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# Metal homeostasis in bacteria: the role of ArsR–SmtB family of transcriptional repressors in combating varying metal concentrations in the environment

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**Abstract** Bacterial infections cause severe medical problems worldwide, resulting in considerable death and loss of capital. With the ever-increasing rise of antibiotic-resistant bacteria and the lack of development of new antibiotics, research on metal-based antimicrobial therapy has now gained pace. Metal ions are essential for survival, but can be highly toxic to organisms if their concentrations are not strictly controlled. Through evolution, bacteria have acquired complex metal-management systems that allow them to acquire metals that they need for survival in different challenging environments while evading metal toxicity. Metalloproteins that controls these elaborate systems in the cell, and linked to key virulence factors, are promising targets for the antibacterial drug development. Among several metal-sensory transcriptional regulators, the ArsR–SmtB family displays greatest diversity with several distinct metal-binding and nonmetal-binding motifs that have been characterized. These prokaryotic metolloregulatory transcriptional repressors represses the expression of operons linked to stress-inducing concentrations of metal ions by directly binding to the regulatory regions of DNA, while derepression results from direct binding of metal ions by these homodimeric proteins. Many bacteria, e.g., *Mycobacterium tuberculosis*, *Bacillus anthracis*, etc., have evolved to acquire multiple metal-sensory motifs which clearly demonstrate the importance of regulating concentrations of multiple metal ions. Here, we discussed the mechanisms of how ArsR–SmtB family regulates the intracellular bioavailability of metal ions both inside and outside of the host. Knowledge of the metal-challenges faced by bacterial pathogens and their survival strategies will enable us to develop the next generation drugs.

**Keywords** ArsR–SmtB family · Metalloprotein · Transcriptional repressor · Allostery · Redox switch · Antibiotic-resistant bacteria

**Abbreviations**

| | |
|---|---|
| O/P | Operator/promoter |
| CDF | Cation diffusion facilitator |
| MD | Molecular dynamics |
| wHTH | Winged helix-turn-helix |
| ORF | Open reading frame |

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| | |
|---|---|
| aa | Amino acids |
| nt | Nucleotides |
| bp | Base pairs |
| HGT | Horizontal gene transfer |

## Introduction

ArsR–SmtB family members possess a highly conserved DNA recognition helix-turn-helix (HTH) motif and bind as homodimers to their operator/promoter (O/P) region, repressing the expression of operons, in absence of metal ions, associated with the metal ion sequestration or efflux in both gram-negative and gram-positive bacteria, while derepresses the operons in presence of toxic concentrations of heavy metal ions, allowing these organisms to survive in challenging environments (Shi et al. 1994; Busenlehner et al. 2003; Osman and Cavet 2010) (Fig. 1). Some of the members also found to control virulence factors (Saha and Chakrabarti 2006; Zhao et al. 2010), sulfur oxidation (Mandal et al. 2007), hypoxia (Guimarães et al. 2011), prodigiosin biosynthesis (Gristwood et al. 2011), oxidative stress response (Ehira et al. 2010), bioluminescence (Gueuné et al. 2008), biofilm formation (Mac Aogáin et al. 2012), etc.

Seven major families of soluble metal-sensing transcriptional regulators have been identified in bacteria (Waldron and Robinson 2009), and are designated based upon their founding member(s): ArsR–SmtB (Huckle et al. 1993; Eicken et al. 2003), MerR (Brocklehurst et al. 1999; Outten et al. 2000), CsoR-RcnR (Liu et al. 2007a; Smaldone and Helmann 2007), CopY (Strausak and Solioz 1997), DtxR (Guedon and Helmann 2003), Fur (Gaballa and Helmann 1998; Ahn et al. 2006) and NikR (Dosanjh and Michel 2006; Wang et al. 2009a). The ArsR–SmtB family displays the greatest diversity among others, with thirteen distinct metal-sensing and two non metal-sensing motifs identified so far (Table 1). These have been designated α3 (Wu and Rosen 1991, 1993; Shi et al. 1994), α3N (Liu et al. 2005), α5 (Huckle et al. 1993; Kuroda et al. 1999; Singh et al. 1999), α3N–α5 (Thelwell et al. 1998; Sun et al. 2001; Busenlehner et al. 2002a), α5c (Campbell et al. 2007), α53 (Cavet et al. 2002), α4c (Cavet et al. 2003; Wang et al. 2005), α4c2 (Wang et al. 2010), α3N–2 (Ordóñez et al. 2008), α5–4 (Qin et al. 2007), α55 (Li et al. 2016a), α2–α52 (Slyemi et al. 2013; Moinier et al. 2014), α3–4 (Wang et al. 2006), α33 (non-metal binding motif) (Ehira et al. 2010) and α2–α5 (non-metal binding motif) (Saha and Chakrabarti 2006; Saha et al. 2006) based upon the location of the site where metal ions bind within the protein fold (Fig. 2; Table 1). The metal-sensing motif names originated from the typical structural fold (α1–α2–α3–α4–β1–β2–α5) observed in SmtB protein (Cook et al. 1998) (Fig. 3).

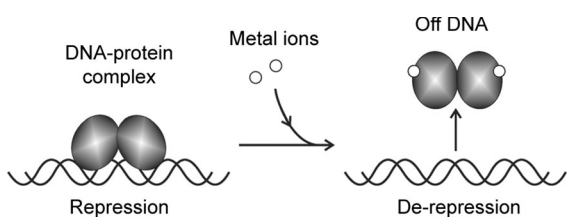



**Fig. 1** Model of the mechanism of gene regulation in the ArsR–SmtB family

ArsR–SmtB family members sense a wide variety of metal ions like As, Sb and Bi (ArsR, *Escherichia coli*), Zn (SmtB, *Synechococcus* sp.; ZiaR, *Synechocystis* sp.), Cd, Pb and Zn (CadC, *Staphylococcus aureus*; AztR, *Anabaena* sp.), Cd and Pb (CmtR, *Mycobacterium tuberculosis*), Zn and Co (CzrA, *Bacillus subtilis*), Ni and Co (NmtR, KmtR, *M. tuberculosis*) or Cu, Ag, Zn and Cd (BxmR, *Oscillatoria brevis*). Among 15 identified motifs in ArsR–SmtB family, only α2–α5 (HlyU, *Vibrio cholerae*; BigR, *Xylella fastidiosa* and *Agrobacterium tumefaciens*; PigS, *Serratia* sp.; SoxR, *Pseudaminobacter salicylatoxidans*; YgaV, *E. coli*) and α33 (CyeR, *Corynebacterium glutamicum*) motifs found not to sense any metal ions, but control transcription via novel redox switches (Saha and Chakrabarti 2006; Mandal et al. 2007; Gueuné et al. 2008; Ehira et al. 2010; Guimarães et al. 2011; Gristwood et al. 2011; Mukherjee et al. 2014, 2015). Some of the ArsR–SmtB family repressors (PagR, *Bacillus anthracis*; PyeR, *Pseudomonas aeruginosa*) do not have apparent metal-sensory sites yet controls transcription via unidentified novel methods (Zhao et al. 2010; Mac Aogáin et al. 2012). More than 82,000 ArsR–SmtB family members are found in the InterPro database (Finn et al. 2017), yet only a handful of the proteins was characterized in this group, indicates the possibility of discovering new and novel metal-sensory sites in this group. Several pathogenic bacteria (e.g., *B. anthracis*, *M. tuberculosis*, *A. tumefaciens*, *B. cereus*,

**Table 1** Summary of known metal- and nonmetal-sensory sites with corresponding sequence patterns found in archaea and bacteria

| No. | Sensory groups | | Sensory site(s) | Inter-/intra-subunit association by metal[c] | Sequence pattern/motif of sensory site(s)[a] |
|---|---|---|---|---|---|
| 1 | Metal binding motifs | α2 group | α2–α52 | Intra[b] | C residue in α2 helix and $Cx_3C$ in α5 helix |
| 2 | | α3 group | α3 | Intra | Any one of the following motifs—(a) $Cx_{0\text{-}5}Cx_{1\text{-}3}C$, (b) $Cx_{1\text{-}2}C(D/x)$ or (c) $Cx_2D$ in the α3 helix |
| 3 | | | α3N | Inter | Any one of (b) or (c) motifs shown in No. 2 and one C or two residues (combinations of C and/H) from amino-terminal |
| 4 | | | α3N–2 | Inter | C between α2 and α3 helix and amino-terminal CC |
| 5 | | | α3–4 | Intra[b] | $Cx_2H$ in the α3 helix and one C between β1 and β2 strands |
| 6 | | α4 group | α4c | Inter | $Cx_3C$ in α4 helix and carboxy-terminal C |
| 7 | | | α4c2 | Inter | Site 1 (same as No. 6) and site 2[b] (C in α2 helix and CC in carboxy-terminal) |
| 8 | | α5 group | α5 | Inter | $DxHx_{10}Hx_2(E/H)$ in α5 helix |
| 9 | | | α5c | Inter | $DxHx_{10}Hx_2(E/H)$ in α5 helix and also possess carboxy-terminal H residues |
| 10 | | | α53 | Inter | $Hx_6Dx_5EHx_7HH$ spanning the α5 helix and carboxy-terminal region |
| 11 | | | α5–4 | Intra[b] | CC at the α5 helix and carboxy-terminal C (motif—$CCx_{4\text{-}6}C$ or $CCx_{15}C$) |
| 12 | | | α55 | Intra[b] | C at the α5 helix and carboxy-terminal CC (motif—$Cx_6CC$) |
| 13 | | multiple | α3N–α5 | Inter | Combination of motifs shown in No. 3 and No. 8 |
| 14 | non-metal binding motifs | | α2–α5 | NA[d] | One C residue each in α2 and α5 helices |
| 15 | | | α33 | NA[d] | $Cx_6C$ in and close to α3 helix |

Helices are numbered according to those found in the SmtB X-ray crystal structure (Cook et al. 1998)

[a] 'x' denotes any amino acid, *C* cysteine, *D* aspartic acid, *H* histidine, *E* glutamic acid

[b] Putative

[c] At the metal binding site

[d] Not applicable

etc.) and non-pathogenic soil bacteria (e.g., *Microbacterium oxydans*, *Amycolatopsis keratiniphila*, etc.) found to possess multiple ArsR–SmtB members in their genomes. This clearly indicates that these bacteria use not only several novel mechanisms to withstand toxic levels of various metal ions in the environment, but may also use them to their advantage to evade host-mediated immunity.

Metals are essential for survival of organisms yet slight changes in the concentration would make them toxic to the cells. Understanding the mechanisms of how bacteria use metals for their advantage would enable us to better prepare for the attacks of pathogenic bacteria, especially from antibiotic-resistant strains, by developing novel methods (e.g., antibacterial metal-nanoparticles, etc.) that use metals at our advantage. Therefore, it is essential to identify new novel metal-sensory sites in ArsR–SmtB family of transcriptional repressors and discover new mechanisms of transcriptional regulation. The knowledge gathered from these studies would help us to develop new-age drugs in response to the attacks of pathogenic microorganisms.

## Characteristics of ArsR–SmtB family of transcriptional regulators

The ArsR–SmtB family of transcriptional metallorepressors represses the expression of genes/operons

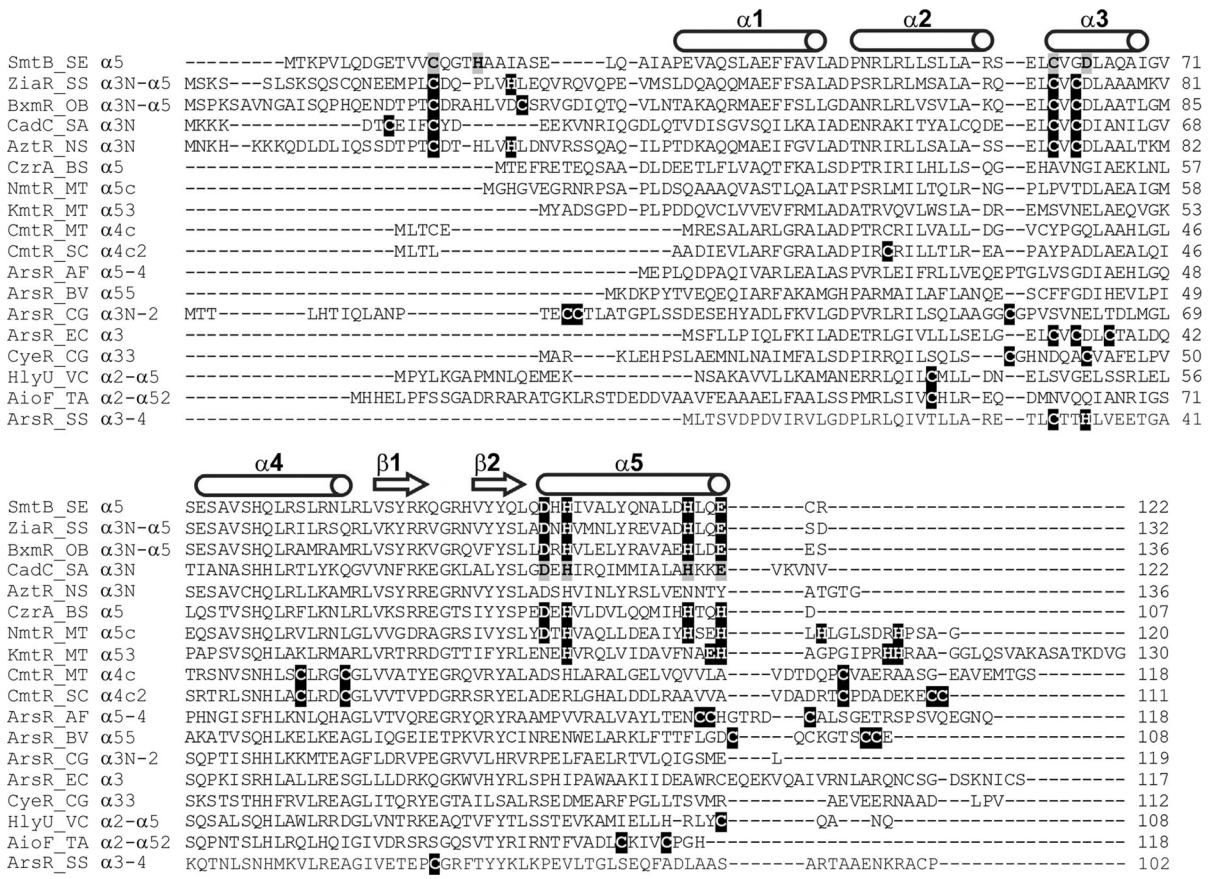



**Fig. 2** Sensory (metal/nonmetal-binding) sites in ArsR–SmtB family repressors. Alignment of representative sequences for the different sensory sites (highlighted in *black*) involving α5 (*Synechococcus elongatus* SmtB, SmtB_SE; *Bacillus subtilis* CzrA, CzrA_BS), α3N–α5 (*Synechocystis* sp. ZiaR, ZiaR_SS; *Oscillatoria brevis* BxmR, BxmR_OB), α3N (*Staphylococcus aureus* CadC, CadC_SA; *Nostoc* sp. AztR, AztR_NS), α5c (*Mycobacterium tuberculosis* NmtR, NmtR_MT), α53 (*M. tuberculosis* KmtR, KmtR_MT), α4c (*M. tuberculosis* CmtR, CmtR_MT), α4c2 (*Streptomyces coelicolor* CmtR, CmtR_SC), α5–4 (*Acidithiobacillus ferrooxidans* ArsR, ArsR_AF), α55 (*Bacteroides vulgatus* ArsR, ArsR_BV), α3N–2 (*Corynebacterium glutamicum* ArsR, ArsR_CG), α3 (*Escherichia coli* ArsR, ArsR_EC), α33 (*C. glutamicum* CyeR, CyeR_CG), α2–α5 (*Vibrio cholerae* HlyU, HlyU_VC), α2–α52 (*Thiomonas arsenitoxydans* AioF, AioF_TA) and α3–4 (*Streptomyces* sp. ArsR1, ArsR_SS) sites are shown. The α3N site in SmtB and the α5 site in CadC are not required for metal-responsiveness (highlighted in *grey*). The secondary structure determined for SmtB is shown on top

associated to stress-inducing concentrations of different heavy metal ions. Direct binding of metal ions by this group of homodimeric metal-sensors, remove them from their cognate O/P DNA, results in the derepression of the corresponding genes/operons (Busenlehner et al. 2003; Osman and Cavet 2010) (Fig. 1).

Among different families of metal-sensing transcriptional regulators that have so far been identified in archaea and bacteria (Wang et al. 2004; Waldron and Robinson 2009; Osman and Cavet 2010), the ArsR–SmtB family displays most diversity with as many as thirteen metal-sensing and two nonmetal-sensing motifs that have been identified till date (end of 2016). These metal-sensing motifs have been designated as α3, α3N, α3N–2, α33, α3–4, α5, α5c, α53, α5–4, α55, α3N–α5, α4c, α4c2, α2–α5 and α2–α52 (Table 1), based upon the locations of sensory amino acids within the known secondary structures of the proteins of the ArsR–SmtB family (Table 1; Fig. 3).

Several studies found that, in both metal-bound and metal-free states, ArsR–SmtB metallorepressors are weakly dissociable homodimers (Kar et al. 1997; Busenlehner et al. 2001, 2002a; Pennella et al. 2003)

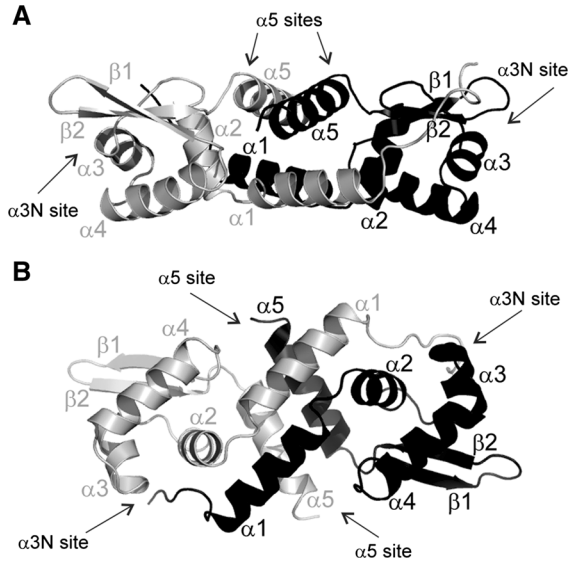



**Fig. 3** **a** Typical structural fold (α1–α2–α3–α4–β1–β2–α5) of an ArsR–SmtB family homodimeric repressor (structure of *Synechococcus elongatus* SmtB shown here). Two sub-units are shown in *black* and *grey* colors respectively. Secondary structural elements are indicated in numbers. **b** 90° rotated image of the above SmtB structure. The position of α3N and α5 sites are indicated by *arrows*. The figures were created with PyMOL (DeLano 2002)

and each homodimer binds two metal ions either in the dimeric interface (inter) or within each monomer (intra) (Table 1).

## α3 motif

One of the founding members of the ArsR–SmtB family is the plasmid-borne (Gladysheva et al. 1994; Bruhn et al. 1996) or chromosomally encoded (Diorio et al. 1995) ArsR that senses As(III), Sb(III) or Bi(III) (Wu and Rosen 1991; Gladysheva et al. 1994; Oden et al. 1994), with the sensory motif $CxCx_2C$ in α3 helix, and represses transcription of the *ars* operon in *E. coli* (Wu and Rosen 1991, 1993; Shi et al. 1994). The *ars* operon in *E. coli* plasmids R46 and R773 contain *arsR*, *arsD*, *arsA*, *arsB* and *arsC* genes, while the chromosomally encodes *ars* operon has all these except *arsD* and *arsA* genes (Rosen 1990; Busenlehner et al. 2003). The ArsC protein catalyzes reduction of arsenate As(V) to arsenite As(III) (Gladysheva et al. 1994) and the metallochaperone ArsD transports As(III) to ArsAB for extrusion (Lin et al. 2006). ArsAB encodes an arsenite-efflux system composed of secondary carrier protein ArsB and an anion-translocating ATPase ArsA (Rosen 1999). ArsA and ArsD proteins are always found together in bacterial and archaeal *ars* operons, which indicates the possibility that *arsRDABC* operon may have evolved from *arsRBC* operon by acquiring *arsA* and *arsD* genes together as a unit (Rosen 1999; Lin et al. 2006). *E. coli* plasmid R773 has three cysteines (Cys32, Cys34 and Cys37) in α3 helix that comprises the $CxCx_2C$ metal-sensory motif and form the trigonal metal-coordination complex (O'Halloran 1993). Only two cysteines (Cys32 and Cys34) are essential to produce the conformational changes, upon metal binding, that help to release the repressor from its cognate DNA and start transcription by RNA polymerase (Shi et al. 1996). Interestingly, the *E. coli* plasmid R773 *ars* operon found to show increased resistance to tellurite (Turner et al. 1992), which is not observed with the chromosomal *ars* operon (Cai et al. 1998), but whether this was due to the failure of tellurite to induce *ars* operon is not clear.

Like *E. coli*, several other bacteria and archaea found to encode ArsR protein (Table 2), e.g., ArsR proteins from *S. aureus* Plasmid pI258 (Ji and Silver 1992), *Staphylococcus xylosus* Plasmid pSX267 (Rosenstein et al. 1992), *Yersinia enterocolitica* Plasmid pYVe227 (Neyt et al. 1997), *B. subtilis* 168 (Sato and Kobayashi 1998), *Pseudomonas aeruginosa* (Cai et al. 1998), *Acidiphilium multivorum* AIU 301 Plasmid pKW301 (Suzuki et al. 1998), *Synechocystis* sp. PCC 6803 (López-Maury et al. 2003), *Serratia marcescens* Plasmid R478 (Ryan and Colleran 2002), *Shigella flexneri* 2457T (Vorontsov et al. 2007), *Lactobacillus plantarum* Plasmid pWCFS103 (van Kranenburg et al. 2005), *Bacillus* sp. CDB3 (Yu et al. 2015), *Ferroplasma acidarmanus* Fer1 (Gihring et al. 2003; Baker-Austin et al. 2007), *Campylobacter coli* (Noormohamed and Fakhr 2013), *C. jejuni* (Wang et al. 2009b), *C. lari* (Matsuda et al. 2016), ArsR1 and ArsR2 proteins from *Pantoea* sp. IMH (Wang et al. 2016), ArsR2 from *Pseudomonas putida* KT2440 (Fernández et al. 2016), ArsR1 and ArsR2 from *Geobacillus kaustophilus* HTA426 (Cuebas et al. 2011), ArsRC protein from *Leptospirillum ferriphilum* (Tuffin et al. 2006) and ArsR1 from *Ochrobactrum tritici* SCII24$^T$ (Branco et al. 2008). While most of these proteins have $CxCx_2C$ sensory motif at α3, some show variations (Table 2). Interestingly, the archaeal protein ArsR from *F. acidarmanus* found to have

**Table 2** Summary of preferred metals, sensory motif, coordination chemistry and functions of characterized ArsR–SmtB family proteins with classification, protein size and unique Uniprot identifier

| No. | Sensory motif | Protein | Organism | Domain | Length (aa) | Motif Signature(s)[h] | Metal ion(s) and coordination chemistry | Function(s) | Uniprot ID[c] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | α3 | ArsR | *Escherichia coli* K12 | Bacteria | 117 | $CxCx_2C$ | As(III), Sb(III), Bi(III)—trigonal | Transcriptional repressor of the *arsRBC* operon. | P37309 |
| | | | *Escherichia coli* Plasmid R46 | | 117 | $CxCx_2C$ | | Transcriptional repressor of the *arsRDABC* operon. | P52144 |
| | | | *Escherichia coli* Plasmid R773 | | 117 | $CxCx_2C$ | | Transcriptional repressor of the *arsRDABC* operon. | P15905 |
| | | | *Staphylococcus xylosus* Plasmid pSX267 | | 104 | $Cx_3CxC$ | As(III), Sb(III)—trigonal | Transcriptional repressor of the *arsRBC* operon. | Q01256 |
| | | | *Staphylococcus aureus* Plasmid pI258 | | 104 | $Cx_3CxC$ | As(III), Sb(III), Bi(III)—trigonal | Transcriptional repressor of the *arsRBC* operon. | P30338 |
| | | | *Yersinia enterocolitica* Plasmid pYVe227 | | 117 | $CxCx_2C$ | As(III)—trigonal | Transcriptional repressor of the *arsHRBC* operon. | P74986 |
| | | | *Bacillus subtilis* 168 | | 105 | $Cx_5CxC$ | As(III), Sb(III)—trigonal | Transcriptional repressor of the *arsR-orf2-arsBC* operon. | P45949 |
| | | | *Pseudomonas aeruginosa* | | 118 | $CxCx_3C$ | As(III), Sb(III)—trigonal | Transcriptional repressor of the *arsRBC* operon. | O68020 |
| | | | *Acidiphilium multivorum* AIU 301 Plasmid pKW301 | | 84 | $CxCx_2C$ | As(III)—trigonal | Transcriptional repressor of the *arsRDABC* operon. | O50591 |
| | | | *Synechocystis* sp. PCC 6803 | | 104 | $CxCx_2C$ | As(III), Sb(III), Bi(III)—trigonal | Transcriptional repressor of the *arsBHC* operon. | P73808 |
| | | | *Serratia marcescens* Plasmid R478 | | 106 | $CxCx_2C$ | As(III)—trigonal | Transcriptional repressor of the *arsHRBC* operon. | Q9L335 |
| | | | *Shigella flexneri* 2457T | | 114 | $CxCx_2C$ | As(III)—trigonal | Transcriptional repressor of the *arsHRBC* operon. | A0A0H2W0U9 |
| | | ArsR1 | *Pantoea* sp. IMH | | 117 | $CxC$ | As(III)—trigonal | Transcriptional repressors of the *ars1* (*arsR1B1C1H1*) and *ars2* (*arsR2B2C2H2*) operons, respectively. | A0A0A7HMJ3 |
| | | ArsR2 | | | 106 | $CxCx_2C$ | | | None[f] |

**Table 2** continued

| No. | Sensory motif | Protein | Organism | Domain | Length (aa) | Motif Signature(s)[h] | Metal ion(s) and coordination chemistry | Function(s) | Uniprot ID[c] |
|---|---|---|---|---|---|---|---|---|---|
| | | ArsR2 | *Pseudomonas putida* KT2440 | | 115 | $CxCx_3C$ | As(III), Sb(III), Bi(III)—trigonal | Transcriptional repressors of the *ars2* (*arsR2B2C2H2*) operon. | Q88JD1 |
| | | ArsR1 | *Geobacillus* | | 115 | CCxC | As(III)—trigonal | Transcriptional repressors of the *ars1* (*arsR1B1C1*) and *ars2* (*arsR2B2C2*) operons, respectively. | Q5KUX7 |
| | | ArsR2 | *kaustophilus* HTA426 | | 115 | CCxC | | | Q5L2F8 |
| | | ArsR | *Lactobacillus plantarum* Plasmid pWCFS103 | | 119 | $Cx_3CxC$ | As(III)—trigonal | Transcriptional repressor of the *ars* (*arsRD1AB-orf4-arsD2*) operon. | Q6LWG7 |
| | | ArsR | *Bacillus* sp. CDB3 | | 115 | CCxC | As(III)—trigonal | Transcriptional repressor of the *ars1* (*arsRYCDAT-orf7-orf8*) operon. | Q9RA93 |
| | | ArsRC | *Leptospirillum ferriphilum* | | 297 | CxCH | As(III)—trigonal | Transcriptional repressor of the *arsRCB* operon. | A5JSW5 |
| | | ArsR | *Campylobacter coli* | | 105 | CxCD | As(III)—trigonal | Transcriptional repressor of the *arsPRC-acr3* operon. | A0A1B3X802 |
| | | ArsR | *Campylobacter jejuni* | | 105 | CxCD | As(III)—trigonal | | B5LWZ9 |
| | | ArsR | *Campylobacter lari* | | 106 | CxCD | As(III)—trigonal | | F8WQU1 |
| | | ArsR1 | *Ochrobactrum tritici* SCII24$^T$ | | 109 | $Cx_2CD$ | As(III), Sb(III)—trigonal | Transcriptional repressor of the *ars1* (*arsR1DA-cbs-arsB*) operon. | A1XP68 |
| | | ArsR | *Ferroplasma acidarmanus* Fer1 | Archaea | 118 | $CxCx_2C$ in carboxy terminal | As(III)—trigonal | Transcriptional repressors of the *arsRB* operon. | S0AS94 |
| 2 | α3N | CadC | *Listeria monocytogenes* Plasmid pLm74 | Bacteria | 119 | $Cx_3C$ in amino terminal and CxCD in α3 | Cd(II)—tetrahedral | Transcriptional repressor of the *cadCA* operon. | Q56405 |
| | | | *Staphylococcus aureus* Plasmid pI258 | | 122 | $Cx_3C$ in amino terminal and CxCD in α3/$DxHx_{10}Hx_2E$ in α5 | Cd(II), Zn(II), Co(II)—tetrahedral; Pb(II), Hg(II)—trigonal; Bi(III)—trigonal/ Tetrahedral | | P20047 |
| | | | *Lactococcus lactis* Plasmid pND302 | | 119 | $Cx_3C$ in amino terminal and CxCD in α3/DxH in α5 | Cd(II)—tetrahedral | | P0A4U3 |

**Table 2** continued

| No. | Sensory motif | Protein | Organism | Domain | Length (aa) | Motif Signature(s)[h] | Metal ion(s) and coordination chemistry | Function(s) | Uniprot ID[e] |
|---|---|---|---|---|---|---|---|---|---|
| | | | *Bacillus firmus* | | 122 | $Cx_3C$ in amino terminal and CxCD in α3/ $DxHx_{10}Hx_2E$ in α5 | ND[e] | | P30339 |
| | | | *Stenotrophomonas maltophilia* D457R | | 122 | $Cx_3C$ in amino terminal and CxCD in α3/ $DxHx_{10}Hx_2E$ in α5 | ND[e] | | Q9JRM3 |
| | | | *Bacillus stearothermophilus* LV | | 122 | C in amino terminal and CxCD in α3/ $DxHx_{10}Hx_2E$ in α5 | Cd(II)—tetrahedral | | Q93GK0 |
| | | | *Streptococcus thermophilus* 4134 | | 119 | $Cx_3C$ in amino terminal and CxCD in α3/DxH in α5 | Cd(II)—tetrahedral | | Q7AY48 |
| | | Rv2642 | *Mycobacterium tuberculosis* | | 126 | C in amino terminal and CxC in α3 | As(III)—trigonal | Transcriptional repressor/ regulator of *cadI-Rv2642-arsC* operon. | P71941 |
| | | ArsR2 | *Streptomyces* sp. FR-008 Plasmid pHZ227 | | 124 | CC in amino terminal and CxC in α3 | As(III)—trigonal | Transcriptional repressors of the *arsR1BO* and *arsCT* operons. | Q1HW04 |
| | | ArsRC2 | *Microbacterium* sp. A33 | | 331 | $Cx_5H$ in amino terminal and CxC in α3 | As(III), As(V), Sb(III)—trigonal | Transcriptional repressors of the *arsC3TX-acr3-arsRC2C1* operon. | B7FAZ7 |
| | | ArsR | *Desulfovibrio desulfuricans* G20 | | 120 | $Cx_5H$ in amino terminal and CxC in α3 | As(III)—trigonal | Transcriptional repressors of the *arsRBC2C3* operon. | Q314U8 |
| | | AztR | *Nostoc* sp. | | 136 | $Cx_5H$ in amino terminal and CxC in α3 | Zn(II), Cd(II)—tetrahedral; Pb(II)—trigonal | Transcriptional repressor of the *aztRA* operon. | Q8ZS91 |
| | | ArsR1 | *Pseudomonas putida* KT2440 | | 128 | C in amino terminal and CxC in α3 | As(III), Sb(III), Bi(III)—trigonal | Transcriptional repressors of the *ars1* (*arsR1B1C1H1*) operon. | Q88LK1 |
| | | AseR | *Bacillus subtilis* 168 | | 111 | C in amino terminal and CxC in α3 | As(III), Sb(III)—trigonal | Transcriptional repressor of the *aseR-ydfA* operon. | P96677 |

**Table 2** continued

| No. | Sensory motif | Protein | Organism | Domain | Length (aa) | Motif Signature(s)[h] | Metal ion(s) and coordination chemistry | Function(s) | Uniprot ID[c] |
|---|---|---|---|---|---|---|---|---|---|
| | | ArsR1 | *Halobacterium salinarum* Plasmid pNRC100 | Archaea | 129 | C in amino terminal and CxC in α3 | As(III), Sb(III)—trigonal | Transcriptional repressor of the *arsADRC* operon. | O52029 |
| | | ArsR2 | | | 119 | CC in amino terminal and CxC in α3 | | Transcriptional repressor of the *arsR2 M* operon. | O52026 |
| 3 | α3N–2 | ArsR1 | *Corynebacterium glutamicum* | Bacteria | 119 | $CCx_{38}C$ | As(III), Sb(III)—trigonal | Transcriptional repressor of the *ars1* (*arsR1B1C1C1′*) and *ars2* (*arsR2B2C2*) operons. | Q6M577 |
| | | ArsR2 | | | 115 | $CCx_{38}C$ | | | Q8NTP5 |
| 4 | α3N–α5 | ZiaR | *Synechocystis* sp. PCC 6803 | Bacteria | 132 | $Cx_5H$ in amino terminal and CxCD in α3/$DxHx_{10}Hx_2E$ in α5 | Zn(II)—tetrahedral | Transcriptional repressor of the *ziaA* gene. | Q55940 |
| | | BxmR | *Oscillatoria brevis* | | 136 | $Cx_3Hx_3C$ in amino terminal and CxCD in α3/ $DxHx_{10}Hx_2E$ in α5 | Cd(II), Zn(II)—tetrahedral; Cu(I), Ag(I)—trigonal | Transcriptional repressor of both *bxa1* gene and *bxmR-bmtA* operon. | Q76L30 |
| 5 | α33[a] | CyeR | *Corynebacterium glutamicum* | Bacteria | 112 | $Cx_6C$ in and close to α3 helix | NA[d] | Repress the expression of *cyeR* and the *cgR_2931 (cye1)-cgR_2932* operon. | A4QI86 |
| 6 | α3–4 | ArsR1 | *Streptomyces* sp. FR-008 Plasmid pHZ227 | Bacteria | 102 | $Cx_2H$ in α3 and one C between β1 and β2 | As(III)—trigonal | Transcriptional repressor of the *arsRBOCT* operon. | Q1HW01 |
| 7 | α5 | SmtB | *Synechococcus elongatus* PCC 7942 | Bacteria | 122 | $Cx_3H$ in amino terminal and CVGD in α3/ $DxHx_{10}Hx_2E$ in α5 | Zn(II), Cd(II), Cu(II)—tetrahedral | Transcriptional repressor of the *smtA* gene. | P30340 |
| | | CzrA | *Staphylococcus aureus* | | 106 | $DxHx_{10}Hx_2H$ | Zn(II), Co(II)—tetrahedral; Ni(II)—octahedral | Transcriptional repressor of *czrAB* operon. | O85142 |
| | | | *Bacillus subtilis* | | 107 | $DxHx_{10}Hx_2H$ | Zn(II)—tetrahedral | Transcriptional repressor of own gene, *cadA* and the *czcD-trkA* operon. | O31844 |
| | | Rv2358 | *Mycobacterium tuberculosis* | | 135 | C in amino terminal and $CxHx_3D$ in α3/ $DxHx_{10}Hx_2E$ in α5 | Zn(II)—tetrahedral | Transcriptional repressor of the *Rv2358-furB(zur)* operon. | P9WMI5 |

**Table 2** continued

| No. | Sensory motif | Protein | Organism | Domain | Length (aa) | Motif Signature(s)[h] | Metal ion(s) and coordination chemistry | Function(s) | Uniprot ID[c] |
|---|---|---|---|---|---|---|---|---|---|
| 8 | α5c | NmtR | *Mycobacterium tuberculosis* | Bacteria | 120 | $DxHx_{10}Hx_2HxHx_6H$ (last two histidines are in carboxy terminal) | Ni(II)—octahedral; Co(II)—penta-/Hepta-coordinate | Transcriptional repressor of *ctpJ/nmtA*. | O69711 |
| 9 | α53 | KmtR | *Mycobacterium tuberculosis* | Bacteria | 130 | $Hx_{12}EHx_7HH$ | Ni(II)—octahedral; Co(II)—penta-coordinate | Represses expression of *Rv2025c* and its own expression. | O53838 |
| 10 | α5–4 | ArsR | *Acidithiobacillus ferrooxidans* | Bacteria | 118 | $CCx_5C$ | As(III), Sb(III)—trigonal | Transcriptional repressor of *arsCRBH* operon. | B7J952 |
| | | ArsR1 | *Agrobacterium* | | 119 | $CCx_6C$ | As(III)—trigonal | Transcriptional repressors of *ars1* (*arsR2-orf2-orf3-arsH1C2-acr3*$_1$*-arsC1C4R1*) operon. | H0HHH0 |
| | | ArsR2 | *tumefaciens* 5A | | 122 | $CCx_{15}C$ | | | H0HHH8 |
| | | ArsR3 | | | 123 | $CCx_{15}C$ | NF[g] | Possible regulator of a *lysR*-type gene. | H0HG04 |
| | | ArsR4 | | | 118 | $CCx_6C$ | As(III)—trigonal | Transcriptional repressors of *ars2* (*arsR3-orf2-arsH2C3-acr3*$_2$*-arsR4*) operon. | H0HG09 |
| | | ArsR | *Pannonibacter indicus* HT23 | | 127 | $CCx_6C$ | As(III)—trigonal | Transcriptional repressors of *arsR-orf2-acr3-arsC* operon. | A0A0A7RS07 |
| | | ArsR | *Chromobacterium violaceum* ATCC 12472 | | 110 | $CCx_4C$ | As(III)—trigonal | Transcriptional repressors of *arsRCB* operon. | Q7NVA5 |
| | | ArsR | *Acidithiobacillus caldus* | | 121 | $CCx_5C$ | As(III)—trigonal | Transcriptional repressors of *arsRBC* operon. | Q0PL59 |
| | | ArsR | *Acidithiobacillus caldus* Tn*AtcArs* | | 125 | $CCx_6C$ | As(III)—trigonal | Transcriptional repressors of *arsRCDADA-orf7-orf8-arsB* operon. | Q3T563 |
| | | ArsR | *Leptospirillum ferriphilum* Tn*LfArs* | | 125 | $CCx_6C$ | As(III)—trigonal | Transcriptional repressors of *arsRCDA-cbs-arsB* operon. | Q2LMN8 |
| | | ArsR | *Sinorhizobium meliloti* Rm1021 | | 137 | $CCx_6C$ | As(III)—trigonal | Transcriptional repressors of *arsR-aqpS-arsC* operon. | Q92R42 |

**Table 2** continued

| No. | Sensory motif | Protein | Organism | Domain | Length (aa) | Motif Signature(s)[h] | Metal ion(s) and coordination chemistry | Function(s) | Uniprot ID[c] |
|---|---|---|---|---|---|---|---|---|---|
| | | ArsR2 | *Ochrobactrum tritici* SCII24$^T$ | | 117 | $CCx_6C$ | As(III)—trigonal | Transcriptional repressors of the *ars2* (*arsR2C1-acr3-arsC2HR3*) operon. | A9X5I3 |
| | | ArsR3 | | | 121 | $CCx_{15}C$ | | | A9X5I8 |
| 11 | α55 | ArsR | *Bacteroides vulgatus* ATCC 8482 | Bacteria | 108 | $Cx_6CC$ | As(III), MAs(III)—trigonal | Transcriptional repressor of *arsR-orf1-orf2-orf3-arsDAC-acr3* operon. | A6L7W8 |
| 12 | α4c | MerR | *Streptomyces lividans* | Bacteria | 125 | $Cx_3C$ in α4 and C in carboxy-terminal | Hg(II)—trigonal | Negatively regulates the mercuric reductase *merA* and the organolyase *merB*. | P30346 |
| | | CmtR | *Mycobacterium tuberculosis* | | 118 | $Cx_3C$ in α4 and C in carboxy-terminal | Cd(II), Pb(II)—trigonal | Transcriptional repressor of the *cmtA-Rv1993c-cmtR* operon. | P9WMI9 |
| 13 | α4c2 | SCO0875/ CmtR | *Streptomyces coelicolor* | Bacteria | 111 | $Cx_3C$ in α4 and C in carboxy-terminal/C in α2 and CC in carboxy-terminal | Cd(II), Pb(II)—trigonal | Transcriptional repressor of cation diffusion facilitator (CDF) family of metal transporters. | Q9RD34 |
| | | SCO3522 | | | | | ND[e] | | Q9X898 |
| 14 | α2–α5[a] | HlyU | *Vibrio cholerae* N16961 | Bacteria | 108 | NA[d] | NA[d] | Up-regulates the expression of the hemolysin gene *hlyA*. | P52695 |
| | | | *Vibrio vulnificus* CMCP6 | | 98 | | | Positive regulator of *rtxA1* gene. | Q8DES3 |
| | | | *Vibrio anguillarum* | | 92 | | | Positive regulator of *vah1-plp* and *rtxACHBDE* gene clusters. | E5KID8 |
| | | BigR | *Xylella fastidiosa* | | 114 | | | Represses an operon that comprises itself, *XF_0764*, *XF_0765*, *XF_0766* and *blh*. | Q9PFB1 |
| | | SoxR | *Pseudaminobacter salicylatoxidans* | | 121 | | | Regulates expression of the sulfur oxidation *sox* operon. | Q5ZQN5 |
| | | YgaV | *Escherichia coli* | | 99 | | | Transcriptional repressor of *ygaVP* operon. | P77295 |

**Table 2** continued

| No. | Sensory motif | Protein | Organism | Domain | Length (aa) | Motif Signature(s)[h] | Metal ion(s) and coordination chemistry | Function(s) | Uniprot ID[c] |
|---|---|---|---|---|---|---|---|---|---|
| | | PigS | *Serratia* sp. | | 118 | | | Represses transcription of *pigS-pmpABC* and *blhA-orfY* operons. | E7BBJ0 |
| | | SqrR | *Rhodobacter capsulatus* SB1003 | | 110 | | | Represses the expression of sulfide responsive genes. | D5AT91 |
| 15 | α2–α52 | AioF | *Thiomonas arsenitoxydans* | Bacteria | 118 | C in α2 and $Cx_3C$ in α5 | As(III), As(V)—trigonal | Positively regulates expression of *aioBA-cyc1-aioF-cyc2* operon. | D6CMI1 |
| 16 | None/ Unknown[b] | NolR | *Rhizobium meliloti* | Bacteria | 118 | NA[d] | Unknown[b] | Negative regulation of the *nod* regulon. | Q83TD2 |
| | | | *Rhizobium leguminosarum* | | 105 | | | Represses nodulation gene *nodD* and *nodABCIJ* operon. | O54057 |
| | | NhlD | *Rhodococcus rhodochrous* | | 112 | | | Negative regulation of *nhlBA* operon. | Q53040 |
| | | PagR | *Bacillus anthracis* Plasmid pXO1 | | 99 | | | Represses the expression of the *pagAR* operon and *atxA* gene. | O31178 |
| | | SdpR | *Bacillus subtilis* | | 90 | | | Represses the transcription of the *sdpIR* operon. | O32242 |
| | | PyeR | *Pseudomonas aeruginosa* | | 100 | | | Negatively regulates *pyeR-pyeM-xenB* operon. | Q9HW47 |
| | | Rv2034 | *Mycobacterium tuberculosis* | | 107 | | | Positively regulates transcription of various genes, such as *phoP*, *groEL2* and *dosR*. Negatively regulates its own transcription. | O53478 |
| | | Ms6762 | *Mycobacterium smegmatis* | | 115 | | | Positively regulates transcription of *phoP*, *groEL2* and *dosR*. | A0R732 |
| | | MJ223 | *Methanococcus jannaschii* | Archaea | 152 | | | Putative HTH-type transcriptional regulator. | Q58958 |

**Table 2** continued

| No. | Sensory motif | Protein | Organism | Domain | Length (aa) | Motif Signature(s)[h] | Metal ion(s) and coordination chemistry | Function(s) | Uniprot ID[c] |
|---|---|---|---|---|---|---|---|---|---|
| | | PH1061 | *Pyrococcus horikoshii* OT3 | | 100 | | | Putative HTH-type transcriptional regulator. | O58788 |
| | | PH1932 | *Pyrococcus horikoshii* OT3 | | 192 | | | Putative HTH-type transcriptional regulator. | O59595 |
| | | Phr | *Pyrococcus furiosus* | | 202 | | | Negative regulation of *hsp20*, an $AAA^+$ ATPase and several other genes. | Q8U030 |

The helices are numbered according to those of SmtB X-ray crystal structure (Cook et al. 1998)

[a] Does not sense metal(s)

[b] No known metal binding residue/motif present

[c] Uniprot identifier (UniProt Consortium 2015)

[d] *NA* not applicable

[e] *ND* not determined

[f] Uniprot ID not available; GenBank Accession (Benson et al. 2013): WP_024968085.1

[g] NF = Not inducible by As(III)

[h] 'x' denotes any amino acid, *C* cysteine, *D* aspartic acid, *H* histidine, *E* glutamic acid

$CxCx_2C$ sensory motif in the C-terminal region instead of usual α3 helix (Gihring et al. 2003; Baker-Austin et al. 2007). *L. ferriphilum arsRC* genes are unusual in that they form one continuous ORF and encodes a 297 aa long fusion protein (Tuffin et al. 2006).

α5 motif

Another founding member of the ArsR–SmtB family, *Synechococcus* sp. PCC 7942 SmtB, functions as a transcriptional repressor that in the absence of Zn(II) represses transcription of *smtA* gene, which encodes a class II metallothionein protein SmtA involved in sequestering excess metal ions inside the cell (Huckle et al. 1993; Turner and Robinson 1995). Although Zn(II) is the preferred metal ion for SmtB, it also senses Cd(II), Cu(II), Co(II), Hg(II), Ni(II), Au(II) and Ag(I) with variable affinities (Turner and Robinson 1995). SmtB has two metal-sensory motifs (α3N and α5; Fig. 3) that binds metal ions, although α5 site is the regulatory one and α3N is the non-regulatory site (VanZile et al. 2002a, b). The α5 site binds Zn(II) ion tightly via Asp104 and His106 residues of one subunit and, His117 and Glu120 from other subunit in a tetrahedral symmetry with a consensus '$DxHx_{10}Hx_2$(-E/H)' sensory motif in α5 (VanZile et al. 2000). The non-regulatory α3N site binds metal ions by Cys61 and Asp64 residues from the α3 helix ($Cx_2D$) of one subunit and, Cys14 and His18 residues (VanZile et al. 2002a) with the motif '$Cx_3H$' in N-terminal region (Table 1). Another well studied member of the α5 group (Table 2) is CzrA from *S. aureus* 912 (Kuroda et al. 1999) and *B. subtilis* (Harvie et al. 2006). Unlike SmtB, CzrA has only α5 sensory site with the typical '$DxHx_{10}Hx_2H$' motif. *S. aureus* CzrA represses *czrAB* operon that codes for the repressor itself and CzrB protein which functions as a cation diffusion facilitator (CDF) antiporter efflux pump (Cherezov et al. 2008). The expression of *czrAB* operon is induced by metal ions with variable affinity, Zn(II)>Co(II)≫Ni(II) (Pennella et al. 2003). The *B. subtilis* CzrA represses its own transcription, *cadA* (a P-type ATPase) and *czcD-trkA* (*czcD* and *trkA* encodes a CDF and a cation exporter, respectively) operon with variable degrees (Harvie et al. 2006). Rv2358 from *M. tuberculosis* is another protein that belongs to the α5 group (Table 2) with the '$DxHx_{10}Hx_2E$' motif, represses *Rv2358-furB* (*zur*) operon, which encodes the repressor itself and a zinc uptake regulator FurB (Zur) (Milano et al. 2004; Canneva et al. 2005; Maciag et al. 2007).

α3N motif

The cadmium resistant *cad* operon, originally identified in *S. aureus* plasmid pI258, has two genes *cadC* (transcriptional repressor) and *cadA* (P-type ATPase) (Novick and Roth 1968; Nucifora et al. 1989). Similar to SmtB, CadC has both α3N (N-terminal $Cx_3C$ and α3 CxC) and α5 ($DxHx_{10}Hx_2E$) sensory sites (Sun et al. 2001). Only α3N site is the regulatory site (Busenlehner et al. 2002a) in CadC, whereas in SmtB α5 is the regulatory one (VanZile et al. 2002a, b). The regulatory α3N site may adopt tetrahedral (Busenlehner et al. 2001) or trigonal (Busenlehner et al. 2002a) coordination complex and senses a wide range of metal ions like Cd(II), Bi(III), Co(II), Zn(II), Pb(II), and Hg(II) (Endo and Silver 1995; Busenlehner et al. 2002a, b), while non-regulatory α5-motif binds Zn(II) and Co(II) metal ions (Busenlehner et al. 2002a; Ye et al. 2005). Residues Cys7 and Cys11 in N-terminal of one subunit, and Cys58 and Cys60 in α3 from other subunit form the inter-subunit association via the metal ions (Wong et al. 2002). Out of four cysteine residues CadC have, only Cys7, Cys58 and Cys60 are required for its biological activity (Busenlehner et al. 2002a).

Other than *S. aureus*, CadC protein was found in several other organisms (Table 2), e.g., *Listeria monocytogenes* Plasmid pLm74 (Lebrun et al. 1994), *Lactococcus lactis* Plasmid pND302 (Liu et al. 1997), *Stenotrophomonas maltophilia* D457R (Alonso et al. 2000), *Bacillus stearothermophilus* LV (Nerey Md Mdel et al. 2002), *Bacillus firmus* (Ivey et al. 1992) and *Streptococcus thermophilus* 4134 (Schirawski et al. 2002). While CadC from *S. aureus*, *B. firmus*, *B. stearothermophilus* and *S. maltophilia* has both α3N and α5 sites, *L. monocytogenes*, *L. lactis* and *S. thermophilus* CadC contain only α3N site (Table 2).

Other bacterial proteins that belong to the α3N group (Table 2) are ArsR from *Desulfovibrio desulfuricans* G20 (Li and Krumholz 2007), ArsR1 from *Pseudomonas putida* KT2440 (Fernández et al. 2016), ArsR2 from *Streptomyces* sp. FR-008 Plasmid pHZ227 (Wang et al. 2006), ArsRC2 from *Microbacterium* sp. A33 (Achour-Rokbani et al. 2010), Rv2642 from *M. tuberculosis* (Li et al. 2016b), AztR from *Nostoc* sp. (Liu et al. 2005) and AseR from *B. subtilis*

168 (Harvie et al. 2006) senses not only Cd(II) but also As(III), Sb(III), Bi(III), Pb(II), Zn(II), etc. ArsR1 and ArsR2 protein from archaeon *Halobacterium* sp. Plasmid pNRC100 (Wang et al. 2004) also have signatures of α3N group and senses As(III) and Sb(III). Sometimes, it is difficult to ensure whether a protein belong to α3 or α3N motif as two cysteine residues in the α3 helix are enough for metalloregulation (Shi et al. 1996). For example, ArsR1 from *P. putida* (Fernández et al. 2016) and AseR from *B. subtilis* (Harvie et al. 2006) both have one N-terminal cysteine residue and CxC motif in α3 helix (Table 2). The position of N-terminal cysteine is not far from α3 helix and compared to SmtB sequence that cysteine residue would fall in α1 helix, unless both α1 and α2 helices are much shorter in length compared to SmtB (Fig. 2). Further experiments are required to correctly ascertain the function of N-terminal cysteine residue in these proteins and place them in the correct group.

The *ars* operon of *Microbacterium* sp. has an unusual *arsRC2* fusion gene (Achour-Rokbani et al. 2010). This kind of fusion of the ArsR and ArsC proteins has been previously described in *L. ferriphilum* (Tuffin et al. 2006). The C-terminal region of 331 aa long ArsRC2 protein is related to putative arsenate reductases while the N-terminal portion has homology to transcriptional repressors of the ArsR–SmtB family. The N-terminal ArsR-domain contains a putative arsenite binding signature (ESCVCDL), almost identical to that of *E. coli* ArsR (ELCVCDL), and a contiguous DNA binding site with wHTH motif (Gladysheva et al. 1994; Achour-Rokbani et al. 2010). This kind of unusual fusion might reduce the problem of the diffusion of arsenic to the inducer attachment site and enhance the efficiency of transcription in response to arsenate.

### α3N–α5 motif

Zn(II)-sensor ZiaR from *Synechocystis* PCC 6803, represses *ziaA* which encodes a heavy metal transporting P-type ATPase, has both α3N (N-terminal $Cx_5H$ and α3 CxC) and α5 ($DxHx_{10}Hx_2E$) metal sensory sites (Thelwell et al. 1998). Another member of α3N–α5 group, *Oscillatoria brevis* BxmR represses the expression of *bxa1* (encoding a CPx-ATPase metal transporter), *bxmR* and *bmtA* (a heavy metal sequestering metallothionein) (Liu et al. 2004). BxmR binds to both monovalent, Ag(I) and Cu(I), and divalent, Zn(II) and Cd(II), metal ions and interestingly, also found to be induced by thiol oxidants diamide and $H_2O_2$ (Hirose et al. 2006). While both α3N and α5 sensory sites are essential for the inducer responsiveness of ZiaR (Thelwell et al. 1998), for BxmR either α3N (senses copper, cadmium, silver and zinc) or α5 (senses zinc) site is sufficient for zinc mediated regulation (Liu et al. 2008). Unlike other ArsR–SmtB sensors, BxmR can adopt an extended range of coordination chemistries (trigonal or tetrahedral) due to the presence of multiple metal-sensing residues in its α3N site ($Hx_7Cx_3Hx_3C$ in N-terminal and CxC in the α3 helix) that can sense a wide range of metals while α5 is primarily restricted to Zn(II) sensing (Liu et al. 2008).

### α5c motif

NmtR, a Ni(II)/Co(II)-sensing repressor, was the first ArsR–SmtB family member that has been characterized in *M. tuberculosis*, represses *nmt* operon that contains *nmtA* gene which encodes a P-type ATPase metal efflux pump (Cavet et al. 2002). NmtR binds to Ni(II), Co(II) and Zn(II) with varying sensitivity, Ni(II)>Co(II)>Zn(II), and Zn(II) is not a potent allosteric regulator of DNA binding as compared to Ni(II) or Co(II) (Pennella et al. 2003). Interestingly, in cyanobacterium *Synechococcus* PCC 7942, NmtR-mediated repression was found to be only alleviated by Co(II) and not Ni(II), despite Ni(II) is known to be the most effective inducer in *M. tuberculosis*, which indicates that cytosolic metal concentrations in different hosts can influence the metal-responsiveness of these transcriptional repressors (Cavet et al. 2002). NmtR requires six residues (Asp91, His93, His104, His107, His109 and His116; Fig. 2) for Ni(II)- or Co(II)-responsiveness in vivo (Cavet et al. 2002). Out of which four (Asp91, His93, His104 and His107) residues are provided by the α5 helices of two monomers and the extra two residues are extended by the C-terminal extensions in NmtR homodimer forming α5c sites with $DxHx_{10}Hx_2HxHx_6H$ motif (Pennella et al. 2003). Interestingly, the N-terminal 'Gly2-His3-Gly4' residues, in *M. tuberculosis* NmtR, are found to form an alternate metal-sensory site with Asp91, His93, His104 and His107 residues, replacing the C-terminal His109 and His116 amino acids (Reyes-Caballero et al. 2011; Lee et al. 2012). The mutant of N-terminal His3 has been found to be

significantly more sensitive to Zn(II)-mediated regulation than the Co(II)-mediated one which indicates that His3 has a direct role in this Ni(II)/Co(II)-mediated allosteric switch (Reyes-Caballero et al. 2011).

α53 motif

After NmtR, KmtR is the second novel Ni(II)/Co(II)-sensing ArsR–SmtB family member characterized from *M. tuberculosis* (Campbell et al. 2007). KmtR represses transcription of *Rv2025c*, encoding a CDF-family metal exporter and its own gene. KmtR-dependent repression was alleviated by binding to Ni(II) or Co(II). Although, both KmtR and NmtR binds Ni(II) and Co(II), KmtR binds these metal ions much tighter than that of NmtR suggesting importance of sensing variable concentrations of these metals by *M. tuberculosis*. In KmtR, His88, Glu101, His102, His110, and His111 form a new sensory site α53 (Table 1) with the motif '$Hx_{12}EHx_7HH$' (Campbell et al. 2007).

α5–4 motif

The *ars* operon in *Acidithiobacillus ferrooxidans* is controlled by an As(III)-responsive transcriptional repressor, ArsR. Interestingly, As(III) binding site in *A. ferrooxidans* ArsR has no resemblance to the traditional α3 sensory motif found in plasmid R773 of *E. coli* and others (Table 1). Instead, it has three cysteine residues, Cys95, Cys96, and Cys102, constituting a unique As(III)-sensory site ($CCx_6C$) at α5-helix designated α5–4 (Qin et al. 2007), where Cys95 and Cys96 residues in the α5 helix form a trigonal coordination metal-binding site with C-terminal Cys102 residue.

Several other bacteria found to possess α5–4 sensory site, e.g., ArsR2 and ArsR3 from *O. tritici* SCII24$^T$ (Branco et al. 2008), ArsR1, ArsR2, ArsR3 and ArsR4 from *A. tumefaciens* 5A (Kang et al. 2016), ArsR proteins from *Pannonibacter indicus* HT23 (Bandyopadhyay and Das 2016), *Chromobacterium violaceum* ATCC 12472 (Azevedo et al. 2008; Arruda et al. 2016), *Acidithiobacillus caldus* (Kotze et al. 2006), *A. caldus* Tn*AtcArs* (Tuffin et al. 2005), *L. ferriphilum* Tn*LfArs* (Tuffin et al. 2006) and *Sinorhizobium meliloti* Rm1021 (Yang et al. 2005). The consensus motif for α5–4 site is either $CCx_{4\text{-}6}C$ or $CCx_{15}C$ at and near α5 helix (Table 1).

α55 motif

ArsR from *Bacteroides vulgatus* ATCC 8482, obligate anaerobe and a common member of the human gut microbiota, is found to be very sensitive to the organoarsenicals methylarsenite MA(III), and arsenite As(III). This arsenic-inducible transcriptional repressor of the *ars* operon in *B. vulgatus* confers high resistance to MAs(III), followed by As(III), suggests that this organism maintains an *ars* operon as the result of dietary exposure to inorganic arsenic (Li et al. 2016a). In *B. vulgatus* ArsR, Cys99 residue from α5, and C-terminal Cys106 and Cys107 residues are predicted to form a new As(III)-sensory site ($Cx_6CC$), designated α55 (Li et al. 2016a), contrary to α5–4 sensory site where two cysteine residues from α5 helix constitute trigonal metal-binding site with one C-terminal cysteine (Qin et al. 2007).

α3N–2 motif

*C. glutamicum* is one of the most arsenic-resistant microorganisms known and can grow in presence of elevated concentrations of arsenite or arsenate (Ordóñez et al. 2005). ArsR1 and ArsR2 proteins from *C. glutamicum*, binds As(III) or Sb(III) by a cysteine triad composed of Cys15, Cys16, and Cys55 residues comprising the $CCx_{38}C$ sensory motif (Table 1) (Ordóñez et al. 2005, 2008). This binding motif is distinctly different from other characterized ArsR–SmtB family regulators (Sun et al. 2001; Gladysheva et al. 1994; Turner and Robinson 1995).

α33 motif (nonmetal-binding)

*C. glutamicum* CyeR, is a unique redox-sensing transcriptional regulator that binds to the intergenic region between *cyeR* and *cye1* (encodes an old yellow enzyme family protein), induced by oxidative stress (Ehira et al. 2010). CyeR does not bind any metal ions, but in the presence of oxidants such as diamide and $H_2O_2$, the DNA-binding activity of CyeR is found to be destabilized (Ehira et al. 2010). It has two cysteine residues (Cys36 and Cys43), with the sensory motif $Cx_6C$ in and close to α3 helix, but only Cys43 found to have a role in redox regulation (Ehira et al. 2010).

α3–4 motif

Plasmid pHZ227 in *Streptomyces* sp., encodes an As(III)-sensing ArsR1 protein, that represses *arsR-BOCT* operon, has a unique metal sensory site designated α3–4, not observed in classical members of ArsR–SmtB family (Wang et al. 2006). ArsR1 is predicted to sense arsenite via $Cx_2H$ motif in the α3 helix and one cysteine residue located between β1 and β2 strands of wHTH DNA-binding region (Fig. 2).

α4c motif

The Cd(II)/Pb(II)-sensing CmtR in *M. tuberculosis* is structurally distinct from the other Cd(II)/Pb(II) sensor CadC of *S. aureus* plasmid pI258 in a way that CmtR binds Cd(II) or Pb(II) via coordination by α4 sensors (Cys57 and Cys61) and C-terminal Cys102 forming a distinct α4c site instead of α3N in CadC (Cavet et al. 2003). CmtR represses *cmt* operon encoding CmtA which is closely related to *S. aureus* CadA (Yoon et al. 1991) and *E. coli* ZntA (Rensing et al. 1997), and encodes Zn(II)/Cd(II)/Pb(II) P-type ATPase efflux pumps (Cavet et al. 2003). C-terminal residue Cys102 functions as a key allosteric metal-sensor in CmtR that influences disassembly of CmtR-*cmt* O/P oligomeric complexes in the presence of metal ions (Wang et al. 2005). Metal-dependent expression from CmtR-*cmt* and NmtR-*nmt* O/P revealed that CmtR is insensitive to Ni(II) and NmtR is insensitive to Cd(II) or Pb(II) (Cavet et al. 2003). MerR of *Streptomyces lividans* 1326 functions as a repressor and has α4c motif (Brünker et al. 1996). MerR binds in the intercistronic region between two operons and negatively regulate several genes, including a mercuric reductase *merA* and an organolyase *merB* (Rother et al. 1999). The repression is alleviated by binding of mercuric ions Hg(II) to the MerR (Brünker et al. 1996). Interestingly, in all the cases of mercury resistances which are mediated by Hg(II) reduction, the genes are usually regulated by activator proteins, except MerR of *S. lividans* that function as a repressor, a hallmark of ArsR–SmtB family regulators (Brünker et al. 1996).

α4c2 motif

*S. coelicolor* CmtR, in contrast to *M. tuberculosis* CmtR, binds Pb(II) or Cd(II) by forming two pairs of sulfur-rich coordination complexes per dimer (Wang et al. 2010), instead of one pair in *M. tuberculosis* (Cavet et al. 2003). While, metal-sensory site 1 resembles exactly to the α4c site of *M. tuberculosis* CmtR, the second metal-binding site is coordinated by the C-terminal Cys110 and Cys111 residues. Site 1 binds Cd(II) tightly than Pb(II) and mediates transcriptional derepression, in contrast, site 2 ligands Cys110 and Cys111 only show Cd(II)-responsiveness (Wang et al. 2010). The residue Cys24 from α2 helix is predicted to be the third thiolate ligand to complete the trigonal coordination structure at metal site 2 with C-terminal Cys110 and Cys111 residues, but Cys24 does not have any regulatory role as its absence has no influence on the Cd(II)-responsiveness at site 2 (Wang et al. 2010).

α2–α5 motif (nonmetal-binding)

HlyU protein from *V. cholerae* and *V. vulnificus* positively regulates the expression of hemolysin *hlyA* (Williams and Manning 1991; Williams et al. 1993) and RTX toxin *rtxA1* (Liu et al. 2007b) genes, respectively, by binding directly to their cognate DNA upstream of the genes (Liu et al. 2007b; Mukherjee et al. 2015). In *V. vulnificus*, HlyU activates transcription of *rtxA1* toxin gene by acting as a repressor of H-NS which negatively regulates the expression of the *rtxA1* gene (Liu et al. 2009a). H-NS not only represses the transcription of the RTX toxin and its transport system, but also found to directly inhibit transcription of hemolysin gene *hlyA*. However, transcriptional silencing of the *hlyA* gene is found to be counteracted by the *V. cholerae* transcriptional activator HlyU (Wang et al. 2015). Therefore, HlyU acts as a repressor of another repressor H-NS (Liu et al. 2009a; Wang et al. 2015). HlyU found to be a member of ArsR–SmtB family, but it does not have any metal-sensory residues or motifs typical of the ArsR–SmtB family and constitutes a unique group, designated α2–α5 that does not sense metals (Saha and Chakrabarti 2006). Molecular dynamics (MD) simulation studies on *V. cholerae* HlyU reveal that the DNA binding residues tend to move away from the DNA bases when the distance between the Cys38 (in α2) and Cys104 (in α5) residues was small. In contrast, in the DNA bound form, the distance between Cys38 and Cys104 increases during simulation indicating the presence of a redox switch. The DNA-bound reduced form is responsible for activating *hlyA* gene and in the

presence of an oxidizing agent repression is established (Mukherjee et al. 2015). Also, under oxygen-limiting conditions (e.g., host intestines, etc.) *V. cholerae* was predicted to use the redox switch for increased expression of virulence genes (Liu et al. 2011).

In *Vibrio anguillarum*, two gene clusters *vah1* and *rtxACHBDE* are found to be responsible for the hemolytic and cytotoxic activities in fish, and are positively regulated by the HlyU protein like other bacterial HlyU proteins (Li et al. 2011). These two gene clusters are again silenced by the negative regulatory action of H-NS protein and *V. anguillarum* HlyU act to alleviate that repression by acting as a repressor of H-NS (Mou et al. 2013).

BigR in *X. fastidiosa* and *A. tumefaciens* is structurally similar to *V. cholerae* HlyU and found to undergo similar DNA-binding and release using a redox switch. In the reduced DNA-bound form of BigR, the two critical cysteine residues (Cys42 and Cys108 in α2 and α5 helices, respectively) found to be wide apart while the oxidized form indicates a reduction in distance between these residues due to the formation of disulfide bridge that results in dissociation of BigR from its cognate DNA (Guimarães et al. 2011). BigR binds to the 'BigR-box' in the *Xylella* and *Agrobacterium* promoters, and strongly represses transcription of an operon (encodes BigR, membrane proteins and beta-lactamase-like hydrolase BLH) responsible for biofilm formation (Barbosa and Benedetti 2007). BigR is found to be easily reduced, but difficult to oxidize as two unbound cysteine residues are not very accessible and a hydrogen sulfide-induced reactive oxygen is predicted to oxidize BigR (Guimarães et al. 2011) (Fig. 4).

In purple photosynthetic bacterium *Rhodobacter capsulatus*, transcriptional repressor SqrR functions as a master regulator of sulfide-dependent gene expressions. In absence of $H_2S$, with two cysteins (Cys41 and Cys107) that are in reduced form, SqrR binds the promoter region and represses the expression of sulfide-responsive genes (SRGs). In presence of $H_2S$, reactive sulfur species (RSS) promotes the formation of a sulfide bond between Cys41 and Cys107 residues, thereby inhibiting the ability of SqrR to bind to the promoter and derepression occurs (Shimizu et al. 2017) (Fig. 4).

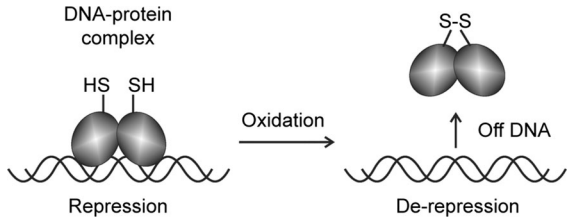



**Fig. 4** Model of redox-responsive gene regulation. The reduced form of the protein binds to the promoter region and represses the expression of downstream genes in the operon. In the presence of an oxidizing agent, oxidized protein displaces from the promoter region. The RNA polymerase binds DNA and subsequently induces gene expression

PigS is another ArsR–SmtB family transcriptional regulator, belong to α2–α5 group, which represses expression of the red-pigmented prodigiosin antibiotic genes via the control of divergent operons in *Serratia* sp. (Gristwood et al. 2011). YgaV is an autoregulated, tributyltin (TBT)-inducible repressor found in *E. coli* that represses *ygaVP* operon. The *ygaVP* operon encodes YgaP protein, which is a membrane-associated protein with sulfur transferase (rhodanese) activity (Gueuné et al. 2008). The dimeric thiosulfate-inducible repressor SoxR binds cooperatively to the promoters regulating expression of the sulfur oxidation *sox* operon in *P. salicylatoxidans* (Mandal et al. 2007).

### α2–α52 motif

Acidophilic and facultative chemoautotrophic bacterium *Thiomonas arsenitoxydans* encodes an ArsR–SmtB family metalloprotein AioF, stabilized by As(III) or As(V), but not Sb(III) or Mo(VI), binds to *aioBA* operon at two distinct places and induces its expression (Slyemi et al. 2013; Moinier et al. 2014). There are three cysteine residues, Cys53 in the α2 helix, and Cys111 and Cys115 in the α5 helix (Fig. 2), in AioF that binds As(III) or As(V) constitute the α2–α52 sensory motif (Slyemi et al. 2013) designated here. ArsR–SmtB family members known to repress the transcription of metal-resistant operons in the absence of the metal ions and derepresses them in their presence. Interestingly, contrary to ArsR–SmtB metalloregulators, AioF specifically activates transcription of the *aioBA* operon in presence of metal ions (Moinier et al. 2014).

### None/unknown motifs

This group includes non-classical ArsR-family regulators that do not have obvious metal-sensory motifs

and involved in processes which are independent of metal-sensing or resistance (Komeda et al. 1996; Ellermeier et al. 2006; Li et al. 2008; Keese et al. 2010; Gao et al. 2011, 2012; Mac Aogáin et al. 2012) (Table 2). Also, these regulators do not have any associated metal resistance genes such as metallothionins, metal reductases, or metal efflux pumps, which are usually linked with classical ArsR–SmtB family proteins (Busenlehner et al. 2003).

In spore-forming bacterium *B. subtilis*, SdpC induces the synthesis of an immunity protein SdpI that protects toxin-producing cells from being killed under starvation. SdpI is encoded by the *sdpIR* operon which is repressed by SdpR (Ellermeier et al. 2006). PyeR in *P. aeruginosa* negatively regulates *pyeR-pyeM-xenB* operon (*pyeM* encodes a major facilitator superfamily membrane transporter and *xenB* encodes an old yellow enzyme family reductase) and is the second ArsR regulator, after BigR (Guimarães et al. 2011), found to be involved in biofilm formation (Mac Aogáin et al. 2012). In *R. meliloti* (Kondorosi et al. 1991) and *R. leguminosarum* (Li et al. 2008) expression of the nodulation genes are repressed by a the NolR protein. In *Rhodococcus rhodochrous*, *nhlBA* operon encodes a nitrile hydratase (L-NHase) whose expression is repressed by the protein NhlD. In the presence of inducer amide, NhlC inhibits the repressor NhlD, leading to the expression of L-NHase, while in the absence of amide NhlC could not inhibit NhlD, leading to the repression of the L-NHase expression by NhlD (Komeda et al. 1996). In *B. anthracis* plasmid pXO1, PagR negatively controls expression of the *pagAR* operon that encodes a toxin gene *pagA*. PagR also represses transcription of *atxA*, a positive regulator of *pagA* (Hoffmaster and Koehler 1999). Rv2034 and Ms6762 proteins in *M. tuberculosis* and *Mycobacterium smegmatis*, respectively, positively regulates the expression of *dosR*, *phoP* and *groEL2* genes and represses own genes (Gao et al. 2011, 2012).

Archaeon *Methanococcus jannaschii* protein MJ223 (Ray et al. 2003), hyperthermophilic archaeon *Pyrococcus horikoshii* proteins PH1061 (Okada et al. 2006) and PH1932 (Itou et al. 2008) have structural features common to ArsR–SmtB family. Phr protein from the hyperthermophilic archaeon *Pyrococcus furiosus* inhibits transcription of its own gene, a small heat shock protein Hsp20 and an AAA+ ATPase (Vierke et al. 2003; Keese et al. 2010).

## Structural studies on ArsR–SmtB family of sensory proteins

ArsR–SmtB family members are included in InterPro database (Finn et al. 2017) with profile IPR001845 (HTH ArsR-type DNA-binding domain), in PROSITE database (Sigrist et al. 2013) with profile PS50987 (HTH_ARSR_2), in Pfam database (Finn et al. 2016) with profile PF01022 (HTH_5), in PRINTS database (Attwood et al. 2012) with profile PR00778 (HTHARSR) and in SMART database (Letunic et al. 2015) with profile SM00418 (HTH_ARSR). Several X-ray crystal and NMR structures of ArsR–SmtB family members have been solved over the years and these 3d structures help us to understand how conformational changes mediated by key sensory residues drive transcriptional regulation in this metallorepressor family (Table 3).

A highly conserved 'ELCV(C/G)D' motif named 'metal-binding box' was originally identified in the members of ArsR–SmtB family (Shi et al. 1994). This motif contains residues that directly involved in binding metal ions and several ArsR–SmtB repressors have been shown to use residues from this 'metal-box' including ArsR and CadC proteins (Endo and Silver 1995; Bruhn et al. 1996). However, with the discovery of several new and unique members the list of metal-sensory motifs found in this family expanded (Table 1). The X-ray structure of apo-SmtB found to contain the 'ELCVGD' motif in the α3 helix as part of the wHTH (α3-turn-α4) DNA-binding motif (Cook et al. 1998). The 2.2 Å resolution structure of SmtB from *Synechococcus* sp. strain PCC7942 is the first three-dimensional (3d) structure of ArsR–SmtB family that was solved by X-ray crystallography (Cook et al. 1998). This apo-SmtB structure showed that the protein is an elongated dimer with a twofold axis of symmetry consisting of 5 α-helices and 2 β-strands arranged into α1–α2–α3–α4–β1–β2–α5 motif (Fig. 5a). The dimeric interface is formed between the two N-terminal α1 and two C-terminal α5 helices. The DNA recognition helix-turn-helix (HTH) domain (α3-turn-α4), specially the α4 helix is highly conserved and is a distinguishing characteristic of the ArsR–SmtB family repressors. Helix 4 (α4) is also

**Table 3** Summary of ArsR–SmtB family proteins whose 3d structures have been solved

| No. | Sensory motif | Protein name | PDB ID(s) | Organism | Domain | Structure type | Structure note | Reference(s) |
|---|---|---|---|---|---|---|---|---|
| 1 | α3N | CadC | 1U2W, 3F72 | *Staphylococcus aureus* | Bacteria | X-ray | 1U2W (two Zn-bound form); 3F72 (D101G/H103A CadC) | Ye et al. (2005) and Kandegedara et al. (2009) |
| 2 | α5 | SmtB | 1SMT, 1R1T, 1R22, 1R23 | *Synechococcus elongatus* PCC 7942 | Bacteria | X-ray | 1SMT and 1R1T (apo-SmtB); 1R22 (two Zn-bound form); 1R23 (Zn-bound form) | Eicken et al. (2003) and Cook et al. (1998) |
| | | CzrA | 1R1U, 1R1V, 2KJB, 2KJC, 4GGG, 2M30 | *Staphylococcus aureus* | Bacteria | X-ray (1R1U, 1R1V, 4GGG); NMR (2KJB, 2KJC, 2M30) | 1R1U (apo-CzrA); 1R1V (Zn-bound form); 2KJC (two Zn-bound form); 2KJB (apo-CzrA); 4GGG (V66A/L68V CzrA, Zn-bound form); 2M30 (two Zn-bound form) | Eicken et al. (2003), Arunkumar et al. (2009) Campanello et al. (2013), and Chakravorty et al. (2013) |
| 3 | α5c | NmtR | 2LKP | *Mycobacterium tuberculosis* | Bacteria | NMR | Apo-NmtR | Reyes-Caballero et al. (2011) |
| 4 | α4c | CmtR | 2JSC | *Mycobacterium tuberculosis* | Bacteria | NMR | Two Cd-bound form | Banci et al. (2007) |
| 5 | α2–α5[a] | HlyU | 4OOI, 4K2E | *Vibrio cholerae* N16961 | Bacteria | X-ray | 4K2E (oxidized form); 4OOI (reduced form) | Mukherjee et al. (2014) |
| | | | 3JTH | *Vibrio vulnificus* CMCP6 | Bacteria | X-ray | Reduced form | Nishi et al. (2010) |
| | | BigR | 3PQJ, 3PQK | *Xylella fastidiosa* | Bacteria | X-ray | 3PQJ (reduced form); 3PQK (oxidized form) | Guimarães et al. (2011) |
| 6 | None/ Unknown[b] | PagR | 2ZKZ | *Bacillus anthracis* | Bacteria | X-ray | Has ArsR–SmtB family signature | Zhao et al. (2010) |
| | | NolR | 4OMY, 4OMZ, 4ON0 | *Sinorhizobium fredii* | Bacteria | X-ray | 4OMY (DNA-bound form); 4OMZ (unliganded); 4ON0 (DNA-bound form) | Lee et al. (2014) |
| | | MJ223 | 1KU9 | *Methanocaldococcus jannaschii* | Archaea | X-ray | Has ArsR–SmtB family signature (partial) | Ray et al. (2003) |
| | | PH1061 | 1UB9 | *Pyrococcus horikoshii* OT3 | Archaea | X-ray | Has ArsR–SmtB family signature | Okada et al. (2006) |
| | | Phr | 2P4W | *Pyrococcus furiosus* | Archaea | X-ray | Has ArsR–SmtB family signature (partial) | Liu et al. (2007c) |
| | | PH1932 | 1ULY | *Pyrococcus horikoshii* OT3 | Archaea | X-ray | Has ArsR–SmtB family signature (partial) | Itou et al. (2008) |

[a] Does not sense metal(s)

[b] No known metal binding residue/motif present

termed as the DNA recognition helix (αR) that binds to the DNA at the major groove, and β1/β2-strands form the wing similar to other winged-HTHs. This wHTH domain (α3-turn-αR) has strong structural similarity to other bacterial transcriptional regulators like catabolite activator protein CAP (Schultz et al. 1991), Fe(III)-regulated diphtheria toxin repressor DtxR (Pohl et al. 1999), LysR-family proteins (Mukherjee et al. 2009; Alanazi et al. 2013), MerR-family proteins (Shewchuk et al. 1989; Huang et al.

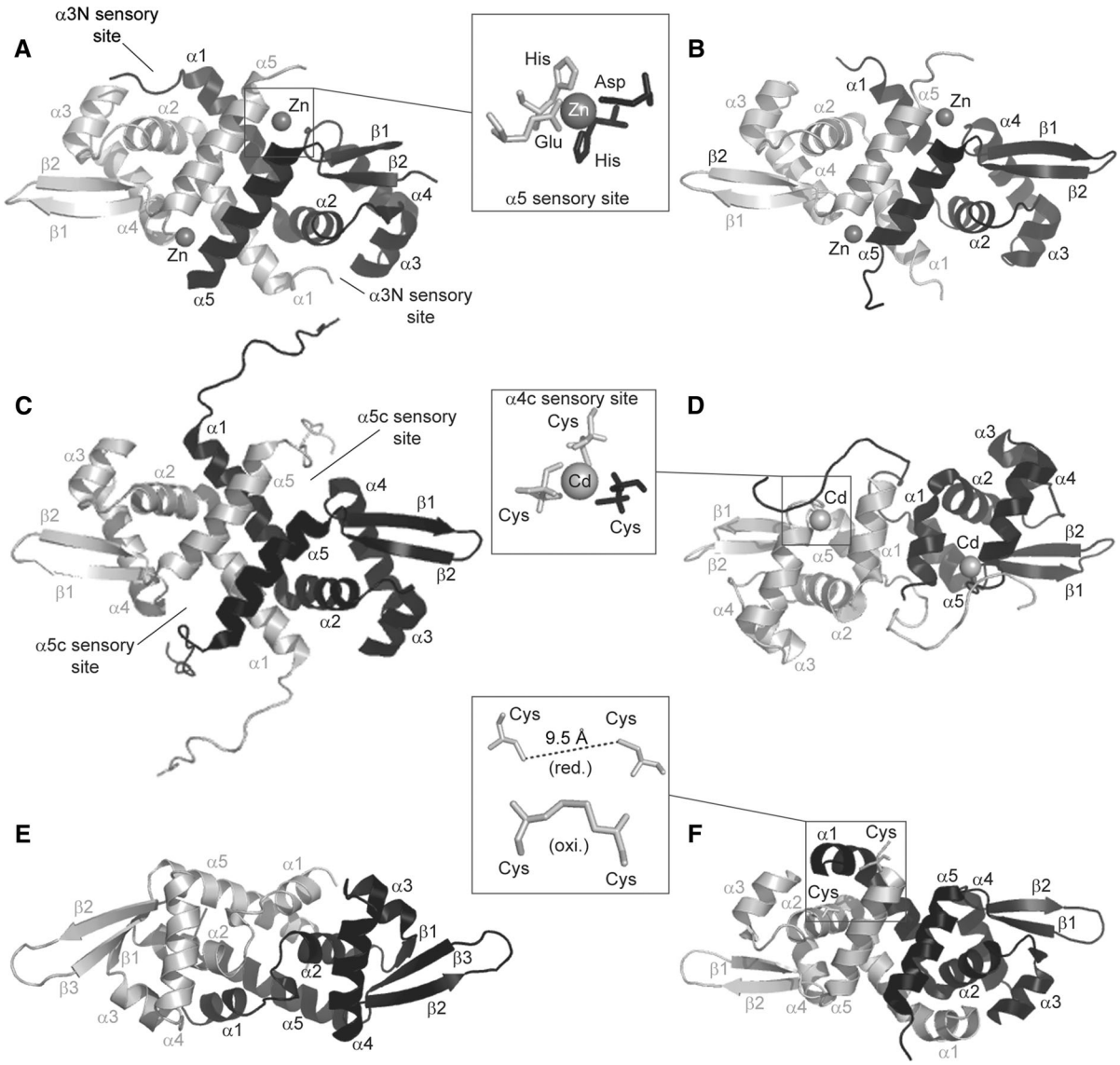



**Fig. 5** Representative 3d structures of ArsR–SmtB family of transcriptional repressors showing different sensory sites. **a** Structure of *S. elongatus* SmtB (PDB ID: 1R22) showing two α5 Zn(II) binding sites shared between two subunits (in *black* and *grey*). One α5 site is shown (*inset*) where histidine and glutamic acid residues (in *grey*) from one subunit and histidine and aspartic acid residues (in *black*) from another subunit binds a single Zn(II) metal ion. Non-regulatory α3N metal binding site is also indicated in the figure. Secondary structural elements of SmtB are indicated in *numbers*. **b** Structure of *S. aureus* CzrA (PDB ID: 2M30) showing two α5 sites which senses Zn(II). **c** Structure of *M. tuberculosis* NmtR (PDB ID: 2LKP) indicating two α5c sites which senses Ni(II). **d** Structure of *M. tuberculosis* CmtR (PDB ID: 2JSC) showing two α4c Cd(II) binding sites. The inset shows two cysteine residues from one subunit (in *grey*) and one cysteine from another subunit (in *black*) binds a single Cd(II) metal ion. **e** Structure of *Pyrococcus horikoshii* PH1061 (PDB ID: 1UB9) showing resemblance of ArsR–SmtB family fold. **f** Structure of *Xylella fastidiosa* BigR showing α2–α5 nonmetal-binding site. The *inset* shows reduced (PDB ID: 3PQJ) and oxidized (PDB ID: 3PQK) cysteine residues

2016), etc. The SmtB crystal structure does not have any metal ion, however, experiments with mercuric acetate derivative suggested a total of four metal-binding sites in the dimeric repressor (Cook et al. 1998). The two metal-sensory motifs in SmtB, α3N (type 1) and α5 (type 2), binds four metal ions

(Fig. 5a), however, Zn(II)-sensing α5 site is the regulatory site that controls derepression while α3N is non-regulatory in nature (VanZile et al. 2002a, b). The structure of the Zn-bound SmtB repressor shows that both α5 Zn(II)-binding sites of the homodimer must be filled in order to change the dynamics of the DNA-bound SmtB that drives the derepression (Eicken et al. 2003).

*S. aureus* CzrA and *Synechococcus* SmtB share nearly 35% sequence identity, but CzrA lacks the N-terminal extension and residues that comprise the α3N metal-sensing site in SmtB (Fig. 5b) (VanZile et al. 2002a, b). In *S. aureus*, upon Zn(II)-binding to α5, CzrA functions to halt the internal dynamics of DNA–protein interaction, such that the Zn(II)-bound form became energetically unfavorable and is no longer binds to the O/P site (Eicken et al. 2003; Chakravorty et al. 2013). The solution structure of CzrA bound to *czr* operator sequence, reveals how two allosteric states of the protein work. Upon Zn(II)-binding, high-affinity DNA-bound closed-state switch to low-affinity open-state and results in derepression (Arunkumar et al. 2009). The allosteric pathway that controls derepression is regulated by several residues (His97, His67, Val66 and Leu68) in and around the sensory α5-helix in CzrA (Campanello et al. 2013).

The X-ray crystal structure of homodimeric *S. aureus* CadC shows that each monomer has six α-helices and three β-strands with α0–α1–α2–β0–α3–α4–β1–β2–α5 motif (Ye et al. 2005). CadC has an extra helix in N-terminal, designated α0, and one extra beta strand, designated β0, in contrast to SmtB (Cook et al. 1998). Although, CadC has both type 1 and type 2 metal-sensory sites like SmtB (Cook et al. 1998), but only type 1 site is required for metalloregulation (Ye et al. 2005) where in SmtB type 2 site is the regulatory one (VanZile et al. 2002a, 2002b). The α3N type 1 metal-binding site in CadC is composed of N-terminal Cys7 and Cys11 residues from one monomer, and Cys58 and Cys60 residues from another monomer. Even though Zn(II)/Cd(II)-sensing type 2 site is not regulatory in CadC, it is similar to type 2 site in SmtB (Turner et al. 1996). Mutagenesis results suggest that the Arg87 residue stabilizes type 2 site in SmtB by forming hydrogen bond and in CadC the residue corresponding to SmtB Arg87 is Gly84. Therefore, in CadC, Gly84 residue do not make any significant changes in the orientation of type 2 site, and hence, binding of Zn(II) to the type 2 site is non-regulatory (Kandegedara et al. 2009). CadC and SmtB might be evolutionary intermediates between ArsR and CzrA. ArsR uses only type 1 site for metalloregulation, while CzrA uses only type 2 site. CadC and SmtB both have type 1 and 2 sites, yet CadC uses only type 1 and SmtB uses only type 2 site for metalloregulation.

Although *M. tuberculosis* Ni(II)/Co(II)-sensor NmtR (Fig. 5c) suggested to bind metal ions with four α5 residues, Asp91, His93, His104, and His107, and two C-terminal residues, His109 and His116 (α5c motif) (Cavet et al. 2002), the recent apo-NmtR NMR structure along with molecular dynamics simulations proposed an alternative Ni(II)-coordination model that involves the N-terminal 'Gly2-His3-Gly4' motif and only four α5 ligands with no contribution from two C-terminal residues His109 and His116 previously suggested (Lee et al. 2012).

*M. tuberculosis* sensor CmtR responds in vivo to Cd(II) or Pb(II) by α4 residues Cys57 and Cys61, and C-terminal Cys102 (α4c motif). The NMR structure (Fig. 5d) shows that CmtR has a relatively weak affinity towards DNA and the unstructured C-terminal tail becomes less mobile in the metal-bound form than the apo-CmtR due to the recruitment of Cys102 as one of the metal–ligand (Banci et al. 2007).

Crystal structures of the transcriptional activator HlyU from *V. vulnificus* (Nishi et al. 2010) and *V. cholerae* (Mukherjee et al. 2014) have been elucidated which suggests the existence of a redox switch in transcriptional regulation instead of metallogeulation typical to ArsR–SmtB family of repressors. In *V. cholerae* HlyU, two cysteines Cys38 (in α2) and Cys104 (in α5) are found in the dimeric interface with a distance between the two being less than 5 Å in the oxidized form (Cys38 was found to be modified as sulfenic acid), while the reduced form shows a distance more than 5 Å. The presence of a redox switch is much more clearly observable in *X. fastidiosa* BigR X-ray crystal structures (Guimarães et al. 2011). In the reduced DNA-bound form of BigR, two cysteine residues (Cys42 and Cys108) are more than 9 Å apart, while in the oxidized form Cys42 and Cys108 are disulfide-linked (Fig. 5f). BigR cannot bind to the DNA in oxidized form due to altered orientation of two important residues Met18 and Tyr104, and more than 30% reduction in interface area compared to the reduced form. Formation of the disulfide bridge involving Cys42 and Cys108 induces conformational changes in the wHTH DNA-binding interface of BigR

homodimer, results in loss of DNA binding (Guimarães et al. 2011).

The crystal structure of the *B. anthracis* PagR was solved at 1.8 Å resolution and the DNA–protein model suggests that the homodimer binds to DNA with a bend of approximately 40̊ (Zhao et al. 2010). The X-ray crystal structures of NolR in apo- and DNA-bound form indicates the importance of conformational switching of Gln56 residue in the DNA-recognition helix that senses target DNA sequence variations and influence nodulation and symbiosis in *S. fredii*. Although like *B. anthracis* PagR, *S. fredii* NolR does not show any substantial change in conformations between apo- and DNA-bound forms (Lee et al. 2014).

*P. furiosus* Phr protein is the first characterized heat shock transcription factor in archaea with ArsR–SmtB family signature. The X-ray crystal structure showed some surprising features. The N-terminal domain of Phr has similarity towards bacterial ArsR–SmtB family, while its C-terminal domain was found to resemble eukaryotic BAG domain (Liu et al. 2007c). X-ray crystal structures of few other proteins from archaea have been solved, e.g., *M. jannaschii* protein MJ223 (Ray et al. 2003), *P. horikoshii* PH1061 (Okada et al. 2006) and PH1932 (Itou et al. 2008), and they all show resemblance to ArsR–SmtB family of bacterial proteins (Fig. 5e).

## Characteristics of DNA-binding region

ArsR–SmtB metallorepressors predominantly exists as homodimers in both metal-bound (weak-affinity towards cognate DNA) and metal-free (high-affinity towards cognate DNA) states in solution (Kar et al. 1997; Busenlehner et al. 2001, 2002a; Pennella et al. 2003). Most of these homodimers bind to at or near O/P sites of repressed operons which contain one or more imperfect inverted repeats with a distinct '12-2-12' architecture (Table 4). The *smt* operon of *Synechococcus* sp. contains two imperfect inverted 12-2-12 repeats, termed 'S1/S2' and 'S3/S4', which overlaps the −10 and −35 regions of the RNA polymerase binding site (Huckle et al. 1993; Turner and Robinson 1995). The S1/S2 inverted repeat is required for the Zn(II)-mediated metalloregulation of *smtA* by α5-sensor SmtB, while the S3/S4 repeat is non-regulatory (Erbe et al. 1995; Turner et al. 1996). Each SmtB homodimer expect to bind to a single 12-2-12 inverted repeat, as both the homodimer and the inverted repeat are approximately two-fold symmetic, with two HTH motifs (α3-turn-α4) of the homodimer interacts with consecutive major grooves in the DNA-bound state, but this scenario would suggests significant bending of the DNA (∼30̊) around the minor groove (Cook et al. 1998). Although the entire O/P region of *smtA* containing both S1/S2 and S3/S4 repeats was found to bind two SmtB homodimers, yet interestingly, both S1/S2 and S3/S4 repeats separately found to bind two homodimers each, which suggest the possibility of *smt* O/P forming a looped-structure stabilized by dimer–dimer interactions (Kar et al. 2001; VanZile et al. 2002b). Other α5 sensors, *S. aureus* and *B. subtilis* CzrA also bind to *czr* O/P with an imperfect 12-2-12 inverted repeat similar to *smt* O/P (Kuroda et al. 1999; Singh et al. 1999; Harvie et al. 2006).

Like the *smtA* and *czr* O/Ps, the *M. tuberculosis nmt* O/P contains a single 12-2-12 inverted repeat where α5c-sensor NmtR has been shown to bind tightly to repress transcription (Cavet et al. 2002; Pennella et al. 2003). Interestingly, another *M. tuberculosis* protein α53-sensor KmtR binds to a '13-4-13' inverted repeat at the O/P region instead of 12-2-12 palindromic sequence (Campbell et al. 2007).

*S. aureus* α3N-sensor CadC protects the O/P site of the *cad* operon with a 12-2-12 imperfect repeat similar to that of the *smt* operon (Endo and Silver 1995; Busenlehner et al. 2003). A single CadC homodimer binds to the *cad* O/P (Busenlehner et al. 2001), however, at low salt concentrations two CadC dimers found to bind the DNA (Busenlehner et al. 2002a). Also, two distinct CadC-DNA complexes found to form at higher concentrations of the homodimer (Endo and Silver 1995). CadC proteins from other bacteria also bind to similar 12-2-12 repeats (Ivey et al. 1992; Lebrun et al. 1994; Liu et al. 1997; Schirawski et al. 2002). Other α3N-sensors like AztR from *Nostoc* sp. and Rv2642 from *M. tuberculosis* also bind to similar inverted repeats (Liu et al. 2005; Li et al. 2016b). Interestingly, *M. tuberculosis* Rv2642 represses several genes by binding to a 16-bp core palindromic region (TTTGATA-TA-TGTCAAA) which could be a part of extended 12-2-12 repeats (Table 4) (Li et al. 2016b). Another α3N-sensor *B. subtilis* AseR shows similar DNA-binding properties (Harvie et al. 2006). *Microbacterium* sp. ArsRC2 fusion-protein binds to larger inverted repeats (17-6-17 and 10-5-10)

**Table 4** Summary of known/putative DNA-binding sites of the ArsR–SmtB family protein regulated O/Ps

| No. | Sensory motif | Protein | Organism | DNA binding motif | DNA binding region | Reference(s) |
|---|---|---|---|---|---|---|
| 1 | α3 | ArsR | *Escherichia coli* Plasmid R773 | 12-2-12, imperfect inverted repeat | ATTAATCATATG-CG-TTTTTGGTTATG | Wu and Rosen (1993) |
| | | | *Escherichia coli* K 12 | | TTAAGTCATATA-TG-TTTTTGACTTAT | Xu et al. (1996) |
| | | | *Staphylococcus xylosus* Plasmid pSX267 | | TCTATATAGATG-TT-AATCTATTAACT[c] | Rosenstein et al. (1994) |
| | | | *Staphylococcus aureus* Plasmid p1258 | | TCTATATAGATG-TT-AATCTATTTAAC[c] | Ji and Silver (1992) |
| | | | *Bacillus subtilis* 168 | | ATCAAAATAAAT-TG-ATTTATTTGCTT[c] | Sato and Kobayashi (1998) |
| | | | *Acidiphilium multivorum* AIU 301 Plasmid pKW301 | | CACACATTCGAT-TT-TCCGAATATATG[c] | Suzuki et al. (1998) |
| | | | *Bacillus* sp. CDB3 | | AAATTAAATATG-AA-TCCATTTAATGA[c] | Yu et al. (2015) |
| | | | *Campylobacter jejuni* | | AATATATCAATA-TA-TATTGATATATT[c] | Wang et al. (2009a, b) |
| | | | *Ferroplasma acidarmanus* Fer1 (Archaea) | | AATAATTACATA-TG-AAATATTATTTA[c] | Baker-Austin et al. (2007) |
| | | ArsR1 | *Pseudomonas putida* | | CATATTCGAATA-GT-CATATATTCGGA[c] | Páez-Espino et al. (2015) |
| | | ArsR2 | KT2440 | | CACATATGGAAA-TA-CGTATATTCGGT[c] | |
| 2 | α3N | CadC | *Listeria monocytogenes* | 12-2-12, imperfect inverted repeat | TCAATTGTTATT-CA-AACGCACTTGAC[c] | Lebrun et al. (1994) |
| | | | *Staphylococcus aureus* | | TACACTCAAATA-AA-TATTTGAATGAA | Endo and Silver (1995) and Busenlehner et al. (2003) |
| | | | *Lactococcus lactis* Plasmid pND302 | | TATATTCAAACA-AA-CATTTGAATGTA[c] | Liu et al. (1997) |
| | | | *Bacillus firmus* | | TATATTCAAGTG-AA-CACTTGAATATC[c] | Ivey et al. (1992) |
| | | | *Streptococcus* | | AACATTCAAACA-TT-CACTTGAATATA (S1)[c] | Schirawski et al. (2002) |
| | | | *thermophilus* 4134 | | TATATTCAAACA-AA-CATTTGAATGTA (S2)[c] | |
| | | AseR | *Bacillus subtilis* | | TGTATATAACGA-TT-TGCTTATATATT[c] | Harvie et al. (2006) |
| | | AztR | *Nostoc* sp. | | TACAATTGAATA-GT-TGTTCAATTGTT | Liu et al. (2005) |
| | | Rv2642 | *Mycobacterium tuberculosis* | 16-nt core palindromic region | cctgaTTCGATA-TT-TGTCAATattga (S1-Rv2642) | Li et al. (2016a, b) |
| | | | | | tcaatATTGATG-TA-TGTCGAAtctgc (S2-Rv2642) | |
| | | | | | atctcTTTGATA-TA-TGTCAAGgtatc (Rv2640c) | |
| | | | | | gccatTTTGATA-TA-AGTCAAAcaact (Rv2641) | |
| | | ArsRC2 | *Microbacterium* sp. A33 | 17-6-17 and 10-5-10, imperfect inverted repeats | TTGTATCGATAAGTGTC-$N_6$-GACACATGTCGATTCAA[c] (S1) | Achour-Rokbani et al. (2010) |
| | | | | | TCCGGCGGGC-$N_5$-GCCCGCCGGA[c] (S2), where N = any nucleotide | |

**Table 4** continued

| No. | Sensory Motif | Protein | Organism | DNA binding motif | DNA binding region | Reference(s) |
|---|---|---|---|---|---|---|
| 3 | α3N–2 | ArsR1 | *Corynebacterium glutamicum* ATCC 13032 | 10-nt palindromic region between 2 sites | TCCACTATATATTGACGAATGTCGATATTG (S1) | Ordóñez et al. (2008) |
| | | | | | GAATATCGACAGGTATCAATATACCGAAAG (S2), palindromic regions are underlined | |
| 4 | α3N–α5 | ZiaR | *Synechocystis* sp. | 12-2-12, imperfect inverted repeat | AATATCTGAGCA-TA-TCTTCAGGTGTT | Thelwell et al. (1998) |
| | | BxmR | *Oscillatoria brevis* | | ATAATATGAACA-TC-TATTCATACTAT (*bxa1*) | Liu et al. (2004) |
| | | | | | AACATCTGAATA-TA-TGTTCAGATGTA (*bxmR/bmtA*) | |
| | | Rv2358 | *Mycobacterium tuberculosis* | | TTGACATGCATC-AT-CATGCATGTGAC[c] | Canneva et al. (2005) |
| | | Ms2358 | *Mycobacterium smegmatis* | | TTGACATGCGAA-TC-AGCGCATGTATT[c] | |
| 5 | α5 | SmtB | *Synechococcus elongatus* PCC 7942 | 12-2-12, imperfect inverted repeat | AACACATGAACA-GT-TATTCAGATATT (S1/S2) | Huckle et al. (1993), Turner and Robinson (1995), Erbe et al. (1995), and Kar et al. (2001) |
| | | | | | CATACCTGAATC-AA-GATTCAGATGTT (S3/S4) | |
| | | CzrA | *Staphylococcus aureus* | | AATATATGAACA-AA-TATTCATATGAA | Kuroda et al. (1999 and Singh et al. (1999) |
| | | | *Bacillus subtilis* | | TATATATGAACA-CA-TGCTCATATATA (*czcD*)[c] | Harvie et al. (2006) |
| | | | | | TATATATGAGTA-TA-TGCTCATATATA (*cadA*)[c] | |
| | | | | | TGAGATTGAAAT-GG-AATACAAGCAGG (*czrA*)[c] | |
| 6 | α5c | NmtR | *Mycobacterium tuberculosis* | 12-2-12, imperfect inverted repeat | AATATATGATCA-TA-TGTTCATTTATT[c] | Cavet et al. (2002) |
| 7 | α53 | KmtR | *Mycobacterium tuberculosis* | 13-4-13, imperfect inverted repeat | CTATTATCTGCGT-ATGA-ATGCAGATAAAAG (*cdf*) | Campbell et al. (2007) |
| | | | | | CTATTGTTTGCGT-ATGT-ACGCAGATAGTGG (*kmtR*) | |
| 8 | α5–4 | ArsR | *Acidithiobacillus ferrooxidans* | 28-nt protected region | ATCCACGAATATTTCTTGCAGTATTGAC | Qin et al. (2007) |
| 9 | α4c | MerR | *Streptomyces lividans* 1326 | 28-31-nt protected region | AGCAGCTATTCGTC-TG-CCCGCATAGTAAGGT (S1) | Rother et al. (1999) |
| | | | | | GGTCTGCAATACGGC-AA-GCCGCATAGGAATG (S2) | |
| | | CmtR | *Mycobacterium tuberculosis* | 4 inverted repeats 14-15 nt | T(A/G)TAA-$N_{4\text{-}5}$-T(T/G)ATA, where N = any nucleotide | Chauhan et al. (2009) |
| 10 | α2–α5[a] | HlyU | *Vibrio cholerae* N16961 | 31-nt imperfect palindrome | TATAAATTAATTCAG-A-CTAAATTAGTTCAAA | Mukherjee et al. (2015) |
| | | | *Vibrio anguillarum* | 18-22 nt regions | TAATAAAAATCTTAAAAA (*rtxH* and *rtxB*) | Li et al. (2011) |
| | | | | | AATAAAAATATCAATAAAATTA (*vah1*), 'TAAAA' repeats are underlined | |
| | | | *Vibrio vulnificus* CMCP6 | 42-nt imperfect palindrome | TGTAATTATTAGTTTTTGTTAA-A-TTAGCATTTTCTTTAAATT | Liu et al. (2009a, b) |
| | | BigR | *Xylella fastidiosa* | 22-nt imperfect palindrome | CAATATATATT-ATTATATATTG | Barbosa and Benedetti (2007) |

**Table 4** continued

| No. | Sensory Motif | Protein | Organism | DNA binding motif | DNA binding region | Reference(s) |
|---|---|---|---|---|---|---|
| | | | *Agrobacterium tumefaciens* | | CATTATATGAT-AATATATACTA | |
| | | SoxR | *Pseudaminobacter salicylatoxidans* | Three variable binding regions | CAACACATATCATTATCTGCATACATAGCC (*sv*)<br>ATATGCAGAAATTCATAT (low affinity-*wx*) AATTGATTGAATCTG (high affinity-*wx*) | Mandal et al. (2007) and Mandal and Das Gupta (2012) |
| | | PigS | *Serratia* sp. | Two inverted repeats | AATATA-CAATAT-TATATT (*blhA*)[c]<br>AATATA-A-TATATT (*pigS*)[c] | Gristwood et al. (2011) |
| | | SqrR | *Rhodobacter capsulatus* SB1003 | 16-nt binding region | ATTC-$N_8$-GAAT | Shimizu et al. (2017) |
| 11 | α2–α52 | AioF | *Thiomonas arsenitoxydans* | 60- and 74-nt long binding regions | GGTCGCGGCGGCGAGAAGGGTTCGGCGGTGC-ATGGTGGCGTCCTCTGCGTTACGAAAACGAAA-CGCTCACGAAC (S1)<br>CCATTGCAAAAGCATGAAGCGCGATTGAGC-GTTTCACAACAACCCCGAGGAGGCATCATG (S2) | Moinier et al. (2014) |
| 12 | None/ Unknown[b] | NolR | *Rhizobium meliloti*<br>*Rhizobium leguminosarum* A34 | 16-nt binding region | (A/T)TTAG-$N_9$-A(T/A)[c], where N = any nucleotide | Cren et al. (1995)<br>Li et al. (2008) |
| | | Phr | *Pyrococcus furiosus* (Archaea) | 24-nt binding region | TTTA-$N_3$-AC-$N_5$-GT-N-A-$N_2$-AAAA[c], where N = any nucleotide | Keese et al. (2010) |
| | | SdpR | *Bacillus subtilis* | Two variable binding regions | TGAAAAT (S1)<br>TA-TACAAAT-A-TCTAAAT-G-TCTAAAT (S2) | Ellermeier et al. (2006) |
| | | Rv2034 | *Mycobacterium tuberculosis* | Variable inverted imperfect repeats | GCAGACTACTGGCAAC (*phoP*)[c]<br>GGTGAGTGCTAGGTCG (*groEl2*)[c]<br>GGTCGCTGAATGCACG (*hspX*)[c]<br>CCGTAAGT-CTAA-ACTTACGG (own promoter)<br>CCGGATGC-GCCGT-ACTTACGG (*dosR*) | Gao et al. (2011, 2012) |
| | | Ms6762 | *Mycobacterium smegmatis* | | GGTGGTGACTCGCATC (*phpP*)[c]<br>CGTGAGTGCTAGGTCG (*groEl2*)[c]<br>GGTCGGATCACTGACG (*hspX*)[c] | |
| | | PyeR | *Pseudomonas aeruginosa* | 40-nt binding region | ATATCG-$N_8$-CGAT-N-TA-$N_3$-TTCG-$N_8$-CGAT, where N = any nucleotide | Mac Aogáin et al. (2012) |

Helices are numbered according to those of SmtB X-ray crystal structure (Cook et al. 1998)

[a] Does not sense metal(s)

[b] No known metal bindings residues present

[c] Putative

(Achour-Rokbani et al. 2010) and neither of these palindromes resemble the ArsR binding regions identified previously (Wu and Rosen 1993; Rosenstein et al. 1994; Xu et al. 1996).

Like α3N- and α5-sensors, α3N–α5 sensory proteins, e.g., *Synechocystis* sp. ZiaR, *O. brevis* BxmR, *M. tuberculosis* Rv2358 and *M. smegmatis* Ms2358, found to have similar DNA-binding characteristics (Table 4) (Thelwell et al. 1998; Liu et al. 2004; Canneva et al. 2005).

The *ars* O/P sequence of *E. coli* and other bacteria also contains an imperfect 12-2-12 repeat, similar to the *cad* O/P, sensed by α3-sensor ArsR proteins (Gralla 1990; Ji and Silver 1992; Wu and Rosen 1993; Rosenstein et al. 1994; Xu et al. 1996; Sato and Kobayashi 1998; Suzuki et al. 1998; Wang et al. 2009b; Yu et al. 2015; Páez-Espino et al. 2015). Gel mobility shift experiments suggest that *E. coli* ArsR form only one DNA–protein complex (Wu and Rosen 1993; Xu et al. 1996). Acidophilic archaeon *F. acidarmanus* also predicted to have an imperfect 12-2-12 repeat adjacent to the TATA-box regions of *arsRB* operon (Baker-Austin et al. 2007).

Interestingly, several ArsR–SmtB family proteins do not conform to the '12-2-12' rule as observed in α3, α3N, α5, α5c, α53 and α3N–α5-sensory repressors. The α3N–2 sensor ArsR1 from *C. glutamicum*, bind to two regions (S1 and S2) of 30 bp each (with 10 bp palindromic region present between S1 and S2) at O/P (from −7 to −37 bp and −47 to −77 bp) of the *arsB* gene (Table 4) (Ordóñez et al. 2008). *S. lividans* MerR (α4c) also binds to two sites spanning 28–31 bp regions with inverted repeats at the O/P site, but not similar to 12-2-12 repeat (Rother et al. 1999). Another α4c-sensor *M. tuberculosis* CmtR binds to an unusually long 90 bp protected region (from −80 to +10 bp) having 4 inverted repeats of 14–15 nt each with a 'T(A/G)TAA-$N_{4-5}$-T(T/G)ATA' consensus at the O/P region (Chauhan et al. 2009). AioF from *T. arsenitoxydans* (α2–α52-sensor) binds to two long regions of 60–74 nt without any inverted repeats in the *aioX-aioB* intergenic region that overlaps O/P sites (Moinier et al. 2014). Similarly, α5–4-sensor *A. ferrooxidans* ArsR protects a 28 nt long region without any inverted repeat (Table 4). Interestingly, the protected region by *A. ferrooxidans* ArsR is found between −60 and −86 nt relative to the start site of the *arsB* gene (outside RNA polymerase binding sites) that it represses (Qin et al. 2007).

Non-metal sensor protein (α2–α5-sensor) like *V. cholerae* HlyU, binds to a region (31-nt long region with a 17-nt core palindrome) of about 150 bp away from the O/P of *hlyA* gene that it controls (Mukherjee et al. 2015). HlyU from *V. vulnificus* also recognizes a 42 nt long region, with imperfect palindrome, about 400 bp away from the *rtxA1* transcription start site (Liu et al. 2009a). Interestingly, *V. anguillarum* HlyU binds to far upstream of the RNA polymerase binding site, but its 18–22 nt protected regions contain 5 bp direct repeats of 'TAAAA' instead of inverted repeats found in HlyU proteins from *V. cholerae* and *V. vulnificus* (Li et al. 2011). Similarly, other α2–α5-sensors like BigR, SoxR, PigS and SqrR binds to variable sized regions at the O/P sites with inverted or direct repeats (Table 4) (Mandal et al. 2007; Barbosa and Benedetti 2007; Gristwood et al. 2011; Mandal and Das Gupta 2012; Shimizu et al. 2017).

The non-classical group of ArsR–SmtB family proteins (NolR, SdpR, Phr, PyeR, Rv2034, etc.) that do not have any metal-sensory motif binds and protect variable regions at O/P sites with or without inverted repeats (Table 4) (Cren et al. 1995; Ellermeier et al. 2006; Li et al. 2008; Keese et al. 2010; Gao et al. 2011, 2012; Mac Aogáin et al. 2012).

## Evolution of metal-sensory motifs

The most important organizing principle in biology is the universal tree of life that separates the living world into three domains—Archaea, Bacteria and Eucarya (Woese et al. 1990). Presently accepted theory of evolution suggests that the life on planet Earth might have evolved from a hot climatic condition (Wächtershäuser 2000, 2002; Schwartzman and Lineweaver 2004) and therefore, a hyperthermophile may have been the last common ancestor of life before the divergence of three primary domains (Schwartzman and Lineweaver 2004).

Though it has been observed that three domains are very dissimilar and the differences that separate them being of a more profound nature, but most of archaeal and bacterial lineages have an extensive history of horizontal or lateral gene transfer. Horizontal gene transfer (HGT), a widely-recognized adaptation mechanism in prokaryotes, can be defined as the sharing of genetic material from one individual to another that are not in a vertical or parent-offspring relationship

(Soucy et al. 2015). Initially HGT was often associated with pathogenicity and antibiotic resistance in a microbial world, but the reach of HGT was far beyond this. It has been interesting to note that how the gene content in different domains of the universal tree of life has been shaped thoroughly by HGT among microbial world, between microbes and eucarya, and even among multicellular eukaryotes (Soucy et al. 2015). The domain archaea comprise of most of the hyperthermophiles while the bacterial kingdom also contains many. Several metabolic processes in archaea are found to be similar to bacterial systems (Laksanalamai et al. 2004) and also, many transcriptional regulators discovered in bacteria have homologs in the archaeal genome (Bell and Jackson 2001; Bell 2005; Geiduschek and Ouhammouch 2005), suggesting the possibility of HGT among domains. Also, archaeal members found to encode a large number of proteins with the HTH DNA-binding motifs whose sequences are highly similar to the bacterial HTH DNA-binding domains rather than to eukaryotic counterparts, and this relationship between archaeal and bacterial transcriptional regulators might have been occurred due to multiple HGT events (Aravind and Koonin 1999).

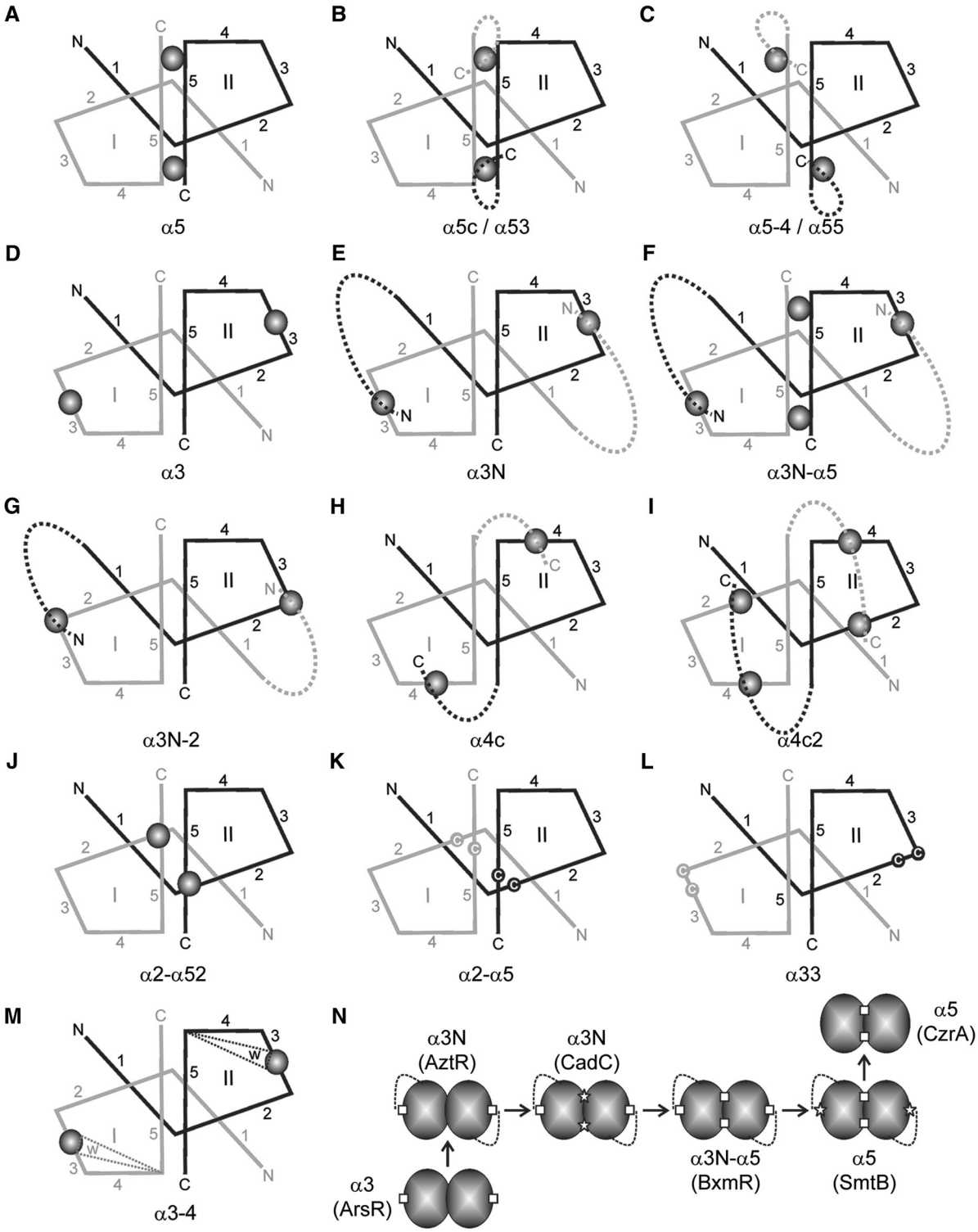
The classical view of the universal tree of life suggests that the Archaea and the Eukarya have a common ancestor, however, the origin of Eucarya remains controversial (Gribaldo et al. 2010). It can be stated that Eucarya are mainly the evolutionary chimeras of bacterial and archaeal cells that arose via endosymbiotic fusion (Soucy et al. 2015), but the mechanism by which eucarya interchange genes with prokaryotes are less clear (Gribaldo et al. 2010). Interestingly, the archaeal heat shock regulator Phr from *P. furiosus* is found to be a molecular chimera having N-terminal wHTH DNA-binding domain resembling bacterial wHTH motif and C-terminal domain that resembles eukaryotic BAG domain, suggesting HGT from hyperthermophiles to mesothermophiles (Liu et al. 2007c).

The ArsR–SmtB family of transcriptional regulators showed a great diversity with different types of sensory sites (Fig. 6) and in future we expect more unique members of this family will be discovered. The founding members of the ArsR–SmtB family repressor proteins (e.g., *E. coli* ArsR) had only type 1 site with α3 motif and binding of As(III) to the protein results in derepression. The As(III) binding site in *E. coli* ArsR is composed of only Cys32 and Cys34,

**Fig. 6** Cartoon representation of functionally characterized ArsR–SmtB family proteins depicting different sensory motifs. **a** α5, **b** α5c/α53, **c** α5–4/α55, **d** α3, **e** α3N, **f** α3N–α5, **g** α3N–2, **h** α4c, **i** α4c2, **j** α2–α52, **k** α2–α5, **l** α33 and **m** α3–4. Metal ions are denoted as *spheres*. Cysteine residues are marked as *filled circles*. Two subunits (I and II) of the protein are indicated in *grey* and *black colors*, respectively. N- and C-terminal ends of each subunit are indicated and for each subunit α-helices are numbered from 1 to 5. *Dotted line* represents N- or C-terminal extensions. The *dotted triangle* with '*w*' letter represents the wing comprising β1–β2 strands in between α4 and α5 helices. **n** Model of the evolution of type 2 metal-binding site from type 1 site. *Square box* represents regulatory metal-binding site, *star* represents non-regulatory metal-binding site and *dotted-line* represents N-terminal extension of the protein

while Cys37 is the non-regulatory residue (Shi et al. 1996), with $CxCx_2C$ motif in α3 helix (Table 1; Fig. 6d). While $CxCx_2C$ motif is the predominant one in α3-sensors, a wide range of variations is also observed in different bacteria (Table 2). A wide array of ArsR–SmtB family repressors sense cysteine residues for metal-mediated derepression, which indicates that the type 1 sites are the ancestral regulatory sites and type 2 sites might have evolved later (Kandegedara et al. 2009).

The α3N-sensory proteins (e.g., *B. subtilis* AseR, *Nostoc* sp. AztR, etc.) may have evolved from the α3-sensory ones by acquiring the N-terminal extension which provide one or two metal sensors apart from two cysteines that are contributed by the α3 helix similar to *E. coli* ArsR (Fig. 6e) (Liu et al. 2005; Harvie et al. 2006). The α3N-sensors have different combinations of residues (one or two cysteine residues or cysteine and histidine residues in N-terminal) contributes to the trigonal or tetragonal α3N site (Table 1; Fig. 2). Another α3N-sensor, *S. aureus* CadC not only has the regulatory α3N site, but also has a non-regulatory type 2 α5 site (Sun et al. 2001; Busenlehner et al. 2002a). Interestingly, CadC from *L. monocytogenes* has only α3N site and no α5 site (Lebrun et al. 1994), while CadC from *L. lactis* or *S. thermophilus* have partial α5 sites (Liu et al. 1997; Schirawski et al. 2002) (Table 2), which suggests a progression of type 2 α5 site in proteins with α3N motif (Fig. 6n). The α3N–α5 sensors (Fig. 6f), *O. brevis* BxmR or *Synechocystis* sp. ZiaR may be considered as intermediates between *E. colil* ArsR (type 1 site) and *S. aureus* CzrA (type 2 site) as they contain both true type 1 and 2 sites (Thelwell et al. 1998; Liu et al. 2004). Contrary to *S. aureus* CadC, although *Synechococcus* sp. SmtB has


A
α5
B
α5c / α53
C
α5-4 / α55
D
α3
E
α3N
F
α3N-α5
G
α3N-2
H
α4c
I
α4c2
J
α2-α52
K
α2-α5
L
α33
M
α3-4
N
α3N (AztR)
α3N (CadC)
α5 (CzrA)
α3 (ArsR)
α3N-α5 (BxmR)
α5 (SmtB)

both α3N and α5 sites, α5 site is the regulatory site and α3N is the non-regulatory one (VanZile et al. 2002a, b). As all these proteins (ArsR, CadC, SmtB, etc.) have true type 1 site or remnants of type 1 site, they might have evolved from a common ancestor (Ye et al. 2005). Although, *Synechocystis* sp. ZiaR, *O. brevis* BxmR and *Synechococcus* sp. SmtB all have α3N and α5 sites, for ZiaR both sites are essential, for BxmR either one and for SmtB only α5 site is required for metal-mediated regulation (Thelwell et al. 1998; VanZile et al. 2002a, b; Liu et al. 2008), again indicating the progression of true type 2 site (e.g., *S. aureus* CzrA; Fig. 6a) by losing the type 1 site from a type 1–2 dual sensor (Fig. 6n) (Ye et al. 2005). The α5c or α53 sensors may have evolved from α5-sensory proteins (Fig. 6b) (Cavet et al. 2002; Campbell et al. 2007).

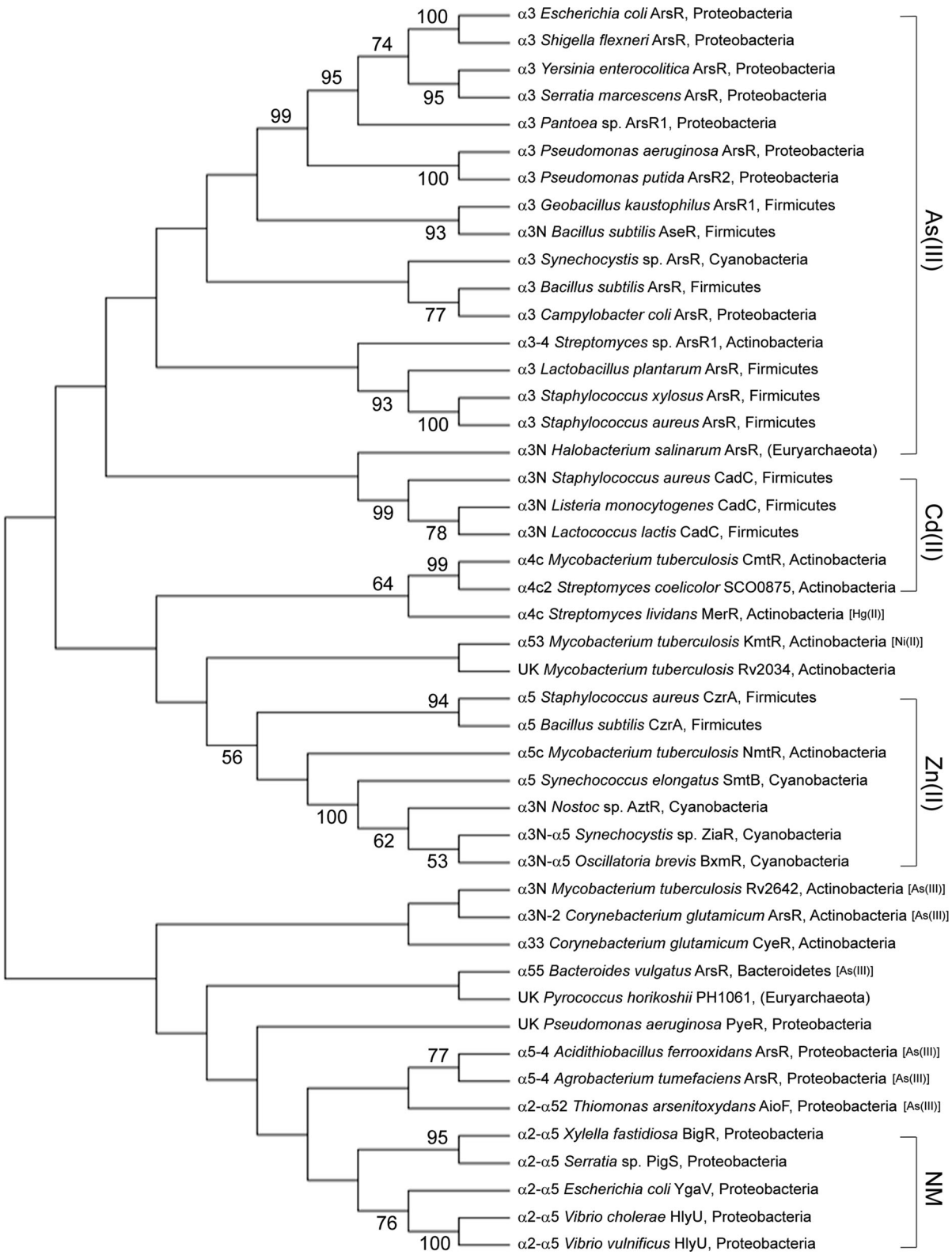
Interestingly, α3-sensor proteins have a length between 84 and 118 aa (e.g., true type 1 sensor *E. coli* ArsR has 117 aa), α3N-sensors have between 119 and 136 aa (e.g., type 1 and pseudo type 2 sensor *S. aureus* CadC has 122 aa), α3N–α5-sensors with 132-136 aa (e.g., dual type 1-2 sensor ZiaR has 132 aa) and α5-sensors have between 103 and 135 aa (e.g., pseudo type 1 and type 2 sensor SmtB has 122 aa; true type 2 sensor CzrA has 106 aa) (Table 2), indicating that the length of a protein has a direct relationship with number of sensory sites. The α3-sensors usually do not have long N-terminal extensions, therefore, length of α3-sensors are less than the α3N sensors with long N-terminal extensions providing metal ligands. Subsequently, α3N–α5 sensors are relatively longer to accommodate both type 1 and type 2 sites. Again, α5-sensors in the course of evolution lost type 1 site and became smaller in terms of protein length (Table 2).

The Arg87 residue in SmtB stabilizes the Zn-sensory type 2 site by forming hydrogen bond and in CadC the corresponding residue is Gly84 which do not make any significant changes in the conformation of type 2 site and subsequently turn into a non-regulatory site (Fig. 2) (Kandegedara et al. 2009). The α5 sensor CzrA also has arginine residue at the corresponding position, while α3/α3N-sensors ArsR, AseR, etc. have glycine residue. Interestingly, a single point mutation (guanine to cytosine) can substitute a glycine residue into an arginine residue suggesting that it is easy for the Nature to convert a non-regulatory type 2 site to a regulatory one (Kandegedara et al. 2009).

**Fig. 7** A phylogenetic tree (Bootstrap consensus tree) of the 46 ▶ sequences (representative sequences taken from Table 2), created using the Neighbor-Joining method (Saitou and Nei 1987). Sequence alignment was done using MUSCLE (Edgar 2004). The percentage of replicating trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown (if the value is 50 or more) next to the branches (Felsenstein 1985). The evolutionary distances were computed using the number of differences method (Nei and Kumar 2000) and are in the units of the number of amino acid differences per sequence. All positions with less than 95% site coverage were eliminated. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016). For each entry sensory site, scientific name, protein name and class (for archaea, classes are shown in *bracket*) are indicated. The metal-binding motifs sense different metals, present in different clusters, are indicated on the *right*. 'UK' stands for the 'unknown' sensory site. 'NM' stands for 'nonmetal-binding' sensory site

A phylogenetic tree of a subset of ArsR–SmtB family proteins show that they have evolved from a common evolutionary ancestor, with three distinct clusters—As(III)-sensor α3-group, Zn(II)-sensor α5-group and non-metal sensor α2–α5 group (Fig. 7). The α3- and α5-sensors, with type 1 and 2 sites respectively, belong to two distinct clusters. Also the redox-sensor α2–α5 proteins form a distinct group apart from the α3/α5-sensors which indicates the possibility of coexistence of primitive ArsR–SmtB family proteins with or without metal-sensory residues. Interestingly, all α2–α5-sensors belong to the phylum proteobacteria (Fig. 7; Table 2) and as proteobacteria is relatively younger compared to other groups (e.g., Firmicutes, Chloroflexi, Actinobacteria, Cyanobacteria, etc.), which suggests that this group may have evolved from ancestral metal-sensory groups by losing metal-binding residues in the course of evolution (Saha and Chakrabarti 2006; Hug et al. 2016). CadC proteins having regulatory type 1 and non-regulatory type 2 sites, clusters with α3/α3N-sensors with type 1 regulatory site, while SmtB which has regulatory type 2 site and vestigial type 1 sites clusters with α5-sensors with type 2 regulatory sites (Fig. 7).

The α3 motif ($CxCx_2C$), instead of its usual place in α3-helix in bacteria, is found at the C-terminal region of acidophilic iron-oxidizing archaeon *F. acidarmanus* ArsR which represses *arsRB* operon and the derepression results from binding of As(III) metal ion to the protein (Gihring et al. 2003; Baker-Austin et al. 2007). Very little information is available about ArsR–SmtB homologs in Archaea and *F. acidarmanus* ArsR


α3 Escherichia coli ArsR, Proteobacteria
α3 Shigella flexneri ArsR, Proteobacteria
α3 Yersinia enterocolitica ArsR, Proteobacteria
α3 Serratia marcescens ArsR, Proteobacteria
α3 Pantoea sp. ArsR1, Proteobacteria
α3 Pseudomonas aeruginosa ArsR, Proteobacteria
α3 Pseudomonas putida ArsR2, Proteobacteria
α3 Geobacillus kaustophilus ArsR1, Firmicutes
α3N Bacillus subtilis AseR, Firmicutes
α3 Synechocystis sp. ArsR, Cyanobacteria
α3 Bacillus subtilis ArsR, Firmicutes
α3 Campylobacter coli ArsR, Proteobacteria
α3-4 Streptomyces sp. ArsR1, Actinobacteria
α3 Lactobacillus plantarum ArsR, Firmicutes
α3 Staphylococcus xylosus ArsR, Firmicutes
α3 Staphylococcus aureus ArsR, Firmicutes
α3N Halobacterium salinarum ArsR, (Euryarchaeota)
α3N Staphylococcus aureus CadC, Firmicutes
α3N Listeria monocytogenes CadC, Firmicutes
α3N Lactococcus lactis CadC, Firmicutes
α4c Mycobacterium tuberculosis CmtR, Actinobacteria
α4c2 Streptomyces coelicolor SCO0875, Actinobacteria
α4c Streptomyces lividans MerR, Actinobacteria [Hg(II)]
α53 Mycobacterium tuberculosis KmtR, Actinobacteria [Ni(II)]
UK Mycobacterium tuberculosis Rv2034, Actinobacteria
α5 Staphylococcus aureus CzrA, Firmicutes
α5 Bacillus subtilis CzrA, Firmicutes
α5c Mycobacterium tuberculosis NmtR, Actinobacteria
α5 Synechococcus elongatus SmtB, Cyanobacteria
α3N Nostoc sp. AztR, Cyanobacteria
α3N-α5 Synechocystis sp. ZiaR, Cyanobacteria
α3N-α5 Oscillatoria brevis BxmR, Cyanobacteria
α3N Mycobacterium tuberculosis Rv2642, Actinobacteria [As(III)]
α3N-2 Corynebacterium glutamicum ArsR, Actinobacteria [As(III)]
α33 Corynebacterium glutamicum CyeR, Actinobacteria
α55 Bacteroides vulgatus ArsR, Bacteroidetes [As(III)]
UK Pyrococcus horikoshii PH1061, (Euryarchaeota)
UK Pseudomonas aeruginosa PyeR, Proteobacteria
α5-4 Acidithiobacillus ferrooxidans ArsR, Proteobacteria [As(III)]
α5-4 Agrobacterium tumefaciens ArsR, Proteobacteria [As(III)]
α2-α52 Thiomonas arsenitoxydans AioF, Proteobacteria [As(III)]
α2-α5 Xylella fastidiosa BigR, Proteobacteria
α2-α5 Serratia sp. PigS, Proteobacteria
α2-α5 Escherichia coli YgaV, Proteobacteria
α2-α5 Vibrio cholerae HlyU, Proteobacteria
α2-α5 Vibrio vulnificus HlyU, Proteobacteria
100
74
95
95
99
100
93
77
93
100
99
78
99
64
94
56
100
62
53
77
95
76
100
As(III)
Cd(II)
Zn(II)
NM

with As(III)-binding ($CxCx_2C$) motif in the C-terminal region may indicate a possibility of the existence of this motif in a different region in the ancestral proteins and bacteria may have acquired it by HGT from archaeal counterparts. ArsR1 and ArsR2 from halophilic archaeon *H. salinarum* have α3N motifs and senses As(III) or Sb(III) to derepress *ars* operons (Table 2) (Wang et al. 2004). Some other archaeon like *M. jannaschii* (Ray et al. 2003), *P. furiosus* (Vierke et al. 2003) and *P. horikoshii* (Okada et al. 2006; Itou et al. 2008) have ArsR–SmtB family members in their genome, but without any identifiable metal-sensory or redox-sensory motifs (Table 2). With very little information about archeal ArsR–SmtB members and a few bioinformatic analysis with limited datasets on bacteria (Busenlehner et al. 2003; Campbell et al. 2007; Harvie et al. 2006), the knowledge on the evolution of ArsR–SmtB family is incomplete and requires further study.

Even though the overall fold in ArsR–SmtB family members is conserved, the location of metal-sensory sites varied on the surface of these proteins. Some of these metal-sensory sites (e.g., α3, α5, etc.) may have evolved in the natural course of evolution from an ancestral protein, but some sensory sites (e.g., α3N–2, α55, etc.) are unrelated to the binding sites of other characterized ArsR–SmtB family members and may have evolved by convergent evolution in response to niche environmental pressures (Ordóñez et al. 2008).

## Identification of new metal sensors

The tree of life is comprised of an enormous number of branches and an approximation of the universal tree of life to full scale is a gigantic task and remains elusive (Woese et al. 1990; Koonin 2014; Hug et al. 2016). The 16s rRNA was used to construct the phylogenetic relationship between microorganisms, but it was soon realized that analyzing different molecular markers or genes may lead to either conflicting phylogenies or phylogenetic incongruence among microorganisms by grouping species that are split by other morphological, physiological or molecular markers. So, to avoid this conflict, it is better to use the whole genome instead of a gene sequence and ample new methods were employed to create genome sequences that illuminate the identity of organisms and place them correctly in the tree of life in the context of their proper ecosystem and community (Brown et al. 2015; Castelle et al. 2015). The genomes of a large number of Proteobacteria, Actinobacteria and Firmicutes, including the environmental strains like *Burkholderia ubonensis* (Price et al. 2013), *Streptomyces antibioticus* (Wang et al. 2017), etc., human pathogens like *M. tuberculosis* H37Rv (Cole et al. 1998), *B. anthracis* (Vilas-Bôas et al. 2007), etc., and plant pathogens like *R. leguminosarum* (Ryu 2015), *A. tumefaciens* (Mansfield et al. 2012), etc., encode a large number of ArsR–SmtB family of transcriptional regulators, mostly of unknown functions (Table 5). Several archaea predominantly from phylum Euryarchaeota, like *Methanosarcina mazei* (Deppenmeier et al. 2002), *Haloarcula amylolytica* (Yang et al. 2007), etc. also express a large number of ArsR–SmtB family proteins (Table 5). This signifies the importance of this family member to modulate varying metal types and concentrations in different environmental conditions for survival and proliferation.

In the last 30 years, only a few different metal-sensing and non-metal binding sites have been characterized in ArsR–SmtB family of transcriptional repressors (Table 2). With the increase in the number of newly characterized genome sequences more proteins now show signature motifs of ArsR–SmtB family in various sequence databases. At present, in InterPro database (Finn et al. 2017), HTH ArsR-type DNA-binding domain (motif number IPR001845) includes more than 82,000 proteins that have ArsR–SmtB signatures which implies the possibility of a large number of undiscovered ArsR–SmtB family proteins with unique sensory motifs. Several bacteria and archaea encodes a large number of putative ArsR–SmtB family regulators (Table 5), many of which do not exactly fit to the α3 or α5 metal binding motifs (Table 5). For example, the Hg(II)-sensor MerR from *S. lividans* (Rother et al. 1999) has been classified as ArsR–SmtB family member based on the basis of having α4c motif like another ArsR–SmtB repressor *M. tuberculosis* CmtR that senses Cd(II) or Pb(II) (Wang et al. 2005). Phylogenetic analysis suggests the possibility of extensive convergent evolution among different groups and strongly argues against the belief which states that proteins sharing overall sequence similarity would sense same metal ions (Ordóñez et al. 2008).

With the discovery of more ArsR–SmtB family members with new sensory sites and based upon the

**Table 5** Representative list of species of archaea, pathogenic and non-pathogenic bacteria having largest number of ArsR–SmtB family members in their genome

| No. | Organism | Phylum, class | Characteristic(s) | Number of ArsR-SmtB members[a] |
|---|---|---|---|---|
| A. Archaea | | | | |
| 1 | *Methanosarcina mazei* LYC | Euryarchaeota, Methanomicrobia | Anaerobic archaeobacter living in semi aquatic environments | 32 |
| 2 | *Haloarcula amylolytica* JCM 13557 | Euryarchaeota, Halobacteria | Starch-hydrolysing and extremely halophilic | 23 |
| 3 | *Haloferax mediterranei* ATCC 33500 | Euryarchaeota, Halobacteria | Extremely halophilic | 22 |
| 4 | *Methanobacterium lacus* AL-21 | Euryarchaeota, Methanobacteria | Autotrophic, hydrogenotrophic methanogen | 19 |
| 5 | *Methanoplanus limicola* DSM 2279 | Euryarchaeota, Methanomicrobia | Mesophilic methanogen | 19 |
| B. Bacteria | | | | |
| 1 | *Burkholderia ubonensis* | Proteobacteria, Betaproteobacteria | Environmental bacterium associated with opportunistic but generally nonfatal infections in healthy individuals | 90 |
| 2 | *Mesorhizobium plurifarium* | Proteobacteria, Alphaproteobacteria | Root nodule bacteria | 51 |
| 3 | *Microbacterium oxydans* | Actinobacteria, Actinobacteria | Involved in efficient treatment of seaweed waste, possess both alginate lyase and laminarinase activities | 51 |
| 4 | *Amycolatopsis keratiniphila* | Actinobacteria, Actinobacteria | Aerobic, gram-positive soil bacteria | 51 |
| 5 | *Streptomyces antibioticus* | Actinobacteria, Actinobacteria | Produces a large number of antibiotic compounds including boromycin, oleandomycin, actinomycin etc. | 49 |

| No. | Organism | Phylum, class | Disease(s) | Number of ArsR-SmtB members[a] |
|---|---|---|---|---|
| C. Pathogenic bacteria (human) | | | | |
| 1 | *Bacillus cereus* VD045 | Firmicutes, Bacilli | Nausea, vomiting, and diarrhea | 29 |
| 2 | *Nocardia asteroides* NBRC 15531 | Actinobacteria, Actinobacteria | Nocardiosis | 23 |
| 3 | *Chlamydia trachomatis* | Chlamydiae, Chlamydiia | Trachoma | 20 |
| 4 | *Bacillus anthracis* H9401 | Firmicutes, Bacilli | Anthrax | 18 |
| 5 | *Mycobacterium tuberculosis* ATCC 25618 | Actinobacteria, Actinobacteria | Tuberculosis | 17 |
| D. Pathogenic bacteria (plant) | | | | |
| 1 | *Streptomyces europaeiscabiei* | Actinobacteria, Actinobacteria | Common scab in potato | 24 |
| 2 | *Sinorhizobium meliloti* CCNWSX0020 | Proteobacteria, Alphaproteobacteria | Citrus greening | 21 |
| 3 | *Rhizobium leguminosarum* WSM2297 | Proteobacteria, Alphaproteobacteria | Induced infection threads in pea root nodules | 21 |
| 4 | *Agrobacterium tumefaciens* 5A | Proteobacteria, Alphaproteobacteria | Crown gall | 19 |
| 5 | *Klebsiella variicola* | Proteobacteria, Gammaproteobacteria | Banana soft rot | 17 |

(A) Archaea, (B) bacteria, (C) pathogenic bacteria (human) and (D) pathogenic bacteria (plant)

[a] Obtained from InterPro database (Finn et al. 2017)

presence or absence of one or more identified metal-sensing motifs, one can more accurately predict their ability to sense specific metals. In general, one metal-sensory motif (e.g., α3 or α5) correspond to specific metal (e.g., As or Zn), but an exception to this rule exists. The α3N proteins usually sense Cd(II) or Zn(II) (e.g., CadC, AztR, etc.), but *M. tuberculosis* Rv2642, *D. desulfuricans* ArsR, *P. putida* ArsR1, *Streptomyces* sp. ArsR2, *B. subtilis* AseR, etc. found to sense As(III) (Table 2). It is not apparent what factors in α3N proteins distinguish between a Cd(II)/Zn(II)-sensing site from a As(III)-sensing one, although it is possible that adjacent residues facilitate this facet of metal selectivity, such as the Arg87 residue that stabilizes the Zn(II)-sensory type 2 site in SmtB while in CadC the equivalent glycine residue convert the site into a non-regulatory one (Kandegedara et al. 2009). Similarly, *M. tuberculosis* CmtR with α4c motif senses Cd(II) or Pb(II), but another α4c group protein *S. lividans* MerR senses Hg(II) (Table 2).

ArsR–SmtB family repressors not only have different metal-sensory motifs, but also several members lack known metal-binding sites (Table 2). Further characterization of new and unique proteins in this family would enable us to understand the factors affecting metal-specificity in vivo. The characterization of ArsR–SmtB members which does not have known metal-sensory sites would help us to assign precise functions to the metal-sensory members in the ever-increasing number of homologues gathering in the sequence databases and metagenome datasets.

## Conclusions

Metals (Na, Mg, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, etc.) are essential for the regular physiology and functions of all organisms. Approximately, half of all known proteins are predicted to require metal atoms for their structure and function (Gaballa and Helmann 1998; Andreini et al. 2004). Metals comprise relatively large portion of the periodic table and have a wide range of chemical properties that govern their sensitivity in the organism. A central metal ion binds to the atoms of donor ligands, such as oxygen, nitrogen and sulfur, through interactions that are often strong and selective (Haas and Franz 2009; Ma et al. 2009). The ability to sense metal ions in the environment is extremely important for the survival of pathogenic bacteria. Host organisms can both restrict access to essential metals from invading bacteria and use their innate toxicity of certain metals for effective bacterial killing (Weinberg 2009; White et al. 2009; Kehl-Fie and Skaar 2010; Shafeeq et al. 2011). In response, bacteria developed complex metal-regulatory systems to evade metal toxicity in hostile environments within the host or outside (Brocklehurst et al. 1999; Busenlehner et al. 2003).

The human body needs many metals like iron, copper, cobalt, zinc etc. for its survival. Most of them are required in a very low concentration and problem may arise if our body receives too much of them (Guengerich 2015). Concentrations of different metals in our body are essentially very low as compared to their occurrence in the environment. For example, the concentration of copper in the environment is about 50 mg/kg (Emsley 2003) but in the human body, it is maintained at a much lower concentration of about 1.7 mg/kg body weight (Velisek 2013). Similarly, the concentration of iron in the environment is nearly 700 fold more than what is found in the human body (Velisek 2013). This metal balance is maintained by homeostasis. A specific set of transporters present in the cell compartments is involved in maintaining the delicate balance of transport activities across the cell membrane. Hemochromatosis, Pica, Wilson's and Menkes diseases are few examples which are associated with improper functioning of the homeostatic mechanism (Nelson 1999). Entry of metals in the body can also be regulated by the process of detoxication (the mechanism of preventing entry of damaging compounds in the body). Also, there are reports showing microbial sequestering of heavy metals by the intestinal microflora, which effectively reduce the metal absorption in the human body (Monachese et al. 2012; Breton et al. 2013).

The metallothioneins, a group of low-molecular-weight proteins, rich in sulfhydryl groups, serve as ligands for several essential and nonessential metals are also involved in regulation of metal concentration (Cherian and Goyer 1995). The expression of metallothionin genes are initiated by binding of metal transcription factor-1 (MTF-1) to the regulative region of metallothionin gene called metal responsive elements (MREs) (Grzywacz et al. 2015). In mammals, different types of metallothioneins are expressed and some are also tissue-specific (Sakulsak 2012). Ferritin is a storage protein for iron in reticuloendothelial cells

of the liver, spleen, and bone. Transferrin is a glycoprotein that binds most of the ferric ion in plasma and plays a role in transporting iron (also aluminium and manganese) across cell membranes (Pillet et al. 2002). ArsR–SmtB family member SmtB from *Synechococcus* sp. regulates a class II metallothionein protein SmtA involved in sequestering excess metal ions inside the cell (Huckle et al. 1993). Another member *O. brevis* BxmR represses *bmtA* gene, which encodes a heavy metal sequestering metallothionein (Liu et al. 2004).

In recent times, the number of infections associated with conventional antibiotic-resistant microorganisms have increased multifold (e.g., multidrug resistant *S. aureus*) that fueled the search for new alternative antimicrobials in absence of new potent antibiotics in the market. Metal-nanoparticles, which use completely different mechanisms of antibacterial activity than the traditional antibiotics, provide a compelling alternative strategy to kill and restrict multidrug-resistant bacteria (Wright et al. 1998; Kaneko et al. 2007; Mikolay et al. 2010). Use of different types of metal-nanoparticles (made up of Zn, Ag, Cu, Fe, Al, Au, Mg, Ti, etc.), especially zinc and silver nanoparticles in particular (Kim et al. 2007; Yoon et al. 2007; Reddy et al. 2007; Padmavathy and Vijayaraghavan 2008; Simon-Deckers et al. 2009; Jiang et al. 2009; Tran et al. 2010), showed substantial reduction in both gram-positive (e.g., *B. subtilis*, *S. aureus*, *Enterococcus faecium*, *B. megaterium*, *L. monocytogenes*, etc.) and gram-negative (e.g., *E. coli*, *klebsiella pneumoniae*, *Salmonella typhi*, *V. cholerae*, *Pseudomonas fluorescens*, *Salmonella enteritidis*, *S. typhimurium*, etc.) bacterial viability (Feng et al. 2000; Koper et al. 2002; Gu et al. 2003; Panacek et al. 2006; Gil-Tomas et al. 2007; Jung et al. 2008; Nanda and Saravanan 2009; Perni 2009; Liu et al. 2009b; Jin et al. 2009; Jiang et al. 2009). Metal nanoparticles also showed substantial antiviral (Elechiguerra et al. 2005; Lu et al. 2008; Pinto et al. 2009; Di Gianvincenzo et al. 2010; Lara et al. 2010) and antifungal activities (Kim et al. 2009; Gajbhiye et al. 2009). The mechanisms of metal-toxicity mediated by metal-nanoparticles mainly relies on the loss of protein function (Calderón et al. 2009; Anjem and Imlay 2012; Xu and Imlay 2012), production of reactive oxygen species (Imlay et al. 1988; Touati et al. 1995; Nunoshiba et al. 1999; Banin et al. 2008; Warnes et al. 2012), impairment of membrane function (Yaganza et al. 2004; Zhang and Rock 2008; Hong et al. 2012), interfere with nutrient uptake (Fauchon et al. 2002; Pereira et al. 2008), or genotoxicity (Keyer and Imlay 1996; Linley et al. 2012).

On the other hand, the unsystematic widespread release of heavy metals into the soil and waters is a major health concern globally, as these cannot be broken down to non-toxic forms and therefore have long lasting effects on the ecosystem. Many of these metals are toxic even at very low concentrations. These are not only cytotoxic but also carcinogenic and mutagenic in nature (Giller et al. 1998; Mclaughlin et al. 1999; Yao et al. 2012). Toxic concentrations of metals, otherwise essential for life, disrupt various body functions and causes severe diseases like renal dysfunction, liver cirrhosis, bone weakness, heart failure, cerebral attack, memory loss, nephrosis, lung damage, chronic anemia, gastrointestinal irritations, vision loss, disability (Vinceti et al. 2001; Neustadt and Pieczenik 2007; Duda-Chodak and Baszczyk 2008; Ainza et al. 2010; Gulati et al. 2010), etc. Although, some heavy metals are essential for microorganisms, some microbes have, however adapted to tolerate high concentrations of metals and use them for their growth. The interactions between microorganisms and metal ions have significant environmental implications especially in bioremediation. That is why bioremediation of heavy metals by microorganisms has received a great deal of interest in recent times because of its beneficial and ecofriendly nature than any other conventional methods. Heavy metal biotransformation done by natural and genetically modified bacteria (e.g., *E. coli*, *Methylococcus capsulatus*, *Pseudomonas* sp., *Ralstonia eutropha*, *Deinococcus radiodurans*, *Alcaligenes eutrophus*, *Bacillus* sp., *Enterobacter cloacae*, *Micrococcus* sp., etc.) is found to be an effective alternative and provides a promising approach for the removal of a wide variety of ecotoxic heavy metals (Diels et al. 1995; Wang et al. 1997; Valls et al. 2000; Brim et al. 2000; Lopez et al. 2002; Ackerley et al. 2004; Zouboulis et al. 2004; Kostal et al. 2004; Iyer et al. 2005; Kiyono and Pan-Hou 2006; Congeevaram et al. 2007; Hasin et al. 2010).

Development of ecofriendly metal bioremediation technology, or metal-nanoparticle based antibacterial therapy are still in early stages of development and better understanding of how bacteria sense metals in various environments are important to develop further technology. Bacterial metal sensors, such as ArsR–

SmtB repressors, detect surplus metal ions and modulate transcription of genes involved in metal uptake, efflux, sequestration, or detoxification (Tottey et al. 2005; Lucarelli et al. 2007). Several bacteria (e.g., *M. tuberculosis*, etc.) harbor not only one but multiple metal sensors in response to extracellular metal ions (Cole et al. 1998). Having several sensory sites for a range of diverse metals allows the pathogen to respond quickly to host mediated metal flux and help them to survive harsh environments. Understanding the mechanisms of how bacteria respond to various metals is of prime importance as this knowledge would benefit us in developing new and unconventional antibacterial treatments and also create a pollution free environment for our future generations.

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