XylS activator and RNA polymerase binding sites at the Pm promoter overlap

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Abstract Transcription from the TOL plasmid *meta*-cleavage pathway operon, Pm, depends on the XylS protein being activated by a benzoate effector. The XylS binding sites are two imperfect 5′-TGCAN$_6$GGNTA-3′ direct repeats located between positions −70/−56 and −49/−35 [González-Pérez et al. (1999) J. Biol. Chem. 274, 2286–2290]. An intrinsic bending of 40°, which is not essential for transcription, is centered at position −43. We have determined the potential overlap between the XylS and RNA polymerase binding sites. The insertion of 2 or more bp between C and T at positions −37 and −36 abolished transcription activation by the wild-type XylS and by XylSS229I, a mutant with increased affinity for the XylS binding sites. In contrast, a 1-bp insertion at −37 was permissible, although when in addition to the 1-bp insertion at −37 the mutant promoter had a point mutation at the XylS binding site (C−47→T), transcription was abolished with the wild-type XylS protein, but not with XylSS229I. The overlap between the proximal XylS binding site and the −35 region recognized by RNA polymerase at positions −35 and −36 appears to be critical for transcription.

Key words: AraC/XylS family; XylS; Transcription; TOL; Activator binding site; *Pseudomonas*

1. Introduction

The sigma subunit of RNA polymerase confers sequence recognition specificity for the binding of the holoenzyme to promoters during transcription initiation [1]. In σ^{70} and related sigma factors in Gram-negative bacteria, this binding is mainly achieved via sequence-specific contacts with bases in the −10 and −35 regions upstream of the transcription start site on promoters [2–5]. In promoters whose expression depends on a positive regulator, in addition to sigma–DNA interactions during initiation, protein–protein contacts also occur between transcriptional activators and subunits of RNA polymerase (see [6] for a review). In fact, in a number of positively regulated promoters, the recognition sequences of transcriptional activator proteins are adjacent to or partly overlap the −35 region contacted by the sigma subunit of RNA polymerase [7]. In the λ P_{RM} promoter, the binding site of λcI overlaps the −35 region for sigma by two nucleotides, and genetic experiments have suggested an interaction between the λcI protein and the −35 region recognized by σ^{70} [8,9]. In other promoters, i.e. the catabolite repression protein-dependent P1*gal* promoter and the PhoB-dependent P*ptsS* promoter, the activator binding site completely overlaps the −35 hexamer, and the activator probably replaces sigma in the recognition of the −35 region [10].

Several research groups have shown that for certain regulators of the AraC/XylS family [11], i.e. AraC, Rob and RhaS, the binding sites for the regulator overlap the −35 region of RNA polymerase by two to four nucleotides in their cognate promoters [7,12]. The situation is different in other members of this family of transcriptional regulators. For instance, the Ada recognition site seems to be separated by 2 or 5 bp from the RNA polymerase binding site in the *ada* and *aidB* promoters, respectively [13]. On the other hand, the MarA binding site can either be upstream from the −35 region (class I promoters), or overlap the RNA polymerase binding site (class II promoters) [14].

The TOL plasmid pWW0 of *Pseudomonas putida* specifies a *meta*-cleavage pathway for the oxidative catabolism of benzoate and toluates. Genes that encode the TOL *meta*-cleavage pathway are grouped in an operon under the control of a single promoter, Pm. Expression from the Pm promoter of the *meta*-cleavage pathway for alkylbenzoate degradation is under the control of the XylS regulator (see [15] for a review). When XylS is activated by effectors such as 3-methylbenzoate (3MB), it stimulates transcription from Pm in a process mediated by RNA polymerase with σ^{32} or σ^{38} depending on the growth phase [16]. The target half-sites for XylS binding at the Pm promoter are two imperfect direct repeats (5′-TGCAN$_6$GGNTA-3′) located between positions −70/−56 and −49/−35 [17,18] (Fig. 1). Therefore, the XylS proximal

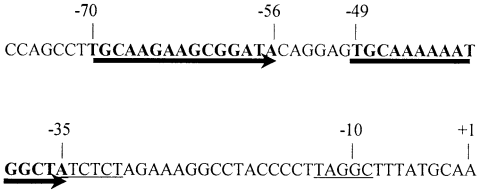

Fig. 1. DNA sequence of the Pm promoter. The +1, −10 and −35 positions of the Pm promoter are indicated. Arrows show the proposed XylS binding sites located between −70 and −56 and between −49 and −35.

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Abbreviations: Ap, ampicillin; Km, kanamycin; LB, Luria–Bertani culture medium; 3MB, 3-methylbenzoate; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; Tc, tetracycline

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site might share one or more nucleotides with the −35 region for RNA polymerase recognition. In addition, a track of six As between positions −41 and −46 in Pm may induce intrinsic curvature in the Pm promoter and thereby influence expression. This study was designed to determine if this structural organization of the promoter is essential for XylS-mediated transcription activation from Pm.

2. Materials and methods

2.1. Bacterial strains, culture medium and plasmids

The strains and plasmids used in this study and their relevant characteristics are shown in Table 1. *Escherichia coli* strains were grown at 30°C in Luria–Bertani (LB) culture medium supplemented, when required, with 100 μg/ml ampicillin (Ap), 25 μg/ml kanamycin (Km) and 10 μg/ml tetracycline (Tc).

2.2. DNA techniques

DNA preparation, digestion with restriction enzymes, analysis by agarose gel electrophoresis, isolation of DNA fragments, ligations, transformations and sequencing reactions were done according to standard procedures [19,20] or the manufacturers' recommendations.

2.3. Construction of Pm mutant promoters

The Pm derivatives with different length spacers between the XylS recognition site and the RNA polymerase binding site were constructed as follows: pBR-Pm101 is the result of cloning Pm101, a Pm mutant promoter bearing a unique *Nco*I site centered at C-42 [21], in pBR322 as a 400-bp *Eco*RI–*Hin*dIII fragment (Table 1). pBR-Pm300 was obtained by inserting a 43-bp (5′-CCATGG-GAGCTCGCTCGAGAGATCTATGCATGTTAACCCATGG-3′) duplex DNA fragment at the unique *Nco*I site of pBR-Pm101. This DNA fragment contains five non-overlapping unique restriction enzymes: *Sac*I, *Xho*I, *Bgl*II, *Nsi*I, and *Hpa*I, flanked by two *Nco*I sites. Pm mutant promoters Pm301 through Pm309 were obtained after double digestion of pBR-Pm300 using different combinations of the five restriction sites mentioned above (Table 1). The linearized plasmids were made blunt-ended by treatment with the Klenow fragment of DNA polymerase, and were religated with T4 DNA ligase. In the resulting mutant promoters the proximal XylS binding site and the −35 region were separated by a DNA sequence that ranged from 37 to 7 bp. All the mutant Pm promoters from Pm300 to Pm309 were subcloned in pMD1405 as a 401-bp *Eco*RI–*Hin*dIII DNA fragment to obtain in-frame Pm*::′*lacZ* fusions, which were designated pMAR-Pm300 to pMAR-Pm309 (Table 1).

To obtain plasmids pMAR-Pm310 to pMAR-Pm319, which contain a spacer region of 5 bp or less between the proximal XylS binding site and the −35 RNA polymerase recognition region, we used overlap extension polymerase chain reaction (PCR) mutagenesis. The template for each mutagenesis was 200 ng of pMT11 (Table 1), and amplification conditions were as described by Higushi [22]. The internal primers used for mutagenesis exhibited 1–5 extra bp between the proximal XylS binding site and the −35 RNA polymerase recognition region with respect to the wild-type sequence. The external oligonucleotides were the so-called M13 reverse primer (5′-CAGGAAA-CAGCTATGACCATG-3′) and a primer complementary to the α fragment of the ′*lacZ* gene (5′-GATGTGCTGCAAGGCGAT-TAAGTTA-3′). After DNA amplification, the resulting DNA was digested with *Eco*RI and *Hin*dIII, and the 401-bp *Eco*RI–*Hin*dIII Pm mutants were inserted between the *Eco*RI–*Hin*dIII sites of pMD1405 to yield plasmids pMAR310 through 319, which carry in-frame Pm*::′*lacZ* fusions (see Fig. 2). All the mutant Pm promoters generated in this study were confirmed by DNA sequencing.

2.4. Construction of pBend2-Pm for cyclic permutation assays

PCR was used to obtain a 401-bp DNA fragment containing the XylS binding sites in the Pm promoter. For the PCR reaction the template was plasmid pJLR100 (Table 1) and the primers used were 5′-GATGTGCTGCAAGGCGATTAAGTTG-3′ and 5′-CGTCTAA-GAAACCATTATTATCATG-3′. The 401-bp amplified PCR product was digested with *Xba*I and *Sal*I and the resulting 86-bp fragment was purified by agarose gel electrophoresis, extracted and ligated between the *Xba*I and *Sal*I sites of pBend2. This plasmid contains two identical DNA segments with 17 restriction sites on either side of *Xba*I and *Sal*I cloning sites [23,24]. The resulting plasmid pBend2-Pm was digested with different enzymes to yield a series of 206-bp DNA fragments, with the position of the DNA sequence carrying the XylS binding sites permutated (Fig. 3A). These fragments were electrophoresed through a continuous 10% (w/v) polyacrylamide gel in TBE buffer. To estimate the intrinsic bend angle (α) and to locate the center of the bend, we used the method of Thompson and Landy [25]. Values given are the average of six independent determinations.

2.5. RNA preparation, analysis and primer extension

RNA was extracted with the guanidinium isothiocyanate–phenol method as described previously [26]. Hybridization of the single-stranded ^{32}P-labelled DNA primer (about 10^5 counts per minute per assay) complementary to the mRNA transcript originated from Pm, and primer extension with avian myeloblastosis virus reverse transcriptase, were carried out as described previously [26]. The cDNA products of the reverse transcriptase reactions were separated and analyzed in urea polyacrylamide sequencing gels.

2.6. β-Galactosidase assays

E. coli bearing the wild-type Pm::′*lacZ* or mutant Pm*::′*lacZ* fusions in pMD1405, plus a compatible plasmid bearing the wild-type *xylS* (pERD103) or mutant alleles encoding XylSS229I (pERD32) and XylSG44S (pERD139), were grown overnight on LB medium

Table 1
Strains and plasmids used in this study

Strains or plasmids	Relevant characteristics	Source or reference
Strains		
E. coli MC4100	F$^-$, *araD*139, (*argF-lac*)U169, *rpsL*150, *relA*1, *flbB*5301, *deoC*1, *ptsF*25, *rpsR*. Sm^R	[20]
E. coli DH5α	*supE*44 *lacU*169 (ø80 *lacZ*Δ M15) hsdR17 ($r_K^-m_K^+$) *recA*1 *endA*1 *gyrA*96 *thi*-1 *relA*1	[39]
Plasmids		
pBend2	Derivative of pBR322 containing 17 restriction sites in a direct repeat surrounding *Xba*I–*Sal*I sites	[23]
pBend2-Pm	pBend2 derivative carrying a 86-bp DNA fragment containing a 75-bp Pm promoter spanning from A-27 to A-100 with respect to the main transcription initiation point, cloned between *Xba*I–*Sal*I sites	This study
pBR322	ColE1, Ap^R, Tc^R	[40]
pBR-Pm101	Mutant Pm101 bearing a *Nco*I site in the proximal XylS binding site, cloned in pBR322	This study
pBR-Pm300 to 309	Pm300–309 in pBR322, Ap^R, Tc^R	This study
pERD32	*xylS* mutant allele encoding XylSS229I, IncQ, Km^R	[41]
pERD103	*xylS*, IncQ, Km^R	[41]
pERD139	*xylS* mutant allele encoding XylSG44S, IncQ, Km^R	[42]
pJLR100	Pm cloned in pEMBL9, Ap^R	[43]
pJLR107	Pm::′*lacZ* in pMD1405, Ap^R	[43]
pMAR-Pm300 to 319	Pm300–319::′*lacZ* in pMD1405, Ap^R	This study
pMD1405	Promoterless ′*lacZ*, Ap^R	[43]
pMT11	Pm101::′*lacZ* in pMD1405, Ap^R	[21]

containing the appropriate antibiotics. Three clones of each strain were used. Cultures were diluted 100-fold in the same medium supplemented or not with 1 mM of 3MB. After 4 h of incubation, cells had reached the logarithmic phase. β-Galactosidase activity was determined in permeabilized whole cells according to Miller [27].

3. Results

3.1. Overlap of the proximal XylS binding site and the −35 region recognized by RNA polymerase

To determine whether the proximal XylS binding site and the RNA polymerase site overlapped in the −35 region, we designed a series of mutant promoters based on the mutant Pm101, in which a *Nco*I site had been introduced by replacing A−42 and A−43 with CC. We used this site to clone a 43-mer linker with several restriction sites. This insertion preserved the promoter sequence up to −40 but moved the critical TGCA from −49/−46 to −86/−83 and the furthest TGCA in the wild-type from −70/−67 to −107/−104. Through a series of internal deletions within the linker, the proximal TGCA was brought to positions −82/−79, −80/−77, −78/−75, −77/−74, −76/−73, −74/−71, −70/−67, −63/−60 and −58/−55. The structure of the last promoter, called Pm309, is shown in Fig. 2.

The different mutant promoters were fused to *′lacZ*. As a control we used the Pm101::*′lacZ* fusion. β-Galactosidase activity was determined in a XylS-proficient background in the absence and in the presence of 3MB. In the control strain β-galactosidase activity reached about 5000 Miller units (Fig. 2); in contrast, levels induced in the Pm mutant promoters were never higher than 100 Miller units (Fig. 2). We therefore concluded that transcription from the mutant Pm promoters was not activatable by the XylS protein with 3MB.

This lack of activity may be attributable to the increased distance between the TGCA motif and the −35 region, or to the modification of the sequence introduced at the 3′-end of the proximal XylS binding site at Pm. To determine the exact cause, we tested the activation of mutant Pm promoters by XylSS229I and XylSG44S. These two mutant regulators, which have increased affinity for Pm binding sites, suppressed mutations in the −49/−46 TGCA critical motif of Pm which otherwise prevented activation of transcription by the wild-type XylS regulator [17]. However, regardless of the presence of 3MB, transcription from Pm309 was not stimulated by either of these two mutant regulators. These results suggest that the lack of activity in the Pm300–309 series was probably due to the insertion of nine or more nucleotides between the XylS binding site and the −35 region.

On the basis of these results we decided to introduce shorter insertions between the −35 region and the XylS binding site. We used overlapping PCR mutagenesis to introduce 5, 4, 3, 2 or 1 bp between C at position −37 and T at position −36. The mutant promoters were designated Pm310 through Pm313 and Pm319 (Fig. 2). Mutants Pm314 and Pm315, in addition to the extra 1-bp insertion located at −37, carried additional mutations and were retained for further studies (see Fig. 2). The mutant promoters were fused to *′lacZ* and then used to test induction with XylS, XylSG44S and XylSS229I in cultures with and without 3MB. Negligible levels of transcription from Pm310 through Pm313, as compared to the wild-type, were found regardless of the regulator used, and regardless of the presence or absence of the effector. These results suggested that the insertion of 2 or more bp between −36 and −37 already prevented transcription stimulation by both wild-type XylS and mutant XylS proteins (Fig. 2). The fact that XylSS229I was not able to suppress the mutations at Pm contrasts with its ability to restore inducibility in Pm promoters with one or more changes in the critical −49/−46 box for XylS recognition [17,21]. This implies that an insertion as short as 2 bp can impair overlap site recognition by the positive regulator and RNA polymerase, or affect the interaction between the two proteins.

In contrast with the above results, the insertion of 1 bp between positions −36 and −37 in Pm319 resulted in a pattern of transcription similar to that obtained for Pm101, i.e. inducible transcription in the presence of 3MB with XylS and constitutive expression in the case of XylSS229I (Fig. 2).

The results with the promoters that had a 1-bp insertion and an additional mutation varied depending on the nature of this mutation and on the regulator used. When in addition to the A insertion at −37 the mutant promoter had a point mutation at the critical XylS recognition box −49/−46 (C−47→T), as in Pm315, only XylSS229I with 3MB was able to stimulate transcription from Pm (Fig. 2), although at a much lower level than in Pm319. These results are in agreement with previous findings by our group that showed that XylSS229I suppresses mutations in the XylS binding sites of Pm when an effector is added to the culture medium

		XylS		XylSS229I		XylSG44S	
		-	+	-	+	-	+
Pm101	TGCAAGAAGCGGATACAGGAGTGCAAACCATGGCTATCTCTAGA	20	4750	5800	7720	185	4200
Pm309	TGCAAGAAGCGGATACAGGAGTGCAAACCATGGC**GACCATGGC**TATCTCTAGA	50	100	30	60	45	40
Pm310	TGCAAGAAGCGGATACAGGAGTGCAAACCATGGC**AAGG**TATCTCTAGA	20	60	35	50	20	40
Pm311	TGCAAGAAGCGGATACAGGAGTGCAAACCATGGC**AAG**TATCTCTAGA	15	140	35	30	30	20
Pm312	TGCAAGAAGCGGATACAGGAGTGCAAACCATGGC**AA**TATCTCTAGA	20	280	20	40	20	30
Pm313	TGCAAGAAGCGGATACAGGAGTGCAAACCATGGC**A**TATCTCTAGA	15	50	30	40	20	30
Pm319	TGCAAGAAGCGGATACAGGAGTGCAAACCATGGCATATCTCTAGA	10	5260	5200	7800	20	4600
Pm314	TGCAAGAAGCGGATACAGGAGTGCAAACCATGGCATATCTCTCGA	10	4300	1400	6250	260	5040
Pm315	TGCAAGAAGCGGATACAGGAGTGTAAACCATGGCATATCTCTAGA	20	330	115	1100	10	20

Fig. 2. Levels of β-galactosidase activity expressed from different mutant Pm promoters with different XylS regulators in the absence and in the presence of 3MB. The figure shows the nucleotides in the mutant Pm promoter from the −27 nucleotide. Each mutant Pm promoter was fused to *′lacZ* in pMD1405. Nucleotides inserted between the original −36 and −37 positions are indicated in bold type; point mutations in Pm314 and Pm315 are underlined. β-Galactosidase activity was determined in triplicate as described in Section 2. Data are the average of six to nine independent determinations, with standard deviations below 15% of the given values. Arrows indicate the XylS binding sites.

[17,21]. However, with Pm314, which in addition to the 1-bp insertion had a point mutation so that A−29 was changed to C, the pattern of transcription was similar to that determined for Pm314, except that the level of expression with XylSS229I without 3MB was only 20% of that achieved in the presence of 3MB.

We determined the transcription initiation point in Pm319, Pm314 and Pm315 under conditions in which expression took place. The transcription initiation point was the same as that found when Pm and XylS were used (not shown). This suggests that the β-galactosidase activity we found reflected activation of transcription from the mutant Pm promoters by the mutant XylS regulators, and not from any artificial newly created promoter.

3.2. Intrinsic curvature of the Pm promoter

The Pm promoter has a track of As in the −41 to −46 region in the top strand (Fig. 1). The presence of AT-rich regions in a DNA sequence normally induces intrinsic bending in the DNA [28]. Dimethyl sulfate assays with Pm in vitro and in vivo revealed hypermethylation of the A located at −42, which indicated that DNA was distorted at this region [17]. Intrinsic bending is frequently found in promoters near the RNA polymerase binding site [29,30] and has been related to contacts between RNA polymerase and the regulator [31]. Because the A-track in Pm was located within the XylS binding site, we decided to determine the intrinsic bending of the Pm promoter with the cyclic permutation assays method [32,33]. We used pBend2-Pm to construct a series of DNA fragments containing the sequence of Pm from −27 to −101 at different positions relative to the ends of the fragments, as described in Section 2 (Fig. 3A). Then we compared the electrophoretic mobility of the different fragments at 4°C in polyacrylamide gel electrophoresis (PAGE) (Fig. 3B). To calculate the intrinsic bending angle, α, we used the equation of Thompson and Landy [25], which gave an intrinsic curvature angle of the Pm promoter of 40.5°, centered in the track of As spanning positions −41 to −46. To assess the role of this curvature in promoter activity, we analyzed two mutant Pm promoters, Pm5U and Pm5D. The first one had a C−47→G mutation that abolished activity [34], whereas the second, Pm5D (A−44→G), bearing a mutation in the A-track, maintained 70% of its wild-type activity. Analysis of the two promoters showed that bending was unaffected by the mutation in Pm5U, whereas both Pm5D had greatly reduced the intrinsic curvature (data not shown). Because there was no correlation between promoter activity and the presence of curvature in the −41/−46 region, we suggest that intrinsic bending around position −43 in Pm is not essential for transcription activation in view of the fact that Pm5D, which lacks this curvature, is still active.

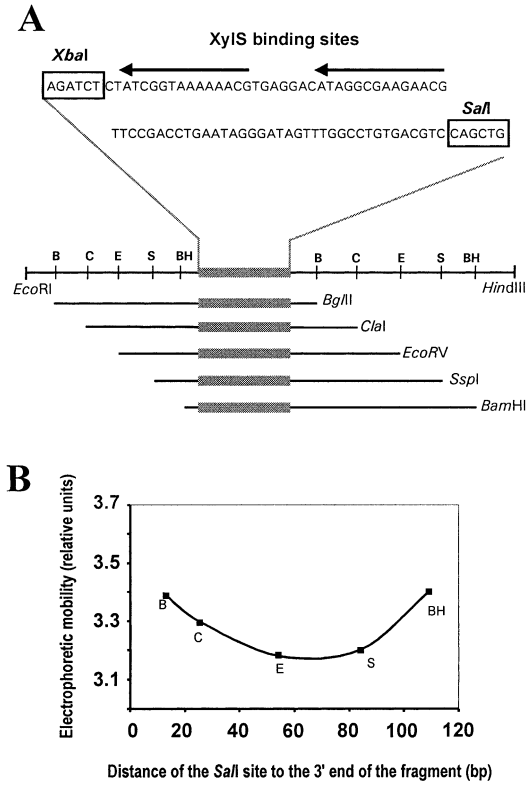

Fig. 3. Cyclic permutation assay constructions. A: The 86-bp DNA fragment from Pm was cloned in pBend2. The DNA segment was cloned between the *Xba*I and *Sal*I sites (boxed); this fragment is shown as a gray box in the different construction. Arrows show the XylS binding site in Pm. The thick line represents the polylinker of pBend2, where the restriction sites used to generate the different 206-bp fragments with permutated locations of Pm are indicated. B: Electrophoretic mobility of the permutated fragments in PAGE. Electrophoresis was carried out as described in Section 2, and the relative mobility of each fragment was plotted against the distance of the *Sal*I site to the 3′ end of the 206-bp fragment. Restriction sites are denoted as follows: B: *Bgl*II; C: *Cla*I; E: *Eco*RV; S: *Ssp*I; BH: *Bam*HI.

4. Discussion

Protein–protein interactions between RNA polymerase and transcriptional regulators that bind adjacent to or overlapping the −35 region are known to occur [6]. These interactions can take place with any of the RNA polymerase subunits, and in some cases they are favored by the intrinsic curvature of DNA upstream from −35 [31] or curvature induced by the binding of the regulator [35]. In this study we show that the Pm promoter exhibits a curvature of about 40° centered in a continuous series of As between −41 and −46. However, this intrinsic curvature is not a sine qua non for transcription activation, in spite of XylS being a class II regulator.

Several other research groups have shown that the AraC, MarA, Rob, and RhaS binding sites overlap the −35 region by two to four nucleotides in their cognate promoters. In contrast, the Ada recognition site seems to be separated by 2 or 5 bp from the RNA polymerase binding site in the *ada* and *aidB* promoters, respectively [7,13,36]. When we began this study we did not know whether the proximal XylS binding site and the −35 region of Pm recognized by the sigma factor of RNA polymerase overlapped. The present results support the hypothesis that a separation of 2 or more bp between the −35 region for RNA polymerase from the XylS binding site has a dramatic effect on XylS-dependent transcription from Pm: transcription from mutant Pm promoters was abolished in the Pm313 promoter, in which 2 bp were inserted at −36/−37. This short 2-bp insertion rendered XylS unable to stimulate transcription. Moreover, mutant regulators with increased affinity for the XylS half-binding sites, i.e. XylSS229I and XylSSG44S, were not able to suppress the mutation at Pm. These findings contrast with the ability of these mutant regulators to restore inducibility in Pm pro-

moters with one or more changes in the critical −49/−46 box for XylS recognition. Fig. 4A shows the alignment of the XylS binding sites as deduced from in vitro and in vivo foot-printing [17,37] and from transcriptional assays with several Pm mutant promoters [17,21]. The extension of these sites suggests that the XylS binding site overlaps that of RNA polymerase, and that these nucleotides are probably located at −35 and −36 with respect to the main transcription initiation point. However, the fact that the insertion of 1 bp at −37 had no significant effect on transcription suggests a 1-bp overlap between XylS and RNA polymerase at position −36 is sufficient to allow full transcription from the promoter (Fig. 4B). For example, Pm319 was activated both by XylS and by a mutant XylS regulator to the same extent as the reference Pm101 promoter. In addition, mutant Pm314, which had the 1-bp insertion at −37/−36 and an additional point mutation outside the RNA polymerase site at −29, was activated by XylS in the presence of 3MB, and by XylSS299I in the presence and absence of 3MB. When the 1-bp insertion at −37 was accompanied by a point mutation at the critical −49/−46 TGCA motif (Pm315), only the mutant XylS with the highest affinity for the XylS binding sites was able to promote a significant level of transcription. It then follows that the pattern of transcription from the mutant Pm with a 1-bp insertion at −37 and an extra mutation behaved similarly to Pm mutants without the 1 extra bp at −37 and with the point mutation as described [17].

We suggest that overlap of the proximal XylS binding site with that of the RNA polymerase may facilitate interactions between XylS and RNA polymerase. In this connection it has recently been established that XylS contacts the α subunit of RNA polymerase [38], and at present we are trying to identify putative specific contacts between the regulator and other subunits of RNA polymerase.

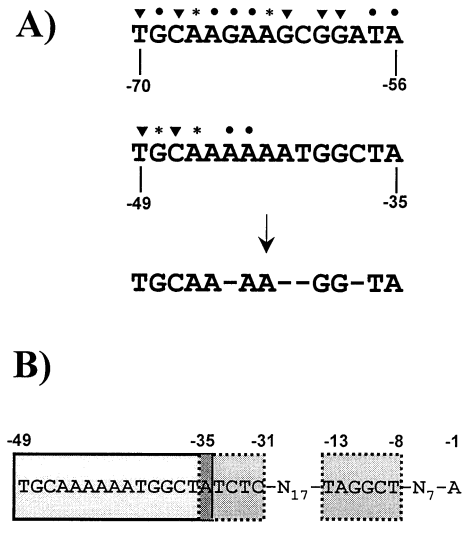

Fig. 4. Alignment of the distal and proximal XylS binding sites. A: The distal and proximal XylS sites were aligned from the highly conserved TGCA submotifs. · indicates critical residues deduced from foot-printing and mutational analysis (mutations in these residues result in a decrease in transcription of more than 90%); 5 indicates positions in which point mutations result in moderate loss of activity, i.e. between 30% and 70%; * indicates residues not contacted by XylS, (point mutations in these positions had no significant effect on activity). B: Physical organization of the proximal XylS binding site and the putative −10/−35 RNA polymerase boxes. Dashed boxes represent the proposed −10 and −35 regions recognized by RNA polymerase. The closed box represents the proximal XylS binding site as deduced from genetic assays and sequence alignments [17,18,37].

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