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Review

Molecular Mechanisms of Ligand-Mediated Attenuation of DNA Binding by MarR Family Transcriptional Regulators

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Bacteria and archaea encode members of the large multiple antibiotic resistance regulator (MarR) family of transcriptional regulators. Generally, MarR homologs regulate activity of genes involved in antibiotic resistance, stress responses, virulence or catabolism of aromatic compounds. They constitute a diverse group of transcriptional regulators that includes both repressors and activators, and the conventional mode of regulation entails a genetic locus in which the MarR homolog and a gene under its regulation are encoded divergently; binding of the MarR homolog to the intergenic region typically represses transcription of both genes, while binding of a specific ligand to the transcription factor results in attenuated DNA binding and hence activated gene expression. For many homologs, the natural ligand is unknown. Crystal structures reveal a common architecture with a characteristic winged helix domain for DNA binding, and recent structural information of homologs solved both in the absence and presence of their respective ligands, as well as biochemical data, is finally converging to illuminate the mechanisms by which ligand-binding causes attenuated DNA binding. As MarR homologs regulate pathways that are critical to bacterial physiology, including virulence, a molecular understanding of mechanisms by which ligands affect a regulation of gene activity is essential. Specifying the position of ligand-binding pockets further has the potential to aid in identifying the ligands for MarR homologs for which the ligand remains unknown.

Keywords: MarR, ligand binding, transcription factor, DNA binding

Introduction

Bacteria and archaea employ a number of efficient mechanisms to affect differential gene expression in response to internal and external stimuli. First characterized in *Escherichia coli*, multiple antibiotic resistance regulator (MarR) family proteins constitute a diverse group of transcriptional regulators that modulate the expression of genes encoding proteins involved in a wide variety of cellular processes including metabolic pathways, stress responses, virulence and degradation or export of harmful chemicals such as phenolic compounds, antibiotics and common household detergents (Cohen et al., 1993; Ariza et al., 1994; Martin and Rosner, 1995; Sulavik et al., 1995; Alekshun and Levy, 1999a). MarR homologs are identified and annotated in genome databases by sequence homology to known MarR proteins (Pfam: PF01047; Finn et al., 2010). There are more than 12000 MarR-like proteins annotated in bacterial and archaeal genomes to date, amongst which around 100 have been studied in terms of their physiological role.

MarR homologs constitute a subgroup of winged helix-turn-helix (wHTH) DNA-binding proteins; they exist as dimers and bind palindromic sequences within target promoters, resulting in either transcriptional repression or activation (and sometimes both). The genomic loci in which the MarR homolog is encoded generally include divergent genes with the MarR homolog functioning as a regulator of both. In addition, distant genes may be regulated by a given homolog. Another defining feature of MarR homologs is that they respond to specific ligands; in absence of ligand, specific DNA binding occurs, most often resulting in repression of gene activity. On binding of the ligand, DNA binding is attenuated, resulting in de-repression of transcription (Wilkinson and Grove, 2006). What has hampered progress in understanding the molecular mechanisms by which ligand-binding causes attenuated DNA binding is that the natural ligand often is unknown.

We are summarizing here recent data that point to general mechanisms by which ligand binding induces a conformation that is incompatible with DNA binding. This analysis is rendered both timely and incisive by the recent publication of several structures and biochemical analyses of MarR homologs in their apo forms and in complex with various ligands. Common features of ligand-binding pockets are emerging, furnishing long-sought clues to the mechanisms of ligand-mediated gene regulation of

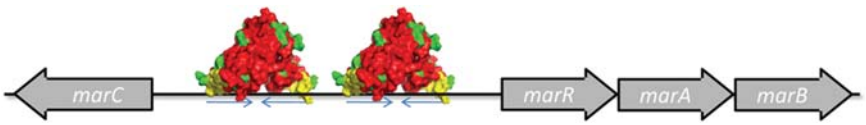

Figure 1 Multiple antibiotic resistance (*mar*) locus from *E. coli*. MarR binds two palindromic sequences in the intergenic region between *marRAB* and *marC* repressing their expression. MarR is shown in space-filled rendering and colored according to secondary structure elements; helix in red, β-sheet in yellow and loop regions in green.

MarR homologs and promising to aid in the identification of ligands for MarR homologs for which ligands still remain elusive.

Transcriptional regulation by MarR homologs

The signal to which MarR homologs respond communicates a change in the environment, for example, in the form of availability of substrates for a specific metabolic pathway or noxious compounds that the cell needs to extrude. Many MarR homologs are encoded adjacent to divergently transcribed genes they regulate, with their binding site(s) residing in the intergenic region spanning those genes. This was first demonstrated for *E. coli* MarR, which binds to the intergenic region between *marC* and *marRAB* at two palindromic sequences, obstructing the −10 and −35 promoter elements (Figure 1; Martin and Rosner, 1995). This mode of binding would hinder the recruitment of RNA polymerase, hence repressing the transcription of both *marC* and *marRAB* genes (Martin et al., 1995). Additional mechanisms of transcriptional repression have been proposed for MarR homologs, including impeding promoter escape or transcriptional elongation by RNA polymerase and competing with a transcriptional activator for promoter binding (Cohen et al., 1993; Stapleton et al., 2002; Galán et al., 2003). Other homologs bind upstream sites, where they may act as an activator by stabilizing the RNA polymerase or by competing for DNA binding by a repressor (Kovacikova et al., 2004; McCallum et al., 2004; Oh et al., 2007; Di Fiore et al., 2009). Thus, the choice of being an activator or a repressor depends on the position of binding relative to the promoter sequence. For example, *Streptomyces coelicolor* OhrR acts as a repressor as well as an activator by binding to the same operator region between *ohrR* and *ohrA* (Oh et al., 2007). The reduced form of scOhrR binds co-operatively to the intergenic operator sequence covering the −10 and −35 promoter elements hindering the recruitment of RNA polymerase. Upon oxidation, scOhrR binds loosely towards *ohrA* (−35) promoting RNA polymerase recruitment to the divergent *ohrR* gene, thus acting as an activator of *ohrR* transcription.

Regulation of catabolic pathways

The metabolic pathways regulated by MarR homologs generally involve catabolism of aromatic compounds. The ligands for these MarR homologs are typically substrates of the regulated metabolic enzymes. For example, 3-chlorobenzoate is a pollutant that is metabolized by *Comamonas testosteroni*. The MarR homolog CbaR represses the *cbaABC* operon, which encodes enzymes involved in the oxidation of 3-chlorobenzoate (Providenti and Wyndham, 2001). Ligand specificity is exemplified by the observation that transcriptional repression is relieved when CbaR binds 3-chlorobenzoate, but DNA binding is enhanced on binding benzoate analogs with a hydroxyl or carboxyl group at the 3-position. *Comamonas testosteroni* also encodes MobR, which functions as a repressor of the *mobA* gene that encodes a 3-hydroxybenzoate 4-hydroxylase. The ligand for MobR is 3-hydroxybenzoate, whose binding attenuates DNA binding and results in efficient induction of *mobA* expression (Yoshida et al., 2007).

The beta-ketoadipate pathway is involved in catabolism of the lignin-derived aromatic compounds protocatechuate and catechol. The enzymes of the protocatechuate branch of this pathway are encoded by the *pca* genes. Transcription of the structural genes in *S. coelicolor* is repressed by a MarR homolog encoded by a divergently oriented gene, and it is induced by protocatechuate and *p*-hydroxybenzoate (Davis and Sello, 2010). Thus, MarR homologs are efficient at detecting the presence of aromatic compounds, which represent a significant nutrient source for soil-dwelling organisms, and induce the expression of enzymes involved in their degradation.

Control of virulence factor expression

The regulatory pathways that govern bacterial virulence are complex, in part as multiple regulators may act on an individual gene. Secondly, gene expression is temporally regulated, with cell wall proteins and surface adhesins expressed during the early, colonizing stages of infection, whereas toxins and secreted proteins are expressed during the subsequent tissue-damaging phase of disease. A number of MarR homologs have been shown to participate in these events, but the signals to which they respond have in many cases proved difficult to ascertain. Examples include *Dickeya dadantii* (*Erwinia chrysanthemi*) PecS and SlyA, both essential for virulence in this phytopathogenic enterobacterium that infects numerous economically important plant species. PecS is involved in global regulation of virulence during host colonization. It was initially described as a regulator of pectinase genes (hence the name) and it has been shown to regulate expression of indigoidine biosynthetic genes and genes involved in flagellum development, virulence factors required for infection (Reverchon et al., 1994; Praillet et al., 1997; Rouanet et al., 1999, 2004; Reverchon et al., 2002; Nasser et al., 2005; Hommais et al., 2008). The ligand for PecS is unknown. More recently, SlyA has also been shown to

contribute to the regulation of pectinase (Haque et al., 2009). SlyA has been primarily characterized in *Salmonella*, where it was shown to regulate, for example, hemolysin and flagella production, both of which are critical for *S. typhimurium* infection (Buchmeier et al., 1997; Stapleton et al., 2002; Wyborn et al., 2004).

An example of a MarR homolog that uses peroxide stress to induce virulence gene expression is SarZ from *Staphylococcus aureus* (Ballal et al., 2009; Chen et al., 2009; Poor et al., 2009). SarZ belongs to the single-cysteine class of OhrR–MgrA proteins that play key roles in oxidative stress resistance and regulation of virulence genes in various bacteria and whose DNA binding is modulated by cysteine oxidation. In contrast, OhrR from *Xanthomonas campestris* pv. phaseoli requires formation of a disulfide bridge between two cysteines for inactivation of its repressor function (Panmanee et al., 2006).

Not only do MarR homologs respond to ligands by altered DNA binding and changes in gene activity, some have been proposed to activate gene expression by competing with the global repressor H-NS. This mode of activity has, for example, been proposed for SlyA in its activation of the hemolysin gene in *E. coli* (Westermark et al., 2000). RovA is a SlyA homolog that was first identified as a positive regulator of the gene coding for invasin in response to temperature and growth phase in *Yersinia* (Cathelyn et al., 2007). Both the *inv* gene that encodes invasin and other genes under RovA control are subject to repression by H-NS, leading to the inference that a principal function of RovA in *Yersinia enterocolitica* is to act as an antagonist of H-NS-mediated transcriptional silencing (Cathelyn et al., 2007).

Response to environmental stress

A number of MarR homologs are involved in multiple antibiotic resistance phenotypes and their control of multidrug efflux pumps have been studied in several species (George and Levy, 1983; Poole et al., 1993; Martin et al., 1995; Seoane and Levy, 1995; Sulavik et al., 1997; Kaatz et al., 2006). *Escherichia coli* MarR was discovered based on genetic selections to identify mutants that conferred resistance to a range of antibiotics (George and Levy, 1983; Cohen et al., 1993). MarR regulates the *marRAB* operon encoding MarA, which is a transcriptional activator of a number of genes responsible for the *mar* phenotype, including a multidrug efflux pump. MarR responds to a range of anionic lipophilic compounds such as 2,4-dinitrophenol, menadione and salicylate (Cohen et al., 1993; Seoane and Levy, 1995). The affinity of MarR for its ligands is low; e.g. salicylate binds MarR with an apparent K_d of 0.5 mM (Martin and Rosner, 1995; Alekshun and Levy, 1999a).

Other MarR homologs have been identified that directly regulate a multidrug efflux system. Examples include the *emrRAB* operon in *E. coli*, which is regulated by EmrR. EmrB pumps drugs across the cytoplasmic membrane whereas EmrA is thought to facilitate their passage through the periplasm. Binding of EmrR is inhibited by structurally unrelated antibiotics, including the MarR ligands salicylate and 2,4-dinitrophenol (Lomovskaya et al., 1995; Xiong et al., 2000). MepR from *S. aureus* represses expression of mepA, which encodes a multidrug efflux pump; MepR is induced by multiple cationic toxins, which are also substrates of MepA (Kaatz et al., 2006; Kumaraswami et al., 2009).

MexR from *Pseudomonas aeruginosa* has been extensively characterized. It is encoded adjacent to the oppositely oriented *mexAB-oprM* operon that encodes a multisubstrate efflux pump that contributes to this organism's intrinsic multidrug resistance; MexR represses its own transcription and that of the *mexAB-oprM* operon (Poole et al., 1993; Srikumar et al., 2000). The signals to which MexR respond have long eluded detection; however, a recent report shows that cysteine oxidation confers a conformational change that is consistent with attenuated DNA binding (Chen et al., 2008).

Several MarR homologs mediate responses to oxidative stress. The gene encoding OhrR from *X. campestris* pv. phaseoli is co-transcribed with the adjacent gene, *ohr*, which is involved in protection against organic peroxides. Transcription is repressed by OhrR, a repression that is relieved by organic peroxides (Mongkolsuk et al., 1998; Sukchawalit et al., 2001). In *Bacillus subtilis*, the *OhrR* and *ohr* genes are divergently encoded; *B. subtilis* OhrR represses transcription of *ohr* and although it is responsive to organic peroxides, its expression does not appear to be autoregulated (Fuangthong et al., 2001). OspR from *P. aeruginosa* likewise senses oxidative stress and induces both antioxidant production and antibiotic resistance as a result (Lan et al., 2010).

A unique homolog has been described in *Deinococcus radiodurans*, which is particularly resistant to agents with the potential to damage cellular components, including that induced by oxidative stress. HucR represses its own expression as well as that of a divergently encoded uricase, a repression that is relieved on binding the ligand urate, the substrate for uricase. As a consequence, cellular levels of urate are intricately regulated, ensuring that optimal concentrations of this potent antioxidant are maintained (Wilkinson and Grove, 2004, 2005; Perera et al., 2009). This genomic locus is unique to *D. radiodurans*, suggesting that control of urate levels contributes to the characteristic oxidative stress resistance in this species.

Structure of MarR family transcriptional regulators

MarR homologs are homodimeric proteins, which generally assume a triangular shape with pseudo-2-fold symmetry (Figure 2). Structures generally comprise six α-helices and three β-strands assuming an α1–α2–β1–α3–α4–β2–β3–α5–α6 topology (Figure 2). An additional N-terminal helix is present in the structure of *D. radiodurans* HucR (Bordelon et al., 2006). Homologs annotated in databases as PecS contain N-terminal extensions, predicting that they also feature this additional secondary structure element. The N- and C-terminal helices interdigitate to create a compact inter-subunit dimerization interface consisting of mainly hydrophobic interactions and intermolecular hydrogen bonds (Alekshun et al., 2001; Hong et al., 2005; Bordelon et al., 2006; Newberry et al., 2007; Saridakis et al.,

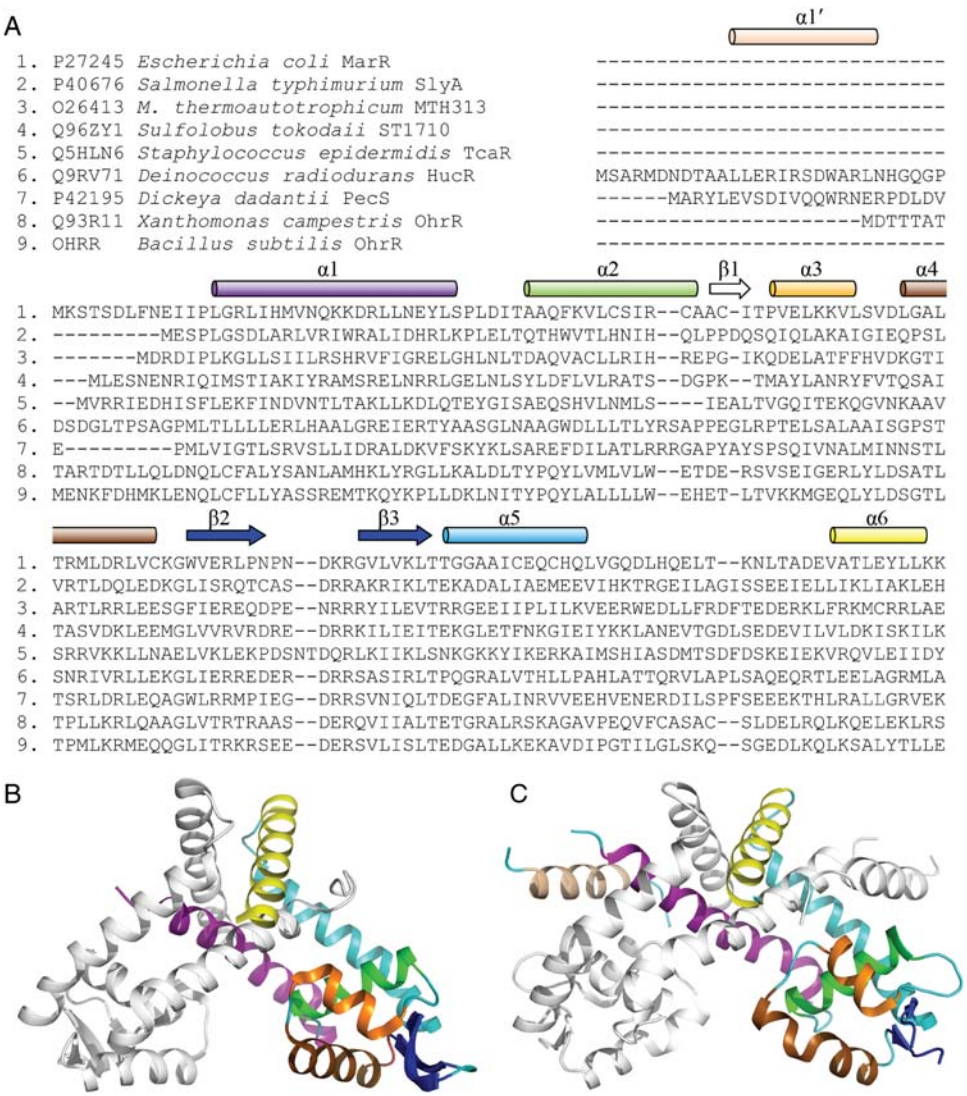

Figure 2 Sequence alignment and general architecture of MarR homologs. (**A**) Sequence alignment of selected MarR homologs. (**B**) Crystal structure of *M. thermoautotrophicum* MTH313 depicts the general MarR scaffold. Secondary structure elements are color coded with reference to the crystal structure of *E. coli* MarR. (**C**) Crystal structure of *D. radiodurans* HucR. The additional N-terminal helix α1′ is only present in HucR.

2008). The dimerization domain dictates the distance between the DNA recognition helices, thus indirectly affecting the affinity of the protein binding for its cognate DNA. Indeed, mutations in the dimer interface have been shown to cause reduced DNA-binding affinity (Andrésen et al., 2010), and C-terminal deletions in MarR homologs have been shown to decrease their ability to form dimers, which correlates with attenuated DNA-binding affinity and elevated multiple antibiotic resistance phenotypes in *E. coli* (Linde et al., 2000).

Crystal structures reveal that each monomer contains a wHTH motif, the DNA-binding domain that is characteristic of MarR homologs (Alekshun et al., 2001). wHTH is a variant of the common helix-turn-helix DNA-binding domain, most of which function as transcriptional regulators (Gajiwala and Burley, 2000). The core of the domain consists of three α-helices (α1, α2 and α3) with α2 and α3 constituting the helix-turn-helix portion. The wing consists of two antiparallel beta sheets (β2 and β3), which is stabilized in part by a short beta strand (β1), creating a three-stranded beta sheet. Although the wing of the DNA-binding domain is not refined well in most crystal structures due to its flexibility, it is generally well resolved when the protein is co-crystallized with the cognate DNA or when it aids in crystal packing. The DNA-binding domains, which are generally well separated and make few direct contacts, are connected to the dimerization domain by helices 1 and 5 (Figure 2).

DNA binding by MarR homologs

The recognition helix of the wHTH domain binds the DNA major groove while the wing contacts the adjacent minor groove (Figure 3A and B). This binding mode generally does not induce major conformational distortions in the DNA. The wing is vital for DNA interaction as evidenced by several mutational studies, which show that the terminally positioned positively charged residues are important for association with cognate DNA. Mutation of Arg94 at the tip of the wing to Cys abolishes the repressor

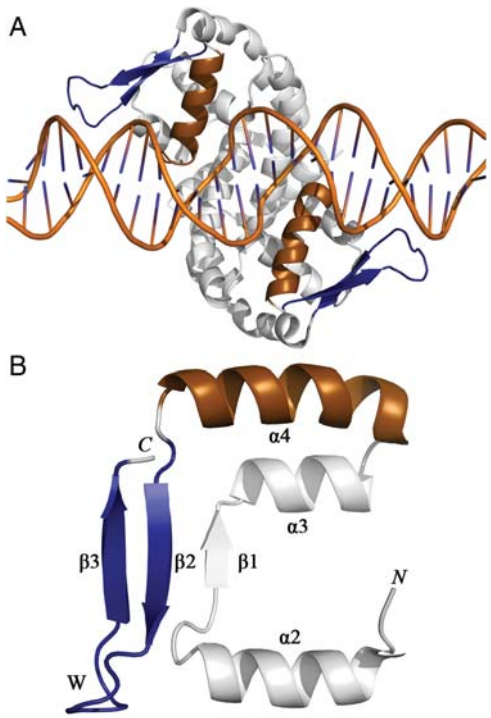

Figure 3 MarR–DNA interactions. (**A**) MarR (1Z9C) interacting with cognate DNA (ventral view). The recognition helix (bronze) interacts with consecutive major grooves while the wing (W: blue) interacts with the adjacent minor groove. (**B**) Topology of the wHTH domain of MarR family transcriptional regulators. Color coding as for (**A**); *N* and *C* denote the amino and carboxyl termini of the domain.

function of *E. coli* MarR, whereas mutation of positively charged amino acids in the wing abolishes DNA binding of ST1710 (Alekshun et al., 2000; Kumarevel et al., 2009). Conversely, manipulation of the wing can also increase DNA-binding affinity, as exemplified by the *E. coli* MarR G95S super repressor whose affinity is increased up to 30-fold *in vitro* (Alekshun and Levy, 1999b). Whereas mutation of conserved arginines in the wing may lower DNA-binding affinity, specificity is not always affected (Wilkinson and Grove, 2005).

MarR proteins associate with 16–20 bp inverted repeats which may or may not be completely palindromic (e.g. Martin and Rosner, 1995; Martin et al., 1996; Evans et al., 2001; Wilkinson and Grove, 2004). These sequences often overlap promoter elements, resulting in transcriptional repression. MarR homologs may bind as a single dimer to an operator or they may associate as multiple dimers at adjacent sequences. *D. radiodurans* HucR exemplifies single dimer binding to the operator sequence (Wilkinson and Grove, 2004). In contrast, DNase I footprinting of *Sulfolobus solfataricus* BldR bound to the operator sequence of gene *Sso2536* shows protection of ~40 bp, suggesting that two protomers associate with this site (Fiorentino et al., 2007), and footprinting of *D. dadantii* PecS binding to different operator sequences within its regulon shows varying spans of protection from 20 to 100 bp, suggesting that PecS may associate as single or multiple dimers at different operators (Hommais et al., 2008). Although they are sequence-specific in their binding, some MarR homologs are capable of associating with highly degenerate sequences (Rouanet et al., 2004).

The spacing between the two halves of the palindrome places the centers of each half-site at two consecutive major grooves, thus the spacing between the two DNA-binding domains, determined by the dimer interface, is critical for binding to cognate DNA. If the two DNA recognition helices are separated by ~34 Å, the protein may associate with cognate *B*-DNA without major conformational rearrangements, an energetically favorable situation. For instance, HucR, having 29 Å between the DNA recognition helices, can be easily modeled *in silico* onto *B*-DNA, perhaps explaining its high affinity (Wilkinson and Grove, 2004; Bordelon et al., 2006). In contrast, MexR is incompatible with contacts to consecutive major grooves in its closed conformation that has 23 Å spacing between recognition helices, whereas recognition helices are separated by 29.2 Å in the open conformation (Lim et al., 2002).

The two co-crystal structures of cognate DNA-bound *B. subtilis* OhrR and ST1710 from the archaeon *Sulfolobus tokodaii* provide insight to how MarR homologs associate with DNA (Hong et al., 2005; Kumarevel et al., 2009). OhrR (with a mutation of C15S to prevent oxidation) was co-crystallized with 29 bp of its operator DNA, which encompasses the −10 region of its promoter. The association of OhrR results in an ~10° DNA bend and a 3.5 Å shortening of the binding site, but it still conforms to *B*-form. Further, the insertion of the binding helix widens the major groove by 5.5 Å. Although the DNA recognition residues between OhrR and ST1710 are conserved, ST1710 binds without causing major conformational changes in its cognate DNA. However, a substantial unequal conformational change between the two subunits occurs in the protein in order to facilitate DNA binding, demonstrating the conformational flexibility of MarR homologs. One of the subunits of ST1710 translocates 13 Å in order to reorient itself on DNA, although the DNA recognition helix is elevated in one subunit and helix 6 is lowered in the other.

In the OhrR–*ohrA* complex, the wing plays a major role in contacting cognate DNA. It is longer than in most prokaryotic wHTH domains and resembles more closely the wing of eukaryotic proteins (Ramakrishnan et al., 1993; Gajiwala et al., 2000). The wing spans over 67 Å with the tip inserted in the minor groove, creating a local overtwisting of ~7°. The wing and recognition helices contact DNA directly or through water-mediated hydrogen bonding. The recognition helices bind to two consecutive major grooves with relatively few contacts, but the N-termini of the helices point towards the base of the groove to make specific contacts. Besides the main DNA contacts to the recognition helix and the wing, the positive dipole of helix 3 and the helix–helix motif consisting of helices α1 and α2 also contribute to DNA binding by contacting the phosphate backbone.

Simultaneous association of multiple MarR homologs with cognate DNA may also be mediated by the wing. Cooperative DNA binding by MarR homologs may occur by lateral association of adjacent proteins *via* their DNA-binding domains, since the topology of the protein may not favor contacts between other parts without major distortions to the bound DNA. When binding sites are separated by ~10 bp the proteins may bind DNA in tandem order on the same face of DNA (Martin and Rosner,

1995). Mutations in the C-terminal helix 5, extending from the wing motif, also affect the DNA-binding affinity of MarR; the mutations were located facing helix 1 of the wHTH domain, which may cause a global conformational change in the DNA-binding domain (Alekshun et al., 2000). This further suggests that subtle local conformational changes can modulate DNA-binding affinity of a MarR homolog.

Structural basis for ligand-mediated attenuation of DNA binding

Ligands for MarR homologs are of two varieties. Many homologs bind small phenolic compounds, resulting in a conformational change that renders the protein incapable of binding DNA. Homologs that regulate metabolic pathways fall in this category, as do many proteins that regulate efflux pumps, as they often share substrate preferences with the enzymes or efflux pumps they regulate. The other variety of ligand interacts transiently with its target to effect a covalent modification, specifically cysteine oxidation; this covalent modification likewise causes attenuated DNA binding due to induced conformational changes, and this mechanism serves an important function in oxidative stress responses, including the production of virulence factors.

Binding of phenolic ligands

The founding member of this protein family, *E. coli* MarR has been studied extensively in terms of its response to various small molecules. Early studies demonstrated that MarR binds salicylate and that a multiple antibiotic resistance-phenotype is induced by salicylate (Martin and Rosner, 1995; Alekshun and Levy, 1999a). Inspired by this observation, subsequent work has focused on the effect of salicylate on other MarR homologs, although a direct physiological relevance may not be readily apparent. The structure of *E. coli* MarR was solved with the proposed aromatic ligand salicylate (PDB ID: 1JGS) (Alekshun et al., 2001). Two binding sites for salicylate were identified in each subunit, each of which were relatively surface exposed and flanking the DNA recognition helix. Ligand-binding at the more buried site, designated SAL-A, includes contacts to T72 of the recognition helix and R86 from the wing, residues that are highly conserved in MarR homologs. The second site, SAL-B, is surface exposed, and bound ligand is contacted by residues from the recognition helix. Although the identified sites were speculated in subsequent literature to be an artifact of using high concentrations of salicylate that facilitated crystal packing, this work led to a number of subsequent co-crystal structures of MarR homologs with salicylate; *Methanobacterium thermoautotrophicum* MTH313, *S. typhimurium* SlyA, *S. tokodaii* ST1710 (PDB IDs: 3BPX, 3DEU and 3GF2, respectively) and *Staphylococcus epidermidis* TcaR (Saridakis et al., 2008; Kumarevel et al., 2009; Chang et al., 2010).

Salicylate associates with MarR homologs and attenuates DNA binding only at high concentrations, questioning its biological relevance as a ligand. Nonetheless, shared features of available co-crystal structures point to the existence of a conserved ligand-binding pocket. The first direct evidence for ligand-mediated conformational changes was furnished by the structures of *M. thermoautotrophicum* MTH313 in its apo form and with the ligand salicylate (Saridakis et al., 2008). MTH313 crystallized with one salicylate molecule per monomer; one salicylate binding pocket (SAL1) was identified between the dimer interface and the DNA-binding domain (Figure 4). This pocket is composed of largely hydrophobic residues, and salicylate forms ionic interactions to K8 and R16 from helix 1, neither of which is conserved in all MarR homologs, suggesting that MTH313 may associate with diverse ligands. The binding site in the other lobe (SAL2) is asymmetrically disposed and consists of a shallow binding site 5 Å away from the symmetrical site; in this site, salicylate was not sufficiently resolved to identify direct contacts. Overlay of apo and salicylate-bound structures suggests that only the deeper binding site (SAL1) is biologically relevant, as its occupancy imparts conformational changes in which the DNA recognition helix is pushed away from its position in the apo structure and rotated 5 Å. However, as previously noted (Wilke et al., 2008), salicylate-bound MTH313 may be superimposed on the DNA-bound OhrR with 2.3 Å rmsd for Cα residues. This is consistent with the significant residual DNA binding seen on addition of salicylate to MTH313 (Saridakis et al., 2008), and it suggests that salicylate may not be the natural ligand for MTH313. That the conformation of apo MTH313 is incompatible with DNA binding further predicts that significant conformational changes will accompany DNA binding.

The structure of the archaeal homolog ST1710 was solved in its apo form and bound to either DNA or salicylate. ST1710 also has low-affinity for salicylate; the ST1710-salicylate dissociation constant is ~20 mM, but attenuation of DNA binding was only observed at 200–250 mM salicylate concentrations (Kumarevel et al., 2009). The ligand-bound structure is only modestly different from the apo structure, with distances between the recognition helices of ~30 Å and only minor conformational changes in the wing. Bound salicylate is located at the interface between the dimerization domain and the wHTH domain with ionic contacts to K17 and R20 of helix 1, comparable interactions to those seen in the MTH313 binding pocket SAL1. In contrast to the MTH313 structure, an asymmetrical binding pocket in the other protein lobe was not seen. These data suggest either that salicylate is not the natural ligand for ST1710 or that binding of salicylate stabilizes the protein in a closed-conformation, restricting its association with DNA; the latter inference is supported by the significant conformational rearrangement associated with DNA binding, while the former is corroborated by the high salicylate concentrations required to disrupt DNA-complex formation (Kumarevel et al., 2009).

Coordinates representing the structure of SlyA in complex with three salicylate molecules per monomer have been deposited. Notably, one of the identified sites approximately corresponds to SAL1 from MTH313 and to the site seen in the structure of salicylate-bound ST1710. The second site is also between the dimerization and DNA-binding domains, but closer to the DNA recognition helix compared with MTH313 site SAL2. The third site is

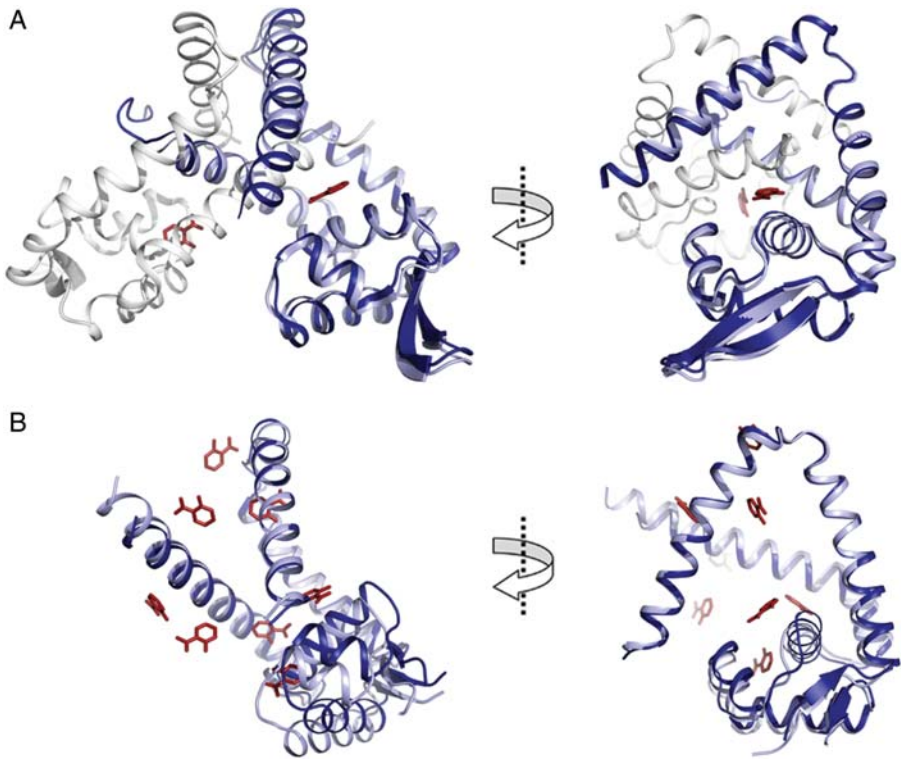

Figure 4 Conformational changes induced on ligand binding to MarR homologs. Superimposed Apo (light blue) and ligand-bound (dark blue) structures of (**A**) MTH313-salicylate (**B**) TcaR-salicylate.

on the solvent-exposed face of helix 5. No apo structure is available, precluding a direct comparison. However, it is notable that occupancy of a deep binding pocket between the dimerization and DNA-binding domains is a recurring feature among available structures of salicylate-bound proteins. It must also be considered, however, that the deprotonated salicylate may interact with MarR homologs non-specifically to equalize charges. This may be the reason behind association of salicylate with highly solvent exposed areas and the facilitation of crystal packing observed for *E. coli* MarR and presumably SlyA. Carrying an aromatic ring would allow salicylate to be drawn into the mostly hydrophobic common binding pocket in MTH313, ST1710 and SlyA.

Initially described as a regulator of the teicoplanin-associated locus, TcaR was subsequently found to regulate a number of distal genes, including the *ica* operon that encodes membrane proteins involved in biofilm formation (Jefferson et al., 2004; McCallum et al., 2004). *Staphylococcus epidermidis*-encoded TcaR was crystallized in its native form and complexed with salicylate and with four antibiotics (Chang et al., 2010). Crystals of ligand-bound protein were obtained by soaking the native crystals in the mother liquor containing the appropriate ligand. This is in contrast to attempts at crystal soaking to obtain the cocrystal structure of HucR–urate, which failed due to cracking of crystals, suggesting a significant conformational change (Bordelon et al., 2006).

Eight salicylate molecules bound to TcaR (Chang et al., 2010). Of these, two salicylate molecules were found in the deep binding pocket corresponding to that described for MTH313 (SAL1), while two were found in the more shallow binding pocket in each monomer; the remainder were solvent exposed and two were asymmetrically disposed in the two subunits. In contrast to the MTH313-salicylate structure, salicylate was found to occupy all four symmetry related sites in TcaR. It would be interesting to assess the cooperativity of salicylate binding to TcaR in such a mode of occupancy, and a systematic mutational analysis of MarR homologs that associate with multiple ligands would be instructive to assess the biological relevance of these sites. Unlike salicylate, the antibiotics interact with the protein at only two sites each. One site is near the DNA recognition helix, and it is highly solvent-exposed. The other site overlaps the shallow binding site. Notably, the drugs bind at only a single symmetry-related binding pocket of the two, which may be due to negative cooperativity of binding. Compared with the apo structure, salicylate- and antibiotic-bound structures show asymmetrical conformational alterations, primarily in the wHTH domain. The distances between the DNA recognition helices have not changed significantly compared with the apo structure. Measuring the distance between Cα of Lys65 to Cα of Lys65′ of the other monomer of the apo structure reveals 24.7 Å distance between the two binding helices, which suggests that TcaR is not preconfigured for DNA binding. Compared with that, TcaR-ampicillin, TcaR-kanamycin, TcaR-methicillin and TcaR-penicillin G have 22.5, 22.0, 21.1 and 26.4 Å distances between the above mentioned Lys residues, respectively. In the energy minimized model of the TcaR-DNA structure, the authors report that the distance between the two Lys residues is 31.2 Å, suggesting a conformational alteration required for binding

DNA. This conformational change may be precluded by ligand-binding, making drug-bound TcaR incompatible with DNA binding.

Taken together, results of structural analyses suggest that a common ligand-binding pocket is found in the crevice between the dimerization domain and the DNA-binding lobe. In the case of MTH313, occupancy of this site results in a conformational change that produces a structure that cannot be docked onto DNA. The data also suggest, however, that ligand-binding may in some cases lock the protein in an apo configuration that is incompatible with DNA binding and that it is instead the interaction with DNA that requires conformational changes, as seen for ST1710 and TcaR. A mechanism by which ligand-binding may induce a conformational change that propagates to the DNA recognition helix was proposed for binding of HucR to its ligand urate (Perera et al., 2009). A blind docking approach was used to predict the ligand-binding site of HucR and mutational analysis used to validate the prediction; urate was found to bind a Trp from the additional N-terminal helix unique to HucR (designated α' in Figure 2A) and Arg from $\alpha 2$. This binding site overlaps the SAL1 binding site of MTH313. The mechanism by which urate-binding is communicated to the recognition helix is *via* a salt bridge between Arg from the recognition helix and Asp in $\alpha 2$; on binding of N3-deprotonated urate, a charge repulsion occurs that is propagated to the recognition helix. On the basis of this model, ligand binding in the deep binding pocket may lock the DNA-binding lobe in place by acting as 'molecular glue' between the dimerization domain and $\alpha 2$ of the wHTH domain, preventing conformational changes required for DNA binding. For proteins such as HucR that appear to be preconfigured for DNA binding, ligand-binding may induce a conformational change that is communicated to the recognition helix through its interaction with $\alpha 2$.

Salicylate binding to MTH313 and to *E. coli* MarR, urate binding to HucR and several other ligand interactions with MarR homologs show negative cooperativity (Wilkinson and Grove, 2005; Saridakis et al., 2008). A single physiologically relevant binding site being filled in MTH313 was attributed to negative cooperativity between the sites on the two subunits with filling of one site preventing the filling of the other. Negative cooperativity in HucR–urate interaction was also attributed to a communication between equivalent binding sites in the two monomers (Wilkinson and Grove, 2005). Binding of different ligands to EmrR was investigated by equilibrium dialysis, which revealed a relatively high affinity for its ligands (2–50 μM) and a stoichiometry of binding of 1:1 (Brooun et al., 1999). This finding was inferred to result from occupancy of only a single site due to negative cooperativity. Negative cooperativity in ligand binding may allow the MarR homolog to be responsive to a wider range of ligand concentration.

Antirepressor-modulation of MexR activity

Efficient efflux mechanisms render the opportunistic human pathogen *P. aeruginosa* resistant to a wide variety of antibiotics (Poole et al., 1993). Although there are a large number of membrane bound efflux pumps found in *P. aeruginosa*, MexAB-OprM operon may be the best characterized (Poole and Srikumar, 2001). Mutations in MexR lead to the overexpression of the tripartite efflux pump system causing the multi drug resistant phenotype (Saito et al., 1999; Srikumar et al., 2000). Along with MexR, which binds to two palindromic sequences in the intergenic region, the expression of *mexAB-oprM* is synergistically regulated by the TetR family proteins NalC and NalD, which regulate the production of ArmR (Evans et al., 2001). The protein modulator ArmR has been identified as an antirepressor of MexR, which lowers the affinity of MexR for its cognate DNA (Cao et al., 2004). A number of small molecule effectors have also been identified as derepressors of the *mexAB-oprM* operon.

The MexR crystal structure reveals considerable flexibility (Lim et al., 2002). Although the DNA-binding domains do not show much flexibility, the dimer interface is highly malleable. This flexibility allows the two binding domains to be displaced by a up to 7.7 Å between the midpoints of the DNA recognition helices. This may also be partly caused by the complete absence of interactions between the two DNA-binding domains. Of the four conformational states observed in unbound MexR, the conformation with 29.2 Å spacing between the two recognition helices (between Arg73 of each monomer) would likely bind DNA with least energetic cost (Lim et al., 2002). The mechanism by which ArmR derepresses MexR-regulated gene expression was revealed from the co-crystal structure of MexR with a fragment of ArmR. The structure shows that the C-terminus of ArmR is inserted through a cleft in one MexR monomer and extends across the dimer interface, reaching the other monomer and occupying both ligand binding pockets (Wilke et al., 2008). Association of ArmR causes a global conformational change in MexR which is pronounced in the DNA binding lobes where the recognition helices and wings are displaced by 13 and 18 Å, respectively, making the ArmR-bound MexR incompatible with DNA binding.

Despite intensive studies, the mechanisms by which genes under MexR control are induced have been elusive. However, a recent report shows that two Cys residues in MexR are redox active and form an inter-monomer disulfide bond (Chen et al., 2008). On mild oxidation, MexR is displaced from its cognate DNA, and mutational analysis established that Cys30 from one monomer and Cys62 from the other participate in a disulfide bond that may decrease the spacing between recognition helices, thus resulting in reduced DNA-binding affinity.

MarR homologs as sensors of oxidative stress

Direct interaction of oxidative agents with MarR homologs constitutes a response to oxidative stress that is independent of the global sensor OxyR (Sukchawalit et al., 2001). Organic peroxide sensing repressor (OhrR) is a repressor of the gene *ohrA*, which encodes a thiol peroxidase that detoxifies organic hydroperoxides (OHPs; Mongkolsuk et al., 1998; Atichartpongkul et al., 2001; Fuangthong et al., 2001; Ochsner et al., 2001; Klomsiri et al., 2005). Oxidation-sensitive Cys residues in the C/N-terminal

helices have been implicated in the mechanism of conformational change in order to attenuate DNA binding in OhrR as well as in MgrA and SarZ (Newberry et al., 2007; Soonsanga et al., 2007; Chen et al., 2009; Poor et al., 2009). *Bacillus subtilis* OhrR (BsOhrR) contains a single reactive Cys residue per monomer whereas *X. campestris* OhrR (XcOhrR) contains two reactive Cys residues per monomer (Panmanee et al., 2006; Lee et al., 2007). OhrR gets oxidized only by OHPs, but not by hydrogen peroxide (Zheng et al., 1998; Fuangthong et al., 2001). The crystal structure of reduced XcOhrR shows a solvent exposed, shallow hydrophobic-channel leading to the reactive Cys residues, which may confer its selectivity towards OHPs (Newberry et al., 2007). The OhrR structure further reveals a hydrophobic OHP-landing patch conserved among its homologs. The conserved hydrophobic residues may aid long-chain non-polar hydroperoxides to properly orient towards the reactive Cys residues in the binding site. Although MgrA shows conservation of these residues, it reacts less selectively with both hydrogen peroxide and OHPs (Luong et al., 2003; Chen et al., 2006). This was attributed to the differences in the residue interactions surrounding the reactive Cys, such as having Ser-Cys hydrogen bonding instead of the conserved Tyr-Cys hydrogen bonding in OhrR.

Although the reactive Cys residues are near the proposed general ligand-binding site of MarR homologs, they present a novel mechanism for attenuation of DNA binding upon oxidation. In OhrR, the reactive Cys residues are 15.5 Å apart and to form a disulfide bond between them requires major conformational rearrangement (Figure 5). Although all three Cys residues per monomer are within 15 Å distance, only Cys 22 and 127 can participate in the intersubunit disulfide bond (Panmanee et al., 2006). Upon oxidation, helix 5 is separated into two helices connected by a loop region. This rearrangement allows C127 to rotate 135° and move 8.2 Å towards the N-terminal α1 which harbors C22′ (where ′ denotes the other subunit). Such movement interchanges positions of α6 and α6′ without a major alteration of the dimer interface. Further, α1 extends by a single turn at the N- and C-termini causing the DNA-binding domain to rotate 28° away from the position in the reduced form, which can be directly related to the attenuated DNA binding in the oxidized OhrR.

In single-Cys proteins such as MgrA and SarZ and a subset of OhrR homologs, a single Cys is oxidized to a sulfenic acid followed by reaction with an external small-molecule thiol to generate a mixed disulfide bond. This Cys oxidation leads to an altered protein conformation, reduced DNA binding and activation of the corresponding regulon (Panmanee et al., 2006; Newberry et al., 2007). The structure of *S. aureus* SarZ shows that the sulfenic acid form of Cys13 is stabilized by hydrogen bonding to neighboring residues without significant conformational changes (relative distances between recognition helices of 32 and 30 Å for reduced and sulfenic acid-modified SarZ, respectively). However, on formation of a mixed disulfide, a conformational change is induced that results in a displacement of the recognition helix by 7 Å, a displacement that would lead to steric clashes with duplex DNA (Poor et al., 2009). Notably, the reactive Cys points directly towards the shared ligand-binding pocket (SAL1 in MTH313), suggesting conservation of effector sites in MarR homologs.

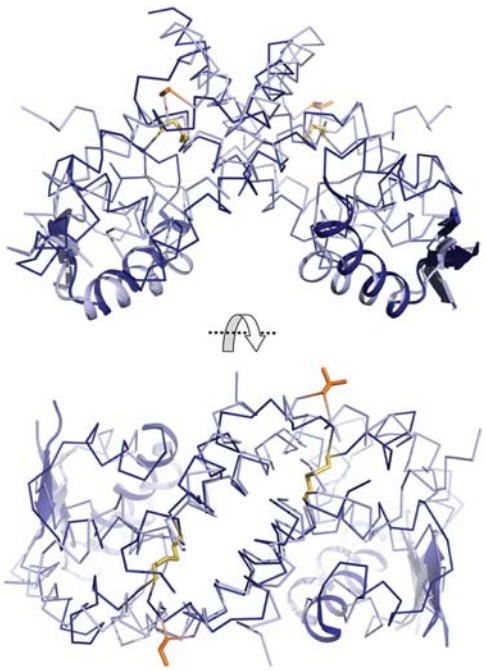

Figure 5 Conformational changes on oxidation of *X. campestris* OhrR. Reduced and oxidized xcOhrR are in ribbon representation, except the wing and recognition helices that are in cartoon representation. Disulfide bond formation between C22 and C127 (yellow stick) is prevented by a C22S mutation in reduced OhrR; C127 is in orange stick representation. Oxidation of reactive Cys residues (dark blue) creates a major conformational change in the DNA-binding domain compared with the reduced form (light blue). Structure was rotated 90° to show the top view of the intersubunit disulfide bond.

Iron sulfur clusters occur in a number of redox-sensitive transcriptional regulators (Kiley and Beinert, 2003). The recent characterization of PqrR from *P. aeruginosa* adds a MarR homolog to the list; PqrR contains an iron sulfur cluster formed by four C-terminal Cys residues and its proper formation is vital for the function of PqrR (Rungrassamee et al., 2009). These Cys residues are conserved in a number of MarR homologs suggesting that a subgroup of MarR-like proteins may contain a redox-sensitive iron sulfur cluster to sense oxidative stress.

Biochemical studies reinforced by structural information are thus finally converging to propose models for the mechanism by which external stimuli cause attenuated DNA binding. Crystal structures reveal a conformational plasticity in MarR homologs that is exploited on binding of either cognate DNA or ligand; the spacing between recognition helices is essential for association with two consecutive DNA major grooves, and ligand-binding appears either to alter a DNA-compatible conformation into one that cannot interact with cognate DNA or to prevent conformational changes required for DNA binding in the case of homologs that do not come preconfigured for DNA binding. What is particularly notable is that such conformational changes originate in the region between the DNA binding lobe and the dimerization interface, whether due to binding of phenolic ligands as exemplified by MTH313 binding pocket SAL1 or due to cysteine modification as seen in the structure of SarZ.

Concluding remarks

MarR family transcriptional regulators constitute a diverse group of proteins that regulate a number of important biological functions, rendering an understanding of the molecular mechanisms governing their response to external stimuli and their transcriptional regulation essential. Despite sequence variations, MarR homologs adopt a shared topology, as evidenced by the numerous structures now available. Although only a limited number of structures exist in which a MarR homolog is co-crystallized with either DNA or ligand, additional shared features are becoming apparent. First, the malleability of the dimer interface is a recurring feature that ensures that requisite conformational changes may be induced on binding of either DNA or ligand. Perhaps even more noteworthy is the emerging picture of a shared effector site, located in the deep crevice between the dimerization domain and the DNA-binding lobe. Although the specifics of the conformational changes induced on ligand binding vary between homologs, the common theme is to maintain a separation between recognition helices that is compatible with DNA binding. For some homologs, it appears that the apo structure is incompatible with contacts to two consecutive DNA major grooves and that significant conformational changes are required for such interaction; in those cases, binding of ligand may freeze the apo configuration, precluding DNA binding. In cases where the apo conformation is preconfigured for DNA binding, ligand-binding is the event that is associated with conformational changes, required to prevent DNA binding. In either case, the conformational changes appear to originate from the shared effector site, whether it is occupied by a phenolic ligand or a modified cysteine. The two-state model of MarR protein function discussed above is based on available structural information; it is possible that an ensemble model may more accurately describe the population of relevant conformational states, however, little experimental data are available on MarR homologs that can inform such models, suggesting a substantive avenue for further research. This issue notwithstanding, what is particularly intriguing about the existence of such shared effector sites is the potential for identifying ligands for homologs whose ligands still remain elusive. The advent of computational docking routines is likely to provide a powerful means for such predictions.

Conflict of interest: none declared.

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References

Alekshun, M.N., and Levy, S.B. (1999a). Alteration of the repressor activity of MarR, the negative regulator of the *Escherichia coli marRAB* locus, by multiple chemicals *in vitro*. J. Bacteriol. *181*, 4669–4672.

Alekshun, M.N., and Levy, S.B. (1999b). Characterization of MarR superrepressor mutants. J. Bacteriol. *181*, 3303–3306.

Alekshun, M.N., Kim, Y.S., and Levy, S.B. (2000). Mutational analysis of MarR, the negative regulator of *marRAB* expression in *Escherichia coli*, suggests the presence of two regions required for DNA binding. Mol. Microbiol. *35*, 1394–1404.

Alekshun, M.N., Levy, S.B., Mealy, T.R., Seaton, B.A., and Head, J.F. (2001). The crystal structure of MarR, a regulator of multiple antibiotic resistance, at 2.3 Å resolution. Nat. Struct. Biol. *8*, 710–714.

Andrésen, C., Jalal, S., Aili, D., Wang, Y., Islam, S., Jarl, A., Liedberg, B., Wretlind, B., Mårtensson, L.G., and Sunnerhagen, M. (2010). Critical biophysical properties in the *Pseudomonas aeruginosa* efflux gene regulator MexR are targeted by mutations conferring multidrug resistance. Protein Sci. *19*, 680–692.

Ariza, R.R., Cohen, S.P., Bachhawat, N., Levy, S.B., and Demple, B. (1994). Repressor mutations in the *marRAB* operon that activate oxidative stress genes and multiple antibiotic resistance in *Escherichia coli*. J. Bacteriol. *176*, 143–148.

Atichartpongkul, S., Loprasert, S., Vattanaviboon, P., Whangsuk, W., Helmann, J.D., and Mongkolsuk, S. (2001). Bacterial Ohr and OsmC paralogues define two protein families with distinct functions and patterns of expression. Microbiology *147*, 1775–1782.

Ballal, A., Ray, B., and Manna, A.C. (2009). sarZ, a sarA family gene, is transcriptionally activated by MgrA and is involved in the regulation of genes encoding exoproteins in *Staphylococcus aureus*. J. Bacteriol. *191*, 1656–1665.

Bordelon, T., Wilkinson, S.P., Grove, A., and Newcomer, M.E. (2006). The crystal structure of the transcriptional regulator HucR from *Deinococcus radiodurans* reveals a repressor preconfigured for DNA binding. J. Mol. Biol. *360*, 168–177.

Brooun, A., Tomashek, J.J., and Lewis, K. (1999). Purification and ligand binding of EmrR, a regulator of a multidrug transporter. J. Bacteriol. *181*, 5131–5133.

Buchmeier, N., Bossie, S., Chen, C.Y., Fang, F.C., Guiney, D.G., and Libby, S.J. (1997). SlyA, a transcriptional regulator of *Salmonella typhimurium*, is required for resistance to oxidative stress and is expressed in the intracellular environment of macrophages. Infect. Immun. *65*, 3725–3730.

Cao, L., Srikumar, R., and Poole, K. (2004). MexAB-OprM hyperexpression in NalC-type multidrug-resistant *Pseudomonas aeruginosa*: identification and characterization of the nalC gene encoding a repressor of PA3720-PA3719. Mol. Microbiol. *53*, 1423–1436.

Cathelyn, J.S., Ellison, D.W., Hinchliffe, S.J., Wren, B.W., and Miller, V.L. (2007). The RovA regulons of *Yersinia enterocolitica* and *Yersinia pestis* are distinct: evidence that many RovA-regulated genes were acquired more recently than the core genome. Mol. Microbiol. *66*, 189–205.

Chang, Y.M., Jeng, W.Y., Ko, T.P., Yeh, Y.J., Chen, C.K., and Wang, A.H. (2010). Structural study of TcaR and its complexes with multiple antibiotics from *Staphylococcus epidermidis*. Proc. Natl. Acad. Sci. USA *107*, 8617–8622.

Chen, P.R., Bae, T., Williams, W.A., Duguid, E.M., Rice, P.A., Schneewind, O., and He, C. (2006). An oxidation-sensing mechanism is used by the global regulator MgrA in *Staphylococcus aureus*. Nat. Chem. Biol. *2*, 591–595.

Chen, H., Hu, J., Chen, P.R., Lan, L., Li, Z., Hicks, L.M., Dinner, A.R., and He, C. (2008). The *Pseudomonas aeruginosa* multidrug efflux regulator MexR uses an oxidation-sensing mechanism. Proc. Natl. Acad. Sci. USA *105*, 13586–13591.

Chen, P.R., Nishida, S., Poor, C.B., Cheng, A., Bae, T., Kuechenmeister, L., Dunman, P.M., Missiakas, D., and He, C. (2009). A new oxidative sensing and regulation pathway mediated by the MgrA homologue SarZ in *Staphylococcus aureus*. Mol. Microbiol. *71*, 198–211.

Cohen, S.P., Hachler, H., and Levy, S.B. (1993). Genetic and functional analysis of the multiple antibiotic resistance (*mar*) locus in *Escherichia coli*. J. Bacteriol. *175*, 1484–1492.

Davis, J.R., and Sello, J.K. (2010). Regulation of genes in *Streptomyces* bacteria required for catabolism of lignin-derived aromatic compounds. Appl. Microbiol. Biotechnol. *86*, 921–929.

Di Fiore, A., Fiorentino, G., Vitale, R.M., Ronca, R., Amodeo, P., Pedone, C., Bartolucci, S., and De Simone, G. (2009). Structural analysis of BldR from *Sulfolobus solfataricus* provides insights into the molecular basis of transcriptional activation in archaea by MarR family proteins. J. Mol. Biol. *388*, 559–569.

Evans, K., Adewoye, L., and Poole, K. (2001). MexR repressor of the *mexAB-oprM* multidrug efflux operon of *Pseudomonas aeruginosa*: identification of MexR binding sites in the *mexA-mexR* intergenic region. J. Bacteriol. *183*, 807–812.

Finn, R.D., Mistry, J., Tate, J., Coggill, P., Heger, A., Pollington, J.E., Gavin, O.L., Gunasekaran, P., Ceric, G., Forslund, K., et al. (2010). The Pfam protein families database. Nucleic Acids Res. *38*, D211–222.

Fiorentino, G., Ronca, R., Cannio, R., Rossi, M., and Bartolucci, S. (2007). MarR-like transcriptional regulator involved in detoxification of aromatic compounds in *Sulfolobus solfataricus*. J. Bacteriol. *189*, 7351–7360.

Fuangthong, M., Atichartpongkul, S., Mongkolsuk, S., and Helmann, J.D. (2001). OhrR is a repressor of ohrA, a key organic hydroperoxide resistance determinant in *Bacillus subtilis*. J. Bacteriol. *183*, 4134–4141.

Gajiwala, K.S., and Burley, S.K. (2000). Winged helix proteins. Curr. Opin. Struct. Biol. *10*, 110–116.

Gajiwala, K.S., Chen, H., Cornille, F., Roques, B.P., Reith, W., Mach, B., and Burley, S.K. (2000). Structure of the winged-helix protein hRFX1 reveals a new mode of DNA binding. Nature *403*, 916–921.

Galán, B., Kolb, A., Sanz, J.M., García, J.L., and Prieto, M.A. (2003). Molecular determinants of the *hpa* regulatory system of *Escherichia coli*: the HpaR repressor. Nucleic Acids Res. *31*, 6598–6609.

George, A.M., and Levy, S.B. (1983). Gene in the major cotransduction gap of the *Escherichia coli* K-12 linkage map required for the expression of chromosomal resistance to tetracycline and other antibiotics. J. Bacteriol. *155*, 541–548.

Haque, M.M., Kabir, M.S., Aini, L.Q., Hirata, H., and Tsuyumu, S. (2009). SlyA, a MarR family transcriptional regulator, is essential for virulence in *Dickeya dadantii* 3937. J. Bacteriol. *191*, 5409–5418.

Hommais, F., Oger-Desfeux, C., Van Gijsegem, F., Castang, S., Ligori, S., Expert, D., Nasser, W., and Reverchon, S. (2008). PecS is a global regulator of the symptomatic phase in the phytopathogenic bacterium *Erwinia chrysanthemi* 3937. J. Bacteriol. *190*, 7508–7522.

Hong, M., Fuangthong, M., Helmann, J.D., and Brennan, R.G. (2005). Structure of an OhrR-*ohrA* operator complex reveals the DNA binding mechanism of the MarR family. Mol. Cell *20*, 131–141.

Jefferson, K.K., Pier, D.B., Goldmann, D.A., and Pier, G.B. (2004). The teicoplanin-associated locus regulator (TcaR) and the intercellular adhesin locus regulator (IcaR) are transcriptional inhibitors of the *ica* locus in *Staphylococcus aureus*. J. Bacteriol. *186*, 2449–2456.

Kaatz, G.W., DeMarco, C.E., and Seo, S.M. (2006). MepR, a repressor of the *Staphylococcus aureus* MATE family multidrug efflux pump MepA, is a substrate-responsive regulatory protein. Antimicrob. Agents Chemother. *50*, 1276–1281.

Kiley, P.J., and Beinert, H. (2003). The role of Fe-S proteins in sensing and regulation in bacteria. Curr. Opin. Microbiol. *6*, 181–185.

Klomsiri, C., Panmanee, W., Dharmsthiti, S., Vattanaviboon, P., and Mongkolsuk, S. (2005). Novel roles of ohrR-ohr in *Xanthomonas* sensing, metabolism, and physiological adaptive response to lipid hydroperoxide. J. Bacteriol. *187*, 3277–3281.

Kovacikova, G., Lin, W., and Skorupski, K. (2004). *Vibrio cholerae* AphA uses a novel mechanism for virulence gene activation that involves interaction with the LysR-type regulator AphB at the *tcpPH* promoter. Mol. Microbiol. *53*, 129–142.

Kumaraswami, M., Schuman, J.T., Seo, S.M., Kaatz, G.W., and Brennan, R.G. (2009). Structural and biochemical characterization of MepR, a multidrug binding transcription regulator of the *Staphylococcus aureus* multidrug efflux pump MepA. Nucleic Acids Res. *37*, 1211–1224.

Kumarevel, T., Tanaka, T., Umehara, T., and Yokoyama, S. (2009). ST1710-DNA complex crystal structure reveals the DNA binding mechanism of the MarR family of regulators. Nucleic Acids Res. *37*, 4723–4735.

Lan, L., Murray, T.S., Kazmierczak, B.I., and He, C. (2010). *Pseudomonas aeruginosa* OspR is an oxidative stress sensing regulator that affects pigment production, antibiotic resistance and dissemination during infection. Mol. Microbiol. *75*, 76–91.

Lee, J.W., Soonsanga, S., and Helmann, J.D. (2007). A complex thiolate switch regulates the *Bacillus subtilis* organic peroxide sensor OhrR. Proc. Natl. Acad. Sci. USA *104*, 8743–8748.

Lim, D., Poole, K., and Strynadka, N.C. (2002). Crystal structure of the MexR repressor of the mexRAB-oprM multidrug efflux operon of *Pseudomonas aeruginosa*. J. Biol. Chem. *277*, 29253–29259.

Linde, H.J., Notka, F., Metz, M., Kochanowski, B., Heisig, P., and Lehn, N. (2000). *In vivo* increase in resistance to ciprofloxacin in *Escherichia coli* associated with deletion of the C-terminal part of MarR. Antimicrob. Agents Chemother. *44*, 1865–1868.

Lomovskaya, O., Lewis, K., and Matin, A. (1995). EmrR is a negative regulator of the *Escherichia coli* multidrug resistance pump EmrAB. J. Bacteriol. *177*, 2328–2334.

Luong, T.T., Newell, S.W., and Lee, C.Y. (2003). Mgr, a novel global regulator in *Staphylococcus aureus*. J. Bacteriol. *185*, 3703–3710.

Martin, R.G., and Rosner, J.L. (1995). Binding of purified multiple antibiotic-resistance repressor protein (MarR) to *mar* operator sequences. Proc. Natl. Acad. Sci. USA *92*, 5456–5460.

Martin, R.G., Nyantakyi, P.S., and Rosner, J.L. (1995). Regulation of the multiple antibiotic resistance (*mar*) regulon by marORA sequences in *Escherichia coli*. J. Bacteriol. *177*, 4176–4178.

Martin, R.G., Jair, K.W., Wolf, R.E., Jr., and Rosner, J.L. (1996). Autoactivation of the *marRAB* multiple antibiotic resistance operon by the MarA transcriptional activator in *Escherichia coli*. J. Bacteriol. *178*, 2216–2223.

McCallum, N., Bischoff, M., Maki, H., Wada, A., and Berger-Bachi, B. (2004). TcaR, a putative MarR-like regulator of *sarS* expression. J. Bacteriol. *186*, 2966–2972.

Mongkolsuk, S., Praituan, W., Loprasert, S., Fuangthong, M., and Chamnongpol, S. (1998). Identification and characterization of a new organic hydroperoxide resistance (*ohr*) gene with a novel pattern of oxidative stress regulation from *Xanthomonas campestris* pv. phaseoli. J. Bacteriol. *180*, 2636–2643.

Nasser, W., Reverchon, S., Vedel, R., and Boccara, M. (2005). PecS and PecT coregulate the synthesis of HrpN and pectate lyases, two virulence determinants in *Erwinia chrysanthemi* 3937. Mol. Plant Microbe Interact. *18*, 1205–1214.

Newberry, K.J., Fuangthong, M., Panmanee, W., Mongkolsuk, S., and Brennan, R.G. (2007). Structural mechanism of organic hydroperoxide induction of the transcription regulator OhrR. Mol. Cell *28*, 652–664.

Ochsner, U.A., Hassett, D.J., and Vasil, M.L. (2001). Genetic and physiological characterization of *ohr*, encoding a protein involved in organic hydroperoxide resistance in *Pseudomonas aeruginosa*. J. Bacteriol. *183*, 773–778.

Oh, S.Y., Shin, J.H., and Roe, J.H. (2007). Dual role of OhrR as a repressor and an activator in response to organic hydroperoxides in *Streptomyces coelicolor*. J. Bacteriol. *189*, 6284–6292.

Panmanee, W., Vattanaviboon, P., Poole, L.B., and Mongkolsuk, S. (2006). Novel organic hydroperoxide-sensing and responding mechanisms for OhrR, a major bacterial sensor and regulator of organic hydroperoxide stress. J. Bacteriol. *188*, 1389–1395.

Perera, I.C., Lee, Y.H., Wilkinson, S.P., and Grove, A. (2009). Mechanism for attenuation of DNA binding by MarR family transcriptional regulators by small molecule ligands. J. Mol. Biol. *390*, 1019–1029.

Poole, K., and Srikumar, R. (2001). Multidrug efflux in *Pseudomonas aeruginosa*: components, mechanisms and clinical significance. Curr. Top. Med. Chem. *1*, 59–71.

Poole, K., Krebes, K., McNally, C., and Neshat, S. (1993). Multiple antibiotic resistance in *Pseudomonas aeruginosa*: evidence for involvement of an efflux operon. J. Bacteriol. *175*, 7363–7372.

Poor, C.B., Chen, P.R., Duguid, E., Rice, P.A., and He, C. (2009). Crystal structures of the reduced, sulfenic acid, and mixed disulfide forms of SarZ, a redox active global regulator in *Staphylococcus aureus*. J. Biol. Chem. *284*, 23517–23524.

Praillet, T., Reverchon, S., and Nasser, W. (1997). Mutual control of the PecS/PecM couple, two proteins regulating virulence-factor synthesis in *Erwinia chrysanthemi*. Mol. Microbiol. *24*, 803–814.

Providenti, M.A., and Wyndham, R.C. (2001). Identification and functional characterization of CbaR, a MarR-like modulator of the *cbaABC*-encoded chlorobenzoate catabolism pathway. Appl. Environ. Microbiol. *67*, 3530–3541.

Ramakrishnan, V., Finch, J.T., Graziano, V., Lee, P.L., and Sweet, R.M. (1993). Crystal structure of globular domain of histone H5 and its implications for nucleosome binding. Nature *362*, 219–223.

Reverchon, S., Nasser, W., and Robert-Baudouy, J. (1994). *pecS*: a locus controlling pectinase, cellulase and blue pigment production in *Erwinia chrysanthemi*. Mol. Microbiol. *11*, 1127–1139.

Reverchon, S., Rouanet, C., Expert, D., and Nasser, W. (2002). Characterization of indigoidine biosynthetic genes in *Erwinia chrysanthemi* and role of this blue pigment in pathogenicity. J. Bacteriol. *184*, 654–665.

Rouanet, C., Nomura, K., Tsuyumu, S., and Nasser, W. (1999). Regulation of *pelD* and *pelE*, encoding major alkaline pectate lyases in *Erwinia chrysanthemi*: involvement of the main transcriptional factors. J. Bacteriol. *181*, 5948–5957.

Rouanet, C., Reverchon, S., Rodionov, D.A., and Nasser, W. (2004). Definition of a consensus DNA-binding site for PecS, a global regulator of virulence gene expression in *Erwinia chrysanthemi* and identification of new members of the PecS regulon. J. Biol. Chem. *279*, 30158–30167.

Rungrassamee, W., Ryan, K.C., Maroney, M.J., and Pomposiello, P.J. (2009). The PqrR transcriptional repressor of *Pseudomonas aeruginosa* transduces redox signals via an iron-containing prosthetic group. J. Bacteriol. *191*, 6709–6721.

Saito, K., Yoneyama, H., and Nakae, T. (1999). nalB-type mutations causing the overexpression of the MexAB-OprM efflux pump are located in the *mexR* gene of the *Pseudomonas aeruginosa* chromosome. FEMS Microbiol. Lett. *179*, 67–72.

Saridakis, V., Shahinas, D., Xu, X., and Christendat, D. (2008). Structural insight on the mechanism of regulation of the MarR family of proteins: high-resolution crystal structure of a transcriptional repressor from *Methanobacterium thermoautotrophicum*. J. Mol. Biol. *377*, 655–667.

Seoane, A.S., and Levy, S.B. (1995). Characterization of MarR, the repressor of the multiple antibiotic resistance (*mar*) operon in *Escherichia coli*. J. Bacteriol. *177*, 3414–3419.

Soonsanga, S., Fuangthong, M., and Helmann, J.D. (2007). Mutational analysis of active site residues essential for sensing of organic hydroperoxides by *Bacillus subtilis* OhrR. J. Bacteriol. *189*, 7069–7076.

Srikumar, R., Paul, C.J., and Poole, K. (2000). Influence of mutations in the *mexR* repressor gene on expression of the MexA-MexB-oprM multidrug efflux system of *Pseudomonas aeruginosa*. J. Bacteriol. *182*, 1410–1414.

Stapleton, M.R., Norte, V.A., Read, R.C., and Green, J. (2002). Interaction of the *Salmonella typhimurium* transcription and virulence factor SlyA with target DNA and identification of members of the SlyA regulon. J. Biol. Chem. *277*, 17630–17637.

Sukchawalit, R., Loprasert, S., Atichartpongkul, S., and Mongkolsuk, S. (2001). Complex regulation of the organic hydroperoxide resistance gene (*ohr*) from *Xanthomonas* involves OhrR, a novel organic peroxide-inducible negative regulator, and posttranscriptional modifications. J. Bacteriol. *183*, 4405–4412.

Sulavik, M.C., Gambino, L.F., and Miller, P.F. (1995). The MarR repressor of the multiple antibiotic resistance (*mar*) operon in *Escherichia coli*: prototypic member of a family of bacterial regulatory proteins involved in sensing phenolic compounds. Mol. Med. *1*, 436–446.

Sulavik, M.C., Dazer, M., and Miller, P.F. (1997). The *Salmonella typhimurium mar* locus: molecular and genetic analyses and assessment of its role in virulence. J. Bacteriol. *179*, 1857–1866.

Westermark, M., Oscarsson, J., Mizunoe, Y., Urbonaviciene, J., and Uhlin, B.E. (2000). Silencing and activation of ClyA cytotoxin expression in *Escherichia coli*. J. Bacteriol. *182*, 6347–6357.

Wilke, M.S., Heller, M., Creagh, A.L., Haynes, C.A., McIntosh, L.P., Poole, K., and Strynadka, N.C. (2008). The crystal structure of MexR from *Pseudomonas aeruginosa* in complex with its antirepressor ArmR. Proc. Natl. Acad. Sci. USA *105*, 14832–14837.

Wilkinson, S.P., and Grove, A. (2004). HucR, a novel uric acid-responsive member of the MarR family of transcriptional regulators from *Deinococcus radiodurans*. J. Biol. Chem. *279*, 51442–51450.

Wilkinson, S.P., and Grove, A. (2005). Negative cooperativity of uric acid binding to the transcriptional regulator HucR from *Deinococcus radiodurans*. J. Mol. Biol. *350*, 617–630.

Wilkinson, S.P., and Grove, A. (2006). Ligand-responsive transcriptional regulation by members of the MarR family of winged helix proteins. Curr. Issues Mol. Biol. *8*, 51–62.

Wyborn, N.R., Stapleton, M.R., Norte, V.A., Roberts, R.E., Grafton, J., and Green, J. (2004). Regulation of *Escherichia coli* hemolysin E expression by H-NS and Salmonella SlyA. J. Bacteriol. *186*, 1620–1628.

Xiong, A., Gottman, A., Park, C., Baetens, M., Pandza, S., and Matin, A. (2000). The EmrR protein represses the Escherichia coli emrRAB multidrug resistance operon by directly binding to its promoter region. Antimicrob. Agents Chemother *44*, 2905–2907.

Yoshida, M., Hiromoto, T., Hosokawa, K., Yamaguchi, H., and Fujiwara, S. (2007). Ligand specificity of MobR, a transcriptional regulator for the 3-hydroxybenzoate hydroxylase gene of *Comamonas testosteroni* KH122–3s. Biochem. Biophys. Res. Commun. *362*, 275–280.

Zheng, M., Aslund, F., and Storz, G. (1998). Activation of the OxyR transcription factor by reversible disulfide bond formation. Science *279*, 1718–1721.