**Inhibition of IS2 transposition by factor for inversion stimulation**

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**Abstract**

The effect of factor for inversion stimulation (Fis) protein on IS2 transposition was investigated. A full-length IS2 was found to transpose at a frequency 64 times lower in a normal *Escherichia coli* than in a fis⁻ mutant. To investigate whether Fis affects IS2 transposition by DNA binding, gel retardation and DNase I footprinting experiments were performed. Analysis of Fis binding to the left terminus of IS2 revealed that Fis binds to nucleotide number 44–60 located between the −35 and −10 regions of the major IS2 promoter. To further determine whether Fis binding affects IS2 transcription, the major IS2 promoter was fused to a luciferase gene and assayed for its transcription efficiency in the presence or absence of Fis. The results showed that Fis reduced transcription from the major IS2 promoter by approximately sixfold. Analysis of Fis binding to the right terminal repeat of IS2 revealed that Fis binds to the inner end of the repeat, which is the same region as the place where the IS2 transposase binds. These results suggest that Fis inhibits IS2 transposition by blocking the binding sites of IS2 transposase and by repressing the transcription of IS2 genes.

**Introduction**

The insertion sequence IS2 is present in multiple copies in *Escherichia coli* K12 and B strains and many other enteric bacteria (Saedler & Heiss, 1973; Nisen et al., 1979; Hu & Deonier, 1981; Lam & Roth, 1983). The entire IS2 is 1331 bp in length with a pair of 42-bp imperfect inverted terminal repeats (Ghosal et al., 1979; Ronecker & Rak, 1987; Hu & Lee, 1988). Five ORF, designated ORF1 to ORF5 (Hu et al., 1994), are present in the IS2 genome, but only two IS2-encoded proteins, InsA (14 kDa) and InsAB' (46 kDa), have been detected (Hu et al., 1994, 1996). InsA is encoded by ORF1 (Hu et al., 1994), and InsAB' is encoded by ORF1 and ORF2 via a −1 frameshift mechanism (Prere et al., 1990; Chandler & Fayet, 1993; Farabaugh, 1996; Hu et al., 1996) (Fig. 1). The transcription of insA and insAB' is initiated from the same promoter, referred to as the major IS2 promoter (Hu et al., 1994), which is located in the left inverted repeat (LIR) and the adjoining region of IS2 (Hu et al., 1994).

InsAB' is the transposase of IS2 (Hu et al., 1996), and InsA is an inhibitor that negatively regulates the transcription of insA and insAB' by binding to the major IS2 promoter (Hu et al., 1994). Overexpression of InsA has a suppressive effect on IS2 transposition (Hu et al., 1994). The global transcriptional regulator cAMP–CRP (cyclic AMP receptor protein) complex has also been shown to bind to the major IS2 promoter and suppress the transcription from the major IS2 promoter, leading to a decreased production of IS2 transposase and frequency of IS2 transposition (Hu et al., 1998).

In addition to InsA and cAMP–CRP, other host factors may also affect IS2 transposition. The factor for inversion stimulation (Fis) protein is a DNA-binding protein found in *E. coli* and *Salmonella typhimurium* (Johnson et al., 1986, 1988; Koch & Kahmann, 1986; Bruist et al., 1987). It enhances the function of the Hin invertase, which is responsible for flagellum phase variation of *S. typhimurium* (Johnson et al., 1986; Bruist et al., 1987). Fis also enhances the function of the Gin invertase, which is required for the inversion of the G segment of bacterophage Mu (Koch & Kahmann, 1986). In addition to stimulating site-specific DNA inversion reactions (Johnson & Simon, 1985; Kahmann et al., 1985) and DNA replication from *oriC* (Gille et al., 1991; Filutowicz et al., 1992), it also acts as a transcriptional activator or repressor (Nilsson et al., 1990; Ross et al., 1990;
Ninnemann et al., 1992; Lazarus & Travers, 1993; Xu & Johnson, 1995; Falconi et al., 1996; Froelich et al., 1996; Gonzalez-Gil et al., 1996; Green et al., 1996; Champagne & Lapointe, 1998; Wu et al., 1998; Hirsch & Elliott, 2005; Huo et al., 2006). Fis has been shown to suppress its own expression (Ninnemann et al., 1992) and the expression of a number of genes including dnaA (Froelich et al., 1996), glnQ (glutamine high-affinity transport), mglA (methyl-galactoside transport), xylf (D-xylose-binding protein), sdhA (succinate dehydrogenase flavoprotein subunit), aldB (aldehyde dehydrogenase) (Xu & Johnson, 1995), and rpoS (Hirsch & Elliott, 2005). In this study, the role of Fis in IS2 transposition was investigated and it was found that Fis negatively regulates IS2 transposition.

Materials and methods

Transposition assay

To follow the transposition event of IS2, an IS2 derivative containing a tetracycline resistance gene (tet) was constructed as follows: the blunt-ended 1.4-kb EcoRI–AvaI fragment from pBR322 containing the tetracycline-resistance gene was ligated with the XhoI-digested pKS1ISF (Hu et al., 1994), resulting in pKS+IS2T (Fig. 2a). pKS+IS2T (Fig. 2a) was introduced into the isogenic E. coli strain BL21(DE3) (fis+) (Novagen) or RJ1852 [BL21(DE3)fis-767] (Yuan et al., 1994). An F-derived plasmid pCJ105 (Guyer et al., 1980) was introduced into these two strains to serve as the target for IS2 transposition. These two E. coli strains also harbor several copies of IS2, which provided the IS2 transposase in this transposition assay. Because pCJ105 carries a chloramphenicol resistance gene, transposition of IS2 onto pCJ105 would render pCJ105 able to confer an E. coli host both tetracycline- and chloramphenicol-resistant phenotypes. To determine the transposition frequency, pCJ105:IS2 were mated from BL21(DE3) (fis+) or RJ1852 (fis−) to DH1 (Hanahan, 1983) by conjugation. The transconjugants were selected on Luria–Bertani agar plates containing chloramphenicol (50 mg L−1), tetracycline (20 mg L−1), and nalidixic acid (50 mg L−1) because DH1 is resistant to nalidixic acid. The transposition frequency was calculated by dividing the number of DH1 cells that were resistant to tetracycline, chloramphenicol, and nalidixic acid by...
by the number of those that were resistant to chloramphenicol and nalidixic acid.

**Luciferase assay**

One hundred microliters of 1% (v/v) n-decyl-aldehyde in ethanol was added to 500 μL of an early log-phase (A₆₀₀ₙₘ = 0.2–0.3) culture of *E. coli* containing an appropriate plasmid. The reaction was incubated at room temperature for 10 s and then measured for luciferase activity using a Luminometer (AutoLumat LB 593; EG & G, Berthold). The luciferase present in each sample converted n-decyl-aldehyde to light, which was recorded as relative light units (RLU) by the Luminometer.

**Purification of Fis protein**

The gene encoding the wild-type Fis was cloned into pRJ1077, a derivative of pET11a (Novagen), in which fis was expressed from the T7 promoter (Pan et al., 1996). The Fis protein was purified by chromatography on a HiTrap SP column (Pharmacia), followed by dialysis against a low-salt buffer to precipitate Fis (Osuna et al., 1991; Pan et al., 1996). Resolublized Fis was concentrated by Centri-plus 3K (Amicon) ultrafiltration and stored at a concentration of 20 mg mL⁻¹ in 20 mM Tris-HCl (pH 8.2), 1 M NaCl, at −70°C.

**Gel retardation assay**

Four different DNA fragments, designated La, Lb, Ra, and Rb, containing the LIR or right inverted repeat (RIR) of IS2 were used. To obtain fragments La and Lb, pSK⁺LIR (Fig. 2b) was constructed by cloning the 89-bp EcoRI–SpeI fragment (Fig. 1), which contained the LIR and the major IS2 promoter, between the EcoRI and SpeI sites of pBlue-script II SK+ (Stratagene). This 89-bp EcoRI–SpeI (fragment La in Fig. 3a) and the 64-bp EcoRI–Msel (fragment Lb in Fig. 3a) fragments were then isolated from pSK⁺LIR (Fig. 3a). The 143-bp HindIII fragment from pSK⁺RIR (Hu et al., 1996, Fig. 3b) was digested with Fnu4HI to obtain the 85-bp Fnu4HI–HindIII fragment Ra (Fig. 3b) and with Msel to generate the 63-bp Msel–HindIII fragment Rb (Fig. 3b); these two fragments contained the RIR of IS2.

To perform the gel retardation assay, fragments La, Lb, Ra, and Rb (Fig. 3a and b) were end labeled with [γ-³²P]dATP by the Klenow enzyme and then incubated with various concentrations of purified Fis at room temperature for 20 min. Each 10 μL reaction mixture contained 25 mM Tris-HCl (pH 7.5), 75 mM NaCl, 0.1 mM EDTA, 5 mM MgCl₂, 1 μg of herring sperm DNA, and 30 mg mL⁻¹ bovine serum albumin (BSA). After addition of 4 μL of a DNA electrophoresis loading buffer (30% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanol), the mixture was electrophoresed on a 5% native polyacrylamide gel (Froelich et al., 1996). Retarded protein–DNA complex bands were visualized by autoradiography of the gel (Fig. 3).

**DNase I footprinting**

The 183-bp fragment Lc containing the LIR and the 265-bp fragment Rc containing the RIR of IS2 were amplified from pSK⁺LIR (Fig. 3a) and pSK⁺RIR (Fig. 3b), respectively. These two fragments were labeled at the 5’ end with ³²P by PCR using the 5’-end-labeled primer KS (Fig. 2b) was constructed by cloning the 89-bp EcoRI–SpeI fragment (Fig. 1), which contained the LIR and the major IS2 promoter, between the EcoRI and SpeI sites of pBlue-script II SK+ (Stratagene). This 89-bp EcoRI–SpeI (fragment La in Fig. 3a) and the 64-bp EcoRI–Msel (fragment Lb in Fig. 3a) fragments were then isolated from pSK⁺LIR (Fig. 3a). The 143-bp HindIII fragment from pSK⁺RIR (Hu et al., 1996, Fig. 3b) was digested with Fnu4HI to obtain the 85-bp Fnu4HI–HindIII fragment Ra (Fig. 3b) and with Msel to generate the 63-bp Msel–HindIII fragment Rb (Fig. 3b); these two fragments contained the RIR of IS2.

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![Fig. 3. Binding of the Fis protein to IS2. DNA fragments dnaAP2, La, Lb, Ra, and Rb of IS2 were end labeled by the Klenow enzyme with ³²P and used in the gel retardation assay. The lowest band in each lane is the free DNA not bound by Fis. The amounts of Fis used for each reaction are as indicated.](https://academic.oup.com/femsle/article-abstract/275/1/98/500684)
(5′-CGAGGTCGCGATCG-3′) and unlabeled primer T3 (5′-ATTAACCTCAGTAAG-3′). The PCR included 40 cycles of 95°C, 1 min; 55°C, 30 s; and 72°C, 30 s with a final extension at 72°C for 2 min. The PCR was performed in a Twin BlockTM thermal cycler (Eppendorf) with Taq DNA polymerase (Bertag). The PCR products were electrophoresed on a 5% acrylamide gel and then isolated from the gel by electrophoresion. The footprinting reaction (Froelich et al., 1996) was performed in a 200 μL mixture containing 25 mM Tris-HCl (pH 7.5), 75 mM NaCl, 5 mM MgCl2, 30 mg L−1 of BSA, 50 fmol of labeled DNA fragments, and various amounts of purified Fis protein. After incubating at room temperature for 20 min, DNase I (0.02 U μL−1, Gibco-BRL) was added and incubated for 5 min at 37°C. The reaction was stopped by adding an equal volume of phenol/chloroform, three volumes of 95% ice-cold ethanol, and 50 μL of saturated ammonium acetate. The DNA fragments were precipitated by centrifugation, dried, and resuspended in 1 μL of water. The sample was heated at 90°C for 5 min and then loaded onto a 6% urea-polyacrylamide sequencing gel. The DNA sequencing ladders of fragments Lc and Rc were loaded onto a 6% urea-polyacrylamide sequencing gel. The radioactivity in the dried gel was detected using the Molecular Dynamics PhosphorImager 425E and the IMAGEQUANT software (Molecular Dynamics).

Results

Effect of Fis on IS2 transposition

A full-length IS2 marked with a tetracycline resistance gene located on pKSIS2T (Fig. 2a) was assayed for transposition in BL21(DE3) (fis−) and RJ1852 [BL21(DE3)fis-767] (fis+) (Yuan et al., 1994) E. coli hosts. These two E. coli strains also contained several copies of IS2, which provided the IS2 transposase in this assay. Therefore, the difference in the transposition frequency of this IS2 in these two strains was due to the presence or absence of Fis. This IS2 was found to transposeto the target pCJ105 at a frequency of 5.0 × 10−8 in BL21(DE3) and 3.2 × 10−6 in RJ1852 (Table 1). This result indicated that the transposition frequency of IS2 is 64 times lower in fis+ than in the fis− host, suggesting that Fis has a suppressive effect on IS2 transposition.

Binding of Fis to the terminal sequences of IS2

Because terminal repeats are the most important sequence of transposable elements, DNA-binding experiments were performed to determine whether Fis affects IS2 transposition by interacting with the terminal repeats of IS2. 32P-labeled DNA fragments La, Lb, Ra, and Rb containing the terminal sequences of IS2 (Fig. 3a and b) were incubated with 25 or 50 ng of Fis and then electrophoresed on a native polyacrylamide gel. The 32P-labeled DNA fragment containing the dnaAP2 promoter region (Froelich et al., 1996) was used as the positive control. Retarded bands were seen in reactions using fragments dnaAP2, La, and Ra incubated with 25 or 50 ng of Fis (Fig. 3, lanes 2, 3, 5, 6, 11, and 12). No binding of Fis to fragments Lb and Rb (Fig. 3) was observed, suggesting that Fis binds to sequences around the MseI site of fragments La (Fig. 3a) and Ra (Fig. 3b) of IS2.

Determination of the Fis-binding sites in the terminal sequences of IS2

To further determine the Fis-binding sites in the terminal sequences of IS2, the DNase I footprinting assay was performed. DNA fragments Lc containing the LIR and Rc containing the RIR were used in the assay. Autoradiograms of Fis footprints in these two DNA fragments are shown in Fig. 4. In the left terminal sequence, Fis bound to IS2 nucleotides 44–60, which include the entire −10 region of the IS2 promoter (Fig. 4a and c). Within this binding region, five nucleotides (as the underscored nucleotides shown below), located in nucleotides 45–59, were found to match with the seven consensus nucleotides of the Fis-binding sequence (G or T)NN(C or T)(A or G)NN(T or A)NN(C or T)(A or G)NN(C or A) determined by Hubner & Arber (1989), while seven nucleotides (nucleotides 45–59) match with the 13 consensus nucleotides of Fis-binding sequence GNT(c or t)(A or G)NN(T or A)NN(C or T)(A or G)NN(C or A) determined by Finkel & Johnson (1992). In the right terminal sequences, Fis bound to IS2 nucleotides 1285–1305, which cover the inner end of the right inverted repeat of IS2. (Fig. 4b and d). Within this binding region, seven nucleotides (as the underscored nucleotides shown below), located in nucleotides 1289–1303, were found to match with the seven consensus nucleotides of Fis-binding sequence (G or T)NN(C or T)(A or G)NN(T or A)NN(C or T)(A or G)NN(C or T)(A or G)NN(C or A).
T)(A or G)NN(C or A) determined by Hubner & Arber (1989), while nine (nucleotides 1289–1303) match with the 13 consensus nucleotides of the Fis-binding sequence
\[
\text{GAt}(\text{C or T})(\text{A or G})(\text{T or G})(\text{T or A})(\text{T or C})(\text{G or A})(\text{G or A})
\]

Effect of Fis on transcription from the major IS2 promoter

The DNase I footprinting assay revealed that Fis binds to the \(-10\) region of the major IS2 promoter (Fig. 4a and c). To investigate whether Fis affects IS2 transposition by regulating transcription from the major IS2 promoter located in the LIR and the adjoining region, pACIS2-Lux (Hu et al., 1998), which contained the major IS2 promoter fused to the coding region of the luciferase gene of Vibrio harveyi, was introduced into BL21(DE3) \((\text{fis}^+)\) and RJ1852 \((\text{fis}^-)\). The transformants were then assayed for the production of luciferase. In the presence of Fis, the major IS2 promoter expressed \(8.8 \times 10^6\) RLU of luciferase. However, in the absence of Fis, a 5.7-fold increase \((49.8 \times 10^6\) RLU\) in the production of luciferase was observed. These results indicate that the major IS2 promoter is less active in the presence of Fis and suggest that Fis is a negative regulator of the major IS2 promoter (Table 2).

Discussion

In this study, the Fis protein was found to suppress IS2 transposition. This suppression could be due to the binding
of Fis to the major IS2 promoter located in the left terminal sequence of IS2. This hypothesis is supported by results of the DNase I footprinting experiment, which revealed that Fis binds to the left terminus of IS2 at nucleotide number 44–60 (Fig. 4a). Because this is the −10 region of the major IS2 promoter (Fig. 4c), it is conceivable that binding of Fis to this region would inhibit transcription from this promoter and thus curb the production of IS2 transposase. Because the IS2 derivative used in this study did not contain a functional transposase gene, its transposition was dependent on the transposase provided by other IS2 elements present in the host. The repression in the transposition of the IS2 derivative is likely due to the inhibition in the production of IS2 transposase by these IS2 elements. Inhibition of IS2 transposition by suppressing the transcription from the IS2 major promoter is consistent with previous reports that Fis is a transcription regulator (Nilsson et al., 1990; Ross et al., 1990; Ninnemann et al., 1992; Lazarus & Travers, 1993; Xu & Johnson, 1995; Falconi et al., 1996; Froelich et al., 1996; Gonzalez-Gil et al., 1996; Green et al., 1996; Champagne & Lapointe, 1998; Wu et al., 1998; Hirsch & Elliott, 2005; Huo et al., 2006). The effect of Fis on DNA transposition has also been observed in Mu and Tn5 (Betermier et al., 1989; Weinreich & Reznikoff, 1992; Betermier et al., 1993; van Drunen et al., 1993). Fis has been shown to modulate the repressor activity and decrease the transposition frequency of Mu (Betermier et al., 1989; Betermier et al., 1993; van Drunen et al., 1993). In contrast, Fis stimulates Tn5 transposition during the exponential growth of E. coli (Weinreich & Reznikoff, 1992).

Fis was found to lead to a six-fold decrease in the transcription activity of the major IS2 promoter but inhibit IS2 transposition by 64 folds, suggesting that the inhibition of transposition by Fis had only partially resulted from the repression of IS2 transcription. The DNase I footprinting assay confirmed that Fis binds to the inner end of the right inverted repeat of IS2 (Fig. 4b and d). It has been demonstrated that this region is the binding site for IS2 transposase (Hu et al., 1996). The binding of Fis to this region would block the binding of transposase to the RIR region, resulting in a repression in IS2 transposition.

It has been demonstrated previously that both IS2-encoded InsA (Hu et al., 1994) and the cAMP–CRP complex (Hu et al., 1998) also suppress the transcription from the major IS2 promoter, resulting in a reduction in transposase production. It is possible that Fis interacts with one or both of these two proteins. However, this possibility was not supported by the gel retardation experiments. In the presence or absence of Fis, InsA or cAMP–CRP complex produced the same banding pattern in the DNA-binding assay (data not shown), suggesting that Fis does not interact with either protein to regulate IS2 transposition. It is known that Fis can repress the transcription of the crp gene (Gonzalez-Gil et al., 1998). On the other hand, Crp can regulate the expression of fis. In the absence of Fis, Crp activates the transcription of fis; however, in the presence of Fis, Crp represses fis gene expression (Nasser et al., 2001). Because Fis is mainly produced during the early log phase of bacterial growth (Ball et al., 1992; Osuna et al., 1995; Talukder et al., 1999), it is likely that Fis regulates IS2 transposition at this stage, while InsA and Crp control IS2 transposition at a later stage of bacterial growth.

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### References


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| Table 2. Effect of Fis protein on transcription from the major IS2 promoter |
|-----------------------------|-----------------|------------------|
| Bacterial strain            | Luciferase activity (RLU)* | Relative fold increase¹ |
| RJ1852 (fis⁻)               | 49.8 ± 7.9 × 10⁶ | 5.7            |
| BL21 (DE3) (fis⁺)            | 8.8 ± 0.7 × 10⁶ | 1              |

*Each value represents the mean of four independent assays ± SD.

¹Fold increase in RLU is relative to that of BL21 (DE3) (fis⁺), which is set as 1. Fis, factor for inversion stimulation; RLU, relative light units.


