Published in final edited form as:
J Mol Biol. 2008 July 4; 380(2): 278–284. doi:10.1016/j.jmb.2008.05.015.

Activation of the *E. coli marA/soxS/rob* regulon in response to transcriptional activator concentration

Robert G. Martin[1,*], **Emily S. Bartlett**[1], **Judah L. Rosner**[1], and **Michael E. Wall**[2]

1*Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0560, USA*

2*Computer, Computational, and Statistical Sciences Division, Bioscience Division, and Center for Nonlinear Studies, Los Alamos National Laboratory, Los Alamos, New Mexico, 87545, USA.*

Summary

The paralogous transcriptional activators, MarA, SoxS and Rob, activate a common set of promoters, the *marA/soxS/rob* regulon of *Escherichia coli*, by binding a cognate site (*marbox*) upstream of each promoter. The extent of activation varies from one promoter to another and is only poorly correlated with the *in vitro* affinity of the activator for the specific *marbox*. Here, we examine the dependence of promoter activation on the level of activator *in vivo* by manipulating the steady-state concentrations of MarA and SoxS in Lon protease mutants and measuring promoter activation using *lacZ* transcriptional fusions. We found that: (i) the MarA concentrations needed for half-maximal stimulation varied by at least 19-fold among the 10 promoters tested; (ii) most *marboxes* were not saturated when there were 24,000 molecules of MarA per cell; (iii) the correlation between MarA concentration needed for half-maximal promoter activity *in vivo* with *marbox* binding affinity *in vitro* was poor and (iv) the two activators differed in their promoter activation profiles. The *marRAB* and *sodA* promoters could both be saturated by MarA and SoxS *in vivo*. However, saturation by MarA resulted in greater *marRAB* and lesser *sodA* transcription than did saturation by SoxS implying that the two activators interact with RNAP in different ways at the different promoters. Thus, the concentration and nature of activator determines which regulon promoters are activated and the extent of their activation.

Keywords

gene regulation; AraC protein family; stress response

Introduction

Cells have to distinguish among different kinds and levels of stress and respond in an appropriate manner. An over-response may be as injurious as an under-response. The MarA, SoxS and Rob transcriptional activators of *Escherichia coli* are interesting in this regard since they activate a common set of about 40 promoters (referred to here as the *marA/soxS/rob* regulon) whose functions engender antibiotic-resistance, superoxide-resistance and organic solvent tolerance.[1–3] Each activator is regulated in response to a different signal: aromatic weak acids (*e. g.*, salicylate) increase the transcription of *marA*; superoxides (*e. g.*, generated by paraquat) increase the transcription of *soxS*; and bile salts, decanoate and dipyridyl activate the abundant Rob protein. The up-regulation of these activators increases antibiotic efflux (*acrAB, tolC*), decreases outer membrane permeability (*micF*), increases superoxide-resistance

*Corresponding author. E-mail address: rgmartin@helix.nih.gov, Tel. (+1) 301 496-5466; Fax (+1) 301 496 0201.

functions (*zwf, fpr, sodA*), substitutes superoxide-resistant proteins for sensitive ones (*acnA, fumC*), enhances DNA repair (*nfo*) and regulates other genes whose functions are not known (*e.g., inaA*). The ability to fine-tune the responses of the regulon components to different magnitudes of diverse signals would appear to be very important.

Although these paralogous activators of the AraC family share substantial amino acid and structural homologies,[4] they bind their cognate DNA sites (*marboxes*) with different affinities as measured *in vitro*. The consensus sequence for the *marbox* is degenerate and asymmetrical (AYnGCACnnWnnRYYAAAY) and there are thousands of such sites in the chromosome.[5–8] However, to enable activation, the *marboxes* have to be configured in a specific orientation and distance relative to the −35 and −10 signals for RNA polymerase.

There is wide variation among the regulon promoters in the extent of their responses to a particular activator and a given promoter may respond very differently (discriminate) to the different activators. Both effects are only partly due to differences in activator affinities for the *marboxes*.[5,7,9] We wished to study the affinity-independent factors for this discrimination between activators by saturating the *marboxes* with different activators, thus eliminating differences due to binding.

We expressed *marA* and *soxS* from a high copy-number plasmid under the control of the $LacI^q$ repressor. Since MarA and SoxS are very sensitive to degradation by Lon protease, we used Lon-deficient cells to further increase the concentration of activators. Then, we determined the relationship between IPTG concentration, intracellular concentration of MarA and the expression of ten regulon promoters. We found that the expression of different members of the regulon required markedly different concentrations of MarA to achieve half-maximal activation. This suggests that activator concentration, determined by environmental signals, is used to tune the extent of regulon response so that it is commensurate with the signal. In addition, promoter saturation by MarA was not achieved for the majority of the promoters.

Results

Quantitation of IPTG-dependent MarA synthesis

We measured the dependence of *marA/soxS/rob* regulon promoter activity on MarA and SoxS activator concentration in *E. coli*. To vary the expression of MarA and SoxS, *marA* and *soxS* were placed under the control of the *lacZ* promoter on a high copy-number plasmid (pUC19-derivative) in a strain carrying F' $lacI^q$. The expression of MarA and SoxS was induced by growth to early logarithmic phase in IPTG for ~10 generations to be sure that equilibrium had been attained. Since MarA and SoxS are known to be sensitive to degradation by Lon protease, these experiments were carried out primarily in *lon− clpP−* strains where these activators are stable.[10] We measured the steady-state promoter transcription (β-galactosidase) levels of regulon *promoter*::*lacZ* fusions and, in parallel, the concentration of MarA as a function of IPTG concentration. We were thus able to correlate promoter activity with the number of MarA molecules per cell.

The relation between IPTG concentration and number of MarA molecules per cell is shown in Figure 1. MarA was measured using the Western blotting technique. Because of the instability of MarA, a number of different extraction techniques were tried, with and without protease inhibitors, and considerable care was taken to collect and lyse the cells rapidly. Despite these precautions, we were unable to detect MarA in the uninduced wild-type cells. The inset to Figure 1 shows a typical Western blot for cells grown in different concentrations of IPTG, with authentic MarA controls used to standardize the measurements. Data from many such gels were compiled in a graph of MarA concentration per cell against IPTG concentration for the wild-type and *lon− clpP−* strains (Fig. 1). The number of MarA molecules per cell increased

from a number too low to estimate in the wild-type cells in the absence of IPTG to ~1,300 at 15 μM IPTG. At higher IPTG concentrations, the rate of increase began to diminish and the number of MarA molecules per cell was close to the asymptotic maximum of ~10,000 by ~50 μM IPTG. In the *lon− clpP−* strains where the numbers were more accurately measured, MarA increased from ~800 molecules per cell in the absence of IPTG to ~24,000 at the highest IPTG concentrations.

It is clear that at concentrations of IPTG beyond about 15 μM, the concentration of MarA changes very little for either *lon+ clpP+* or protease-deficient cells (Fig. 1). The levels of MarA in wild-type cells are clearly lower as expected but, surprisingly, not by a constant factor over the entire range. Despite the fact that MarA is reported to have a very short half-life, the relatively modest (~2.4-fold) difference seen between the concentration of MarA in wild-type and *lon− clpP−* cells at high concentrations of IPTG nonetheless can be accounted for on the assumption that the amount of MarA per cell exceeds the K_M for the Lon protease. Indeed, modeling of the data presented and turnover data (unpublished) are consistent with a Lon protease K_M for MarA of 33 μM, consistent with the K_Ms found for other Lon protease substrates.[11]

Activation of promoter transcription as a function of MarA concentration *in vivo*

Even in Lon protease-deficient cells, the majority of regulon promoters did not achieve saturation at the highest MarA concentrations attained *in vivo* (24,000 molecules per cell). Of the 10 promoters studied in detail the activation profiles for only three achieved plateaus: *marRAB, sodA* (Fig. 2A) and *micF* (Fig. 2C). Two others, *mdaB* (Fig. 2A) and *ybjC* (Fig. 2B), may be near saturation but the profiles for the remaining five promoters showed no plateau.

The correlation between the concentration of MarA required for half-activation of the promoter activity *in vivo* and values for the dissociation constants (K_D) of MarA for the *marbox* sequences of the same promoters *in vitro*[9,12] is poor (Table 1). *marRAB* and *micF* are among the five promoters that approach saturation of activity *in vivo* and their *marboxes* have the highest affinity for MarA *in vitro*. However, *sodA* and *ybjC* are also in this group yet MarA affinity for their *marboxes* was indetectable or weak, respectively. Thus the affinity of MarA for the *marbox* as measured *in vitro* is not the only factor that determines the concentration required for activation of regulon promoters *in vivo*.

Differential activation by MarA and SoxS of promoters that are saturated by activator *in vivo*

As indicated in the previous section, only *marRAB, sodA* and *micF* were fully saturated by MarA *in vivo*. Although we do not know the number of SoxS molecules per cell that correspond to a given concentration of IPTG used in our experiments, we argue that *marRAB* and *sodA* promoters are also saturated with SoxS. First, there was little difference between the wild-type and *lon− clpP−* cells in the IPTG-stimulated activation of the *marRAB* and *sodA* promoters despite the greater concentration of activators in the protease-deficient hosts (Fig. 3). This suggests that SoxS was not limiting. Second, the plateaus for these promoter activites were achieved by SoxS and MarA at low or intermediate concentrations of IPTG whereas higher concentrations were required for promoters that did not achieve saturation by MarA (data not shown). This also suggests that SoxS was not limiting.

Thus, it is interesting that the profiles for MarA and SoxS were significantly different from one another relative to two of these promoters. For the *marRAB* promoter, the maximal activity with MarA was higher than that with SoxS (Fig. 3A and 3B). This would suggest that MarA is a better activator than SoxS. However, for the *sodA* promoter the maximal activity with MarA was lower than that with SoxS (Fig. 3C and 3D). Little difference was observed between the profiles for MarA and SoxS stimulation of *micF* (Fig. 3E and 3F). Since the differences

between the behaviors of MarA and SoxS at the *marRAB* and *sodA* promoters cannot be explained by differences in binding affinity, we suggest that the interaction between activator and RNAP must be different at the different promoters.

Discussion

We have shown here that the extent to which genes of the *marA/soxS/rob* regulon are activated is a function of MarA concentration. Under steady-state conditions, intermediate levels of MarA fully activate some members of the regulon (e.g., *marRAB, sodA*) without significant activation of other members of the regulon (e.g., *acnA, pqi5*, Fig. 2). While we have not measured the number of molecules of SoxS per cell that were produced by IPTG-treatment, the data (Fig. 2 and unpublished) indicate that the response of the regulon to SoxS concentration is similar.

We call this phenomenon "commensurate regulon activation" because it enables *E. coli* to mount a proportionate response of the *marA/soxS/rob* regulon to a stress signal, activating the minimum number of genes necessary for overcoming a prolonged threat. When there is a low level of signal, a low level of activator is made and only a few genes are activated (e.g., *micF* which decreases outer membrane permeability and *sodA* which converts superoxides to peroxides). When the stress is greater, more activator is made and additional genes are activated (e.g., *mdaB, zwf, fpr*). Only at the highest stress levels are the highest activator levels made and the full panoply of genes brought into play (e.g., *acrAB, tolC, pqi5*). Commensurate activation therefore enables the level of threat to be matched to the cost of a response. For example, over-production of the AcrAB–TolC efflux pump, essential for the removal of multiple antibiotics and organic solvents, may also deplete the cell of energy and vital constituents. If overexpression of these genes were not costly to the cell, we would expect wild-type, unthreatened cells to have higher basal levels of MarA, SoxS and Rob and/or higher basal levels of transcription of the regulon promoters. We presume that the comparatively low levels of basal transcription for each promoter is optimal in the absence of threat. Indeed, it has been observed that overexpression of MarA and SoxS or activation of Rob can lead to severe growth inhibition.[13,14]

Commensurate activation is likely relevant to transcriptional regulation of systems other than the *marA/soxS/rob* regulon. For example, it has previously been suggested that different promoters of the CRP modulon are activated at different levels of cAMP-CRP, leading to an observed hierarchy of response to cAMP concentration[15,16] although parallel measurements of promoter activity and cAMP-CRP concentration were not measured in those studies. In a further example, the response of different recA-dependent promoters to the same signal is diverse; however, in this case the diversity cannot be completely explained by differences in the way RecA acts at promoters.[17]

Because of the connections between the mechanisms of the *steady-state* phenomenon of commensurate activation and the *dynamic* phenomenon of temporally ordered activation,[18–20] we would also expect activation of the *marA* regulon to exhibit temporal ordering in response to a slow rise in MarA. However, salicylate and paraquat induce a rapid rise in MarA and SoxS levels.[13,21] Furthermore, because promoters of the regulon control expression of functionally diverse genes, the temporal ordering might not lead to the advantages in efficiency proposed for temporal ordering of functionally coherent regulons.[18–20]

We also note that the first promoter activated by either activator is *marRAB* itself.[21] This has two consequences: 1) MarA autocatalytically increases its own synthesis, which is a rare feature among transcription factors in *E. coli*.[22,23] Positive self-regulation is associated with multistability [24,25] but we have seen no evidence for multistability in activation of the

marA regulon. It will be important to determine the functional consequences of positive autoregulation of MarA. 2) The SoxS signal is also converted into a MarA signal, tying the two responses together. Nevertheless, because of promoter discrimination, overexpression of MarA leads to greater antibiotic resistance and less superoxide resistance than does overexpression of SoxS.[9] Thus, commensurate activation enables the cell to bring many different defenses into play depending on the kind of signal, its amplitude and its duration, providing a flexible defense against different levels of threat. It is also likely that in a population of cells there will be substantial heterogeneity in terms of the extent of activation of any particular regulon member and this may provide further advantages for survival.

We can use the relation between promoter activation and MarA concentration (Fig. 2) to back-calculate from the β-galactosidase activity of a cell to an equivalent concentration in MarA for any regulon promoter. Thus, our standard treatment of wild-type cells with 5 mM salicylate for 1 hr is the net equivalent of achieving a steady-state concentration of about 9,000 molecules of MarA per cell, far higher than the 750 molecules measured previously. This is likely due to a systematic error in the Western blot analyses of *lon*+ *clpP*+ strains.

We estimate that, at a minimum, there is a 19-fold variation in the amount of MarA needed for half-saturation of the different promoters. This variation is only poorly correlated with the binding affinity of MarA with these promoters *in vitro* (Table 1). For example, as previously reported, the *marboxes* of *pqi-5* and *acrAB* bind very tightly to purified MarA but *pqi-5* and *acrAB* require relatively high concentrations of MarA for activation. In contrast, MarA binds the *sodA* promoter very weakly but *sodA* is activated by low concentrations of MarA. This suggests that other factors present *in vivo* such as DNA supercoiling and/or global regulators (Fis, H-NS, *etc.*) may play important roles in determining promoter response to activator.

We have used the term “discrimination” for differences in activation of a single promoter by the paralogous activators and have shown a rough correlation of activation with the affinity (K_D) of a particular activator for a particular *marbox*.[9] However, at the highest activator concentrations attained here, both MarA and, as we have argued above, SoxS saturate the *marRAB* and *sodA marboxes* (Fig. 2A and 2B) so K_D is not a limiting factor. Nevertheless, the *marRAB* promoter shows greater activity with MarA than with SoxS whereas the opposite is true for the *sodA* promoter. One possibility is that the two activators differ in how they interact with RNAP at different promoters and that the specific ternary complex is critical.

Materials and Methods

Bacterial strains and plasmids

All strains are derivatives of *Escherichia coli* K-12. Their genotypes are given in Table 2. All *lon*− strains were derived by P1 transduction from strain SG12079 (*lon*Δ510 *clpP*::*cat*), kindly provided by S. Gottesman. Transductants were selected for chloramphenicol-resistance and then screened for the mucoidy phenotype of *lon*− cells. Because of this selection, all of the *lon*− strains are also *clpP*::*cat*. Strains designated as “wild-type” are *lon*+ *clpP*+.

β-galactosidase assays

Bacteria were grown overnight in LB (Lennox) medium at 32°C with appropriate antibiotics, diluted 1:4000 in antibiotic-free medium and grown to an A_{600} of 0.15 (generally 4–6 hrs) with the indicated concentrations of IPTG. β-galactosidase was measured according to Miller[26] and all assays agreed to ± 5%. All assays presented were performed at least 3 times in duplicate and the standard errors of the mean were <± 10%.

Western blotting technique

Cells were grown, extracts prepared and Western blots analyzed as previously described[12] using both our anti-MarA antibody and that kindly provided by Laura McMurray and Stuart Levy.[27] The addition of protease inhibitors did not enhance the recovery of MarA from the wild-type cells.

Acknowledgements

We thank Laura McMurray and Stuart Levy for generously providing us with anti-MarA antibody and Susan Gottesman for strain SG12079. This research was supported by the Intramural Research Program of the NIH and by the U.S. Department of Energy through the LANL/LDRD Program.

References

1. Aono R. Improvement of organic solvent tolerance level of *Escherichia coli* by overexpression of stress-responsive genes. Extremophiles 1998;2:239–248. [PubMed: 9783171]
2. Demple B. Redox signaling and gene control in the *Escherichia coli soxRS* oxidative stress regulon--a review. Gene 1996;179:53–57. [PubMed: 8955629]
3. White, DG.; Alekshun, MN.; McDermott, PF. Frontiers in Antimicrobial Resistance. Washington, D.C.: ASM Press; 2005.
4. Martin, RG.; Rosner, JL. Structure and function of MarA and its homologs. In: White, DG.; Alekshun, MN.; Mcdermott, PF., editors. Frontiers In Antimicrobial Resistance: A Tribute To Stuart B. Levy. Washington, DC: ASM Press; 2005. p. 235-246.
5. Griffith KL, Wolf RE Jr. 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 2001;40:1141–1154. [PubMed: 11401718]
6. Martin RG, Gillette WK, Rosner JL. 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 1999;34:431–441. [PubMed: 10564485]
7. Martin RG, Rosner JL. 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 2002;44:1611–1624. [PubMed: 12067348]
8. Wood TI, Griffith KL, Fawcett WP, Jair KW, Schneider TD, Wolf RE Jr. 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 1999;34:414–430. [PubMed: 10564484]
9. Martin RG, Gillette WK, Rosner JL. Promoter discrimination by the related transcriptional activators MarA and SoxS: differential regulation by differential binding. Mol. Microbiol 2000;35:623–634. [PubMed: 10672184]
10. Griffith KL, Shah IM, Wolf RE Jr. 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 2004;51:1801–1816. [PubMed: 15009903]
11. Maurizi M. Degradation *in vitro* of bacteriophage λ N protein by Lon protease from *Escherichia coli*. J. Biol. Chem 1987;262:2696–2703. [PubMed: 2950089]
12. Martin RG, Gillette WK, Martin NI, Rosner JL. Complex formation between activator and RNA polymerase as the basis for transcriptional activation by MarA and SoxS in *Escherichia coli*. Mol. Microbiol 2002;43:355–370. [PubMed: 11985714]
13. Griffith KL, Shah IM, Myers TE, O'Neill MC, Wolf RE Jr. Evidence for "pre-recruitment" as a new mechanism of transcription activation in *Escherichia coli*: the large excess of SoxS binding sites per cell relative to the number of SoxS molecules per cell. Biochem. Biophys. Res. Commun 2002;291:979–986. [PubMed: 11866462]
14. Rosner JL, Dangi B, Gronenborn AM, Martin RG. Posttranscriptional activation of the transcriptional activator Rob by dipyridyl in *Escherichia coli*. J. Bacteriol 2002;184:1407–1416. [PubMed: 11844771]

15. Alper MD, Ames BN. Transport of antibiotics and metabolite analogs by systems under cyclic AMP control: Positive selection of *Salmonella typhiumurium cya* and *crp* mutants. J. Bacteriol 1978;133:149–157. [PubMed: 201606]

16. Busby, S.; Kolb, A. The CAP Modulon. In: Lin, ECC.; Lynch, AS., editors. Regulation of Gene Expression in *E. coli*. New York: Chapman and Hall; 1996. p. 255-279.

17. Smith CL. Response of *recA*-dependent operons to different DNA damage signals. J. Biol. Chem 1985;260:10069–10074. [PubMed: 3894363]

18. Alon U. Network motifs: theory and experimental approaches. Nat. Rev. Genet 2007;8:450–461. [PubMed: 17510665]

19. Kalir S, McClure J, Pabbaraju K, Southward C, Ronen M, Leibler S, Surette MG, Alon U. Ordering genes in a flagella pathway by analysis of expression kinetics from living bacteria. Science 2001;292:2080–2083. [PubMed: 11408658]

20. Zaslaver A, Mayo AE, Rosenberg R, Bashkin P, Sberro H, Tsalyuk M, Surette MG, Alon U. Just-in-time transcription program in metabolic pathways. Nat. Genet 2004;36:486–491. [PubMed: 15107854]

21. Martin RG, Jair K-W, Wolf RE Jr, Rosner JL. Autoactivation of the *marRAB* multiple antibiotic resistance operon by the MarA transcriptional activator in *Escherichia coli*. J. Bacteriol 1996;178:2216–2223. [PubMed: 8636021]

22. Thieffry D, Huerta AM, Pérez-Rueda E, Collado-Vides J. From specific gene regulation to genomic networks: a global analysis of transcriptional regulation in *Escherichia coli*. Bioessays 1998;5:433–440. [PubMed: 9670816]

23. Wall ME, Hlavacek WS, Savageau MA. Design of gene circuits: lessons from bacteria. Nat. Rev. Genet 2004;5:34–42. [PubMed: 14708014]

24. Atkinson MR, Savageau MA, Myers JT, Ninfa AJ. Development of genetic circuitry exhibiting toggle switch or oscillatory behavior in *Escherichia coli*. Cell 2003;113:597–607. [PubMed: 12787501]

25. Savageau MA. Alternative designs for a genetic switch: analysis of switching times using the piecewise power-law representation. Math. Biosci 2002;180:237–253. [PubMed: 12387925]

26. Miller, JH. Experiments in Molecular Genetics. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press; 1972.

27. Nicoloff H, Perreten V, McMurry LM, Levy SB. Role for tandem duplication and Lon protease in AcrAB-TolC- dependent multiple antibiotic resistance (Mar) in an *Escherichia coli* mutant without mutations in *marRAB* or *acrRAB*. J. Bacteriol 2006;188:4413–4423. [PubMed: 16740948]

28. Martin RG, Rosner JL. Transcriptional and translational regulation of the *marRAB* multiple antibiotic resistance operon in *Escherichia coli*. Mol. Microbiol 2004;53:183–191. [PubMed: 15225313]

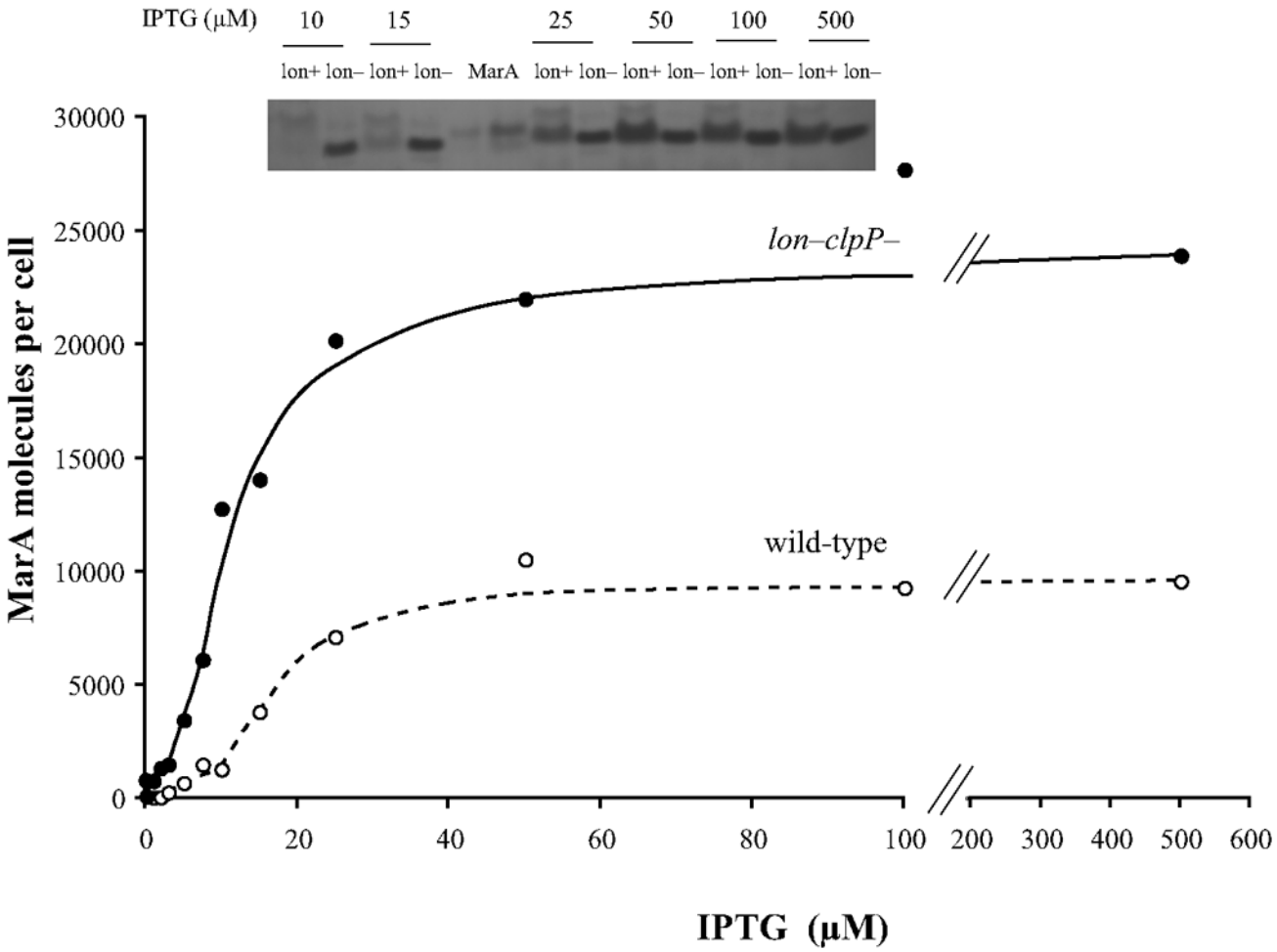

Figure 1.

MarA molecules per cell as a function of IPTG concentration in wild type and *lon− clpP −*cells. The inset is one of many Western blots from which the values in the graph were calculated. As previously noted,[12] MarA made *in vivo* migrates slightly faster than the purified material from the plasmid construct which contains an additional 5 amino acids at its N-terminus. There is a band also present with pre-immune serum that moves slightly less rapidly than the MarA. Each lane contained the extract from $4.5*10^7$ *lon− clp−* cells (M3897) or $1.4*10^8$ wild-type cells (M3723). The two MarA lanes contained 11 (left lane) and 33 ng (right lane) of purified MarA.

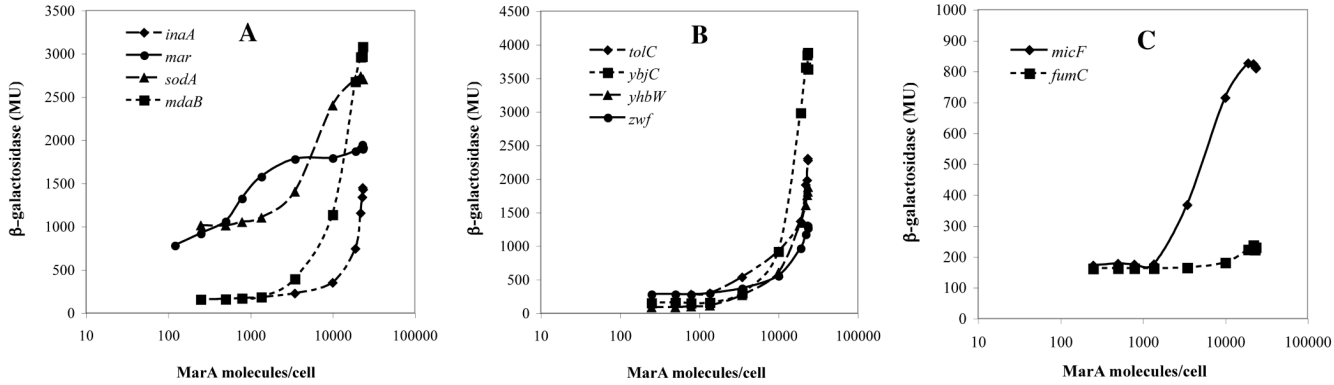

Figure 2.

The β-galactosidase activities of 10 promoters of the *marA/soxS/rob* regulon as a function of the calculated number of MarA molecules in *lon− clpP−* cells. The *promoter*::*lacZ* fusions are indicated.

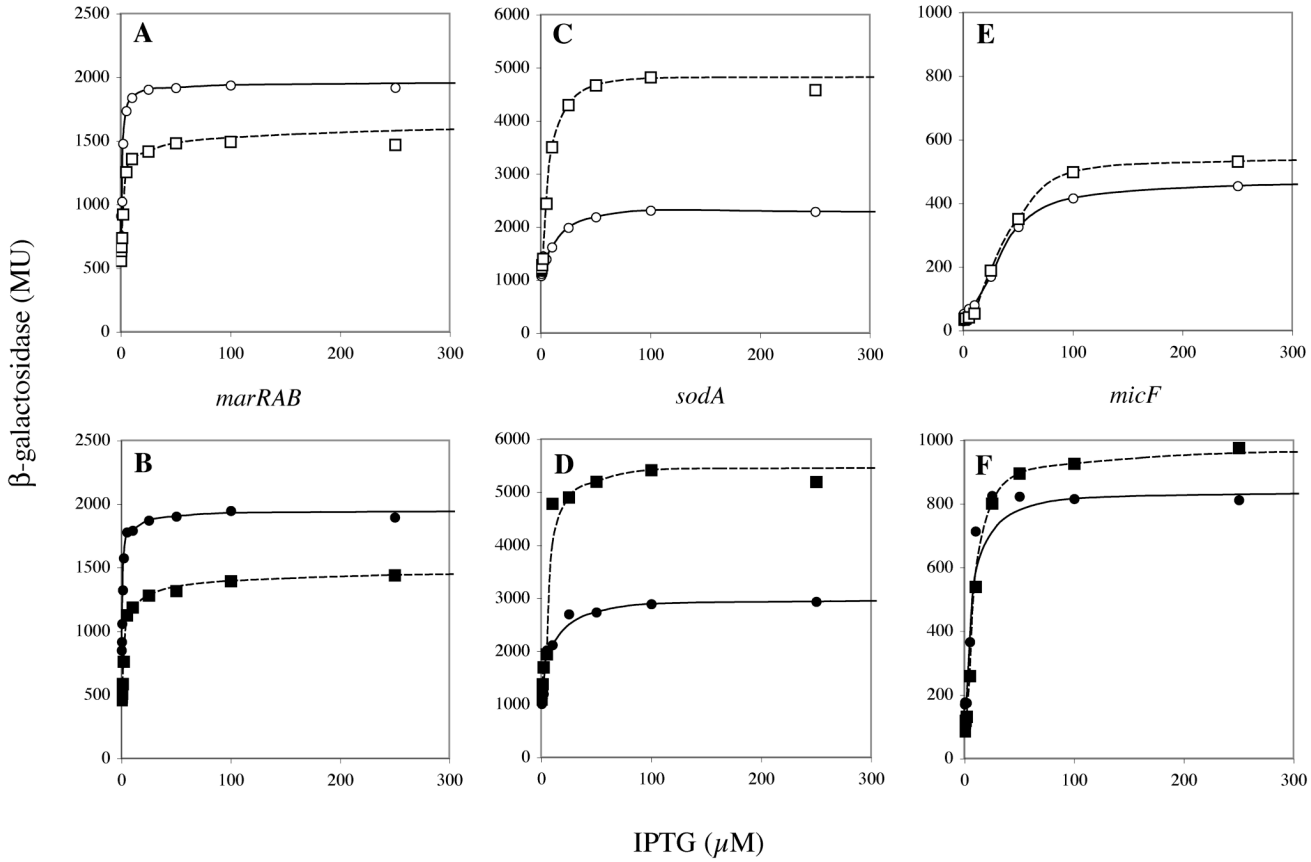

Figure 3.

Activation of three promoters by MarA or SoxS as a function of IPTG concentration in wild-type (A, C, E) and in *lon− clpP−* cells (B, D, F). *marRAB* promoter (A, B); *sodA* promoter (C, D); *micF* promoter (E, F). Circles, MarA; squares, SoxS; open symbols, wild-type cells; closed symbols, *lon− clpP−* cells.

Table 1

Comparison of *in vivo* concentrations of MarA required for half-maximal activity of 10 regulon promoters and the *in vitro* dissociation constants of MarA and the 20 bp promoter *marbox* sequences

Promoter	MarA (μM)[a]	K_D (μM)[b]
marRAB	1.5	0.075
sodA	13	>1
micF	17	0.05
mdaB	~25	ND[c]
ybjC	~25	0.32
fumC	>28	0.32
inaA	>28	0.5
tolC	>28	0.8
yhbW	>28	ND
zwf	>28	>1

[a] *In vivo* concentration of MarA providing half-maximal activation of the indicated promoter was calculated from Fig. 1 and Fig. 2 and assumes that the internal volume of an *E. coli* cell is ~10^{-15} liters.

[b] *In vitro* dissociation constants from references [9] and [12].

[c] ND, not done.

Table 2

Strains used in these studies.

Promoter fused to *lacZ*	strain #[a]	Plasmid[b]	Mutation	*lacZ* fusion from	
				Strain	Reference
fumC	M3957	*marA*	*lon*− *clpP*−	N9083	6
inaA	M3905	*marA*	*lon*− *clpP*−	N9246	6
marRAB	M2971	none		M2971	28
	M3687	none		M2971	this work
	M3720	*marA*		M3687	this work
	M3938	*soxS*	*lon*− *clpP*−	M3687	this work
	M3941	*soxS*		M3687	this work
	M4161	none	*lon*− *clpP*−	M3687	this work
	M4543	*marA*	*lon*− *clpP*−	M4161	this work
mdaB	M3899	*marA*	*lon*− *clpP*−	M1095	7
micF	M3944	*soxS*		M9084	6
	M3710	*marA*		M9084	6
	M3943	*soxS*	*lon*− *clpP*−	M9084	6
	M3893	*marA*	*lon*− *clpP*−	M9084	6
sodA	M3713	*marA*		N9086	6
	M3908	*marA*	*lon*− *clpP*−	N9086	6
	M3939	*soxS*	*lon*− *clpP*−	N9086	6
	M3940	*soxS*		N9086	6
tolC	M4266	*marA*		M4263	Martin, in prepartion
	M4267	*marA*	*lon*− *clpP*−	M4263	Martin, in prepartion
	M4427	none		M4263	Martin, in prepartion
ybjC	M3732	*marA*		M1108	7
yhbW	M3723	*marA*		M1071	7
	M3897	*marA*	*lon*− *clpP*−	M1071	7
zwf	M3895	*marA*	*lon*− *clpP*−	N9214	6

[a] All strains are derivatives of N8452 (Δ*marRAB rob*::*kan*).[28] All but M2971 carry F' *lacI*^{q} (TetR) (from strain XL-1 Blue (Stratagene, La Jolla, CA). The *lon*Δ*510 clpP*::*cat* strains were prepared by P1 transduction, using lysates grown on strain SG12079 and selecting for CamR.

[b] pRGM9817 is the vector, pRGM9818 carries *marA* and pJLR70 carries *soxS*. All are pUC19 derivatives (AmpR) described previously.[9]