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A Unifying Model for the Role of Polyamines in Bacterial Cell Growth, the Polyamine Modulon*[S]

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We reported previously that the synthesis of specific proteins such as OppA, Cya, and RpoS (σ^{38}), which are important for cell growth and viability, is stimulated by polyamines at the level of translation. In this study we found that the synthesis of FecI and Fis was also stimulated by polyamines at the level of translation. The FecI and Fis proteins enhance the expression of mRNAs that are involved in iron uptake and energy metabolism and the expression of rRNA and some tRNAs. The Shine-Dalgarno (SD) sequence of their mRNAs was not obvious or was not located at the usual position. When the SD sequences were created at the normal position on these mRNAs, protein synthesis was no longer influenced by polyamines. Thus, the common characteristic of these mRNAs was to have a weak or ineffective SD sequence. We propose that a group of genes whose expression is enhanced by polyamines at the level of translation be referred to as a "polyamine modulon." By DNA microarray, we found that 309 of 2,742 mRNA species were up-regulated by polyamines. Among the 309 up-regulated genes, transcriptional enhancement of at least 58 genes might be attributable to increased levels of the transcription factors Cya, RpoS, FecI, and Fis, which are all organized in the polyamine modulon. This unifying molecular mechanism is proposed to underlie the physiological role of polyamines in controlling the growth of *Escherichia coli*.

Polyamines (putrescine, spermidine, and spermine) are present at millimolar concentrations in both prokaryotic and eukaryotic cells and play regulatory roles in cell growth (1–3). The intracellular levels of polyamines are elaborately regulated at various steps including synthesis, degradation, uptake, and excretion (4, 5). Polyamines exist mostly as polyamine-RNA complexes and thus affect translation at various steps (6, 7). In fact, polyamines stimulate the synthesis of some proteins *in vitro* (8–10) and *in vivo* (11, 12) and increase the fidelity of translation (13, 14). Polyamines also induce the *in vivo* assembly of 30 S ribosomal subunits (15, 16).

At present, however, it remains unresolved whether polyamines function as specific regulators that control the translation of a defined set of proteins in addition to their roles controlling the overall rate and fidelity of protein synthesis. In an attempt to get insight into this problem, we have carried out a systematic comparison of the gene expression pattern in *Escherichia coli* MA261, a polyamine-requiring mutant, in the presence and absence of polyamines. The strain MA261 is unable to synthesize putrescine, and cell growth slows down in the absence of exogenous putrescine (17). When putrescine is added to the culture medium, it is taken up into cells from which spermidine is synthesized, leading to the recovery of cell growth. By comparing the protein expression pattern in MA261 cultured with or without putrescine, we reported previously that the synthesis of OppA, a periplasmic substrate-binding protein of the oligopeptide uptake system, is strongly stimulated by polyamines. We also made the following findings. (i) The stimulation of OppA synthesis takes place at the level of translation. (ii) The position and secondary structure of the Shine-Dalgarno (SD)[1] sequence in *oppA* mRNA are correlated with this stimulation (18). (iii) Polyamines induce structural changes of RNA at the SD sequence and the initiation codon AUG of *oppA* mRNA, facilitating formation of the initiation complex (19). Later, we also found that polyamines increase the efficiency of translation of *cya* (adenylate cyclase) mRNA and *rpoS* (σ^{38}) mRNA by facilitating UUG codon-dependent initiation of *cya* mRNA translation (20) and UAG amber codon-dependent Gln-tRNA^{supE} binding to ribosomes at the 33rd position of *rpoS* mRNA (21). The amber codon at the 33rd position of *rpoS* mRNA was observed in several *E. coli* strains (22).

Because both Cya (adenylate cyclase) and RpoS (σ^{38}) are involved in the global regulation of transcription, a number of genes should be affected indirectly by the presence or absence of polyamines. The microarray analysis of whole mRNA from the strain MA261, one of the subjects of this report, indeed showed the expected changes of transcription pattern in the presence and absence of polyamines, *i.e.* the expression of many genes regulated by Cya and RpoS is enhanced by polyamines. We then looked for other transcription factors or nucleoid proteins whose synthesis is stimulated by polyamines at the level of translation. Here we found that the synthesis of both the FecI sigma factor (σ^{18}), one of the σ subunits of RNA polymerase, and Fis, a global regulator of transcription of some growth-related genes (23), is enhanced by polyamines at the level of translation. From these results, we propose the novel concept of a "polyamine modulon" that is involved in the control of cell proliferation.

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental data in the form of tables containing detailed information on genes up- or down-regulated in the presence of putrescine.

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[1] The abbreviations used are: SD, Shine-Dalgarno; nt, nucleotide(s).

EXPERIMENTAL PROCEDURES

Bacterial Strains and Culture Conditions—The polyamine-requiring mutant *E. coli* MA261 (*speB speC gly leu thr thi*) (17) was kindly provided by Dr. W. K. Maas, New York University School of Medicine. MA261*lacZ*::*Em* was constructed as described previously (24). *E. coli* MA261 and MA261*lacZ*::*Em* cells 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) or absence of putrescine (12). Cell growth was monitored by measuring the absorbance at 540 nm.

Plasmids—Total chromosomal DNA from *E. coli* W3110 was prepared according to the method of Ausubel and co-workers (25). To make the *fecI-lacZ* fusion gene, PCR was performed using total chromosomal DNA as template and 5′-ATGGAAATGGAACCCGGGCAAGCACCTTAA-3′ (P1) and 5′-GCAAAAAAGTGTCCCGGGAATGTCATCTGC-3′ (P2) as primers. The amplified *fecI* gene (a 293-nt 5′-upstream region and a 128-nt open reading frame) was digested with SmaI and inserted into the SmaI site of pMC1871 (26) to make a pMC*fecI-lacZ* fusion plasmid. For construction of pMW*fis-lacZ,* the *fis* gene was amplified using total chromosomal DNA as the template and 5′-GATTACGCCAAGCTTGACTTTTATGGTCCG-3′ (P3) and 5′-CCTCTAGAGTCGACCTGCAGTTTGAGCAAA-3′ (P4) as primers. After digesting the fragment with HindIII and PstI, the *fis* gene (a 195-nt 5′-upstream region and a 125-nt open reading frame) was inserted into the HindIII-PstI sites of pUC119 (Takara Shuzo, Co. Ltd) together with the 3.1-kb PstI fragment of pMC1871 to make a pUC*fis-lacZ* plasmid. The HindIII-XbaI fragment of pUC*fis-lacZ* was inserted into the same restriction sites of the low copy number vector pMW119 (Nippon Gene, Tokyo) to make pMW*fis-lacZ.* Site-directed mutagenesis for the construction of mutated fusion genes with modified SD sequences was performed by overlap extension using PCR (27).

Dot Blotting and DNA Microarray Analysis—*E. coli* MA261 cells were cultured at A_{540} = 0.03 in the presence and absence of putrescine and harvested at A_{540} = 0.2. Total RNA was prepared from these cells by the method of Emory and Belasco (28). Dot blot analysis was performed according to the method of Sambrook *et al.* (29). PCR products of the *fecI* gene (primers 5′-TTAACTTTGGAGGCACTCCACATGTCTGAC-3′ and P2) and the *fis* gene (primers P3 and P4) were labeled with [α-^{32}P]dCTP using the BcaBEST™ Labeling Kit (Takara Shuzo Co. Ltd.) and used as probes. The radioactivity on the blot was quantified by a BAS2000II imaging analyzer (Fuji Film, Japan). DNA microarray experiments and data analysis were carried out according to the method of Oshima *et al.* (30) with total RNA isolated from MA261 cultured in the presence and absence of putrescine and harvested at A_{540} = 0.5 using TaKaRa IntelliGene *E. coli* CHIP, version 1.0 (Takara Shuzo Co. Ltd.).

Western Blot Analysis—Rabbit polyclonal antibodies against transcription factors were raised as described previously (23, 31, 32). MA261 cells were cultured in the presence and absence of putrescine, and cell lysate was prepared as described previously (12) from cells harvested at A_{540} = 0.2 or 0.5. Cell lysate (10 to 100 μg of protein) was separated by SDS-PAGE according to the method of Laemmli (33) and transferred to Immobilon transfer membrane (Millipore), and proteins were detected using specific antibodies followed by ECL™ Western blotting detection reagents (Amersham Biosciences). The level of proteins was quantified by a LAS-1000 Plus luminescent image analyzer (Fuji Film, Japan). Protein was determined by the method of Bradford (34).

Measurement of FecI-LacZ or Fis-LacZ Fusion Proteins—*E. coli* MA261*lacZ*::*Em* cells containing pMC*fecI-lacZ* or pMW*fis-lacZ* were cultured at A_{540} = 0.03 in medium A without putrescine. At the cell density of 0.2 A_{540}, the culture was divided into 5-ml aliquots and continued to grow in the presence (100 μg/ml) or absence of putrescine. After 10 min, [^{35}S]methionine (1 MBq) was added to each 5-ml aliquot, and the cells were allowed to grow for additional 20 min. After the addition of unlabeled methionine at a final concentration of 20 mM, the cells were harvested, resuspended in 1 ml of buffer A (10 mM sodium phosphate, pH 7.4, 100 mM NaCl, 1% Triton X-100, and 0.1% SDS), and disrupted with a French pressure cell at 20,000 p.s.i. The amount of radioactive FecI-LacZ or Fis-LacZ was determined using whole cell lysates containing 1,000,000 cpm of [^{35}S]methionine-labeled proteins and antiserum against β-galactosidase (Sigma) as described previously (20). After SDS-PAGE, the radioactivity associated with FecI-LacZ or Fis-LacZ was quantified using a Fujix Bas 2000II imaging analyzer.

RESULTS

Identification of mRNAs Whose Synthesis Is Up-regulated by Polyamines—To find genes whose expression is stimulated by polyamines, we compared mRNA levels in *E. coli* MA261 cells

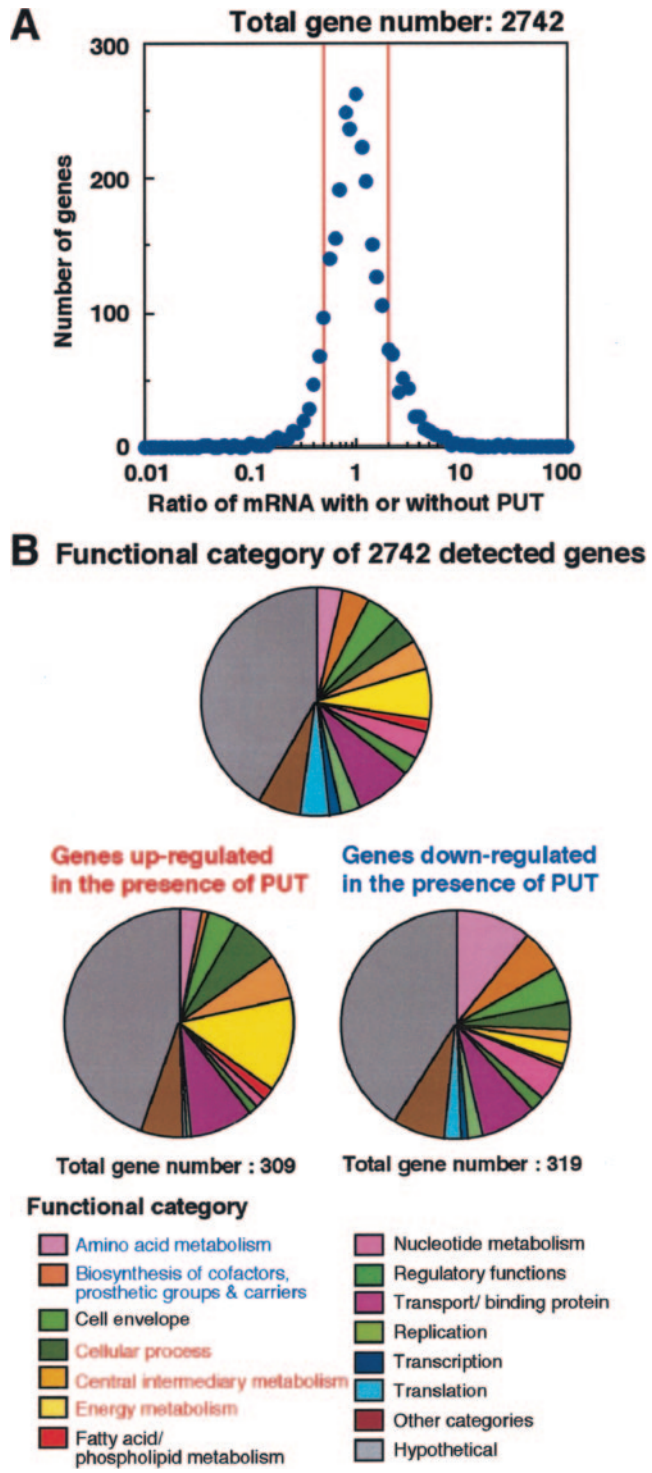

FIG. 1. **Identification of genes whose expression was regulated by polyamines at the level of transcription.** *A*, DNA microarray experiments and data analysis were carried out as described under "Experimental Procedures." Genes were classified into three groups, namely genes up-regulated by polyamines, genes not regulated by polyamines, and genes down-regulated by polyamines. Each range of the ratio of mRNA with or without putrescine (*PUT*) (0.01–0.1, 0.1–1, 1–10, and 10–100) was divided into 20, and the number of genes belonging to the divided range was shown in the graph. *B*, classification of genes by functional category was carried out using the data base of GenoBase (ecoli.aist-nara.ac.jp/gb4/search/function/orffunc.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 to the percentage of the category in 2,742 detected genes, and a category labeled with *blue letters* means that the percentage of the category in genes down-regulated in the presence of putrescine increased >1.5-fold.

TABLE I
Typical up-regulated genes in the presence of putrescine

Genes shown by boldfaced italic are those regulated by *rpoS* and *cya*. Genes underlined are those regulated by *fecI* (σ^{18}) and *fis*, which were identified as new members of polyamine modulon in this report.

	Gene
Genes regulated by σ^{38} (σ^{S})	***acnA, aidB, aldB, bolA, cbpA, dps, fic, frdD, gadA, hdeA, hdeB, katE, ldcC, osmC, osmE, osmY, otsA, otsB, poxB, sodC, treA, treF, uspB, wrbA, ybhP, yfcG, yhiU, yohF***
Genes regulated by *cya*	
1) Genes regulated by σ^{28} (σ^{F})	***flgA, flgM, flhB, fliA, fliD, fliG, fliH, fliI, fliM, fliN, fliQ***
2) Energy production	***malP, malQ, manX, manY, manZ, ptsG[a], sdhA, sdhB, sdhD, ugpQ***
3) Nucleotide metabolism	***cdd, udp***
Genes regulated by FecI (σ^{18})	*fecA, fecB, fecC, fecD, fecE*
Genes regulated by Fis	*adhE, nuoH, ptsG[a]*

[a] Regulated by both *cya* and *fis*.

cultured with or without putrescine. The transcript profiles of the exponential phase culture of MA261 with or without polyamines were determined by a two-color (Cy3 and Cy5) cDNA microarray analysis (35) using a DNA chip that contains 4,028 gene spots from a total of 4,236 genes in *E. coli*. In three separate experiments, the expression of 2,742 genes was always detected in cells cultured with or without putrescine. Among these 2,742 genes, 309 genes were up-regulated (>2-fold increase), and 319 genes were down-regulated (>2-fold decrease) by polyamines (Fig. 1*A*). The functional category of these genes was identified through the GenoBase data base (Fig. 1*B*). Details of the 309 up-regulated and 319 down-regulated genes and the ratio of mRNA with or without putrescine is given in the supplemental data found in the on-line version of this article. As shown in Fig. 1*B*, genes that were up-regulated by polyamines were relatively centered in the functional categories labeled "Cellular process," "Central intermediary metabolism," and "Energy metabolism" (in *red letters*), and the down-regulated genes were in the categories labeled "Amino acid metabolism" and "Biosynthesis of cofactors, prosthetic groups, and carriers" (in *blue letters*). Typical genes whose expression is enhanced by polyamines are summarized in Table I. Some of these are genes transcribed by RNA polymerase containing RpoS (σ^{38}) or RpoF (σ^{28}). Enhancement of the expression of these genes by polyamines is in good agreement with our previous observations that the translation of *rpoS* mRNA and *cya* mRNA is enhanced by polyamines (20, 21) (note that the flagella regulon genes including *rpoF* are under the control of cAMP-dependent master regulators FlhDC). The genes related to energy metabolism were up-regulated (Table I). Within the genes related to energy production, the genes reported to be regulated by cAMP were seen (36, 37). Two genes related to nucleotide metabolism (*cdd* and *udp*) were also up-regulated by polyamines probably through the increase in cAMP (38). Genes regulated by FecI (σ^{18}), a transcription factor for the iron transport operon (39), and Fis, a global regulator of transcription of some growth-related genes (23), were also seen, and effects of polyamines on the synthesis of FecI (σ^{18}) and Fis will be described later.

Search for Transcription Factors Up-regulated by Polyamines—Enhanced transcription of >300 genes is not necessarily a direct result of polyamine effect. Rather, it may reflect indirect regulation by the altered level or activity of some transcription factors as occurs in the case of Cya and RpoS. We then looked for transcription factors whose synthesis is stimulated by polyamines at the level of translation. Based on the search for the transcription factors, which have been reported to be involved in transcriptional regulation of the up-regulated genes such as those for energy metabolism and iron and zinc transport, we analyzed, by Western blotting, the intracellular levels of 12 kinds of transcription factors, namely FecI (σ^{18}), Fis, CRP, DcuR, DnaA, H-NS, IHF, Lrp, Mlc, Rob, Rsd, and Zur. As shown in Table II, the level of FecI (σ^{18}) and the global transcription regulator Fis was found to increase significantly after the addition of polyamines. The levels of the other 10 transcription factors were not influenced by polyamines. Because the level of *fecI* and *fis* mRNAs in cells cultured in the presence of putrescine was lower than that in the absence of putrescine (Table II), it is thought that the increase in the level of FecI (σ^{18}) and Fis proteins by polyamines is at the post-transcriptional level, most probably at the level of translation as in case of OppA, Cya, and RpoS (18–21).

TABLE II
Levels of mRNA and protein of transcription factors in MA261 cultured in the presence and absence of putrescine

Levels of mRNA and protein of transcription factors were estimated by cDNA microarray and Western blotting, respectively, and the ratio of these levels in cells cultured with or without putrescine (PUT) was shown together with the SD sequence and initiation codon of mRNAs (−27 to +6 nt). The SD sequence is shown in white letters with a black background, and the initiation codon is underlined.

Transcription factor	Ratio (PUT +/-)		Nucleotide sequence of mRNA at the region of SD sequence and initiation codon		Number of nt between SD and initiation codon
	mRNA	Protein	SD sequence	Initiation codon	
FecI (σ^{18})	0.5	2.8	UUA ACU UUU GAU GCA CUC CGC AUG UCU		10 nt
Fis	0.8	3.8	AAA UAA AGA GCU GAC AGA ACU AUG UUC		11 nt
CRP	1.1	1.0	UAU AAC AGA GGA UAA CCG CGC AUG GUG		9 nt
DcuR	0.9	1.0	GGA CGG GGA GAG GUC GAA CAG AUG AUC		7 nt
DnaA	0.8	1.0	UUU GUU CGA GUG GAG UCC GCC GUG UCA		6 nt
H-NS	1.5	1.0	UAU AAG UUU GAG AUU ACU ACA AUG AGC		9 nt
IHF	0.8	0.9	CAU CAU UGA GGG AUU GAA CCU AUG GCG		8 nt
Lrp	1.2	1.3	GAA UAC AGA GAG ACA AUA AUA AUG GUA		9 nt
Mlc	1.0	0.7	CGA AAA UAU AGG GAG UAU GCG GUG GUU		6 nt
Rob	0.7	0.6	UUG AAA GGA UGA GGA UAU UUU AUG GAU		6 nt
Rsd	1.3	1.0	UAC AAA CUU GCG GAG UCA AUC AUG CUU		6 nt
Zur	0.8	1.0	CGG CAA CAA UAA GGG UUC UCG GUG UUU		6 nt

Stimulation of FecI (σ^{18}) and Fis mRNA Translation by Polyamines—FecI (σ^{18}) is involved in the expression of the iron uptake operon (*fecABCDE*) (Fig. 2*A*) (39). The level of FecI protein was significantly higher in cells cultured in the presence of putrescine than in its absence, as determined by Western blot analysis (Fig. 2*B*). Accordingly, the level of *fecABCDE* mRNAs transcribed by an RNA polymerase holoenzyme containing FecI (σ^{18}) was 2.2–3.8 times higher in the presence of putrescine (see Fig. 2*A*). The level of *fecI* mRNA in cells cultured in the presence of putrescine was, however, ~70% of the level in the absence of putrescine (Fig. 2*C*). This apparent disparity between mRNA and protein levels suggests that the efficiency of *fecI* mRNA translation is high in the presence of polyamines.

One unique characteristic of *fecI* mRNA is the lack of consensus SD sequence (GGAGG) in the expected position relative to the initiation codon AUG. This is similar to a characteristic of *oppA* mRNA, the translation of which is enhanced by poly-

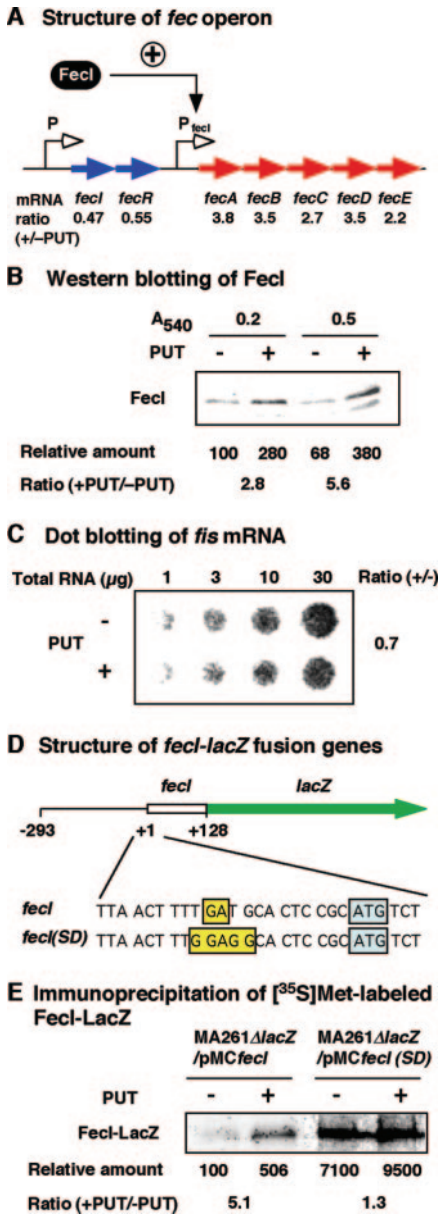

FIG. 2. **Polyamine stimulation of FecI (σ^{18}) synthesis at the level of translation.** *A*, schematic of the structure of *fecABCDE* genes and positive regulation by FecI (39). The level of mRNAs in the absence and presence of putrescine (*PUT*) was determined by DNA microarray analysis. *B*, a cell lysate was prepared from cells harvested at $A_{540} = 0.2$ or 0.5. Western blot analysis was carried out using antisera against FecI with 100 μg of protein of the cell lysate (33) as described under "Experimental Procedures." *C*, dot blot analysis of *fecI* mRNA was carried out as described under "Experimental Procedures". *D*, schematic of the *fecI-lacZ* fusion genes. The *fecI* gene containing a 293-nt 5′-upstream region with an unmodified or a modified SD sequence, and a 128-nt open reading frame was fused to the *lacZ* gene in the pMC1871 fusion vector. *E*, synthesis of FecI-LacZ fusion protein was measured as described under "Experimental Procedures." The level of *fecI-lacZ* mRNA in cells cultured with or without 100 μg/ml putrescine was nearly equal judging from the dot blot analysis.

amines (19). In *fecI* mRNA there is no obvious SD sequence, but a candidate for the SD sequence is GA, which is located 10 nucleotides upstream from the initiation codon AUG (Fig. 2*D*). To determine whether the weak SD-like sequence is related to polyamine stimulation of FecI synthesis, the SD-like sequence was replaced by the typical SD sequence GGAGG (Fig. 2*D*), and the translation efficiency of *fecI-lacZ* fusion mRNA was measured by immunoprecipitation of [^{35}S]methionine-labeled proteins with anti-β-galactosidase serum followed by fluorography. Synthesis of the FecI-LacZ fusion protein from the original weak SD-like sequence-containing mRNA was stimulated 5.1-fold by polyamines, whereas the polyamine stimulation was reduced to only 1.3-fold after replacement with the consensus SD sequence even though the basal level of protein synthesis in the absence of polyamines was strongly enhanced (71-fold) (Fig. 2*E*). These results indicate that the synthesis of FecI was enhanced by polyamines at the translational level due to a weak SD-like sequence in the *fecI* mRNA.

Next, we also analyzed the mechanism of polyamine stimulation of the synthesis of Fis protein. The nucleoid-associated Fis protein enhances the transcription of rRNA, some tRNAs, and some genes involved in energy production (40). These genes are *adhE* encoding alcohol dehydrogenase (41), *ptsG* encoding glucose-specific permease (42), and *nuoH* encoding the NADH dehydrogenase I chain H (43). These genes were up-regulated by polyamines (Fig. 3*A*). The intracellular level of the Fis protein significantly increased in the presence of putrescine (Fig. 3*B*), but the level of *fis* mRNA was nearly equal in cells cultured with or without putrescine as determined by dot blot analysis (Fig. 3*C*). These observations again suggest that polyamines enhance the translation of *fis* mRNA.

A weak SD sequence, GAG, is present at 11 nucleotides upstream of the initiation codon AUG of *fis* mRNA (Fig. 3*D*). To determine whether this weak SD sequence is responsible for polyamine stimulation of Fis synthesis, analogous to the case of FecI synthesis, the consensus SD sequence (GGAGG) was inserted at the normal position, seven nucleotides upstream from the initiation codon AUG of *fis* mRNA (Fig. 3*D*), and the level of Fis-LacZ fusion protein synthesis was measured. Synthesis of the fusion protein from the *fecI-lacZ* mRNA containing the unmodified SD sequence was strongly stimulated by polyamines. In contrast, when using the mRNA containing the consensus SD sequence, the degree of polyamine stimulation decreased from 2.9- to 1.2-fold, even though the basal activity increased 8.8-fold (Fig. 3*E*). These results indicate that the synthesis of Fis is also enhanced by polyamines at the translational level.

DISCUSSION

Polyamines selectively enhance the expression of a set of genes at the level of translation. Accumulated data indicate that most of the genes enhanced by polyamines are not under the direct control of polyamines but are activated indirectly as a result of increased levels of transcription factors whose synthesis is enhanced by polyamines at the level of translation. From these studies, we propose that the genes whose expression is enhanced by polyamines at the level of translation are classified as part of the polyamine modulon. In this study, we identified two new members of the polyamine modulon, *fecI* and *fis*. Both *fecI* and *fis* mRNAs have non-consensus SD sequences with low translational efficiency. Genes encoding transcription factors are good targets for finding new members of polyamine modulon. About 150 transcription factors have been identified in *E. coli* to date (44). When we searched the nucleotide sequences of the mRNAs encoding transcription factors, ~15% of these mRNAs have non-consensus SD sequences. We are looking for new members of the polyamine modulon among these mRNAs.

Polyamines induce conformational changes in RNA through the binding of, on average, 2 mol of spermidine and 4 mol of putrescine to each 100 nucleotide-long RNA in *E. coli* (7) and about 1 mol each of spermidine and spermine to each 100 nucleotide-long RNA in rat liver (6). We have shown previously that polyamines cause a structural change of relatively unstable double-stranded RNA (19, 45). It has been also reported that 2 mol of spermine bound to the anti-codon stem of $tRNA^{Phe}$

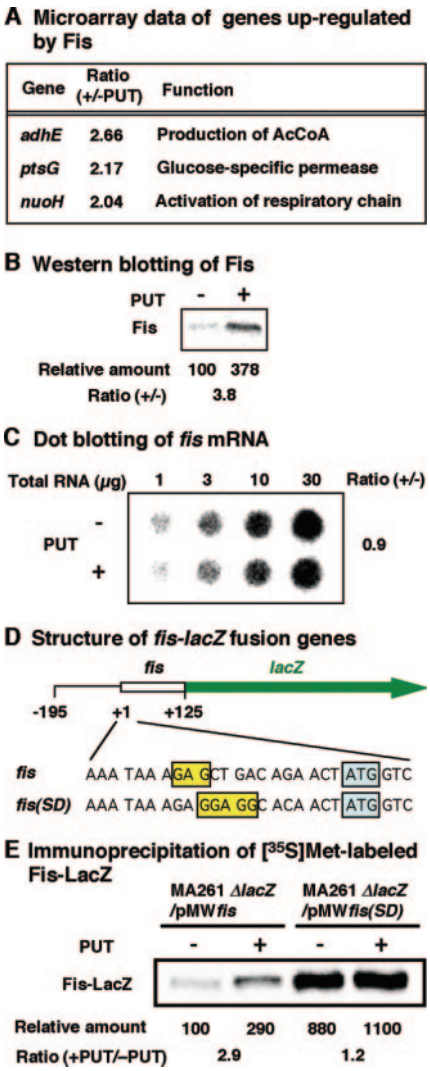

FIG. 3. **Polyamine stimulation of Fis synthesis at the level of translation.** *A*, DNA microarray data of genes up-regulated by Fis. *B*, a cell lysate was prepared from cells harvested at A_{540} = 0.2. Western blot analysis was carried out using 20 μg of protein of the cell lysate and antisera against Fis (23). *C*, dot blot analysis of *fis* mRNA was carried out as described under "Experimental Procedures." *D*, schematic of the *fis-lacZ* fusion genes. A fusion gene containing a 195-nt 5′-upstream region with an unmodified or a modified SD sequence and a 125-nt open reading frame of the *fis* gene fused to the *lacZ* gene of pMC1871 was inserted into pMW119. *E*, the amount of the Fis-LacZ fusion protein synthesized was measured as described under "Experimental Procedures." The level of *fis-lacZ* mRNA in cells cultured with or without 100 μg/ml putrescine (*PUT*) was nearly equal judging from the dot blot analysis.

(46). Thus, it is thought that polyamines induce a conformational change in relatively unstable double-stranded RNA at the region of SD sequence and initiation codon, leading to an increase in the rate of translation initiation.

To date, we have identified five members of the polyamine modulon, *oppA*, *cya*, *rpoS*, *fecI*, and *fis*. Except for *oppA*, all polyamine modulon members play regulatory roles in transcription, and we identified thus far 58 genes up-regulated by Cya, RpoS, FecI, and Fis. We expect that Cya, together with cAMP receptor protein (CRP), regulates more genes than those we reported, because the cAMP-CRP complex plays a role in regulating gene expression, not only for classic inducible catabolic operons but also for other categories (37). We are also looking for new members of the polyamine modulon. Accordingly, the expression of a number of *E. coli* genes is activated indirectly by the transcription factors belonging to the polyamine modulon (Fig. 4). As for polyamine stimulation of cell

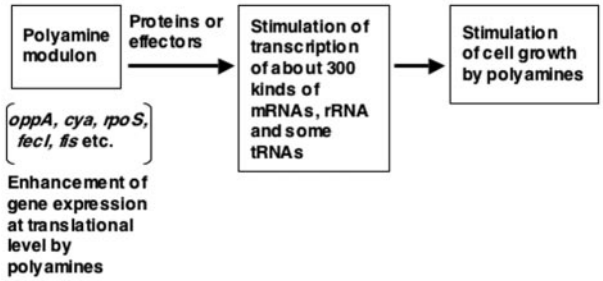

FIG. 4. **Proposed role of the polyamine modulon in cell proliferation.**

growth, Fis plays important roles by increasing the level of rRNA and some tRNAs in addition to the increase in the transcription of *adhE*, *ptsG*, and *nuoH* genes. Our experimental data altogether support a unifying molecular mechanism defined by the polyamine modulon underlying the role of polyamines in cell growth.

Our hypothesis about the effect of polyamine on cell growth is as follows. Polyamines probably do not function as an on/off switch of gene expression of specific proteins. Polyamines "modulate" the level of many kinds of proteins, 10-fold at most, to maintain optimal conditions for cell growth (Fig. 4). In this way, polyamines function as an important factor for cell growth. Under conditions in which a polyamine-requiring mutant is cultured in the absence of putrescine, polyamine content becomes very low (47) and modulation by polyamines is weakened. Thus, cells grow slowly. If cells are cultured further in the absence of putrescine, polyamine content in cells becomes negligible, and cell growth would stop as a response to the decrease in the level of many proteins that are involved in cell growth.

We also identified ~300 genes down-regulated by polyamines. Many genes that are involved in the categories labeled "Amino acid metabolism" and "Biosynthesis of cofactors, prosthetic groups & carriers" in Fig. 1*B* are down-regulated. These results may be related to the slight increase in the level of amino acids in cells cultured in the presence of polyamines (data not shown). One possible mechanism, arising as an extension of our model, is that transcription factors encoded by as yet unidentified members in the polyamine modulon repress transcription of these down-regulated mRNAs. Another possibility is that the transcription of these mRNAs is directly regulated by the increased polyamines or some amino acids. We have shown previously that the complex of spermidine and PotD, a substrate-binding protein of the spermidine uptake system, inhibits the transcription of the *potABCD* operon, which encodes the spermidine uptake system (48). The decrease in the level of the *potABCD* mRNA in cells cultured with putrescine was ~40% by microarray analysis (data not shown). Experiments are underway to define the underlying mechanism of polyamine-mediated repression.

This kind of polyamine modulation (stimulation or inhibition) of the synthesis of specific proteins at the translational level has been also observed in eukaryotic cells. However, we have not yet succeeded in clarifying its detailed mechanism.

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A Unifying Model for the Role of Polyamines in Bacterial Cell Growth, the Polyamine Modulon

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