Mutational studies of the conserved Lys693 in ZntA from *Escherichia coli*

Sandhya Muralidharan, Bharati Mitra

Department of Biochemistry and Molecular Biology, School of Medicine, Wayne State University, Detroit, Michigan.

ZntA from *Escherichia coli*, is a member of the P_{1B}-type ATPase family of transporters. These pumps maintain cellular homeostasis of heavy metals such as Zn^{2+}, Co^{2+}, Cu^{2+}, Cu^{+}, and mediate resistance to toxic metals such as Pb^{2+}, Cd^{2+} and Ag^{+}. Despite a high level of sequence homology, transporters in the P_{1B} family of ATPases show stringent metal selectivity. They can be divided into subgroups that are selective for $Zn^{2+}/Pb^{2+}/Cd^{2+}$, Cu^{+}/Ag^{+}, Co^{2+}, Cu^{2+} and Ni^{2+}. We are investigating the molecular determinants of metal selectivity of the metal site in the transmembrane region. P_{1B}-type ATPases have eight transmembrane helices. A CP(C/H) motif present in the sixth transmembrane helix, TM6, of all P_{1B}-type pumps, provide two ligands to the metal ion. In addition, studies in our laboratory have shown that an Asp residue in the middle of TM8, which is strictly conserved in the $Zn^{2+}/Pb^{2+}/Cd^{2+}$ subgroup but not in the Cu^{+}/Ag^{+}, Co^{2+}, and Cu^{2+} subgroups, supplies ligands to the metal site. This Asp residue is conserved in P_2 and P_3-type ATPases, including the Ca^{2+}-ATPase from sarcoplasmic reticulum and the plasma membrane H^{+}-ATPase.

Sequence alignments indicate that a Lys residue, K693, in the middle of TM7, is strictly conserved in the $Zn^{2+}/Pb^{2+}/Cd^{2+}$ subgroup, but not in any other subgroups of P_{1B}-type ATPases. Additionally, this residue is not conserved in P_2 and P_3-type ATPases. This residue may be important in maintaining a salt bridge to the conserved Asp residue in TM8, or it may be directly involved in providing a ligand to the metal. Preliminary mutational studies of K693 indicate that this residue is important for overall activity of the transporter. The possible involvement of this residue in metal binding and selectivity will be described.