Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 5182–5186, August 1987
Biochemistry

Expression of the regulatory gene *xylS* on the TOL plasmid is positively controlled by the *xylR* gene product

(*Pseudomonas putida*/reverse transcriptase mapping/S1 nuclease mapping/transcriptional regulation/cascade response)

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Communicated by I. C. Gunsalus, April 13, 1987

ABSTRACT **The regulatory gene *xylS* on the TOL plasmid of *Pseudomonas putida* activates the transcription of the *xylDLEGF* operon for the *m*-toluate-degrading pathway in the presence of *m*-toluate. The gene also activates the transcription of the same operon in the presence of *m*-xylene or *m*-methylbenzyl alcohol, but for this activation another regulatory gene, *xylR*, is required. In this study we examined the *xylS* expression by determining the mRNA by reverse transcriptase mapping and by monitoring the enzyme activity of the *xylE* gene product, which was expressed under the control of the *xylS* promoter. The results of the above experiments provide evidence that *xylR* positively controls the transcription of *xylS* in the presence of *m*-xylene or *m*-methylbenzyl alcohol. The *xylS* product thus amplified may in turn activate the *xylDLEGF* operon. The nucleotide sequence of the *xylS* promoter resembles that of the promoter of the *xylCAB* operon for the *m*-xylene-degrading pathway, which is also activated by *xylR* in the presence of *m*-xylene or *m*-methylbenzyl alcohol. In addition, we have demonstrated that the expression of *xylR* is negatively controlled by its own product. On the basis of these findings, we propose a revised model for the regulation of expression of *xyl* genes on the TOL plasmid.**

Bacteria of the genus *Pseudomonas* have the capacity to utilize a variety of aromatic compounds as a sole source of carbon and energy. Some of this capacity depends on enzymes encoded by plasmids (1). The TOL plasmid from *Pseudomonas putida* mt-2 encodes enzymes for the degradative pathway of toluene and xylenes (2). The genes for the enzymes are organized in two operons; one is the *xylCAB* operon, which is responsible for the "upper" catabolic pathway from toluene and xylenes to aromatic carboxylic acids, and the other is the *xylDLEGF* operon, which is required for the "lower" catabolic pathway from the aromatic carboxylic acids to compounds that enter the tricarboxylic acid cycle. Two regulatory genes, *xylR* and *xylS*, positively control these operons. In 1978, Worsey *et al.* proposed a regulation model based on genetic analysis (3); the *xylR* gene activates both the first and second operons in the presence of *m*-xylene (*m*Xyl) or *m*-methylbenzyl alcohol (*m*MBA), whereas the *xylS* gene activates the second operon in the presence of *m*-toluate (*m*Tol). By molecular cloning of *xyl* genes in *Escherichia coli*, we presented evidence that the two regulatory genes are in fact present on the TOL plasmid, but with respect to the function of regulatory genes, some modification was necessary; i.e., both the *xylR* gene and the *xylS* gene are required for the activation of the second operon by *m*Xyl or *m*MBA (4, 5). Essentially the same results were obtained with *P. putida* (6–8). On the basis of the above results, we previously proposed a provisional scheme that the *xylS* and *xylR* products work together to activate the *xylDLEGF* operon (5).

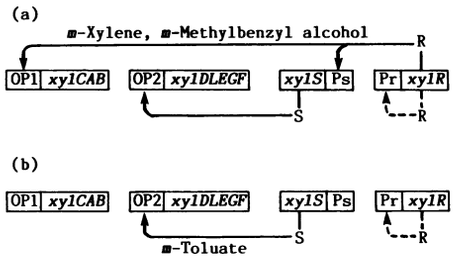

FIG. 1. Model for the regulation of *xyl* genes on the TOL plasmid. *xylCAB* and *xylDLEGF* are structural gene clusters for enzymes. The enzymes encoded by *xylCAB* catalyze the oxidation of *m*Xyl to *m*Tol, and those encoded by *xylDLEGF* catalyze the oxidation of *m*Tol to pyruvate and propionaldehyde. OP1 and OP2 refer to the operator–promoter regions of the *xylCAB* operon and the *xylDLEGF* operon, respectively. *xylS* and *xylR* are regulatory genes, and R and S represent their products. Ps and Pr refer to the promoter regions of *xylS* and *xylR*, respectively. Arrows with solid and broken lines indicate positive and negative control, respectively. (*a*) In the presence of *m*Xyl or *m*MBA, R activates both *xylCAB* and *xylS*. The induced S activates the *xylDLEGF* operon in the absence of inducers. (*b*) In the presence of *m*Tol, S at a noninduced level activates the *xylDLEGF* expression. R represses its own expression.

In the subsequent analysis by S1 nuclease and reverse transcriptase mapping we determined the transcription start sites of the *xylCAB* and *xylDLEGF* operons, and demonstrated that the regulation of the two operons occurs at the transcriptional level (9, 10). Concerning the regulatory genes, we identified the *xylR* and *xylS* products by the maxicell method (11, 12). Furthermore, the transcription start site of the *xylR* gene was determined (11). However, because of a low transcriptional activity of the *xylS* gene, we were unable to analyze the transcription at a molecular level.

In this study, by reverse transcriptase mapping using a synthetic oligonucleotide primer we determined the transcription start site of *xylS* and demonstrated that the expression of *xylS* is positively controlled by *xylR* in the presence of *m*Xyl or *m*MBA. Furthermore, we have recently obtained evidence that the amplified *xylS* product can activate the second operon (unpublished results). These results could account for the previous observations that both *xylR* and *xylS* are required for the *m*Xyl- or *m*MBA-dependent activation of the second operon. In addition, we showed that the expression of *xylR* is autogenously repressed. Thus regulatory genes themselves are subject to fine regulation under certain

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Abbreviations: kb, kilobase pair(s); bp, base pair(s); *m*Xyl, *m*-xylene; *m*MBA, *m*-methylbenzyl alcohol; *m*Tol, *m*-toluate.

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circumstances in the TOL system. A revised model for the regulation of *xyl* gene expression is proposed (Fig. 1).

MATERIALS AND METHODS

Materials. Restriction endonucleases were purchased from Bethesda Research Laboratories, New England Biolabs, or Takara Shuzo. T4 DNA polymerase, T4 polynucleotide kinase, T4 DNA ligase, and S1 nuclease were obtained from Takara Shuzo. Reverse transcriptase was purchased from Life Sciences (St. Petersburg, FL) or Takara Shuzo. [γ-^{32}P]ATP (5000 Ci/mmol; 1 Ci = 37 GBq) was obtained from the Radiochemical Centre. The 22-mer oligodeoxynucleotide 5′-GATCTGACTTTTCTCGTTCAAT-3′ (Fig. 2) was synthesized by an automated DNA synthesizer (Applied Biosystems model 381A) and purified by HPLC.

Bacterial Strains and Plasmids. Strains used were *E. coli* 20SO, *P. putida* TN2100, and *P. putida* TN1126 (11). Plasmid pTN2 was a recombinant of TOL and RP4, which confers inducible synthesis of TOL enzymes in a manner similar to the wild-type TOL plasmid (15, 16). Plasmids pTS41 (4), pTS145, pTS146, pTS174 (8), and pTS1133 (11) were described previously.

Preparation and Analysis of Nucleic Acids. Plasmid DNA preparation, gel electrophoresis, restriction endonuclease mapping, 5′-end-labeling, ligation, and transformation were carried out as described (17) unless otherwise indicated. Conditions for preparation of crude RNA were described (10).

Reverse Transcriptase Mapping. The method described previously (9) was modified as follows. Crude RNA (2–20 μg) was mixed with the 5′-end-labeled 22-mer oligodeoxynucleotide (0.2–0.5 pmol), and nucleic acids were precipitated with ethanol. The resulting pellet was dissolved in 20 μl of primer extension buffer (50 mM Tris·HCl, pH 8.0/50 mM KCl/8 mM $MgCl_2$/20 mM dithiothreitol), and the mixture was incubated successively for 2 min at 95°C and 1 hr at 60°C and then cooled on ice. After addition of 5 μl of 8 mM dNTPs (2 mM dATP/2 mM dCTP/2 mM dGTP/2 mM dTTP), the reaction mixture was incubated with 20 units of reverse transcriptase for 1 hr at 37°C. The reaction was terminated by the addition of 2 μl of 250 mM EDTA followed by extraction with phenol. The nucleic acids were precipitated with ethanol and dissolved in 20 μl of a solution containing 80% (vol/vol) formamide, 10 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol. Electrophoresis was carried out in an 8% polyacrylamide/8.3 M urea gel in TBE buffer (80 mM Tris·HCl, pH 8.3/90 mM sodium borate/2.5 mM EDTA).

S1 Nuclease Mapping. S1 nuclease protection analysis was performed as reported (10) except for the hybridization conditions, which were the same as for the reverse transcriptase mapping described above.

FIG. 2. Nucleotide sequence of the region containing the *xylS* and *xylR* promoters (11–13). Nucleotides are numbered from the transcription start site of *xylS* determined in this study, which is represented as 1. Arrows with a closed circle indicate the orientation and transcription start sites of *xylR* (mRNA-1, mRNA-2) (11) and *xylS* (mRNA). The underlined nucleotides are complementary to the 3′ end of the 16S rRNA of *Pseudomonas aeruginosa* (14). The initiation codons (ATG) are both overscored and underlined. Sequences that are homologous to the consensus sequence of *E. coli* promoters (−35 and −10 regions) and of *ntr*/*nif* promoters (−24 and −12 region) are boxed. Restriction sites are shown with brackets above the sequence. The primer sequence used for reverse transcriptase mapping is doubly underlined.

RESULTS

Reverse Transcriptase Mapping Analysis of the *xylS* Transcript in *P. putida* and *E. coli*. We previously cloned the *xylS* gene and determined its nucleotide sequence (4, 12). In Fig. 2 is shown the nucleotide sequence of the 0.6-kilobase pair (kb) *Bgl* II fragment that contains the NH_2-terminal coding sequence of *xylS* and the upstream region. To detect the transcript of *xylS*, we used a synthetic oligonucleotide primer for reverse transcriptase mapping. The 22-mer homologous with the coding sequence from position 156 to 177 (Fig. 2) was labeled at the 5′ end and hybridized with RNAs extracted from *P. putida* or *E. coli* cells harboring pTN2. After primer extension by reverse transcriptase, products were analyzed by polyacrylamide gel electrophoresis.

A band corresponding to about 170 nucleotides was seen with RNAs from either *P. putida* or *E. coli* cells grown in the presence of *m*Xyl or *m*MBA (Fig. 3, lanes 3, 4, 7, and 8). After prolonged exposure of autoradiography, the 170-nucleotide

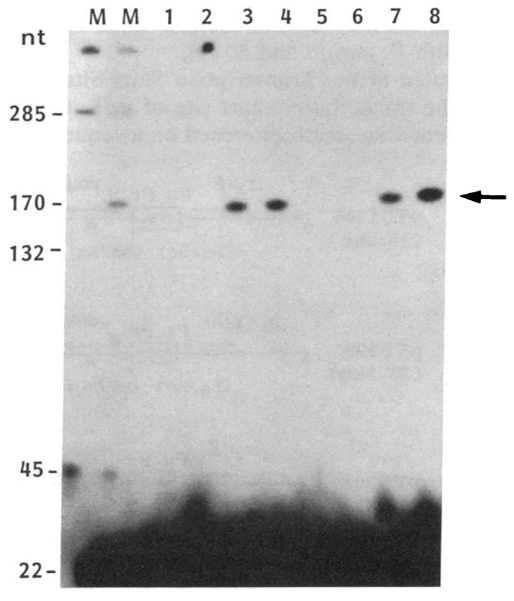

FIG. 3. Reverse transcriptase mapping of the *xylS* transcript in *P. putida* and *E. coli*. The 5′-end-labeled primer (0.3 pmol) was hybridized with 10 μg of RNA. After extension of primer, products were analyzed by electrophoresis in an 8% polyacrylamide/8.3 M urea gel. Lanes M are single-stranded marker DNA fragments; an arrow indicates the extended fragment; nt, nucleotides. RNA was extracted from the following strains grown in the absence (lanes 1 and 5) or presence of *m*Tol (lanes 2 and 6), *m*MBA (lanes 3 and 7), or *m*Xyl (lanes 4 and 8). Lanes 1–4, *E. coli* 20SO carrying pTN2; lanes 5–8, *P. putida* TN2100 carrying pTN2. The extended DNA fragments were located by autoradiography and cut out from the gel, and their radioactivities were determined in a liquid scintillation counter.

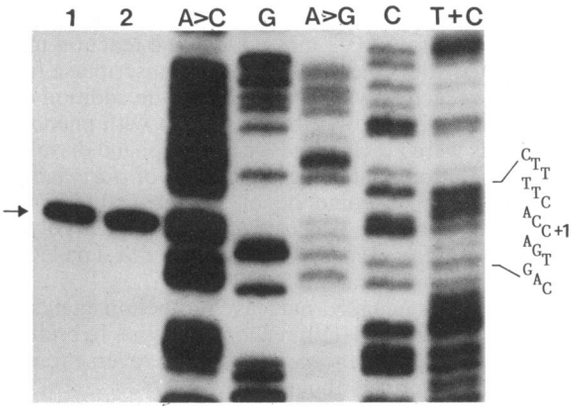

FIG. 4. Determination of the transcription start site of *xylS* by reverse transcriptase mapping. The 5'-end-labeled primer (0.5 pmol) was hybridized with 20 μg of RNA prepared from *E. coli* 20SO carrying pTN2 (lane 1) or *P. putida* TN2100 carrying pTN2 (lane 2) grown in the presence of *m*MBA. The products were subjected to electrophoresis on an 8% sequencing gel next to the chemically cleaved sequencing products (lanes A>C to T+C) (18). DNA used for sequencing was the 395-bp *Stu* I–*Bgl* II DNA fragment labeled at the *Bgl* II site. A part of the sequence deduced from the "ladders" is shown on the right. An arrow indicates the extended fragment, which corresponds to cytosine at position 1 (Fig. 2). The amount of sample applied in lane 2 was half of that in lane 1.

band was also detected with RNAs from cells grown in the absence of inducers or presence of *m*Tol (data not shown). The amount of transcripts as determined by radioactivity associated with the 170-nucleotide band increased 5- to 10-fold with *m*Xyl or *m*MBA, and both induced and noninduced levels of *xylS* mRNA were essentially the same in *P. putida* and *E. coli*. *m*Tol had no effect on the transcription. These results indicate that the synthesis of *xylS* mRNA is dependent on the inducers for the activation of the *xylCAB* operon in both *P. putida* and *E. coli*.

Determination of the Transcription Start Site of *xylS*. To determine the transcription start site of *xylS*, the extended DNA fragment was electrophoresed on a sequence gel next to the chemically cleaved products of the 395-base pair (bp) *Stu* I–*Bgl* II fragment labeled at the *Bgl* II site (Fig. 4). The 5' end of the *Bgl* II site of the fragment was identical with the 5' end of the primer DNA (Fig. 2). When RNAs from *m*MBA-induced cells of either *P. putida* or *E. coli* harboring pTN2 were used, a single band was observed, which corresponded to cytosine at position 1 in Fig. 2.

Regulation of the *xylS* Expression. From the results presented in Fig. 3, a possibility arose that the *xylR* gene, which activates the *xylCAB* operon in the presence of *m*Xyl or *m*MBA, is also required for *xylS* transcription. To test this possibility, a set of recombinant plasmids was constructed as shown in Fig. 5. The plasmid pTS1144 contained the 0.6-kb *Bgl* II fragment (Fig. 2) on a broad host range vector pTS1133 (11). In pTS1144, the *xylS* promoter was fused to the *xylE* gene, which encodes catechol 2,3-dioxygenase (19). Plasmids derived from pTS1144 were pTS296 (containing both the *xylS* and *xylR* genes), pTS301 (containing the *xylR* gene), and pTS303 (containing the *xylS* gene).

Specific activities of catechol 2,3-dioxygenase directed by the plasmids in *P. putida* are shown in Table 1. The *xylE* expression under the control of the *xylS* promoter was activated by *m*Xyl or *m*MBA in the plasmids containing intact *xylR* (pTS296 and pTS301). The *xylS* gene showed neither stimulatory nor inhibitory effect (pTS303 vs. pTS1144). Copy numbers of the plasmids in the cells under these conditions were essentially the same, as determined by staining plasmid DNAs extracted by the rapid boiling method (17) and subjected to gel electrophoresis. These results indicate that the expression of *xylS* is positively controlled by the *xylR* product in the presence of *m*Xyl or *m*MBA and is not autogenously regulated. These results were further confirmed by reverse transcriptase mapping (Fig. 6). RNAs were prepared from cultures that were subjected to the enzyme assay. Transcripts of *xylS* were detected in cells carrying pTS296 or pTS301 grown in the presence of *m*Xyl or *m*MBA. Thus, the activation of *xylS* by *xylR* occurs at the transcriptional level.

Autogenous Repression of *xylR*. In plasmid pTS1144, the *amp* gene was downstream from the promoter of *xylR* (Fig. 5). The β-lactamase expression was enhanced 7-fold in

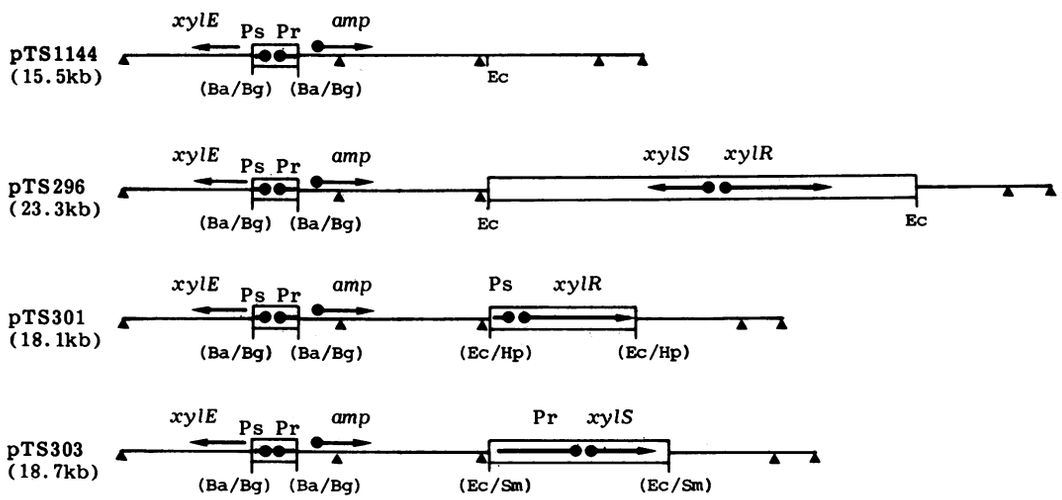

FIG. 5. Structures of constructed plasmids. Thin lines and open boxes represent the vector pTS1133 (11) and the regions cloned in this study, respectively. *xylE* and *amp* refer to the genes for catechol 2,3-dioxygenase and β-lactamase, respectively. Closed circles and arrows indicate the promoters and the direction of transcription, respectively. Closed triangles indicate the *Pst* I site. Other restriction sites are abbreviated as follows: Ba, *Bam*HI; Bg, *Bgl* II; Ec, *Eco*RI; Hp, *Hpa* I; and Sm, *Sma* I. Restriction sites in parentheses correspond to the hybrid sites formed after ligation or repair-ligation. Other symbols are the same as in Fig. 1. Construction of plasmids was as follows: pTS1144, the 0.6-kb *Bgl* II fragment was isolated from pTS145 (8) and ligated with *Bam*HI-cleaved pTS1133 (11); pTS296, the 7.8-kb *Eco*RI fragment containing both *xylS* and *xylR* was isolated from pTS41 (4) and ligated with *Eco*RI-cleaved pTS1144; pTS301, the 2.6-kb *Hpa* I fragment containing intact *xylR* was isolated from pTS174 (8) and ligated with *Eco*RI-cleaved and T4 DNA polymerase-treated pTS1144; pTS303, the 3.2-kb *Sma* I fragment containing intact *xylS* was isolated from pTS146 (8) and ligated with *Eco*RI-cleaved and T4 DNA polymerase-treated pTS1144.

Table 1. Synthesis of catechol 2,3-dioxygenase from the *xylS* promoter and β-lactamase from the *xylR* promoter in *P. putida* TN1126 carrying recombinant plasmids

Plasmid	Inducer	Specific activity, mU*/mg of protein: Catechol 2,3-dioxygenase	Specific activity, mU*/mg of protein: β-Lactamase
pTS1144	None	18	680
	*m*Tol	16	710
	*m*MBA	17	700
	*m*Xyl	23	700
pTS296	None	10	400
	*m*Tol	40	350
	*m*MBA	890	410
	*m*Xyl	1210	380
pTS301	None	25	270
	*m*Tol	43	250
	*m*MBA	320	260
	*m*Xyl	890	200
pTS303	None	30	820
	*m*Tol	42	810
	*m*MBA	21	780
	*m*Xyl	29	800
pTS1133†	None	44	106

Cells were grown in L broth at 27°C for 5 hr in the absence or presence of 5 mM *m*MBA or *m*Tol. *m*Xyl was added in vapor.
*One milliunit corresponds to formation of 1 nmol of the product or degradation of 1 nmol of the substrate per min at 27°C.
†Data are from ref. 11.

pTS1144 as compared with that in pTS1133 that did not have the *xylR* promoter (Table 1). On the other hand, specific activities of β-lactamase directed by pTS296 and pTS301 were lower than those directed by pTS1144 or pTS303. These findings suggest that *xylR* negatively controls its own expression. To test this possibility, S1 nuclease protection analysis of the *xylR* transcript was carried out (Fig. 7). RNA preparations were the same as those used in reverse transcriptase mapping. The sense strand of the 460-bp *Taq* I–*Bgl* II

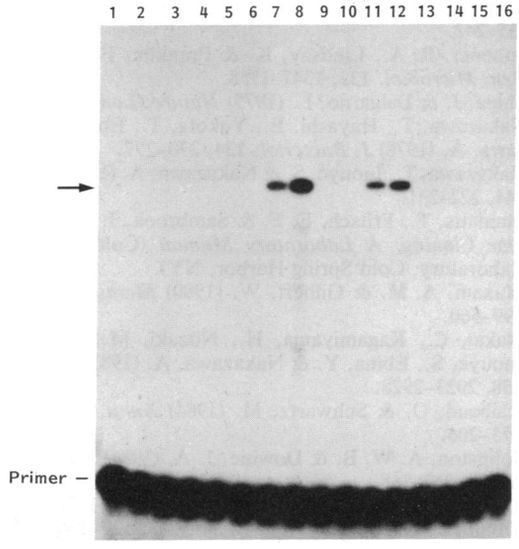

FIG. 6. Reverse transcriptase mapping of the *xylS* transcript in *P. putida* TN1126 carrying recombinant plasmids. The 5'-end-labeled primer (0.2 pmol) was hybridized with 10 μg of RNA. RNA was extracted from *P. putida* TN1126 carrying the following plasmids in the absence (lanes 1, 5, 9, and 13) or presence of *m*Tol (lanes 2, 6, 10, and 14), *m*MBA (lanes 3, 7, 11, and 15), or *m*Xyl (lanes 4, 8, 12, and 16). Lanes 1–4, pTS1144; lanes 5–8, pTS296; lanes 9–12, pTS301; lanes 13–16, pTS303.

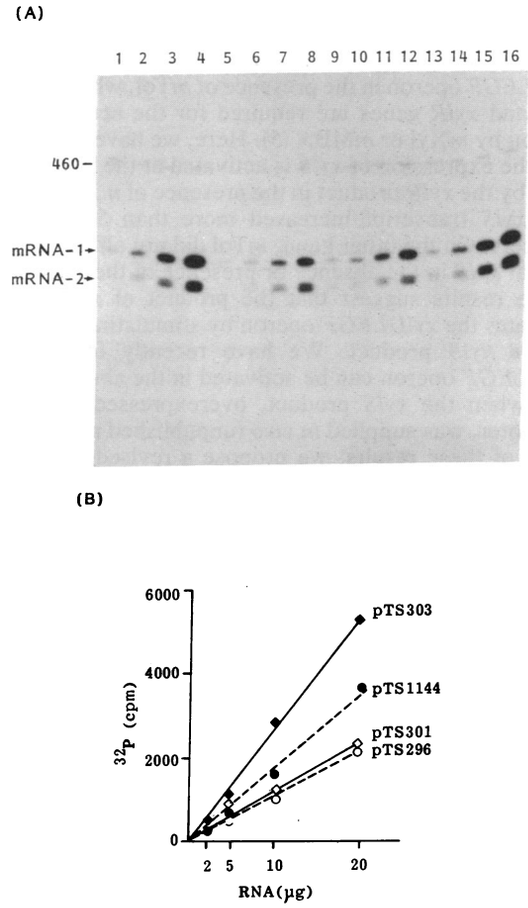

FIG. 7. S1 nuclease protection analysis of the *xylR* transcripts in *P. putida* TN1126 carrying plasmids. The DNA probe was the sense strand of the 460-bp *Taq* I–*Bgl* II fragment (0.1 pmol) labeled at the *Bgl* II site. After hybridization of RNA with the DNA probe, the mixture was treated with 480 units of S1 nuclease for 30 min at 37°C. The products were subjected to electrophoresis on a 5% polyacrylamide/7 M urea gel. (*A*) RNA was extracted from *P. putida* carrying the following plasmids grown in the absence of inducers: lanes 1–4, pTS1144; lanes 5–8, pTS296; lanes 9–12, pTS301; lanes 13–16, pTS303. Amounts of RNA were 2 μg (lanes 1, 5, 9, and 13), 5 μg (lanes 2, 6, 10, and 14), 10 μg (lanes 3, 7, 11, and 15), or 20 μg (lanes 4, 8, 12, and 16). mRNA-1 and mRNA-2 indicate the DNA fragments protected by two species of *xylR* transcripts. A band corresponding to 460 nucleotides represents the probe DNA protected by the contaminated antisense strand. (*B*) After autoradiography, S1-protected bands (mRNA-1, mRNA-2) in each lane were cut out from the gel. Radioactivities were determined in a liquid scintillation counter and plotted against quantities of RNA used in the experiment. ●, pTS1144; ○, pTS296; ◇, pTS301; ◆, pTS303.

fragment (Fig. 2) labeled at the *Bgl* II site was purified and hybridized with various amounts of RNA, and radioactivities of S1-protected DNA bands were determined. In accord with the previous results (11), we observed two species of transcripts, mRNA-1 and mRNA-2, which started at −164 and −192, respectively, with all the preparations (Figs. 2 and 7*A*). As shown in Fig. 7*B*, the amount of *xylR* mRNA directed by plasmids containing intact *xylR* (pTS301 and pTS296) was estimated to be ⅓ of that by the plasmid containing truncated *xylR* (pTS303). The amount of *xylR* mRNA in cells carrying pTS1144 was about ½ of that in cells carrying pTS303, because the latter had two copies of the *xylR* promoter that could produce probe-hybridizable transcripts (Fig. 5). Inducers did not affect the transcription of *xylR* on these plasmids (data not shown). These results suggest that the *xylR* product represses its own transcription, which is consistent with the results of the enzyme assay shown in Table 1.

DISCUSSION

We previously showed that the *xylS* gene can activate the *xylDLEGF* operon in the presence of *m*Tol, whereas both the *xylS* and *xylR* genes are required for the activation of the operon by *m*Xyl or *m*MBA (5). Here, we have demonstrated that the expression of *xylS* is activated at the transcriptional level by the *xylR* product in the presence of *m*Xyl or *m*MBA. The *xylS* transcript increased more than 5-fold with the inducers. On the other hand, *m*Tol did not affect the expression of *xylS* in the absence or presence of the *xylR* product. These results suggest that the product of *xylR* indirectly activates the *xylDLEGF* operon by stimulating the synthesis of the *xylS* product. We have recently found that the *xylDLEGF* operon can be activated in the absence of inducers, when the *xylS* product, overexpressed from the *tac* promoter, was supplied *in vivo* (unpublished results). On the basis of these results, we propose a revised model for the regulation of *xyl* genes. As shown in Fig. 1*a*, when *m*Xyl or *m*MBA is present, the *xylR* product activates both the *xylCAB* operon and the *xylS* gene. Then the *xylS* product at the induced level activates the *xylDLEGF* operon. The activation of *xylDLEGF* by *xylS* is further enhanced by *m*Tol, which is formed by degradation of *m*Xyl or *m*MBA. On the other hand, when *m*Tol is present (Fig. 1*b*), the *xylS* product, even at a noninduced level, interacts with *m*Tol and activates the expression of the *xylDLEGF* operon. Thus, the induction mechanism of the *xylDLEGF* operon involves a cascade response of gene expression. Another point of this model is the autogenous regulation of *xylR*. The expression of *xylR* is repressed by its own product. By this mechanism the cellular concentration of the *xylR* product could be maintained at a relatively constant level, as proposed for other regulatory systems (20).

We determined the transcription start site of *xylS* by reverse transcriptase mapping. The nucleotide sequences of the promoters of *xylS* and *xylCAB*, both of which are activated by *xylR*, have homology to the consensus sequence of nitrogen-regulated (*ntr*) and nitrogen-fixation (*nif*) promoters (21, 22) (Fig. 8). It has been shown that transcription of the *xylCAB* operon in *E. coli* is activated by the heterologous regulatory genes *ntrC* or *nifA* as well as the homologous regulatory gene *xylR* (23). In all cases, the activation is dependent on the *ntrA* gene, whose product is a specific σ factor (σ^{60}) for the *ntr/nif* promoters (24, 25). A mechanism proposed for the transcriptional activation of the *ntr/nif* promoters involves recognition of the −12 and −24 regions by σ^{60}–RNA polymerase and interaction of *ntrC* product either with the promoter sequence or with RNA polymerase to initiate transcription (24, 26). The *xylR* gene might have a role similar to *ntrC* in transcriptional activation of the *xylCAB* operon and possibly in activation of *xylS* in *E. coli* as well. It may be reasonable to assume that a σ^{60}-like factor exists in *P. putida* that recognizes the promoter sequence shown in Fig. 8.

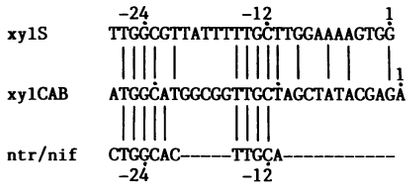

FIG. 8. Sequence homology of the promoter region of *xylS* with that of *xylCAB* and with the *ntr/nif* consensus sequence. The transcription start site is designated as 1. Identical bases are joined by vertical lines.

In contrast to the promoters of *xylCAB* and *xylS*, the promoter of *xylR* shows homology with the consensus sequence for *E. coli* promoters, which probably accounts for a high expression of *xylR* in *E. coli* as in *P. putida* (11). The promoter of *xylDLEGF* may be a representative of another type of the *Pseudomonas* promoter. It has no similarity to either the *E. coli* consensus sequence or the *ntr/nif* consensus sequence. Although little information is so far available on *Pseudomonas* RNA polymerase, it is plausible that bacteria of the genus *Pseudomonas* also have more than one species of RNA polymerase with different promoter specificities.

Note. We found that the *ntrA* gene product is required for the *m*Xyl-dependent *xylS* activation by the *xylR* gene product in *E. coli*.

We thank Hiromi Kumahara for his help in oligonucleotide synthesis. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

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