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The amino-terminal domain of the prokaryotic enhancer-binding protein XylR is a specific intramolecular repressor

(*Pseudomonas*/σ^{54} factor/promoters/NtrC family/protein trans-complementation)

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ABSTRACT **The mechanism under which the signal-reception amino-terminal portion (A domain) of the prokaryotic enhancer-binding protein XylR controls the activity of the regulator has been investigated through complementation tests *in vivo*, in which the various protein segments were produced as independent polypeptides. Separate expression of the A domain repressed the otherwise constitutive activity of a truncated derivative of XylR deleted of its A domain (XylRΔA). Such inhibition was not released by *m*-xylene, the natural inducer of the system. Repression caused by the A domain was specific for XylR because it did not affect activation of the σ^{54} promoter *PnifH* by a derivative of its cognate regulator, NifA, deleted of its own A domain. The A domain was also unable to repress the activity of a NifA–XylR hybrid protein resulting from fusing two-thirds of the central domain of NifA to the carboxyl-terminal third of XylR, which includes its DNA-binding domain. The inhibitory effect caused by the A domain of XylR on XylRΔA seems, therefore, to result from specific interactions in trans between the two truncated proteins and not from mere hindering of an activating surface.**

Prokaryotic promoters dependent on the alternative σ^{54} factor resemble their eukaryotic counterparts in their requirement of distant (>100 bp) upstream activation sequences, which stimulate transcription in an enhancer-like fashion and are the target of cognate regulatory proteins (1). The regulators that activate this class of promoters also resemble eukaryotic transcriptional factors in their modular organization in distinct functional domains (2). These include an activating central domain (C domain), believed to interact with the σ^{54}-containing form of the RNA polymerase (σ^{54}-RNAP), the carboxyl-terminal DNA-binding domain (D domain), and a very variable amino-terminal domain (A domain) that is connected to the C domain through a hinge domain (B domain) spanning a specific sequence named the Q-linker (3). The A domain recognizes directly or indirectly (frequently through phosphorylation) the signal to which the promoter is ultimately programmed to respond (1). Such signal is translated into stimulation of an ATPase activity generated in the C domain, which eventually initiates transcription at the corresponding promoter through a poorly understood mechanism (4, 5). At least in the case of the archetypical protein NtrC, this process seems to be concomitant with the oligomerization of the activator (6).

Similarly, to other regulators of the family, the XylR protein (63.7 kDa), which controls expression of the σ^{54}-promoters *Pu* and *Ps* of the TOL (toluene, *m*-xylene and *p*-xylene degradation) plasmid of *Pseudomonas putida* (7), also possesses a modular structure (see Fig. 1). While the C and D domains of XylR functionally resemble those of other related regulators of the NtrC family (2), the A domain of XylR seems to have specific properties. Genetic data (8) indicate that the A domain of XylR interacts directly with the aromatic effectors of the TOL system (xylene and related compounds), an event that is finally translated into activation of its cognate promoters *Pu* and *Ps*. The fact that an A-domain deletion of XylR causes the activator to be fully constitutive (9) suggests that the A module of the protein acts as a repressor domain. Furthermore, disruptive amino acid changes at the interdomain hinge domain (B domain or Q-linker) result in a semiconstitutive phenotype (9). The sequence of events leading to the activation of XylR seems, therefore, to involve a significant displacement of the A domain in respect to the rest of the protein. In this work, we examine whether the repression exerted by the A domain in XylR results from a simple steric hindrance of protein regions involved in activation or engages specific intramolecular interactions. Our results are consistent with the second alternative—i.e., repression is caused by specific contacts between the A domain and a distinct protein surface on the C domain of the regulator.

MATERIALS AND METHODS

Bacterial Strains and General Methods. *Escherichia coli* strains S90C and MC4100, all of them Δ*lac*, have been described (10). pRSPu (10), encoding the reporter *Pu–lacZ* fusion used for monitoring XylR activity, is a transcriptional *lacZ* fusion plasmid containing a 312-bp *Eco*RI–*Bam*HI fragment spanning positions −205 to +93 of the *Pu* promoter region of the TOL plasmid. Where indicated, *lacZ* fusions and adjacent insertions of the genes for native and hybrid regulators were integrated into the chromosome of *E. coli* MC4100 or *E. coli* S90C by growing λRS45 phage on *E. coli* CSH50 transformed with the corresponding pRS551 derivative and then using the resulting lysates to transduce the kanamycin-resistance marker of the hybrid phage into the target strain, as described in ref. 11. For detection of XylR protein and its derivates, the corresponding *E. coli* cultures were grown under the conditions specified in each case. Cells were collected, lysed, and adjusted to the same protein concentration; this sample was then run in denaturing 10% polyacrylamide gels and subjected to immunoblot analysis with a polyclonal anti-XylR serum. The bands corresponding to XylR and its derivatives were developed with the use of protein A coupled to horseradish peroxidase and H_2O_2/diaminobenzidine in the presence of $NiCl_2$ at 0.8 mg/ml for enhancing the reaction. Unless otherwise indicated, promoter activity was monitored by assaying the accumulation of β-galactosidase (12) in cells carrying transcriptional *lacZ* fusions to the promoter of interest in monocopy gene dosage. β-Galactosidase activity values represent the average of at least three independent experiments, each of which was conducted in duplicate samples, with

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Abbreviations: IPTG, isopropyl β-D-thiogalactoside; A domain, amino-terminal portion; B domain, hinge domain; C domain, central domain; D, domain, DNA-binding domain.

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deviations being <15%. Specific conditions are specified in each case in the legend to the corresponding figure.

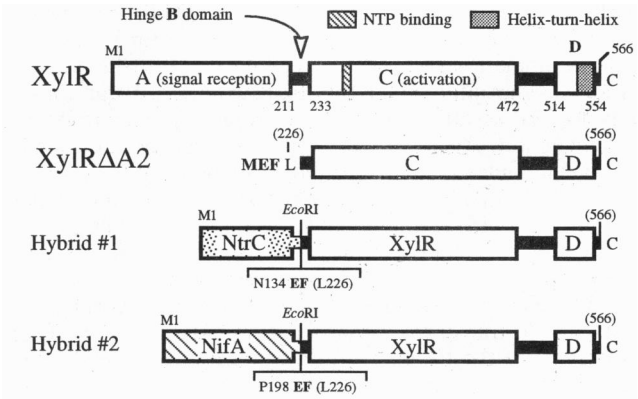
Construction and Expression of XylR Derivatives and Hybrid Proteins. The plasmid encoding resistance to ampicillin pEZ6 (pBR322 replicon, ref. 13) is an expression plasmid encoding the wild-type XylR that uses the $LacI^q$-regulated *Ptrc* promoter and the translation initiation region of expression vector pTrc99A (14). A construction equivalent to pEZ6 but expressing the truncated protein XylRA2 (see Fig. 1) was made by amplifying by PCR the DNA sequence-spanning positions equivalent to aa 226–566 of XylR, as an *Eco*RI–*Bam*HI fragment that was then cloned at the same sites of expression vector pTrc99A. The resulting plasmid (pJPV02) was used as the basis for constructing the NtrC–XylR and NifA–XylR hybrids described below as hybrids 1 and 2. In one case, the DNA sequence-spanning aa 1–198 of the NifA sequence of *Klebsiella pneumoniae* was amplified from plasmid pMC71A (15) as a PCR product flanked by *Nco* I and *Eco*RI sites and subsequently cloned at the same sites of pJPV02. This procedure gave rise to a plasmid (pJPV03), expressing a fusion protein consisting of the A domain of NifA followed by the C and D domains of XylR (hybrid 2). In the other case, the A domain of the NtrC-encoding gene of *K. pneumoniae* was similarly inserted in front of the C and D domains of XylR by amplifying from pNH31 (16) the DNA sequence equivalent to aa 1–134 of the NtrC sequence flanked by *Nco* I and *Eco*RI sites. Cloning of the resulting PCR product in the equivalent sites of pJPV02 resulted in plasmid pJPV04, which expressed the NtrC–XylR fusion hybrid 1 (see Fig. 1).

For expression of the A domain of XylR as an isolated protein, a *Bam*HI site was generated within the *xylR* sequence of pEZ16 (9) at the sequence corresponding to aa 219–220. The new *Bam*HI site and the *Nco* I site at the leading ATG was used to excise the sequence between positions 1 and 219 and place it at the corresponding sites of pTrc99A, resulting in plasmid pJPV05, in which the A domain of XylR is expressed through a *Ptrc*/$lacI^q$ system.

The DNA sequences encoding the truncated and hybrid proteins along with their cognate reporter *lacZ* fusions used in the experiments of Fig. 3 were assembled on vector pRS551 as follows. The DNA segment spanning *xylRΔA2* and a divergent *Pu* promoter in transposon mini-Tn*5*/MAD2 (9) was excised as a *Bam*HI restriction fragment and cloned at the *Bam*HI site of pRS551, so that the *Pu* promoter points toward the *lacZ* reporter gene of the plasmid as shown in Fig. 3, resulting in plasmid pRS/MAD2. This insertion inherits various restriction sites that were subsequently used to exchange, in the same plasmid, several DNA fragments encoding the regulators and promoters of interest. An internal *Eco*RI deletion of pRS/MAD2 eliminated the portion of the construction carrying *xylRΔA2* and gave rise to control plasmid pRSPu/Pr. For exchange of the *xylRΔA2* sequence with that of *dmpRΔA*, the DNA segment-spanning positions equivalent to aa 225–563 of DmpR were amplified from pUC-DmpR (17) as a *Nco* I–*Eco*RI fragment, which was then cloned at the corresponding sites of pRSPu/Pr, resulting in plasmid pRSdmpRΔA/Pu. Exchange of *xylRΔA2*/*Pu* by *nifAΔA*/*PnifH* occurred in two steps. (*i*) The *xylRΔA2* gene of pRS/MAD2 was exchanged with that of *nifAΔA* as a *Nco* I–*Eco*RI restriction fragment spanning equivalent aa 187–524 of the NifA sequence, obtained by PCR on plasmid pMC71A (15). This intermediate construction is called pRSnifAΔA/Pu. (*ii*) The remaining *Pu* promoter was replaced with the *PnifH* promoter by inserting the 190-bp *Eco*RI–*Bam*HI fragment of pJES384 (18) at the *Sph*I and *Bam*HI sites of pRSnifAΔA/Pu, previous blunt-ending of the *Eco*RI and the *Sph* I sites. This procedure gave rise to plasmid pRSnifAΔA/PnifH. The strategy used ensured that the three truncated regulators (XylRΔA, DmpRΔA, and NifAΔA) were expressed through the very same translation initiation region and *Pr* promoter that drives expression of the native *xylR* genes. Finally, a chimera between NifAΔA and XylRΔA was constructed by replacing the internal *Eco*RV fragment of pRS/MAD2 with that of pRSnifAΔA/Pu, resulting in plasmid pRShyb3/Pu. The inserts in all pRS551 derivatives were then recombined *in vivo* into λRS45 phage, as described in ref. 11, to place the corresponding DNA segments in monocopy gene dosage in the chromosome of *E. coli* strain S90C.

RESULTS AND DISCUSSION

Steric Hindrance and Intramolecular Repression of XylR Activity. Deletion of the A domain of XylR results in a fully constitutive activator (9). On the contrary, exchange of the same protein segment and the domain-isolator Q-linker (Fig. 1) by a heterologous polypeptide derived from the MS2 virus



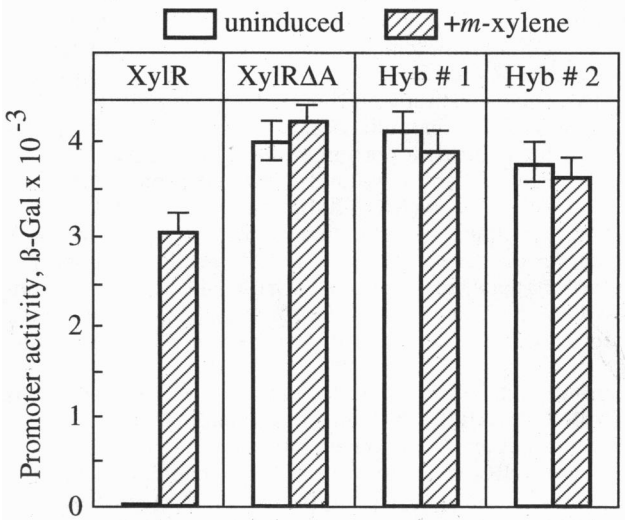


FIG. 1. Activation of *Pu* promoter by hybrid NtrC–XylR and NifA–XylR proteins. (*Upper*) Organization of native XylR, showing the boundaries between the functional domains and the localization of relevant functions within the protein sequence. Leading residues of reference XylRΔA2 protein (9) are indicated with the position formerly occupied by the nearest unchanged residue (in parentheses). Structures of fusion proteins containing the A domains of either NtrC (hybrid 1) or NifA (hybrid 2) and the rest of XylR are shown also, the junctions between domains being specified in each case. (*Lower*) *E. coli* strain MC4100λPu-lacZ (10) was independently transformed with plasmids pEZ6 ($xylR^+$), pJPV02 ($xylR\Delta A^+$), pJPV04 ($ntrC$::$xylR^+$), and pJPV03 ($nifA$::$xylR^+$). Each transformant was grown at 37°C to an OD_{600} = 1.0 in M9 minimal medium/0.1% glucose/0.1% Casamino acids and exposed for 3-hr to saturating vapors of *m*-xylene. The expression system of each of the *xylR* varieties ensured an equivalent expression level for all of them. Values for β-galactosidase (β-Gal) activity are shown. Inducer had no significant effect on growth rate under the conditions used.

polymerase, gives rise to a hybrid protein unable to stimulate transcription but still able to efficiently bind DNA (9). Because the configuration of MS2pol::XylR was designated to lock the hinge between the A domain and the C domain of XylR in a configuration less flexible than the native one, our early observations suggested that the A domain could down-regulate the activity of XylR through simple steric hindrance of a protein surface involved in transcriptional activation. This result, however, does not rule out the idea that repression in the native protein requires the amino-terminal module to interact specifically with a matching protein surface within XylR. To study this possibility, we reasoned that if the inhibition caused by the A domain is the consequence of a simple steric hindrance, any other heterologous protein domain should have the same effect. In contrast, if specific interactions occur, the precise swapping of the amino-terminal module of XylR by the A domain of another regulator of the σ^{54}-related family will create a constitutive phenotype. This result would be due to the anticipated structural tolerance for a heterologous A domain and also to the absence of the specific intramolecular interactions that fix the repressed state of the activator. To scrutinize these predictions experimentally, we exchanged the A domain of XylR for each of the amino-terminal modules of two unrelated σ^{54}-dependent activators—namely, NtrC and NifA—so that besides the protein modules, the XylR Q-linker domain would remain intact. The phenotypes endowed by the different hybrids were monitored *in vivo* by assaying the accumulation of β-galactosidase as described. For this, plasmids encoding the corresponding hybrid genes were used to transform *E. coli* strain MC4100λ*Pu–lacZ* (10). Expression of the fusion proteins at equivalent levels *in vivo* was confirmed through immunoblotting of cell extracts from each strain revealed with anti-XylR polyclonal antiserum (data not shown). Fig. 1 shows that the ability of either NtrC–XylR (hybrid 1) or NifA–XylR (hybrid 2) protein to activate the reporter *Pu–lacZ* system was indistinguishable from that of wild-type XylR protein under *m*-xylene induction or from the activity of the constitutive XylRΔA derivative, which has lost its amino-terminal module. Because swapping the same domain for the equivalent portion of the XylR-related regulator DmpR results in an active and regulated protein (19), the constitutive activity of NtrC–XylR (hybrid 1) and NifA–XylR (hybrid 2) proteins suggested that repression exerted by the A domain on the rest of the XylR protein results from specific interactions between surfaces in each domain and not just from gross steric hindrance, as previously suggested. The following sections offer various lines of evidence in support of this hypothesis.

Inhibition in Trans of the Constitutive Activity of an A-Domain-Deleted XylR by the Isolated A Domain. The next step to ascertain whether the A domain interacts specifically with the rest of the XylR protein was to check the possibility of specific repression in trans when a constitutive XylR deleted of its A domain (XylRΔA) was coexpressed *in vivo* with an excess of A domain produced as an independent polypeptide. We had previously shown that a truncated XylR derivative deleted of the whole A module (i.e., spanning only former amino acid positions 226–566, Fig. 1) was produced as a stable and active regulator (9). Similarly, we sought to produce the A domain as an isolated protein. To do this, we constructed an expression plasmid (pJPV05) in which the polypeptide spanning aa 1–219 of the XylR sequence was expressed through the isopropyl β-D-thiogalactoside (IPTG)-inducible promoter *Ptrc*. pJPV05 was transformed into an *E. coli* strain that was a λ lysogen carrying a DNA segment with a *Pu–lacZ* fusion and the gene encoding the A-domain-deleted derivative XylRΔA1, as sketched on top of Fig. 2. This strain was subjected to β-galactosidase assays at different concentrations of IPTG, while the production of the two independent truncated proteins, XylRΔA and DomA, was followed through immunoblot. The results shown

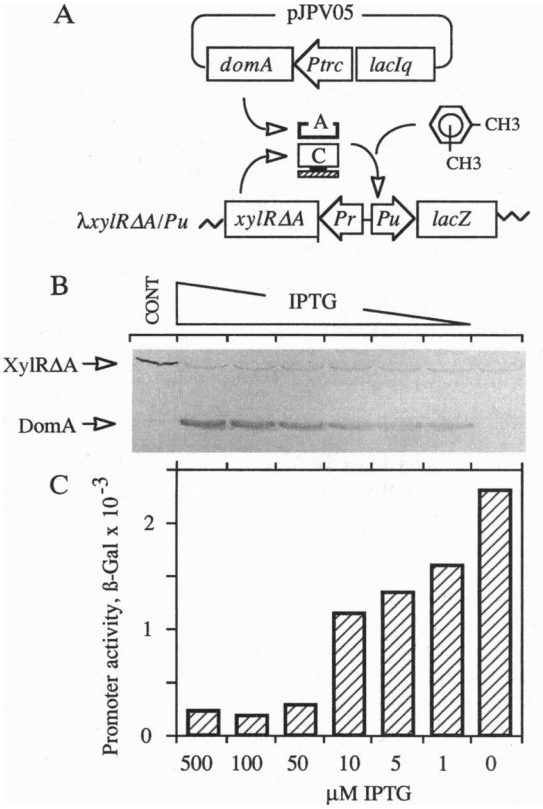


FIG. 2. Repression in trans of the constitutive activity of XylRΔA by the isolated signal reception domain. (*A*) Strategy for monitoring repression in trans of the XylR A domain. The basic genetic test used to detect protein complementation between A and C–D domains of XylR is sketched at top. A specialized *E. coli* S90C lysogen was constructed with a λ phage harboring a DNA segment that included a *Pu–lacZ* reporter system and the sequence for expression of *xylRΔA2* transcribed through the native, constitutive promoter of *xylR, Pr*. The resulting λ*xylRΔA/Pu*-containing strain was then transformed with pJPV05, which expresses the A domain of XylR (DomA) through a *lacI*^{q}*/Ptrc* promoter. Repression in trans can be monitored by examining β-galactosidase levels of the resulting strain upon induction of DomA expression with increased amounts of IPTG. (*B*) Conditional expression of the A domain of XylR *E. coli* strain S90Cλ*xylRΔA/Pu-lacZ* (10) transformed with pJPV05 was grown at 37°C to an OD_{600} = 0.2 in M9 minimal medium/0.1% glucose/0.1% Casamino acids, at which point IPTG was added at the indicated concentrations. Seven hours later, protein extracts of *E. coli* cells form the cultures were immunoblotted with anti-XylR serum. IPTG addition had no effect on growth rate. Note that XylRΔA expression is not affected by overproduction of the isolated A domain. The control (CONT) lane at left was loaded with extract of a strain overproducing XylRΔA. Position of each truncated protein is indicated. (*C*) β-Galactosidase (β-Gal) accumulated by the same cells in each case is represented graphically.

in Fig. 2 indicate that overproduction of the A domain significantly decrease the activity of the *Pu–lacZ* reporter fusion. Immunoblot analysis of the same cells (Fig. 2*B*) rule out that such decrease could be due to a lower expression of XylRΔA1, which remains constant under the various growth conditions. Interestingly, the XylR effector *m*-xylene did not restore the inducibility of the system by aromatic molecules (data not shown; see below). This result suggested that the induction mechanism requires an intact B domain connecting the signal-reception A domain with the rest of the protein.

Specificity of the Repression Caused by the Signal Reception A Domain of XylR. Although these experiments indicated that repression of the A domain on the remaining XylR protein could be reproduced in trans, we wondered about the specificity of the effect on other regulators of the σ^{54}-dependent family. To explore this question, we used two such regulators—

namely, DmpR and NifA—which are constitutively active when deleted of their A domains. The first regulator is closely related to XylR in the overall organization of its domains (20) and its responsiveness to aromatic effectors, although the specific profile of inducer molecules is totally different (19, 20). Furthermore, A domains can be swapped between the two proteins, thus resulting in hybrid proteins with different effector profiles (19). Specific interdomain interactions between the A domain of XylR and the rest of the protein, should they exist, must be maintained in DmpR, and repression in trans of a DmpRΔA truncated protein should be detected. On the contrary, although the overall organization of NifA in functional domains generally matches that of XylR, the A domain of NifA differs markedly (2), and this domain does not affect XylR when swapped for the native receiver domain of the protein (see Fig. 1). The prediction in this case is, therefore, that the isolated A domain of XylR will have little, if any, effect on the constitutive activity of a NifAΔA protein.

To examine these predictions, we designed an experimental system *in vivo* in which the effect of the A domain of XylR on the activities of the truncated DmpRΔA and NifAΔA derivatives could be faithfully compared. For this, we constructed isogenic *E. coli* strains in which a reporter *lacZ* fusion was engineered in a λ lysogen along with the gene for the cognate regulator expressed through the very same promoter and the same translation initiation region. This procedure ensured that expression of the corresponding truncated regulators and their effect on the reporter *lacZ* fusion was maintained within equivalent conditions (data not shown). Each resulting strain was then transformed with plasmid pJPV05, which drives expression of the A domain of XylR under the control of an IPTG-inducible promoter.

Fig. 3 shows that while expression in trans of the signal reception module of XylR decreased the activity of XylRΔA2 and DmpRΔA on the *Pu* promoter by 25-fold under the assay conditions, it had no detectable effect on the ability of NifAΔA to stimulate transcription from its cognate *PnifH* promoter. These results fit the predictions of the hypothesis that the A domain is an intramolecular repressor that interacts specifically with other portions of the XylR protein and not simply a bulky domain that hinders access to a protein surface required for activation. As mentioned above, exposure of the cells to the XylR inducer *m*-xylene had no influence on the repression caused by the isolated A domain on XylRΔA2. The implications of this effect in explaining the activation of XylR by *m*-xylene are discussed below.

A NifA-XylR Hybrid Devoid of Signal Reception Domain Is Not Repressed by the Isolated A Domain of XylR. The above results indicated that the A domain of XylR can repress other portions of the protein, but they say nothing about the target of such interactions. Because the NifAΔA protein used in the experiments of Fig. 3 was active but nonrepressible by the A domain of XylR, we reasoned that a hybrid between this protein and XylRΔA would inherit or lose the portion of the protein involved in the specific interactions, depending on the segments used for its construction. To test this concept, we took advantage of an *Eco*RV restriction site that is conserved in equivalent positions of the C domain of XylR and NifA to construct a hybrid protein (hybrid 3, see Fig. 3), which included about two-thirds of the A domain of NifAΔA and one-third of the carboxyl-terminal part of XylR. This protein maintains the DNA-binding domain of XylR and can activate the *Pu* promoter, although more weakly than the XylRΔA protein. Fig. 3 shows that when subjected to the same repression assay as the one above, the NifAΔA–XylRΔA fusion (hybrid 3) was unaffected by the A domain of XylR. This result suggested that the target of the intramolecular repression is located in the C domain of the regulator and not the D domain of the protein. This result is consistent with the observation that the DNA-binding activity of this regulator is not affected by the inducer (13, 21). Interestingly, overproduction of the A domain of XylR did not repress the activity of either wild-type XylR or NifA–XylR hybrid 2 (Fig. 1), whereas it did repress the otherwise constitutive NtrC–XylR hybrid 1 (data not shown).

Activation of XylR by *m*-Xylene: A Plausible Model. Control of the activity of a transcriptional regulator by intramolecular repression has been described in a number of prokaryotic proteins, such as the transcriptional activators FixJ (22), SpoOA (23), LuxR (24), and the σ^{70} subunit of the RNA polymerase (25). The case of XylR, however, seems to possess distinct characteristics. The picture that emerges from the data presented here and in previous reports (8, 9, 13) predicts the XylR protein to be organized in three independent functional modules (Fig. 4). Within this scheme, the A domain interacts specifically with a surface involved in activation present at the central segment of the protein that is released upon *m*-xylene

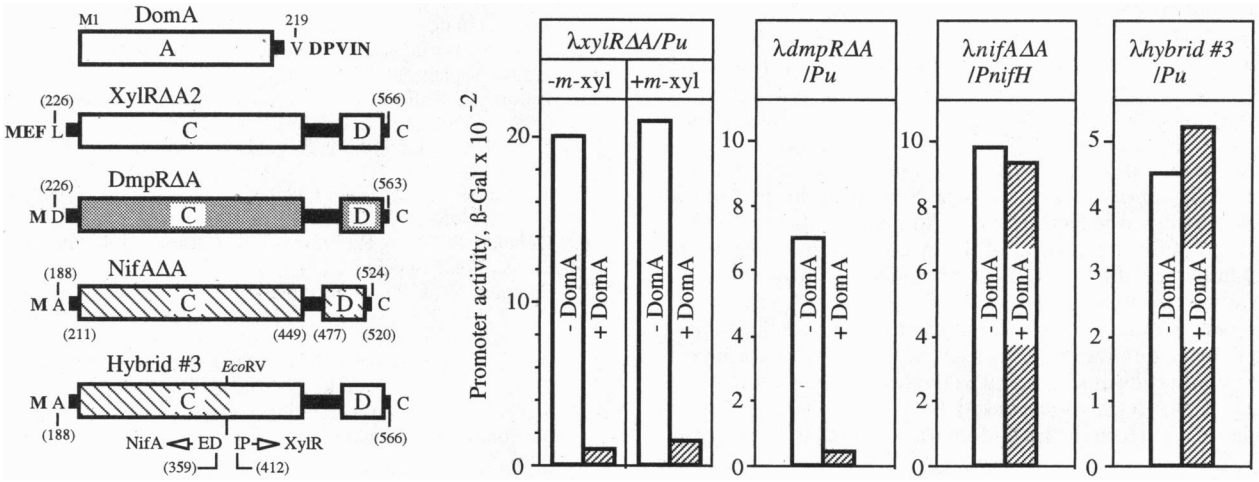


FIG. 3. Effect of the A domain of XylR on truncated σ^{54}-dependent regulators. Each *E. coli* lysogen expressing the truncated and hybrid proteins represented at left, along with their cognate *lacZ* fusion to their corresponding promoters, was transformed with pJVP05 and assayed for β-galactosidase (β-Gal) under various conditions. Each strain was grown as described in the Fig. 1 legend to an OD_{600} = 1.0, after which 0.1 mM IPTG was added and these strains were exposed, where indicated, to saturating vapors of *m*-xylene (*m*-xyl). Neither the aromatic effector nor the IPTG affected growth rate significantly. Accumulation of β-galactosidase was examined 3 hr after induction. Note the different vertical scales. The basal level of *Pu* promoter without any activator was ≈20 Miller units of β-galactosidase. The basal level of *PnifH* promoter without NifAΔA was <20 units. Note the strong repression of XylRΔA and DmpRΔA activities by the A domain but not of NifAΔA or fusion protein NifA–XylR (hybrid 3).

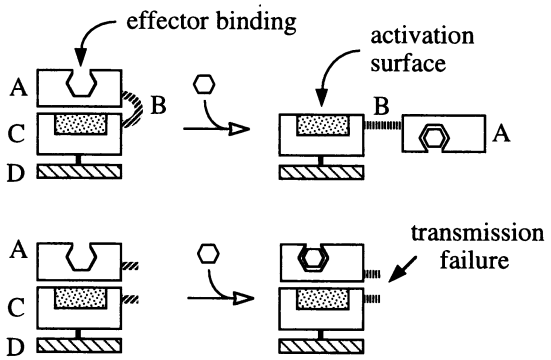


FIG. 4. Model of activation of XylR by *m*-xylene. The model assumes that XylR is a protein organized in three distinct modules—namely, A (reversible repression), C (activation), and D (DNA binding). Domains A and C are connected through the linker B domain. In the absence of aromatic inducer, the A domain blocks an activating surface in the D domain that is released upon *m*-xylene binding. Interactions of the A domain with the central part of the protein are maintained through intramolecular protein–protein contacts and further reinforced by positioning caused by the B region. Release of repression requires an intact hinge, which may provide a molecular fulcrum to move the A domain into a nonrepressor position.

binding. The A domain is assisted in its positioning in an efficient intramolecular inhibitory conformation through the B domain. That the repression caused by the A domain in trans cannot be reverted by *m*-xylene suggests that the de-repression caused by the inducer in the native system requires physical connection between the A and C domains. When the two domains are in trans, *m*-xylene seems unable to cause their separation (i.e., the protein does not bind the inducer or the effect cannot be transmitted to the rest of the protein), and their interaction results in an intermediate phenotype of no activity/no inducibility. Activation should, therefore, involve a major structural change in the B domain that turns unproductive when the A and C portions of the proteins are produced separately. This hypothesis is supported by the observation that disruptive mutations at the B domain lead to a partial constitutivity (i.e., mimic the effect of the inducer), and its removal leads to a fully constitutive phenotype independent of *m*-xylene (9).

The model of Fig. 4 predicts the constitutive or semiconstitutive phenotypes can be produced upon mutations in the A domain, the C domain, or the B domain. Besides those already observed at the A–C linker (9), Delgado *et al.* (26) have reported a partially constitutive mutant at the carboxyl-proximal part of the A domain. Furthermore, Shingler and Pavel (27) have also observed a variety of mutations at the A, B, and C domains of the XylR-related protein DmpR, leading to a partially constitutive phenotype. Similar to the case of the σ^{54}-related activator DctD (28), A-domain deletions of XylR protein pinpoint the portion of the A domain that is closer to the Q-linker as the segment responsible for intramolecular repression (unpublished work).

Our results raise the question of whether or not all prokaryotic enhancer-binding proteins that act in concert with the σ^{54} factor follow the same general activation pathway, involving release of the repression caused by the signal reception A domain on a surface at the C domain required for activation. The DctD protein of *Rhizobium* also becomes constitutive upon deletion of its A domain (28, 29) and may, in fact, have the same activation mechanism as XylR. Other members of the family, including the archetypical NtrC regulator seem, however, to lose activity when the amino-terminal segment is deleted (30). These differences between regulators of the same family deserve further study.

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