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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/rRNA 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), Tn*5* 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: FIS^{G72S} binds

and bends DNA normally and yet is defective for activation of transcription at *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 *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 *rrnB* P1; (ii) test whether the RNAP α subunit has a role in FIS-mediated activation of transcription at *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 α subunit, is remarkably similar to that proposed for activation complexes containing *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.

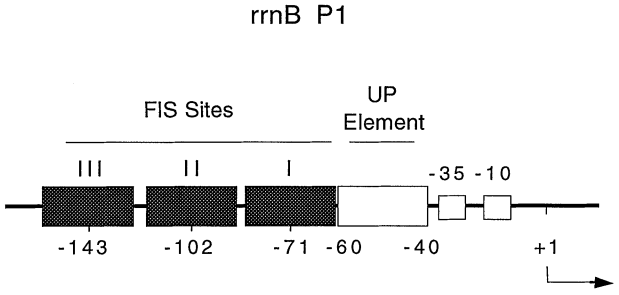

Fig. 1. Organization of the *rrnB* P1 promoter. The core promoter region (containing the –10 and –35 recognition hexamers) and the UP element (–40 to –60 region) are represented by open boxes. The FIS binding sites (I–III, filled rectangles) are centered at –71, –102 and –143 respectively. +1 is the transcription start site.

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 Cβ (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 *fis* alleles *in vivo* to identify those defective for transcription activation. A hybrid promoter, containing FIS Site I and the UP element from *rrnB* P1 and the –10/–35 region from the *lac* P1 promoter, was fused to *lacZ* as a reporter of transcription. The *lac* core promoter was used in place of the *rrnB* P1 core promoter to avoid potential complications arising from other regulatory events, since the core *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 *rrnB–lac* hybrid promoter to the same extent as at *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^{G72A}, FIS^{N73A} and FIS^{Q74A} proteins activated

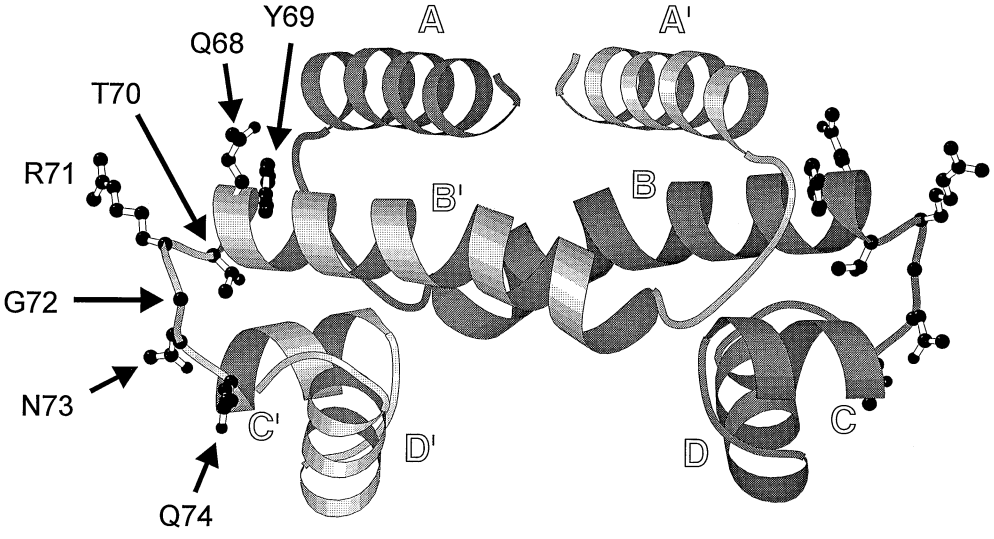

Fig. 2. Ribbon diagram of the FIS dimer, adapted from Yuan *et al.* (1991). The α helices of each monomer are represented by ribbons labeled A–D and A′–D′. Residues 68–74, within and flanking the loop between helices B and C, were replaced one at a time with alanines and the wild-type side chains are represented as stick models. The two monomers are shaded differently. The N-terminal 25 amino acids have been omitted since they were not resolved in the crystal structure (Yuan *et al.*, 1991).

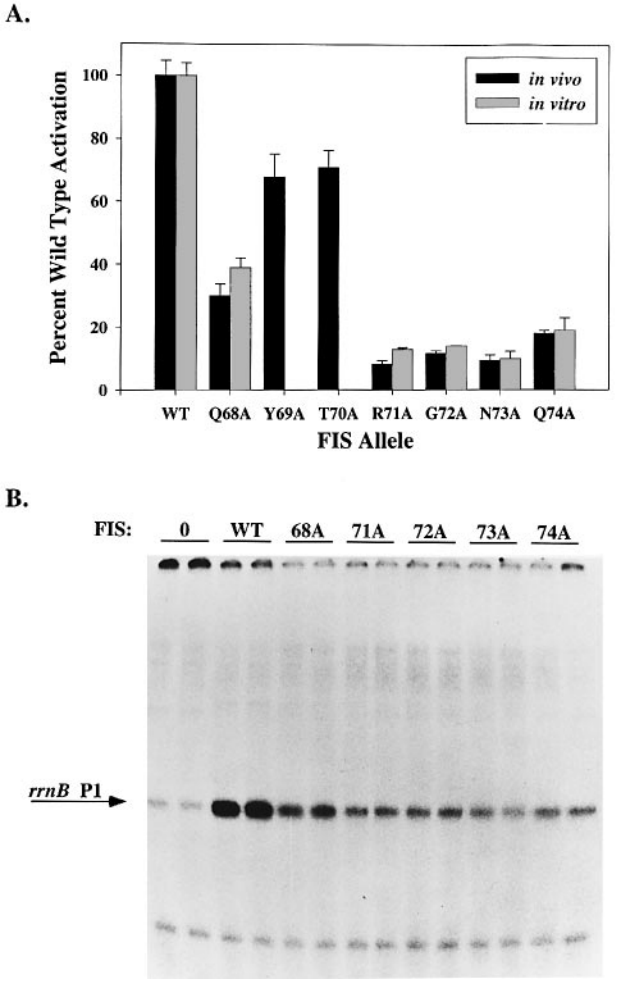

Fig. 3. (**A**) Effects of alanine substitutions in and adjacent to the FIS B–C loop on transcription activation *in vivo* (black bars) and *in vitro* (lighter bars). Percent activation was determined from *in vivo* measurements of β-galactosidase activities of a *fis*::*kan* host containing a chromosomal *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 FIS^{WT} at 75 nM, FIS^{Q68A} at 75 nM, FIS^{R71A} at 100 nM, FIS^{G72A} at 150 nM, FIS^{N73A} at 1.1 μM or FIS^{Q74A} 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 FIS^{WT} (3.3-fold *in vivo* or 9.0-fold *in vitro* in these experiments).

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 FIS^{Q68A}, FIS^{R71A}, FIS^{G72A} and FIS^{Q74A} proteins exhibited significantly reduced transcription activation at *rrnB* P1 *in vitro*

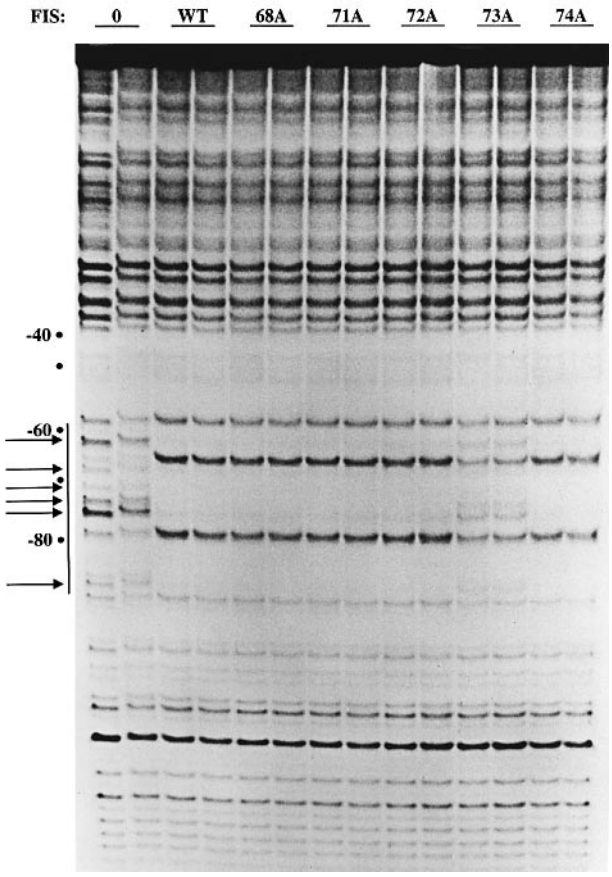

Fig. 4. DNase I footprints of purified FIS^{WT}, FIS^{Q68A}, FIS^{R71A}, FIS^{G72A}, FIS^{N73A} and FIS^{Q74A} bound at Site I. A DNA template containing Site I was incubated with FIS^{WT} at 75 nM, FIS^{Q68A} at 75 nM, FIS^{R71A} at 100 nM, FIS^{G72A} at 150 nM, FIS^{N73A} at 1.1 μM or FIS^{Q74A} at 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.

(2.5- to 10-fold reduction; Figure 3A), even though the proteins had similar apparent binding constants (~10^{-7} M; Figure 4 and data not shown). The N73A substitution also reduced transcription from *rrnB* P1, but FIS^{N73A} bound with such low affinity that Site I could not be saturated (Figure 4). We conclude that the effects of the Q68A, R71A, G72A and Q74A substitutions on transcription activation cannot be explained by defects in DNA binding, but the effect of the N73A protein on transcription could be a consequence of its weak binding at Site I.

A circular permutation assay was used to ask whether the reduced activation mediated by FIS^{Q68A}, FIS^{R71A}, FIS^{G72A}, FIS^{N73A} and FIS^{Q74A} could be ascribed to a reduction in the magnitude of the bend induced in Site I (Figure 5). FIS^{Q68A}, FIS^{G72A} and FIS^{Q74A} bent Site I DNA to the same degree as the wild-type protein (i.e. ~75°; see Materials and methods). However, FIS^{R71A} and FIS^{N73A} 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 FIS^{Q68A} and FIS^{Q74A} in transcription activation likely reflect direct

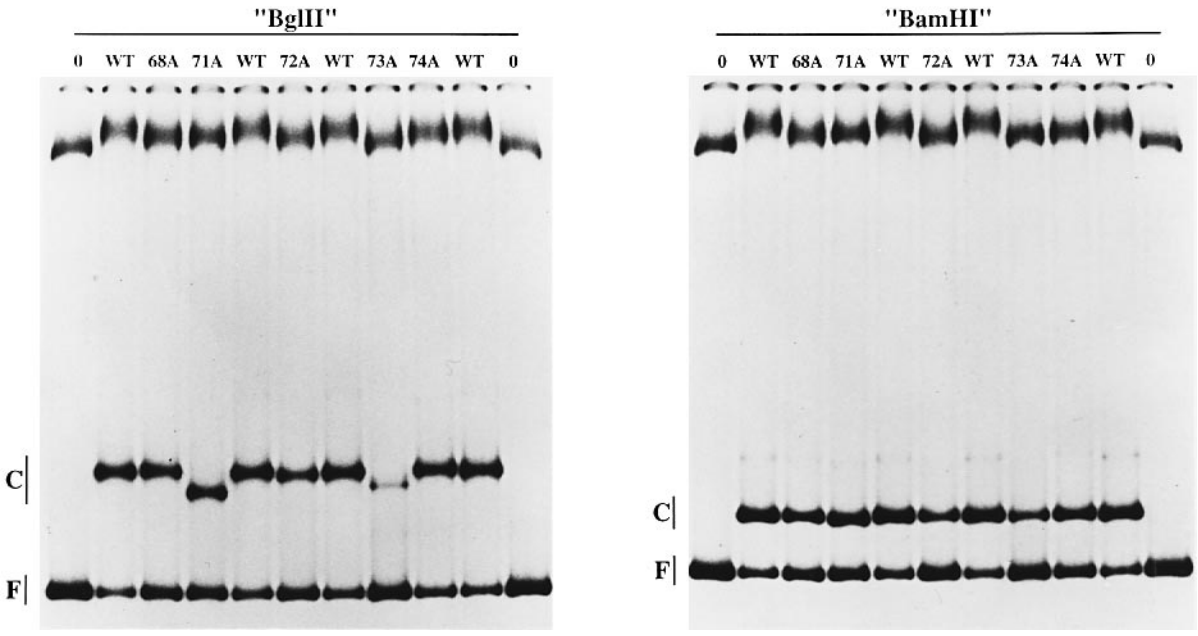

Fig. 5. DNA bending by purified FIS^{WT}, FIS^{Q68A}, FIS^{R71A}, FIS^{G72A}, FIS^{N73A} and FIS^{Q74A} bound at Site I. Two different 269 bp DNA fragments, '*Bgl*II' or '*Bam*HI', were complexed with FIS^{WT} at 5 nM, FIS^{Q68A} at 5 nM, FIS^{R71A} at 5 nM, FIS^{G72A} at 20 nM, FIS^{N73A} at 400 nM or FIS^{Q74A} 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 '*Bgl*II' fragment, where the complex is positioned near the center of the fragment. The complex is positioned near the end of the '*Bam*HI' 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 FIS^{G72A} 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, FIS^{R71K}, using the assays described above. Substitution of lysine for arginine preserves the long basic side chain but substitutes the ξ 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

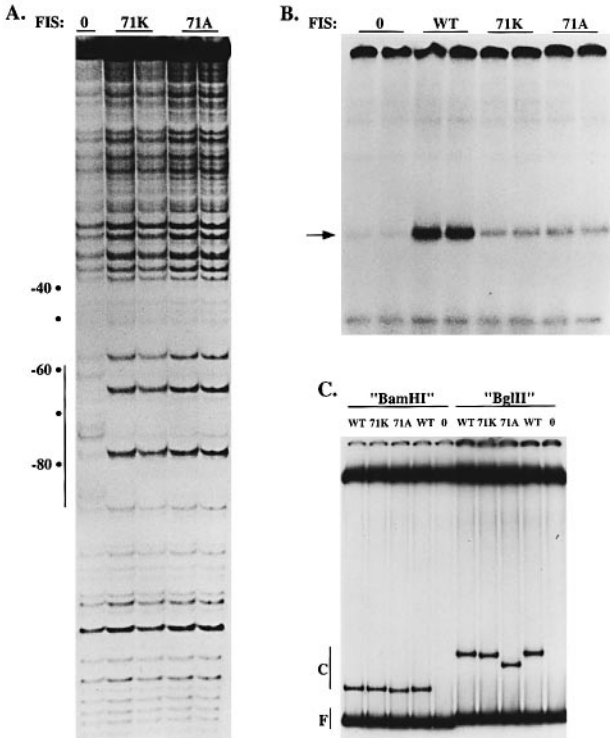

Fig. 6. *In vitro* analysis of FIS^{R71K}. DNase I footprinting (**A**), multiple round transcription (**B**) and circular permutation assays (**C**) were performed as described in the legends to Figures 3–5. In (A) and (B), FIS^{WT} was used at 75 nM and FIS^{R71A} and FIS^{R71K} were used at 100 nM. 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.

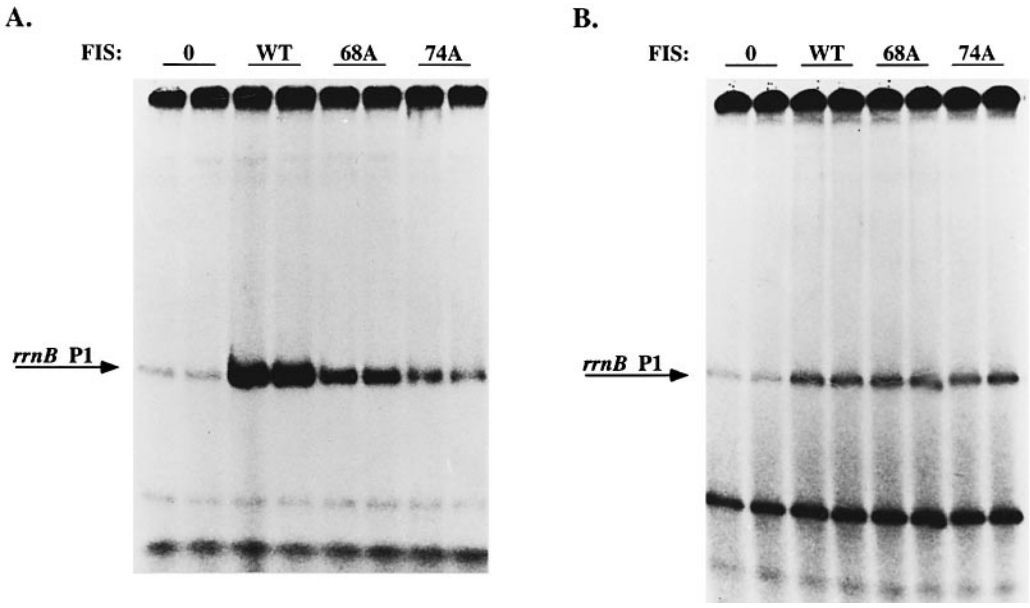

Fig. 7. Effect of the α235 deletion mutation on activation of transcription by FIS. Multiple round *in vitro* transcription assays were performed with $RNAP^{WT}$ at 0.5 nM (**A**) or with $RNAP^{\alpha\Delta 235}$ at 40 nM (**B**) in the presence or absence of FIS^{WT} at 75 nM, FIS^{Q68A} at 75 nM or FIS^{Q74A} at 100 nM. RNAP concentrations were chosen to give the same amount of transcription in the absence of FIS. The increased RNAP concentration needed with $RNAP^{\alpha\Delta 235}$ results from the loss of UP element utilization by the mutant enzyme. The *rrnB* P1-directed transcript is indicated by the arrow. The autoradiogram shown is a representative of several replicate experiments.

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′, σ^{70} 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 FIS^{Q68A} and FIS^{Q74A} compared with FIS^{WT}. However, if FIS activates transcription by αΔ235 RNAP by an alternative mechanism, then transcription by αΔ235 RNAP should be similar in the presence of FIS^{WT}, FIS^{Q68A} and FIS^{Q74A}. As shown in Figure 7B, FIS^{Q68A}, FIS^{Q74A} and FIS^{WT} all activated transcription by the reconstituted αΔ235 RNAP with the same efficiency, while FIS^{Q68A} and FIS^{Q74A} 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 $RNAP^{WT}$ an average of 8.5-fold (100% activation). FIS activated transcription by $RNAP^{\alpha D259A}$ and $RNAP^{\alpha E261A}$ almost normally (89 ± 12% and 79 ± 11% respectively of the activation observed with $RNAP^{WT}$), but FIS activated transcription by $RNAP^{\alpha D258A}$ 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

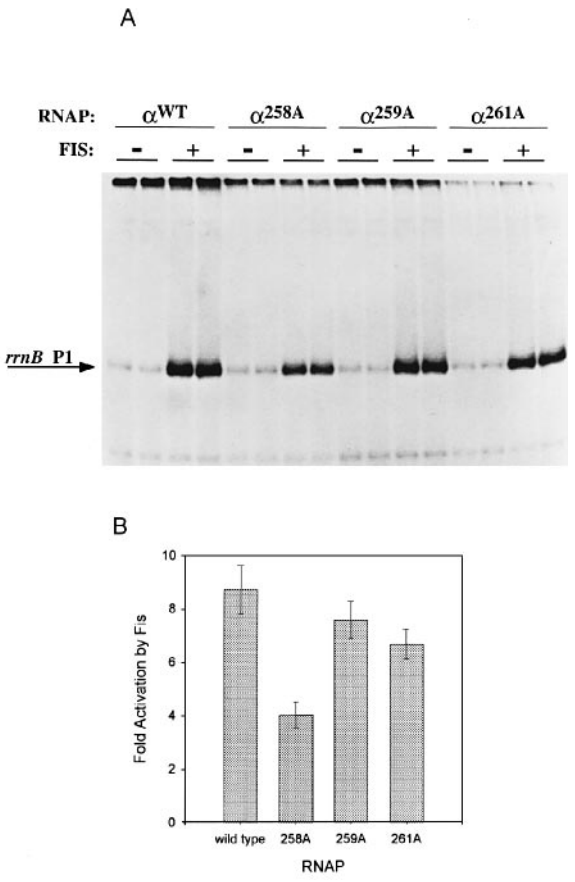

Fig. 8. Effect of RNAP αCTD alanine substitutions on activation by FIS. (**A**) A multiple round *in vitro* transcription assay performed with $RNAP^{WT}$ at 0.5 nM, $RNAP^{\alpha D258A}$ at 0.2 nM, $RNAP^{\alpha D259A}$ at 1 nM or $RNAP^{\alpha E261A}$ at 0.9 nM in the presence or absence of FIS^{WT} 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 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, $RNAP^{\alpha D258A}$ 4.0 ± 0.5-fold, $RNAP^{\alpha D259A}$ 7.6 ± 0.7-fold and $RNAP^{\alpha E261A}$ 6.7 ± 0.6-fold.

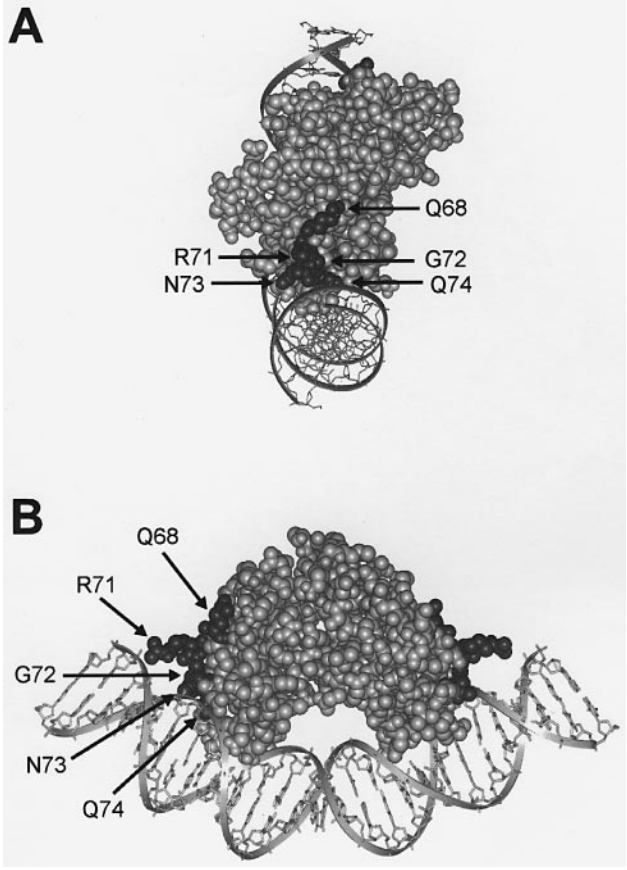

Fig. 9. The FIS 'activation patch'. The space-filling model of FIS is adapted from Yuan *et al.* (1991) and the DNA (strands) is modeled to fit the FIS structure. In (**A**), the modeled complex is viewed end on, so that the surface of FIS adjacent to RNAP is visible. In (**B**), the modeled complex is viewed from the side. Side chains of residues Q68, R71, G72, N73 and Q74 are labeled and shaded black to indicate the 'activation patch'. G72 and N73 are included, although the roles of these residues in RNAP interactions could be indirect (see text).

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 near normal DNA bend induced by FIS^{R71K} implies that its interactions with the sequences within and flanking the central recognition sequence in *rrnB* P1 FIS Site I 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 RNAP$^{\alpha D258A}$ 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 α mutants defective in FIS-dependent transcription. However, screens for mutants in α affecting activation 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; Artsimovitch *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

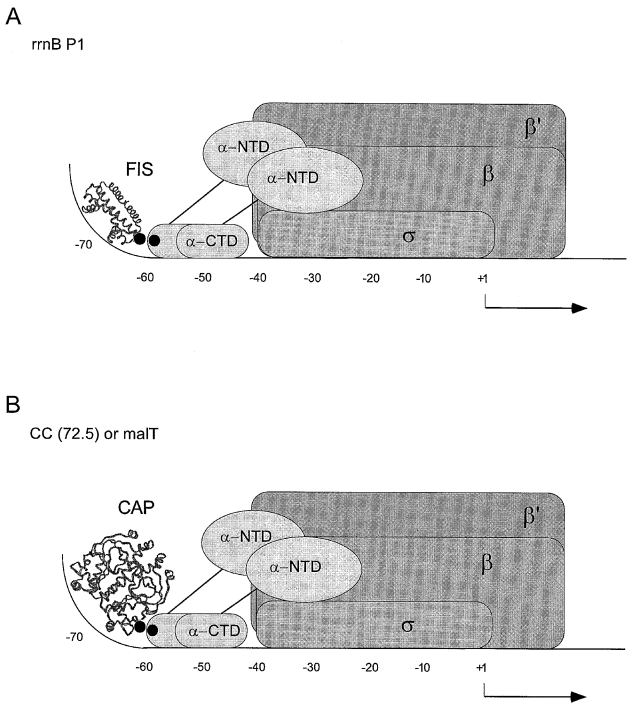

Fig. 10. Cartoon model of the FIS–RNAP activation complex at *rrnB* P1 (**A**) compared with the CAP–RNAP activation complex at CC(–72.5) or *malT* (**B**) (Gaston *et al.*, 1990; Chapon and Kolb, 1983). The structures of CAP and FIS are from Schultz *et al.* (1991) and Yuan *et al.* (1992) respectively. 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.

B–C loop just upstream of its helix–turn–helix DNA binding motif and αD258 maps to a short unstructured loop just upstream of the αCTD DNA binding helix, α_1 (Gaal *et al.*, 1996). Although we have not shown that the FIS B–C loop and the loop containing αD258 interact directly, their charges are complementary, and footprinting studies indicate they are likely to be positioned in close proximity (Bokal *et al.*, 1995). Thus, we envision interactions between FIS and the RNAP αCTD at *rrnB* P1 that involve side chains immediately adjacent to their respective DNA binding motifs (Figure 10A). The architecture of the FIS–RNAP activation complex fits generally with that suggested for the CAP–RNAP complex at the *lac* P1 promoter (Ebright and Busby, 1995; Gaal *et al.*, 1996) and almost exactly to that observed for promoters where activators bind at about –71, such as CC(–72.5) and *malT* (Chapon and Kolb, 1983; Gaston *et al.*, 1990; Zhou *et al.*, 1994; Savery *et al.*, 1996) (Figure 10B). It is striking that FIS and CAP, two non-homologous proteins of very different size and structure, appear to bend DNA similarly, to present similar surface loops to RNAP and to target a similar surface on the αCTD. We suspect that this overall architecture, with activators binding upstream of RNAP and RNAP and activators interacting near their respective DNA binding surfaces, may be a common one at bacterial promoters.

Materials and methods

Bacterial strains and plasmids

Strain RLG1739 (MG1655 Δ*lacX74 fis*::*kan*, F′ *proAB lacI*^{q}*Z*$_{u118}$*fzz*::Tn*5-320*) is a monolysogen for λ carrying the *rrnB* P1[–88 to –37]–*lac*[–36 to +2]–*lacZ* transcriptional fusion (Gosink *et al*., 1996). (The numbers in brackets refer to the limits of *rrnB* 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 *tac* promoter. The plasmid pRLG589 (Ross *et al*., 1990) contains the *rrnB* P1[–88 to +50] promoter. The circular permutation plasmid pSL9 (Gosink *et al.* 1993) contains the *rrnB* P1[–88 to +50] promoter.

Alanine scanning mutagenesis

Mutant *fis* 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′-GTTACCACGGGTGTATGCCATCACCATGTC-3′; A69, 5′-GTTACCACGGGTGGCTTGCATCACCATGTC-3′; A70, 5′-GTTACCACGGGCGTATTGCATCACCATGTC-3′; A71, 5′-CGGGTCTGGTTACCAGCGGTGTATTGCATC-3′; A72, 5′-CGGGTCTGGTTAGCACGGGTGTATTGCATC-3′; A73, 5′-GCGCAGCACGGGTCTGGGCACCACGGGTGT-3′; A74, 5′-GCGCAGCACGGGTCGCGTTACCACGGGTGT-3′. Primers annealing to pKG18 sequences flanking the *fis* gene were: pHD, 5′-CTGAAAATCTTCTCTCATCCGCC-3′; pRI, 5′-GTGTGGAATTGTGAGCGGATAACAA-3′. PCR products and M13RF DNAs were digested with *Eco*RI and *Hin*dIII 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^{R71K} 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 αΔ235 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 *rrnB* P1[–88 to –37]–*lac*[–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 A_{600} 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 nM supercoiled pRLG589, 170 mM NaCl, 10 mM $MgCl_2$, 12 mM Tris–HCl, pH 7.7, 1 mM DTT, 100 µg/ml BSA, 4% glycerol, 500 µM ATP, 50 µM CTP, 5 µM GTP, 5 µM UTP and 40 Ci/mmol [α-^{32}P]UTP. FIS was allowed to bind for 20 min as indicated, transcription was then initiated by the addition of RNAP and terminated after 20 min by the addition of an equal volume of 10 mM EDTA, 1% SDS, 7 M urea, 1× TBE, 0.025% xylene cyanol and 0.05% bromophenol blue. Samples were electrophoresed on 5% polyacrylamide, 0.5× TBE gels containing 7 M urea. Autoradiograms were exposed for ~24 h with intensifying screens and transcripts were quantified by phosphorimaging (Molecular Dynamics).

DNase I footprinting

FIS–DNA complexes were prepared at 22°C in 25 µl reactions containing 170 mM NaCl, 10 mM $MgCl_2$, 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 [*Xho*I(–168)–*Nhe*I(+75)] from pSL9 was ^{32}P-labeled in the *Xho*I 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).

DNA bend angle determinations

We measured the electrophoretic mobilities of FIS–Site I complexes formed on two different 269 bp DNA fragments derived from *Bgl*II or *Bam*HI digests of the circular permutation vector pSL9. The FIS–Site I complex is positioned near the center of the *Bgl*II fragment (i.e. Site I centered 128 bp from one end of the fragment) and near the end of the *Bam*HI fragment (i.e. Site I centered 31 bp from one end of the fragment). Reaction mixtures of 10 µl contained 0.3 nM ^{32}P-labeled DNA, 200 mM NaCl, 10 mM $MgCl_2$, 12 mM Tris–HCl, pH 7.7, 1 mM DTT, 100 µg/ml BSA, 5% glycerol and purified FIS. After 20 min at 22°C, reactions were loaded directly onto a pre-run 6% polyacrylamide, 0.5× TBE gel and electrophoresed for ~4 h at 220 V. Autoradiographs were exposed for ~4 h without intensifying screens.

Apparent DNA bend angles in the FIS–Site I complexes were estimated using the cosine formula as described by Thompson and Landy (1988). Although this analysis may not precisely predict absolute bend angles, it does nevertheless detect alterations in bend angles relative to those resulting from binding by wild-type FIS. We note that this analysis might not detect a change in the direction of a bend unless the mutation also changed the bend angle.

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