Effects of FIS Overexpression on Cell Growth, rRNA Synthesis, and Ribosome Content in *Escherichia coli*

Richard Richins and Wilfred Chen*

Department of Chemical and Environmental Engineering, University of California, Riverside, California 92521

The *Escherichia coli* DNA binding protein FIS is a transcriptional modulator involved in the regulation of many cellular processes, including the activation of rRNA synthesis. High-level overproduction of FIS in early, mid, or late log cultures resulted in growth-phase- and media-specific variations in cell growth, rRNA synthesis, and ribosome content. FIS overproduction caused a pronounced increase in rRNA synthesis for late-exponential cultures but a substantial reduction in cell growth and ribosome content. The addition of simple sugars such as glucose or fructose reversed these phenomena, consistent with the functional role of FIS in carbon metabolism.

Introduction

The regulation of ribosomal RNA (rRNA) synthesis plays a key role in the coordination of ribosome synthesis (*1*). Under fast-growing conditions, rRNA may constitute over half of the total cellular RNA (*2*). A number of activating and repressing mechanisms such as growth-rate control and stringent response are coordinately involved in the global control network that modulates rRNA transcription (*3, 4*). The nucleotide ppGpp is one such regulatory signal responsible for reducing rRNA synthesis during the transition from exponential to stationary phase growth (*5*). However, even in the absence of ppGpp, other growth-rate-mediated regulations are sufficient to modulate rRNA synthesis (*6*).

FIS is a 11.2 kDa, heat stable, DNA binding protein in *Escherichia coli* that has the ability to stimulate site-specific DNA inversion reactions by binding to an enhancer sequence and bending the DNA (*7, 8*). FIS can stimulate stable RNA synthesis both in rich medium and under conditions of nutrient upshift (*9, 10*). An upstream activator sequence (UAS) appears to be the target. FIS-dependent activation appears to be crucial for providing the high rate of rRNA synthesis required for rapid cell growth. Although in vitro experiments have demonstrated the direct enhancement in transcription from the rRNA promoters in the presence of FIS, in vivo demonstrations have so far been limited to comparisons between *fis*$^+$ and *fis*$^-$ strains.

In addition to activating the rRNA promoters, FIS is also active in many cellular processes. Even though the *fis* gene is not essential for viability, the expression of a diverse set of genes is negatively regulated by FIS, including some that are specifically inhibited during the transition from exponential to stationary growth (*11*). This suggests that even very low levels of FIS may be sufficient to reduce gene expression either directly or by an inhibitory cascade. A similar activation effect on rRNA synthesis by FIS during the stationary phase has been suggested (*12*). FIS has also been shown to be an important transcriptional modulator involved in the regulation of both sugar and nucleic acid metabolism (*13*), suggesting that FIS may play a global role in the adaptation of *E. coli* to changing environmental and nutritional conditions.

Previously, we have shown that a low level of elevated FIS production resulted in enhanced rRNA promoter activity and recombinant protein synthesis (*14*). However, the precise mechanism for this activation is still unknown. Since the cellular level of FIS normally declines to only a few percent of its peak concentration after the early exponential phase, very little is known about the effect of FIS on ribosome synthesis and cell physiology under these conditions. In this study, we describe the overproduction of FIS to varying degrees in order to investigate the differential activation in rRNA synthesis and the physiological effects on *E. coli* during the entire growth cycle.

Materials and Methods

Plasmids, Strains, and Media. Plasmids pKG18 (the source of the *fis* gene) was generously provided by R. Gourse (*15*). pKG18 was digested with *Ssp*I + *Bsp*HI. The fragments were then treated with mung bean nuclease digestion prior to ligation into *HincII* cut pK184 (*16*). *E. coli* strain JM105 (F′ *traD36 lacI*^{q} Δ(*lacZ*)*M15 proA*$^+$*B*$^+$|*thi rpsL* (Str^r) *endA sbcB15 sbcC hsdR4*($r_k^-m_k^+$) Δ(*lac-proAB*)) and DH5a F′ (F′ *ϕ80dlac* Δ*M15* Δ(*lacZYA-argF*)-*U169 deoR recA1 endA1 hsdR17*($r_k^-m_k^+$) *supE44*λ^- *thi-1 gyr*A96 *relA1*) served as the recipient cell line for construction of the plasmids. The resulting plasmid, pKFis4, contained an optimal context for expression of Fis from a *tac* promoter.

A pK184-based control plasmid expressing the chloramphenicol acetyltransferase (CAT) gene was also constructed. A CAT cartridge isolated from pKC6 (*17*) was treated with *Hind*III and ligated into similarly digested pKK223 to give pKK233-CAT2. The *tac* promoter and CAT gene were excised by treatment with *Bsp*H1 (followed by treatment with Mung Bean Nuclease), then isolated by gel purification. The DNA fragment was then partially digested with *Ssp* I. The 1312 bp *tac*-CAT fragment was finally gel isolated and ligated into *Hinc*II-cut (CIP-treated) pK184 to give pKCAT5.

E. coli strain W1485ΔH [*rrn*H P1P2 − CAT] (*18*) was used for the growth rate studies and the *rrn* promoter

* Ph: (909) 787-2473. Fax: (909) 787-2425. E-mail: Wilfred@engr.ucr.edu.

10.1021/bp000170f CCC: $20.00
Published on Web 02/15/2001

analysis. Since this cell line does not express appreciable amounts of the *lac* repressor, a *lacI*^{q} expressing plasmid pKQV4 (*19*) was used to reduce leaky expression of the FIS constructs. Media utilized for this investigation include YT [8 g Bacto-Tryptone; 5 g yeast extract; 5 g NaCl]; YTG [YT + 0.4% (w/v) glucose]; BYT [YT + 25 mM KPO_4, pH 7.0]; BYTG [BYT + 0.4% (w/v) glucose]; and M9 (*20*).

Activation of *rrnH* Promoter (CAT Reporter). *E. coli* strain W1485ΔH [*rrn*H P1P2 − CAT] cells that were doubly transformed with pKQV4 and either pK184 or pKFis4 were cultured in selected media. Media was supplemented with kanamycin (50 μg/mL) and ampicillin (100 μg/mL) to ensure retention of the pK184-based constructs and pKQV4 plasmid, respectively. Isopropylthiogalactoside (IPTG) was added at varying concentrations and/or at various times post-inoculation to induce production of FIS. CAT activity was assayed following the procedure described in Rodriguez and Tait (*21*).

Quantification of rRNA Production by Primer Extension. *E. coli* cells transformed with plasmids pKFis4 and pKQV4 (or pK184 and pKQV4 control) were grown to an OD (600 nm) of 0.4 and then induced with IPTG. Primer Extension (*20*) was used to monitor rRNA accumulation 0, 1, 2, and 3 h post-induction. A 17-base oligonucleotide (dGAGCAGTGCCGCTTCGC) was annealed to isolated, total cellular RNA prior to reverse transcription. Samples were electrophoresed through 8% sequencing gels prior to autoradiography.

Ribosome Quantification. Ribosomes were quantified by the procedure of Dong et al. (*22*) except for the following modifications. A total of 100 μg/mL lysozyme was included in the poly-mix buffer used to initially resuspend the cells. The cells were incubated in the lysozyme-containing buffer for 15 min at room temperature prior to being sonicated for 10 s. The cells were centrifuged two times for 10 min at 13,000 rpm in an Eppendorf 5415C microcentrifuge. A total of 600 μL of the supernatants were layered atop 12 mL of 10%−40% sucrose density gradients. Gradients were centrifuged in a Beckman SW 41 rotor for 4 h at 41,000 rpm. Absorbance profiles were determined for each gradient at 254 nm with an ISCO gradient fractionator.

Purification and Analysis of FIS. FIS purification was performed as described by Gosink et al. (15). Samples (1 mL) of *E. coli* cultures were transferred into 1.5 mL microcentrifuge tubes. Cells were collected by centrifugation and resuspended in 0.375 mL of FIS cell lysis buffer [0.3 M NaCl; 50 mM Tris, pH 8.0, 1 mM EDTA, 2 mM DTT, 0.1 mM PMSF, 200 μg/mL lysozyme, 5 mg/mL spermadine, 10% (v/v) glycerol]. Following a 15 min incubation at room temperature, the samples were cooled to 0 °C and sonicated for 10 s. Next, 63 μL of 5 M NaCl was mixed thoroughly into the lysed cells; the mixture was then centrifuged for 10 min at 14,000 rpm. Then, 454 μL of the supernantent was transferred into a clean microcentrifuge tube containing 1.05 mL of 5% (v/v) SP-Sepharose [in 20 mM Tris-HCl, pH 8.0; 0.1 mM EDTA; 10% (v/v) glycerol]. This mixture was gently mixed for about 10 min. The mixture was then gently passed through a Promega Wizard minicolumn (attached to a 3 mL syringe). The minicolumn was washed with 2 mL of Fis wash buffer [0.3 M NaCl; 20 mM Tris, pH 8.0; 0.1 mM EDTA; 10% (v/v) glycerol]. The minicolumn was placed in a microcentrifuge tube and centrifuged for 4−5 min at 2500 rpm. The minicolumn was transferred to a clean microcentrifuge tube. 40 μl of FIS elution buffer [1.0 M NaCl; 20 mM Trips, pH 8.0; 0.1 mM EDTA; 10% (v/v) glycerol] was added to the column. Following a 1−2 min incubation, the minicolumn was centrifuged for 4−5 min.

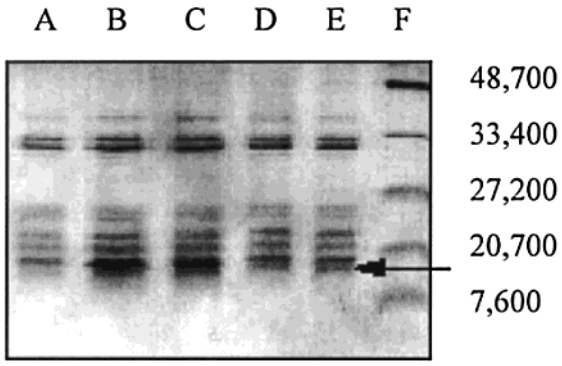

Figure 1. FIS expression in induced W1485ΔH cells. FIS proteins were purified as described in Materials and Methods prior to electrophoresis through a 17.5% SDS−PAGE gel. Samples were collected 3 h postinduction. Lane A: cells carrying pK184 induced with 1 mM IPTG. Lanes B−E: cells expressing pKFis4 induced with 1, 0.1, 0.01, or 0 mM IPTG, respectively. Lane F: molecular weight markers (Bio-Rad Broad Range SDS−PAGE Standards). The approximate sizes of the molecular weight markers are shown. The arrow indicates the position of the FIS protein on the gel.

Samples (10−20 μL of the eluted protein sample) were analyzed on 17.5% SDS−PAGE gels. Gels were silver stained using the BioRad Silver Stain Plus Kit (BioRad, Hercules, CA).

Results and Discussion

FIS Overproduction Enhances rRNA Synthesis during the Entire Growth Cycle. To test the effects of FIS overproduction on the activation of rRNA promoters, *E. coli* strain W1845ΔH carrying pKFIS and pKQV4 was grown at 37 °C in YT medium. FIS was overproduced to varying degrees by the addition of different amounts of IPTG. Induction with as little as 0.1 mM was sufficient to produce a significant amount of FIS (Figure 1).

Differential FIS activation of the rRNA promoters during the growth cycle was investigated by taking advantage of the chromosomal *rrnH-cat* fusion in the *E. coli* strain W1485ΔH. The inherent strength of the *rrnH* promoter is directly reflected from the measured CAT activity (*18*). As expected, CAT activity in uninduced cultures (or in the pK184 control) rose sharply during early and mid-exponential growth (Figure 2A). Upon transition into the late log and stationary phases, the specific CAT activities declined 3- to 4-fold, reflecting the decreasing *rrnH* promoter activity. Conversely, CAT activity in induced cultures containing pKFis4 was substantially increased with increasing IPTG concentrations. CAT activity was almost 3-fold higher in cultures induced with 1 mM IPTG. This relatively high level of CAT activity was maintained even during the stationary phase.

To ensure that the increased CAT activity truly corresponds to an increase in rRNA promoter activity, rRNA synthesis was measured directly by primer extension. As depicted in Figure 2A, rRNA accumulation increased with increasing FIS overexpression, thus supporting the CAT expression data. For cultures induced with 0.1 and 1 mM IPTG, significant rRNA synthesis was still evident 3 h postinduction, while only minimal synthesis was observed for cultures without FIS overproduction. This finding demonstrates that cessation of rRNA synthesis during the transition from exponential growth to station-

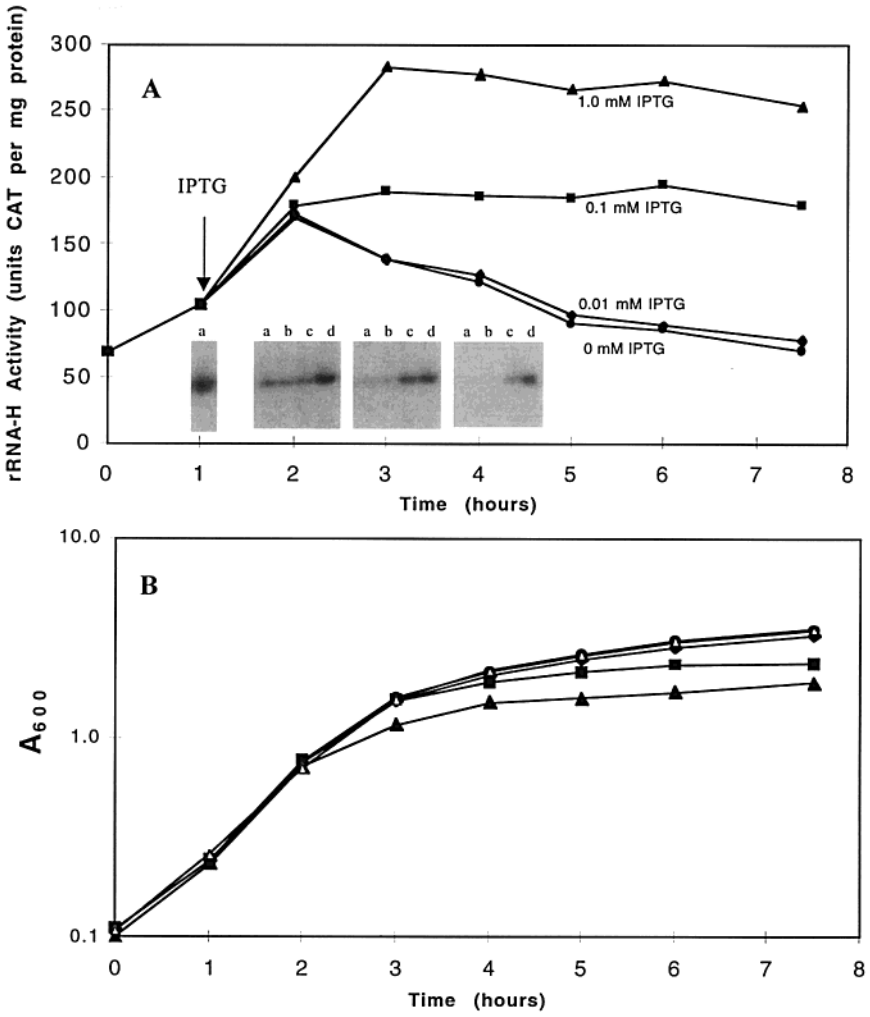

Figure 2. (A) FIS overexpression enhances rRNA production in W1485ΔH cells. Normalized rRNA-H promoter activities (CAT reporter) are shown for cells carrying pKFis4 and induced with various concentrations of IPTG. Inset shows primer extension results (rRNA quantification) for samples up to 4 h post-induction (lanes a−d: cultures induced with 0 (●), 0.01 (◆), 0.1 (■), or 1 mM IPTG (▲), respectively). (B) Effect of FIS overproduction on cell growth. Cell density (A_{600}) for W1485ΔH cells overexpressing FIS: pKCAT5 (1 mM IPTG) (△), pKFis4 (0 mM IPTG) (●), pKFis4 (0.01 mM IPTG) (◆), pKFis4 cells (0.1 mM IPTG) (■), pKFis4 (1 mM IPTG) (▲). Arrow indicates the time of induction. Cultures were grown in YT medium at 37 °C and 300 rpm.

ary growth can be prevented by increasing the intracellular levels of FIS. Stimulation of rRNA synthesis during late exponential and early stationary growth is significant because the effects of FIS-dependent activation has so far been observed primarily with rapidly growing cultures (*9, 23*). These results suggest that high levels of FIS may be able to counteract the inhibitory effects of ppGpp and H-NS, which are responsible for down-regulating rRNA synthesis during slow-growing condition (*6*). In vitro inhibition of rRNA synthesis by ppGpp and H-NS has already been demonstrated to be less effective in the presence of modest concentrations of FIS (*24*−*26*).

FIS Overproduction Reduces Cell Growth in a Growth-Rate-Specific Manner. In contrast to the observed increase in rRNA synthesis, cell growth was reduced when FIS was overproduced to a high level (Figure 2B). The final cell density was reduced by more than 2-fold in cultures induced with 1 mM IPTG. As a control, CAT was overproduced with a similar construct (pKCAT5), but the effect on cell growth was negligible compared with the effect of overproducing FIS. There was little difference in cell growth or CAT expression for the sample induced with 2.5 mM IPTG compared to that of the culture induced with 1 mM IPTG, indicating a limit to FIS expression and/or a saturation of FIS interactions with its target sequences (data not shown). The reduction in growth was directly correlated with the elevated level of rRNA synthesis, an observation consistent with other reports that indicated anywhere from a 10% to 40% decrease in growth rates when rRNA was overexpressed (*27*−*29*). The global effector molecule ppGpp did not appear to contribute to the slow in cell growth since similar reduction in growth was also observed in cells devoid of ppGpp (data not shown).

Because the intracellular level of FIS varies widely with growth rate and growth phase, reaching its highest level during early log phase, it is possible that the effect of FIS overproduction may be growth rate (phase) specific. To determine this possibility, IPTG was added (to 1 mM) to induce FIS overproduction either during early log, mid log, or late log phase. Independent of when induction occurred, minimal inhibitory effect on cell growth was observed in rapidly growing cultures (Figure 3). The level of growth inhibition became significant only when cell growth began to decline. Because maximal occupancy of the FIS binding sites normally occurs during early log phase (*30*), it is unlikely that any further binding/promoter regulation could occur even in the presence of elevated FIS production. This may explain the higher tolerance of FIS for rapidly growing cells and why FIS overexpression was highly deleterious to the cell growth upon the onset of the late log phase. During slow-

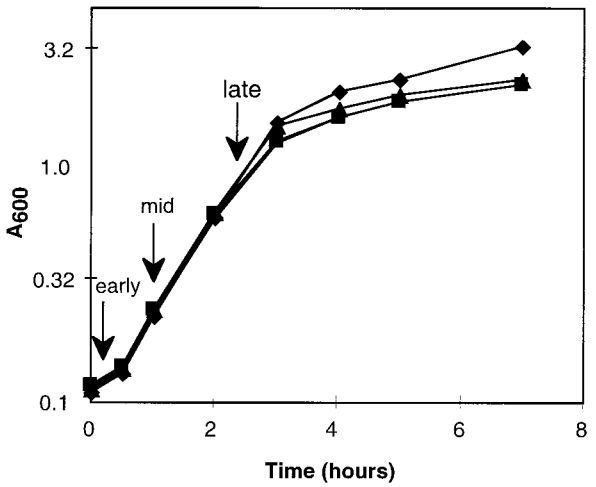

Figure 3. Growth-phase-specific inhibition of cell growth. Cell growth (A_{600}) is shown for W1485ΔH cells carrying the pK184 plasmid induced with 1 mM IPTG during early (■), mid (●), and late (▲) exponential phase. Uninduced cells (◆) are shown for comparison.

growing conditions, the balance between rRNA synthesis and nutrient availability may be adversely affected by the gross overproduction of FIS.

Effects of FIS Overproduction on Ribosomes. In previous studies, overexpression of rRNA has always been associated with a corresponding accumulation of ribosomes (*27*, *31*). To investigate whether the increase in rRNA synthesis as a result of FIS overproduction during late log or stationary phase can also lead to higher intracellular ribosome concentrations, the cellular compliment of ribosomes were quantified by sucrose gradient analysis. In contrast to rRNA synthesis, the intracellular compliment of 70S ribosomes declined substantially as a function of increasing FIS overexpression (Figure 4). As a control, no reduction in the 70S ribosome content was observed when CAT (pKCAT5) was overexpressed (data not shown). Since the synthesis rates of rRNA and ribosome are typically correlated (*1*), such an inverse correlation was unexpected. Since the ribosome content is a function of growth rate, roughly increasing as μ^2, the dramatic reduction in growth rate may be responsinble for the decline in ribosomes.

The Effects of FIS Overproducing Cells Are Affected by Carbon Source. It has been established that FIS is a transcriptional modulator involved in the regulation of carbon metabolism in *E. coli* (*13*). To assess whether carbon metabolism plays a role in the global effect of FIS on cell growth, rRNA and ribosome synthesis, we repeated the growth experiments described above in media supplemented with either glucose, fructose or glycerol. Under all conditions, cultures induced with 1 mM IPTG produced a similar level of FIS as in YT medium. For media supplemented with 0.2% glucose, cell growth was virtually unaffected (Figure 5). A similar effect was observed when fructose was added to the growth medium (data not shown). On the other hand, addition of a non-PTS carbon source such as glycerol did not prevent the reduction in cell growth caused by FIS overproduction. In addition, the *rrnH* promoter was only slightly activated by FIS overproduction in glucose-supplemented medium, while it was activated by more than 3-fold in glycerol-supplemented medium (Figure 5). A comparison of the cellular ribosome compliment also revealed no noticeable difference between cells with or without FIS overexpression when grown in glucose-supplemented medium (data not shown). Because the intracellular cAMP concentration is generally low in the presence of glucose and fructose but not glycerol, these results suggest a potential link between the intracellular cAMP concentration and the inhibitory effects of FIS.

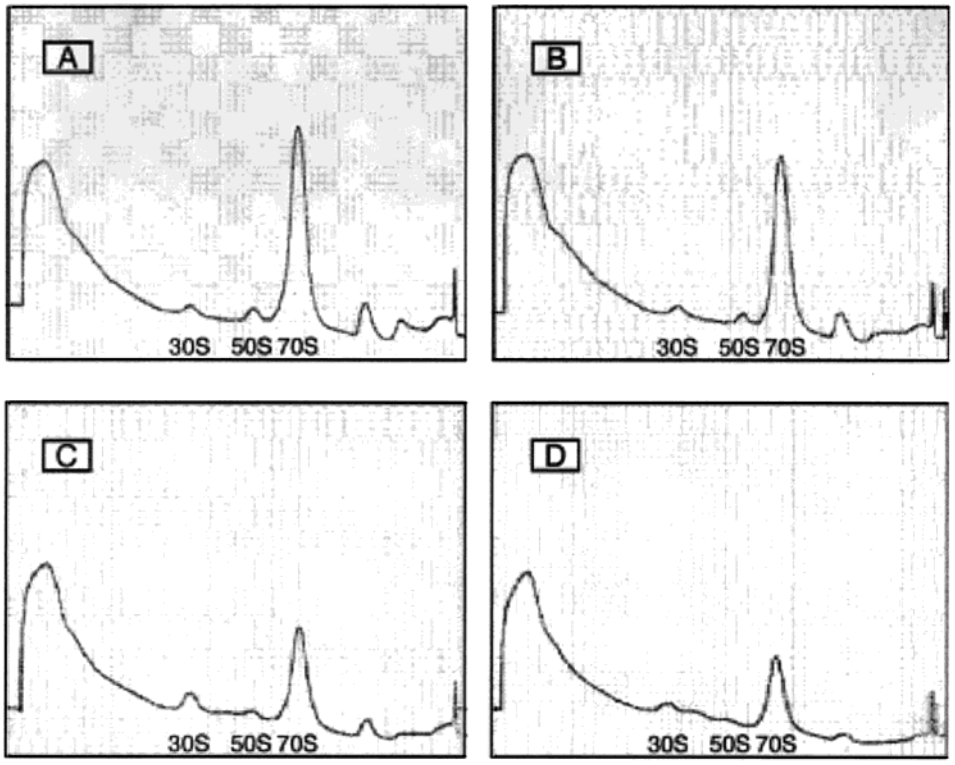

Figure 4. Effect of FIS overproduction on the cellular ribosome content. Ribosomes were fractionated as described in Materials and Methods. The profiles obtained from the sucrose gradients (A254) are shown. Panels A−D: cells carrying the pKFis4 plasmid induced with 0, 0.01, 0.1 and 1.0 mM IPTG, respectively. Samples were collected 4 h after induction as shown in Figure 2B.

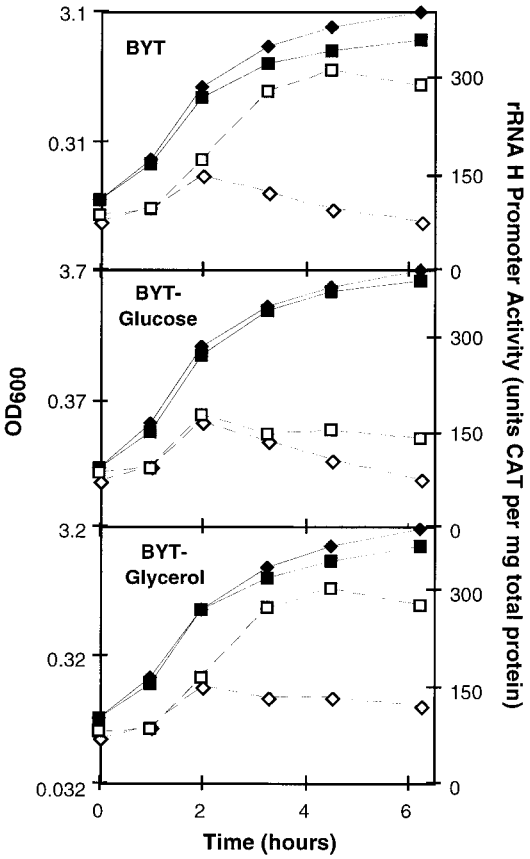

Figure 5. Carbon source affects cell growth and FIS-mediated activation of rRNA promoter. The effects of carbon source on cell growth (solid lines) and rRNA-H activity (dashed lines) of cells carrying pK184 (◆, ◇) and pKFis4 (■, □) are shown for cells grown in BYT (top panel), BYT containing 0.2% glucose (middle panel), and BYT containing 0.2% glycerol.

Conclusions

In conclusion, high levels of FIS are sufficient to activate rRNA synthesis even in the presence of other inhibitory mechanisms, demonstrating that the relative contribution of FIS-dependent mechanism to $rrnp_1$ activity may be more dominant than other overlapping regulatory mechanisms. A similar effect may also be true for other FIS-regulated processes. A more detailed study of the mechanisms by which FIS influences cell growth and ribosome content will provide more insights for assessing the precise role of FIS in slow-growing cultures.

Acknowledgment

This work was supported by an NSF Career Award (BES9700981). We thank Dr. R. L. Gourse for providing the plasmid pKG18.

References and Notes

(1) Zengel, J. M.; Lindahl, L. Diverse mechanisms for regulating ribosomal protein synthesis in *Escherichia coli*. In *Progress in Nucleic Acid Research and Molecular Biology*; Cohn, W. E., Moldave, K., Eds.; Academic Press: San Diego, 1994; Vol. 47, pp 331−370.

(2) Jinks-Robertson, S.; Nomura, M. Ribosomes and tRNA. In *Escherichia coli and Salmonella typhimurium*: *Cellular and Molecular Biology*; Neidhardt, F. C., Curtiss, R., Ingraham, J. L., Lin, E. C. C., Low, B., Magasanik. B., Eds.; American Society for Microbiology: Washington, DC, 1996; pp 1358−1385.

(3) Cashel, M.; Gentry, D. R.; Hernandez, V. J. and Vinella, D. The stringent response. In *Escherichia coli and Salmonella typhimurium*: *Cellular and Molecular Biology*; Neidhardt, F. C., Curtiss, R., Ingraham, J. L., Lin, E. C. C., Low, B., Magasanik. B., Eds.; American Society for Microbiology: Washington, DC, 1996; pp 1458−1496.

(4) Gausing, K. Regulation of ribosome production in *Escherichia coli*: Synthesis and stability of ribosomal RNA and ribosomal protein messenger RNA at different growth rates. *J. Mol. Biol.* **1977**, *115*, 335−354.

(5) Ryals, J.; Little, R.; Bremer, H. Control of ribosomal RNA and transfer RNA synthesis in *Escherichia coli* by guanosine tetraphosphate. *J. Bacteriol.* **1982**, *151*, 1261−1268.

(6) Aviv, M.; Giladi, H.; Oppenheim, A. B.; Glaser, G. Analysis of the shut-off of ribosomal RNA promoters in *Escherichia coli* upon entering the stationary phase of growth. *FEMS Microbiol. Lett.* **1996**, *140*, 71−76.

(7) Johnson, R. C.; Bruist, M. F.; Simon, M. I. Host protein requirements for in vitro site-specific DNA inversion. *Cell* **1986**, *46*, 531−539.

(8) Kahmann, R.; Rudt, F.; Koch, C.; Merlens, G. G inversion in bacteriophage Mu DNA is stimulated by a site within the invertase gene and a host factor. *Cell* **1985**, *41*, 771−780.

(9) Nilsson, L.; Vanet, A.; Vijgenboom, E.; Bosch, L. The role of Fis in *trans*-activation of stable RNA operons of *E. coli. EMBO J.* **1990**, *9*, 727−734.

(10) Nilsson, L.; Verbeek, H.; Vijgenboom, E.; van Drunen, C.; Vanet, A.; Bosch, L. Fis-dependent *trans* activation of stable RNA operons of *Escherichia coli* under various growth conditions. *J. Bacteriol.* **1992**, *174*, 921−929.

(11) Xu, J.; Johnson, R. C. Identification of genes negatively regulated by FIS: FIS and RpoS comodulate growth-phase-dependent gene expression in *Escherichia coli*. *J. Bacteriol.* **1995**, *177*, 938−947.

(12) Afflerbach, H.; Schroder, O.; Wagner, R. Effects of the *Escherichia coli* DNA-binding protein H-NS on rRNA synthesis in vivo. *Mol. Microbiol.* **1988**, *28*, 641−653.

(13) Gonzalez-Gil, G., Bringmann, P. and Kahmann, R. FIS is a regulator of metabolism in *Escherichia coli*. *Mol. Microbiol.* **1996**, *22*, 21−29.

(14) Richins, R.; Htay, T., Kallio, P. T., Chen, W. Elevated Fis expression enhances recombinant protein synthesis in *Escherichia coli*. *Biotechnol. Bioeng.* **1997**, *56*, 138−144.

(15) Gosink, K. K.; Gaal, T.; Bokal, A. J.; Gourse, R. L. A positive control mutant of the transcription activator protein FIS. *J. Bacteriol.* **1996**, *178*, 5182−5187.

(16) Jobling, M. G.; Holmes, R. K. Construction of vectors with the P15a replicon. kanamycin resistance inducible Lac-Z-α and pUC18 or pUC19 multiple cloning sites. *Nucleic Acids Res.* **1990**, *18*, 5315−5316.

(17) Chen W.; Kallio, P.; Bailey, J. E. Construction and characterization of a novel cross-regulation system for regulating cloned-gene expression in *E. coli*. *Gene* **1993**, *130*, 15−22.

(18) Condon, C.; Philips, J.; Fu, Z. J.; Squires, C.; Squires, C. L. Comparison of the expression of the seven ribosomal RNA operons in *Escherichia coli. EMBO J.* **1992**, *11*, 4175−4185.

(19) Strauch, M. A.; Spiegelman, G. B.; Perego, M.; Johnson, W. C.; Burbulys, D.; Hoch, J. A. The transition state transcription regulator *abrB* of *Bacillus subtilis* is a DNA binding protein. *EMBO J.* **1989**, *8*, 1615−162.

(20) Ausubel, F. M.; Brent, R.; Kingston, R. E.; Moore, D. D.; Seidman, J. G.; Smith, J. A.; Struhl, K. *Current Protocols in Molecular Biology*; Greene Publishing Associates and Wiley-Interscience: New York, 1987.

(21) Rodriguez, R. L.; Tait, R. C. *Recombinant DNA Techniques: An Introduction*; The Benjamin/Cummings Publishing Company, Inc.: Menlo Park, CA, 1983.

(22) Dong, H.; Nilsson, L.; Kurland, C. G. Gratuitous overexpression of genes in *Escherichia coli* leads to growth inhibition and ribosome destruction. *J. Bacteriol.* **1995**, *177*, 1497−1504.

(23) Ball, C. A.; Osuna, R.; Ferguson, K. C.; Johnson, R. C. Dramatic changes in Fis levels upon nutrient upshift in *Escherichia coli*. *J. Bacteriol.* **1992**, *174*, 8043−8056.

(24) Lazarus, L. R.; Travers, A. A. The *Escherichia coli* Fis protein is not required for the activation of *tyrT* transcription on entry into exponential growth. *EMBO J.* **1993**, *12*, 2483−2494.
(25) Tippner, D.; Afflerbach, H.; Bradaczek, C.; Wagner, R. Evidence for a regulatory function of the histone-like *E. coli* protein H-NS in ribosomal RNA synthesis. *Mol. Microbiol.* **1994**, *11*, 589−604.
(26) Travers, A. A. *DNA-Protein Interactions*; Chapman & Hall: London, 1993.
(27) Baracchini, E.; Bremer, H. Control of rRNA synthesis in *Escherichia coli* at increased *rrn* gene dosage. *J. Biol. Chem.* **1991**, *266*, 11753−11760.
(28) Jinks-Robertson, D.; Gourse, R. L.; Nomura, M. Expression of rRNA and tRNA genes in *Escherichia coli*: evidence for feedback regulation byproducts of rRNA operons. *Cell* **1983**, *33*, 865−876.
(29) Stevenson, B. and Schmidt, T. M. Growth-rate-dependent accumulation of RNA from plasmid-borne rRNA operons in *Escherichia coli. J. Bacteriol.* **1998**, *180*, 1970−1972.
(30) Appleman, J. A.; Ross, W.; Salomon, J.; Gourse, R. L. Activation of *Escherichia coli* rRNA transcription by FIS during a growth cycle. *J. Bacteriol.* **1998**, *180*, 1525−1532.
(31) Gourse, R. L.; Takebe, Y.; Sharrock, R. A.; Nomura, M. Feedback regulation of rRNA and tRNA synthesis and accumulation of free ribosomes after conditional expression of rRNA genes. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1069−1073.

Accepted for publication December 22, 2000.

BP000170F