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Letter to the Editor

The *marC* Gene of *Escherichia coli* Is Not Involved in Multiple Antibiotic Resistance[▽]

The *marC* gene of *Escherichia coli* is divergently transcribed from the *marRAB* operon involved in resistance to multiple antibiotics (5, 8), oxidative stress agents (2), and organic solvents (3, 16). Previous data from our laboratory had suggested a role for *marC* in intrinsic *m*ultiple *a*ntibiotic *r*esistance (4, 9, 16), and the gene has been so annotated in most databases.

Because that earlier work had suggested that *marC* was regulated by the repressor MarR and induced by tetracycline, we sought the transcriptional start site for *marC* to see if the *marC* promoter might overlap the MarR binding sites within the *marRAB* promoter (5′ rapid amplification of cDNA end [RACE] system of Gibco/BRL Life Technologies, cells grown with 2 μg/ml tetracycline to increase the amount of mRNA). Transcription of *marC* started 30 nucleotides upstream from the putative ATG initiation codon of MarC (bp 1266 of Cohen et al. [4]). Therefore, the *marC* promoter does not contain the MarR binding sites. Moreover, Northern blot analysis of AG100 and its isogenic *marR* mutant AG112 (in which MarR is inactive [8, 12]) showed no differences in levels of *marC* mRNA between the two strains (data not shown), nor was expression of *marC* induced by salicylate, which inactivates the repressor MarR (1) (Fig. 1). We conclude that *marC* is not regulated by MarR. Since chloramphenicol does not bind to MarR (1), the apparent up-regulation by tetracycline and chloramphenicol (Fig. 1) (4) likely reflects stabilization of mRNA rather than true induction (11, 13, 14).

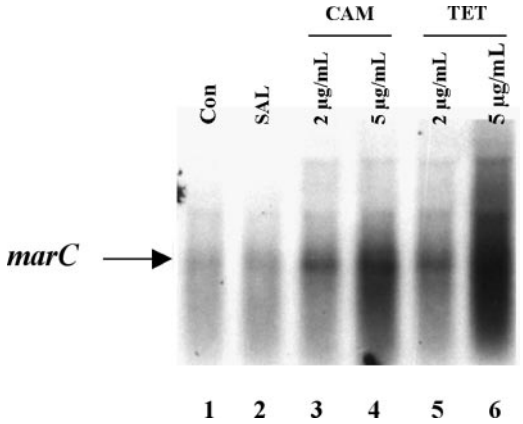

FIG. 1. Northern blot analysis of *marC* from *E. coli*. RNA was isolated from mid-exponential-phase cells grown in LB broth at 30°C and treated for 1 h with the specified compounds. Separate cultures of *E. coli* AG100 following exposure to 5 mM salicylate (SAL), tetracycline (TET), or chloramphenicol (CAM) at the indicated concentrations were probed with *marC*. Controls (Con) represent RNA from nontreated cultures. Total cellular RNA was prepared by cesium chloride gradient as previously described (15). RNA (20 μg) was separated by electrophoresis in a 1.5% agarose gel containing 20 mM guanidinium thiocyanate, transferred to Hybond N+ nylon membranes (Amersham), and probed using ^{32}P-radiolabeled *marC*. Hybridization signals were visualized using a phosphorimager (Molecular Dynamics, Sunnyvale, CA).

We replaced the *marC* locus in *E. coli* AG100 with a kanamycin cassette as described previously (10) and looked for any increase in susceptibilities to antimicrobials. MICs were determined on LB agar by use of Etest strips (AB Biodisk, Sölna, Sweden) with about three dozen different agents, including beta lactams, tetracyclines, fluoroquinolones, cephalosporins, imipenem, macrolides, aminoglycosides, chloramphenicol, fusidic acid, trimethoprim, and rifampin. Gradient plates (6) were used for oxidative stress agents (plumbagin, paraquat, phenylmethylsulfonate, dinitrophenol, and menadione) and for ethidium bromide. No differences in susceptibilities were seen for the *marC*::*kan* deletion mutant relative to the wild type. We then replaced the genes for the *E. coli* MarC paralogs YchE and YhgN in the *marC*::*kan* strain by use of spectinomycin and gentamicin cassettes, respectively, to create a triple knockout mutant, but again no differences in susceptibilities were seen.

We also used three plasmid constructs designed to overexpress *marC* via the *araBAD*, T7, or native *marC* promoter. Plasmid pHA-1::marC (obtained from D. Daley) specifies membrane-bound MarC-PhoA regulated by *Salmonella enterica* serovar Typhimurium pBAD/AraC (7). We constructed pETmarC11 (T7 promoter/lac operator with lac repressor; specifying MarC-6H) and pACmarC1 (*marC* promoter starting 58 bp upstream of the transcriptional start site; specifying native MarC) by cloning PCR-amplified DNA into vectors pET21b (Novagen) and pACYC184, respectively. None of these three plasmids led to a change in susceptibility of cells to a variety of antibiotics and oxidative stress agents.

We suggest that MarC no longer be classified as a multiple antibiotic resistance protein. However, we do not advise a name change until a function is found.

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