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# *CUE1*: A Mesophyll Cell-Specific Positive Regulator of Light-Controlled Gene Expression in Arabidopsis

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**Light plays a key role in the development and physiology of plants. One of the most profound effects of light on plant development is the derepression of expression of an array of light-responsive genes, including the genes encoding the chlorophyll *a/b* binding proteins (CAB) of photosystem II. To understand the mechanism by which light signals nuclear gene expression, we developed a genetic selection to identify mutants with reduced *CAB* transcription. Here, we describe a new Arabidopsis locus, *CUE1* (for *CAB* underexpressed). Mutations at this locus result in defects in expression of several light-regulated genes, specifically in mesophyll but not in bundle-associated or epidermis cells. Reduced accumulation of *CAB* and other photosynthesis-related mRNAs in the mesophyll was correlated with defects in chloroplast development in these cells, resulting in a reticulate pattern with veins greener than the interveinal regions of leaves. Moreover, chalcone synthase mRNA, although known to be regulated by both phytochrome and a blue light receptor, accumulated normally in the leaf epidermis. Dark basal levels of *CAB* expression were unaffected in etiolated *cue1* seedlings; however, induction of *CAB* transcription by pulses of red and blue light was reduced, suggesting that CUE1 acts downstream from both phytochrome and blue light photoreceptors. CUE1 appears to play a role in the primary derepression of mesophyll-specific gene expression in response to light, because *cue1* mutants are severely deficient at establishing photoautotrophic growth. Based on this characterization, we propose that *CUE1* is a cell-specific positive regulator linking light and intrinsic developmental programs in Arabidopsis leaf mesophyll cells.**

## INTRODUCTION

Plant development is plastic and flexible in response to external stimuli. Of the environmental factors that influence plant development, light has an especially important role as a substrate for photosynthesis and as a stimulus for many developmental processes. Light effects on plant growth and development have been observed at almost every stage of the plant life cycle, from germination to floral induction (or suppression). Seedlings are particularly responsive to light, which causes profound changes in the morphology of the developing seedling (reviewed in Chory and Susek, 1994). These changes include the derepression of several nuclear- and chloroplast-encoded light-regulated genes, production of chlorophyll, differentiation of leaves, expansion of cotyledons, inhibition of the rate of stem growth, stimulation of root growth, and the development of photosynthetically competent chloroplasts from undifferentiated proplastids. This light-dependent development of plants is a complex process resulting from the combined action of several photoreceptors.

Higher plants have at least three photoreceptor systems that mediate responses to light: a UV-B photoreceptor of unknown chemistry (Beggs and Wellman, 1985), a blue/UV-A photoreceptor(s), which may be a flavoprotein (Ahmad and Cashmore, 1993), and the most intensively studied red/far-red–absorbing photoreceptors, the phytochromes (reviewed in Quail et al., 1995). Despite many significant advances in recent years, the molecular mechanisms by which these photoreceptors initiate a signal transduction cascade are not known, and a complete signal transduction chain from photoreceptor excitation to light-regulated response has not been elucidated.

Numerous observations suggest that light signals are coupled to endogenous developmental programs in plants. For instance, in Arabidopsis, light-regulated chloroplast differentiation is restricted to the leaf and stem tissues and, within leaves, to certain cell types: the mesophyll, bundle sheath, and guard cells. Thus, the differentiation of proplastids to chloroplasts is related to leaf development in higher plants and must also involve cell-specific signals. In addition, many studies designed to dissect the *cis*-regulatory sequences of photoregulated nuclear target genes have been unsuccessful in separating the upstream light regulatory sequences from those that confer tissue specificity of gene expression (for example, see

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Gilmartin et al., 1990). Several light-regulated nuclear genes have been characterized, including ones that are both positively and negatively regulated by light (Gilmartin et al., 1990; Li et al., 1993). These genes are expressed in different cell types within the plant, and they share common *cis*-regulatory sequence motifs. Their expression is regulated by phytochrome (Silverthorne and Tobin, 1987; Reed et al., 1994), yet their expression can also be modulated by other photoreceptors, including a blue/UV-A photoreceptor (Fluhr and Chua, 1986; Schulze-Lefert et al., 1989; Gao and Kaufman, 1994). Moreover, the responsiveness of a particular gene to a given wavelength of light varies with the light intensity as well as the developmental stage of the plant (Ehmann et al., 1991; Frohnmeyer et al., 1992). These observations further reinforce the interplay of light and development in the regulation of gene expression in plants.

Potentially, one of the simplest light signaling pathways to dissect will be the light-regulated expression of nuclear genes. The best studied of these target genes encode chloroplast-destined proteins, such as the chlorophyll *a/b* binding protein of photosystem II (CAB) and the small subunit of the ribulose bisphosphate carboxylase/oxygenase (RBCS). *CAB* and *RBCS* are positively regulated by light and show tight cell-type specificity, being expressed exclusively in chloroplast-containing cells and at their highest levels during early leaf development (reviewed in Chory and Susek, 1994). Using pharmacological agonists and antagonists that disrupt phytochrome-mediated events, it has been shown recently that phytochrome may act through a signal transduction pathway involving heterotrimeric G proteins and calcium-activated calmodulin to derepress *CAB* and *RBCS* expression (Bowler et al., 1994a, 1994b). Other nuclear genes whose expression is positively regulated by light are not involved in chloroplast functions and are expressed in cells that do not contain chloroplasts. These include the anthocyanin biosynthetic genes, the best characterized of which is the gene encoding chalcone synthase (CHS). The *CHS* gene is highly expressed in the epidermis very early during leaf development (Schmelzer et al., 1988; Chory and Peto, 1990). The pharmacological studies of Bowler et al. (1994a, 1994b) implicate a heterotrimeric G protein and cGMP in mediating phytochrome-regulated *CHS* gene expression. Interpreted in the context of the intact seedling, these studies suggest that phytochrome acts through different downstream effectors, which may be specific to the cell type in which light is eliciting a molecular response.

Genetics is an alternative tool for studying light-regulated gene expression, with the potential to identify genes on the basis of their function. A morphological screen for mutants with reduced responses to light (manifested as seedlings with an elongated hypocotyl) has been undertaken by several laboratories to identify positively acting components of the light signaling pathways. A number of mutants have been identified that affect photoperception, including phytochrome and a putative blue/UV-A photoreceptor; however, with the possible exception of the long hypocotyl mutant, *hy5*, and the far-red long hypocotyl mutants, *fhy1* and *fhy3*, these screens have not been fruitful in identifying positively acting components in light signal transduction (reviewed in Bowler and Chua, 1994; Chory and Susek, 1994; Reed and Chory, 1994). In contrast, genetic screens for mutations that uncouple the light signal from downstream light-regulated developmental and gene expression responses have identified numerous genes for negatively acting light signaling intermediates (reviewed in Bowler and Chua, 1994; Chory and Susek, 1994; Reed and Chory, 1994).

To identify new mutants in positively acting components of light signaling pathways and to close the current gap between the pharmacological and genetic studies, we devised a genetic screen that has allowed us to identify mutations that reduce the expression of genes in response to light. A stably transformed line of Arabidopsis was constructed that contained a transgene with two *CAB3* (also called *CAB180* [Leutwiler et al., 1986] and *Lhcb1*2* [Jansson et al., 1992]) promoters fused to different reporter genes (Chory et al., 1993). Seeds from transgenic plants homozygous for the transgene were mutagenized and used to screen for mutants that expressed both reporter genes at aberrantly low levels in the light. We report here the characterization of several mutant alleles at a locus designated *CUE1* (for *CAB* underexpressed). Mutations at this locus result in defects in light-regulated expression of photosynthesis genes in mesophyll cells. We show that the expression of other light-regulated genes, such as *CHS*, is not altered in *cue1* mutants and that *CAB* expression is normal in bundle sheath cells aligning the leaf veins. Furthermore, unlike the wild type, *CAB* expression in etiolated *cue1* seedlings is not induced by pulses of red, blue, or white light. These results suggest that *CUE1* acts downstream from multiple photoreceptors in mesophyll cells, linking light to cell-type–specific gene expression in Arabidopsis.

## RESULTS

### Mutant Isolation and Genetic Characterization

We designed a reporter construct to identify mutations in positive effectors of light-regulated transcription of *CAB*, as shown

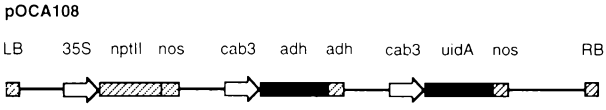


**Figure 1.** Schematic Representation of the pOCA108 Transgene Construct.

The neomycin phosphotransferase II gene (*nptII*) used for transformation selection was driven by the constitutive cauliflower mosaic virus 35S promoter (35S). One *CAB3* promoter was fused to the *ADH* gene. The second *CAB3* promoter was fused to the *E. coli uidA* gene. The *uidA* and *nptII* genes were fused to the nopaline synthase (*nos*) terminator; the *ADH* gene had its own termination sequences. Details of the construct are described in Methods and by Susek et al. (1993). LB and RB, left and right borders of the T-DNA, respectively.

in Figure 1. The reporter construct, herein called pOCA108, contains two full-length *CAB3* promoters (see details in Susek et al., 1993) fused to either the alcohol dehydrogenase (*ADH*) gene from Arabidopsis (Chang and Meyerowitz, 1986) or the *Escherichia coli* β-glucuronidase (*uidA*, or *GUS*) gene (Jefferson, 1987). We chose ADH because both positive and negative genetic selections exist for this enzyme (Bonner et al., 1984). This was important to our screen because we could select against ADH activity using allyl alcohol, which is converted into the toxic aldehyde acrolein. We transformed pOCA108 into an Arabidopsis Bensheim line, R002, that contains a null mutation in the endogenous *ADH* gene (Jacobs et al., 1988). All ADH activity in the resulting transformants was under the control of the *CAB3* promoter of the transgene. More than 20 transgenic lines were generated and characterized. One line (pOCA108-1) that was homozygous for a single insertion site of the transgene on chromosome 2 (see Methods) was used for further studies. This line displayed proper light-regulated and tissue-specific expression of the *CAB3–ADH* and *CAB3–uidA* transgenes (data not shown). Seeds from the pOCA108-1 line were mutagenized with ethyl methanesulfonate (EMS) or γ-rays, and plants ($M_1$) were allowed to self-fertilize ($M_2$). In our initial selection for mutants with reduced *CAB3* expression, 25,000 $M_2$ seedlings (derived from 28 γ-ray–mutagenized $M_1$ families) were grown for 5 days in liquid medium under continuous white light and then treated with 3 mM allyl alcohol for 1 hr (see Methods). Seedlings with reduced *CAB3* promoter activity, resulting in reduced amounts of ADH, are more resistant to the treatment. Of the 25,000 seedlings treated, 172 plants survived the allyl alcohol treatment and produced progeny.

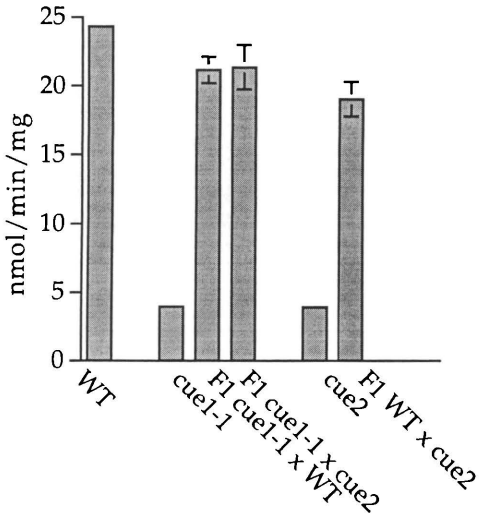


**Figure 2.** Specific GUS Activity of the Two *cue* Mutants, of $F_1$ Seedlings from Backcrosses to the Wild Type, and of $F_1$ Seedlings from the Complementation Cross.

Seedlings were grown for 5 days under continuous light on MS medium with 2% sucrose. The standard deviations for several samples are too small to be seen in the scale plotted. WT, wild type.

**Table 1.** Genetic Analysis of *cue1* Mutants

| Cross[a] | Mutant[b,c] | Wild Type[c] |
|---|---|---|
| *cue1-1/+* × *cue1-1/+* | 28 | 84 |
| *+/+* × *cue1-2/cue1-2* | 0 | 12 |
| *cue1-1/cue1-1* × *cue1-2/cue1-2* | 15 | 0 |
| *cue1-2/cue1-2* × *cue1-1/cue1-1* | 14 | 0 |
| *cue1-1/cue1-1* × *cue1-3/cue1-3* | 24 | 0 |

[a] Crosses are expressed as female × male.
[b] Mutants were scored with the phenotype of reticulate veins on leaves (see Figure 8).
[c] Number of seedlings.

To eliminate false positives and *cis*-acting mutations in the *CAB3–ADH* transgene, survivors of the allyl alcohol treatment were further screened for the reduced expression of the second transgene, *CAB3–uidA*, by assaying GUS activities of $M_3$ progeny of individual self-fertilized mutants. Among the 172 plants identified by resistance to allyl alcohol, 26 plants also had reduced GUS activities when compared with the wild type. Two mutants with the lowest GUS activities in the light were chosen for further characterization.

To determine the nature of the mutations in these two lines, each was backcrosssed to the unmutagenized parent pOCA108-1 line and to each other. Figure 2 shows that $F_1$ seedlings from the two mutants had wild-type levels of GUS activity when backcrossed to the wild type. In addition, $F_1$ seedlings from a complementation cross between the two mutants also had wild-type GUS activity in the light. These data indicated that the two mutations were recessive and that each allele defined a distinct locus named *cue1* and *cue2*. Further analysis of *cue1* confirmed that it was a single gene recessive mutation because the $F_2$ progeny from a backcross to the wild type segregated in a wild type/mutant ratio of 3:1 (Table 1). We mapped *cue1* to the upper portion of chromosome 5, between the markers *DFR* and *ASA1*, ~11 centimorgans (cM) proximal to the *transparent testa, glabra* (*ttg*) locus (see Methods).

Two additional *cue1* alleles were identified in subsequent screens. As shown in Table 1, these two mutants were crossed to *cue1* and failed to complement the *cue1* allele, indicating that these mutants contained new alleles of *cue1*. We designated these mutants *cue1-2* and *cue1-3*, and the original allele *cue1-1*. *cue1-2* is in the Columbia ecotype background and was identified by its similar visible phenotype to *cue1-1* (see below). *cue1-3* is in the Bensheim R002 background and was identified using the allyl alcohol selection on EMS-mutagenized seeds (E. Lopez and J. Chory, unpublished data). All three alleles had an identical visible phenotype that is described in detail below. Backcrossed *cue1-1* and *cue1-2* were chosen for further molecular and physiological characterization.

### *cue1* Mutations Reduce the Expression of Light-Regulated Genes in Leaf Mesophyll Cells, Whereas the Expression of *CHS* Is Unaffected

To investigate whether the endogenous *CAB* promoters are affected by the *cue1* mutations in a manner similar to the *CAB3* promoters in the transgene, we performed RNA gel blot analysis with mutants and wild-type plants. Figure 3A shows that light-grown *cue1-1* and *cue1-2* accumulated lower amounts of *CAB*, *RBCS*, and *psbA* (the chloroplast gene encoding the D1 protein of photosystem II) RNAs than did the wild type (from 5 to 25% of wild-type levels). The *cue1-1* mutant was further analyzed for accumulation of chloroplast-encoded *rbcL* mRNA (encoding the large subunit of ribulose bisphosphate carboxylase/oxygenase) and an epidermis-specific light-regulated mRNA, *CHS* (Chory and Peto, 1990). Whereas *rbcL* accumulation is also reduced in the *cue1-1* mutant, the expression of *CHS* was not affected (Figure 3A).

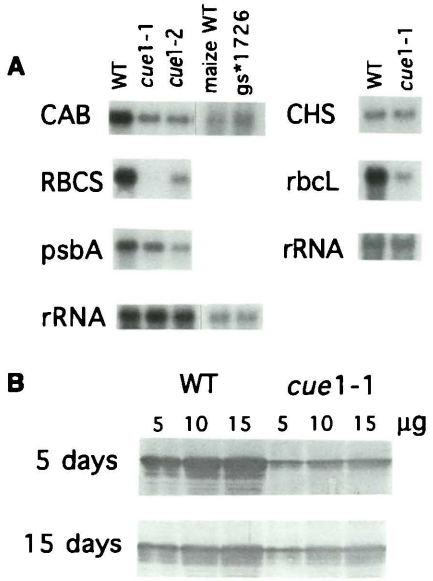


**Figure 3.** Effect of the *cue1* Mutations on the Expression of Several Light-Regulated Genes.

**(A)** Accumulation of mRNAs as determined by an RNA gel blot. Two micrograms of total RNA from the wild type (WT) or individual mutants, as indicated above, was loaded per lane. The blot was hybridized to specific probes as indicated at left. Also shown is *CAB* mRNA accumulation from RNA prepared from wild-type and *gs**1726 maize seedlings.

**(B)** Levels of LHCP in *cue1-1*. Total proteins were extracted from 5- or 15-day-old wild-type or *cue1-1* seedlings. Five to 15 µg of total protein, as indicated, was loaded per lane. The gel was transferred to a nitrocellulose membrane and probed with an antibody raised against the CAB protein. The first three lanes from the left were proteins extracted from the wild type. The three lanes on the right were proteins extracted from the mutant.

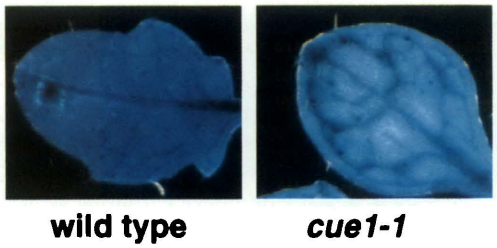


**Figure 4.** Histochemical Localization of GUS in Leaves of pOCA108-Transformed Wild-Type and *cue1-1* Plants.

Whole seedlings were stained overnight in 200 µg/mL X-gluc. The green-blue precipitate represents regions of high GUS activity. Note the reticulate staining pattern in the *cue1* leaf versus the uniform staining pattern in the wild type.

We analyzed the pattern of tissue and cell-type specificity of *CAB3* expression in *cue1* mutants by histochemical analysis of GUS activity in situ. Figure 4 shows that the *CAB3–uidA* transgene is expressed at low levels in the mesophyll cell layers of *cue1-1* leaves but at wild-type levels in the bundle sheath cells that align the veins, resulting in a reticulate pattern of *CAB* expression (Figure 4). This is in contrast to the wild type, for which GUS activity was more or less uniform throughout the leaf (Figure 4). Although these results are strictly qualitative, they suggest, together with the *CHS* results, that *CUE1* plays a role in light-regulated gene expression specifically in leaf mesophyll cells.

Previous studies using mutants (Reiter et al., 1994) and antisense transgenic plants (Flachmann et al., 1995) have revealed that the level of *CAB* mRNA does not necessarily correlate with the level of LHCP (light-harvesting chlorophyll proteins encoded by *CAB*). We investigated whether the reduced *CAB* mRNA levels in the *cue1* mutant were reflected by a similar reduction of LHCP. Figure 3B shows that *cue1-1* had reduced LHCP compared with the wild type. LHCP in 5-day-old *cue1* seedlings was reduced four- to eightfold when compared with 5-day-old wild-type seedlings; this parallels the reduction in *CAB* mRNA (Figure 3A).

The gene expression results shown in Figure 3A were obtained from seedlings germinated and grown for 5 days in continuous white light. To investigate the effect of the *cue1* mutation on *CAB* expression in plants of different developmental stages, we determined a time course of *CAB3–uidA* expression over a 15-day period. Figure 5 shows that GUS activity increased gradually with similar kinetics in both the mutant and wild type between 3 and 15 days of growth. The most striking difference between the mutant and the wild type was the low level of GUS activity at early time points. This difference was not due to a decrease in the dark basal activity of the *CAB3* promoter (pOCA108-1, 388 ± 39 nmol $min^{-1}$ $mg^{-1}$; *cue1-1*, 401 ± 33 nmol $min^{-1}$ $mg^{-1}$) but to differences in the very earliest stages of derepression of *CAB* gene expression by light. Therefore, a major function for CUE1 in Arabidopsis plants may

be to activate or allow the derepression of the expression of *CAB* and other photosynthesis-related genes in mesophyll cells at an early stage of leaf differentiation. However, *CAB3–uidA* activity in *cue1* remained lower than that in the wild type, even after 15 days of growth, suggesting that CUE1 is required throughout the life of the leaf.

### *cue1* Mutants Are Insensitive to Pulses of Red, Blue, and White Light

It has been shown that *CAB* transcription is greatly increased when plants are transferred from dark to light (Tobin, 1981). However, light-grown plants are developmentally different from etiolated seedlings. The reduced photosynthetic gene expression in *cue1* might be due to defects in photosynthetic physiology or chloroplast development rather than in light signaling. To discriminate between these possibilities, light pulse experiments on etiolated seedlings were performed. The light pulse treatments can induce gene expression, without significantly altering the development or physiology of the etiolated seedling. This induction is mediated at least in part by the red/far-red light receptor phytochrome (Karlin-Neumann et al., 1988; Reed et al., 1994) and a blue light receptor (Gao and Kaufman, 1994). We wanted to know which photoreceptor(s), if any, might be involved in the reduced *CAB* expression in continuous white light in *cue1*. We treated etiolated wild-type and *cue1* seedlings with pulses of light of specific wavelengths as shown in Figure 6. The results of two independent experiments indicated that dark accumulation of *CAB* mRNAs was similar in etiolated *cue1* and wild-type seedlings (data not shown), which correlates well with the similar *CAB3–uidA* activities measured in *cue1* and wild-type etiolated seedlings, as discussed above. In etiolated wild-type seedlings, the expression of *CAB* was induced by a pulse of red, blue, or white light as expected, and the induction by red light could be partially reversed by an additional pulse of far-red light, indicating that phytochrome is at least in part responsible for the observed induction by red light (Figure 6). In contrast, etiolated *cue1-1* seedlings did not accumulate significant amounts of *CAB* mRNA in response to pulses of red, blue, or white light. A similar quantitative reduction of *CAB* mRNA accumulation in *cue1* was observed in two separate experiments, one of which is shown in Figure 6. Thus, CUE1 may function downstream of several photoreceptors after their signal transduction pathways have converged.

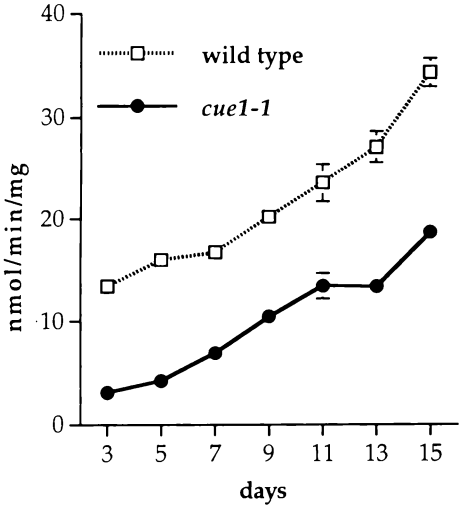


**Figure 5.** *CAB3–uidA* Transgene Expression Time Course in *cue1-1* and the Wild Type.

Specific GUS activity was assayed on seedlings grown on MS medium with 2% sucrose under continuous light.

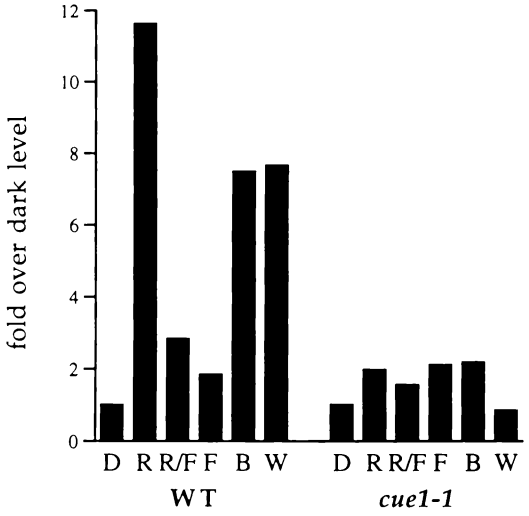


**Figure 6.** *CAB* mRNA Accumulation after Different Light-Pulse Treatments.

Seedlings were grown for 5 days in the dark on MS medium without sucrose and then treated with light as indicated. Fluence rates are as given in Methods. Seedlings were returned to darkness for 4 hr before they were harvested for RNA analysis. D, dark control; R, 2 min of red light; R/F, 2 min of red light followed by 4 min of far-red light; F, 4 min of far-red light alone; B, 30 sec of blue light; W, 1 min of white light; WT, wild type.

### *cue1* Requires Exogenously Supplemented Sucrose To Establish Growth

The *CAB3–uidA* expression time course experiment (Figure 5) and the light pulse experiments (Figure 6) suggest that CUE1 may be important for the induction of photosynthesis genes when plants are initially exposed to light. If so, one might expect CUE1 to be important in establishing autotrophic growth. We tested this hypothesis by germinating *cue1-1* seeds on media with or without exogenously supplemented sucrose. *cue1-1*

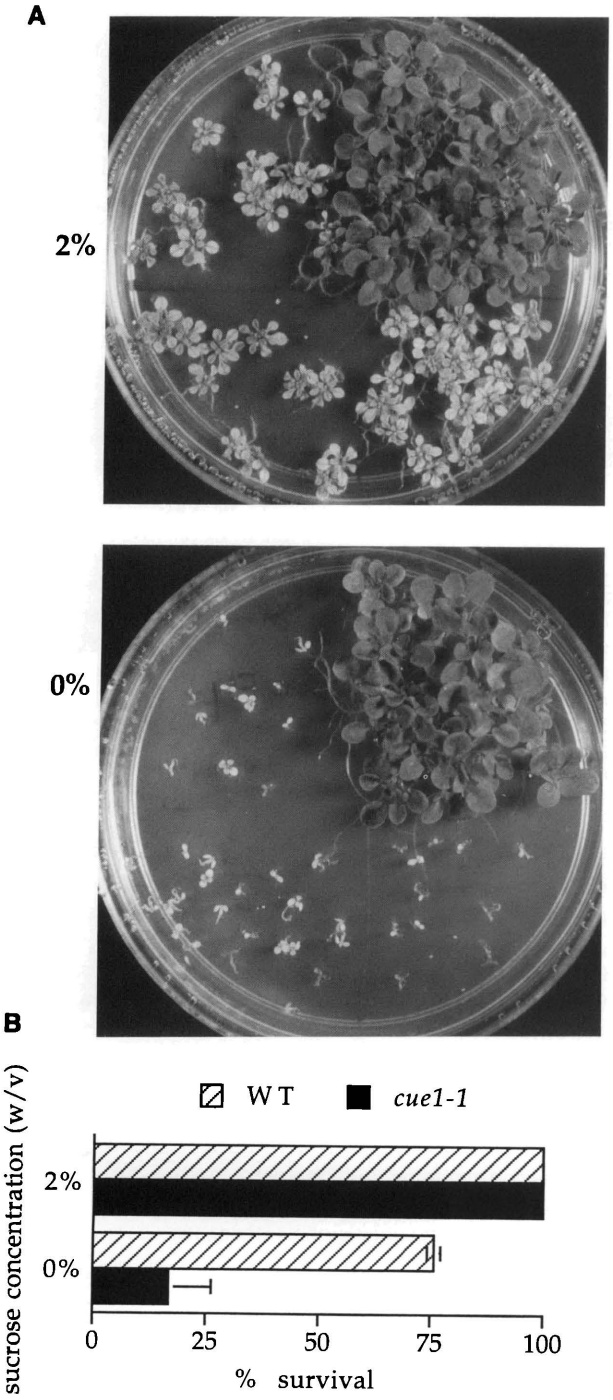


**Figure 7.** Sucrose Is Required for the Development of *cue1-1* Seedlings.

**(A)** Development of *cue1-1* and wild-type seedlings on medium with or without sucrose. Seeds were sown on MS agar plates supplemented with 2% sucrose (top) or without sucrose supplementation (bottom). On each plate, the top right corner was sown with wild-type seeds and the other three corners were sown with *cue1-1* seeds harvested at different times. Photographs were taken after the plants had been growing for 13 days under continuous light.

failed to develop significantly on media without exogenously supplemented sucrose (Figure 7A, bottom). Without sucrose, development of most *cue1-1* seedlings was arrested at a stage in which the seedlings had expanded cotyledons but short roots and no leaves. The cotyledons stayed green on the media for more than 17 days. A minority (16%) of *cue1* seedlings grown on media without sucrose could develop one pair of leaves and several millimeters of roots after 13 days (Figure 7B). The same result was obtained with *cue1-1* seeds harvested at different times (Figure 7A) and with the *cue1-2* allele, although *cue1-2* seedlings had a higher survival rate (data not shown). On sucrose-containing media, *cue1-1* seedlings developed the same number of leaves at the same rate as the wild type, although they were only approximately one-third the fresh weight of wild type after 17 days of growth (data not shown).

Similar results were obtained with an $F_2$ population segregating the mutant and wild type, excluding a maternal effect on seed reserves (data not shown). Furthermore, dark-grown *cue1* seedlings on synthetic medium lacking sucrose grew equally well as the wild type (data not shown). These data indicated that the apparent sucrose-requiring phenotype of the *cue1* mutants was not due to reduced seed reserve but rather to the effect of the *cue1* mutation on seedling growth. In addition, *cue1-1* seedlings developed normally if transferred to media without sucrose after being germinated on media with sucrose for as few as 2 days (data not shown). This again supports the hypothesis that CUE1 functions very early in seedling development, perhaps by its involvement in the establishment of photoautotrophic growth.

### Visible Phenotypes of *cue1* Mutants

*cue1* mutants have a visible phenotype as seen in Figure 8. The most striking aspect of this phenotype is that the paraveinal regions of the leaves are dark green and the interveinal regions are pale green, resulting in a reticulate pattern (Figure 8). This same phenotype was observed in each of the three alleles and correlates with the pattern of *CAB* transcription shown in Figure 4. The pale color of the interveinal regions in *cue1* mutant plants was due to a reduction of total chlorophylls and carotenoids (Figure 9). The extent of this reduction correlated well with the level of reduction of *CAB* transcripts and LHCP in these cells (Figure 3).

We performed light and electron microscopy on leaf sections of the *cue1-1* mutant and wild type, as shown in Figures 10 and 11. Cross-sections of leaves from *cue1* seedlings clearly showed the smaller size of the mesophyll cells and an increase in the volume of air space between these cells when compared with wild-type Arabidopsis (Figures 10A and 10B). Paradermal sections documented further the underdeveloped mesophyll

**(B)** Quantitation of the results presented in **(A).** Seedlings with two or more leaves and roots longer than 3 mm were scored as survivors. WT, wild type.


**Figure 8.** Phenotype of a *cue1-1* Mutant.

**(A)** A 4-week-old *cue1-1* plant.
**(B)** Magnified leaf from a *cue1-1* plant.

layer of *cue1* mutants and showed that bundle sheath cells aligning the veins appeared normal in the mutant (Figures 10C and 10D). We also examined chloroplast ultrastructure in *cue1* and wild-type mesophyll and paraveinal cells (Figure 11). Wild-type mesophyll chloroplasts were typically larger than *cue1* chloroplasts (Figures 10A and 10B, and 11A and 11B; data not shown). In contrast, in the bundle sheath cells associated with the veins, chloroplasts from *cue1* and the wild type were of similar size and had approximately the same amount of granal membranes (Figures 11C and 11D). In both cases, however, the size and amount of membranes in chloroplasts of the bundle sheath cells were considerably smaller than in either wild-type or *cue1* mesophyll chloroplasts.

### Not All Reticulate Mutants Have Reduced *CAB* Expression

Several other mutants have been isolated that have paraveinal regions that are greener than the interveinal regions, for example, the yellow-stripe mutants of maize (Bell et al., 1958; von Wirén et al., 1994) and the *reticulata* mutant of Arabidopsis (Redei and Hirono, 1964). We were thus interested in knowing whether other mutants with the phenotypic characteristics of *cue1* also have reduced *CAB* transcripts. Unlike *cue1*, the maize *gs**1726 yellow-stripe line (Figure 3A) and the Arabidopsis *reticulata* mutant (data not shown) both accumulated wild-type levels of *CAB* mRNAs. Complementation and mapping analyses further indicated that *cue1* and *reticulata* were not allelic (data not shown).

## DISCUSSION

We developed a genetic selection to identify positively acting components in photoreceptor signaling pathways in Arabidopsis. Here, we describe *CUE1*. Mutations at this locus result in defects in expression of nuclear- and chloroplast-encoded genes in response to light. For several reasons, *CUE1* appears

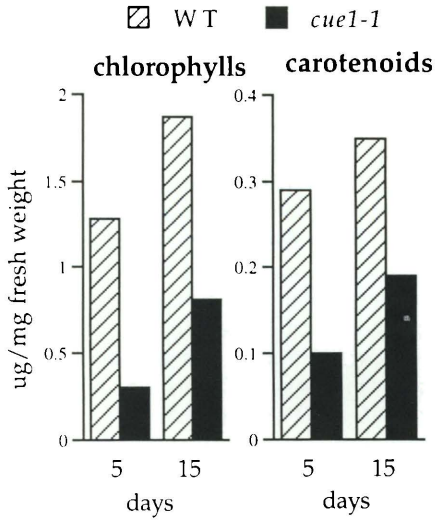


**Figure 9.** Amounts of Total Chlorophylls and Carotenoids in Wild-Type and *cue1-1* Seedlings.

Seedlings were grown for 5 or 15 days under continuous light on MS medium with 2% sucrose. WT, wild type.

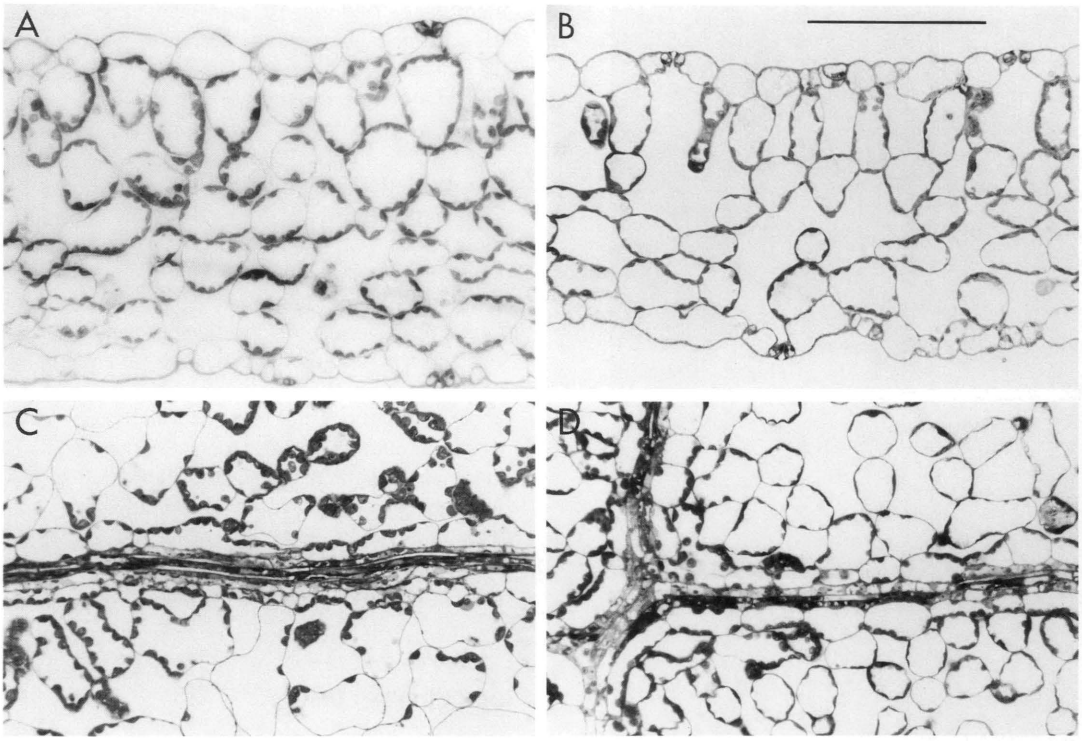


**Figure 10.** Light Microscopy of 1-μm Cross-Sections and Paradermal Sections from Wild-Type and *cue1* Leaves.

**(A)** and **(B)** Cross-sections from wild-type and *cue1* leaves, respectively.
**(C)** and **(D)** Paradermal sections from wild-type and *cue1* leaves, respectively.
Note the small size of the mesophyll cells and increased volume of air space in the *cue1* leaves in **(B)** versus the wild type in **(A).** Also, plastids in the *cue1* mesophyll cell layers appear smaller than in the wild type. The paradermal sections in **(C)** and **(D)** show the bundle sheath cells associated with the vasculature. Unlike the mesophyll cells, there are no major differences in cell or plastid size in the bundle sheath cell layer of *cue1* in **(D)** versus the wild type in **(C).** The bar in **(B)** = 100 μm.

to be a key positive regulator linking light to cell-type–specific gene expression in Arabidopsis. First, *cue1* mutations do not affect the dark basal level of expression of the *CAB3* promoter, but the accumulation of mesophyll cell–specific light-regulated mRNAs was diminished in light-grown *cue1* mutant seedlings (Figure 3). The *CAB3–uidA* transgene was expressed at low levels in the mesophyll layers but at wild-type levels in the bundle sheath cells that align the veins (Figure 4). This and the reduced expression of other photosynthesis-related genes are correlated with defects in chloroplast development specifically in the mesophyll but not in the bundle sheath layer of the *cue1* leaf (Figures 8, 10, and 11). In addition, *CHS* mRNA, although known to be regulated by both phytochrome and a blue light receptor, accumulated normally in the leaf epidermis (Figure 3; Chory and Peto, 1990), corroborating the hypothesis that CUE1 functions primarily in mesophyll-specific gene expression. Moreover, CUE1 appears to be responsible for the primary derepression of mesophyll-specific light-regulated gene expression, because *cue1* mutants were severely deficient at establishing photoautotrophic growth (Figure 7), and the most severe defects in *CAB* promoter activity were seen during the initial stages of deetiolation (Figure 5). In addition, *CAB* mRNA accumulation in etiolated *cue1* seedlings was reduced in response to the red, blue, and white light pulses that are required to initiate the processes of coordinate leaf and chloroplast differentiation, suggesting that the *cue1* mutations do not affect photosynthetic physiology or chloroplast development. Together, these results suggest that CUE1 functions early during leaf and chloroplast development in response to light.

A collection of maize yellow-stripe mutants, which in many ways are similar in phenotype to the *cue1* mutant alleles, has been described (Bell et al., 1958; von Wirén et al., 1994). The phenotype of the maize mutants appears to be caused by iron deficiency due to a defect in uptake of iron phytosiderophores (for example, von Wirén et al., 1994). It is unlikely that the green vein phenotype of *cue1* mutants is caused by nutrient deprivation. We examined the effect on *CAB* gene expression in two other "green-vein" mutants, *reticulata* of Arabidopsis (Redei and Hirono, 1964) and *gs**1726 of maize (R. Martienssen, personal communication), and found that these mutants have

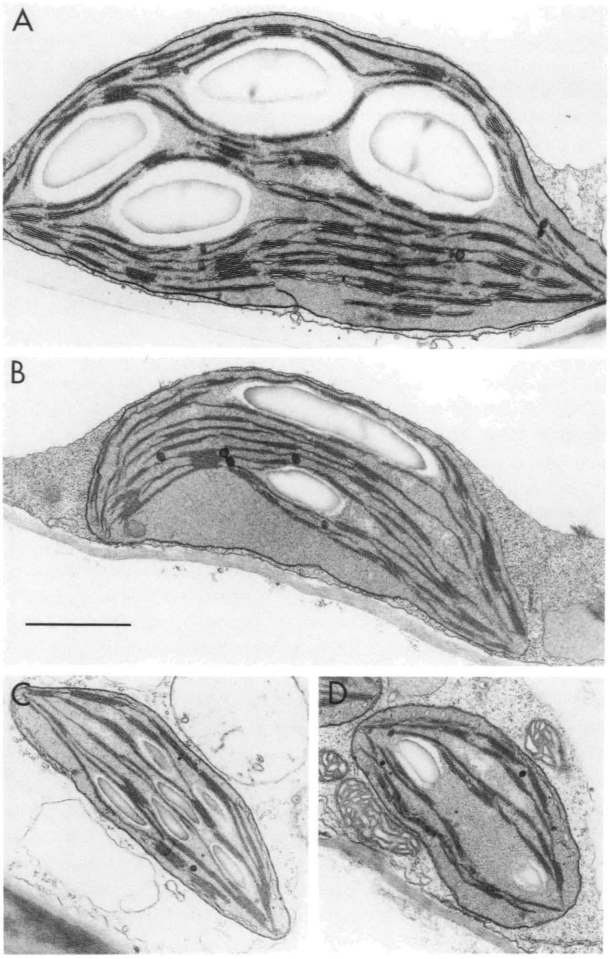


**Figure 11.** Electron Microscopy of Representative Chloroplasts from Wild-Type and *cue1* Mesophyll Cells and Wild-Type and *cue1* Bundle Sheath Cells.

**(A)** and **(B)** Representative chloroplasts from wild-type and *cue1* mesophyll cells, respectively.
**(C)** and **(D)** Representative chloroplasts from wild-type and *cue1* bundle sheath cells, respectively.
In mesophyll cells, *cue1* chloroplasts were significantly smaller than those of the wild type. In contrast, the bundle sheath chloroplasts from both wild type and *cue1* were similar, being smaller than mesophyll chloroplasts. The bar in **(B)** = 1 μm.

normal levels of *CAB* transcripts. Also, the pale green phenotype of *cue1* leaves is not rescued by nutrient supplements. These results suggest that reduced photosynthetic gene expression in *cue1* may be the direct cause of the green-vein phenotype rather than an indirect consequence of nutrient deprivation.

In addition to its postulated role in linking light to tissue-specific gene expression, *CUE1* may also define a phytochrome pathway–specific regulator. Using microinjection techniques, Bowler et al. (1994a, 1994b) have shown that the *CHS* gene is regulated by phytochrome via a cyclic GMP–dependent pathway and that the *CAB* and *RBCS* genes are regulated by a different downstream pathway that involves calcium and activated calmodulin. These differential effects correlate well with the gene expression pattern of the *cue1-1* mutant: both *CAB* and *RBCS* gene expression are severely reduced in *cue1*, whereas *CHS* expression is normal. One might hypothesize that *CUE1* encodes a mesophyll cell–specific positive regulator that functions in the calcium/calmodulin branch of a phytochrome signaling pathway.

A 78-bp domain in the Arabidopsis *CAB2* promoter confers both phytochrome and circadian regulation upon a heterologous promoter in tobacco (Anderson et al., 1994). Because *cue1* seedlings have a reduced response to phytochrome-mediated induction of *CAB* transcription, it is possible that the *CUE1* locus encodes a factor that either interacts directly with this 78-bp domain or regulates other factors that interact with this domain. Photomorphogenetic mutants such as *deetiolated1* (*det1*; Chory et al., 1989) and *constitutive photomorphogenic1* (*cop1*; Deng et al., 1991) have high-level expression of *CAB* and *RBCS* in the dark, similar to light-grown wild-type plants. Because CUE1 is required for the expression of *CAB* and *RBCS* in the light, it is formally possible that the high-level expression of these genes in dark-grown *det1* and *cop1* mutants is also mediated by CUE1. We think, however, that this is unlikely because *det1 cue1* double mutants still have high levels of *CAB* expression in the dark (H-m. Li and J. Chory, unpublished results). This result suggests that CUE1 acts only in the light and may indicate that CUE1 does not interact with DET1 in the dark. These results also imply that *CUE1* does not encode one of the transcription factors, such as ATH1 (Quaedvlieg et al., 1995) or CA-1 (Sun et al., 1993), that were proposed previously to interact with DET1.

The levels of mRNAs and proteins of photosynthetic genes are regulated by many developmental and environmental factors through complex processes. The reduced RNA expression of one gene does not necessarily result in the reduced expression of other closely related genes. For example, the *doc2* and *doc3* mutations of Arabidopsis separate genetically the regulated expression of *CAB* and *RBCS* (Li et al., 1994). In other studies, it has been shown that reduced expression of a specific light-regulated RNA by an antisense RNA is not necessarily correlated with a reduction of the protein. For instance, transgenic antisense plants that result in reduced accumulation of RNAs for two components of the oxygen-evolving complex and the Rieske iron/sulfur protein do not result in reduced accumulation of these proteins (Palomares et al., 1993). In addition, an antisense *CAB* plant has severely reduced *CAB* mRNA (5% of the wild-type levels), but LHCP accumulates to 100% of the wild-type levels (Flachmann and Kühlbrandt, 1995). In contrast, an antisense *RBCS* plant of tobacco has reduced *RBCS* mRNA and protein (Jiang and Rodermel, 1995). No common theme has emerged from these studies because sometimes mRNA and protein levels are reduced in parallel with each other (for example, see Jiang and Rodermel, 1995), whereas in other

situations, post-transcriptional control mechanisms appear to compensate for the reduced mRNA levels in the antisense-expressing plants (Palomares et al., 1993; Flachmann and Kühlbrandt, 1995). In our studies, we observed a direct relationship between the accumulation of *CAB* mRNAs and proteins during development in the *cue1* mutant as well as reduced expression from the *CAB3* promoter (Figure 5). Although we cannot explain the disparate results observed in the antisense RNA studies, our studies are different in that we required reduced transcription from the *CAB* promoter for mutant selection. Moreover, *RBCS* mRNA accumulation was also reduced. As such, *CUE1* formally encodes a *trans*-acting regulatory factor that acts either directly or indirectly to control transcription of light-regulated promoters.

In conclusion, we developed a genetic selection to identify positively acting components in the photoreceptor action pathways in Arabidopsis. The mutant screen is based on the selection for survivors of allyl alcohol treatment of mutagenized Arabidopsis lines containing a *CAB3* promoter fused to the *ADH* gene, thus allowing the identification of mutants—independent of phenotype—in which a tightly regulated light-controlled promoter is expressed at low levels in the light. Our data indicate that the screen is robust, resulting in mutations in at least 11 independent *cue* loci (E. Lopez and J. Chory, unpublished results), including loci encoding the apoprotein component of phytochrome B (Reed et al., 1993), and the synthesis of the linear tetrapyrrole common to all phytochromes, *hy1* (Koornneef et al., 1980; Parks and Quail, 1991). The fact that we found photoreceptor mutations indicates that we might recover alleles in all of the positively acting components of phytochrome signaling of *CAB* gene expression. We should also find mutations in genes that exert their effects on light-regulated promoters in response to cell- and developmental stage–specific signals. Because the selection can be adjusted so that we can recover mutants that have *CAB* levels reduced by only two- to threefold (E. Lopez and J. Chory, unpublished results), we should be able to isolate leaky mutations in genes encoding components in the photoreceptor action pathways, overcoming the potential problem that the activity of these genes may be required for viability. Our strategy is to isolate a large number of mutants to identify all possible genes that affect the positive control of *CAB* gene expression. Together with other mutants, this should give a complete picture of the complexity of the signal transduction network controlling photoregulated gene expression in plants.

## METHODS

### Construction of the Arabidopsis pOCA108-1 Transgenic Line

We created the pOCA108 transgenic construct by modification of pOCA107 (Susek et al., 1993). In pOCA108, the hygromycin phosphotransferase gene–nopaline synthase terminator of pOCA107 was replaced with the Arabidopsis alcohol dehydrogenase (*ADH*) genomic sequence (−14 to ATG, including 3′ untranslated region) (Chang and Meyerowitz, 1986). We introduced pOCA108 into an *adh* mutant of Arabidopsis ecotype Bensheim (R002; Jacobs et al., 1988) using a modified version of the standard *Agrobacterium tumefaciens*–mediated root transformation protocol (Chory and Peto, 1990) and generated >20 independent transgenic lines. We selected one line, pOCA108-1, for which the segregation of kanamycin resistance indicated a single pOCA108 insertion site.

### Plant Material, Growth Conditions, and Mutant Screening

Unless otherwise noted, the wild type is the unmutagenized R002 line (ecotype Bensheim) that is homozygous for a single insertion of the pOCA108 transgene on chromosome 2. Plants grown in the light on synthetic MS medium (Gibco, Grand Island, NY) (Murashige and Skoog salt mix, Gamborg's vitamin mix, with or without 2% sucrose) were maintained in growth chambers at 21°C with continuous light of ~200 $\mu E\ m^{-2}\ sec^{-1}$. Plants grown in the greenhouse on soil were maintained as described previously (Somerville and Ogren, 1982).

Seeds from the pOCA108-1 line were imbibed overnight at 4°C and then mutagenized with $\gamma$-ray (30 krad). Mutagenized $M_2$ seeds (56,000) from 28 pools were screened for allyl alcohol resistance by growing the seedlings in batches of 100 in liquid MS medium for 5 days with shaking under continuous white light. Seedlings were treated with 3 mM allyl alcohol for 1 hr, rinsed twice in fresh MS medium, and transferred to MS agar plates. Cotyledons of all seedlings bleached out initially. Resistant seedlings then developed new green leaves. Seeds from self-fertilized plants that survived the allyl alcohol treatment were grown for 5 more days under continuous light on MS agar plates. Seedlings were then harvested and analyzed for specific β-glucuronidase (GUS) activity (Jefferson, 1987). The *cue1-2* mutant was identified from batch 92D of fast-neutron mutagenized seed population M2F-1A-1, in the Columbia ecotype with the *glabra1* (*gl1*) mutation, purchased from Lehle Seeds (Tucson, AZ). The *cue1-3* allele was identified from ethyl methanesulfonate (EMS)–mutagenized (0.3% for 16 hr) pOCA108-1 transformant seeds. These were the same seeds as those used for $\gamma$-ray mutagenesis. Additional details about the screen can be found in Chory et al. (1995).

### Molecular Characterization and Mapping of *cue1*

All molecular characterizations of the mutants were performed with lines that had been backcrossed at least once. RNA isolation, RNA gel blotting, and probe synthesis were performed as described by Li et al. (1994). Quantification of samples was performed using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Histochemical staining of wild-type and *cue1-1* leaves was performed by incubating whole seedlings in 200 μg/mL 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-gluc) for 16 hr after fixing the seedlings for 30 min in 0.3% formaldehyde. Light-pulse treatments of etiolated seedlings were performed as described by Reed et al. (1994). The blue light glass filter used was type 5-57 from Kopp (purchased from Newport Industrial Glass, Costa Mesa, CA), with transmission from 320 to 580 nm and >60% transmission from 380 to 460 nm. The fluence rates used were $382\ \mu E\ m^{-2}\ sec^{-1}$ for red light, $38\ \mu E\ m^{-2}\ sec^{-1}$ for blue light, and $760\ \mu E\ m^{-2}\ sec^{-1}$ for white light. Total chlorophyll and carotenoids were extracted with 80% acetone and measured as described by Lichtenthaler (1987).

*cue1* and the pOCA108-1 insertion site were mapped using codominant ecotype-specific polymerase chain reaction–based markers as described by Konieczny and Ausubel (1993). Although the markers were originally established between the ecotypes Landsberg *erecta* and Columbia, we found that the restriction pattern of Bensheim DNA at a particular marker can be the same as either that of Columbia or that of Landsberg *erecta*. The *cue1-1* mutant and the pOCA108-1 transformant were thus crossed to both wild-type Landsberg *erecta* and Columbia plants. DNA was isolated from mutant $F_2$ seedlings of the crosses. Based on 75 $F_2$ samples (150 chromosomes), *cue1* was mapped to chromosome 5, ~27 centimorgans (cM) from the marker *ASA1* and 18 cM from the marker *DFR*. *cue1-1* was then crossed to the *transparent testa*, *glabra* (*ttg*) mutant. Of 83 *cue1* mutants from the $F_2$ segregating population, only one plant also showed the *ttg* mutation. Using the neomycin phosphotransferase gene as the selection marker, the pOCA108-1 insertion site was positioned between the markers m429 and *GPA1* on the middle portion of chromosome 2, ~4.8 cM from *GPA1* and 15.6 cM from m429.

### Light and Electron Microscopy

Six wild-type leaves and 11 *cue1* leaves were processed for light and transmission electron microscopy by fixing in 4% paraformaldehyde, 2.5% glutaraldehyde, 0.1 M sodium phosphate buffer, pH 7.2, using intermittent vacuum for 30 min at room temperature, followed by 21 hr at 4°C. This was followed by fixing in 1% osmium tetroxide, 1.5% potassium ferricyanide in phosphate buffer for 70 min at 4°C (Langford and Coggeshall, 1980), and a third fixative consisting of 2% uranyl acetate (aqueous) for 60 min at 4°C. The samples were dehydrated in ethanol and propylene oxide, and embedded in Spurr resin. Five blocks each of the wild type and *cue1* were stained in 1 μM toluidine blue and examined by light microscopy. One representative block from each was selected for thin sectioning; they were uranium and lead stained, and examined in an electron microscope (EM109; Zeiss, Thornwood, NY).

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