

Evolution of a Naturally Removable Protein Subdomain

Bijal Patel and Anne O. Summers *Microbiology Dept, Univ. of Georgia, Athens GA, 30602.*

Background Mercuric ion reductase (MerA), the flavin-disulfide reductase (FDR) of bacterial mercury resistance operons (*mer*) consists of a catalytic FDR Core, which reduces Hg(II), linked via ~ 30 residues to an N-terminal domain (NmerA) homologous to heavy-metal-associated (HMA) transport proteins, metallochaperones, and the periplasmic Hg(II) binding protein, MerP. Surprisingly, NmerA is cleaved from ~40% of MerA during normal or over-expression. The catalytic Core remains active and whether attached or free, NmerA enhances survival when cells deficient in thiols are stressed with Hg(II). The site of *in vivo* cleavage of NmerA from Core was not defined, so *in vitro* NmerA work has used a 68 residue recombinant protein defined by its alignment with the periplasmic homolog, MerP. The NMR structure of recombinant NmerA closely resembles that of MerP and the HMA domains of the Cu(I) transporter CopA and the copper chaperone CopZ. How did an ancestral HMA gene become fused with an ancestral FDR gene and how is the protein product of this fused gene cleaved? **Methods** Phylogenetic analyses were used on 68 alignable residues of MerA, MerP, and bacterial Cu(I) HMA domains. The MerA cleavage point was determined by MALDI-MS of purified MerA which always contains full length and cleaved molecules. **Results** The phylogenetic method Neighbor Joining (NJ) shows NmerA is related to a cytosolic Cu(I) chaperone (Cop) and Core to glutathione reductase. Gram negative (G-) NmerA is closer by ~0.1 changes per residue (cpr) to Cop than to Gram positive (G+) NmerAs (0.32 cpr). The G+ NmerA to Cop distance is ~ 0.2 cpr, suggesting either that G- and G+ NmerAs arose separately from Cop or they evolved at different rates from an ancestral two domain MerA. The G+ and G- MerPs group at 0.1 cpr from Cop, like G- NmerAs, but G+ NmerAs and MerPs diverge by ~0.5 cpr so the two MerPs evolved from Cop independently of NmerA. Maximum likelihood generally confirms the NJ clades and discrepancies are likely due to the small protein sizes. For NmerA-Core cleavage we saw major peaks of ~59 kDa (Full-length), ~52 kDa (Core), ~9 kDa (NmerA) and ~19 kDa (possible NmerA S-S dimer). The ~9 kDa fragment places the major cleavage 22 residues into the linker, beyond the C-terminus of homolog MerP; however, minor peaks suggest some cleavage adjacent to the MerP homology and to the Core. **Conclusion** NmerA and MerP evolved separately from an ancestral Cop rather than from each other. *In vivo* cleavage of MerA occurs near the middle of the linker rather than adjacent to the MerP homology terminus as previously assumed.