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Transcriptional activation by protein-induced DNA bending: Evidence for a DNA structural transmission model

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ABSTRACT **Integration host factor (IHF) is a DNA-bending protein that binds to an upstream activating sequence (UAS1) and, on a negatively supercoiled DNA template, activates transcription from the *ilv*P_G promoter of the *ilvGMEDA* operon of *Escherichia coli*. The transcriptional initiation site of the *ilvGMEDA* operon is located 92 bp downstream of UAS1. Activation is still observed when the orientation of the upstream IHF binding site is reversed. This manipulation places the IHF binding site on the opposite face of the DNA helix, directs the IHF-induced DNA bend in the opposite direction, and presents the opposite face of the nonsymmetrical, heterodimeric, IHF molecule to the downstream RNA polymerase. Lymphoid enhancer-binding factor, LEF-1, is a DNA-bending, lymphoid-specific, mammalian transcription factor that shares no amino acid sequence similarity with IHF. When the IHF site in UAS1 is replaced with a LEF-1 site, LEF-1 activates transcription from the downstream *ilv*P_G promoter in *E. coli* as well as it is activated by its natural activator, IHF. These results suggest that specific interactions between IHF and RNA polymerase are not required for activation. The results of DNA structural studies show that IHF forms a protein–DNA complex in the UAS1 region that, in the absence of RNA polymerase, alters the structure of the DNA helix in the -10 hexanucleotide region of the downstream *ilv*P_G promoter. The results of *in vitro* abortive transcription assays show that IHF also increases the apparent rate of RNA polymerase isomerization from a closed to an open complex. We suggest, therefore, that IHF activates transcription by forming a higher-order protein–DNA complex in the UAS1 region that structurally alters the DNA helix in a way that facilitates open complex formation at the downstream *ilv*P_G promoter site.**

Integration host factor (IHF) is a member of a family of DNA-bending proteins that share amino acid similarity with the bacterial HU protein. These are small, basic, minor-groove, nonspecific, DNA-binding proteins that play an architectural role in determining the structure of the bacterial nucleoid (1). IHF is a sequence-specific DNA-binding protein which induces a severe bend in the DNA helix of about 140° (2). While IHF was initially isolated as a protein of *Escherichia coli* involved in the higher-order assembly of a nucleoprotein complex required for the integrative recombination of bacteriophage λ into the bacterial chromosome, it is now known that IHF is involved in a diverse set of cellular functions, including the regulation of gene expression (1). For example, IHF increases the expression of the *ilvGMEDA* operon of *E. coli*, which encodes four of the five enzymes required for the biosynthesis of L-isoleucine and L-valine (3).

The *ilv*P_G promoter and the upstream regulatory sequences of the *ilvGMEDA* operon are shown in Fig. 1. Transcription initiation from the *ilv*P_G promoter is activated about 60-fold by two UASs, UAS1 and UAS2 (3). UAS2 is a 21-bp DNA sequence centered at base pair -50 that contains two tracts of five and six adenines in helical phase. A DNA bend centered between these phased adenine tracts activates transcription from the *ilv*P_G promoter, in a face-of-the-helix-dependent manner, about 15-fold even in the absence of the upstream IHF site (3). Similar A+T-rich DNA sequences have been observed in the -50 region of several other promoters (5). These other sequences also activate transcription in a face-of-the-helix-dependent manner about 10- to 30-fold. Ross *et al.* (5) have suggested that this activation is correlated with the interaction of these curved, upstream, DNA sequences with the α subunit of RNA polymerase, and they have proposed that these "UP" promoter sequences constitute a third promoter recognition element for strong σ^{70} promoters.

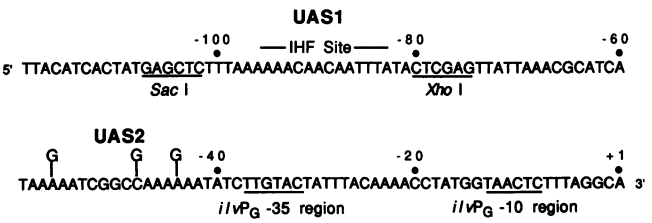

FIG. 1. The nucleotide sequence of the promoter region of the *ilvGMEDA* operon from base pair −118 to base pair +1. The IHF binding site, 5′-AAACAACAATTTA-3′, in upstream activating sequence (UAS)1 is located between base pairs −82 and −96 (3). UAS2, located between base pairs −41 and −59, contains a set of helically phased adenine residues. To disrupt the DNA bend in UAS2, the two adenine residues at positions −44 and −56 and the cytosine residue at −48 were changed to guanine residues by site-directed mutagenesis. To facilitate the reversal of the IHF binding site and replacement of the IHF binding site by the lymphoid enhancer-binding factor (LEF)-1 binding site, the 5′-TTTATT-3′ sequence between base pairs −108 and −101 and the 5′-TTGAAA-3′ sequence between base pairs −81 and −74 of the wild-type *ilv* promoter region (4) were changed to *Sac* I and *Xho* I endonuclease restriction sites by site-directed mutagenesis. The creation of the *Xho* I site eliminates the transcriptional activity of a weak, *in vitro*, promoter (referred to in previous publications as *ilv*P_G1) in UAS1 (3). The loss of this promoter does not affect either the intrinsic DNA bend-mediated or the IHF-induced (Table 1) activation of transcription from the downstream, *in vivo*, *ilv*P_G promoter [referred to in previous publications as *ilv*P_G2 (3)].

The second UAS (UAS1) contains a binding site for IHF. Binding of IHF at this site, on a negatively supercoiled DNA template, activates transcription from the downstream *ilv*P_G promoter an additional 3 to 4-fold (3). In this report, we show that this activation occurs in the absence of specific protein–protein interactions and that the formation of an IHF–DNA nucleoprotein structure in the UAS1 region of a negatively supercoiled DNA template causes a conformational change in the DNA helix at the downstream promoter site and increases

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Abbreviations: IHF, integration host factor; UAS, upstream activating sequence; LEF, lymphoid enhancer-binding factor; GST, glutathione *S*-transferase; HMG, high mobility group; IPTG, isopropyl β-D-thiogalactoside; ONP, *o*-nitrophenol.

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the rate of the isomerization step of the transcription initiation reaction.

MATERIALS AND METHODS

Plasmids and Bacterial Strains. All recombinant DNA methods were standard (6). Plasmids containing either wild-type or variant *ilv* promoter fragments were created by ligating a 272-bp *Eco*RI–*Bst*BI (end-filled) fragment [*ilvGMEDA* sequence from base pair −248 to base pair +6 (4) containing *Sac* I and *Xho* I sites at base pairs −103 and −76, respectively] into the *Eco*RI and *Bam*HI (end-filled) sites of the *lacZ*-truncated transcriptional fusion plasmid pRS551Δ (7). To replace the IHF site with the LEF-1 site and to reorient and reposition the IHF site, 27-bp double-stranded *Sac*I–*Xho* I DNA fragments containing the appropriate sequences were synthesized and ligated into these sites in the promoter fragment. The GST/LEF-HMG translational fusion gene, under the control of an isopropyl β-D-thiogalactoside (IPTG)-inducible *tac* promoter, contained on a 1071-bp *Eco*RI–*Ssp* I fragment (ref. 8; GST, glutathione *S*-transferase; HMG, high mobility group), was ligated into the unique *Sal* I (end-filled) site of plasmids containing either the IHF or the LEF-1 binding site.

The plasmid pDHΔwt, used in the *in vitro* abortive transcription and chemical probing reactions, was constructed by ligating a 251-bp *Hin*fI–*Bst*BI DNA fragment containing the $ilvP_G$ promoter region from base pair −245 to base pair +6 (4) inserted into the *Bam*HI (end-filled) site of plasmid pDD3 (9). The *Bam*HI site in pDD3 is flanked by tandem *rrnB* T1 transcriptional terminator sequences.

Each $ilvP_G$::*lacZ'* plasmid was integrated, in single copy, into the chromosomal copy of the *lacZ* gene in a *himA*$^+$ or *himA*$^-$ strain of the *polA*-deficient strain NO3434 (10) as previously described (22). Correct single-copy integration events were verified in each strain by Southern blot analysis.

Abortive Transcription Initiation Assay. Abortive transcription initiation assays were performed in the presence or absence of IHF according to the procedures of Hawley *et al.* (11). Reactions were initiated by the addition of 5 μl of RNA polymerase (final concentrations of 40, 60, 80, 100, and 120 nM) to a 95-μl, 37°C, reaction mixture containing the following: buffer A [0.04 M Tris·HCl (pH 8.0), 0.1 M KCl, 0.01 M $MgCl_2$, 1.0 mM dithiothreitol, 0.1 mM EDTA, and bovine serum albumin (BSA) at 100 μg/ml]; 0.5 mM CpA, 40 μM UTP, 100 μCi (1 μCi = 37 kBq) of [α-^{32}P]UTP, 10 nM purified IHF, and 2 nM supercoiled DNA plasmid template, pDHΔwt. After the initiation of the reaction, 5-μl samples were removed after 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0, 10.0, 15.0, 20.0, and 40.0 minutes and transferred to 5 μl of transcription stop buffer (8.0 M urea/0.1 M EDTA/0.4% SDS/0.04 M Tris·HCl, pH 8.0/0.025% bromophenol blue/0.025% xylene cyanol). The abortive transcription product, CpApUpU, produced at each time point, was isolated by electrophoresis in a 25% polyacrylamide (19:1 acrylamide to bisacrylamide) gel. The products were visualized by autoradiography, excised from the gel, and quantitated by Čerenkov counting in a Beckman LS230 liquid scintillation counter. The inclusion of the dinucleotide CpA in the reaction mixture restricts transcription initiation to the $ilvP_G$ promoter of the plasmid DNA template. τ is a measure of the lag time required for open complex formation observed in a product vs. time plot. τ_{obs} values were determined by a nonlinear least-squares analysis of the kinetic data as described by Goodrich and McClure (12). The RNA polymerase apparent binding affinity (K_B) and the first-order isomerization rate constant (k_2) were determined by plotting τ_{obs} as a function of 1/[RNA polymerase] on the basis of a least-squares fit to the equation $\tau_{obs} = (1/k_2) + (1/k_2K_B[\text{RNA polymerase}])$.

$KMnO_4$ Analysis of IHF-Induced DNA Conformational Changes in the *ilv* Promoter Region. Negatively supercoiled plasmid pDHΔwt (17 nM) was incubated in the absence and in increasing concentrations of IHF (3.5, 7, 14, 28, and 56 nM) in 17 μl of buffer A at 37°C for 10 min. The reaction of the DNA template with $KMnO_4$ was initiated by the addition of 2 μl of an 80 mM solution of $KMnO_4$. After exactly 2 min, the $KMnO_4$ reaction was quenched by the addition of 1.5 μl of 2-mercaptoethanol (14.6 M). The $KMnO_4$-treated DNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1, vol/vol) and passed through a Sephadex G-50 spin column pre-equilibrated with TE buffer (10 mM Tris·HCl, pH 8.0/1 mM EDTA). The DNA was precipitated with ethanol, dried under reduced pressure, cleaved at the $KMnO_4$-modified bases with piperidine, and lyophilized. The lyophilized DNA pellet was resuspended in 35 μl of TMD buffer (50 mM Tris·HCl, pH 7.2/10 mM $MgSO_4$/0.2 mM dithiothreitol). The sites of piperidine cleavage were mapped by primer extension using the Klenow fragment of DNA polymerase I and an *ilv*-specific oligonucleotide that anneals to *ilv* base pairs −147 to −132 (4).

RESULTS

Effect of the Intrinsic DNA Bend in UAS2 on IHF-Mediated Activation. To determine if the intrinsic DNA bend in UAS2 is required for the IHF-mediated activation of transcription from the $ilvP_G$ promoter, the DNA curvature in this region was disrupted by site-directed mutagenesis without deleting or displacing the IHF binding site in UAS1. The three base pair changes identified above the DNA sequence in Fig. 1 resulted in a complete loss of the DNA curvature in UAS2 (data not shown) and a 16-fold decrease in transcription from the $ilvP_G$ promoter (compare strains IH100 and IH150; Table 1). However, even in the absence of the DNA bend in UAS2, IHF remains able to activate transcription from the downstream promoter (compare strains IH150 and IH155; Table 1). This demonstrates that the intrinsic DNA bend in UAS2 is not required for IHF-mediated activation from UAS1 and that the transcriptional activation properties of these two regions are functionally independent.

Effect of Reversing the IHF-Binding Site in UAS1. We previously showed that IHF remains able to activate transcription when its binding site is rotated to the opposite face of the DNA helix by the insertion of 6 bp of DNA between the IHF binding site and the downstream $ilvP_G$ promoter (3). This observation suggested that, unlike most activator proteins, IHF might not require a specific protein interaction with RNA polymerase for activation. Thus, IHF might also be able to activate when the orientation of its binding site in UAS1 is reversed and the opposite face of the nonsymmetrical, heterodimeric, IHF molecule is directed toward the downstream RNA polymerase. To test this idea, the asymmetrical IHF binding site, 5′-AAACAACAATTTA-3′, encoded in the DNA sequence between the *Sac* I and *Xho* I sites shown in Fig.

Table 1. Effect of adenine tracts in UAS2 on IHF-mediated activation of transcription from the $ilvP_G$ promoter

Strain	Relevant genotype	β-Galactosidase specific activity, nmol of ONP per min per mg of protein
IHRS551	Δ$ilvP_G$::*lacZ*	ND
IH-100	$ilvP_G$::*lacZ*	8995 ± 560
IH-105	$ilvP_G$::*lacZ*, *himA* (IHF$^-$)	2925 ± 240
IH-150	$ilvP_G$::*lacZ*, UAS2$^-$	550 ± 60
IH-155	$ilvP_G$::*lacZ*, UAS2$^-$, *himA* (IHF$^-$)	130 ± 10

Each strain was grown in M63 minimal salts/glucose medium, and β-galactosidase was measured by hydrolysis of *o*-nitrophenyl β-D-galactoside to produce *o*-nitrophenol (ONP) in permeabilized cells obtained from logarithmic-phase cultures as described by Miller (13). Specific activity values are the mean ± SD of at least three separate assays, each performed at several assay times and cell concentrations. ND, not detectable.

1 was reversed. This manipulation also places the IHF binding site on the opposite face of the DNA helix and orients the IHF-induced DNA bend in the opposite direction. While the basal level of promoter activity is decreased 2-fold in this construct, the data in Table 2 show that IHF retains its ability, at least partially (2-fold), to function as a transcriptional activator (compare strains IH175 and IH176). IHF also retains its ability to activate when the IHF site is brought back to the same face of the DNA helix as the RNA polymerase binding site by the insertion of 5 bp immediately adjacent to the 5′-terminal dC residue of the *Xho* I site (Fig. 1). In this construct, the basal level of promoter activity is improved and a wild-type level of IHF activation is observed (compare strains IH185 and IH186; Table 2). The up to 2-fold variations in β-galactosidase expression observed in Tables 2 and 3 might be due to sequence alterations in the extended promoter region and/or a steric inhibition of RNA polymerase binding due to the positioning of the IHF binding site. Indeed, in the absence of any three-dimensional information about the structure of the IHF–RNA polymerase transcription complex on a negatively supercoiled DNA template, the possibility of nonspecific interactions between these proteins cannot be excluded. Nevertheless, the results of these experiments suggest that the IHF-mediated activation of transcription from the *ilv*P_G promoter does not require *specific* protein contacts between IHF and RNA polymerase.

LEF-1-Mediated Activation of Transcription from *ilv*P_G. If, indeed, specific protein interactions are unimportant, and if IHF-mediated activation of transcription from the *ilv*P_G promoter is influenced by the IHF-induced DNA bend in the UAS1 region, then any heterologous protein that bends the DNA helix to the same approximate extent as IHF might be expected to activate transcription from the downstream *ilv*P_G promoter site. An ideal candidate for testing the ability of a heterologous DNA-bending protein to activate transcription from this promoter is the mammalian transcription binding factor LEF-1.

LEF-1 is a member of a family of nonspecific DNA-binding proteins that share amino acid similarity with the eukaryotic non-histone protein HMG-1. Like the bacterial HU family of proteins, HMG-1 proteins are small, basic, minor-groove-binding, DNA-bending proteins that play an architectural role in chromatin structure (14). LEF-1 binds to DNA in a sequence-specific manner in the human T-cell receptor α enhancer region of pre-B and -T lymphocytes. Upon binding to its target DNA site, LEF-1 bends the DNA helix about 130° (14). Giese *et al.* (15) have demonstrated that LEF-1, or its HMG DNA-binding domain, can substitute for bending by IHF at the phage λ *att*P site. In spite of the remarkable functional similarities between IHF and LEF-1, however, these two proteins possess no amino acid similarities.

Table 2. Effect of the reversal of the IHF binding site on IHF-mediated activation of transcription from the *ilv*P_G promoter

Strain	Relevant genotype	β-Galactosidase specific activity, nmol of ONP per min per mg of protein
IHRS551	Δ*ilv*P_G::*lacZ*	ND
IH-175	*ilv*P_G::*lacZ*, IHF site reversed	4125 ± 360
IH-176	*ilv*P_G::*lacZ*, IHF site reversed, *himA* (IHF$^-$)	2035 ± 210
IH-185	*ilv*P_G::*lacZ*, IHF site reversed, additional 5 bp	6740 ± 560
IH-186	*ilv*P_G::*lacZ*, IHF site reversed, additional 5 bp, *himA* (IHF$^-$)	2130 ± 190

Each strain was grown in M63 minimal salts/glucose medium, and β-galactosidase activity was measured as described for Table 1. ND, not detectable.

To determine if IHF could be functionally replaced by LEF-1, the core IHF binding site in the UAS1 region was replaced by the core LEF-1 binding site as explained in *Materials and Methods*. When the GST/LEF-HMG protein was induced in cells containing the LEF-1 site, β-galactosidase levels increased about 4-fold [compare strains IH601 (−IPTG) and IH601 (+IPTG); Table 3]. However, in strains that do not contain the GST/LEF-HMG coding region, no increase in the level of β-galactosidase was observed, with or without the addition of IPTG, in either a wild-type strain [compare strains IH600 (−IPTG) and IH600 (+IPTG); Table. 3], or in a *himA* mutant strain [compare strains IH605 (−IPTG) and IH605 (+IPTG); Table. 3]. The comparable β-galactosidase activities in strains IH900 (±IPTG) and IH950 (±IPTG) demonstrate that the induction of the GST/LEF-HMG gene in these constructs does not influence transcription from the nearby *ilv*P_G promoter containing an IHF site in UAS1. Thus, although the GST/LEF-HMG fusion protein shares no amino acid sequence similarity with IHF, it is able to activate transcription from the downstream *ilv*P_G promoter. This result further argues that the activation of transcription from the *ilv*P_G promoter is not mediated by specific protein interactions between IHF and RNA polymerase and suggests a role for the protein-induced DNA bend in the activation mechanism.

Kinetic Effects of IHF on Transcriptional Initiation from *ilv*P_G. To determine the effects of IHF on the kinetic step(s) of the transcription initiation reaction at the *ilv*P_G promoter, we used closed-circular, negatively supercoiled, DNA templates containing the *ilv*P_G promoter region to perform abortive transcription initiation assays in the presence and absence of purified IHF protein. The results presented in Table 4 show that IHF decreases the binding affinity of the polymerase (K_B) at this promoter about 2-fold and increases the rate of the slow step of the abortive transcription reaction, presumably the isomerization step (k_2), approximately 4-fold. The observation that IHF enhances the rate of open complex formation is consistent with the idea that IHF influences the structure of the DNA helix at the downstream promoter site.

Effect of IHF on the Structure of the DNA Helix in the *ilv*P_G Promoter Region. We have provided evidence that transcription from the *ilv*P_G promoter can be activated by heterologous DNA-bending proteins (IHF and LEF-1) and that this activation is associated with an increase in the rate of isomerization of RNA polymerase from a closed to an open complex. This suggests that the expression of the *ilvGMEDA* operon of *E. coli* might be increased by an allosteric DNA mechanism in which a severe protein-induced DNA bend in UAS1 activates transcription by effecting a conformational change that facilitates an unwinding of the DNA helix in the −10 region of the downstream *ilv*P_G promoter. Thus, we used $KMnO_4$ to examine the structure of the DNA helix in the *ilv*P_G promoter region with and without IHF at its upstream binding site.

$KMnO_4$ serves as a probe for detecting sharply distorted and single-stranded DNA structures and is routinely used to detect open-complex formation at promoters (16). The data presented in Fig. 2 show that, on a negatively supercoiled DNA template and in the absence of IHF, $KMnO_4$ reacts with a (presumably distorted) thymidine residue located at base pair position −85 in the IHF binding site (Fig. 2, lane 1; Fig. 1). No $KMnO_4$ reactivity at this site is observed on a linear DNA fragment or a closed-circular relaxed DNA template (data not shown). This $KMnO_4$-reactive site is protected by the binding of IHF, which enhances the $KMnO_4$ reactivity of a pair of adjacent thymine residues at base pair positions −11 and −12 in the downstream −10 hexanucleotide region of the *ilv*P_G promoter (Fig. 2, lanes 2–6; Fig. 1). These data show a reciprocal relationship between the protection of the $KMnO_4$-reactive site in the IHF binding region (IHF occupancy) and the appearance of the $KMnO_4$-reactive sites in the downstream promoter region. Thus, these experiments demonstrate that

Table 3. Activation of transcription from the *ilv*P_G promoter by LEF-1

Strain	Relevant genotype	β-Galactosidase specific activity, nmol of ONP per min per mg of protein
IHRS551	Δ*ilv*P_G::*lacZ*	ND
IH-100	*ilv*P_G::*lacZ*	8,995 ± 560
IH-105	*ilv*P_G::*lacZ*, *himA* (IHF$^-$)	2,925 ± 240
IH-601 (−IPTG)	LEF-1 binding site, GST/LEF-HMG coding region under control of *tac* promoter	2,508 ± 175
IH-601 (+IPTG)	LEF-1 binding site, GST/LEF-HMG coding region under control of *tac* promoter	10,903 ± 820
IH-600 (−IPTG)	LEF-1 binding site, no GST/LEF-HMG coding region	2,134 ± 190
IH-600 (+IPTG)	LEF-1 binding site, no GST/LEF-HMG coding region	2,303 ± 130
IH-605 (−IPTG)	LEF-1 binding site, no GST/LEF-HMG coding region, *himA*	2,240 ± 190
IH-605 (+IPTG)	LEF-1 binding site, no GST/LEF-HMG coding region, *himA*	2,190 ± 115
IH-900 (−IPTG)	IHF binding site, GST/LEF-HMG coding region under control of *tac* promoter	8,475 ± 620
IH-900 (+IPTG)	IHF binding site, GST/LEF-HMG coding region under control of *tac* promoter	8,620 ± 690
IH-950 (−IPTG)	IHF binding site, GST/LEF-HMG coding region under control of *tac* promoter, *himA*	2,140 ± 175
IH-950 (+IPTG)	IHF binding site, GST/LEF-HMG coding region under control of *tac* promoter, *himA*	2,235 ± 160

The cells were grown in M63 minimal salts/glucose medium, and β-galactosidase activity was measured as described for Table 1. IPTG was added at a final concentration of 1 mM. ND, not detectable.

the binding of IHF to UAS1 causes a change in the structure of the DNA helix at the downstream *ilv*P_G promoter region.

DISCUSSION

In prokaryotes, activation of transcription can be effected by the binding of an activator protein to a site either immediately adjacent to or tens to hundreds of base pairs away from the promoter site. In most cases, the activator site lies adjacent to the promoter site and the activation mechanism is thought to involve specific interactions between the activator protein and RNA polymerase. When the activator site is located some distance away from the promoter the interaction is accompanied by a looping out of the intervening DNA sequence. This DNA looping is often facilitated by the action of a DNA-bending protein which binds to a site between the promoter and the activator binding sites. For example, IHF facilitates the interaction between activator proteins and RNA polymerase in this manner at many σ^{54} promoters (17). We know, however, that IHF does not function to activate transcription from the *ilv*P_G promoter by looping an upstream transcription factor(s) around to interact with RNA polymerase at the downstream promoter site because (*i*) IHF-mediated activation is retained, *in vivo* and *in vitro*, when the DNA sequences upstream of the IHF binding site are replaced with heterologous sequences; and (*ii*) IHF-mediated activation is observed in an *in vitro* transcription reaction, containing purified IHF and RNA polymerase, in the absence of any additional proteins (3). It has also been proposed that intrinsic or protein-induced DNA bends immediately upstream of a promoter site can activate transcription by looping nonspecific DNA sequences around to interact with the backside of RNA polymerase (18). However, in this case, unlike the case reported here, a strict face-of-the-helix dependence is required.

The structural organization of the control elements upstream of the *ilv*P_G promoter are strikingly similar to the organization of analogous control regions upstream of the λ P_L

Table 4. Effect of IHF on the kinetics of the transcription initiation reaction from the *ilv*P_G promoter

Conditions	Kinetic parameters from τ plots	
	K_B, 10^6 M^{-1}	k_2, 10^2 sec^{-1}
IHF absent	3.13 ± 0.44	1.02 ± 0.12
IHF present	1.44 ± 0.20	4.77 ± 0.67

Abortive transcription initiation assays were performed according to the procedures of Hawley *et al.* (11). τ is a measure of the lag time required for open complex formation observed in the product vs. time plot. The RNA polymerase apparent binding affinity (K_B) and the first-order isomerization rate constant (k_2) were determined by plotting τ_{obs} as a function of 1/[RNA polymerase] on the basis of a least-squares fit to the equation $\tau_{obs} = (1/k_2) + (1/k_2K_B[\text{RNA polymerase}])$. The kinetic values are the mean ± SD for five separate experiments.

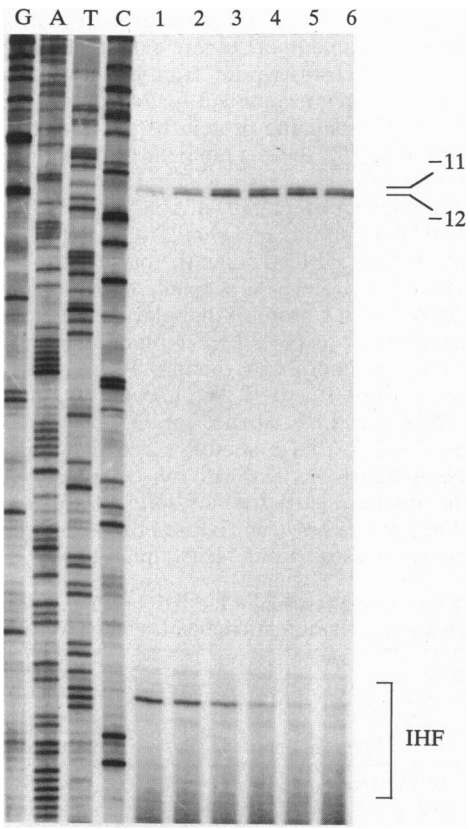

FIG. 2. $KMnO_4$ probing of the DNA structure in the *ilv*P_G promoter region in the presence and absence of IHF. Negatively supercoiled DNA templates were treated with $KMnO_4$ (no IHF, lane 1, and increasing concentrations of IHF in lanes 2–6). The $KMnO_4$-modified sites were cleaved by piperidine treatment and mapped by primer extension using an *ilv*-specific oligonucleotide that binds to *ilv* base pairs −147 to −132 (4). The DNA sequence shown in the left four lanes was determined by Sanger dideoxynucleotide sequencing using the same oligonucleotide primer.

and *rrnB* P1 promoters. The λ P_L promoter is activated by the binding of IHF at a site 80 bp upstream of the transcription initiation site (19). Transcription from the *rrnB* P1 promoter is activated by the binding of another DNA-bending protein (factor for inversion stimulation, FIS) at a site 71 bp upstream of the transcription initiation site (20). In both of these cases a specific interaction between the DNA-bending protein and the α subunit of RNA polymerase is required for activation. Also, the activation of these promoters is dependent on the helical phasing of the DNA-bending protein binding site. Activation of transcription from the *ilv*P_G promoter by a similar protein–protein interaction mechanism is unlikely because IHF-mediated activation of *ilv*P_G is partially retained when the IHF binding site is reversed and placed on the opposite face of the DNA helix and because a heterologous DNA-bending protein, LEF-1, can activate transcription from *ilv*P_G as well as IHF (Table 3).

These observations, coupled with the fact that *ilv*P_G activation requires a negatively supercoiled DNA template, led us to consider the possibility that the severe IHF-induced DNA bend might be more important than protein interactions for the activation of this promoter. Thus, we considered the possibility that the severe LEF-1 or IHF-induced DNA bend in UAS1 results in the formation of a higher-order protein–DNA structure which activates transcription by causing a conformational change in the DNA helix at the downstream promoter site. The results of the chemical probe experiments shown in Fig. 2 show that the binding of IHF in the UAS1 region of a negatively supercoiled DNA template, but not a relaxed template or a linear DNA fragment (data not shown), does result in a distortion of the DNA helix in the −10 hexanucleotide region of the *ilv*P_G promoter. Furthermore, the results of the abortive transcription initiation assays performed in the presence and absence of IHF (Table 4) show that IHF binding in UAS1 increases the rate of open complex formation at *ilv*P_G four-fold. These results suggest that IHF activates transcription from *ilv*P_G by a novel structural transmission mechanism. We propose that IHF forms a higher-order protein–DNA complex in the UAS1 region that structurally alters the DNA helix at the downstream *ilv*P_G promoter site in a way that facilitates open complex formation.

In conclusion, it is interesting to note that the LEF-1 protein used in the experiments reported here is a member of a growing number of DNA-bending proteins containing an HMG DNA-binding domain that are involved in the transcriptional activation of eukaryotic genes (14). It is conceivable that these architectural proteins might add three-dimensional diversity to the enhancer nucleoprotein complex, not only by facilitating specific interactions among neighboring transcription factors (21) but also by affecting the binding and/or activity of neighboring transcription factor(s) by altering the topography of the DNA as suggested by the results reported here.

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