ArsR-SmtB metal-sensing transcriptional-repressors

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Metal-sensing transcriptional regulators are useful for probing the disposition of metals in cells, for exploring the kinetic and thermodynamic factors that allocate metals to the correct proteins, because (i) they can reveal metal-occupancy *in vivo* via the association of reporter genes with their target promoters, and (ii) in comparison to the binding sites of many other metalloproteins, metal-selectivity is likely to have been a dominant factor driving the divergence from common ancestors of the metal-binding sites of related sensors that detect different elements. Many bacterial genomes encode metal-sensing ArsR-SmtB transcriptional repressors. De-repression of ArsR-SmtB regulated promoters occurs when the proteins bind the respective metal(loid) resulting in a weaker affinity for DNA. There are eleven documented *in vivo* effectors (As, Zn, Cd, Pb, Co, Ni, Sb, Cu, Hg, Ag or Bi), five alternative intra/inter-molecular sensory sites (α3, α3N, α5, α5C or α4C) exploiting three, four or six Cys, His, Asp or Glu ligands. Using pair-wise comparisons of ArsR-SmtB sensors of differing metal-selectivities, contributions to specificity of (i) metal-partitioning based upon metal-affinity, (ii) metal-specific allostery and (iii) differential access to metals *in vivo* have been documented. This research will be summarized and analyses of two new sensors from *B. subtilis* described. Expression profiling of mutants deleted in genes encoding deduced ArsR-SmtB sensors, *ydeT*, *yozA* (now *aseR*, *czrA,* respectively) from *B. subtilis* confirmed de-repression of predicted target genes, while purified AseR and CzrA formed specific complexes with these promoters in gel retardation, and fluorescence anisotropy, assays. A candidate (i) partly-thiolate, α3 site was predicted in AseR, (ii) tetrahedral, non-thiolate, α5 site in CzrA, which we hypothesised would respond to oxyanions of As, Sb (AseR); or Zn (CzrA). These hypotheses were tested *in vivo* and *in vitro* and on this occasion found correct. Although AseR does not sense Zn *in vivo*, it binds one molar equivalent of Zn *in vitro* exploiting α3-thiols, but Zn-AseR retains DNA-binding and As-sensing. Thus, selectivity relies upon discriminatory triggering of allostery, not solely metal-partitioning based on affinities, even for an α3 sensory site which is proximal to the helix-turn-helix DNA-binding region. Cu(II) does not trigger CzrA but prevents Zn-sensing *in vitro* indicating that access to copper *in vivo* must be controlled to avoid aberrant formation of copper-CzrA.