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Growth Phase-Dependent Transcription of the σ^{54}-Dependent Po Promoter Controlling the *Pseudomonas*-Derived (Methyl)phenol *dmp* Operon of pVI150

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Transcription from *Pseudomonas*-derived −24, −12 Po promoter of the pVI150-encoded *dmp* operon is mediated by the σ^{54}-dependent DmpR activator in response to the presence of aromatic pathway substrates in the medium. However, global regulatory mechanisms are superimposed on this regulatory system so that the specific response to aromatic effectors is absent in cultures until the stationary phase is reached. Here we genetically dissect the system to show that the growth phase response is faithfully mimicked by a minimal system composed of the *dmpR* regulatory gene and the Po promoter regulatory region and can be reproduced in heterologous *Escherichia coli*. Using this system, we show that the growth phase-dependent DmpR-mediated response to aromatic compounds is limited to fast-growing cultures. Thus, during exponential growth of cultures in minimal media containing different carbon sources, the response to aromatics is immediate, while the response is suppressed in cultures grown on rich media until the exponential-to-stationary phase transition. Elements known to be involved in the DmpR-mediated transcription from Po were analyzed for the ability to influence the growth phase response. Most dramatically, overexpression of DmpR was shown to completely abolish the growth phase response, suggesting that a negatively acting factor may mediate this level of regulation. The possible mechanism of action and integration of the specific regulation of the *dmp* operon-encoded catabolic enzymes with the physiological status of the bacteria are discussed.

Pseudomonas sp. strain CF600 harboring the IncP-2 catabolic megaplasmid pVI150 can efficiently grow on phenol, monomethylated phenols, and 3,4-dimethylphenol as sole sources of carbon and energy (40). The 15 structural genes for the enzymes of the catabolic pathway are located within the plasmid-encoded *dmp* operon (Fig. 1A) (43). Complete mineralization of pathway substrates is achieved by hydroxylation of the phenolic ring to form catechol, followed by a conversion to Krebs cycle intermediates pyruvate and acetyl coenzyme A via the sequential steps of the *meta*-cleavage pathway (reviewed in reference 35). Transcription of the *dmp* operon from the operon promoter, Po, is tightly regulated by the divergently transcribed *dmpR* gene product so that the enzymes of the pathway are expressed only in the presence of pathway substrates or structural analogs (Fig. 1) (33, 39, 41).

DmpR belongs to the prokaryotic enhancer-binding family of σ^{54}-dependent regulators, which function to positively control transcription from −12, −24 promoters recognized by RNA polymerase utilizing the alternative sigma factor, σ^{54}, encoded by *rpoN* or its analogs (reviewed in references 29 and 30). Close physical contact between the regulators bound to the enhancer-like sequences (also known as upstream activation sequences) and the cognate promoter-bound σ^{54}-RNA polymerase involves looping out of the intervening DNA. This process has, in some cases, been shown to be assisted by binding of the DNA-bending protein integration host factor (IHF) or by intrinsic bends (reviewed in reference 34).

Like other members of the σ^{54}-dependent family, DmpR has a distinct domain structure, with each module performing a specific function(s) in signal reception, transcriptional activation, and DNA binding (Fig. 1B; reviewed in reference 38). The central C domain of the regulators is the mostly highly conserved domain in the family and contains a nucleotide-binding motif. This domain mediates ATP binding and hydrolysis essential for transcriptional activation (1, 45) and is also believed to encompass the region involved in interaction with σ^{54}-RNA polymerase. The carboxy-terminal D domain contains a helix-turn-helix DNA-binding motif analogous to those found in a number of transcriptional activators and repressors. The amino-terminal signal reception A domains are tethered to the C domains by means of a short flexible B domain (Q-linker).

Many members of the family are part of so-called two-component regulatory systems in which the activity of the regulator is controlled by the status of a conserved Asp residue of the A domain that is phosphorylated via a sensor histidine kinase in response to an environmental signal. DmpR, however, belongs to a different mechanistic subgroup, including Xy1R (8) and Fh1A (21), that responds directly to small effector molecules (reviewed in reference 38). Chimeric proteins (41) and isolation of effector specificity mutants (33, 42) have been used to show that the specificity of activation of DmpR by its aromatic effectors resides within its A domain. Direct interaction of DmpR with its effector molecule allows expression of its otherwise repressed ATPase activity (42). Repression of the C-domain-mediated ATPase activity is relieved by deletion of the A domain of DmpR, leading to a constitutively active, effector-independent regulator (42).

In addition to the specific DmpR-mediated control mechanism described above, at least one global regulatory system is superimposed on the expression of the *dmp* operon-encoded catabolic enzymes. In this work, we demonstrate that it is the action of effector-activated DmpR on Po that is subject to modulation by the physiological status of the cell, since DmpR

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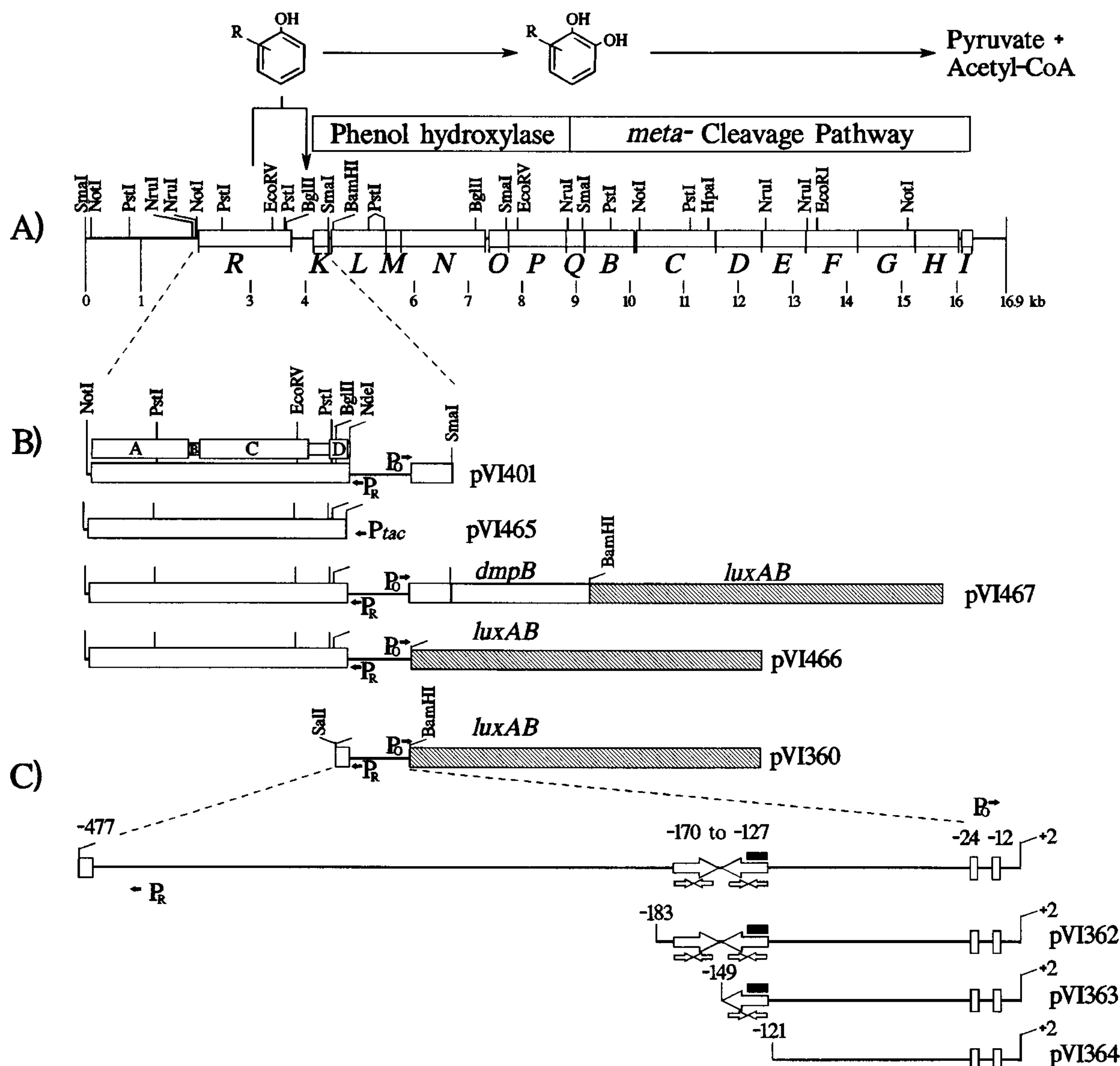

FIG. 1. (A) Restriction map of a 16.9-kb pVI150-derived DNA fragment encoding the *dmp* system. The locations and extents of the divergently transcribed *dmpR* regulatory gene and the genes of the *dmp* operon (*dmpKLMNOPQBCDEFGHI*) are indicated. The upper part illustrates the biochemical route for (methyl)phenol catabolism via hydroxylation catalyzed by the *dmpKLMNOP*-encoded enzyme and a subsequent *meta*-cleavage pathway encoded by *dmpQBCDEFGHI*. Horizontal arrows indicate the biochemical route, while the vertical arrow indicates that phenol is also required to activate DmpR and thus transcription from the operon promoter. Acetyl-CoA, acetyl coenzyme A. (B) Schematic representation of the extent of insert DNA carried on key plasmids used in this study. The domain structure of the 563-amino-acid DmpR is also shown above its coding region. The functions of domain A (residues 1 to 211), B (residues 212 to 233), C (residues 234 to 472), and D (residues 519 to 558) are discussed in the text. P_R, P_O, and P*tac* denote the *dmpR*, *dmp* operon, and vector-located promoters, respectively. (C) Schematic representation of the Po regulatory region present in different plasmids. The locations of the −24 TGGC, −12 TTGC promoter and the WATCAANNNNTTR core consensus IHF-binding sequence (black bars) (18) are shown. The open arrows indicate inverted repeat sequences; each half of the large inverted repeat, which is believed to be encompass the binding sites for DmpR (39), is itself an inverted repeat (14). Coordinates are given relative to the transcriptional start of the*dmp* operon mRNA (39).

expression is constant over the growth curve. In rapidly growing cultures, transcriptional activation is not observed until the exponential/stationary phase transition, while in slowly growing cultures, the response to aromatics is immediate. Here we dissect the minimal components of the *dmp* system needed to faithfully reproduce the growth phase response and present evidence suggesting that growth phase-dependent regulation involves a factor acting negatively at the level of DmpR activity during exponential phase.

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains and plasmids used are listed in Table 1. Plasmids were constructed by standard recombinant DNA techniques (37), and the extent of insert DNA is illustrated in Fig. 1. Plasmid pVI401 harbors the *dmpR* gene expressed from its native promoter with an *Nde*I site manipulated to overlap the ATG initiation codon (33). pVI465 expressing DmpR from the P*tac* promoter was constructed by cloning the *Nde*I-to-*Not*I fragment from pVI401 into pMMB66EH manipulated to contain these sites within the polylinker. Plasmid pVI466 was constructed by regenerating *dmpR* in its native configuration in pVI360, which already harbors the operon promoter region controlling expression of *luxAB*. Plasmids pVI362 to pVI364 were generated by replacing the complete Po regulatory region of pVI360 with deletion derivatives generated by PCR as *Sal*I-to-*Bam*HI fragments. The nucleotide sequence of each PCR-generated fragment was confirmed to ensure that no mutations had been introduced. Plasmid pVI467 contains *dmpR*, expressed from its native promoter, in its wild-type configuration with respect to the Po operon promoter controlling transcription of two promoterless reporter genes, *dmpB* and the *luxAB* gene of *Vibrio harveyi* (32) (Fig. 1). The *dmpB* gene was cloned

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant properties	Reference or source
E. coli strains		
MC4100	RpoS$^+$ strain, Δ(*arg lac*) *araD rpsL ptsF flbB rbsR deoC relA*	44
RH90	RpoS$^-$ strain, *rpoS*::Tn*10* derivative of MC4100	26
S90C	IHF$^+$ strain, Δ(*lac pro*) *rpsL*	4
DPB101	IHF$^-$ strain, *himD*::mini-*tet* derivative of S90C	4
MC1000	Fis$^+$ strain, Δ*lacX74 araD135* Δ(*ara-leu*)*7697 galU galK strA*	5
RJ1617	Fis$^-$ strain, MC1000 *fis*::767	23
P. putida strains		
KT2440	Prototrophic Res$^-$	16
PB2701::Tn*5*	Sm^r, Tn*5* derivative of KT2440	M. Bagdasarian
KT2440::Po-*luxAB*	Chromosomally located Po operon promoter controlling transcription of *luxAB* gene	33
KT2440::*dmpR*	Chromosomally located *dmpR* gene transcribed from its native promoter	39
KT2440::*dmpR*-Po-*dmpB*/*luxAB*	Chromosomally located *dmpR* gene in its native configuration with respect to Po controlling transcription of the *dmpB* and *luxAB* genes	This study
Plasmids		
pUT-mini-Tn*5* Km2	Ap^r Km^r, defective mini-Tn*5* suicide donor plasmid	11
pVLT31	Tc^r, broad-host-range *lac*I^q P*tac* expression vector	10
pFH30	Tc^r, P*tac rpoN* expression plasmid, pVLT31 based	5
pMMB66EH and -HE	Ap^r, broad-host-range *lac*I^q P*tac* expression vectors	17
pVI397	Ap^r, pMMB66HE-based broad-host-range vector deleted for *lac*I^q and P*tac*	41
pVI150	Hg^r, wild-type catabolic plasmid of *Pseudomonas* sp. strain CF600	40
pVI360	Ap^r, −477 to +2 Po region controlling transcription of *luxAB*	41
pVI362	Ap^r, −183 to +2 Po region controlling transcription of *luxAB*	This study
pVI363	Ap^r, −149 to +2 Po region controlling transcription of *luxAB*	This study
pVI364	Ap^r, −121 to +2 Po region controlling transcription of *luxAB*	This study
pVI401	Ap^r, *dmpR* expressed from its native promoter, pVI397 based	33
pVI465	Ap^r, *dmpR* expressed from P*tac*, pMMB66EH based	This study
pVI466	Ap^r, *dmpR* gene in its native configuration with respect to Po controlling transcription of *luxAB*, pVI397 based	This study
pVI467	Ap^r Km^r, *dmpR* gene in its native configuration with respect to Po controlling transcription of the *dmpB* and *luxAB* genes, pUT-mini-Tn*5* Km2 based	This study

downstream of the *dmpR*-Po fragment, at the *Sma*I site +288 relative to the transcriptional start site, and subsequently transcriptionally fused to the *luxAB* genes. A fragment spanning the entire region was cloned into the unique *Not*I site within the defective transposon, mini-Tn*5* Km2 (11) carried on a suicide plasmid, to generate pVI467. The transposon carrying the insertion was introduced into the chromosome of *Pseudomonas putida* KT2440 to generate KT2440::*dmpR*-Po-*dmpB*/*luxAB* as previously described (11).

Culture conditions. *Escherichia coli* strains were grown at 37°C, and *P. putida* strains were grown at 30°C. Luria broth (LB) and M9 salts (37) were used as rich and minimal media. Minimal M9 salts were supplemented with the following carbon sources: Casamino Acids at 0.2% (wt/vol) or 10 mM glucose, gluconate, succinate, or acetate. Prior to assay determinations of reporter genes, cells were grown overnight in the indicated media containing appropriate antibiotics for strain and resident plasmid selection. To ensure balanced growth, cultures were then diluted 1:30, grown for 2 h, diluted, grown into early to mid-exponential phase, and then diluted once more before initiation of the experiment. IPTG (isopropyl-β-D-thiogalactopyranoside) was added at the indicated concentrations after dilution of overnight cultures when induction of the P*tac* promoter was required. Unless otherwise stated, the effector 2-methyphenol was added at a final concentration of 2 mM when the experiment was initiated.

Assay procedures. Luciferase assays of the *luxAB* gene product were performed on whole cells, using a 1:2,000 dilution of the decanal substrate as described previously (32). Data for light emission are the average of triplicate determinations from at least two independent experiments, values for which did not differ by more than 20%. The specific activity (in units per milligram of protein) of the catechol 2,3-dioxygenase (C23O) *dmpB* gene product was determined as previously described (31). Cells from 2 to 40 ml of culture (depending on the A_{650}) were collected, washed in ice-cold 0.1 M phosphate buffer (pH 7.5), resuspended in the same buffer, and sonicated. Cell debris was removed by centrifugation to give the crude extract used in the assays. The variability between duplicate samples did not exceed 10%.

Western blot (immunoblot) analysis. Crude extacts were prepared as described above, and proteins were separated by sodium dodecyl sulfate–11% polyacrylamide gel electrophoresis. Proteins were transferred to nitrocellulose filters and Western blotted with an anti-DmpR serum as previously described (42). Anti-DmpR-decorated bands were revealed by using ^{125}I-protein A and quantified with a Molecular Dynamic PhosphorImager.

RESULTS

The *dmp* operon is growth phase regulated. Previous determinations of enzyme levels encoded by the genes of the *dmp* operon had suggested that maximal expression was achieved subsequent to exponential growth. To determine the kinetics of expression of the *dmp* operon genes on the native plasmid pVI150 (one to two copies per cell [33]), expression of the *dmpB* gene product, C23O, was monitored in *P. putida*. Cells were grown in LB containing the effector 2-methylphenol to activate DmpR and induce the system, and C23O was monitored throughout the growth curve. As can be seen in Fig. 2A, expression of C23O was restricted until late exponential/early stationary phase, approximately 4 h after addition of effector. In a similar experiment, cells were grown under the same conditions but effector was excluded from the medium. Instead, samples were removed at different time points and exposed to effector for 2 h prior to C23O determinations. The results in Fig. 2A show that this treatment resulted in an expression curve similar to that obtained when effector was present throughout the experiment; i.e., expression was still growth phase responsive. These results demonstrate that it is the physiological status of the cells rather than the length of time of exposure to the effector that determines the expression level.

DmpR is constitutively expressed. One possible mechanism mediating the growth phase response may be regulated expression of the specific DmpR regulator itself. To test this possibility, protein from crude extracts of cultures at different time points was subjected to Western analysis and the level of

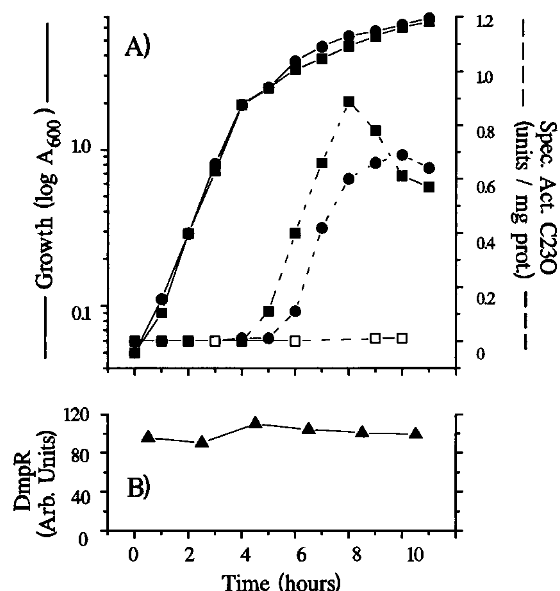

FIG. 2. (A) PB2701::Tn*5*(pVI150) growth and C23O expression in the presence (filled symbols) or absence (open symbols) of 2 mM 2-methylphenol. Cells were grown in LB in the continuous presence of effector (squares), or samples were removed and exposed to effector for 2 h prior to C23O determinations (circles). Results are representative of two independent experiments. (B) DmpR levels as determined by quantitative Western blot analysis of proteins from cells grown in the continuous presence of effector as described above.

DmpR was quantified (see Materials and Methods). The results shown in Fig. 2B demonstrate that DmpR is present at approximately the same level in all phases of the growth curve. This finding is consistent with our observation that transcription of *dmpR* on pVI150 is at a low constitutive level throughout the growth curve, as monitored by a transcriptional fusion of the *luxAB* genes (data not shown). Hence, we conclude that in the wild-type system, that variation of the expression levels of DmpR does not mediate the growth phase response.

Growth phase regulation in a minimal system. The catabolic plasmid pVI150 exceeds 200 kb in size (40). To investigate the minimal plasmid-encoded factors required to reproduce growth phase regulation, we used a previously constructed *P. putida* strain, KT2440::*dmpR*, in which DmpR is expressed from its native promoter in monocopy on the host chromosome. Plasmid pVI360 has a copy number of 16 (33) and carries the Po operon promoter region controlling transcription of the *luxAB* luciferase reporter gene (Fig. 1B). This plasmid was introduced into KT2440::*dmpR*, and the resulting strain was grown in LB in the presence and absence of 2 mM 2-methylphenol. The results shown in Fig. 3A demonstrate that this minimal system consisting of *dmpR* and the Po promoter region faithfully reproduces both the tight effector-dependent DmpR-mediated transcription from the Po operon promoter and the growth phase response.

The Po regulatory region present in pVI360 contains DNA upstream of the −24, −12 consensus promoter sequence, including a region containing a large inverted repeat believed to be the binding site for DmpR (Fig. 1C). Each half of the repeat is itself an inverted repeat (39), and this region has 70% homology with the binding sites of XylR, a regulator analogous to DmpR from the TOL toluene/xylene catabolic plasmid (14). To determine if this entire region is required, we constructed a deletion series of the Po region in the reporter plasmid pVI360 in which the repeat upstream region or one or both halves of the inverted repeat was deleted (Fig. 1C). The resulting plasmids, pVI362 to pVI364, were introduced into KT2440::*dmpR*, and the strains were monitored for DmpR-activated transcription from Po as described above. Deletion of the repeat upstream region while leaving the entire large inverted repeat intact in pVI362 resulted in an expression profile similar to that with the original reporter pVI360 except that the basal level was somewhat elevated (Fig. 3B). Deletion to the center of the large inverted repeat in pVI363 resulted in approximately 75% of the peak activity (Fig. 3C), while deletion of both halves of the large inverted repeat in pVI364 totally eliminated any DmpR-mediated response (data not shown). These results demonstrate that both halves of the large inverted repeat are required for maximal DmpR-mediated transcription from Po, but that DNA sequences between position −149 and +2 bp relative to the start of transcription (Fig. 1C), spanning just the promoter-proximal half of the large inverted repeat, are sufficient to mimic the growth phase regulation.

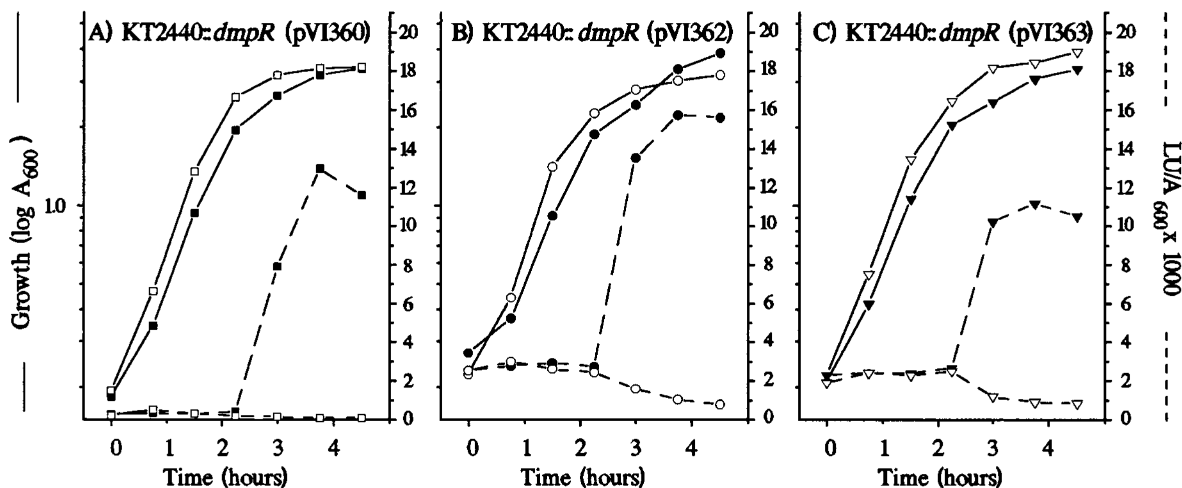

FIG. 3. Growth of and luciferase *luxAB* expression in the indicated strains upon growth in LB in the presence (filled symbols) or absence (open symbols) of 2 mM 2-methylphenol. Results are representative of two to five independent experiments. LU, luciferase units.

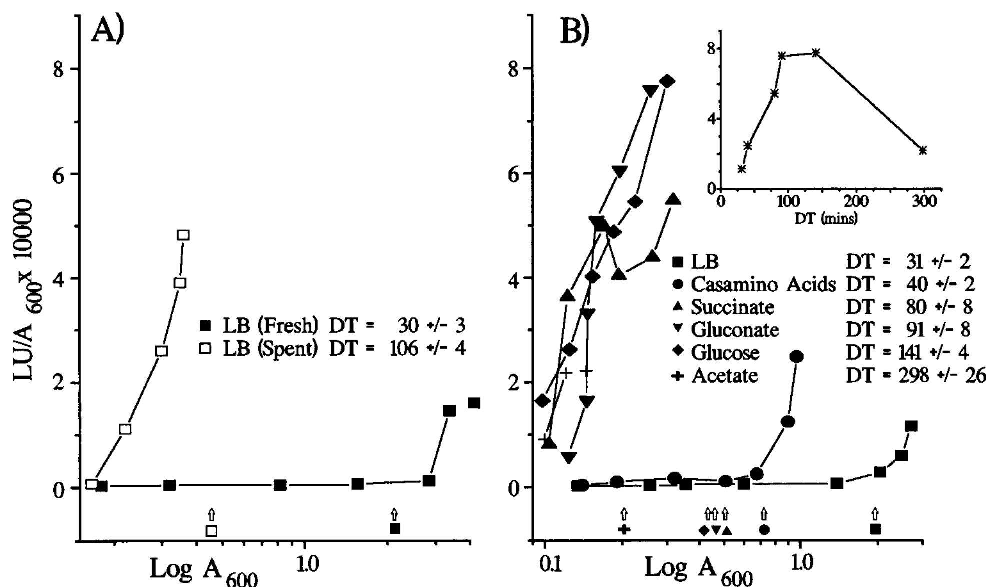

FIG. 4. Transcriptional response of KT2440::*dmpR*(pVI360) when grown on different media. (A) LB-grown cells were grown into exponential phase (A_{600} = 0.6), diluted, and resuspended in either effector-supplemented fresh LB or spent LB (filter-sterilized medium from a stationary-phase culture), and the luciferase response was monitored. (B) Cultures were grown in LB or carbon source-supplemented minimal M9 containing 2 mM 2-methylphenol as described in Materials and Methods. Arrows indicate the approximate A_{600} at which the initiation of the exponential-to-stationary phase transition occurs in each culture. Results are representative of two to six independent experiments for each medium. The insert shows the peak response value in a given medium plotted against the doubling time (DT) during exponential phase. LU, luciferase units.

The growth phase response is dependent on the culture media. The growth phase response is observed during the transition from exponential to stationary phase in LB. This type of response has previously been found with the analogous XylR-regulated Pu promoter system of TOL (13, 20, 28). Marqués and coworkers (28) showed, using spent medium, that the XylR-mediated Pu growth phase response contained a nutritional component. To test if this was also the case for DmpR-mediated regulation of Po, we used the minimal luciferase reporter system of KT2440::*dmpR*(pVI360) described above. This strain was grown into mid-exponential phase (A_{600} = 0.6), at which point no response of Po to DmpR is observed (Fig. 3A). The cells were then harvested and resuspended in either fresh LB or spent LB (filter-sterilized medium from a culture at an A_{600} of 3.0), each containing 2 mM effector. The results shown in Fig. 4A demonstrate that the response of exponential cells to fresh LB was as previously seen (Fig. 3A), while the same cells exposed to spent medium grew much more slowly but responded immediately.

One obvious variable at this transition point is the nutritional status of the culture. To examine if the carbon source had any influence on the growth phase response, we used the minimal luciferase reporter system of KT2440::*dmpR*(pVI360) described above. This strain was grown into exponential phase in a variety of media prior to dilution in these same media and growth in the presence of 2 mM 2-methylphenol. The results from these experiments (Fig. 4B) demonstrate that growth phase regulation is a phenomenon observed only in rapidly growing cultures such as those grown in LB and minimal medium supplemented with Casamino Acids as the carbon and energy source. In the case of growth on minimal medium containing Casamino Acids, the shift of the curve relative to that of cultures grown in LB primarily reflects the lower A_{600} at which exponential-to-stationary phase transition occurs. Growth with minimal medium containing succinate, gluconate, glucose, or acetate as the sole carbon and energy source results in cultures with doubling times of 80 to 298 min that exhibit a rapid immediate response to the presence of the DmpR effector 2-methylphenol in the medium irrespective of the A_{600} of the exponential-to-stationary phase transition. The insert in Fig. 4 shows a bell-shaped curve found upon plotting the peak luciferase reporter response values obtained after growth on the different media against the doubling times during exponential phase. It is notable that the maximal activation achieved in the fast-growing cultures is only 15 to 25% of that achieved by slow-growing cultures grown at the expense of glucose, gluconate, or succinate.

The growth phase response can be reproduced in *E. coli*. Since the growth phase response in *P. putida* required only the plasmid-derived *dmpR* regulatory gene and the Po promoter regulatory region, we considered if these components were sufficient for the response to be mediated in *E. coli*. To address this question, we constructed a reporter plasmid, pVI466, in which *dmpR* was placed in its native configuration relative to Po, which controls transcription of *luxAB*. This plasmid was introduced into two *E. coli* strains, MC4100 and S90C, that serve as the parents of specific mutants used in later experiments. The data in Fig. 5 demonstrate that the growth phase response is faithfully reproduced in both *E. coli* strains, although the specific genetic background influences the absolute levels of the reporter gene expression. Hence, we conclude that any host factors involved in mediating the growth phase response in *P. putida* have functional analogs in *E. coli.*

Many genes that exhibit a growth phase response are directly or indirectly regulated by the stationary-phase sigma factor RpoS (σ^s) (reviewed in reference 27). The constant levels of DmpR over the growth curve suggest that *dmpR* transcription is not directly influenced by RpoS but do not exclude the possibility of indirect regulation of its activity or its action at the Po promoter. Therefore, to test for any role of RpoS in growth phase regulation, the response from the *dmpR*-Po-*luxAB* reporter plasmid pVI466 in MC4100 ($RpoS^+$) was com-

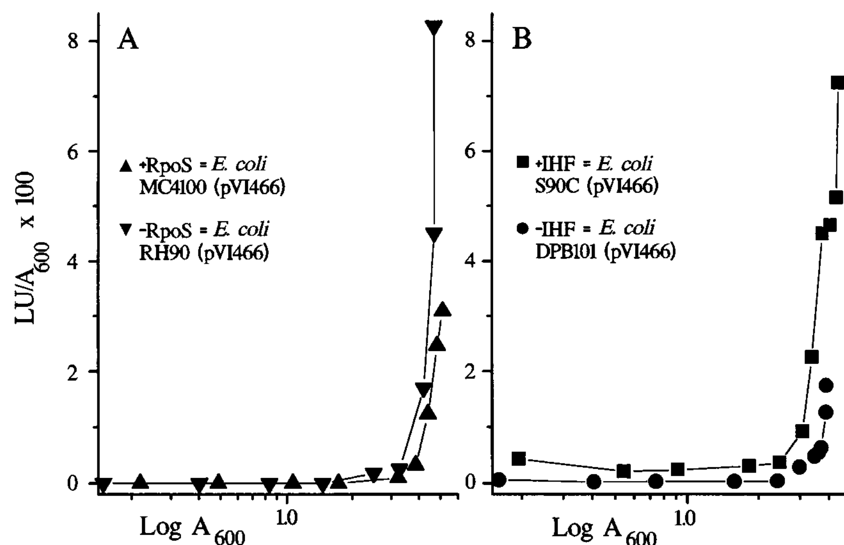

FIG. 5. Luciferase transcriptional response of *E. coli* strains harboring the *dmpR*-Po-*luxAB* reporter plasmid pVI466. Cells were grown as described in Materials and Methods prior to addition of 2-methylphenol at the start of the experiment. Results are representative of two to three independent experiments. LU, luciferase units.

pared with that of pVI466-harboring RH90 (RpoS$^-$). The results depicted in Fig. 5A show that the luciferase response in the RpoS mutant strain is approximately twofold higher than that in the RpoS$^+$ parent strain. This could be due to the greater availability of RNA polymerase core enzyme to other sigma factors (e.g., σ^{70} and σ^{54}) in the absence of competition by RpoS. The level of luciferase response in the RpoS mutant is about that achieved in S90C (Fig. 5B). Laboratory strains of *E. coli* have been found to frequently harbor mutations in *rpoS* (24), presumably since some mutations lead to a selective advantage in survival (46). Hence, it is possible that S90C has an unidentified *rpoS* mutation, thus explaining its different luciferase value relative to MC4100. Nevertheless, the data in Fig. 5A clearly demonstrate that RpoS does not influence the growth phase response.

A role for IHF but not Fis in DmpR-mediated transcription. Since the minimal system of DmpR and the Po promoter region is sufficient to mimic growth phase regulation, this regulation is probably mediated at the level of DmpR activity or factors involved in the transcription initiation from Po. DNA bending induced by binding of IHF has been implicated in altering promoter topology to achieve optimal transcription (18, 34). The Po regulatory region has a sequence homologous to the core consensus for IHF binding (Fig. 1C). Intracellular levels of IHF have been reported to vary in a growth phase-dependent manner both in *E. coli* and *P. putida* (2, 6, 12). Therefore, we examined if (i) IHF is required for DmpR-mediated activation of transcription of the Po operon promoter and (ii) IHF has a role in the observed growth phase response. To examine these possibilities, the *dmpR*-Po-*luxAB* reporter plasmid pVI466 was introduced into an IHF$^-$ strain of *E. coli*, DPB101, and its isogenic parental IHF$^+$ strain, S90C, and the *luxAB* transcriptional response in the presence of 2-methylphenol was monitored. The results shown in Fig. 5B demonstrate that lack of IHF results in a 75 to 90% decrease in transcription from Po. Thus, regulation by DmpR is IHF dependent. However, even in the absence of IHF, the residual transcription is still subject to growth phase regulation. Therefore, we conclude that limitation of the level of IHF during exponential phase cannot account for the growth phase regulation.

The Fis protein (factor for inversion stimulation) is a small DNA-binding protein whose cellular concentration varies tremendously under different growth conditions, with high levels during exponential growth and low levels during stationary phase (15). Four considerations prompted us to consider if Fis may compete with DmpR at or near its DNA-binding sites and thus mediate growth phase regulation: (i) DNA topology appears important for Po function (see above), (ii) Fis recognizes a degenerate DNA-binding consensus sequence (15), (iii) the DNA-binding regions of σ^{54}-dependent regulators have some homology to that of Fis (30), and (iv) Fis is inversely regulated with respect to the growth phase regulation of the *dmp* system. However, using the reporter plasmid pVI466 and isogenic Fis$^+$ and Fis$^-$ strains (see Materials and Methods), we found that the growth phase response is seen in the presence or absence of Fis (data not shown). Hence, Fis does not appear to play a role in DmpR-regulated transcription from Po.

Overexpression of DmpR overcomes the growth phase regulation in CF600. The reporter plasmid pVI466 used in the experiments outlined above has *dmpR* linked to Po controlling *luxAB* and is present at a copy number of 16 in both *E. coli* and *P. putida*. This plasmid in *E. coli* mimics the growth phase regulation of the wild-type *dmp* system at one to two copies per cell in *P. putida* KT2440. However, when pVI466 was introduced into *P. putida* KT2440 and transcription from Po was monitored in the presence of 2-methylphenol, a growth phase-dependent response was not observed. As shown in Fig. 6, this plasmid at its 16 copies in *P. putida* (33) gave a rapid immediate response to effectors in the media. Since the copy number of the plasmid is the same in *E. coli* and *P. putida*, this result suggested that differences in DmpR expression levels might account for the different results in the two species. To test this idea, Western blot analysis was performed with anti-DmpR antiserum on extracts from cells in mid-exponential phase. As shown in the insert in Fig. 6 (lanes 1 and 2), the level of DmpR expressed from pVI466 in *E. coli* is undetectable under conditions which show an intense DmpR band in *P. putida*.

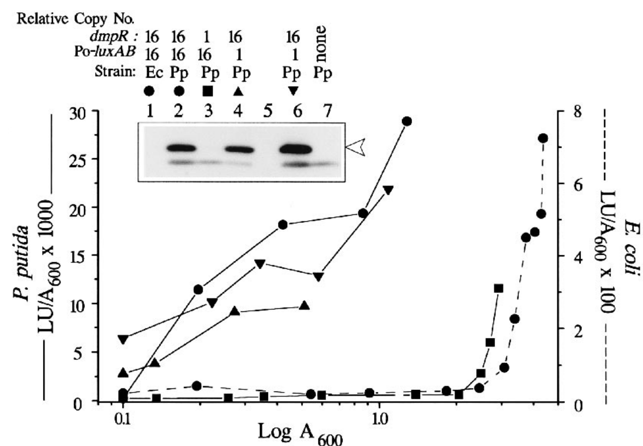

FIG. 6. Luciferase transcriptional response in the presence of 2 mM 2-methyphenol of *E. coli* and *P. putida* strains harboring *dmpR* and the Po-*luxAB* reporter fusion at different copy numbers: *E. coli* S90C(pVI466), *dmpR*/Po-*luxAB* ratio of 16:16 (--●--); *P. putida* KT2440(pVI466), *dmpR*/Po-*luxAB* ratio of 16:16 (—●—); *P. putida* KT2440::*dmpR* (pVI360), *dmpR*/Po-*luxAB* ratio of 1:16 (■); *P. putida* KT2440::Po-*luxAB* (pVI401), *dmpR*/Po-*luxAB* ratio of 16:1 (▲); *P. putida* KT2440::Po-*luxAB*(pVI465), *dmpR*/Po-*luxAB* ratio of 16:1 (▼). Cells were grown as described in Materials and Methods prior to addition of 2-methylphenol at the start of the experiment. For expression of DmpR from the vector P*tac* promoter of pVI465, IPTG was added 0.05 mM during growth of the cells. Note the change of scale for *E. coli* (pVI466) to facilitate comparison of transcriptional response patterns. Results are representative of two to three independent experiments for each strain. The insert shows Western analysis of 50 μg of crude extract proteins derived from exponentially growing cells, using an anti-DmpR antibody as described in Materials and Methods. Lanes 1 to 4 and lane 6, *E. coli* (Ec) or *P. putida* (Pp); lane 7, KT2440(pMMB66HE) vector control; lane 5, molecular mass standards. The arrowhead indicates DmpR; the lower, less intense band is a *Pseudomonas*-derived cross-reacting protein. LU, luciferase units.

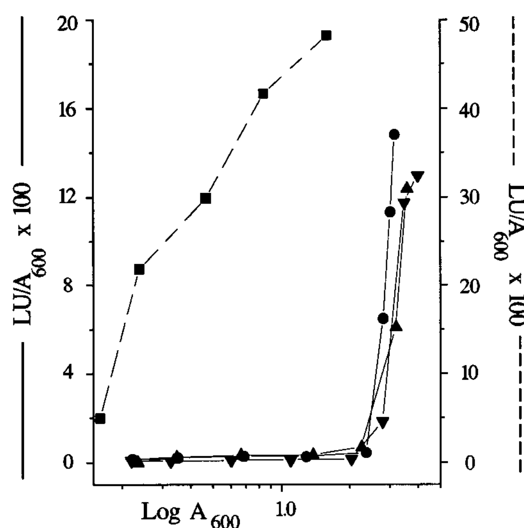

FIG. 7. Luciferase transcriptional response of *P. putida* KT2440::*dmpR*-Po-*dmpB*/*luxAB* harboring various plasmids in the presence of 2 mM 2-methylphenol. Cells were grown as described in Materials and Methods prior to addition of 2-methylphenol at the start of the experiment. IPTG was added to 0.5 mM for induction of the vector P*tac* promoter. Shown are results for KT2440::*dmpR*-Po-*dmpB*/*luxAB* harboring no plasmid (▼); pFH30 P*tac*-*rpoN* (●); pVLT31 vector control (▲); and pVI465 P*tac*-*dmpR* (■). The pMMB-based vector control for pVI465 gave results similar to those for the pVLT31 vector control (data not shown). Note the change of scale for pVI465 to facilitate comparison of transcriptional response patterns.

The upstream region of *dmpR* contains numerous regions with poor homology to *E. coli* and *Pseudomonas* promoters. However, the promoter sequence itself remains elusive (39), and so we cannot speculate as to why DmpR is expressed at such different levels in the two species. Nevertheless, the results suggest that overexpression of DmpR can overcome the growth phase dependence. To further test this idea, different reporter strain and plasmid systems were used to examine the growth phase regulation, with the components at different copy numbers: (i) KT2440::*dmpR*(pVI360) (*dmpR*/Po-*luxAB* ratio of 1:16), *dmpR* expressed from its native promoter; (ii) KT2440::Po-*luxAB*(pVI401) (*dmpR*/Po-*luxAB* ratio of 16:1), *dmpR* expressed from its native promoter; and (iii) KT2440::Po-*luxAB* (pVI465) (*dmpR*/Po-*luxAB* ratio of 16:1), *dmpR* expressed from the vector P*tac* promoter. As can be seen from the data shown in Fig. 6, high expression of DmpR at 16 copies per cell, either from its native promoter (on pVI401) or from the IPTG-induced P*tac* promoter (on pVI465), results in an immediate response to the presence of effector in the media. Conversely, low levels of DmpR expression at monocopy in *P. putida* or at 16 copies in *E. coli* results in suppression of transcriptional activation until the exponential-to-stationary phase transition. The DNA insert of plasmid pVI401 spans both *dmpR* and the Po promoter regulatory region (Fig. 1). Hence, KT2440::Po-*luxAB*(pVI401) has, in addition to the *luxAB*-fused copy of Po, 16 extra copies of the Po promoter and regulatory region. This is not the case with KT2440::Po-*luxAB*(pVI465), in which *dmpR* is expressed from the vector-located P*tac* promoter. Despite this difference, the two strains behave similarly (Fig. 6). This result suggests that the number of copies of the Po promoter has a minimal impact on the ability of high levels of DmpR to overcome the growth phase response.

The observation that overexpression of DmpR can overcome the growth phase regulation effectively rules out the trivial explanation that physiological changes in the membrane as cells progress through the growth curve (7) could mediate growth phase regulation by restricting access of aromatic effector molecules until late in growth. Rather, this result suggests that some negative factor, active in exponential phase, can be compensated for by the excess DmpR.

Effect of σ^{54} on growth phase regulation. The Po promoter belongs to the −24, −12 class recognized by RNA polymerase utilizing σ^{54}. To date, a number of these types of promoters, including two promoters from the toluene/xylene catabolic TOL plasmid, Pu and Ps (6, 9, 22, 28), P*nifH*, involved in nitrogen assimilation (6), the DctD-controlled promoter involved in dicarboxylic acid transport (19), and a carbon starvation survival gene promoter (25), have been reported to be growth phase regulated. A unifying theme of these specific regulatory systems is their dependence on σ^{54}. Although the levels of RpoN (σ^{54}) do not vary along the growth curve in *P. putida* (6), this observation did not rule out the possibility that the activity of σ^{54} itself could be modulated as has been suggested for σ^{E} (36). Cases and coworkers tested the effect of overexpression of *rpoN* from the P*tac* promoter of pFH30 on the XylR-mediated transcriptional response from Pu when the components of the system were cloned at monocopy (6). They found that overexpression of σ^{54} partially alleviated the growth phase regulation of Pu, shifting the curve towards earlier phases of growth. To test if a similar effect was observed with the *dmp* system, we constructed a similar reporter strain, KT2440::*dmpR*-Po-*dmpB*/*luxAB*, which carries the minimal system in monocopy on the chromosome (see Materials and Methods). This strain was used in conjunction with vector control or plasmids with either *rpoN* or *dmpR* expressed from the P*tac* promoter which was induced with 0.5 mM IPTG as described previously (6). Figure 7 summarizes the results of

these experiments and demonstrates that in the *dmp* system, overexpression of σ^{54} causes a small but reproducible shift in the transcriptional response curve. However, the effect is minor compared with that of overexpressing DmpR.

DISCUSSION

Bacteria respond to specific signals with the appropriate adaptive transcriptional response. However, the appropriate response must not only reflect the response to a specific signal but also incorporate more broad-based, and sometimes conflicting, signals such as the nutritional and physiological status of the cell. In this report, we show that the transcription of the Po *dmp* operon promoter that mediates transcription of (methyl)phenol catabolic enzymes in response to the presence of aromatic effectors of the its regulator, DmpR, is modulated by both the growth medium and growth phase of the culture.

Transcription from Po was found to be suppressed in fast-growing cultures until the exponential-to-stationary phase transition point (Fig. 2). This response was shown to be dependent on the physiological status of the cell and could be mimicked by a minimal system consisting of the *dmpR* regulatory gene and a 150-bp region of DNA spanning the binding sites for the regulator and the Po promoter (Fig. 3). However, by growing the cells in a variety of different media, the growth phase response was shown to be a property of only fast-growing cultures cultivated on carbon-rich media. Conversely, a rapid response to the presence of aromatic compounds was detected in cultures with slow growth rates as a result of cultivation on minimal media supplemented with different single carbon sources (Fig. 4B). With the exception of acetate, the transcriptional response was found to be higher in cultures grown on media that elicit slow growth (Fig. 4). Since depletion of rich media at the exponential-to-stationary phase transition also results in an immediate response to aromatic compounds by exponential-phase cells (Fig. 4A), these results imply that the regulatory system is probably designed for maximal ability to respond under conditions that would be anticipated to be prevalent in the bacteria's natural soil environment, namely, carbon source deprivation and slow growth rate.

One consequence of suppression of transcription from Po in fast-growing cultures would be to effectively keep the expression of the specialized catabolic enzymes silent when other, more energetically favorable carbon sources are present. Suppression of transcription of the Pu promoter from an analogous system of the TOL plasmid for toluene/xylene catabolism by yeast extract, glucose, Casamino Acids, and some other readily metabolized carbon sources has been reported (13, 20, 28). Where tested, this phenomenon has been shown to be different from the classical cyclic AMP-dependent catabolite repression mechanism of *E. coli* (20). The finding that *dmpR* and the Po regulatory region are sufficient to mimic the growth phase response in both *P. putida* and *E. coli* (Fig. 5) demonstrates that any host-encoded factors involved in this response are functionally common to both strains.

The signal(s) mediating the regulatory mechanisms underlying the growth phase response must act through some component of the *dmpR*-Po promoter minimal system. A number of different levels of possible action can be envisioned: the level or activity of DmpR, the topology of the Po promoter, the availability of Po for the transcriptional machinery, or the level/activity of the alternative sigma factor, σ^{54}. A number of these possibilities have been tested here.

Both transcription of *dmpR* and the level of DmpR were found to be constant over the growth curve, demonstrating that variations in the levels of this key protein are unlikely to account for the growth phase regulation (Fig. 2). However, this finding does not exclude the possibility that the activity of DmpR is modulated during growth. Using *E. coli* mutants and their isogenic parental strains, we excluded a role for RpoS and Fis in mediating the growth phase response (Fig. 5). The DNA-bending protein IHF was found to be essential for maximal transcription from Po but not for the growth phase response per se (Fig. 5). The sequence homologous to the IHF core consensus sequence is located in an unusual position in the Po regulatory region. The IHF-binding sites identified cognate to -24, -12 promoters are generally located approximately equidistant between the promoter sequence and the activator-binding site, and the mode of action of IHF is believed to be predominantly at the level of promoter topology, allowing protein-protein interaction with the transcriptional apparatus (reviewed in reference 34). In the case of the Po region, the potential IHF-binding site completely overlaps the promoter-proximal portion of the DmpR-binding site. Hence we cannot exclude the possibility that IHF has an additional role in stabilizing the DmpR-DNA interaction.

Overexpression of DmpR was found to have a dramatic effect, completely abolishing the growth phase regulation and leading to an immediate response to the presence of aromatic compounds in the medium (Fig. 6 and 7). Although this observation does not identify the regulatory signal, it strongly suggests that some negative factor, active or produced during rapid exponential growth, is titrated out or compensated for by excess DmpR in the cytosol. Possibilities include unfavorable DNA topology that changes with growth conditions (3) and production of a negatively acting RNA or protein during rapid growth. The mode of action of the negative factor could block one of the activities of DmpR, i.e., DNA binding, activation by aromatics, or interaction with σ^{54}-RNA polymerase transcriptional apparatus. A number of -24, -12 σ^{54}-dependent promoters in addition to Po are subject to growth phase regulation, including those regulated by XylR, NifA, and DctD (6, 9, 19, 22, 28). The alternative sigma factor, σ^{54}, is normally expressed at constant levels in *P. putida* (6). A notable shift in the growth phase-regulated response curve of the XylR-regulated Pu promoter was observed upon overexpression of σ^{54} (6). This observation led Cases and coworkers to suggest that the activity of σ^{54} may be regulated, thereby accounting for the growth phase regulation (6). Overexpression of σ^{54} under the same conditions in the similar DmpR-Po minimal monocopy system resulted in a minor but reproducible shift in the growth phase-regulated response curve (Fig. 7). While this result may suggest that activity of σ^{54} plays a role in the growth phase response of the *dmp* system, the dramatic effect of overexpression of DmpR suggests that some additional factor is involved or that DmpR can titrate out a negative regulator of σ^{54} by competition. We are currently addressing the level at which growth phase regulation occurs by analyzing the effects of mutants of both DmpR and the *Pseudomonas* host chromosome.

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REFERENCES

1. **Austin, S., and R. Dixon.** 1992. The prokaryotic enhancer binding protein NtrC has an ATPase activity which is phosphorylation and DNA dependent. EMBO J. **11:**2219–2228.

2. **Aviv, M., H. Giladi, G. Schreiber, A. B. Oppenheim, and G. Glaser.** 1994. Expression of the genes encoding for the *Escherichia coli* integration host factor are controlled by growth phase, *rpoS*, ppGpp and by autoregulation. Mol. Microbiol. **14:**1021–1031.
3. **Balke, V. L., and J. D. Gralla.** 1987. Changes in the linking number of supercoiled DNA accompanying growth transition in *Escherichia coli*. J. Bacteriol. **169:**4499–4506.
4. **Biek, D. P., and S. N. Cohen.** 1989. Involvement of integration host factor (IHF) in maintenance of plasmid pSC101 in *Escherichia coli*: characterization of pSC101 mutants that replicate in the absence of IHF. J. Bacteriol. **171:**2056–2065.
5. **Casadaban, M. J., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J. Mol. Biol. **138:**179–207.
6. **Cases, I., V. de Lorenzo, and J. Pérez-Martín.** 1996. Involvement of sigma factor σ^{54} in exponential silencing of the *Pseudomonas putida* TOL plasmid Pu promoter. Mol. Microbiol. **19:**7–17.
7. **Cronan, J. E.** 1968. Phospholipid alterations during growth of *Escherichia coli*. J. Bacteriol. **95:**2054–2061.
8. **Delgado, A., and J. L. Ramos.** 1994. Genetic evidence for activation of the positive transcriptional regulator XylR, a member of the NtrC family of regulators, by effector binding. J. Biol. Chem. **269:**8059–8062.
9. **de Lorenzo, V., I. Cases, M. Herrero, and K. N. Timmis.** 1993. Early and late response of TOL promoters to pathway inducers: identification of postexponential promoters in *Pseudomonas putida* with *lacZ-tet* bicistronic reporters. J. Bacteriol. **175:**6902–6907.
10. **de Lorenzo, V., L. Eltis, B. Kessler, and K. N. Timmis.** 1993. Analysis of *Pseudomonas* gene products using *lacI*^{q}/*Ptrp-lac* plasmids and transposons that confer conditional phenotypes. Gene **123:**17–24.
11. **de Lorenzo, V., M. Herrero, U. Jakubzik, and K. N. Timmis.** 1990. Mini-Tn*5* transposon derivatives for insertional mutagenesis, promoter probing, and chromosomal insertion of cloned DNA in gram-negative eubacteria. J. Bacteriol. **172:**6568–6572.
12. **Ditto, M. D., D. Roberts, and R. A. Weisberg.** 1994. Growth phase variation of integration host factor level in *Escherichia coli*. J. Bacteriol. **176:**3738–3748.
13. **Duetz, W. A., S. Marqués, C. de Jong, J. L. Ramos, and J. G. van Andel.** 1994. Inducibility of the TOL catabolic pathway in *Pseudomonas putida* (pWW0) growing on succinate in continuous culture: evidence of carbon catabolite repression control. J. Bacteriol. **176:**2354–2361.
14. **Férnandez, S., V. Shingler, and V. de Lorenzo.** 1995. Cross-regulation by XylR and DmpR activators of *Pseudomonas putida* suggest that transcriptional control of biodegradation operons evolves independently of catabolic genes. J. Bacteriol. **176:**5052–5058.
15. **Finkel, S. E., and R. C. Johnson.** 1992. The Fis protein: it's not just for DNA inversion anymore. Mol. Microbiol. **6:**3257–3265.
16. **Franklin, F. C. H., M. Bagdasarian, M. M. Bagdasarian, and K. N. Timmis.** 1981. Molecular and functional analysis of the TOL plasmid pWW0 from *Pseudomonas putida* and cloning of genes for the entire regulated aromatic ring *meta*-cleavage pathway. Proc. Natl. Acad. Sci. USA **78:**7458–7462.
17. **Fürste, J. P., W. Pansegrau, R. Frank, H. Blöcker, P. Scholz, M. Bagdasarian, and E. Lanka.** 1987. Molecular cloning of the RP4 DNA primase region in a multirange *tacP* expression vector. Gene **55:**19–28.
18. **Goosen, N., and P. van de Putte.** 1995. The regulation of transcription by initiation host factor. Mol. Microbiol. **16:**1–7.
19. **Gu, B., J. H. Lee, T. R. Hoover, D. Scholl, and B. T. Nixon.** 1994. *Rhizobium meliloti* DctD, a σ^{54}-dependent transcriptional activator, may be negatively controlled by a subdomain in the C-terminal end of its two-component receiver module. Mol. Microbiol. **13:**51–66.
20. **Holtel, A., S. Marqués, I. Möhler, U. Jakubzik, and K. N. Timmis.** 1994. Carbon source-dependent inhibition of *xyl* operon expression of the *Pseudomonas putida* TOL plasmid. J. Bacteriol. **176:**1773–1776.
21. **Hopper, S., and A. Böck.** 1995. Effector-mediate stimulation of ATPase activity by the σ^{54}-dependent transcriptional activator FHLA from *Escherichia coli*. J. Bacteriol. **177:**2798–2803.
22. **Hugouvieux-Cotte-Pattat, N., T. Köhler, M. Rekik, and S. Harayama.** Growth-phase-dependent expression of the *Pseudomonas putida* TOL plasmid pWW0 catabolic genes. J. Bacteriol. **172:**6651–6660.
23. **Johnson, R. C., C. A. Ball, D. Pfeffer, and M. I. Simon.** 1988. Isolation of the Hin recombinational enhancer binding protein. Proc. Natl. Acad. Sci. USA **85:**3484–3488.
24. **Kaasen, I., P. Falkenberg, O. B. Styrvold, and A. Strom.** 1992. Molecular cloning and physical mapping of the *otsBA* genes, which encode the osmoregulatory trehalose pathway of *Escherichia coli*: evidence that transcription is activated by KatF (AppR). J. Bacteriol. **174:**889–898.
25. **Kim, Y., L. S. Watrud, and A. Matin.** 1995. A carbon starvation survival gene of *Pseudomonas putida* is regulated by σ^{54}. J. Bacteriol. **177:**1850–1859.
26. **Lange, R., and R. Hengge-Aronis.** 1991. Growth phase-regulated expression of *bolA* and morphology of stationery-phase *Escherichia coli* cells are controlled by the novel sigma factor σ^{s}. J. Bacteriol. **173:**4474–4481.
27. **Loewen, P. C., and R. Hengge-Aronis.** 1994. The role of sigma factor σ^{s} (KatF) in bacterial global regulation. Annu. Rev. Microbiol. **48:**53–80.
28. **Marqués, S., A. Holtel, K. N. Timmis, and J. L. Ramos.** 1994. Transcription induction kinetics from the promoters of the catabolic pathways of TOL plasmid pWW0 of *Pseudomonas putida* for metabolism of aromatics. J. Bacteriol. **176:**2517–2524.
29. **Morett, E., and L. Segovia.** 1993. The σ^{54} bacterial enhancer-binding protein family: mechanism of action and phylogenetic relationship of their functional domains. J. Bacteriol. **175:**6067–6074.
30. **North, A. K., K. E. Klose, K. M. Stedman, and S. Kustu.** 1993. Prokaryotic enhancer-binding proteins reflect eukaryotic-like modularity: the puzzle of nitrogen regulatory protein C. J. Bacteriol. **175:**4267–4273.
31. **Nozaki, M.** 1970. Metapyrocatechase (*Pseudomonas*). Methods Enzymol. **17:**522–525.
32. **Olsson, O., C. Konez, and A. A. Szalay.** 1988. The use of the *luxA* gene of the bacterial luciferase operon as a reporter gene. Mol. Gen. Genet. **215:**1–9.
33. **Pavel, H., M. Forsman, and V. Shingler.** 1994. An aromatic effector specificity mutant of the transcriptional regulator DmpR overcomes the growth constraints of *Pseudomonas* sp. strain CF600 on *para*-substituted methylphenols. J. Bacteriol. **176:**7550–7557.
34. **Pérez-Martin, J., F. Rojo, and V. de Lorenzo.** 1994. Promoters responsive to DNA bending: a common theme in prokaryotic gene expression. Microbiol. Rev. **58:**268–290.
35. **Powlowski, J., and V. Shingler.** 1994. Genetics and biochemistry of phenol degradation by *Pseudomonas* sp. strain CF600. Biodegradation **5:**1–18.
36. **Rouviere, P. E., A. De Las Penas, J. Mecsas, C. Z. Lu, K. E. Rudd, and C. A. Gross.** 1995. *rpoE*, the gene encoding the second heat-shock sigma factor, sigma E, in *Escherichia coli*. EMBO J. **14:**1032–1042.
37. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. **Shingler, V.** 1996. Signal sensing by σ^{54}-dependent regulators: derepression as a control mechanism. Mol. Microbiol. **19:**409–416.
39. **Shingler, V., M. Bartilson, and T. Moore.** 1993. Cloning and nucleotide sequence of the positive regulator, DmpR, of the phenol catabolic pathway of pVI150: a member of the NtrC family of transcriptional activators. J. Bacteriol. **175:**1596–1604.
40. **Shingler, V., F. C. H. Franklin, M. Tsuda D. Holroyd, and M. Bagdasarian.** 1989. Molecular analysis of a plasmid encoded phenol hydroxylase of *Pseudomonas* CF600. J. Gen. Microbiol. **135:**1083–1092.
41. **Shingler, V., and T. Moore.** 1994. Sensing of aromatic compounds by the DmpR transcriptional activator of phenol catabolizing *Pseudomonas* sp. strain CF600. J. Bacteriol. **176:**1555–1560.
42. **Shingler, V., and H. Pavel.** 1995. Direct regulation of the ATPase activity of the transcriptional activator DmpR by aromatic compounds. Mol. Microbiol. **17:**505–513.
43. **Shingler, V., J. Powlowski, and U. Marklund.** 1992. Nucleotide sequence and functional analysis of the complete phenol/3,4-dimethylphenol catabolic pathway of *Pseudomonas* sp. strain CF600. J. Bacteriol. **174:**711–724.
44. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
45. **Weiss, D. S., J. Batut, K. E. Klose, J. Keener, and S. Kustu.** 1991. The phosphorylated form of enhancer-binding protein NTRC has an ATPase activity that is essential for activation of transcription. Cell **67:**155–167.
46. **Zambrano, M. M., D. A. Siegel, M. Almirón, A. Tormo, and R. Kolter.** 1993. Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures. Science **259:**1757–1760.