Enhancement of +1 Frameshift by Polyamines during Translation of Polypeptide Release Factor 2 in *Escherichia coli*

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Polyamines (putrescine, spermidine, and spermine), aliphatic cations that mostly exist as polyamine-RNA complexes in cells (4, 5), their proliferative effects are presumed to be caused by stimulation of nucleic acid growth (1–3). Because polyamines interact with nucleic acids and are necessary for normal cell growth, it is concluded that genes whose expression is enhanced by polyamines at the level of translation can be classified as a “polyamine modulon.”

In the case of *RpoS* (σB) synthesis, UAG amber codon-dependent Gln-tRNA<sup>ω</sup> binding to ribosomes at the 33rd position of open reading frame (ORF) of *rpoS* mRNA was enhanced by polyamines (19). The termination codon in the ORF of mRNA can be decoded not only by readthrough but also by +1 frameshift. In mammalian cells, it has been reported that antizyme (AZ) mRNA has the termination codon UGA at the 69th position of its ORF, and the +1 frameshift is strongly enhanced by polyamines (21, 22). AZ is involved in the negative regulation of polyamine content in cells by stimulating the degradation of ornithine decarboxylase and by inhibiting the polyamine uptake (23–26). In *E. coli*, polypeptide release factor 2 (RF2), which recognizes UAA as termination codons, is known to be synthesized by a +1 frameshift at the 26th UGA termination codon of RF2 mRNA (27, 28). In this study, we examined whether the +1 frameshift of RF2 mRNA is enhanced by polyamines.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Culture Conditions—Polyamine-requiring mutants** *E. coli* DR112 (speA speB thi) (29) and MA261 (speB specA thr leu thi) (16) were kindly provided by Dr. D. R. Morris (University of Washington) and by Dr. W. K. Maas (New York University School of Medicine), respectively. For growth of DR112, medium A (4 g of glucose, 6 g of Na<sub>2</sub>HPO<sub>4</sub>, 3 g of KH<sub>2</sub>PO<sub>4</sub>, 500 mg of NaCl, 1 g of NH<sub>4</sub>Cl, 250
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mg of MgSO₄·7H₂O, 13 mg of CaCl₂, 2 mg of thiamine, 100 mg each of alanine, asparagine, aspartic acid, glutamic acid, glycin, methionine, proline, serine, and threonine, and 50 mg each of cysteine, histidine, isoleucine, leucine, phenylalanine, tyrosine, and valine, 5 mg of tryptophan, and 1 g of arginine/liter of water) was used. An ornithine deficiency was achieved by the addition of 1 mg of arginine/ml to the medium. For growth of MA261, medium B (4 g of glucose, 7 g of K₂HPO₄, 3 g of KH₂PO₄, 500 mg of sodium citrate, 1 g of (NH₄)₂SO₄, 100 mg of MgSO₄·7H₂O, 2 mg of thiamine, 10 mg of biotin, 100 mg each of leucine, threonine, methionine, serine, glycine, and ornithine/liter of water) was used. The cells were cultured in the absence or presence of 100 µg/ml putrescine dihydrochloride. Cell growth was monitored by measuring the absorbance at 540 nm.

**Plasmids**—Plasmid pMAL-c2 to express foreign proteins by fusion to maltose-binding protein (30) was kindly supplied by Dr. S. Yasuda (National Institute of Genetics, Mishima, Japan). Total chromosomal DNA from *E. coli* W3110 was prepared according to the method of Ausbel et al. (31). To make the *malE-prfB* fusion gene, PCR was performed using total chromosomal DNA as template, and 5′-CAGACCATGTGTTAGTTCAATCCGGTA-3′ (P1) and 5′-TGGTGCGCCTTCGTTGGGACATGTTCACTGC-3′ (P2) as primers. To make the *malE-dnaX* fusion gene, PCR was performed using total chromosomal DNA as template, and 5′-ACTGTTACGGCGCCGGAATTCCAGACACGCGCATGGATCCTAGCTC-3′ (P3) and 5′-AACCGGATCGTCCGACGGAGCCGAGCTTTC-3′ (P4) as primers. The amplified *prfB* gene (a 6-nucleotide 5′-upstream region and a 162-nucleotide open reading frame) and *dnaX* gene (nucleotides 1204–1371; nucleotide 1 is defined as the A in the initiation codon ATG) were digested with EcoRI and SalI and inserted into the same restriction sites of pMAL-c2 to make pMAL-RF2 and pMAL-DnaX. For construction of pUC-malE-RF2 (UGA), pUC-malE-DnaX, *malE-prfB* and *malE-dnaX* fusion genes were amplified using pMAL-RF2 and pMAL-DnaX as templates and 5′-TCAGGATCCCTTCACCAA-3′ (P5) and 5′-CTGGATCCGGCTGAAATTAC-3′ (P6) as primers. After cutting with BamHI, the 1.6-kb PCR fragment was inserted into the same restriction sites of pUC119 (Takara Shuzo Co.). Site-directed mutagenesis by overlap extension using PCR (32) was performed to prepare pUC-malE-RF2 (UAG), pUC-malE-RF2 (UAA), pUC-malE-RF2 (UGG), pUC-malE-RF2 (SD+), and pUC-malE-RF2 (SD−). To make pUC-malE-RF2 (UAG), the first PCR was performed using pMAL-RF2 as template and P5 and 5′-TTGGTGCGTCTAGCTAAGATACCCCTCT-3′ (underlined base for UGA substitution with UAG) as primers. The second PCR was performed using the first PCR products as templates and P5 and P6 as primers. To make pUC-malE-RF2 (UAA), pUC-malE-RF2 (UGG), pUC-malE-RF2 (SD+), and pUC-malE-RF2 (SD−), PCR was performed using the same method. After cutting with BamHI, the 1.6-kb PCR fragment was inserted into the same restriction site of pUC119. Plasmids in which *prfB* or the fusion gene is under the control of lacUV5 promoter were selected.

For preparation of pBS-RF2, PCR was performed using total chromosomal DNA as template and 5′-TCACCGCATGTCCCTAGCTCAATAAAAAGA-3′ (P7) and 5′-TGGTGCTGGATCCGACATGTTCGTTCTCA-3′ (P8) as primers. After cutting with BamHI, the 1.1-kb PCR fragment was inserted into the same restriction site of pBluescript II SK+. (Toyobo). To make pBS-malE-RF2 (UGA), pBS-malE-RF2 (SD+), and pBS-malE-DnaX, PCR was performed using pMAL-RF2, pUC-malE-RF2 (SD−), and pMAL-DnaX as templates and P5 and P6 as primers. After cutting with BamHI, the 1.6-kb PCR fragments were inserted into the same restriction site of pBluescript II SK+. Plasmids in which *prfB* or the fusion gene is under the control of T7 promoter were selected.

The plasmid pMW-OppA(UGA) encoding *oppA* having UGA as termination codon was prepared from pMW-OppA(UAA) having UAA as termination codon (pMW975 in Ref. 14) with site-directed mutagenesis by overlap extension using PCR (32). The nucleotide sequence of the plasmids was confirmed by the Gene Rapid System (Amersham Biosciences).

**Western Blot Analysis**—Western blot analysis was performed by the method of Nielsen et al. (33) using ECL™ Western blotting reagents (Amersham Biosciences). Antibody against RF2 (34) was kindly sup-

![Figure 1](image-url)
Polyamines Enhance the Frameshift of RF2 Synthesis

A

**malE-reporter system**

*malE-RF2 (UGA)*

mRNA

frameshift site (133-mer)

translation

+1 frameshift (+1 FS)

readthrough (RT)

termination (TERM)

55 KDa

50 KDa

45 KDa

B

|                | MA261/ pUC-malE-RF2 (UGA) | DR112/ pUC-malE-RF2 (UGA) |
|----------------|---------------------------|---------------------------|
| **A540**       | 0.2                       | 0.2                       |
| **PUT**        | +                        | +                        |
| **RT**         | +                        | +                        |
| **TERM**       | +                        | +                        |
| **Efficiency (%)** |                  |                  |
| **+1FS/total** | 7.3 14.7                  | 9.1 20.4                  |
| **Polyamine Stimulation (-fold)** | 2.0                  | 2.2                       |

 identification of spermidine cross-linked to 30 S ribosomal proteins through dimethyl suberimidate—Preparation of salt (0.5 m N4HCl)-washed ribosomes from *E. coli* Q13 (rna pnp) and 30 S ribosomal subunits from ribosomes were carried out as described previously (42, 43). The reaction mixture (0.1 ml) for spermidine binding to 30 S ribosomal subunits contained 20 mM Tris-HCl (pH 7.5), 10 mM magnesium acetate, 100 mM N4HCl, 3 A260 units of 30 S ribosomal subunits, 1 mM [14C] spermidine (148 kBq), and antibiotics at the specified concentration. After incubation at 30 °C for 30 min, an equal volume (0.1 ml) of cross-linking reagent containing 66 mM sodium borate, 10 mM magnesium acetate, 100 mM N4HCl, and 45 mM dimethyl suberimidate, was added to the reaction mixture, the final pH being 8.2. Further incubation was performed at 30 °C for 30 min. Ribosomal proteins were extracted with 66% acetic acid and 30 mM magnesium acetate (44). The supernatant, obtained by centrifugation at 15,000 rpm for 10 min, was then treated with 5 volumes of acetone. The precipitate thus obtained was dissolved in the sample loading buffer containing 6 M urea and 50 mM 2-mercaptoethanol. Ribosomal proteins were analyzed by two-dimensional gel electrophoresis; the first dimension gel electrophoresis was run in an acidic system, and the second dimension separation was carried out in the presence of SDS (45). Identification of the individual proteins was according to the nomenclature proposed by Baltzschmidt and Wittman (45). The radioactivity incorporated into each of the ribosomal protein was quantified using a Fuji BAS-2000II imaging analyzer (Fuji Film).

Identification of Spermidine Cross-linked to 30 S Ribosomal Proteins

**RESULTS**

Effect of Polyamines on in Vivo +1 Frameshift of RF2 Synthesis—It is well known that AZ synthesis involves a +1 frameshift at the 69th termination codon of ORF of AZ mRNA in mammalian cells, and the efficiency of this frameshift is enhanced by polyamines (21, 22). Because RF2 in *E. coli* is also synthesized by a +1 frameshift at the 26th termination codon (Fig. 1A) (27, 28), the effect of polyamines on the efficiency of this frameshift was examined using the polyamine-requiring mutants MA261 and DR112. When these two mutants were cultured in the presence of 100 μg/ml putrescine, polyamines (putrescine and spermidine) in cells increased, and cell growth became faster (data not shown). The level of RF2 was increased ~1.7-fold by polyamines only at the early

Effect of polyamines on +1 frameshift of RF2 synthesis in the translational termination/frameshift assay system. A, three products in the translation termination/frameshift assay system. B, MA261 and DR112 cells transformed with pUC-malE-RF2 (UGA) were cultured with or without putrescine and harvested at A540 = 0.2. Three products were analyzed by Western blotting using antibody against maltose-binding protein, and 8 or 24 μg of protein of cell lysate prepared from cells cultured with or without putrescine. The experiments were repeated three times, and the S.E. was within ±10%.

**FIGURE 2. Effect of polyamines on +1 frameshift of RF2 synthesis in the translational termination/frameshift assay system.** A, three products in the translation termination/frameshift assay system. B, MA261 and DR112 cells transformed with pUC-malE-RF2 (UGA) were cultured with or without putrescine and harvested at A540 = 0.2. Three products were analyzed by Western blotting using antibody against maltose-binding protein, and 8 or 24 μg of protein of cell lysate prepared from cells cultured with or without putrescine. The experiments were repeated three times, and the S.E. was within ±10%.
logarithmic phase (Fig. 1B), which is the important phase with the regard to the overall rate of cell growth. The level of RF2 increased with the progression of cell growth and the effect of polyamines declined. The level of RF2 mRNA was nearly equal in cells cultured with or without putrescine (Fig. 1C). As a control, the level of /H9268, one of the /H9268 subunits of RNA polymerase, was measured, and it was not increased by polyamine (data not shown). The results suggest that the 1 frameshift of RF2 synthesis is enhanced by polyamines and negatively regulated by RF2 (28).

The effects of polyamines were then examined using translational termination/frameshift assay system (46). In this system, three kinds of products can be identified by antibody against maltose-binding protein; those are: a 1 frameshift product (55 kDa), a readthrough product (50 kDa), and a termination product (45 kDa) (Fig. 2A). Efficiency of the +1 frameshift is shown in Fig. 2B.

FIGURE 3. Effect of codon variation at the 26th position of RF2 ORF (A and B) and the SD-like sequence of RF2 mRNA (C and D) on polyamine stimulation of +1 frameshift in the translational termination/frameshift assay system in DR112 cells. A, amino acid sequence around the termination codon encoded by various mutated malE-RF2 mRNAs. B and D, DR112 cells transformed with pUC-malE-RF2 (UGA), pUC-malE-RF2 (UAG), pUC-malE-RF2 (UAA), pUC-malE-RF2 (UGG), pUC-malE-RF2 (SD+), and pUC-malE-RF2 (SD−) were cultured with or without putrescine and harvested at A540 = 0.2. Western blotting was performed as described in the legend to Fig. 2. The experiments were repeated three times, and the S.E. was within ±10%.
frameshift was calculated as the percentage of the level of +1 frameshift product divided by the total level of three products. As shown in Fig. 2B, the +1 frameshift of RF2 was enhanced by polyamines 2.0–2.2-fold. The polyamine effect was greater in this assay system, probably because of the existence of higher level of mRNA synthesized from the multi-copy plasmid. The results confirm that the +1 frameshift of RF2 synthesis is enhanced by polyamines.

Factors that influence polyamine enhancement of +1 frameshift were then examined using *E. coli* DR112. The termination codon (UGA) at the 26th codon of ORF of RF2 mRNA was changed to other termination codons, UAG and UAA, or to UGG for Trp (Fig. 3A). However, polyamine enhancement of the +1 frameshift was not influenced by changing this codon (Fig. 3B). The influence of a SD-like sequence at the 5′-end of the termination codon was also studied. If the SD-like sequence was present, polyamine enhancement of the +1 frameshift was observed regardless of the nucleotide sequence (AGGGGG and AGGAGG). When the SD-like sequence was changed to a non-SD-like sequence (AGGGAG), the +1 frameshift decreased greatly (Fig. 3, C and D). Thus, it remains to be clarified whether polyamines are involved in the interaction between the SD-like sequence and 16 S rRNA.

**Effect of Spermidine, RF2, and SD-like Sequence on in Vitro +1 Frameshift of RF2 Synthesis**—An in vitro DNA-dependent protein synthetic system (39) was used to study the factors influencing the +1 frameshift of RF2 synthesis. As shown in Fig. 4A, RF2 synthesis was enhanced 1.8-fold by 1.5 mM spermidine. Confirming this, spermidine, but not Mg2+, enhanced the +1 frameshift of RF2 synthesis in a translational termination/frameshift assay (Fig. 4, B and C). The effect of RF2 on the +1 frameshift of RF2 synthesis was then examined. As shown in Fig. 4 (D and E), the addition of RF2 reduced +1 frameshift of RF2 synthesis and reduced the polyamine enhancement of this frameshift.

Because it was not clear in an *in vivo* assay system whether polyamines function at the level of the interaction between the SD-like sequence and 16 S rRNA, this was examined in an *in vitro* system. When the SD-like sequence was absent, the efficiency of the +1 frameshift greatly decreased. However, polyamine enhancement of the frameshift was still observed (Fig. 4F). The results suggest that polyamines may act on 30 S ribosomal subunits rather than on RF2 mRNA. We next tested whether AZ synthesis was enhanced by polyamines in an *in vitro* *E. coli* cell-free system. Although AZ mRNA does not have the SD-like sequence (22) and efficiency of +1 frameshift was low, spermidine
FIGURE 5. Effect of various antibiotics on polyamine stimulation of +1 frameshift in the translational termination/frameshift assay system in an E. coli cell-free system. The malE-RF2 (UGA) mRNA derived protein synthesis in DNA-dependent transcription and translation reactions was performed in the presence and absence of antibiotics and spermidine shown in the figure, and the efficiency (%) of the +1 frameshift of RF2 synthesis was shown. Exposure time (h) in a Fuji BAS-2000II imaging analyzer and total protein synthetic activity (%) are also shown in the figure. The experiments were repeated three times, and the S.E. was within ±15%. A, streptomycin; B, neomycin; C, tetracycline; D, spectinomycin; E, pactamycin; F, edeine; G, the binding site of antibiotics shown on 16S ribosomal RNA was cited from Refs. 45–47 and 51.
clearly enhanced +1 frameshift of synthesis (data not shown), suggesting again that polyamines may act on 30 S ribosomal subunits.

Effect of Various Antibiotics on Polyamine Stimulation of in Vitro +1 Frameshift of RF2 Synthesis—This was examined using an in vitro translational termination/frameshift assay system. As shown in Fig. 5, polyamine stimulation of the +1 frameshift of RF2 was decreased by the addition of streptomycin, neomycin, tetracycline, and spectinomycin, which act at the A site of 30 S ribosomal subunits (47–49), in parallel with the decrease in protein synthesis (Fig. 5, A–D). However, polyamine stimulation of frameshifting was not affected by edeine and pactamycin, which act at the P or E site of 30 S ribosomal subunits (49, 50), although protein synthetic activity decreased greatly (Fig. 5, E and F). The results suggest that structural change at the A site of 30 S ribosomal subunits by polyanines is important for polyamine stimulation of the +1 frameshift of RF2 synthesis.

Polynamine stimulation of the +1 frameshift of RF2 was more strongly affected by spectinomycin and tetracycline than by streptomycin and neomycin (Fig. 5, A–D). It has been reported that spectinomycin and tetracycline bind close to the A site (Fig. 5G) (47–49). Ribosomal proteins S4 and S5 are also located close to the A site (Fig. 6E) (52, 53). Thus, the level of spermidine bound to the A site of 30 S ribosomal subunits was measured in the presence and absence of spectinomycin through spermidine cross-linking to 30 S ribosomal proteins by dimethyl suberimidate (44). As shown in Fig. 6 (A–D), spermidine cross-linked to S4 was decreased in the presence of spectinomycin. However, we could not estimate the spermidine level cross-linked to S5 because spermidine cross-linked to S5 was very low. Similar results were obtained with 1 mM tetracycline (data not shown). Along these lines, the inhibition of growth of a polyamine requiring mutant DR112 by spectinomycin and tetracycline was reduced by polyamines (data not shown). These results indicate that the level of spermidine bound to the A site of 30 S ribosomal subunits decreased in the presence of spectinomycin and tetracycline and vice versa. Thus, it is deduced that the decrease in polyamine stimulation of the +1 frameshift of RF2 synthesis by these antibiotics is due to a decrease in spermidine bound to the A site of 30 S ribosomal subunits.
Polyamines Enhance the Frameshift of RF2 Synthesis

A. Functional category of 2714 detected genes

B. Usage frequency of termination codon (%)

Analysis of mRNA Species Enhanced by Polyamines at the Early Logarithmic Phase—Experiments were carried out to study the physiological significance of the stimulation of +1 frameshift of RF2 synthesis at the early logarithmic phase. In these studies, mRNAs that are up-regulated by polyamines were identified by DNA microarray analysis. It has been reported that recognition of the termination codon UAA by RF2 is weaker than that of UGA (51). At the early logarithmic phase, expression of 347 genes was enhanced by polyamines more than 2-fold among 2,714 detected genes at $A_{540}=0.15$. In particular, expression of the genes in the categories of energy production and translation were enhanced by polyamines (Fig. 7A). Because expression of genes involved in translation such as ribosomal proteins and elongation factors of protein synthesis was not significantly enhanced by polyamines at the middle logarithmic phase ($A_{540}=0.5$) (20), the usage frequency of UAA as a termination codon was investigated in the mRNAs involved in translation. As shown in Fig. 7B, the usage frequency of UAA as a termination codon among the mRNAs expressed at the early logarithmic phase was 65.1%, and that among the mRNAs up-regulated by polyamines was 69.5%. However, the usage frequency of UAA as a termination codon among the mRNAs involved in translation was 82.3%, and that in the mRNAs up-regulated by polyamines was 93.9%. Thus, it was tested whether protein synthesis from mRNA having UAA as termination codon was more strongly enhanced by polyamines at the level of translation than that from the mRNA having UGA as termination codon. The level of both OppA mRNAs was enhanced by polyamines by 1.8–1.9-fold (Fig. 8). These results suggest that the mRNAs having UAA as a termination codon were more efficiently translated through quick termination of the synthesis of these proteins. Synthesis of ribosomal protein L20 and EF-G from the mRNAs having UAA termination codon was actually enhanced by polyamines at the level of not only transcription but also translation, i.e. the level of these mRNAs was enhanced by polyamines 2.1-fold (Fig. 8B). As a control, synthesis of $\sigma^{24}$ subunit of RNA polymerase from the mRNA having a UGA termination codon was measured. The level of both $\sigma^{24}$ subunit protein and its mRNA was enhanced by polyamines 1.2-fold (data not shown), i.e. synthesis of the $\sigma^{24}$ subunit protein was not enhanced by polyamines at the level of translation.

Effect of Polyamines on +1 Frameshift of DnaX mRNA—In E. coli, one more protein is synthesized by the frameshift, that is, the $\gamma$ subunit of DNA polymerase III holoenzyme is synthesized by the +1 frameshift of DnaX mRNA (54). The effect of polyamines on +1 frameshift of DnaX mRNA was examined using in vivo and in vitro systems. As shown in Fig. 9, efficiency of the +1 frameshift in vivo is very high even in the absence of polyamines (80–90% in both MA261 and DR112 strains), and polyamines did not influence the +1 frameshift of DnaX mRNA in vivo and in vitro.

FIGURE 7. Identification of genes whose expression was enhanced by polyamines at the level of transcription (A) and the usage frequency of termination codon (B). A, DNA microarray analysis was performed as described under “Experimental Procedures.” The data were the average of three experiments. Classification of genes by functional category was carried out using the data base of GenoBase (ecoli.aist-nara.ac.jp/gb4/search/function/offfunc.php). Among the 15 categories, a category labeled with red letters means that the percentage of the category in genes up-regulated in the presence of putrescine increased >1.5-fold compared with the percentage in 2,714 detected genes. B, the usage frequency of termination codons. The percentage of three kinds of termination codon used in the mRNAs was calculated using the data base of GenoBase.
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**A**  
Protein synthesis

| Protein synthesis | mRNA |
|--------------------|------|
| **Polyamines Stimulus (fold)** | **mRNA** |
| PUT OppA | PUT OppA |
| MA261 ΔoppA/pMW-OppA | MA261 ΔoppA/pMW-OppA |
| UAA | UGA | PUT | PUT |
| 100 | 710 | 134 | 675 |

**DISCUSSION**

Our results indicate that the +1 frameshift of RF2 synthesis in *E. coli* was enhanced by polyamines. However, this was only observed at the early logarithmic phase of cell growth, because synthesis of RF2 is autoregulated by RF2 protein (28), and the level of RF2 was increased with the progress of cell growth. It is also well known that polyamines enhance the +1 frameshift of AZ synthesis in mammalian cells (22) and yeast (55). Polyamine enhancement of the +1 frameshift in synthesis of RF2 and AZ seems rational. When the level of RF2 is low at the early logarithmic phase, polyamines stimulate RF2 synthesis to release the completed protein from ribosomes rapidly. When high levels of polyamines become potentially toxic to cells, AZ is induced to decrease the level of polyamines in eukaryotic cells. AZ is known to stimulate degradation of ornithine decarboxylase, a rate-limiting enzyme in polyamine biosynthesis (23, 24), and to inhibit polyamine uptake (25, 26). It should be noted, however, that the +1 frameshift at CUU codon of *gag-pol* protein for transposition of Ty1 in yeast is decreased by polyamines (56).

With regard to −1 frameshifting, the synthesis of γ subunit of DNA polymerase III holoenzyme (this work) and *gag-pol* fusion protein of the L-A double-stranded RNA virus in yeast (56) is not affected by polyamines. The results suggest that the mechanism of +1 and −1 frameshifting on ribosomes is different.

For the +1 frameshift in RF2 synthesis, an SD-like sequence was necessary to slow down the speed of translation. However, an SD-like sequence did not affect polyamine stimulation of the +1 frameshift. Therefore, we thought that polyamines may act on ribosomes rather than mRNA to stimulate +1 frameshifting. Indeed, spectinomycin and tetracycline, which bind to the A site of 30 S ribosomal subunits (47–49), reduced the polyamine enhancement of +1 frameshifting. However, pactamycin and edeine, which bind closely to the interaction site with an SD-like sequence of RF2 mRNA on 30 S ribosomal subunits, did not influence the +1 frameshifting. The results strongly suggest that spermidine binds to and causes a structural change in the A site to enhance the +1 frameshift.

It has been reported that premature release of the E site tRNA from the ribosomes is coupled with high level frameshifting (57). Our results suggest that polyamines do not stimulate premature release of the E site tRNA, because edeine and pactamycin, which act at the P or E site of 30 S ribosomal subunits, did not influence polyamine stimulation of the +1 frameshift of RF2 synthesis.

Polyamines preferentially bind to double-stranded RNA, especially GC-rich double-stranded RNA (4, 58). We have also shown that the existence of a GC-rich double-stranded region close to SD sequence of OppA mRNA is important for spermidine stimulation of fMet-tRNA binding to ribosomes (59). It is also estimated that ~10–15% of phosphate in RNA interacts with polyamines in cells (4, 5), and the binding site of polyamines on 16 S rRNA has been determined (60). Several binding sites of polyamines and those of antibiotics acting at the A site of 30 S ribosomal subunits were close to each other, and the binding of spermidine at the A site was inhibited by these antibiotics judging from the decrease in cross-linked spermidine to S4 protein. Our results suggest that spermidine binding to the A site is important for spermidine stimulation of +1 frameshifting during RF2 synthesis. Because RF2 also binds close to the A site (61), the binding of RF2 probably inhibits the +1 frameshift by competing with spermidine binding to the A site. These results, taken together,
suggest that a structural change of the decoding site by polyamines is important for the +1 frameshift of RF2 synthesis, and antibiotics acting on the A site and RF2 disturb the polyamine stimulation of the +1 frameshift through their binding to the decoding site.

It is noted that expression of many mRNAs encoding ribosomal proteins and elongation factors was enhanced by polyamines at the early logarithmic phase. Because these mRNAs contain the UAA termination codon, it is quite possible that the efficiency of translation from these mRNAs is increased by polyamine enhancement of RF2 synthesis judging from the results of OppA synthesis and polyamine enhancement of the synthesis of ribosomal protein L20 and EF-G at the levels of both transcription and translation shown in Fig. 8. The mechanism of polyamine stimulation of the synthesis of these mRNAs is now under investigation.

It is known that polyamines stimulate cell growth through enhancement of the assembly of 30 S ribosomal subunits (13–15) and stimulation of the synthesis of specific kinds of proteins that are important for cell growth (20, 62). We proposed that the genes whose expression is enhanced by polyamines at the level of translation are classified as polyamine modulon. Those are oppA, cya, rpoS,
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