Characterization of an Early-Light Induced Protein in *Chlamydomonas Reinhardtii*

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Photosynthesizing since 1997

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 (Engelkan 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.

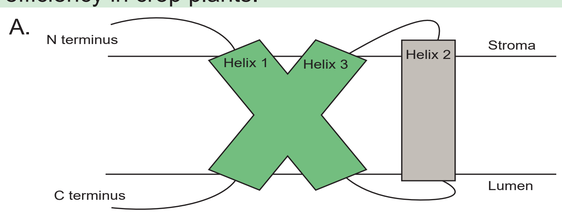

Figure 1: ELIPs in *Chlamydomonas reinhardtii*. A. The predicted protein structure of ELIPs, with conserved chlorphyll binding helices 1 and 3 highlighted in green. Adapted from Adamska 2001. **B.** Conservation of the helix 1 chlorohyll binding domain in Chlamydomonas. LHCBM, LHCA, and LHCB are light-harvesting proteins (LHC) while LHCSR, PSBS, OHP, and ELI are stress response proteins (LHC-like). Conserved chlorophyll binding residues marked A4 and A1.

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.

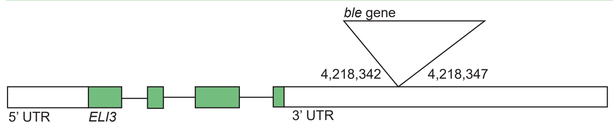

Figure 2: Insertion site of the *ble* gene in *eli3*. The *ELI3* gene on chromosome 9 is depicted with the zeocin-resistance conferring *ble* gene inserted between coordinates 4,218,342 and 4,218,342 of chromosome 9, in the 3' untranslated region of *ELI3*.

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. Transforming *eli3* with an endogenous, functional version of 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 glass beads transformation into *eli3*. In parallel, *eli3* was crossed with wildtype to determine if antibiotic resistance co-segregates with the Fv/Fm phenotype.

Quantifying *ELI3* Expression

To both verify 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* was used as an expression control and *LHCSR 3.1* 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 and co-expression data, elucidating networks involved in ELI3 function and regulation.

Results

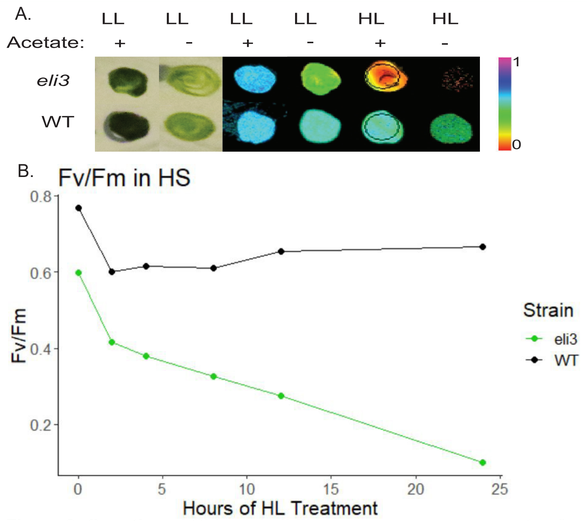

Figure 3: Growth and Chlorophyll Fluorescence measurements of *eli3* and wildtype. A. Comparative growth of cells on acetate replete and deplete medium grown for two weeks in low light (LL, 70 µE m-2 s-1). Fv/Fm represented as false color colonies before and after a 24 hour high light treatment (HL, 500 µE m-2 s-1). **B.** Fv/Fm with respect to hours of HL treatment. Fv/Fm take by FMS of cultures grown in liquid acetate deplete media after various lengths of high light treatment.

In low light, *eli3* can grow without acetate (Figure 3A). This proves that the insertion does not prevent photosynthesis as the strain is using exclusively atmospheric carbon for growth. However, after exposure to high light stress, the quantum yield of photosystem II (F_V/F_m) 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.

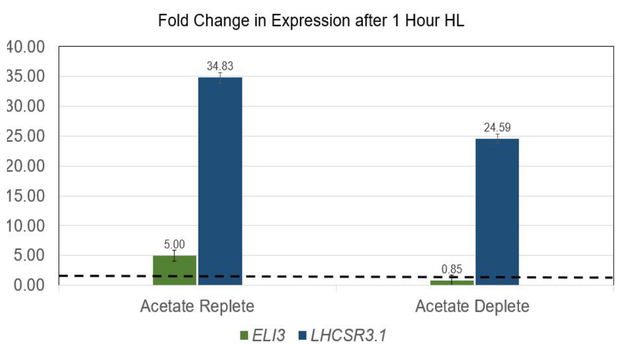

Figure 4: Fold Change in expression of ELI3 and LHCSR3.1 in wildtype after 1 hour of high light treatment. CBLP control shown as 1 fold change by the dashed line. Fold change calculated by the $\Delta\Delta C_T$ method.

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 dwarfed 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 replications 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 performace 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

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