Optimizing the Metalloregulator MerR for Metallosequestration and Metallosensing

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This is a new project based on our previous work supported by another agency. Its objective is to re-engineer the metal binding domain (MBD) of MerR to recognize metals besides Hg(II), especially those of interest in DOE bioremediation.

Expression of the Tn21 mercury resistance (mer) operon is regulated by a high affinity, high specificity metal-sensing repressor-activator, MerR, which represses transcription of the structural genes, merTPCAD, in the absence of Hg(II) and activates their transcription in the presence of Hg(II). MerR contains three domains: An N-terminal DNA binding domain, a C-terminal Hg(II)-metal-binding domain (MBD), and a coupling domain which lies between them. The MBD consists of a helical region from Cys82 to Cys117 followed by a loop from Cys117 to Cys126. Dimerization of MerR is effected by the formation of a coiled-coil employing these C-terminal helical regions of each monomer. Independent, Hg(II)-binding centers lie at each end of the coiled-coil and are comprised of Cys82 from one monomer and Cys117 and Cys126 from the other. This novel inter-subunit, trigonal metal binding center is highly specific for Hg(II) over other Group 12 metals, Cd(II) and Zn(II). We have previously described 11 single point mutants which allow MerR to respond to Cd(II), but not to Zn(II).

As the next step in manipulating the metal-responsiveness of MerR, we are asking how the loop region between Cys117 and Cys126 influences metal specificity by replacing the loop region of MerR with the ZntR loop region. ZntR, is a MerR-like regulator that is more responsive to Zn(II) and Cd(II) than to Hg(II), but unlike MerR, it contains a histidine residue in the middle of its loop region. Histidines are very often found as ligands to Zn(II) in other proteins, so we hypothesize that the ZntR histidine residue may provide a fourth ligand required for Zn(II)-or Cd(II)-binding. To avoid possible artifacts when using metals other than Hg(II), we have switched from using a hexa-Histidine affinity tag to using a streptavidin affinity tag (Sigma-Genosys) for protein purification. We are screening these mutant proteins for their ability to bind Hg(II), Cd(II), Zn(II), and ions of metals involved in radiation bioremediation, including Co and Pb, using a facile DNA gel mobility shift assay to detect metal-induced allosteric changes in MerR. When mutants exhibiting optimum metal affinity characteristics are identified, their MBD domains will be produced alone (lacking the DNA-binding and coupling domains) and subject to precise metal-binding quantification via equilibrium dialysis and isothermal calorimetry. Further optimization of these minimal MBDs will employ phage display technology.