Investigation of Tha4 oligomerization based on the hydrophobicity of transmembrane helix during the active cpTAT translocation.

Vidusha Weesinghe[1], Carole Dabney-Smith[2]

[1]*Department of Chemistry and Biochemistry, Miami University. 701 E. High St., Oxford, OH 45056. weesinvs@miamioh.edu.*

Most of the proteins required by chloroplasts for proper metabolic and photosynthetic function are encoded by genes in the nucleus and translated in the cytosol of plant cells. These newly synthesized proteins contain an N-terminal amino acid extension that serve to direct or target the protein, called a precursor, to the chloroplast envelope and thylakoids. Protein transport complexes in membranes provide the conduit through which precursors are transported across the membrane. One such transport complex is the Twin Arginine Transport (cpTAT) pathway. The cpTAT pathway transports fully folded proteins by using the proton motive force (PMF) as the only source of energy.[1]

The cpTAT pathway consists of three membrane-bound proteins namely, Tha4, Hcf106, and cpTatC. From previous studies, it was found that the conserved glutamate residue (E10) in the transmembrane domain (TMD) of Tha4 is essential for function in the translocation of precursor proteins and for Tha4 assembly.[2] It was found that the substitution of alanine for the glutamate prevents transport while an aspartate substitution partially recovers the transport. We predict that the E10 of Tha4 TMD acts as a sensor of the formation of PMF to assemble into large oligomers, which aid the transport of the precursor. First, oligomer formation of each Tha4 E10/A/D variant, in the presence and absence of PMF was investigated by substituting cystines to the lumen proximate positions in the transmembrane helix of Tha4 by employing by radiolabeled crosslinking assays. Tha4 oligomer formation increases when alanine is substituted for the glutamate in the Tha4 TMD, while an aspartate substitution showed a lower degree of oligomerization. From these studies, we show that modulations to helix hydrophobicity of Tha4 impact monomer and oligomer stability and their packing, thereby altering precursor transport via cpTAT pathway.

References-

01. New, C. P.; Ma, Q.; Dabney-smith, C. 2018 Routing of Thylakoid Lumen Proteins by the Chloroplast Twin Arginine Transport Pathway. *Photosynth. Res.*, 138(3), 289-301
02. Dabney-Smith, C., H. Mori, and K. Cline. 2003. Requirement of a Tha4-conserved transmembrane glutamate in thylakoid Tat translocase assembly revealed by biochemical complementation. J Biol Chem. 278:43027-4303