GUEST COMMENTARY

Form and Function in Metal-Dependent Transcriptional Regulation: Dawn of the Enlightenment

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Metal homeostasis is a complicated process involving uptake, efflux, and sequestration of metals. The processes have to be very carefully regulated at different levels. Metal-dependent transcriptional regulation is the major mechanism of the cellular response to changing metal concentrations. It is therefore crucial to understand how metalloregulators are able to differentiate between metals and how this information is translated into transcriptional control. The ArsR/SmtB family of metalloregulators is present in many bacteria and archaea, and its members respond to a variety of different metals. However, even in this well-studied regulator family, the determinants conferring metal specificity are only beginning to be understood. The elegant crystal structure of CadC by Ye et al. (26) published in this issue of the *Journal of Bacteriology* advances our knowledge on two crucial points. First, it reinforces the notion that coordination of a metal and subsequent specific conformational changes or steric hindrance is responsible for metal specificity. In other words, nature builds its protein structure around the inherent chemical properties of a metal. Second, the structure of CadC may shed light on the evolutionary history of the ArsR/SmtB family. The members most closely related to a common ancestor, represented by the ArsR family, contain only a type 1 metal binding site, while the more divergent homologue SmtB possesses only a type 2 metal binding site. CadC, with both types of metal-binding sites, might represent an evolutionary intermediate between ArsR and SmtB.

Members of the ArsR/SmtB family of metalloregulatory proteins possess a highly conserved DNA recognition helix-turn-helix domain and bind as homodimers to their operator/promoter (O/P) sequences, repressing transcription of the operon. Metal binding to some family members presumably leads to derepression by inducing a conformational change leading to the release of the metalated repressor from the O/P sequence. In the case of CadC, Ye et al. postulate that binding of the metal brings the N terminus of one subunit into position to sterically block the DNA binding site of the other subunit (Fig. 1). Members of the ArsR/SmtB family respond to an amazing array of different metals and metalloids; these include As^{3+}-, Sb^{3+}-, and Bi^{3+}-responsive ArsR (22, 25), Zn^{2+}-responsive SmtB (11), Co^{2+}- and Zn^{2+}-responsive CzrA (12, 23),

Ni^{2+}- and Co^{2+}-responsive NmtR (6), Hg^{2+}-responsive MerR (18), Cd^{2+}-, Pb^{2+}-, and Zn^{2+}-responsive CadC (10, 17), and, most recently, Cu^+-, Ag^+-, Zn^{2+}-, and Cd^{2+}-responsive BxmR (13). One of the main questions concerning this and other families of metal-regulatory proteins is how these proteins are able to differentiate between metals or metalloids.

It has become clear that simple knowledge of the affinity of a repressor for metal is not enough to predict in vivo functionality. A good example is *Escherichia coli* NikR. NikR is a repressor that binds to the O/P sequence of the *nik* operon, thereby inhibiting transcription of the nickel uptake transporter Nik in the presence of nickel (7, 8). One would therefore expect metal binding by NikR to be selective for nickel. On the contrary, NikR binds several divalent metals, such as Zn^{2+}, Cd^{2+}, and Cu^{2+}, at least as tightly as nickel (20). However, only nickel is able to stabilize the folded structure of NikR, indicating that the inherent chemical properties of nickel affect protein conformation (20). Three side chains from one NikR monomer (His87, His89, and Cys96) and one side chain from another monomer (His76) are arranged in a square-planar coordination and might be the basis for nickel specificity (19). However, upon NikR binding to operator DNA, a novel six-coordinate nickel site lacking cysteines is formed (4). Whether this corresponds to a postulated second, lower-affinity binding site remains to be determined (2). A six-coordinate environment would favor binding of nickel over zinc, which would probably be the most physiologically relevant cation to interfere with selective binding of nickel. In another example, two members of the ArsR/SmtB family, CzrA from *Staphylococcus aureus* and NmtR from *Mycobacterium tuberculosis*, show a high degree of similarity yet respond to metals differently in vivo (16). CzrA is selective for Co^{2+} and Zn^{2+} but not Ni^{2+} by creating an environment that leads to formation of a tetrahedral complex with Co^{2+} and Zn^{2+}. This tetrahedral complex is required for release of the repressor from the DNA. In contrast, Ni^{2+} forms an octahedral complex that does not lead to the release of CzrA. NmtR responds to the same metals differently, being selective for Ni^{2+} and Co^{2+} but not for Zn^{2+}, even though the binding affinities of Zn^{2+} and Ni^{2+} are almost the same. In contrast to CzrA, NmtR has an octahedral coordination environment when binding to Ni^{2+} and Co^{2+} and not the tetrahedral environment formed when binding Zn^{2+}. Binding of Ni^{2+} and Co^{2+} results in a decrease in DNA binding affinity and subsequent release. A short C-terminal extension in NmtR contain-

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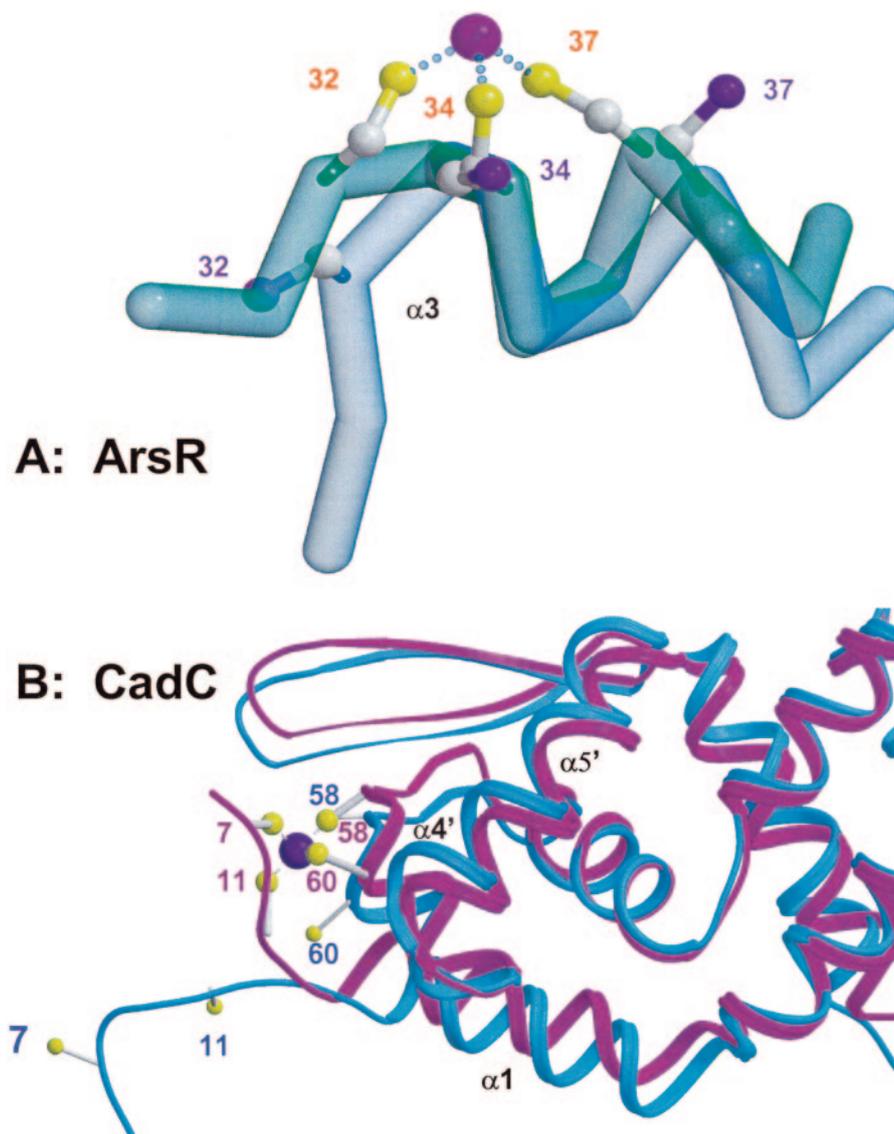
FIG. 1. Metal binding to ArsR and CadC produces derepression by different mechanisms. Derepression in ArsR is the result of a conformational change in the DNA binding domain by binding arsenic, while in CadC the DNA binding domain is shielded by the N terminus as a result of metal binding. (A) Least-squares overlap between models of the $\alpha 3$ helix of apo-ArsR and arsenic-bound ArsR based on the $\alpha 3$ helix of SmtB. The arsenic-bound model was made by adjusting the phi/psi angles of residues 32, 34, and 37 in a suitable position to bind arsenic with the bond distances of the S3 complex determined by X-ray absorption spectroscopy. The models predict that binding of arsenic would substantially distort the $\alpha 3$ helix such that it might not be able to bind to DNA. (B) Least-squares overlap between the crystal structure of apo-CadC (cyan) in which residue 11 was made a cysteine and a model with bound zinc (purple sphere) made by adjusting the phi/psi angles of the N-terminal residues to place Cys-7 and Cys-11 in a suitable position to bind zinc with Cys-58 and Cys-60 of the $\alpha 4$ helix according to the bond distances determined by X-ray absorption spectroscopy. The model was placed in a 9.8-nm box and solvated (simple point charge water model; solute-wall distance, 0.9 nm). The model was then energy minimized by steepest descents and subjected to a 20-ps relaxation "soak" with positional restraints (particle mesh Ewald electrostatics, Berendsen temperature/pressure coupling, Gromos 43A1 force field). This was followed by a 100-ps molecular-dynamics simulation in a completely free system. The model predicts that the conformation of the $\alpha 4$ helix is not substantially changed. On the other hand, there is a large difference in the buried area between the N terminus and the helix-turn-helix motif before (416 $Å^2$) and after (675 $Å^2$) zinc binding. The model suggests that $\alpha 4$ is shielded and may not be able to bind to DNA.

ing as many as three additional potential ligands (His109, Asp114, and His116) is responsible for this difference (5).

It is in this context that determination of the crystal structure of CadC gains importance. The subgroup most closely related to a common ancestor is the ArsR group, responding to As^{3+} and Sb^{3+}. This group contains a conserved metalloid binding site, called $\alpha 3$ of ArsR (because the metal binding ligands are

all in the third α-helix) or $\alpha 4N'$ in CadC (because two of the metal binding ligands are in the fourth α-helix of one monomer and the other two are in the N terminus of the other monomer) according to the terminology of Giedroc (3). For simplicity, Ye et al. have termed these type 1 metal binding sites. In the ArsR repressor from *E. coli* plasmid R773, this corresponds to Cys32, Cys34, and the nonessential Cys37 (Fig. 1). This inducer

binding site is located in α3, which is the first helix of the putative helix-turn-helix DNA binding domain. It is thought that binding of As^{3+} or Sb^{3+} leads to distortion of this helix, resulting in dissociation of ArsR from the O/P sequence (24). Since only Cys32 and Cys34 are required to form the type 1 metal binding site in ArsR, one has to predict that $As-S_2$ is sufficient to produce the desired conformational change. A type 1 metal binding site is also present in CadC as Cys58 and Cys60. However, CadC has an N-terminal extension containing an essential Cys7 and a nonessential Cys11. The addition of this extension is the basis for metal selectivity, with Cys7 and Cys11 (if present) from one monomer and Cys58 and Cys60 from the other monomer responsible for metal binding. These residues form a trigonal S_3 complex with Pb^{2+} or a distorted tetrathiolate complex with Cd^{2+} and Bi^{3+}. However, data from nuclear magnetic resonance chemical shifts of CadC induced by the presence of Cd^{2+} are more in agreement with a trigonal S_3 complex (1, 14). In addition, replacement of Cys11 with a glycine residue did not affect the function of CadC, indicating that formation of a trigonal S_3 complex is sufficient to induce the desired conformational change in CadC.

CadC also contains a second metal binding site that is conserved in SmtB and other members mostly involved in regulation of borderline soft/hard metals such as Zn^{2+}, Co^{2+}, and Ni^{2+} that are biologically required (3). Ye et al. have termed this a type 2 metal binding site, or α5 of SmtB and α6 of CadC according to the terminology of Giedroc; it consists of four conserved ligands from the C-terminal α5 helices. In SmtB these ligands (Asp104 and His106 from one monomer and His117 and Glu120 from another) bind two Zn^{2+} ions with distorted tetrahedral coordination geometry (9). This binding of two zinc ions causes SmtB to become much more globally compact, thereby stabilizing a low DNA-binding conformation. Similar binding sites exist in CzrA from a variety of bacteria and NmtR with a C-terminal extension (3). Clearly, a type 2 metal binding site favors binding of borderline metals compared to thiolate-rich type 1 metal binding sites favoring large thiophilic soft metals. CadC and many other members of the ArsR/SmtB family contain both metal binding sites. In the case of CadC, the type 2 metal binding is not necessary for function, as determined by mutagenesis (21, 26). Nevertheless, the site is occupied by two zinc ions in the apo structure (26). In other proteins, such as BxmR from *Oscillatoria brevis*, which responds to both Cu^+ and Ag^+ as well as Zn^{2+} and Cd^{2+}, one would predict the presence of two different effective metal binding sites due to the difference in configuration preference of these metals (13).

One can model evolutionary processes in the ArsR/SmtB family using current models of evolution of microbial genomes. Genes in any given organism can be subdivided into core, operational, and accessory genetic elements. The major forces driving the evolution of microbial genomes are genome reduction, horizontal gene transfer, and environmental conditions. The core genetic elements are replaced at a very low rate, if at all, since this is most often lethal. The operational and accessory genetic elements can be (and often are) replaced over time. For example, comparative analysis of the obligately symbiotic *Buchnera* genome with those of related enteric bacteria reveals that extensive genomic changes must have occurred. Early on, large deletions, chromosomal rearrangements, and

repetitive-element proliferation took place. These events were followed by extreme stasis in gene order and the slow decay of additional genes (15). However, this can occur only in a symbiotic environment devoid of changing environmental challenges and other organisms, enabling horizontal gene transfer. In a more natural setting, microorganisms will pick up or retain genetic elements if these prove useful in their environmental setting. In analogy, members of the ArsR/SmtB family contain a conserved DNA-binding region that can be considered the core. Other domains, such as the metal binding sites, can be exchanged, deleted, or extended, allowing an enormous variety of possible sites. This, in my opinion, leads to the astonishing observation that binding sites for a specific metal are not conserved. The Pb^{2+} and Cd^{2+} binding site in CadC is not conserved in Pb^{2+}- and Cd^{2+}-responsive CmtR from *M. tuberculosis*; there are As^{3+}-responsive members without enough cysteine to form an S_2 type 1 metal binding site; and Zn^{2+}-responsive regulation can occur at different binding sites in different ArsR/SmtB family members. The overarching theme appears to be the ability of metalloregulatory proteins to exploit the inherent chemical properties, such as inorganic coordination preferences, of a given metal to cause a specific conformational change upon binding that then leads to the desired change in affinity for the O/P sequence. In this regard, CadC teaches us a valuable lesson about the ingenuity and beauty of nature and offers the prospect of more exiting discoveries to come in this area.

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