Metal sensor proteins: nature’s metalloregulated allosteric switches

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Metalloregulatory proteins control the expression of genes that allow organisms to quickly adapt to chronic toxicity or deprivation of both biologically essential metal ions and heavy metal pollutants found in their microenvironment. Emerging evidence suggests that metal ion homeostasis and resistance defines an important tug-of-war in human host–bacterial pathogen interactions. This adaptive response originates with the formation of “metal receptor” complexes of exquisite selectivity. In this perspective, we summarize consensus structural features of metal sensing coordination complexes and the evolution of distinct metal selectivities within seven characterized metal sensor protein families. In addition, we place recent efforts to understand the structural basis of metal-induced allosteric switching of these metalloregulatory proteins in a thermodynamic framework, and review the degree to which coordination chemistry drives changes in protein structure and dynamics in selected metal sensor systems. New insights into how metal sensor proteins function in the complex intracellular milieu of the cytoplasm of cells will require a more sophisticated understanding of the “metallome” and will benefit greatly from ongoing collaborative efforts in bioinorganic, biophysical and analytical chemistry, structural biology and microbiology.

Introduction

Transition metal ions are required for life, functioning as cofactors for myriad metalloenzymes that catalyze an extraordinary diversity of biological reactions. As such, cells concentrate metal ions through the activity of specific membrane-associated transporters. Conversely, all metal ions are toxic in excess, and the intracellular availability of each is tightly regulated. Microorganisms have evolved a tremendous sophistication and dedicate considerable regulatory machinery to acquiring, utilizing, trafficking, detoxifying and otherwise managing, the intracellular and extracellular concentrations and types of metal ions encountered in their microenvironments.1 Toxic heavy metal resistance systems for HgII and the oxyanions of As are likely billions of years old,2,3 and evolutionary tinkering of these regulatory systems likely now allows soil- and water-dwelling bacteria to adapt to environments that are heavily polluted with heavy metals from human industrial activity.4 Understanding how magnetotactic bacteria biomineralize sufficient FeIII to build magnetosomes required for direction availabil...
sensing or how Fe-reducing *Geobacter metallireduens* thriving on Fe\(^{III}\)-oxides also promises to uncover novel mechanisms of iron scavenging and homeostasis.

For pathogenic bacteria, the challenge may well be greater. These organisms face an ever-changing landscape of metal deprivation and toxicity in the human host, depending on the stage of infection, that is compounded by the oxidative and nitrosative stress associated with survival inside the phagosomal compartment of infected macrophages. To illustrate, *Mycobacterium tuberculosis*, a human pathogen responsible for two million deaths a year, encodes 28 metal transporter systems and conservatively, \(\approx 15\) metal sensor proteins.\(^7\) Not surprisingly, proteins responsible for the acquisition, homeostasis and intracellular trafficking of biologically required metal ions as well as their regulators, *i.e.*, metal sensor proteins, are known virulence factors, deletion of which diminishes or abrogates bacterial survival and/or pathogenicity. Historically, this case has been most strongly made for Fe,\(^8,9\) which is severely limiting and sequestered by host lipocalins, which bind Fe-siderophores secreted by bacteria to scavenge Fe\(^{III}\). Recent work illustrates the gymnastics that *Staphylococcus aureus*, the causative agent of most wound and hospital-acquired infections, goes to to obtain and utilize heme Fe from the host,\(^12,13\) which itself is superimposed on multiple siderophore-based systems. Genes encoding metal transporter (uptake) components for both Cu\(^{I}\) and Mn\(^{II}\) are, respectively, virulence factors in food borne infections caused by *Listeria monocytogenes*,\(^16\) *Bacillus anthracis*,\(^17\) the causative agent of anthrax, and *Streptococcus pneumoniae*.\(^18\)

Zn\(^{II}\) uptake systems may also play a role in bacterial virulence since the availability of free Zn\(^{II}\), like free Fe\(^{III}\), may be limiting or may fluctuate dramatically depending on the specific microenvironment and stage of infection.\(^23–25\) Zn\(^{II}\) metalloenzymes are found in biosynthetic pathways required to synthesize complex extracellular structures\(^25\) and maintain intracellular redox status\(^26\) that influence virulence; secreted extracellular zinc metalloproteinases, *e.g.*, lethal factor from *B. anthracis*,\(^27–28\) disable the immune system by blocking intracellular signaling pathways associated with the host defense. Clearly, the Zn\(^{II}\) status of the cell is efficiently monitored since multiple Zn\(^{II}\)-containing ribosomal proteins (*e.g.*, L31 and S14 in *B. subtilis*) are replaced with a non-Zn\(^{II}\) paralog under conditions of Zn\(^{II}\) starvation.\(^29,30\) Finally, maintaining Ni\(^{II}\) homeostasis is known to be crucial for the gastric colonization of *Helicobacter pylori*, largely due to the action of two Ni\(^{II}\) metalloenzymes, hydrogenase, which allows *H. pylori* to use hydrogen as an energy source,\(^31\) and urease, which generates ammonia to maintain a zone of extracellular neutrality in the otherwise acidic environment of the gut;\(^32\) mycobacterial ureases may function similarly by neutralizing the phagosomal pH and inhibiting lysosomal–phagosomal fusion.\(^33–35\)

These few examples illustrate the degree to which pathogenic bacteria manipulate, exploit and evade host metal-ion status is an important adaptive response to intracellular survival in the mammalian host. This adaptive response is mediated by gene regulatory proteins, coined metalloregulatory proteins,\(^13–16\) or metal sensor proteins. These specialized “metal receptor” proteins have evolved metal coordination sites that “sense” specific metals or ion(s) by forming specific coordination complexes; this, in turn, functions to activate or inhibit DNA binding or transcription activation, thereby controlling the expression of genes that mediate what must be an exquisitely selective adaptive response (Fig. 1).

An emerging consensus summarized in this perspective is that metal selectivity of sensor proteins is dictated strongly by the coordination chemistry of the chelate,\(^35–39\) coupled to the ability of that chelate to drive changes in protein structure and/or dynamics to effect biological regulation.\(^40\)

This perspective will focus on intra-cytoplasmic sensing of transition metal ions in bacteria that employ a single protein that is responsible for both binding metals and regulating gene expression. As such, we will not touch on membrane-associated two-component signaling systems that mediate homeostasis of Mg\(^{II}\),\(^41\) transition metal ions, *e.g.*, Cu\(^{I}\),\(^42,43\) and resistance to Cd\(^{II}\)/Zn\(^{II}\)/Co\(^{II}\),\(^44\) nor will we discuss an exciting developing story on a Mg\(^{II}\)-sensing riboswitch RNA that regulates Mg\(^{II}\) uptake.\(^45–47\)

In addition, the reader is referred to several recent reviews that

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**Fig. 1** Cartoon representation of how a generic metal sensor protein affects resistance to toxic concentrations of a particular transition metal ion in the cytoplasm.

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place much of what is discussed below in a more comprehensive biological context.\textsuperscript{48–50}

### The importance of the metallome

Metal-responsive control of gene expression occurs within the context of an organism’s metallome, defined as the distribution, identity and quantity of both free (uncomplexed aqueous ions) and complexed metal ions within the cell.\textsuperscript{81} Metallomics refers to the study of how the metallome changes in response to some stimulus,\textsuperscript{82} and sensitive analytical methods including inductively coupled plasma mass spectrometry (ICP-MS),\textsuperscript{83} X-ray fluorescence microscopic imaging,\textsuperscript{84} and hybrid metalloproteomics-based strategies for metalloprotein characterization\textsuperscript{85,86} are emerging tools to obtain temporal or spatial resolution of the absolute quantities and speciation of multiple elements in a single experiment. Although the metallome remains largely unexplored for the vast majority of organisms and cells, recent evidence suggests that the metallome may well be relatively constant across a range of bacterial species.\textsuperscript{56}

The cytoplasm of all cells is a strongly reducing environment maintained by a high ratio of reduced vs. oxidized low molecular weight thiols, e.g., glutathione (γ-glutamyl l-cysteinyl glycine) and its conjugates or mycothiols in \textit{M. tuberculosis},\textsuperscript{57} that detoxify reactive oxygen species (ROS) and reactive nitrogen species (RNS).\textsuperscript{58,59} In the absence of oxidative stress, these reduced intracellular thiols represent an abundant (\textasciitilde{\textmu}M) competing ligand that has a substantial affinity for thiophilic metal ions, in particular Zn\textsuperscript{II} and Cu\textsuperscript{II} and to a lesser degree Ni\textsuperscript{II} and Co\textsuperscript{II}. This reducing environment will further ensure that Mn\textsuperscript{II}, Fe\textsuperscript{II} and Cu\textsuperscript{II} are maintained in the cytoplasm in their reduced oxidation states, Mn\textsuperscript{II}, Fe\textsuperscript{II} and Cu\textsuperscript{II}. This has several consequences. One is that concentrations of free or uncomplexed Cu\textsuperscript{II} and Fe\textsuperscript{II} are kept extremly low so as not to catalyze the production of highly damaging hydroxyl radical.\textsuperscript{90} Cu\textsuperscript{II} ions are carefully trafficked inside cells by Cu\textsuperscript{II} chaperones\textsuperscript{65,62} which deliver Cu\textsuperscript{II} to acceptor proteins, enzymes and transporters; Fe\textsuperscript{II}, on the other hand is stored as Fe\textsuperscript{III} in ferric hydroxide (ferritin)\textsuperscript{63} and may be trafficked by Dps-like proteins.\textsuperscript{64} Secondly, the higher affinity of Cu\textsuperscript{II} relative to Zn\textsuperscript{II} and all other transition metal ions as dictated by the Irving–Williams series,\textsuperscript{65,66} is likely of little consequence in the cytoplasm. In contrast, the periplasmic space of gram-negative bacteria is far more oxidizing and this intracellular compartment can be used to sequester excess metal ions, e.g., copper in the more oxidized less toxic Cu\textsuperscript{I} form, either as Cu\textsuperscript{II}/Cu\textsuperscript{I} bimetallated complexes\textsuperscript{67,69} or bound to Cu\textsuperscript{II} or Cu\textsuperscript{I}.\textsuperscript{70} As required by a more oxidizing environment, Cu\textsuperscript{II} complexes in the periplasm often employ low coordination number thioether (Met) ligation, rather than cysteine thiolates utilized in the cytoplasm.\textsuperscript{59,71}

The unique cytoplasmic environment is further shaped by the bacterial metal quota\textsuperscript{72} or metallome. Mg\textsuperscript{II} is the most abundant divalent cation, at \textasciitilde{0.1} M total concentration, with free concentrations in the mM range. Total concentrations of Fe and Zn are next most abundant and are comparable to Cu\textsuperscript{II}, present at \textasciitilde{10}\textsuperscript{−4} M. Estimates of Cu and Mn are \textasciitilde{10}\textsuperscript{−50}-fold lower (1–10 \textmu M) than Fe/Zn with Co and Ni lower still, in the low \textmu M (Ni\textsuperscript{III}) or sub-\textmu M (Co\textsuperscript{I}) range. A remarkable feature of this picture is that the relative concentrations of free or weakly chelated, kinetically labile aqueous ions, is radically different. Any free Fe\textsuperscript{II} and Mn\textsuperscript{II} will be weakly chelated in the cytoplasm due to their relatively low affinity for thiolate ligands and rapid exchange with other adventitious N/O sites and thus might be present at \textasciitilde{10}\textsuperscript{−7} M free metal.\textsuperscript{74} In order for a cell to detect potential Fe\textsuperscript{II} or Mn\textsuperscript{II} toxicity, the Fe\textsuperscript{II}/Mn\textsuperscript{II}-sensing metalloregulatory proteins might operate at a “set-point” in the \textmu M (or higher) range of free metal; this is, by and large, what has been observed.\textsuperscript{73,74} In other words, Mn\textsuperscript{II} and trace Fe\textsuperscript{II}, like Mg\textsuperscript{II} and Ca\textsuperscript{II}, may well equilibrate between free and bound states,\textsuperscript{71} and intracellular sensors may well be “tuned” to changes in intracellular concentrations around a particular set-point, reminiscent of the classical Ca\textsuperscript{II} sensor in mammalian cells, calmodulin.\textsuperscript{75}

In contrast, the cytoplasm seems to have a tremendous overcapacity to chelate both Cu\textsuperscript{II} and Zn\textsuperscript{II} such that the free concentrations of these metal ions is vanishingly small.\textsuperscript{41,72} For toxic Cu\textsuperscript{II}, the reasons for this are generally well-understood,\textsuperscript{64} but not so for Zn\textsuperscript{II} which has a relatively low toxicity due to a stable +2 oxidation state and a corresponding lack (to date) of Zn\textsuperscript{II}-specific metallochaperones in the cell. On the other hand, given the considerable extent to which cells concentrate Zn\textsuperscript{II} (vide supra), a sizable cytoplasmic pool of free Zn\textsuperscript{II} might compromise the specificity of Fe\textsuperscript{II}/Mn\textsuperscript{II}-sensing and metalloenzyme systems. In any case, the affinity of Cu\textsuperscript{II}/Zn\textsuperscript{II}-specific sensing proteins for their cognate metal might have to be quite high to sense the metal ion in a large pool of competing small molecule and biomolecule (RNA polymerase, ribosome, metalloenzyme) ligands.\textsuperscript{88} This is exactly what has been found, with \textit{K} \textsubscript{50} for \textit{boda fide} zinc sensors in the 10\textsuperscript{3} to 10\textsuperscript{4} M\textsuperscript{−1} range,\textsuperscript{72,76} and \textit{K} \textsubscript{50} in the 10\textsuperscript{−3}–10\textsuperscript{−4} M\textsuperscript{−1} range.\textsuperscript{83,77} As such, Zn\textsuperscript{II} and Cu\textsuperscript{II} will not readily equilibrate between free and bound states, rendering the “set-point” paradigm proposed for Mn\textsuperscript{II} and perhaps Fe\textsuperscript{II} almost certainly not operative. This, in turn, has fueled speculation that Cu\textsuperscript{II}/Zn\textsuperscript{II} sensors, like those that detect heavy metal pollutants Cd\textsuperscript{II}, Hg\textsuperscript{II} and Pb\textsuperscript{II} operate under kinetic control, but it is not exactly clear in molecular terms what this means. One possibility is that these extraordinary \textit{K} \textsubscript{50} (association equilibrium constant for metal ion, M) values simply ensure that metal binding is rapid and essentially irreversible in the absence of a metal ligand exchange partner, \textit{e.g.}, a metallochaperone,\textsuperscript{40,43} thus protecting the cell by turning on an adaptive response, while minimizing dissociation of the metal from the sensor;\textsuperscript{41} a recent investigation of the kinetics of metal binding and dissociation by the Cd\textsuperscript{II}/Pb\textsuperscript{II} sensor CadC is consistent with this picture.\textsuperscript{79}

It is not yet clear if the same scenario applies to Co\textsuperscript{II} and Ni\textsuperscript{II}, although it may well be the case for Ni\textsuperscript{II}, given that Ni\textsuperscript{II}-specific metallochaperones are required to metallate Ni\textsuperscript{II} enzymes urease and hydrogenase.\textsuperscript{84,85} Furthermore, recent work from \textit{E. coli} suggests that a cytoplasmic Ni\textsuperscript{II} pool detected by the Ni\textsuperscript{II}-specific sensor NikR does not readily equilibrate with a dedicated Ni\textsuperscript{II} pool that enters the cell through the high-affinity \textit{nikABCDE} Ni\textsuperscript{II} transport system that is used exclusively to metallate Ni/Fe hydrogenase.\textsuperscript{87} This observation is consistent with the idea, first articulated by Robinson’s group,\textsuperscript{50} that suggests that intracellular trafficking, sequestration, uptake and efflux of individual metal ions may well vary from cell to cell, and the ability of a sensor to specifically respond to a particular metal ion may be dictated by the intracellular milieu, \textit{i.e.}, the “metallome” characteristic of that organism. A particularly striking demonstration of this is the Ni\textsuperscript{II}/Co\textsuperscript{II}-specific ArsR family sensor \textit{M. tuberculosis} \textit{NmtR}.\textsuperscript{86} \textit{NmtR}
binds metal ions roughly in accord with the Irving–Williams series, \( \text{Zn}^{II} \geq \text{Ni}^{II} \geq \text{Co}^{II} \); nonetheless, \( \text{Ni}^{II} \) and \( \text{Co}^{II} \) derepress NmtR-induced gene expression in a mycobacterial host, \( M. \text{smegmatis} \), while \( \text{Co}^{II} \) is the exclusive inducer in cyanobacterial cells (\( \text{Synechococcus} \)). This cytosol-specific selectivity of \( \text{Co}^{II} \) over \( \text{Zn}^{II} \) in mycobacteria was found to be determined by two specific features: 1) \( M. \text{smegmatis} \) does not accumulate sufficient \( \text{Zn}^{II} \) to “turn on” a known \( \text{Zn}^{II} \) sensor, \( \text{Synechococcus} \text{SmtB} \); and 2) \( \text{Zn}^{II} \) is a poor allosteric negative regulator of DNA binding relative to \( \text{Co}^{II} \) or \( \text{Ni}^{II} \); X-ray absorption spectroscopy studies reveal that this is because \( \text{Zn}^{II} \) fails to adopt the octahedral coordination geometry adopted by \( \text{Co}^{II} / \text{Ni}^{II} \).

Families of metal sensor proteins

There are currently seven major families of metal-sensing transcriptional regulators that have been identified in prokaryotes, and we now have crystallographic structures of one or more representative members in a variety of metallated states for six of these (Fig. 2). In only two families, however, are protein–DNA operator complex structures available, and include the \( \text{Ni}^{II} \)-DtxR:DNA, \( \text{Ni}^{II} \)-IdeR:DNA, and \( \text{Ni}^{II} \)-NikR:DNA complexes. Five of the seven sensor families utilize the ubiquitous winged helix domain to bind DNA, \(^{30} \) formed by an \( \alpha_2\beta \) secondary structure (Fig. 3a) in which the \( \alpha_2\)-turn–\( \alpha_3 \) (HTH) element just N-terminal to the \( \beta \)-wing (\( \beta_1\)-\( \beta_2 \)) makes nucleotide base-specific contacts with the major groove of the DNA operator; the \( \beta \)-wing often makes electrostatic phosphate contacts with the adjacent minor grooves (see Fig. 3b). CsoR has a novel DNA binding fold, \(^{27} \) while NikR utilizes a dimeric ribbon–helix–helix motif \(^{31} \) that “reads out” bases in the major groove of the \( \text{nik} \) operator with two N-terminal antiparallel \( \beta \)-strands (ribbons) from each protomer (\( \text{vide infra} \)).

These seven sensor families span the detection of the six primary biologically essential first row transition elements Mn, Fe, Co, Ni, Cu and Zn, as well as Group II d\(^9\) and d\(^{10}\) heavy metals Ag/Au and Cd/Hg, respectively. In addition, ArsR and MerR family sensors have been identified that detect the Group IV heavy metal Pb\(^{II} \), as well as Group V trivalent ions As \(^{III} \)/Sb \(^{III} \) (or As \(^{V} \) arsenates) and Bi \(^{III} \). Sensor families are named for the founding member(s) that gave rise to the family. These are the As/Sb sensor \( E. \text{coli} \) R774 ArsR, \(^{92} \) also referred to as the ArsR/SmtB family, the latter referring to the \( \text{Zn}^{II} \) sensor \( \text{Synechococcus} \text{SmtB} \), \(^{93} \) the Cu sensor \( \text{Mycobacterium tuberculosis} \text{CsoR} \), \(^{77} \) the Cu sensor \( \text{Enterococcus hirae} \text{CopY} \), \(^{95} \) the Fe sensor \( \text{E. coli} \text{Fur} \), \(^{96} \) the Fe sensor \( C. \text{diphtheriae} \text{DtxR} \), \(^{97} \) and the Ni sensor \( E. \text{coli} \text{NikR} \). \(^{91} \)

![Fig. 2](image-url)
elements as well as heavy metal ion xenobiotics, and organic
wider range of metal ions, including both first row transition
contain representative members that have evolved to sense a far
this, the Fur, DtxR and NikR structural scaffolds have evolved
metal ion functions as an obligate co-repressor. Consistent with
metal ion(s) allosterically activates DNA binding; in this case the
response to metal excess (Fig 1. scheme 4), where direct binding of
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Metal sensor families above the dotted line in Fig. 2 are involved
in metal resistance and function to increase the expression of genes
involved in metal detoxification, storage and efflux (see Fig. 1,
schemes 1–3); here, the direct binding of a specific ion(s) to a
repressor either allosterically inhibits DNA binding, leading to
de-repression of regulated genes (ArsR, CsoR and CopY), or
allosterically activates transcription initiation by RNA polymerase
by remodeling the promoter structure (MerR).
Those grouped below this line turn off the expression of uptake systems in
response to metal excess (Fig 1. scheme 4), where direct binding of
metal ion(s) allosterically activates DNA binding; in this case the
metal ion functions as an obligate co-repressor. Consistent with
this, the Fur, DtxR and NikR structural scaffolds have evolved to
sense only the first row transition elements that are required for
biological function; in contrast, the ArsR and MerR families
contain representative members that have evolved to sense a far
wider range of metal ions, including both first row transition elements as well as heavy metal ion xenobiotics, and organic
As/Sb/Hg metalloid compounds. ArsR is likely an ancient
protein and evolutionary progenitor of the entire ArsR family
which conservatively numbers ≥500 members in ≈200 sequenced
bacterial genomes, the vast majority of which remain functionally
uncharacterized; when one considers the biogeochemical cycling
of Hg. E. coli MerR may also be a progenitor protein.

Fig. 2, right, shows a ribbon representation of a representative
member from individual sensor families, with bound regulatory
and structural metal ions highlighted. Only in the case of the Cu'
sensor CopY\textsuperscript{102,103} is a high resolution structure not yet available.
However, CopY is almost certainly a member of the MecI/BlaI
family\textsuperscript{104} of winged helical β-lactamase repressors (see Fig. 2),\textsuperscript{105}
uniquely decorated with a C-terminal extension that conserves
a pair of Cys–X–Cys sequences projected to coordinate a very
stable solvent shielded Cu' \textsubscript{2}–S\textsubscript{2} binuclear cluster. Inspection
of Fig. 2 reveals several recurring structural features. Firstly,
all sensors adopt oligomeric, mostly dimeric, assembly states,
with the exception of tetrameric NikR, which functions as a
dimer of dimers. This two-fold rotational symmetry allows sensor
proteins to interact with two-fold symmetric DNA operator sites
(see Fig. 3b). Secondly, metals bind at or near dimer (oligomer)
interfaces, and are often coordinated by metal ligand donor
atoms that are contributed, or shared, by different protomers
(subunits) within the oligomer. Here, metal binding might “freeze
out” a high affinity DNA binding conformation, stabilizing a
low DNA-binding affinity state(s).\textsuperscript{48} If not bound at a protomer
interface, regulatory metal ions are coordinated by residues
within a single chain, but positioned at an interfacial region
between physically separated N-terminal DNA-binding and more
C-terminal oligomerization domains. Metal binding here is ideally
positioned to anchor the interdomain orientation of the two
domains, thereby “freezing in” a high affinity (Fur, DtxR, and
NikR families) or allosterically activated (MerR) DNA-binding
conformation. The structural and energetic determinants of metal-induced conformational changes are discussed in more detail
below.

**Coordination chemistry and metal selectivity**

Accumulating evidence from a variety of metal-sensing systems
is consistent with the proposal that coordination number, ge-
ometry, and nuclearity dictates the biological specificity of these
metal-regulated switches.\textsuperscript{53,59,107} Metal ions that readily adopt a
coordination structure compatible with known preferences from
fundamental coordination chemistry will drive conformational
changes in protein structure and/or dynamics to effect regulation;
other metal ions may bind, but form non-native chelate structures
that are poorly regulatory. On the other hand, it is now generally
accepted that the intrinsic metal ion affinity is a poor predictor of
biological metal specificity.\textsuperscript{46,100}

A recently reported illustration of this is the Mn\textsuperscript{II}-sensor
*B. subtilis* MntR. Like the square planar site\textsuperscript{94,50} in Ni\textsuperscript{II}-specific
regulator NikR,\textsuperscript{100} the regulatory site in Fe\textsuperscript{II}-regulated Fur,\textsuperscript{93}
and other metalloproteins that bind divalent transition metal ions,\textsuperscript{110}
divalent metal ion binding affinities of free MntR tend to roughly
follow the Irving–Williams series, with Mn\textsuperscript{II} binding far more
weakly than all other divalent metal ions (\(K_{Mn} \approx 1 \times 10^{-5} \text{M}^{-1}\)),
and Zn\textsuperscript{II} binding over ≈1000-fold more tightly (\(K_{Zn} \approx 5 \times 10^{-1} \text{M}^{-1}\)).\textsuperscript{24}
The MntR homolog from pathogenic *B. anthracis*, AntR, also
binds Mn\textsuperscript{II} quite weakly.\textsuperscript{111} However, the absolute affinities of

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**Fig. 3** (a) Ribbon representation of a superposition of the winged helix
DNA binding domains (α3ββ) of Zn\textsuperscript{II} S. aureus p125S CadC (1U2W;
shaded coral), Cu' E. coli CueR (1Q05; blue), apo PerR (2FE3; green), and
Mn–Mn. *B. subtilis* MntR (2F5F; violet). The helix–turn–helix motifs are
shaded black in each case, with the β-wing toward the back. CueR
does not contain an α-helix analogous to N-terminal el helix; instead, the
α-helix immediately C-terminal to the β-wing is found here. (b) Ribbon
representation of the structure of a two-domain version of the Fe-sensor
*Mtb* IdeR dimer complexed with two regulatory (Ni) and two structural
(Ni\textsuperscript{II}) Ni\textsuperscript{II} ions and operator DNA (2ISZ).\textsuperscript{88} The helix–turn–helix motif is
shaded black with the α3 helix positioned deep in the major groove; the
β-wing is close to the adjacent minor groove.
these ions are such that only Mn

is likely to reach an intracellular concentration required to activate MntR for DNA binding so as to repress the expression of the Mn

uptake system;\textsuperscript{113} the fact that \( K_m \) is six orders of magnitude weaker than bona fide Zn

specific sensors may render intracellular Zn

sensing ineffective. Furthermore, recent structural studies establish that Zn

is unable to form a binuclear Mn

cluster and binds tightly to just one of the two sites (site A) in MntR (see Fig. 6, below).\textsuperscript{109} An MntR mutant with relaxed specificity for Mn

over Fe

in \textit{vivo}\textsuperscript{113} also binds a single Fe

atom to the A-site.\textsuperscript{114} In contrast with Zn

, Cd

which also binds nearly 1000-fold more tightly than Mn

and forms the binuclear complex, is an efficient co-repressor \textit{in vivo};\textsuperscript{109} this may provide \textit{B. subtilis} with a secondary defense against Cd

Toxicy.\textsuperscript{112} Thus, the relative magnitudes of \( K_m \) coupled with the distinct structures of metalloderivatives of MntR collaborate to create a sensor that is selective for Mn

/Cd

over other divalent cations.

On the other hand, the recent discovery of \textit{Salmonella} GolS, a Au

-selective MerR sensor that exhibits 42\% amino acid identity to a \textit{Salmonella} CueR in the same organism, provides an illustration of the extent to which nature goes to fine-tune the metal selectivity of a simple S

metal coordination chelate.\textsuperscript{115} Crystallographic studies of \textit{E. coli} CueR reveal that the coinage metals Cu

, Ag

and Au

, form isostructural coordination complexes\textsuperscript{38} and \textit{E. coli} CueR is capable of sensing Cu

and Au

equally well. Remarkably, GolS discriminates between the larger Au

over Cu

/Ag

by over 100-fold, and surgical replacement of the GolS metal binding loop with that of CueR gives rise to significant Cu
-sensing \textit{in vivo}.\textsuperscript{116} These studies provide compelling support for the idea that relatively subtle modifications in those residues that make up the metal binding pocket also lead to the evolution of MerR sensors that preferentially detect Cd

(CadR) or Pb

(PbrR) over Zn

, Hg

(MerR\textsuperscript{118}) over Cd

(see Fig. 2), and even superoxide via a \([2\text{Fe}-2\text{S}]\) cluster (\textit{E. coli} SoxR).\textsuperscript{38,117,118}

The nearly coincident discoveries of \textit{M. tuberculosis} CsoR\textsuperscript{77} and \textit{E. coli} RcnR\textsuperscript{119,120} also speak of the evolution of distinct metal sensor proteins on a relatively simple all \(\alpha\)-helical scaffold. CsoR is thought to represent the founding member of a large class of Cu
-sensing repressors; indeed, genetic deletion of \textit{csoR} in \textit{B. subtilis} abolishes Cu
-sensing in that organism.\textsuperscript{121} CsoR regulates the expression of the Cu-efflux pump that protects \textit{Mtb} from Cu
-toxicity and Cu

stabilizes an allosterically inhibited form of the protein that has weak DNA-binding activity (Fig. 2). Although the mechanism of DNA binding and allosteric inhibition by Cu

is poorly understood,\textsuperscript{77} the Cu

is coordinated by one Cys from one protomer (Cys36') and Cys61 and His65 from the other, to form a subunit-bridging S,N trigonal coordination complex.\textsuperscript{77} Substitution of His61 with Ala stabilized a non-native digonal Cys36'Cys65 S complex reminiscent of \textit{E. coli} CueR,\textsuperscript{38} in this case however, H61A CsoR is inactive in Cu
-mediated regulation of the \textit{cso} operator-promoter binding.

We propose that \textit{E. coli} RcnR is a Ni

/Co

-selective member of the fledging CsoR family that metalloregulates the expression of \textit{rcnA} to maintain Ni
-homeostasis in \textit{E. coli}.\textsuperscript{119} Although \textit{Mtb} CsoR and \textit{E. coli} RcnR are only distantly related, they are likely derived from a common evolutionary progenitor, a possibility for which is the \textit{nre} metal resistance determinant on megaplasmid pTOM9 from \textit{Achromobacter xylosoxidans} 31A.\textsuperscript{122} Although direct metal binding by RcnR has yet to be reported, the work predicts that Cu

and Ni

sensing sites of distinct coordination geometries have evolved on this simple helical scaffold that employ a subset of common metal donor ligands, much like pairs of metal sensors from the ArsR\textsuperscript{77} and MerR\textsuperscript{38} families.

A variation on this theme occurs in the ArsR family of repressors.\textsuperscript{123} Inspection of Fig. 2 reveals that ArsR family proteins collectively sense the widest range of metal ion effectors of any metal sensor protein family. Part of the reason for this is that no fewer than \textit{three} physically separated, subunit-bridging metal receptor sites have evolved on this scaffold (Fig. 4), each named for the secondary structural elements that provide donor atoms to individual metals. These are denoted \(\alpha3N/\alpha3\) (also designated metal site 1 in \textit{S. aureus} pI258 CadC\textsuperscript{124}), \(\alpha4C\) and \(\alpha5\) (denoted metal site 2 in \textit{S. aureus} pI258 CadC). A remarkable feature of what appears to be something of a “scatter-shot” picture, is that each metal site, although structurally independent, may well exploit convergent strategies to effect allosteric inhibition of DNA
binding. In the case of the α4C sensor Mtb CmrR,\textsuperscript{128} Cys61 of the Cys57–X7–Cys61 core Cd\textsuperscript{II}/Pb\textsuperscript{II} binding sequence\textsuperscript{127} corresponds precisely to His67 in the α5 Zn\textsuperscript{II} sensor S. aureus CzrA (Arg87 in SmtB), whose backbone carbonyl oxygen atom accepts a hydrogen bond from the non-ligating Nz2 face of His97 (His117 in SmtB) of the opposite protomer (see Fig. 5b below).\textsuperscript{40} Likewise, our NMR structural studies of CzrA bound to DNA reveal that Val42, the residue in CzrA that corresponds to Val59 next to Cys60, a key allosteric residue in the α3N Cd\textsuperscript{II}/Pb\textsuperscript{II} sensor S. aureus pL258 CadC,\textsuperscript{129} lies at the protein–DNA interface;\textsuperscript{129} if CadC binds to operator DNA in a similar fashion, then Cd\textsuperscript{II}/Pb\textsuperscript{II} binding here might be expected to disrupt key protein–DNA contacts.

Some ArsR sensors, including SmtB and CadC, harbor more than one pair of metal sites, with one pair playing a metalloregulatory role, with the other playing some other role, perhaps structural, but currently not well defined.\textsuperscript{128,129} The lone characterized Cu\textsuperscript{II}-sensor in the ArsR family, O. brevis BxmR, forms a novel α3N Cu–S, binuclear cluster with three Cys anchoring this complex.\textsuperscript{130} BxmR also contains an α5 site that is exquisitely sensitive to Zn\textsuperscript{II} regulation. BxmR is therefore distinguishable from other closely related ArsR-family sensors in having evolved a metalloregulatory α3N site that can adopt an expanded range of coordination chemistries, while maintaining redundancy in the Zn\textsuperscript{II}-sensing response. This relaxed metal selectivity allows the cyanobacterium Oscillatoria to adapt to both monovalent (Cu\textsuperscript{I}/Ag\textsuperscript{I}) and divalent (Zn\textsuperscript{II}/Cd\textsuperscript{II}) metal ion toxicity both in vivo and in vitro.\textsuperscript{131}

### Allosteric switching in metal sensor proteins

#### Theory

Excluding MerR-family sensors, regulatory metal ions allosterically activate or inhibit operator–promoter binding by metal sensor proteins. From classical linkage theory, DNA (D) and metal ion (M) are /th and /th allosteric ligands, respectively, of the sensor protein, P. Here, P is the minimal oligomeric assembly state that is capable of binding a D molecule containing a single two-fold symmetric operator site, i.e., a homodimer or a NikR tetramer.\textsuperscript{132} If P is a homodimer that contains two regulatory metal binding sites per dimer, a simplified coupled equilibrium can be constructed that is characterized by four allosteric “end” states, free P, singly ligated PD, PM\textsubscript{2} species, and “doubly ligated” PDM\textsubscript{2} states (Scheme 1).

![Scheme 1](#)

The free energy of allosteric coupling between the binding of the /th (D) and /th (M ligands) is denoted \(\Delta G_{ij}\) and is simply given by eqn (1)

\[
\Delta G_{ij} = -RT\ln(K_{i}/K_{j}) = -RT\ln(K_{Mi}/K_{Mj})
\]  

with the equality simply a restatement of the conservation of free energy required by the thermodynamic cycle shown. This equality also illustrates a fundamental feature of all such coupled equilibria, that linkage is reciprocal, i.e., measuring the effect of M on the binding of D to P (the ratio of the vertical manifolds boxed \textit{in green}; Scheme 1) is equal to measuring the influence of D on M binding (defined by the ratio of the horizontal manifolds boxed \textit{in blue}, Scheme 1). In this case, eqn (1) becomes eqn (2)

\[
\Delta G_{c} = -RT\ln(K_{c}/K_{1})
\]  

The sign on \(\Delta G_{c}\) dictates whether the binding of ligands D and M to P antagonize or enhance the binding of the opposite ligand. If D and M are antagonistic, then \(\Delta G_{c}\) will be positive, indicative of an unfavorable heterotropic coupling free energy; this is clearly the case for the ArsR,\textsuperscript{133} CsoR\textsuperscript{77} and Cop\textsuperscript{134} repressors. On the other hand, if D and M enhance the affinity of one another relative to the P reference state, then \(\Delta G_{c}\) will have a negative sign, indicative of a favorable heterotropic coupling free energy. This clearly characterizes the Fur, DtxR and NikR metal sensor families. The magnitude (positive or negative) is a quantitative indication of the extent to which the two ligands inhibit or reinforce the binding of the other, i.e., the extent to which the binding of the two ligands are thermodynamically linked.

A companion view of \(\Delta G_{c}\) is that this parameter is defined by eqn (3)

\[
\Delta G_{c} = -RT\ln(K_{c})(3)
\]

where \(K_{c}\) is a unitless disproportionation equilibrium constant which defines the extent to which the two singly ligated species PD and PM\textsubscript{2} are populated relative to the free P and doubly ligated PM\textsubscript{2}D states (for \(i = 1\) and \(j = 2\)) governed by eqn (4)

\[
P + D + PM_{2} \rightleftharpoons 12K_{c} \rightleftharpoons P + PM_{2} + D
\]

If this equilibrium lies to the right, then \(K_{c} > 1\) and \(\Delta G_{c} < 0\) indicative of allosteric positive coupling; on the contrary, \(K_{c} < 1\) and \(\Delta G_{c} > 0\) reflects allosteric negative coupling of D and M binding to P. Using these simple relationships, it becomes possible to quantify the allosteric impact that various cognate and non-cognate metal ions have on DNA binding by a particular metal sensor protein. For example, the relative magnitudes of \(\Delta G_{c}\) (Ni\textsuperscript{II}) (+3.3 kcal mol\textsuperscript{-1}) vs. \(\Delta G_{c}\) (Zn\textsuperscript{II}) (+2.2 kcal mol\textsuperscript{-1}) for the Ni\textsuperscript{II}/Co\textsuperscript{II}-sensing ArsR family repressor NmtR are opposite to that of the Zn\textsuperscript{II}/Co\textsuperscript{II}-sensor CzrA, as expected for a Ni\textsuperscript{II} sensor that does not detect Zn\textsuperscript{II} \textit{in vivo}.\textsuperscript{137} How NmtR tunes its allosteric switch to respond preferentially to Ni\textsuperscript{II} vs. Zn\textsuperscript{II} is still under investigation; however, the switch is more effectively turned when the metal binds in an octahedral coordination geometry since Zn\textsuperscript{II} is known to bind tetrahedrally.\textsuperscript{137}

A particularly powerful use of this approach allows one to deconvolute the extent to which individual metal ligand donor atoms are required to simply stabilize the metal complex (\(K_{M}\) in Scheme 1) vs. driving the allosteric switching mechanism (embodied in \(\Delta G_{c}\)). Using such an approach, we uncovered a “division of labor” among metal ligand donor atoms in the α5 chelate in CzrA: Asp84 and His97 were found to be key allosteric residues while His86 and His100 could be substituted with nonligating residues with substantial decreases in \(K_{M}\), as expected, but with little or no quantitative effect on \(\Delta G_{c}\).\textsuperscript{134} These studies
established a 1 : 1 correlation between the ability to form a metal site of the native coordination geometry, *i.e.*, tetrahedral, with structural switching within the dimer as revealed by NMR studies, and functional coupling to DNA binding. Similar features appear to characterize the Cd\textsuperscript{2+}/Pb\textsuperscript{2+} chelates of two other ArsR sensors, CadC\textsuperscript{20} and CmtR.\textsuperscript{27}

This linkage approach also allows direct elucidation of the underlying energetics associated with the magnitude and sign of \(\Delta G^\circ\), *i.e.*, the magnitudes and signs of \(\Delta H^\circ\) and \(\Delta S^\circ\) which correspond to the enthalpic and entropic contributions to the allosteric coupling free energy, respectively. This is important because resolution of \(\Delta H^\circ\) and \(\Delta S^\circ\) allows one to determine the relative contributions that structural changes (\(\Delta H^\circ\)) vs. changes in dynamics (\(\Delta S^\circ\)) influence the coupling.\textsuperscript{18} Isothermal titration calorimetry provides a measure of the global enthalpy change for a reaction, with \(\Delta H^\circ\) determined from eqn (5) (see Scheme 1, right)

\[
12\Delta H^\circ_c = \Delta H^\circ_m - \Delta H^\circ_S = \Delta H^\circ_m - \Delta H^\circ_m
\]

and the global \(\Delta S^\circ_c\) determined from the Gibbs relationship \(\Delta S^\circ_c = (\Delta H^\circ_m - \Delta G^\circ_m) / T\). Residue-specific contributions to the global \(\Delta S^\circ_c\) can be investigated from an analysis of the residue-specific dynamics over very short (ps–ns),\textsuperscript{136,137} intermediate (ms–ms)\textsuperscript{138,139} and long (ms–s)\textsuperscript{140} timescales by NMR spectroscopy; these experiments allow direct assessment of the relative contributions that backbone conformational entropy, correlated domain motions, and perturbations in the native state conformational ensemble, respectively, make in stabilizing distinct allosteric states accessible to P. This powerful approach can thus be used to define both global and local origins of allosteric regulation, and as discussed below, can provide support for or against mechanistic models that emerge from crystallographic studies.

**Recent structural insights into allosteric switching**

Below we summarize recent structural findings that provide insights into how metal ions induce conformational changes in protein structure to effect biological regulation embodied in \(\Delta G^\circ\).

**ArsR family \(\alpha5\) sensors.** The \(\alpha5\) metal site in ArsR family sensors is a tetrahedral, subunit-bridging site composed of either an N\textsubscript{2}O or N\textsubscript{2}O\textsubscript{2} ligand donor set derived from residues on opposite ends of the C-terminal \(\alpha5\) helix (Fig. 5).\textsuperscript{40,124} The crystallographic structures of apo- and Zn\textsuperscript{2+} forms of SmtB and CzrA reported by Eicken *et al.*,\textsuperscript{40} as well as companion NMR studies, are consistent with a plausible quaternary structural switching model for allosteric regulation of DNA binding by Zn\textsuperscript{2+}/Co\textsuperscript{2+} (Fig. 5b). This involves the formation of two successive side chain–main chain, main chain–main chain interprotomer hydrogen bonds that link the \(\alpha5\) metal binding site with the DNA binding \(\alpha4\) (or \(\alpha3\)) helix thereby stabilizing a “closed” conformation with low DNA binding affinity (Fig. 5a). The key allosteric residue in this model is His\textsubscript{117} (His\textsubscript{97}) whose N\textsubscript{ε1} forms a coordination bond with the metal, the N\textsubscript{ε2} face donating a hydrogen bond to the carbonyl oxygen of Arg\textsubscript{87} (His\textsubscript{67}) across the subunit interface (Fig. 5b).\textsuperscript{40} This hydrogen bonding pathway is predicted to make a substantial contribution to the observed \(\Delta G^\circ\) of \(\approx +6\) kcal mol\textsuperscript{−1}, which occurs with a concomitant rigidification or dampening of the internal dynamics (on the ms–s timescale) of the entire core of the molecule; in striking contrast, the \(\beta\)-wings become highly dynamic.\textsuperscript{40} Characterization of single chain, covalently fused dimers of CzrA revealed that filling just one of the two \(\alpha5\) sites stabilized a structurally asymmetric state that is characterized by \(\approx 80\%\) of the allosteric coupling free energy of wild-type CzrA; this reveals that the CzrA switch is not concerted\textsuperscript{141} in contrast to conclusions drawn from crystallographic studies alone.\textsuperscript{40}

In fact, the degree to which structural changes (embodied in \(\Delta H^\circ\)) vs. changes in residue-specific dynamics (\(\Delta S^\circ\)) participate in driving the ArsR-family \(\alpha5\) site allosteric switch is still an open question, largely due to the lack of understanding of the structure and dynamics of the apo-CzrA-DNA complex (PD in Scheme 1) as well as the ternary PM\textsubscript{2}D state. Without this key information, there is considerable uncertainty in making mechanistic projections concerning the molecular basis of allosteric coupling free energy on the basis of one or a few crystal structures of a subset of allosteric states (P and PM\textsubscript{2} in this case). Toward this end, we have recently identified solution conditions that allow us to extensively interrogate the structure and residue-specific dynamics of all four allosteric end states of CzrA by NMR spectroscopy.\textsuperscript{142}

**B. subtilis MntR.** MntR binds two metal ions in a binuclear cluster, in either an Mn\textsubscript{n}/Mn\textsubscript{o} or Mn\textsubscript{o}/Mn\textsubscript{n} configuration. The Mn\textsubscript{n}/Mn\textsubscript{c} configuration is thought to be the biologically relevant conformer since these crystals were grown at room

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**Fig. 5** Allosteric structural changes induced by the binding of regulatory Zn\textsuperscript{2+} ions to a representative \(\alpha5\) ArsR-family sensor, *Synechococcus* SmtB. (a) Apo-SmtB (1R1T) superimposed on Zn\textsubscript{o}-SmtB (1R25).\textsuperscript{46} The green-shaded region comprises the \(\alpha1\) and \(\alpha5\) dimerization \(\alpha\)-helices, with the winged helix domains shaded green (Zn\textsubscript{o}) and coral (apo) for the two structures. (b) The \(\alpha5\) chelate structure is shown, as is a quaternary structural hydrogen-bonding pathway that defines a plausible allosteric switch.
temperature and solution EPR experiments are more consistent with a longer (∼4.4 Å) internuclear Mn–Mn distance (Fig. 6) relative to the Mns/Min conformer.145 A holo-MntR-DNA operator complex structure is not yet available so the possibility exists that DNA might stabilize or enforce a well-defined geometry around the metal ion(s); on the other hand, this was found not to be the case for the related DtsR/IdeR sensors.143 A recent mechanistic proposal posits that the A-site metal functions as a selectivity filter, which recognizes the coordination geometry of the bound A-site metal and determines whether the C site will be filled; in this model, Mnκ is the activating metal ion in MntR.147

Fig. 6 Allosteric structural changes induced by the binding of regulatory Mn ions to a representative DtxR-family repressor, B. subtilis MntR. Two crystal forms of apo-MntR (2HYF shaded coral; 2HYG shaded green) and Mnκ/Mnµ MntR (2FSF shaded blue) are shown,146,148 as is the binuclear cluster chelate. This superposition limits were defined by the dimerization domain (shaded grey).

Two recent structures of apo-MntR compared to the ZnII, MnII, CaII and CdII structures suggests a quaternary structural mechanism of activation of MntR by metal ions.146 In particular, the relative dispositions of the N-terminal winged helix and C-terminal dimerization domain change greatly with a pivot point centered at residue 75, mediated by a slight underwinding of residues 72–75 of the α4 linker helix (Fig. 6). This results in a lateral displacement of the winged helix domains by ∼3.4 Å relative to the molecular two-fold axis, coupled with a rigid body rotation of one domain relative to the other. Metal ligands Glu11 (to Mnκ) and Asp8 coordinated to Mnκ/Mnµ from the N-terminal α1 helix play major roles in driving this conformational shift (see Fig. 6).144 The N-terminus of the α1 helix also appears to adopt a fully helical structure, as a result of forming a coordination bond to Asp8, relative to the apo- and poorly activating ZnII states. A similar proposal has been made for the FeII-selective ReoR (base-specific contacts derived from the DNA recognition α3 helix, and electrostatic stabilization provided by residues from the α2 helix and β-wing (see Fig. 3 above).148

These structural changes in metal-activated MntR are consistent with the results from hydrogen–deuteration exchange mass spectrometry recently mapped by Cohen and co-workers,145 although it remains unclear if the two apo-MntR structures trapped by crystallography span the full range of relative orientations of the winged helical and dimerization domains in solution (Fig. 6). As might be expected, the α1, α4 and α5 helices that donate metal ligands to the binuclear cluster become strongly protected from solvent exchange. The greatest effect, however, is in the α4 linker helix, which suggests a high degree of interdomain mobility with metal binding greatly restricting this range of motions. These studies taken collectively are consistent with a model in which the enthalpy of formation of metal ligand coordination bonds is used to drive an unfavorable reduction in the conformational entropy of the repressor, which does not have to be paid upon operator DNA binding. A direct measure of the allosteric coupling enthalpy (∆Hc) and entropy (∆Sc) would provide strong support for this model of activation.

Fur family regulators. Fur proteins are found in a wide variety of gram negative prokaryotes and are named for the founding member of this family of proteins, the Fe-regulated repressor, E. coli Fur.146 E. coli Fur is a global transcriptional regulator that controls the transcription of over 90 genes which function in iron homeostasis and acquisition, the oxidative stress response, and acid tolerance.146 The Fur family is now known to include members that sense other metals in the cell, including the ZnII-sensor Zur,146 the MnII/FeII-sensing regulator Mur,147 the NiII-selective regulator S. coelicolor Nur,148 and a hydrogen peroxide stress-sensing repressor, PerR, from B. subtilis.149 PerR is capable of binding FeII or MnII to a metalloregulatory site which represses the expression of genes that control a response to H2O2 and oxidative stress.149 The mechanism for peroxide-sensing was recently worked out by Lee and Helmann in an elegant series of biochemical experiments.150 These studies demonstrated that bound FeII reacts with peroxide, leading to rapid and irreversible metal-catalyzed oxidation of two coordinated His residues, His37 from the DNA binding domain and His91 from the dimerization domain, to 2-oxo-histidine, weakening the affinity of the regulatory site for metal and leading to derepression of regulated genes.

Fig. 7 shows the structure of the apo-PerR dimer, with the winged helical domains shaded blue and the C-terminal dimerization domains in grey. The structures of one of the two subunits of Pa Fur (coral) and Mtb Zur (green) are superimposed on apo-PerR in the C-terminal dimerization domain. PerR,156 like E. coli Zur,157 Mtb Zur and E. coli Fur, but not Pa Fur, contains what is now known to be a structural tetrathiolate S4 site (denoted Zn4; Fig. 7) formed by a pair of Cys residues derived from the β-hairpin loop between the first two β-strands of the dimerization domain (β3–β4) and a C-terminal Cys–X2–Cys sequence. In E. coli Fur, NMR studies reveal that formation of this Zn4 S4 chelate strongly stabilizes a functional form of the dimer.158

Both Zur and Pa Fur have two additional bound metals, one of
which (Zn') is nicely positioned between the two domains. In Zur, Zn' forms a tetrahedral SN₂O coordination complex, with the two His residues (His81, His83) derived from the first β-strand in the dimerization domain, β3, connected to the β-wing via a linker, and Asp62 and Cys76 derived from β1 and β2 strands in β-wing itself of the DNA binding domain (Fig. 7). Strikingly, Pa Fur contains a bound metal here as well (metal site 2), which is also tetrahedral when bound to Zn²⁺ (the Fe²⁺ coordination geometry is unknown). Nonetheless, His32 and His89 in this site in Pa Fur are absolutely essential for Fe-sensing in vivo, and correspond precisely to the two Fe ligands in PerR that are oxidized as a result of peroxide stress. Strikingly, metal binding here appears to orient the two domains into a specific orientation, driven by formation of metal-ligand bonds and the inherent flexibility of the connecting linker between the two domains. Apo-PerR, which lacks a bound metal ion in this site, is in a rather extended conformation, relative to the others; this likely corresponds to an inactive DNA binding conformation. Interestingly, both Mtb Zur and Pa Fur contain a bound metal (Zn3 in Fig. 7) whose functional role is unclear, but may be adventitiously bound.

These structures are consistent with a simple model of metal-ligation of operator DNA binding in which regulatory metal ions fix the relative dispositions of the DNA-binding and dimerization domains thereby stabilizing a conformation that allows the helix–turn–helix motif to make base-specific contacts with the DNA (see Fig. 3). This model is superficially exactly analogous to the proposal for metal activation in MntR/DtxR/ IdeR, although the range of conformational space accessible by apo-Fur family regulators may well be much larger. Forming such a complex in the absence of regulatory metals, while structurally possible, would be strongly opposed by a substantial entropic penalty, i.e., a large −ΔS°, (see Scheme 1). This might be paid for by the formation of metal coordination bonds that drive the formation of the correct chelate geometry in each case, tetrahedral for Zn²⁺–Zur and distorted octahedral for Fe³⁺–Fur/PerR. Another component of the activation mechanism perhaps unique to Fe³⁺–Fur/PerR invokes folding of the α-helix, which is an N-terminal appendage on the ααββ winged helix motif (see arrow, Fig. 7). NMR studies of the unactivated E. coli Zn³⁺ Fur dimer reveal that this helix is not formed, in contrast to the free DNA binding domain and in Zn-bound Pa Fur; this folding positions three conserved basic side chains on the same face of the helix that are well positioned to make contacts with the DNA. Recent studies clearly establish that the regulatory metal must by bound to E. coli Zn³⁺ Fur to activate DNA binding.⁷³

It will be very interesting to determine the coordination structure around the Ni³⁺ in nickel uptake regulator S. coelicolor Nur, since unlike Fur, Nur is exquisitely selective for Ni³⁺ in vitro and in vivo. Regulation by Ni³⁺–Nur not only shuts off high affinity Ni³⁺ uptake in S. coelicolor, but also represses transcription of an Fe–SOD (encoded by sodF) allowing Ni–SOD⁷⁹ (encoded sodN) to function as the major superoxide dismutase under these conditions. Interestingly, in a related Streptomyces strain, S. grissius, the expression of the sodF gene is repressed by an ArsR family repressor (SrnR) that forms an oligomeric complex with a Ni³⁺-sensing protein (SrnQ) of unknown structure; biochemical experiments seem to suggest that SrnR does not bind Ni³⁺ directly.⁶⁰

E. coli NikR. NikR is a ribbon–helix–helix DNA binding protein that functions formally as a dimer of dimers. NikR contains a central mixed αβ tetrameric oligomerization domain flanked by homodimeric ribbon–helix–helix DNA binding domains. The tetramerization domain exhibits four-fold rotational symmetry into which is incorporated four Ni³⁺ ions bound in a square planar coordination geometry from three His (His87, His89 and His76) and a cysteine residue (Cys95) across the tetramer interface (Fig. 8). This site binds Ni³⁺ with pM affinity and allosterically activates operator–promoter binding. E. coli NikR also harbors a second set of regulatory or lower affinity Ni³⁺ binding sites that further enhance DNA binding affinity and substantially change the structure of the NikR–DNA complex.⁶¹ The coordination structure of these regulatory Ni³⁺ sites has recently been probed by element-selective X-ray absorption spectroscopy of two “bimetallic” derivatives of NikR, a Cu²⁺/Ni³⁺ hybrid, with Cu²⁺ bound to the square planar high affinity site, and Ni³⁺ bound to the low affinity sites, and a Ni³⁺/Co³⁺ hybrid, with Ni³⁺ and Co³⁺ bound in the C-terminal and regulatory sites, respectively.⁹ These and other experiments clearly establish that only when the C-terminal site adopts a square planar coordination structure, e.g., with Cu²⁺ or Ni³⁺, can a unique conformation be induced in NikR such that the lower affinity regulatory sites be filled in a way that they strongly activate nik operator–promoter binding.⁹ The coordination geometry in the regulatory sites was found to be octahedral with six N/O donors in the presence of DNA.⁹⁹ Since 2003 three groups have reported crystallographic structures for NikRs from E. coli,⁸⁹,¹¹³ H. pylori¹⁶⁵ and P. horikoshii¹⁶⁴ in a number of crystal forms, including the E. coli holo-NikR–operator DNA complex.⁸⁹ These structures taken collectively reveal that Ni³⁺ binding to the low affinity sites dramatically biases the conformational ensemble to adopt a more “closed” cis-type
conformation relative to trans-type or more “open” conformations that are obtained in the absence of regulatory metal ions, but that DNA-binding is required to fully enforce a “closed” cis conformation (Fig. 8). Furthermore, the chelate structure of the low-affinity metal sites are not well formed unless holo-NikR is crystallized in the presence of \( \text{PO}_4^- \) anion\(^{164} \) or bound to DNA.\(^9\) Remarkably, however, in the DNA complex, a \( \text{K}^+ \) ion is bound to each of the two regulatory sites, coordinated by two conserved carboxylates (E30, D34) from the ribbon–helix–helix DNA binding domain, and three backbone carbonyl donor atoms (I116, Q118, V121) from the tetramerization domain (see Fig. 8); the conserved Glu30 also helps move a neighboring Arg33 from a “locked” to an “unlocked” conformation to enable specific binding to the operator DNA (Fig. 8).\(^{164} \) Mutagenesis experiments\(^{89,164} \) that target E30 and/or D34 are consistent with the identification of these \( \text{K}^+ \) sites as the regulatory \( \text{Ni}^{II} \) sites in NikR, as first hypothesized on the basis of the \( P. \ horikoshii \) NikR–\( \text{PO}_4^- \) structure.\(^{164} \) However, it must be pointed out that this \( \text{O}_6 \) coordination site, with long (\( \approx 3.0 \) Å) metal–ligand bonds, is a poor match for known \( \text{Ni}^{II} \) chelates,\(^{165} \) which also tend to incorporate one or more histidine ligands into an \( (\text{N/O})_6 \) octahedral coordination complex. Such an atypical coordination geometry for a regulatory metal ion is not unprecedented in metal sensor or metal trafficking proteins,\(^{71} \) with other metalloregulatory sites characterized by coordination numbers that are lower (CuI–S\(_2 \) in CueR\(^{38} \)) or higher (Hg\(^{II} \)-S\(_3 \) in MerR\(^{116} \)) than expected. In addition, Cd\(^{II} \) complexes of two ArsR-family Cd\(^{II}/\text{Pb}^{II} \)-sensing repressors CadC (S\(_3 \))\(^8 \) and CmrR (S\(_3 \), or S\(_2 \), O\(_2 \))\(^{127} \) are characterized by at least one cysteine thiolate ligand that only weakly deshields the \(^{113} \text{Cd} \) nucleus; this is consistent with considerable distortion from regular trigonal planar or tetrahedral symmetry in each case.

In any case, the primary effect of \( \text{Ni}^{II} \) binding to the low affinity sites in NikR may be functionally analogous to that which occurs in DtxR- and Fur-family regulators, where metal binding quenches interdomain mobility, induces local folding around the chelate, \( i.e. \), near subunit-bridging high affinity \( \text{Ni}^{II} \) ligand His76 in the \( \alpha 3 \) helix, and stabilizes a conformation of the oligomer that is well-matched to interact specifically with individual half-sites of the DNA operator. As discussed above, this scenario makes the prediction that a large favorable \( \Delta G_c \) in NikR is driven principally by a large and favorable \( \Delta S_c \).

**Conclusions**

Recent structural insights from a wide range of bacterial metal sensor proteins collectively emphasize several common features that characterize these bioinorganic switches. One is that the coordinate covalent nature of metal–ligand bonding is harnessed to drive changes in tertiary and/or quaternary structure and/or dynamics, the full extent of which is not yet fully established for any metal sensor protein. In addition, the central importance of metal homeostasis and resistance to bacterial fitness coupled with the ease with which allostery can apparently be evolved,\(^{165,167} \) virtually ensures that additional structural scaffolds beyond the seven discussed here will emerge that could accommodate novel “metal receptor” sites. A second emerging theme is that biological metal selectivity, while dictated chiefly by the coordination chemistry of the chelate, is further refined by the metallome within...
which a particular switch operates; this allows an organism to mount a specific adaptive response. Future efforts that more critically evaluate the role that protein motions (dynamics) in solution play in mediating allostery in these systems would permit a more sophisticated understanding of these fascinating molecules. Concurrently with these efforts, whole organism systems biology and metallomics-based approaches to understand the “inorganic chemistry of the cell” will require to fully appreciate the extraordinary lengths to which nature has gone to harness the power and minimize the potential damage of metal ions in biology.

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