Introduction

Light drives life on earth by powering photosynthesis. While the chloroplast and most primary photosynthetic proteins and pigments have been well characterized, the regulation of photosynthesis in response to dynamic environmental conditions remains complex and partially unknown. The extended light harvesting complex (LHC) protein family form the photosynthetic antennae where these reactions take place. LHC proteins transfer light energy to the reaction center of photosystems while ancestrally related LHC-like proteins perform a photoprotective role in the presence of excess light energy, but both groups share a common ancestor and conserved chlorophyll binding domains (Engelken et al., 2012).

Early-light-induced proteins (ELIPs) represent a subfamily of the LHC-like proteins present in green algae and land plants. They are characterized by two conserved chlorophyll binding domains in the first and third of three helices and expression induced in response to high light stress, suggesting a photoprotective role. However, the mechanism by which ELIPs function as photoprotective proteins is unknown. To investigate this function, an insertional mutant of an early light induced protein (ELI3, Cre09.g394325) in the model organism Chlamydomonas reinhardtii will be characterized. Characterization of ELI3 will improve understanding of photosynthetic regulation in response to light stress with potential applications in improving photosynthetic efficiency in crop plants.

Materials and Methods

Establishing the eli3 phenotype

The mutant strain eli3 (Dent et al. 2005) was sequenced by whole genome shotgun sequencing (Wakao et al. in preparation) to determine the insertion site of the antibiotic resistance marker (Figure 2). Growth of eli3 was evaluated on acetate-containing medium and acetate-free medium in low light conditions to determine if the mutant could still photosynthesize. The maximal quantum yield of photosystem II (Fv/Fm) was then measured by a video imager and a fluorescence monitoring system after increasing durations of high light stress.

Connecting Phenotype to ELI3 Disruption

Because eli3 was generated by insertional mutagenesis, it must be proven that the insertion in Cre09.g394325 (Figure 2) is solely responsible for its photosynthetic phenotype. Transgenic eli3 colonies were used to express, functionally complement, the ELI3 gene should restore photosynthetic capacity. An optimized version of the ELI3 gene with C and N-terminal FLAG tags was synthesized and cloned into the pOptimized vector system for transformation (Figure 3). This proves that the insertion does not prevent photosynthesis as the strain is using exclusively dephosphorylated carbon for growth. However, after exposure to high light stress, the quantum yield of photosystem II (Fv/Fm) decreases to 0 in eli3 while wildtype recovers (Figure 3B). This inability to recover from stress supports the hypothesis of ELI3 as a photoprotective protein.

Results

Quantifying ELI3 Expression

To verify both absence of ELI3 expression in the insertional mutant and gain an improved understanding of ELI3 expression in wildtype, qPCR was performed on RNA extracted from eli3 and wildtype samples grown in low light and after an hour in high light, and on acetate replete and deplete medias. CBLP control strain was measured to see how other LHC-like stress proteins respond to this treatment. This will be expanded upon by RNA-seq whole transcriptome studies after various timepoints of high light treatment to allow for differential gene expression analysis and genetic cross and RNA-seq experiments were disrupted and indefinitely postponed, but would support that this insertion is solely responsible for the observed phenotype and provide a global view of expression in response to high light treatment and in the absence of ELI3. Future high performance liquid chromatography comparisons, pigment binding studies, and protein localization and quantification will all provide clues to the function of ELI3 as a photoprotective protein.

References & Acknowledgements


By Chandler Sutherland, Dr. Setsuko Wakao, and Dr. Krishna Niyogi

The expression of endogenous ELI3 in wildtype increased 5x after an hour of high light treatment in acetate replete media (Figure 4). This increase was confirmed by the fold change of LHCSR3.1, a related stress-induced protein tested for comparison. In acetate deplete media, expression of ELI3 unexpectedly decreased after high light exposure. This contradicts previously published expression studies of ELI3 and more repressions and exploration of different high light exposure time is necessary.

Conclusions & Future Directions

The insertion of the ble gene, despite its location in the 3’ UTR, is sufficient to prevent expression of ELI3 in the insertional mutant. This mutant can still successfully harvest light and fix carbon dioxide, but decreases in photosynthetic yield in response to high light stress, pointing towards a photosystem II-related photoprotective role of ELI3.

Because of COVID-19 related closures, complementation, genetic crosses, and RNA-seq experiments were disrupted and indefinitely postponed, but would support that this insertion is solely responsible for the observed phenotype and provide a global view of expression in response to high light treatment and in the absence of ELI3. Future high performance liquid chromatography comparisons, pigment binding studies, and protein localization and quantification will all provide clues to the function of ELI3 as a photoprotective protein.

References


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