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Polyamine Modulon in *Escherichia coli*: Genes Involved in the Stimulation of Cell Growth by Polyamines

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We have recently proposed an idea to explain how polyamines enhance cell growth in *Escherichia coli*. Since most polyamines exist as polyamine-RNA complexes, our idea is that polyamines stimulate several kinds of protein synthesis which are important for cell growth at the level of translation. We found that synthesis of oligopeptide binding protein (OppA), which is important for nutrient supply, adenylate cyclase (Cya), RNA polymerase σ^{38} subunit (RpoS), transcription factor of iron transport operon (FecI), and transcription factor of growth-related genes including rRNA and some kinds of tRNA synthesis (Fis) was enhanced by polyamines at the level of translation. We proposed 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. This unifying molecular mechanism is proposed to underlie the physiological role of polyamines in controlling the growth of *Escherichia coli*.

Key words: cell growth, cell viability, polyamines, polyamine–RNA interactions protein synthesis.

Polyamines (putrescine, spermidine and spermine) are present at millimolar concentration in both prokaryotic and eukaryotic cells and play important roles in cells (*1*, *2*). This review focuses on the effect of polyamines on cell growth in *Escherichia coli*. Since decrease or increase in polyamine content greatly diminishes cell growth (*3*, *4*), the intracellular levels of polyamines are closely regulated at various steps including synthesis, degradation, uptake and excretion (*5*, *6*). To study the effect of polyamines on cell growth, we first estimated the cellular distribution in cells (*7*, *8*). In *E. coli*, about 50% of putrescine and 90% of spermidine exist as polyamine-RNA complexes (Table 1). Accordingly, the effect of polyamines on cell growth was mainly studied at the level of translation.

Table 1. **Polyamine distribution in *Escherichia coli*.**

	Putrescine		Spermidine	
	(mM)	(%)	(mM)	(%)
Total	32.2	100	6.88	100
Free	12.5	38.8	0.26	3.8
DNA	3.0	9.3	0.35	5.1
RNA	15.4	47.8	6.17	89.7
Phospholipids	0.46	1.4	0.05	0.7
ATP	0.84	2.6	0.05	0.7

Dissociation constants were determined in the presence of Tris-HCl, pH 7.5, 10 mM magnesium acetate, and 150 mM KCl. Polyamine distribution was calculated using dissociation constants and concentration of macromolecules, ATP and polyamines.

Polyamine stimulation of general protein synthesis

To study the effect of polyamines on protein synthesis, we used a polyamine-requiring mutant MA261, which cannot synthesize putrescine. Cell growth in this mutant was very slow when putrescine was absent in the medium. When 100 μg/ml putrescine was added to the medium, cell growth recovered, and polyamine (putrescine and spermidine) content in cells became close to that in normal cells (Fig. 1A). To compare ribosome activity, cells were collected at the logarithmic phase ($A_{540} = 0.2$). The activity of ribosomes obtained from cells cultured with putrescine was about double that of ribosomes obtained from cells cultured without putrescine. The mechanism of polyamine enhancement of ribosome activity was studied in detail. It was found that association of certain 30S ribosomal proteins with 23S core particles (precursor particles of 30S ribosomal subunits) was enhanced by polyamines. This enhancement occurred through stimulation by polyamines of the methylation of two adjacent molecules of adenine located close to the 3′-end of 16S ribosomal RNA in 23S core particles (*9*, *10*). However, the effect of polyamines on cell growth was not completely explained by the enhancement of ribosome activity, because cell growth of a polyamine-requiring mutant was enhanced about 3- to 5-fold by polyamines (Fig. 1A).

Polyamine stimulation of OppA synthesis at the level of translation

We next studied whether polyamines can enhance the synthesis of specific kinds of proteins which are important for cell growth. For this purpose, proteins were labeled with [^{35}S]methionine and analyzed by fluorography. We

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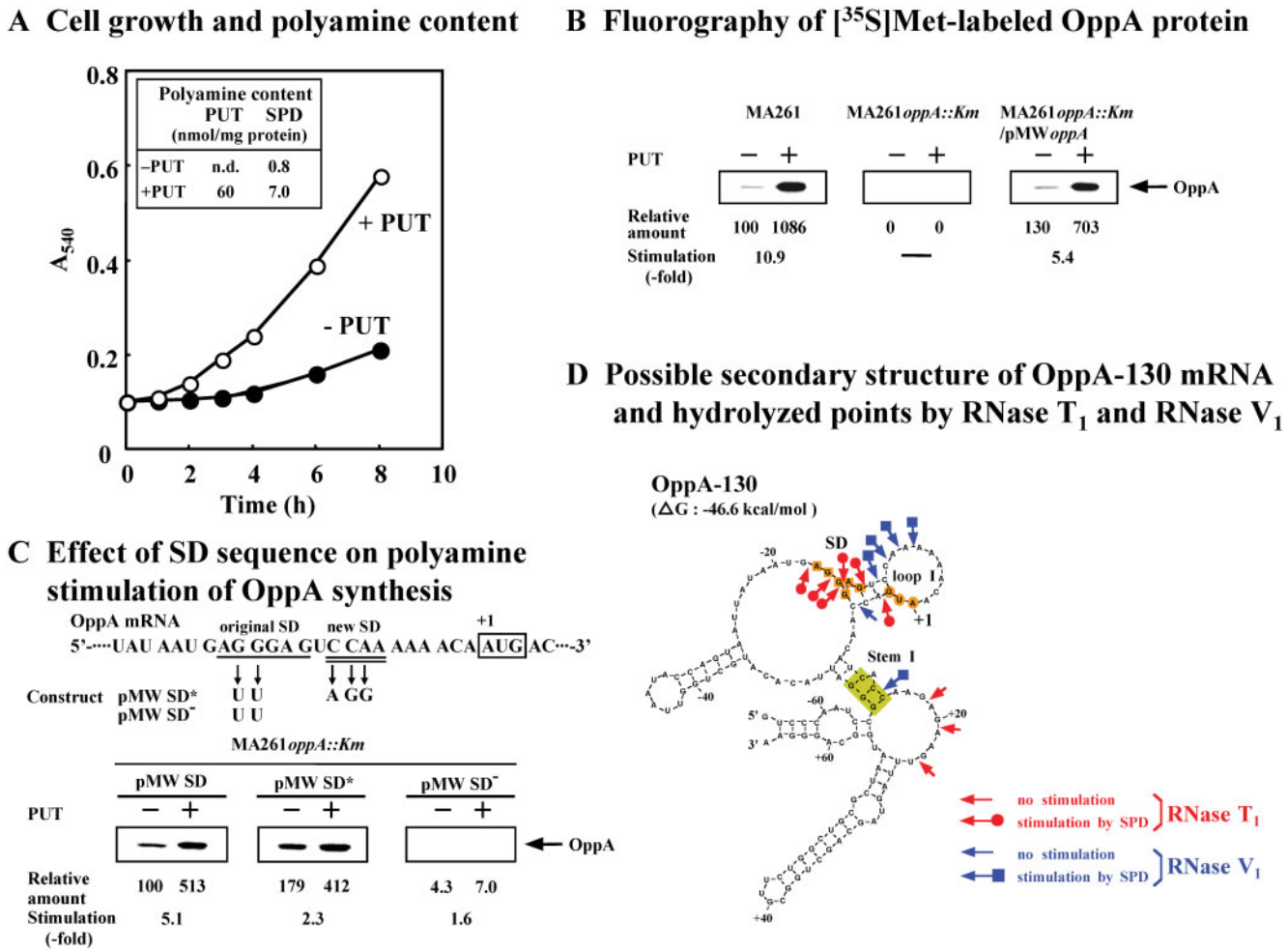

Fig. 1. **Polyamine stimulation of cell growth and OppA synthesis**. A: *Escherichia coli* MA261 was cultured in the presence or absence of 100 μg/ml putrescine. Cell growth was monitored at A_{540}, and polyamine content was measured using HPLC. B: OppA protein synthesized was measured by an immunoprecipitation method. C: Effect of SD sequence on polyamine stimulation of OppA synthesis. D: Possible secondary structure of OppA-130 mRNA and the hydrolyzed points by RNase T_1 and RNase V_1. PUT, putrescine; SPD, spermidine.

found that synthesis of a protein with molecular mass of 62 kDa was strongly enhanced by polyamines. The protein was purified and identified by amino acid sequencing as an oligopeptide-binding protein (OppA), which is a component of oligopeptide transporter (*11*). The mechanism of polyamine stimulation of OppA synthesis was studied by measuring the levels of OppA mRNA and OppA protein. It was found that polyamine stimulation of OppA synthesis occurred at the level of translation (Fig. 1B). One feature of the nucleotide sequence of OppA mRNA is that the Shine-Dalgarno (SD) sequence, important for the initiation of protein synthesis (*12*), is more distant (12 nucleotides) from the initiation codon AUG than that in other mRNAs, in which the SD sequence is typically located 7 nucleotides upstream from the AUG. Thus, a new SD sequence was inserted 7 nucleotide upstream from the AUG. When OppA was synthesized from the mRNA with the new SD sequence, a lower degree of polyamine stimulation was observed (2.3-fold) (Fig. 1C). In the absence of putrescine, however, OppA synthesis from the mRNA with the new SD sequence was greater than that from the normal OppA mRNA. These results suggest that the position of the SD sequence may influence polyamine stimulation of OppA synthesis (*13*).

We have shown that the SD sequence of OppA mRNA is absolutely necessary for OppA synthesis (Fig. 1C). We next determined the size of OppA mRNA necessary for efficient recognition of the SD sequence. When the OppA mRNA was a 30-mer, the optimal Mg^{2+} concentration for fMet-tRNA binding to ribosomes was 9 mM, and maximal binding of fMet-tRNA was obtained at a molar ratio of mRNA to ribosomes of 30 to 1. Furthermore, 1 mM spermidine did not stimulate fMet-tRNA binding to ribosomes. When the OppA mRNA was a 130-mer, the optimal Mg^{2+} concentration for fMet-tRNA binding to ribosomes was 3 mM, and maximal binding of fMet-tRNA was obtained at a molar ratio of mRNA to ribosomes of 3 to 1. Under these conditions, 1 mM spermidine strongly enhanced fMet-tRNA binding to ribosomes in the presence of 1 to 3 mM Mg^{2+}, suggesting that relatively long nucleotides are required for

efficient recognition of the OppA SD sequence during initiation complex formation (*14*).

The structural change of OppA mRNA induced by spermidine was examined by limited digestion of the mRNA with a single-stranded G-specific RNase T_1. Hydrolysis of the five Gs in the SD sequence and the G in the initiation codon AUG was stimulated greatly by spermidine (Fig. 1D). The optimal concentration of spermidine was 0.4–0.8 mM. Hydrolysis of Gs positioned at 18, 20, and 23 was not influenced by spermidine. The results suggest that spermidine relaxes the structure of both the SD sequence and the initiation codon AUG. As stated above, OppA mRNA is unusual in that the SD sequence is 12 nucleotides upstream from the initiation codon AUG. Thus, in the presence of spermidine, the SD sequence and the initiation codon may become closer during formation of the initiation complex, and this may be important for initiation. This possibility was studied using RNase V_1, which recognizes double-stranded RNA or stacked RNA (*15*). The region between the SD sequence and the initiation codon AUG became sensitive to RNase V_1 in the presence of spermidine (Fig. 1D). This result suggests that the distance between the SD sequence and the initiation codon AUG of OppA mRNA contracts in the presence of spermidine (*14*).

Mechanism of polyamine stimulation of the synthesis of adenylate cyclase and RNA polymerase σ^{38}

We next looked for another protein whose synthesis is enhanced by polyamines at the level of translation and found that adenylate cyclase (Cya) is one such protein (*16*). After addition of putrescine, cell growth was stimulated and interacellular cAMP increased. The SD sequence of Cya mRNA was positioned normally and relatively exposed, but the initiation codon was UUG instead of AUG. Although the amount of fMet-tRNA binding to ribosomes was much greater with AUG than with UUG, spermidine stimulated only UUG-dependent fMet-tRNA binding significantly. If the UUG codon is replaced by the initiation codon AUG, the cells are nonviable (*17*). Given that an increase in Cya causes cell death, polyamines may contribute to maintenance of an optimal cAMP level by facilitating the UUG codon-dependent initiation (Fig. 2A). UUG is the initiation codon for 34 identified genes in *E. coli* (*18*). If polyamines stimulate the interaction between the initiation codon UUG and the anticodon of fMet-tRNA, CAU, protein synthesis from all mRNAs having UUG as the initiation codon would be stimulated by polyamines. However, this was not the case. The secondary structure of the initiation region of mRNA is probably important for the stimulation of the UUG codon–dependent initiation by polyamines. Analysis of RNA secondary structure suggests that exposure of the SD sequence is a prerequisite for polyamine stimulation of UUG codon–dependent initiation.

We also found that synthesis of the RNA polymerase σ^{38} subunit (RpoS) was stimulated by polyamines at the level of translation (*19*). Polyamine stimulation was observed only in strains in which the 33rd codon of RpoS mRNA is a UAG amber termination codon instead of the CAG codon for glutamine in wild-type *E. coli*. We found that a mutation at the 33rd position of the ORF of RpoS mRNA occurs frequently. Readthrough of the termination codon

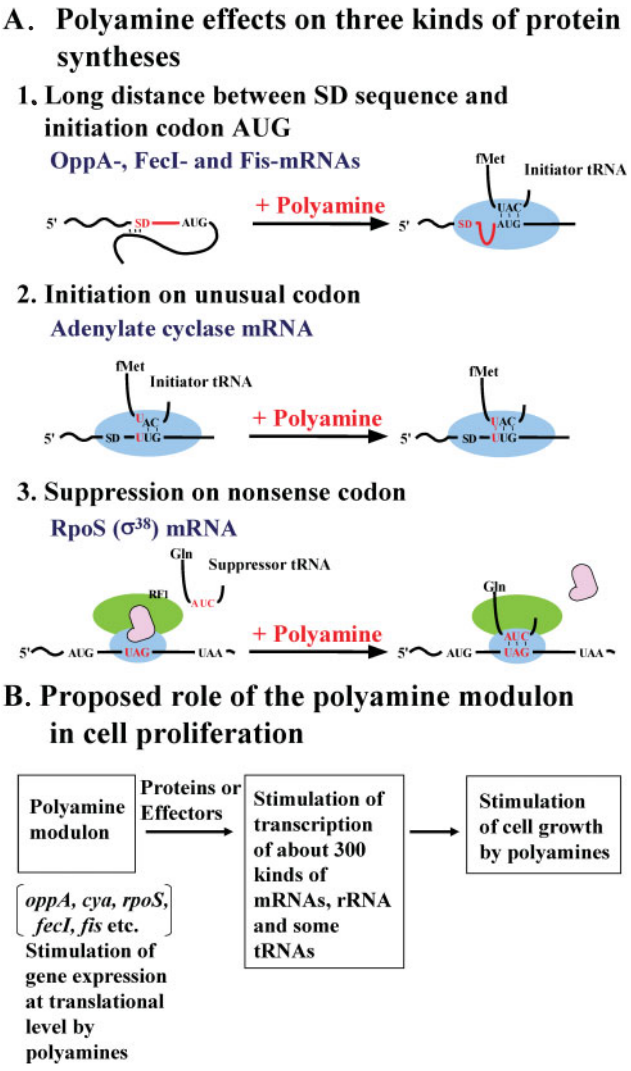

Fig. 2. **Polyamine modulon**. A: Three mechanisms of polyamine stimulation of five kinds of protein syntheses. B: Proposed role of the polyamine modulon in cell proliferation.

by Gln-$tRNA^{supE}$ was stimulated by polyamines (Fig. 2A). The 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 or UAA codons. One of the W3110 strains carries a UAG termination codon at the 270th position of the RpoS mRNA. Readthrough of the UAG termination codon at the 270th amino acid position was also stimulated by polyamines. 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 (*18*). Because relatively few 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. Examination of cell viability of *E. coli* MA261 having a termination codon in the 33rd position of RpoS mRNA revealed that it was higher in cells cultured with putrescine than in those cultured without putrescine. The results

confirm that elevated expression of the *rpoS* gene is important for cell viability at the late stationary phase. The increase in readthrough of the amber codon by polyamines was also found for translation of the mutant gene 1 protein mRNA from T7 phage (*20*).

Up to now, three different mechanisms by which polyamines stimulate protein synthesis have been identified: a structural change of OppA mRNA, leading to enhanced template activity for translation, stimulation of initiation codon UUG–dependent fMet-tRNA binding to Cya mRNA-ribosome complex; and stimulation of readthrough of the amber codon UAG–dependent Gln-tRNA^{supE} on ribosome-associated RpoS mRNA (Fig. 2A). Thus, polyamines modulate protein synthesis not only at the level of initiation but also at the level of elongation of translation.

Polyamine modulon

We propose that genes whose expression is modulated by polyamines at the level of translation be referred to as a "polyamine modulon." Among three proteins whose syntheses are enhanced by polyamines, Cya and RpoS are transcription factors. We also thought that synthesis from mRNAs having a weak SD sequence may be enhanced by polyamines. About 150 transcription factors have been identified in *E. coli* to date (*21*). Accordingly, we looked for new members of the polyamine modulon among the genes encoding transcription factors and having weak SD sequence in the mRNAs.

FecI (σ^{18}) is involved in the expression of the iron uptake operon (*fecABCDE*) (*22*). The level of FecI protein was significantly higher in cells cultured in the presence of putrescine than its absence. The level of FecI mRNA in cells cultured in the presence of putrescine was, however, ~70% of the level in the absence of putrescine. This apparent disparity between mRNA and protein levels suggests that the efficiency of FecI mRNA translation is high in the presence of polyamines. One characteristic of FecI mRNA was the lack of the consensus SD sequence (GGAGG) in the expected position relative to the initiation codon AUG. In fact, FecI mRNA has no obvious SD sequence, but a candidate is the GA located 10 nucleotides upstream from the initiation codon AUG. To determine whether this weak SD-like sequence is related to polyamine stimulation of FecI synthesis, it was replaced by the typical SD sequence (GGAGG), and translational efficiency of FecI-LacZ-fusion mRNA was measured. 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 introduction of the consensus SD sequence, even though the basal level of protein synthesis in the absence of polyamines was strongly enhanced (71-fold). 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 (Fig. 2A) (*23*).

We also found that synthesis of Fis protein, a transcription factor of rRNA, some tRNAs, and some genes involved in energy production (*24*), was enhanced by polyamines about 3- to 4-fold at the level of translation. A weak SD sequence, GAG, is present 11 nucleotides upstream of the initiation codon AUG of Fis mRNA. Using the method described above, it was shown that the synthesis of Fis was enhanced by polyamines due to the existence of a weak SD sequence in the Fis mRNA (Fig. 2A) (*23*).

Since four of the five proteins (OppA, Cya, RpoS, FecI and Fis) encoded by the polyamine modulon are transcription factors, we compared mRNA levels in *E. coli* MA261 cells cultured with or without putrescine. The transcription profiles of the exponential-phase culture of MA261 with or without putrescine were determined by a two-color (Cy3 and Cy5) cDNA microarray analysis (*25*). Expression of 2,742 genes was detected in cells cultured with or without putrescine. Among these, 309 genes were up-regulated (>2-fold increase), and 319 genes were down-regulated (>2-fold decrease) by polyamines. Among the 309 up-regulated genes, 28, 23, 4 and 3 genes were under the control of RpoS, Cya, FecI and Fis, respectively. The results suggest that transcriptional enhancement of at least these 58 genes might be attributable to increased level of the transcription factors RpoS, Cya, FecI and Fis. 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. 2B). Our experimental data together support a unifying molecular mechanism defined by the polyamine modulon underlying the role of polyamines in cell growth.

Our hypothesis about the effect of polyamines on cell growth in *E. coli* is as follows. Polyamines probably do not function as an on/off switch of gene expression of specific proteins but, rather, "modulate" the level of many kinds of proteins, by 10-fold at most, to maintain optimal conditions for cell growth. In this way, polyamines function as an important factor for cell growth. When a polyamine-requiring mutant was cultured in the absence of putrescine, polyamine content became very low (Fig. 1A). Thus, modulation by polyamines would be weakened, causing cells to grow slowly. Upon further activation in the absence of putrescine, polyamine content in cells would become negligible, and cell growth would stop as a result of the decreased levels of many proteins that are involved in cell growth.

Effect of polyamines on cell growth at acidic pH

Having examined the effect of polyamines on cell growth at neutral pH, we next examined the effect at acidic pH (4.5 to 5.5). At acidic pH, the functions of two operons (*speF-potE* and *cadBA*) are important. These genes encode acid-inducible ornithine decarboxylase, putrescine-ornithine antiporter, cadaverine-lysine antiporter and acid inducible lysine decarboxylase (*26–28*). If lysine and ornithine are present in the acidic medium, expression of these genes is greatly enhanced. As the result, proton motive force is formed due to the consumption of H^+ through the production of putrescine and cadaverine by the two decarboxylases and the electrogenic antiport of putrescine with ornithine or cadaverine with lysine by the two antiporters, and the medium is neutralized through the excretion of putrescine and cadaverine by the two antiporters. Furthermore, CO_2, one of the substrates for the formation of ATP, is produced during the production of putrescine and cadaverine. Thus, the function of two operons (*speF-potE* and *cadBA*) regulated by amino acids at the level of transcription is important for cell growth at acidic pH, together with the polyamine modulon.

Further perspectives

Polyamines are also essential for cell growth in eukaryotes. We succeeded in identifying several proteins whose syntheses are enhanced by polyamines at the level of translation in eukaryotes. However, we have not yet succeeded in clarifying the detailed mechanism, because protein synthesis is initiated by different mechanisms in prokaryotes and eukaryotes. Furthermore, eIF5A, which contains the butylamine moiety of spermidine, is present in eukaryotes (*29*, *30*). eIF5A is essential for cell growth, but its function is still unclear. Polyamines, especially spermine, also modulate the function of inward rectification of K^+-channel and NMDA receptors in mammalian cells (*31–34*). Further studies are necessary to clarify the function of polyamines in eukaryotes at the molecular level.

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REFERENCES

1. Cohen, S.S. (1988) *A Guide to Polyamines*, Oxford University Press, New York
2. Igarashi, K. and Kashiwagi, K. (2000) Polyamines: mysterious modulators of cellular functions. *Biochem. Biophys. Res. Commun.* **271**, 559–564
3. Igarashi, K., Kashiwagi, K., Kishida, K., Kakegawa, T., and Hirose, S. (1981) Decrease in the S1 protein of 30-S ribosomal subunits in polyamine-requiring mutants of *Escherichia coli* grown in the absence of polyamines. *Eur. J. Biochem.* **114**, 127–131
4. Raj, V.S., Tomitori, H., Yoshida, M., Apirakaramwong, A., Kashiwagi, K., Takio, K., Ishihama, A., and Igarashi, K. (2001) Properties of a revertant of *Escherichia coli* viable in the presence of spermidine accumulation: increase in L-glycerol 3-phosphate. *J. Bacteriol.* **183**, 4493–4498
5. Pegg, A. (1988) Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. *Cancer Res.* **48**, 759–774
6. Igarashi, K. and Kashiwagi, K. (1999) Polyamine transport in bacteria and yeast. *Biochem. J.* **344**, 633–642
7. Watanabe, S., Kusama-Eguchi, K., Kobayashi, H., and Igarashi, K. (1991) Estimation of polyamine binding to macromolecules and ATP in bovine lymphocytes and rat liver. *J. Biol. Chem.* **266**, 20803–20809
8. Miyamoto, S., Kashiwagi, K., Ito, K., Watanabe, S., and Igarashi, K. (1993) Estimation of polyamine distribution and polyamine stimulation of protein synthesis in *Escherichia coli*. *Arch. Biochem. Biophys.* **300**, 63–68
9. Igarashi, K., Kashiwagi, K., Kishida, K., Watanabe, Y., Kogo, A., and Hirose, S. (1979) Defect in the split proteins of 30-S ribosomal subunits and under-methylation of 16-S ribosomal RNA in a polyamine-requiring mutant of *Escherichia coli* grown in the absence of polyamines. *Eur. J. Biochem.* **93**, 345–353
10. Igarashi, K., Kishida, K., Kashiwagi, K., Kakegawa, T., and Hirose, S. (1981) Relationship between methylation of adenine near the 3′ end of 16-S ribosomal RNA and the activity of 30-S ribosomal subunits. *Eur. J. Biochem.* **113**, 587–593
11. Kashiwagi, K., Yamaguchi, Y., Sakai, Y., Kobayashi, H., and Igarashi, K. (1990) Identification of the polyamine-induced protein as a periplasmic oligopeptide binding protein. *J. Biol. Chem.* **265**, 8387–8391
12. Shine, J. and Dalgarno, L. (1974) The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding site. *Proc. Natl. Acad. Sci. USA* **71**, 1342–1346
13. Igarashi, K., Saisho, T., Yuguchi, M., and Kashiwagi, K. (1997) Molecular mechanism of polyamine stimulation of the synthesis of oligopeptide-binding protein. *J. Biol. Chem.* **272**, 4058–4064
14. Yoshida, M., Meksuriyen, D., Kashiwagi, K., Kawai, G., and Igarashi, K. (1999) Polyamine stimulation of the synthesis of oligopeptide-binding protein (OppA). Involvement of a structural change of the Shine-Dalgarno sequence and the initiation codon AUG in OppA mRNA. *J. Biol. Chem.* **274**, 22723–22728
15. Ehresmann, C., Baudin, F., Mougel, M., Romby, P., Ebel, J.P., and Ehresmann, B. (1987) Probing the structure of RNAs in solution. *Nucleic Acids Res.* **15**, 9109–9128
16. Yoshida, M., Kashiwagi, K., Kawai, G., Ishihama, A., and Igarashi, K. (2001) Polyamine enhancement of the synthesis of adenylate cyclase at the translational level and the consequential stimulation of the synthesis of the RNA polymerase σ^{28} subunit. *J. Biol. Chem.* **276**, 16289–16295
17. Reddy, P., Peterkofsky, A., and McKenny, K. (1985) Translational efficiency of the *Escherichia coli* adenylate cyclase gene: mutating the UUG initiation codon to GUG or AUG results in increased gene expression. *Proc. Natl. Acad. Sci. USA* **82**, 5656–5660
18. Blattner, F.R., Plunkett III, G., Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F., Gregor, J., Davis, N.W., Kirkpatrick, H.A., Goeden, M.A., Rose, D.J., Mau, B., and Shao, Y. (1997) The complete genome sequence of *Escherichia coli* K-12. *Science* **277**, 1453–1462
19. Yoshida, M., Kashiwagi, K., Kawai, G., Ishihama, A., and Igarashi, K. (2002) Polyamines enhance synthesis of the RNA polymerase σ^{38} subunit by suppression of an amber termination codon in the open reading frame. *J. Biol. Chem.* **277**, 37139–37146
20. Tabor, H. and Tabor, C.W. (1982) Polyamine requirement for efficient translation of amber codons *in vivo*. *Proc. Natl. Acad. Sci. USA* **79**, 7087–7091
21. Ishihama, A. (2000) Functional modulation of *Escherichia coli* RNA polymerase. *Annu. Rev. Microbiol.* **54**, 499–518
22. Visca, P., Leoni, L., Wilson, M.J., and Lamont, I.L. (2002) Iron transport and regulation, cell signalling and genomics: lessons from *Escherichia coli* and *Pseudomonas*. *Mol. Microbiol.* **45**, 1177–1190
23. Yoshida, M., Kashiwagi, K., Shigemasa, A., Taniguchi, S., Yamamoto, K., Makinoshima, H., Ishihama, A., and Igarashi, K. (2004) A unifying model for the role of polyamines in bacterial cell growth, the polyamine modulon. *J. Biol. Chem.* **279**, 46008–46013
24. Hirvonen, C.A., Ross, W., Wozniak, C.E. Marasco, E., Anthony, J.R., Aiyar, S.E., Newburn, V.H., and Gourse, R.L. (2001) Contributions of UP elements and the transcription factors FIS to expression from the seven *rrn* P1 promoters in *Escherichia coli*. *J. Bacteriol.* **183**, 6305–6314
25. Schena, M., Shalon, D., Davis, R.W., and Brown, P.O. (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* **270**, 467–470
26. Kashiwagi, K., Kuraishi, A., Tomitori, H., Igarashi, A., Nishimura, K., Shirahata, A., and Igarashi, K. (2000) Identification of the putrescine recognition site on polyamine transport protein PotE. *J. Biol. Chem.* **275**, 36007–36012
27. Neely, M.N. and Olson, E.R. (1996) Kinetics of expression of the *Escherichia coli cad* operon as a function of pH and lysine. *J. Bacteriol.* **178**, 5522–5528
28. Soksawatmaekhin, W., Kuraishi, A., Sakata, K., Kashiwagi, K., and Igarashi, K. (2004) Excretion and uptake of cadaverine by CadB and its physiological functions in *Escherichia coli*. *Mol. Microbiol.* **51**, 1401–1412

29. Park, M.H., Lee, Y.B., and Joe, Y.A. (1997) Hypusine is essential for eukaryotic cell proliferation. *Biol. Signals* **6**, 115–123
30. Nishimura, K., Murozumi, K., Shirahata, A., Park, M.H., Kashiwagi, K., and Igarashi, K. (2005) Independent roles of eIF5A and polyamines in cell proliferation. *Biochem. J.* **385**, 779–785
31. Lopatin, A.N., Makhina, E.N., and Nichols, C. G. (1994) Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification. *Nature* **372**, 366–369
32. Yan, D.-H., Nishimura, K., Yoshida, K., Nakahira, K., Ehara, T., Igarashi, K., and Ishihara, K. (2005) Different intracellular polyamine concentrations underlie the difference in the inward rectifier K^+ currents in atria and ventricles of the guinea-pig heart. *J. Physiol.* **563**, 713–724
33. Williams, K. (1997) Interactions of polyamines with ion channels. *Biochem. J.* **325**, 289–297
34. Masuko, T., Kashiwagi, K., Kuno, T., Nguyen, N.D., Pahk, A.J., Fukuchi, J., Igarashi, K., and Williams, K. (1999) A regulatory domain (R1-R2) in the amino terminus of the *N*-methyl-D-aspartate receptor: Effects of spermine, protons, and ifenprodil, and structural similarity to bacterial leucine/isoleucine/valine binding protein. *Mol. Pharmacol.* **55**, 957–969