The Escherichia coli mar Locus—Antibiotic Resistance and More

The mar locus and related systems confer multiple antibiotic resistance and control expression of virulence factors and genes for metabolizing small molecules

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The multiple antibiotic resistance (mar) locus of Escherichia coli was first described in the early 1980s during efforts to characterize the genetic basis of tetracycline-resistant mutants derived in vitro. Originally selected on sub-inhibitory concentrations of tetracycline, these mutants also exhibited cross-resistance to other antibiotics, including fluoroquinolones and β-lactams. The Mar phenotype is inducible by salicylate, salicylate-like compounds, and aryloxoalcanoic acids. Inducible Mar and Mar-like systems are found in Yersinia spp., Salmonella enterica serovars Choleraesuis, Typhimurium, and Paratyphi B, Enterobacter aerogenes, Pseudomonas aeruginosa, Burkholderia cepacia, Mycobacterium tuberculosis, and Staphylococcus aureus.

The Mar phenotype in E. coli depends on the activity of two transcription factors, MarR (multiple antibiotic resistance repressor) and MarA (multiple antibiotic resistance activator), which are members of the helix-turn-helix (HTH) superfamily of proteins. MarR is a representative of the winged-helix subfamily, and MarA is a member of the AraC subfamily. There are numerous MarR and AraC orthologs/paralogs that are widely distributed among both the Bacteria and the Archaea, among which they play roles in regulating multiple and single antibacterial resistances, microbial virulence, and bacterial physiology.

The mar Locus: Its Components and Regulation

The E. coli marCRAB locus consists of four genes specifying MarC, MarR, MarA, and MarB (Fig. 1). Expression of marRAB is regulated by the activity of MarR and MarA. When MarR is active, expression is repressed; when it is inactivated by small molecules or mutated, marRAB transcription increases. Of prokaryotic and eukaryotic genomes examined, more than 1,400 AraC family members were found among prokaryotes and only isolated representatives were reported among eukaryotes (Fig. 2). Whether the latter represent true genome members or prokaryotic contaminants is unknown. AraC proteins can be divided into two general classes. One class includes small (∼15-kDa) proteins such as E. coli MarA and SoxS that contain only a DNA-binding domain (DBD). Other AraC-like proteins, such as E. coli Rob and AraC and P. aeruginosa ExsA, are much larger (∼30 kDa) and contain both a DBD and another domain of either known or unknown function.

The marC gene, which specifies a 221-amino-acid (aa) inner membrane protein of unknown function, is well conserved in a number of bacterial genera. marB codes for a small putative protein (72 aa) also of unknown function; the gene has so far been found only in E. coli, Shigella flexneri 2a, and S. enterica serovar Typhimurium (S. typhimurium).

Regulons: a Panoply of Genes Regulated by MarA and other AraC Family Members

MarA, SoxS, and Rob act as pleiotropic transcription factors. Each protein directly regulates the expression of multiple genes, termed the MarA, SoxS, and Rob regulons (Fig. 3A). These
gene products are responsible for protecting the cell from a variety of stress conditions. Because of redundancy among these regulons, the cell is well poised to respond to toxic insults such as antibiotics, even in the absence of any one transcription factor.

Researchers using genomic arrays, molecular genetics, bioinformatics, and other methods to characterize the E. coli MarA, SoxS, and Rob and P. aeruginosa ExsA regulons have reached similar, but also different conclusions about how these regulons work.

For instance, in a strain of E. coli with a plasmid constitutively expressing marA this regulon involved expression of 62 genes, according to our former colleague Teresa Barbosa. Taking two different approaches, Bruce Demple and his collaborators at the Harvard School of Public Health, in Boston, Mass., analyzed Rob-responsive genes and also characterized the MarA and SoxS regulons as well as the cell’s response to sodium salicylate (a MarR antagonist and inducer of marRAB expression) and paraquat (an inducer of the soxRS system). Despite identifying differences, they also concluded that the number of genes whose expression increases outnumbered those with reduced expression by a ratio of 3:1 in response to MarA. In contrast, SoxS down-regulates approximately three times as many genes as it up-regulates. We also find several intergenic regions responsive to MarA.

MarA directly activates genes, according to Richard Wolf, Jr., at the University of Maryland and Baltimore County, Baltimore, Md., and Robert Martin and Lee Rosner at the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Md. Moreover, MarA can also act directly as a repressor, and this function is apparently affected by the position of the MarA binding site (the marBox), according to our collaborator Thamarai Schneiders. Similarly Demple and his group report that Rob has repressor activity.

Martin and Rosner recently used both standard molecular biology techniques and bioinformatics to cross-validate a number of the genes identified in the MarA and SoxS regulons. Their data suggest that microarray experiments tend to overestimate the numbers of genes within these regulons, thus lowering the estimate for the combined MarA/SoxS/Rob regulon to about 50 genes.

**Clinical and Environmental Relevance of mar**

Some clinical isolates of E. coli, Enterobacter cloacae, Klebsiella pneumoniae, and S. typhimurium constitutively express MarA or a related transcription factor. Several research groups use the organic solvent tolerance (OST) phenotype exhibited by Mar mutants to identify clinical strains that overexpress MarA or a MarA ortholog.

In a survey of 138 fluoroquinolone-susceptible (FQs) and -resistant (FQR) clinical isolates, we found that about 2% of the FQs but 30% of the FQR isolates were cyclohexane tolerant; 40% of the latter isolates constitutively expressed either marA or soxS. Among 19 FQR clinical Klebsiella isolates, 52% were tolerant to organic solvents, while about 16% of those strains overexpressed RamA, a MarA ortholog.

According to Robert Tibbetts and colleagues at Purdue University in West Lafayette, Ind., about 34% of S. enterica serovar Choleraesuis isolates (n = 53) from pigs were OST and multiple-drug resistant (MDR); several overexpressed marA. Similarly, S. typhimurium expressed resistance to an E. coli-secreted microcin following exposure to salicylate, and MarR modulated this phenotype, according to

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**FIGURE 1**

Genetic organization of the E. coli mar locus. The marRAB operon is negatively (−) regulated by MarR (multiple antibiotic resistance repressor) and positively (+) regulated by MarA (multiple antibiotic resistance activator and global transcription regulator) via their binding to the mar promoter/operator region. The functions of MarB and MarC are unknown.
Steve Carlson and his collaborators at the U.S. Department of Agriculture Agricultural Research Service, Ames, Iowa. Approximately 10% of 388 *Salmonella* serotypes of animal origin were OST and bore a MDR phenotype that included resistance to antibiotics and disinfectants such as triclosan and cetrimide, according to Martin Woodward and his collaborators at Veterinary Laboratories Agency in Weybridge, Surrey, United Kingdom.

Resistance to household disinfectants is associated with MarA and SoxS overexpression. Because so many antibiotics used in the United States are also administered for purposes of animal husbandry, isolates of animal as well as human origin could serve as reservoirs of MDR strains.

**MarA Paralogs and Orthologs: Roles in Antibiotic Resistance and Virulence**

Many AraC family members play roles in antibiotic resistance and microbial virulence (Table 1). For example, overexpression of MarA in *E. coli* produces drug resistance and attenuates the rapid bactericidal activity of fluoroquinolones.

MarA-mediated drug resistance in these and other organisms is in large part achieved through increased expression of a multidrug efflux system. While a correlation between drug efflux and resistance was first suggested in studies involving both susceptible and resistant isolates, Hiroshi Nikaido at the University of California, Berkeley, and his collaborators found that MarA-mediated resistance depends on increased expression of AcrAB, a multidrug efflux pump. AcrAB likely protects against biliary salts, an obvious advantage to a microbe passing through the digestive tract of a mammalian host.

Nonionic detergents, such as triton X-100 and nonoxynol-9, can induce the MtrCDE efflux system of *Neisseria gonorrhoeae*, and this inducibility is dependent on MtrA (another AraC protein), according to William Shafer of Emory University School of Medicine, Atlanta, Ga. He and his colleagues find that MtrCDE protects pathogens from antimicrobial agents present on mucosal surfaces of infected mice.

The dramatic effects on pathogenesis (Table 1) following the deletion of a gene specifying an AraC-like protein is in part attributed to the ability of these proteins to coordinate the expression of large numbers of genes in “virulence regulons.” The genes within these regulons specify proteins that regulate the expression of membrane proteins involved in MDR, type III secretion systems, microbial toxins (e.g., cholera toxin), biofilm for-
mation, carbohydrate transport and metabolism, cell envelope synthesis, and lipid metabolism (Fig. 3A).

Deleting the gene specifying LcrF in Yersinia pestis KIMS3 results in a substantial decrease in the infectivity of this organism (Table 1), according to Yehuda Flashner and colleagues at the Israel Institute for Biological Research, Ness Ziona, Israel. Similarly, removing either exsA in P. aeruginosa or toxT in Vibrio cholerae renders each of these organisms virtually avirulent in mice (Table 1), according, respectively, to Joanne Engel at the University of California, San Francisco and Victor DiRita at the University of Michigan Medical School in Ann Arbor.

In a mouse model of pyelonephritis, we recently found that E. coli lacking MarA, SoxS, and Rob cannot colonize kidneys. Also, Proteus mirabilis UreR (an AraC protein) is required to infect the urinary tracts of mice, according to Harry Mobley at the University of Maryland School of Medicine, Baltimore, Md. When tested in chickens, S. typhimurium DT104 strains lacking marA are less frequently isolated from the spleens and caecal contents of infected chicks than are parent strains containing marA, according to Martin Woodward and his colleagues in the United Kingdom.

### Three-Dimensional Structures of MarA and Rob

The three-dimensional structures for both the E. coli MarA and Rob proteins in complex with their DNA substrates are now known (Fig. 4). These structural data directly support a wealth of genetic and biochemical evidence that first suggested the presence of the unique dual helix-turn-helix (HTH) DNA binding motif that defines this protein family. Surprisingly, this motif also occurs in the \( \lambda \) integrase, a protein that is not a transcription factor.

In the crystal structures, MarA engages its target with both N- and C-terminal HTH motifs, thus bending the DNA (Fig. 4A), whereas Rob shows only a N-terminal HTH in its interaction with a linear DNA fragment (Fig. 4B). Although these two crystal forms differ, each is supported by either additional biochemical data or further biophysical characterizations.

### AraC Family Members as Targets of New Anti-infection Therapeutics

Antibiotic resistance represents a growing public health problem. For instance, methicillin-resistant Staphylococcus aureus (MRSA) and multidrug-resistant uropathogenic E. coli are
now widespread in communities. Some clinical isolates of MRSA also contain high-level resistance to vancomycin, which is considered a “drug of last resort.”

Although the fluoroquinolones are the mainstay for treating common urinary tract infections, resistance to these agents among gram-negative bacilli is climbing in parallel to the increased use of these drugs. Several large pharmaceutical companies have stopped their antibiotic research and development programs, in part because of economic concerns, leaving the burden of drug development to smaller companies.

A novel approach for preventing or treating infections without risking development of drug resistance is needed to meet the threat of future MDR infectious diseases. For instance, Michael Givskov and colleagues at the Technical University of Denmark, Lyngby, Denmark, recently identified small molecules that inhibit quorum sensing in P. aeruginosa, and these compounds exhibit activity in vivo.

The targeting of a family of bacterial transcription factors like AraC family members for anti-infection chemotherapy, however, would represent a unique opportunity. While most previous attempts to curtail bacterial virulence focused on single virulence factors, including microbial adhesins such as pili and MSCRAMMs to prevent adherence of the pathogen, the targeting of a regulatory protein offers the advantage of simultaneously affecting expression of a number of virulence factors (Fig. 3B).

We recently identified groups of low-molecular-weight organic compounds that target AraC family proteins from E. coli, S. typhimurium, P. mirabilis, and P. aeruginosa. These novel agents inhibit the DNA binding activity in vitro of multiple AraC transcription factors. Of note, these agents produce the attenuated virulence phenotype exhibited by mutant E. coli in the murine pyelonephritis model.

The therapeutic potential of transcription factor modulators is validated in cancer chemotherapy, atherosclerosis, inflammation, diabetes, Parkinson’s disease, and in rheumatic and other autoimmune diseases. Examples of clinically effective products from these efforts include small molecules that target peroxisome proliferator-activated receptors, Hedgehog, and liver X receptor. The potential of this technology in anti-infective therapy remains to be further explored.

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SUGGESTED READING


