Chemical & Physical Sciences
UNIVERSITY OF TORONTO
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COLLOQUIUM SEMINAR SERIES

Structural and Biochemical Characterization of the Effects of Post-Translational Modifications in the Regulation of ATP-Binding Cassette Proteins

featuring Sarah Bickers

Recipient of the Fraser Code PhD Thesis Award, Physics, CPS Dept

The Yeast cadmium factor 1 protein (Ycf1p) is an ATP Binding Cassette (ABC) membrane protein responsible for actively transporting glutathione conjugated heavy metals from the yeast cytoplasm into the vacuole, to detoxify the yeast cell. Like other ABC proteins, Ycf1p is a multidomain protein composed of three transmembrane domains (TMDs) and two nucleotide binding domains (NBDs) arranged as TMD0-TMD1- NBD1-TMD2-NBD2. Ycf1p also contains the cytoplasmic loop (L0) which connects TMD0 to TMD1 and an intrinsically disordered regulatory (R) region that links NBD1 to TMD2. The activity of Ycf1p, and almost all ABC proteins, is regulated through post-translational modifications occurring at loops and disordered regions. Specifically in Ycf1p, proteolytic cleavage at a vacuolar luminal loop alters Ycf1p substrate affinity and phosphorylation of the R region increases Ycf1p activity. Despite the prevalence of regulatory proteolytic cleavage and phosphorylation post-translational modifications of proteins across the ABC transporter superfamily, the underlying molecular mechanisms for these regulatory modes remains largely unknown. This dissertation details structural and biochemical investigations into the molecular mechanism of regulatory post-translational modifications in Ycf1p, a member of the C subfamily of ABC proteins. I determined that the association of TMD0 with the L0 and TMDs differs between ABC proteins of subfamily C. This structural plasticity may serve to regulate ABC protein-protein interactions that are hypothesized to occur through TMD0. I also identified that proteolytic cleavage promotes stable dimerization of Ycf1p, which is mediated by membrane and cytosolic protein-protein as well as protein-lipid interactions involving TMD0. Further multidisciplinary Ycf1p investigations highlighted that the R region makes transient interactions with NBD1, and that phosphorylation of the R region alters its transient interdomain associations. Subsequent characterization of the R region ensemble changes due to phosphorylation revealed localized structural changes. My studies overall greatly expand our understanding of ABC structure and regulation, and provide the foundation for future investigations into post-translational modifications of ABC proteins

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