Analysis of *Arabidopsis thaliana* Red-Light Response Mutants Identified in a Genetic Enhancer Screen

Allison Welter Faculty Mentor: Derek Gingerich
Department of Biology, University of Wisconsin-Eau Claire, Eau Claire, WI

For more information contact:
Derek Gingerich
Email: gingerdj@uwec.edu

Introduction

Plants require light for survival and have developed sophisticated pathways to respond and adapt to the light in their environment[1]. Photoreceptors allow plants to sense specific wavelengths of light, one family being the red /far-red-absorbing phytochromes (phys). Phys have two conformations: inactive (P_r) and active (P_{fr}) forms. Absorption of red light promotes a shift to active P_{fr}, while far-red light absorption promotes assumption of inactive P_r[2]. P_{fr} can also shift back to P_r independent of light (known as dark reversion)[3]. Upon activation by red light and transition to the P_{fr} form, the phytochromes are translocated into the nucleus from the cytosol to regulate gene expression[4] (Figure 1).

Work by our lab and others has shown two genes called *Light-Response BTB 1* and *2* (*LRB1* and *LRB2*) regulate the phy light-response pathway in the model plant *Arabidopsis thaliana*[5,6]. *LRB1* and *LRB2* encode BTB (Bric-a-Brac, Tramtrack and Broad Complex) proteins that are target adaptors in E3 ubiquitin-ligase complexes that lead to the degradation of the phys through ubiquitylation under light treatment (Figure 1)[5,6]. Plants containing mutations within *LRB* genes (*lrb1 lrb2* mutants) display hypersensitivity to red light due to elevated levels of the phys[5,6].

To discover additional genes involved in light responses, previous members of the Gingerich lab conducted genetic enhancer screens utilizing the red light-hypersensitive *lrb1 lrb2* mutants. They mutagenized *lrb1 lrb2* seed with ethyl methanesulfonate (EMS) and identified individuals that exhibited further enhanced red light hypersensitivity. I have been working to map the mutations responsible for the highly increased red-light hypersensitivity in a subset of the lines (Figure 2). Our initial hope was that these mutations may occur in genes not previously known to be involved in light responses.

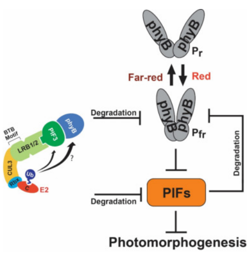


Figure 1. Structure and function of the LRB/CUL3 E3 ubiquitin ligase complex in the red light response pathway in *Arabidopsis thaliana*. Red light activates phytochrome B, which blocks the activity of PIF proteins, which are negative regulators of red light responses. The complex mediates ubiquitylation and degradation of both PIF3 and phyB. Figure is based partly on model of LRB action presented by Ni *et. al.*[6]

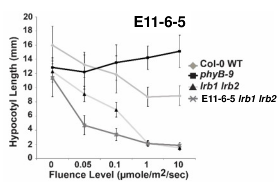


Figure 2. Hypocotyl elongation response to red light of the E11-6-5 line, one of the enhancer mutants identified in the Gingerich lab genetic screen. Hypocotyl lengths of wild-type (Col-0 WT), *lrb1 lrb2*, *phyB-9*, and E11-6-5 mutants grown in darkness or in various levels of red light are shown. The E11-6-5 line contains the *lrb1-1 lrb2-1* insertion mutations of the *lrb1 lrb2* parent line. Seeds were germinated under white light and then grown in the dark or under continuous red light for 4 days. Error bars indicate ± SD.

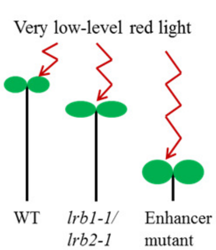
Screen to Identify Enhancer Mutants

Mutagenize population of *lrb1-1 lrb2-1* seed with ethyl methanesulfonate (EMS)

Germinate seeds and grow plants, 2000 individuals total

Collect seed from these individuals

Germinate and grow this next generation (M2) under red filtered light; identify individuals that have increased red light sensitivity compared to the *lrb1-1 lrb2-1* double mutant parents.



Strategy to Map Enhancer Mutants by Whole-Genome Sequencing

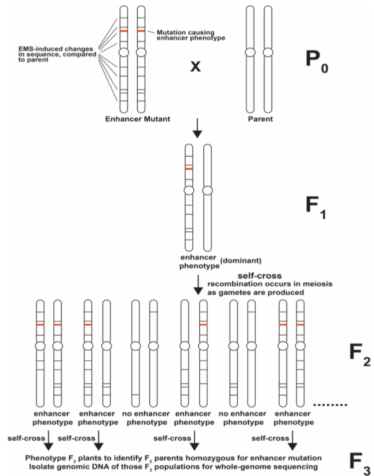


Figure 3. Generation of plants for whole-genome sequencing. In the P_0 generation the *lrb1-1 lrb2-1* double mutant parent is crossed with original enhancer mutant, which also contains the *lrb1-1/lrb2-1* insertion mutations (not shown).

The E2-1-2 and E11-6-5 Enhancer Mutations are Dominant

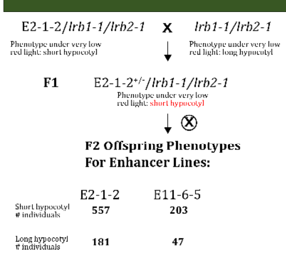


Figure 4. The E2-1-2 and E11-6-5 enhancer mutations act in a dominant fashion. Seedlings were grown for four days under low level (0.075 µmole/m²/sec) red light. F1 individuals showed the phenotype of the enhancer mutation and F2 populations displayed an approximately 3:1 enhancer phenotype: wild-type phenotype ratio.

WGS/Mapping Results

E2-1-2

Chromosome	Position	Reference	Change	Gene_ID	Gene_name	old_AA/new_AA
chr1	6598789	G	A	AT1G19100	AT1G19100	R/H
chr1	10847738	G	A	AT1G30610	EMB2279	E/K
chr2	890071	G	A	AT2G03030	AT2G03030	V/M
chr2	7402342	G	A	AT2G17033	AT2G17033	G/E
chr2	7411044	G	A	AT2G17050	AT2G17050	Q/*
chr2	8111222	G	A	AT2G18700	ATTPS11	S/N
chr2	8141622	G	A	AT2G18790	PHYB	G/E
chr2	8378739	G	A	AT2G19360	AT2G19360	E/K
chr2	8848502	G	A	AT2G20560	AT2G20560	P/S
chr2	9915638	G	A	AT2G23300	LRR-RLK	G/E
chr2	10121722	G	A	AT2G23770	LYK4	T/I
chr2	10171848	G	A	AT2G23890	AT2G23890	R/H
chr2	12552986	G	A	AT2G29200	APUM1	A/V
chr2	12564147	G	A	AT2G29220	LECRK31	P/L
chr2	12644793	G	A	AT2G29550	TUB7	P/L
chr2	13168951	G	A	AT2G30940	AT2G30940	R/H
chr2	13660433	G	A	AT2G32150	AT2G32150	A/T
chr2	16283136	G	A	AT2G38995	AT2G38995	R/K
chr2	17979359	G	A	AT2G43250	AT2G43250	A/T
chr2	18042367	G	A	AT2G43445	AT2G43445	G/E
chr2	18792991	G	A	AT2G45620	AT2G45620	A/T
chr5	12223176	C	T	AT5G32590	AT5G32590	S/N

E11-6-5

Chromosome	Position	Reference	Change	Gene_ID	Gene_name	old_AA/new_AA
chr2	2581713	G	A	AT2G06500	AT2G06500	T/I
chr2	7556311	G	A	AT2G17390	AKR2B	G/E
chr2	8142670	G	A	AT2G18790	PHYB	G/E
chr2	11190164	G	A	AT2G26280	CID7	A/V
chr2	17234822	G	A	AT2G41360	AT2G41360	R/Q
chr5	6574139	C	T	AT5G19485	AT5G19485	G/E

Figure 5. Whole-genome sequencing and analysis of our samples were performed by the Washington University in St. Louis Genome Technology Access Center. Samples were submitted and processed as part of a laboratory consortium led by Dr. Lucia Strader of Washington University. Shown are lists of mutations present in the enhancer lines that may be responsible for the enhancer phenotype. Notice that in both lines a similar region in chromosome 2 is linked to the phenotype and in both lines, there is a mutation in the *PHYB* gene. Since both lines show very similar phenotypes and *PHYB* is known to be involved in red light response, we believe these mutations are likely causing the phenotype.

Whole-genome sequencing and downstream analysis of three samples cost less than $700!

Putative Casual Mutations Identified in the *PHYB* Gene

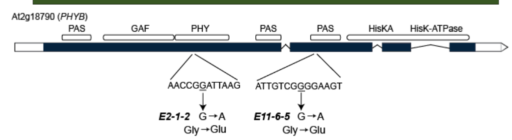


Figure 6. Locations of putative enhancer mutations in the *PHYB* gene in lines E2-1-2 and E11-6-5. Boxes indicated exons, lines indicate introns. Domains of the phyB protein are indicated. Both mutations in enhancer lines lead to a single amino acid change of glycine to glutamic acid.

Western Blot Analysis of phyB Protein Levels in Line E2-1-2

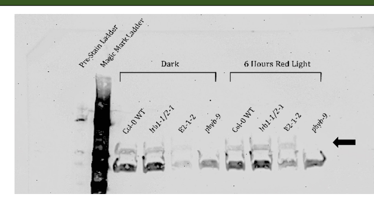


Figure 5. Preliminary Western Blot analysis of phyB protein levels in enhancer line E2-1-2. Controls included Col-0 wild-type, *lrb1-1/2-1*, and *phyB-9* plants. Seedling germination was induced by 8 hours of white light. Seedlings were then grown for 5 days in the dark. One group of plants were kept in the dark, while a second was treated with 6 hours of red light (20 µmole/m²/sec. red light). The bands indicated by the arrow are the phyB protein. The band below is likely another protein reacting to the antibody. While lanes were likely not loaded with equal amounts of total protein, these results suggest that the E2-1-2 line does not have significantly increased levels of phyB protein compared to wild-type or the *lrb1 lrb2* parent line.

Future Work

- Repeat Western Blot analyses for both E2-1-2 and E11-6-5 to determine the amount of phyB protein present under different light conditions.
- Prove the mutations in the *PHYB* genes in lines E2-1-2 and E11-6-5 are responsible for the enhancer phenotype by expressing these mutant forms in plants containing a null mutation in the endogenous gene (*phyB-9*) and analyzing the red light phenotype of the plants.
- Conduct red/far-red light pulse experiments to test the ability of the mutant phyB proteins in E2-1-2 and E11-6-5 to photoconvert between the P_r and P_{fr} forms.
- Sequence/map additional enhancer mutants lines (genomic DNA for a third line is already awaiting sequencing at the Washington University GATC).

References

1. Mathews S (2006) Phytochrome-mediated development in land plants: red light sensing evolves to meet the challenges of changing light environments. *Molecular Ecology* 15: 3483-3503.
2. Kretsch T, Poppe C, Scäfer E (2000) A new type of mutation in the plant photoreceptor phytochrome B causes a loss of photoreversibility and an extremely enhanced light sensitivity. *Plant J* 22(3): 177-186.
3. Ádám É, Hussong A, Bindics J, Wüst F, Viczián A, et al. (2011) Altered dark- and photoconversion of phytochrome B mediate extreme light sensitivity and loss of photoreversibility of the phyB-401 mutant. *PLoS ONE* 6(11): e27250.
4. Jiao Y, Lau O, Deng X (2007) Light-regulated transcriptional networks in higher plants. *Nature Reviews Genetics:* 217-230.
5. Christians M, Gingerich D, Hua Z, Lauer T, Vierstra, R (2012) The light-response BTB1 and BTB2 proteins assemble nuclear ubiquitin-ligases that modify Phytochrome B and D signaling in *Arabidopsis*. *Plant Physiology* 160: 118-34.
6. Ni W, Xu S, Tepperman J, Stanley D, Maltby D, Gross J, Burlingame A, Wang Z, Quail P (2014) A mutually assured destruction mechanism attenuates light signaling in *Arabidopsis*. *Science* 344(6188): 1160-1164.

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