

**Final Report: DE-FG03-89ER13993**  
**Signal Transduction Pathways that Regulate CAB Gene Expression**  
**Period Covered by Report: 2/25/1989 -12/31/2004**

**A. Introduction**

This project is concerned with the identification of new genes that regulate light-controlled development and gene expression in *Arabidopsis thaliana*. When this proposal was originally submitted in 1988, we understood very little about the events that occurred between photoreceptor excitation and the regulated expression of nuclear and chloroplast genes. At that time, I proposed a broad strategy to isolate and characterize photomorphogenic mutants based on phenotypic screens. During the funding period, our work has identified *Arabidopsis thaliana* mutants in both signal perception and transduction elements of the photoreceptor pathways. In the original proposal, I also outlined a second strategy, using promoter fusions to select trans-acting regulatory mutations that would allow us to isolate new mutants independent of a predetermined phenotype. For our studies, we have utilized the *Arabidopsis cab3* (now called *lhcb*) promoter to select for new mutants in which the *cab3* promoter is aberrantly expressed with respect to light, tissue-specificity of expression, and in response to signals from the chloroplast.

Our long-term goal is to understand in detail the molecular mechanisms by which organisms perceive environmental signals and respond to them. Our model is light signaling in the small mustard plant, *Arabidopsis thaliana*, an ideal organism for in-depth genetic and molecular investigations of signal transduction networks. These studies should ultimately influence our abilities to manipulate plant growth and development, and will aid in the understanding of the developmental control of photosynthesis.

**B. Major Findings**

**1. Molecular genetic dissection of the light signaling network.** (*This was the major project during the first 5 years*) (Chory et al., 1989a, 1989b, 1993, 1994, 1995, 1996; Chory, 1991, 1992, 1997; Reed et al., 1993, 1994, 1996; 1998; Li et al., 1993, 1995; Elich and Chory, 1994; Fankhauser and Chory, 1997; Nemhauser and Chory, 2002). In the early years of this award, we used phenotypic screens to identify light regulatory mutants of *Arabidopsis*. Our mutant screens identified seedlings that were insensitive to light, as well as seedlings that developed as light-grown plants in the absence of light. We went on to show that some of the light-insensitive mutants correspond to the red/far-red light photoreceptors, phytochromes A and B. The second class of mutants led to the elucidation of the *DET/COP/FUS* class of negative regulators. These genetic studies indicated that light responses were not simply endpoints of linear signal transduction pathways, but result from the integration of information from a network of interacting signaling components. As with other signal transduction systems, our data suggested that a small number of signaling components regulated a multitude of responses by co-opting a larger number of specific regulatory molecules.

Our genetic studies identified many of the regulatory switches controlling light-regulated gene expression and development in *Arabidopsis*. We used genetic epistasis analyses to develop a working hypothesis for light-regulated development. In this model, the action of multiple photoreceptors is integrated through global repressors (*DET1*, *COP1*, *FUS*). These act in turn through specific regulators that activate or repress expression of certain sets of genes including those required for morphogenesis. The light-dependent regulators interact with cell-type specific positive regulators. While this model did not address the actual mechanisms involved, it suggested a framework from which to address the mode of action and the interactions of the

various gene products. Since 1990, many of these mechanisms have been elucidated, by both my lab and others.

**2. Auxin plays a role in light-regulated seedling development** (Li et al., 1994; Christensen et al., 2000; Gil et al., 2001). Auxin plays a major role in promoting hypocotyl elongation and acts as a primary target for the photoreceptors' signal to inhibit this growth. In mutant screens designed to find genes that inappropriately express light-regulated genes in the dark, we identified *doc1* and showed *doc1* mutants are allelic to the polar auxin transport mutant, *tir3*. Expression profiling experiments indicated that altered expression of multiple light-regulated genes in *doc1* mutants is suppressed by elevated levels of auxin, suggesting that normal auxin distribution is required to maintain low-level expression of these genes in the dark. *DOC1* encodes the *Arabidopsis* homolog of calossin, a huge protein whose function in other systems is not understood. *DOC1* is required to correctly localize the auxin efflux carrier, PIN1, explaining the observed defect in auxin transport in *doc1/tir3* mutants (collaboration with Mark Estelle and Klaus Palme). These results indicate an unexpected role for auxin in the repression of light-regulated genes in dark-grown seedlings.

**3. Plastid protein import** (Jarvis et al., 1998, 1999, 2000). We screened in excess of 7000 T-DNA lines that we had generated in the lab for pale or virescent mutants in which *CAB* was underexpressed. We obtained sequence data from 6 of the tagged mutant lines, one of which, *ppi*, was characterized in detail. *ppi* is a virescent mutant that underexpresses *CAB* in response to either continuous light or to light pulses. The gene disrupted by the T-DNA insertion encodes a 33 kD protein (Toc33) that is very similar to Toc34 from *Arabidopsis* and pea. Toc34 is a GTP-binding protein of the plastid outer envelope membrane that co-purifies with other components of the plastid general protein import apparatus. We showed that Toc33 (encoded by the *PPI* gene) inserts into the plastid outer envelope membrane and that *ppi* mutants are defective in plastid protein import. *PPI* mRNA accumulates to high levels in young seedlings and in newly emerging leaves, thereby suggesting an important role for Toc33 during the early stages of plastid and leaf development. Our data imply that there are multiple different translocon complexes in plastids and that these complexes must be functioning efficiently for optimal transcription of nuclear genes encoding plastid-destined proteins.

How might a reduction of import of proteins into the plastid negatively feedback regulate *CAB* transcription in the *ppi* mutant? One possibility is that the accumulation of precursors of enzymes involved in Chl biosynthesis might impact the flux of metabolites through the Chl/heme biosynthetic pathway. As we (see below) and others have shown, the disruption of this tightly regulated pathway leads to the misregulation of nuclear genes encoding plastid-destined proteins. Indeed, we showed that the precursor for protochlorophyllide oxidoreductase accumulates outside of the plastid in the *ppi* mutant, thereby disrupting the Chl branch of this pathway. Thus, the *ppi* mutant points to a role for the Chl/heme biosynthetic pathway in the regulation of *CAB* transcription, a hypothesis that we continue to explore with current DOE funding.

**4. Retrograde signaling from the chloroplast regulates the expression of nuclear light-responsive genes.** (*This was the major project from 1995-2004*) (Susek and Chory, 1992; Chory et al., 1993; Susek et al., 1993; Chory and Susek, 1994; Mochizuki et al., 1996; 2001; Lopez-Juez et al., 1998a, 1998b; Vinti et al., 2000; Surpin et al., 2002; Strand et al., 2003, 2006; Larkin et al., 2003; Verdecia et al., 2005; Larkin and Chory, 2006; Nott et al., 2006). Plant cells coordinately regulate the expression of nuclear and plastid genes that encode components of the photosynthetic apparatus. Nuclear genes that regulate chloroplast development and chloroplast gene expression provide part of this coordinate control. There is also evidence that information flows in the opposite direction, from chloroplasts to the nucleus. We designed a mutant screen to analyze this retrograde signaling pathway. Five non-allelic loci were identified as genome uncoupled mutants (*gun1-5*); these mutants express nuclear-encoded photosynthetic genes in the absence of proper chloroplast development. Three of these loci encode enzymes in the tetrapyrrole pathway. We used these mutants and new mutants identified in reverse genetic studies to demonstrate that the chlorophyll intermediate Mg-protoporphyrin (Mg-ProtoIX) acts

as a signaling molecule from damaged chloroplasts. Accumulation of Mg-ProtoIX is both necessary and sufficient to repress the expression of a large number of nuclear genes encoding chloroplastic proteins. These studies also identified GUN4, a chloroplast-localized protein. GUN4 binds the product and substrate of Mg-chelatase, an enzyme that produces Mg-Proto. GUN4 also activates Mg-chelatase. GUN4's role in the retrograde signaling pathway is suggested by its 3-D structure and its localization to multiple chloroplast sub-compartments, suggesting that GUN4 may promote Mg-Proto export from the plastid by stimulating Mg-Proto synthesis and/or by recruiting Mg-Proto to the plastid envelope.

### **C. Education and Human Resources.**

#### **FORMER POSTDOCTORAL FELLOWS SUPPORTED BY DOE FUNDS**

<b><u>NAME</u></b>	<b><u>YEARS IN LAB</u></b>	<b><u>CURRENT POSITION</u></b>
Altschmied, Lothar	03/89-05/92	Assistant professor, Institut of Plant Genetics and Crop Plant Research, Gatersleben, Germany
Gil, Pedro	07/95-08/99	Senior scientist, Akadix
Jarvis, Paul	11/96-10/98	Lecturer, U. of Leicester, U.K.
Koussevitzky, Shai	11/00-08/05	Post-doc, Univ of Nevada-Reno
Larkin, Robert	09/97-03/03	Assistant professor, DOE-PRL, Michigan State Univ
Li, Hsou-min	03/92-01/94	Professor, Academia Sinica, Taipei, Taiwan
Lopez-Juez, Enrique	02/94-05/96	Lecturer, Royal Holloway, Univ of London
Mochizuki, Nobuyoshi	05/93-09/95	Assistant professor, Kyoto University
Reed, Jason	07/91-12/94	Associate professor, U. North Carolina
Strand, Asa	09/00-12/02	Assistant professor, Agricultural Univ of Umea, Sweden
Streatfield, Stephen	08/95-10/97	Senior scientist, Prodigene, College Station, TX
Surpin, Marci	10/95-12/01	Assistant research biologist, UC-Riverside
Susek, Ronald	12/89-06/93	Business Development, Chenon

#### **FORMER GRADUATE STUDENTS SUPPORTED BY DOE FUNDS**

<b><u>NAME</u></b>	<b><u>YEARS IN LAB</u></b>	<b><u>CURRENT POSITION</u></b>
Nguyen, Linh	01/96-05/98	Researcher, biotech industry
Poole, Daniel	02/92-09/97	Senior research tech, Univ of Wisconsin

#### **D. Publications Acknowledging DOE Support.**

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