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# Identification of Genes Negatively Regulated by Fis: Fis and RpoS Comodulate Growth-Phase-Dependent Gene Expression in *Escherichia coli*

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**Fis is a nucleoid-associated protein in *Escherichia coli* that has been shown to regulate recombination, replication, and transcription reactions. It is expressed in a transient manner under batch culturing conditions such that high levels are present during early exponential phase and low levels are present during late exponential phase and stationary phase. We have screened a random collection of transposon-induced *lac* fusions for those which give decreased expression in the presence of Fis. Thirteen different Fis-repressed genes were identified, including *glnQ* (glutamine high-affinity transport), *mglA* (methyl-galactoside transport), *xylF* (D-xylose-binding protein), *sdhA* (succinate dehydrogenase flavoprotein subunit), and a newly identified aldehyde dehydrogenase, *aldB*. The LacZ expression patterns revealed that many of the fusions were maximally expressed at different stages of growth, including early log phase, mid- to late log phase, and stationary phase. The expression of some of the late-exponential- and stationary-phase genes was dependent on the RpoS sigma factor, whereas that of others was affected negatively by RpoS. We conclude that Fis negatively regulates a diverse set of genes and that RpoS can function to both activate and inhibit the expression of specific genes.**

Fis is a small DNA-binding protein that was originally identified because of its prominent role in site-specific DNA inversion reactions (18, 19). It has a relaxed target specificity and has been shown to bind to many different sites throughout the *Escherichia coli* chromosome (8). DNA binding is mediated by helix-turn-helix motifs located in the carboxy termini of the Fis dimer and results in a high degree of bending of the DNA (22, 50). A region in the amino terminus is required to stimulate Hin- and Gin-mediated DNA inversion and may play a role in transcriptional activation but does not influence λ excision (12, 20, 35).

The expression of Fis is growth phase dependent (4, 31, 32, 46). Its intracellular levels increase rapidly in a transient manner in response to nutritional upshift. The number of Fis dimers per cell increases about 500-fold within the first cell division when a stationary-phase culture is inoculated into a rich medium, reaching a peak level of 40,000 to 50,000 dimers per cell. Under standard batch culturing conditions, Fis expression is largely turned off in mid-exponential phase and the intracellular levels of Fis decline rapidly as a function of each cell division. Fis levels in cells maintained in stationary phase are extremely low.

In addition to functioning in site-specific recombination reactions, Fis has been shown to enhance transcription of rRNA and certain tRNA operons (29, 30, 38, 41) and to stimulate DNA replication from *oriC* (7, 11). The activation of the rRNA and tRNA operons by Fis is associated with the binding of the protein to specific DNA sequences upstream of the promoters (31, 38). Fis has also been shown to negatively regulate its own expression by binding to multiple sites within the *fis* promoter region (4, 32). In addition, levels of certain tRNAs are elevated in *fis* mutant cells (29). A broad role for Fis in gene regulation is indicated by a comparison of the protein profiles of *fis* mutant cells and *fis*$^+$ cells resolved by a two-dimensional gel electrophoresis (36). More than 20 spots disappeared from the protein profile of *fis* mutant cells, and a similar number of new spots appeared. These results suggest that many Fis-regulated genes remain to be identified, especially those repressed by Fis.

To identify genes whose expression is modulated by Fis, transposon-generated LacZ protein and operon fusions were screened for differential expression as a function of Fis levels. In this study, we characterize 14 *lacZ* fusions whose expression is increased in the absence of Fis. Most of these genes display variable expression patterns with respect to growth phase, suggesting that they function under specific physiological conditions. A significant number of the fusions repressed by Fis were found to be regulated positively or negatively by RpoS, a sigma factor required for the expression of many stationary-phase genes. As opposed to those of Fis, levels of RpoS increase in late exponential phase and stationary phase (10, 45).

## MATERIALS AND METHODS

**Bacterial strains, plasmids, phages, and media.** The bacterial strains, plasmids, and phages used in this study are described in Table 1. The *fis-985* mutation contains the *str*/*spc* cassette substituted in place of the central half of the *fis* gene (4), and *katF13*::Tn*10* from UM122 is a Tn*10* insertion in *rpoS* (27). Luria Bertani (LB) broth and LB agar plates were used as rich media, and M9 media were used as defined media throughout the study (25). Antibiotics were used at the following concentrations in plates and in broth: ampicillin, 100 μg/ml; chloramphenicol, 20 μg/ml; kanamycin, 20 μg/ml; spectinomycin, 50 μg/ml; and tetracycline, 10 μg/ml. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) was used at a concentration of 40 μg/ml.

**General molecular and genetic techniques.** Standard recombinant DNA methodologies were as described (40). Bacterial conjugation and P1*vir*-mediated transduction were performed according to the protocols described by Miller (25). The presence of the *katF13*::Tn*10* mutation in tetracycline-resistant transductants was confirmed by testing for decreased catalase activity by $H_2O_2$ bubbling (27).

**Generation of lacZ fusions.** LacZ operon and protein fusions to chromosomal genes were isolated by infecting *E. coli* K-12 strain RJ4000 *fis-985* (pRJ4000, pRJ823) with phage λTn*phoA′-1* or λTn*phoA′-4* to generate *lacZ* operon and protein fusions, respectively (47). pRJ4000 contains *fis* under *lacP* control on a pBR322-derived plasmid, and its expression is repressed by the *lacI*$^q$ gene product carried by the pACYC184 derivative pRJ823. In the absence of IPTG (iso-

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TABLE 1. Bacterial strains, plasmids, and phages

| Strain, plasmid, or phage | Relevant characteristics[a] | Source or reference |
|---|---|---|
| Strains | | |
| BW5104 | Mu-1 *lac-169 creB510 hsdR514* | B. Wanner (47) |
| BW14879 | pMW11/Mu *c*ts62 DE3 Δ*lacX74* Δ(*phoA532 Pvu*II) *phn* (*EcoB*) *arcA1655 fnr-1655* | B. Wanner (47) |
| CAG4000 | MG1655 Δ*lacX74* | C. Gross |
| RJ1801 | CAG4000 *fis-985* | This lab (4) |
| RJ4000 | RJ1801 pRJ823 pRJ4000 | This study |
| RJ4001 | RJ4000 *frg-15*::Tn*phoA′-4* | This study |
| RJ4002 | RJ4000 *xylF-103*::Tn*phoA′-4* | This study |
| RJ4003 | RJ4000 *sdhA-133*::Tn*phoA′-4* | This study |
| RJ4004 | RJ4000 *frg-502*::Tn*phoA′-1* | This study |
| RJ4005 | RJ4000 *frg-509*::Tn*phoA′-1* | This study |
| RJ4006 | RJ4000 *frg-541*::Tn*phoA′-4* | This study |
| RJ4007 | RJ4000 *mglA-543*::Tn*phoA′-4* | This study |
| RJ4008 | RJ4000 *frg-566*::Tn*phoA′-4* | This study |
| RJ4009 | RJ4000 *frg-567*::Tn*phoA′-4* | This study |
| RJ4010 | RJ4000 *frg-725*::Tn*phoA′-4* | This study |
| RJ4011 | RJ4000 *aldB-731*::Tn*phoA′-4* | This study |
| RJ4012 | RJ4000 *glnQ-732*::Tn*phoA′-4* | This study |
| RJ4013 | RJ4000 *frg-733*::Tn*phoA′-4* | This study |
| RJ4014 | RJ4000 *frg-734*::Tn*phoA′-4* | This study |
| RJ4116 | CAG4000 *xylF-103*::Tn*phoA′-3* | This study |
| RJ4120 | CAG4000 *frg-566*::Tn*phoA′-3* | This study |
| RJ4122 | CAG4000 *frg-725*::Tn*phoA′-3* | This study |
| UM122 | HfrH *thi-1 katF13*::Tn*10* | 27 |
| Plasmids | | |
| pRJ823 | *lacI*$^q$ Tc$^r$ p15A | This lab |
| pRJ4000 | *lacP-fis* Ap$^r$ ColE1 | This lab (49) |
| pMW11 | Mini-Mu cloning vector, Sm$^r$/Sp$^r$ | B. Wanner (47) |
| Phages | | |
| λ::Tn*phoA′-1* | Generating *lacZ* operon fusion, Tnp$^+$ Km$^r$ | B. Wanner (47) |
| λ::Tn*phoA′-3* | *lacZ* operon fusion, Tnp$^-$ Km$^r$ | B. Wanner (47) |
| λ::Tn*phoA′-4* | Generating LacZ protein fusion, Tnp$^+$ Km$^r$ | B. Wanner (47) |
| λ::Tn*phoA′-7* | For fusion switch, Tnp$^-$ Cm$^r$ | B. Wanner (47) |

[a] Ap$^r$, ampicillin resistant; Tc$^r$, tetracycline resistant; Sm$^r$, streptomycin resistant; Km$^r$, kanamycin resistant; Sp$^r$, spectinomycin resistant.

propyl-β-D-thiogalactopyranoside), Fis was undetectable by Western blotting (immunoblotting) with Fis antibody; in the presence of 1 mM IPTG, Fis levels were slightly less than that expressed from the chromosomally encoded *fis* gene during early-log-phase growth (49). The phage-infected cells were plated on LB agar plates containing kanamycin, tetracycline, ampicillin, and X-Gal in the presence or absence of IPTG. Blue colonies were replica plated or streaked onto the same kind of plates with or without IPTG. Colonies which displayed differences in the darkness of the blue color between the two types of plates were selected for a second screening in liquid media. Overnight cultures of candidates were diluted 1/100 and grown with and without IPTG in LB for 5 h, and β-galactosidase assays were performed. In this paper we focus on fusions whose expression was elevated greater than twofold in the absence of IPTG, implying that Fis is negatively regulating their expression. Approximately 300 candidates of about 15,000 Lac$^+$ fusions were assayed in liquid culture, and 14 of these are characterized in this study. The analysis of genes whose expression was decreased in the absence of IPTG, suggesting that Fis activates their expression, will be described in a subsequent communication.

Recombinational switching from protein fusions to operon fusions was done by a two-step protocol as previously described (47). The protein fusions were first converted into a kanamycin-sensitive (Km$^s$), chloramphenicol-resistant (Cm$^r$), Lac$^-$ intermediate with Tn*phoA′-7*, a Cm$^r$ Tnp$^-$ element that has only the left and right ends in common with other Tn*phoA′* elements. The resulting recombinants were then converted into a Tn*phoA′-lacZ* operon fusion by phage infection with Tn*phoA′*-3 (Km$^r$ Tnp$^-$).

**Genetic mapping of the Tn*phoA′* mutations.** Genetic mapping of the LacZ fusions on the *E. coli* chromosome was performed by a two-step procedure as described by Singer et al. (43). The approximate locations of the Tn*phoA′* mutations were determined with an Hfr mapping set (43). This was followed by P1 transduction analysis with P1 lysates generated from a series of mapping strains containing Tn*10* in the appropriate regions (43). Cotransduction frequencies between the Tn*10* and the wild-type alleles of the transposon-generated *lacZ* fusions were determined by scoring the frequency of LacZ$^-$ colonies from the total number of transductants.

**DNA sequencing and analysis.** To sequence the various *lacZ* fusion junctions, the fusions were first cloned by the mini-Mu in vivo cloning method with the cloning vector and strains kindly provided by B. Wanner (13, 47). *lacZ* fusions were transduced into BW14879, and mini-Mu phage lysates were prepared and used to infect BW5104. Plasmid DNAs were isolated from spectinomycin-resistant (Sp$^r$), Km$^r$, and X-Gal$^+$ colonies and sequenced with a *phoA*-specific primer (5′-AATATCGCCCTGAGCA-3′) (47). DNA sequences were determined by the dideoxy method with Sequenase (Amersham) as recommended by the supplier. The sequences of most of the fusion junctions were determined from at least two independent clones. DNA sequences were analyzed for homology with the sequences in the database by using Blastmail (2).

**β-Galactosidase activity assays.** Stationary-phase cultures (grown for 14 h) were subcultured 1/100 into fresh LB broth with appropriate antibiotics and grown with shaking at 37°C. Samples were taken at the times specified in the individual experiments, and β-galactosidase activities were assayed in duplicate or triplicate as described by Miller (25). The data presented in the tables are the means and standard deviations from at least three separate experiments, and the data in the figures are representative results from at least two experiments.

## RESULTS

**Isolation of mutants expressing reduced LacZ levels as a function of Fis.** Transposons Tn*phoA′-1* and Tn*phoA′-4* were used to obtain chromosomal *lacZ* operon and protein fusions, respectively, as described in Materials and Methods. Fusions whose expression was modulated by Fis were initially identified in strain RJ4000, which contains *fis* under the control of the *lacUV5* promoter. A total of 14 fusions with decreased LacZ levels in the presence of IPTG (Fis$^+$) were isolated. Unless the genes had been previously identified (see below), the loci identified by the fusions were designated *frg* (stands for Fis-regulated genes). *frg-502* and *frg-509* were operon fusions, and all others were protein fusions. As shown in Table 2, LacZ levels of each of these fusions in the absence of IPTG (Fis$^-$) were 2- to 13-fold greater than in the presence of IPTG (Fis$^+$) when measured at a time in the growth cycle when the effect of altering Fis expression was maximal. Thus, we presume that these fusions identify genes that are directly or indirectly repressed by Fis.

**Chromosomal location of the *lacZ* fusions.** The *lacZ* fusions were mapped on the *E. coli* chromosome by Hfr crosses; this step was followed by P1 transduction with the ordered transposon set of Singer et al. (43). The estimated locations based on the linkage data are given in Table 3. In some cases, such as *frg-566*, *aldB-731*, and *frg-734*, the transduction linkage to the closest Tn*10* was quite low, and for *xylF-103*, no cotransduction with transposons within the Hfr interval (65 to 82 min) was obtained. The location of *xylF-103* was determined, however, to be at 80 min from the DNA sequence information as described below. The location of *aldB-731*, which gave the lowest cotransduction frequency to its nearest marker, was confirmed by hybridization to the Kohara *E. coli* library (data not shown). Three fusions whose loci have been identified by DNA sequences (*sdhA-133*, *mglA-543*, and *glnQ-732*; see below) were located at the expected positions of 16, 45, and 18 min, respectively (3).

**Identification of *lacZ* fusion junctions by DNA sequencing.** To identify the genes containing the transposon insertions, the fusion junctions were cloned into plasmids by the in vivo mini-Mu procedure and sequenced. Four fusions were found to be located in known genes encoding subunits of glutamine permease (*glnQ-732*) (33), β-methyl-galactoside transporter (*mglA-543*) (16), succinate dehydrogenase (*sdhA-133*) (48), and a D-xylose-binding protein (*xylF-103*) (44). The exact locations of the fusion junctions of these four genes are given in

TABLE 2. Effects of Fis on the expression of the *frg-lacZ* fusions

| Strain | *lacZ* fusion[a] | $OD_{600}$[b] | β-Galactosidase activity | | |
|---|---|---|---|---|---|
| | | | +IPTG ($Fis^+$)[c] | −IPTG ($Fis^-$)[c] | $Fis^-/Fis^+$[d] |
| RJ4001 | *frg-15* (P) | 1.5–1.8 | 22 ± 1 | 138 ± 5 | 6.3 |
| RJ4002 | *xylF-103* (P) | 3.2–3.5 | 107 ± 10 | 431 ± 33 | 4.0 |
| RJ4003 | *sdhA-133* (P) | 1.8–2.0 | 545 ± 37 | 1,127 ± 79 | 2.1 |
| RJ4004 | *frg-502* (O) | 3.2–3.6 | 21 ± 3 | 118 ± 18 | 5.6 |
| RJ4005 | *frg-509* (O) | 1.4–1.9 | 20 ± 1 | 155 ± 8 | 7.8 |
| RJ4006 | *frg-541* (P) | 3.1–3.4 | 578 ± 69 | 1,161 ± 41 | 2.0 |
| RJ4007 | *mglA-543* (P) | 3.1–3.5 | 34 ± 2 | 85 ± 2 | 2.5 |
| RJ4008 | *frg-566* (P) | 1.2–1.9 | 16 ± 1 | 206 ± 12 | 12.9 |
| RJ4009 | *frg-567* (P) | 1.4–1.8 | 26 ± 2 | 224 ± 13 | 8.6 |
| RJ4010 | *frg-725* (P) | 0.8–0.9 | 115 ± 4 | 250 ± 6 | 2.2 |
| RJ4011 | *aldB-731* (P) | 3.3–3.6 | 134 ± 15 | 654 ± 27 | 4.9 |
| RJ4012 | *glnQ-732* (P) | 1.2–2.0 | 13 ± 1 | 41 ± 7 | 3.2 |
| RJ4013 | *frg-733* (P) | 0.7–1.0 | 45 ± 1 | 120 ± 3 | 2.7 |
| RJ4014 | *frg-734* (P) | 3.2–3.5 | 162 ± 14 | 476 ± 26 | 2.9 |

[a] O, *lacZ* operon fusion; P, LacZ protein fusion.
[b] Range of cell densities at the time of the assay for both induced and uninduced cells. $OD_{600}$, optical density at 600 nm.
[c] *fis* is under control of the *lac* promoter. In the presence of IPTG, *fis* is induced. Means ± standard deviations are given.
[d] Difference in β-galactosidase levels between −IPTG and +IPTG samples.

Table 4. The *frg-566* insertion was found to be located 114 bp upstream of the *frg-567* transposon insertion in the same unknown gene. A match to the *frg-731* fusion junction was found in the latest *E. coli* sequence release (GenBank accession number ECOUM76 [44]) in the GenBank database from nucleotide 169858 to 169964. The DNA and amino acid sequences of the *frg-731* fusion junction showed very high homology to those of the *acoD* gene of *Alcaligenes eutrophus* (Fig. 1). *acoD* encodes an NAD-dependent acetaldehyde dehydrogenase (AcDH-II) which is induced during growth on ethanol or acetoin (37). This enzyme is believed to be responsible for the oxidation of acetaldehyde to acetate in the catabolic pathways for both ethanol and acetoin. The deduced amino acid sequence from *acoD* shows high degrees of homology with a group of aldehyde dehydrogenases from prokaryotes to eukaryotes. The partial DNA and amino acid sequences of the *aldB-731* gene also show significant homology to a previously identified *E. coli* aldehyde dehydrogenase gene, *aldH*, which is located at min 29.3 on the *E. coli* chromosome (14, 39). Thus, we believe that *frg-731* identifies an aldehyde dehydrogenase gene in *E. coli* and designate it *aldB*. DNA sequences of other *lacZ* fusion junctions showed no significant homology to sequences currently in the databases (data not shown).

TABLE 3. Chromosomal locations of *lacZ* fusions

| *lacZ* fusion | Location of selected marker (min)[a] | Cotransduction frequency[b] | Location of *lacZ* fusion (min)[c] |
|---|---|---|---|
| *frg-15* | *zjh-920* (96.75) | 0.11 | 97–98 |
| *xylF-103* | | | 80 |
| *sdhA-133* | *nadA57* (16.75) | 0.77 | 16 |
| *frg-502* | *metC162* (65) | 0.74 | 65 |
| *frg-509* | *zjh-920* (96.75) | 0.11 | 97–98 |
| *frg-541* | *zde-235* (32.00) | 0.14 | 31–33 |
| *mglA-543* | *zeg-722* (46.5) | 0.02 | 45–47 |
| *frg-566* | *zjh-920* (96.75) | 0.08 | 97–98 |
| *frg-725* | *zbf-3057* (16.25) | 0.93 | 16 |
| *aldB-731* | *zic-4901* (81.75) | 0.01 | 80–83 |
| *glnQ-732* | *zbh-29* (18.75) | 0.41 | 18 |
| *frg-733* | *zfb-1* (51) | 0.27 | 50 |
| *frg-734* | *zee-3129* (44.25) | 0.05 | 43–45 |

[a] Only the Tn*10* marker giving the highest cotransduction efficiency with the *lacZ* fusions is shown.
[b] Phage P1 grown on the Tn*10* marker strain was used to transduce the *lacZ* fusion strain. Values are the fraction of $Tc^r$ $Km^s$ X-Gal$^-$ transductants from the total number of $Tc^r$ transductants.
[c] The locations of the *lacZ* fusions are approximate, especially those based on low cotransduction frequencies with the selective markers. For *sdhA* and *glnQ*, the published locations (3), which are consistent with our data, are given. The location of *xylF-103* is based on DNA sequence analysis as described in the text. Hfr mapping placed *xylF-103* between min 65 and 82.

**Expression of the Fis-regulated genes.** The fusions were initially identified in strains where Fis expression is controlled by the *lac* promoter. Because wild-type *fis* expression varies tremendously with growth conditions, exhibiting maximal levels in early to mid-exponential phase, the true physiological effects of Fis on expression of the fusions required assaying in a wild-type *fis* background. The *frg-lacZ* fusions were transferred into CAG4000 ($fis^+$) as well as its isogenic *fis* deletion mutant, RJ1801, by P1 transduction. The transductants were grown in LB broth, and β-galactosidase activities were assayed over time. Of the 14 fusions, 10 expressed their highest levels of β-galactosidase at different stages of exponential growth, while the remaining 4 were induced as cells entered stationary phase (for a summary, see Table 6). In some cases the growth phase regulation is most prominent in *fis* mutant cells. Representative results are shown in Fig. 2 and are described below. The effects of the chromosomally encoded Fis on the expression of all of the fusions were qualitatively similar to those observed with *lacP*-controlled *fis* for most of the fusions, though fusions expressed maximally in stationary phase were more affected by *lacP-fis*.

TABLE 4. Positions of *lacZ* fusion junctions in known genes

| *lacZ* fusion | Position of fusion junction[a] | | | Reference |
|---|---|---|---|---|
| | Gene | Nucleotide | Amino acid | |
| *sdhA-133* | *sdhA* | 1921 | 35 | 48 |
| *mglA-543* | *mglA* | 2460 | 347 | 16 |
| *glnQ-732* | *glnQ* | 1734 | 65 | 33 |
| *xylF-103* | *xylF* | 145427 | 16 | 44 |

[a] The locations of the *lacZ* fusion junctions within sequences of known genes. The nucleotide numbering is based on the sequence given in the reference, and the amino acid position in the coding region of the designated gene at the fusion boundary is given.

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AldH  264 VWLEAGGKSANIVFADCPDLQQAASATAAGIF----YNQ 298
          | || |||| || |||  |   |    |   |      ||
AldB      VTLELGGKSPNIFFADVMDEEDAFFDKALEGFALFAFNQ
          ||||||||||||||| ||    ||||||||||||| || ||
AcoD  259 VTLELGGKSPNIFFEDVLAADDAFFDKALEGFAMFALNQ 297
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FIG. 1. Amino acid sequence alignment of the fusion junction of AldB-731 and segments of two aldehyde dehydrogenases, AcoD (*A. eutrophus*), and AldH (*E. coli*). Vertical lines denote identity with AldB. A gap is introduced to improve the alignment with AldH. The sequence given for AldB-731 extends from the boundary of the fusion site. The 39-amino-acid sequence of AldB shows 82% identity with that of AcoD and 46% identity with that of AldH.

**(i) Log-phase genes negatively regulated by Fis.** Fusions represented by *frg-733* and *frg-725* exhibited maximal expression levels in either *fis*$^+$ or *fis* mutant cells during early exponential growth, reaching their peak levels 1 to 2 h after subculturing (Fig. 2A and B). In the absence of Fis, three- to fourfold-higher peak levels of LacZ expression were obtained and higher levels persisted into stationary phase. In the presence of Fis, expression levels of these protein fusions were relatively low and decreased in mid-exponential-growth phase. These fusions may identify genes which encode products that could be most useful under poor growth conditions when Fis levels are low. Consistent with this, the *frg-733* insertion mutant displays extremely long lags when subcultured into defined media with a poor carbon source such as glycerol or acetate (data not shown). Neither the *frg-733* nor the *frg-725* insertion mutant display any altered growth properties in LB or M9-glucose medium.

A number of the fusions displayed low expression levels at early exponential growth, but the levels increased during mid- to late exponential growth in the presence or absence of Fis. LacZ levels in *frg-566* (Fig. 2C), *sdhA-133* (Fig. 2D), *frg-567*, *mglA-543*, *glnQ-732*, *frg-15*, and *frg-509* (data not shown) were lowest approximately 1 h after subculturing but then increased, particularly in the *fis* mutant cells, where a 3- to 10-fold increase in levels was obtained in mid- to late exponential phase. LacZ levels then slowly decreased in the *fis* mutant cells but remained relatively constant in *fis*$^+$ cells. *frg-566*, *frg-567*, *frg-15*, and *frg-509* show a particularly striking repression by Fis with about five to seven times more LacZ activity present in *fis* mutant versus *fis*$^+$ cells at peak expression times. In addition, these four fusions displayed very similar expression patterns, exhibiting very low LacZ activities in both rich medium (Fig. 2C) and M9-glycerol medium (data not shown) in *fis*$^+$ cells. As noted above, sequence analysis indicated that *frg-566* and *frg-567* represent different insertions in the same gene and that *frg-509* and *frg-15* map to the same region in the chromosome as *frg-566*/*567*. Thus, it is possible that these four fusions all represent insertions into the same gene or operon, although the restriction patterns of the mini-Mu clones for *frg-509* and *frg-566*/*567* are completely different (data not shown).

Expression of *xylF-103* increased during late exponential growth in *fis*$^+$ cells and then decreased slightly (Fig. 2E). However, in *fis* mutant cells its expression continued to increase sharply well into stationary phase, reaching over seven times the level present in *fis*$^+$ cells.

**(ii) Stationary-phase genes negatively regulated by Fis.** *frg-734* (Fig. 2F), *frg-502* (Fig. 2G), *frg-541* (Fig. 2H), and *aldB-731* (data not shown) represent fusions to genes which are turned on as cells enter stationary phase. LacZ levels of these fusions increased from 7-fold (*frg-502*) to over 25-fold (*frg-541* and *frg-734*) in stationary phase. In *fis* mutant cells, about twofold-higher levels were obtained, with the difference being maximal after 8 h of growth. For the fusions in this category, the effects of chromosomally encoded Fis were usually less than those observed with Fis expressed from the *lac* promoter, where much higher levels of Fis are present in stationary phase (Table 2 and Fig. 2).

**Regulation of the *lacZ* fusions by Fis occurs at the RNA level.** While most of the Fis-regulated genes were isolated as protein fusions, *frg-502* (a stationary-phase gene) and *frg-509* (maximally expressed in late exponential phase in *fis* cells) were operon fusions generated with Tn*phoA′-1*, suggesting that the reduced expression by Fis was mediated at the RNA level. To further substantiate this, three of the protein fusions were switched to *lacZ* operon fusions with Tn*phoA′-3* as described in Materials and Methods. These included *frg-725* (an early-exponential-phase gene) (Fig. 3A), *frg-566* (a mid- to late-exponential-phase gene) (Fig. 3B), and *xylF-103* (a late-exponential-phase gene) (Fig. 3C). These three resulting operon fusions showed expression patterns similar to those of their corresponding protein fusions, although the *frg-725* and *xylF-103* operon fusions exhibited higher levels of LacZ activities in both *fis*$^+$ and *fis* mutant backgrounds. Moreover, they displayed a similar pattern of control by Fis, though the magnitude of the difference was somewhat reduced, particularly for *xylF-103*.

**Effects of RpoS on the expression of the Fis-regulated *lacZ* fusions.** RpoS (or KatF) has been reported to be a sigma factor (23, 26, 45) essential for the expression of many genes expressed in stationary phase (for reviews, see references 15 and 21). Since many of the *frg* genes are maximally expressed in stationary phase, we tested whether any were also regulated by RpoS. An *rpoS* mutation (*katF13*::Tn*10*) was introduced into the *fis*$^+$ fusion strains by P1 transduction, and β-galactosidase activities of the mutants were assayed and compared with those of their isogenic *rpoS*$^+$ parent strains. Among the stationary-phase genes, the expression of fusions *frg-502*, *aldB-731*, and *frg-734* was found to be RpoS dependent. In the presence of the *rpoS* mutations, β-galactosidase activities of these fusions were reduced from 6- to over 30-fold (Tables 5 and 6). Expression of *frg-541*, which also is maximally expressed in stationary phase, was elevated 1.5-fold in the presence of the *rpoS* mutation, suggesting that RpoS may have a slight negative effect on its expression.

While the effects of RpoS on expression of stationary-phase genes are well documented, any effects on the expression of log-phase genes have not been established. As expected, none of the *frg* fusions which are maximally expressed in log phase were activated by RpoS. However, the levels of expression of *xylF-103*, *sdhA-133*, and *mglA-543* were found to be elevated 2.6- to 7.7-fold by the *rpoS* mutation (Tables 5 and 6). Figure 4A and B shows the difference in expression of *xylF-103* and *sdhA-133*, respectively, as a function of RpoS during the growth cycle. In the *rpoS*$^+$ background the expression of both fusions peaked at approximately 4 h after subculturing and decreased or remained constant over the next 3 h. In the *rpoS* mutant, the LacZ activities of both fusions continued to increase such that after 7 h of growth, LacZ levels were three to six times greater than those of their *rpoS*$^+$ parents. This difference was maintained through approximately 14 h of growth (the zero time point in Fig. 4). Thus, RpoS is reducing the expression of these genes during late log and stationary phase. Inhibition of *xylF-103* expression by RpoS is at the RNA level, since LacZ levels in the *xylF-103* operon fusion are also elevated in the *rpoS* mutant (Fig. 4C).

**Combinatorial effect of *fis* and *rpoS* on expression of *xylF-103*.** The relationship between Fis and RpoS in reducing expression of the *xylF-103* protein fusion was examined by comparing LacZ levels in an *rpoS* mutant (RJ4107) and in the *rpoS fis* double mutant strain (RJ4128). LacZ levels in the RpoS-

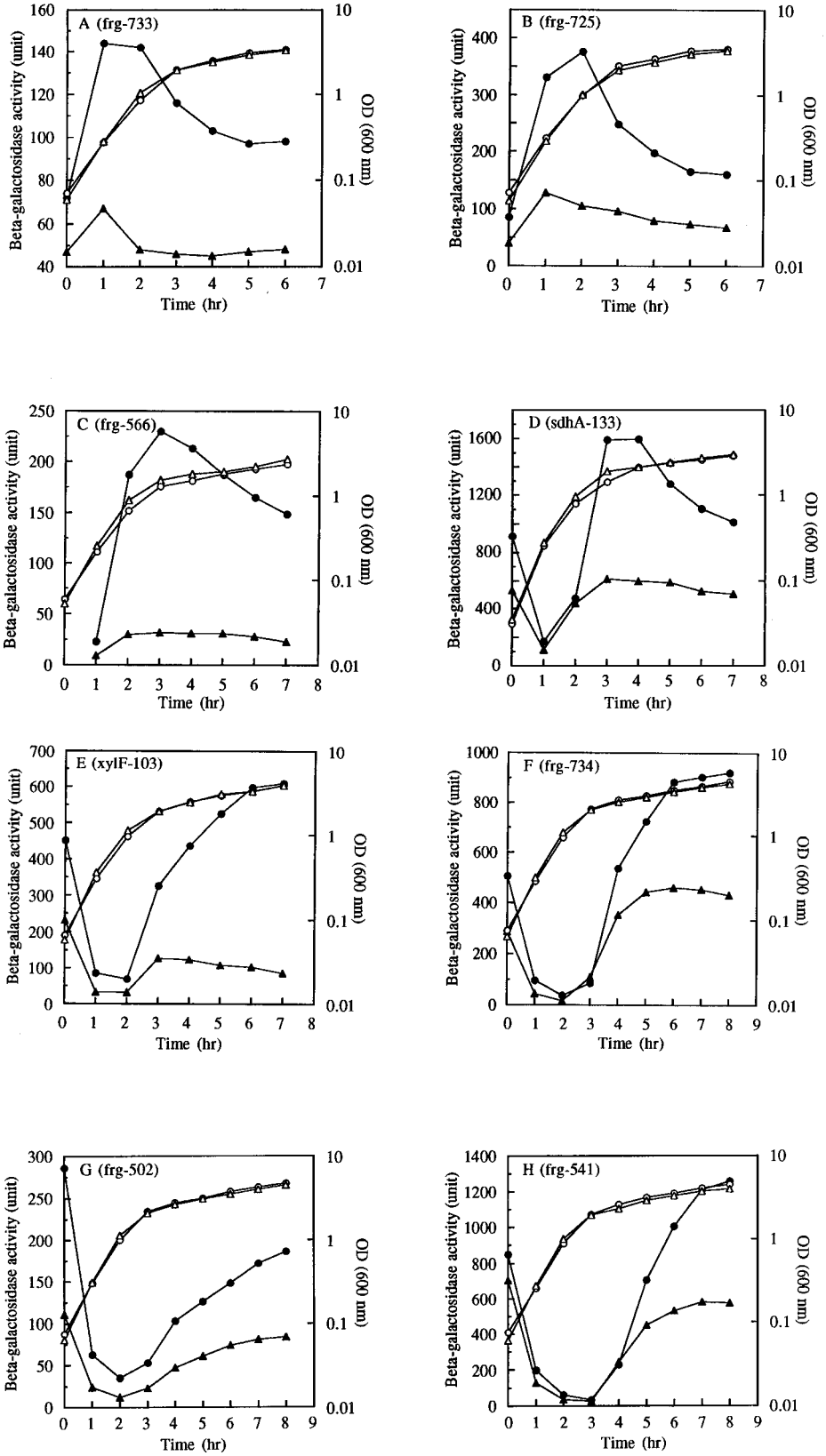


FIG. 2. Effects of Fis on the temporal expression patterns of the *frg-lacZ* fusions. LB overnight (14-h) cultures of the *fis*$^+$ strain CAG4000 (triangles) and its isogenic *fis* mutant RJ1801 (circles) containing the fusions were diluted (1/100) into fresh LB medium and incubated at 37°C with agitation. Samples were removed at 1-h intervals, and cell densities (open symbols) and β-galactosidase activities (closed symbols) were measured. OD, optical density.

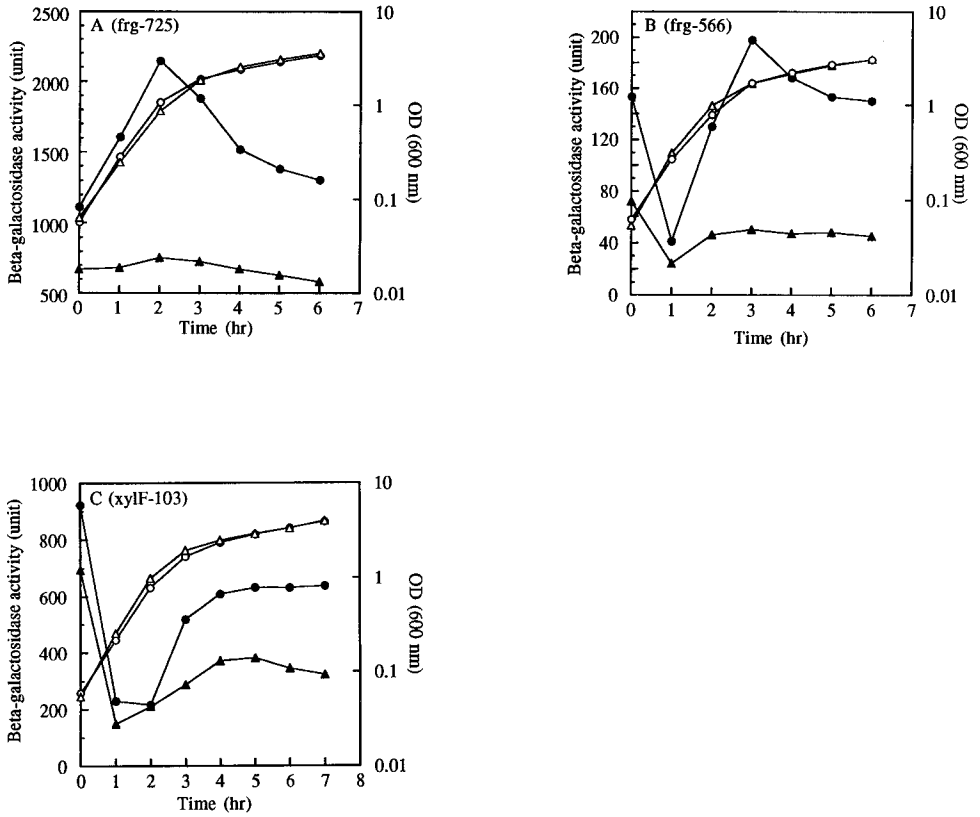


FIG. 3. Effects of Fis on the temporal expression of selected *frg-lacZ* operon fusions. Culture growth and assays were performed as described in the legend to Fig. 2 on the *frg-725* (A), *frg-566* (B), and *xylF-103* (C) Tn*phoA'-3 lacZ* operon fusions in the *fis*$^+$ strain CAG4000 (triangles) and its isogenic *fis* mutant RJ1801 (circles). Samples were removed at 1-h intervals, and cell densities (open symbols) and β-galactosidase activities (closed symbols) were measured. OD, optical density.

deficient strains increased about twofold when Fis was also absent (Fig. 5), suggesting that Fis and RpoS function at least partially independently to inhibit *xylF-103* expression.

## DISCUSSION

Fis was originally identified because of its essential role in the Hin- and Gin-mediated site-specific inversion reactions (18, 19). These reactions largely depend on Fis, since in vivo and in vitro inversion rates are reduced over 1000-fold in its absence. More recently, Fis has been shown to modulate gene expression. In this capacity, it was initially identified as a positive activator of a number of operons encoding products involved in translation, such as rRNAs, tRNAs, and EF-Tu (29, 30, 38, 41). These operons are expressed at very high levels and are regulated coordinately with the growth rate. Fis is believed to contribute to their high-level expression, particularly under conditions of rapid growth when Fis levels in the cell are high.

Other than the *fis* operon itself, only the expression of certain tRNA genes in *E. coli* have been reported to be elevated in the absence of Fis (4, 29, 32). We have identified 13 different loci whose expression is enhanced in *fis* mutant cells, thus implying that Fis directly or indirectly negatively regulates their expression. Because transposons were used to generate these fusions, only genes whose products are not essential for standard laboratory growth are included in this collection. The genes show a remarkable diversity in both their levels and temporal expression patterns as measured by β-galactosidase activities programmed by the protein or operon fusions. Three of the identified genes encode products that are required for growth under nutrient-poor conditions, in which Fis levels are expected to be low. These include proteins involved in the transport of D-xylose (XylF), glutamine (GlnQ), and methylgalactosides (MglA). Together with the flavoprotein subunit of succinate dehydrogenase (SdhA), each of these proteins is

TABLE 5. Effects of *rpoS* on the expression of Fis-regulated *lacZ* fusions

| *lacZ* fusion[a] | $OD_{600}$[b] | β-Galactosidase activity[c] | | |
|---|---|---|---|---|
| | | *rpoS*$^+$ | *rpoS* | Ratio |
| *frg-502* (O) | 3.3–3.8 | 73 ± 6 | 12 ± 2 | +6.1 |
| *aldB-731* (P) | 3.1–3.9 | 453 ± 7 | 16 ± 1 | +28.3 |
| *frg-734* (P) | 3.2–3.8 | 463 ± 14 | 13 ± 1 | +35.6 |
| *xylF-103* (P) | 3.2–3.9 | 67 ± 5 | 523 ± 21 | −7.8 |
| *sdhA-133* (P) | 2.8–2.9 | 466 ± 10 | 1,317 ± 123 | −2.8 |
| *frg-541* (P) | 3.0–3.8 | 555 ± 26 | 836 ± 27 | −1.5 |
| *mglA-543* (P) | 3.1–3.6 | 44 ± 3 | 154 ± 6 | −3.5 |
| *glnQ-732* (P) | 3.1–3.6 | 23 ± 2 | 69 ± 4 | −2.7 |
| *frg-566* (P) | 3.1–3.8 | 21 ± 2 | 23 ± 2 | NE |
| *frg-733* (P) | 2.4–3.5 | 48 ± 1 | 42 ± 1 | NE |

[a] O, *lacZ* operon fusion; P, LacZ protein fusion.
[b] Ranges of cell densities for both *rpoS*$^+$ and *rpoS* cultures at the time of the assay are given. $OD_{600}$, optical density at 600 nm.
[c] An *rpoS* (*katF13*::Tn*10*) mutation was introduced into the *lacZ* fusion derivatives of CAG4000 (*fis*$^+$) by P1 transduction. Overnight cultures were diluted 1/100 into LB medium and incubated at 37°C for 7 h. The effects of the *rpoS* mutation on the expression of the fusions were determined by LacZ activity assay. A positive ratio (*rpoS*$^+$/*rpoS*) indicates the gene is positively regulated by RpoS; a negative ratio (*rpoS*/*rpoS*$^+$) indicates the gene is negatively regulated by RpoS. NE, no effect. Mean and standard deviations of the β-galactosidase values are given.

TABLE 6. Summary of expression patterns and effects of RpoS on the Fis-repressed *lac* fusions

| Locus | Expression[a] | Effect of RpoS[b] |
|---|---|---|
| *frg-725* | Early log | None |
| *frg-733* | Early log | None |
| *frg-15* | Mid log | ND |
| *frg-509* | Mid log | ND |
| *frg-566/567* | Mid log | None |
| *glnQ-732* | Mid log | Inhibits slightly |
| *mglA-543* | Mid log | Inhibits |
| *sdhA-133* | Mid log | Inhibits |
| *xylF-103* | Late log | Inhibits |
| *frg-502* | Stationary | Activates |
| *frg-541* | Stationary | Inhibits slightly |
| *aldB-731* | Stationary | Activates |
| *frg-734* | Stationary | Activates |

[a] Time of maximum expression in *fis*$^+$ cells: early log is considered approximately 1 h after subculture, mid log is 2 to 3 h after subculture, late log is 3 to 4 h after subculture, and stationary is ≥6 h after subculture (Fig. 2).
[b] Activation or inhibition of expression by RpoS. ND, not determined.

associated with the cell membrane or periplasm. We have observed that *fis* mutant cells display increased sensitivity to certain basic dyes and hydrophobic compounds, reflecting an increased permeability of the membrane, and that they have a propensity to clump (49). Thus, the absence of Fis may result in changes in the cell membrane.

In all cases tested, Fis-mediated inhibition of gene expression can also be observed with the corresponding operon fusions, suggesting that the control by Fis is largely at the RNA level. In the case of *xylF-103*, the effect of Fis on the operon fusion expression was significantly less than that measured with the protein fusions, implying that a posttranscriptional control mechanism may be operating. The molecular mechanisms responsible for Fis-mediated repression of these genes remain to be investigated, although previous studies on autorepression of *fisP* may be applicable (4, 32). Six Fis-binding sites are present in the extended promoter region of *fis*. Binding of Fis at two of the high-affinity sites was shown to exclude the binding of RNA polymerase in vitro (4). A similar mechanism may be involved in mediating Fis repression of some of the genes involved in this study. For example, we have recently found that multiple Fis-binding sites are present in the *aldB* promoter region (49). One of the high-affinity Fis-binding sites is located in a region directly overlapping the *aldB* promoter sequence, which presumably would interfere with binding of the RpoS form of RNA polymerase. Modulation of gene expression by Fis could also occur by an indirect mechanism, such as by affecting expression of genes whose products regulate the expression of the identified genes or by affecting the local topology of DNA by its promiscuous binding and bending properties. Indeed, the derepression of Mu early gene expression in the absence of *fis* is believed to be due to a decreased function of the repressor,

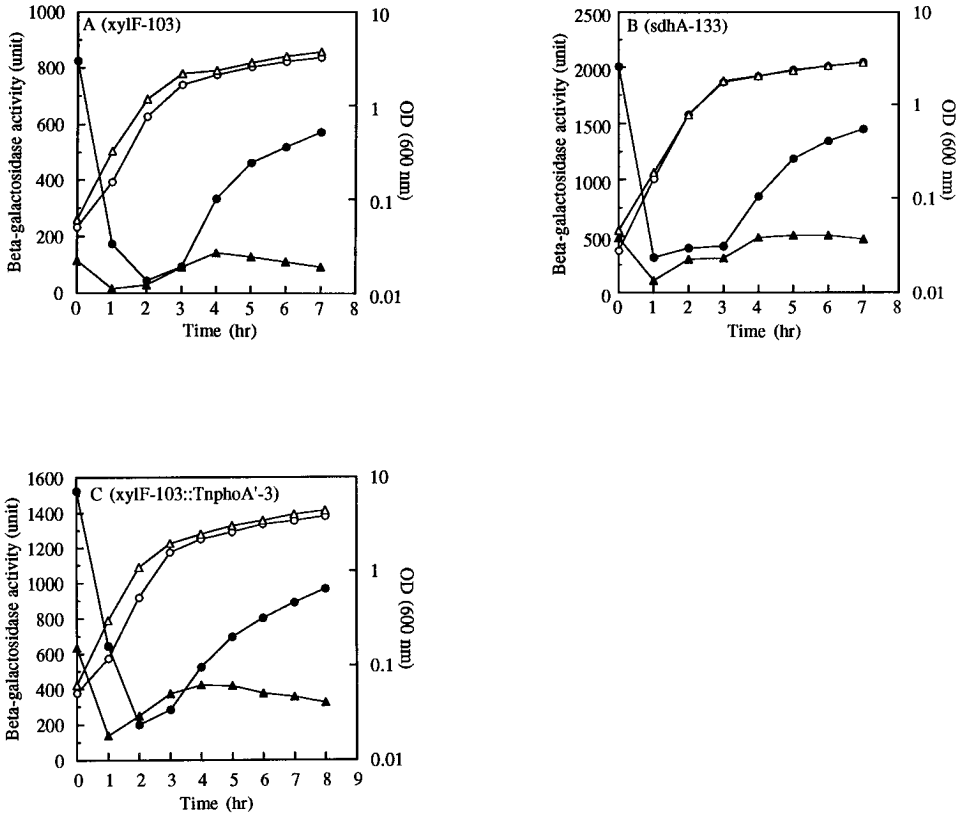


FIG. 4. Effects of RpoS on the expression of *xylF-103* and *sdhA-133*. Levels of expression of the XylF-103 LacZ protein fusion (A), SdhA-133 LacZ protein fusion (B), and *xylF-103 lacZ* operon fusion (C) in the presence (triangles) or absence (circles) of RpoS were determined. The *rpoS* mutation (*katF13*::Tn*10*) was introduced into CAG4000 containing the fusions by P1 transduction. Fourteen-hour cultures were subcultured (1/100) into fresh LB medium and incubated at 37°C with agitation. Samples were removed at 1-h intervals, and cell densities (open symbols) and β-galactosidase activities (closed symbols) were measured. OD, optical density.

as opposed to a direct effect on repressor synthesis (5). A general effect of a *fis* mutation on osmoregulation causing the increase in LacZ levels of the *frg* fusions is considered unlikely, since most of the *frg* fusions do not display increased expression levels upon a shift to high medium osmolarity in *fis*$^+$ cells (49).

**Expression patterns of genes negatively regulated by Fis.** Many of the fusions that are negatively regulated by Fis are expressed differentially during the course of the growth cycle (summarized in Table 6). The growth phase regulation is often most prominent in *fis* mutant cells in which their overall expression levels are elevated, resulting in an amplification of the temporal expression changes. Thus, Fis contributes to the control of their expression as a function of the growth phase, though in no case is Fis the sole regulator in this regard.

Maximal expression of two of the fusions (*frg-725* and *frg-733*) occurs immediately after subculturing, particularly in *fis* mutant cells. Fis may be normally functioning to inversely regulate the levels of these genes with respect to growth rate. Under nutrient-rich conditions when Fis levels are high, expression levels of these genes are low. On the other hand, under nutrient-poor conditions when Fis levels are low, transcription of these genes may be induced. Identification of the products of these genes may reveal them to be important for growth under suboptimal conditions.

The other *frg* genes that are maximally expressed from mid- to late exponential phase show a high diversity in both functions and expression patterns. *sdhA*, encoding the flavin-containing subunit of succinate dehydrogenase, which is a critical component of the tricarboxylic acid cycle, is regulated by many factors. The general regulators Fnr and ArcA have previously been shown to inhibit its expression (17). We have found that both Fis and RpoS also negatively regulate *sdhA* expression. Fis has the largest repressing effect on *xylF-103* and the *frg-566*/*567*, *frg-15*, and *frg-509* family of genes, in which there are up to 10-fold differences in expression of the protein fusions. Clearly, Fis is a very important regulator of these genes.

A few loci whose expression is inhibited by Fis are induced during the transition from exponential phase to stationary phase. The effects of Fis at this time in the growth cycle were unexpected, since cellular Fis levels are decreasing rapidly to a few percent of their peak concentration (4). However, it is apparent that this level must be sufficient to either directly reduce expression of these genes or establish an inhibitory cascade. The high levels of Fis present in exponentially growing cells may contribute to the very low level of transcription of these genes at this time. However, Fis cannot be the only factor responsible for the low expression of these genes during exponential growth, since low levels are present in both *fis*$^+$ and *fis* mutant cells at early time points (e.g., *frg-502*, *frg-541*, and *frg-734* in Fig. 2). Some of these genes (e.g., *aldB-731* and *frg-734*) require the stationary-phase sigma factor RpoS, which may be primarily responsible for controlling their growth phase expression.

The influence of Fis on the expression of these fusions implies an important role by this protein in controlling the balanced expression of a group of late-exponential- and stationary-phase genes which may be required for survival under stress conditions. The regulation of Fis expression with respect to the growth phase is most likely critical in mediating this effect. In support of this, we have found that constitutive expression of Fis in stationary phase markedly reduces long-term survival potential (36). On the other hand, the complete absence of Fis compromises cells with respect to resistance to certain stress conditions, since *fis* mutant cells in stationary phase are more sensitive to high-temperature treatment than wild type (49). We expect that many of the late-exponential- and stationary-phase genes that we have identified may encode products which are important for coping with various stress conditions.

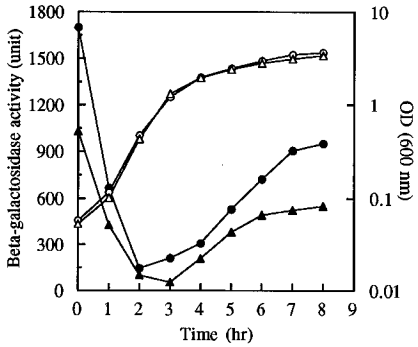


FIG. 5. Combinatorial effects of Fis and RpoS on the expression of the XylF-103 LacZ protein fusion. LB overnight (14-h) cultures of RJ4107 (CAG4000 *katF13*::Tn*10 xylF-103*::Tn*phoA'-4*; triangles) and RJ4128 (RJ4107 *fis-985*; circles) were subcultured (1/100) into fresh LB medium and incubated at 37°C with agitation. Samples were removed at 1-h intervals, and cell densities (open symbols) and β-galactosidase activities (closed symbols) were measured. OD, optical density.

**RpoS can inhibit gene expression.** RpoS was originally identified as a sigma factor required for the expression of a number of stationary-phase genes or genes turned on under starvation conditions (15, 21, 24). The ability of RpoS to inhibit expression of certain genes in *E. coli* has been implied from two-dimensional gel analysis (23). Very recently, expression of the *stiB* gene of *Salmonella typhimurium* was reported to be reduced by RpoS under phosphate and carbon starvation (34). We have identified five additional genes in this paper whose expression is reduced by the presence of RpoS (Table 5 and 6). Limited expression of these genes in late exponential and stationary phases may help conserve valuable components needed for long-term survival. For example, the inhibitory activity of RpoS appears to be functioning to maintain a constant expression level of *sdhA* in stationary phase. Without RpoS, levels of β-galactosidase produced from the *sdhA-133 lacZ* protein fusion are elevated three- to fourfold during stationary phase (Fig. 4B). During the latter part of exponential growth, the inhibitory function of Fis is playing a more prominent role (Fig. 2D). Thus, these two regulatory proteins are coordinately acting to regulate the temporal expression of *sdhA*. RpoS and Fis are also both functioning to decrease transcription of *xylF-103* during the late exponential and stationary phases. The absence of either RpoS or Fis results in about a 7-fold increase in LacZ activity after 7 h of growth, while the absence of both regulatory proteins results in a 12-fold increase in LacZ activity (Fig. 2E, 4A, and 5). Thus, for *xylF-103*, the inhibitory effect of Fis is not mediated exclusively through RpoS. It is apparent that additional factors are functioning in the growth phase-dependent expression of *xylF-103*, since the overall temporal pattern is maintained in the absence of both RpoS and Fis.

There are several mechanisms by which RpoS could be reducing the expression of these genes. Since RpoS is a sigma factor, its inhibitory activity on specific genes could be indirect by activating transcription of repressor genes. Alternatively, RpoS may be able to bind DNA without the associated core polymerase, as has been shown for RpoN (6), and thus function directly as a repressor. Another possibility is that the elevated levels of RpoS in stationary phase may result in competition of RpoS with RpoD for binding to core polymerase

molecules whose levels remain relatively constant through the growth cycle (10). This may cause a modest decrease in the transcription of certain RpoD-dependent genes, such as has been observed with *frg-541* and *glnQ-732*.

**Conclusion.** The accumulating evidence indicates that Fis modulates the expression of a variety of genes which function under different physiological conditions. It activates promoters controlling genes such as rRNAs and tRNAs which need to be expressed at maximal levels during rapid exponential growth. Fis also reduces the expression of specific genes whose normal expression is maximal in early exponential phase, mid- to late exponential phase, or stationary phase. Many of these genes are related, in that they are required for growth under suboptimal nutrient conditions. Fis can regulate promoters which are RpoD ($\sigma^{70}$) dependent (30, 38, 41), RpoS ($\sigma^{38}$) dependent (this study), and RpoH ($\sigma^{32}$) dependent (28). The magnitude of the effect of Fis on gene expression varies and may be small relative to a specific regulator(s) for the particular gene. However, its ability to affect the expression of such a wide variety of genes indicates that Fis is an important global regulator of gene expression that has been recruited to play disparate roles. Other abundant nucleoid-associated proteins such as IHF, H-NS, and Dps/PexB also have been shown to control the expression of a variety of specific genes (1, 9, 42).

While Fis is expressed maximally in early to mid-exponential phase or in rapid growth conditions in steady-state cultures, expression of RpoS is turned on in late exponential phase and stationary phase (10, 45). RpoS is required for the transcription of a set of genes that are important in stationary phase (15, 21), and it directly or indirectly inhibits expression of some genes which are presumably not required in large amounts during stationary phase (this study and reference 23) but may be beneficial for rapid exponential growth. High levels of expression of such genes may, in fact, be deleterious to long-term survival under starvation conditions. Neither RpoS nor Fis is essential under standard laboratory culturing conditions, yet artificially altering their cellular levels can have profound pleiotropic effects. With respect to prolonged survival in stationary phase, overexpression of Fis (36) or underexpression of RpoS is detrimental (15, 21).

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## REFERENCES

1. **Almirón, M., A. J. Link, D. Furlong, and R. Kolter.** 1992. A novel DNA-binding protein with regulatory and protective roles in starved *Escherichia coli*. Genes Dev. **6:**2646–2654.
2. **Altschul, S. F., W. Gish, W. Miller, E. W. Myer, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
3. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54:**130–197.
4. **Ball, C. A., R. Osuna, K. C. Ferguson, and R. C. Johnson.** 1992. Dramatic changes in Fis levels upon nutrient upshift in *Escherichia coli*. J. Bacteriol. **174:**8043–8056.
5. **Bétermier, M., I. Poquet, R. Alazard, and M. Chandler.** 1993. Involvement of *Escherichia coli* FIS protein in maintenance of bacteriophage Mu lysogeny by the repressor: control of early transcription and inhibition of transposition. J. Bacteriol. **175:**3798–3811.
6. **Buck, M., and W. Cannon.** 1992. Specific binding of the transcription factor sigma-54 to promoter DNA. Nature (London) **358:**422–424.
7. **Filutowicz, M., W. Ross, J. Wild, and R. L. Gourse.** 1992. Involvement of Fis protein in replication of the *Escherichia coli* chromosome. J. Bacteriol. **174:** 398–407.
8. **Finkel, S. E., and R. C. Johnson.** 1992. The Fis protein: it's not just for DNA inversion anymore. Mol. Microbiol. **6:**3257–3265.
9. **Freundlich, M., N. Ramani, E. Mathew, A. Sirko, and P. Tsui.** 1992. The role of integration host factor in gene expression in *Escherichia coli*. Mol. Microbiol. **6:**2557–2563.
10. **Gentry, D. R., V. J. Hernandez, L. H. Nguyen, D. B. Jensen, and M. Cashel.** 1993. Synthesis of the stationary-phase sigma factor $\sigma^s$ is positively regulated by ppGpp. J. Bacteriol. **175:**7982–7989.
11. **Gille, H., J. B. Egan, A. Roth, and W. Messer.** 1991. The Fis protein binds and bends the origin of chromosomal DNA replication, *oriC*, of *Escherichia coli*. Nucleic Acids Res. **19:**4167–4172.
12. **Gosink, K. K., W. Ross, S. Leirmo, R. Osuna, S. E. Finkel, R. C. Johnson, and R. L. Gourse.** 1993. DNA binding and bending are necessary but not sufficient for Fis-dependent activation of *rrnB* P1. J. Bacteriol. **175:**1580–1589.
13. **Groisman, E. A., and M. J. Casadaban.** 1986. Mini-Mu bacteriophage with plasmid replicons for in vivo cloning and *lac* gene fusing. J. Bacteriol. **168:** 357–364.
14. **Heim, R., and E. E. Strehler.** 1991. Cloning an *Escherichia coli* gene encoding a protein remarkably similar to mammalian aldehyde dehydrogenases. Gene **99:**15–23.
15. **Hengge-Aronis, R.** 1993. Survival of hunger and stress: the role of *rpoS* in early stationary phase gene regulation in *E. coli*. Cell **72:**165–168.
16. **Hogg, R. W., C. Voelker, and I. V. Carlowitz.** 1991. Nucleotide sequence and analysis of the *mgl* operon of *Escherichia coli* K12. Mol. Gen. Genet. **229:** 453–459.
17. **Iuchi, S., A. Aristarkhov, J. M. Dong, J. S. Taylor, and E. C. C. Lin.** 1994. Effects of nitrate respiration on expression of the Arc-controlled operons encoding succinate dehydrogenase and flavin-linked L-lactate dehydrogenase. J. Bacteriol. **176:**1695–1701.
18. **Johnson, R. C., M. F. Bruist, and M. I. Simon.** 1986. Host protein requirements for *in vitro* site-specific DNA inversion. Cell **46:**531–539.
19. **Koch, C., and R. Kahmann.** 1986. Purification and properties of the *Escherichia coli* host factor required for inversion of the G segment in bacteriophage Mu. J. Biol. Chem. **261:**15673–15678.
20. **Koch, C., O. Ninnemann, H. Fuss, and R. Kahmann.** 1991. The N-terminal part of the *E. coli* DNA binding protein Fis is essential for stimulating site-specific DNA inversion but is not required for specific DNA binding. Nucleic Acids Res. **19:**5915–5922.
21. **Kolter, R., D. A. Siegele, and A. Tormo.** 1993. The stationary phase of the bacterial life cycle. Annu. Rev. Microbiol. **47:**855–874.
22. **Kostrewa, D., J. Granzin, C. Koch, H. W. Choe, S. Raghunathan, W. Wolf, J. Labahn, R. Kahmann, and W. Saenger.** 1991. Three-dimensional structure of the *E. coli* DNA-binding protein Fis. Nature (London) **349:**178–180.
23. **Lange, R., and R. Hengge-Aronis.** 1991. Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. Mol. Microbiol. **5:**49–59.
24. **McCann, M. P., J. P. Kidwell, and A. Matin.** 1991. The putative σ factor KatF has a central role in development of starvation-mediated general resistance in *Escherichia coli*. J. Bacteriol. **173:**4188–4194.
25. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Mulvey, M. R., and P. C. Loewen.** 1989. Nucleotide sequence of *katF* of *Escherichia coli* suggests KatF protein is a novel σ transcription factor. Nucleic Acids Res. **17:**9979–9991.
27. **Mulvey, M. R., P. A. Sorby, B. L. Triggs-Raine, and P. C. Loewen.** 1988. Cloning and physical characterization of *katE* and *katF* required for catalase HPII expression in *Escherichia coli*. Gene **73:**337–345.
28. **Newlands, J. T., T. Gaal, J. Mecsas, and R. L. Gourse.** 1993. Transcription of the *Escherichia coli rrnB* P1 promoter by the heat shock RNA polymerase ($E\sigma^{32}$) in vitro. J. Bacteriol. **175:**661–668.
29. **Nilsson, L., and V. Emilsson.** 1994. Factor for inversion stimulation-dependent growth rate regulation of individual tRNA species in *Escherichia coli*. J. Biol. Chem. **269:**9460–9465.
30. **Nilsson, L., A. Vanet, E. Vijgenboom, and L. Bosch.** 1990. The role of Fis in trans activation of stable RNA operons of *E. coli*. EMBO J. **9:**727–734.
31. **Nilsson, L., H. Verbeek, E. Vijgenboom, C. van Drunen, A. Vanet, and L. Bosch.** 1992. FIS-dependent *trans* activation of stable RNA operons of *Escherichia coli* under various growth conditions. J. Bacteriol. **174:**921–929.
32. **Ninnemann, O., C. Koch, and R. Kahmann.** 1992. The *E. coli fis* promoter is subject to stringent control and autoregulation. EMBO J. **11:**1075–1083.
33. **Nohno, T., T. Saito, and J.-S. Hong.** 1986. Cloning and complete nucleotide sequence of the *Escherichia coli* glutamine permease operon (*glnHPQ*). Mol. Gen. Genet. **205:**260–269.
34. **O'Neal, C. R., W. M. Gabriel, A. K. Turk, S. J. Libby, F. C. Fang, and M. P. Spector.** 1994. RpoS is necessary for both the positive and negative regulation of starvation survival genes during phosphate, carbon, and nitrogen starvation in *Salmonella typhimurium*. J. Bacteriol. **176:**4610–4616.
35. **Osuna, R., S. E. Finkel, and R. C. Johnson.** 1991. Identification of two functional regions in Fis: the N-terminus is required to promote Hin-mediated DNA inversion but not λ excision. EMBO J. **10:**1593–1603.
36. **Osuna, R., D. Lienau, K. T. Hughes, and R. C. Johnson.** Sequence, regulation, and function of *fis* in *Salmonella typhimurium*. Submitted for publication.

37. **Priefert, H., N. Krüger, D. Jendrossek, B. Schmidt, and A. Steinbüchel.** 1992. Identification and molecular characterization of the gene coding for acetaldehyde dehydrogenase II (*acoD*) of *Alcaligenes eutrophus*. J. Bacteriol. **174:** 899–907.
38. **Ross, W., J. F. Thompson, J. T. Newlands, and R. L. Gourse.** 1990. *E. coli* Fis protein activates ribosomal RNA transcription *in vitro* and *in vivo*. EMBO J. **9:**3733–3742.
39. **Rudd, K. E.** 1992. Alignment of *E. coli* DNA sequences to a revised, integrated genomic restriction map, p. 2.3–2.43. *In* J. H. Miller (ed.), A short course in bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
40. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
41. **Sander, P., W. Langert, and K. Mueller.** 1993. Mechanisms of upstream activation of the *rrnD* promoter P1 of *Escherichia coli*. J. Biol. Chem. **268:** 16907–16916.
42. **Schmid, M. B.** 1990. More than just "histone-like" proteins. Cell **63:**451–453.
43. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53:**1–24.
44. **Sofia, H. J., V. Burland, D. L. Daniels, G. Plunkett III, and F. R. Blattner.** 1994. Analysis of the *Escherichia coli* genome. V. DNA sequence of the region from 76.0 to 81.5 minutes. Nucleic Acids Res. **22:**2576–2586.
45. **Tanaka, K., Y. Takayanagi, N. Fujita, A. Ishihama, and H. Takahashi.** 1993. Heterogeneity of the principal σ factor in *Escherichia coli*: the *rpoS* gene product, $\sigma^{38}$, is a second principal σ factor of RNA polymerase in stationary-phase *Escherichia coli*. Proc. Natl. Acad. Sci. USA **90:**3511–3515.
46. **Thompson, J. F., L. Moitoso de Vargas, C. Koch, R. Kahmann, and A. Landy.** 1987. Cellular factors couple recombination with growth phase: characterization of a new component in the lambda site-specific recombination pathway. Cell **50:**901–908.
47. **Wilmes-Riesenberg, M. R., and B. L. Wanner.** 1992. Tn*phoA* and Tn*phoA′* elements for making and switching fusions for study of transcription, translation, and cell surface localization. J. Bacteriol. **174:**4558–4575.
48. **Wood, D., M. G. Darlison, R. J. Wilde, and J. R. Guest.** 1984. Nucleotide sequence encoding the flavoprotein and hydrophobic subunits of the succinate dehydrogenase of *Escherichia coli*. Biochem. J. **222:**519–534.
49. **Xu, J., and R. C. Johnson.** Unpublished data.
50. **Yuan, H. S., S. E. Finkel, J. A. Feng, M. Kaczor-Grzeskowiak, R. C. Johnson, and R. E. Dickerson.** 1991. The molecular structure of wild-type and a mutant Fis protein: relationship between mutational changes and recombinational enhancer function or DNA binding. Proc. Natl. Acad. Sci. USA **88:**9559–9562.