JOURNAL OF BACTERIOLOGY, Dec. 2000, p. 7075–7077 Vol. 182, No. 24
0021-9193/00/$04.00+0
Copyright © 2000, American Society for Microbiology. All Rights Reserved.

Transcription Activation by a Variety of AraC/XylS Family Activators Does Not Depend on the Class II-Specific Activation Determinant in the N-Terminal Domain of the RNA Polymerase Alpha Subunit

SUSAN M. EGAN,[1]* ANDREW J. PEASE,[2]† JEFFREY LANG,[2] XIANG LI,[2] VYDEHI RAO,[1] WILLIAM K. GILLETTE,[3]‡ RAQUEL RUIZ,[1,4] JUAN L. RAMOS,[4] AND RICHARD E. WOLF, JR.[4]

Department of Molecular Biosciences, University of Kansas, Lawrence, Kansas 66045[1]; Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, Maryland 21250[2]; and Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0560[3]; and Consejo Superior de Investigaciones Cientificas, Estación Experimental del Zaidín, Department of Plant Biochemistry and Molecular and Cellular Biology of Plants, Granada, Spain[4]

Received 21 August 2000/Accepted 4 October 2000

The N-terminal domain of the RNA polymerase α subunit (α-NTD) was tested for a role in transcription activation by a variety of AraC/XylS family members. Based on substitutions at residues 162 to 165 and an extensive genetic screen we conclude that α-NTD is not an activation target for these activators.

The AraC/XylS family is a large family of transcription regulators, many of whose members activate virulence factors in bacterial pathogens and hence are of interest as potential targets of antibacterial agents (9). Virtually all AraC/XylS family members are capable of transcription activation, and thus it is likely the mechanisms used by these proteins to activate transcription have been conserved, although subsets of family members may use different mechanisms. A variety of AraC/XylS family members have been shown to require the RNA polymerase α subunit C-terminal domain (α-CTD) and the C-terminal end of the σ subunit for full activation (4, 12–19; S. M. Egan and C. C. Holcroft, unpublished results; R. Ruiz, J. L. Ramos, and S. M. Egan, unpublished results). However, for several family members it is believed that one or more additional activation targets have yet to be identified. For example, SoxS has been shown to be capable of activating in vitro transcription of a class II promoter when the reconstituted RNA polymerase lacks both the α-CTD and the C-terminal residues of σ^{70} (K.-W. Jair and R. E. Wolf, Jr., unpublished results). There is also evidence that additional activation targets may exist in the cases of MarA, RhaS and RhaR (4, 12, 13) (Holcroft and Egan, unpublished results; R. Martin, personal communication).

Effect of α-NTD derivatives on activation by RhaS, RhaR, XylS, MarA and SoxS. Niu et al. (24) have demonstrated that residues 162 to 165 of the RNA polymerase α subunit N-terminal domain (α-NTD) are required for transcription activation at class II cyclic AMP (cAMP) receptor protein (CRP)-dependent promoters where the CRP binding site overlaps the promoter −35 hexamer. To test whether α-NTD plays a role in transcription activation by a variety of AraC/XylS family activators, we transformed strains carrying a wild-type chromosomal copy of *rpoA* with plasmids overexpressing either wild-type α or previously described alanine substitution derivatives of α (24).

Activation of the *rhaBAD* promoter requires RhaS bound to a site that overlaps the −35 hexamer, CRP bound at −92.5, and α-CTD (6, 7, 12). Activation of the divergent *rhaSR* operon requires RhaR bound to a site that overlaps the −35 hexamer, CRP bound at −111.5 and α-CTD (29, 30) (Holcroft and Egan, unpublished). We grew strains carrying the α-NTD derivatives and *rhaB-lacZ* or *rhaS-lacZ* fusions in MOPS (morpholinepropanesulfonic acid) minimal medium with 0.4% glycerol, 0.2% L-rhamnose, and 125 μg of ampicillin per ml and then assayed for β-galactosidase expression as previously described (3). We found that none of the substitutions produced a significant defect in activation (Table 1).

In the presence of an effector such as 3-methylbenzoate, XylS activates expression of the *Pseudomonas putida* TOL plasmid *meta* promoter, Pm, from a site that overlaps the −35 hexamer (10). The Pm promoter system was reconstituted in *Escherichia coli* MC4100 (28) by transformation with pERD100, which carries Φ(Pm-′*lacZ*) (1), a derivative of pLOW2 (11) encoding *xylS*, and the plasmid encoding wild-type α or alanine substitution derivatives. These strains were grown in Luria-Ber-

TABLE 1. RhaS and RhaR activation with alanine substitutions in α-NTD

α-NTD derivative	β-Galactosidase sp act (% of wild-type activity)[a]	
	Φ(*rhaB-lacZ*)Δ110	Φ(*rhaS-lacZ*)Δ128
Wild type	617 ± 45 (100)	132 ± 6 (100)
162A	576 ± 51 (93)	138 ± 10 (104)
163A	566 ± 38 (92)	131 ± 10 (99)
164A	597 ± 50 (97)	135 ± 6 (102)
165A	592 ± 48 (96)	122 ± 9 (93)
162–165A	575 ± 56 (93)	113 ± 9 (86)

[a] β-Galactosidase specific activity (in Miller units) was measured from single-copy *rhaB-lacZ* or *rhaS-lacZ* fusions in a wild-type strain background transformed with plasmids encoding either wild-type or substitution derivatives within the N-terminal domain of α. Φ(*rhaB-lacZ*)Δ110 (in strain SME1035) includes the binding sites for both the RhaS and CRP activators (7), while Φ(*rhaS-lacZ*)Δ128 (in strain SME2503) includes sites for both RhaR and CRP (Holcroft and Egan, unpublished). Cultures were grown in the presence of the inducer L-rhamnose. Values are the averages of three independent experiments.

* Corresponding author. Mailing address: Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045. Phone: (785) 864-4294. Fax: (785) 864-5294. E-mail: sme@ukans.edu.
† Present address: Villa Julie College, Stevenson, MD 21153.
‡ Present address: Life Technologies, Inc., Rockville, MD 20850.

TABLE 2. XylS activation at Φ(Pm-'*lacZ*) with alanine substitutions in α-NTD

α-NTD derivative	β-Galactosidase sp act (% of wild-type activity)[a]	
	−3MBz	+3MBz
Wild type	70 ± 3 (100)	1520 ± 46 (100)
162A	70 ± 2 (100)	1625 ± 42 (107)
163A	70 ± 2 (100)	1790 ± 66 (118)
164A	70 ± 2 (100)	1900 ± 15 (125)
165A	75 ± 2 (107)	1745 ± 90 (115)
162–165A	75 ± 1 (107)	1760 ± 17 (116)

[a] β-Galactosidase specific activity (in Miller units) was measured from plasmid borne (Pm-'lacZ) in *E. coli* MC4100 transformed with pLOW2 encoding wild-type XylS and plasmids encoding either wild-type or substitution derivatives within the N-terminal domain of α. Cultures were grown either in the absence (−) or the presence (+) of the inducer 3-methylbenzoate (3MBz). Values are the averages of three independent experiments.

tani (LB) medium with 100 μg of ampicillin, 25 μg of kanamycin, and 10 μg of tetracycline per ml, and β-galactosidase assays were performed as previously described (23, 26). We found that expression of the α-NTD derivatives had no significant effects on activation by XylS (Table 2) or the related activator (25) XylS1 (data not shown).

The structure of the single domain MarA protein has been determined in complex with DNA (27). MarA is capable of activating transcription of a large variety of promoters (2), in some cases from a site that overlaps the −35 hexamer (class II), and in other cases from a site further upstream (class I) (20). We tested the effect of the α-NTD derivatives on MarA-dependent activation at *lacZ* fusions to two class I (*fpr* and *zwf*, data not shown) and three class II (*inaA*, *fumC*, and *micF*) promoters (Table 3). Cultures were grown in LB medium-ampicillin (100 μg/ml) and induced with 5 mM salicylate for 1 h, and β-galactosidase activity was assayed as described previously (22, 23). We found no significant defects at any of the MarA-dependent promoters.

Similar to MarA, SoxS consists of a single domain and can activate class I and class II promoters (8). Activation of class II promoters was not significantly decreased upon deletion of α-CTD (15), and residues at the C-terminal end of σ^{70} are not essential for transcription activation by SoxS (Jair and Wolf, unpublished). To test for a role of α-NTD in SoxS activation, we assayed strains bearing translational fusions of four class II SoxS-dependent promoters (*fumC*, *micF*, *nfo*, and *sodA*) (Table 4) and two class I SoxS-dependent promoters (*zwf* and *fpr*) (data not shown). Cultures were grown in LB medium-ampicillin (125 μg/ml), induced for 1 h with 0.5 mM paraquat, and then assayed as previously described (23). The α-NTD derivatives conferred no significant effects on transcription activation of the class I or class II SoxS-dependent promoters.

TABLE 3. MarA activation at class II promoters with alanine substitutions in α-NTD

α-NTD derivative	β-Galactosidase sp act (% of wild-type activity)[a]		
	Φ(*inaA-lacZ*)	Φ(*fumC-lacZ*)	Φ(*micF-lacZ*)
Wild type	88/107 (100)	326/376 (100)	702/559 (100)
162A	87/110 (101)	338/309 (92)	648/556 (95)
163A	88/107 (100)	339/373 (101)	719/504 (97)
164A	95/113 (107)	303/394 (99)	686/581 (100)
165A	126/115 (124)	317/394 (101)	659/586 (99)
162–165A	102/113 (110)	312/436 (107)	669/529 (95)

[a] β-Galactosidase specific activity (in Miller units) was measured from single-copy *inaA*-, *fumC*- and *micF-lacZ* fusions in strains N8457, N9638 and N9639 (21), respectively, transformed with plasmids encoding either wild-type or substitution derivatives within the N-terminal domain of α. Cultures were grown in the presence of the inducer salicylate. Data from experiment 1 are shown before the slash, and data from experiment 2 are shown after the slash.

TABLE 4. SoxS activation at class II promoters ith alanine substitutions in α-NTD

Promoter	*rpoA* mutation	Activity (Miller units)[a]		Induction ratio	% of wild-type activity
		Uninduced	Induced		
fumC	Wild type	66	2210	33	100
	162A	72	2540	35	115
	163A	74	2120	29	96
	164A	71	2000	28	90
	165A	74	2400	32	109
	162–165A	82	2920	36	132
micF	Wild type	43	325	7.5	100
	162A	44	390	8.8	120
	163A	45	335	7.4	104
	164A	45	400	8.9	123
	165A	47	290	6.2	89
	162–165A	63	345	5.5	107
nfo	Wild type	225	2400	10.6	100
	162A	205	2000	9.7	84
	163A	225	2070	9.2	86
	164A	215	2100	9.7	88
	165A	205	2460	12.1	103
	162–165A	205	2440	12.0	102
sodA	Wild type	1,080	9,530	8.8	100
	162A	915	8,990	9.8	94
	163A	1,105	8,770	7.9	92
	164A	905	9,560	10.6	100
	165A	880	7,930	9.0	83
	162–165A	805	8,960	11.2	94

[a] β-Galactosidase specific activity was measured from single-copy *lacZ* fusions in a wild-type strain background transformed with plasmids encoding either wild-type or substitution derivatives within the N-terminal domain of α. Cultures were grown either in the absence or the presence of the inducer paraquat. The percent wild-type value was calculated from the induced Miller unit value in the presence of the α-NTD mutant compared with the induced Miller unit value obtained with wild-type α. Values are the averages of three independent experiments.

Genetic screen for mutations in *rpoA* resulting in SoxS activation defects. Given that the mutations at residues 162 to 165 had no effect, a screen for other *rpoA* mutations affecting activation of class II SoxS-dependent promoters was designed. To construct the screening strain, we moved a *soxR* constitutive mutation (31), which provided an intermediate level of SoxS, into a strain that contained a *fumC-lacZ* fusion (21). This strain was transformed with derivatives of plasmid pREIIα (5) in which the entire *rpoA* gene had been subjected to PCR mutagenesis with *Taq* polymerase (32) using primers with the same sequence as those used by Niu et al. (24). The transformants were screened on lactose-tetrazolium plates (23) containing ampicillin (100 μg/ml) and kanamycin (20 μg/ml). The strain carrying the $soxR^{C1}$ allele produced white colonies with light pink centers whereas a similarly uninduced isogenic strain with the wild-type allele of *soxR* produced red colonies. In the presence of paraquat, both strains produced white colonies. We demonstrated that less than a twofold reduction in *lacZ* expression in this strain resulted in reddish colonies that were

clearly distinguishable from the pink-centered wild-type colonies. Approximately 24,000 transformants were screened from 26 independent mutagenesis reactions. No mutations in α-NTD were isolated that conferred a defective phenotype; however, the screen readily yielded mutations in α-CTD.

We next confined a genetic screen to α-NTD (by PCR mutagenesis of only the *Xba*I-to-*Hin*dIII fragment of pREIIα) to determine whether any α-NTD substitutions conferred activation defects. Twenty independent PCR mutagenesis mixtures were ligated into pREIIα, and 18,000 transformants were screened. Only one transformant with an activation-deficient phenotype was identified, and this plasmid turned out to have a rearrangement that produced an α-CTD deletion. As a control, we also screened for mutations in α-CTD and found 16 apparent activation-deficient mutants among just two independent PCRs and 2,000 transformants. Therefore, while we readily obtained mutations in the α-CTD by using this mutagenesis strategy, we were again unable to isolate any mutations in the α-NTD that influenced SoxS activation.

Remarks. From the results of this work, we conclude that transcription activation by RhaS, RhaR, XylS, MarA, and SoxS does not require contact with the 162-to-165 determinant of α-NTD, nor, most likely, any other portion of α-NTD. The activators tested in this study represent a diverse set of AraC/XylS family proteins, which, with the exception of particularly related pairs (MarA/SoxS and RhaS/RhaR), share only 24 to 28% amino acid sequence identity. It is likely, therefore, that our conclusions apply to many other AraC/XylS family members.

We are very grateful to Richard H. Ebright for providing the plasmids encoding α-NTD derivatives and Robert Martin and Judah Rosner for providing strains.

Work in the laboratory of S.M.E. was supported by Public Health Service grant GM55099 from the National Institute of General Medical Sciences and the Franklin Murphy Molecular Biology Endowment. Work in the laboratory of R.E.W. was supported by Public Health Service grant GM27113 from the National Institutes of General Medical Sciences. R.R. received a travel fund from the Spanish Ministry of Education to visit the laboratory of S.M.E. at the University of Kansas. Work in the laboratory of J.L.R. was funded by grant BIO-97-0641.

REFERENCES

1. **Abril, M. A., C. Michan, K. N. Timmis, and J. L. Ramos.** 1989. Regulator and enzyme specificity of TOL plasmid-encoded upper pathway for degradation of aromatic hydrocarbons and expansion of the substrate range of the pathway. J. Bacteriol. **171:**6782–6790.
2. **Barbosa, T. M., and S. B. Levy.** 2000. Differential expression of over 60 chromosomal genes in *Escherichia coli* by constitutive expression of MarA. J. Bacteriol. **182:**3467–3474.
3. **Bhende, P. M., and S. M. Egan.** 1999. Amino acid-DNA contacts by RhaS: an AraC family transcription activator. J. Bacteriol. **181:**5185–5192.
4. **Bhende, P. M., and S. M. Egan.** 2000. Genetic evidence that transcription activation by RhaS involves specific amino acid contacts with sigma 70. J. Bacteriol. **182:**4959–4969.
5. **Blatter, E. E., W. Ross, H. Tang, R. L. Gourse, and R. H. Ebright.** 1994. Domain organization of RNA polymerase α subunit: C-terminal 85 amino acids constitute a domain capable of dimerization and DNA binding. Cell **78:**889–896.
6. **Egan, S. M., and R. F. Schleif.** 1994. DNA-dependent renaturation of an insoluble DNA binding protein. Identification of the RhaS binding site at *rhaBAD*. J. Mol. Biol. **243:**821–829.
7. **Egan, S. M., and R. F. Schleif.** 1993. A regulatory cascade in the induction of *rhaBAD*. J. Mol. Biol. **234:**87–98.
8. **Fawcett, W. P., and R. E. Wolf, Jr.** 1994. Purification of a MalE-SoxS fusion protein and identification of the control sites of *Escherichia coli* superoxide-inducible genes. Mol. Microbiol. **14:**669–679.
9. **Gallegos, M.-T., R. Schleif, A. Bairoch, K. Hofmann, and J. L. Ramos.** 1997. AraC/XylS family of transcriptional regulators. Microbiol. Mol. Biol. Rev. **61:**393–410.
10. **Gonzalez-Perez, M. M., J. L. Ramos, M. T. Gallegos, and S. Marques.** 1999. Critical nucleotides in the upstream region of the XylS-dependent TOL meta-cleavage pathway operon promoter as deduced from analysis of mutants. J. Biol. Chem. **274:**2286–2290.
11. **Hansen, L. H., S. J. Sorensen, and L. B. Jensen.** 1997. Chromosomal insertion of the entire *Escherichia coli* lactose operon, into two strains of *Pseudomonas*, using a modified mini-Tn*5* delivery system. Gene **186:**167–173.
12. **Holcroft, C. C., and S. M. Egan.** 2000. Roles of cyclic AMP receptor protein and the carboxyl-terminal domain of the α subunit in transcription activation of the *Escherichia coli rhaBAD* operon. J. Bacteriol. **182:**3529–3535.
13. **Jair, K., R. G. Martin, J. L. Rosner, N. Fujita, A. Ishihama, and R. E. Wolf, Jr.** 1995. Purification and regulatory properties of MarA protein, a transcriptional activator of *Escherichia coli* multiple antibiotic and superoxide resistance promoters. J. Bacteriol. **177:**7100–7104.
14. **Jair, K.-M., X. Yu, K. Skarstad, B. Thony, N. Fujita, A. Ishihama, and R. E. Wolf, Jr.** 1996. Transcriptional activation of promoters of the superoxide and multiple antibiotic resistance regulons by Rob, a binding protein of the *Escherichia coli* origin of chromosomal replication. J. Bacteriol. **178:**2507–2513.
15. **Jair, K.-W., W. P. Fawcett, N. Fujita, A. Ishihama, and R. E. Wolf, Jr.** 1996. Ambidextrous transcriptional activation by SoxS: requirement for the C-terminal domain of the RNA polymerase alpha subunit in a subset of *Escherichia coli* superoxide-inducible genes. Mol. Microbiol. **19:**307–317.
16. **Landini, P., J. A. Bown, M. R. Volkert, and S. J. W. Busby.** 1998. Ada protein-RNA polymerase σ subunit interaction and α subunit-promoter DNA interactions are necessary at different steps in transcription activation at the *Escherichia coli ada* and *aidB* promoters. J. Biol. Chem. **273:**13307–13312.
17. **Landini, P., and S. J. Busby.** 1999. The *Escherichia coli* Ada protein can interact with two distinct determinants in the σ^{70} subunit of RNA polymerase according to promoter architecture: identification of the target of Ada activation at the *alkA* promoter. J. Bacteriol. **181:**1524–1529.
18. **Landini, P., T. Gaal, W. Ross, and M. R. Volkert.** 1997. The RNA polymerase α subunit carboxyl-terminal domain is required for both basal and activated transcription from the *alkA* promoter. J. Biol. Chem. **272:**15914–15919.
19. **Lonetto, M. A., V. Rhodius, K. Lamberg, P. Kiley, S. Busby, and C. Gross.** 1998. Identification of a contact site for different transcription activators in region 4 of the *Escherichia coli* RNA polymerase σ^{70} subunit. J. Mol. Biol. **284:**1353–1365.
20. **Martin, R. G., W. K. Gillette, S. Rhee, and J. L. Rosner.** 1999. Structural requirements for marbox function in transcriptional activation of mar/sox/rob regulon promoters in *Escherichia coli*: sequence, orientation and spacial relationship to the core promoter. Mol. Microbiol. **34:**431–441.
21. **Martin, R. G., W. K. Gillette, and J. L. Rosner.** 2000. Promoter discrimination by the related transcriptional activators MarA and SoxS: differential regulation by differential binding. Mol. Microbiol. **35:**623–634.
22. **Martin, R. G., and J. L. Rosner.** 1997. Fis, an accessorial factor for transcriptional activation of the *mar* (multiple antibiotic resistance) promoter of *Escherichia coli* in the presence of the activator MarA, SoxS, or Rob. J. Bacteriol. **179:**7410–7419.
23. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
24. **Niu, W., Y. Kim, G. Tau, T. Heyduk, and R. H. Ebright.** 1996. Transcription activation at class II CAP-dependent promoters: two interactions between CAP and RNA polymerase. Cell **87:**1123–1134.
25. **Osborne, D. J., R. W. Pickup, and P. A. Williams.** 1988. The presence of two homologous *meta* pathway operons on TOL plasmid pWW53. J. Gen. Microbiol. **134:**2965–2975.
26. **Ramos, J. L., A. Stolz, W. Reineke, and K. N. Timmis.** 1986. Altered effector specificities in regulators of gene expression: TOL plasmid *xylS* mutants and their use to engineer expansion of the range of aromatics degraded by bacteria. Proc. Natl. Acad. Sci. USA **83:**8467–8471.
27. **Rhee, S., R. G. Martin, J. L. Rosner, and D. R. Davies.** 1998. A novel DNA-binding motif in MarA: the first structure for an AraC family transcriptional activator. Proc. Natl. Acad. Sci. USA **95:**10413–10418.
28. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
29. **Tobin, J. F., and R. F. Schleif.** 1990. Purification and properties of RhaR, the positive regulator of the L-rhamnose operons of *Escherichia coli*. J. Mol. Biol. **211:**75–89.
30. **Tobin, J. F., and R. F. Schleif.** 1990. Transcription from the *rha* operon p_{sr} promoter. J. Mol. Biol. **211:**1–4.
31. **Tsaneva, I. R., and B. Weiss.** 1990. *soxR*, a locus governing a superoxide response regulon in *Escherichia coli* K-12. J. Bacteriol. **172:**4197–4205.
32. **Zhou, Y. H., X. P. Zhang, and R. H. Ebright.** 1991. Random mutagenesis of gene-sized DNA molecules by use of PCR with Taq DNA polymerase. Nucleic Acids Res. **19:**6052.