Communication

Identification of the Repressor Subdomain within the Signal Reception Module of the Prokaryotic Enhancer-binding Protein XylR of Pseudomonas putida*

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In the presence of m-xylene, the protein XylR encoded by the TOL plasmid of Pseudomonas putida, activates the α54-dependent promoter Pu. Early activation stages involve the release of the intramolecular repression caused by the signal reception N-terminal (A domain) of XylR on the central module of the protein. A genetic approach has been followed to locate the specific segment within A domain of XylR that is directly responsible for its down-regulation in the absence of inducer, as compared to that involved in effector (m-xylene) binding. For this, a reporter Escherichia coli strain carrying a monocopy transcriptional fusion of Pu to lacZ was transformed with a collection of plasmids encoding equivalent truncated varieties of XylR, consisting of nested and internal deletions throughout the entire A domain. Examination of the resulting phenotypes allowed the assignment of the A domain region near the central activation domain, as the portion of the protein responsible for the specific repression of XylR activity in the absence of m-xylene.

Strains of the genus Pseudomonas harboring the TOL plasmid pWW0 can grow on toluene, m-xylene, and p-xylene as the only carbon source owing to the activity of a complex catabolic pathway (summarized in Fig. 1) that proceeds in two major biochemical steps (Nakazawa et al., 1990). These are determined by two independent operons that become coordinately transcribed when bacteria face pathway substrates such as m-xylene (see Marqués and Ramos (1993) for a review). The main regulator of the system is the so-called XylR protein, a member of the family of prokaryotic enhancer-binding regulators that act in concert with the alternative α factor α54 (Morett and Segovia, 1993; North et al., 1993). In the presence of xylens, XylR bound to upstream sequences activates the Pu promoter of the upper-TOL operon, thus triggering expression of the corresponding catabolic genes (de Lorenzo et al., 1991; Abril et al., 1991). The very early chain of events that translates the presence of xylens into activation of Pu, involve effector-mediated conversion of XylR into a transcriptionally competent form. For this, the inducer binds directly to the N-terminal, signal reception module of XylR termed the A domain (Dalgado and Ramos, 1994; Fernández et al., 1995; see Fig. 1) and triggers the release of the repression caused by this domain on the central portion of the protein that is involved in the contact and activation of the α54-containing RNA polymerase (Pérez-Martín and de Lorenzo, 1995). This notion is based on the observation that deleting the entire A domain of XylR gives rise to a truncated protein that activates constitutively Pu in the absence of aromatic effectors, both in vivo (Fernández et al., 1995) and in vitro (Pérez-Martín and de Lorenzo, 1996). It seems, therefore, that the A domain of XylR has at least two functions: (a) recognition of the aromatic inducers and (b) intramolecular repression. Since these two are obviously connected, the question arises as whether discrete subdomains within the N-terminal module can be assigned to each of them.

To explore the presence and the location of specific portions within the A module of XylR that are accountable for the central domain repression, we chose a reporter system in which the effect of changes in the regulator could be related immediately to a distinct phenotype in activation of Pu. Since all transcriptional control elements can be faithfully reproduced in E. coli, this reporter system employs a derivative of E. coli YMC10 that had been lysogenized with an specialized λ phage containing a transcriptional Pu-lacZ fusion (Pérez-Martín and de Lorenzo, 1995). This strain (E. coli λRSPu) was transformed in vivo with each one of a collection of plasmids bearing truncated xylR alleles that differed only in A domain sequences (Fig. 1). These were generated with a polymerase chain reaction-based strategy (PCR) described in the legends of Figs. 2 and 3. The activity of the truncated products expressed in trans was measured as the accumulation in vivo of β-galactosidase in the presence or absence of the XylR effector m-xylene. Simultaneously to each activity assay, we examined the level of expression of each XylR-derived protein through Western blot assays (Fernández et al., 1995) to ensure that the proteins were produced at similar levels (not shown).

To have a preliminary indication on the portion of the A domain of XylR involved in intramolecular repression, we sought to divide the domain (211 amino acids, Inouye et al., 1988) and Shingler et al. (1993); see Fig. 2) in 6 large segments, that were progressively deleted from the N terminus (Fig. 2). These deletions were generated by amplifying with PCR the sequences of interest with an adequate collection of primers, so that the resulting products were flanked by EcoRI and BamHI sites as specified in the legend to Fig. 1. Transfer of the resulting DNA fragments to the specialized expression vector pPR (Pérez-Martín and de Lorenzo (1995); see legend to Fig. 2) provided a translation initiation sequence and a leading ATG for expression of the six xylR deletion alleles shown in Fig. 2. These were named, respectively, Δ30, Δ120, Δ150, Δ180, Δ210, and Δ226. The corresponding proteins were produced in vivo at levels comparable to those of the wild-type XylR with
Functional Subdomains of the N Terminus of XylR

Figure 1. The TOL system of plasmid pWW0 and domain organization of XylR. The TOL system for degradation of toluene and m-xylene includes two gene clusters, the upper-operator and the meta-operator, as well as two regulatory genes, xylS and xylR, downstream of the meta-operator. The ρ-independent promoters Pu and Ps (underlined) are activated by the cognate activator XylR in the presence of m-xylene, while the Prm promoter is activated by XylS in the presence of benzylate or toluates. Functional domains of XylR are shown expanded, with an indication of the amino acid positions corresponding to the boundaries of each domain (Inouye et al., 1988; Shinger, 1996). These include a signal reception N-terminal module (A domain), the central (C) activation domain, and the D-terminal segment (D domain) containing a helix-turn-helix motif for DNA binding. The lower part of the figure sketches the strategy used to amplify with the polymerase chain reaction (PCR) specific DNA segments corresponding to different portions of the XylR protein for expression of truncated variants of the A domain. These include N-terminal deleted or internally truncated proteins (see legends to Figs. 2 and 3).

Figure 2. Effect of sequential N-terminal deletions through the A domain in the transcriptional activity of XylR. The activity of the N-terminal truncated derivatives of the A module of XylR (211 amino acids, Fig. 1) indicated in the figure was monitored as accumulation of β-galactosidase in the E. coli strain XRSpu, that carried a chromosomal Pu-lacZ fusion. For construction and expression of each of the truncated proteins, the following strategy was pursued. A DNA fragment containing the wild-type XylR sequence was subjected to a PCR reaction using as direct primers (Fig. 1) various oligonucleotides (33-mers) that targeted an EcoRI site to the left of the site of deletion desired. For the reverse priming of the reaction, the same oligonucleotide was used in all cases, that generated a BamHI site following the STOP codon of the XylR sequence. TAG. The amplified products were cloned as EcoRI-BamHI fragments of different sizes at the same sites of vector pPr. This is a derivative of pCG1 (Myers et al., 1987) in which the native Pr promoter of xylR within the TOL plasmid (Fig. 1) has been engineered in front of the same polynucleotide as pTrc99A (Amman et al., 1988), that is led by an NcoI site overlapping a first structural ATG. This allowed all truncated proteins to be expressed through the very same native promoter and translation initiation regions as the wild-type XylR. The use of the direct EcoRI primers in the PCR reaction introduced in all cases amino acid residues EF (corresponding to 5′-GAA TTC-3′), next to the leading methionine of the truncated products. Replacement of the second (Ser) and third (Leu) amino acid residue of the wild-type XylR protein by EF had no effect on protein activity (not shown). For the experiment of the figure, each of the E. coli XRSpu transformants were grown in LB medium (Miller, 1972) at 30°C up to an A600 = 0.5, after which they were exposed, as indicated, to saturating vapors of m-xylene. Accumulation of β-galactosidase (Miller, 1972) was then measured 5 h of induction. The figure for the reporter proteins are indicated with respect to the site of the deletions corresponding to each truncated regulator. The values shown are the average of independent experiments carried out with duplicate samples. The approximate localization of the repression subdomain suggested by the results is indicated on top.
The ability of XyI R alleles carrying the internal deletions within the A domain indicated in the figure was examined as for the sequential N-terminal deletions (Fig. 2). The strategy to generate the internally truncated proteins involved the production by PCR of two restriction fragments that were sequentially assembled in vector pPr (see legend to Fig. 2). The left restriction fragments (flanked by EcoRI-XbaI sites) were produced by amplifying the desired part of the A domain sequence with a direct EcoRI primer and a reverse XbaI primer (Fig. 1). The right restriction fragments were similarly produced with a direct XbaI primer, targeted to the site of interest within the sequence of the A domain, and a reverse BamHI primer at the end of the XyI R sequence (Fig. 1). Their assembly downstream of the Pr promoter in pPr gave rise to the truncated proteins under examination. Accumulation of β-galactosidase by each of the reporter strains, transformed with the corresponding plasmids, was examined as described in the legend to Fig. 2. The hatched portion of the A module shows the position of the repression subdomain.

The A domain of XyI R is the key component of this activator that endows specificity in the response to m-xylene (Delgado and Ramos, 1994; Shingler and Moore, 1994), so that direct effector binding is translated into release of intramolecular repression (Delgado et al., 1995; Pertz-Martín and de Lorenzo, 1995). Although the precise mechanism by which this happens remains unsolved, the data presented in this work suggest that different portions of the A domain have specific roles in the process. It is possible that, similarly to what may happen to NtrC (Fiedler and Weiss, 1995), derepression could involve the dimerization of the N-terminal module in response to the receptor activator. The different predictions raised by this hypothesis and other alternatives are currently under study in our laboratory.

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