Novel Physiological Modulation of the Pu Promoter of TOL Plasmid

NEGATIVE REGULATORY ROLE OF THE TURA PROTEIN OF PSEUDOMONAS PUTIDA IN THE RESPONSE TO SUBOPTIMAL GROWTH TEMPERATURES*

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From crude protein extracts of Pseudomonas putida KT2440, we identified a small protein, TurA, able to bind to DNA fragments bearing the entire Pu promoter sequence of the TOL plasmid. The knock-out inactivation of the turA gene resulted in enhanced transcription initiation from the Pu promoter, initially suggesting a negative regulatory role of TurA on Pu expression. Ectopic expression of TurA both in P. putida and in Escherichia coli reporter strains and transcription in vitro of the Pu promoter in the presence of purified TurA confirmed the TurA repressor role on Pu activity. turA gene inactivation did not significantly alter two well characterized physiological regulations of the Pu expression in routine conditions of cultivation, exponential silencing, and carbon-mediated repression, respectively. However, the growth at suboptimal temperatures resulted in a TurA-dependent increase of Pu repression. These results strongly suggest that a physiological significance of the negative role of TurA on Pu activity could be limitation of the expression of the toluene-degrading enzymes at suboptimal growth temperatures. Therefore, the identification of TurA as Pu-binding protein revealed a novel physiological modulation of Pu promoter that is different from those strictly nutritional described previously.

The number of genes that must be expressed for the aerobic degradation of aromatic compounds is usually high (1), and their well timed silencing increases host bacterium fitness in environmental conditions where the exploitation of the aromatic source is inefficient or unessential for bacterial growth (2, 3). In terms of evolution, to reach such physiological control, specific carbon regulatory devices have supposedly integrated in global networks able to sense the environmental stimuli. One example of this is the degradation of the aromatic hydrocarbons, toluene and m-p-xylene, by the Pseudomonas putida, encoded by two large operons, upper and meta, harbored by the TOL mega-plasmid (for a review, see Ref. 4). Expression from the upper operon is driven by the σ^54-dependent promoter Pu (Fig. 1C), activated at a distance in the presence of aromatic substrates of the catabolic pathway by the toluene-responsive regulator XylR (5, 6), belonging to the family of bacterial enhancer-binding transcriptional activators that act upon the σ^54-containing RNA polymerase (σ^54-RNAP) for reviews, see Refs. 7–10). In addition to the specific response to aromatic substrates, it is conceivable that the TOL system had integrated additional co-regulatory devices to avoid the expression of its energetically expensive pathway, under unfavorable physiochemical conditions (temperature, pH, osmolarity, etc.) and/or in the presence of more appetizing carbon and energy sources than toluenes. The influence of the physiochemical conditions on Pu expression has not been extensively characterized. On the contrary, the expression performance of Pu appears to be strictly dependent on the energetic status of the cell (2, 11). An abundance of nutrients results in strong repression of the Pu promoter during the exponential growth phase, a phenomenon that has been referred to as exponential silencing (12). On the other hand, the presence of carbon sources, such as glucose, gluconate, or α-chetoglutarate, exerts a form of carbon-mediated inhibition on Pu activity (13, 14). Furthermore, the small signal molecule (p)pGpp may participate in the Pu nutrient-responsive regulatory network (15). By contrast, the search for Pu regulatory protein components, which may be the effector molecules of environmental stimuli, has not been examined in any great depth to date. In this study, we aimed to identify novel protein factors involved in the physiological and/or environmental regulation of Pu expression. We envisaged the existence of host protein factor(s) that possibly played a co-regulatory role through direct protein-Pu DNA interactions. We decided to screen the P. putida proteome for proteins able to interact with Pu DNA. Using this approach, we identified a small Pu-interacting protein, which we named TurA. It shares in silico a degree of structural similarity with H-NS, the major protein constituent of the Escherichia coli nucleoid, widespread in Gram-negative bacteria (for reviews, see Refs. 16 and 17). H-NS is involved in the condensation of the bacterial chromosome (18, 19) and pleiotropically modulates the transcription of many environmentally regulated genes (e.g. by osmolarity, temperature, anaerobiosis, pH, growth phase, etc.), including those related to environment adaptation and/or virulence (16, 20, 21). H-NS inhibits transcription at most of the target promoters, and hns gene inactivation generally results in increased expression of the cognate

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§ The abbreviations used are: RNAP, RNA polymerase; DTT, dithiothreitol; IHF, integration host factor; X-gal, 5-bromo-4-chloro-3-indolylyl-β-D-galactopyranoside.
gene products. Furthermore, a number of genes negatively affected by H-NS are regulated by specific positive transcription factors. In this study, we show that the knock-out of the turA gene leads to increased expression of the Pu promoter. Furthermore, we present evidence, both in vivo and in vitro, demonstrating that TurA strongly represses XylR-mediated transcription of the Pu promoter. Inactivation of turA gene does not affect either exponential silencing or carbon-mediated inhibition of Pu expression. On the contrary, we present evidence showing that TurA-mediated repression of Pu activity can strengthen at suboptimal growth temperatures. Therefore, this study revealed a novel physiological modulation of Pu promoter that is different from those strictly nutritional described previously. Furthermore, the identification of TurA also represents a novelty in the field of the regulation of aromatic degradation pathways. In fact, to date, only protein regulatory components involved in the specific response to the aromatic inducers have been described.

**MATERIALS AND METHODS**

**Bacterial Strains, Plasmids, and General Procedures**—The *P. putida* strain MAD1 (Pu- lacZ, xylR) is a derivative of *P. putida* KT2442 (ribitol-2-epimerase derivative of *P. putida* KT2440) (22) carrying a chromosomal Pu-lacZ fusion along with the xylR gene assembled within a tellurite resistance mini-transposon (23). MAD1 turA:: Km, the MAD1 derivative carrying a Km\(^n\) cassette insertion in the PuI366 locus of KT2440 genome sequence (turA gene), was constructed by allelic exchange with a Km\(^n\)-inserted allele as follows. The DNA region containing the PuI366 locus was amplified as an 832-bp BamHI-XbaI fragment from *P. putida* KT2440 genome by PCR utilizing the direct primer 5'-CGGGATCC/BamHI underlined)-GCC-GCCTATTCCTAGGCCTGCACC-3' and the reverse primer 5'-CTGCTGTAAGA/XbaI underlined-CGAAAAAGGGCGGACAGCAGCTCC-3'. This 832-bp fragment was cloned into the pCR® 2.1-TOPO® plasmid vector (Invitrogen), giving rise to pTBA1 plasmid. For an efficient selection of the integration event of a Km\(^n\) cassette into the turA gene, the pTBA1 native Km\(^n\) gene was deleted by digestion with both BglII and NcoI enzymes, treatment with Klenow, and religation, giving rise to pTBA2 plasmid. The plasmid pTBA::Km, carrying a Km\(^n\) cassette into the turA gene, was constructed by digestion of pTBA2 DNA with BclI, cutting at the third codon of the turA gene, was purified as described previously (30). For TurA purification, *E. coli* DH5a strain cells harboring pUC- turA were grown overnight at 37 °C in 200 ml of LB medium, collected by centrifugation at 8000 × g for 10 min, resuspended in 10 ml of lysis buffer (20 mM sodium phosphate buffer, 4% glycerol, pH 6.8), and disrupted in a French press cell. Cell debris was separated from the soluble protein crude extract by centrifugation at 20,000 × g for 30 min at 4 °C. About 600 μg of soluble proteins were separated by 15% polyacrylamide SDS-PAGE and stained with Tangerine Green (Molecular Probes Inc.). Bands corresponding to TurA were cut out of the SDS-PAGE gel and eluted by incubation for 16 h at room temperature in elution buffer (50 mM Tris-HCl, pH 7.9, 100 mM EDTA, 0.1% SDS, 5 mM DTT, and 150 mM NaCl). To remove SDS and allow renaturation, the eluted TurA protein was dialyzed in Spectra- Por Micro-Dispalyzer tubes at 4 °C for 2 h against dialysis buffer (50 mM Tris-HCl, pH 7.9, 100 mM EDTA, 50 mM NaCl, 5 mM DTT, 0.1% Nonidet P-40, and 5% glycerol). The purity of the TurA preparation was estimated in SDS-PAGE stained with Brilliant Blue G- Colloidal Concentrate (Sigma).

**In Vitro Transcription Assays**—The linear template for in vitro transcription of Pu promoter was obtained as a 310-bp Smal-HincII restriction fragment from the pUC41I Pu-lacZ plasmid. The transcription reactions were carried out on a total volume of 25 μl of transcription buffer containing 35 mM Tris acetate, pH 8.0, 5 mM MgAc2, 70 mM KAc, 20 mM NH4Ac, 1 mM DTT. The DNA template, added at the buffer to a final concentration of 40 μM, was incubated at 37 °C for 20 min with 4 μM ATP, 50 μM core RNAp, 150 μM dNTPs, 100 μM XyIβA, and 600 units of RNAP. Pu-TurA concentrations of 5-3.5, and 0.5 μg were used. Transcription reactions were initiated by adding a mixture of ATP, CTP, GTP (400 μM each), UTP (50 μM), and [α-32P]UTP (5 μCi; 3000 Ci/ mmol). After incubation for 10 min at 37 °C, the reactions were stopped by the addition of 25 μl of STOP mix containing 0.1 M EDTA and 4 M
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RESULTS

Screening for Novel Regulatory Proteins of the Host Strain

P. putida KT2440 Able to Bind to the Pu Promoter of the TOL Plasmid—To identify P. putida KT2440 proteins involved in Pu co-regulation, we aimed to screen for novel Pu DNA-protein interactions by protein chromatography coupled with South-Western blotting using a radioactive Pu probe. To this end, a crude cell extract derived from an overnight culture of the TOL plasmid host strain P. putida KT2440 was fractionated through binding to a heparin affinity chromatography column, eluted from the heparin column by concentrations of NaCl ranging from 0.82 to 0.95 M. Among the proteins of these fractions collected along the elution gradient were analyzed by 10% SDS-PAGE for a longer period of time. As shown in Fig. 1A, this procedure confirmed that the Pu DNA could interact with a protein species of about 15 kDa. However, the 15-kDa band of Fig. 1A could be resolved into two protein species of very similar molecular weights, which we initially called P15 and P16, respectively. It is interesting to note that P16 alone could interact efficiently with labeled Pu DNA in a South-Western blotting assay (Fig. 1B).

Identification and in Silico Analysis of TurA Protein—The amino acid sequence of P16 amino-terminal end was determined, searched through the KT2440 deduced protein data base at The Institute for Genomic Research (TIGR) Comprehensive Microbial Resource (31, 32), and found at the amino-terminal of a small deduced protein of 125 amino acids, accession number PP1366, annotated as sharing a high degree of similarity with the transcriptional regulator MvaT, P16 subunit (33). Standard BLAST search (34) also found in KT2440 genome four open reading frames (loci PP3765, PP0017, PP3693, PP2947, respectively) that code for protein paralogues of P16. Furthermore, P16 orthologue proteins, organized in paralogue pairs, can be also found in Pseudomonas aeruginosa and Pseudomonas fluorescens genomes. No significant sequence similarity in bacterial species not belonging to the genus Pseudomonas was found. To extend our search of similarities from primary to tertiary structure, we adopted predictive software packages able to detect structural similarity between a query protein and experimentally determined structural domains. Firstly, preliminary architecture domain analysis of P16 both by SMART server and by COILS program (35–37) predicted a structure that resembled the E. coli nucleoid-associated protein H-NS. Secondly, the combined use of ORFeus, a profile-profile comparison program (38), and PSI-BLAST search (39) validated the structural similarity between the P16 protein and the cluster of orthologous groups (COGs) COG2916 (40), which was annotated as an H-NS-like protein family. Thirdly, this structural similarity was further confirmed by confident transitive similarity searches conducted with ORFeus on protein families extracted from Part B of the Pfam data base (41).

Since the data presented (see below) strongly indicated that P16 protein binds to the Pu promoter and represses its activity, we called P16 “TOL upper operon repressor A” (TurA). The amino acid sequence of P15 amino-terminal end was also determined. Interestingly, it corresponded to the amino-terminal of P16 paralogue PP3765 (see above). This could explain the tight similarity of molecular weights and elution profiles in heparin affinity chromatography, respectively, between P15 and P16. Inactivation of the PP3765 gene did not influence Pu activity (data not shown). However, by analogy with TurA, we named P15 TurB.

Deficiency of TurA Protein Causes an Increase in Pu Promoter Activity at the End of the Exponential Silencing Phase—To determine the role of TurA protein in Pu regulation, we generated, by kanamycin-resistance (KmR) cassette insertion, the knock-out mutant allele of the turA gene in the P. putida KT2442 derivative strain MAD1 (KT2442::Pu-lacZ, xylR+), which harbors both a Pu-lacZ transcriptional fusion
and a xylR gene stably integrated in the chromosome (23). Fig. 2A shows the comparison of β-galactosidase accumulation by MAD1 and isogenic MAD1 turA::kmR strains bearing a Pu-lacZ transcriptional fusion, stably inserted into the chromosome, were grown in LB medium at 30 °C until A600 of 0.1 and then divided into two subcultures. Saturating toluene vapors were supplemented in one of them to induce Pu expression. Incubation at 30 °C was then continued for each subculture. Accumulation of β-galactosidase (β-gal) activity expressed by the cells of each subculture after A600 = 0.1 is shown as an average of samples taken during three independent experiments. A Western blot with anti-TurA antibodies on culture samples taken in the mid-logarithmic phase is shown in the inset. As shown in B, cultures of MAD1 turA::kmR, harboring either pVLT31 or pVLT31-turA, were grown in LB medium supplemented with 20 µg/ml tetracycline and then induced with saturating toluene vapors and monitored for β-galactosidase accumulation as in A.

Fig. 2. Effects of inactivation or overexpression of turA gene on Pu activity. As shown in A, cultures of both MAD1 and isogenic MAD1 turA::kmR strains bearing a Pu-lacZ transcriptional fusion, stably inserted into the chromosome, were grown in LB medium at 30 °C until A600 of 0.1 and then divided into two subcultures. Saturating toluene vapors were supplemented in one of them to induce Pu expression. Incubation at 30 °C was then continued for each subculture. Accumulation of β-galactosidase (β-gal) activity expressed by the cells of each subculture after A600 = 0.1 is shown as an average of samples taken during three independent experiments. A Western blot with anti-TurA antibodies on culture samples taken in the mid-logarithmic phase is shown in the inset. As shown in B, cultures of MAD1 turA::kmR, harboring either pVLT31 or pVLT31-turA, were grown in LB medium supplemented with 20 µg/ml tetracycline and then induced with saturating toluene vapors and monitored for β-galactosidase accumulation as in A.

and a xylR gene stably integrated in the chromosome (23). Fig. 2A shows the comparison of β-galactosidase accumulation by MAD1 and MAD1 turA::KmR strains, respectively, when cultured in rich LB medium, with and without toluene induction. Growth in LB medium in the absence of an inducer resulted in no or very low, basal expression of Pu (23). In the case of the toluene-supplemented cultures, both induction curves, wild-type and mutant, displayed the typical exponential silencing phenomenon, consisting of very low levels of Pu activity during exponential growth followed by rapid induction at the onset of the stationary phase (12). However, in terms of the magnitude of the Pu expression after the exponential silencing, MAD1 and MAD1 turA::KmR behaved differently. In fact, after exponential silencing, the expression curves of MAD1 and MAD1
TurA:KmR diverged consistently, with levels of β-galactosidase accumulation three times higher in the mutant strain. Thus, the deficiency of TurA protein revealed unexpected repression on Pu that appeared to be different from the exponential silencing phenomenon described previously (12). To test whether TurA might be involved in the regulatory cascade behind the carbon catabolite repression exerted by glucose on Pu that was shown to be distinct from Pu exponential silencing (11, 14), we examined the Pu performance in vivo in both MAD1 and MAD1 turA::KmR when cultured in M9 minimal medium, supplemented with 0.2% casamino acids as the main carbon source, in the presence and in the absence of 10 mM glucose. The results of these experiments (not shown) clearly suggested that TurA does not participate in the mechanism of carbon catabolite repression of Pu by glucose. In fact, the higher β-galactosidase Pu expression levels in the MAD1 turA::KmR derivative continued to be down-regulated by glucose administration to the same extent as in the parental MAD1.

The Ectopic Expression of TurA Silences the Pu Promoter Both in P. putida and in E. coli—To directly assess the negative role of TurA in Pu regulation, we set out to monitor the Pu activity in the MAD1 turA::KmR strain, transformed with either the lacIPRmu∥-based broad host range expression vector pVLT31 (26) or its TurA-expressing derivative pVLT-turA, with or without toluene administration. As shown in Fig. 2B, the amount of TurA expressed from uninduced Pmu of pVLT-turA could completely silence the toluene-mediated increase of Pu activity at the onset of the stationary phase. Furthermore, to obtain stronger evidence of direct TurA involvement in Pu regulation, the influence of TurA expression on Pu activity was also evaluated in the heterologous genetic background of CC118 Pu-lacZ/pACYCxylR, a XylR-expressing E. coli derivative harboring in the chromosome the same Pu-lacZ transcriptional fusion as MAD1, transformed either with pVLT31 or with pVLT-turA. To prevent a possible less efficient TurA expression in E. coli, we set out to monitor Pu activity also in the presence of isopropyl-1-thio-β-D-galactopyranoside. The quantitative evaluation of Pu expression along the growth of CC118 Pu-lacZ/pACYCxylR pVLT-turA was hindered by the fact that TurA expression resulted in extremely long growth lags following inoculation of fresh liquid medium. Thus, we assayed Pu activity by the amount of blue pigmentation accumulated by cell spots grown on LB plates supplemented with X-gal. As shown in Fig. 3 (lanes 1, 3, 5, and 7), CC118 Pu-lacZ/pACYC-xylR cells harboring pVLT31 displayed a comparable intense blue pigmentation both in the absence and in the presence of toluene administration. This can be attributed to a saturating accumulation of blue pigmentation during prolonged growth on agar surface, even in the case of uninduced Pu activity, which was at least 10-fold lower when tested by standard Miller’s assay on liquid cultures (data not shown). However, both toluene-independent and toluene-stimulated Pu activity could be repressed by TurA expression (Fig. 3, lanes 2, 4, 6, and 8). Apparently, Pmu promoter induction could improve TurA expression from pVLT-turA in E. coli since isopropyl-1-thio-β-D-galactopyranoside addition seemed to enhance the repression of Pu activity, although to a limited extent only. These results represent further evidence that TurA plays a negative regulatory role on Pu activity. Furthermore, the fact that TurA repression on Pu activity can be reproduced in E. coli strongly corroborated the notion that TurA directly modulates the Pu activity. Finally, these results also suggested that Pu activity modulation by TurA could be achieved by the fine-tuning of TurA abundance in vivo.

TurA Protein Represses XylR-mediated Activation of the Pu Promoter in Vitro—The results presented above indicated that TurA protein directly represses Pu activity. To assess this rigorously, we purified TurA protein (Fig. 4A) to evaluate its influence on the transcription of the Pu promoter in vitro. With this objective, the 310-bp Smal-HincII DNA fragment, spanning the Pu sequence from positions −203 to +93, was transcribed in vitro by XylRΔA-activated σ70-RNAP (23, 30), in the presence of increasing concentrations of purified TurA, 2, 3.5, and 7 nM, respectively. As a control, to monitor possible generic effects of purified TurA on transcriptional activity, RNAI (42) was transcribed in the presence of the same gradient of TurA concentrations. As shown in Fig. 4B, the addition of TurA at 7 nM could almost completely repress Pu transcription. The same TurA concentration could not repress the transcription of RNAI by σ70-RNAP when the pUC18-based plasmid pEZ9 was used as a template. The pattern of the TurA-mediated repression did not have linear correlation with TurA concentration. In fact, at TurA concentrations less than or equal to 3.5 nM, the production of the 93-nucleotide transcript did not decrease significantly (Fig. 4B, lanes 2–4). On the contrary, the addition of twice the amount of TurA (7 nM) was sufficient to achieve a strong reduction of Pu transcription (Fig. 4B, lanes 2 and 5). Therefore, the behavior of purified TurA protein appeared to be consistent with the notion presented above, that TurA plays a negative regulatory role on Pu expression.

TurA Can Modulate Pu Expression in Response to Suboptimal Growth Temperatures—None of the results presented above directly suggested a role for the negative modulation of TurA on Pu activity. Indirectly, since TurA inactivation did not affect either exponential silencing or glucose repression, it indicated that TurA could play a role on Pu activity not strictly correlated to availability of more appetizing carbon and energy sources than toluenes. Therefore, we speculated, also on the basis of the putative structural TurA similarity with H-NS (see above), which modulates the transcription of many environmentally regulated genes, that TurA could co-regulate the expression of the toluene-degrading enzymes in response to suboptimal physiochemical conditions. To address this issue, we set out to compare β-galactosidase accumulation by MAD1 and MAD1 turA::KmR strains, respectively, when cultured with...
with and even higher than those at 30 °C. Taken together, these results strongly suggested that the TurA-mediated repression of Pu activity can strengthen in response to suboptimal environmental physicochemical conditions, such as, for example, a temperature 14 °C lower than the optimal for growth.

**DISCUSSION**

The main protein components of the toluene-responsive genetic switch assembled at the Pu promoter are the σ^{54}-dependent activator XylR and σ^{54}-RNAP. It is currently believed that, in the regulatory cascade leading from toluene sensing by XylR protein to the escape of σ^{54}-RNAP, there are at least three limiting multistep events able to influence the rate of transcription initiation: (i) the process of intramolecular derepression of XylR on toluene binding that permits the switch from an inactive to an active form capable of establishing productive contacts with σ^{54}-RNAP (6, 43) (for this step in the related DmpR protein, see Refs. 44–47); (ii) the closed complex formation of σ^{54}-RNAP with the Pu promoter DNA that involves both σ^{54} and a subunits of σ^{54}-RNAP (48–50); and (iii) the open complex formation that requires contacts between a toluene-activated XyR and the σ^{54} subunit of σ^{54}-RNAP, both prebound at a distance to upstream activating sequences and −12/−24 motif, respectively (51). It was already known that at least two of these three limiting steps, the formation of both closed and open complex, are positively influenced by the promoter geometry imposed by the IHF protein (48–51). Limiting steps of transcription initiation are potential targets for gene regulation (52). However, IHF seems to provide a uniquely structural aid for overcoming these limiting steps and is not a key regulator involved in the physiological control of Pu expression (53). Therefore, other factors must contribute to the fine modulation of toluene-responsive Pu expression by environmental stimuli.

For these reasons, we aimed to identify novel modulator proteins of Pu activity. To do this, we envisaged that some of the putative Pu modulators may have a regulatory role through direct protein-Pu DNA interactions. Therefore, we fractionated the P. putida-soluble proteins by heparin affinity chromatography, able to enrich the DNA-binding proteins, and then screened the different protein fractions for their ability to bind to the Pu DNA by South-Western blotting. Using this method, we identified a small protein, TurA, that could interact with the Pu promoter DNA. Moreover, we presented evidence that strongly supports the notion that TurA-Pu DNA interactions participate in direct negative modulation of Pu activity. Firstly, in vivo, (i) the inactivation of the turA gene gave rise to an increase in Pu expression (Fig. 2A), and (ii) the overexpression of turA both in P. putida and in E. coli resulted in strong decrease of Pu activity (Figs. 2B and 3). Secondly, in vitro, purified TurA is able to efficiently repress Pu transcription by XyIR-activated σ^{54}-RNAP (Fig. 4).

It is interesting to note that our results suggest that the TurA protein has no role in either exponential silencing or glucose repression of Pu activity (11). Therefore, with this study, for the Pu physiological tuning, we identified a novel regulatory device that is different from those responding to growth phase and/or nutrient availability. Our results clearly show that, in routine conditions of cultivation, Pu-induced expression is higher in the absence of TurA (Fig. 2A). This suggests that Pu-induced expression might be maintained by TurA at levels below those that can be sustained purely by the currently available cellular quantities of XylR, IHF, and σ^{54}. TurA may have the role of limiting an otherwise unessential Pu extra-expression, and it may possibly produce efficient rates of promoter switching-off when toluene runs out. Moreover, we cannot rule out the possibility of a restrictive role of TurA (referred to previously as "restrictor") to either prevent...
or at least minimize both spurious (i.e. by other σ54-dependent activators) and gratuitous (i.e. not by toluene addition) Pu activations (54). Furthermore, we present evidence that TurA-mediated repression of Pu activity can strengthen at suboptimal growth temperatures. Therefore, under suboptimal conditions of cultivation, the role of TurA could be limited by the expression of the toluene-degrading enzymes. At suboptimal temperatures, the physiological significance of such modulation could be the prevention of (i) energy-consuming biosynthesis of a large set of toluene-degradative enzymes under unfavorable conditions, (ii) toxicity associated with the activity of the degradative enzymes, and (iii) enzyme bottlenecks leading to accumulation of toxic intermediates of the toluene degradation pathway. Therefore, our results strongly suggest that TurA can play the role of environmental rather than strictly nutritional modulator of Pu activity.

BLAST search analysis indicated that TurA belongs to the MvaT protein family, identified by sequence similarity in the Pseudomonas species (55). Remarkably, TurA matches the behavior of the negative transcription factor of MvaT in P. aeruginosa (55). However, in P. aeruginosa, MvaT appears to modulate the expression of a different class of genes (namely, quorum sensing-controlled virulence genes) and participates in a growth phase-dependent control system (55). In this study, we extended the comparative analysis to the structural features of TurA. This analysis indicated that TurA and, possibly, the other members of the MvaT family are structurally related to the nucleoid-associated protein H-NS (16, 17). Surprisingly, no gene product with significant sequence similarity with H-NS can be identified by BLAST search in the currently available Pseudomonas genome sequences. Based on these observations, it can be suggested that the members of the MvaT family have roles in the Pseudomonas species, analogous to H-NS-like proteins in other Gram-negative species (17). Thus, the functional features of both P. putida TurA and P. aeruginosa MvaT appear to match the H-NS behavior as a global modulator of gene expression principally through a negative effect on transcription, and acting, in many cases, on environmental signal-responsive promoters that are positively regulated by specific transcription factors (16).

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