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The Dark-Adaptation Response of the De-Etiolated Pea Mutant *lip1* Is Modulated by External Signals and Endogenous Programs[1]

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The *lip1* mutant of pea (*Pisum sativum* L.) exhibits a de-etiolated phenotype. When grown in darkness, *lip1* plants have several characteristics normally associated only with light-grown plants. Young wild-type (WT) seedlings accumulate high levels of transcripts from plastid-related genes (such as those encoding chlorophyll *a/b*-binding proteins, ferredoxin, and the small subunit of Rubisco) only in the light. In contrast, regardless of the light conditions under which the plants are grown, young mutant seedlings accumulate transcript levels equal to or greater than those seen in light-grown WT seedlings of the same age. Under some conditions, light-grown *lip1* seedlings failed to respond to dark treatment. The largest response to darkness observed in the mutant occurred when older seedlings were first grown under low-light conditions before transfer to darkness. The mutant's inability to respond to darkness is not due to a gross disturbance in the circadian clock. We conclude that environmental signals (light) and endogenous programs (developmental and circadian) regulate gene expression in both WT and mutant plants. However, mutant seedlings exhibit a developmentally regulated and exaggerated response to light. In addition, the effect of the mutation may be greatest during a brief period early in development.

In general, plants are dependent on sunlight for metabolic energy. The developmental program that plants follow in the light is referred to as photomorphogenesis and it affects many aspects of plant growth, such as stem elongation, leaf and chloroplast development, and transcript accumulation from light-regulated genes. Plants have evolved mechanisms to cope with periods of darkness, when they are deprived of light energy. Etiolation, or skotomorphogenesis, is the developmental program followed by plants grown in complete darkness (Mohr and Shropshire, 1983). The etiolated habit most likely evolved to give seedlings a better chance of surviving in an initial period of darkness, e.g. under soil or leaf litter, until phototrophic growth is possible. A genetic analysis of etiolation has shown that skotomorphogenesis is a result of repression of photomorphogenesis (Chory et al., 1989).

Plants must also contend daily with the darkness of night. An endogenous clock regulates several phenomena, such as leaf movements and transcript abundance oscillations, presumably allowing plants to anticipate and respond effectively to daily periods of darkness (for review, see Kay and Miller, 1996). In addition, light-grown plants exhibit a response to prolonged exposure (e.g. >24 h) to darkness. This response is referred to as dark adaptation and often includes an arrest of growth and a reduction in transcript accumulation from several light-regulated genes. Although dark adaptation and etiolation have several similar aspects, little is known about the relationship of the mechanisms involved.

Much of what has been learned about skotomorphogenesis has come from the analysis of mutants that fail to exhibit an etiolated phenotype in the dark (for reviews, see Chory and Susek, 1994; McNellis and Deng, 1995). De-etiolated mutants have been isolated from three plant species: Arabidopsis (Chory et al., 1989; Deng et al., 1991; Castle and Meinke, 1994), pea (*Pisum sativum* L.; Frances et al., 1992), and tobacco (Traas et al., 1995), and all mutations identified to date are recessive. Several mutants, such as *lip1* of pea and *det1* (for *de-et*iolated), *cop1* (for constitutive photomorphogenic), *cop8, cop9, cop10,* and *cop11* of Arabidopsis, are strikingly pleiotropic, affecting both light- and dark-grown phenotypes (Chory et al., 1991; Frances et al., 1992; Kwok et al., 1996). Indeed, severe mutant alleles of several of these genes (e.g. *cop1* and *det1*) are lethal. This observation suggests that these genes are essential for Arabidopsis (McNellis et al., 1994; Kwok et al., 1996). Evidence exists for the participation of some of these genes in other aspects of plant growth, such as carbohydrate metabolism and plant hormone function (Castle and Meinke, 1994). Therefore, it is likely that these WT gene products are repressors of photomorphogenesis that couple light-signal transduction and other signal transduction pathways to morphogenesis (Chory et al., 1989; Deng et al., 1991; Castle and Meinke, 1994).

In contrast, de-etiolated mutants, such as *det3, cop2, cop3,* and *cop4* of Arabidopsis, show only a subset of phenotypes exhibited by the more pleiotropic mutations. These mutants are probably lesions in the downstream branches of

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Abbreviations: CAB, chlorophyll *a/b*-binding protein RNA; FED, Fd RNA; RBCS, ribulose bisphosphate carboxylase small subunit RNA; WT, wild type.

the control of photomorphogenesis (Chory et al., 1991, Kwok et al., 1996). Several de-etiolated mutants of Arabidopsis, such as the *det2* mutant (Li et al., 1996) and others (Clouse, 1996), are thought to represent lesions in brassinosteroid synthesis or action. Brassinosteroids may control photomorphogenesis through a pathway that is independent of that controlled by the *DET1/COP1* genes (Li et al., 1996). The existence of de-etiolation mutants in several species allows the comparison of the various skotomorphogenic strategies used by different plants.

The light-independent photomorphogenesis 1 mutant *lip1* of the garden pea fails to produce a typical etiolated phenotype and has several characteristics of light-grown plants even when grown in complete darkness (Frances et al., 1992). Unlike etiolated WT plants, dark-grown *lip1* plants have short epicotyls and expanded leaves that contain partially developed chloroplasts. Transcripts from light-regulated genes, such as those encoding chlorophyll *a/b*-binding proteins (*Cab*), Fd (*Fed1*), and the small subunit of ribulose bisphosphate carboxylase (*RbcS*), accumulate to higher levels in dark-grown *lip1* seedlings than they do in etiolated WT seedlings. In addition, the *lip1* mutation also affects the light-grown phenotype. Mutant plants are shorter and darker green and have reduced fertility compared with WT plants.

In a previous study (Frances et al., 1992) we attempted to determine whether the *lip1* mutation also affects the dark response of light-grown plants. Seedlings were grown to different ages under various light conditions. It was observed that the dark-adaptation response of *lip1* plants depended on the conditions under which the plants were grown prior to the dark treatment. It was not possible to determine from those experiments which of the conditions was responsible for the difference in the response. In this paper we describe experiments designed to determine the effect of each of the parameters, seedling age and light conditions, on the response of WT and *lip1* mutant plants to dark treatment. The data show that both parameters modulate the phenotype of *lip1* plants and give evidence that *lip1* is a developmental mutant.

MATERIALS AND METHODS

Plant Material and Growth Conditions

WT pea (*Pisum sativum* L. cv Alaska) seeds were obtained from W. Atlee Burpee Seed (Warminster, PA) or N.K. Rogers (Twin Falls, ID). The *lip1* mutant was originally isolated as a spontaneous mutation from the Alaska seed stock (Frances et al., 1992). Seeds were grown in the Southeastern Plant Environmental Laboratory (Raleigh, NC) in temperature-controlled chambers kept at a constant 22°C (for description, see Downs and Thomas, 1991). Seeds were surface-sterilized in a 10% (v/v) solution of commercial bleach (0.525% hypochlorite final concentration) for 10 min, rinsed with three washes of distilled water, and allowed to imbibe in distilled water for 4 h.

For all experiments except the circadian study, seeds were sown onto three layers of wet Kimpak (Kimberley Clark, Roswell, GA) soaked with water containing 10 mg/L Benomyl (methyl 1-butyl-carbamoyl-2 benzimidazole-carbamate from Charles H. Lilly, Portland, OR). Distilled water was added to the Kimpak as necessary during the experiment. High-intensity light (294 μmol photons s^{-1} m^{-2}) was provided by 6 100-W incandescent and 16 4-foot VHO cool-white fluorescent lamps in a C-type plant growth chamber (3- × 4- × 4-foot reach-in cabinet). Medium-intensity light (188 μmol photons s^{-1} m^{-2}) was provided in an identical chamber by three incandescent and eight cool-white fluorescent lamps. Two different low-intensity light conditions were used and the results from these two conditions were identical. For one experiment, low-intensity light (27 μmol photons s^{-1} m^{-2}) was provided by two 8-foot slim-line, cool-white fluorescent lamps in a photoperiod room. In another experiment, low-intensity light (33 μmol photons s^{-1} m^{-2}) was provided by one 100-W incandescent and four 4-foot VHO cool-white fluorescent lamps in a C-type plant chamber. Dark-grown plants were grown in complete darkness in a photoperiod room. For etiolated WT seedlings the buds were harvested for RNA extraction. For *lip1* and light-grown WT plants, all tissue at the first leaf-bearing node and above was harvested. Dark-grown tissue was harvested in complete darkness or under a dim-green safelight.

For the circadian study, seeds were surface-sterilized as above, sown into a standard substrate (gravel/peat), and watered with one-half-strength Hoagland solution (Hoagland and Aron, 1950) once a day. The plants were grown for 10 d in a 9-h light/15-h dark cycle in an A-type chamber (8 × 12 × 7 feet) under fluorescent and incandescent lamps at an intensity of 700 μmol m^{-1} s^{-2}. On the 10th d after imbibition, at the beginning of the normal dark period (5 PM), the plants were transferred to complete darkness in a photoperiod room. Both leaflets from the second leaf-bearing node were harvested under a dim-green safelight. At the time of harvest, all unharvested plants were exposed to the safelight.

Light measurements were taken with an ESR-1 hand-held light meter (P.K. Morgan Instruments, Andover, MA). Intensities are reported as units of PAR (wavelengths between 380 and 780 nm).

Northern Analysis

Total RNA was isolated and glyoxylated as previously described (Elliott et al., 1989). Five micrograms of glyoxylated RNA per lane was fractionated on 1.25% (w/v) agarose gels, transferred to a MagnaGraph membrane (Micron Separations, Westboro, MA) by capillary blotting with 25 mM Na_2HPO_4 buffer, and cross-linked with 120 μJ of UV light (245 nm) in a Stratagene cross-linker. RNA probes were prepared with RNA polymerase T3 or T7 (Promega) according to the manufacturer's protocol. Templates for probe preparation were as follows: FED (an *Alu*I-*Ava*II restriction fragment [Elliott et al., 1989] inserted into pBSM13-, Stratagene); CAB (a *Pst*I restriction fragment of pAB96 [Coruzzi et al., 1983] inserted into pBSM13-, Stratagene), and RBCS (pDMT as described by Thompson et al., 1992).

Membranes were prehybridized and hybridized at 59°C (FED and RBCS) or 64°C (CAB) in a buffer containing 50% formamide, 5× SSC (1× SSC is 150 mM NaCl and 15 mM

sodium citrate), 50 mM Tris-HCl (pH 8.0 at room temperature), 5 mM EDTA, 0.1% sodium PPi, 1% (w/v) SDS, 0.2% PVP, 0.2% Ficoll, and 40 μg/mL calf-liver RNA. After a 16- to 24-h incubation, filters were washed once at room temperature for 1 min in 2× SSC and 1% (w/v) SDS and then at the hybridization temperature in 1× SSC and 1% (w/v) SDS for 20 min. Autoradiographic images were obtained with X-Omat AR film (Kodak) at room temperature or at −70°C with Kodak Lanex intensifying screens. Hybridization signals were quantitated with a radioanalytic imaging system (AMBIS Systems, San Diego, CA). Blots were subsequently reprobed with an oligonucleotide complementary to the 18S rRNA (Gallo-Meagher et al., 1992) to control for variations in loading and transfer efficiency. All data presented are the results of single experiments. However, in each case, very similar data were obtained in at least one (circadian) or two other independent experiments.

RESULTS

Phenotypes of WT and *lip1* Plants under Different Light Conditions

In Figure 1A WT and mutant plants grown under high- and low-intensity light are compared. Mutant plants were always shorter than WT plants grown under the same conditions. Both WT and mutant plants grown under high-intensity light exhibited reduced plant height compared with control plants grown under low-intensity light. This difference in height was due to a reduction of internode length rather than a decrease in the number of nodes. WT plants grown under high-intensity light had an appearance similar to mutant plants grown under low-intensity light. Thus, high-intensity light can produce a morphological phenocopy of *lip1* in WT seedlings.

In Figure 1B WT and *lip1* plants that were grown in the light for 10 d and then transferred to darkness for 2 d are compared. Apical portions of these seedlings continued to expand after transfer to darkness. The newly expanded tissue was yellow rather than green, presumably because the biosynthesis of chlorophyll failed to occur in the dark (Griffiths, 1978). After transfer to darkness, some WT plants developed a bend in the stem immediately below the stipule of the youngest expanded node (Fig. 1B). This bent stem is reminiscent of the apical hook seen in etiolated seedlings. Conditions under which the plants are grown can influence the formation of the apical hook (degree of bending and percentage of seedlings exhibiting a hook) in WT etiolated seedlings. However, mutant plants never exhibited a similar structure in any of our experiments. The observation that dark-adapted WT plants form a structure similar to the apical hook may indicate that some aspects of the etiolated growth pattern are re-established when light-grown WT plants are exposed to prolonged darkness.

The Dark-Adaptation Response of WT and Mutant Plants Is Affected by Seedling Age and Light Intensity

Since both WT and *lip1* plants respond to differences in light intensity (see above), it seemed appropriate to determine whether differences in the amount of illumination could influence the dark-adaptation response in mutant plants. Figure 2 shows an analysis of FED1 transcript accumulation in WT and mutant plants of different ages grown under continuous light of high, medium, or low intensity. In each case, WT plants contained lower levels of transcript in the dark compared with the levels that were seen in the light. However, the extent of decline in the dark was greater in older plants and in plants grown under lower light intensities. Mutant seedlings grown under low-intensity light showed a similar decline in transcript abundance when placed in the dark. However, *lip1* plants grown under high- and medium-intensity light maintained high levels of mRNA in darkness. From these data it is clear that both light conditions and the developmental state of the WT and mutant plants influence the response to darkness.

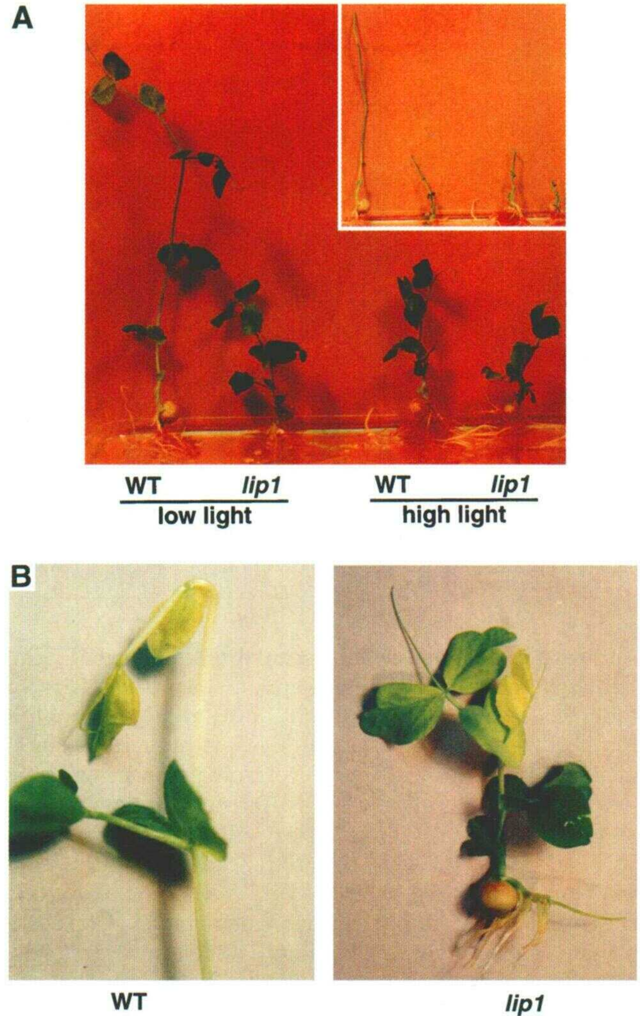

Figure 1. A, Seedlings were grown in continuous light for 15 d under low- and high-irradiance light (27 and 294 μmol s^{-1} m^{-2}, respectively). Inset, Same seedlings with leaves removed to illustrate differences in stem lengths. B, Plants were grown in continuous light for 10 d and then transferred to darkness for 2 d. Only the upper portion of the WT seedling is shown. Note the re-formation of the apical hook in the WT plant.

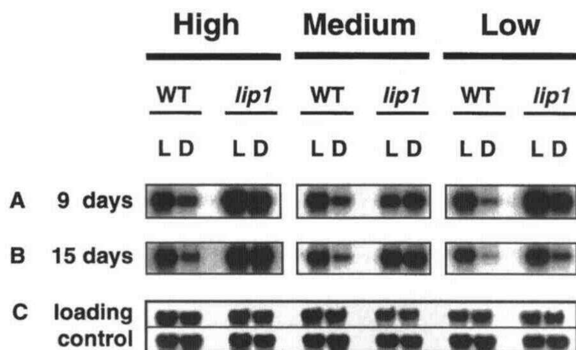

Figure 2. Dark-adaptation responses of Fd mRNA accumulation in WT and *lip1* plants grown under high, medium, or low irradiances. A, Seedlings were harvested after 9 d of growth in continuous light (L) or following an additional 2 d in the dark (D). B, Seedlings were harvested after 15 d of growth in continuous light (L) or following an additional 2 d in the dark (D). C, Same lanes as in A and B probed with an oligonucleotide complementary to the 18S rRNA. Light intensities: High, 294 μmol s^{-1} m^{-2}; Medium, 188 μmol s^{-1} m^{-2}; and Low, 27 μmol s^{-1} m^{-2}. Five micrograms of total RNA was loaded per lane.

To further examine developmental modulation of light-regulated gene expression, WT and *lip1* plants were grown under constant conditions (light and darkness). In addition, to determine the effect of seedling age on the dark-adaptation response, plants were grown in constant light for various lengths of time before transfer to constant darkness for 48 h. Figure 3 shows the results of this experiment for FED1 mRNA. In WT plants transcript levels were always low in the dark, regardless of whether the plants had been previously exposed to light. Transcript levels in light-grown WT seedlings were high until d 13, when they began to decline. In contrast, mutant plants showed similar patterns of transcript accumulation in both light and darkness. Transcript levels in the mutant were similar to those in light-grown WT plants at the beginning and end of the experiment. However, maximal accumulation in mutant plants was much higher than in WT plants. Thus, it can be concluded that the level of expression from light-regulated genes is developmentally modulated in both the mutant and WT plants. However, the mutant exhibits a somewhat different pattern of transcript accumulation, and this accumulation is largely unresponsive to light conditions during the first few days after germination.

Circadian Rhythms in WT and Mutant Plants

Young *lip1* seedlings exhibit high levels of expression from light-regulated genes under both light and dark conditions (Figs. 2 and 3). To determine whether gene expression in the mutant is truly constitutive, we asked whether it was uncoupled from the endogenous circadian clock under conditions in which circadian cycling had been initiated by prior exposure to a light/dark cycle. Previous work has shown that CAB mRNA, in particular, shows a strongly circadian pattern of expression in WT plants (Kloppstech, 1985). This experiment differed from the others in two significant ways. First, the seedlings were 10 d old at the start of the experiment. Second, unlike the other experiments in which seedlings were grown in constant conditions, the seedlings for this experiment were grown in a light/dark cycle. Figure 4 shows the pattern of CAB, FED1, and RBCS expression in WT and mutant plants that were grown in a light/dark cycle and then transferred to constant darkness. In both WT and mutant plants the relatively high levels of FED1 and RBCS transcript abundance seen in the light decreased to low levels in the dark. This result indicates that, under the conditions of this experiment, the expression of FED1 and RBCS in *lip1* seedlings is not constitutive but rather decreases upon transfer to darkness.

CAB mRNA abundance was observed to have an apparently circadian oscillation in both WT and mutant plants (Fig. 4). The pattern of CAB expression did not differ greatly between *lip1* and WT plants. This suggests that CAB expression in the mutant is correctly regulated by a circadian clock and that the oscillators of both WT and mutant plants exhibit similar periods under constantly dark conditions. (However, it should be noted that, because samples were taken every 4 h, small differences in period length might not have been detected in this experiment.) We conclude that the failure to respond to dark adaptation, observed in the *lip1* mutant (Figs. 2 and 3), is not due to a gross alteration in the circadian clock.

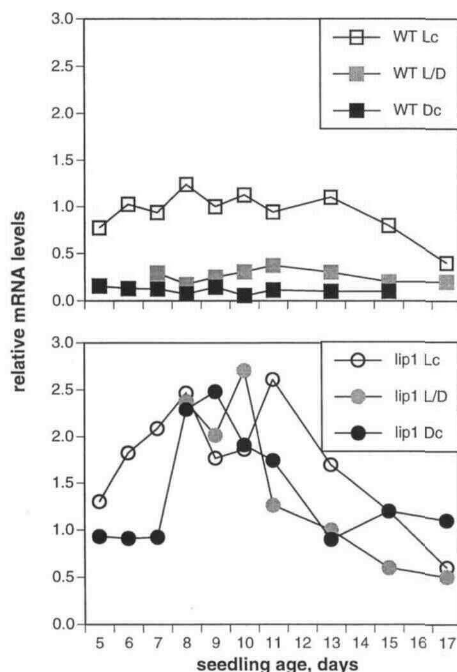

Figure 3. Developmental regulation of FED accumulation in WT and *lip1* seedlings. Seedlings were grown in continuous darkness (Dc), 33 μmol m^{-1} s^{-2} continuous light (Lc), or continuous light followed by 48 h of continuous darkness (L/D) prior to harvest. Total RNA was isolated from apical portions (buds of etiolated seedlings, buds and all nodes containing expanded leaves from de-etiolated seedlings) and northern-blot analysis was performed. Hybridization signals from the blots were quantitated with an AMBIS radioanalytic imaging system. Data have been normalized to the WT signal at 6 d under constant light. Black symbols, Continuous darkness; gray symbols, continuous light transferred to darkness; and white symbols, continuous light.

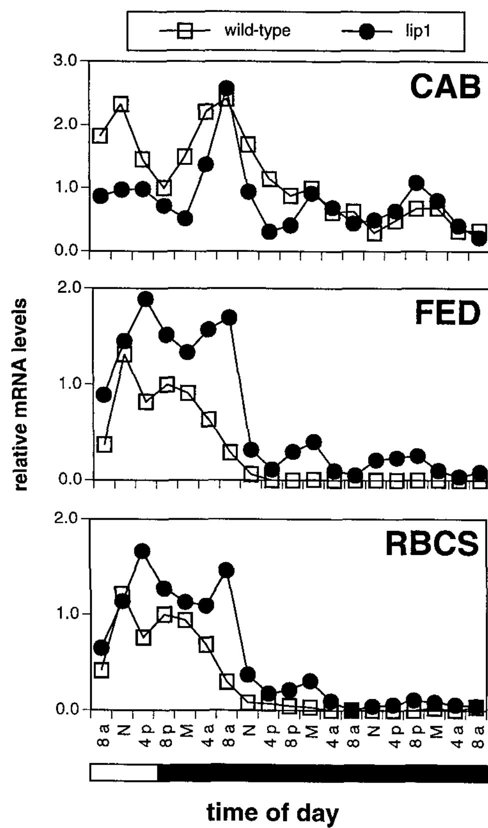

Figure 4. Accumulation of FED, CAB, and RBCS transcripts in WT and *lip1* seedlings grown in a light/dark cycle and transferred to continuous darkness. Seedlings were grown in a light/dark cycle with illumination (700 μmol m^{-1} s^{-2}) from 8 AM to 5 PM. At the beginning of the normal dark period (5 PM) on the 10th d after imbibition the plants were transferred to continuous darkness. Tissue was harvested every 4 h, beginning at 8 AM on the 10th d. Total RNA was isolated from the leaflets from the second leaf-bearing node and northern-blot analysis was performed. Hybridization signals from the blots were quantitated with an AMBIS radioanalytic imaging system. Data have been normalized to the first WT 8 PM value. The bar beneath the graph indicates the actual light conditions. N, Noon; M, midnight.

DISCUSSION

The degree of difference between the etiolated and light-grown habits of plants varies greatly between species. For example, the degree to which darkness inhibits cotyledon development and hypocotyl elongation varies in dark-grown seedlings of different pine species (J. Silverthorne, personal communication). In the legume family leaf development is arrested at different stages in the dark. The etiolated buds of garden pea seedlings contain small, unexpanded leaves (Frances et al., 1992). However, the etiolated buds of some legume species contain large leaves that have undergone extensive development in the absence of light. These observations suggest that skotomorphogenesis involves several pathways and that in the dark these pathways are repressed to different extents in different plant species.

The dark repression that occurs when light-grown plants are transferred to darkness for prolonged periods may have features in common with etiolation processes. In this paper we show that several features seen in etiolated seedlings, such as reduced expression from some light-regulated genes, developmental arrest, and morphological changes resembling the apical hook, are re-established in dark-adapted plants. A genetic relationship between etiolation and dark-adaptation mechanisms may be amenable to genetic analysis. Some Arabidopsis de-etiolated mutants, such as *det1*, have been shown to exhibit a normal dark-adaptation response (Chory et al., 1989). However, it has been reported that other mutants, such as *cop1*, exhibit an aberrant response to prolonged dark treatment (Deng et al., 1991). It has not yet been determined whether the stage of seedling development influences the dark adaptation in these Arabidopsis mutants in a manner similar to what is reported here for pea.

The dark-adaptation response of the *lip1* mutant is variable and depends on the conditions under which the plants are grown (Frances et al., 1992). When WT and mutant plants were grown in a light/dark cycle for 15 d and then transferred to darkness for 2 d, FED1 transcript levels declined in both WT and mutant plants. However, if the plants were grown for 9 d in continuous light before transfer to darkness, FED1 transcripts decreased in WT plants but not in the mutant. It was not clear from these experiments whether the differences in the mutants' response to dark adaptation was due to differences in age and/or light conditions between the two experiments. From this study we have determined that seedling age and the light intensity under which the seedlings were grown prior to transfer influence the extent of the dark-adaptation response (Figs. 2 and 3).

Eight to 10 d after imbibition, light- and dark-grown *lip1* plants exhibit a peak of transcript accumulation strikingly greater than that seen in light-grown WT plants (Fig. 3). Thereafter, transcript levels in mutant seedlings are more similar to those seen in light-grown WT plants. These data suggest that the effect of the mutation is strongest early in development. Several phenomena could contribute to the observed pattern of expression.

First, transcript levels measured in the apical portion of the plant are an average of the expression in individual leaves. Transcript levels are likely to be highest in young, expanding leaves and decline as the leaves mature (He et al., 1994). The increase and decline of expression seen in *lip1* seedlings (Fig. 3) may therefore simply reflect the changing percentage of tissue that is actively expanding in the apical portion of the plant. Thus, transcripts accumulate to higher levels in mutant plants because the level of expression in each leaf is higher in the mutant than the WT. According to this hypothesis, expression patterns of dark- and light-grown mutant seedlings would be similar because they contain similar percentages of expanding material. Thus, the effect of the *lip1* mutation on the expression of these genes is not related to the light conditions but rather to leaf development.

Furthermore, the *lip1* mutation might affect the expression of genes that contribute more extensively to early vegetative development. This hypothesis would suppose that the function of the WT *Lip1* gene product was temporally regulated or that the complement of target genes affected by *Lip1* is

different in early and late development. For example, the mutant pattern of expression may be related to the pattern observed in dark-grown WT seedlings. The expression of several genes that are normally considered to be light-induced, including CAB genes (Brusslan and Tobin, 1992), and genes encoding enzymes of chlorophyll and heme biosynthesis (He et al., 1994) is also developmentally regulated. Low but detectable levels of expression from these genes can be observed in dark-grown WT seedlings. This developmentally regulated peak of expression may be amplified in the *lip1* mutant. Amplification of a light-independent expression pattern would result in increased transcript accumulation in the dark as well as in the light.

It is clear that the failure of light-grown *lip1* seedlings to respond to darkness is not due to a gross disturbance of the circadian time keeper. When *lip1* plants grown in a light/dark cycle are transferred to continuous darkness, transcripts from photosynthesis-related genes accumulate in a circadian fashion similar to that observed in WT plants (Fig. 4). These results indicate that the high levels of expression in dark-grown mutant plants do not result from a deregulation of the clock that would lead to constitutive expression.

Mutant *lip1* plants exhibit pleiotropic phenotypic differences from WT plants throughout development. The fact that *lip1* plants respond to differences in light intensities (Figs. 1 and 2) indicates that the mutant retains its ability to perceive light quantity and can mediate light responses based on this perception. However, the mutant behaves differently than the WT plant and, in general, exhibits light responses of greater magnitude than observed in WT plants. In addition, light-regulated phenomena in the *lip1* mutant are influenced by endogenous developmental and circadian programs. Taken together, our results suggest that *lip1* plants are capable of perceiving and responding to light, but they have a reduced light requirement for photomorphogenic responses. This interpretation is consistent with the hypothesis that the mutant is deficient in a repressor of photomorphogenesis, which functions in both dark- and light-grown plants.

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LITERATURE CITED

Brusslan JA, Tobin EM (1992) Light-independent developmental regulation of *cab* gene expression in *Arabidopsis thaliana* seedlings. Proc Natl Acad Sci USA **89:** 7791–7795

Castle LA, Meinke DW (1994) A *FUSCA* gene of Arabidopsis encodes a novel protein essential for plant development. Plant Cell **6:** 25–41

Chory J, Nagpal P, Peto CA (1991) Phenotypic and genetic analysis of *det2*, a new mutant that affects light-regulated seedling development in *Arabidopsis*. Plant Cell **3:** 445–459

Chory J, Peto CA, Feinbaum R, Pratt L, Ausubel F (1989) *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. Cell **58:** 991–999

Chory J, Susek RE (1994) Light signal transduction and the control of seedling development. *In* EM Meyerowitz, CR Somerville, eds, Arabidopsis. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp 579–614

Clouse SD (1996) Molecular genetic studies confirm the role of brassinosteroids in plant growth and development. Plant J **10:** 1–8

Coruzzi G, Broghi R, Cashmore A, Chua N-H (1983) Nucleotide sequences of two pea cDNA clones encoding the small subunit of ribulose 1,5-bisphosphate carboxylase and the major chlorophyll *a/b* binding thylakoid polypeptide. J Biol Chem **258:** 1399–1402

Deng X-W, Caspar T, Quail PH (1991) *cop1*: a regulatory locus involved in light-controlled development and gene expression in *Arabidopsis*. Genes Dev **5:** 1172–1182

Downs RJ, Thomas JF (1991) Phytotron Procedural Manual for Controlled-Environment Research at the Southeastern Plant Environmental Laboratory. NCARS Technical Bulletin 244 (revised). North Carolina State University Agricultural Research Service, Raleigh, NC

Elliott RC, Pedersen TJ, Fristensky B, White MJ, Dickey LF, Thompson WF (1989) Characterization of a single copy gene encoding ferredoxin I from pea. Plant Cell **1:** 681–690

Frances S, White MJ, Edgerton MD, Jones AM, Elliot RC, Thompson WF (1992) Initial characterization of a pea mutant with light-independent photomorphogenesis. Plant Cell **4:** 1519–1530

Gallo-Meagher M, Sowinski DA, Elliott RC, Thompson WF (1992) Both internal and external regulatory elements control expression of the pea *Fed-1* gene in transgenic tobacco seedlings. Plant Cell **4:** 389–395

Griffiths WT (1978) Reconstitution of protochlorophyllide formation by isolated etioplast membranes. Biochem J **174:** 681–692

He Z-H, Li J, Sundqvist C, Timko MP (1994) Leaf developmental age controls expression of genes encoding enzymes of chlorophyll and heme biosynthesis in pea (*Pisum sativum* L.). Plant Physiol **106:** 537–546

Hoagland DR, Aron DI (1950) California Agriculture Experimental Station Circular 347, Berkeley, CA

Kay SA, Miller AJ (1996) New models in vogue for circadian clocks. Cell **83:** 361–364

Kloppstech K (1985) Diurnal and circadian rhythmicity in the expression of light-induced plant nuclear messenger RNAs. Planta **165:** 502–506

Kwok SF, Piekos B, Misera S, Deng X-W (1996) A complement of ten essential and pleiotropic Arabidopsis *COP/DET/FUS* genes is necessary for repression of photomorphogenesis in darkness. Plant Physiol **110:** 731–742

Li J, Nagpal P, Vitart V, McMorris TC, Chory J (1996) A role for brassinosteroids in light-dependent development of Arabidopsis. Science **272:** 398–401

McNellis TW, Deng X-W (1995) Light control of seedling morphogenetic pattern. Plant Cell **7:** 1749–1761

McNellis TW, von Arnim AG, Araki T, Komeda Y, Misera S, Deng XW (1994) Genetic and molecular analysis of an allelic series of *cop1* mutants suggests functional roles for the multiple protein domains. Plant Cell **6:** 487–500

Mohr H, Shropsire W (1983) An introduction to photomorphogenesis for the general reader. *In* A Pirson, MH Zimmermann, eds, Encyclopedia of Plant Physiology, 16A. Springer-Verlag, Heidelberg, pp 22–38

Thompson WF, White MJ (1991) Physiological and molecular studies of light regulated nuclear genes in higher plants. Annu Rev Plant Physiol Mol Biol **42:** 423–466

Traas J, Laufs P, Jullien M, Caboche M (1995) A mutation affecting etiolation and cell elongation in *Nicotiana plumbaginifolia* causes abnormal division plane alignment and pattern formation in the root meristem. Plant J **7:** 785–796