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The σ^{54}-Dependent Promoter *Ps* of the TOL Plasmid of *Pseudomonas putida* Requires HU for Transcriptional Activation In Vivo by XylR

JOSÉ PÉREZ-MARTÍN AND VÍCTOR DE LORENZO*

Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, 28006 Madrid, Spain.

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In the presence of toluene and xylenes, the σ^{54}-dependent *Ps* promoter of the TOL (toluene biodegradation) plasmid pWW0 of *Pseudomonas putida* is activated at a distance by the XylR protein, of the NtrC family of transcriptional regulators. Since contacts between XylR bound to upstream activating sites and the RNA polymerase require the looping out of the intervening DNA segment, the intrinsic curvature, the bendability of the corresponding sequence, and the spatial effects of protein-induced DNA bending have an influence on promoter activity. Unlike other σ^{54}-dependent promoters, *Ps* does not require the structural aid of the integration host factor to assemble a specific promoter geometry required for transcriptional initiation. In vivo analysis of transcriptional activity in various genetic backgrounds suggests, instead, that the looping out of intervening DNA sequences in *Ps* would result from the exacerbation of a preexisting static bend within the region, assisted by the histone-like protein HU.

The TOL plasmid pWW0 of *Pseudomonas putida* for biodegradation of toluene and *m*- and *p*-xylenes carries two σ^{54}-dependent promoters (2, 19). One of them, named *Pu*, drives expression of the so-called *upper* operon for bioconversion of toluene and xylenes to benzoate and toluates. The other, termed *Ps*, transcribes a second regulatory gene, *xylS*, as specified in Fig. 1. When cells encounter toluene or xylenes in the growth medium, both promoters are activated at a distance by the cognate regulator of the system, the XylR protein, of the NtrC family of transcriptional regulators (17, 25). Interactions between the activator bound to distant upstream sites and the σ^{54}-containing RNA polymerase (σ^{54}-RNAP) bound to the $-12/-24$ region require the looping out of the intervening DNA (31). Thus, the physical properties of the nucleotide sequence and the binding proteins which alter DNA topology are predicted to have a substantial effect on promoter activity (27). Most σ^{54}-dependent promoters known so far (including the *Pu* promoter of the TOL plasmid [Fig. 1]) contain target sequences for the integration host factor (IHF) protein between the upstream activating sequences (UAS) and the site of attachment of σ^{54}-RNAP (1, 8, 18). It is currently believed that the major role of IHF in these promoters is that of providing a structural aid to improve contacts between the σ^{54}-RNAP bound to the $-12/-24$ positions and the activator protein attached to the UAS, thereby enhancing promoter activity (1, 8, 18, 26, 28). However, other σ^{54}-dependent systems, such as the *glnAp2* promoter of *Escherichia coli* (24), have no apparent IHF binding sites. In these cases, specific protein-induced changes of the intervening region of DNA between the UAS and $-12/-24$ appear not to be required. The lack of IHF seems to be balanced in these instances by a high-affinity sequence for binding σ^{54}-RNAP at $-12/-24$ (18).

The *Ps* promoter of the TOL plasmid seems to represent an intermediate case between IHF-dependent and IHF-independent σ^{54} systems. Analysis of the DNA sequence of the region (14, 15) reveals the presence of at least two potential IHF binding sites, one of them at position -140 to -153 (i.e., overlapping the UAS) and a second one at position -35 to -47, proximal to the binding site for the polymerase at $-12/-24$ (10, 15) (Fig. 1). IHF-*Ps* interaction can be detected at high concentrations of IHF in vitro (8, 10, 15), but the role of the protein in vivo is uncertain (10, 11, 15). While the absence of IHF has been reported to decrease *Ps* activity somewhat (15), its overproduction seems to inhibit *xylS* transcription (10). In the present work, we address the issue of how looping out of distant UAS in *Ps* occurs. Our results present genetic evidence that the histone-like protein HU, and not IHF, is the key element that assists the assembly of the correct promoter geometry for the initiation of transcription at *Ps*.

MATERIALS AND METHODS

Strains, plasmids, and general procedures. *E. coli* N99 and its isogenic derivatives *E. coli* A5475 (Δ*himA82 hip157*), *E. coli* A5196(*hupA16*::*km hupB11*::*cat*) and *E. coli* A5477 (*hupA16*::*km hupB11*::*cat* Δ*himA82 hip157*) have been described previously (20). *him* and *hip* genes encode each of the two subunits of the IHF protein. *hupA* and *hupB* encode each of the two subunits of the HU protein. *E. coli* S90C and its *him* (IHF$^-$) derivative *E. coli* DBP102 have also been described (8). *rpoN*, formerly called *ntrA*, encodes the structural sequence for the σ^{54} factor. *E. coli* MC4100 was made *rpoN* minus through P1 phage transduction of the *ntrA208*::Tn*10* marker from *E. coli* ET8045 (28) and, where required, was made also IHF minus through transduction of the *hip*[Δ3::cat] marker from *E. coli* MC252 (6). Hybrid promoters *Pu-AT4*, *Pu-AT5*, and *Pu-ST* (28) are artificial constructs in which the naturally occurring IHF binding site of the *Pu* promoter of the TOL plasmid has been replaced by either a segment of DNA intrinsically bent by ca. 120° (*Pu-AT4* and *Pu-AT5*) or by an equivalent noncurved DNA sequence (*Pu-ST*). These hybrid promoters maintain the overall orientation of core elements on the DNA helix surface present in the wild-type *Pu*, but they are devoid of IHF sites (28). pRS551 plasmid carrying transcriptional fusions of *lacZ* to the *Pu* promoter (pRS*Pu*) or its hybrid derivatives *Pu-AT4* (pRS*Pu*/*AT4*) and *Pu-ST* (pRS*Pu*/*ST*) is described elsewhere (28). A transcriptional fusion of *Ps* to *lacZ* was assembled also in pRS551 vector (30). For this fusion, a DNA segment from the TOL pWW0 plasmid spanning positions -219 to $+65$ of the *Ps* promoter region (16) was flanked by a synthetic polylinker bounded by *Bam*HI sites at both ends, and the resulting 346-bp restriction fragment was inserted at the *Bam*HI site of pRS551, thus giving rise to pRS*Ps*. Where indicated, *lacZ* fusions

* Corresponding author. Mailing address: Centro de Investigaciones Biológicas (CSIC), C/. Velázquez 144, 28006 Madrid, Spain.

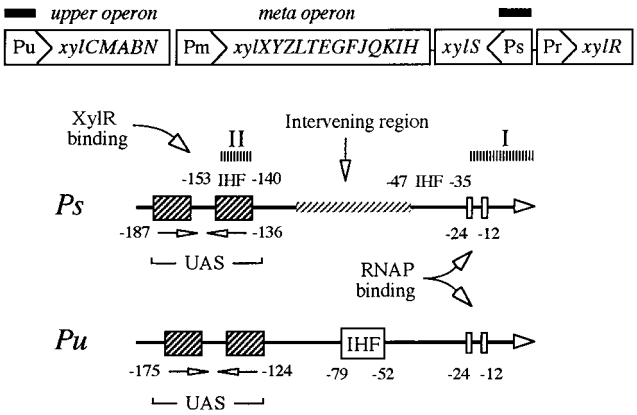

FIG. 1. Organization of the *xyl* genes and their σ^{54} promoters in the TOL plasmid pWW0. The TOL system for degradation of toluene and *m*- and *p*-xylene includes two gene clusters as well as two regulatory genes, *xylS* and *xylR*. The system has two σ^{54}-dependent promoters, *Pu* and *Ps* (indicated by solid bars at the top), which are activated by the cognate activator XylR in the presence of pathway substrates (19). Below the scheme of the operons, both promoter regions are expanded and lined up, showing the locations of relevant DNA sequences, including upstream binding sites (UAS) for XylR and the −12/−24 motif recognized by σ^{54}-RNAP. The *Ps* sequence includes at least two potential IHF binding sites. One of them (site I) is proximal to the −12/−24 region, while the other (distal site II) overlaps the UAS (10). Site II spans a consensus IHF binding sequence (positions −140 to −153), and it is footprinted in vitro by purified IHF (10). In contrast, the proximal site I defined by the protection in vitro of the sequences from position +1 to −27 (10) is considerably displaced in respect to the nearest sequence similar to the consensus IHF site at positions −35 to −47 (15). Unlike *Ps*, *Pu* has only one IHF binding site that is functional in vivo and in vitro (1, 8), and this site is located within the intervening region between the promoter core elements.

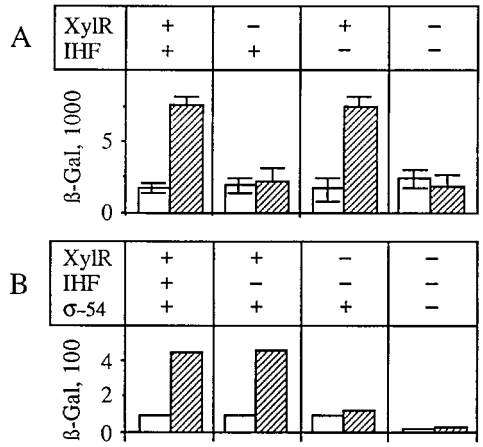

FIG. 2. IHF-independent activity of the *Ps* promoter. (A) Multicopy gene dosage. *E. coli* S90C and its isogenic derivative *E. coli* DBP102 *himA* (IHF$^-$) were cotransformed with the *xylR*$^+$ plasmid pTS174 (or with the vector pACYC184 for *xylR* mutants) and the *Ps-lacZ* plasmid pRS*Ps*. (B) Monocopy gene dosage. Specialized λ phages carrying a *lacZ* fusion to *Ps* were employed to lysogenize isogenic strains *E. coli* MC4100 *xylR*$^+$ *hip*$^+$ (IHF$^+$) *rpoN*$^+$, *E. coli* MC4100 *xylR*$^+$ *hip* (IHF$^-$) *rpoN*$^+$, *E. coli* MC4100 *xylR hip* (IHF$^-$) *rpoN*$^+$, and *E. coli* MC4100 *xylR hip* (IHF$^-$) *rpoN* in order to have all the regulatory elements of the system integrated into the chromosome. Both for multicopy and monocopy dosages, cultures of each strain were exposed to saturating vapors of *m*-xylene as explained in Materials and Methods and β-galactosidase (β-Gal) accumulation (in Miller units) was recorded after 4 h of induction. The aromatic inducer and the absence of IHF and/or σ^{54} had no significant effect on cell growth under the conditions used. □, uninduced; ▨, plus *m*-xylene.

were integrated into the chromosomes of *E. coli* MC4100 and its *rpoN* and IHF$^-$ derivatives by recombination with λRS45 phage and further lysogenization of the strain of interest (30). When required, the *xylR* gene was introduced into the chromosome of *E. coli* by using the specialized hybrid minitransposon mini-Tn*10* Ptt *xylR*/*xylS* (8). pTS174 (Cm^r) is a *xylR*$^+$ derivative of pACYC184 (11). An additional *xylR*$^+$ plasmid equivalent to pTS174 but carrying instead a Tc^r marker (pAX13) was constructed by inserting a 1.7-kb *Eco*RI-*Sca*I fragment of the TOL operon spanning the *xylR* sequence (17) into pACYC184 vector.

Detection of DNA bends and measurement of bending angles. Circular permutation tests (36) were performed to identify nonlinear DNA structures within the sequences of interest. A tandem duplication of the *Ps* sequence was generated as follows. A DNA segment spanning positions −219 to +65 of the *Ps* promoter region (16) was cloned at the *Hin*cII site of pUC18 (resulting in plasmid pEZ2a) and duplicated internally by blunt-ending by separation of the *Hin*dIII and *Bam*HI sites followed by religation of the *Sca*I-digested products. The resulting plasmid (named pB*Ps*) contains a tandem duplication of a 318-bp *Xba*I fragment spanning *Ps* as shown in Fig. 3. Circular permutations of the promoter sequences were produced by digesting pB*Ps* with each of the enzymes indicated in Fig. 3, purifying the promoter-containing segments flanked by different lengths of DNA, loading approximately 200 ng of each purified fragment in a nondenaturing 7% polyacrylamide gel with 40 mM Tris acetate [pH 8]–1 mM EDTA buffer at 5 to 10 V/cm, and visualizing their relative positions after the run with ethidium bromide stain. The bending angle was estimated from the ratio between mobilities of the fastest-migrating and most slowly migrating complexes, combined with linear interpolation of points obtained with A-tract DNA standards (32). Tridimensional images of DNA sequences were generated with the DNASTAR computer program (DNASTAR, Inc., London, United Kingdom), which employs a simplified algorithm assigning 8.6° to A-A wedge angles in A-T tracts (34).

Quantification of promoter activity in vivo. Transcriptional activity was monitored by assaying accumulation of β-galactosidase in cells carrying *lacZ* gene fusions to the promoters indicated in each case. For this purpose, cells were grown at 37°C to an optical density at 600 nm of 1.2 in Luria-Bertani liquid medium (21) supplemented with appropriate antibiotics and exposed for 4 h to saturating vapors of the XylR effector *m*-xylene. β-Galactosidase assays were made on cells permeabilized with chloroform and sodium dodecyl sulfate (21). The linearity of the assay with *o*-nitrophenyl-β-D-galactoside was verified in all cases. β-Galactosidase values were the averages of at least three independent measurements, each of which was from duplicate samples.

RESULTS

IHF does not influence *Ps* promoter activity in vivo. Since there have been somewhat conflicting reports (10, 11, 15) concerning the effect of IHF on the *Ps* promoter of the TOL plasmid, we started by reexamining the issue with our own assay system. This system uses *lacZ* reporter technology to reproduce in *E. coli* strains all the regulatory elements which control transcriptional activity of *Ps* in vivo. Figure 2A shows different β-galactosidase profiles raised by a *Ps-lacZ* fusion plasmid in IHF-plus and IHF-minus genetic backgrounds. The results indicated that the absence of IHF does not significantly affect *Ps* activity either under uninduced conditions or upon exposure of the cells to the XylR effector *m*-xylene. Since potential regulatory effects could be masked by multicopy effects, we examined also in monocopy gene dosage all the genetic elements relevant to the system. For this examination, the same *Ps-lacZ* fusion was introduced into the chromosome of the *E. coli* strains by using a specialized phage lambda vector, while *xylR* was placed in single copy by inserting a hybrid transposon carrying the corresponding sequence. Figure 2B shows that the system behaves identically with multicopy and monocopy gene dosages—yet, expectedly, within different ranges of promoter activity. Under the very same conditions, the IHF-containing *Pu* promoter, used as a control, lost >60% of its activity in IHF$^-$ strains (Fig. 3; also see below). The data shown in Fig. 2 confirm that transcription from *Ps* is XylR dependent and σ^{54} (*rpoN*) dependent but not affected by IHF, either positively or negatively, at least within the variation limits of intracellular IHF levels in *E. coli* (3, 9). Although the potential IHF binding sites are located at key positions in the *Ps* promoter sequence (Fig. 1), it is very unlikely that IHF binds them at all under normal physiological conditions. Therefore, the observed binding in vitro of IHF to *Ps* when the protein is present at high concentrations (8, 10, 15) does not seem have much significance in vivo. Instead, the

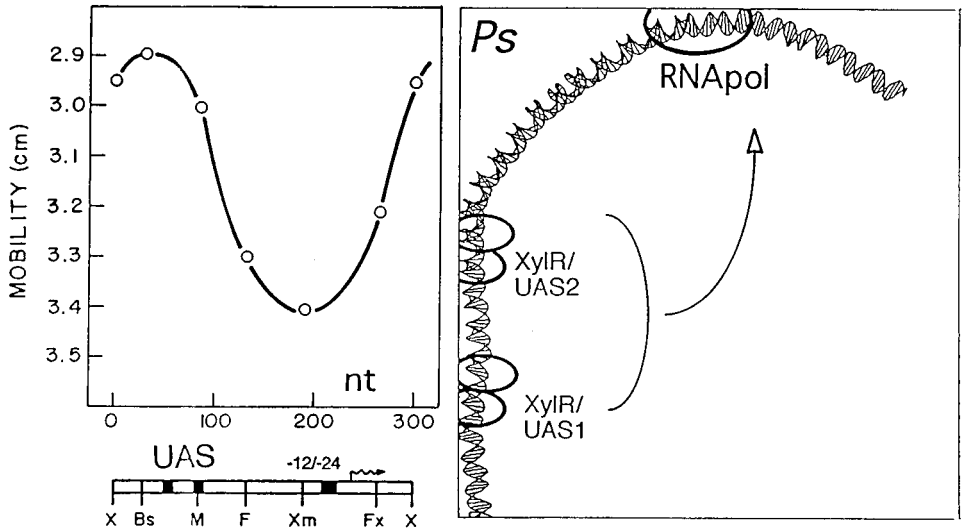

FIG. 3. Static curvatures in *Ps* DNA sequence. The plot on the left shows the result of the circular permutation assay performed on restriction fragments spanning DNA segments with the *Ps* sequence as indicated below the graph. The graph indicates the mobilities in a nondenaturing gel (in centimeters) of the DNA fragments released after digestion of the tandem sequences with each of the restriction enzymes indicated at the bottom. The relative positions (in numbers of nucleotide residues [nt]) of the restriction sites used for the permutation are indicated on the horizontal axis. Restriction sites: X, *Xba*I; Bs, *Bsm*I; M, *Mun*I; F, *Fnu*4HI; Xm, *Xmn*I; Fx, *Fok*I. In the right panel, the predicted distribution of intrinsic DNA bends within the *Ps* promoter sequences, based on a simplified modeling of dinucleotide wedge angles (see Materials and Methods), is shown. Projection of the overall shape of the promoter uses as a reference the upstream −213 (*Ps*) position, indicated by a small square symbol in the lower part of the right panel. The locations of relevant elements within the region are shown. The UAS of *Ps* are represented at the two DNA portions of maximum homology to those of *Pu*. The direction of the loop predicted to occur during activation of *Ps* is indicated with an arrow in the right panel.

putative IHF sites may have been merely inherited as the remains of an ancestor promoter sequence which has evolved to adjust *Ps* performance within a certain window of activity in the TOL system.

The *Ps* sequence is endowed with a significant static bend. Bend swapping experiments carried out with the σ^{54}-dependent promoters *Pu* (28) and *PnifH* (22) have shown that the looping out of the UAS bound to their cognate regulators (XylR and NifA, respectively) into the RNAP bound to −12/−24 region requires that the intervening sequence possess the ability to bend in a particular orientation. Substitution of the IHF binding site of *Pu* (Fig. 1) by an unbent DNA sequence resulted in a promoter virtually irresponsive to *m*-xylene and XylR (28). It thus seems unlikely that in the absence of IHF-assisted bending a somewhat straight intervening DNA sequence could sustain the geometry required for the activation of the *Ps* promoter by XylR. It is probable, instead, that the intervening sequence in *Ps* has structural features (i.e., intrinsic curvatures, bendability, or flexibility) that help DNA to adapt the precise geometry required for the activation of the promoter. On these premises, the next question to address was whether a structural feature of this kind could be present in *Ps* to assist formation of the required DNA loop. Circular permutation assays (36) were carried out with the *Ps* promoter to detect regions possessing significant curvatures. The results shown in Fig. 3 indicate that the *Ps* sequence is intrinsically curved, the apparent overall center being at −50/−60 in respect to the transcription start site. The bending angle was estimated to be in the range of 60 to 70°. This experimental result is compatible with the tridimensional imaging of the corresponding sequences obtained with a simplified Trifonov's algorithm (34) as shown in Fig. 3. Inspection of the predicted structure of the region and the distribution of the different promoter elements (Fig. 3) suggested that the intrinsic bend in *Ps* appears predisposed to move the UAS-XylR complex towards the σ^{54}-RNAP. Yet the degree of curvature detected in circular permutation assays (Fig. 3) seems insufficient by itself to generate a loop closed enough to position all promoter elements in a productive configuration. This raised the possibility that other proteins could account for additional structural effects within the *Ps* promoter region.

***Ps* behaves like *Pu* in the absence of IHF.** One plausible candidate to cause changes in DNA structure, functionally equivalent to those produced by IHF binding, was the histone-like protein HU. IHF and HU are known to be exchangeable in a number of cases (5, 12, 20, 23), but HU does not seem to have preferred attachment sites. Therefore, the structural changes caused by its nonspecific binding do not have a specific directionality (4, 29). Instead, HU seems to mediate a general flexibility-enhancing effect (29), compared with the rigid and localized bends produced by IHF and other DNA-bending proteins (23). To address this issue in the case of *Ps*, we monitored the activity of the *Ps-lacZ* construct mentioned above in various IHF-minus and HU-minus genetic backgrounds. The results shown in Fig. 4 indicate that HU plays a role in *Ps* activity, since the *Ps-lacZ* fusion used in the experiment becomes virtually irresponsive to inducer *m*-xylene in HU^- and IHF^- HU^- strains. As a control, we used a *Pu-lacZ* fusion, the activity of which became HU dependent also in *E. coli* cells lacking IHF. In fact, *Ps* appeared to resemble *Pu* in the absence of IHF (Fig. 4) in its having an intrinsic curvature at the intervening region (8, 28) and in its dependence on HU for XylR- and *m*-xylene-induced activity (Fig. 4).

Hybrid promoters endowed with intrinsically curved intervening sequences become HU independent. The genetic results with *Pu* (in the absence of IHF) and *Ps* discussed above are consistent with the notion that HU assists formation of the local DNA conformation that is required for transcription initiation within the region (16). However, they do not prove a direct effect of HU on the intervening sequences. Since HU mutations are known to be pleiotropic, the data presented thus far could be, alternatively, the results of indirect effects and not of a genuine structural aid equivalent to that of IHF in other

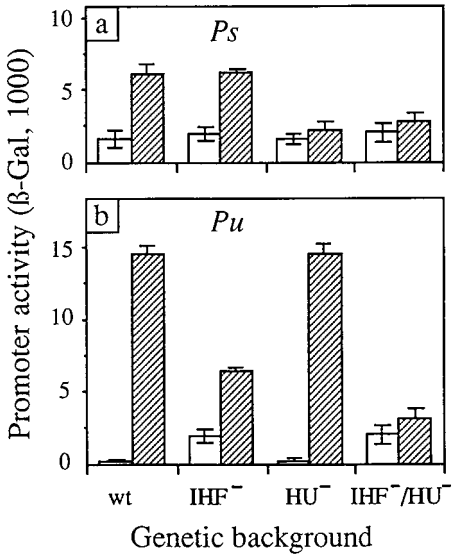

FIG. 4. Effect of IHF and HU mutations on *Ps* and *Pu* promoter activities. *E. coli* N99 (wt) and its IHF-minus (*him hip*) and/or HU-minus (*hupA hupB*) isogenic derivatives were cotransformed with the *xylR*$^+$ plasmid pAX13 and either pRS*Ps* or (as a control) pRS*Pu*, carrying *lacZ* fusions to each of the two promoters. Cotransformants were grown in Luria-Bertani medium and induced with *m*-xylene vapors as explained in Materials and Methods. The bars represent, in each case, the Miller units of β-galactosidase (β-Gal) accumulated at 4 h after induction. ☐, uninduced; ▨, plus *m*-xylene.

σ^{54}-type promoters. To test this alternative explanation, we reasoned that if the role of HU in the two XylR-dependent promoters is to assist directly the bending of the intervening sequence, then functional substitutions of such a DNA segment by a sharply curved nucleotide sequence should result in an HU-independent phenotype.

On these bases, the role of HU was ascertained by examining the phenotypes endowed by hybrid promoters *Pu-AT4*, *Pu-AT5*, and *Pu-ST* in the presence or absence of HU. These are artificial XylR-dependent and σ^{54}-dependent promoters which contain upstream binding sites for XylR (UAS), the σ^{54}-RNAP binding site at −12/−24 of *Pu*, and an intervening sequence of identical size and base composition but with very different structural features (Fig. 5). *Pu-AT4* and *Pu-AT5* include a sharply bent DNA structure resulting from phasing six times of a motif of six adenine nucleotides, that results in an intrinsic curvature of about 120° (28) (Fig. 5). This arrangement forces the promoter region into a curved conformation in which the looping out of the upstream region is maintained by the DNA structure itself. In contrast, *Pu-ST* contains a heterologous noncurved nucleotide sequence at the intervening region, thus maintaining the promoter region in a nonbent conformation. The activities of *Pu-AT4*, *Pu-AT5*, and *Pu-ST* fusions to *lacZ* were measured in IHF$^-$, HU$^-$, and HU$^-$ IHF$^-$ genetic backgrounds. The results shown in Fig. 5 clearly indicate that, unlike *Ps* and *Pu*, the hybrid promoters *Pu-AT4* and *Pu-AT5* maintain their full activity regardless of the presence or absence of IHF, HU, or both. This ensured that for XylR-dependent promoters HU assists the bending of intervening sequence, because the presence of a static curvature at the correct position within the promoter region totally eliminated the requirement for HU. In contrast, the hybrid promoter *Pu-ST*, which has a noncurved intervening DNA segment (but the same spatial arrangement of the core promoter elements [28]), showed only a residual

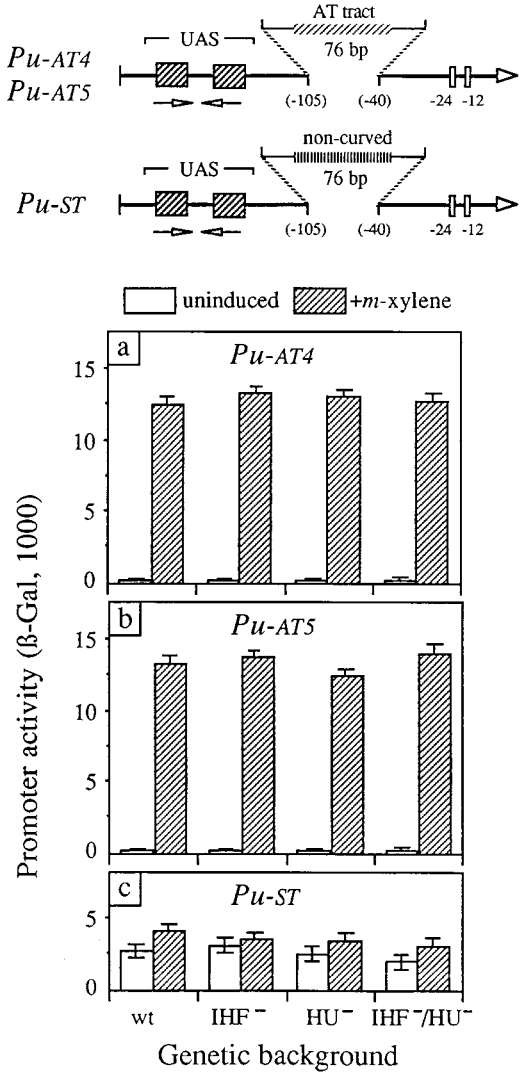

FIG. 5. Hybrid XylR-dependent promoters containing static DNA curvatures are not affected by HU. The organizations of the hybrid promoters used in this experiment are shown at the top of the figure. *Pu-AT4* (28) is an artificial promoter which contains sequences for binding of XylR (UAS) and σ^{54}-RNAP separated by an intervening DNA segment that includes a tract of 76 bp endowing an intrinsically curved sequence which substitutes for the structural effect of IHF. *Pu-AT5* is formally similar to *Pu-AT4*, but the functional substitution of the IHF binding site by a bent DNA segment is made through an alternative distribution of the promoter core elements (UAS and −12/−24 sequences). The *Pu*-ST promoter is equivalent to *Pu-AT4* in its base composition and in the distances between the promoter elements, but the 76-bp AT tract has been replaced by a noncurved DNA sequence from pBR322 (28). For the experiments whose results are shown, *E. coli* N99 (wt) and its IHF-minus (*him hip*) and/or HU-minus (*hupA hupB*) isogenic strains were cotransformed with the *xylR*$^+$ plasmid pAX13 and the pRS551 derivative carrying the promoter indicated in each case, fused transcriptionally to *lacZ*. Cotransformants were grown in Luria-Bertani medium and induced with *m*-xylene vapors as explained in Materials and Methods. The bars represent the Miller units of β-galactosidase (β-Gal) accumulated after 4 h of induction. Note that neither IHF nor HU had any significant influence on promoter activity.

responsiveness to XylR activation, regardless of the genetic background used. This last observation suggests that besides the assistance of additional proteins, looping out of the intervening segment in this type of promoters requires that the nucleotide sequence possess a certain predisposition to bend in a particular orientation.

DISCUSSION

Although *Pu* and *Ps* are both σ^{54} promoters responsive to the same regulator (XylR), the former requires IHF for full activity (1, 8), while the latter does not. In this work, we show that HU assists activation of the IHF-independent *Ps* promoter in vivo. IHF and HU are known to be exchangeable in a number of cases, including transposition (23), DNA replication (5), site-specific recombination (12), and phage maturation (20). In addition, as shown in this work, HU seems to bypass in part the requirement for IHF in vivo of the *Pu* promoter. While both proteins have similar tridimensional structures, share homologous regions (35), and are able to deform the DNA helix upon binding (13, 37), the basis for their functional interchangeability is still unclear. IHF binds and bends DNA at specific sequences (37), whereas HU does not seem to have preferred attachment sites. Hence, the structural changes of the DNA helix caused by HU binding do not have a specific directionality (29, 33). This is important, since promoter geometry (i.e., the right tridimensional assembly of all functional elements) is essential for transcription initiation at σ^{54} promoters (7). In this context, HU is likely to produce a "structural trapping" effect like that proposed for the integration of λ phage (29). This effect would involve binding to *Ps* DNA and bending of this DNA into a shape that brings the UAS and the σ^{54}-RNAP together, thus promoting stable contacts that fix the correct architecture of the whole promoter region.

Since DNA is a generally flexible polymer, it could be argued that protein-assisted or sequence-directed DNA bending should not be strictly necessary if sufficient DNA-protein (XylR and RNAP) and protein-protein (RNAP-XylR) interaction energies are available. This seems clearly not to be the case in the limited number of examples in which this issue has been addressed with σ^{54}-dependent systems. For both the *Pu* (28) and the *PnifH* (22) promoters, the requirement of a bent DNA structure at the intervening region between the core promoter elements has been clearly substantiated. At least for *Pu*, the substitution of a curved intervening region by an equivalent nonbent DNA sequence resulted in a XylR-irresponsive promoter (28). This suggested that potential XylR-RNAP-DNA interactions are not strong enough to occur in vivo unless the intervening DNA segment is either bent or bendable by additional proteins. This notion is fully consistent with the structural role of HU with the σ^{54}-dependent promoters reported in this article. In view of the low activity of the *Pu-ST* promoter containing an equivalent but nonbent DNA segment (Fig. 5), it is likely that the effect of HU becomes significant only when the DNA sequence is already curved or prone to bend in a particular direction, so that the histone-like protein may become instrumental in exacerbating a preexisting curvature. The results shown above indicate that the intrinsic ca. 60° curvature between promoter core elements in *Ps* (Fig. 3) appears to be insufficient to facilitate by itself the contacts between XylR bound to the UAS and the σ^{54}-RNAP. It is therefore plausible that, in vivo, besides the effect of the static bend present in *Ps*, HU binding further increases the general flexibility of the intervening region and facilitates bending in the direction predetermined by the DNA sequence.

An intriguing sidelight of the experiments whose results are shown in Fig. 2 and 4 is that *Ps* seems to have a substantial level of activity even in the absence of XylR, HU, or IHF. However, the relatively high basal activity observed for both monocopy and multicopy gene dosage appears to depend strongly on σ^{54} (Fig. 2). This suggests that other proteins of the NtrC family (25) could act on the promoter as illegitimate activators. Whether this effect may have a physiological meaning is currently under investigation.

In summary, the results presented in this paper support the view that the structure and properties of the intervening regions between the UAS and the −12/−24 promoter elements in σ^{54}-dependent systems not only provide a somewhat passive structural aid for the assembly of the transcription initiation machinery, but that they can also become targets of additional coregulation levels mediated by protein-induced changes in DNA structure which modulate and even determine the final outcome of promoter activity.

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