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Involvement of IHF protein in expression of the Ps promoter of the Pseudomonas putida TOL plasmid
Involvement of IHF Protein in Expression of the Ps Promoter of the Pseudomonas putida TOL Plasmid

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Regulation of the xyl gene operons of the Pseudomonas putida TOL plasmid is mediated by the products of the downstream clustered and divergently oriented xylR and xylS regulatory genes. The xylR-xylS intergenic region contains the xylR and xylS promoters Pr and Ps, respectively. A binding site for the XylR activator protein is located upstream of Ps and overlapping Pr. DNase I footprint experiments showed that one of these sites, which overlaps the recognition site for XylR activator, as well as an AT-rich region comprising the Ps promoter consensus were protected by integration host factor (IHF). IHF was found to act negatively in the in vivo activation of the Ps promoter, since the activity of a Ps promoter::lacZ fusion was elevated in an Escherichia coli mutant lacking IHF. In contrast, no alteration in the synthesis of XylR protein in the E. coli IHF-deficient mutant was detected.

The degradation of toluene and related aromatic compounds by Pseudomonas putida pWW0 is determined by four xyl gene operons on TOL plasmid pWW0 (3, 19). Two catabolic operons (upper and meta pathway operons, preceded by their cognate promoters Pu and Pm, respectively) contain the pathway structural genes, and two others contain the regulatory genes xylR and xylS (Fig. 1), which are adjacent but divergently transcribed from their respective promoters Pr and Ps. Transcription from the Pu and Ps promoters depends on the RNA polymerase sigma factor σ54 as well as XylR activator protein, which binds to recognition sequences (upstream activation sequences [UASs]) present in the Pu and Ps promoter upstream regions (1, 5, 9, 12, 13, 16). The current model for the activation of σ54-dependent promoters suggests loop formation between upstream-bound activator and the promoter-bound RNA polymerase-σ54 complex to bring about transcriptional activation (21, 23). In the case of Pu, the DNA-bending protein integration host factor (IHF) (for a review, see reference 7) binds to a specific recognition site between the upstream XylR-target UAS and the promoter to provide the required loop. The promoter is not activated in Escherichia coli mutants lacking IHF (1, 2, 5). Like Pu, the xylS gene promoter Ps requires σ54 and XylR activator protein, in concert with a specific effector, for activation. Ps forms part of a complex xylR-xylS intergenic region, with the XylR activator-binding site of Ps overlapping the xylR tandem Pr promoters (Fig. 2A and 4). Two potential IHF-binding sites have been identified, one with consensus sequences on both strands that overlaps the XylR-UAS/Pr promoter region, and another located close to the Ps promoter (13). Fragments containing these sites exhibited IHF binding in gel retardation assays, but IHF is not essential for activation of Ps in an E. coli background. Here we present data showing that IHF binds to recognition sites in the Ps promoter in vitro and that IHF negatively affects Ps activity.

IHF binds to two target sites in the xylR-xylS intergenic region. To identify IHF-binding sites, the region between the StuI and the SphI sites (Fig. 2A and 4) containing the Pr promoter was isolated as a 150-bp EcoRI-SphI fragment from plasmid pAH96 (13). End labeling was performed with [α-32P]ATP and avian myeloblastosis virus reverse transcriptase. To test for binding of IHF in the Ps promoter region, a 370-bp fragment containing the region from the BglII site in the xylS gene (Fig. 2A) to the SphI site at −87 bp of Ps (Fig. 2A and 4) was synthesized by PCR with [γ-32P]ATP-end-labeled M13 reverse primer, unlabeled universal primer, and, as the template, plasmid pAH94 (13), previously deleted between its

FIG. 1. Regulation of xyl gene operons. Operons are presented as boxes; shaded areas indicate promoter regions. Dashed arrows indicate induction by the XylR or XylS regulatory protein in concert with the respective aromatic effectors.

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SphI sites. DNase I footprint experiments were carried out as described by Mengeritsky et al. (20) with 0.5 ng of the labeled fragment and *E. coli* IHF protein at not less than 0.3 µM.

Figure 3A shows protection from DNase I digestion of a region between -137 and -156 bp at all IHF concentrations tested (0.3 to 1.5 µM). IHF may interact with a larger DNA region; however, this could not be determined since no corresponding digestion products beyond -156 bp are present in the control lane without IHF. Typically, the adjacent regions appear hypersensitive to DNase I digestion. The protected region corresponds to the previously identified IHF site, with good consensus sequences on both strands (13), which overlap at the same time the XylR activator recognition sequence and the Pr promoter consensus sequences (Fig. 4). In the Ps promoter-proximal region, IHF did not bind to the previously predicted imperfectly conserved consensus site between -35 and -47 bp (13), but rather to an adjacent region between -2 and -30 bp (Fig. 3B). This region does not exhibit a perfect IHF consensus site (10) but is typically AT-rich (8, 11) (Fig. 4). Specific protection of this sequence was observed with IHF concentrations of up to 1 µM, while at higher IHF concentrations, protection became unspecific. In summary, we have identified two IHF-binding sites. The locations of both of these sites imply a negative role for IHF in the control of the Ps promoter.

IHF reduces the activity of the Ps promoter but does not affect the Pr promoter. To assess the role of IHF in the transcriptional activity of the Ps and Pr promoters, isogenic *E. coli* strains producing or lacking IHF protein were transformed with a set of xyl promoter-*lacZ* fusion plasmids based on the low-copy vector pJEL122 Ap' (25). Plasmids pAH120 and pAH100 carry the xylR-xylS intergenic region on a 0.6-kb BglII fragment (Fig. 2A and B) in both possible orientations, providing the respective Pr:*lacZ* and Ps:*lacZ* promoter-reporter gene fusions (13). Construct pUJ100, like pAH100, carries the Ps promoter:*lacZ* fusion and, in addition, the entire xylR activator gene in its native configuration in cis (14) (Fig. 2B). High-copy-number plasmid pTS174 (15) bearing the xylR activator gene was used for activation of the Ps:*lacZ* fusion (on pAH100) in trans. The *E. coli* strains used were N99 (supO) (6) and K1299 (N99 Tn10-himAA82, Tc'; D. Friedman). Since activation of the Ps promoter was recently found to be inhib-

FIG. 2. xylR-xylS intergenic region. (A) The Ps and Pr promoters are indicated by a solid (Ps) and two open (Pr tandem promoters) boxes. The three shaded boxes represent the previously identified IHF consensus recognition sites. The inverted half arrows show the location of the invertedly repeated recognition motif of the XylR target UAS upstream of the Ps promoter. (B) Plasmid constructs carrying the Ps or Pr promoter:*lacZ* fusions described in the text. Solid arrowheads mark the promoter, and open arrows show the transcribed genes. Abbreviations: Ba, BamHI; Bg, BglII; C, CiaI; E, EcoRI; H, HpaII; S, SmaI; Sp, SphI; St, StuI.

FIG. 3. Footprints of IHF on the xylR-xylS intergenic region containing the Pr and Ps promoters. Samples of end-labeled fragments were mixed with different amounts of IHF protein prior to DNase I digestion. The regions showing specific protection by IHF are indicated by vertical bars; all numberings refer to the transcriptional start from the Ps promoter. (A) Coding strand from the Pr promoter region. Lane 1, no IHF added; lane 2, 0.3 µM IHF; lane 3, 0.6 µM IHF; lane 4, 0.9 µM IHF; lane 5, 1.5 µM IHF. (B) Coding strand from the Ps promoter region. Lane 1, no IHF added; lane 2, 0.5 µM IHF; lane 3, 1 µM IHF; lane 4, 2.5 µM IHF; lane 5, 5 µM IHF.
FIG. 4. Localization of protein-binding sites in the xylR-xylS intergenic region. Conserved nucleotides of the Pr tandem promoters (PPr, -35 and -10 bp consensus of σ70-dependent standard promoters) and of the Ps promoter (−24 and −12 bp consensus of σ54-dependent promoters) are marked by brackets below the sequence. The inverted repeat of the XyIR binding site overlapping the Pr promoter is indicated by inverted arrows above the sequence. Potential consensus motifs of IHF-binding sites in the sequence are boxed. Shaded bars denote regions found to be protected by IHF in the DNase I footprint experiments. The SplI (Sp) and Smal (St) restriction sites are represented as shaded areas in the sequence. The numbering refers to the transcriptional start from the Ps promoter.

TABLE 1. Activation of Ps and Pr promoters in isogenic E. coli wild-type and IHF-deficient mutant strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>IHF</th>
<th>Plasmids</th>
<th>β-Galactosidase activity (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Protein factor</td>
<td>Promoter fusion</td>
</tr>
<tr>
<td>N99</td>
<td>+</td>
<td>None</td>
<td>pAH100 (Ps::lacZ)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
<td>pAH100 (Ps::lacZ)</td>
</tr>
<tr>
<td>N99</td>
<td>+</td>
<td>pTS174 (xylR)</td>
<td>pAH100 (Ps::lacZ)</td>
</tr>
<tr>
<td>K1299</td>
<td>−</td>
<td>pTS174 (xylR)</td>
<td>pAH100 (Ps::lacZ)</td>
</tr>
<tr>
<td>N99</td>
<td>+</td>
<td>None</td>
<td>pUJ100 (xylR-Ps::lacZ)</td>
</tr>
<tr>
<td>K1299</td>
<td>−</td>
<td>None</td>
<td>pUJ100 (xylR-Ps::lacZ)</td>
</tr>
<tr>
<td>N99</td>
<td>+</td>
<td>None</td>
<td>TnUT85 (xylR-Ps::lacZ)</td>
</tr>
<tr>
<td>I299</td>
<td>−</td>
<td>None</td>
<td>TnUT85 (xylR-Ps::lacZ)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−</td>
<td>pHX3-8 (HIF+)</td>
</tr>
<tr>
<td>N99</td>
<td>+</td>
<td>None</td>
<td>pAH120 (Pr::lacZ)</td>
</tr>
<tr>
<td>K1299</td>
<td>−</td>
<td>None</td>
<td>pAH120 (Pr::lacZ)</td>
</tr>
</tbody>
</table>

chromosomally integrated on a minitransposon (TnUT85; Table 1, lines 7 and 8). The activity of the Ps promoter was decreased to the level of activity seen in the wild type when plasmid pHX3-8 (17) carrying the IHF-encoding structural genes (himA4 and hip) was introduced into the IHF-deficient strain K1299 (line 9).

In a previous study (13), we assayed Ps promoter activation with plasmids pAH100 and pTS174 in cells grown in LB medium. More recently, LB was found to inhibit expression from the Ps but not from the Pr promoter (18), and in such experiments, we could never obtain as strong an activation of the Ps promoter as seen here with low-LB-content medium; the increase in Ps activity in the absence of IHF had thus previously been concealed.

Since IHF may affect XyIR-mediated activation of the Ps promoter indirectly by altering the rate of synthesis of XyIR activator, we examined expression of the xylR activator gene.

The results show that IHF has no effect on transcription from the xylR tandem Pr promoters (Table 1, lines 10 and 11).

In conclusion, IHF acts differently in the regulation of two xyl gene operons of the P. putida TOL plasmid. While in the Pu promoter IHF was previously shown to bend the DNA so as to allow activation of Pu by the XyIR activator, we have demonstrated here that in the Pr promoter IHF negatively affects promoter activity. The importance of the modulation of Ps activity by IHF remains to be investigated.

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REFERENCES