGAAGATCAGTCAACCAAACATCGAC-3′ (P5) and 5′-GAAGGATCCGACGTGTCAA-3′ (P4) as primers. After cutting with BamHI, the 0.9-kb fragment was inserted into the same restriction sites of pCMV119 (Takara Bio Inc., Japan) and pACYC184 (PerkinElmer Life Sciences).

Western Blot Analysis—Antisera against ρ30 and σ70 subunits were prepared as described previously (29). Western blot analysis was performed by the method of Nielsen et al. (30) using ProtoArray Western blotting kit (Promega).

Dot Blot and Nucleotide Sequencing of RNA—Total RNA was prepared from various E. coli strains according to the method of Emory and Belasco (31). Dot blot analysis of RpoS mRNA was performed according to the method of Sambrook et al. (32). The 1.1-kb PCR product was digested as described above with SalI and was labeled with [α-32P]dATP using BcaBEST labeling kit (Takara Bio Inc.) and used as a probe. Northern blot analysis of tRNAs encoded by the metT operon was performed using the 0.9-kb PCR product produced similarly to the probe described above (19).
**Fig. 2.** Effect of polyamines on the synthesis of $\sigma^{38}$ subunit derived from RpoS mRNA containing sense codon or nonsense codon at the 33rd position. A, structures of pMW33CAG, pMW33TCG, pMW33TAG, pMW33TGA, and pMW33TAA are shown. B, Western blotting of $\sigma^{38}$ subunit was performed using 10 $\mu$g of cell lysate protein. Cell lysates were prepared from cells cultured with or without 100 $\mu$M putrescine (PUT) and harvested at A$_{540}$ = 0.2.

**Fig. 3.** Effect of polyamines on the synthesis of $\sigma^{38}$ subunit derived from RpoS mRNA containing amber codon at the 270th position. A, structures of pMW33CAG, pMW33TCG, pMW33TAG, pMW33TGA, and pMW33TAA are shown. B, Western blotting of $\sigma^{38}$ subunit was performed using 10 $\mu$g of cell lysate protein. Cell lysates were prepared from cells cultured with or without 100 $\mu$M putrescine (PUT) and harvested at A$_{540}$ = 0.2.

**RESULTS**

Polyamine Stimulation of the Synthesis of RNA Polymerase $\sigma^{38}$ Subunit Based on the Readthrough of an Amber Codon in the Open Reading Frame (ORF) of RpoS mRNA—We reported previously that synthesis of the RNA polymerase $\sigma^{38}$ subunit was stimulated by polyamines using one of the polyamine-requiring mutants, MA261 (19). To confirm this observation, we reported previously that synthesis of the RNA polymerase $\sigma^{38}$ subunit was stimulated by polyamines using one of the polyamine-requiring mutants, MA261 (19). To confirm this observation,
the effect of polyamine addition on the synthesis of $\sigma^{38}$ subunit was examined using three independent isolates of polyamine-requiring E. coli mutants, MA261, HT283, and DR112 (20–22). As shown in Fig. 1A, the increased synthesis of $\sigma^{38}$ subunit was observed for MA261 and HT283 but not for DR112. The level of $\sigma^{70}$ subunit was, however, nearly equal for all three mutants and was not affected by the addition of polyamines (Fig. 1A). The level of RpoS mRNA in E. coli MA261, as measured by dot blotting, was nearly equal in the presence and absence of polyamine addition (Fig. 1B), indicating that the stimulation of $\sigma^{38}$ synthesis by polyamines takes place at a post-transcriptional step(s).

To understand why the polyamine stimulation takes place in only two strains (MA261 and HT283) among three polyamine-requiring mutants, the nucleotide sequence of the $rpoS$ gene was determined. As shown in Fig. 2, a TAG termination codon (instead of CAG codon for glutamine) was present at the 33rd position of the ORF of the $rpoS$ gene in MA261 and HT283. We also determined the nucleotide sequence of the $rpoS$ gene in two kinds of E. coli W3110 from our laboratory stock. One (type D) has a CAG codon for glutamine, but the other (type F, new type) has the termination codon TAG. Together these results suggest that a high incidence of mutations are accumulated at the 33rd codon of the $rpoS$ gene.

The three E. coli strains, MA261, HT283, W3110 (type F), carry the TAG amber codon at the 33rd position of the $rpoS$ gene, suggesting that these strains carry a suppressor for UAG and that the efficiency of suppression is enhanced by polyamines. To test the UAG suppression of the mutant $rpoS$ gene in MA261 (one of the three mutants) we constructed an MA261 derivative with $rpoS$ disrupted by insertion of the tet gene, and we tested the expression of a plasmid-encoded $\sigma^{38}$ after transfection of various plasmids containing the $rpoS$ gene with various mutations at the 33rd codon. In the presence of polyamine addition, the level of $\sigma^{38}$ subunit increased only for the $rpoS$ mutant with a UAG amber termination codon at the 33rd position of RpoS mRNA (Fig. 3B, 33TAG), whereas the $\sigma^{38}$ level remained unaltered for the $rpoS$ mutant with a UGA codon at the same position (Fig. 3B, 33TGA). The $\sigma^{38}$ subunit was not detected for the mutant $rpoS$ gene with the UAA termination codon (Fig. 3B, 33TAA), suggesting that the MA261 strain does not carry the UAA suppressor.

The expression level of $\sigma^{38}$ was essentially the same in the presence and absence of polyamines for the $rpoS$ gene with CAG (Gln) or UCG (Ser) codons at the 33rd position of RpoS mRNA (Fig. 3B, 33CAG and 33TGC). Taken together we conclude that neither CAG-dependent Gln-tRNA nor UCG-dependent Ser-tRNA binding to ribosomes nor readthrough of the UGA termination codon were influenced by polyamines.

One of the W3110 strains (type B) carries a UAG termination codon at the 270th position of the RpoS mRNA (25). We checked whether polyamines enhance the level of suppression at this position. As shown in Fig. 4, polyamines enhanced the synthesis of full-length $\sigma^{38}$ subunit from the mutant RpoS mRNA.
about 2-fold in MA261 rpoS::tet carrying pMW270TAG, indicating that polyamines stimulate the suppression of the UAG codon on the rpoS gene regardless of the position of UAG codon. The percentage of readthrough at the 270th position, i.e. the ratio of full-length σ^{38} to the COOH-terminal truncated σ^{38}, in the presence and absence of polyamines was −18 and 11%, respectively. The stimulation of the amber mutant rpoS expression by polyamines was, however, not observed when the same
quences of the tRNAGln genes for all three mutants. The nature of the amber suppressor in each of the three polyamine-requiring mutants, we determined the nucleotide sequences of the tRNA Gln genes for all three mutants. The nucleotide sequences of the tRNA GlnU, tRNAGlnW, and tRNAGlnV, exist in the metT operon (40). A commonly occurring suppressor tRNA for the amber termination codon in E. coli is encoded by the supE gene, which can be generated after mutation in either glnV or glnX of the metT operon. To identify the nature of the amber suppressor in each of the three polyamine-requiring mutants, we determined the nucleotide sequences of the tRNA Gln genes for all three mutants. The nucleotide sequences of the tRNA GlnU, tRNAGlnW, and tRNAGlnV genes were the same among the three polyamine-requiring mutants (data not shown). However, the tRNA GlnX gene was changed to the tRNA supE gene in both MA261 and HT283 but not in DR112 (Fig. 5). These results indicate that tRNA supE is involved in the stimulation of $\sigma^{38}$ subunit synthesis by polyamines.

**Mechanism of Polyamine Stimulation of Readthrough of the Amber Termination Codon in RpoS mRNA**—One possible mechanism of the enhancement of amber suppression by polyamines is an increase in the level of suppressor tRNA. To test this possibility, we first measured the level of tRNA supE in E. coli MA261. Total cellular RNA was subjected to Northern blot hybridization using a probe that hybridizes to both tRNA supE and tRNA GlnV. As shown in Fig. 6A, the combined level of tRNA supE and tRNA GlnV in E. coli MA261 was higher for the culture with polyamines than that without polyamines. This was confirmed by Northern blot analysis of the combined levels of all seven species of tRNA (see Fig. 5A) encoded by the metT operon (Fig. 6B). The level was found to be 1.8× higher in the presence of polyamines than that found in its absence. Most of the tRNA supE and tRNA GlnV was aminoacylated in E. coli MA261 cultured with or without the addition of polyamines (Fig. 6A). These results suggest that polyamines stimulate transcription of the metT operon or stabilize the tRNAs.

The effects of high-level tRNA supE on the polyamine stimulation of the synthesis of $\sigma^{38}$ subunit were then examined. As shown in Fig. 6C, the degree of stimulation of the synthesis of $\sigma^{38}$ subunit by polyamines became smaller in both early and middle logarithmic phases by transforming a high-copy number plasmid (pUC184) but not in DR112 (Fig. 5). These results indicate that tRNA supE is involved in the stimulation of $\sigma^{38}$ subunit synthesis by polyamines.

**Possible effects of polyamines on mutant RpoS(33UAG)**

mRNA-dependent synthesis in vitro of the $\sigma^{38}$ subunit were then analyzed by measuring the incorporation of [35S]methionine into the TCA-insoluble fraction. As shown in Fig. 7B, 1 mM spermidine significantly stimulated the overall activity of protein synthesis. Because the protein synthesis activity in the in vitro translation system employed depends on the addition of externally added mRNA (data not shown), the result indicates the stimulation of $\sigma^{38}$ subunit synthesis by spermidine. To confirm this interpretation the protein products were analyzed by SDS-PAGE, and the gels were subjected to fluorography. Fig. 7C shows one of the fluorograms, which indicates that the band intensity of $\sigma^{38}$ subunit increased, albeit at low levels, in the presence of spermidine.

Stimulation of poly(U)-dependent polyphenylalanine synthesis by polyamines is attributable to the increase in aminoacyl-tRNA binding to ribosomes but not at the levels of peptide bond formation and translocation (5). Thus, we first examined the effect of polyamines on mRNA-independent binding of Gln-tRNA supE to ribosomes. Although the binding activity was low, it was clearly stimulated by 1 mM spermidine (Fig. 7D), indicating that the affinity of Gln-tRNA supE to ribosomes is increased by polyamines. Then, the effect of polyamines on AUGUAG-dependent binding of Gln-tRNA supE was examined. Polyamines also enhanced Gln-tRNA supE binding to ribosomes (data not shown). These results, taken together, suggest that polyamines stimulate the synthesis of $\sigma^{38}$ subunit through at least two steps; (i) the increased level of tRNA supE and (ii) the increased binding of Gln-tRNA supE to ribosomes.

**Physiological Significance of Polyamine Stimulation of Suppression of Amber Mutation in RpoS mRNA**—The rpoS gene is essential for cell viability in the stationary phase (41). Thus, we compared cell viability between MA261 cells cultured with or without putrescine. The rate of cell growth was enhanced by...
Putrescine as reported (17). When rpoS was disrupted, cell growth slowed slightly, but polyamine enhanced cell growth greatly (Fig. 8A). As for cell viability, determined by colony formation on a rich plate, it increased greatly when the cells were cultured in the presence of putrescine. However, cell viability of MA261 cultured in the absence of putrescine was higher than that of MA261 in which rpoS was disrupted (Fig. 8B). Cell viability was parallel with the level of \( \sigma^{38} \) subunit (Fig. 8C). The results suggest that elevated expression of rpoS gene is important for cell viability.

**DISCUSSION**

Polyamines stimulate the synthesis of a set of proteins such as oligopeptide-binding protein (OppA) (17), adenylate cyclase (Cya) (19), and \( \sigma^{38} \) subunit (RpoS) (this paper). Up to now two different mechanisms have been identified: a structural change of OppA mRNA, leading to enhanced template activity for translation (17), and stimulation of initiation codon UUG-dependent fMet-tRNA binding to Cya mRNA-ribosomes (19). The results herewth described show a novel mode of the polyamine stimulation of protein synthesis. Polyamines stimulate readthrough of the amber codon by enhancing the binding of amber codon UAG-dependent Gln-tRNA\textsuperscript{supE} on ribosome-associated RpoS mRNA (see Fig. 9). Thus polyamines modulate protein synthesis not only at the level of initiation but also at the level of elongation of translation. We propose that genes whose expression is modulated by polyamines at the level of translation are referred to as “polyamine modulon.” Experiments are in progress to find other members of the polyamine modulon.

The increase in readthrough of the amber codon by polyamines has been found for translation of the mutant gene 1 protein mRNA from T7 phage (42). Stimulation of readthrough of both UAG amber and UGA opal codons by polyamines also has been reported in E. coli and eukaryotic cell-free systems (43–45). In these cases, however, the mechanism was not studied in detail. As for polyamine stimulation of the synthesis of \( \sigma^{38} \) subunit, the translation suppression was found to be caused by stimulation of Gln-tRNA\textsuperscript{supE} binding to ribosomes via two processes, i.e. an increase in the level of tRNA\textsuperscript{supE} and an increase in the binding affinity of Gln-tRNA\textsuperscript{supE} to ribosomes. As to the increase in tRNA\textsuperscript{supE} level, it is of interest to know whether polyamines stimulate transcription of the metT operon or stabilize tRNA\textsuperscript{supE}. If the latter is the case, the increase in both intracellular level and intrinsic function of tRNA\textsuperscript{supE} can be explained by a structural change of tRNA\textsuperscript{supE} by polyamines.

Readthrough in vivo of the UGA opal codon at the 33rd codon of RpoS mRNA was not stimulated by polyamines in cells under our experimental conditions (see Fig. 3). The frequency of the use of UAG, UGA, and UAA as the termination codon in E. coli is 7.6, 29.3, and 63.1%, respectively (46). Furthermore, 316 among 4288 genes in E. coli K12 use tandem termination codons (47). Because not so many genes use UAG as the real termination codon at the end of full-length reading frames, the...
gene expression as a whole may not be influenced strongly by polyamines even if polyamines stimulate the readthrough of UAG at the natural termination sites.

We found that a mutation at the 33rd position of the ORF of RpoS mRNA occurs frequently. In such strains, polyamines enhance cell viability. The results confirmed that the σ38 subunit is important for cell viability (41) and indicate that polyamines play an important role in cell viability of E. coli cells having an amber codon at the 33rd position of the ORF of RpoS mRNA.

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