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Susana K. Checa · Fernando C. Soncini

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Abstract Gold ions are mobilized and disseminated through the environment and enter into the cells by non-specific intake. To avoid deleterious effect that occurs even at very low concentrations, bacteria such as *Salmonella enterica* and *Cupriavidus metallidurans* use Au-specific MerR-type transcriptional regulators to detect the presence of these toxic ions, and control the expression of specific resistance factors. In contrast to the related copper sensor CueR, the Au-selective metalloregulatory proteins are able to distinguish Au(I) from Cu(I) or Ag(I). This is achieved by finely tuning a single dithiolate metal coordination with conserved cysteine residues at the metal binding site of the proteins to lower the affinity for Cu(I) in comparison to the Cu-sensors, while maintaining or even increasing the affinity for Au(I). In *Salmonella*, GolS not only privileges the binding of Au(I) over Cu(I) or Ag(I), but also distinguishes its target recognition sites in its regulated promoters minimizing cross-activation of CueR-controlled operators. In this sense, the presence of a selective Au sensory devise would allow species harbouring resident Cu-homeostasis systems to eliminate the toxic ion without affecting Cu acquisition in Au rich environments.

Keywords Gold sensing and resistance · MerR-regulators · *Salmonella* Typhimurium · *Cupriavidus metallidurans* · GolS · CueR · CupR

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Introduction

Gold (Au) along with copper (Cu) and silver (Ag) constitute the Group IB of transition metals in the Periodic Table of the Elements. Like the other members of this group, Au has one s-orbital electron on top of a filled *d* electron shell (Housecroft and Constable 2006). This electronic configuration, plus the large size of the Au atom, confers a large ionization potential and electron affinity. Au can exist in its metallic form as well as in six other oxidation states, but only the +1 (Au(I)) and +3 (Au(III)) states are commonly found in nature. Like the other members of the group, Au is a soft Lewis acid, thus it forms stable complexes with ligands that have free electron pairs also known as soft Lewis bases, such as S and N containing groups (Nies 2007).

The antibacterial capacity of Au ions has been exploited since the nineteenth century with the discovery by Robert Koch that gold cyanide has bacteriostatic effects towards the tubercle bacillus

(Benedek 2004). It is predicted that under the reducing conditions present in the cytoplasm, Au would be found mainly as Au(I). This ion has a tendency to tightly coordinate with sulfhydryl groups from cysteine and methionine protein residues (Shaw 1999). Thus, either by chemical modification or by displacing essential metals, Au(I) may cause disruption of the redox equilibrium, increase in cell permeability, and interference of metabolic pathways that eventually results in cell death (Carotti et al. 2000; Karthikeyan and Beveridge 2002; Witkiewicz and Shaw 1981).

Au has also been recently reported as an efficient cofactor for a nicotinamide adenine dinucleotide oxidase in the Gram positive actinomycete *Micrococcus luteus* (Levchenko et al. 2004). Furthermore, lithoautotrophic Gammaproteobacteria and Cyanobacteria gain metabolic energy by utilizing Au-containing complexes (review by Southam et al. 2009).

The average Au concentration in natural waters is about 1–5 parts per trillion or ng/l (Williams-Jones et al. 2009), but it can reach more than 100 parts-per-billion (ppb) in soil solution from auriferous soils (Reith et al. 2007). Since the redox potential of both Au(I) and Au(III) ions exceeds that of water (Nies 2007; Williams-Jones et al. 2009), the existence of free gold ions is thermodynamically unfavorable in aqueous media. Despite its low solubility, its high mobility in soils and placers can account for its toxic effects on nearby organisms. This turned out to be evident near metal deposits where total and bioavailable amounts of gold increase substantially (Karthikeyan and Beveridge 2002; Nies 2007; Reith et al. 2007). Thus, the ability to develop detoxifying mechanisms would certainly be advantageous for microorganisms living in such contaminated niches.

Two different strategies for the elimination of Au have been proposed in bacteria and archaea. One of these strategies involves the active efflux of Au ions through metal transporters (see below). The other mechanism implies the reductive precipitation of bioavailable Au complexes either in the cytoplasm or at the cell surface (Kashefi et al. 2001; Reith et al. 2007).

The secretion of a number of metabolites able to complex and solubilize gold such as thiosulphate, amino acids and cyanide, plus the active precipitation of dissolved Au by some bacterial species have been linked to bioaccumulation and biomineralization of Au (Southam et al. 2009). Recently, a study performed on gold grains from Queensland, Australia, showed the presence of a bacterial biofilm containing Au nanoparticles associated with extracellular polymeric substances, as well as bacterioform Au (Karthikeyan and Beveridge 2002; Nies 2007; Reith et al. 2007). This finding revealed the importance of bacterial biofilms in the formation and growth of secondary Au deposits, as well as the mobilization and dispersion of Au in the environment. The microbe-driven biogeochemical cycle for gold has been recently reviewed (Reith et al. 2007; Southam et al. 2009).

The purpose of this review is to summarize the recent advances in our understanding of the molecular basis of Au-sensing and response by Gram negative bacteria, as well as the strategies that these microorganisms employ to survive in contaminated niches.

Au-resistance by active efflux

Active efflux from either the cytoplasm or the periplasm is well-known as a major mechanism of bacterial heavy metal resistance (Nies 2007; Summers 2009). Recent evidence shows that this mechanism is also employed by certain bacteria to alleviate the toxic effect produced by the presence of Au ions in their niches. This was first reported in *Salmonella enterica* serovar Typhimurium (Checa et al. 2007; Pontel et al. 2007), and later proposed in *Cupriavidus metallidurans* CH43 (Jian et al. 2009; Julian et al. 2009), a *β*-proteobacterium highly resistant to metals, found forming biofilms on gold grains (Reith et al. 2009).

The Au-resistance *gol* cluster from *Salmonella* is composed of three transcriptional units organized in a divergon, *gesABC* and *golTS*, and a separate monocistronic gene, *golB* (Fig. 1). This cluster codes for one metal exporter, GolT, a CBA efflux system, GesABC, a small metal-binding protein, GolB, and GolS, the transcriptional regulator that coordinates the response to gold ions. While the deletion of any of these components affects survival in the presence of Au ions, the more severe phenotype is attained by either the inactivation of the whole *gol* locus or the deletion of the gene coding for the transcriptional regulator, *golS* (Checa et al. 2007; Pontel et al. 2007).

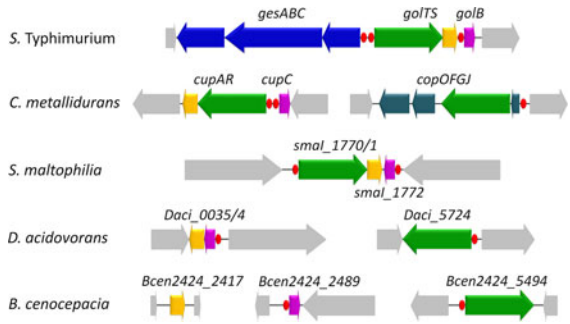

Fig. 1 Au-inducible gene clusters are present in *Salmonella enterica* as well as in bacterial species associated to Au-grain surfaces. Schematic representation of the genetic organization of *gol*-like loci in *S. enterica* serovar Typhimurium LT2, *Cupriavidus metallidurans* CH34, *Stenotrophomonas maltophilia* R551-3, *Delftia acidovorans* SPH-1 and *Burkholderia cenocepacia* HI2424. Genes coding for the Au sensor, the P-type ATPase and the metal-binding chaperone are colored in *yellow*, *green* and *purple*, respectively. Other genes under the putative regulation of the Au-responsive transcriptional regulator are shown in different tones of *blue*. *Red* ovals indicate the position of predicted Au-responsive promoters

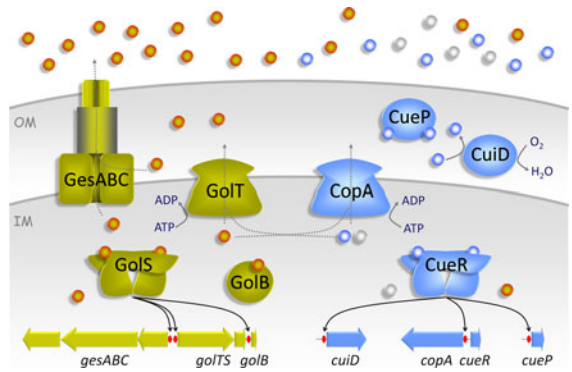

Fig. 2 The *Salmonella gol* and *cue* regulons. In the presence of Au, GolS induces transcription of genes encoding the P-type ATPase GolT, the metal binding protein GolB and the GesABC efflux system to reduce the free metal ion from the cytoplasm or the periplam. The Cu-sensor CueR, activated by either Cu, Ag or Au, directs the expression of the P-type ATPase CopA, the multicopper oxidase CuiD and the periplasmic metal binding protein CueP. Both GolT and CopA can transport Cu, Ag or Au ions overlapping in their function

The *gol* locus is present in the two species of *Salmonella*, *bongori* and *enterica*, but absent in other enteric bacteria such as *E. coli*, *Shigella* or *Yersinia*. It forms part of a larger *Salmonella* specific region of approximately 45 kbp (http://www.sanger.ac.uk/Projects/Salmonella/), probably acquired by horizontal gene transfer. Interestingly, most of the locus sequence is absent in the human-adapted *S. enterica* serovars Typhi and Paratyphi A. The presence of a 5′-CGG(C/A)GGCGCG-3′ scar in these genomes that matches the sequences present downstream of the *gesC* and *golS* genes at positions 392964 and 401260 in the LT2 chromosome sequence, is indicative of the deletion of this region by recombination in the human-adapted serovars (Florea et al. 2003). The absence of the *gol* locus in these pathogens suggests that it is not required for human infection and favours the role for this locus during *Salmonella* survival outside its hosts.

GolT and GolB are rapidly expressed after GolS activation by Au as the first line of defense against the toxic ion (Fig. 2, see also Checa et al. 2007). GolT is homologous to P_{1B}-type metal ion transporters, suggesting that it can export monovalent metal ions from the cytoplasm, while GolB has the conserved MXCXXC signature at its N-terminus characteristic of metal-chaperones (Harrison et al. 2000; Robinson and Winge 2010). GolB is required for full Au-resistance, suggesting that it may act as an intracellular Au-chaperone. Alternatively, its high expression levels after exposure to Au may reduce the free cytosolic metal ion by chelation (Checa et al. 2007 and our unpublished results). GolT, and probably GolB, can also contribute to Cu(I) resistance (Fig. 2), but only in a strain that lacks the Cu(I)-transporter CopA or the *copA*-activator CueR (Checa et al. 2007; Espariz et al. 2007). Conversely, the main Cu-transporter CopA (Fig. 2) can also contribute to Au efflux in *Salmonella*, but only at high Au concentrations (Checa et al. 2007; see also below).

The redundancy in function between the monovalent metal transporters GolT and CopA was also reported by Osman et al. (Osman et al. 2010), although these authors argued against a role of these transporters in Au(I) efflux. The nature of the apparent discrepancy with our previous results (Checa et al. 2007; Pontel et al. 2007) is not clear, although this may be caused by differences in the genetic background of the strains employed by both groups.

The transcriptional activation of the *gesABC* operon differs from the other two GolS-controlled transcriptional units in that it requires higher Au concentrations. This creates a delay in the expression of *gesABC* compared to *golTS* or *golB* (Pontel et al. 2007). Based on similarities with other members of the CBA family (Lobedanz et al. 2007; Murakami 2008; Nikaido and Takatsuka 2009; Pos 2009), GesABC is likely to span

the cell envelope from the cytoplasm to the outer space, directing its substrate from either the periplasm or the cytoplasm to the outside milieu (Fig. 2). Elimination of periplasmic Au ions may serve to avoid damage caused by the displacement of essential Cu(I) ions from their binding sites. In cells lacking the main drug transporter AcrAB, GesABC can also confer resistance to different antimicrobial agents and chemical compounds, but only by GolS-mediated induction (Pontel et al. 2007) or ectopic overexpression (Conroy et al. 2010; Nishino et al. 2006). In view of these results and the similarity of GesB with the HEA1 group of resistance-nodulation-cell division (RND) proteins that export organic substances, such as AcrB and MexF (Saier et al. 2009), it is tempting to hypothesize that this system is involved in the elimination of Au-complexes or cellular metabolites damaged by the toxic metal, rather than free Au ions. Moreover, GesB which is responsible for the substrate specificity of the CBA complex, has a conserved and essential (double) Asp-Asp motif in the middle of transmembrane alpha-helix IV, characteristic of HEA1-RND proteins and has been proposed to be required for proton import in these proton-driving antiporters (Nies 2003).

Recent studies indicate that *C. metallidurans* CH34 has a Au-inducible *gol*-like cluster in the CHR1 chromosome (Fig. 1 and Janssen et al. 2010). The Rmet_3523/Rmet_3525 *cup* locus codes for the P_{1B}-ATPase CupA, the metal chaperone CupC and a GolS-xenolog CupR that directs the response to Au. Transcriptional analysis shows that *cupC* is the strongest $AuHCl_4$-upregulated gene at the lowest metal concentration tested (Jian et al. 2009). This finding is in agreement with the transcriptional profile reported in *Salmonella*, in which the GolS-dependent gene encoding the metal chaperone is the strongest upregulated gene in the presence of Au (Checa et al. 2007; Perez Audero et al. 2010; Pontel et al. 2007).

Other genes from the *cop* region involved in Cu resistance localized in the *C. metallidurans* plasmid pMOL30 (Monchy et al. 2006) were also strongly upregulated in the presence of Au ions (Reith et al. 2009). The *in silico* analysis performed in our laboratory predicts the presence of a CupR operator upstream the *copOFGJ* operon, whose genes have been reported to be under the control of the CopRS two-component system (Monchy et al. 2006). Because the operator sequence upstream *copOFGJ* is less conserved than those present upstream *cupAR* and *cupC*, its CupR-dependent induction is predicted to take place under long-term gold exposure, similarly to the GolS-dependent induction of the *Salmonella gesABC* operon (Perez Audero et al. 2010; Pontel et al. 2007).

The genomes of the other bacterial species identified in Au-associated biofilm communities (Reith et al. 2010) also carry genes encoding for GolS/CupR-like proteins, as well as putative GolS-dependent promoters upstream of P-type ATPases and metal chaperones coding sequences (Perez Audero et al. 2010; see also Fig. 1), highlighting the conservation of Au resistance pathways in bacteria associated with Au-grain surfaces. Horizontal transfer of genes involved in metal resistance and degradation of aromatic compounds has been demonstrated to occur between *Delftia acidovorans* and *C. metallidurans*, two of the species that coexist in most of the Au-grain associated biofilms (Reith et al. 2010; Van et al. 2009). Thus, this mechanism may be responsible for the dissemination of the gold-resistance genes within the biofilm.

Dissecting the molecular bases for bacterial Au sensing

GolS and CupR belong to the MerR family of bacterial regulators responsive to heavy metal ions, including both the essential (Zn(II), Co(II) and Cu(I)) and harmful (Cd(II), Pb(II), Hg(II) and Au(I)) transition elements. Mer-like regulators also respond to a wide spectrum of stress signals including aromatic and organic toxic compounds, as well as oxidative stress (Brown et al. 2003; Helmann et al. 2007; Hobman et al. 2005; Summers 2009). Each protomer in the active dimer can be divided into three functional domains: the N-terminal DNA-binding region that includes a winged helix–turn–helix structure, a long anti-parallel coiled coil helix involved in both dimerization and signal transduction, and the C-terminal inducer binding domain that differs in size and structure among the members of the family (Brown et al. 2003; Hobman et al. 2005). Structural studies performed on *E. coli* CueR bound to Cu(I) and ZntR bound to Zn(II) indicate that the metal ion is buried in a solvent-inaccessible site at the dimer interface in two symmetry-related metal-binding sites (Changela et al. 2003). Selectivity in metal recognition is mainly determined

by the number and organization of the ligands within the folded sensor protein (Chen and He 2008; Ma et al. 2009). Coordination of divalent metals in ZntR is achieved by conserved Cys/His residues from the C-terminal inducer-binding domain of one protomer and a conserved Cys residue at the N-terminus of the dimerization helix of the other protomer (Changela et al. 2003). In CueR, Cu(I) is coordinated in an intramolecular diagonal array with C112 and C120 residues from the metal binding loop (Changela et al. 2003). The conserved S77 residue at the N-terminus of the dimerization helix in the other protomer provides a shielded hydrophobic environment for the metal ion and at the same time avoids binding of +2 metal ions that typically require higher coordination numbers (Changela et al. 2003; Ma et al. 2009).

In spite of the progress made to understand the mechanisms that dictate selectivity between monovalent versus divalent metal ions in members of this family of regulators, it still remains unclear how a specific sensor can distinguish between metal ions that are similar in charge and coordination chemistry. In fact, biochemical and structural studies have shown that CueR binds Cu(I), Ag(I) and Au(I) with similar affinity (Changela et al. 2003; Stoyanov and Brown 2003). By contrast, GolS and CupR prefer Au(I) over the other coinage metal ions (Checa et al. 2007; Jian et al. 2009). GolS and CupR exhibit 42 and 46% amino acid identity to *E. coli* and *Salmonella* CueR respectively. Furthermore, both Au-sensors are phylogenetically linked and separated from CueR and its homologues (Checa et al. 2007; Perez Audero et al. 2010). Like CueR, GolS and CupR conserve the C112 and C120 metal binding residues, and the S77 residue that distinguishes monovalent metal sensors (Changela et al. 2003). A number of in vivo and in vitro experiments indicate that Au selectivity in these sensors is ensured by lowering their affinity for Cu(I) in comparison to the Cu-sensors, while maintaining or even increasing the affinity for Au(I) (Checa et al. 2007; Jian et al. 2009).

X-ray absorption spectroscopy performed on the Cu(I)-CupR complex (Jian et al. 2009) shows differences with the Cu(I)-centre determined in CueR (Changela et al. 2003; Chen et al. 2003). The data from the metal-binding environment of the Cu(I) ion bound to CupR best fit to a three-coordinate Cu(I)-complex composed of two short sulfur ligands, the conserved C112 and C120, and one long unidentified ligand assumed to be the Cys residue from the "CHH" motif at the C-terminus of the protein (Jian et al. 2009). CueR has also an analogous Cys/His rich "CCHH" motif at its C-terminus, however, both structural and spectroscopic studies performed for the Cu-sensor reflect a lineal di-coordinated geometry for the binding of Cu(I), Ag(I) or Au(I) (Changela et al. 2003; Chen et al. 2003). Interestingly, both the C-terminus Cys/His rich motif truncated versions of CueR and CupR still recognize Au(I) and Cu(I), albeit with altered affinities (Jian et al. 2009; Stoyanov and Brown 2003), suggesting that this region may modulate, but not determine metal selectivity. Furthermore, the presence of a "CHH" or "CCHH" motif adjacent to the metal binding loop is rather an exception among MerR monovalent metal sensors (Perez Audero et al. 2010).

The *Salmonella* GolS sensor does not have any Cys residue at its C-terminal region other than the conserved C112 and C120, yet, it is selective towards the larger Au(I) ion over Cu(I) (Checa et al. 2007). Moreover, the replacement of the very C-terminal region of GolS (after the I122) for the equivalent region of CueR did not affect metal selectivity (Checa et al. 2007). On the other hand, a chimaeric protein with a surgical exchange of the GolS Cys-encompassing metal binding loop (from I109 to C120) with that present in CueR mimics the wild type Cu-sensor in its responsiveness to Cu(I) and Au(I) ions (Checa et al. 2007). Interestingly, GolS and CupR share similarities in the loop sequence that could account for their metal preference. This includes a conserved Pro residue at position 118 that is replaced by Ala in CueR homologues. Conversely, CueR harbors a Pro residue at position 113 while GolS and CupR have an Ala or a Thr residue in this position, respectively. It remains to be established whether these subtle modifications at the metal binding loop are indeed the cause of Au-selectivity.

Promoter selectivity and transcription factor availability contribute to maintain monovalent metal homeostasis in *Salmonella*

Typically, MerR-like regulators recognize inverted repeat sequences spanning σ^{70}-dependent promoters with unusually longer distance between the −35 and −10 promoter elements than the normal 17 ± 1 bp (Brown et al. 2003; Hobman et al. 2005). The current

model, seemingly common to most of the members of the family, emerges from the pioneer work of Ansari et al. on the mercury sensor MerR (Ansari et al. 1992, 1995), and subsequent biochemical, genetic and structural studies performed on different MerR homologues (reviewed by Brown et al. 2003; Chen et al. 2010; Ma et al. 2009; Summers 2009). The model predicts that the affinity of the regulator for its target DNA binding site is not substantially affected by the inducer i.e. the metal ion. Interaction of the regulator with the operator sequence in the absence of the metal ion produces a curvature in the DNA, that captures the RNA polymerase in a stable but inactive pre-initiation complex. The interaction of the inducer with the sensing domain provokes a conformational change in the sensor protein, allosterically affecting the distal DNA binding domain (Chen et al. 2010; Ma et al. 2009). As a result, the activated regulator untwists the DNA by localized base-pair breaking and base sliding at the center of the operator, realigning the −35 and −10 promoter elements, which in turn leads to the formation of an open complex between the RNA polymerase and the initiation of transcription. In this regard, it has been postulated that only coordination of the right metal can direct suitable allosteric changes in the sensor protein to ensure proper biological regulation (Song et al. 2007).

All monovalent metal-binding MerR regulators characterized to date have very similar operator sequences, a TTGACCTTCCC inverted repeat conserved sequence, particularly at the hemisite that overlaps with the −35 promoter element (Perez Audero et al. 2010; Checa et al. 2007; Espariz et al. 2007; Jian et al. 2009; Pontel et al. 2007; Pontel and Soncini 2009; Stoyanov and Brown 2003). Therefore, the mechanism that prevents cross-recognition of target binding sites when two highly homologous transcription factors are simultaneously expressed in a single bacterial cell poses an intriguing dilemma. Recent work carried out in our laboratory shows that, in spite of the similarities in the operator sequences recognized by *Salmonella* GolS and CueR, in vivo cross-activation is prevented in two ways: the presence of bases at the centre of the operators sequences that confer selectivity towards their innate regulator, and tight control of the cytoplasmic concentration of the regulators (Fig. 3, see also Perez Audero et al. 2010).

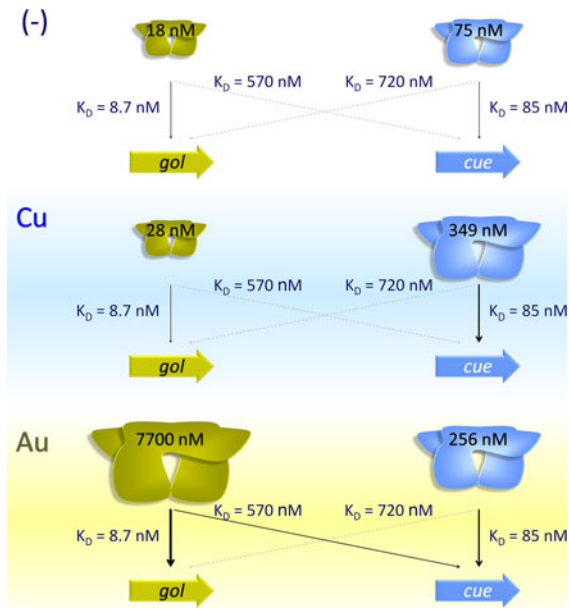

Fig. 3 *Salmonella* defence against group 1B monovalent metals depends on the interplay between regulator/operator binding affinity and the intracellular concentration of GolS and CueR. The figure shows the intracellular concentration of both GolS (*yellow*) and CueR (*blue*), and their dissociation constants (K_D) with the *gol* and *cue* promoters determined in the absence of metal (-), in the presence of Cu (Cu), or in the presence of Au (Au) (Perez Audero et al. 2010). *Filled lines* show demonstrated intra- and inter-regulon interactions. Unlikely inter-regulon interactions are shown with *dashed lines*. In the absence of inducing metal or in the presence of Cu only intra-regulon interactions occurred. In the presence of Au, GolS increases its concentration more than 400-fold (Perez Audero et al. 2010), outcompeting CueR for binding to the *cue* promoters

Phylogenetic analysis of putative MerR monovalent metal-ion regulators binding sites from 45 selected genomes of Gram-negative species reveals a tight correlation between the phylogenetic association of each regulator and its predicted binding sites. All GolS-xenologues are predicted to control the expression of their target genes by recognizing GolS-like target binding sites, i.e., sequences harbouring an A and a T at positions 3′ and 3 from the center of the operators (Perez Audero et al. 2010). Conversely, CueR-like regulators cluster together and are predicted to control genes with operators harbouring either a C or a G at the same position. In *Salmonella*, swapping these bases in the operators of GolS-controlled *golB* and CueR-controlled *copA* promoters results in a switch of the in vivo regulator's dependency and the in vitro regulator/operator

affinity (Perez Audero et al. 2010). It is important to note that the predicted *C. metallidurans* CupR operators harbor an A and a T at positions 3′ and 3, respectively (Perez Audero et al. 2010). Both phylogenetic clustering of the regulators and differences in their predicted target binding sites confirm the divergence between GolS xenologs and CueR-like regulators. It remains to be established whether differences in the operators sequences plus modulation of the cytoplasmic regulator availability operating in *Salmonella* (Fig. 3). are shared by other species harbouring more than one MerR paralog regulator. Likewise, they could also apply to other families of highly homologous transcription factors.

Crystallographic data of the drug-binding BmrR and MtaN, as well as the oxidative stress sensor SoxR bound to their target operators indicate that amino acid residues within the α2 helix of the winged helix-turn-helix motif and those from the loop between the α3 and α4 helixes at the N-terminal region interact with the DNA, causing a distortion of the centre of the operator that leads to transcriptional activation (Heldwein and Brennan 2001; Kumaraswami et al. 2010; Newberry and Brennan 2004; Watanabe et al. 2008). GolS-xenologues and CueR-like sensors are strikingly similar at this region and only few differences are apparent in the amino acid residues encompassing the α2 helix. In particular, all GolS homologues harbour a conserved Met at position 16, while Thr, Ala, or Ser, but not Met are found in CueR homologues. Unpublished observations from our laboratory indicate that replacement of the Met16 for Ala in GolS switches promoter selectivity. Conversely, the Ala16→Met mutation of CueR leads to an increased affinity for *gol* promoters and a reduced recognition of *cue*-like operators, indicating that this position is essential for specificity in the binding to cognate target operators. A more detailed analysis is underway to evaluate the role of these conserved residues in the operator's selectivity.

Concluding remarks

Microorganisms are known to be active in the formation and mobilization of gold deposits (Southam et al. 2009). Thus, the presence of Au-specific sensing/resistance devices in species isolated from biofilms on Au-grain surfaces, such as *Cupriavidus*, *Stenotrophomonas*, *Delftia* and *Burkholderia* (Fig. 2) is not surprising. On the other hand, the identification of a functional Au-responsive genetic cluster in *Salmonella* as well as in other bacterial pathogens (Checa et al. 2007; Perez Audero et al. 2010) is somehow intriguing. It has been demonstrated that several invertebrates, animals and plants can absorb and accumulate Au ions (Eisler 2004; Southam et al. 2009). Thus, the ability to detoxify Au may also confer an advantage for microorganisms that colonize these hosts. In this sense, we speculate that the evolution of a selective Au adaptive mechanism from a somehow promiscuous ancestral resistance system could be of great importance to bacteria harbouring resident Cu-homeostasis systems in order to properly detoxify Au ions without affecting Cu acquisition. In *Salmonella*, GolS not only privileges the binding of Au(I) over Cu(I) or Ag(I), but also distinguishes its target recognition sites in order to minimize cross-activation of CueR-controlled operators (Checa et al. 2007; Perez Audero et al. 2010). A similar situation may occur in other bacteria such as *Rhizobium leguminosarum* bv. Viciae 3841 and *Mesorhizobium sp.* BNC1 that contain genes coding for the two groups of MerR-monovalent metal ion regulators (Perez Audero et al., 2010). Further experimental evidence is required to support this hypothesis and to thoroughly elucidate the role of gold in the biosphere.

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