This is the final report for grant **DE-FG02-02ER15333** "Functional Analysis of Chloroplast Early Light Inducible Proteins (ELIPs)", PI **Carolyn M. Wetzel, Smith College**. The project is a continuation of work originally funded under grant DE-FG02- 99ER20333 (same title and objectives) at East Tennessee State University, and this report will not repeat information filed in the final report for that grant submitted to DOE in November 2002 (hereafter referred to as Report 1). Several of the objectives were met in the work at ETSU, so only the objectives addressed in research at Smith College are described in this report.

Objective I: Characterize *ELIP* gene structure and expression

I.A. Gene Structure

Results related to the structure of genes in *Arabidopsis thaliana* were described in Report 1. Since then, Arianna Bruno (a Masters student at Smith College) and I analyzed the *ELIP* gene in *Lycopersicon esculentum* (Bruno and Wetzel, 2004). We determined that is a single copy gene in tomato with a 3-exon structure typical for the gene in other species. Both cDNA and genomic sequences were determined, and 5'-RACE was carried out to verify the cDNA start.

I.B. *ELIP* Expression

1. Expression in tomato

Expression of the *ELIP* gene in tomato was analyzed in de-etiolating seedlings and in ripening fruit (Bruno and Wetzel, 2004). The pattern of *ELIP* mRNA expression in seedlings was as described for *ELIP* in other species and was similar to what we observe for Arabidopsis *ELIP1* in our lab. Its abundance increases from undetectable in the dark to highest after 2-4 hours of illumination, then drops back to low levels by 8 hours light treatment. In ripening fruit, expression is low but detectable in developing green fruit but then undergoes a dramatic increase in abundance at the breaker stage of ripening, when the chloroplasts are converting into chromoplasts. From our results we conclude that the ELIP protein may have a role in the chloroplast-to-chromoplast transition, a novel observation.

We are in the process of analyzing ELIP expression during fruit development using several well-characterized ripening mutants of tomato, which include disruption of light signaling, ethylene perception, a MADS-box factor, and pigmentation. We are developing anti-tomato ELIP antisera to extend the expression analysis to protein levels.

2. Expression in Arabidopsis

An undergraduate in the lab, Laura Harmacek, continues the analysis of the effect of salt on *ELIP* mRNA expression. Some data related to this effect have come out in microarray results from other labs, but Laura is doing a more precise and in-depth study of the combined effects of light, salt, stage of development, and salt versus osmotic potential. She is away for Junior Year Abroad and plans to finish her work next year.

Work on the effect of redox status and pigmentation on ELIP expression continues. We routinely use quantitative real-time PCR to measure *ELIP1* and *ELIP2* abundance in Arabidopsis. Results related to analysis of various signaling pathways are coming together and will soon be submitted. Abundance of ELIP protein is also being measured in some of these analyses.

I.C. Generation of Anti-ELIP Antibodies

Report 1 described our work with anti-Arabidopsis ELIP antisera. We are now working on generating anti-tomato ELIP antisera and thus far have cloned the cDNA in-frame into an expression vector (pET28) for overexpression of antigen.

Objective II: Analyzing the Role of ELIP in Antisense *ELIP* Transformed *A. thaliana* and in *ELIP* Gene Knockout Mutants

Screening of the Wisconsin populations of T-DNA transformed knock-out (KO) plants (described more fully in Report 1) has led to identification of an ELIP1 KO. The insertion is at the beginning of exon II. Thus far we have been unable to grow a homozygous mutant on regular soil/light conditions which suggests that the gene is essential for normal development. We are currently trying alternative growing conditions that may be more permissive.

Screening putative insertion lines from the Salk collection has yielded an ELIP2 KO plant with an insertion in the beginning of exon I. Phenotypic characterization of the homozygous mutant is underway.

Publication from this work:

Bruno, AK and Wetzel CM. 2004. The early light-inducible protein (*ELIP*) gene is expressed during the chloroplast-to-chromoplast transition in ripening tomato fruit. *J Exp Bot* 55:2541-2548