Genetic and Biophysical Insights into How MerR Distinguishes Hg(II), Cd(II), and Zn(II)

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MerR, the metalloregulator of the bacterial mercury resistance (*mer*) operon, is the matriarch of an eponymous family of transcriptional repressor-activator proteins with an entirely novel mechanism of transcriptional control. MerR-type regulators function as dimers whose monomers are joined at the interface of an anti-parallel coiled coil that is flanked by N-terminal DNA-binding domains and C-terminal inducer-recognition domains. They bind DNA in an over-long spacer region between the -10 and -35 recognition hexamers for sigma-70 RNA polymerase (RNAP) in the promoters of their respective structural gene transcripts. Prior to exposure to Hg(II) MerR sequesters RNAP in a stable, pre-initiation complex. Subsequently, MerR is provoked by Hg(II)-binding to underwind the spacer DNA, allowing RNAP to fully engage and melt the -10 region forming an open complex and initiating transcription. The various members of the metalloregulator branch of the MerR family include proteins responsive to Cd(II), Zn(II), Pb(III), Co(II), and superoxide via Fe(II/III) redox cycling. In in vivo and in vitro transcription these proteins respond specifically to their preferred metal ion inducers. Recently we found that in vitro purified MerR and a small derivative containing only the metal binding domain will bind several different thiophilic metals, notably Zn(II) and Cd(II) even in the presence of millimolar competing thiols such as 2-mercaptoethanol. Thus, induction specificity does not reside simply in metal binding. Employing x-ray absorption spectroscopy, titration calorimetry, ultracentrifugation, equilibrium dialysis, mobility shift assays, and NMR of ^{19}F-substituted wildtype and mutant MerR proteins we have observed dramatic differences in the structure and behavior of MerR with preferred vs. non-preferred inducers. Consideration of these observations in light of homology modelling with the 3D structures of homologs BmrR, CueR, and ZntR allows us to begin to map the allosteric trajectory from the metal-binding domain through a heretofore undefined domain to the ca. 25 Å distant DNA binding domain.