Investigation of Hsp70 functions in *Physcomitrella patens* chloroplasts

Lan-Xin Shi*, Christina Reginaldo and Steven M. Theg

Section of Plant Biology, One Shields Avenue, University of California, Davis, CA 95616
*e-mail: lshi@ucdavis.edu, phone: 1 530 752 8859

Heat shock protein (Hsp) 70s are encoded by multi-gene families and are localized in different cellular compartments. Hsp70s require co-chaperones, such as GrpE, a nucleotide exchange factor and J-domain proteins to fulfill their functions. The Hsp70s have a number of functions among which is a role in protein trafficking. For example, a mitochondrial matrix Hsp70 has been shown to be involved in protein translocation across the mitochondrial membranes. The role of chloroplast stromal Hsp70 systems in protein import into that organelle is unclear. We are trying to resolve this question using the moss *Physcomitrella patens* as a model system.

In this study, we isolated two Hsp70 and two GrpE genes from *P. patens*. The products of these genes are shown to be localized in chloroplast stroma. We have generated *Pphsp70-1* and *PpgrpE* null mutants by gene targeting. The *hsp70-1* knockout displays a slightly slower growth rate as its sole phenotype. In contrast, deletion of PpHsp70-2 gene causes lethality and plants must be rescued by co-transformation with a rescuing cDNA copy of the disrupted gene product. We have adopted a number of strategies to obtain conditional mutants aimed at revealing the function of this latter essential gene. First, we have sought, with limited success, to use a temperature-sensitive protein to rescue the gene-disrupted *Pphsp70-2* mutant plants. Second, we have used a tetra-cysteine tagged PpHsp70-2 (TC-Hsp70-2) as the rescuing protein. We are seeking to eliminate the functional copy of this protein by chromophore-assisted light inactivation. Finally, we are attempting to use the mitochondrial matrix Hsp70 to complement PpHsp70-2, because temperature-sensitive alleles of this gene are already known. Characterization of some of these conditional *hsp70-2* mutants and *grpE* mutants will be presented.