The Plant Cell, Vol. 12, 199–210, February 2000, www.plantcell.org

Genetic Interactions between the Chlorate-Resistant Mutant *cr88* and the Photomorphogenic Mutants *cop1* and *hy5*

Dongsun Cao, Yun Lin,[1] and Chi-Lien Cheng[2]

Department of Biological Sciences, University of Iowa, Iowa City, Iowa 52242

The chlorate-resistant mutant *cr88* is defective in photomorphogenesis, as shown by the phenotypes of long hypocotyls in red light and yellow cotyledons under all light conditions. A subset of light-regulated genes is expressed at subnormal levels in *cr88.* To analyze further the role that CR88 plays in photomorphogenesis, we investigated the genetic interactions between *cr88* and mutants of two other loci affecting photomorphogenesis, *CONSTITUTIVE PHOTOMORPHOGENIC1* (*COP1*) and *LONG HYPOCOTYL5* (*HY5*). COP1 represses the expression of light-regulated genes in the dark, and HY5 inhibits hypocotyl elongation in the light. Using morphological, cellular, and gene expression criteria for epistasis analyses to position *CR88* in the genetic hierarchy of the photomorphogenesis pathway, we determined that *CR88* acts downstream of *COP1* but in a branch separate from *HY5.* In the course of our analysis, we discovered that light causes extensive destruction of plastids in dark-grown *cop1* seedlings and that *cr88* prevents this destruction.

INTRODUCTION

Light signals are essential environmental cues for seedlings to elaborate their developmental program during the transition from heterotrophy to autotrophy. The effects of light on seedling development can be observed at many levels, including gene expression, cell differentiation, and plant morphology. During seedling development, higher plants, such as Arabidopsis, can adopt two contrasting development schemes: skotomorphogenesis, which takes place in the dark, and photomorphogenesis, which occurs in the light (Kendrick and Kronenberg, 1994). Dark-grown seedlings undergoing skotomorphogenesis exhibit etiolated morphologies, including long hypocotyls, apical hooks, and closed and undeveloped cotyledons. The genes required for chloroplast development and photosynthesis are expressed at low or undetectable levels. When exposed to light, seedlings undergo photomorphogenesis and exhibit deetiolated morphologies, such as short hypocotyls and open and developed cotyledons. Expression of genes encoding photosynthetic proteins is strongly induced, and chloroplasts develop from etioplasts (Mullet, 1988; Susek et al., 1993; Deng, 1994; Reiter et al., 1994).

Genetic analyses have identified components in the photomorphogenesis pathway. In addition to mutants defective in the phytochrome photoreceptors PhyA (Dehesh et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993) and PhyB (Koornneef et al., 1980; Reed et al., 1993), and the blue light photoreceptor cryptochrome (CRY1) (Koornneef et al., 1980; Ahmad and Cashmore, 1993), two classes of regulatory mutants have been identified. One class consists of mutants that exhibit an etiolated morphology when germinated in the light, suggesting a positive role for the wild-type gene products in photomorphogenesis. These mutants define loci, such as *LONG HYPOCOTYL5* (*HY5*) (Koornneef et al., 1980); *PHYTOCHROME SIGNALING–EARLY FLOWERING (PEF1, PEF2*, and *PEF3*) (Ahmad and Cashmore, 1996); *RED ELONGATED1* (*red1*) (Wagner et al., 1997); and *ELONGATED HYPOCOTYL IN FAR-RED LIGHT* (*FHY1* and *FHY3*) (Whitelam et al., 1993; Johnson et al., 1994). The *hy5* mutant displays long hypocotyls under red, far-red, and blue light conditions (Koornneef et al., 1980), suggesting that *HY5* encodes a component that functions after convergence of the pathways that transduce signals triggered by red, far-red, and blue light (Koornneef et al., 1980). The predicted protein sequence encoded by *HY5* resembles a basic leucine zipper transcription activator (Oyama et al., 1997). HY5, which is required for light-induced expression of the gene encoding chalcone synthase (CHS), binds to the *CHS* promoter (Ang et al., 1998). The remaining mutants in this class are defective in the photomorphogenic response specific to either or both of the PhyA and PhyB signaling pathways (Whitelam et al., 1993; Johnson et al.,

[1] Current address: Department of Genetics, Harvard Medical School, and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

[2] To whom correspondence should be addressed. E-mail chi-liencheng@uiowa.edu; fax 319-335-2583.

1994; Ahmad and Cashmore, 1996; Wagner et al., 1997). Except for *hy5*, all mutants in this class appear to define early steps of light signaling (Ahmad and Cashmore, 1996; Wagner et al., 1997). Recently, these loci, together with *SPA1* (Hoecker et al., 1998, 1999) and *PHYTOCHROME SIGNALING2* (*PSI2)* (Genoud et al., 1998), which cause a hypersensitive photomorphogenic response when mutated, have been proposed to act downstream of photoreceptors and upstream of the protein encoded by *CONSTITUTIVE PHOTOMORPHOGENIC1* (*COP1*) (Ni et al., 1998).

Another class of mutants displays deetiolated morphologies when grown in the dark. The recessive nature of the mutants suggests that the gene products are required to repress photomorphogenesis in the dark. These mutants include at least 16 *cop*/*deetiolated*/*fusca* loci (Chory et al., 1989; Deng et al., 1991; Wei and Deng, 1992; Misera et al., 1994; Wei et al., 1994; Kwok et al., 1996). Genetic analyses suggest that these genes act at or after the convergence of the PhyA, PhyB, and CRY1 signaling pathways (Chory, 1993; Ang and Deng, 1994). Among these loci, *COP1* encodes a key repressor that is hypothesized to interact with specific transcription factors in the nucleus to repress their activities in the dark. One of these transcription factors is HY5, which has been shown to interact directly with the COP1 WD-40 repeats (Ang et al., 1998; Chattopadhyay et al., 1998). Exposure to light causes COP1 to be excluded from the nucleus, thereby allowing the expression of downstream genes (reviewed in Osterlund et al., 1999).

While selecting chlorate-resistant mutants that were defective in the expression of genes encoding nitrate reductase (NR), we identified a novel chlorate-resistant mutant, *cr88*, that displays long hypocotyls in red light but not in far-red or blue light. Cotyledons and young leaves of light-grown *cr88* are yellow-green, correlating with delayed chloroplast development. The expression of light-induced genes encoding the chlorophyll *a*/*b* binding protein (CAB), the small subunit of ribulose bisphophate carboxylase (RBCS), and NR2—but not the expression of *CHS* and *NR1*—was altered.

On the basis of these observations, we hypothesized that CR88 acts in the pathways for transduction of light signals to regulate a subset of photomorphogenesis responses that are repressed by COP1 and, moreover, that CR88 plays both a major role in controlling the greening process and a minor role in controlling hypocotyl elongation (Lin and Cheng, 1997). By analyzing epistatic relationships between mutants *cr88* and *cop1* and between mutants *cr88* and *hy5*, we found that *CR88* is likely to act downstream of *COP1* but in a branch separate from that affected by *HY5.* During the course of the analysis, we discovered that the loss of greening ability of *cop1* mutants after prolonged growth in the dark (Ang and Deng, 1994) is caused by the extensive destruction of plastids and other cellular structures when exposed to light. However, the *cr88* allele is able to prevent the destruction caused by *cop1.* In addition, we show that the long hypocotyls in red light and the impaired light induction of gene expression in *cr88* are unlikely to be secondary effects caused by a delay in the greening process.

RESULTS

Inhibition of Photosynthesis Neither Increases the Hypocotyl Length nor Decreases the Expression of Photosynthetic Genes in Wild-Type and *cr88* Seedlings

We showed previously that *cr88* exhibits reduced expression of *NR2*, *CAB*, and *RBCS* genes as well as impaired deetiolation in red light. In addition, the chloroplasts of *cr88* cotyledons and young leaves are not as well developed as those of the wild type (Lin and Cheng, 1997). These observations raised the possibility that the lack of inhibition of hypocotyl elongation and reduced expression of *NR2*, *CAB*, and *RBCS* mRNA in *cr88* might be caused by a decrease in the photosynthetic activity of the underdeveloped chloroplasts. Therefore, we tested whether inhibiting photosynthesis in wild-type seedlings would result in an increase in hypocotyl length in red light and a decrease of the expression of *NR2*, *CAB*, and *RBCS* genes. To determine whether inhibiting photosynthesis would result in longer hypocotyls, we grew the wild-type and *cr88* seedlings in continuous red light for 4 days on media containing 0, 0.1, 1, 5, or 10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), a photosynthesis inhibitor, or on media containing 0, 1, 5, 10, or 20 mM norflurazon, which causes photooxidative damage. Neither compound increased the hypocotyl lengths of the seedlings, even though cotyledons of the norflurazon-treated seedlings exhibited photobleaching (data not shown).

We also asked whether inhibition of photosynthesis would affect light-induced gene expression. Wild-type and *cr88* seedlings were grown for 4 days in the dark in media containing 0 or 10 μM DCMU and were then exposed to red light for 2, 4, or 8 hr. Similar to our previous observations on plants grown in white light (Lin and Cheng, 1997), the amounts of red light–induced *NR2*, *CAB*, and *RBCS* mRNAs were less in *cr88* than in the wild-type seedlings (Figure 1). DCMU did not cause a decrease in the steady state amounts of mRNAs of these genes; to the contrary, DCMU may have increased their expression. The increase in gene expression caused by DCMU is consistent with a previous report in which DCMU was shown to increase *CAB* transcription rates (Escoubas et al., 1995). In contrast, the expression of the gene encoding ferredoxin (*FEDA*) was decreased in response to DCMU, as also reported previously (Petracek et al., 1997).

These results demonstrate that inhibition of photosynthesis did not produce a phenocopy of the Hy phenotype of *cr88* in red light, nor did it cause a decrease in light-induced expression of *NR2*, *CAB*, and *RBCS* genes. Thus, these defects of *cr88* are unlikely to be secondary effects caused by decreased photosynthetic activity.

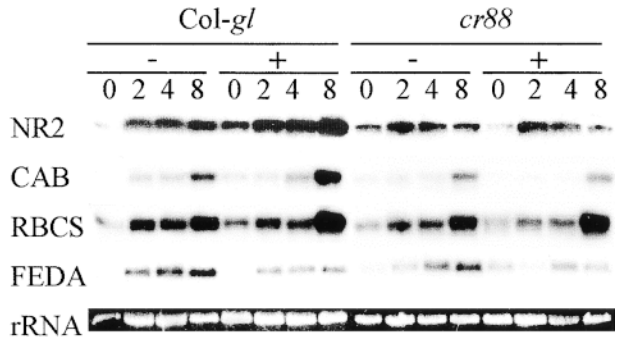

Figure 1. Effects of DCMU on Red Light Induction of *NR2*, *CAB*, and *RBCS* mRNA.

Each lane contains 5 μg of total RNA isolated from seedlings grown on medium in the dark for 4 days and then exposed to red light. The number above each lane indicates the hours of red light induction in the absence (−) or presence (+) of 10 μM DCMU. At left, NR2, CAB, RBCS, and FEDA indicate the RNAs hybridizing with their respective probes. Ethidium bromide–stained 28S rRNA served as a loading control.

CR88 Is Located on Chromosome 2

CR88 was mapped to chromosome 2 by using the set of Arabidopsis restriction fragment length polymorphism markers described by Fabri and Schaffner (1994). Use of additional markers on chromosome 2 located *cr88* between m246 and CDs3. Complementation tests between *cr88* and other known *hy* mutants suggested that *CR88* defines a new *HY* locus (Lin and Cheng, 1997). The map position of *CR88* eliminates the possibility that it encodes one of the minor phytochromes, *PHYC* (Schmidt et al., 1997), *PHYD* (Schmidt et al., 1996), or *PHYE* (Schmidt et al., 1996), all of which map to other chromosomes. It also precludes *CR88* being an allele of the recently identified long hypocotyl mutants *elongated* (*elg*) (Halliday et al., 1996), *pef1* (Ahmad and Cashmore, 1996), and *red1* (Wagner et al., 1997) or of the *CAB underexpressed* (*cue*) mutants (Li et al., 1995; Lopez-Juez et al., 1998) because none of these maps to chromosome 2.

Light Induction of *NR2*, *CAB*, and *RBCS* Is Defective in Red, Far-Red, and Blue Light Conditions

We showed previously that light-induced expression of *NR2*, *CAB*, and *RBCS*, but not *NR1* and *CHS*, is impaired in *cr88* after 8 hr of exposure to white light (Lin and Cheng, 1997). To further understand this defect, we examined the induction of light-regulated gene expression in different light spectra. Wild-type and *cr88* seedlings were grown for 4 days in the dark and then exposed to red, blue, or far-red light for 4 or 8 hr. The steady state amounts of mRNAs for *NR1*, *NR2*, *CAB*, *RBCS*, and *CHS* in *cr88* were compared with those in the wild type (Figure 2). In *cr88*, the mRNAs for *NR2*, *CAB*, and *RBCS* were less than those in the wild type in all light conditions, whereas the amounts of the mRNAs for *NR1* and *CHS* at steady state were similar to or greater than those in the wild type. This result further confirms our earlier conclusion that the light regulation of *NR2*, *CAB*, and *RBCS* is in a pathway different from that of *NR1* and *CHS* (Lin and Cheng, 1997) and that *CR88* controls the expression of a subset of light-regulated genes that includes *NR2, CAB*, and *RBCS.* Neuhaus et al. (1997) also reported that the light-signaling pathway regulating *CHS* gene expression differs from the one that regulates *CAB* and *RBCS.* In addition, the reduction of light-regulated gene expression in broad spectra in *cr88* is consistent with *CR88* acting downstream of the convergence of the pathways that transduce light signals from different photoreceptors.

Epistatic Relationships between *cr88* and *cop1* Mutations in Hypocotyl Elongation Are Allele Specific and Light Condition Dependent

Previous physiological and genetic characterization of *cr88* suggests that CR88 regulates a subset of COP1-repressed photomorphogenesis responses (Lin and Cheng, 1997). We hypothesized that *CR88* plays a minor role in inhibiting hypocotyl elongation; the Hy phenotype of *cr88* in red light is the result of the inability of *COP1* to suppress *HY5* completely under this condition. HY5 is known to play a major role in inhibiting hypocotyl elongation in the light and was shown recently to interact directly with the COP1 protein (Koornneef et al., 1980; Oyama et al., 1997). We examined the epistatic relationships between *cr88* and two *cop1* alleles, *cop1-4* and *cop1-6*, to gain insight into the relationship

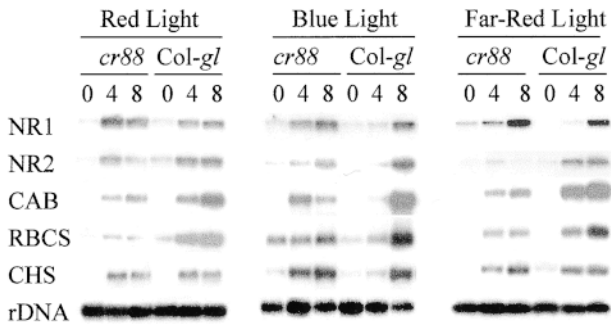

Figure 2. RNA Gel Blot Analysis of Induction of *NR1*, *NR2*, *CAB*, *RBCS*, and *CHS* mRNA in *cr88* and the Wild-Type Col-*gl* Seedlings after Exposure to Red, Blue, and Far-Red Light.

Each lane contains 5 μg of total RNA isolated from seedlings that were grown in the dark for 6 days before exposure to red, blue, or far-red light. The number above each lane indicates the hours of light exposure. At left, NR1, NR2, CAB, RBCS, CHS, and rDNA indicate the RNAs hybridizing with their respective probes.

between CR88 and COP1 in the light signal transduction pathway. Mutations in the *cop1* and *cr88* loci cause opposite phenotypes with respect to hypocotyl length in red light. Being constitutively photomorphogenic, *cop1* exhibits short hypocotyls in all light conditions (Deng et al., 1991), whereas *cr88* exhibits long hypocotyls only in red light (Lin and Cheng, 1997). The *cr88 cop1-4* double mutant resembles the *cop1-4* single mutant in all conditions, indicating that *cop1-4* is epistatic to *cr88* in deetiolation. In contrast, the epistatic relationship between *cr88* and *cop1-6* is dependent on the light conditions. In dark and white light, the hypocotyl lengths of the *cr88 cop1-6* double mutant are equal to that of the *cop1-6* single mutant. In red light, however, the double mutant exhibits intermediate hypocotyl length, indicating that *cr88* and *cop1-6* mutations partially suppress each other in red light (Figure 3). The allele-specific interaction of *cr88* and *cop1* mutations suggests that these two gene products may function in close proximity. Allele-specific and light condition–dependent epistatic relationships have also been observed between the *hy5* and *cop1* mutations (Ang and Deng, 1994).

cr88 and *cop1* Mutations Show Contrasting Epistatic Relationships in Expression of *NR2*, *RBCS*, and *CAB*

We examined the epistatic relationship of *cr88* and *cop1* with respect to expression of *NR2*, *CAB*, and *RBCS* genes.

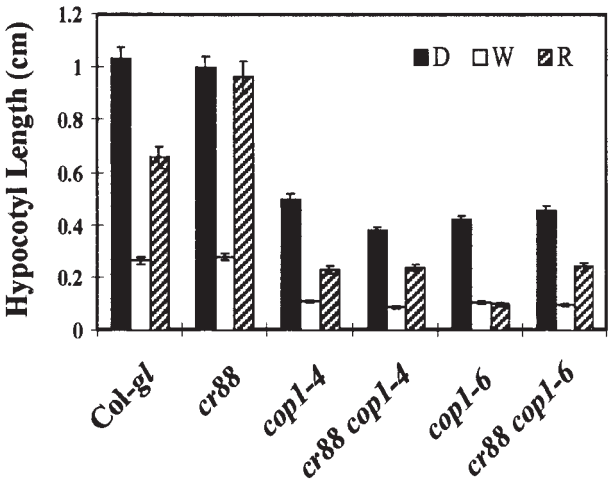

Figure 3. Hypocotyl Lengths of Col-*gl*, *cr88*, *cop1-4*, *cr88 cop1-4*, *cop1-6*, and *cr88 cop1-6* Grown in the Dark and in White Light and Red Light.

Seedlings were grown on medium in the dark (D) or in white (W) or red (R) light for 4 days. The hypocotyl lengths of 25 seedlings from each treatment were measured and used to calculate the mean. The error bars represent the standard deviations.

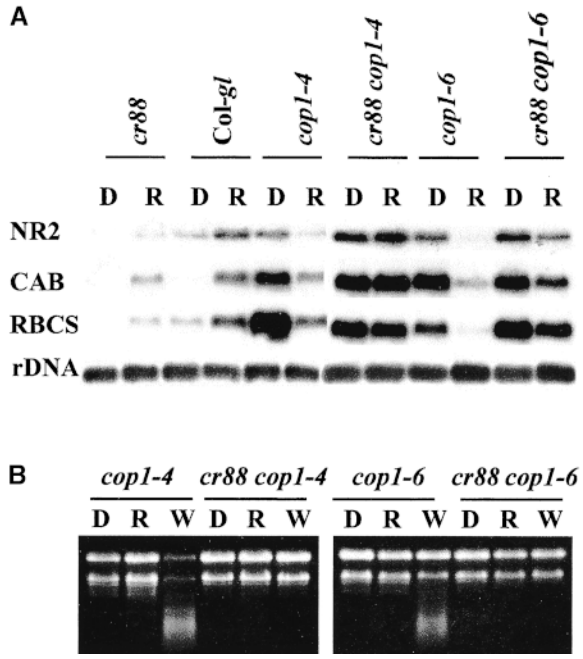

Figure 4. Total RNA Analysis and RNA Gel Blot Analysis of *NR2*, *CAB*, and *RBCS* mRNA in Dark-Grown Col-*gl*, *cr88*, *cop1-4*, *cop1-6*, *cr88 cop1-4*, and *cr88 cop1-6* Seedlings after Exposure to Light.

(A) Each lane contains 5 μg of total RNA isolated from seedlings grown on medium in the dark for 4 days and harvested before (D) or 8 hr after (R) exposure to red light. At left, NR2, CAB, RBCS, and rDNA indicate the RNAs hybridizing with their respective probes.
(B) Each lane contains 5 μg of total RNA isolated from seedlings grown on medium in the dark for 4 days and harvested before (D) or 8 hr after exposure to red (R) or white (W) light. RNA was stained with ethidium bromide for visualization.

Seedlings were grown in the dark for 4 days and were then exposed to red light for 8 hr. The amounts of mRNA for the light-regulated genes *NR2*, *CAB*, and *RBCS* were compared (Figure 4A). All of these genes were expressed in dark-grown *cop1-4* and *cop1-6* and in the double mutants *cr88 cop1-4* and *cr88 cop1-6.* Eight hours of red light exposure led to a considerable decrease in the mRNAs for these genes in the *cop1-4* and *cop1-6* mutants. In contrast, light-induced expression of these genes was restored in the double mutants *cr88 cop1-4* and *cr88 cop1-6*, the amounts being as high as those in dark-grown *cop1* mutants and much higher than that in *cr88.* The constitutive expression of these genes in the double mutants revealed contrasting epistatic relationships between *cr88* and *cop1* mutations. On the one hand, *cop1* mutations suppressed the decreased expression of *NR2*, *RBCS*, and *CAB* in *cr88.* On the other hand, *cr88* suppressed the light-triggered decreased expression of these genes in *cop1* mutants.

cr88 Mutation Restores the Greening Ability of the Dark-Grown *cop1* Seedlings

After growing in the dark for 4 days, most of the *cop1* seedlings, unlike wild-type seedlings, are incapable of greening when transferred to light. The *hy5* mutant can rescue this *cop1*-triggered defect (Ang and Deng, 1994). To examine whether *cr88* could also rescue this defect, we compared the greening ability after transfer to light of 4- and 6-day-old dark-grown mutant seedlings. Consistent with the report of Ang and Deng (1994), only 2.5% of *cop1-4* seedlings and 56% of *cop1-6* seedlings retained the greening ability after 4 days in the dark (Table 1). Six days of growth in the dark further decreased the percentage of *cop1-4* and *cop1-6* individuals that retained the greening ability to 1.5 and 38.6%, respectively. The double mutants turned green in light after 4 or 6 days of growth in the dark. After 4 days in the dark, 97% of the double mutant seedlings turned green in the light. After 6 days in the dark, 90.7% of *cr88 cop1-4* and 80.5% of *cr88 cop1-6* seedlings still retained greening ability. Nevertheless, although the double mutants restored the greening ability, their cotyledons exhibited the yellow-green phenotype of *cr88* (data not shown).

Light Exposure of Dark-Grown *cop1* Causes Plastid Destruction and RNA Degradation That Can Be Rescued by *cr88*

The loss of greening ability in dark-grown *cop1-6* and *cop1-4* on transfer to light suggested a defect in chloroplast development, and the restoration of greening ability to these mutants by *cr88* suggested suppression of this defect. The defect and the restoration of plastid development may be revealed by examining the ultrastructure of the developing plastids. No plastid development was observed in dark-grown wild-type (Figure 5A) and *cr88* seedlings (Figure 5B). Apparent development of the thylakoid membrane was found in the dark-grown seedlings of *cop1-4* (Figure 5E) and *cop1-6* (Figure 5F), as reported previously (Deng et al., 1991). The thylakoid membrane of the double mutants *cr88 cop1-4* (Figure 5C) and *cr88 cop1-6* (Figure 5D), although exhibiting less stacking than that of the *cop1* single mutants, was partially developed, suggesting that the constitutive plastid development of *cop1* was not suppressed by *cr88.* Chloroplasts were fully developed 6 days after transfer of the 6-day-old dark-grown seedlings of the wild type (Figure 5G) and of *cr88* (Figure 5H) into light. Consistent with the previous report (Lin and Cheng, 1997), *cr88* showed less thylakoid stacking. Transfer of the dark-grown seedlings of *cop1-4* (Figure 5E) and *cop1-6* (Figure 5F) to white light led to destruction of the chloroplasts and other cellular structures (Figures 5K and 5L, respectively). The destruction was not restricted to chloroplasts; indeed, almost no organelle within the cell could be recognized. In contrast, chloroplasts were well developed in the double mutants (Figures 5I and 5J), albeit with less stacking of the thylakoid membrane than in the wild-type (Figure 5G) chloroplasts. The destruction occurred as early as 8 hr after transfer to white light (Figure 5N) and was rescued by *cr88* (Figure 5M).

During the investigation, we discovered that exposure to light of dark-grown *cop1* mutants also causes general RNA degradation in addition to chloroplast destruction. We treated the 4-day-old dark-grown seedlings for 8 hr with white light or red light and then compared the integrity of the total RNA afterward (Figure 4B). The RNA extracted from 4-day-old dark-grown *cop1-4* and *cop1-6* seedlings was intact. In contrast, 8 hr of white light exposure led to degradation of the RNA. At this time, the plastids in *cop1-4* seedlings were already showing severe degradation (Figure 5M), but no degradation was observed in the *cr88 cop1-4* and *cr88 cop1-6* double mutants. Although a general degradation of the RNA extracted from red light–treated *cop1* seedlings was not visible by ethidium bromide staining (Figure 4B), a decrease in the amount of mRNA from specific genes was evident (Figure 4A).

Taken together, analyses at the phenotypic, ultrastructural, and RNA levels suggest that the *cr88* mutation suppresses the loss of the greening ability caused by *cop1* mutations by preventing the destruction of chloroplasts and other cellular structures in response to light.

Table 1. Greening Ability of Dark-Grown Col-*gl*, *cr88*, *cop1-4*, *cop1-6*, *cr88 cop1-4*, and *cr88 cop1-6* Seedlings after Exposure to Light

	4 Days in the Dark		6 Days in the Dark	
Lines	Total Plants	Greened (%)[a]	Total Plants	Greened (%)[a]
Col-*gl*[b]	43	43 (100)	81	81 (100)
cr88	45	45 (100)	73	73 (100)
cop1-4	58	2 (3.5)	64	1 (1.5)
cr88 cop1-4	87	85 (97.0)	65	59 (90.7)
cop1-6	54	30 (56.0)	67	26 (38.5)
cr88 cop1-6	71	69 (97.0)	36	29 (80.5)

[a] The percentage of green seedlings in total plants scored is shown in parentheses.
[b] Col-*gl* serves as the wild-type control.

cr88 Partially Suppresses the Loss of Negative Gravitropic Response of the Dark-Grown Seedlings of *cop1*

Hypocotyls of the dark-grown wild-type Arabidopsis seedlings grow upward. This phenomenon, called the negative gravitropic response, is attenuated in light (Hangarter, 1997). Although *cop1* seedlings exhibit similar degrees of negative gravitropism as the wild type in light (Hou et al., 1993; D. Cao and C.-L. Cheng, unpublished results), these seedlings lost the negative gravitropic response in the dark (Figure 6),

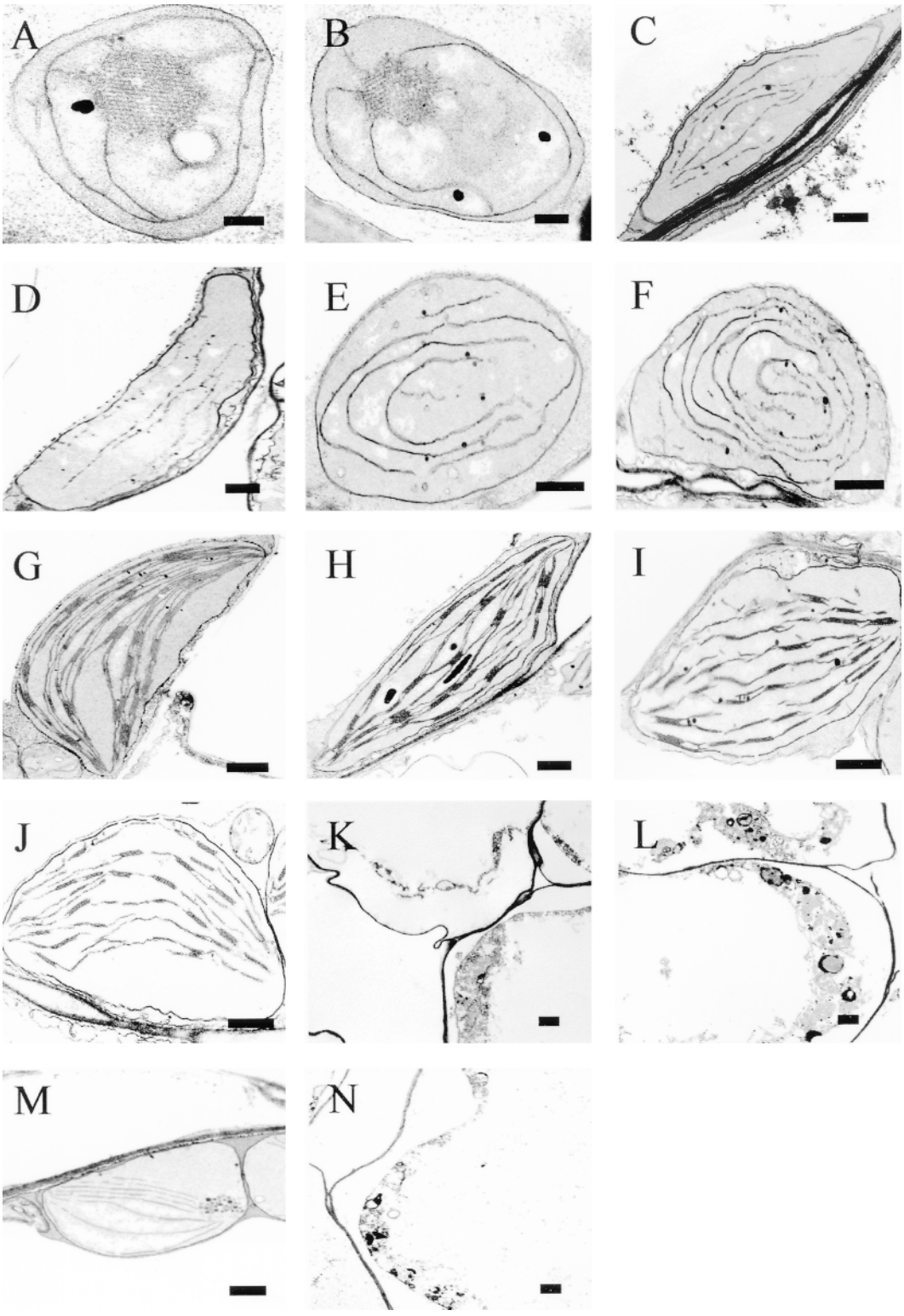

Figure 5. Plastid Development of Dark-Grown Col-*gl*, *cr88*, *cop1-4*, *cr88 cop1-4*, *cop1-6*, and *cr88 cop1-6* Seedlings after Exposure to Light.

Representative plastids from seedlings grown in the dark for 4 days (**[A]** to **[F]**) and then transferred to white light for 4 days (**[G]** to **[L]**) or 8 hr (**[M]** and **[N]**). Bars in **(A)** to **(N)** = 1 μm.

whereas *cr88* seedlings retain the negative gravitropic response (Figure 6). To determine whether the *cr88* mutation suppresses the loss of the negative gravitropic response caused by *cop1* mutations, we grew single and double mutant seedlings in the dark for 4 days and then measured the growth angles of the seedlings relative to the horizontal level (Figure 6). Performing this experiment with both horizontal and vertical plates yielded consistent results. Figure 6 shows the results from seedlings grown on horizontal plates. Eighty-two percent of *cr88* and 90% of the wild-type seedlings grew at angles between 75 and 90°. In contrast, 90% of *cop1-4* and 80% of *cop1-6* lay flat on the plates (growth angle at 0°). For the double mutant *cr88 cop1-4*, approximately 30% of the seedlings grew at angles ranging from 75 to 90°, whereas only 2% of *cop1-4* grew at angles in this range. Similar but less pronounced reversal was observed between the double mutant *cr88 cop1-6* and *cop1-6.* These results indicate that *cr88* can partially suppress the loss of negative gravitropic response of the dark-grown *cop1* seedlings.

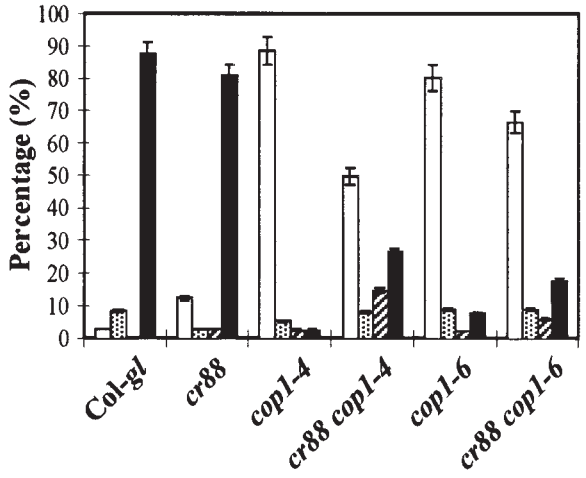

Figure 6. Hypocotyl Orientations of Dark-Grown Col-*gl*, *cr88*, *cop1-4*, *cop1-6*, *cr88 cop1-4*, and *cr88 cop1-6* Seedlings.

Angles relative to the agar surface are 0° (white bars), 1 to 29° (stippled bars), 30 to 74° (cross-hatched bars), and 75 to 90° (black bars). Error bars represent standard deviations of the mean in percentage of seedlings that exhibited the indicated range of angles.

CR88 Controls Hypocotyl Length in Red Light Independent of HY5

To test the hypothesis that *CR88* and *HY5* act in separate pathways to regulate hypocotyl length, we constructed a double mutant of *cr88* and *hy5.* The seedlings were grown in the dark or in red or white light for 4 days, after which the hypocotyl lengths of the double and single mutants were compared (Figure 7). As expected, *cr88* seedlings exhibited hypocotyls as long as those of the wild-type in white light and longer hypocotyls in red light; *hy5* seedlings exhibited hypocotyls longer than wild-type hypocotyls in both white and red light. In white light, the hypocotyl length of the *cr88 hy5* double mutant was similar to that of the *hy5* single mutant. In red light, however, the hypocotyl length of *cr88 hy5* was significantly greater than that of either *hy5* ($P = 0.000168$) or *cr88* ($P = 0.00054$). These results support our hypothesis that for inhibition of hypocotyl length, the function of CR88 is redundant to HY5 in all but red light spectra.

New Phenotypes Arise in *cr88 hy5*

The overall morphology of the mature *cr88 hy5* double mutant is different from that of either parent (Figure 8). The mature *cr88 hy5* plants were thinner and weaker and had fewer leaves than either *cr88* or *hy5* plants. The double mutants were spindly and could not stand upright when grown in soil. Seeds in 25 siliques sampled randomly from 10 plants for each mutant line were counted, and the standard deviations (±SD) were calculated. The *cr88 hy5* double mutant yielded fewer seeds (26.4 ± 2.7) per silique, whereas the parents *hy5* and *cr88* yielded 35.8 ± 3.4 and 33.4 ± 3.4 seeds per silique, respectively. Although, like *cr88*, the *cr88 hy5* double mutant exhibited the yellow-green phenotype, greening was delayed more severely than in *cr88* (data not shown). Taken together, the new, additive morphology of the double mutant at mature stages suggests that *CR88* and *HY5* act in separate yet interacting pathways.

cr88 hy5 Flowers Early

The time to flowering was measured as the number of rosette leaves present when the inflorescence was 2 cm

Figure 5. (continued).

(A) and **(G)** Col-*gl.*
(B) and **(H)** *cr88.*
(C), **(I)**, and **(M)** *cr88 cop1-4.*
(D) and **(J)** *cr88 cop1-6.*
(E), **(K)**, and **(N)** *cop1-4.*
(F) and **(L)** *cop1-6.*

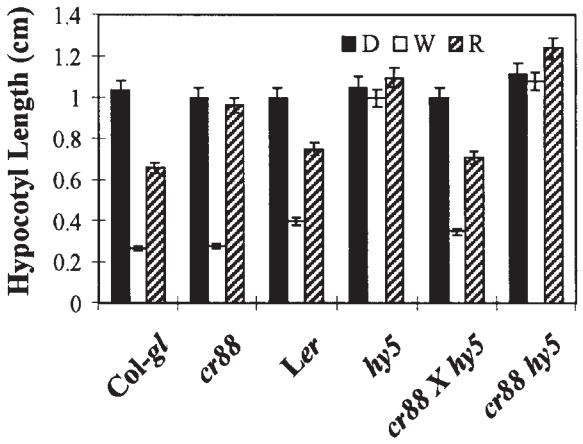

Figure 7. Hypocotyl Lengths of Col-*gl*, *cr88*, Landsberg *erecta*, *hy5*, and *cr88 hy5* Grown in the Dark and in White and Red Light.

Col-*gl* and Landsberg *erecta* (L*er*) are wild types for *cr88* and *hy5*, respectively. Seedlings were grown on medium in the dark (D) or in white (W) or red (R) light for 4 days. The hypocotyl lengths of 25 seedlings from each line were measured for calculating the mean. The error bars represent standard deviations.

long. Table 2 shows that *cr88* flowered at a later developmental stage (10 leaves) than did the wild-type ecotype Columbia *glabrous* (Col-*gl*; 8.3 leaves), whereas *hy5* flowered at a similar stage (7.8 leaves), and so did the wild-type ecotype Landsberg *erecta* (7.6 leaves). Although *cr88* flowered later than *hy5*, the double mutant *cr88 hy5* flowered when the plants only had four rosette leaves, a much earlier developmental stage than that of either parents or the F_1 plants. Again, these results demonstrate that a new phenotype arises in the double mutant, suggesting that *CR88* and *HY5* act in separate yet interacting pathways.

DISCUSSION

Inhibiting Photosynthesis Does Not Produce a Phenocopy of the Photomorphogenic Defects of *cr88*

cr88 plants exhibit a typical etiolated morphology in red light: long hypocotyls and closed and yellow cotyledons. They also exhibit a slow-greening phenotype in all light conditions. In addition, the light-induced expression of certain genes is impaired in red, blue, far-red, and white light (Lin and Cheng, 1997; Figure 2). We showed previously that 1% sucrose cannot eliminate differences in the extents of expression of *NR2*, *RBCS*, and *CAB* mRNA between *cr88* and the wild-type seedlings (Lin and Cheng, 1997). However, the photomorphogenic defects and the impaired gene expression could be secondary effects of a possible decrease of photosynthetic activity in *cr88.* Neither blocking photosynthesis with DCMU nor photobleaching with norflurazon could abolish the inhibition of hypocotyl elongation in the wild-type seedlings grown in red light. Thus, simply inhibiting photosynthesis did not produce a phenocopy of the photomorphogenic defect of *cr88.* These results also suggest that no plastidic signal is required for the inhibition of hypocotyl elongation mediated by PhyB—although one apparently is required for phytochrome-mediated nuclear gene expression (Oelmueller and Mohr, 1986; Oelmueller et al., 1986; Oelmueller and Briggs, 1990; Susek et al., 1993; Lopez-Juez et al., 1998). Thus, the proposed plastidic signal probably acts downstream of *CR88.*

DCMU also inhibits the expression of *FEDA* mRNA (Figure 1), as has been reported (Petracek et al., 1997). In contrast, the red light–induced expression of *NR2*, *CAB*, and *RBCS* is not inhibited. Therefore, photosynthesis is required only for the light-induced expression of a subset of nuclear-encoded genes, the gene products of which function in chloroplasts. *CAB* and *RBCS* are not in this group. The impaired induction of *NR2*, *CAB*, and *RBCS* in *cr88* by light is therefore unlikely to be caused by a presumed decrease in photosynthesis.

Figure 8. Morphologies of Mature *hy5*, *cr88*, and *cr88 hy5* Plants.

hy5 (left), *cr88* (middle), and *cr88 hy5* (right) plants were grown in soil for 1 month and then photographed.

Table 2. Comparison of Rosette Leaf Number for Double Mutant *cr88 hy5* and the Parents

Lines[a]	Leaf Number[b]
Col-*gl*[c]	8.3 ± 0.3
cr88	10.0 ± 0.6
L*er*[d]	7.6 ± 0.2
hy5	7.8 ± 0.3
cr88 × *hy5*; F_1	11.2 ± 0.4
cr88 hy5	4.0 ± 0.5

[a] Plants were grown in soil under 16-hr-light/8-hr-dark cycles at 21°C.
[b] Rosette leaf numbers were recorded at bolting. Numbers are the means of 20 to 25 plants ±SD.
[c] The wild type for *cr88*.
[d] L*er*, Landsberg *erecta*, the wild type for *hy5*.

Epistatic Relationships between *cr88* and *cop1* and between *cr88* and *hy5*

The only known null allele used in these analyses is *hy5* (Koorneef et al., 1980). The *cop1-4* and *1-6* alleles are weak mutants (Ang and Deng, 1994), and the nature of the *cr88* mutation is unknown. Meaningful interpretation of the results for determining epistatic relationships was made possible by taking advantage of the pleiotropic phenotypes of *cop1* and *cr88* and the information of COP1 (Deng et al., 1992; Ang et al., 1998) and HY5 (Oyama et al., 1997; Chattopadhyay et al., 1998) at the structural and functional levels.

The epistatic relationships between *cr88* and *cop1* vary with respect to *cop1* alleles chosen, physiological responses analyzed, and light conditions used. Similar variations have been observed in the analysis of epistasis between *hy5* and *cop1* mutations (Ang and Deng, 1994). Such similarities suggest that *CR88* may be located at a similar hierarchical position as *HY5*, acting in close proximity with *COP1*, in the photomorphogenesis pathway.

Because CR88 and HY5 each regulates a subset of COP1-controlled processes, they are likely to function downstream of COP1. The additive relationship between *cr88* and *hy5* with respect to hypocotyl length in red light, together with the new phenotypes such as early flowering, suggest that *CR88* and *HY5* act in separate yet interacting pathways. An alternative explanation for the arising of the new phenotypes is that the two genes may interact differently in different developmental stages. The phenotypic differences between *hy5* and *cr88* indicate that *HY5* plays a major role in controlling hypocotyl elongation, whereas *CR88* plays a major role in promoting the greening process. The requirement for both genes in the red light inhibition of hypocotyl elongation and the ability of both *cr88* and *hy5* mutations to restore the greening ability of dark-grown *cop1-4* and *cop1-6* seedlings indicate that *CR88* and *HY5* are also required in certain common processes. The function of CR88 in inhibition of hypocotyl elongation is required in red light, possibly because of incomplete derepression of HY5 by COP1. Red light is less efficient than blue and far-red light in inhibiting hypocotyl elongation (McNellis and Deng, 1995). In the blue and far-red light spectra, the derepression of HY5 is complete, and the function of CR88 in inhibition of hypocotyl elongation becomes dispensable.

The above interpretation is consistent with the allele-specific interactions between *cr88* and the two *cop1* alleles with respect to hypocotyl length in red light. *cop1-4* has a nonsense mutation that produces a truncated protein lacking the C-terminal domain of WD-40 repeats, a domain that is required for the interaction between COP1 and HY5 (McNellis et al., 1994; Ang et al., 1998). When HY5 is fully derepressed, the ability of CR88 to inhibit hypocotyl elongation is masked. Whereas the *cop1-6* allele encodes a full-length protein with a five–amino acid insertion 5′ of the WD-40 domain (McNellis et al., 1994), this protein may still partially repress HY5 in red light. In this situation, a complete inhibition of hypocotyl elongation requires a functional CR88. The nonlinear relationship of *COP1*, *CR88*, and *HY5* that we proposed in the pathway for transduction of light signals may partly explain the complex epistatic interactions between *cr88* and *cop1* and between *hy5* and *cop1* with respect to hypocotyl length.

The light-induced expression of *NR2*, *CAB*, and *RBCS* genes is defective in *cr88* (Lin and Cheng, 1997; Figure 2). However, the constitutive expression of these genes caused by *cop1* mutations is not suppressed by *cr88* (Figure 4A). In view of the broad pleiotropism of *cop1* mutants (Deng et al., 1992) and the role of COP1 as a repressor of photomorphogenesis (Torii et al., 1998; Stacey et al., 1999), COP1 most likely interacts with additional factors other than HY5 and CR88 to control downstream pathways. Some of these factors may override the control of CR88 over the expression of *NR2*, *CAB*, and *RBCS* genes in *cop1* mutants in the dark.

Loss of Greening Ability of Dark-Grown *cop1* and Its Reversal by *cr88*

Our results show that the loss of greening ability of *cop1* seedlings after prolonged growth in the dark is accompanied by chloroplast destruction and degradation of RNA. Eight hours of white light treatment degraded all of the RNA (Figure 4B) and destroyed chloroplasts (Figure 5N). Although a causal relationship between RNA degradation and chloroplast destruction has not been established, chloroplast destruction probably causes a general degradation of the RNA. The cause of light-induced destruction seen in *cop1* is unclear, but studies of protochlorophyllide reductase (POR) suggest that this light-activated enzyme may play a role. Barnes et al. (1996) showed that far-red light blocks the greening of cotyledons and inhibits POR synthesis. The plastids of dark-grown *cop1* mutants lack an organized

prolamellar body, a defect that can be restored by overexpressing POR (Sperling et al., 1998). We do not see an organized prolamellar body in the dark-grown plastids in double mutants of *cr88* and *cop1* (Figures 5C and 5D). It will be interesting to determine whether transgenic *cop1*plants that overexpress POR are protected from photodestruction.

The ability of *cr88* to rescue light-induced chloroplast destruction is consistent with *CR88* acting downstream of *COP1.* In addition to *NR2*, *CAB*, and *RBCS*, CR88 is likely to control the expression of other genes in the greening process. Some of the genes controlled by CR88 may play a role in causing the light-induced destruction in dark-grown *cop1.* Mutant alleles of *HY5* also can restore the greening ability of dark-grown *cop1* (Ang and Deng, 1994), probably also by preventing light-induced destruction of chloroplasts. HY5 is known to interact with COP1 and is likely to activate light-regulated gene expression of a subset of genes, including *CHS* (Ang et al., 1998). Interestingly, genes expressed in subnormal amounts in *cr88*, such as *CAB* and *RBCS*, are not affected in *hy5* (Ang and Deng, 1994). Conversely, *CHS* expression is not affected in *cr88* (Lin and Cheng, 1997) but is lower in *hy5* (Ang et al., 1998). Mutation in either *CR88* or *HY5* can rescue the loss of greening ability of *cop1*, suggesting that both gene products contribute to the loss of greening ability in *cop1.*

In conclusion, we have further characterized *cr88* with respect to long hypocotyls in red light and defective gene expression. Using morphological, cellular, and gene expression criteria for epistasis analyses, we determined that *CR88* acts downstream of *COP1* and in a branch separate from *HY5* in the genetic hierarchy of the photomorphogenesis pathway. Most strikingly, *cr88* rescues the light-induced destruction of chloroplast in dark-grown *cop1* seedlings. The map position of *CR88* demonstrates that it defines a new photomorphogenesis locus.

METHODS

Plant Material and Growth Conditions

The chlorate-resistant mutant line *cr88* was isolated previously (Lin and Cheng, 1997). Seeds of *Arabidopsis thaliana* Columbia *glabrous* (Col-*gl*) were obtained from Lehle Seeds (Round Rock, TX). The two constitutive photomorphogenic *cop1* mutant alleles, *cop1-4* and *cop1-6* (McNellis et al., 1994), were a gift from X.-W. Deng (Yale University, New Haven, CT). The *hy5* mutant (Koornneef et al., 1980) used in all experiments has been renamed long hypocotyl *hy5-1* and was obtained from the Arabidopsis Biological Research Center (Ohio State University, Columbus).

For growth on media, seeds were surface-sterilized by treating with 95% ethanol for 2 min and 1.5% sodium hypochlorite for 5 min. Seeds were then rinsed six times with sterile water. Seeds were sown on Petri dishes containing half-strength Murashige and Skoog salts (Sigma), 0.7% agar (Difco, Detroit, MI), and 1% sucrose. To achieve uniform germination, we incubated the plates in the dark at room temperature for 1 day, moved them to 4°C for 2 days, and grew them at 21°C. For treatments with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and norflurazon (Sandoz 9789; Novartis Crop Protection, Greensboro, NC), the stock solutions were filter-sterilized and added to the media.

For growth in soil, seeds were imbibed in 10 mM KNO_3 in the dark at 4°C for 2 days before being sown on Jiffy Mix Plus (Jiffy Products of America, Inc., Batavia, IL). Plants were grown at 21°C under a 16-hr-light/8-hr-dark cycle in 800 μmol m^{-2} sec^{-1} white light for the indicated time.

Light sources used in light induction experiments were as described previously (Lin and Cheng, 1997). The fluence rates used were as follows: red, 19 μmol m^{-2} sec^{-1}; blue, 11 μmol m^{-2} sec^{-1}; far red, 10 μmol m^{-2} sec^{-1}; and white, 100 μmol m^{-2} sec^{-1}.

Construction of Double Mutants

To construct *cr88 hy5-1*, *cr88 cop1-4*, and *cr88 cop1-6* double mutants, we crossed individuals homozygous for each mutant allele and allowed the F_1 progeny to self-fertilize. Double mutants of *cr88* and *cop1* were identified easily from the F_2 population because a new phenotype of short stature and yellow-green color arose in one-sixteenth of the population. These plants were considered to be double mutants, and their seeds were used directly in the analysis. No segregation of the new phenotype in the F_3 population was observed from the 25 selected F_2 individuals. To select the double mutant *cr88 hy5-1*, we identified F_2 individuals homozygous for the phenotype of the *cr88* mutant (yellow-green) and allowed them to self-fertilize. The F_3 seedlings that exhibited long hypocotyls in white light (the phenotype of *hy5* but not *cr88*) were considered homozygous for *cr88* and *hy5*; no segregation of the yellow-green phenotype was observed in the F_3 generation.

RNA Gel Blot Analysis

The procedure for RNA gel blot analysis and the sources for Arabidopsis nitrate reductase *NR1* and *NR2* and the small subunit of ribulose bisphosphate carboxylase (*RBCS*) were described by Cheng et al. (1991). The sources for cDNAs of Arabidopsis chlorophyll *a*/*b* binding protein (*CAB*), chalcone synthase (*CHS*), and the soybean 28S rRNA were as described previously (Lin and Cheng, 1997). The ferredoxin gene (*FEDA*) was described by Somers et al. (1990).

Linkage Analysis

The genetic location of *cr88* was established by determining linkage between *cr88* and restriction fragment length polymorphisms (Fabri and Schaffner, 1994). A homozygous *cr88* plant (ecotype Col) was crossed to a wild-type plant (ecotype Landsberg *erecta*). The resulting F_1 progeny were allowed to self-pollinate, generating F_2 plants. For rough mapping, seeds were collected from 20 *cr88* F_2 plants to establish F_3 families. Genomic DNA was isolated as described by Dellaporta et al. (1983). DNA was digested with diagnostic restriction enzymes, transferred to membranes, and hybridized with probes made from a set of markers described by Fabri and Schaeffner (1994). DNA gel blot analysis was performed according to standard procedure (Ausubel et al., 1987). Additional DNA samples were isolated from 150 F_2 individuals exhibiting the phenotype of the *cr88* mutant and used in linkage analysis. Markers used were Mi320, Mi421 (Liu et al.,

1996), and CDs3. All markers were obtained from the Arabidopsis Biological Research Center except for CDs3, which was kindly provided by G. Picard (University of Blasé Pascal, Aubière cedex, France).

Transmission Electron Microscopy

Fixation and embedding of Arabidopsis seedlings were performed as described previously (Lin and Cheng, 1997). Briefly, cotyledons were fixed in 2.5% glutaraldehyde in 100 mM sodium cacodylate buffer, pH 7.2, kept at room temperature for 1 hr, and then transferred to 4°C overnight. After postfixation in 1% osmium tetroxide for 1 hr, samples were stained with 2.5% uranyl acetate for 20 min. Samples were then dehydrated in acetone and embedded in Spurr's resin (Polysciences Incorporated, Warrington, PA). After microtomy, 95-nm-thick sections were poststained with 5% uranyl acetate for 8 min, followed with lead citrate for 7 min. Specimens were observed with a transmission electron microscope (model H7000; Hitachi Scientific Instruments, Mountain View, CA).

ACKNOWLEDGMENTS

We thank Xing-Wang Deng for *cop1* seeds; George Picard and Peter H. Quail for DNA probes; Mark A. Conkling, Wayne Carlson, and Rodney N. Nagoshi for critical reading of the manuscript; and Jean Ross for assisting with the electron microscopy work. This work was supported by grants from the Carver Foundation and by National Science Foundation Grant No. IBN 98-08823.

Received August 30, 1999; accepted November 30, 1999.

REFERENCES

Ahmad, M., and Cashmore, A.R. (1993). *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue light photoreceptor. Nature **360,** 162–166.

Ahmad, M., and Cashmore, A.R. (1996). The *pef* mutants of *Arabidopsis thaliana* define lesions early in the phytochrome signaling pathway. Plant J. **10,** 1103–1110.

Ang, L.-H., and Deng, X.-W. (1994). Regulatory hierarchy of photomorphogenic loci: Allele-specific and light-dependent interaction between the *HY5* and *COP1* loci. Plant Cell **6,** 613–628.

Ang, L.-H., Chattopadhyay, S., Wei, N., Oyama, T., Okada, K., Batschauer, A., and Deng, X.-W. (1998). Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of *Arabidopsis* development. Mol. Cell **1,** 213–222.

Ausubel, F.M., Brent, R., Kinston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K., eds (1987). Current Protocols in Molecular Biology. (New York: Green Publishing/Wiley Interscience).

Barnes, S.A., Nishizawa, N.K., Quaggio, R.B., Whitelam, G.C., and Chua, N.-H. (1996). Far-red light blocks greening of Arabidopsis seedlings via a phytochrome A–mediated change in plastid development. Plant Cell **8,** 601–615.

Chattopadhyay, S., Ang, L.-H., Puente, P., Deng, X.-W., and Wei, N. (1998). Arabidopsis bZIP protein HY5 directly interacts with light-responsive promoters in mediating light control of gene expression. Plant Cell **10,** 673–683.

Cheng, C.L., Acedo, G.N., Dewdney, J., Goodman, H.M., and Conkling, M.A. (1991). Differential expression of the two *Arabidopsis* nitrate reductase genes. Plant Physiol. **96,** 275–279.

Chory, J. (1993). Out of darkness: Mutants reveal pathways controlling light-regulated development on plants. Trends Genet. **9,** 167–172.

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

Dehesh, K., Francl, C., Parks, B.M., Seeley, K.A., Short, T.W., Tepperman, J.M., and Quail, P.H. (1993). Arabidopsis *HY8* encodes phytochrome A. Plant Cell **5,** 1081–1088.

Dellaporta, S.L., Wood, J., and Hicks, J.B. (1983). A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. **1,** 19–21.

Deng, X.-W. (1994). Fresh view of light signal transduction in plants. Cell **76,** 423–426.

Deng, X.-W., Caspar, T., and Quail, P.H. (1991). *COP1*: A regulatory locus involved in light-controlled development and gene expression in *Arabidopsis.* Genes Dev. **5,** 1172–1182.

Deng, X.-W., Matsui, M., Wei, N., Wagner, D., Chu, A.M., Feldmann, K.A., and Quail, P.H. (1992). *COP1*, an Arabidopsis regulatory gene, encodes a protein with both a zinc-binding motif and a G_{β} homologous domain. Cell **71,** 791–801.

Escoubas, J.M., Lomas, M., Laroche, J., and Falkowski, P.G. (1995). Light intensity regulation of *cab* gene transcription is signaled by the redox state of the plastoquinone pool. Proc. Natl. Acad. Sci. USA **92,** 10237–10241.

Fabri, C.O., and Schaffner, A.R. (1994). An *Arabidopsis thaliana* RFLP mapping set to localize mutations to chromosomal regions. Plant J. **5,** 149–156.

Genoud, T., Millar, A.J., Nishizawa, N., Kay, S.A., Schafer, E., Nagatani, A., and Chua, N.-H. (1998). An Arabidopsis mutant hypersensititive to red and far-red light signals. Plant Cell **10,** 889–904.

Halliday, K., Devlin, P.F., Whitelam, G.C., Hanhart, C., and Koornneef, M. (1996). The *ELONGATED* gene of *Arabidopsis* acts independently of light and gibberellins in the control of elongation growth. Plant J. **9,** 305–312.

Hangarter, R.P. (1997). Gravity, light and plant form. Plant Cell Environ. **20,** 796–800.

Hoecker, U., Xu, Y., and Quail, P.H. (1998). *SPA1*: A new genetic locus involved in phytochrome A–specific signal transduction. Plant Cell **10,** 19–33.

Hoecker, U., Tepperman, J.M., and Quail, P.H. (1999). SPA1, a WD-repeat protein specific to phytochrome A signal transduction. Science **284,** 496–499.

Hou, Y., von Arnim, A.G., and Deng, X.-W. (1993). A new class of Arabidopsis constitutive photomorphogenic genes involved in regulating cotyledon development. Plant Cell **5,** 329–339.

Johnson, E., Bradley, M., Harberd, N.P., and Whitelam, G.C. (1994). Photoresponses of light-grown phyA mutants of Arabidopsis: Phytochrome A is required for the perception of daylength extensions. Plant Physiol. **105,** 141–149.

Kendrick, R.E., and Kronenberg, G.H.M., eds (1994). Photomorphogenesis in Plants, 2nd ed. (Dordrecht, The Netherlands: Kluwer Academic Publishers).

Koornneef, M., Rolff, E., and Spruit, C.J.P. (1980). Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana.* Z. Pflanzenphysiol. **100,** 147–160.

Kwok, S.F., Piekos, B., Misera, S., and 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, H.M., Culligan, K., Dixon, R.A., and Chory, J. (1995). *CUE1*: A mesophyll cell–specific positive regulator of light-controlled gene expression in Arabidopsis. Plant Cell **7,** 1599–1610.

Lin, Y., and Cheng, C.L. (1997). A chlorate-resistant mutant defective in the regulation of nitrate reductase gene expression in Arabidopsis defines a new *HY* locus. Plant Cell **9,** 21–35.

Liu, Y.G., Mitsukawa, N., Lister, C., Dean, C., and Whittier, R.F. (1996). Isolation and mapping of a new set of 129 RFLP markers in *Arabidopsis thaliana* using recombinant inbred lines. Plant J. **10,** 733–736.

Lopez-Juez, E., Jarvis, R.P., Takeuchi, A., Page, A.M., and Chory, J. (1998). New Arabidopsis *cue* mutants suggest a close connection between plastid and phytochrome regulation of nuclear gene expression. Plant Physiol. **118,** 803–815.

McNellis, T.W., and Deng, X.-W. (1995). Light control of seedling morphogenic pattern. Plant Cell **7,** 1749–1761.

McNellis, T.W., von Arnim, A.G., Araki, T., Komeda, Y., Misera, S., and Deng, X.-W. (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.

Misera, S., Muller, A.J., Weiland-Heidecker, U., and Jurgens, G. (1994). The *FUSCA* genes of Arabidopsis: Negative regulators of light responses. Mol. Gen. Genet. **244,** 242–252.

Mullet, J.E. (1988). Chloroplast development and gene-expression. Annu. Rev. Plant Physiol. Plant Mol. Biol. **39,** 475–502.

Neuhaus, G., Bowler, C., Hiratsuka, K., Yamagata, H., and Chua, N.-H. (1997). Phytochrome-regulated repression of gene expression requires calcium and cGMP. EMBO J. **16,** 2554–2564.

Ni, M., Tepperman, J.M., and Quail, P.H. (1998). PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix-loop-helix protein. Cell **95,** 657–667.

Oelmueller, R., and Mohr, H. (1986). Photooxidative destruction of chloroplasts and its consequences for expression of nuclear genes. Planta **167,** 106–113.

Oelmueller, R., and Briggs, W.R. (1990). Intact plastids are required for nitrate-induced and light-induced accumulation of nitrate reductase activity and messenger RNA in squash cotyledons. Plant Physiol. **92,** 434–439.

Oelmueller, R., Levitan, I., Bergfeld, R., Rajasekhar, V.K., and Mohr, H. (1986). Expression of nuclear genes as affected by treatments acting on the plastids. Planta **168,** 482–492.

Osterlund, M.T., Ang, L.-H., and Deng, X.-W. (1999). The role of COP1 in repression of *Arabidopsis* photomorphogenic development. Trends Cell Biol. **9,** 113–117.

Oyama, T., Shimura, Y., and Okada, K. (1997). The *Arabidopsis HY5* gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. Genes Dev. **11,** 2983–2995.

Parks, B.M., and Quail, P.H. (1993). *hy8*, a new class of Arabidopsis mutant deficient in functional phytochrome A. Plant Cell **5,** 39–48.

Petracek, M.E., Dickey, L.F., Huber, S.C., and Thompson, W.F. (1997). Light-regulated changes in abundance and polyribosome association of ferredoxin mRNA are dependent on photosynthesis. Plant Cell **9,** 2291–2300.

Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M., and Chory, J. (1993). Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis. Plant Cell **5,** 147–157.

Reiter, R.S., Coomber, S.A., Bourett, T.M., Bartley, G.E., and Scolnik, P.A. (1994). Control of leaf and chloroplast development by the Arabidopsis gene *pale cress.* Plant Cell **6,** 1253–1264.

Schmidt, R., West, J., Gerda, C., Love, K., Balestrazzi, A., and Dean, C. (1996). Detailed description of four YAC contigs representing 17 Mb of chromosome 4 of *Arabidopsis thaliana* ecotype Columbia. Plant J. **9,** 755–765.

Schmidt, R., Love, K., West, J., Lenehan, Z., and Dean, C. (1997). Description of 31 YAC contigs spanning the majority of *Arabidopsis thaliana* chromosome 5. Plant J. **11,** 563–572.

Somers, D.E., Caspar, T., and Quail, P.H. (1990). Isolation and characterization of ferredoxin gene from *Arabidopsis thaliana.* Plant Physiol. **93,** 572–577.

Sperling, U., Franck, F., van Cleve, B., Frick, G., Apel, K., and Armstrong, G.A. (1998). Etioplast differentiation in Arabidopsis: Both PORA and PORB restore the prolamellar body and photoactive protochlorophyllide-F655 to the *cop1* photomorphogenic mutant. Plant Cell **10,** 283–296.

Stacey, M.G., Hicks, S.N., and von Arnim, A.G. (1999). Discrete domains mediate the light-responsive nuclear and cytoplasmic localization of Arabidopsis COP1. Plant Cell **11,** 349–363.

Susek, R., Ausubel, F., and Chory, J. (1993). Signal transduction mutants of Arabidopsis uncouple nuclear *CAB* and *RBCS* gene expression from chloroplast development. Cell **74,** 787–799.

Torii, K.U., McNellis, T.W., and Deng X.-W. (1998). Functional dissection of *Arabidopsis* COP1 reveals specific roles of its three structural modules in light control of seedling development. EMBO J. **17,** 5577–5587.

Wagner, D., Hoecker, U., and Quail, P.H. (1997). *RED1* is necessary for phytochrome B–mediated red light–specific signal transduction in Arabidopsis. Plant Cell **9,** 731–743.

Wei, N., and Deng, X.-W. (1992). *COP9*: A new genetic locus involved in light-regulated development and gene expression in Arabidopsis. Plant Cell **4,** 1507–1518.

Wei, N., Kwok, S.F., von Arnim, A.G., Lee, A., McNellis, T.W., Piekos, B., and Deng, X.-W. (1994). Arabidopsis *COP8*, *COP10*, and *COP11* genes are involved in repression of photomorphogenic development in darkness. Plant Cell **6,** 629–643.

Whitelam, G.C., Johnson, E., Peng, J., Carol, P., Anderson, M.L., Cowl, J.S., and Harberd, N.P. (1993). Phytochrome A null mutants of Arabidopsis display a wild-type phenotype in white light. Plant Cell **5,** 757–768.