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Inhibition of DNA Supercoiling-dependent Transcriptional Activation by a Distant B-DNA to Z-DNA Transition*

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Negative DNA superhelicity can destabilize the local B-form DNA structure and can drive transitions to other conformations at susceptible sites. In a molecule containing multiple susceptible sites, superhelicity can couple these alternatives together, causing them to compete. In principle, these superhelically driven local structural transitions can be either facilitated or inhibited by proteins that bind at or near potential transition sites. If a DNA region that is susceptible to forming a superhelically induced alternate structure is stabilized in the B-form by a DNA-binding protein, its propensity for transition will be transferred to other sites within the same domain. If one of these secondary sites is in a promoter region, this transfer could facilitate open complex formation and thereby activate gene expression. We previously proposed that a supercoiling-dependent, DNA structural transmission mechanism of this type is responsible for the integration host factor-mediated activation of transcription from the *ilv*P_G promoter of *Escherichia coli* (Sheridan, S. D., Benham, C. J. & Hatfield, G. W. (1998) *J. Biol. Chem.* 273, 21298–21308). In this report we confirm the validity of this mechanism by demonstrating the ability of a distant Z-DNA-forming site to compete with the superhelical destabilization that is required for integration host factor-mediated transcriptional activation, and thereby delay its occurrence.

Negative supercoiling within DNA molecules can destabilize the B-form duplex at locations where its thermodynamic stability is least. If this superhelically induced DNA duplex destabilization (SIDD)[1] is sufficiently strong, it can drive transitions to locally unpaired structures such as denaturation or cruciform extrusion (1, 2). Negative superhelicity also can drive transitions to other helical conformations, such as to the left-handed Z-form that requires an alternating purine-pyrimidine DNA sequence (3).

In a negatively superhelical molecule containing two or more sites that are susceptible to destabilizations or other transitions, superhelicity induces a global competition among all the possible structural alterations, with the energetically most favorable transition being the first to occur (4–7). By absorbing negative superhelical turns, the first transition induces a partial relaxation of the domain that delays other transitions to more extreme superhelicities than would be needed to drive them were the first transforming site not present. In molecules containing a site that can occur in the Z-form, the B-Z transition generally will be energetically favored over other alternatives, primarily because the change from right-handed helix to left-handed helix accommodates more negative superhelicity, and thereby allows the balance of the domain to relax by a correspondingly larger amount. So the insertion of a first Z-susceptible site into a molecule will offset the threshold superhelix density required to drive other destabilizations or structural transitions by an amount corresponding to the number of negative superhelical turns absorbed by the B-Z transition (7). In this way one can alter the destabilization characteristics of other local regions without changing their base sequences.

Theoretical analyses predict that SIDD occurs within the A + T rich DNA sequence extending from base pair +1 to base pair −160 in the regulatory region of the *ilv*P_G promoter of *Escherichia coli* (8–10). Osmium tetroxide binding experiments show that significant destabilization of the B-form DNA duplex initiates at superhelical density $\sigma = -0.038 \pm 0.003$ around base pair −98 within UAS1, the upstream activating sequence present in the 5′ portion of this SIDD region (8, 11). As the negative superhelix density of the DNA template becomes more extreme, destabilization spreads in the 5′ direction to base pair −153. When this destabilization (or another transition) occurs, the free energy associated with superhelicity is no longer uniformly distributed along the sequence. Rather, the destabilized site constitutes a local concentration of free energy. If this destabilization is sufficient to drive a local transition, the resulting alternate structure also is a local concentration of negative superhelical turns. In principle, such local accumulations of free energy and torsional deformation could serve functional purposes.

In previous studies (8, 12), we have investigated the role of destabilization within the UAS1 region in the IHF-mediated transcriptional activation of the *ilv*P_G promoter. This activation was shown to require a supercoiled DNA template having superhelical density $\sigma \leq -0.035 \pm 0.005$, coincident with the onset of UAS1 destabilization. Although IHF binds to a site centered at base pair position −92 within UAS1, immediately downstream of this superhelically destabilized region, its strength of binding does not depend upon DNA superhelicity (8, 12, 13). IHF-mediated activation of transcription from this promoter also does not involve protein-protein interactions between IHF and the RNA polymerase. However, IHF binding has been shown to stabilize the B-form DNA helix within the UAS1 region (8). In the presence of IHF no duplex destabiliza-

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[1] The abbreviations used are: SIDD, superhelically induced DNA duplex destabilization; IHF, integration host factor; bp, base pair(s).

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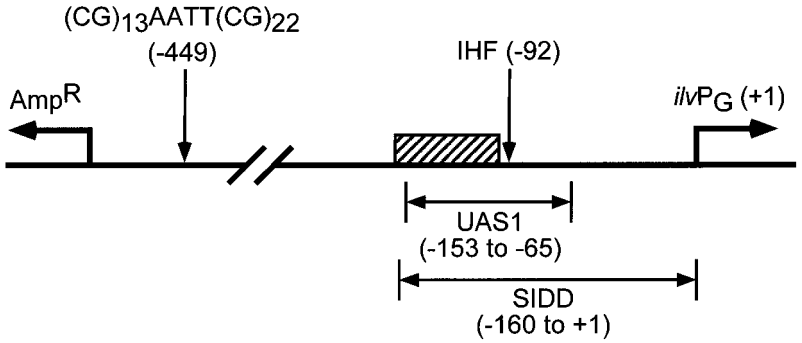

FIG. 1. **The *ilv*P_G regulatory region and construction of plasmid pSSΔZ.** Plasmid pSSΔZ was constructed by the insertion of a 74-bp *Bst*Y1 fragment from plasmid pRW1554 (10) containing the sequence $(CG)_{13}AATT(CG)_{22}$ into the unique *Ssp*I site 449 bp upstream of the *ilv*P_G transcriptional start site in plasmid pDHΔwt (2). *Arrows* indicate the transcriptional start sites of promoters in these plasmids. IHF binding site in the UAS1 region is indicated. The superhelically destabilized structure in the 5′ portion of the SIDD region is indicated by a *slashed box*. Numbers in parentheses indicate base pair positions relative to the start of *ilv*P_G transcription.

tion is observed to occur in this region, even in highly negatively supercoiled DNA templates. As a result, the free energy that was localized by destabilization of this region in the absence of IHF must, in the presence of IHF, be redistributed to other sites. In particular, IHF binding to a supercoiled DNA template has been shown to cause DNA helix destabilization in the −10 region of the downstream promoter. This suggested a mechanism of activation whereby IHF binding displaces superhelical destabilization from the UAS1 region to the downstream 3′ portion of the SIDD region that contains the *ilv*P_G promoter site (8). This supercoiling-dependent, IHF-mediated destabilization of the DNA helix in the −10 region lowers the energy of open complex formation, which increases the rate of transcriptional initiation from this promoter (8, 12, 14).

The experiments reported here were designed to test this DNA structural transmission mechanism of IHF-mediated transcriptional activation. We introduce a sequence with strong Z-DNA-forming potential into a site distant from the UAS1 region, which alters the destabilization characteristics of the UAS1 region without changing its base sequence. If this structural transmission model for IHF-dependent transcriptional activation is correct, then the presence of the Z-forming site should delay activation until more extreme superhelicities corresponding to a change of linking difference equal to the number of turns absorbed by the B-Z transition.

MATERIALS AND METHODS

Chemicals and Reagents—Restriction endonucleases and T4 DNA ligase were purchased from New England Biolabs. *E. coli* RNA polymerase was purchased from Amersham Pharmacia Biotech. Pancreatic RNasin was purchased from Promega. *Drosophila melanogaster* topoisomerase II was purchased from U. S. Biochemical Corp. Osmium tetroxide (OsO_4) and 2,2′-bipyridine were purchased from Sigma Chemical Co. Radiolabeled nucleotides were obtained from NEN Life Science Products. DNA sequencing was performed using the Sequenase kit of U. S. Biochemical Corp. DNA oligonucleotides were purchased from Operon Technologies. IHF was purified in this laboratory by the method of Nash *et al.* (15).

Plasmids—Plasmid DNA isolation, recombinant DNA manipulations and construct verifications were carried out using standard methods (16). Plasmid DNA containing the $(CG)_{13}AATT(CG)_{22}$ Z-DNA forming sequence, pRW1554 (17), was isolated from the *rec*A^- *E. coli* strain XL-1 blue. Plasmid pDHΔwt contains a 272-bp *Eco*RI-*Bst*BI (end-filled) restriction endonuclease DNA fragment (*E. coli ilv* bp positions, −248 to +6) ligated into the unique *Eco*RI and *Bam*HI (end-filled) sites of pDD3 (8) containing *rrnB*T_1T_2 transcription terminating sequences located 157 bp downstream of the *ilv*P_G transcriptional start site (12). The construction of pSSΔZ is described in Fig. 1. pDHΔwt and pSSΔZ contain 4,203 and 4,277 bp, respectively.

Generation of Plasmid DNA Topoisomers—10 μg of each plasmid was relaxed with *D. melanogaster* topoisomerase II in 40 μl reaction mixtures containing 0–60 μM ethidium bromide, as described previously (8). Topoisomers were purified and their linking number deficiencies (ΔLk) and superhelical densities ($-\sigma$) were determined by agarose gel electrophoresis (8, 18).

Two-dimensional Gel Electrophoresis of Plasmid DNA Topoisomers—Two-dimensional agarose gel electrophoresis was performed with pooled topoisomers in 1.4% agarose gels, as described previously (8). The first dimension (top to bottom) was run at 37 °C in 0.5× TBE. The second dimension (from left to right) was performed in 1× TAE buffer containing 0.10 μg/ml ethidium bromide. DNA supercoil-dependent structures were evaluated as described by Bowater *et al.* (19).

In Vitro Transcriptions—Closed-circular supercoiled plasmids were used as DNA templates for *in vitro* transcription assays performed in the absence and presence of purified IHF protein. RNA polymerase-plasmid DNA complexes were formed by preincubating 0.5 units (1.2 pmol) RNA polymerase and 250 ng of plasmid DNA (0.1 pmol) in a 45 μl reaction mixture (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, 200 μM CTP, 20 μM UTP, 10 μCi of [α-^{32}P]UTP (3,000 Ci/mmol), 100 μg/ml bovine serum albumin, and 40 units of RNasin) for 10 min at 25 °C. Transcription reactions were initiated by the addition of 5 μl of a 2 mM ATP, 2 mM GTP solution. Reactions were terminated after 2, 4, 6, 8, and 10 min by removing a 10-μl sample into 10 μl of stop solution (95% formamide, 0.025% bromphenol blue, 0.025% xylene cyanol). The *rrnB*T_1T_2-terminated 157-base pair reaction products were separated by electrophoresis on an 8% denaturing polyacrylamide gel (7.6% acrylamide, 0.4% *N,N′*-methylenebisacrylamide) containing 8 M urea in TBE buffer and visualized by autoradiography following exposure of the gels to Kodak XAR-5 film at −70 °C in the presence of a Cronex Quanta III intensifying screen (NEN Life Science Products). Transcriptional rates were determined by quantitation of band intensity *versus* reaction time using the public domain NIH IMAGE gel quantitation software (ftp://zippy.nimh.nih.gov).

RESULTS

If the mechanism of IHF-mediated activation of the *ilv*P_G promoter involves modulation of destabilization within the UAS1 region as proposed (8), then this activation should be offset to a more extreme superhelix density by the presence of a competing site that undergoes a B- to Z-DNA transition. To test this prediction, the Z-DNA forming sequence $(CG)_{13}AATT(CG)_{22}$ (10) was inserted into a unique *Ssp*I site 449 base pairs upstream of the transcriptional start site of the *ilv*P_G promoter in pDHΔwt to create pSSΔZ (Fig. 1).

Characterization of DNA Supercoiling-dependent Structural Transitions in pSSΔZ and pDHΔwt as Functions of Superhelical Stress—To determine the threshold superhelical densities required for transitions in the pSSΔZ and pDHΔwt plasmids, sets of DNA topoisomers of each plasmid were constructed having defined linking number deficiencies in the range $-62 \leq \Delta Lk \leq 0$ ($-0.16 \leq \sigma \leq 0.00$). These topoisomer sets were pooled for each plasmid and analyzed by two-dimensional gel electrophoresis. The gel migration pattern of the pDHΔwt topoisomers is shown in Fig. 2*A*. The superhelically induced destabilization of the A + T rich sequence in the UAS1 region commences at linking difference $\Delta Lk = -15$, ($\sigma = -0.038$), which is comparable with the previously determined threshold superhelical

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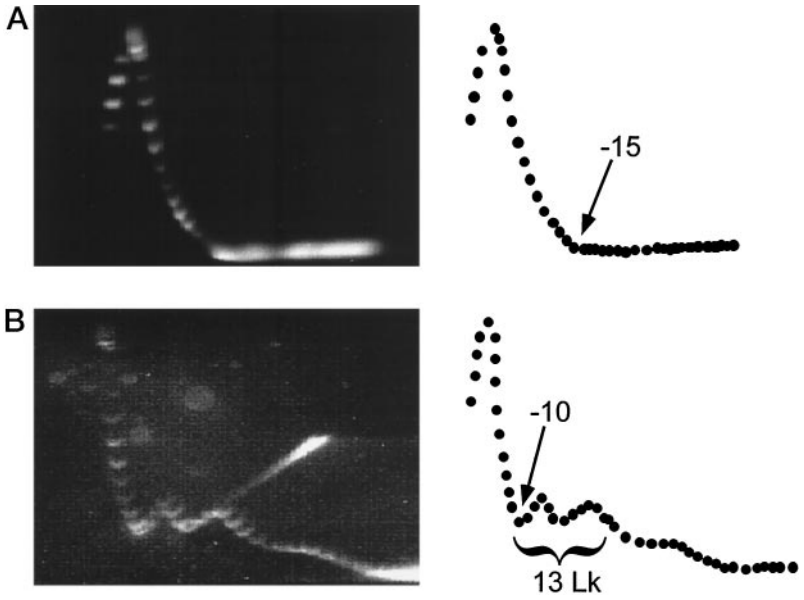

FIG. 2. **Characterization of structural transitions in pDHΔwt and pSSΔZ by two-dimensional agarose gel electrophoresis.** Topoisomers of plasmids pDHΔwt (*A*) and pSSΔZ (*B*) were analyzed by two-dimensional agarose gel electrophoresis on 24-cm square 1.4% agarose gels. The first dimension (top to bottom) was run at 37 °C in 0.5× TBE. The second dimension (from left to right) was performed in 1× TAE buffer containing 0.1 μg/ml ethidium bromide. Graphical representations are presented on the right. *Arrows* indicate threshold linking number deficiency (−ΔLk) for structural transitions. The *bracket* in *panel B* indicates the linking number range of the B- to Z-DNA transition.

density required for this structure (8, 17). In pSSΔZ, however, the B- to Z-DNA transition is observed to initiate at ΔLk = −10, (σ = −0.025; Fig. 2*B*), a significantly less extreme superhelix density than that required for the destabilization observed in the UAS1 region (σ = −0.038). The migration pattern of the topoisomers in this gel also shows that, as described previously (17), the B- to Z-DNA transition within the 74-base pair insert occurs in two steps, and absorbs approximately 13 superhelical turns. This observation is consistent with the loss of 1.8 turns of right-handed twist for every 10.5 base pairs of B-DNA that are converted to Z-DNA (3). This shows that the B-Z transition in pSSΔZ occurs at a significantly less extreme threshold superhelical density than does duplex destabilization of the SIDD site in UAS1.

The Effects of the B-Z Transition on the Topology of the ilvP_G Promoter Regulatory Region—Because the B-Z transition absorbs approximately 13 negative superhelical turns in pSSΔZ, it is predicted to offset the global plasmid linking number required for duplex destabilization in the UAS1 region of that plasmid by that amount. This prediction has been tested in experiments in which OsO_4 was used to probe both plasmids for destabilized sites. The results of these experiments, shown in Fig. 3, confirm the prediction. In pDHΔwt, the plasmid lacking the Z-DNA insert, destabilization of the A + T rich SIDD-susceptible site in the UAS1 region starts at linking differences between ΔLk = −11 ± 2 (σ = −0.028 ± 0.005; Fig. 3*A*, *lane 2*) and ΔLk = −18 ± 2 (σ = −0.044 ± 0.005; Fig. 3*A*, *lane 3*). In pSSΔZ, the plasmid containing the Z-DNA insert, destabilization of this site is observed to commence at linking differences between ΔLk = −26 ± 2 (σ = −0.064 ± 0.005; Fig. 3*B*) and ΔLk = −31 ± 2 (σ = −0.076 ± 0.005; Fig. 3*B*). Thus, the DNA template containing the Z-DNA insert, pSSΔZ, must be untwisted by 14 ± 2 additional turns to reach a global superhelical density that initiates destabilization of the A + T rich SIDD-susceptible sequence in UAS1.

Z-DNA Formation Increases the Global Superhelix Densities Required for Both Basal Level and IHF-activated Transcription from the ilvP_G Promoter—We have shown that superhelically induced duplex destabilization within the UAS1 region is offset to more extreme superhelical densities in the pSSΔZ plasmid that contains the Z-DNA forming sequence. If, as we have proposed (8), destabilization of this region of UAS1 is required for IHF-mediated transcriptional activation, then activation in that plasmid should be offset to the same extent. To test this prediction, we performed *in vitro* transcription assays in the presence and absence of IHF on the same DNA topoisomer sets of pDHΔwt and pSSΔZ that were used in the experiments reported in Figs. 2 and 3. The results of these experiments are shown in Fig. 4. In the absence of both the Z-DNA insert and IHF, basal level transcription from the *ilv*P_G promoter in pDHΔwt increases approximately 40-fold as the negative superhelicity of the DNA is changed from ΔLk = −18 ± 2 to the optimum value of ΔLk = −49 ± 2 (Fig. 4*A*). In the presence of IHF, activation of transcription from the *ilv*P_G promoter in pDHΔwt is observed over this same range of superhelix densities (12) (Fig. 4*B*). However, in the plasmid containing the Z-DNA insert, the half-maximal level of basal transcription is delayed by 11 ± 4 linking numbers (Fig. 4*A*), and IHF-activated transcription is delayed by 12 ± 4 linking numbers (Fig. 4, compare *B* and *C*). Thus, as predicted, the presence of the Z-susceptible insert offsets the threshold superhelicity required for IHF-mediated activation by approximately 13 turns, the amount of twist absorbed by the B-Z transition.

DISCUSSION

Transitions to superhelically induced non B-DNA structures can either be facilitated or inhibited by proteins that bind at or near potential transition sites (8, 9). If a DNA region that is favored to form a superhelically induced alternate structure is stabilized in the B-form by a DNA-binding protein, its propensity for transition will be transferred to other sites within the same superhelical domain. If one of these secondary sites is in a promoter region, where strand separation is required for transcriptional initiation, this transfer could facilitate open complex formation and thereby activate transcription. We have previously demonstrated that IHF binding inhibits the superhelically induced destabilization of the B-form DNA helix in an upstream activating sequence (UAS1) of the *ilv*P_G promoter of *E. coli*, and simultaneously facilitates duplex destabilization of its downstream −10 region (8, 14). We also have shown that this downstream destabilization decreases the energy required for open promoter complex formation, which activates transcriptional initiation (14). We have proposed that the superhelical energy required for this transcriptional activation was derived from the IHF-mediated inhibition of superhelically induced duplex destabilization within the upstream UAS1 region (8). This novel DNA structural transmission mechanism explains the observations that IHF-mediated activation of tran-

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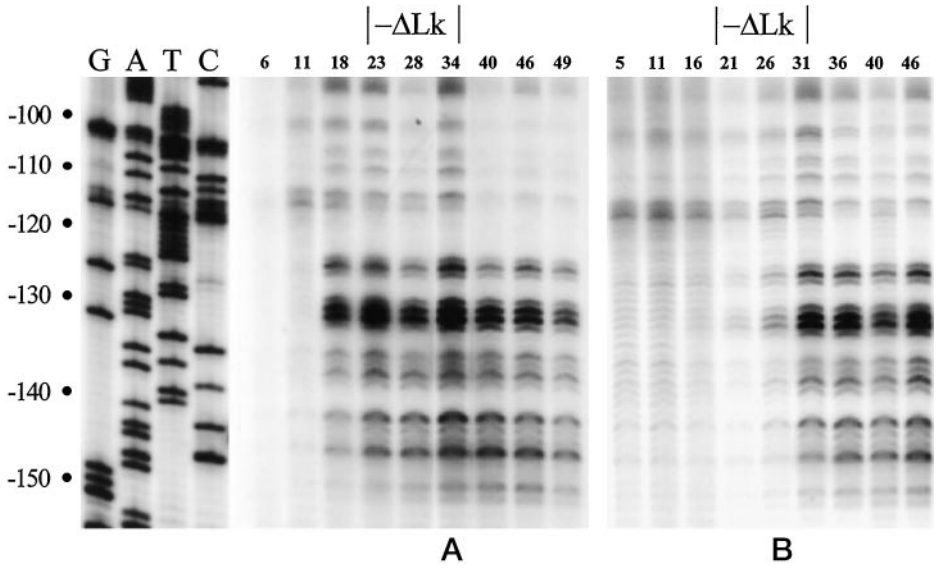

FIG. 3. **Effect of template superhelicity on helix destabilization in the UAS1 region of *ilv*P_G.** Topoisomers of plasmids pDHΔwt (*A*) or pSSΔZ (*B*) were treated with 2 mM osmium tetroxide and 2 mM 2,2-bipyridine, and sites of modification were mapped by primer extension analysis. Base pairs positions are shown with respect to the transcriptional start site of the *ilv*P_G promoter. *Lanes 1–9* contain negatively supercoiled DNA topoisomers with average linking number deficiencies (ΔLk ± 2).

scription from the *ilv*P_G promoter requires a supercoiled DNA template, and occurs in the absence of specific interactions between IHF and RNA polymerase.

We emphasize that, although this model involves the transfer of the free energy of destabilization, it does not require DNA denaturation, either within the SIDD site of UAS1 before IHF binding or around the −10 region after binding. Indeed, transcriptional activation was assayed at moderate salt concentrations, a condition where denaturation at normal superhelix densities is inhibited (20). (We note that OsO_4 binds to structures in which the B-form is destabilized but not necessarily denatured (21).) We have performed sample calculations to illustrate the transmission of destabilization energy under these conditions. The methods used in these calculations have been described previously (10, 22, 23). Their results are plotted in Fig. 5. Fig. 5*A* displays the calculated destabilization energy profiles for a 500-bp portion of the plasmid sequence that contains the UAS1 region, both in the absence (*dotted line*) and in the presence (*solid line*) of bound IHF. Here $G(x)$ is the incremental free energy that is required to guarantee that the base pair at position x is open (23). Smaller values of $G(x)$ correspond to more strongly destabilized sites. This calculation assumes that the plasmid has an overall superhelix density of $\sigma = -0.035$. In the absence of IHF binding, the low basal level of transcription is regarded as slightly reducing the local superhelix density upstream of the polymerase, to $\sigma = -0.045$. IHF binding is modeled as constraining the DNA to B-form throughout the 30-bp region where DNA-protein contacts are seen in the co-crystal structure (24). This binding causes an extreme bend, which we regard as strongly affecting the manner in which transcriptionally generated incremental negative superhelicity is accommodated. Specifically, when the wake of negative superhelicity generated by transcription (25, 26) encounters the strongly bent region, its untwisting torsional deformations (the component of superhelicity that directly drives transitions) act to physically rotate the DNA·IHF complex, thereby transducing superhelical torsional deformations into writhing deformations. Because this transduction requires rotation of a relatively large complex through a medium that is highly viscous on this size scale, the torsional stresses that drive it must accumulate on the 3′ side of the IHF. Thus, although bound IHF is not a barrier to the passage of superhelicity (26), the torsional component that drives transitions will still be lower on its 5′ side and higher on its 3′ side. The increased rate of transcription consequent on activation will produce a further augmentation of the negative superhelicity 5′ to the RNA polymerase complex. We model these events in our calculation by assuming that the component of superhelicity that drives transitions reverts to its basal level on the 5′ side of the IHF·DNA complex, and has twice that value in the region between that complex and the polymerase. Fig. 5*B* shows the calculated change in the destabilization free energy $G(x)$ in this region consequent on IHF binding. The largest decrease, corresponding to the greatest destabilization, occurs around the −10 and −35 regions of the promoter. (The −10 region is denoted by a bar in both parts of the graph in Fig. 5.) Under the conditions assumed by this calculation, approximately 4 kcal/mol less free energy is needed to open this region when IHF is bound than when it is not bound.

This model predicts that superhelically induced DNA structural destabilization, not the presence of specific DNA sequences, is the primary determinant of IHF-mediated activation. To directly test this prediction, we inserted a distant, superhelically induced Z-DNA-forming sequence that is capable of inhibiting the destabilization of UAS1 without altering the DNA sequence in any part of the *ilv*P_G regulatory-promoter region. The inserted $(CG)_{13}AATT(CG)_{22}$ sequence undergoes a superhelically driven B-Z transition, which absorbs 13 negative superhelical turns (Fig. 2), and thereby relaxes the global superhelix density of the remainder of the supercoiled DNA template by a corresponding amount (17). Because this B-Z transition occurs at a lower threshold superhelical density than does the destabilization of UAS1, it inhibits UAS1 destabilization until approximately 13 additional negative superhelical turns are added to the DNA template (Fig. 3). If the energy required for IHF-mediated transcriptional activation is indeed derived by transfer from the initially destabilized UAS1 region upon IHF binding, then the superhelicity required for IHF activation should be offset by approximately 13 turns in the plasmid containing the Z-susceptible site. The results of transcription assays on DNA templates of defined superhelix densities show this to be the case (Fig. 4). The superhelicities required both for half-maximal basal level and for IHF-activated transcription are indeed offset by approximately 13 turns. These experiments clearly demonstrate the involvement of superhelically induced DNA duplex destabilized structure of UAS1 in IHF-mediated activation. Because we have previously shown that IHF binding to its target site in UAS1 is unaffected by superhelical density (12), these experiments: (i) support our previous demonstration that IHF-mediated activation occurs in

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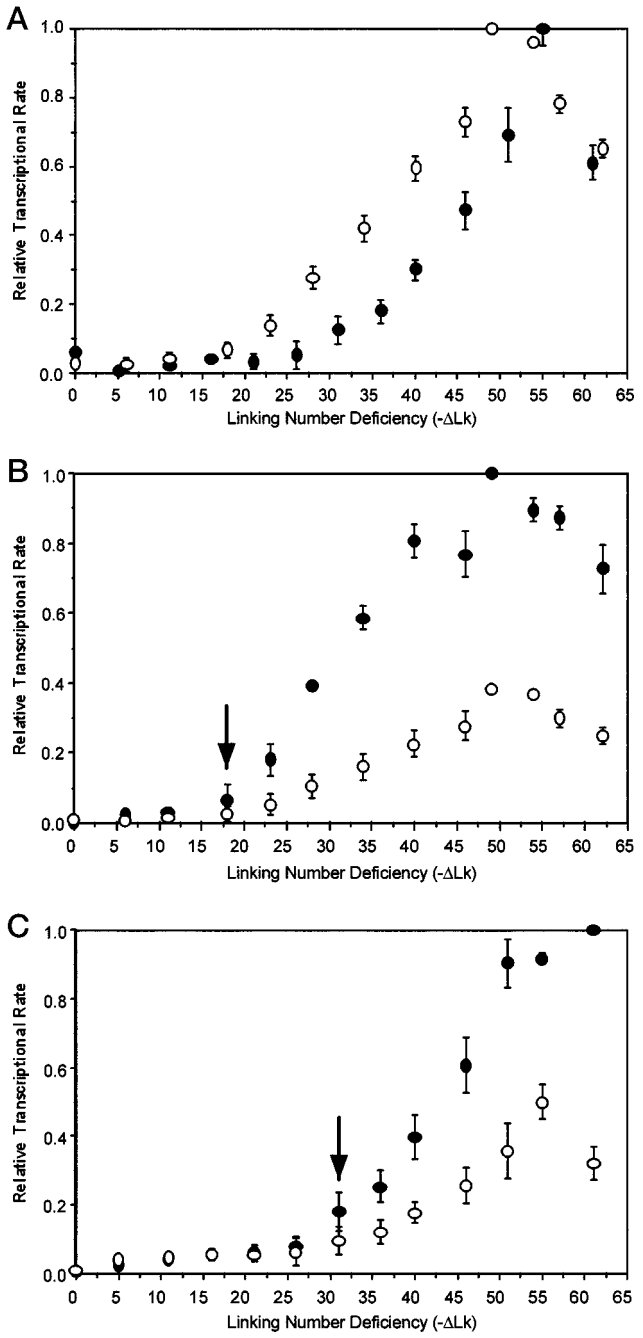

FIG. 4. **Effects of superhelicity on basal level and IHF-activated transcription from the *ilv*P_G promoter.** *Panel A*, the *ilv*P_G basal transcriptional rates determined on topoisomers of pDHΔwt (○) or pSSΔZ (●) are plotted as a function of average linking number deficiency (−ΔLk ± 2). *Panel B,* the *ilv*P_G basal and IHF-activated transcriptional rates determined on topoisomers of pDHΔwt in the absence (○) and presence (●) of IHF. *Panel C*, the *ilv*P_G basal and IHF-activated transcriptional rates determined on topoisomers of pSSΔZ in the absence (○) and presence (●) of IHF. *Arrows* indicate DNA templates of the lowest |ΔLk| that demonstrate OsO_4 reactivity in the UAS1 region. Transcriptional rates (±S.D. of three experiments) were normalized by setting the maximum level of transcription for each DNA template in each plot equal to 1.0.

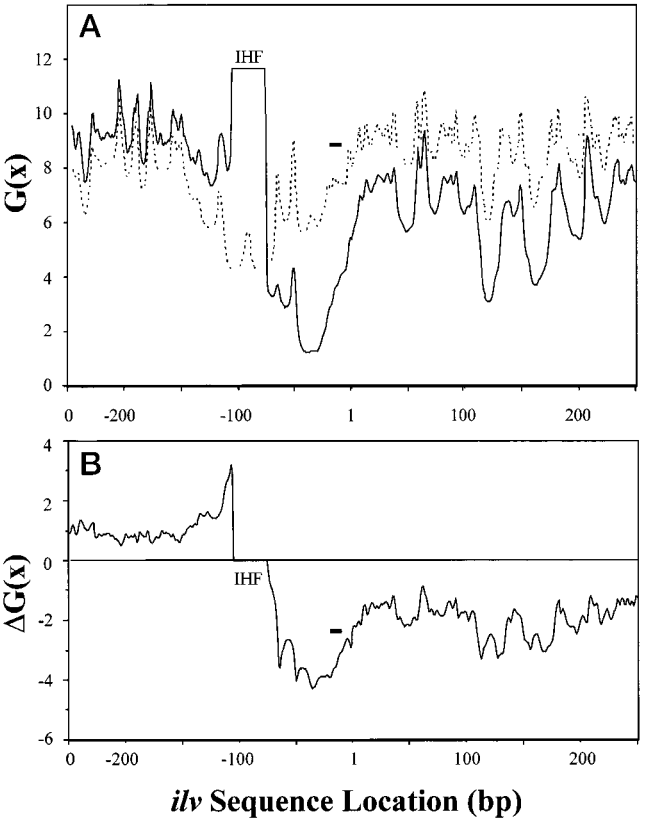

FIG. 5. **Sample calculations were performed to illustrate the transmission of destabilization energy consequent on IHF binding from the UAS1 region to the −10 region.** The energies of denaturation used were experimentally measured at [Na] = 0.1 M (30), close to the ionic strength used in the transcription assays reported here. *Panel A* displays the calculated destabilization energy profiles for a 500-bp portion of the plasmid sequence that contains the UAS1 region, both in the absence (*dotted line*) and in the presence (*solid line*) of bound IHF. Here $G(x)$ is the incremental free energy that is required to guarantee that the base pair at position x is open (23). Smaller values of $G(x)$ correspond to more strongly destabilized sites. This calculation assumes the plasmid has an overall superhelix density of $\sigma = -0.035$. In the absence of IHF binding, the low basal level of transcription further reduces the local superhelix density upstream of the polymerase to $\sigma = -0.045$. IHF binding constrains the DNA to B-form throughout the 30-bp region where DNA-protein contacts occur. The torsional stress component that drives superhelical transitions is modeled as being lower on the 5′ side of the bound IHF and higher on its 3′ side, as described in the text. *Panel B* shows the calculated change in the destabilization free energy $G(x)$ in this region consequent on IHF binding. A substantial decrease in the stability around the −10 and −35 regions of the promoter arise when IHF binds, even though no region is predicted to denature under these circumstances. In both parts of this figure the −10 region is denoted by a bar.

the absence of interactions between IHF and RNA polymerase (14), even on a supercoiled DNA template; (ii) explain the requirement for a supercoiled DNA template for IHF-mediated activation; and (iii) confirm the predictions of the protein-mediated DNA structural transmission mechanism of transcriptional activation (8).

It is important to emphasize that the effects of the Z-DNA structure on basal level transcription and IHF-mediated activation reported here are facilitated by altering the structure of the DNA helix in the *ilv*P_G promoter-regulatory region without altering its natural DNA sequence. This type of experiment establishes a competition between DNA structures to separate the regulatory roles of structural transitions from those of base sequence (9). We suggest that this approach can be applied to dissect the effects of these factors in a wide variety of other DNA supercoiling-dependent biological processes.

The biological significance of superhelically induced DNA secondary structures is becoming an increasingly active area of research, and a great deal of information suggesting that DNA assumes a variety of structures in living cells is emerging (27–29). Our work demonstrates that these DNA supercoiling-dependent biological processes can be regulated by distant or nearby DNA supercoiling-dependent structures, and that these

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effects can be modulated by DNA-binding proteins through their influence on the formation of these structures (8). This realization presents an important paradigm for future studies on the regulation of many biological processes, such as gene expression, that are sensitive to the superhelical state of DNA.

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