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Arabidopsis *COP8*, *COP10*, and *COP11* Genes Are Involved in Repression of Photomorphogenic Development in Darkness

Ning Wei,[a] Shing F. Kwok,[b] Albrecht G. von Arnim,[a] Angela Lee,[a] Timothy W. McNellis,[a] Barry Piekos,[a] and Xing-Wang Deng [a,1]

[a]Department of Biology, Osborn Memorial Laboratories, OML 301, Yale University, 165 Prospect Street, New Haven, Connecticut 06520-8104

[b]Department of Genetics Graduate Program, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520

Wild-type Arabidopsis seedlings are capable of following two developmental programs: photomorphogenesis in the light and skotomorphogenesis in darkness. Screening of Arabidopsis mutants for constitutive photomorphogenic development in darkness resulted in the identification of three new loci designated *COP8*, *COP10*, and *COP11*. Detailed examination of the temporal morphological and cellular differentiation patterns of wild-type and mutant seedlings revealed that in darkness, seedlings homozygous for recessive mutations in *COP8*, *COP10*, and *COP11* failed to suppress the photomorphogenic developmental pathway and were unable to initiate skotomorphogenesis. As a consequence, the mutant seedlings grown in the dark had short hypocotyls and open and expanded cotyledons, with characteristic photomorphogenic cellular differentiation patterns and elevated levels of light-inducible gene expression. In addition, plastids of dark-grown mutants were defective in etioplast differentiation. Similar to *cop1* and *cop9*, and in contrast to *det1* (*deetiolated*), these new mutants lacked dark-adaptive change of light-regulated gene expression and retained normal phytochrome control of seed germination. Epistatic analyses with the long hypocotyl *hy1*, *hy2*, *hy3*, *hy4*, and *hy5* mutations suggested that these three loci, similar to *COP1* and *COP9*, act downstream of both phytochromes and a blue light receptor, and probably *HY5* as well. Further, *cop8-1*, *cop10-1*, and *cop11-1* mutants accumulated higher levels of COP1, a feature similar to the *cop9-1* mutant. These results suggested that COP8, COP10, and COP11, together with COP1, COP9, and DET1, function to suppress the photomorphogenic developmental program and to promote skotomorphogenesis in darkness. The identical phenotypes resulting from mutations in *COP8*, *COP9*, *COP10*, and *COP11* imply that their encoded products function in close proximity, possibly with some of them as a complex, in the same signal transduction pathway.

INTRODUCTION

Light signals are captured by at least three different photoreceptor systems in higher plants: phytochromes, blue/UV-A light receptors, and UV-B light receptors (Quail, 1991; Young et al., 1992; Kaufman, 1993), each absorbing a different spectral quality of light. Following light perception, the signals are transduced and integrated into the developmental programs to influence a diverse range of processes, such as seed germination, seedling development, and flowering. Seedling development of Arabidopsis has been used as a model system to study the mechanisms of light signal transduction leading to photomorphogenesis in plants (Chory, 1993; Deng, 1994). Like most higher plants, Arabidopsis seedlings exhibit drastically different morphologies depending on whether they grow in the presence or absence of light. The dark-grown seedlings have long hypocotyls, apical hooks, and small and closed cotyledons that contain etioplasts and undergo developmental arrest at the seedling stage. The light-grown seedlings, in contrast, have little hypocotyl elongation, but have open, expanded, and green cotyledons that contain chloroplasts, have high-level expression of light-inducible genes, and proceed to adult and reproductive development (Kendrick and Kronenberg, 1993).

To understand the mechanism of light regulation of plant development, mutants with altered seedling morphology in response to light have been isolated. One group of mutants in Arabidopsis shows the dark-grown characteristic of long hypocotyls when grown in the light. These include seven long hypocotyl (*HY*) loci (*HY1*, *HY2*, *HY3*, *HY4*, *HY5*, *HY6*, and *HY8* [or *FRE1* or *FHY2*, both for far-red–elongated hypocotyl]) (Koornneef et al., 1980; Chory, 1989b; Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993), two additional *FHY* loci (*FHY1* and *FHY3*) (Whitelam et al., 1993), and three blue light–uninhibited loci (*BLU1*, *BLU2*, and *BLU3*) (Liscum and Hangarter, 1991). Recent molecular analyses revealed that

[1] To whom correspondence should be addressed.

many of these mutations lead to functionally defective photoreceptors, which result in the mutants with reduced sensitivity to light (Quail, 1991; Ahmad and Cashmore, 1993; Parks and Quail, 1993; Reed et al., 1993). Genetic studies on other loci such as *HY5*, *FHY1*, and *FHY3* implied that they may encode downstream components involved in the transmission of light signals from a particular photoreceptor or multiple photoreceptors (Koornneef et al., 1980; Whitelam et al., 1993).

The other group of mutants displays the light phenotype when grown in the dark. The reported Arabidopsis mutants of this type belong to three deetiolated loci (*DET1*, *DET2*, and *DET3*) (Chory et al., 1989a, 1991a; Cabrera y Poch et al., 1993) and five constitutively photomorphogenic loci (*COP1*, *COP9*, *COP2*, *COP3*, and *COP4*) (Deng et al., 1991; Deng and Quail, 1992; Wei and Deng, 1992; Hou et al., 1993). Among these mutants, *det1*, *cop1*, and *cop9* exhibit the most pleiotropic phenotypes: the dark-grown mutants have short hypocotyls, open and enlarged cotyledons, and altered patterns of cell differentiation and gene expression. These phenotypes indicate that DET1, COP1, and COP9 may be involved in early steps of the signal transduction pathway before it splits to branched pathways controlling individual responses of seedling development. The recessive nature of all of these mutants suggests that the normal functions of the gene products are to suppress the photomorphogenic development and direct skotomorphogenesis in darkness.

The repressor hypothesis is consistent with the molecular nature of COP1. The COP1 protein has a novel structural combination of an N-terminal zinc binding motif; a C-terminal WD-40 repeat, which is homologous to the β subunit of trimeric G proteins; and a potential coiled-coil helix structure in the middle (Deng et al., 1992; von Arnim and Deng, 1993; Deng, 1994; McNellis et al., 1994). This structure implies that COP1 has the potential to bind nucleic acids with its zinc binding motif, as well as to interact with protein factors through the coiled-coil or G_β protein domains. Interestingly, a subunit of the Drosophila TFIID transcription complex, $dTAF_{II}80$, has been reported to share homology with COP1 over the entire region of the protein except for the zinc binding domain (Dynlacht et al., 1993). Hence, COP1 may be able to interact with the transcriptional machinery on one hand, and on the other hand, to sense the incoming signal by interacting with other components of the light signaling network.

To gain further insight into the mechanisms of light signaling, it is necessary to identify other components in the circuitry. For this purpose, we have conducted an extensive screen of Arabidopsis mutants for a *cop1*-like phenotype. *COP9* was the first such locus reported and was postulated to function in proximity to *COP1* in the same pathway (Wei and Deng, 1992). Here, we report the identification and characterization of three new loci, *COP8*, *COP10*, and *COP11*, mutations of which result in phenotypes similar to those of the *cop1* and *cop9* mutants. For a better understanding of the phenotypes of the mutants, we have completed a detailed description of the temporal progression of the morphologies and cellular differentiation patterns of dark- and light-grown wild-type Arabidopsis seedlings. Our results suggested that in darkness, the new mutants are defective in suppressing photomorphogenic development and in promoting skotomorphogenesis. In addition, epistasis studies with other photomorphogenic loci provided information on the genetic hierarchy of these three loci in the light signaling circuitry. Recently, the characterization of the same three mutant strains in the context of the *FUSCA* phenotype and the molecular cloning of one of the loci have been reported (Castle and Meinke, 1994).

RESULTS

New Constitutive Photomorphogenic Mutants Define Three Genetic Loci

Three recessive mutants that exhibit pleiotropic constitutive photomorphogenic phenotypes were identified after further screening of the T-DNA–transformed Arabidopsis lines (Feldmann, 1991). Figure 1 shows dark-grown mutant seedlings (Figures 1B, 1C, 1D, and 1E) in comparison with dark- and light-grown wild-type seedlings (Figures 1A and 1F, respectively). All of the mutant seedlings displayed light-grown characteristics, such as short hypocotyls and open and expanded cotyledons when grown in the dark, similar to *cop1*, *cop9*, and *det1* mutants. Complementation tests were performed between these new mutants and the previously reported mutants of similar phenotype, namely *cop1-4*, *cop9-1*, *det1-1*, and *det2-1*. The results, as summarized in Table 1, clearly indicated that the three mutants define three new complementation groups. We named the new loci *COP8*, *COP10*, and *COP11*, and their corresponding mutant alleles *cop8-1*, *cop10-1*, and *cop11-1*. The light-grown *cop8-1*, *cop10-1*, and *cop11-1* mutants are extremely small; when full sized, they are less than 0.5 cm in diameter. The *cop8-1* and *cop11-1* mutants usually developed one or two tiny true leaves and accumulated very high levels of anthocyanin, and they died ∼2 weeks after germination. The *cop10-1* plants were able to develop up to six tiny true leaves and died in ∼3 to 4 weeks before reaching the reproductive stage. This adult lethal phenotype indicated that *COP8*, *COP10*, and *COP11* are essential for normal development of Arabidopsis in the light. Although these mutants were isolated from T-DNA insertional mutagenized lines, the mutations did not cosegregate with kanamycin resistance conferred by the T-DNA insert (data not shown).

It was first noted during the course of analyzing different alleles of *cop1* mutations that the lethal alleles of *cop1*, but not the strong and weak alleles, produce dark purple seeds (Deng et al., 1992; McNellis et al., 1994). *cop8-1*, *cop9-1*, *cop10-1*, and *cop11-1* mutations are also lethal, and their seeds are dark purple as well. Because the purple seed color was the basis for screening *fusca* (*fus*) mutants (Müller, 1963), it is not surprising that some of the *fus* mutants turned out to be allelic to the *cop* mutants. Complementation tests (McNellis et al., 1994; Miséra et al., 1994) revealed that *cop8* is allelic

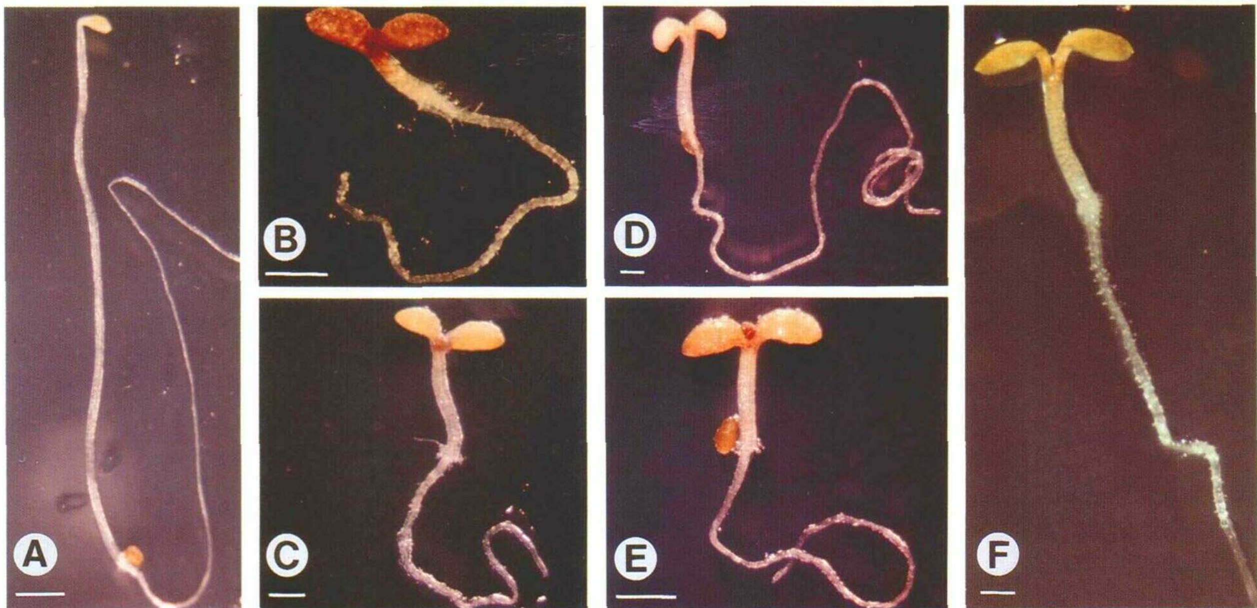

Figure 1. Morphologies of Dark-Grown Mutants in Comparison with Wild-Type Seedlings.

(A) Six-day-old dark-grown wild-type etiolated seedling.
(B) Seven-day-old dark-grown *cop8-1* mutant.
(C) Seven-day-old dark-grown *cop9-1* mutant.
(D) Eight-day-old dark-grown *cop10-1* mutant.
(E) Seven-day-old dark-grown *cop11-1* mutant.
(F) Five-day-old light-grown wild-type seedling.
The magnifications are different in **(A)** to **(F)**; bars = 1 mm in **(A)** to **(F)**.

to *fus8*, *cop10* is allelic to *fus9*, and *cop11* is allelic to *fus6*. In addition, Castle and Meinke (1994) have recently described the same three mutant lines in the context of *fus* mutants (*cop8-1* as *fus8-1*, *cop10-1* as *fus9-1*, and *cop11-1* as *fus6-2*). Among them, *COP11* has been cloned (Castle and Meinke, 1994), and it encodes a novel hydrophilic protein of 50.5 kD predicted to be rich in α-helical structures.

Table 1. Complementation Test of *cop8*, *cop10*, and *cop11* with *cop1*, *cop9*, *det1*, and *det2*

Crosses	*cop8-1*	*cop10-1*	*cop11-1*	*cop1-4*	*cop9-1*	*det1*	*det2*
cop8-1	No	Yes	Yes	Yes	Yes	Yes	Yes
cop10-1	Yes	No	Yes	Yes	Yes	Yes	Yes
cop11-1	Yes	Yes	No	Yes	Yes	Yes	Yes

The female parents are listed in the upper row, and the male parents are listed in the left column. Plants heterozygous for *cop8-1*, *cop9-1*, *cop10-1*, and *cop11-1* mutations and plants homozygous for *cop1-4*, *det1-1*, and *det2-1* mutations were used. For each cross, 47 to 200 F_1 seedlings were scored. The "No" complementation scores indicate that a quarter of the mutants in the F_1 populations were observed. The "Yes" scores indicate that all progeny in F_1 populations were wild type.

Cellular Basis for Skotomorphogenic and Photomorphogenic Development of Wild-Type Arabidopsis Seedlings

Explicit understanding of the defects caused by the cop mutations on seedling development requires knowledge of skotomorphogenic and photomorphogenic seedling development of wild-type Arabidopsis on a cellular basis. Although Arabidopsis seedling development has been widely used as a model system for genetic dissection of light signal transduction (Chory, 1993; Deng, 1994), basic information, such as a detailed description of the sequential changes in morphologies and in cellular differentiation patterns of developing seedlings in darkness and light, was not yet available. Thus, we have examined the overall morphology and the pattern of cell differentiation of developing wild-type Arabidopsis seedlings during germination in both light and dark conditions using an electron microscope.

Figure 2 shows the morphological changes of the developing seedlings in either complete darkness or light. No visible change was observed from day 0 to day 1 (Figures 2A, 2B, and 2C); at this stage the "seedling" was essentially an imbibed mature embryo inside its seed coat. From day 1 (24 hr

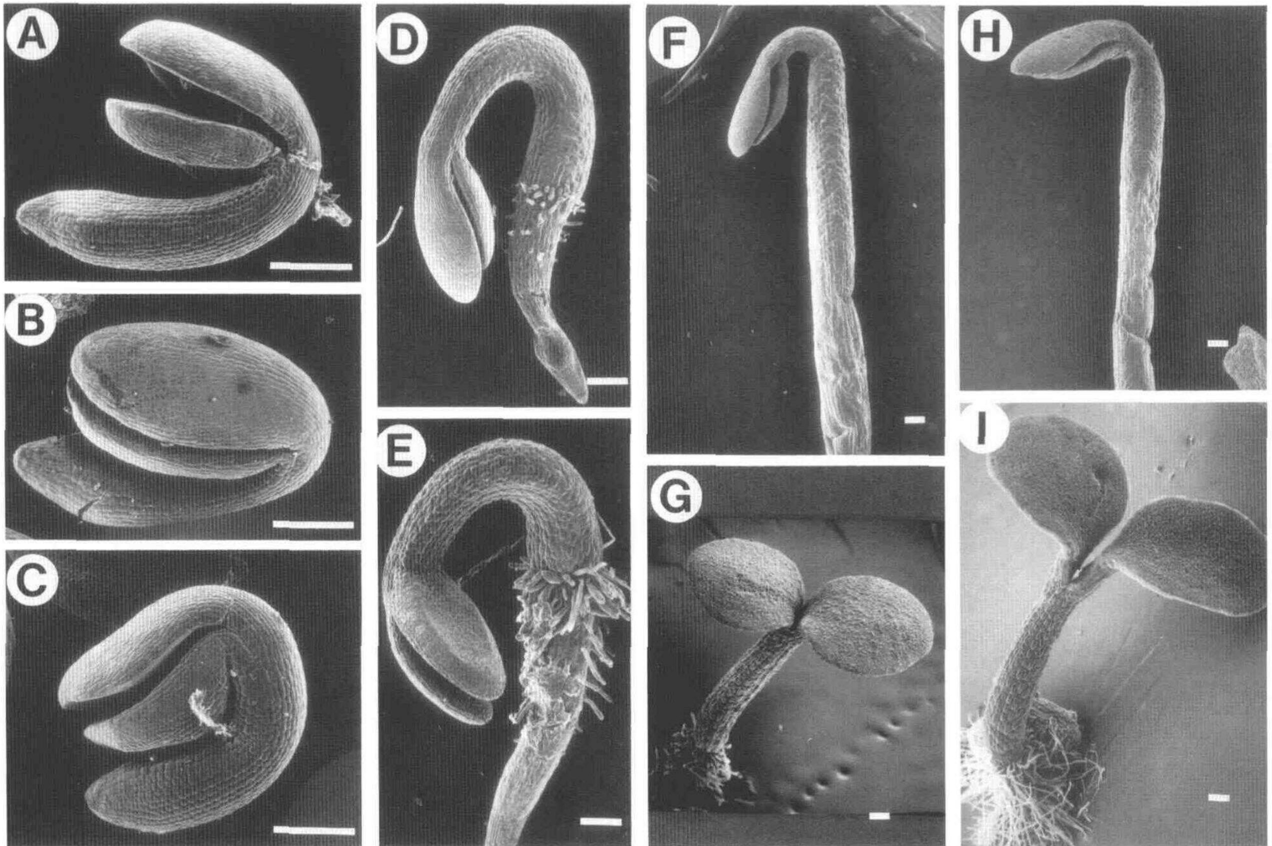

Figure 2. The Temporal Progression of Morphogenetic Changes of Developing Arabidopsis Seedlings in the Presence or Absence of Light as Examined by Scanning Electron Microscopy.

(A) Embryo from a seed at day 0.
(B) Seedling grown in the dark for 1 day.
(C) Seedling grown in the light for 1 day.
(D) Seedling grown in the dark for 2 days.
(E) Seedling grown in the light for 2 days.
(F) Seedling grown in the dark for 3 days.
(G) Seedling grown in the light for 3 days.
(H) Seedling grown in the dark for 4 days.
(I) Seedling grown in the light for 4 days.
The same magnification was used for the dark- and light-grown seedlings of the same age. Bars = 0.1 mm in **(A)** to **(I)**.

after germination) to day 2, the seedlings started to unwrap their seed coats. At day 2, seedlings emerged from their seed coats and root hairs appeared, delineating clearly the boundary between hypocotyl and root (Figures 2D and 2E). Both dark- and light-grown seedlings showed obvious hypocotyl and root growth but retained small and closed cotyledons, such that the dark- and light-grown seedling morphologies were hardly distinguishable from each other at this stage of development. Dramatic changes occurred between day 2 and day 3 after germination. At day 3, the dark-grown seedlings exhibited elongated hypocotyls with apical hooks and closed and undeveloped cotyledons (Figure 2F). The light-grown seedlings, in contrast, had short hypocotyls without apical hooks and had opened and expanded cotyledons (Figure 2G). By day 4, the distinct characteristics of dark/light morphologies were fully displayed (Figures 2H and 2I). No significant changes occurred in the later stage of seedling development, except further elongation of hypocotyls for the dark-grown seedlings and fast vegetative growth for the light-grown seedlings, which is symbolized by the appearance of true leaves.

Figure 3 shows the progressive changes in the pattern of cell differentiation in the hypocotyl surface of dark- and light-grown seedlings. Again, no difference was observed between dark- and light-grown seedlings during the first 2 days of germination, although there was cell enlargement from day 1 to day 2 in both cases (Figures 3A, 3B, 3C, and 3D). By day 3,

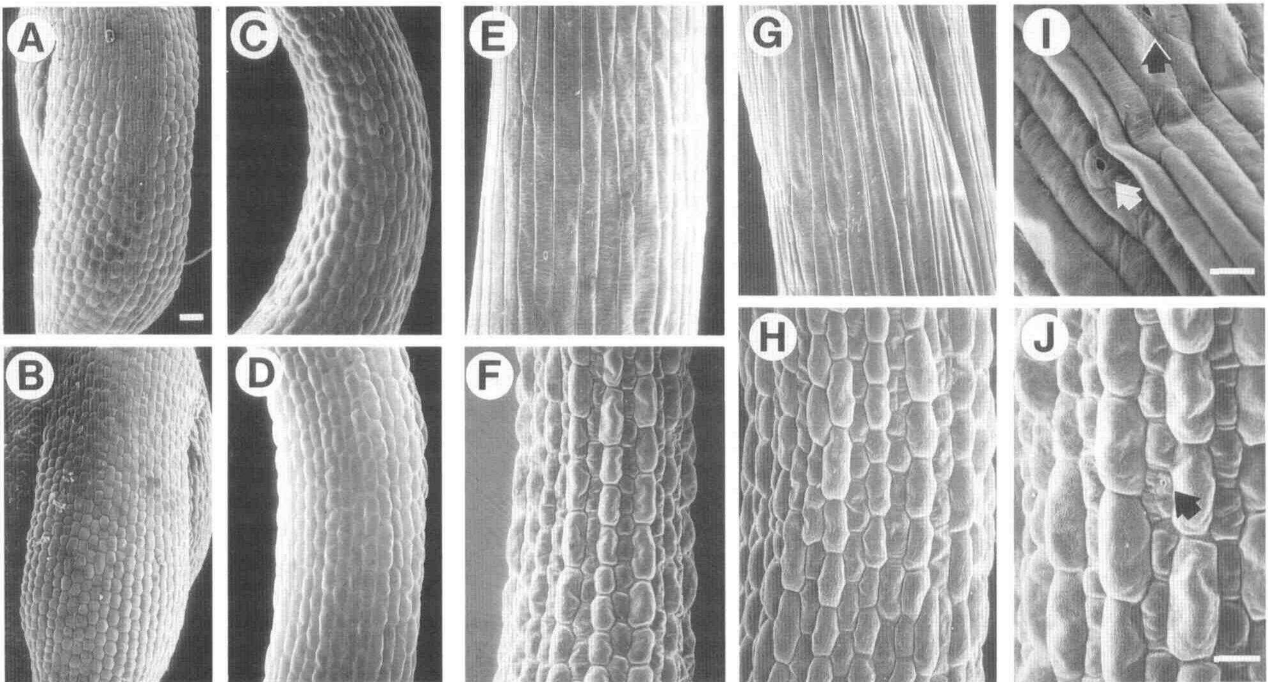

Figure 3. Hypocotyl Epidermal Cell Differentiation of Wild-Type Arabidopsis during Germination in the Dark or Light as Examined by Scanning Electron Microscopy.

(A) Seedling grown in the dark for 1 day.
(B) Seedling grown in the light for 1 day.
(C) Seedling grown in the dark for 2 days.
(D) Seedling grown in the light for 2 days.
(E) Seedling grown in the dark for 3 days.
(F) Seedling grown in the light for 3 days.
(G) Seedling grown in the dark for 4 days.
(H) Seedling grown in the light for 4 days.
(I) Seedling grown in the light for 10 days.
(J) Seedling grown in the light for 3 days [same as **(F)**].
Bars = 0.02 mm in **(A)**, **(I)**, and **(J)**. The same magnification was used in **(A)** to **(H)**; the scale was doubled in **(I)** and **(J)**. Stomatal structures in **(I)** and **(J)** are indicated by arrows.

the dark-grown hypocotyls showed extensively elongated cells and a smooth surface (Figure 3E), whereas the light-grown hypocotyl cells were only enlarged, which gave rise to a ridged surface (Figure 3F). In addition, stomatal structures appeared in the light-grown hypocotyls starting from day 3 (Figure 3J). Extended dark growth resulted in further elongation of hypocotyl cells (Figure 3G), but the stomatal structure differentiation was not observed even 10 days after germination (data not shown). Longer periods of growth in the light beyond day 3 led to more cell enlargement and cell division, as well as initiation and development of additional stomata (Figures 3H and 3I).

Figure 4 illustrates the changes in cotyledon surface cell differentiation during germination in darkness and light. Remarkably, even at day 0 (Figure 4A), there were some round cells evenly distributed among the epidermal cells (as indicated by arrows), which would eventually develop into stomatal guard cells. The same pattern was observed in the cotyledons of newly matured seeds before vernalization (data not shown). This result suggests that guard cell differentiation on the cotyledons is initiated at a late embryonic stage during seed maturation. Limited cell divisions were observed during the first day of germination regardless of light conditions (Figures 4B and 4C). Most of these divisions were uneven, resulting in a larger cell and a small round cell, the latter being a guard cell progenitor. The stomatal structure differentiation was the most obvious event on the second day of germination. The round progenitor cells divided into two guard cells to form immature stomatal structures that were unopened (Figures 4D and 4E). Up to this stage, the differentiation of guard cells is a light-independent process. By day 3, the light-grown cotyledons had significantly enlarged epidermal cells and stomatal

guard cells (Figure 4G). Notice the presence of stomata at different stages of development (indicated by arrows in Figure 4G): the large mature stomata have opened, and in the meantime, small immature stomata have been produced by active cell divisions. Cell enlargement of light-grown cotyledons continued until day 4 (Figure 4I) and beyond, producing larger and more irregularly shaped epidermal cells as compared with those from day 3. Therefore, both cell division and cell enlargement led to cotyledon expansion of light-grown seedlings. In contrast, cotyledon development in darkness was arrested after 2 days of germination; cotyledon surface cells at day 3, day 4, and later on (Figures 4F, 4H, and data not shown, respectively) looked very similar to those at day 2. The absence of mature and opened stomatal structure in dark-grown seedlings suggested that maturation and opening of stomatal structures require the presence of light.

Mutations in *COP8*, *COP10*, and *COP11* Result in Photomorphogenic Cell Differentiation in Darkness

The resemblance of dark-grown *cop8-1*, *cop10-1*, and *cop11-1* mutants to light-grown wild-type seedlings (Figure 1) prompted us to examine the pattern of cell differentiation in cotyledons and hypocotyls of mutant seedlings during germination in darkness. After seedlings had emerged from their seed coats, the apical hooks quickly unfolded, and the hypocotyls did not elongate even after an extended period of dark growth. Furthermore, cell division, differentiation, and enlargement, which are characteristics of light-grown seedlings, occurred in dark-grown mutant seedlings.

Figure 5 shows the scanning electron micrographs of cotyledons and hypocotyls of 8-day-old dark-grown mutants. It is worth mentioning that the mutants germinate and grow

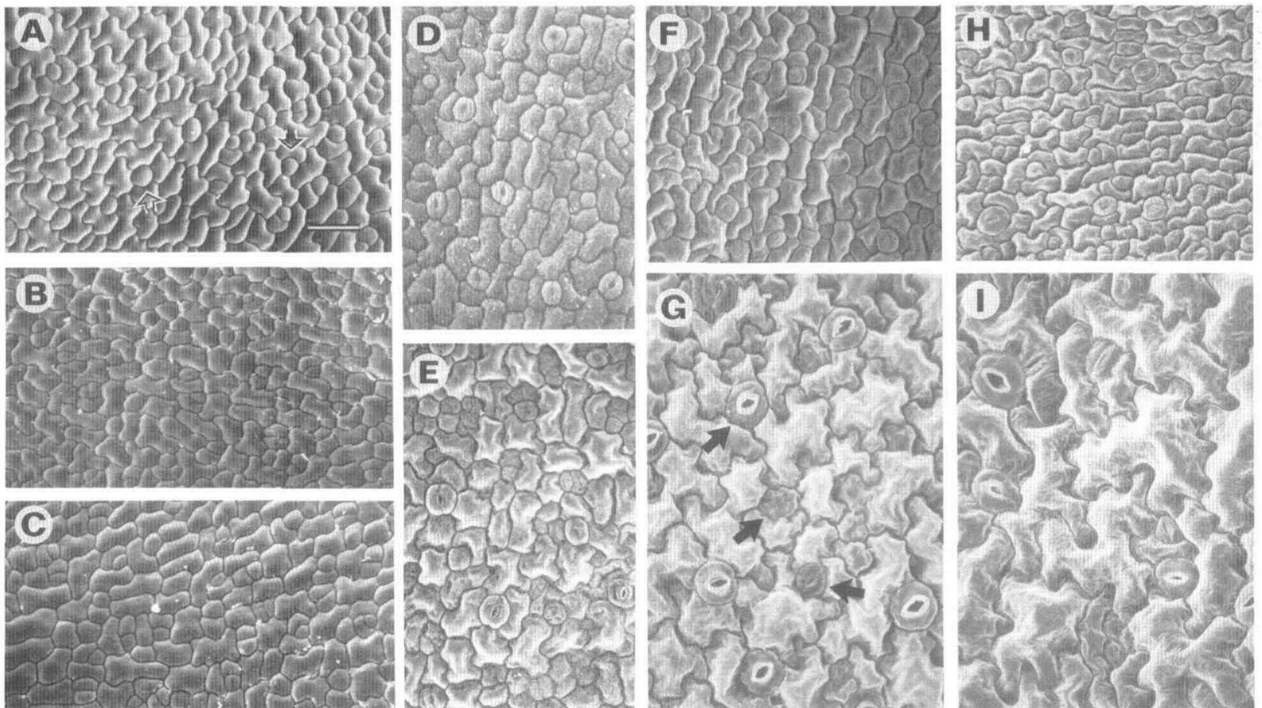

Figure 4. Cotyledon Epidermal Cell Differentiation of Wild-Type Arabidopsis during Germination in the Dark or Light as Examined by Scanning Electron Microscopy.

(A) Embryo from a seed at day 0.
(B) Seedling grown in the dark for 1 day.
(C) Seedling grown in the light for 1 day.
(D) Seedling grown in the dark for 2 days.
(E) Seedling grown in the light for 2 days.
(F) Seedling grown in the dark for 3 days.
(G) Seedling grown in the light for 3 days.
(H) Seedling grown in the dark for 4 days.
(I) Seedling grown in the light for 4 days.
The same magnification was used in **(A)** to **(I)**. Bar in **(A)** = 0.02 mm. Representative guard cell progenitors and stomatal structures in **(A)** and **(G)** are indicated by arrows.

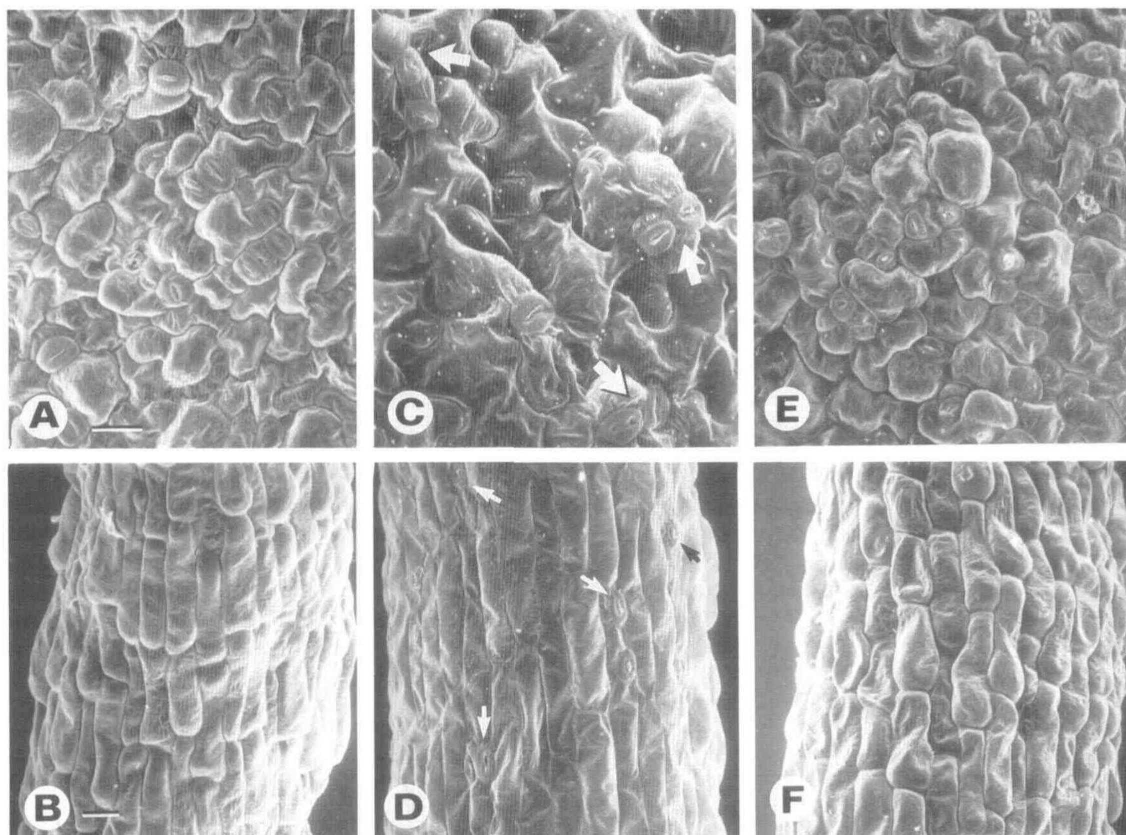

Figure 5. Epidermal Surfaces of 8-Day-Old Dark-Grown Mutant Seedlings as Examined by Scanning Electron Microscopy.

(A) *cop8-1* cotyledon.
(B) *cop8-1* hypocotyl.
(C) *cop10-1* cotyledon.
(D) *cop10-1* hypocotyl.
(E) *cop11-1* cotyledon.
(F) *cop11-1* hypocotyl.
Bars = 0.02 mm in **(A)** and **(B)**. **(A)**, **(C)**, and **(E)** are at the same magnification, and **(B)**, **(D)**, and **(F)** have the same magnification. Representative stomatal structures in **(C)** and **(D)** are indicated by arrows.

relatively slowly, and 8-day-old mutants are comparable to 4- to 5-day-old wild-type seedlings. The cotyledon epidermal cells of the mutants were enlarged to various degrees as compared to those of etiolated wild type (compare Figures 5A, 5C, and 5E to Figure 4H). The cell enlargement was especially evident in *cop10-1* (Figure 5C), which also exhibited the most cotyledon expansion (Figure 1D). Unlike the wild type, spacing of stomatal structures in the mutant cotyledons was uneven, with many of them being clustered in a group of two or more (representative groups are indicated by arrows in Figure 5C). Such uncoordinated stomata differentiation and cell enlargement resulted in a rough surface in the mutant cotyledons. In addition, some stomatal structures in the mutants were open in the dark. The hypocotyl cells of *cop8-1*, *cop10-1*, and *cop11-1* mutants showed very limited elongation in the dark (Figures 5B, 5D, and 5F, respectively), similar to those of light-grown wild-type seedlings (Figures 2I and 3H), and in contrast to the etiolated wild-type seedling (Figures 2H and 3G). Moreover, mature and opened stomatal structures were observed in the hypocotyls of dark-grown *cop10-1* mutants (indicated by arrows in Figure 5D), which were quite densely distributed as in the light-grown seedling of similar stage. These results suggest that the mutations in *COP8*, *COP10*, and *COP11* resulted in seedling development that proceeds according to the photomorphogenic program regardless of ambient light conditions.

Plastids of the Dark-Grown *cop8-1*, *cop10-1*, and *cop11-1* Mutants Are Aberrant

In angiosperms, the normal sequence of plastid development in light is from proplastid through amyloplastidic intermediate

stages to chloroplast. In the dark, the plastids develop from proplastids to etioplasts, which then convert to chloroplasts once exposed to light (Virgin and Egneus, 1983). We examined the effect of the *cop8-1*, *cop10-1*, and *cop11-1* mutations on plastid development.

Figure 6 shows representative plastids from the dark-grown mutants in comparison with the typical etioplast and chloroplast from wild-type Arabidopsis seedlings. The majority of plastids in all three dark-grown mutants (Figures 6C, 6D, and 6E) contained neither the characteristic prolamellar bodies of etioplasts (Figure 6A) nor thylakoid membrane stacking, which is a distinctive feature of chloroplasts (Figure 6B). With round shape and the invaginative circular membranes, these plastids closely resemble proplastids or their partially developed form, which prevail in embryonic or meristematic tissues (Schnepf, 1980). It is worth noting that in a recent report, Castle and Meinke (1994) showed that the plastids of dark-grown *fus6-1*, allelic to *cop11*, typically exhibit smaller prolamellar bodies. This observation suggests that the *fus6-1* allele of *cop11* has a less severe defect than *fus6-2* in plastid etiolation in darkness. Although we did not observe smaller prolamellar bodies in plastids of our dark-grown mutants, we cannot rule out their presence in a small fraction of plastids due to the limited number of sections examined. Collectively, our results indicated

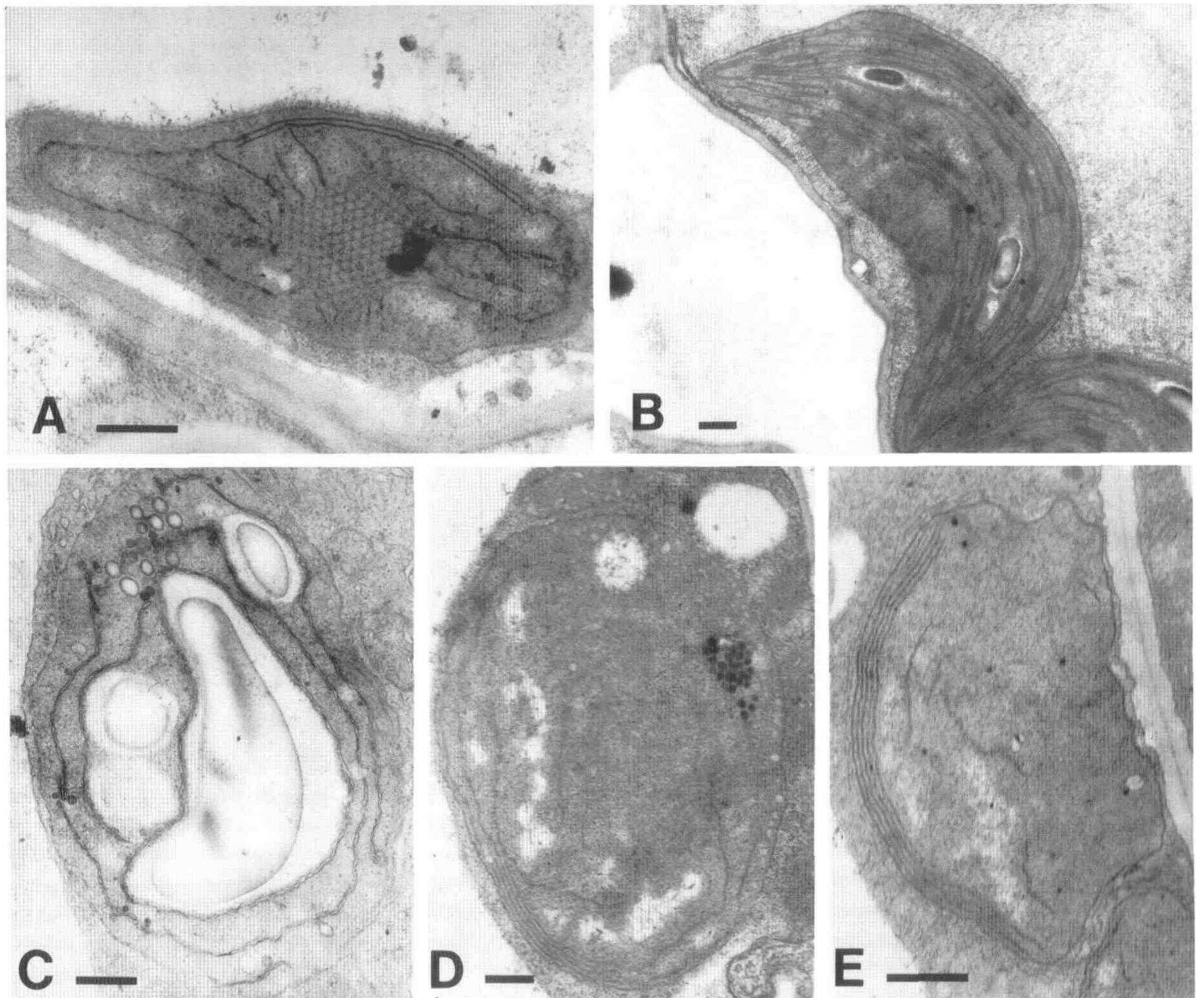

Figure 6. Plastid Development in 6-Day-Old Wild-Type and Mutant Seedlings.

(A) Etioplast from a dark-grown wild-type seedling.
(B) Chloroplast from a light-grown wild-type seedling.
(C) Plastid from a dark-grown *cop8-1* mutant.
(D) Plastid from a dark-grown *cop10-1* mutant.
(E) Plastid from a dark-grown *cop11-1* mutant.
Bars = 0.6 μm in **(A)** to **(E)**.

that the pathway leading to etioplast development in the dark is impaired to different degrees in these mutants. Because the light-grown mutants contained structurally normal chloroplasts (data not shown), the pathway leading to chloroplast differentiation in the light did not seem to be directly affected by these mutations.

Light-Inducible Genes Are Actively Expressed in Dark-Grown and Dark-Adapted Mutants

The expression of many photosynthesis-related genes is tightly regulated by light. This regulation is absent in the *cop1*, *cop9*, and *det1* mutants, which results in a more or less constitutive expression of normally light-inducible genes (Chory et al., 1989a; Deng et al., 1991; Wei and Deng, 1992). In *cop1* and *cop9*, the capability of dark adaptation in light-grown plants is also affected. Here, we have investigated the effect of *cop8-1*, *cop10-1*, and *cop11-1* mutations on the expression of light-inducible genes; the results are shown in Figure 7. In all three mutants, both nuclear-encoded genes (*rbcS*, *cab*, and *fedA*) and a chloroplast-encoded gene (*psbA*) are expressed in the dark (Figure 7, lanes D) at levels similar to those in the light (Figure 7, lanes L). Similar results for *rbcS* gene expression in another *cop11* allele, *fus6-1*, was recently reported (Castle and Meinke, 1994). For the wild type, however, the mRNA levels of these genes are much reduced in dark-grown plants. These results indicate that the mutants failed to repress both nuclear- and chloroplast-encoded light-inducible genes in the dark. In addition, there is no down-regulation of *rbcS*, *cab*, and *fedA* expression in dark-adapted mutants, in contrast to the pattern of expression found in wild-type plants (Figure 7, lanes DA), which indicates that the mutations abolish the ability of the plants to undergo dark adaptation. We also noted that, regardless of light conditions, the mutants have an approximately five- to 10-fold lower level of *cab* gene expression than do the light-grown wild-type plants. Consequently, the RNA gel blot was exposed longer for mutant sets to get equivalent signals. The same phenomenon was observed previously with the *cop9-1* mutant (Wei and Deng, 1992). It is likely that *fus6-1* (allelic to *cop11-1*) also has a lower level of *cab* gene expression. This may explain the result from Castle and Meinke (1994), in which *cab* expression was not detected in either dark- or light-grown *fus6-1* mutants. It is likely that, in addition to light, *cab* expression is regulated by other developmental signals that may also be affected by the mutations.

To determine whether the altered pattern of gene expression is controlled at the level of transcription, we introduced the Arabidopsis *rbcS-1A* promoter–β-glucuronidase (*GUS*) chimeric gene (Wei and Deng, 1992) into the mutants so that GUS activity could be used as an indicator of promoter activity. Figure 8 shows the comparison of the GUS activities in dark- and light-grown mutants with wild-type seedlings. In wild-type Arabidopsis, the GUS activity of dark-grown seedlings was less than half the value of light-grown seedlings, which is consistent with light induction of the promoter activity reported (Wei and Deng, 1992). In the *cop8-1* and *cop10-1* mutants, however, the dark-grown seedlings had comparable or slightly higher GUS activities than the light-grown siblings (Figures 8A and 8B). Due to the close linkage of the *cop11-1* mutation with the genomic location of the T-DNA carrying the *rbcS-1A* promoter–*GUS* chimeric gene, direct comparison of GUS activities between mutant and wild-type seedlings in the F_2 population was impractical. However, GUS activities of dark- and light-grown *cop11-1* mutants were very much alike (data not shown) and were similar to *cop8-1* and *cop10-1* mutants. In conclusion, the data suggested that the introduced *rbcS-1A* promoter is as active in the dark-grown *cop8-1*, *cop10-1*, and *cop11-1* mutants as in their light-grown siblings. It is worth noting that the GUS activities in both dark- and light-grown mutants were lower than that of light-grown wild-type seedlings (Figure 8), whereas their *rbcS* mRNA levels were similar (Figure 7). This is most likely due to the fact that GUS activity was based on equal amounts of total proteins (not total RNA as is the case for mRNA

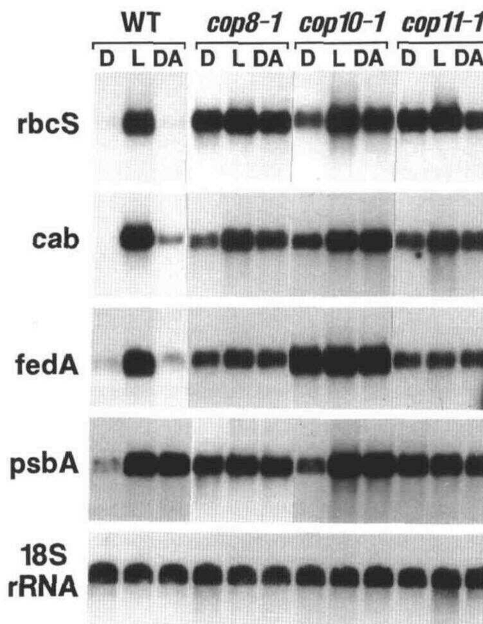

Figure 7. RNA Gel Blot Analysis of the Steady State mRNA Levels of Representative Light-Inducible Genes in the Wild Type and Mutants.

Seedlings of the wild type (WT) and *cop8-1*, *cop10-1*, and *cop11-1* mutants were grown in the dark (D) or continuous light (L) for 7 days or for 7 days under continuous light followed by 2 days of dark adaptation (DA). The hybridization probes are the following: *rbcS*, gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase (Krebbers et al., 1988); *fedA*, ferredoxin type A gene (Somers et al., 1990); *cab*, gene encoding the chlorophyll *a/b* binding protein of photosynthetic light-harvesting complexes (Leutwiler et al., 1986); *psbA*, plastid gene encoding the 32-kD protein of photosystem II (Zurawski et al., 1982); and 18S rRNA, cytoplasmic 18S ribosomal RNA (Jorgensen et al., 1987). Two micrograms of total RNA was used in each lane for hybridization with *rbcS* and *psbA*; 4 μg was used for *cab* and *fedA*; and 1 μg was used for the 18S probe.

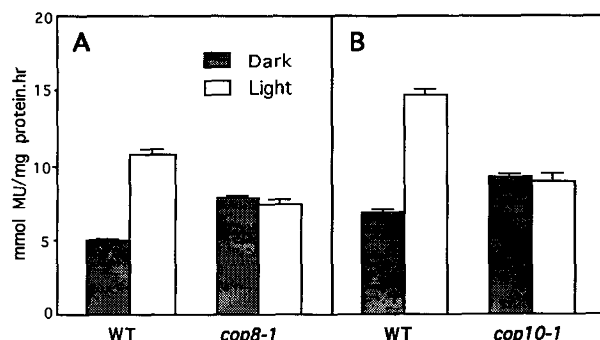

Figure 8. GUS Activity Assays Resulting from *rbcS-1A* Promoter–*GUS* Fusion in Wild-Type or Mutant Seedlings Grown in the Dark or Light.

(A) *cop8-1* mutant compared with the wild type (WT).
(B) *cop10-1* mutant compared with the wild type (WT).
GUS activities shown (millimoles of 4-methylumbelliferone [MU] per milligram of total protein per hr) are the average of three independent measurements; error bars represent standard deviation.

levels), and the protein content per seedling (or per cell basis) is quite different between mutant and wild-type seedlings because of their physiological differences. Nevertheless, it was reasonable to conclude that the high level of gene expression in the dark, as shown in Figure 7, was due to transcriptional activation or derepression of the corresponding promoter.

Mutations at *COP8*, *COP10*, and *COP11* Loci Do Not Affect Phytochrome Control of Seed Germination

In many plant species, the Pfr form of phytochromes, which usually exist in seeds prior to any light treatment, stimulates germination (reviewed in Frankland and Taylorson, 1983). Among the photomorphogenic mutants reported so far, only *det1* results in photoinsensitivity of seed germination (Chory et al., 1989a). We conducted the germination tests for *cop8-1*, *cop10-1*, and *cop11-1* mutants, and the results are summarized in Table 2. Clearly, the red light treatment, which converts Pr to Pfr, increased germination rates over the corresponding dark controls or almost equaled the control in the case of *cop10-1*. In all cases, the far-red light treatment, which converts the preexisting Pfr to Pr, caused a decrease in germination rates. Again, this effect could be reversed by an additional red light treatment, which resulted in an increase in the germination rates in all cases. In conclusion, seed germination of *cop8-1*, *cop10-1*, and *cop11-1* mutants remained responsive to phytochrome, similar to *cop1* and *cop9* mutants.

cop8-1, *cop10-1*, and *cop11-1* Mutations Are Epistatic to *hy1*, *hy2*, *hy3*, *hy4*, and *hy5* Mutations

The phenotypes of *cop8-1*, *cop10-1* and *cop11-1* suggested that the mutations uncouple the photomorphogenic responses from light signals. To define the regulatory relationships of these newly identified genetic loci to the photoreceptors and other light regulatory components, double mutants homozygous for each of the three mutations and individual *hy* (*hy1*, *hy2*, *hy3*, *hy4*, and *hy5*) mutations were constructed. In all cases, the overall morphologies of double mutants were very similar to those of the parental *cop* mutants rather than the *hy* mutants. The hypocotyl lengths of dark- and light-grown parental mutants and the double mutants were measured, and the result is depicted in Figure 9.

In both light and dark conditions, the hypocotyl lengths of *hy1/cop8-1*, *hy2/cop8-1*, *hy3/cop8-1*, and *hy4/cop8-1* double mutants were short, similar to the *cop8-1* single mutants. The *hy* mutants, in contrast, displayed elongated hypocotyls that were more than 18-fold longer in the dark and more than ninefold longer in the light than were the hypocotyls of the double and *cop8-1* single mutants (Figures 9A and 9B). Similar results were also obtained for the *hy1/cop10-1*, *hy2/cop10-1*, *hy3/cop10-1*, *hy4/cop10-1*, *hy1/cop11-1*, *hy2/cop11-1*, *hy3/cop11-1*, and *hy4/cop11-1* double mutants (Figures 9A and 9B). These data established that *cop8-1*, *cop10-1*, and *cop11-1* are epistatic to *hy1*, *hy2*, *hy3*, and *hy4*. Because *hy1*, *hy2*, and *hy3* are phytochrome mutants (Quail, 1991; Chory, 1993) and *hy4* is probably a blue light receptor mutant (Ahmad and Cashmore, 1993), these epistatic relationships imply that *COP8*, *COP10*, and *COP11* act downstream of both phytochromes and a blue light receptor.

HY5 most likely encodes a signal transduction component downstream of both phytochromes and a blue light receptor (Koornneef et al., 1980; Deng, 1994). The double mutants of *hy5/cop8-1*, *hy5/cop10-1*, and *hy5/cop11-1* all had short hypocotyls with their lengths about the same as, or only slightly

Table 2. Germination Rates of *cop8-1*, *cop10-1*, and *cop11-1* Mutant Seeds after Different Light Treatments

Light Treatment	Number of Seedlings/Number of Seeds (Germination Rate %)			
	Wild Type	*cop8-1*	*cop10-1*	*cop11-1*
Dark	192/244 (79)	13/69 (19)	92/92 (100)	1/77 (1)
Red	450/473 (95)	44/146 (30)	149/158 (94)	23/167 (14)
Far-Red	72/460 (16)	10/144 (7)	47/153 (31)	0/150 (0)
Far-Red/Red	409/440 (93)	59/148 (40)	149/153 (97)	62/152 (41)

Wild-type and mutant seeds were planted on solid growth medium plates, and the total number of seeds was counted at the end of planting. Cold treatment at 4°C in darkness was 4 days for wild-type and *cop10-1* seeds and 7 days for *cop8-1* and *cop11-1* seeds to improve their germination rates. The light treatments were a 10-sec saturating far-red light pulse (Far-Red), a 5-sec saturating red light pulse (Red), or a 10-sec far-red light pulse immediately followed by a 5-sec red light pulse (Far-Red/Red). After light treatments, the plates were kept in the dark at 22°C for 6 days before scoring for germination.

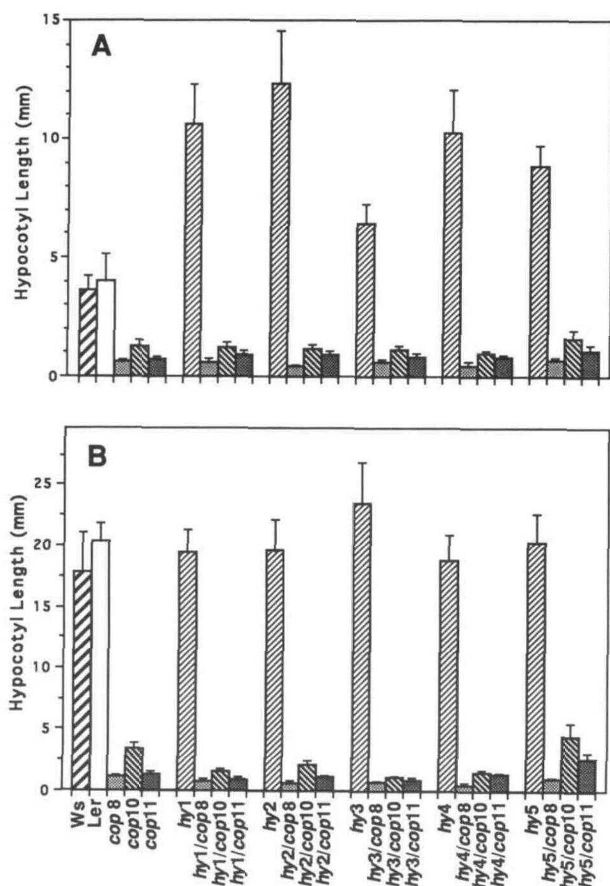

Figure 9. Comparisons of Hypocotyl Lengths of 8-Day-Old Dark- and Light-Grown Wild-Type, *hy*, *cop*, and *hy/cop* Double Mutant Seedlings.

(A) Seedlings grown under white light.

(B) Seedlings grown in the dark.

All values represent the average of more than 30 seedlings for each sample, and the error bars represent the standard deviation. Ws and Ler indicate wild types of Wassilewskija and Landsberg *erecta* ecotypes. *cop8* = *cop8-1*; *cop10* = *cop10-1*; *cop11* = *cop11-1*.

longer than, the corresponding *cop* single mutants in both light and dark conditions (Figures 9A and 9B). Moreover, the overall morphology, color, and lethality of the double mutants all resembled their parental *cop* mutants. These results suggested that the mutations in *cop8-1*, *cop10-1*, and *cop11-1* are epistatic to *hy5*. Therefore, *COP8*, *COP10*, and *COP11* may function downstream of *HY5*, in a position similar to *COP1* and *COP9* (Ang and Deng, 1994; N. Wei, D. Chamovitz, and X.-W. Deng, manuscript in preparation).

cop8-1, *cop10-1*, and *cop11-1* Mutants Accumulate Higher Levels of COP1 in a Light-Independent Manner

As described earlier, *cop8-1*, *cop10-1*, and *cop11-1* have a very similar phenotype to *cop1* and *cop9*, which partially overlaps with *det1*. We asked whether expression of COP1 is negatively affected by these mutations, because this would help to explain the almost identical phenotypes of these mutants with the lethal alleles of *cop1* mutants. We examined the COP1 protein levels in these mutants by protein gel blot analysis, and the result, as shown in Figure 10, indicates that COP1 protein accumulated to a higher level in *cop8-1*, *cop10-1*, and *cop11-1* mutants than in wild-type seedlings. In addition, this overexpression is light independent, because both light-grown (lanes 1 to 4) and dark-grown (lanes 5 to 8) seedlings had similar results. Interestingly, COP1 has also been found to accumulate at higher levels in both dark- and light-grown *cop9* and *det1* mutants at both mRNA and protein levels (Wei and Deng, 1992; A.G. von Arnim and X.-W. Deng, data not shown). Thus, all of the complete pleiotropic photomorphogenic mutants examined, except for most *cop1* mutant alleles, had higher levels of COP1.

DISCUSSION

In this study, we report the isolation and detailed characterization of three mutants that define three new loci involved in light-regulated development. We also make several observations about the mechanism of light regulation of seedling development.

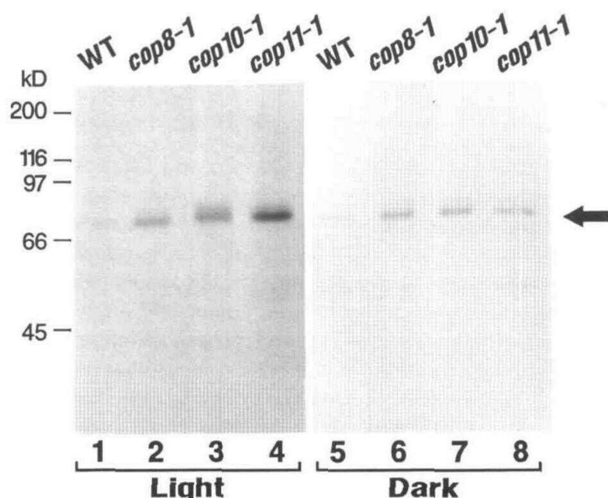

Figure 10. Protein Gel Blot Analysis of COP1 Protein in Dark- and Light-Grown Wild-Type and Mutant Seedlings.

Approximately 6 μg of total protein per lane was loaded for the "light" samples (lanes 1, 2, 3, and 4); ~2 μg per lane was loaded for the "dark" samples (lanes 5, 6, 7, and 8). Wild-type (WT) or mutant (*cop8-1*, *cop10-1*, and *cop11-1*) extracts are indicated above each lane. The arrow on the right indicates the position of COP1 protein. The positions of protein size markers (in kilodaltons) are indicated on the left.

Dark- and Light-Grown Seedlings Display Distinct Patterns of Cellular Differentiation after Two Days of Germination

To better understand the effects of these mutations, we studied the temporal progression in morphologies and cellular differentiation patterns during germination and seedling development of Arabidopsis in the presence and absence of light. Our results showed that dark- and light-grown seedlings follow similar patterns of cellular and morphological differentiation during the first 2 days of germination. After this point, the differentiation patterns dramatically diverged according to the light environments, which resulted in contrasting morphologies of dark- and light-grown seedlings. In darkness, cotyledon development was completely arrested 2 days after germination, whereas the hypocotyl rapidly grew longitudinally as a result of extensive cell elongation but exhibited no sign of stomatal structure differentiation. In the light, cell division, enlargement, and differentiation accelerated in both hypocotyl and cotyledons, accompanied by a loss of the apical hook.

Plastid development during germination was at a pace similar to the progression of the overall morphology. The transitions from proplastid to etioplast in darkness, or to chloroplast in the light, occurred approximately between day 2 and day 3 after germination (data not shown). This is consistent with the observation that in barley the distinct patterns of plastid differentiation become apparent after 48 hr of germination (Schnepf, 1980). It is possible that seedlings have to develop to a certain stage to become physiologically competent to respond to light signals. Alternatively, seedlings are already determined to one of the two developmental pathways, and the first 2 days of germination may reflect the time needed to initiate the distinct biochemical processes, which in turn lead to contrasting cellular differentiation patterns.

***COP1*, *COP8*, *COP9*, *COP10*, *COP11*, and *DET1* Are Crucial for the Developmental Switch between Skotomorphogenesis and Photomorphogenesis**

In the *cop8*, *cop10*, and *cop11* mutants, the seedling developmental program in darkness was completely altered. The dark-grown mutants seemed to bypass the requirement of light stimuli and followed the photomorphogenic developmental pathway constitutively with respect to morphology, the pattern of cell differentiation, and gene expression. This pleiotropic phenotype suggests that, like *COP1*, *COP9*, and *DET1*, *COP8*, *COP10*, and *COP11* are involved in controlling the primary switch of whether to follow photomorphogenic or skotomorphogenic pathways according to the ambient light conditions. However, their roles during photomorphogenic development do not exclude functions in other fundamental processes. All of the strong alleles from this group were adult lethal even under light conditions, indicating that their gene products are essential for normal growth and development. Recently, it has been shown (Castle and Meinke, 1994) that mutations in all these loci also led to altered plant responses to both endogenous factors (sugars and phytohormones) and environmental signals (nutrients and light). Therefore, their gene products may constitute or regulate a developmental switch, which is subjected to regulation by light as well as other signals.

Other photomorphogenic mutants, including *det2* (Chory et al., 1991a), *det3* (Cabrera y Poch et al., 1993), and *cop2*, *cop3*, and *cop4* (Hou et al., 1993) display only partially pleiotropic phenotypes. For example, all of these mutants develop normal etioplasts in the dark. Some of them (*cop2*, *cop3*, and *cop4*) have elongated hypocotyls in the dark; some of the mutations (*cop2*, *cop3*, and *det3*) do not affect light-regulated gene expression. The existence of these mutants has three implications. First, the individual processes of photomorphogenic development can be uncoupled from each other. Second, different combinations of the components may be required for different light-regulated processes. Third, this group of loci (*DET2*, *COP2*, *COP3*, *COP4*, and *DET3*) is only involved in a subset of functions, whereas *DET1*, *COP1*, *COP8*, *COP9*, *COP10*, and *COP11* may be involved in controlling the primary switch between the two distinct developmental programs: photomorphogenesis or skotomorphogenesis.

Genetic Hierarchy of Photomorphogenic Loci

Phenotypic analyses of double mutants between mutations in *COP* or *DET* loci and mutations of phytochromes and a blue light receptor revealed that *det1*, *det2*, *cop1*, *cop8*, *cop9*, *cop10*, and *cop11* are epistatic to both classes of photoreceptor mutants (Deng et al., 1991; Chory, 1992; Ang and Deng, 1994; N. Wei, D. Chamovitz, and X.-W. Deng, manuscript in preparation). This relationship, together with the pleiotropic phenotype of these mutants, implies that *DET1*, *COP1*, *COP8*, *COP9*, *COP10*, and *COP11* are each essential for both phytochrome and blue light receptor–mediated photomorphogenic responses. Therefore, light signals perceived by phytochromes and blue light receptors somehow converge to common regulatory steps defined by the above mentioned mutations before the pathway branches for individual responses.

Our data showed that *cop8-1*, *cop10-1*, and *cop11-1* mutations are epistatic to *hy5*, and therefore the three new loci, just like *COP9* (N. Wei, D. Chamovitz, and X.-W. Deng, manuscript in preparation), are placed downstream of *HY5*. Chory (1992) reported that *det1* mutations were partially suppressed by *hy5* and suggested that *DET1* acts either in an independent pathway or upstream of *HY5*. Detailed epistasis analysis between *hy5* and different alleles of *cop1* revealed that they interact in an allele-specific and light-dependent manner, suggesting the possibility of direct interaction of HY5 and COP1 (Ang and Deng, 1994). Since the null allele of *cop1*, *cop1-5*, is completely epistatic to *hy5*, *COP1* is placed immediately downstream of *HY5*. Therefore, *COP1*, *COP8*, *COP9*, *COP10*, and *COP11* all act downstream of *HY5* and are distinct from the hierarchical position of *DET1* in the light signaling circuitry. This idea is

also supported by the fact that *det1* mutants differ from *cop8*, *cop10*, *cop11*, as well as *cop1* and *cop9* mutants in two physiological properties. First, mutations in *det1* do not affect dark-adaptive regulation of gene expression in light-grown plants. Second, mutations in *det1* abolish phytochrome control of seed germination and result in germination irresponsive to red/far-red light treatment (Chory et al., 1989a).

The COP1 protein accumulated to higher levels in *cop8-1*, *cop9-1*, *cop10-1*, and *cop11-1* mutants (Figure 10; A.G. von Arnim and X.-W. Deng, unpublished data). It is unlikely that the "*cop1*-like" phenotype in *cop8*, *cop9*, *cop10*, and *cop11* is caused by the elevated COP1 level because it is the loss of COP1 function that causes the *cop1* phenotype. Conversely, overexpression of COP1 should have, if any, an opposite effect—dark-grown seedling characteristics in the light. Therefore, the *cop* phenotype must be conferred directly by *cop8*, *cop9*, *cop10*, and *cop11* mutations, and it cannot be compensated for or suppressed by merely raising the COP1 level per se or by the observed overexpression of the COP1 protein. It is possible that *COP8*, *COP9*, *COP10*, and *COP11* are somehow involved, directly or indirectly, in repression of *COP1* gene expression by a feedback control mechanism. However, such regulation of *COP1* expression is unlikely a key step in the light signal transduction pathways, because the regulation is independent of light signals. All evidence points to the fact that *cop8*, *cop10*, and *cop11* mutants closely resemble the *cop9* mutant. This supports the hypothesis that *COP8*, *COP10*, and *COP11* may function in proximity to *COP9* in the same signal transduction pathway, with the possibility that some of them may encode subunits of a functional complex.

Is Photomorphogenesis a Default Pathway of Seedling Development?

Until now, loss-of-function mutations in three *DET* and eight *COP* loci of Arabidopsis have been described (Chory, 1993; Deng, 1994). All of these mutations result in either complete or partial photomorphogenic development of dark-grown seedlings. Additional mutants are likely to be found, because the mutagenesis screens have not reached saturation. Therefore, a large number of genes are involved in suppressing photomorphogenic development in darkness. Besides the numerous mutations, external stimuli other than light can also cause photomorphogenic responses in darkness. Chory et al. (1991b) reported that cytokinins, when applied to the dark-germinated Arabidopsis, enabled the wild-type seedlings to display some phenotypic features of *det1* mutants. Recently, Araki and Komeda (1993) reported that constant shaking of liquid-cultured Arabidopsis seedlings in the dark can induce some photomorphogenic traits and eventually lead to flowering. In addition, cyclic heat treatment was reported to direct photomorphogenesis-like development in dark-grown pea (Kloppstech et al., 1991) and barley (Beator et al., 1992).

All of these observations are consistent with a hypothesis that photomorphogenesis is the default pathway of seedling development. To undergo skotomorphogenesis, many gene products are involved in repressing the photomorphogenic development, and the repression is released in response to light. Consequently, loss-of-function mutations in any one of these genes would result in the default pathway of development: photomorphogensis. Certain external stimuli, such as the presence of a phytohormone, cyclic heat treatment, or mechanical stimulation, may somehow reduce the activities of some of the suppressive components and result in the default pathway.

This hypothesis is also consistent with the evolutionary history of green plants. The early stages of evolution of all plants occurred in the sea, where they are naturally exposed to diurnal sunlight. It is logical that photomorphogenic development was initially selected by the ancestor of green plants. This idea is further supported by the fact that most of the lower plants do not have perfect skotomorphogenic programs. For example, gymnosperms and the great majority of algae form chloroplasts in the dark. Among those that do not, such as *Euglena* and *Ochromonas*, proplastid-like structures developed. These proplastid-like structures do not contain the extensive prolamellar bodies usually associated with etioplasts (Kirk and Tilney-Bassett, 1978). Later with the advance of territorial plants, which encountered dark growth conditions due to the presence of soil and dense canopy, the dark-adaptive seedling developmental pathway or skotomorphogenesis evolved. Therefore, this scenario supports our hypothesis that photomorphogenesis is the default pathway and that skotomorphogenesis is a dark-adaptive pathway of seedling development in higher plants.

METHODS

Plant Materials and Growth Conditions

Wild-type Arabidopsis plants used in Figures 2, 3, and 4 are in the Columbia ecotype. The *c*onstitutive *p*hotomorphogenic (*cop*) mutants and the wild-type plants used in all other experiments are in the Wassilewskija background. Because the mutants are lethal, seeds of heterozygous plants for individual mutations were used as seed stock in all experiments. The homozygous *cop8-1*, *cop10-1*, and *cop11-1* mutant seeds are distinct from wild-type seeds due to their dark purple color and can be readily recognized. The growth conditions have been described previously (Wei and Deng, 1992). The seeds were surface sterilized, rinsed, and plated on solid growth medium (Valvekens et al., 1988) containing 1% sucrose. Cold treatment was usually for 4 to 7 days for the mutants. The seedlings were grown in the 22°C growth chamber in complete darkness or in a cycle of 16-hr light/8-hr dark unless otherwise specified. Day 0 after germination is defined as the point in time when the plates are moved from 4°C to the 22°C growth chamber. In the time course experiment shown in Figures 2, 3, and 4, samples were taken at the same time each day.

Light and Electron Microscopy

The light microscopy and scanning electron microscopy were performed as described previously (Hou et al., 1993). For transmission electron

microscopy, cotyledons of seedlings were dissected and fixed overnight at 4°C in a solution containing 4% glutaraldehyde, 0.2 M sucrose in 0.1 M sodium phosphate buffer, pH 6.8. After washing in the sodium phosphate buffer for three times of 5 min each, the samples were incubated in the same phosphate buffer containing 2% OsO_4 solution overnight at 4°C. The samples were then washed five times with distilled water and dehydrated in a graded ethanol series. Next, the samples were washed three times with propylene oxide for 10 min and then incubated overnight in a mixture of propylene oxide and the "firm" recipe of Spurr's embedding media (Electron Microscopy Sciences, Fort Washington, PA) in a 1:1 ratio. The samples were finally embedded in fresh Spurr's by vacuum baking at 80°C overnight. The embedded material was sectioned using an ultramicrotome. The section was stained with uranyl acetate and lead citrate and viewed in an electron microscope (model 300; Philips Electronic Instruments Co., Mahwah, NJ).

Double Mutant Construction

The long hypocotyl (*hy*) mutant alleles used for double mutant analysis were *hy1* (21.84N), *hy2* (To76), *hy3* (Bo64), *hy4* (2.23NO), and *hy5* (Ci88), all in the Landsberg *erecta* background (Koornneef et al., 1980). The crosses were performed using homozygous *hy* mutants and heterozygous *cop8-1*, *cop10-1*, and *cop11-1* mutants. The F_1 plants were selfed, and F_2 plants that displayed a *hy* phenotype and contained approximately a quarter of dark purple seeds in their siliques were selected because the dark seed color is due to the homozygous *cop8-1*, *cop10-1*, and *cop11-1* mutations. The F_3 seedlings segregated approximately a quarter double mutants in a *hy* homozygous background and were used for the experimentation.

RNA and Protein Gel Blot Analyses and GUS Activity Assays

The mutant seedlings used for RNA gel blot analysis were grown in complete darkness or continuous light for 7 days. Plants for dark adaptation were first grown in continuous light for 7 days and then transferred to darkness for 2 additional days. The dark-grown and dark-adapted tissues were harvested under a dim green safelight. Total RNA extraction, electrophoresis and blotting, and hybridization with radiolabeled probes were performed as previously described (Sharrock and Quail, 1989). Hybridization probes for *rbcS*, *fedA*, *cab*, and 18S rRNA (Figure 7) were made from the purified DNA fragments, as described by Deng et al. (1991). Probes were labeled with ^{32}P-dCTP using a random priming DNA labeling kit (U.S. Biochemical Corp.).

The transgenic line carrying the *rbc*S promoter–β-glucuronidase (*GUS*) fusion construct has been described previously (Wei and Deng, 1992). The promoter was derived from the 1.7-kb fragment of the Arabidopsis *rbcS-1A* promoter (−1700 to +2) (Donald and Cashmore, 1990). The reporter construct was introduced into *cop8-1*, *cop10-1*, and *cop11-1* mutants by standard crosses as described previously for *cop9-1* (Wei and Deng, 1992). The F_2 seeds, which consisted of one-quarter mutants and three-quarters wild type, were used for the GUS assay. The ratio of plants carrying *rbcS-1A* promoter–*GUS* constructs among F_2 mutant and wild-type populations was monitored by histochemical staining, as described by Deng et al. (1991), and the value of the GUS activity was adjusted accordingly. The GUS activity measurement was as described by Wei and Deng (1992).

The protein extraction and the immunoblot analysis with purified polyclonal antibody against COP1 produced by overexpression in *Escherichia coli* were performed as described by McNellis et al. (1994).

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