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Regulatory Hierarchy of Photomorphogenic Loci: Allele-Specific and Light-Dependent Interaction between the *HY5* and *COP1* Loci

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Previous studies suggested that the *CONSTITUTIVE PHOTOMORPHOGENIC 1* (*COP1*) gene product represses photomorphogenic development in darkness and that light signals reverse this action. In this report, we used genetic analysis to investigate the regulatory hierarchical relationship of *COP1* and the loci encoding the photoreceptors and other signaling components. Our results showed that *cop1* mutations are epistatic to the long hypocotyl mutations *hy1*, *hy2*, *hy3*, and *hy4*, suggesting that *COP1* acts downstream of the phytochromes and a blue light receptor. Although epistasis of a putative null *cop1-5* mutation over a *hy5* mutation implied that *COP1* acts downstream of *HY5*, the same *hy5* mutation can suppress the dark photomorphogenic phenotypes (including hypocotyl elongation and cotyledon cellular differentiation) of the weak *cop1-6* mutation. This, and other allele-specific interactions between *COP1* and *HY5*, may suggest direct physical contact of their gene products. In addition, the synthetic lethality of the weak *deetiolated1* (*det1*) and *cop1* mutations and the fact that the *cop1-6* mutation is epistatic to the *det1-1* mutation with respect to light control of seed germination and dark-adaptative gene expression suggested that *DET1* and *COP1* may act in the same pathway, with *COP1* being downstream. These results, together with previous epistasis studies, support models in which light signals, once perceived by different photoreceptors, converge downstream and act through a common cascade(s) of regulatory steps, as defined by *DET1*, *HY5*, *COP1*, and likely others, to derepress photomorphogenic development.

INTRODUCTION

The light environment plays a crucial role in plant growth and development. Besides serving as a source of energy, light provides signals to regulate many complex developmental processes (Mohr and Shropshire, 1983; Kendrick and Kronenberg, 1993). At least three photoreceptor families—phytochromes (red and far-red light), blue light receptors, and UV light receptors—mediate these light-regulated developmental processes. Light signals perceived by specific photoreceptors are transduced via signaling components to bring about the diverse downstream physiological responses, including seed germination, stem elongation, chloroplast and leaf development, floral induction, and coordinated expression of many light-regulated nuclear- and chloroplast-encoded genes.

Early seedling development in Arabidopsis provides an excellent model system to dissect the light signal transduction pathway in plants (Chory, 1993; Deng, 1994). As a typical dicotyledonous plant, Arabidopsis seedlings follow two distinct developmental programs, skotomorphogenesis in darkness and photomorphogenesis in light. Dark-grown Arabidopsis seedlings have elongated hypocotyls, apical hooks, and small and unopen cotyledons with etioplasts, and largely undifferentiated cells. There is little or no expression of so-called light-inducible genes encoded by both nuclear and plastid genomes. In contrast, seedlings grown in the light have short hypocotyls, no apical hooks, and open and expanded cotyledons with developed chloroplasts and differentiated cell types. There is also a dramatic increase in the expression of light-inducible genes. The environmental light signals perceived by all three known photoreceptor families apparently contribute to the decision to follow either the skotomorphogenic or photomorphogenic developmental program.

Genetic analysis of Arabidopsis seedling development has led to the identification of many light regulatory loci, mutations which result in two general classes of contrasting phenotypes (Adamse et al., 1988; Chory, 1993; Deng, 1994). One class comprised *hy* (long hypocotyl) and *blu* (blue light–uninhibited hypocotyl elongation) mutants that exhibit reduced responses to light inhibition of hypocotyl elongation. Briefly, *hy1*, *hy2*, and *hy6* mutants are defective in biosynthesis of the phytochrome chromophore, and are thus deficient in all functional phytochromes (Koornneef et al., 1980; Chory et al., 1989b; Parks and Quail, 1991). *hy3* and *hy8* are defective in phytochrome B and phytochrome A (Dehesh et al., 1993; Reed et al., 1993;

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Whitelam et al., 1993), and therefore do not exhibit red and far-red light inhibition of hypocotyl elongation (Koornneef et al., 1980; Parks and Quail, 1993), respectively. Other members of this class are *hy4*, *blu1*, *blu2*, and *blu3* mutants, which are specifically defective in blue light inhibition of hypocotyl elongation (Koornneef et al., 1980; Liscum and Hangarter, 1991). Recent sequence analysis of the *HY4* locus suggested that it encodes one of the blue light receptors (Ahmad and Cashmore, 1993). The *hy5* mutant is unique in that it is defective in inhibition of hypocotyl elongation mediated by red, far-red, and blue light (Koornneef et al., 1980). Thus, the *HY5* locus likely encodes a signaling component that functions downstream of the red, far-red, and blue light photoreceptors.

The other class of mutants shows constitutive photomorphogenic responses in the absence of light. In Arabidopsis, mutations in several different loci lead to this phenotype. These include the *DEETIOLATED* ([*DET*]; *det1*, *det2*, and *det3*, Chory et al., 1989a, 1991; Cabrera y Poch et al., 1993) and the *CONSTITUTIVE PHOTOMORPHOGENIC* ([*COP*]; *cop1*, Deng et al., 1991; *cop9*, Wei and Deng, 1992; *cop2*, *cop3*, and *cop4*, Hou et al., 1993; *cop8*, *cop10*, and *cop11*, Wei et al., 1994) loci. The recessive nature of the *DET* and *COP* loci suggested that the wild-type gene products act to repress the photomorphogenic development in darkness and that light reverses this repressive action. Mutations in the *COP1*, *COP8*, *COP9*, *COP10*, *COP11*, and *DET1* loci result in the most pleiotropic phenotypes. Therefore, these six loci may be involved in the early steps of light signaling before the branched pathways that control individual developmental processes (Deng, 1994). Recently, it was found that severe mutations in all six loci lead to purple seed color; therefore, they were also named *FUSCA* (*FUS*) loci (Castle and Meinke, 1994; McNellis et al., 1994; Miséra et al., 1994). However, the regulatory relationship among these loci is unclear at present.

The *COP1* locus represents the first among these loci that has been characterized molecularly and biochemically (Deng et al., 1992; von Arnim and Deng, 1993; McNellis et al., 1994). *COP1* encodes a novel protein that consists of a zinc binding motif at the N terminus, a putative coiled-coil region, and several WD40 repeats in the C-terminal half that exhibit homology to the β subunit of the trimeric G protein. The structure of the COP1 protein is consistent with its predicted negative regulatory role based on the mutant phenotype. Intriguingly, the recently cloned $dTAF_{II}80$ subunit of *Drosophila* TFIID, a key component of the RNA polymerase II transcriptional apparatus, shows sequence similarity to COP1 throughout the entire protein, except that it has no zinc binding domain (Dynlacht et al., 1993). Together, these data implicate the potential of COP1 to act as a negative transcriptional regulator and its capability to interact with other light signal transduction components.

In this study, we have performed a detailed genetic analysis in an effort to define the regulatory hierarchy between *COP1* and other described photomorphogenic loci, including those that encode photoreceptors and downstream signaling components. Specifically, we intended to address two questions. First, how does *COP1* relate to the phytochromes and a blue light receptor? Second, how does *COP1* interact with two other downstream light signaling components, *HY5* and *DET1*? Together with recently published epistasis studies involving *det1*, *cop8*, *cop10*, *cop11*, as well as *hy1* to *hy5* mutants (Chory, 1992; Wei et al., 1994), our results reveal new insights and lead to specific models for the regulatory hierarchy among the phytochromes, a blue light photoreceptor, and *HY5*, *DET1*, *COP1*, *COP8*, *COP10*, and *COP11* during light-regulated plant development.

RESULTS

Experimental Design and Summary of Mutant Strains Used

To establish the regulatory hierarchy between *COP1* and other light signaling components, we chose to analyze the genetic relationships between *cop1* mutations and mutations of the photoreceptors or the downstream signaling components. This approach involves construction of double mutant lines for both a *cop1* mutation and a mutation in another locus of interest, and analysis of the double mutant phenotype. Six loci that encode the phytochromes (*HY1*, *HY2*, and *HY3*), a putative blue light receptor (*HY4*), and the downstream signaling components (*HY5* and *DET1*) were chosen to study their interactions with the *COP1* locus.

Representative alleles of those six loci, *hy1-21.84N*, *hy2-To76*, *hy3-Bo64*, *hy4-2.23N*, *hy5-Ci88* (Koornneef et al., 1980), and *det1-1* (Chory et al., 1989a), respectively, were used to construct double mutant lines with different *cop1* mutations. Recently, it has been shown that the *hy3-Bo64* mutation creates an in-frame stop at codon 448 of the phytochrome B gene (Reed et al., 1993) and that the *hy4-2.23N* mutation results from an ~2-kb deletion at the 3′ end of the *HY4* gene, which includes both coding and noncoding sequences (Ahmad and Cashmore, 1993). The phenotypes of *hy3-Bo64* and *hy4-2.23N* are similar to putative null mutations at these loci. The nature of the other four mutations is not clear at present. Recently, the *fusca2* mutation was found to be allelic to the *det1* mutation (Castle and Meinke, 1994; Miséra et al., 1994). Because the *fusca2* mutation exhibits a much stronger phenotype than *det1-1* and is adult lethal, the moderate phenotype of *det1-1* indicates that it is a weak (leaky) mutation.

The four mutant alleles of the *COP1* locus selected for this study represent all three phenotypic classes of *cop1* mutations (McNellis et al., 1994). These include two weak alleles (*cop1-4* and *cop1-6*), a strong allele (*cop1-1*), and a likely null allele (*cop1-5*). The *cop1-4* allele has a missense mutation that converts codon 283 into a stop codon and results in a truncated protein of 33 kD that is expressed at a reduced level (McNellis et al., 1994). The *cop1-6* allele has a point mutation at the 3′ end of intron 4, which leads to a 15-bp insertion due to cryptic splicing. Therefore, the COP1 protein in *cop1-6* mutants has a five–amino acid in-frame insertion between codons 301 and

302 (McNellis et al., 1994). The strong allele, *cop1-1*, has an in-frame 22-amino acid deletion (codon 355 to 376) just before the Gβ protein homology domain and results in a protein of smaller size (74 kD; McNellis et al., 1994). The lethal (null) *cop1-5* allele has a large T-DNA insertion at the beginning of the Gβ protein homology domain and does not accumulate COP1 protein at all (McNellis et al., 1994). The phenotypes of these four alleles have been described previously (Deng and Quail, 1992; McNellis et al., 1994) and will only be briefly summarized.

Dark-grown seedlings of *cop1-4* and *cop1-6* develop short hypocotyls and expanded cotyledons. Similarly, dark-grown *cop1-1* seedlings have expanded cotyledons, but the hypocotyls are much shorter than *cop1-4* and *cop1-6.* As adults, they are smaller than the wild type, with *cop1-1* being the smallest and with the poorest seed production. The *cop1-5* seedlings exhibit the most severe phenotype among the existing *cop1* alleles, and the plants die after the seedling stage.

cop1 Mutations Are Epistatic to *hy1*, *hy2*, *hy3*, and *hy4* Mutations

The single and double mutants of *hy1* to *hy4* and *cop1-1* and *cop1-4* were germinated in both dark and light. As shown in Figure 1, the hypocotyl lengths of 6-day-old *hy/cop1* double mutants were identical (*hy/cop1-1*) or very similar (*hy/cop1-4*) to those of the *cop1* single mutants under both dark and light growth conditions. Slightly longer hypocotyls in *hy1/cop1-4*, *hy2/cop1-4*, and *hy3/cop1-4* double mutants than in *cop1-4* mutants in the light have been observed, possibly because *cop1-4* is a weak mutation and is unable to completely suppress the long hypocotyl phenotype of the *hy* mutations in the light. In addition, the overall seedling morphology of dark- and light-grown *hy/cop1* double mutants resembled that of *cop1* single mutants. An example is illustrated by the *hy4/cop1-1* double mutant in Figure 2. Further, the adult morphology of *hy/cop1* double mutants also resembled that of *cop1* single mutants (data not shown). These data extend our previous study (Deng and Quail, 1992) in which we demonstrated that *cop1-1* was able to suppress the phenotype of *hy1*. Together, the results indicate that *cop1* mutations are clearly epistatic to *hy1*, *hy2*, *hy3*, and *hy4* mutations, suggesting that *COP1* acts downstream of the phytochromes and a blue light receptor.

Interactions between the *COP1* and *HY5* Loci Are Light Dependent and Allele Specific

To study the genetic relationship of *COP1* and *HY5*, we initially constructed double mutant lines homozygous for a *hy5* mutation and each of the three chosen viable *cop1* mutations, *cop1-6*, *cop1-4*, and *cop1-1*. Figures 3 and 4 summarize the hypocotyl elongation properties and the overall morphology of 6-day-old single and double mutants in comparison with the wild-type plants. Depending on the light conditions, opposite

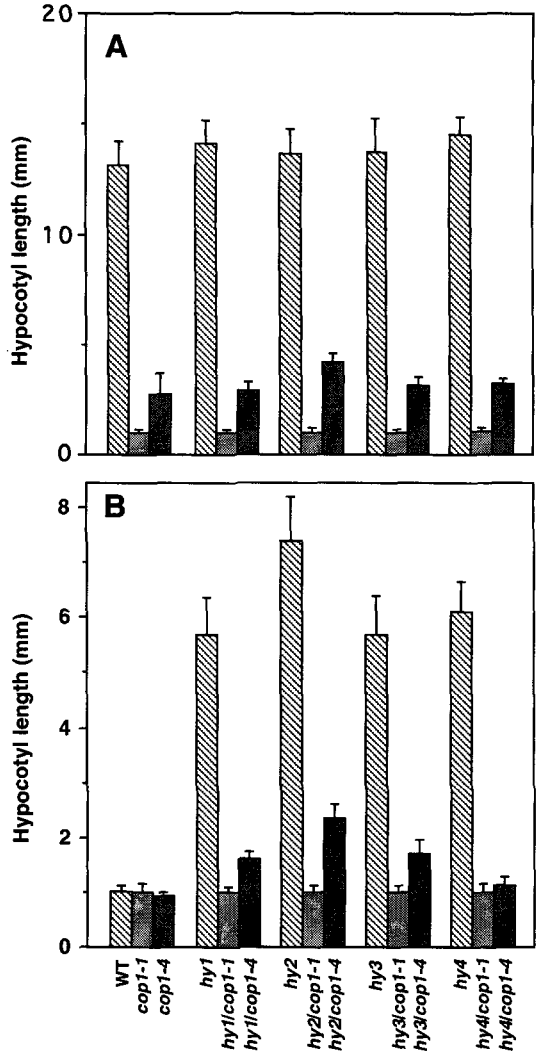

Figure 1. Comparison of the Hypocotyl Lengths of Wild-Type and Mutant Seedlings.

(A) Dark-grown seedlings of the wild type, *cop1-1*, *cop1-4*, *hy1* to *hy4*, and the *hy/cop1* double mutants.

(B) Light-grown seedlings of the wild type, *cop1-1*, *cop1-4*, *hy1* to *hy4*, and the *hy/cop1* double mutants.

The seedlings were grown either in complete darkness or continuous light for 6 days. Hypocotyl lengths of 30 seedlings from each strain were measured, and the mean values are shown. The standard deviations are represented by error bars.

epistatic relationships between *cop1-6* and the *hy5* mutation can be inferred from the phenotypes of the *hy5/cop1-6* double mutants. In darkness, *hy5/cop1-6* double mutant seedlings showed equal hypocotyl elongation to *hy5* (Figure 3A) and developed much smaller cotyledons compared to *cop1-6* mutants, except that they were open and had no apical hook (Figures 4A, 4B, 4F, and 4G). In contrast, light-grown seedlings of the

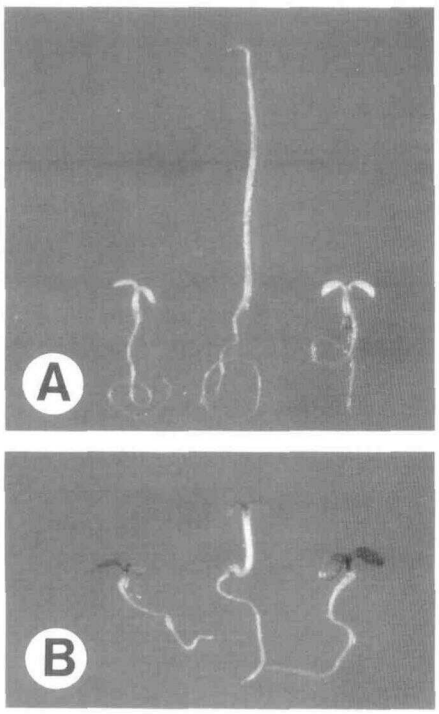

Figure 2. Morphology of 6-Day-Old *cop1-1*, *hy4*, and *hy4/cop1-1* Double Mutant Seedlings.

(A) From left to right, dark-grown *cop1-1*, *hy4*, and *hy4/cop1-1* double mutant seedlings.
(B) From left to right, light-grown *cop1-1*, *hy4*, and *hy4/cop1-1* double mutant seedlings.
The seedling phenotypes of double mutants between *cop1-1* and *hy1* to *hy3* as well as between *cop1-4* and *hy1* to *hy4* resembled that of *hy4/cop1-1* (data not shown).

hy5/cop1-6 double mutant had short hypocotyls, although they were slightly longer than those of the *cop1-6* single mutant (Figures 3B, 4L, 4P, and 4Q) and wild-type (Figures 3B and 4K) seedlings. These results demonstrated that in darkness the *hy5* mutation fully suppresses the short hypocotyl phenotype of the *cop1-6* mutation and at least partially suppresses *cop1-6* with regard to cotyledon development. In the presence of light, however, the *cop1-6* mutation partially suppresses the *hy5* mutation.

The *cop1-1* and *cop1-4* mutant alleles showed similar interactions with the *hy5* mutation, but these interactions were different from that between *cop1-6* and *hy5*. For the dark-grown seedlings, *hy5/cop1-1* and *hy5/cop1-4* double mutants exhibited intermediate hypocotyl elongation in comparison to *cop1* and *hy5* single mutants (Figure 3A), but developed open and expanded cotyledons resembling the *cop1* mutants (for *hy5/cop1-1*, see Figures 4D, 4F, and 4I; for *hy5/cop1-4*, see Figures 4C, 4F, and 4H). In contrast, the light-grown *hy5/cop1-1* and *hy5/cop1-4* double mutants had short hypocotyls and open and expanded cotyledons either identical or similar to the *cop1* single mutants (for *hy5/cop1-1*, see Figure 3B and Figures 4N, 4P, and 4S; for *hy5/cop1-4*, compare Figure 3B and Figures 4M, 4P, and 4R). These results indicated that in the dark the short hypocotyl phenotypes of *cop1-1* and *cop1-4* can be partially suppressed by the *hy5* mutation. In the light, however, the *cop1-1* and *cop1-4* mutations can completely or partially suppress the *hy5* mutation, respectively. With respect to

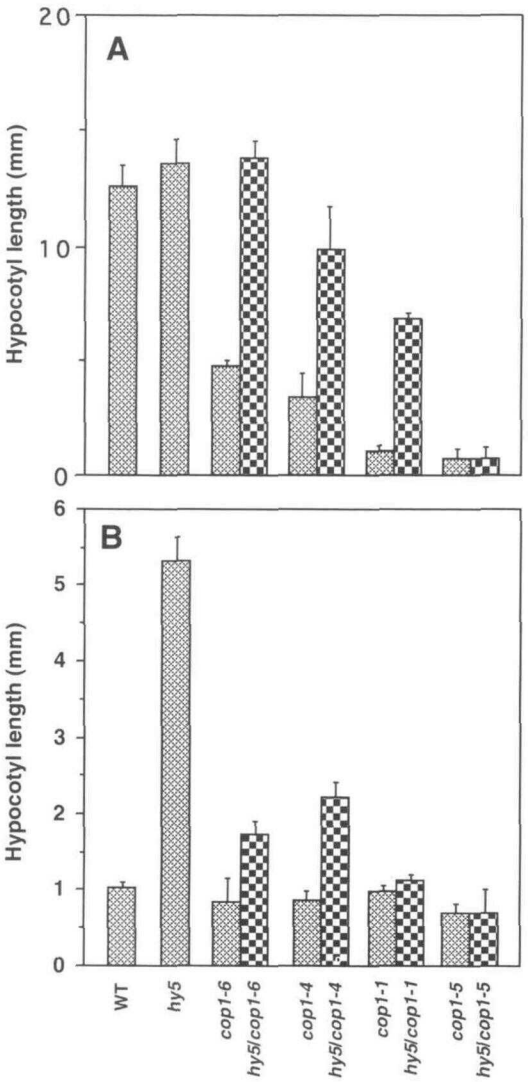

Figure 3. Comparison of the Hypocotyl Lengths of Wild-Type, *cop1*, *hy5*, and *hy5/cop1* Double Mutant Seedlings.

(A) Six-day-old dark-grown wild-type (WT), *cop1-6*, *cop1-4*, *cop1-1*, *cop1-5*, *hy5*, and the respective *hy5/cop1* double mutant seedlings.
(B) Six-day-old light-grown wild-type (WT), *cop1-6*, *cop1-4*, *cop1-1*, *cop1-5*, *hy5*, and the respective *hy5/cop1* double mutant seedlings.
The hypocotyls of 30 seedlings from each strain were measured, and the means are shown on the chart. Error bars represent standard deviations.

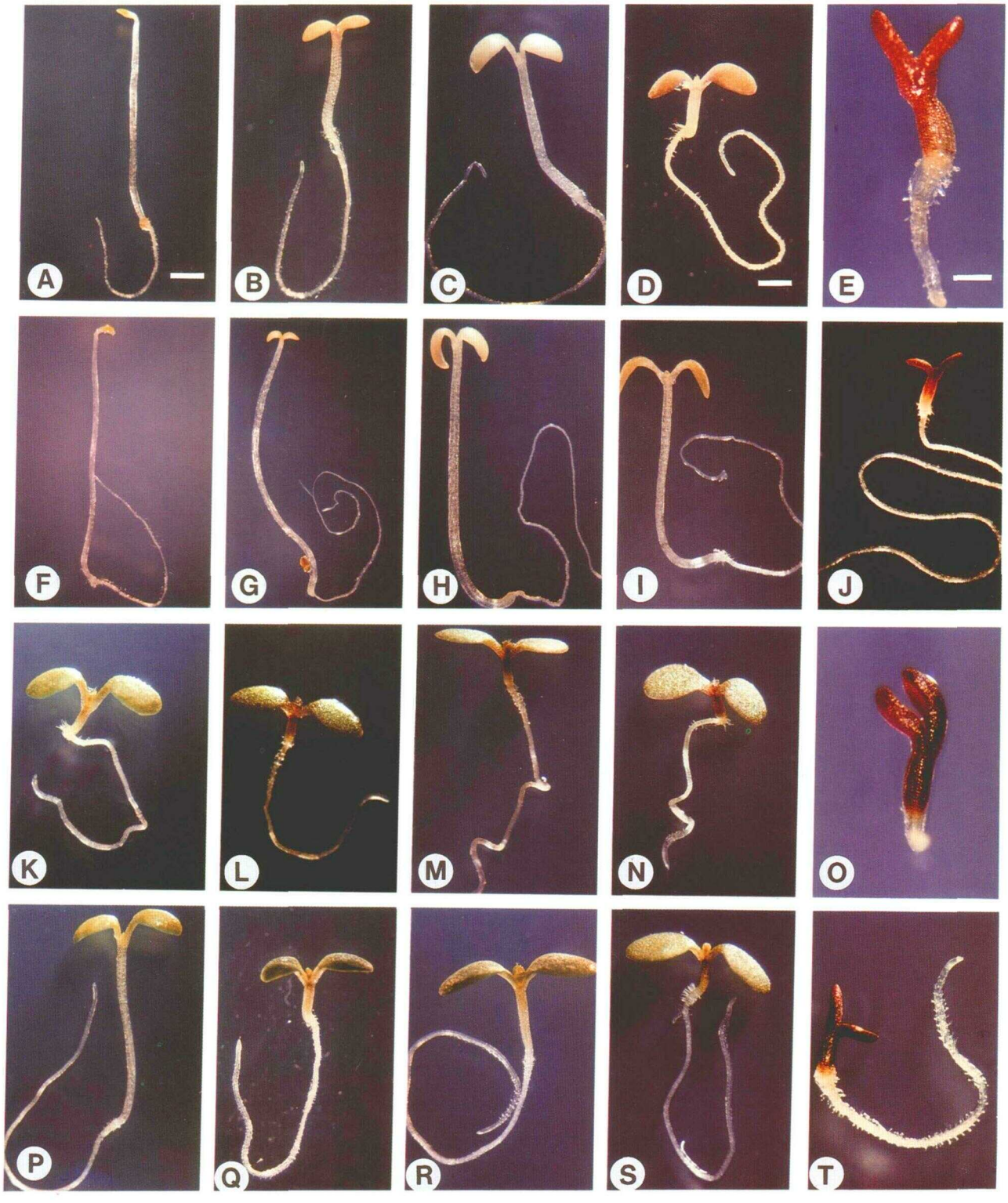

Figure 4. Morphogenetic Comparison of 6-Day-Old *hy5/cop1* Double Mutants with Their Parental Strains.

(A) to **(E)** Dark-grown wild-type, *cop1-6*, *cop1-4*, *cop1-1*, and *cop1-5* seedlings, respectively.
(F) to **(J)** Dark-grown *hy5* seedling and *hy5/cop1-6*, *hy5/cop1-4*, *hy5/cop1-1*, and *hy5/cop1-5* double mutant seedlings, respectively.
(K) to **(O)** Light-grown wild-type, *cop1-6*, *cop1-4*, *cop1-1*, and *cop1-5* seedlings, respectively.
(P) to **(T)** Light-grown *hy5* mutant and *hy5/cop1-6*, *hy5/cop1-4*, *hy5/cop1-1*, and *hy5/cop1-5* double mutant seedlings, respectively.
The same magnification was used for **(A)**, **(B)**, **(F)**, **(G)**; the scale bar (= 2 cm) is shown in **(A)**. **(E)** and **(O)** were of the same magnification; the scale bar (= 0.5 cm) is shown in **(E)**. **(D)**, **(G)**, **(H)**, **(I)**, **(J)**, **(K)**, **(L)**, **(M)**, **(N)**, **(O)**, **(P)**, **(Q)**, **(R)**, **(S)**, and **(T)** were at the same magnification; the scale bar (= 1 cm) is shown in **(D)**.

cotyledon development, the *hy5* mutation has no effect on the *cop1-1* and *cop1-4* phenotype.

Figure 5 compares the adult morphology of *hy5/cop1* double mutants with *cop1* and *hy5* single mutants. Adult plants of all three *cop1* alleles (Figures 5B, 5C, and 5D) were much smaller in size than the wild type and *hy5* that were grown under identical growth conditions (Figures 5A and 5E), with *cop1-1* adult plants being the smallest. In overall morphology, *hy5/cop1* double mutants (Figures 5F, 5G, and 5H) resembled *cop1* single mutants, except that they were relatively bigger and had higher seed production, indicating that the *hy5* mutation partially compensates for the effect of *cop1* mutations on the adult plant sizes.

These allele-specific and light-dependent interactions between the three partial loss-of-function *cop1* alleles and the *hy5* mutation suggest that the products encoded by *HY5* and *COP1* loci act in close proximity, possibly involving either direct interaction or competitive regulation of a common downstream effector to control early seedling development. To further reveal the regulatory hierarchy between *HY5* and *COP1* in the light signaling pathway, we examined the interaction between the *hy5* mutation and *cop1-5*, a putative null allele of the *COP1* locus.

cop1-5 Is Epistatic to the *hy5* Mutation

Double mutants homozygous for *cop1-5* and the *hy5* mutation were constructed and their phenotypes examined. As shown in Figure 3, 6-day-old *hy5/cop1-5* double mutants developed

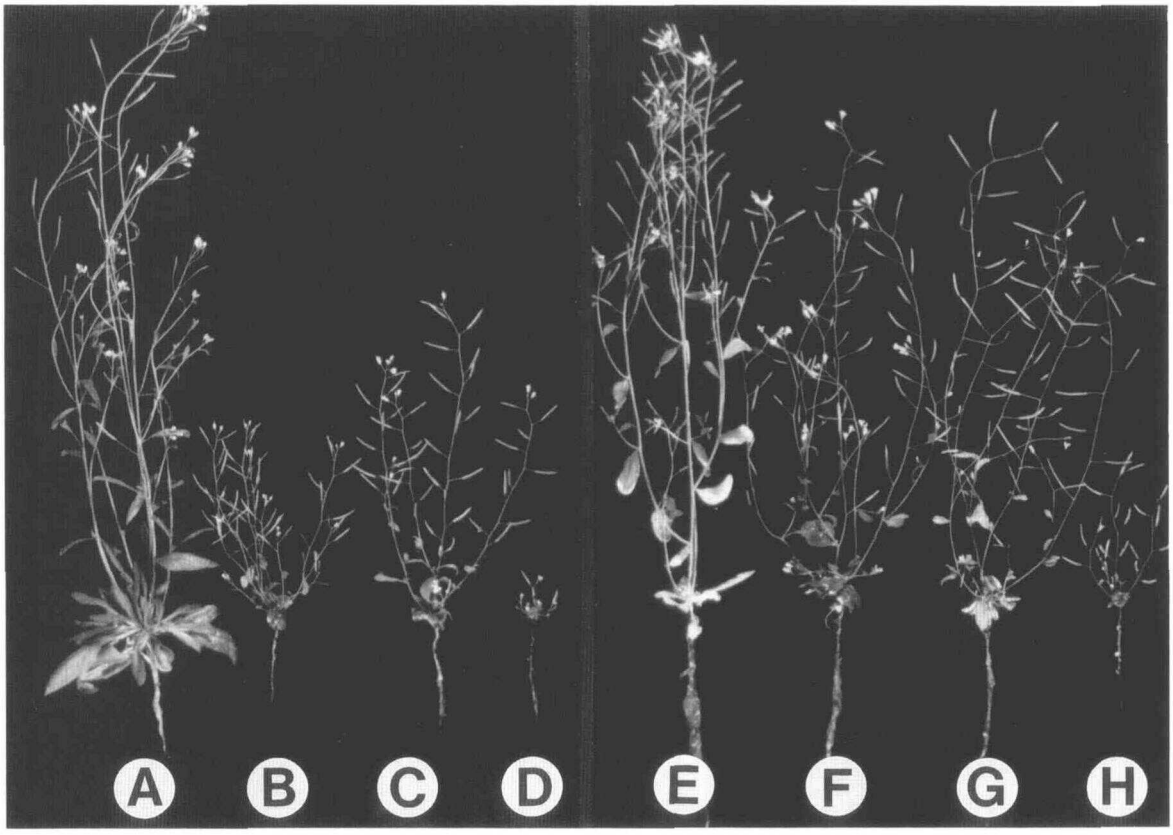

Figure 5. Comparison of the Adult Morphology between *hy5/cop1* Double Mutants and the Wild-Type, *cop1*, and *hy5* Mutants.

(A) Wild type.
(B) *cop1-6* mutant.
(C) *cop1-4* mutant.
(D) *cop1-1* mutant.
(E) *hy5* mutant.
(F) *hy5/cop1-6* double mutants.
(G) *hy5/cop1-4* double mutants.
(H) *hy5/cop1-1* double mutants.
The plants were grown under the same long-day (16-hr light/8-hr dark) conditions for 1 month.

short hypocotyls and open cotyledons that accumulated a high level of anthocyanin, resembling the *cop1-5* single mutants under both dark (Figures 4E, 4F, and 4J) and light (Figures 4O, 4P, and 4T) conditions. We also noted that the *hy5/cop1-5* double mutants (Figures 4J and 4T) developed longer roots than the *cop1-5* single mutants (Figures 4E and 4O) in darkness and light, although the basis for this light-independent phenomenon is not clear. Nevertheless, our results indicate that the *cop1-5* mutation can completely suppress the *hy5* mutation and is therefore epistatic to it with respect to hypocotyl elongation and cotyledon development in both darkness and light. Because all available evidence indicates that the *cop1-5* mutation is a null *cop1* allele (McNellis et al., 1994), we conclude that *COP1* acts downstream of *HY5* to regulate hypocotyl elongation and cotyledon development. It is worth noting that since the nature of the *hy5* mutation used is not known, we cannot completely rule out the alternative possibility that *HY5* and *COP1* act in parallel pathways but competitively regulate a common downstream effector.

hy5 Suppresses the Cotyledon Cell Differentiation Caused by *cop1-6*

The clear allele-specific interactions between the three partial loss-of-function *cop1* alleles and the *hy5* mutation during seedling development in darkness prompted us to examine the cellular differentiation patterns of these single and double mutants using scanning electron microscopy. The results are summarized in Figure 6. Cotyledons of dark-grown wild-type and *hy5* seedlings had small and regularly shaped epidermal cells, and small and immature stomata with no detectable openings (Figure 6A and data not shown), whereas cotyledons of the light-grown seedlings consisted of enlarged and differentiated epidermal cells of irregular shape and mature stomata (Figure 6B and data not shown). Cotyledons of dark-grown *cop1-6*, *cop1-4*, and *cop1-1* mutants had enlarged and irregularly shaped epidermal cells and mature stomata (Figures 6C, 6E, and 6G), similar to those of light-grown wild-type seedlings (see also Deng et al., 1992; McNellis et al., 1994). Interestingly, clustering of two or three stomata was commonly observed in the *cop1-1* cotyledons, whereas it was rarely seen in either dark- or light-grown *cop1-4*, *cop1-6*, or wild-type seedlings. Obviously, the strong mutation (*cop1-1*) also affects the initiation and thus the spacing of stomata. Dark-grown *hy5/cop1-1* and *hy5/cop1-4* double mutants developed enlarged and irregularly shaped epidermal cells and mature stomata similar to *cop1* single mutants, and in contrast to wild-type and *hy5* mutants (Figures 6A, 6E, 6F, 6G, and 6H). However, the clustering of stomata observed in *cop1-1* seedlings was not detected in the *hy5/cop1-1* double mutants. In contrast, the cotyledon epidermal cells and stomata of dark-grown *hy5/cop1-6* double mutants (Figure 6D) were much less developed than those of *cop1-6* (Figure 6C) and very similar to the wild type (Figure 6A). This observation is consistent with the small cotyledons of dark-grown *hy5/cop1-6* double mutant seedlings shown in Figure 4G. These results indicate that in darkness the *hy5* mutation can suppress the photomorphogenic cotyledon cell differentiation caused by the *cop1-6* mutation, although the *hy5* mutation itself does not cause any obvious defect in cotyledon development in both darkness and light.

hy5 Has No Effect on Constitutive Expression of Light-Inducible Genes Caused by *cop1*

We further examined whether the *hy5/cop1* double mutants constitutively express the light-inducible genes like *cop1* single mutants. The expression of three well-characterized light-regulated genes (Deng et al., 1991), two nuclear-encoded genes (*CAB* and *RBCS*), and a plastid-encoded gene (*PSBA*), was analyzed. Figure 7 summarizes the RNA gel blot analysis. As expected, all three genes were expressed at very low levels in the dark-grown wild-type and *hy5* seedlings, but at greatly elevated levels in the light-grown seedlings, whereas constitutive high levels of expression were observed in the dark-grown *cop1-1*, *cop1-4*, and *cop1-6* mutants. The *hy5/cop1* double mutants expressed these three light-regulated genes constitutively, identical to the *cop1* single mutants (Figure 7). Thus, the *hy5* mutation had no detectable effect on the dark activation of light-inducible genes caused by *cop1* mutations, even in the case of *cop1-6* in which the seedling morphogenic development was extensively suppressed by *hy5*. This result again supports the notion that photomorphogenic cellular differentiation can be uncoupled from the expression of genes encoding plastid proteins (Cabrera y Poch et al., 1993; Hou et al., 1993).

Allele-Specific Interaction between *COP1* and *HY5* during Greening of Dark-Grown Seedlings

We noted that after 6 days of growth in the dark, most of the *cop1* mutant seedlings were incapable of greening when transferred to light, whereas the wild-type seedlings were able to deetiolate, turn green, and become photosynthetically competent. Table 1 summarizes a typical greening experiment, with variations on the ability to green being evident among the three different *cop1* alleles. To check whether the *hy5* mutation has a specific effect on individual *cop1* mutations during greening, we examined the greening ability of different dark-grown *hy5/cop1* double mutant seedlings. As shown in Table 1, the *hy5* mutation had quite different effects on the three *cop1* mutant alleles examined. For *cop1-6*, the presence of the *hy5* mutation improved the ability to green after 4 and 6 days of dark growth. For *cop1-4*, the improvement of the ability to green was restricted to 4-day-old dark-grown plants and had no apparent effect on 6-day-old dark-grown seedlings. For *cop1-1*, however, the presence of the *hy5* mutation decreased the ability of 4-day-old plants to green and had no detectable effect on 6-day-old dark-grown plants.

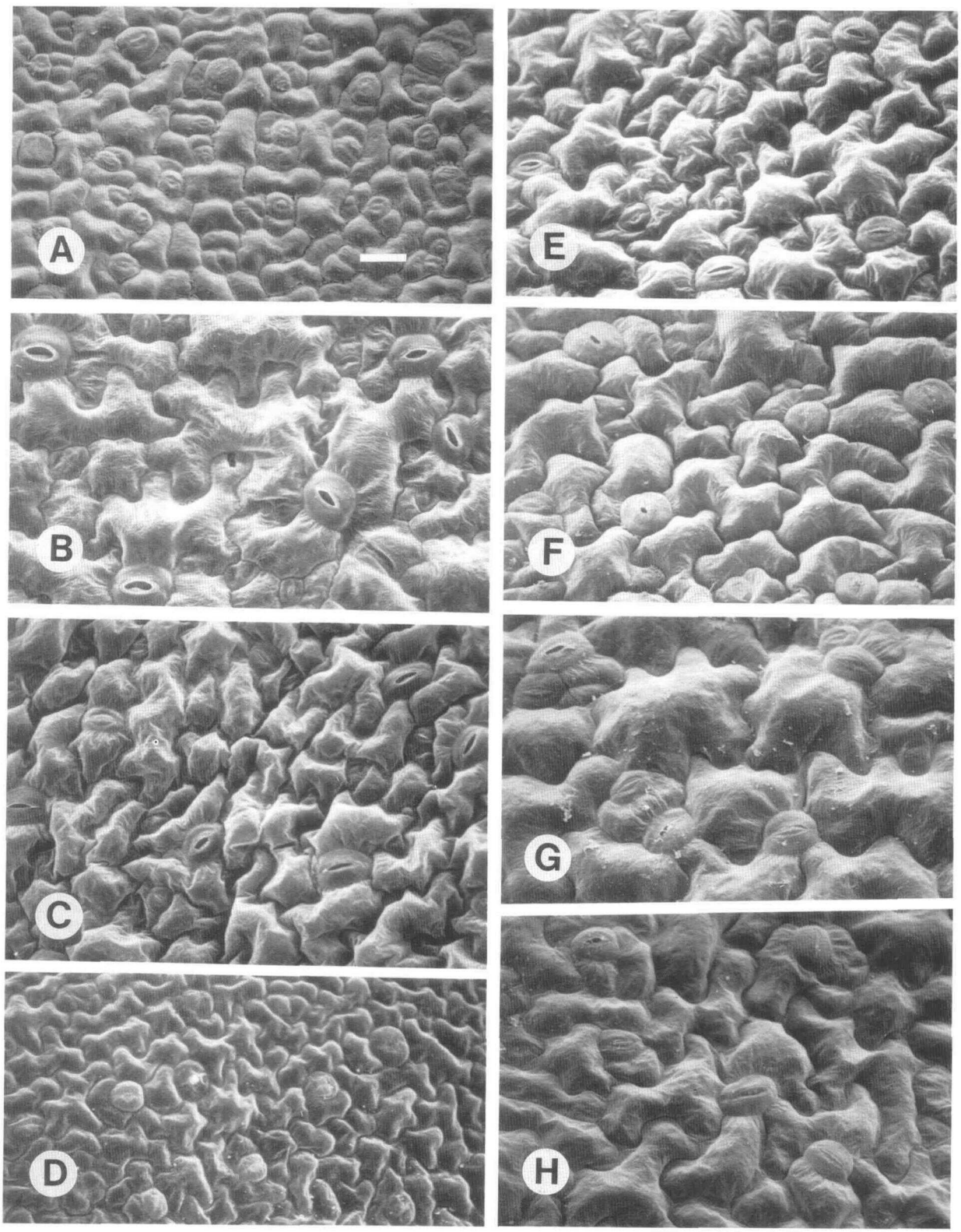

Figure 6. Effects of the *hy5* Mutation on the Photomorphogenic Cell Differentiation Patterns Caused by Different *cop1* Mutations.

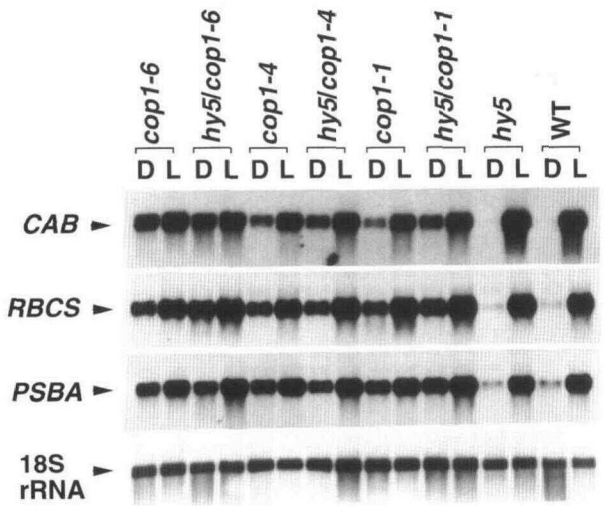

Figure 7. Comparison of the Expression Patterns of Light-Inducible Genes in *hy5/cop1* Double Mutants with Their Parental Mutants and the Wild Type.

Total RNA was isolated from 6-day-old seedlings grown either in complete darkness (D) or continuous light (L). The RNA blots were probed with ^{32}P-labeled *CAB* (encoding the chlorophyll *a/b* binding protein of the light-harvesting complex), *RBCS* (encoding the ribulose bisphosphate carboxylase small subunit), *PSBA* (encoding the photosystem II reaction center D1 protein), and the 18S rRNA gene. The blot probed for 18S rRNA serves as a control to show that equal amounts of total RNA were loaded into each lane. WT, wild type.

hy5/cop1 Double Mutants Are Able To Flower in Complete Darkness

A previous study (McNellis et al., 1994) demonstrated that the *cop1-6* mutants were able to complete their entire photomorphogenic development, including flowering, in complete darkness, whereas the wild-type plants arrested development before growing any true leaves. Therefore, we examined whether the *hy5* mutation affects the dark-flowering phenotype of *cop1* mutations. Table 2 summarizes the results.

When grown in complete darkness on growth medium supplemented with 1% sucrose, most of the plants homozygous for any of the three *cop1* alleles examined developed six to eight true leaves after 1 month. All of the *cop1-6* mutants that developed true leaves also bolted and developed flower buds. However, only half of the *cop1-1* and *cop1-4* mutants that developed true leaves formed tiny flower buds, but none bolted. Similar to the wild type, *hy5* developed neither true leaves nor flower buds under the same growth conditions. *hy5/cop1-6* double mutants were able to develop true leaves, bolt, and develop flower buds in the dark, like *cop1-6* and unlike *hy5*, although at a reduced frequency. *hy5/cop1-1* and *hy5/cop1-4* double mutants, on the other hand, developed flower buds not only at an increased frequency but the flower buds were also larger than those of the *cop1-1* and *cop1-4* single mutants. Furthermore, a significant fraction of the *hy5/cop1-1* double mutants

Table 1. Comparison of the Greening Capability of Dark-Grown Wild Type, *hy5*, *cop1* Mutants, and *hy5/cop1* Double Mutant Seedlings after Transfer to Light

	4 Days in the Dark[a]		6 Days in the Dark[a]	
Strains	Total Plants	Greened (%)	Total Plants	Greened (%)
WT Col.[b]	156	156 (100)	115	61 (53)
WT Ler.[c]	87	87 (100)	47	32 (68)
hy5	104	104 (100)	74	52 (70)
cop1-6	211	6 (3)	167	3 (2)
hy5/cop1-6	107	93 (87)	111	44 (40)
cop1-4	205	1 (1)	77	0 (0)
hy5/cop1-4	149	69 (46)	125	0 (0)
cop1-1	185	70 (38)	172	3 (2)
hy5/cop1-1	143	26 (18)	151	3 (2)

[a] The number of total seedlings examined (total plants) and seedlings turned green (greened) with percentages indicated in parentheses were recorded for seedlings grown in darkness for either 4 or 6 days; they were then transferred to continuous light for at least 4 days before the seedlings were scored for their ability to green. The criteria of scoring is whether their cotyledons turned green or completely bleached in the light. The experiment was repeated and similar results were obtained.

[b] WT Col., wild-type Columbia.

[c] WT Ler., wild-type Landsberg *erecta*.

Figure 6. (continued).

(A) Dark-grown wild type.
(B) Light-grown wild type.
(C) Dark-grown *cop1-6* mutant.
(D) Dark-grown *hy5/cop1-6* double mutant.
(E) Dark-grown *cop1-4* mutant.
(F) Dark-grown *hy5/cop1-4* double mutant.
(G) Dark-grown *cop1-1* mutant.
(H) Dark-grown *hy5/cop1-1* double mutant.

The cotyledon epidermal cells of 4-day-old seedlings were examined by scanning electron microscopy. The same magnification was used for all panels. Bar = 2 mm.

Table 2. Flowering in Darkness in the Wild Type, *hy5*, *cop1-6*, *cop1-4*, and *cop1-1* Mutants, and the *hy5/cop1* Double Mutants[a]

Strains	Total Plants Examined	Plants with True Leaves (%)	Plants with Flower Buds (%)	Plants Bolted (%)
WT Col.[b]	57	0 (0)	0 (0)	0 (0)
WT Ler.[c]	24	0 (0)	0 (0)	0 (0)
hy5	57	0 (0)	0 (0)	0 (0)
cop1-6	69	44 (64)	44 (64)	44 (64)
hy5/cop1-6	50	25 (50)	15 (30)	15 (30)
cop1-4	55	40 (73)	21 (38)	0 (0)
hy5/cop1-4	19	15 (79)	15 (79)	0 (0)
cop1-1	56	42 (75)	21 (36)	0 (0)
hy5/cop1-1	72	70 (97)	59 (82)	23 (32)

[a] The numbers of plants examined, plants that developed true leaves, plants with flower buds, and plants that bolted were recorded, with percentages for the latter three indicated in parentheses. It should be noted that none of the plants that failed to develop true leaves formed flower buds and that bolting only occurred in those plants with flower buds. The experiment was repeated and similar results were obtained.

[b] WT Col., wild-type Columbia.

[c] WT Ler., wild-type Landsberg *erecta*.

bolted as well, producing short floral stems separating the rosettes from the flower buds (data not shown). Together, these results indicate that the ability of the *hy5/cop1* double mutants to bolt and flower in complete darkness is similar to the respective *cop1* single mutants. However, the *hy5* mutation modifies this characteristic in an allele-specific manner, ranging from partial suppression of *cop1-6* to enhancement of *cop1-1* and *cop1-4*.

Combination of Weak *cop1* and *det1* Mutations Led to Synthetic Lethality

Severe (putative null) mutations in *COP1* and *DET1* loci are lethal after the seedling stage (Castle and Meinke, 1994; McNellis et al., 1994; Miséra et al., 1994), suggesting that they encode gene products essential for a common developmental function. If *COP1* and *DET1* act in the same regulatory pathway, knowledge from numerous studies in other systems (Guarente, 1993) would predict that in combination their weak alleles should enhance each other's phenotype and result in synthetic lethality. Therefore, a weak *det1* allele (*det1-1*) was chosen to construct double mutant lines with two representative partial loss-of-function *cop1* alleles, a strong (*cop1-1*) and a weak (*cop1-6*) mutation. In Figure 8, we compare a *cop1-6/*

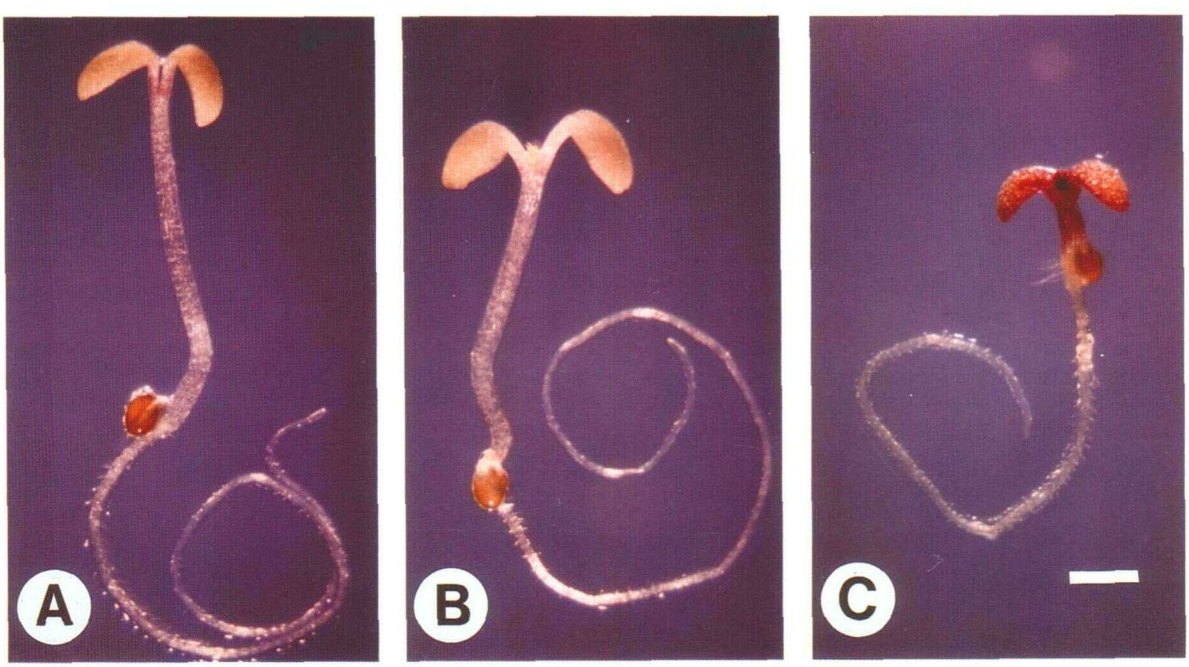

Figure 8. Morphogenetic Comparison of 6-Day-Old Dark-Grown *cop1-6*, *det1-1*, and *cop1-6/det1-1* Mutant Seedlings.

(A) *cop1-6* mutant.
(B) *det1-1* mutant.
(C) *cop1-6/det1-1* double mutant.
All three pictures were taken under the same magnification, and a scale bar is shown in **(C)**. Bar = 1 cm.

det1-1 double mutant with the parental mutants. The dark-grown seedlings of the *cop1-6* and *det1-1* parental mutants were very similar; both developed short hypocotyls and open and expanded cotyledons (Figures 8A and 8B). In contrast, both *cop1-6/det1-1* and *cop1-1/det1-1* double mutants (Figure 8C and data not shown) had morphological characteristics very similar to the lethal alleles of both *DET1* or *COP1* loci (Castle and Meinke, 1994; McNellis et al., 1994; Miséra et al., 1994). These phenotypes include dark purple cotyledon color in both mature seeds and seedlings and severely retarded seedling development, as illustrated by the very short hypocotyls and small cotyledons as well as by adult lethality. The severe phenotype and adult lethality of the *cop1/det1* double mutants demonstrate that weak mutations in *DET1* and *COP1* loci are able to enhance each other's phenotype and lead to synthetic lethality. Therefore, these results support the possibility that *DET1* and *COP1* act in the same pathway to regulate photomorphogenic development. The fact that both putative null alleles of *COP1* and *DET1* loci lead to adult lethality would argue against an alternative possibility in which *COP1* and *DET1* act in parallel pathways that converge downstream to a common effector (see also Discussion).

COP1 May Act Downstream of *DET1*

To further delineate the regulatory hierarchy between *DET1* and *COP1*, we have examined the dark-adaptive changes in expression of the light-regulated genes and the seed germination of *cop1-6/det1-1* double mutants. Previously, it has been shown that although *cop1* and *det1* mutations result in very similar effects on photomorphogenic development of Arabidopsis, there are two major features that distinguish these two mutations (Chory et al., 1989a; Deng et al., 1991). First, mutations in *COP1*, but not *DET1*, affect the normal changes of gene expression in light-grown adult plants during dark adaptation. Second, *cop1* mutations do not affect phytochrome control of seed germination, whereas *det1* seeds germinate even in the absence of active phytochrome. We reasoned that examination of these two properties of the *cop1/det1* double mutants may help to resolve the epistatic relationship of *DET1* and *COP1*.

Total RNA from continuous light-grown double mutants and their 2-day-old dark-adapted counterparts was extracted, and the expression of three light-inducible genes (*CAB*, *RBCS*, and *Ferredoxin A*) was examined by RNA gel blot analysis. The *cop1-6/det1-1* double mutants were completely defective in dark-adaptational changes in expression of the three light-inducible genes (data not shown), and thus were similar to that reported for *cop1* mutants (Deng et al., 1991) yet different from the wild type and *det1* mutants (Chory et al., 1989a). This result is consistent with the possibility that *COP1* acts downstream of *DET1*.

Arabidopsis seed germination is under the control of phytochrome. Far-red light, which reduces active phytochrome, greatly reduces the germination frequency of wild-type seeds, whereas red light, which converts inactive phytochrome to active phytochrome, reverses the inhibitory effect of far-red light (Chory et al., 1989a; Deng et al., 1991). Here, we have examined the influence of various light treatments on the germination of *cop1-6/det1-1* double mutants, and the results are summarized in Table 3. Seed germination of *cop1-6* was clearly controlled by phytochrome, similar to the results for the wild type and other *cop1* alleles (Deng et al., 1991; Table 3). In contrast, seed germination of *det1* mutants was independent of light treatment, as reported previously (Chory et al., 1989a; Table 3). Germination of *cop1-6/det1-1* double mutants was induced by red light from 89 to 99%, and a pulse of far-red light reduced the germination frequency from 89 to 52%. Further, the effect of far-red light treatment could be completely reversed by a subsequent red light treatment. These results show that seed germination of *cop1-6/det1-1* double mutants is clearly under the control of phytochrome, similar to *cop1-6* and in contrast to *det1-1*. Thus, with respect to phytochrome control of seed germination, the *cop1-6* mutation is epistatic to *det1-1*.

Table 3. Seed Germination Frequencies of *cop1-6*, *det1-1*, and *cop1-6/det1-1* Double Mutants under Different Light Treatments[a]

	Seed Germinated/Total Seed Planted (%)				
Strains	Dark	Red	Red/ Far-Red	Far-Red	Far-Red/ Red
WT Col.[b]	192/243 (79)	211/218 (97)	76/200 (38)	37/231 (16)	202/224 (90)
cop1-6	194/247 (79)	240/242 (99)	214/274 (78)	104/325 (32)	241/254 (95)
det1-1	214/220 (97)	174/180 (97)	184/190 (97)	169/173 (98)	201/201 (100)
cop1-6/det1-1	73/82 (89)	96/97 (99)	59/92 (64)	49/94 (52)	97/97 (100)

[a] The seeds were imbibed at 4°C in darkness for 4 days before they were given the respective light treatments. The red light pulse (Red) was 5 sec and the far-red light pulse (Far-Red) was 10 sec. For treatments involving two different pulses, the first light pulse was followed immediately by the second pulse. Following the light treatments, the seeds were grown at 22°C in complete darkness for 6 days before the germination frequencies were determined.
[b] WT Col., wild-type Columbia.

DISCUSSION

In recent years, the isolation of new photomorphogenic mutants and molecular cloning of some of these loci have provided insights into the complex network of light-regulated early seedling development (Chory, 1993; Deng, 1994). Mutants defective in the perception of light signals, namely the *hy* and *blu* mutants, provide genetic evidence that there are specific red and blue light photoreceptors and that red/far-red and blue light signal transduction pathways are genetically separable. It has been well recognized that photomorphogenic seedling development in wild-type plants requires the concerted action of multiple photoreceptors (Kendrick and Kronenberg, 1993).

Thus, these individual pathways are likely to converge downstream upon common regulatory steps that transmit the information to the cellular effectors, which in turn control different aspects of seedling development. The pleiotropic phenotypes caused by mutations in the *COP1*, *COP8*, *COP9*, *COP10*, *COP11*, and *DET1* loci implied that these genes may encode products involved in these common regulatory steps. To better understand this complex network would require knowledge of the regulatory hierarchy among the photoreceptors and different downstream components. Recent reports have started to address these questions. An epistasis study between *hy* and *det1* and *det2* mutations had placed *DET1* and *DET2* downstream of *HY1*, *HY2*, *HY3*, and *HY4* and either upstream of *HY5* or in a separate pathway from *HY5* (Chory, 1992). On the other hand, characterization of *hy1/cop1-1* double mutants led to the conclusion that *COP1* acts downstream of *HY1* (Deng and Quail, 1992). Most recently, double mutant analysis between mutations in the three new *COP* loci (*COP8*, *COP10*, and *COP11*) and *hy1* to *hy5* suggested that *COP8*, *COP10*, and *COP11* act downstream of all five *HY* loci examined (Wei et al., 1994). In this work, we have expanded those studies with molecularly and genetically defined *cop1* mutant alleles and revealed possible regulatory hierarchies among those genes in the light signaling circuitry of Arabidopsis. The conclusions drawn from this genetic analysis should provide a framework for future molecular study toward understanding the mechanism of light signaling.

Regulatory Hierarchy of Photomorphogenic Loci

Analysis of double mutants homozygous for *cop1-1* or *cop1-4* and *hy1*, *hy2*, *hy3*, and *hy4* loci showed that *hy/cop1* double mutants clearly resembled *cop1* single mutants at the seedling and adult stages. Thus, *cop1* mutations are epistatic to *hy1*, *hy2*, *hy3*, and *hy4* mutations. These results have two implications. Because *HY4* likely encodes an apoprotein of a blue light photoreceptor (Ahmad and Cashmore, 1993), the ability of *cop1* mutations to suppress *hy4* mutation suggests that *COP1* acts downstream of the blue light photoreceptor defined by *HY4*. On the other hand, as *hy3* mutants are defective in phytochrome B and *hy1* and *hy2* mutants are defective in the synthesizing chromophore for all types of phytochromes, our results suggest that COP1 protein functions downstream of the red/far-red light photoreceptor(s) (at least phytochrome B and possibly others).

Clear epistasis of a putative null allele, *cop1-5*, over *hy5* suggests that *COP1* acts downstream of *HY5*. Because *hy5* mutants have normal photoreceptors, but reduced sensitivity to red, far-red, and blue light signals, *HY5* presumably encodes a light signaling component downstream of the multiple photoreceptors. Thus, the *HY5* gene product must play a role somewhere between the photoreceptors and COP1 in the light signaling network.

Recently, it has been shown that *COP1* and *DET1* loci are identical to *FUSCA1* and *FUSCA2* loci, respectively (Castle and Meinke, 1994; McNellis et al., 1994; Miséra et al., 1994). The *fusca* mutants were isolated based on their high levels of anthocyanin in the cotyledons of both mature seeds and young seedlings (Müller, 1963; Miséra et al., 1994). Both *fusca1* and *fusca2* mutants share common characteristics, including retarded but constitutive photomorphogenic seedling development and lethality after the seedling stage (Miséra et al., 1994). The similar seedling phenotype and adult lethality observed in severe (possibly null) mutations of both loci indicate that they are essential for a common developmental function. If the two genes act in the same pathway, one would predict that partial loss-of-function alleles, such as *cop1-1*, *cop1-6*, and *det1-1*, would enhance each other's phenotype and lead to synthetic lethality (Guarente, 1993). Our results demonstrated that both *cop1-1/det1-1* and *cop1-6/det1-1* double mutants exhibit a much stronger phenotype than the parental mutants and are similar to the lethal (null) alleles of these loci. The synthetic lethality caused by interactions between these otherwise viable partial loss-of-function alleles supports the notion that *COP1* and *DET1* are involved in the same pathway. The adult lethality of the putative null mutations of both *COP1* and *DET1* loci strongly argues against an alternative possibility that *COP1* and *DET1* act in parallel pathways but converge downstream to a common effector, because if this were so, a complete loss of function in either one of the pathways should not cause lethality.

To establish the epistatic relationship and to rule out the alternative possibility that *COP1* and *DET1* act in parallel pathways, we further investigated two properties of *cop1-6/det1-1* double mutants, namely phytochrome control of seed germination and dark-adaptational changes in gene expression, that are different in the parents. Seed germination of *cop1-6/det1-1* double mutants was still under the control of phytochrome, as in *cop1-6* and in contrast to *det1-1*. The fact that the *cop1-6* mutation could suppress the *det1-1* mutation with respect to phytochrome control of seed germination was inconsistent with the possibility that *COP1* and *DET1* act in parallel pathways. What was not immediately clear, and calls for further study, was how, in biochemical terms, the *cop1-6* mutation achieved its suppression of the germination defect caused by the *det1-1* mutation. On the other hand, the *cop1-6/det1-1* double mutants were defective in altering the expression of light-regulated genes during dark adaptation, a property they shared with *cop1* rather than *det1* mutants. Both observations were consistent with a hypothesis that *COP1* acts downstream of *DET1*, at least with respect to seed germination and dark adaptation. Therefore, it is reasonable to hypothesize that *COP1* also acts downstream of *DET1* during light-regulated seedling development.

Based on our genetic studies, we propose in Figure 9 two possible models that depict how light signals perceived by the red/far-red and blue light receptors are transduced through a common cascade(s) of regulatory steps, as defined by *HY5*, *COP1*, *COP8*, *COP9*, *COP10*, *COP11*, and *DET1*, leading to the derepression of photomorphogenic development. In model I, *DET1* and *HY5* are purported to act upstream of *COP1* (see

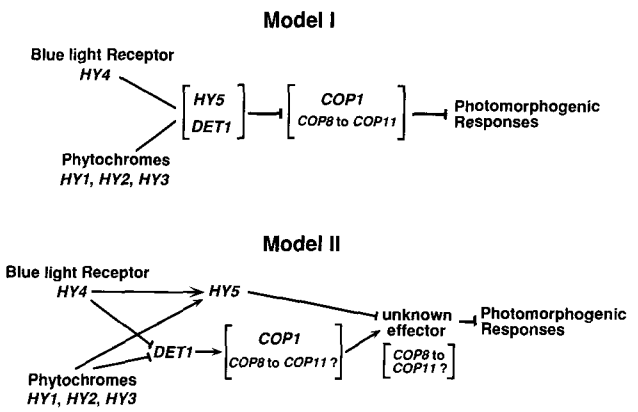

Figure 9. Two Possible Models Representing the Hierarchical Relationship among the Photoreceptors, *HY5*, *DET1*, *COP1*, *COP8*, *COP9*, *COP10*, and *COP11* in Controlling the Primary Commitment of Photomorphogenic Development.

In both models, it is proposed that light signals perceived by the red/far-red and blue light receptors converge to a common cascade(s) of regulatory steps, leading to the derepression of photomorphogenic development. It is worth noting that in model II, some of the pleiotropic *COP* genes (except *COP1* itself) could either themselves constitute the unknown effector or act upstream. For simplicity, genes involved in controlling specific subsets of photomorphogenic development are not shown. The lines (with arrows to indicate positive action or activation, with "T" bars to indicate negative action or repression, or not yet defined) between each proposed step reflect only the flow of information and may involve intermediate components. The hierarchical relationships between *DET1* and *HY5*, or among the five *COP* genes, are not clear at present. Recently, *COP1*, *COP8*, *COP9*, *COP10*, *COP11*, and *DET1* were found to be identical to *FUS1*, *FUS8*, *FUS7*, *FUS9*, *FUS6*, and *FUS2*, respectively (Castle and Meinke, 1994; McNellis et al., 1994; Miséra et al., 1994). For more details, see Discussion.

discussion above) in the same regulatory circuitry. Based on their *cop1*-like phenotype and similar epistatic relationship with the *hy* mutations, *COP8*, *COP9*, *COP10*, and *COP11* are placed in the same hierarchical position as *COP1*. Recently, Chory proposed that *DET1* acts either upstream of *HY5* or in a separate pathway from *HY5* (Chory, 1992, 1993). However, because the *det1-1* allele used in their study is a weak allele and in view of the complicated allele-specific interactions observed between *cop1* and *hy5* mutations, it is also possible that a complete loss-of-function (null) *det1* allele might exhibit a clear epistasis over *hy5*.

Therefore, *HY5* and *DET1* could interact in one of three possible ways: (1) *HY5* upstream of *DET1*, (2) *DET1* upstream of *HY5*, or (3) *DET1* and *HY5* in parallel pathways but converging downstream. In this model, light signals are perceived by multiple photoreceptors, transduced by way of specific early steps, and then converge to either inactivate *DET1* and/or activate *HY5*, which in turn abrogates the suppressive action of *COP1*, *COP8*, *COP9*, *COP10*, and *COP11* to bring about the downstream photomorphogenic responses. Currently, it is not clear how the five *COP* (or *FUSCA*) genes, and possibly more (Miséra et al., 1994), relate to each other. They could define sequential steps or form a multimeric protein complex, or a combination of both of these. In model II, we take into consideration that there is an alternative possibility that *HY5* may act in a separate pathway from *DET1* and *COP1* genes but converge to competitively regulate a common downstream effector. In this case, it is possible that some of the pleiotropic *COP* genes (except *COP1*) could either constitute the unknown effector or act upstream in close proximity to *COP1*.

In both models, it is expected that because of its hierarchic position in the signaling process, *HY5* would be able to regulate both cotyledon and hypocotyl development. Although the *hy5* mutation itself does not cause visible defects in the cotyledon development of Arabidopsis seedlings, it can affect the photomorphogenic development in the dark caused by the *cop1* mutations, including cotyledon development, greening of dark-grown seedlings, and flowering. These results indicate that *HY5* not only regulates hypocotyl elongation but cotyledon development as well, a property that is consistent with the expectation of the models. It is important to note that these models are a simplification of the complex regulatory circuitry involved in light control of development. For example, the interactions between each of the proposed steps only reflect the flow of information and may involve intermediate steps. Further, there are probably many branches and parallel pathways for specific processes that are separable, such as hypocotyl elongation, cotyledon development, plastid development, and gene expression. The identification of less pleiotropic photomorphogenic mutants, like *det2*, *det3*, *cop2*, *cop3*, and *cop4*, that were affected in specific subsets of these developmental processes supports this notion.

Evidence for Direct Interaction between COP1 and HY5

Analysis of double mutants between three classes of *cop1* alleles—a putative null allele (*cop1-5*), a strong (*cop1-1*) mutation, and weak (*cop1-4* and *cop1-6*) mutations—and a *hy5* mutation revealed that *COP1* and *HY5* interact in an allele-specific and light-dependent manner. In the light, the *cop1* mutations either completely (*cop1-5* and *cop1-1*) or partially (*cop1-4* and *cop1-6*) suppress the long hypocotyl phenotype of the *hy5* mutant. In darkness, however, different interactions with respect to cotyledon and hypocotyl development were observed. For hypocotyl elongation in darkness, the *hy5* mutation can suppress the defect of the three weak *cop1* mutations either partially (*cop1-1* and *cop1-4*) or completely (*cop1-6*), but not that of a putative null allele, *cop1-5*. Although the *hy5* mutation has no apparent effect on the defects in repression of cotyledon development resulting from *cop1-1* and *cop1-4* mutations, it clearly suppresses photomorphogenic cotyledon development caused by the *cop1-6* mutation. Furthermore, allele-specific interactions between *COP1* and *HY5* were also evident during greening of dark-grown seedlings. The presence of the *hy5* mutation has different effects, ranging from partial suppression to enhancement, on the greening ability

of the different dark-grown *cop1/hy5* double mutants examined (Table 1). In addition, the *hy5* mutation also modifies the dark-flowering characteristic of *cop1* mutations in an allele-specific manner (Table 2).

The simplest explanation for these allele-specific interactions between *HY5* and *COP1* is that their encoded products interact directly. Sequence analysis of the *COP1* gene (Deng et al., 1992; Deng, 1994) revealed that it encodes a novel protein of 675 amino acids that includes three recognizable domains, a ring finger–type zinc binding motif, a potential coiled-coil region, and a domain with multiple WD40 repeats homologous to the β subunit of the trimeric G proteins. The coiled-coil domain and the WD40 repeats have been implicated in mediating protein–protein interactions in other regulatory proteins involved in diverse biological processes, such as signal transduction, cell-cycle regulation, splicing, and transcriptional repression (Deng, 1994). Thus, the *HY5* gene product may represent an interactive partner that contacts COP1 possibly through one of these interactive motifs. A recent study from our laboratory showed that the *cop1-4* mutation leads to the deletion of the C-terminal 392 amino acids of the COP1 protein, including all WD40 repeats (McNellis et al., 1994). The fact that the *hy5* mutation can partially suppress the hypocotyl elongation defect of *cop1-4* in darkness would suggest that the N-terminal 283 residues of COP1 are able to interact with the mutant HY5 protein to promote hypocotyl elongation.

Due to the limited *hy5* alleles available for this study and to the lack of knowledge about the nature of the *hy5* mutation, our data cannot completely rule out alternative mechanisms that may give rise to the observed allele-specific interactions between *COP1* and *HY5* loci. For example, it is formally possible that *COP1* and *HY5* may be involved in parallel pathways but converge to competitively regulate a common downstream effector (Figure 9, model II). Obviously, the direct test of the proposed regulatory relationship between *HY5* and *COP1* awaits the molecular cloning of *HY5*.

METHODS

Plant Material and Growth Conditions

The wild type and the three *constitutive photomorphogenic1* (*cop1*) mutant alleles, *cop1-1*, *cop1-4*, and *cop1-6*, used in this study are in the *Arabidopsis thaliana* Columbia ecotype, and *cop1-5* is in the Wassilewskija ecotype. The long hypocotyl (*hy*) mutants alleles used in this study were *hy1* (*21.84N*), *hy2* (*To76*), *hy3* (*Bo64*), *hy4* (*2.23N*), and *hy5* (*Ci88*) (Koornneef et al., 1980). All *hy* mutants are in the Arabidopsis Landsberg *erecta* ecotype and were provided by Dr. Maarten Koornneef (Wageningen Agricultural University, The Netherlands). The *deetiolated1-1* (*det1-1*) allele is in the Columbia ecotype (Chory et al., 1989a) and was provided by Dr. Joanne Chory (The Salk Institute, San Diego, CA).

The seeds were surface sterilized for 10 min in 30% bleach (Clorox), rinsed at least five times, and sown on Petri dishes (150 × 25 mm) or Magenta boxes (Sigma) (for flowering in darkness) containing growth medium supplemented with 1% sucrose (Valvekens et al., 1988). After cold treatment at 4°C for 2 to 4 days in the dark, the plates or boxes were incubated in a growth chamber at 22°C in complete darkness, in a 16-hr light/8-hr dark photoperiod, or in continuous light (only for the dark-adaptation experiment). Plants on Petri dishes were either used directly for experiments or transferred to soil to grow to maturity. The light source in the growth chamber was a combination of cool-white fluorescent light and incandescent lights with intensity ranging from 50 to 200 μmol m^{-2} sec^{-1}. In the seed germination experiment, light sources of specific wavelengths (red, far-red, and green safelight) were used as described previously by Deng et al. (1991). For the flowering in darkness experiment, the Magenta boxes containing the planted seeds were wrapped with two layers of aluminum foil before they were grown in complete darkness for 1 month.

Construction of Double Mutants

To generate Arabidopsis strains homozygous for two different mutations, we usually crossed plants homozygous for two individual mutations and allowed the F_1 progeny to self. Ten to 20 F_2 plants that were homozygous for one mutation were selected and grown to maturity, and the F_3 seeds from these plants were harvested individually. F_2 individuals that were heterozygous for the second mutation were identified by examining the phenotypic segregation in the F_3 progeny. The F_3 plants that showed a new phenotype among the progeny of these selected F_2 families were considered homozygous for both parental mutations. Double mutants that were unable to reproduce were picked from the F_3 population and used directly for phenotypic characterization. Otherwise, they were selected and selfed to produce individual double mutant lines. Because *cop1-5* is a homozygous lethal mutation, a heterozygous *cop1-5* plant was used to construct *hy5/cop1-5* double mutants. The F_1 progeny were selected on growth medium containing 50 μg/mL kanamycin to identify those F_1 plants that carried a copy of the *cop1-5* mutation (*cop1-5* was caused by a T-DNA insertion in the exon of *COP1* which conferred kanamycin resistance). The rest of the selection is similar to that described above.

For double mutants that involved mutations originating from two different ecotypes (*cop1* and *hy* mutants), multiple double mutant lines were generated for each pair, and their phenotypes were examined in parallel. In each case, identical phenotypes among the multiple double mutant lines derived from the same parental pair were observed, implying that ecotype background had a negligible effect on these double mutant phenotypes.

Scanning Electron Microscopy

Four-day-old dark- and light-grown seedlings were fixed in a fixative solution containing 5% acetic acid, 4% formaldehyde, and 50% ethanol at room temperature in darkness for at least 2 hr and dehydrated in a graded ethanol series. Dehydrated materials were critical point dried in liquid carbon dioxide. Individual seedlings were mounted on stubs and sputter coated with gold palladium. Specimens were examined under a scanning electron microscope, as described by Hou et al. (1993).

RNA Gel Blot Analysis

Six-day-old dark- and light-grown seedlings of wild type, *cop1*, *hy* single mutants, and *hy/cop1* double mutants were used for the RNA analysis. The dark-grown seedlings were harvested under dim-green

safe light in the dark room. Isolation of total RNA, electrophoresis and blotting, as well as hybridization with radioactively labeled DNA probes were as described previously by Deng et al. (1991). The probes were labeled with ^{32}P-dCTP using a random priming DNA labeling kit (U.S. Biochemical Corp.).

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