Molecular anatomy of a transcription activation patch: FIS–RNA polymerase interactions at the *Escherichia coli* *rrnB* P1 promoter

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FIS, a site-specific DNA binding and bending protein, is a global regulator of gene expression in *Escherichia coli*. The ribosomal RNA promoter *rrnB* P1 is activated 3- to 7-fold *in vivo* by a FIS dimer that binds a DNA site immediately upstream of the DNA binding site for the C-terminal domain (CTD) of the α subunit of RNA polymerase (RNAP). In this report, we identify several FIS side chains important specifically for activation of transcription at *rrnB* P1. These side chains map to positions 68, 71 and 74, in and flanking a surface-exposed loop adjacent to the helix–turn–helix DNA binding motif of the protein. We also present evidence suggesting that FIS activates transcription at *rrnB* P1 by interacting with the RNAP αCTD. Our results suggest a model for FIS-mediated activation of transcription at *rrnB* P1 that involves interactions between FIS and the RNAP αCTD near the DNA surface. Although FIS and the transcription activator protein CAP have little structural similarity, they both bend DNA, use a similarly disposed activation loop and target the same region of the RNAP αCTD, suggesting that this is a common architecture at bacterial promoters.

Keywords: FIS/protein–protein interactions/RNA polymerase/tRNA transcription/transcription activation

**Introduction**

In rapidly dividing *Escherichia coli* cells, the P1 promoters of the seven ribosomal RNA (*rrn*) operons direct more transcription than all of the other promoters in the cell combined (Bremer and Dennis, 1987). Three factors contribute to the remarkable strength of the best characterized of the *rrn* P1 promoters, *rrnB* P1 (Figure 1). First, the –10 and –35 recognition hexamers for RNA polymerase (RNAP) differ from consensus at only one position (Harley and Reynolds, 1987). Second, the AT-rich UP element (Figure 1) immediately upstream of the –35 recognition hexamer interacts directly with the C-terminus of the RNAP α subunit (αCTD) and increases transcription 30-fold (Ross et al., 1993; Rao et al., 1994). Third, the dimeric FIS protein binds to a site adjacent to the UP element (Site I) and increases transcription an additional 3- to 7-fold (Ross et al., 1990). FIS binds to two additional sites further upstream (Sites II and III), increasing transcription another 30% (Ross et al., 1990; Bokal et al., 1995).

FIS is a site-specific DNA binding protein and a global regulator of gene expression in *E. coli*. In addition to its roles as an activator (tRNA, Nilsson et al., 1990; rRNA, Ross et al., 1990; *proP*, Xu and Johnson, 1995a) and repressor (Xu and Johnson, 1995b) of transcription, FIS also plays roles in site-specific DNA inversion (Johnson and Simon, 1985; Kahmann et al., 1985; Johnson et al., 1986; Koch and Kahmann, 1986; Haffter and Bickle, 1987), phage λ excision (Thompson et al., 1987; Ball and Johnson, 1991), Tn5 transposition (Weinreich and Reznikoff, 1992) and DNA replication at oriC (Gille et al., 1991; Filutowicz et al., 1992).

The crystal structure of the FIS homodimer (monomer mol. wt 11.2 kDa) has been determined (Kostrewa et al., 1991; Yuan et al., 1991). Each 98 amino acid subunit in the dimer consists of four α helices (A, B, C and D) that are separated by unstructured spans of 3–7 residues (Figure 2). FIS binds a 15 bp degenerate DNA consensus sequence (Hubner and Arber, 1989; Finkel and Johnson, 1992) through the helix–turn–helix motifs in the C-terminal regions of its subunits (Koch et al., 1991; Osuna et al., 1991). DNA bound by FIS is bent at an angle of 40–90° (Thompson and Landy, 1988; Gille et al., 1991; Finkel and Johnson, 1992). This is primarily because the DNA recognition helices in FIS, C and D (Figure 2) are too closely spaced relative to the periodicity of the major groove to permit binding to linear B-DNA (Kostrewa et al., 1991; Yuan et al., 1991). In addition, amino acids just upstream of helix C (i.e. in the B–C loop) interact with DNA flanking the 15 bp recognition sequence and promote further wrapping of DNA around FIS (Pan et al., 1994).

Several lines of evidence suggest a role for FIS–RNAP contact in FIS-mediated transcription activation at *rrnB* P1. (i) DNA contacts made by FIS to Site I and RNAP to the UP element map to the same face of the DNA helix and are separated by less than one helical turn, suggesting that the protein surfaces are in close proximity (Bokal et al., 1995). (ii) FIS-mediated activation of transcription at *rrnB* P1 is strongly dependent on the rotational orientation of Site I with respect to the core promoter (Newlands et al., 1992; Zacharias et al., 1992). (iii) FIS and RNAP bind cooperatively: RNAP enhances the affinity of FIS for Site I and FIS enhances the affinity of RNAP for *rrnB* P1 (Bokal et al., 1995).

Determinants of FIS-mediated activation of transcription at *rrnB* P1 have been localized to the vicinity of the B–C loop (Gosink et al., 1996). This region of the protein has been implicated in DNA bending (Pan et al., 1994). However, DNA bending *per se* is not sufficient for activation. A glycine to serine substitution at position 72 results in a positive control (PC) phenotype: FISSer72G binds...
and bends DNA normally and yet is defective for activation of transcription at \textit{rrnB} P1 (Gosink et al., 1996). Although the glycine residue itself cannot contribute a side chain for interaction with RNAP, introduction of a side chain at position 72 might prevent the interaction of RNAP with a nearby residue (e.g. by interference or by limiting conformational flexibility of the peptide backbone in the B–C loop).

We have investigated the FIS–RNAP interaction in greater detail to gain insight into the molecular architecture of the activation complex at \textit{rrnB} P1. In this report we: (i) target alanine scanning mutagenesis to the FIS B–C loop to identify amino acid side chains important specifically for activation of \textit{rrnB} P1; (ii) test whether the RNAP $\alpha$ subunit has a role in FIS-mediated activation of transcription at \textit{rrnB} P1. The results provide insight into the mechanism by which FIS activates transcription. The overall geometry of the FIS–RNAP–DNA complex, including the positions of the DNA binding sites and of the interacting regions of FIS and of the $\alpha$ subunit, is remarkably similar to that proposed for activation complexes containing \textit{E.coli} CAP (catabolite gene activator protein), even though FIS and CAP have little structural similarity. Thus, the data support a general model for transcription activation (Ebright and Busby, 1995; Gaal et al., 1996) in which activator–RNAP interactions near their respective DNA binding surfaces stimulate transcription.

### Results

#### FIS side chains required specifically for transcription activation

The FIS transcription activation domain has been localized to the vicinity of the B–C loop (Gosink et al., 1996). In order to determine precisely which side chain(s) FIS uses to activate transcription, we replaced the seven amino acids in and immediately flanking the B–C loop one at a time with alanine (Figure 2). Alanine scanning mutagenesis yields a chemically consistent set of substitutions in which all side chain atoms beyond C$_\beta$ (and interactions made by these atoms) are eliminated (Cunningham and Wells, 1989). Our alanine scanning mutagenesis resulted in side chain removal at each position except at position 72, a glycine in the wild-type protein, where alanine substitution actually increased the size of the side chain.

We screened this library of \textit{fis} alleles \textit{in vivo} to identify those defective for transcription activation. A hybrid promoter, containing FIS Site I and the UP element from \textit{rrnB} P1 and the −10/−35 region from the \textit{lac} P1 promoter, was fused to \textit{lacZ} as a reporter of transcription. The \textit{lac} core promoter was used in place of the \textit{rrnB} P1 core promoter to avoid potential complications arising from other regulatory events, since the core \textit{rrnB} P1 promoter is subject to a feedback derepression mechanism that can compensate for the loss of activation by FIS (Ross et al., 1990). FIS activates transcription at this \textit{rrnB}–\textit{lac} hybrid promoter to the same extent as at \textit{rrnB} P1 (J.A.Appleman, W.Ross, J.Salomon and R.L.Gourse, in preparation). We found that introducing alanine at five of the seven tested positions strongly reduced the ability of FIS to activate transcription (Figure 3A, black bars). The FIS$^{Q68A}$, FIS$^{R71A}$, FIS$^{Y69A}$, FIS$^{Q74A}$ and FIS$^{Q74A}$ proteins activated...
A.

Fig. 3. (A) Effects of alanine substitutions in and adjacent to the FIS B–C loop on transcription activation in vivo (black bars) and in vitro (lighter bars). Percent activation was determined from in vivo measurements of β-galactosidase activities of a far-kan host containing a chromosomal rrnB–lac hybrid promoter–lacZ fusion and the indicated FIS alleles on plasmids or from measurements of rrnB P1-directed transcription in vitro in the presence of the indicated purified FIS proteins. (B) An autoradiogram from a representative in vitro transcription experiment. The transcript from rrnB P1 is indicated. Reactions were performed with RNAP at 0.4 nM in the presence or absence of FISWT at 75 nM, FISQ68A at 75 nM, FISR71A at 100 nM, FISG72A at 150 nM, FISN73A at 1.1 μM or FISQ74A at 100 nM and the transcription products were separated by polyacrylamide electrophoresis (see Materials and methods). Percent wild-type activation was calculated by interpolation; 0% activation corresponds to that observed in the absence of FIS and 100% activation corresponds to that observed in the presence of FISWT (3.3-fold in vivo or 9.0-fold in vitro in these experiments).

B.

Fig. 4. DNase I footprints of purified FISWT, FISQ68A, FISR71A, FISG72A, FISN73A and FISQ74A bound at Site I. A DNA template containing Site I was incubated with FISWT at 75 nM, FISQ68A at 75 nM, FISR71A at 100 nM, FISG72A at 150 nM, FISN73A at 1.1 μM or FISQ74A at 100 nM for 20 min and then probed with DNase I (see Materials and methods). The limits of Site I protection (vertical line) and specific positions protected from DNase I cleavage (arrows) are indicated.

transcription at the rrnB–lac hybrid promoter only 10–30% as well as wild-type FIS. These proteins were therefore purified for further characterization in vitro.

To ask whether the effects of the alanine replacements at positions 68, 71, 72, 73 and 74 were direct, we tested their effects on FIS-dependent activation of transcription of the native rrnB P1 promoter in vitro (Figure 3A, lighter bars; a representative experiment is shown in Figure 3B). We used DNase I footprinting to determine the concentration of each purified FIS protein required for complete Site I occupancy (Figure 4). The FISQ68A, FISR71A, FISG72A and FISQ74A proteins exhibited significantly reduced transcription activation at rrnB P1 in vitro (2.5- to 10-fold reduction; Figure 3A), even though the proteins had similar apparent binding constants (~10⁻⁷ M; see Materials and methods). However, FISN73A and FISQ74A bent the DNA 10–20% less than wild-type FIS (Figure 5). This is consistent with previous observations that these residues are minor contributors to DNA bending (Pan et al., 1994). The experiments shown above indicated that the two glutamine side chains, Q68 and Q74, have no role in DNA binding or bending. Therefore, the defects of FISQ68A and FISQ74A in transcription activation likely reflect direct
Fig. 5. DNA bending by purified FISWT, FISQ68A, FISR71A, FISG72A, FISN73A and FISQ74A bound at Site I. Two different 269 bp DNA fragments, ‘BglII’ or ‘BamHI’, were complexed with FISWT at 5 nM, FISQ68A at 5 nM, FISR71A at 5 nM, FISG72A at 20 nM, FISN73A at 400 nM or FISQ74A at 100 nM for 20 min and then resolved on polyacrylamide gels (see Materials and methods). Alterations in DNA bending are more apparent on the ‘BglII’ fragment, where the complex is positioned near the center of the fragment. The complex is positioned near the end of the ‘BamHI’ fragment, where alterations in DNA bending have little or no effect. This template serves as an internal control for changes in protein charge or overall structure. The positions of the free DNA and the FIS–Site I complexes are indicated by the letters F and C respectively.

interactions of those side chains with RNAP. Although FISG72A also bound and bent DNA normally, its effect on interactions with RNAP may be indirect, since glycine cannot contribute a side chain (see also Discussion). However, the arginine side chain at position 71 in the B–C loop reduces both DNA bending and transcription activation. Therefore, we suspected that the role of R71 was to bend DNA so that RNAP is properly positioned to contact the other side chains. However, it was also possible that the multivalent arginine side chain of FIS R71 has the unusual property of participating in intermolecular interactions with both DNA and another protein, RNAP.

To distinguish between these potential roles of the R71 side chain, we characterized a different substitution, FISR71K, using the assays described above. Substitution of lysine for arginine preserves the long basic side chain but substitutes the ζ amino group for the δ guanidido group. Under conditions where FIS R71K fully occupied Site I (Figure 6A), it activated transcription at rrnB P1 only 10–20% as well as wild-type FIS (Figure 6B). Yet, FIS R71K reduced the angle of the DNA bend at Site I only very slightly (~1–2%; Figure 6C). These results suggest that the arginine side chain plays a major role in activation by contacting both DNA and RNAP.

Role of the RNAP α subunit in FIS-mediated transcription activation at rrnB P1

Considering (i) that the DNA contacts made by FIS to Site I and by the RNAP αCTD to the UP element are on the same face of the DNA and are separated by less than one helical turn (Bokal et al., 1995) and (ii) that the FIS transcription activation region defined above maps adjacent to its DNA binding domain, it seems likely that FIS-mediated activation of transcription at rrnB P1

Fig. 6. In vitro analysis of FIS R71K. DNase I footprinting (A), multiple round transcription (B) and circular permutation assays (C) were performed as described in the legends to Figures 3–5. In (A) and (B), FISWT was used at 75 nM and FIS R71A and FIS R71K were used at 100 nM. RNAP was used at 0.4 nM in (B). In (A), the limits of Site I protection (vertical line) are indicated. In (B), the rrnB P1-directed transcript is indicated by the arrow. In (C), the positions of the free DNA and the FIS–Site I complexes are indicated by the letters F and C respectively.

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involves interactions between the side chains in and flanking the FIS B–C loop and the RNAP αCTD. To test this hypothesis, we used an in vitro transcription assay to ask if FIS can activate transcription by RNAP that lacks the αCTD (αΔ235 RNAP). RNAPs reconstituted from β, β′, σ70 and either wild-type α or αΔ235, which lacks the C-terminal domain, were calibrated to achieve equivalent FIS-independent rrnB P1 transcription (Figure 7A, lanes 1 and 2 versus Figure 7B, lanes 1 and 2). FIS activated transcription by αΔ235 RNAP only ~25% as well as it activated transcription by wild-type RNAP (Figure 7A and 7B, lanes 1 and 2 versus lanes 3 and 4). The residual activation observed with αΔ235 RNAP was independent of RNAP concentration from 4 to 80 nM (data not shown; see also Discussion).

To address whether the weak FIS-mediated activation of transcription by αΔ235 RNAP occurred by an alternative mechanism (e.g. via different interactions between FIS and RNAP), we used an in vitro transcription assay to ask if the Q68 and Q74 side chains (shown above to be likely to contact wild-type RNAP) are important for the residual activation of transcription by αΔ235 RNAP. If FIS activates transcription by αΔ235 RNAP using the same amino acid side chains as it uses to activate wild-type RNAP, then transcription by αΔ235 RNAP should be reduced in the presence of FISQ68A and FISQ74A compared with FISWT. However, if FIS activates transcription by αΔ235 RNAP by an alternative mechanism, then transcription by αΔ235 RNAP should be similar in the presence of FISWT, FISQ68A and FISQ74A. As shown in Figure 7B, FISQ68A, FISQ74A and FISWT all activated transcription by the reconstituted αΔ235 RNAP with the same efficiency, while FISQ68A and FISQ74A reduced activation by wild-type reconstituted RNAP (Figure 7A). We conclude that the Q68 and Q74 side chains are likely to interact with the RNAP αCTD, but that the residual activation observed when RNAP lacks the αCTD works by an alternative mechanism, independent of interaction with these FIS side chains.

If FIS does indeed interact with the RNAP αCTD, specific mutations in the αCTD should affect activation by FIS. Like FIS, CAP uses a surface-exposed loop next to its DNA binding surface to contact the αCTD (Niu et al., 1994). D258, D259 and E261 in the RNAP αCTD were implicated as a contact site for CAP at the lac P1 promoter (Tang et al., 1994). D258 has also been proposed to interact with the phage Mu transcription activator protein Mor (Artsimovitch et al., 1996). Furthermore, these side chains are complementary in charge to those in the FIS transcription activation region defined above. Therefore, we asked if any of these three αCTD side chains have a role in FIS-mediated activation of transcription at rrnB P1 in vitro (Figure 8). FIS was tested on the reconstituted wild-type and mutant RNAP preparations at RNAP concentrations resulting in equivalent transcription in the absence of FIS. FIS activated transcription by RNAPWT and an average of 8.5-fold (100% activation). FIS activated transcription by RNAPD258A and RNAPP6259A and RNAPD258A almost normally (89 ± 12% and 79 ± 11% respectively of the activation observed with RNAPWT), but FIS activated transcription by RNAPP259A only 47 ± 8% as well as it activated the wild-type enzyme. We conclude that FIS likely interacts with the side chain at position 258 in the RNAP αCTD. Since deletion of the entire αCTD reduced the effect of FIS more than did D258A, D258 is unlikely to be the only αCTD side chain that interacts with FIS. A more systematic analysis will be required to fully elucidate the residues in the αCTD constituting the activation target.

Discussion

We have identified several amino acid residues that constitute an ‘activation patch’ on FIS. Figure 9A displays a
Fig. 8. Effect of RNAP αCTD alanine substitutions on activation by FIS. (A) A multiple round in vitro transcription assay performed with RNAP^{WT} at 0.5 nM, RNAP^{D258A} at 0.2 nM, RNAP^{D259A} at 1 nM or RNAP^{D261A} at 0.9 nM in the presence or absence of FIS^{WT} at 75 nM. RNAP concentrations were chosen to give the same amount of transcription in the absence of FIS. The rrnB P1-directed transcript is the ‘activation patch’. G72 and N73 are included, although the roles of indicated by the arrow. (B) Quantitation of results from several experiments such as that illustrated in (A). Fold activation is the ratio of transcript observed in the presence/absence of FIS. FIS activated RNAP^{WT} 8.5 ± 0.9-fold, RNAP^{D258A} 4.0 ± 0.5-fold, RNAP^{D259A} 7.6 ± 0.7-fold and RNAP^{D261A} 6.7 ± 0.6-fold.

model for the activation patch (shaded black) end on. In this view, the FIS surface adjacent to RNAP is pictured from the perspective of the αCTD bound to the rrnB P1 UP element. Mutant proteins containing alanine substitutions at Q68 and Q74 activate transcription inefficiently, even though the mutant proteins bind and bend DNA normally. Therefore, the Q68 and Q74 side chains flanking the FIS B–C loop most likely interact directly with RNAP. Replacement of the glycine residue at position 72 in the B–C loop with alanine also resulted in a PC phenotype. While G72 might supply a peptide backbone interaction with RNAP, it is possible that introduction of an alternative residue at this position limits the conformational flexibility of FIS so that the Q68, Q74 or R71 (see below) side chains cannot interact productively with RNAP. Alanine substitution for N73 reduced transcription activation but also substantially reduced DNA binding. Therefore, we cannot distinguish whether N73 plays a direct or indirect role in activation.

The side view (Figure 9B) emphasizes the prominent position of Arg71 at the center of the B–C loop. R71 appears to interact with DNA at rrnB P1 FIS Site I (as well as at other FIS binding sites; Gosink et al., 1993; Pan et al., 1994). The role of this side chain in DNA bending does not appear to be sufficient to explain the requirement for R71 for transcription activation: replacement of R71 with lysine severely impairs the ability of FIS to activate transcription at rrnB P1, even though the mutant proteins bind and bend DNA normally. Therefore, the Q68 and Q74 side chains flanking the FIS B–C loop are very similar to those of wild-type FIS. Therefore, we suggest that the multivalent arginine at position 71 makes contacts with both DNA and RNAP, although it is not known whether these contacts occur simultaneously or alternatively.

We also present evidence suggesting that FIS-mediated activation of transcription at rrnB P1 involves direct interactions between FIS and the RNAP αCTD, but that an alternative or additional mechanism of activation can be detected when RNAP lacks the αCTD. This second mechanism of activation was inefficient in comparison with that observed with wild-type RNAP. Although the second mechanism still depended on FIS binding to Site I (data not shown), the FIS side chains important for activation of transcription by wild-type RNAP were not...
important for activation of transcription by RNAP lacking the αCTD.

In a previous report (Ross et al., 1993), we also detected FIS-mediated activation of αΔ235 RNAP in vitro, but under the conditions used in those experiments, FIS activated wild-type and αΔ235 RNAP similarly, ~3.5-fold, similar to the 3-fold activation of transcription by αΔ235 RNAP observed here. More recently, we showed that FIS facilitates the initial binding step of transcription initiation (i.e. the RNAP concentration-dependent step; Bokal et al., 1995). Therefore, in the experiments reported here, we modified the reaction conditions to increase the magnitude of transcription activation by FIS: we used wild-type RNAP at a relatively low concentration and increased the salt concentration slightly (from 150 to 170 mM NaCl). As a result, in the experiments reported here, FIS activated transcription by wild-type RNAP 12-fold. In order to achieve equivalent FIS-independent transcription at rrnB P1, we used more αΔ235 RNAP than wild-type RNAP, since the αCTD is required for UP element utilization (Ross et al., 1993; Blatter et al., 1994). However, unlike with wild-type RNAP, FIS-mediated activation of transcription by αΔ235 RNAP was not a function of RNAP concentration in the experimentally accessible range. Activation of transcription with αΔ235 RNAP was ~3-fold at all concentrations tested, further indicating that the residual activation observed in the absence of the αCTD occurs by a different mechanism than that observed with wild-type RNAP. We have not investigated the surfaces on RNAP and FIS involved in this αCTD-independent activation mechanism.

Additional evidence supporting a role for the αCTD in FIS-mediated activation of transcription at rrnB P1 was obtained through the identification of a mutation in the αCTD that reduces the efficiency of transcription activation by FIS at rrnB P1. Removal of the side chain at position 258 in the αCTD reduces the stimulatory effect of FIS on RNAP ~2-fold. Since removal of the α258 side chain does not appear to reduce UP element utilization (Gaal et al., 1996), the reduction in FIS-mediated transcription activation is unlikely to be an indirect effect of altered α–DNA interactions. Because the reduction in FIS-mediated activation is less for RNAPαDD258 than for αΔ235 RNAP, other side chains in the αCTD, in addition to the side chain at position 258, are likely to contact FIS, i.e. it is unlikely that we have defined all the residues that contribute to the activation patch in α. In theory, genetic screens could be used to identify mutants defective in FIS-dependent transcription. However, screens for mutants in α affecting transcription by a variety of transcription factors have often resulted in the identification of residues known to affect DNA–α interactions (Zou et al., 1992; Tang et al., 1994; Tao et al., 1995; Murakami et al., 1996; Artsimovich et al., 1996). Since many substitutions in the αCTD affect interactions with UP element DNA (Gaal et al., 1996), it will be necessary to distinguish mutations in α that reduce transcription by directly altering interactions with FIS from those that reduce transcription by affecting interactions with the DNA.

Our experiments suggest a model for FIS-mediated activation of transcription at rrnB P1 that involves FIS–RNAP interactions near the DNA surface. The FIS transcription activation region defined above includes the

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Fig. 10. Cartoon model of the FIS–RNAP activation complex at rrnB P1 (A) compared with the CAP–RNAP activation complex at CC(–72.5) or malT (B) (Gaston et al., 1990; Chapon and Kolb, 1983). The structures of CAP and FIS are from Schultz et al. (1991) and Yuan et al. (1992) respectively. The proposed interacting regions on the activators and the α subunit surfaces are represented by black dots (Tang et al., 1994; Zhou et al., 1994; see also text). Only one monomer of the α dimer is proposed to interact with one monomer of the activator dimer (Zhou et al., 1993, 1994). The positioning of the α subunits on DNA is presented for schematic reasons only and has not been proven.
Materials and methods

Bacterial strains and plasmids
Strain RGL1739 (MG1655 lacI74 lacI:kan, F' prodB lacI434ΔZ183F1:: Tn-5-320) is a monosony for λ carrying the rnb P1[-88 to -37] lacI-[36 to -2] lacZ transcriptional fusion (Gosink et al., 1996). The numbers in brackets refer to the limits of rnb P1 sequence relative to the transcription start site. There is no lac operator in this reporter fusion.

The Fis expression vector pKG18 (Gosink et al., 1996) is a pKK223-3 (Pharmacia) derivative that expresses Fis from the lac promoter. The plasmid pRLL589 (Ross et al., 1990) contains the rnb P1[-38 to +50] promoter. The circular permutation plasmid pSFL9 (Gosink et al. 1993) contains the rnb P1[-88 to +50] promoter.

Alanine scanning mutagenesis
Mutant fs alleles were created by incorporation of a mutagenic oligonucleotide during PCR amplification of the fis gene from pKG18 (codons 69 and 71–74; Michael, 1994) or by the Kunkel method (codons 68 and 70; Kunkel, 1985). Mutagenic oligonucleotides (mutations are underlined) were: A68, 5'-GTACCCAGGGTTATTCCATACCATC-3'; A69, 5'-GTACCCAGGGTTATTCCATACCATC-3'; A70, 5'-GTACCCAGGGTTATTCCATACCATC-3'; A71, 5'-GGGCTTGTTACCAACCCGTGATTGCATC-3'; A72, 5'-CGGCTTGTACACCGGTTATGATGACATC-3'; A73, 5'-GCGGACCGGTTACCCGAGCGTTGTGACATC-3'; A74, 5'-GGCCGACCGGTTACCCGAGCGTTGTGACATC-3'. Primers annealing to pKG18 sequences flanking the fis gene were: phd, 5'-CTGAAACTTCTTCTCATCTCCGCC-3'; phdh, 5'-CTGAAACTTCTTCTCATCTCCGCC-3'. PCR products and M13RF DNAs were digested with EcoRI and HindIII to yield fis gene-containing fragments that were gel purified and ligated into pKK223-3. Ligation reactions were transformed into RLG1739 and plasmids obtained from single colony purified transformants were sequenced to verify that the fis gene was mutated at only the intended position(s).

Purified proteins
Wild-type and mutant FIS proteins were purified as described (Gosink et al., 1996). The FIS protein was constructed and purified by R.C. Johnson (our unpublished data). Native RNAP was generously provided by Peter Schlax. Reconstituted mutant and wild-type RNAPs containing N-terminal hexahistidine-tagged α subunits were prepared as described (Gaal et al., 1996). The α235 RNAP preparation, made by the denaturing method, was judged by silver staining to be free of wild-type α subunits (0.5% contamination with wild-type α would have been detected in the analysis).

β-Galactosidase determinations
Determinations of β-galactosidase synthesis directed by the hybrid rnb P1[-88 to -37] lacI-[36 to -2] lacZ transcriptional fusion were made as described (Miller, 1972). lysogens were grown logarithmically for four generations in LB at 37°C and assayed at an A600 of ~0.5. Duplicate measurements were made for each of two independent cultures of each lysogen and standard errors were <20%.

In vitro transcription
Multiple round transcription reactions were performed at 22°C in 25 μl reactions containing 0.4 mM nucleoside triphosphates, 170 mM NaCl, 10 mM MgCl2, 12 mM Tris–HCl, pH 7.7, 1 mM DTT, 100 μM each of α,β,γ and δ phosphates, 0.5X TBE gel and electrophoresed for ~4 h at 220 V. Autoradiographs were exposed for ~4 h without intensifying screens.

DNase I footprinting
FIS–DNA complexes were prepared at 22°C in 25 μl reactions containing 170 mM NaCl, 10 mM MgCl2, 12 mM Tris–HCl, pH 7.7, 1 mM DTT, 100 μg/ml BSA, 4% glycerol and purified FIS. The DNA template containing Site I [XhoI (–168)–Hsal (+75)] from pSL9 was 32P-labeled in the XhoI site. After 20 min, reactions were treated with 7 μg/ml DNase I for 30 s, processed and electrophoresed as described previously (Ross et al., 1990).


Received on July 24, 1996; revised on September 27, 1996