

W0339

Metal Preference of DEDDh vs DEDDy Exonucleases? A Structural and Mutagenic Study of *E. coli* Oligoribonuclease. Adviye A. Tolun, Yuhong Zuo, Jianwei Zhang, Yong Wang, Arun Malhotra, Univ. of Miami School of Medicine, Miami, FL.

Proteins of the DEDD family, including the proof-reading domains of DNA polymerases and many other DNA and RNA exonucleases, have a characteristic core comprised of four highly conserved acidic residues embedded within three sequence motifs. They share a common catalytic mechanism involving two metal ions. The DEDD exonucleases fall into two subgroups, DEDDh and DEDDy, based on whether they contain a histidine (h) or a tyrosine (y) in motif III. The conserved tyrosine has been shown in the Klenow fragment structure to direct and activate a water molecule for nucleophilic attack. A similar role has also been proposed for the DEDDh histidine. Oligoribonuclease (ORN), the only essential exoribonuclease in *E. coli*, is a DEDDh enzyme. ORN action requires Mg²⁺ or Mn²⁺, but is inhibited by Zn²⁺ or Co²⁺. Recently we solved a new structure of *E. coli* ORN from crystals obtained in the presence of Cd²⁺/Zn²⁺. In the DEDD active center of this structure, we find three bound metal ions rather than the usual two. The extra metal ion interacts with the conserved histidine H157. Enzymatic analysis with H157 mutants suggests an essential role of H157 for ORN activity and its inhibition by Zn²⁺/Co²⁺. A metal preference of DEDDh vs DEDDy exonucleases is hypothesized.