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Activation of *Escherichia coli leuV* Transcription by FIS

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The transcription factor FIS has been implicated in the regulation of several stable RNA promoters, including that for the major $tRNA^{Leu}$ species in *Escherichia coli*, $tRNA_1^{Leu}$. However, no evidence for direct involvement of FIS in $tRNA_1^{Leu}$ expression has been reported. We show here that FIS binds to a site upstream of the *leuV* promoter (centered at −71) and that it directly stimulates *leuV* transcription in vitro. A mutation in the FIS binding site reduces transcription from a *leuV* promoter in strains containing FIS but has no effect on transcription in strains lacking FIS, indicating that FIS contributes to *leuV* expression in vivo. We also find that RNA polymerase forms an unusual heparin-sensitive complex with the *leuV* promoter, having a downstream protection boundary of ~−7, and that the first two nucleotides of the transcript, GTP and UTP, are required for formation of a heparin-stable complex that extends downstream of the transcription start site. These studies have implications for the regulation of *leuV* transcription.

The *leuV* operon encodes three of the four genes for $tRNA_1^{Leu}$, one of the most abundant *Escherichia coli* tRNA species (12, 21). The promoter for *leuV* is strong, with activity similar to that of the rRNA promoter *rrnB* P1 (6, 7); like many other rRNA and tRNA promoters, it is regulated in response to growth rate and amino acid starvation (6, 37). The *leuV* promoter has several features similar to those of rRNA promoters, including near-consensus −10 and −35 hexamers spaced at the nonconsensus distance of 16 bp, a G+C-rich sequence (the discriminator region) between the −10 element and the transcription start site, and an upstream sequence that contributes to promoter activity (Fig. 1). However, the effect of upstream sequence at *leuV* is smaller than that at *rrnB* P1 (~10- to 40-fold versus ~300-fold) (6, 7, 34), and the mechanism(s) responsible for its effects has not been fully characterized.

The *leuV* upstream sequence has two components, and their contributions to promoter strength are similar (6, 7). The region just upstream of the −35 hexamer (−39 to −47) is likely to increase transcription by interacting with the C-terminal domain of the α subunit of RNA polymerase (RNAP), since it is quite similar to the promoter-proximal region of the UP element consensus (13) and since in vitro transcription of *leuV* in the absence of proteins other than RNAP is reduced by an RNAP α-subunit mutation that abolishes UP element recognition (αΔ235) (35). The second region, between −47 and −107, affects transcription by a previously uncharacterized mechanism. It was originally suggested that a T tract in this region (at −69 to −73) influences *leuV* promoter activity through its effects on DNA bending (7). However, a 2-bp substitution within this T tract (T−71G T−72G) abolished the upstream effect on transcription without affecting the anomalous electrophoretic mobility (bending) of the promoter fragment (7).

FIS is a 12-kDa DNA binding protein that directly activates transcription from a number of promoters by binding to sites upstream of the core promoter (e.g., *rrnB* P1, *thrU*/*tufB*, *tyrT*, *proP*, and *mar* [26, 29, 31, 32, 36, 40]). FIS also plays a role in other cellular processes, including repression of transcription (41), site-specific recombination (15), transposition (39), and DNA replication (14). It was suggested that FIS contributes to $tRNA_1^{Leu}$ transcription, since at higher growth rates in *fis* mutant strains the concentration of $tRNA_1^{Leu}$ (as well as of some other tRNAs) is reduced relative to that of 16S rRNA (30). However, it was not known whether this effect of *fis* was direct or indirect.

The concentration of FIS in the cell varies dramatically as a function of growth rate and growth phase (2, 3), and the extent of activation by FIS at some promoters varies as a function of growth rate (1, 11). However, regulation of *rrnB* P1 with growth rate appears to involve a different mechanism that involves sensing of the initiating nucleotide concentration (16). The extent of activation of *rrnB* P1 by FIS does not vary substantially with growth rate in wild-type strains (1), although FIS is responsible for growth rate-dependent regulation of *rrnB* P1 in strains with RNAP mutations that alter the nucleoside triphosphate (NTP)-sensing mechanism (4). Thus, the contribution of FIS to promoter activity and regulation can vary, depending on the specific kinetic properties of a promoter and other regulatory mechanisms that affect it.

In this work, we have identified a FIS binding site in the *leuV* promoter upstream region and we have examined the effects of FIS on *leuV* expression both in vivo and in vitro by using promoter derivatives with mutant or wild-type FIS binding sites. We have also identified an unusual heparin-sensitive RNAP complex with the *leuV* promoter. These studies support the proposal that multiple mechanisms, including activation by FIS and NTP sensing, contribute to the transcription and regulation of *leuV*.

Identification of a FIS binding site upstream of the *leuV* promoter. FIS binds to the upstream region in several stable RNA promoters (*rrnB* P1, *tyrT*, *tufB*, and *valU* [10, 31, 36, 38]), and putative FIS binding sites have been identified upstream of many others, including *leuV* (24, 25, 30). The proposed *leuV* FIS site contains a one-base mismatch from the consensus (Fig. 1) (15). However, the degeneracy of the FIS consensus sequence has limited its predictive value, and not all consensus sequences actually bind purified FIS (14, 15). We therefore

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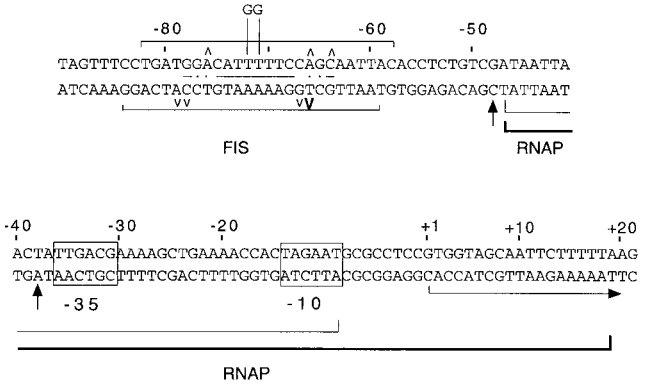

FIG. 1. Sequence of the *leuV* promoter region. Positions protected by FIS in DNase I footprints and positions of enhanced DNase I cleavage within the FIS site (carets) are indicated. Enhanced DNase I cleavage at −38 and −48 in the presence of RNAP is indicated by vertical arrows. The 2-bp substitution mutant T−71G T−72G reduces FIS binding (Fig. 2). Boundaries of protection by RNAP in the absence (thin underline, −47 to −7) or the presence (thick underline, −47 to +20) of the initiating nucleotides GTP and UTP are indicated. Similarity of the FIS site to a consensus derived from information in references 15 and 20 [Gnn(c/t)(A/g)(a/t)(a/t)(T/A)(t/a)(t/a)(T/c)(g/a)nnC] is indicated by lines between the top and bottom strands. Dots between strands in the FIS site indicate poorly conserved positions in different FIS sites.

determined the location of FIS binding sites in the *leuV* promoter region experimentally.

In a DNase I footprinting experiment, FIS protected a site in the *leuV* promoter centered at −71 (Fig. 1 and 2A). The

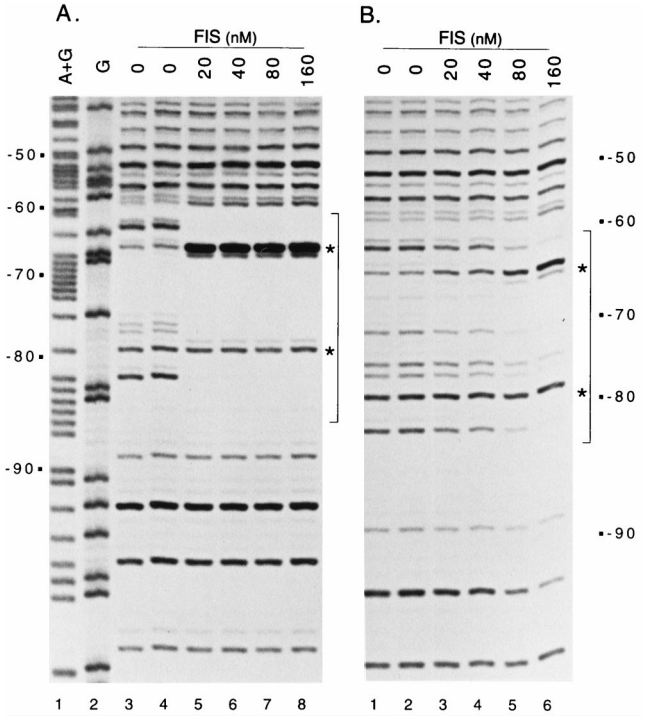

FIG. 2. DNase I footprints of FIS bound to wild-type (A) or mutant (B) *leuV* promoter fragments. *Bgl*II-*Hin*dIII *leuV* promoter fragments were obtained from p*leu*D9 (*leuV* −109 to +33 [7]) or pHEB3 (*leuV* −109 to +11, T−71G T−72G [7]) and were ^{32}P labeled in the bottom (template) strand at the *Bgl*II site, approximately 20 bp upstream from *leuV* position −109. Footprinting reactions were carried out at 22°C, essentially as described previously (35), in a solution of 10 mM Tris-Cl (pH 7.9), 10 mM $MgCl_2$, 150 mM NaCl, 1 mM dithiothreitol, and 100 μg of bovine serum albumin per ml. FIS was present at the concentrations indicated. Sequence markers were prepared by the method of Maxam and Gilbert (27).

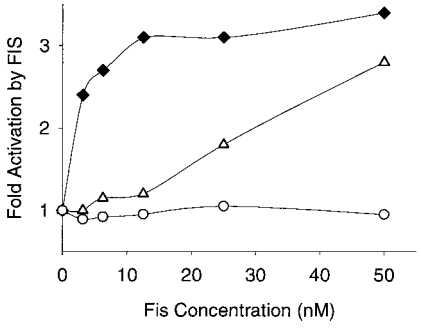

FIG. 3. Effects of FIS on in vitro transcription of wild-type and mutant *leuV* promoters (wild type, diamonds; T−71G T−72G mutant, triangles; FIS site deletion [−47 endpoint], circles). Transcription was carried out in the absence of FIS or in the presence of the indicated concentrations of FIS by using supercoiled plasmid templates with *leuV* promoter fragments inserted into the *Eco*RI and *Hin*dIII sites of pRLG770, upstream of the *rrnB* T1 terminator (36). Plasmids used were pRLG927 (wild-type *leuV* −109 to +33, obtained from p*leu*D9 [7]), pRLG930 (*leuV* −109 to +11, T−71G T−72G, obtained from pHEB3 [7]), and pRLG931 (*leuV* −47 to +55, obtained from pLC118 [7]). Multiple-round transcription was carried out essentially as described previously (36) except that nucleotide concentrations were 100 μM (for ATP, CTP, and GTP) or 10 μM (for UTP, with [α-^{32}P]UTP [DuPont, NEN]). Purified FIS was a gift from Reid Johnson (University of California at Los Angeles). *leuV* transcripts were analyzed on 6.5% acrylamide–7 M urea gels and quantified with a Molecular Dynamics PhosphorImager. The effect of FIS is shown as a ratio of transcript values in the presence and absence of FIS. Results from a representative experiment are shown.

position of the site and the apparent K_d, approximately 2 to 4 nM (determined from additional DNase I footprint titrations [not shown]), were similar to that of FIS site I in the *rrnB* P1 promoter (17, 36). Sites of enhanced DNase I cleavage within the *leuV* site (−66, −67, −78, and −79 on the bottom strand; −64, −66, and −76 on the top strand [Fig. 1 and 2A]) are likely to reflect FIS-induced DNA distortion or kinking, as noted for other FIS sites (15). At much higher FIS concentrations (~160 nM), there was partial occupancy of a second site, overlapping the core promoter region (data not shown). Similar weak binding sites for FIS also occur in the *rrnB* P1 core promoter (34), but no function has been ascribed to these sites.

A previously constructed 2-bp substitution mutation in the *leuV* promoter is located within the FIS binding site (pHEB3, T−71G T−72G [Fig. 1] [7]). This mutation reduced the affinity for FIS by at least 10-fold (apparent K_d, ~40 to 80 nM [Fig. 2B and data not shown]).

Multiple FIS binding sites contribute to activation at some other promoters (8, 29, 32, 36). However, at *leuV* only one FIS site was observed within the sequence extending to −109. Since the sequence upstream of ~−76 was previously found not to contribute to *leuV* promoter activity (7), it seems unlikely that FIS sites upstream of −109 have a major effect on *leuV* transcription.

FIS activates transcription from the *leuV* promoter in vitro. Transcription of the wild-type *leuV* promoter and of two mutant *leuV* promoters that lack a functional FIS binding site (T−71G T−72G and Δ−47, a *leuV* derivative with a deletion of sequences upstream of −47) was carried out in vitro in the presence of increasing concentrations of FIS. FIS stimulated transcription from the wild-type *leuV* promoter, with maximal activation (about threefold) observed at approximately 10 nM FIS (Fig. 3). Transcription from the promoter deleted for the FIS binding site (Δ−47) was not affected by FIS at any concentration tested, while that of the 2-bp substitution mutant promoter (T−71G T−72G) was stimulated only by much higher concentrations of FIS (>50 nM) than that required for the wild-type promoter (Fig. 3), consistent with the differences in

TABLE 1. Effects of FIS site substitution or deletion mutations on *leuV* promoter activity in wild-type *fis* and *fis*::*kan* strains

Strain	Promoter	*fis* allele	Activity[a]	% Activity[b]
RLG4043	*leuV* (−105 to +11)	Wild type	4,962	100
RLG4045	*leuV* (−105 to +11, T−71G T−72G)	Wild type	2,173	44
RLG4044	*leuV* (−47 to +11)	Wild type	1,393	28
RLG3274	*leuV* (−105 to +11)	*fis*::*kan-767*	6,869	100
RLG3276	*leuV* (−105 to +11, T−71G T−72G)	*fis*::*kan-767*	6,895	100
RLG3285	*leuV* (−47 to +11)	*fis*::*kan-767*	5,065	74

[a] β-Galactosidase levels were determined in promoter-*lacZ* fusion-containing strains grown for several generations in Luria-Bertani medium (28) and are averages of duplicate determinations differing by less than 10%. Strains were monolysogenic for phage λ system I (34), carrying fusions of the indicated *leuV* promoters to *lacZ* (Table 2). Promoter-*lacZ* fusions were constructed from *leuV* promoter fragments obtained by PCR from the MG1655 chromosome. *fis*::*kan-767* derivatives of these strains (RLG3274, RLG3276, and RLG3285 [Table 2]) were constructed by P1 transduction from strain RJ1617 (23).

[b] Activity expressed as a percentage of that of the wild-type *leuV* promoter (−105 to +11) in the appropriate strain background (wild type or *fis*::*kan-767*).

affinity for FIS of the wild-type and mutant promoters observed by DNase I footprinting (Fig. 2).

FIS activates transcription from the *leuV* promoter in vivo. The effect of FIS on *leuV* transcription in vivo was determined by comparing the activity of the wild-type promoter with that of the promoter containing the FIS site mutation T−71G T−72G. Promoter activities were determined in strains containing single-copy chromosomal promoter-*lacZ* fusions. The FIS site mutation reduced promoter activity to about 44% of its wild-type activity in a strain containing FIS (Table 1 [see also Table 2]), a result consistent with previous observations with similar constructs (7). However, this mutation did not reduce *leuV* promoter activity in a strain lacking FIS (a *fis*::*kan* strain [Table 1]). This result indicates that the effect of the mutation in the wild-type strain is attributable to loss of FIS binding and activation.

Deletion of the entire FIS site (Δ−47) had a slightly larger effect on promoter activity than the 2-bp substitution, reducing it to 28% of wild-type activity (Table 1). This suggests either that the 2-bp substitution does not fully eliminate activation by FIS (consistent with the weak affinity of the 2-bp mutant DNA for FIS [Fig. 2B and 3]), that sequences upstream of −47 contribute slightly to the *leuV* UP element, or both. The latter possibility is consistent with the slight reduction in activity of the Δ−47 promoter (74% of the wild type) in a *fis*::*kan* strain (Table 1).

As observed previously for other promoters (26, 36), the activity of each of the *leuV* promoters was greater in *fis*::*kan* strains than in wild-type *fis* strains (Table 1). This increase may reflect contributions from at least two factors. First, some of the increase in *leuV* activity is likely to result from a compensating effect of the rRNA feedback system acting on core promoter function, as described previously for the *rrnB* P1 and *tufB* promoters (32, 36). This feedback effect is thought to result from loss of activation of the *rrn* operons by FIS and may operate through the recently described NTP-sensing mechanism for growth rate regulation of *rrn* promoters (16). Transcription from growth rate-regulated *rrn* P1 promoters lacking FIS sites is increased to a greater extent (approximately four- to fivefold) than transcription from control promoters (see below) in *fis*::*kan* strains (34b, 36). An effect of the feedback system on the *leuV* promoter is consistent with previously described effects of altered *rrn* gene dosages on tRNA expression (19, 22).

In addition, some of the increase in *leuV* activity in *fis*::*kan* strains is likely to derive from a promoter-independent effect on the *lacZ* reporter system, since all promoter-*lacZ* fusions that we have tested (including non-growth-rate-regulated promoters such as *lacUV5* and growth rate-defective mutant derivatives of *rrnB* P1) show some degree of increase in activity in *fis*::*kan* strains (~1.5- to 2-fold) (34a). Since FIS has many roles in the cell and *fis* mutants have pleiotropic effects (14), this nonspecific effect is not surprising.

Although transcription of the *leuV-lacZ* fusion appears to be as active in *fis*::*kan* strains as in wild-type strains (Table 1), reduced levels of $tRNA_1^{Leu}$ have been reported (relative to 16S rRNA) in *fis* mutant strains (30). These observations are consistent with the proposed contribution of a nonspecific increase in promoter-*lacZ* fusion activity in *fis*::*kan* strains, together with a feedback derepression of the *leuV* core promoter activity that may not be as great as the derepression observed for *rrnB* P1. This suggests that the *leuV* promoter may not be as responsive to the NTP-sensing mechanism as is *rrnB* P1 (see also references 5 and 33). Alternatively, the apparent discrepancy between p*leuV-lacZ* fusion activity and reduced $tRNA_1^{Leu}$ levels in *fis*::*kan* strains may reflect either an overestimate of $tRNA_1^{Leu}$ production from the *leuV* operon (which encodes three tandem $tRNA^{Leu}$ genes) with the promoter-*lacZ* fusion or reduced tRNA production from the *argT* operon, which encodes the fourth $tRNA_1^{Leu}$ gene.

Properties of RNAP-*leuV* promoter complexes. Since RNAP forms an unstable, heparin-sensitive complex with the *rrnB* P1 promoter, a feature responsible at least in part for its regulation by the NTP-sensing mechanism, we also characterized the properties of complexes formed between RNAP and the *leuV* promoter by using DNase I footprinting. RNAP formed a heparin-sensitive complex with the *leuV* promoter in the absence of NTPs (Fig. 4, lanes 4 and 5). The boundaries of this complex are somewhat unusual, extending from −47 to about −7, thus not including the transcription start site. At *rrnB* P1, a closed, heparin-sensitive complex with protection extending approximately to the transcription start site (−60 to +1) was observed under similar conditions (5, 8). These complexes differ from the open, heparin-stable complexes formed at most other promoters in the absence of nucleotides.

A heparin-stable *leuV* promoter-RNAP complex, in which protection extended downstream to ~+20, was formed in the

TABLE 2. Strains and plasmids

Strain or plasmid	Genotype	Reference or source
Strains		
RLG4006	MG1655 *lacZΔ145 thi-39*::Tn*10*	5
RLG4043	RLG4006 λ*leuV* (−105 to +11)-*lacZ*	This work
RLG4044	RLG4006 λ*leuV* (−47 to +11)-*lacZ*	5
RLG4045	RLG4006 λ*leuV* (−105 to +11, T−72G T−71G)-*lacZ*	This work
RLG3274	RLG4043 *fis*::*kan-767*	This work
RLG3276	RLG4045 *fis*::*kan-767*	This work
RLG3285	RLG4044 *fis*::*kan-767*	This work
RJ1617	MC1000 *fis*::*kan-767*	23
Plasmids		
pRLG927	p*leuV* (−109 to +33) in pRLG770	This work
pRLG930	p*leuV* (−109 to +11, T−72G T−71G) in pRLG770	This work
pRLG931	p*leuV* (−47 to +33) in pRLG770	This work

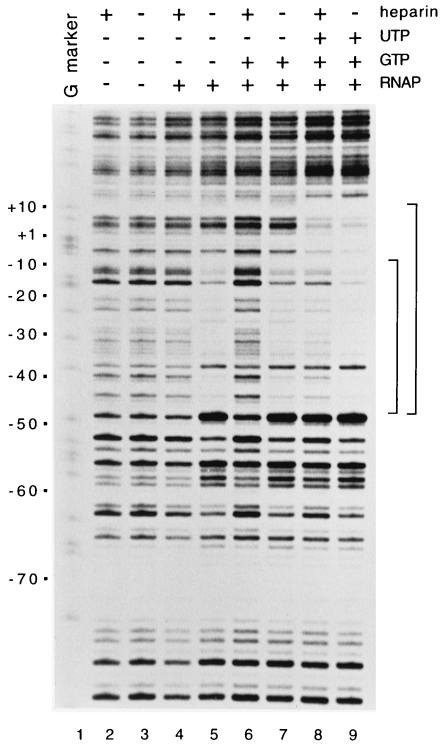

FIG. 4. DNase I footprints of RNAP bound to the wild-type *leuV* promoter. Complexes were formed with RNAP (10 nM) and the *leuV* promoter fragment (described in the legend to Fig. 2) in the presence or absence of the initiating nucleotides (500 μM GTP or 500 μM GTP and 50 μM UTP) at 22°C in buffer described in the legend to Fig. 2, except that it contained 30 mM KCl rather than 150 mM NaCl. Where indicated, heparin (10 μg/ml) was added prior to DNase I digestion.

presence of the initiating nucleotides GTP and UTP but not with GTP alone (Fig. 4, lanes 6 to 9). These results are similar to those obtained with *rrnB* P1, where the initiating nucleotides ATP and CTP, generating a 5-mer slipped transcript (9, 18), were required for a heparin-stable complex. At *leuV*, the presence of GTP and UTP would be predicted to result in formation of a template-directed 5-mer transcript.

The proximal UP element region of the *leuV* promoter (~−40 to −47) was protected by RNAP in both the heparin-sensitive (−47 to −7) and heparin-stable (−47 to +20) complexes, although the region upstream of −47 was not protected. This protection pattern is consistent with stimulation of transcription by the sequence between −39 and −47 (7). Sites of enhanced DNase I cleavage occurred at positions −38 and −48 (Fig. 1 and 4), suggesting that RNAP may bend or distort the DNA at these sites. Similar enhanced cleavage was observed at position −38 in the *rrnB* P1 promoter (35).

Implications of these findings for the regulation of *leuV* promoter activity. The results presented here are consistent with the model that multiple mechanisms, including activation by FIS and an NTP concentration-sensing mechanism, may contribute to regulation of *leuV* transcription. We find that *leuV* transcription is directly activated by FIS and that, like *rrn* P1 promoters, it responds to a feedback regulation signal generated by mutation of the *fis* gene. However, the response of the *leuV* promoter to the feedback signal may not be as great as that observed for *rrnB* P1, since $tRNA_1^{Leu}$ levels are somewhat reduced in *fis*::*kan* strains (30). Consistent with this hypothesis, RNAP mutations that alter the NTP-sensing mechanism at *rrnB* P1 also affect *leuV* transcription but to a lesser degree than *rrnB* P1 (5). Other findings are also consistent with the possibility that the NTP-sensing mechanism described for *rrnB* P1 affects *leuV* transcription. These include the formation of unusual heparin-sensitive complexes of the *leuV* promoter with RNAP (Fig. 4), the moderate level of growth rate-dependent regulation of *leuV* promoter derivatives lacking a FIS site (6, 33), and the dependence of *leuV* transcription in vitro on the concentration of the initiating nucleotides GTP and CTP (some transcripts were observed to initiate with CTP [33]). Thus, *leuV* transcription most likely reflects multiple regulatory inputs (33).

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REFERENCES

1. **Appleman, J. A.** 1998. Ph.D. thesis. University of Wisconsin—Madison.
2. **Appleman, J. A., W. Ross, J. Salomon, and R. L. Gourse.** 1998. Activation of *Escherichia coli* rRNA transcription by FIS during a growth cycle. J. Bacteriol. **180:**1525–1532.
3. **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.
4. **Bartlett, M. S.** 1997. Ph.D. thesis. University of Wisconsin—Madison.
5. **Bartlett, M. S., T. Gaal, W. Ross, and R. L. Gourse.** 1998. RNA polymerase mutants that destabilize RNA polymerase-promoter complexes alter NTP-sensing by *rrn* P1 promoters. J. Mol. Biol. **279:**331–345.
6. **Bauer, B. F., R. M. Elford, and W. M. Holmes.** 1993. Mutagenesis and functional analysis of the *Escherichia coli* tRNA(1Leu) promoter. Mol. Microbiol. **7:**265–273.
7. **Bauer, B. F., E. G. Kar, R. M. Elford, and W. M. Holmes.** 1988. Sequence determinants for promoter strength in the *leuV* operon of *Escherichia coli*. Gene **63:**123–134.
8. **Bokal, A. J., IV, W. Ross, and R. L. Gourse.** 1995. The transcriptional activator protein FIS: DNA interactions and cooperative interactions with RNA polymerase at the *Escherichia coli rrnB* P1 promoter. J. Mol. Biol. **245:** 197–207.
9. **Borukhov, S., V. Sagitov, C. A. Josaitis, R. L. Gourse, and A. Goldfarb.** 1993. Two modes of transcription initiation in vitro at the *rrnB* P1 promoter of *Escherichia coli*. J. Biol. Chem. **268:**23477–23482.
10. **Champagne, N., and J. Lapointe.** 1998. Influence of FIS on the transcription from closely spaced and non-overlapping divergent promoters for an aminoacyl-tRNA synthetase gene (*gltX*) and a tRNA operon (*valU*) in *Escherichia coli*. Mol. Microbiol. **27:**1141–1156.
11. **Dong, H., L. A. Kirsebom, and L. Nilsson.** 1996. Growth rate regulation of 4.5S RNA and M1 RNA the catalytic subunit of *Escherichia coli* RNase P. J. Mol. Biol. **261:**303–308.
12. **Duester, G., R. K. Campen, and W. M. Holmes.** 1981. Nucleotide sequence of an *Escherichia coli* tRNA (Leu 1) operon and identification of the transcription promoter signal. Nucleic Acids Res. **9:**2121–2139.
13. **Estrem, S. T., T. Gaal, W. Ross, and R. L. Gourse.** 1998. Identification of an UP element consensus sequence for bacterial promoters. Proc. Natl. Acad. Sci. USA **95:**9761–9766.
14. **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.
15. **Finkel, S. E., and R. C. Johnson.** 1992. The Fis protein: it's not just for DNA inversion anymore. Mol. Microbiol. **6:**3257–3265.
16. **Gaal, T., M. S. Bartlett, W. Ross, C. L. Turnbough, Jr., and R. L. Gourse.** 1997. Transcription regulation by initiating NTP concentration: rRNA synthesis in bacteria. Science **278:**2092–2097.
17. **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.
18. **Gourse, R. L.** 1988. Visualization and quantitative analysis of complex formation between *E. coli* RNA polymerase and an rRNA promoter in vitro. Nucleic Acids Res. **16:**9789–9809.
19. **Gourse, R. L., and M. Nomura.** 1984. The level of rRNA, not tRNA, synthesis controls transcription of rRNA and tRNA operons in *Escherichia coli*. J. Bacteriol. **160:**1022–1026.
20. **Hengen, P. N., S. L. Bartram, L. E. Stewart, and T. D. Schneider.** 1997. Information analysis of Fis binding sites. Nucleic Acids Res. **25:**4994–5002.
21. **Ikemura, T.** 1981. Correlation between the abundance of *Escherichia coli* transfer RNAs and the occurrence of the respective codons in its protein genes. J. Mol. Biol. **146:**1–21.

22. **Jinks-Robertson, S., R. L. Gourse, and M. Nomura.** 1983. Expression of rRNA and tRNA genes in *Escherichia coli*: evidence for feedback regulation by products of rRNA operons. Cell **33:**865–876.
23. **Johnson, R. C., C. A. Ball, D. Pfeffer, and M. I. Simon.** 1988. Isolation of the gene encoding the Hin recombinational enhancer binding protein. Proc. Natl. Acad. Sci. USA **85:**3484–3488.
24. **Josaitis, C. A., T. Gaal, W. Ross, and R. L. Gourse.** 1990. Sequences upstream of the −35 hexamer of *rrnB* P1 affect promoter strength and upstream activation. Biochim. Biophys. Acta **1050:**307–311.
25. **Keener, J., and M. Nomura.** 1996. Regulation of ribosome synthesis, p. 1417–1431. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, vol. 1. ASM Press, Washington, D.C.
26. **Martin, R. G., and J. L. Rosner.** 1997. Fis, an accessorial factor for transcriptional activation of the *mar* (multiple antibiotic resistance) promoter of *Escherichia coli* in the presence of the activator MarA, SoxS, or Rob. J. Bacteriol. **179:**7410–7419.
27. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65:**499–560.
28. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Plainview, N.Y.
29. **Muskhelishvili, G., M. Buckle, H. Heumann, R. Kahmann, and A. A. Travers.** 1997. FIS activates sequential steps during transcription initiation at a stable RNA promoter. EMBO J. **16:**3655–3665.
30. **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.
31. **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.
32. **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.
33. **Pokholok, D. K., M. Redlak, C. L. Turnbough, Jr., S. Dylla, and W. M. Holmes.** The *Escherichia coli leuV* tRNA promoter utilizes multiple mechanisms for responding to growth rate dependent or stringent control. Submitted for publication.
34. **Rao, L., W. Ross, J. A. Appleman, T. Gaal, S. Leirmo, P. J. Schlax, M. T. Record, Jr., and R. L. Gourse.** 1994. Factor independent activation of *rrnB* P1. An "extended" promoter with an upstream element that dramatically increases promoter strength. J. Mol. Biol. **235:**1421–1435.
34a. **Ross, W.** Unpublished observations.
34b. **Ross, W., and V. Newburn.** Unpublished observations.
35. **Ross, W., K. K. Gosink, J. Salomon, K. Igarashi, C. Zou, A. Ishihama, K. Severinov, and R. L. Gourse.** 1993. A third recognition element in bacterial promoters: DNA binding by the alpha subunit of RNA polymerase. Science **262:**1407–1413.
36. **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.
37. **Rowley, K. B., R. M. Elford, I. Roberts, and W. M. Holmes.** 1993. In vivo regulatory responses of four *Escherichia coli* operons which encode leucyl-tRNAs. J. Bacteriol. **175:**1309–1315.
38. **Verbeek, H., L. Nilsson, and L. Bosch.** 1992. The mechanism of transactivation of the *Escherichia coli* operon *thrU*(*tufB*) by the protein FIS. A model. Nucleic Acids Res. **20:**4077–4081.
39. **Weinreich, M. D., and W. S. Reznikoff.** 1992. FIS plays a role in Tn*5* and IS*50* transposition. J. Bacteriol. **174:**4530–4537.
40. **Xu, J., and R. C. Johnson.** 1995. Fis activates the RpoS-dependent stationary-phase expression of *proP* in *Escherichia coli*. J. Bacteriol. **177:**5222–5231.
41. **Xu, J., and R. C. Johnson.** 1995. Identification of genes negatively regulated by Fis: Fis and RpoS comodulate growth-phase-dependent gene expression in *Escherichia coli*. J. Bacteriol. **177:**938–947.