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ABSTRACT TITLE: Defining the Molecular-Cellular-Field Continuum of Hg Detoxification

ABSTRACT: Very effective Hg detoxifying proteins have evolved in many bacteria and archaea. Exploiting removal of Hg^{2+}/$MeHg^{+}$ in wetlands by such naturally Hg resistant (HgR) bacteria requires knowing how these proteins work. We study HgR genes of α- and γ-proteobacteria and actinobacteria abundant in the ORR and report here on the mechanisms of the *mer* reductase (MerA) and demethylase (MerB) and regulatory proteins that optimize their expression. We also report on novel specific, high affinity Hg binding to natural, cellular nucleic acids.

hπPROTEOBACTERIAL FUSED MerB-MerA: *Ochrobactrum anthropi*, a relative of α-proteobacteria found in the East Fork Poplar Creek, have a *merBmerA* gene encoding a novel fused MerB-MerA with demethylase and *mer* reductase activities. We cloned the full fusion and its separate *merB* and *merA* genes and properties of all three constructs will be presented.
JπPROTEOBACTERIAL MerA: Reduction of Hg^{2+} in all MerA proteins occurs deep within the catalytic domain. Two cysteines near the C-terminus transfer Hg^{2+} from dithiol complexes in solution to a pair of cysteines in the active site. Altered rates for Hg^{2+} acquisition and reduction with a K449A mutation suggest K449 electrostatically controls dynamic motions of the C-terminal tail. Computational studies implicate two additional residues. Effects of mutations of these on kinetic and dynamic properties of the enzyme will be presented.
ACTINOBACTERIAL MerA/MerB: Relatives of *Streptomyces lividans* are also found in high Hg regions of the ORR. *S. lividans* MerA lacks the tethered NmerA domain found in γ-proteobacterial MerA, and its MerB lacks a cysteine of proteobacterial MerB essential for Hg^{2+} transfer to NmerA, but has a distinct C-terminal cysteine pair whose role in RHg^{+} binding and Hg^{2+} release is being probed by mutagenesis.
Hg ISOTOPE FRACTIONATION BY PURIFIED MerA: Distinct signatures in Hg isotope fractionation are important for distinguishing biotic and abiotic contributions to natural Hg cycling. Kritee et al (2007) measured Hg isotope fractionation by intact cells expressing *mer* genes and proposed that MerA is largely responsible for the observed effects. To test this in vitro, we're measuring Hg isotope fractionation with a purified γ-proteobacterial MerA. Technical challenges and progress will be presented.
PROTEOBACTERIAL REGULATION OF *mer* EXPRESSION: In gamma-proteobacteria, repressor-activator MerR holds RNA polymerase (RNAP) at the *mer* operator-promoter (MerOP) until Hg^{2+} stimulates it to let RNAP transcribe. We find that Hg^{2+} also binds the 38 bp MerOP DNA in the absence of thiols (as in acute Hg^{2+} exposure) at 2-3 high affinity sites. The EXAFS spectrum of Hg-MerOP is indistinguishable from pure thymidine(T)+Hg and best fits two N ligands. Calorimetry reports similar high affinity sites in the upper and lower MerOP single strands and in the Hg + T interaction. Once formed, Hg-DNA adducts are only slowly removed by a two-fold excess of cysteine. T's do not abut in B-form DNA, but MerOP may adopt a cruciform structure where two Ts abut in the lower strand loop and we are currently testing relevant mutants. Intact *E.coli* cells bind >3-fold more Hg^{2+} than their available thiols could bind and EXAFS indicated N or O ligands are involved. MerOP-Hg binding reveals Hg binding distinct from the classic model based on slippage in AT runs, so DNA and RNA (uracil) may be an unrealized sink for Hg^{2+}

with unknown turnover. We are pursuing this broader aspect of Hg biology with larger defined DNA and RNA molecules.

ACTINOBACTERIAL REGULATION OF *mer* EXPRESSION: In contrast, Actinobacteria control the *mer* operon with a simple ArsR-type repressor. The *S. lividans* MerR (SLMerR) is a distinct clade of the ArsR family which, by homology, shows a possible Hg^{2+} binding site near its DNA recognition helix, very different from metal sites in other ArsR regulators. SLMerR over-expression was toxic in *E.coli* due to codon incompatibility, so we synthesized a codon-optimized *S.lividans merR* gene with a His-tagged SUMO fusion for facile purification of a tagless wildtype protein. This has worked beautifully with 100% cleavage yielding completely pure, soluble SLMerR at approximately 12-13 mg/L, ready to scale up for biochemical and biophysical studies.