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Promoter Discrimination at Class I MarA Regulon Promoters Mediated by Glutamic Acid 89 of the MarA Transcriptional Activator of *Escherichia coli*▽†

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Three paralogous transcriptional activators MarA, SoxS, and Rob, activate >40 *Escherichia coli* promoters. To understand why MarA does not activate certain promoters as strongly as SoxS, we compared MarA, MarA mutants, and SoxS for their abilities to activate 16 promoters and to bind their cognate marbox binding sites. Replacement of the MarA glutamic acid residue 89 with alanine greatly increased the marbox binding and activation of many class I promoters. Like cells constitutive for SoxS, cells expressing the MarA with the E89A mutation were more resistant to superoxides than those harboring WT MarA. The activities of several other E89 substitutions ranked as follows: E89A > E89G > E89V > WT > E89D. Increased binding and activation occurred only at class I promoters when the 12th base of the promoter's marbox (a position at which there is no known interaction between the marbox and MarA) was not a T residue. Furthermore, WT MarA binding to a synthetic marbox *in vitro* was enhanced when the phosphate group between positions 12 and 13 was eliminated on one strand. The results demonstrate that relatively minor changes in a single amino acid side chain (e.g., alanine to valine or glutamic acid to aspartic acid) can strongly influence activity despite any evidence that the side chain is involved in positive interactions with either DNA or RNA polymerase. We present a model which attributes the differences in binding and activation to the interference between the β- and γ-carbons of the amino acid at position 89 and the phosphate group between positions 12 and 13.

The three paralogous *Escherichia coli* AraC/XylS family activators MarA, SoxS, and Rob are regulated by three different systems (*marRAB*, *soxRS*, and *rob*, respectively) in response to different stresses (29). These activators transcriptionally activate the same set of >40 promoters (the MarA-SoxS-Rob regulon) but to different extents (2, 26, 33, 43). This work was undertaken to discern structural differences between MarA and SoxS that might be responsible for this "promoter discrimination."

Treatment of *E. coli* with phenolic derivatives, such as salicylate, inactivates MarR, leading to derepression of the *marRAB* operon (7, 24). The resultant increase in cellular resistance to low levels of diverse antibiotics and organic solvents is due primarily to activation of the efflux pump genes *acrAB* and *tolC* (3, 32).

Treatment with superoxide-generating compounds, such as paraquat, activates SoxR, which, in turn, transcriptionally activates the expression of *soxS* (5, 10, 31, 46). Upregulation of *soxS* renders the cells resistant to the same levels of antibiotics as but to greater levels of superoxides than does *marRAB* derepression (23). This is likely due to the greater extent of activation by SoxS of promoters involved in superoxide defense, e.g., *acnA*, *fpr*, *zwf*, *fumC*, and *sodA*. The Rob protein is primarily regulated posttranslationally (37, 38) and was not studied further here.

The basis for the activation of these promoters is the presence of a 20-bp binding site for MarA and SoxS (the marbox or soxbox). The consensus sequence for this site is highly degenerate, and the marbox must be in one of several configurations relative to the binding signals for RNA polymerase (RNAP) in order to be functional (17, 21, 22, 45). From X-ray crystallographic analysis of the cocrystal of MarA with the marbox from the *marRAB* promoter, two helix-turn-helix (HTH) motifs were identified that make 34 contacts with the DNA and bend it by 35° (36). While no physical structure is available for SoxS, it is likely to resemble MarA in how it binds DNA: at the MarA positions where amino acid side chains make DNA contact, the identity between MarA and SoxS is 60% (87% homology) even though the overall identity between the two proteins (1) is only 40% (56% homology). The structure of Rob bound to the *micF* marbox (19) is not pertinent here as it appears to be, in part, an artifact of crystal packing.

Among the promoters activated by MarA, the correlation between the strength of binding and the extent of activation is poor (23). For example, MarA binds tightly to the *marRAB* promoter *in vitro* yet stimulates transcription by only ~3-fold; no significant binding to *inaA* can be demonstrated by gel shift experiments, yet transcription is stimulated >5-fold. This finding may be related to the fact that the basal transcription of *marRAB* is high, whereas that of *inaA* is low. A similar lack of correlation between binding strength and activation is found for SoxS (23).

In contrast, there is a correlation between the relative strengths of binding of MarA and SoxS to the marbox of a

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TABLE 1. DNA fragments used in binding studies[a]

Marbox type	Sequence of one strand of the marbox fragment at the indicated position																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Consensus	A	Y	N	G	C	A	C	N	N	W	N	N	R	Y	Y	A	A	A	C	N
Promoter																				
acnA	A	A	C	C	C	A	A	A	T	T	G	A	T	A	A	A	A	G	A	G
acrAB	A	T	G	G	C	A	C	G	A	A	A	A	A	C	C	A	A	A	C	A
fpr	A	A	G	G	C	T	C	A	A	T	C	G	A	T	C	A	A	A	T	C
fumC	A	T	G	G	C	A	C	G	A	A	A	G	A	C	C	A	A	A	C	A
inaA	A	C	G	A	C	A	C	G	T	T	T	C	A	T	T	A	A	G	A	T
marRAB	A	T	G	C	C	A	C	G	T	T	T	**T**	G	C	T	A	A	A	T	C
mdaB	T	T	T	G	C	A	C	A	T	T	T	**T**	G	C	T	A	A	T	T	T
mdtG	A	G	A	G	C	T	T	T	T	A	T	C	G	C	T	A	A	A	T	C
micF	A	C	A	G	C	A	C	T	G	A	A	**T**	G	T	C	A	A	A	A	C
nfsB	A	G	C	G	C	A	T	T	T	T	T	C	T	C	G	C	T	T	A	C
pqiA	A	A	A	G	C	A	G	A	A	A	C	**T**	G	T	A	A	A	A	C	G
poxB	G	A	G	G	C	A	C	T	A	A	C	G	G	T	T	A	A	A	T	A
sodA	A	C	G	G	C	A	T	T	G	A	T	A	A	T	C	A	T	T	T	T
ybjC	A	A	A	G	C	T	A	T	A	A	C	**T**	G	T	T	A	A	A	C	A
yhbW	A	T	A	G	C	T	C	A	C	T	T	**T**	G	T	T	A	A	C	A	A
zwf	A	T	C	G	C	A	C	G	G	G	T	G	G	A	T	A	A	G	C	G

[a] Both strands of the 20-mer marbox from each promoter were synthesized and annealed and used in the binding experiments. Each marbox is shown in the forward orientation (22). Although there is no base preference at position 20, which is why we refer to the marbox as being 19 bp in length, binding *in vitro* requires a 20-bp fragment (22). At position 12, 6 of the 16 marbox sequences have a T at this position (boldface). At positions 17 and 18, 9 of the 16 marboxes have an A at both positions; underlining shows where there is no A; only the *sodA* and *nfsB* marboxes have no A at either position.

particular promoter and the relative abilities of these activators to stimulate that promoter (23). Since MarA activates some promoters more effectively and SoxS activates others more effectively, we wished to determine what structural differences might account for this promoter discrimination (23) and whether these differences were related to DNA binding at the corresponding marboxes.

In this report, we extend our earlier alanine-scanning mutagenesis studies (13) to a large number of promoters. Our principal finding is that MarA with the mutation E89A [MarA(E89A)] behaves more like SoxS than MarA in exhibiting greater activation and binding at class I promoters. We propose a model for the role of E89 in promoter discrimination.

MATERIALS AND METHODS

Bacterial strains. Table S1 in the supplemental material provides a list of the strains used in this study. The *lacZ* transcriptional fusions have been described previously (12, 13, 20, 26, 27) except for *acrAB*::*lacZ*. This fusion contains the *acrAB* promoter from nucleotides 485037 to 484920 fused to *lacZ* and was constructed as described previously (39); the transcription start site is at 484922 (11). Most of the fusions were introduced into, and assayed in, strain M3997 (Δ*marRAB rob*::*kan* Δ*lon clpP*::*cat* [F′ *proAB*$^+$ Tn*10 lacI*^{q}]). The *lon* mutation prevents degradation of the proteolysis-sensitive MarA and SoxS proteins (15). However, the *mdtG*::*lacZ* fusion was introduced into, and assayed in, the wild-type (WT) strain GC4468, and the *acrAB*::*lacZ* fusion was introduced into, and assayed in, M4436, an *acrR*::*cat* derivative of GC4468. In experiments shown in Fig. 3, *fpr*::*lacZ* and *mdtG*::*lacZ* fusions were analyzed in strain GC4468. Because of the instability of the strain carrying E89A in GC4468, each strain (see Fig. 3) carrying the MarA wild type or mutant or the SoxS plasmid was reconstructed and purified immediately prior to assay. Since we have demonstrated that the MarA concentrations attained here (20) exceed the K_m for the Lon protease, the levels of MarA in *lon*$^+$ *clpP*$^+$ cells are ~65% of those in *lon clpP* cells and should not affect the results significantly. Both M3997 and N8452 have null mutations in *marRAB* and *rob* to eliminate the basal levels of expression of the activators; the WT *soxS* present on the chromosome is negligibly expressed (our unpublished observations).

The plasmids carrying *marA*, *soxS*, and most of the *marA* mutations used in this study are derivatives of pUC19 and have been described previously (13, 23). However, MarA(E89A) was reconstructed because plasmid recovered from frozen stocks (13) did not have the correct sequence. Strains carrying this plasmid were found to be highly unstable in the absence of *lacI*^{q}. Other plasmids containing mutants of MarA or SoxS not previously described were constructed in a manner analogous to that of Gillette et al. (13). These include MarA mutants E25R, E77R, Y81D, E89D, E89G, E89V, R85D, E25R R85D, and E77R R85D and the SoxS mutant V83E. All mutations were verified by sequence analysis of both DNA strands.

β-Galactosidase assays. β-Galactosidase activity was assayed using the SDS-$CHCl_3$ method described by Miller (30). Cells containing a plasmid that expresses wild-type or mutant MarA or SoxS under *lacI*^{q} control were grown to mid-log phase and derepressed for 1 h with 500 μM isopropyl-β-D-thiogalactopyranoside (IPTG) prior to assay. The *poxB* promoter, which requires RpoS function, was assayed in stationary-phase cultures. See Materials and Methods in the supplemental material for a discussion of the statistical analysis of the data. The data presented in Tables 2 and 3 and in Fig. 1 are normalized to the activity of the promoter-*lacZ* fusion carrying the WT MarA plasmid according to the following calculation: (activity of the mutant MarA − activity of the no-activator control)/(activity of WT MarA − activity of the no-activator control).

The data for Fig. 3 are from overnight cultures diluted 100-fold and allowed to grow 2 h to mid-logarithmic phase before assay.

DNA binding assays. DNA binding assays were performed as described previously (25). Briefly, MarA, MarA mutants, and SoxS containing the N-terminal fragment encoded in pET15b were purified as previously described (13, 18) and serially diluted 1.67-fold in buffer containing 50 mM HEPES, pH 8.0, 25% glycerol, 0.5 M NaCl, 100 μg/ml bovine serum albumin, and 500 nM poly(dA-dT) (20 bp in length). One microliter of activator was mixed with 9 μl of 5′ ^{32}P end-labeled double-stranded DNA (dsDNA) fragment (20 bp in length; ~5 pmol/ml, one strand of which is listed in Table 1) in Tris-acetate-EDTA (TAE) buffer with 25% glycerol and subjected to electrophoresis at 150 V on 6% gels for 35 min. Gels were dried and analyzed with a Molecular Dynamics PhosphorImager as previously described (21). Assays were performed in duplicate and had variances of <1 dilution, i.e., ± 1.67-fold. For the experiments employing DNA with one chain interrupted, the complementary 36-nucleotide (nt) fragment (including the marbox of *fpr* flanked by the 7 nt to its 5′ end and 9 nt to its 3′ end as listed in Table 1, i.e., CCTCTGATTGATTTGATCGATT GAGCCTTCCAGTCC) was end labeled and annealed either to a single unlabeled fragment having the sequence GGACTGGAAGGCTCAATCGATCAA ATCAATCAGAGG (marbox underlined) or to two unlabeled fragments (both 5′ and 3′ ends dephosphorylated) together containing the appropriate sequence

TABLE 2. Activation of class I promoters by MarA, 13 MarA mutants, and SoxS[a]

Activator	Activity relative to that of MarA promoter-*lacZ* fusion[b]						
	marRAB	*acnA*	*acrAB*	*fpr*	*mdtG*	*poxB*	*zwf*
MarA							
WT	1.00	1.00	1.00	1.00	1.00	1.00	1.00
E25R	1.03	0.71	1.2	0.95	1.10	1.2	0.70
E77A	0.98	0.80	1.02	0.91	1.08	0.78	1.03
E77R	1.02	0.39	0.81	0.59	1.03	1.2	0.53
L80A	0.97	1.3	**3.7**	1.3	1.2	0.36	1.2
Y81A	1.01	1.4	**9.5**	1.4	1.5	0.24	1.2
Y81D	0.96	0.83	1.2	0.95	1.2	0.13	0.70
E84A	0.99	1.10	1.5	0.98	1.81	0.55	1.9
R85A	1.00	0.89	0.98	0.39	0.57	1.5	0.61
R85D	0.17	0.19	0.51	1.3	0.54	0.42	0.19
E89A	1.10	**5.4**	1.8	**4.9**	**4.1**	0.16	**2.5**
Q91A	0.92	0.10	1.03	0.45	0.57	0.52	0.85
E25R R85D	0.98	0.92	0.93	0.55	0.89	0.44	0.52
E77R R85D	1.00	0.85	0.81	0.67	0.90	1.00	0.53
SoxS	1.10	**3.2**	1.8	**23.**	**3.7**	0.22	**2.3**

[a] All strains are derivatives of M3997 and have null mutations in *marRAB*, *rob*, *lon*, and *clpP* except that those containing the *acrAB*::*lacZ* or *mdtG*::*lacZ* transcriptional fusion are derivatives of M4435 (*acrR*) or GC4468 (wild type), respectively, and are wild type for *lon* and *clpP*. The wild-type MarA β-galactosidase activities (Miller units), control and induced, respectively, for each of the promoters were as follows: for *acnA*, 50 and 140; *acrAB*, 80 and 450; *fpr*, 80 and 180; *marRAB*, 750 and 2000; *mdtG*, 7 and 37; *poxB*, 35 and 85; and *zwf*, 200 and 1.200. For a discussion of the statistical significance of these values see the supplemental material.

[b] Numbers in bold show activation >2-fold. See Table S1 for promoter-*lacZ* fusion references.

(e.g., the two oligonucleotides GGACTGGAAGGCTCAATCG and ATCAAATCAATCAGAGG).

Superoxide sensitivity assays. Bacteria were assayed for superoxide sensitivity on gradient plates as described previously (23, 41).

RESULTS

Detailed examination of the crystal structure of MarA suggested that the two regions of the molecule containing the two helix-turn-helix motifs were likely to be less flexible than those of SoxS (see Fig. S1 in the supplemental material). We therefore considered the possibility that the existence of bridging contacts formed between these two regions by E25 and R85 but absent in SoxS might account in part for the differences between MarA and SoxS with regard to the activation of different promoters. Thirteen MarA mutants (seven single-alanine substitutions, four charge inversion mutations [acidic to basic side chains or *vice versa*] and two double-charge inversions) were compared with MarA and SoxS for activation of 16 MarA-SoxS-Rob regulon promoters. We reasoned that class I promoters were more likely to reveal a correlation between activity and flexibility, if it existed, since they depend on only a single interaction of MarA with the marbox and a single interaction with RNAP, whereas class II promoters involve additional interactions with RNAP. (The amino acids chosen for this study were known not to involve the interaction of MarA with RNAP [8]).

Only the E89A variant showed a consistent increase in activation of class I promoters that paralleled the activation by SoxS (Table 2). This was surprising since E89A is unlikely to have any direct effect on the flexibility of MarA and was intended only as a control. As expected, there was no correlation between the activation of class II promoters by MarA(E89A) and by SoxS (Fig. 1).

To ascertain whether the greater activation of the *acnA*, *acrAB*, *fpr*, *mdtG*, and *zwf* promoters by MarA(E89A) was related to binding, SoxS, MarA, MarA(E89A), and MarA(Q91A) proteins were purified and assayed by gel retardation for their ability to bind the 20 bp marboxes (listed in Table 1). In general, marbox binding (Fig. 2) paralleled promoter activation for the class I promoters (Table 3). Both SoxS and MarA(E89A) showed considerable binding to the marboxes of all of these promoters, whereas MarA and MarA(Q91A) showed significant binding to only the *marRAB* and *acrAB* marboxes. MarA(E89A) showed large increases in binding (relative to WT MarA), concomitant with large increases in relative activation for the *acnA*, *fpr*, *mdtG*, and *zwf* promoters, and showed modest increases in both binding and activation of the *acrAB* promoter. All four activators bound the *marRAB* marbox with similar affinities and activated *marRAB* to similar extents. SoxS bound the *acrAB* marbox with similar affinity to MarA but, like MarA(E89A), marginally increased *acrAB* transcription (~1.8-fold). Binding of the *poxB* marbox was seen only for MarA(E89A) (data not shown). We note that, of these promoters, *acnA*, *fpr*, and *zwf* are necessary for optimal superoxide resistance (1, 2, 5, 26, 31, 33).

The binding of MarA, the two MarA mutants, and SoxS to

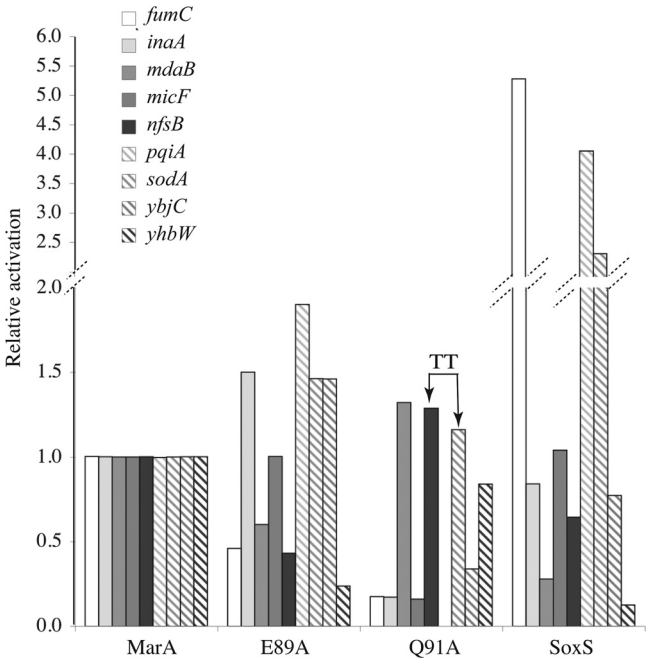


FIG. 1. Activation of nine class II promoters by MarA, MarA mutants E89A and Q91A, and SoxS (Table 3 gives details.) The bases present in the *sodA* and *nfsB* marboxes at positions 17 and 18 of the consensus marbox are both Ts rather than As (indicated as TT at the top of the bars) (26). The relative activities were calculated as described in Materials and Methods. The WT MarA β-galactosidase activities (Miller units), control and induced, respectively, for the indicated promoters were as follows: for *fumC*, 75 (control) and 220 (induced); *inaA*, 100 and 1,400; *mdaB*, 150 and 3,000; *micF*, 80 and 800; *nfsB*, 80 and 1,000; *pqiA*, 25 and 100; *sodA*, 1,200 and 3,000; *ybjC* 150 and 3,800; and *yhbW*, 400 and 1,800.

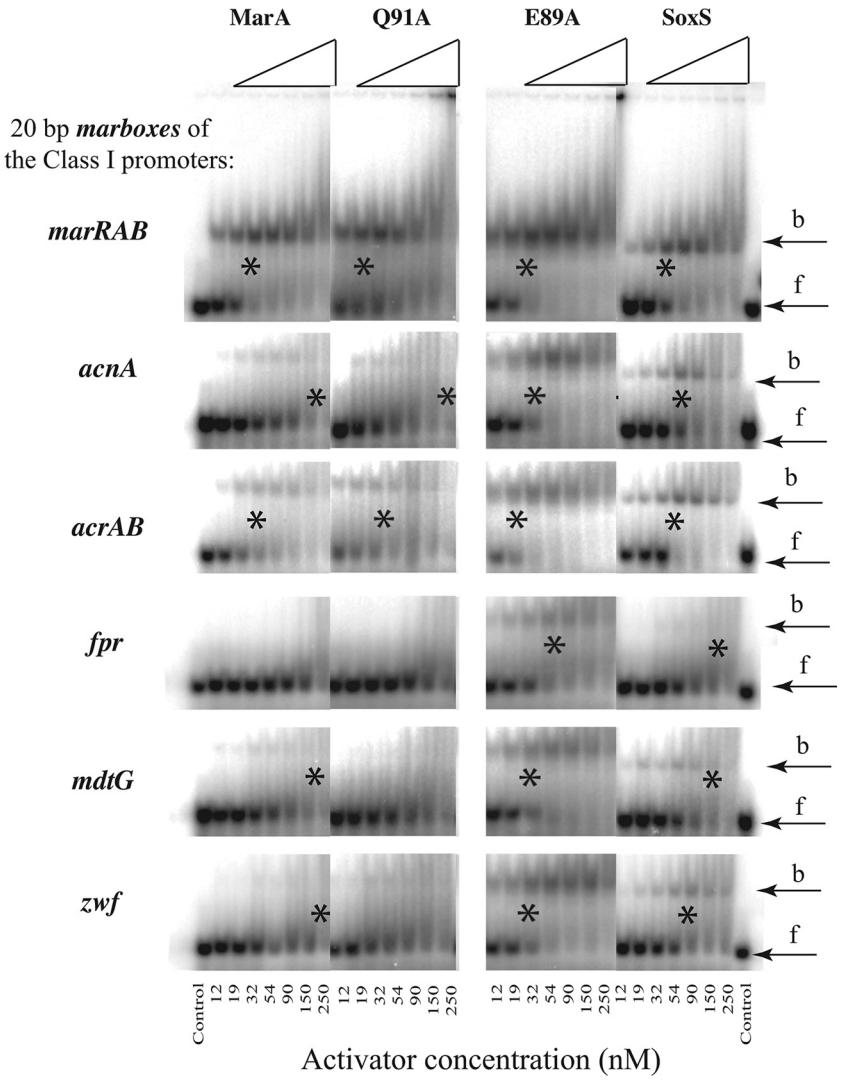


FIG. 2. Autoradiographs of gel electrophoretic mobility assays using MarA, MarA mutants E89A and Q91A, and SoxS with ^{32}P-labeled 20-bp DNA fragments (Table 1) corresponding to the marbox binding sites at different class I promoters. Partially purified (~80%) MarA, MarA(E89A), MarA(Q91A), or SoxS starting at a concentration of 250 nM was serially diluted 3:2 and mixed with the DNA, and the amount of protein that bound 50% of the DNA (*) was used to estimate the dissociation complex (K_D). When only a weak band was seen at the highest concentration of protein used, the K_D was estimated as >250. The positions of the free (f) and bound (b) DNA are indicated. The calculated K_Ds from these gels are listed in Table 3.

the marbox sequences of nine class II promoters was also determined by gel mobility assays and is summarized in Table 3. In every case, MarA(E89A) bound as well as or more tightly (i.e., had a lower K_D [equilibrium dissociation constant) than WT MarA. For several promoter marboxes this change was dramatic: the K_D dropped from ≫150 to 50 for *inaA*, from 200 to 75 for *nfsB*, and from ≫150 nM to ~100 nM for both *ybjC* and *yhbW*. In spite of this, MarA(E89A) activation was greater than that of WT MarA for *inaA*, *sodA*, and *ybjC*; it was comparable to that of WT MarA for *micF* and less than that of WT MarA for *fumC*, *mdaB*, *nfsB*, and *ybhW* (Table 3 and Fig. 1). We conclude that the WT glutamic acid of MarA at position 89 is an inhibitor of MarA binding to many marboxes.

In an effort to understand why E89 is inhibitory, we examined the sequences of the 14 marboxes for which we have data (Table 3). We noticed that five have a T at position 12 and that the binding to none of these sequences is increased by the E89A substitution (less than 1.7-fold). In contrast, of the remaining nine sequences that do not have a T at this position, the binding of E89A was increased for six of them by >2.5-fold and for the seventh by 1.7-fold; only two do not show increased binding. Although the cocrystal structure of MarA with the *marRAB* marbox DNA (with a T at position 12) indicates no interaction between the two molecules at this position (36), we considered the possibility that steric hindrance between the marbox DNA and MarA could limit activation by MarA when position 12 is not a T residue (see the Discussion for a fuller treatment).

To examine one facet of this possibility, namely, that the glutamic acid side chain of E89 sterically inhibits interaction with marbox DNAs lacking a T at position 12, we tested the effects of several amino acid substitutions at residue 89 on the

TABLE 3. Relative activation compared with relative binding for activators and mutants at MarA-SoxS-Rob regulon promoters[a]

Promoter class and name	WT MarA			MarA(Q91A)			Position 12[c]	MarA(E89A)			SoxS		
	RA	RB	K_D (nM)[b]	RA	RB	K_D (nM)		RA	RB	K_D (nM)	RA	RB	K_D (nM)
Class I													
marRAB	1.0	1.0	25	0.9	~0.8	30	T	1.1	1.0	25	1.1	0.8	30
acnA	1.0	1.0	180	0.1	0.7	250	A	5.4	6.0	30	3.2	3.0	60
acrAB	1.0	1.0	30	1.0	0.6	50	A	1.8	1.5	20	1.8	0.8	40
fpr	1.0	—	>250	0.5	—	>250	G	4.9	~5.0	50	23.0	>2.0	200
mdtG	1.0	1.0	200	0.6	<0.8	>250	C	4.1	6.7	30	3.7	1.3	150
zwf	1.0	1.0	200	0.9	<0.8	>250	G	2.5	6.7	30	2.3	2.7	75
Class II													
fumC	1.0	1.0	50	0.2	<0.3	>150	G	**0.5**	**~1.7**	30	**5.3**	**~1.0**	50
inaA	1.0	—	≫150	0.2	—	≫150	C	1.5	~3.0	50	0.8	—	≫150
mdaB	1.0	1.0	50	1.3	0.8	63	T	**0.6**	**1.4**	35	0.3	0.5	100
micF	1.0	1.0	25	0.2	1.0	25	T	1.0	1.0	25	1.0	~2.0	50
nfsB	1.0	1.0	200	1.3	~.8	~250	C	**0.4**	**2.7**	75	**0.6**	**1.3**	150
pqiA	1.0	—	≫150	0.0	—	≫150	T	1.9	—	≫150	4.0	—	≫150
sodA	1.0	—	≫150	1.2	>1.0	>150	A	1.5	~1.0	>150	2.3	—	≫150
ybjC	1.0	—	≫150	0.3	—	≫150	T	1.5	~1.5	100	0.8	—	≫150
yhbW	1.0	—	>150	0.8	<1.0	≫150	T	**0.2**	**~1.5**	100	0.1	—	>150

[a] The data for the activation relative to that of WT MarA are from Table 2 and Fig. 1. RA, relative activation; RB, relative binding constant. Where values are shown in boldface, the relative activation did not parallel the relative binding. —, value could not be calculated.
[b] The dissociation constant (K_D) was calculated from gel shift assays similar to those shown in Fig. 2.
[c] The nucleotide at position 12 for this marbox sequence.

fpr::*lacZ* fusion and the *mdtG*::*lacZ* fusion, the two fusions that showed the greatest effects of E89A on activation (see above). Plasmids carrying WT MarA, the MarA variant MarA(E89A) (with a nonpolar single methyl group side chain), MarA(E89G) (with no side chain), MarA(E89D) (with a side chain one methylene group shorter than glutamic acid), MarA(E89V) (with the amino acid present at the corresponding position of SoxS and having a dimethyl methylene side chain,), WT SoxS, and SoxS(V83E) were introduced into these fusions and assayed for β-galactosidase. Again (Table 2), activation of these promoters by SoxS was much greater than that by MarA (Fig. 3). Similarly, MarA(E89A) was considerably more active than WT MarA. In contrast, the E89D variant was less active than WT MarA for *fpr* and completely inactive (indistinguishable from the control plasmid) for *mdtG*. E89G was approximately twice as active as WT MarA for *fpr* and 4-fold more active for *mdtG* although for both promoters it was considerably less active than E89A. The E89V variant was marginally more active than the WT for both promoters but substantially less so than E89A. SoxS(V83E) reduced the activation of these promoters relative to WT SoxS, but SoxS(V83E) was still more active than WT MarA. (The activations shown here are greater than those apparent in Table 2 because the expression of the plasmids carrying MarA, SoxS, and their mutants is not entirely shut off by *lacI*^{q} so that the increases expressed in Table 2 appear smaller.) As outlined in the Discussion, these results, namely, (i) that variant E89D is virtually inactive, (ii) that E89G is very active although to a lesser extent than E89A, (iii) that E89V is only marginally more active than the WT, and (iv) that SoxS(V83E) has reduced activation although not to the low levels expressed by WT MarA, are consistent with the possibility that that the side chain of E89 sterically inhibits interaction with the DNA for marbox sequences lacking a T at position 12.

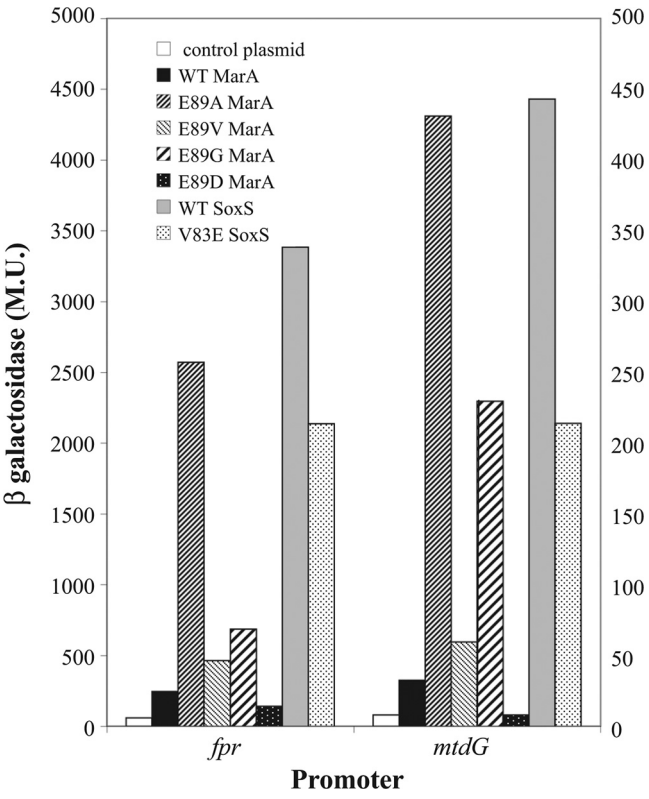


FIG. 3. Activation of class I promoters by MarA and SoxS and their mutants at position E89 (MarA) or V83 (SoxS). The absolute β-galactosidase values (Miller units [MU]) for the *fpr*::*lacZ* fusion (left-hand scale) and the *mdtG*::*lacZ* and *acrAB*::*lacZ* fusions (right-hand scale) are plotted for the plasmid control, MarA, MarA(E89A), MarA(E89V), SoxS, and SoxS(V83E).

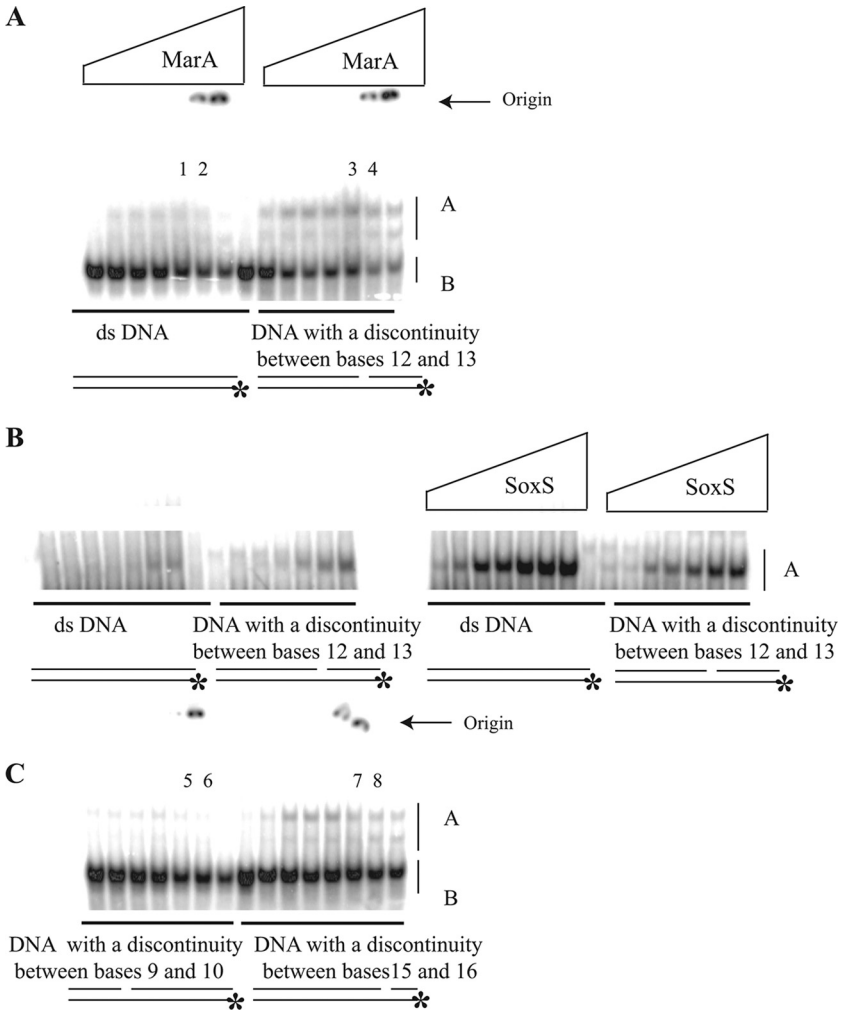


FIG. 4. Autoradiographs of gel retardation assays as in Fig. 2 except that the DNA fragment was 36 nt long and corresponds to the 7 nt upstream and 9 nt downstream of the native *fpr* marbox (GGACTGG**AAGGCTCAATCGATCAAATC**AATCAGAGG; the marbox is in boldface). Gels A and C were run at the same time using the same preparation of highly purified MarA with the His_6 tag removed. We have no explanation for the slower-moving band seen only with this preparation of MarA. The ratios of the bound to unbound DNA (intensity of the A/B bands) in the indicated lanes were as follows: lane 1, 0.19; lane 2, 0.21; lane 3, 0.34; lane 4, 0.44; lane 5, 0.05; lane 6, 0.08; lane 7, 0.20; and lane 8, 0.22. Gel B (showing only the bound material) employed a different preparation of MarA from which the His_6 tag had not been removed (the same as in Fig. 2) and the comparable preparation of SoxS. The concentrations of MarA and SoxS are as described in the legend of Fig. 2.

If, as outlined above and presented in greater detail in the Discussion, steric interference with the phosphate between positions 12 and 13 and the glutamic acid side chain at position 89 is responsible for the very poor activation of promoters such as *fpr*, then it would be predicted that binding of WT MarA to the *fpr* marbox would be enhanced if that phosphate were absent. We therefore compared the binding affinity of WT MarA to either a 36-bp double-stranded DNA containing the marbox sequence of *fpr* or with dsDNA of the same sequence and length but prepared so that the phosphate linkage between positions 12 and 13 of the marbox was eliminated (see Materials and Methods). Again (Fig. 2 and Table 3), MarA bound very poorly to the *fpr* marbox (Fig. 4). However, binding increased significantly when the *fpr* DNA lacked the phosphate group between positions 12 and 13 (Fig. 4A and B). In contrast, SoxS bound more tightly to dsDNA than to the discontinuous DNA (Fig. 4B). When the phosphate located 3 nt farther upstream (between positions 9 and 10) was absent, no significant alteration in binding was observed (Fig. 4) although a small increase was observed when the phosphate between positions 15 and 16 was absent. We conclude that the phosphate group between nt 12 and 13 of the consensus sequence inhibits the ability of MarA to bind.

Activation and marbox binding by MarA(Q91A). The only other MarA mutation found here to differ significantly from WT MarA in the activation of class I promoters was Q91A (Table 2). Of the seven class I promoters examined, MarA(Q91A) activated the *acrAB*, *marRAB*, and *zwf* promoters to similar extents as WT MarA but activated *acnA*, *fpr*, *mdtG*, and *poxB* to only 60% or less of MarA WT levels (Table 2; see also below).

For the nine class II promoters, MarA(Q91A) significantly reduced the activation of *fumC*, *inaA*, *micF*, *pqiA*, and *ybjC* but had no significant effect on *nfsB*, *mdaB*, *sodA*, or *yhbW*. MarA(Q91) has been identified as forming van der Waals interac-

TABLE 4. Superoxide resistance of strains carrying MarA, MarA mutations, or SoxS

Plasmid	Strain no.	Resistance to the indicated superoxide-generating compound[a]			
		Phenazine methosulfate		Menadione	
		MIC (μM)	Relative increase in MIC	MIC (mM)	Relative increase in MIC
Vector	M5390	12		0.5	
WT MarA	M5391	35	1.0	1.8	1.0
MarA(E25R)	M5392	31	0.89	1.6	0.91
MarA(E77A)	M5393	22	0.65	1.2	0.68
MarA(L80A)	M5394	42	1.2	1.4	0.76
MarA(E89A)	M5395	57	1.7	2.4	1.3
MarA(Q91A)	M5396	35	1.0	0.9	0.49
WT SoxS	M5397	55	1.6	3.5	2.0

[a] The MIC of each chemical for each strain was estimated from gradient plates that were performed two times. To determine the relative increase, the MIC for each strain was divided by that of the strain carrying the WT MarA plasmid.

tions with the methyl groups of the two thymidines that are complementary to the adenines at positions 17 and 18 of the consensus sequence (36). Thus, it would be expected that the Q91A substitution might reduce activation of the seven class II promoters that have at least one A at position 17 or 18 but not the two promoters, *nfsB* and *sodA*, that have no A residues at these positions. Indeed, *nfsB* and *sodA* are among the promoters that Q91A activated to the same extent as WT MarA. Since Q91A reduced the expression of four of the seven class I promoters and four of the nine class II promoters, our results are inconsistent with the proposition that Q91 is principally required for interactions at class II promoters, as has been proposed for the analogous site (Q85) in SoxS (16).

For the majority of these promoters, the gel mobility assay for binding of MarA to marboxes was insufficiently precise to determine whether there is a correlation between binding and activation by MarA(Q91A) relative to MarA (Table 3). Among the class I promoters that exhibited measurable binding to MarA, MarA(Q91A) showed no greater binding or activation for *acrAB*, a modest reduction in activation and binding for *mdtG* and *zwf*, and a reduction in binding but not in activation for *marRAB*. A modest reduction in binding with a small increase in activation was seen for the class II *mdaB* promoter. Although not observed in these experiments, a more detailed analysis of the binding of MarA(Q91A) to the *micF* marbox (using protein without the His_6 tag) showed a very small reduction in binding concomitant with the reduced ability of MarA(Q91A) to activate the class II *micF* promoter (13). Thus, relative to MarA, there may be a correlation between activation and binding for Q91A at class I promoters, but none is obvious with regard to the class II promoters.

Activation of superoxide resistance by MarA(E89A). If the glutamic acid at position 89 of MarA is a major determinant *in vivo* of the reduced activity of MarA at many promoters where SoxS is more active, we would expect cells carrying MarA(E89A)to be more resistant to superoxides than cells carrying WT MarA. This was tested with gradient plate assays of sensitivity to two superoxide-generating compounds, phenazine methosulfate (PMS) and menadione (Table 4). Cells constitutively expressing MarA(E89A)were more resistant than WT MarA to PMS (1.7-fold) and, to a lesser extent, to menadione (1.3-fold). Comparable MICs with SoxS for PMS were 1.6-fold and for menadione 2.0-fold. Clearly, MarA(E89A)activates superoxide resistance to a greater extent than WT MarA. Curiously, the Q91A substitution had no effect on resistance to the superoxide generator PMS (MIC of 35 μM for both the Q91A mutant and the WT MarA) but lowered resistance to the superoxide generator menadione (MIC of 0.9 mM for the Q91A mutant and 1.8 mM for the WT).

DISCUSSION

The MarA-SoxS-Rob regulon of *E. coli* consists of a variety of genes that enable cells to adapt to multiple stresses. It contains genes that render the cell multidrug and organic-solvent resistant (*acrAB*, *tolC*, and *micF*) and that defend against superoxide stress (e.g., *acnA*, *fpr*, *fumC*, *nfsA*, *sodA*, and *zwf*) (1, 2, 5, 26, 31, 33). Not surprisingly, there are quantitative differences in the extents of activation of particular promoters by the paralogous activators so that the phenotypic outcomes depend on which activator is upregulated (2, 26, 34, 43).

E89 inhibition. We have shown here that the MarA glutamic acid residue E89 is responsible for decreasing the binding of WT MarA relative to SoxS for the class I marbox promoters, *acnA*, *mdtG*, *fpr*, and *zwf*, thereby decreasing the relative activation of these promoters and hence the resistance engendered to superoxides by MarA. We think the following may explain these results.

A detail of the MarA structure (Fig. 5) shows that E89 is oriented in the cocrystal with its acidic groups exposed to the solvent away from the DNA backbone. In so doing, methyl hydrogens on the β- and γ-carbons are positioned restrictively close to an oxygen of the phosphate group between bases T12 and G13 of the *marRAB* marbox. Even this orientation of E89 is only possible as the result of a small "clash" (as predicted by the MolProbity program [http://molprobity.biochem.duke.edu/]) between E89 and S90 that permits the rotation of E89 into the MarA core and away from the DNA backbone. This suggests that any further displacement of the phosphate group closer to MarA would be unfavorable. Indeed, Dangi et al. (9) have shown that there are no differences detectable (shift difference of <0.45 ppm) by nuclear magnetic resonance (NMR) in the backbone chemical shifts for E89 of MarA when it binds to the marboxes of *marRAB*, *fumC*, *fpr*, or *micF* although such shifts are detectable at other positions.

We suggest that a thymidine at position 12 (see Results) might optimize the location of the phosphate group so as to minimize its interference with E89 (Fig. 6). Rhee et al. (36) have noted that the displacement of the DNA to achieve the 35° bend required for binding is not uniform and is primarily limited to the regions of the DNA between bases 7 and 9 and bases 11 and 13 of the marbox. Hydrogen bonds between the bases are not significantly disrupted at these points (i.e., there is no melting of the DNA), but significant stacking energy must be lost between bases on the convex face of the DNA since they are separated to a greater extent than those on the concave face. Thus, it is not simply the base pair at this position that is critical but the specific base on the convex surface. While there is still disagreement as to how to estimate base

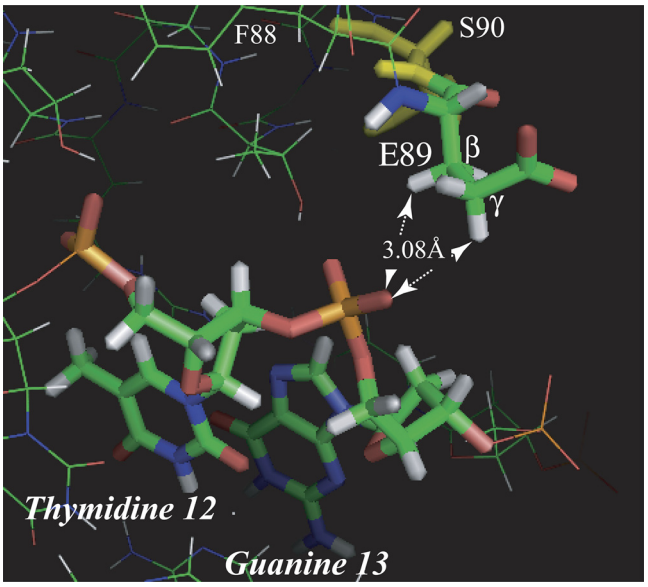
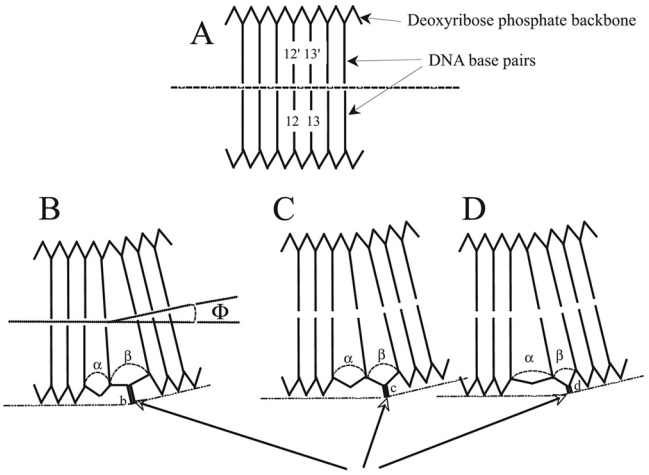


FIG. 5. Detail of the MarA DNA cocrystal structure (36) showing the close proximity of hydrogens of the β- and γ-carbons of E89 to one of the oxygens of the phosphate in the DNA backbone between positions 12 and 13 of the MarA marbox. The other γ-hydrogen is 3.20 Å from the same oxygen. The MolProbity program (http://molprobity.biochem.duke.edu/) indicates a clash between the oxygen of the peptide bond of E89 with a β-hydrogen of S90 (in yellow) of 0.445Å, which has the effect of allowing E89 to rotate slightly into the MarA structure, thereby moving the β- and γ-hydrogens to permissible separations from the phosphate oxygen. At the same time, the oxygens of E89 can face away from the DNA into the solvent.



Displacements of the phosphate between bases 12 and 13 associated with DNA bending

FIG. 6. (A) Schematic representation of a portion of a marbox DNA sequence showing the bases stacked at a 3.2-Å separation, the ribose-phosphate backbone connecting them, and a dotted line to indicate that the DNA is linear. The numbers (12 and 13) indicate the positions of the bases in the consensus marbox sequence, and 12′ and 13′ are their complements. (B, C, and D) To allow for a bend of angle 2Φ (35°) in the marbox DNA, Rhee et al. (36) have pointed out that the DNA does not distribute the necessary distortion over the entire 20-bp length but, rather, limits the distortion to the regions between bases 7 to 9 and 11 to 13 as illustrated in these three diagrams. The distortion of the backbone between the bases at positions 11 to 13 results in separations of α and β. The relative sizes of these displacements and the absolute energy required to generate them will depend on the stacking energies of the particular bases occupying positions 11, 12, and 13. As a consequence, the position of the phosphate between bases 12 and 13 will be altered relative to the surface of the DNA. We postulate that the presence of T at position 12 of the consensus sequence in general results in the most favorable of these structures to accommodate the glutamic acid at position 89.

stacking energies, there is general agreement on the following: that stacking energies may vary from one base to another by several kcal, that there are energetic differences between which base is to the 5′ side, and that there are additional "many-body effects" (i.e., effects on the stacking energy between bases 1 and 2 by base 3) and nonadditive effects (40, 47).

Consistent with this model, we find that, first, WT MarA binds more tightly to DNA of the *fpr* marbox lacking a phosphate between positions 12 and 13 than to uninterrupted dsDNA. This is not simply the result of compensation for the energy lost on bending normal DNA with this sequence since an interruption in the ribose-phosphate backbone at the other stress point (position 9) does not enhance DNA binding. Furthermore, SoxS binds more tightly to the dsDNA if it has the phosphate between positions 12 and 13. Thus, the model offers an explanation as to why there is a strong correlation between E89A activation and the lack of a T at position 12 of the marboxes, namely, to prevent interference with the phosphate. Second, for two of the promoters most restricted by E89 (*fpr* and *mdtG*), there is a significant difference between different MarA substitutions. As expected from the model, since E89A and E89G lack γ-carbons, they are the most effective in binding and activation. E89V, with two γ-carbons that could clash with the phosphate between 12 and 13 but are still somewhat free to rotate, is less effective. WT E89 is even less effective since, if the model were correct, the rotation of its γ-carbon would be severely limited by the hydrophilicity of its acidic group. Finally, E89D is almost inactive since its γ-carbon is part of the acidic group, and we would predict a significant clash with the phosphate leading to distortions of the structure.

Implications of these results for the mechanism of activation by MarA. The differences in behavior of the E89A and Q91A mutants relative to WT MarA at class I and class II promoters is instructive with regard to the mechanism of transcriptional activation. MarA(E89A) exhibited increased binding relative to WT MarA for 13 of the 16 promoter marboxes examined although the increase was small for *acrAB* (Table 3). Only two promoters, *marRAB* and *micF*, showed no increase (no binding to *pqiA* was found at even the highest activator concentrations). In the case of the six *rpoD*-stimulated class I promoters, the increase in binding by all except *marRAB* was associated with increased activation. The implication of this is that an increase in the interaction between activator and binding site is associated with increased RNAP activity at these five promoters. This, in turn, implies that an important part of the mechanism for activation is recruitment of RNAP by the bound activator (35). A further conclusion is that recruitment is not an element of the mechanism for activation of the *marRAB* promoter, as has been demonstrated elsewhere (42).

Furthermore, MarA may have a special role at the *marRAB* promoter since it appears to be a competitive inhibitor of MarR (at least in solution [28]), thereby freeing the −10 and −35 signals for RNAP binding. This effect would not have

been seen here or in the experiments of Wall et al. (42) since they were carried out in the absence of MarR. Similarly, since the activation experiments with the *acrAB* promoter were carried out in an *acrR* null mutant, the small increases in binding and activation of *acrAB* by MarA(E89A) compared to levels of the WT may have masked any competition between MarA and AcrR for the *acrAB* promoter.

The lack of correlation between marbox binding by MarA (E89A) and activation at class II promoters (Table 3) is consistent with a large body of information indicating that additional interactions between activator and RNAP are essential at class II promoters (4, 6, 14, 44). Like the E89A variant, MarA(Q91A) reduced the activation of and, to a limited extent, the binding to about half of both the class I and class II promoters. Thus, we see no indication of a specific role for Q91 in class II promoter activation. This is contrary to the finding that the corresponding amino acid in SoxS, Q85, interacts with the σ subunit of RNAP (16).

Finally, we note that discrimination between SoxS and MarA is not entirely the result of increased binding of SoxS at class I promoters. While a number of the principal functions required for superoxide resistance are controlled at class I promoters, others are at class II promoters (e.g., *fumC* and *sodA*). The greater ability of SoxS over MarA to activate these class II promoters appears to have a different basis and will be the subject of a future communication.

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REFERENCES

1. **Ariza, R. R., Z. Li, N. Ringstad, and B. Demple.** 1995. Activation of multiple antibiotic resistance and binding of stress-inducible promoters by *Escherichia coli* Rob protein. J. Bacteriol. **177:**1655–1661.
2. **Barbosa, T., and S. Levy.** 2000. Differential expression of over 60 chromosomal genes in *Escherichia coli* by constitutive expression of MarA. J. Bacteriol. **182:**3467–3474.
3. **Baucheron, S., et al.** 2004. AcrAB-TolC directs efflux-mediated multidrug resistance in *Salmonella enterica* serovar Typhimurium DT104. Antimicrob. Agents Chemother. **48:**3729–3735.
4. **Bhende, P., and S. Egan.** 2000. Genetic evidence that transcription activation by RhaS involves specific amino acid contacts with sigma 70. J. Bacteriol. **182:**4959–4969.
5. **Blanchard, J., W. Wholey, E. Conlon, and P. Pomposiello.** 2007. Rapid changes in gene expression dynamics in response to superoxide reveal SoxRS-dependent and independent transcriptional networks. PLoS One **2:**e1186.
6. **Busby, S., and R. Ebright.** 1999. Transcription activation by catabolite activator protein (CAP). J. Mol. Biol. **293:**199–213.
7. **Cohen, S. P., S. B. Levy, J. Foulds, and J. L. Rosner.** 1993. Salicylate induction of antibiotic resistance in *Escherichia coli*: activation of the *mar* operon and a *mar*-independent pathway. J. Bacteriol. **175:**7856–7862.
8. **Dangi, B., A. M. Gronenborn, J. L. Rosner, and R. G. Martin.** 2004. Versatility of the carboxy-terminal domain of the α subunit of RNA polymerase in transcriptional activation: use of the DNA contact site as a protein contact site for MarA. Mol. Microbiol. **54:**45–59.
9. **Dangi, B., et al.** 2001. Structure and dynamics of MarA-DNA complexes: an NMR investigation. J. Mol. Biol. **314:**113–127.
10. **Demple, B.** 1996. Redox signaling and gene control in the *Escherichia coli soxRS* oxidative stress regulon—a review. Gene **179:**53–57.
11. **Eguchi, Y., et al.** 2003. Transcriptional regulation of drug efflux genes by EvgAS, a two-component system in *Escherichia coli*. Microbiology **149:**2819–2828.
12. **Fàbrega, A., R. Martin, J. Rosner, M. Tavio, and J. Vila.** 2010. Constitutive SoxS expression in a fluoroquinolone-resistant strain with a truncated SoxR protein and identification of a new member of the *marA-soxS-rob* regulon, *mdtG*. Antimicrob. Agents Chemother. **54:**1218–1225.
13. **Gillette, W., R. Martin, and J. Rosner.** 2000. Probing the *Escherichia coli* transcriptional activator MarA using alanine-scanning mutagenesis: residues important for DNA binding and activation. J. Mol. Biol. **299:**1245–1255.
14. **Grainger, D. C., C. L. Webster, T. A. Belyaeva, E. I. Hyde, and S. J. Busby.** 2004. Transcription activation at the *Escherichia coli melAB* promoter: interactions of MelR with its DNA target site and with domain 4 of the RNA polymerase sigma subunit. Mol. Microbiol. **51:**1297–1309.
15. **Griffith, K., I. Shah, and R. J. Wolf.** 2004. Proteolytic degradation of *Escherichia coli* transcription activators SoxS and MarA as the mechanism for reversing the induction of the superoxide (SoxRS) and multiple antibiotic resistance (Mar) regulons. Mol. Microbiol. **51:**1801–1816.
16. **Griffith, K. L., and R. E. Wolf, Jr.** 2002. A comprehensive alanine scanning mutagenesis of the *Escherichia coli* transcriptional activator SoxS: identifying amino acids important for DNA binding and transcription activation. J. Mol. Biol. **322:**237–257.
17. **Griffith, K. L., and R. E. Wolf, Jr.** 2001. Systematic mutagenesis of the DNA binding sites for SoxS in the *Escherichia coli zwf* and *fpr* promoters: identifying nucleotides required for DNA binding and transcription activation. Mol. Microbiol. **40:**1141–1154.
18. **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.
19. **Kwon, H. J., M. H. Bennik, B. Demple, and T. Ellenberger.** 2000. Crystal structure of the *Escherichia coli* Rob transcription factor in complex with DNA. Nat. Struct. Biol. **7:**424–430.
20. **Martin, R., E. Bartlett, J. Rosner, and M. Wall.** 2008. Activation of the *Escherichia coli marA/soxS/rob* regulon in response to transcriptional activator concentration. J. Mol. Biol. **380:**278–284.
21. **Martin, R., W. Gillette, N. Martin, and J. Rosner.** 2002. Complex formation between activator and RNA polymerase as the basis for transcriptional activation by MarA and SoxS in *Escherichia coli*. Mol. Microbiol. **43:**355–370.
22. **Martin, R., W. Gillette, S. Rhee, and J. Rosner.** 1999. Structural requirements for marbox function in transcriptional activation of *mar/sox/rob* regulon promoters in *Escherichia coli*: sequence, orientation and spatial relationship to the core promoter. Mol. Microbiol. **34:**431–441.
23. **Martin, R., W. Gillette, and J. Rosner.** 2000. Promoter discrimination by the related transcriptional activators MarA and SoxS: differential regulation by differential binding. Mol. Microbiol. **35:**623–634.
24. **Martin, R., and J. Rosner.** 1995. Binding of purified multiple antibiotic-resistance repressor protein (MarR) to *mar* operator sequences. Proc. Natl. Acad. Sci. U. S. A. **92:**5456–5460.
25. **Martin, R., and J. 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.
26. **Martin, R., and J. Rosner.** 2002. Genomics of the *marA/soxS/rob* regulon of *Escherichia coli*: identification of directly activated promoters by application of molecular genetics and informatics to microarray data. Mol. Microbiol. **44:**1611–1624.
27. **Martin, R., and J. Rosner.** 2004. Transcriptional and translational regulation of the *marRAB* multiple antibiotic resistance operon in *Escherichia coli*. Mol. Microbiol. **53:**183–191.
28. **Martin, R. G., K. W. Jair, R. E. Wolf, Jr., and J. L. Rosner.** 1996. Autoactivation of the *marRAB* multiple antibiotic resistance operon by the MarA transcriptional activator in *Escherichia coli*. J. Bacteriol. **178:**2216–2223.
29. **Martin, R. G., and J. L. Rosner.** 2005. Structure and function of MarA and its homologs, p. 235–246. *In* D. G. White, M. N. Alekshun, and P. F. McDermott (ed.), Frontiers in antimicrobial resistance: a tribute to Stewart B. Levy. ASM Press, Washington, DC.
30. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
31. **Nunoshiba, T., E. Hidalgo, C. Amábile Cuevas, and B. Demple.** 1992. Two-stage control of an oxidative stress regulon: the *Escherichia coli* SoxR protein triggers redox-inducible expression of the *soxS* regulatory gene. J. Bacteriol. **174:**6054–6060.
32. **Okusu, H., D. Ma, and H. Nikaido.** 1996. AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multiple-antibiotic-resistance (Mar) mutants. J. Bacteriol. **178:**306–308.
33. **Pomposiello, P., M. Bennik, and B. Demple.** 2001. Genome-wide transcriptional profiling of the *Escherichia coli* responses to superoxide stress and sodium salicylate. J. Bacteriol. **183:**3890–3902.
34. **Pomposiello, P. J., and B. Demple.** 2001. Redox-operated genetic switches: the SoxR and OxyR transcription factors. Trends Biotechnol. **19:**109–114.
35. **Ptashne, M., and A. Gann.** 1997. Transcriptional activation by recruitment. Nature **386:**569–577.
36. **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. U. S. A. **95:**10413–10418.
37. **Rosenberg, E. Y., D. Bertenthal, M. L. Nilles, K. P. Bertrand, and H. Nikaido.** 2003. Bile salts and fatty acids induce the expression of *Escherichia*

coli AcrAB multidrug efflux pump through their interaction with Rob regulatory protein. Mol. Microbiol. **48:**1609–1619.
38. **Rosner, J., B. Dangi, A. Gronenborn, and R. Martin.** 2002. Posttranscriptional activation of the transcriptional activator Rob by dipyridyl in *Escherichia coli*. J. Bacteriol. **184:**1407–1416.
39. **Simons, R., F. Houman, and N. Kleckner.** 1987. Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. Gene **53:**85–96.
40. **Sponer, J., H. Gabb, J. Leszczynski, and P. Hobza.** 1997. Base-base and deoxyribose-base stacking interactions in B-DNA and Z-DNA: a quantum-chemical study. Biophys. J. **73:**76–87.
41. **Szybalski, W., and V. Bryson.** 1952. Genetic studies on microbial cross resistance to toxic agents. I. Cross resistance of *Escherichia coli* to fifteen antibiotics. J. Bacteriol. **64:**489–499.
42. **Wall, M. E., D. A. Markowitz, J. L. Rosner, and R. G. Martin.** 2009. Model of transcriptional activation by MarA in *Escherichia coli*. PLoS Comput. Biol. **5:**e1000614.
43. **White, D. G., M. N. Alekshun, and P. F. McDermott (ed.).** 2005. Frontiers in antimicrobial resistance: a tribute to Stewart B. Levy. ASM Press, Washington, DC.
44. **Wickstrum, J., and S. Egan.** 2004. Amino acid contacts between sigma 70 domain 4 and the transcription activators RhaS and RhaR. J. Bacteriol. **186:**6277–6285.
45. **Wood, T. I., et al.** 1999. Interdependence of the position and orientation of SoxS binding sites in the transcriptional activation of the class I subset of *Escherichia coli* superoxide-inducible promoters. Mol. Microbiol. **34:**414–430.
46. **Wu, J., and B. Weiss.** 1992. Two-stage induction of the *soxRS* (superoxide response) regulon of *Escherichia coli*. J. Bacteriol. **174:**3915–3920.
47. **Yakovchuk, P., E. Protozanova, and M. Frank-Kamenetskii.** 2006. Base-stacking and base-pairing contributions into thermal stability of the DNA double helix. Nucleic Acids Res. **34:**564–574.