Genetic Evidence for Interdomain Regulation of the Phenol-responsive σ^{54}-dependent Activator DmpR*

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The σ^{54}-dependent DmpR activator regulates transcription of the dmp operon that encodes the enzymes for catabolism of (methyl)phenols. DmpR is expressed constitutively, but its transcriptional promoting activity is controlled positively in direct response to the presence of aromatic pathway substrates (effectors). DmpR has a distinct domain structure with the amino-terminal A-domain controlling the specificity of activation of the regulator by aromatic effectors (signal reception), a central C-domain mediating an ATPase activity essential for transcriptional activation, and a carboxyl-terminal D-domain involved in DNA binding. Deletion of the A-domain has been shown previously to result in an effector-independent transcriptional activator with constitutive ATPase activity. These results, in conjunction with the location of mutations within the A- and C-domains which exhibit an effector-independent (semiconstitutive) property, have led to a working model in which the A-domain serves to mask the ATPase and transcriptional promoting property of the C-domain in the absence of effectors. To investigate the mechanism by which the A-domain exerts its repressive effect, we developed a genetic system to select positively for intramolecular second site revertants of DmpR. The results demonstrate (i) that mutations within the A-domain can suppress the semiconstitutive activity of C-domain located mutations and vice versa; (ii) that the C-domain located mutations do not influence the intrinsic ATPase and transcriptional promoting property of the C-domain in the absence of the A-domain; and (iii) that semiconstitutive mutations of the A- and C-domain have an additive effect. Taken together these results support a model in which the A-domain represses the function(s) of the C-domain by direct interactions between residues of the two domains.

The pV150 plasmid-encoded dmp system of Pseudomonas sp. strain CF600 confers the ability to utilize phenol, monomethylated phenols, and 3,4-dimethylphenol as sole carbon and energy source. The dmp system is composed of the closely linked but divergently transcribed dmp regulatory gene and the 15-gene dmp operon that encodes the catabolic enzymes required for conversion of substrates to central metabolites (1, 2). Transcription of the dmp operon from the operon promoter Po is regulated positively by DmpR, resulting in expression of the specialized catabolic enzymes only in the presence of pathway substrates or structural analogs (3, 4).

DmpR belongs to the prokaryotic enhancer-binding family of σ^{54}-dependent regulators. These activators function to control transcription positively from −12, −24 promoters that are recognized by RNA polymerase utilizing the alternative sigma factor, σ^{54}, encoded by rpoN or its analogs (for review, see Refs. 5 and 6). Close physical contact between the regulators bound to their enhancer-like sequences and the cognate promoter-bound σ^{54}-RNA polymerase is believed to be brought about by a common mechanism involving looping out of the intervening DNA. In some σ^{54}-dependent systems, including the dmp system (7), this process has been suggested to be assisted by binding of the DNA-bending protein host factor, whereas in others, binding of the HU protein or intrinsic bends have been implicated (for review, see Refs. 8 and 9).

Members of the σ^{54}-dependent regulator family have distinct domains that mediate specific function(s) and exhibit varying degrees of homology (for review, see Ref. 5). The amino-terminal signal reception A-domains are joined to the central activation C-domains by means of short flexible B-domain (Q-linker). A region of variable length separates the C-domains from the conserved carboxyl-terminal D-domains that contain a helix-turn-helix DNA binding motif analogous to those found in a number of transcriptional regulators (see Fig. 1A; for review, see Ref. 10). The highly conserved central C-domains of the regulators contain a nucleotide binding motif and are also believed to encompass the region involved in direct interaction with σ^{54}-RNA polymerase. The C-domain mediates ATP binding and hydrolysis, essential for transcriptional activation of and open complex formation by σ^{54}-RNA polymerase (11, 12). In the case of NtrC, this domain has also been shown to mediate oligomerization required prior to ATPase activity and thus transcriptional activation (13, 14), a process that is facilitated by binding to its cognate enhancer sequence (15). The mechanism underlying the coupling of ATP hydrolysis and transcriptional activation is not yet understood fully but may involve a process by which ATP hydrolysis allows the regulator to interact successfully with σ^{54} and thereby relieve the repressive effect of σ^{54} on the ability of σ^{54}-RNA polymerase to form open transcriptional complexes (16).

The A-domain signal reception module of the regulators is the least conserved domain among the different members of the family. Different mechanistic subgroups, which reflect the mode of activation, have been identified (for review, see Ref. 10). Many members of the family, including two archetypal members, NtrC and DctD, are part of so-called two-component regulatory systems. The activities of these regulators are controlled by the phosphorylation status of a conserved Asp residue of the A-domain which is modulated by a sensor histidine kinase in response to an appropriate environmental signal. DmpR, however, belongs to a different mechanistic subgroup that also includes XylR (17, 18) and FhIA (19), which responds

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directly to small effector molecules (for review, see Ref. 10).

The transcriptional promoting activity of DmpR is activated in the presence of the dmp pathway substrates and some, but not all, structural analogs (4). Chimeric proteins (4) and isolation of effector specificity mutants (20, 21) 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 (21). Moreover, deletion of the signal reception amino-terminal A-domain of DmpR results in an effector-independent regulator with full in vivo constitutive transcriptional promoting activity and in vitro ATPase activity (21). Thus, effector-mediated repression of the C-domain ATPase and transcriptional promoting activity can be mimicked by deletion of the A-domain.

Within DmpR, single amino acid changes in the A-domain (e.g. E135D, E135A), the central activation C-domain (e.g. V276A, V276G), and the short flexible B-linker domain (e.g. L219P), lead to a semiconstitutive (sc) activity with varying degrees of transcriptional promoting ability in the absence of effector (21). These mutations, therefore, partially mimic the activated state of the protein. The location of the mutations within the A-, B-, and C-domains suggests that the A-domain may serve to repress the function(s) of the C-domain by A/C interdomain interactions that are tethered via the normally flexible B-domain. Here, using a genetic system designed to select intramolecular second site suppressor mutations of DmpR-sc derivatives, we investigate the mechanism by which A-domain repression of the C-domain is achieved. Genetic data are presented supporting the model described above, i.e. that repression is mediated via direct interactions between residues of the A- and C-domains rather than by steric hindrance.

MATERIALS AND METHODS

Construction of SacB Selection Strain and Isolation of Second Site Mutations—A promoterless sacb gene of Bacillus subtilis (22) was generated as a BamHI to NdeI fragment as follows. First, the ribosome binding site and the S' portion of the gene to an internal EcoR1 site were amplified by polymerase chain reaction and sequenced to ensure that no mutations were introduced; the complete sacb was subsequently reconstituted in its native configuration by cloning of an EcoR1-NdeI fragment spanning the remainder of the gene. The promoterless sacb gene was then cloned under the control of the dmp operon promoter region (23) relative to the start of transcription (3), in a modified pBluescript(Strategene) derivative that contained an NdeI and two NotI sites in the polylinker. The resulting fusion was cloned as an NotI fragment into the defective transposon, mini-Tn5, and carried on a suicide vector (23). The p-sock sacb fusion of the resulting plasmid, pVI468, was inserted into the chromosome of P. putida KT2440::Po-luxAB to generate KT2440::Po-sacb, as described previously (25). The broad host range plasmid pVI401 harbors the dmp gene from its native promoter (20). Semiconstitutive mutant derivatives of DmpR, derived from pVI401, DmpR-V276A (pVI444), DmpR-E135A (pVI447), and DmpR-E135D (pVI448), and the effector specificity mutant DmpR-E135K (pVI428) have been described previously (20, 21). Plasmids expressing dmpR-sc derivatives were introduced into KT2440::Po-sacb to generate strains that will lyse in the presence of 5% sucrose (26) due to the mutant DmpR-mediated repression is mediated via direct interactions between residues of the A- and C-domains rather than by steric hindrance.

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RESULTS

Isolation of Second Site Suppressor Mutations of Semiconstitutive DmpR Derivatives—The simplest interpretation of the semiconstitutive activity mediated by A- and C-domain located mutations, and the proline substitution in the flexible linker that separates them, is that in each case the mutation leads to a weakening of a putative A/C-domain interactions. If this is indeed the case then the model would predict that compensatory mutations in either domain should be selectable. To test this prediction, we constructed a positive selection system to isolate second site revertants. The genetic selection system developed utilizes the conditional lethal effects of the sacB gene product in the presence of sucrose (26). A promoterless sacB gene was constructed and placed under the control of the dmp operon promoter Po and subsequently inserted into the chromosome of a Pseudomonas host to generate KT2440::Po-sacB as described under "Materials and Methods." Trans activation of the Po promoter by plasmid-encoded semiconstitutive derivatives of DmpR (E135A, E135D, or V276A), active even in the absence of aromatic effectors, completes the system to give a strain that will lyse in the presence of sucrose. Positive selection of second site revertants was achieved by isolation of mutants that survived on sucrose-containing plates, i.e. those that inefficiently or no longer activate Po and thus do not express SacB. Five DmpR mutants that had this property, but which were still capable of activation in the presence of aromatic compounds, were analyzed further to locate the mutated residue(s) that mediated the suppression.

The results, summarized in Fig. 1A, revealed two cases of interdomain suppression, where the second mutation resides in a domain distinct from the original mutation. Starting with the A-domain mutant DmpR-E135D, the suppressor mutation P297R was selected. The C-domain mutant DmpR-V276A was used to select the A-domain located second site mutation W193R. In addition to the two cases of interdomain suppression, three examples of intra-A-domain second site revertants were isolated: the semiconstitutive activity of DmpR-E135D was found to be suppressed by either F93L or K188E, whereas that of DmpR-E135A was suppressed by L83P.

Transcriptional Activation by DmpR Derivatives—To quantify the transcriptional response mediated by DmpR derivatives, we used the previously constructed luciferase reporter strain KT2440::Po-luxAB. Plasmids expressing dmpR derivatives harboring the original semiconstitutive mutations, the genetically selected double mutations, or the second site mutations alone were introduced into KT2440::Po-luxAB, and the mutant DmpR-mediated transcriptional response from Po was monitored in the absence and presence of its effector 2-methylphenol. The transcriptional response and protein expression levels are compared with those of wild type DmpR in Fig. 1, B and C. DmpR-E135D, the interdomain suppressed mutant E135D/P297R, and the derivative with the suppressor mutation P297R alone are all expressed at similar levels (Fig. 1C, lanes 2–4). The P297R mutation clearly suppresses the semiconstitutive activity of DmpR-E135D; the transcriptional response L83P causes a 2-fold reduction in the protein level of P297R alone is indistinguishable from wild type DmpR in the presence of effector. Values are the average of triplicate determinations from at least two independent experiments; error ranges are indicated. Panel C, expression levels of DmpR derivatives. Western analysis of 30 μg of crude extract derived from cells used in experiments shown in panel B were separated by 11% SDS-polyacrylamide gel electrophoresis and treated with anti-DmpR as described under "Materials and Methods."

by at least 3.4-fold relative to that of V276A, it appears that W193R genuinely suppresses the semiconstitutive activity of V276A rather than just bringing the level and thus activity of the protein below threshold levels. It is notable that the W193R substitution alone results in an even lower expression level, i.e. less stable polypeptide, than the W193R/V276A mutant. This observation provides additional support for the interdependence of this pair of mutations.

The intra-A-domain E135D/F93L mutant is expressed at the same level as derivatives harboring each mutation in isolation (Fig. 1C, lanes 2, 11, and 12). The F93L mutation once again clearly suppresses the semiconstitutive activity of E135D with a reduction of 12.8-fold in the absence effectors. In contrast to P297R, which has wild type effector-activated activity, the F93L mutation alone has a 2.1-fold reduced effector-activated activity. In the second example of intradomain suppression, DmpR-E135D/K188E and the K188E mutant are expressed at 50–75% of the level of E135D and are phenotypically indistinguishable. The K188E mutation reduces the transcriptional response of E135D by 11.3-fold and 2.4-fold in the absence and presence of effector, respectively. Thus, K188E also suppresses the semiconstitutive activity of E135D. In E135A/L83P, the final example of intradomain suppression, the second site mutation L83P causes a 2-fold reduction in the protein level of E135A/L83P but does not influence the expression of the pro-
tein harboring the mutation alone (Fig. 1C, lanes 8–10). E135A/L83P compared with E135A has a 5.2- and 3.2-fold reduced transcriptional response in the absence and presence of effector, respectively. Thus, despite differences in protein levels of three derivatives harboring two substitutions, in all five cases the transcriptional promoting ability in the absence of effector is affected more severely than that in the presence of effector. Therefore we conclude that all five cases are genuine second site suppressors but that the degree with which they suppress the semiconstitutive activities of their respective primary mutation varies between 3.4- and 12.8-fold.

C-domain Located Mutations Do Not Influence the Intrinsic ATPase and Transcriptional Promoting Activities of DmpR—The identification of two interdomain suppressor mutations, E135D/P297R in which the C-domain located P297R mutation suppressed the semiconstitutive activity of the A-domain located E135D mutation, and V276A/W193R in which the A-domain located mutation suppressed the semiconstitutive activity of the C-domain located V276A mutation, strongly suggests that interdomain interactions mediate A-domain repression of the transcriptional promoting ability of DmpR. However, as outlined in the Introduction, once activated, the transcriptional promoting properties of the regulator are mediated by the C-domain. Therefore, it is possible that each C-domain located mutation may in itself influence the ability of the mutant regulator to promote transcription. To test this possibility we made use of the fact that deletion of the A-domain of DmpR results in a protein, ΔA2-DmpR, that is fully active in intact cells in terms of transcriptional activation and in vitro ATPase activity (21). Thus, deletion of the A-domain of DmpR allows assessment of the transcriptional promoting property in the absence of A-domain-mediated effector activation and A/C-domain interactions. Plasmids expressing ΔA2-DmpR derivatives harboring the C-domain located mutations involved in the second site revertants (V276A and P297R) were constructed and introduced into the luciferase reporter strain. Transcription activation by mutant ΔA2-DmpR derivatives was compared with that of wild type and a negative control protein, an inactive derivative harboring a mutation within the ATP binding site (G268S). As shown in Fig. 2A, the two C-domain mutant derivatives are expressed and mediated transcription at levels comparable to wild type in this reporter system.

To analyze the in vitro ATPase activity of ΔA2-DmpR derivatives we employed the eight-amino acid Flag epitope (29). In-frame carboxyl-terminal fusion of this tag to wild type DmpR does not influence the specificity or transcriptional response of the protein and allows rapid purification from contaminating ATPase activity (21). ΔA2-DmpR-Flag derivatives were constructed and expressed at high levels using a promoter from phage T7 (see "Materials and Methods"). Monoclonal Flag M2 antibodies coupled to beads were used to affinity purify the individual ΔA2-DmpR-Flag derivatives and the resulting preparations used in in vitro ATPase activity assays. The results shown in Fig. 2B demonstrate that neither the V276A nor the P297R mutation altered the ATPase activity of the protein in vitro.

The Role of Residue Charge in Second Site Suppression—Both interdomain second site mutations, P297R and W193R, involve a residue charge change. This observation led us to investigate the role of charge in mediating the observed suppression of the semiconstitutive mutant activity. Since the P297R mutation did not influence the protein expression level, either alone or in combination with E135D which it suppressed (Fig. 1C, lanes 3 and 4), this residue was chosen for further analysis. Mutagenesis of codon 297 of DmpR to introduce positive, negative, or neutral amino acids was performed, and the effects of these mutations in isolation or in combination with differentially charged residues at position 135 were monitored in the luciferase reporter system as described above. All mutations used in the analysis are expressed at comparable levels (data not shown), and the results are summarized in Table I. With the exception of P297E, which introduces a negative charge, none of the mutants of P297 influenced the regulator-mediated response in isolation. The P297E mutation slightly increased the level of transcription in the absence of effectors. The P297K mutation, which like P297R has a positive charge, similarly also suppressed the semiconstitutive activity of E135D, whereas the neutral exchange P279G and the negatively charged mutation P297E could not. Therefore, we conclude that the charge of the residue at position 279 plays a major role in suppression of the E135D.

The activity mediated by changes in the charged residue at position 135 does not follow a charge-related pattern; substitution of the negatively charged E135 by the alternative negatively charged D residue or neutral A residue results in a semiconstitutive activity, whereas substitution with a positively charged residue results in an effector specificity mutant which, unlike the wild type, can be activated by the effectors 4-ethylphenol and 2,4-dimethylphenol (20, 21; see Table I). The P297R mutation, which was originally isolated on the basis of suppression of the semiconstitutive activity of E135D, can also suppress the same activity of E135A (Table I) but did not influence the novel effector specificity of E135K (data not shown). These results suggest that the P297R mutation is an independent mutation that compensates for a weakening in the A/C-domain interaction in E135D and E135A, rather than residue P297R interacting directly with corresponding residue at position 135. That this is also the case for A-domain located second site suppressors is suggested below.

![Fig. 2. Transcriptional activation and ATPase activity of ΔA2-DmpR derivatives.](image-url)
The absence of effectors so close to basal levels (Fig. 1) suggests that E135D and V276A mutations are likely to be independent and that E135D (42% of effector-activated) are additive in the mutant alone. As shown in Fig. 3, the prediction holds true; the semiconstitutive activities of V276A (16% of effector-activated) and E135D (42% of effector-activated) are additive in V276A/E135D (63% of effector-activated). These data also suggest that E135D and V276A mutations are likely to be independent mutations that affect different A/C-domain interactions.

Additive effects of different second site suppressor mutations could not be tested since each mutation brought the activity in the absence of effectors so close to basal levels (Fig. 1B). However, a second prediction from the model outlined above could be tested; namely, that if each second site mutation mediated a tightening of the A/C-domain interaction, then each mutation should also be able to suppress the activity of semiconstitutive mutations other than the one on the basis of which it was isolated. To test this prediction we combined the A-domain E135D and the C-domain V276A mutations and compared the activity of the double substituted DmpR derivative with that of each mutant alone. As shown in Fig. 3A, the prediction holds true; the semiconstitutive activities of V276A (16% of effector-activated) and E135D (42% of effector-activated) are additive in V276A/E135D (63% of effector-activated). These data also suggest that E135D and V276A mutations are likely to be independent mutations that affect different A/C-domain interactions.

The simplest interpretation of the data described above is that mutations leading to semiconstitutive activity in the absence of effectors weaken interactions between the A- and C-domains, whereas second site suppressor mutations mediate compensatory tightening interactions. If this is indeed the case then the model would predict that combining mutations of different domains that mediate semiconstitutive activity should result in an additive effect. To test this prediction we combined the A-domain E135D and the C-domain V276A mutations and compared the activity of the double substituted DmpR derivative with that of each mutant alone. As shown in Fig. 3A, the prediction holds true; the semiconstitutive activities of V276A (16% of effector-activated) and E135D (42% of effector-activated) are additive in V276A/E135D (63% of effector-activated). These data also suggest that E135D and V276A mutations are likely to be independent mutations that affect different A/C-domain interactions.

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**DISCUSSION**

Repression appears to be a common mechanism by which the intrinsic activity of different families of prokaryotic transcriptional activators is controlled. For example, truncated derivatives of FixJ (31), SpoOA (32), AraC (33), and LuxR (34) are active in the absence of their normal activating signal. Similarly, the DNA binding properties of $\sigma^{70}$ and related $\sigma$ factors are also under repression control (36). In this work we have investigated the mechanism by which the transcriptional promoting ability of DmpR is regulated in response to the presence of aromatic compounds. As outlined in the Introduction, previous work has demonstrated that the activity of DmpR is regulated by direct interaction with its aromatic effectors leading to expression of its C-domain-mediated ATPase and transcriptional promoting activity (21). Deletion of the amino-terminal A-domain results in a protein that no longer requires effectors to achieve the fully active state. This finding suggested that the A-domain, in the absence of effectors, represses the activity of the C-domain (21), but did not provide any information on how the repression may be mediated. Two possible mechanisms could be envisaged: (i) steric hindrance of the C-domain by the A-domain or (ii) direct A/C interdomain interactions via specific pairs of amino acid residues. Here we used a genetic approach to investigate which mechanism operates. Using a positive genetic selection system we successfully isolated both inter- and intradomain suppressors of DmpR derivatives which mimic the activated state of the regulator in the absence of effector (Fig. 1A). The fact that interdomain suppressors could be isolated, i.e. that mutations of the A-domain of DmpR could compensate for the effect of a mutation in the C-domain and vice versa (Figs. 1A and 3B) strongly supports the suggestion that specific residues of the two domains interact to mediate repression of C-domain function(s). In this model the original semiconstitutively active mutants would cause a weakening of the A/C-domain interaction, whereas the suppressor mutation would provide a compensatory tightening of the interaction. This model does not necessarily imply that the second site mutation has to influence the same A/C residue/residue interaction as the semiconstitutive mutation that was selected against (see Fig. 3). It could still be argued, however, that the steric hindrance model cannot be excluded since the original mutations could cause distortions of the overall shape of one domain, which could possibly be compensated for by accommo-
interdomain regulation of DmpR. Due to significant distortions of the reciprocal domain. To distinguish between these alternative possibilities we constructed novel combinations of the genetically selected mutants. The A/C-domain interaction model would predict that a suppressor (tightened interaction) of one semiconstitutively active mutant (weakened interaction) would also be able to suppress the same activity of an independent semiconstitutively active mutant, a situation that would not be predicted for the steric hindrance model. All three intra-A-domain suppressors (L83P, F93L, and K188E) were found to also be capable of suppressing the C-domain located semiconstitutively mutant (V276A), once again supporting the A/C-domain interaction model.

Like DmpR, truncated derivatives of the highly related aromatic responsive regulator XylR are constitutively active in vivo (36). By coexpressing different domains of XylR, it has been shown recently that the A-domain of XylR can specifically inhibit the constitutive activity of A-domain-deleted derivatives of XylR and DmpR (37). Although not responsive to aromatic effectors, the inhibition again supports a model in which interdomain interactions mediate repression. This observation, in conjunction with the finding that swapping the A-domain of DmpR for that of the XylR results in a protein with fully regulated XylR effector specificity (4), suggests that the interactions mediating the repression and thus activity are conserved in the two proteins. Sequential deletion into the A-domain of XylR has pinpointed the C-domain proximal portion as the region responsible for interdomain repression (37). Therefore, the DmpR A-domain suppressors (L83P, F93L, K188E, and W193R) of the C-domain semiconstitutive mutant (V276A) may reflect interactions that can compensate for, but are not necessarily normally involved in, the interdomain repression.

In addition to DmpR and XylR, regulatory domain deletions of a number of other α54-dependent regulators have been shown to be constitutively active, including DctD (38) and LevR (39), whose activities are normally controlled by phosphorylation, and NifA and NifA-like regulators (40, 41), whose activities are modulated by protein:protein interactions (for review, see Ref. 10). However, this is not the case for α54-dependent NtrC. Deletion of the A-domain of NtrC results in an inactive protein, whereas the same deletion of a partially constitutive mutant retains the same level of activity (14). Hence, the phosphorylation of the A-domain of NtrC seems to serve a genuine stimulatory function. Despite this difference, interdomain repression may be the key control system. Recently, on the basis of chimeric proteins, the central C-domain of NtrC has been postulated to repress the formation of A-domain dimers in the absence of phosphorylation (42). Promotion or inhibition of A-domain dimers may serve as a role model for the future challenge of elucidating how aromatic effectors control the A/C-domain repression of DmpR.

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REFERENCES

1. Shingler, V., Powolowski, J., and Marklund, U. (1992) J. Bacteriol. 174, 711–724.
2. Powolowski, J., and Shingler, V. (1994) Biodegradation 5, 1–18.
3. Shingler, V., Bartilson, M., and Moore, T. (1993) J. Bacteriol. 175, 1596–1604.
4. Shingler, V., and Moore, T. (1994) J. Bacteriol. 176, 1555–1560.
5. Morett, E., and Segovia, L. (1993) J. Bacteriol. 175, 6067–6074.
6. North, A. K., Kloze, K. E., Stedman, K. M., and Kustu, S. (1993) J. Bacteriol. 175, 4267–4273.
7. Sze, C. C., Moore, T., and Shingler, V. (1996) J. Bacteriol. 178, in press.
8. Pérez-Martin, J., Rojo, F., and de Lorenzo, V. (1994) Microbiol. Rev. 58, 268–290.
9. Pérez-Martin, J., and de Lorenzo, V. (1995) J. Bacteriol. 177, 3758–3763.
10. Shingler, V. (1996) Mol. Microbiol. 19, 409–416.
11. Austin, S., and Dixon, R. (1992) EMBO J. 11, 2219–2228.
12. Weiss, D. S., Balat, J., Kloze, K. E., Keener, J., and Kustu, S. (1991) Cell 67, 157–167.
13. Porter, S. C., North, A. K., Wedel, A. B., and Kustu, S. (1993) Genes Dev. 7, 2258–2273.
14. Porter, S. C., North, A. K., and Kustu, S. (1995) in Two-component Signal Transduction (Sillibry, T., and Hoch, J., eds) pp. 147–158, American Society for Microbiology, Washington, D. C.
15. Mettke, I., Fiedler, U., and de Lorenzo, V. (1995) J. Bacteriol. 177, 5056–5061.
16. Wang, J. Y., Syed, A., Hsieh, M., and Graila, J. D. (1995) Science 270, 592–594.
17. Abril, M. A., Michan, C., Timmis, K. N., and Ramos, J. L. (1989) J. Bacteriol. 171, 6782–6790.
18. Delgado, A., and Ramos, J. L. (1994) J. Biol. Chem. 269, 8059–8062.
19. Hopper, S., and Böck, A. (1995) J. Bacteriol. 177, 2798–2803.
20. Pavel, H., Forsman, M., and Shingler, V. (1994) J. Bacteriol. 176, 7550–7557.
21. Shingler, V., and Pavel, H. (1995) Mol. Microbiol. 17, 505–513.
22. Steinmetz, M., LeCoq, D., Aymerich, S., Gonzyl-Théboul, G., and Gay, P. (1985) Mol. & Gen. Genet. 200, 220–228.
23. de Lorenzo, V., Herrero, M. J., Jakubzik, U., and Timmis, K. N. (1990) J. Bacteriol. 172, 6568–6572.
24. Franklin, F. C. H., Bagdasarian, M., Bagdasarian, M. M., and Timmis, K. N. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 7458–7462.
25. de Lorenzo, V., and Timmis, K. N. (1994) Methods Enzymol. 235, 386–405.
26. Gay, P., Le Coq, D., Steinmetz, M., Berkelman, T., and Kado, C. I. (1985) J. Bacteriol. 164, 918–921.
27. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., pp. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
28. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., and Pease, L. R. (1989) Gene (Amst.) 77, 51–59.
29. Horwich, T. P., Pritchett, K. S., Price, V. L., Libby, R. T., March, C. J., Cerretti, D. P., Urdal, D. L., and Conlon, P. J. (1988) BioTechnology 6, 204–212.
30. Rosenberg, A. H., Lada, B. N., Chui, D.-S., Lin, S.-W., Dunn, J. J., and Studier, F. W. (1987) Gene (Amst.) 56, 125–135.
31. Kahn, D., and Ditta, G. (1991) Mol. Microbiol. 5, 87–97.
32. Ireson, K., Rudner, D. Z., Sironasio, K. J., and Grossman, A. D. (1993) Genes Dev. 7, 283–294.
33. Menon, K. P., and Lee, N. I. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3708–3712.
34. Cho, S. H., and Greenberg, E. P. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 11115–11119.
35. Deleted in proof.
36. Dombroski, A. J., Walter, W. A., and Gross, C. A. (1993) Genes Dev. 7, 2446–2455.
37. Pérez-Martin, J., and de Lorenzo, V. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9392–9396.
38. Huala, E., and Ausubel, F. M. (1992) J. Bacteriol. 174, 1428–1431.
39. Martin-Verastraete, I., Désbarbouilli, M., Klier, A., and Rappoport, G. (1994) J. Mol. Biol. 241, 178–192.
40. Austin, S., and Lambert, J. (1994) J. Biol. Chem. 269, 18141–18148.
41. Berger, D. K., Naberhause, F., and Kustu, S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 103–107.
42. Fiedler, U., and Weiss, V. (1995) EMBO J. 14, 3696–3705.