The Chromosomal arsR Gene of Escherichia coli Encodes a trans-acting Metalloregulatory Protein*

(Received for publication, August 23, 1995, and in revised form, November 20, 1995)

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Plasmid-encoded arsenical resistance (ars) operons confer high level resistance to arsenicals and antimonials, while the chromosomally encoded ars operon of Escherichia coli bestows low level resistance. The transcriptional start site of the chromosomal ars mRNA was mapped by primer extension, and putative −10 and −35 promoter recognition sites were identified. The arsR gene, the first gene in this operon, was cloned using polymerase chain reaction. The arsR gene product, the ArsR repressor, was expressed and purified. The results of gel mobility shift assays indicated that the repressor is a DNA binding protein that binds to a fragment of DNA containing the chromosomal ars promoter. The specific binding site, as determined by DNase I footprint analysis, spans 33 nucleotides in the promoter region, including the putative −35 promoter element. By construction and expression of a series of in-frame fusions between truncated arsR genes and the coding region for the mature form of β-lactamase (blaM), it was shown that ArsR is a trans-acting repressor that regulates expression of the chromosomal ars operon. In addition, the chromosomally-encoded repressor can regulate expression of the ars operon of plasmid R773, and the R773 repressor can cross-regulate expression from the chromosomal operon.

The Escherichia coli chromosomal ars operon was identified first by analysis of the E. coli genome (Sofia et al., 1994) and later by examination of metal-responsive gene fusions (Diorio et al., 1995). It was shown to have three open reading frames, originally termed arsFG that were subsequently renamed arsRBC (Carlin et al., 1995) because of their high degree of sequence similarity to the chromosomal R773 homologues (Chen et al., 1986; San Francisco et al., 1990). The chromosomal operon was shown to confer resistance to arsenite and antimonite in E. coli, with resistance correlated with increased extrusion of arsenite (Carlin et al., 1995), as has been shown for the plasmid-encoded resistances (for reviews, see Dey and Rosen (1995) and Rosen et al. (1995)). The level of resistance conferred by the chromosomal operon was considerably less than the high level of resistance produced by the operons of the staphylococcal plasmids p258 and pSX267 (Jia and Silver, 1992; Rosen et al., 1992) or the E. coli plasmids R773 or R46 (Hedges and Baumberg, 1973; Silver et al., 1981; Mobley et al., 1984). In all plasmid-borne ars operons, transcription was controlled by the ArsR repressor, the product of the first gene of each operon. These are all members of the ArsR family of regulatory proteins (Shi et al., 1994). Other members of the ArsR family include Cd²⁺/Zn²⁺ regulatory proteins (Yoon et al., 1991; Ivey et al., 1992; Morby et al., 1993). All are believed to be metal-inducible repressors that control the basal level expression of the respective operons (Wu et al., Rosen, 1991, 1993; Morby et al., 1993; Rosenstein et al., 1994).

The E. coli chromosomal arsR gene encodes a 13-kDa protein, ArsR, in which 75% of the residues (88 of 117) are identical to those of the plasmid R773 repressor in primary amino acid sequence but only 26% (34 of 137) are identical to the staphylococcal plasmid p258 or pSX267 ArsR proteins (Carlin et al., 1995). In this study, the chromosomal protein was shown to be a trans-acting regulator of both the E. coli chromosomal and the R773 plasmid ars operons. The gene was expressed at a high level, and ArsR was purified. The purified protein eluted from a gel filtration at a position corresponding with that of a 26-kDa homodimer. In gel shift DNA binding assays, the purified protein retarded the migration of DNA fragments containing either the chromosomal or R773 plasmid ars promoters. From DNase I footprint analysis, the ArsR binding site was found to span nucleotides −64 to −31 of the chromosomal operon.

MATERIALS AND METHODS

E. coli Strains, Plasmids, and Media—The bacterial strains and plasmids used in this study are described in Table I. E. coli cells were grown in LB medium at 37 °C. Ampicillin (100 μg/ml), kanamycin (80 μg/ml), tetracycline (15 μg/ml) or chloramphenicol (20 μg/ml) were added as required. For protein expression, 0.5 mM isopropyl-1-thio-β-D-galactopyranoside or 20 μM sodium arsenite were used as inducer, except where otherwise noted. Plasmid pCX1 was made by cloning the SphI and XmaI fragment from pWPS1 (Carlin et al., 1995) into vector plasmid pBluescript (Broome-Smith and Spratt, 1986) that had been digested with both SphI and XmaI. This fragment contained 630 bp upstream of the translational initiation point of the arsR gene, the entire arsR gene, and part of the arsB gene. To remove the PvuII site upstream of the ars promoter but within the insert, a XcmI fragment was deleted from plasmid pCX1, producing plasmid pCX2. Removal of the PvuII site allowed the PvuII site in front of the blaM gene to be used for construction of arsR:blaM fusions (see below). To delete the tet promoter in the vector sequence, plasmid pCX2 was digested with BssHII and EcoRV, filled in with the Klenow fragment of DNA polymerase I, and intramolecularly ligated to produce plasmid pCX3. Plasmid pCX1 was created by ligating the HindIII-EcoRV fragment which consists of 630 bp upstream of the translational initiation point of arsR, the entire arsR gene, and part of the arsB gene from plasmid pWPS1 into pBluescript that had been digested with both HindIII and EcoRV. Plasmid pCX12 was derived from plasmid pCX11 by cloning the EcoRI-ScaI fragment containing the chromosomal ars promoter and arsR gene insert into EcoRI-ScaI-digested plasmid pACYC184 (Chang and Cohen, 1978).

* This work was supported by United States Public Health Service Grant AI19793. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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1 The abbreviations used are: bp, base pair(s); DTT, dithiothreitol; EDTA, ethylenediaminetetraacetate; PAO, phenylarsine oxide; PCR, polymerase chain reaction.

Vol. 271, No. 5, Issue of February 2, pp. 2427-2432, 1996
Printed in U.S.A.
DNA Manipulation and Sequencing—Preparation of plasmid DNA was performed by using a Wizard DNA purification kit (Promega). Endo- and exonuclease digestions, DNA fragments separations and isolations, ligations, transformations, and Klenow fragment fill in were performed according to standard procedures (Sambrook et al., 1989) unless otherwise noted. The Sequenase kit (version 2.0, U.S. Biochemical Corp.) was employed for sequencing of double-stranded DNA.

Polymerase Chain Reaction—Polymerase chain reactions (PCR) (Mullis and Faloona, 1987) were run on a Stratagene Robocycler 40 thermal cycler. The reactions contained in a final volume of 50 μl of 2 mM Tris-HCl (pH 8), 1 mM EDTA, 0.1 mM dithiothreitol, 0.5 mg/ml bovine serum albumin, 5% (v/v) glycerol, 1.5 mM MgCl₂, 2.5 μM units of Taq DNA polymerase, 0.1 μM each deoxynucleoside triphosphate, 0.25 μg of each primer, and 20 μg of DNA. Each reaction was overlaid with 20 μl of light mineral oil and cycled 30 times: 94°C for 1 min, 60°C for 0.5 min, 72°C for 1 min, followed by a single cycle at 72°C for 10 min.

Cloning of the arsR Gene—Two PCR primers were used for PCR cloning of the arsR gene. The forward primer had the sequence 5'-TCCCGGATAAAACACATCTG-3' for 1 h. The soluble fraction was loaded onto a 2.5-cm diameter column filled to 45 cm with Superose 12 (Pharmacia) and eluted with buffer A containing 0.2 M NaCl. Fractions were analyzed by SDS-polyacrylamide gel electrophoresis, and those containing ArsR were pooled, concentrated, and stored at −70°C until use. From the intensity of staining, ArsR was judged to be approximately 80–90% pure.

Construction of arsR::blam' Gene Fusions—To create a series of chromosomal arsR::blam' gene fusions, plasmid pCX3 was linearized by digestion at the unique XmalI site followed by exonuclease III digestion. The digestion products were removed at various times during Exon III digestion and pooled. The ends were flushed with S1 nuclease, followed by digestion with PvuI. The resulting linear fragments were self-ligated with T4 DNA ligase, and the ligation mixture was transformed into cells of E. coli strain J M109 made competent by the method of Chung et al. (1989). Kanamycin-resistant transformants were selected. Cells with in-frame fusions of arsR gene to the portion of the blam' gene containing the coding region of the mature form of the TEM β-lactamase were identified by growth as patches on LB agar containing 100 μg/ml ampicillin and 50 μM sodium arsenite, and confirmed by digestion of BamHI and PstI. To determine the fusion site of blam' with arsR, plasmids were sequenced using a primer with the sequence 5'-GTGTGACCCCAAAGCTGA-3', which is complementary to codons 14–18 of the mature form of TEM β-lactamase. The resulting arsR::blam' fusion plasmids were named as the pCRB series, with the numbers representing the amino acid residue number of the ArsR protein to which the mature TEM β-lactamase was fused.

Expression of ArsR-β-Lactamase Fusion Proteins—Cells of E. coli strain AW10 bearing each arsR::blam' fusion plasmid alone or both the fusion plasmid and plasmid pCX12, which contains a wild-type arsR gene, were grown overnight at 37°C in LB medium containing kanamycin or both kanamycin and chloramphenicol, respectively. The cultures were diluted 100-fold into fresh LB medium and grown at 37°C to an absorbance of 0.6 at 600 nm, at which time 50 μM sodium arsenite was added as inducer. Growth was continued at 37°C for 2 h following induction. The cells were collected by centrifugation and suspended in 0.1 ml of SDS sample buffer for analysis. For immunoblotting, the proteins were transferred to a nitrocellulose membrane using a transblot apparatus (Bio-Rad) according to the manufacturer’s instructions. Proteins were electrophoretically transferred to a nitrocellulose sheet (0.2 μm pore size) and immunoblotted as described previously (Tisa and Roso, 1990). A chemiluminescent assay was used to detect the antigen-antibody reaction. The filter was incubated with 10 ml of the enhanced chemiluminescence solution (Amersham Corp.) and exposed on x-ray.
film for 1 min at room temperature.

Isolation of RNA—TRI Reagent RNA/DNA/protein isolation reagent (MRC, Inc.) was used to isolated total cellular RNA according to the manufacturer's directions from cells of E. coli strain J M109 carrying plasmid pWP51 induced with 20 μM sodium arsenite. RNA was suspended in ethanol and stored at −70 °C until use.

Primer Extension—A primer, 5'-CCAGACGGTTCATCAGCAA-GAATTTCG-3', corresponding to a region within the coding sequence of arsR, end-labeled with [γ-32P]ATP was used for primer extension analysis (Sambrook et al., 1989). Total RNA was mixed with end-labeled primer, dNTPs, and Superscript II RNaseH reverse transcriptase (Life Technologies, Inc.). The primer extension product was loaded on an 8% polyacrylamide, 8 M urea sequencing gel. A size ladder produced by dideoxy sequencing of plasmid pWP51 with the same primer used in the primer extension reaction was used to measure the length of the primer extension product.

Gel Mobility Shift and DNase I Footprinting Assays—Gel mobility shift and DNase I footprinting assays were performed as described previously (Wu and Rosen, 1993). A fragment including the regulatory region DNA and start of the arsR gene was generated by PCR using the primer extension digonucleotide and a second primer with the sequence 5'-CCGAATTCGACGCAAAGTCG-3', containing an EcoRI site at the 5'-end. The purified PCR product was digested with MunI, labeled with [α-32P]dATP and the Klenow fragment of DNA polymerase, and purified using a Wizard DNA purification kit (Promega). This 208-bp labeled fragment was prepared for gel mobility shift assay and coding strand footprinting. For footprinting the non-coding strand, a 203-bp fragment was prepared by digesting the purified PCR product with TfiI and was labeled as described above. A DNA probe carrying the plasmid R773 ars promoter was prepared as described previously (Wu and Rosen, 1993). A mixture of two DNA probes containing the lac promoter (211 bp) and a nonspecific DNA fragment (111 bp) were obtained by digestion of plasmid pUC19 with BamHI and PvuII, and labeled as described above. The nucleotide size ladders were created by dideoxy sequencing of the plasmid pXC11 using primer 5'-ATTGGAGTG-GGTAAACGAA-3' for DNase I footprinting of the coding strand and primer 5'-ATTCACCTCTTTATAG-3' for footprinting the non-coding strand.

RESULTS

Phenylarsine Oxide Is an Inducer of the ars Operon—Phenylarsine oxide (PAO) is an organoarsenical containing As3+ that interacts strongly with proteins containing vicinal cysteine pairs (Hoffman and Lane, 1992). Its ability to induce the chromosomal ars operon was examined using plasmid pCRBB91, which carried a reporter construct in which the blaM gene was fused to the arsR gene and expression of the operon was under control of the ars promoter. Cells with this plasmid became ampicillin resistance when induced from the lac promoter (211 bp) and a nonspecific DNA fragment (111 bp) were obtained by digestion of plasmid pUC19 with BamHI and PvuII, labeled as described above. The nucleotide size ladders were created by dideoxy sequencing of the plasmid pXC11 using primer 5'-ATTGGAGTG-GGTAAACGAA-3' for DNase I footprinting of the coding strand and primer 5'-ATTCACCTCTTTATAG-3' for footprinting the non-coding strand.

To study expression of the ArsR-β-lactamase chimeras, the eight fusions were individually transformed into cells of E. coli strain AW10, in which the chromosomal ars operon has been deleted (Carlin et al., 1995). Proteins from uninduced cells or cells induced with arsenite were analyzed by SDS-polyacrylamide gel electrophoresis, followed by immunoblotting with antibody to β-lactamase (Fig. 3A). Chimeras in which the fusion sites were at residues 20, 69, 74, and 79 of the ArsR proteins were produced in both arsenite-induced and uninduced cells, indicating that these chimeric proteins were constitutively expressed. In contrast, the hybrid ArsR-β-lactamase proteins from plasmids with fusions at codons 92, 101, 104, or 114 of the arsR gene were visible only in arsenite-induced cells. Thus expression of the chimeric proteins in these four fusions remained inducible by arsenite.

The ArsR Protein Is a trans-acting Repressor—To demonstrate that ArsR is a trans-acting repressor, the arsR gene was cloned into vector plasmid pACYC184, yielding plasmid pCX12. Four arsR::blaM fusion plasmids, pCBBR69, pCBBR79, pCBBR92, and pCBBR104 were individually co-transformed with plasmid pCX12 into E. coli strain AW10, and expression of these four chimeric proteins with or without the inducer arsenite was monitored by immunoblotting. Immunoreactive hybrid proteins were only observed in arsenite-induced cells (Fig. 3B). Although the chimeric proteins in pCBB69 and pCBB79 were constitutively expressed in the absence of an arsR gene (Fig. 3A), in the presence of an arsR gene in trans they became inducible. There was no trans effect of a wild-type arsR gene on expression of the fusions in pCBB92 and pCBB104, in which the chimeric ArsR proteins still functioned as repressors (Fig. 3B). These results demonstrate that ArsR is a trans-acting regulatory protein. Furthermore, the sequence from the 92nd aminoacyl residue to the C terminus is not required for the regulatory activity of the protein.
Regulation of the Chromosomal ars Operon

![Diagram](image)

**Regulatory region of the chromosomal ars operon.** A, promoter region of the R773 ars operon. The contact points between the R773 ArsR repressor and DNA are enclosed in boxes (Wu and Rosen, 1993). B, promoter region of the chromosomal ars operon. The deduced amino acid sequence for the open reading frame corresponding to the ArsR is shown below the coding strand. The shaded sequence indicates the predicted mass of the ArsR-BlaM chimera from plasmid pCRB20. The boxed sequences are identical to those identified to the contact points between the R773 ArsR repressor and DNA. The locations of fusion sites between arsR and blaM are indicated below the sequence by the arsR codon numbers at the fusion sites. For both A and B, 1 indicates the start site of arsR transcription, with the presumed −10 and −35 promoter elements and the most likely Shine-Dalgarno sequence sites underlined.

**Fig. 2.** Primer extension analysis of the transcription start site of the chromosomal ars operon. Primer extension and nucleotide sequencing assays were performed as described under "Materials and Methods." Lane 1 shows the primer extension product; lanes 2-5 show the nucleotide sequence ladders generated with the same primer. The arrow indicates the position of the transcriptional initiation site. The corresponding DNA sequence of the coding strand is shown on the left. The start codon of the arsR gene, the Shine-Dalgarno sequence, and the first nucleotide of the chromosomal ars transcript are indicated.

Localization of the Transcription Initiation Site of the Chromosomal ars Operon—To identify the promoter responsible for chromosomal ars transcription, total RNA was isolated from cells containing plasmid pWP51, which contains the entire chromosomal ars operon, grown in LB medium with sodium arsenite as inducer. The 5'-end of the RNA was determined by primer extension. The same primer was used in a DNA sequencing reaction with plasmid pWP51 as template to determine the nucleotide to which the primer extension product mapped. Only one primer extension product was observed; it corresponded to a cytosine nucleotide located 16 nucleotides upstream from the ATG start codon of arsR gene (Fig. 4). Putative −10 and −35 promoter sequences were identified as GACACT and TTGACT.

The ArsR Protein Is a DNA Binding Protein—Gel mobility shift assays were used to examine the DNA binding activity of ArsR. Both the chromosomal and R773 ArsR proteins were able to retard the migration of DNA fragments containing either the chromosomal (Fig. 5A) or the plasmid R773 (Fig. 5B) ars promoters. However, with neither protein was a mobility shift observed with DNA fragments carrying either the lac promoter or nonspecific DNA (data not shown). The chromosomal ArsR protein could be dissociated from its promoter by addition of inducer (Fig. 6). However, neither arsenite and antimonite were very effective in vitro, requiring nonphysiological concentrations of inducer to effect dissociation. Although the explanation is unknown, a similar unresponsiveness to arsenite had been noted for the R773 and pSX267 ArsR proteins (Wu and Rosen, 1993; Rosenstein et al., 1994). On the other hand, the organoarsenical PAO was extremely effective, with 1 μM PAO sufficient to completely dissociate the repressor.

**Fig. 3.** Expression of the arsR::blaM fusion genes and their regulation by arsR in trans. Equal amounts of total cell protein from cells of E. coli strain AW3110 harboring the indicated plasmids were separated on 10% SDS-polyacrylamide gels followed by immunoblotting with antiserum against TEM-1 foot printing. The boxed sequences are identical to those identified to the contact points between the R773 ArsR repressor and DNA. The locations of fusion sites between arsR and blaM are indicated below the sequence by the arsR codon numbers at the fusion sites. For both A and B, 1 indicates the start site of arsR transcription, with the presumed −10 and −35 promoter elements and the most likely Shine-Dalgarno sequence sites underlined.

**Fig. 4.** Primer extension analysis of the transcription start site of the chromosomal ars operon. Primer extension and nucleotide sequencing assays were performed as described under "Materials and Methods." Lane 1 shows the primer extension product; lanes 2-5 show the nucleotide sequence ladders generated with the same primer. The arrow indicates the position of the transcriptional initiation site. The corresponding DNA sequence of the coding strand is shown on the left. The start codon of the arsR gene, the Shine-Dalgarno sequence, and the first nucleotide of the chromosomal ars transcript are indicated.
FIG. 5. ArsR protein-DNA interaction. Mobility shift assays were performed as described under "Materials and Methods." Two different DNA fragments were radiolabeled with [α-32P]dATP and incubated with the indicated repressor proteins. The binding mixtures were analyzed on 6% polyacrylamide gels. A, the radiolabeled DNA was the PCR product containing the chromosomal ars promoter region digested with Muni. B, the probe was a 153-bp EcoRI-DraI DNA fragment containing the R773 ars promoter region. The middle lanes contained DNA incubated with 3 μg of purified chromosomal ArsR. The right lanes contained DNA incubated with 3 μg of purified R773 ArsR.

FIG. 6. Effect of inducers of the chromosomal ars operon on ArsR-DNA complex formation. Mobility shift assays were performed as described under "Materials and Methods." The PCR product containing the chromosomal ars promoter region was digested with Muni, radiolabeled with [α-32P]dATP, and incubated with 3 μg of purified ArsR. Potential inducers were added individually to the binding mixtures. The binding mixtures were analyzed on 6% polyacrylamide gel. All lanes contained probe DNA; lanes 2–13 also contained ArsR; lane 3, 0.5 mM potassium antimonial tartrate; lane 4, 4.2 mM potassium antimonial tartrate; lane 6, 0.5 mM sodium arsenite; lane 7, 5 mM sodium arsenite; lane 8, 0.1 μM PAO; lane 10, 0.5 μM PAO; lane 12, 0.5 mM sodium arsenite; lane 13, 5 mM sodium arsenite.

purified chromosomal ArsR, the site of binding to the chromosomal ars regulatory region was analyzed by DNase I protection assays. A protected region of approximately 33 bp (from nucleotides –64 to –31) in both the coding strand (Fig. 7A) and the noncoding strand (Fig. 7B) was observed. Protection was prevented by addition of the inducer PAO (data not shown). R773 ArsR protected this same region of the DNA, and chromosomal ArsR protected the same region of the R773 promoter DNA as did the plasmid repressor (data not shown).

DISCUSSION

The chromosomal ars operon of E. coli was originally identified by sequencing of the E. coli genome (Sofia et al., 1994). This operon is responsible for the basal level resistance to arsenite, antimonite, and arsenate in plasmidless strains of E. coli (Carlin et al., 1995). The operon has three genes, arsR, arsB, and arsC. From the 75% sequence similarity of chromosomal ArsR with the ArsR repressor encoded by the ars operon of plasmid R773 (San Francisco et al., 1990), a regulatory function was proposed for the chromosomal ArsR (Sofia et al., 1994; Carlin et al., 1995).

In this study, the chromosomal arsR gene was shown to regulate expression of reporter arsR::blam' genes in trans (Fig. 3B). Purified chromosomal ArsR, which was found to elute from a gel filtration column at a size corresponding to that of a homodimer, bound to promoter DNA (Fig. 5). Arsenite and antimonite did not dissociate the complex in concentrations at which they induce in vivo, which may suggest that dissociation is not required for induction. On the other hand, phenylarsine oxide, the only organoarsenic found thus far to induce, prevented retardation of the promoter DNA (Fig. 6) and reversed protection from DNase I digestion at 1 μM (data not shown), the same concentration at which PAO is an inducer in vivo (Fig. 1). These results indicate that induction results when the repressor dissociates from the DNA.

The region of the DNA protected from DNase I digestion by ArsR overlaps with the putative –35 element of the chromosomal ars promoter and covers the region from nucleotides –64 to –31 (Figs. 2 and 4). The plasmid R773 ArsR repressor has been shown to bind to the R773 ars promoter at a region of imperfect dyad symmetry just upstream of the –35 site (Wu and Rosen, 1993). Although the overall sequences of the two promoters and the location of the protected sequences are different between the two ars operons, higher resolution analysis of the R773 ArsR binding site revealed that only two small regions of 4 bp each (TCAT and TTTG of the coding strand) are protected separated by 7 bp (Wu and Rosen, 1993). Since the chromosomal ars sequence that was protected from DNase I by chromosomal ArsR also contained the TCAT and TTTG elements separated by 7 bp, it was possible that the two repressors could bind to the other 's promoter. As shown in Fig. 5, the chromosomal ArsR repressor retarded the migration of DNA containing the R773 ars promoter, and R773 ArsR retarded the corresponding chromosomal ars DNA. Both proteins protected the same regions of both promoters from DNase I digestion (data not shown). Thus the sequence TCAT-NNNNNNTTTG appears to represent a consensus binding site for the two ArsR repressors. It is interesting that the chromosomal and plasmid R773-encoded ArsR proteins from E. coli are essentially interchangeable, even though the two proteins are 25% dissimilar, and their promoter regions contain significant differences in sequence and placement of the regulatory elements. On the other hand, the homologous ars repres-
sor from the staphylococcal plasmid pSX267 protects two regions within the promoter region from DNase I digestion (Rosenstein et al., 1994), but this region does not contain TCAATNNNNNNTTGG, suggesting that it is a Gram-negative consensus sequence. The binding of the ArsR repressors to each other promoters may have physiological significance; in vivo when the chromosomal arsR gene was carried on a compatible plasmid with lacZ gene fused to the R773 ArsR promoter, expression of the reporter gene became arsenite inducible (data not shown).

We would propose that all members of the ArsR family of metalloregulatory proteins contain at least four domains. First, we have shown that a putative DNA binding domain in the R773 repressor is required for repression (Shi et al., 1994). Second, we have shown that Cys-32 and Cys-34 of R773 ArsR are part of a metal binding domain involved in induction (Shi et al., 1994). However, in the ArsR family of transcriptional repressors, both arsenic/antimony and cadmium/zinc responsive repressors have this cysteine pair. To account for differential recognition of metals, we would propose the existence of an additional metal binding domain in the cadmium/zinc responsive regulatory proteins. In those repressors, there is an additional N-terminal sequence with two cysteinyl residues that might provide this function.

Finally, the ArsR repressors are most likely functional homodimers, which indicates the existence of a dimerization domain. When the mature form of β-lactamase was fused to the ArsR protein at residue 92, 101, 104, or 114, expression of the chimeric protein was still inducible, indicating that residues 92 to the C terminus are not required for ArsR function. Similar results were obtained for C-terminal chimeras of R773 ArsR (Wu and Rosen, 1991), and those chimeras were shown to be bind to the promoter DNA as dimers (Wu and Rosen, 1993). These results demonstrate that information required for dimerization is not contained in residues from 92 to the C terminus. On the other hand, chimeras with fusions at residues 79 or closer to the N terminus were constitutively expressed. Similar chimeras in R773 ArsR were unable to bind to DNA. These results suggest that residues in the region of 79–92 may be involved in dimerization. In conclusion, members of the ArsR family of repressor proteins are postulated to have a metal binding domain, followed by a DNA binding domain. In some members there may also be an N-terminal metal discrimination domain. Finally, there is most likely a dimerization domain that may require residues C-terminal to the DNA binding domain. A more detailed analysis of these proteins will be necessary to identify this domain.

REFERENCES

Braun-Smith, J. K., and Spratt, B. G. (1986) Gene (Amst.) 49, 341–349
Carlin, A., Shi, W. P., Dey, S., and Rosen, B. P. (1995) J. Bacteriol. 177, 981–986
Chang, A. C. Y., and Cohen, S. N. (1978) J. Bacteriol. 134, 1141–1156
Chen, C. M., Misra, T., Silver, S., and Rosen, B. P. (1986) J. Bid. Chem. 261, 15030–15038
Chung, C. T., Niemela, S. L., and Miller, R. H. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2172–2175
Dey, S., and Rosen, B. P. (1995) in Drug Transport in Antimicrobial and Anticancer Chemotherapy (Georgopapadakou, N. H., ed) pp. 103–132, Dekker, New York
Doria, C., Cai, J., Marmor, J., Shinder, R., and Dubow, M. S. (1995) J. Bacteriol. 177, 2056–2066
Hedges, R. W., and Baumberg, S. (1973) J. Bacteriol. 115, 459–460
Hoffman, R. D., and Lane, M. D. (1992) J. Biol. Chem. 267, 14005–14011
Ivey, D. M., Guffanti, A. A., Shen, Z., Kudyan, N., and Krulwich, T. A. (1992) J. Bacteriol. 174, 4878–4874
Ji, G., and Silver, S. (1992) J. Bacteriol. 174, 3684–3694
Laemmli, U. K. (1970) Nature 227, 680–685
Mobley, H. L. T., Silver, S., Porter, F. D., and Rosen, B. P. (1984) Antimicrob. Agents Chemother. 25, 157–161
Morby, A. P., Turner, J. S., Huckle, J. W., and Robinson, N. J. (1993) Nucleic Acids Res. 21, 921–925
Mullis, K. B., and Faloona, F. A. (1987) Methods Enzymol. 155, 335–350
Rosen, B. P., Bhattacharjee, H., and Shi, W. P. (1995) J. Biogenet. Biomembr. 27, 85–91
Rosenstein, R., Peschel, A., Wierland, B., and Götz, F. (1992) J. Bacteriol. 174, 3676–3683
Rosenstein, R., Nikolet, K., and Götz, F. (1994) Mol. & Gen. Genet. 242, 566–5728
Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
San Francisco, M. J. D., Hope, C. L., Owolabi, J. B., Tisa, L. S., and Rosen, B. P. (1990) Nucleic Acids Res. 18, 619–624
Shi, W., Wu, J., and Rosen, B. P. (1994) J. Biol. Chem. 269, 19826–19829
Silver, S., Budd, K., Leahy, K. M., Shaw, W. V., Hammond, D., Novick, R. P., Willisky, G. R., Malorny, M. H., and Rosenberg, H. (1983) J. Bacteriol. 154, 983–996
Sofia, H., J., Burland, V., Daniels, D. L., Plunkett, G., III, and Blattner, F. R. (1994) Nucleic Acids Res. 22, 2576–2586
Tabo, S., and Richardson, C. C. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 1074–1078
Tisa, L. S., and Rosen, B. P. (1990) J. Biol. Chem. 265, 190–194
Wu, J., and Rosen, B. P. (1991) Mol. Microbiol. 5, 1331–1336
Wu, J., and Rosen, B. P. (1993) J. Biol. Chem. 258, 52–58
Yoon, K. P., Misra, T. K., and Silver, S. (1991) J. Bacteriol. 173, 7643–7649