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*COP*9: A New Genetic Locus Involved in Light-Regulated Development and Gene Expression in Arabidopsis

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We report here the identification and characterization of a new Arabidopsis light-regulatory locus, *COP*9, mutation that leads to a *c*onstitutive *p*hotomorphogenic phenotype. Dark-grown *cop*9 seedlings exhibit many morphological characteristics of light-grown seedlings, including short hypocotyls and open and enlarged cotyledons with cell-type and chloroplast differentiation. Furthermore, the *cop*9 mutation leads to high-level expression of light-inducible genes in the absence of light, probably by altering the promoter activities of these genes. These properties imply that the mutation in the *COP*9 locus uncouples the light/dark signals from morphogenesis and light-regulated gene expression. In addition, light-grown *cop*9 mutants are severely dwarfed and are unable to reach maturation and flowering. This adult-lethal phenotype indicates that the *COP*9 locus also plays a critical role for normal development of the light-grown plant. Similar to *cop*1 mutants, but not *det*1, the *cop*9 mutants show (1) no effect on the phytochrome control of seed germination and (2) deficiency in the dark-adaptive change of expression of light-regulated genes. Our results suggest that the *cop*9 and *cop*1 mutations result in the same range of phenotypes and therefore *COP*9 and *COP*1 loci may encode closely related components in the same regulatory pathway.

INTRODUCTION

Higher plants, such as Arabidopsis, are intrinsically programmed with two developmental plans: skotomorphogenesis and photomorphogenesis (for review, see Kendrick and Kronenberg, 1986). Dark-grown seedlings have long hypocotyls, apical hooks, and unopened and undeveloped cotyledons with etioplasts (skotomorphogenesis). Conversely, light-grown seedlings have short hypocotyls and opened and enlarged cotyledons with distinct chloroplast and cell-type differentiation (photomorphogenesis). At the molecular level, the expression of a group of genes, such as most photosynthesis-related genes, is significantly increased; whereas others, such as genes encoding type A phytochrome, are repressed in the light-grown plants (Kendrick and Kronenberg, 1986; Sharrock and Quail, 1989; Gilmartin et al., 1990; Bruce et al., 1991; Thompson and White, 1991). The switch between skotomorphogenesis and photomorphogenesis in early plant development is naturally triggered by light signals. It is known that the light signals are perceived by at least three types of photoreceptors in higher plants: phytochromes, blue light (UV-A) receptors, and UV-B receptors (Kendrick and Kronenberg, 1986; Quail, 1991). However, the mechanisms of how the signals, once perceived, are integrated to dictate the switch between these two morphogenic pathways are just beginning to be unraveled.

Genetic approaches have been taken to identify the signaling components involved in the light-regulated development of Arabidopsis (recently reviewed by Okada and Shimura, 1992). Two general groups of mutants defective in coupling light signals with morphogenesis have been isolated. One group of mutants, such as those derived from *hy* and *blu* loci, shows dark-grown seedling characteristics (long hypocotyls) when grown under either white or blue light conditions (Koornneef et al., 1980; Chory et al., 1989b; Liscum and Hangarter, 1991). Mutants derived from several of these loci are defective in the functional photoreceptors (Chory et al., 1989b; Parks and Quail, 1991; Somers et al., 1991). The other group of mutants exhibits light-grown seedling characteristics when grown in the dark. Three such genetic loci have been described and are as follows: *dee*tiolated-1 (*DET*1) (Chory et al., 1989a), *DET*2 (Chory et al., 1991), and *c*onstitutive *p*hotomorphogenic-1 (*COP*1) (Deng et al., 1991; Deng and Quail, 1992). Mutations in these three loci also lead to constitutive expression of normally light-regulated genes in dark-grown seedlings. These phenotypes suggest that these genetic loci are directly involved in coupling light perception to morphogenesis and gene expression during different stages of plant development. The recessive nature of all three mutations implies that their wild-type gene products act to repress photomorphogenic development in the absence of light.

Several interesting features distinguish these three constitutive photomorphogenetic mutants from one another. First, mutations in *COP*1 and *DET*2, but not *DET*1, result in lack of dark-adaptive change of gene expression in light-grown adult plants. Second, *cop*1 mutations do not affect phytochrome

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control of seed germination, whereas *det*1 seeds germinate even in the absence of active phytochromes. Third, both *det*1 and *cop*1 mutations, but not *det*2, lead to chloroplast differentiation of dark-grown seedlings.

The *COP*1 gene encodes a protein with a Zn binding motif and a domain homologous to the β subunit of trimeric G-proteins (Deng et al., 1992). This novel structure suggests that the *COP*1 gene product not only has the potential to directly bind DNA through its Zn binding domain, it may also have the ability to interact with other protein components through its G-protein homologous domain. These interactions can be twofold: that is, the other regulatory components could either modulate COP1 activity in response to light signals or be controlled by COP1. Depending on the nature of interaction, mutations in the genes encoding these components may give a *cop*1-like phenotype. To understand the molecular mechanism of COP1 function in the light signal transduction pathway, it will be necessary to identify the regulatory components that interact with COP1. Toward this goal, we report here the characterization of a new genetic locus, designated *COP*9, mutation that leads to the *c*onstitutive *p*hotomorphogenic phenotype which is identical to that of *cop*1 mutants.

RESULTS

Identification of the *COP*9 Locus

A new Arabidopsis mutant line, carrying a single recessive *cop* mutation, was isolated during our screening of new *cop* mutants (see Methods). Complementation tests between this new *cop* mutation and *cop*1, *det*1, and *det*2, as shown in Table 1, indicated that it was able to complement the mutant phenotype of each of the three mutations. We have further shown that this new *cop* mutant is also distinct from other *COP* loci (*COP*2 to *COP*8) thus far identified in our laboratory (Y. Hou, A.G. Arnim, and X.-W. Deng, manuscript submitted; N. Wei and X.-W. Deng, unpublished results). These results establish that the new *cop* mutation defines a new locus, designated as *COP*9. This mutant line, designated as the *cop*9-1 allele, is used in all of the experiments described in this report.

Table 1. Phenotypic Segregation in the F_1 Progeny of Crosses between *cop*9 Heterozygotes and *cop*1 and *det* Mutations

Parental Genotype	No. of Seeds	No. of Seedlings	
		Wild Type	Mutant
*COP*9-1/*cop*9-1 × *cop*1-4/*cop*1-4	104	99	0
*COP*9-1/*cop*9-1 × *det*1/*det*1	65	63	0
*COP*9-1/*cop*9-1 × *det*2/*det*2	34	27	0
*COP*9-1/*cop*9-1 × *COP*9-1/*cop*9-1	NA[a]	3594	798

[a] NA, data not available.

Homozygous *cop*9-1 seedlings growing in the dark exhibit many morphological characteristics normally associated with light-grown seedlings. Figure 1 shows 10-day-old dark- and light-grown *cop*9 seedlings (Figures 1A and 1B) as compared with 6-day-old dark- and light-grown wild-type seedlings (Figures 1C and 1D). It is obvious that dark-grown, as well as light-grown, *cop*9 seedlings develop opened and enlarged cotyledons and short hypocotyls. These features are typical of a light-grown wild-type seedling and are in sharp contrast to the dark-grown wild-type seedlings. Both dark- and light-grown mutant seedlings are substantially smaller in size than wild-type light-grown seedlings, and they accumulate high levels of anthocyanins as evidenced by the purple color.

Dark-Grown *cop*9-1 Seedlings Display Characteristics of Light-Grown Seedlings at Cellular and Subcellular Levels

Figure 2 shows cotyledon cross-sections of mutant and wild-type seedlings growing in the dark and light. The cotyledon expansion in dark-grown *cop*9 seedlings (Figure 2A) is similar to that of their light-grown siblings (Figure 2B) and is significantly greater than that of dark-grown wild-type (Figure 2C). Further, the overall cellular differentiation in the dark-grown mutant cotyledons, as evidenced by air space between mesophyll cells and by cell-type differentiation, is more similar to those found in cotyledons of light-grown seedlings than to those of dark-grown wild-type seedlings.

The cellular differentiation pattern of the epidermal cell layer was further examined by scanning electron microscopy, as shown in Figure 3. The dark- and light-grown (Figures 3C and 3D) *cop*9 mutant cotyledons have enlarged and differentiated epidermal cells of irregular shape, and mature opened stomatas. These properties are similar to those found in light-grown wild-type cotyledons (Figure 3B). By contrast, the dark-grown wild-type seedlings have smaller and more regularly shaped epidermal cells and immature stomatal structures with no detectable openings (Figure 3A). Close examination of an immature stomatal structure under transmission electron microscopy (Figure 3E) revealed that there is a substantial amount of intercellular matrix between the two guard cells. A functional stomatal structure would at least require the removal of this intercellular matrix. We also noticed that the hypocotyl cells in dark-grown *cop*9 mutants (Figure 3F) are significantly shorter (approximately fivefold to eightfold) than those of dark-grown wild-type seedlings (Deng et al., 1992), and are slightly longer than that of light-grown mutants (Figure 3G). This suggests that lack of cellular elongation rather than cell division is primarily responsible for the short hypocotyl of dark-grown *cop*9-1 mutant seedlings as shown in Figures 1 and 3F.

The effect of the *cop*9 mutation on plastid development was examined by transmission electron microscopy. Figure 4A

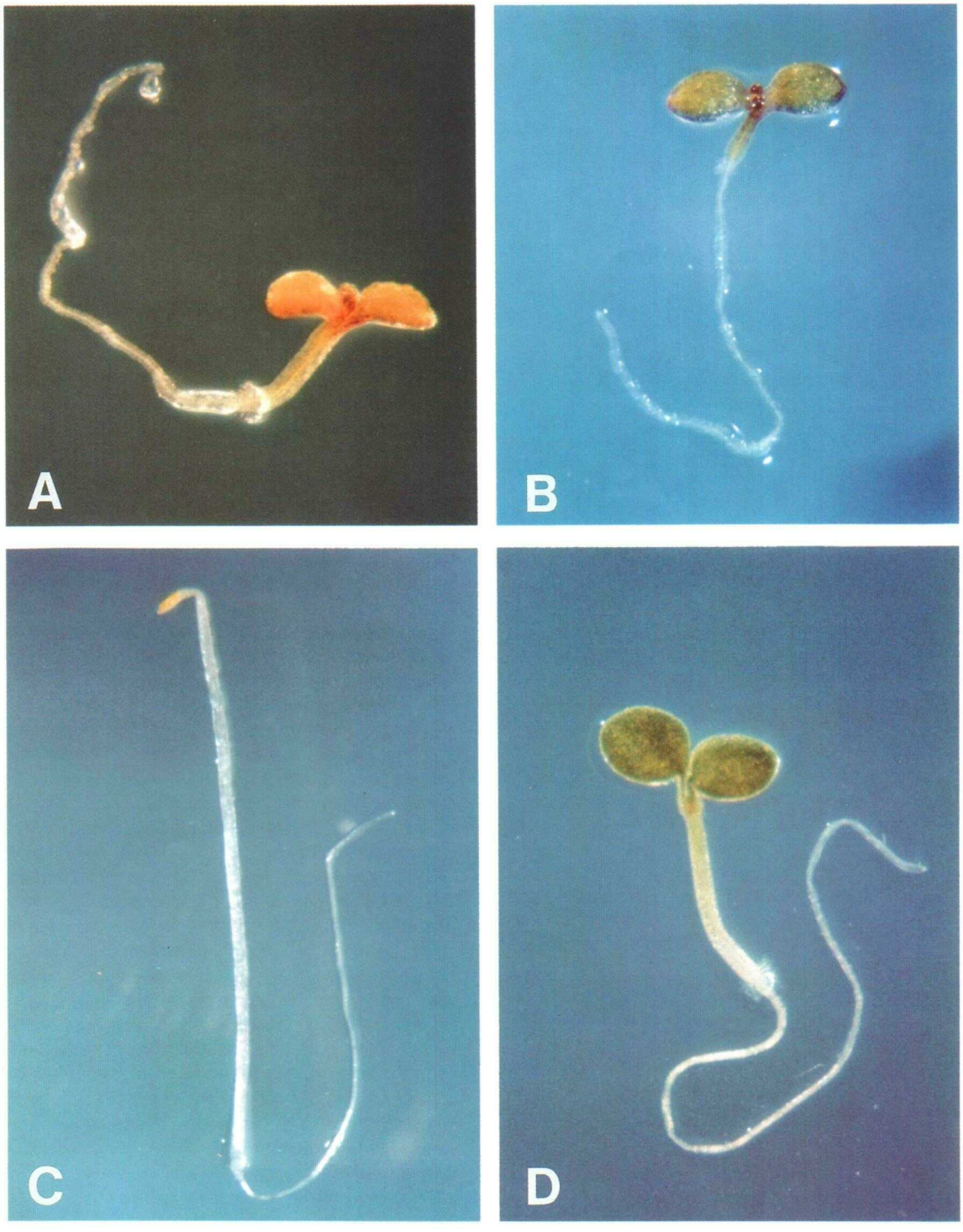

Figure 1. Morphologies of Dark- and Light-Grown *cop*9-1 and Wild-Type Arabidopsis Seedlings.

(A) Ten-day-old dark-grown *cop*9-1 mutant seedling.
(B) Ten-day-old light-grown *cop*9-1 mutant seedling.
(C) Six-day-old dark-grown wild-type seedling.
(D) Six-day-old light-grown wild-type seedling.
The same magnification (×7.5) was used for all panels with the exception of **(C)**, which has two-thirds (×5) of the magnification used for the others.

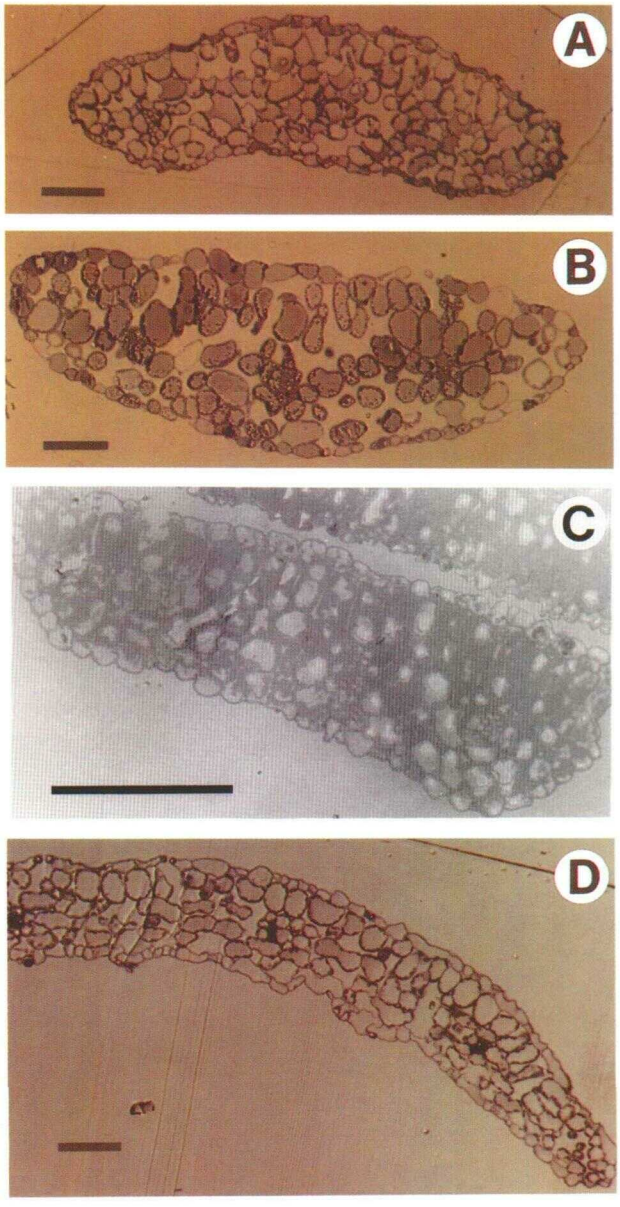

Figure 2. Morphogenetic Comparison of Cotyledon Cross-Sections.

(A) Cotyledon cross-section of a 9-day-old dark-grown *cop*9-1 mutant seedling.

(B) Cotyledon cross-section of a 9-day-old light-grown *cop*9-1 mutant seedling.

(C) Cotyledon cross-section of a 6-day-old dark-grown wild-type seedling.

(D) Cotyledon cross-section of a 6-day-old light-grown wild-type seedling.

Light micrographs were taken from 1-μm sections of the fixed and embedded seedlings. Bars = 0.2 mm.

shows the morphology of a typical etioplast of dark-grown wild-type seedlings. The most characteristic feature of an etioplast is the presence of a large paracrystalline assembly of tubules, termed the prolamellar body (Kirk and Tilney-Bassett, 1978). Mature chloroplasts, as found in the cotyledons of normal light-grown seedlings, contain parallel and highly stacked membrane systems. These fully developed chloroplasts are predominant in light-grown *cop*9-1 mutants (Figure 4B). In the dark-grown *cop*9-1 mutant seedlings, most of the plastids do not have prolamellar bodies but are filled with parallel and unstacked membranes instead (Figure 4C). This morphology resembles the plastids found in the dark-grown *cop*1 mutants (Deng et al., 1991; Deng and Quail, 1992). Occasionally, however, a prolamellar body of significantly reduced size was observed in small fractions of plastids of dark-grown mutants (Figure 4D). These observations indicate that the chloroplast differentiation has been initiated, although not completed, in the dark-grown *cop*9-1 mutants.

Mutation in the *COP*9 Gene Results in Constitutive Expression of Light-Inducible Genes in the Dark

The expression pattern of a group of light-regulated genes in wild-type Arabidopsis seedlings changes dramatically following the switch from skotomorphogenesis to photomorphogenesis upon exposure to light. Because the *cop*9-1 mutation leads to photomorphogenic development in the absence of light, it was interesting to see whether it would also uncouple light signals from the regulation of gene expression. As shown in Figure 5, we examined the expression of four nuclear-encoded genes (*rbc*S, *fed*A, *cab*, and *chs*) and one plastid-encoded gene (*psb*A), all of which are positively regulated by light in wild-type plants (see legend to Figure 5 for explanation of abbreviations). The mRNA levels of *rbc*S, *fed*A, *cab*, and *psb*A were very low, yet detectable, in dark-grown wild-type seedlings, and elevated drastically in light-grown seedlings. In the *cop*9-1 mutant, however, no change in the level of expression was observed between dark- and light-grown seedlings. It appeared that the expression of *cab* genes in both dark- and light-grown *cop*9-1 seedlings is greatly repressed compared with light-grown wild-type seedlings. We have previously observed a similar suppression of *cab* expression in *cop*1 mutants. The degree of suppression seems to correlate with the severity of individual alleles (Deng et al., 1991, 1992). Because the *cop*9-1 mutants have a more severe phenotype (see sections below) than most of the *cop*1 mutations, a strong suppression of the *cab* mRNA levels may not be unexpected. We also noticed an abnormally higher chalcone synthase mRNA level in the mutant seedlings growing under either dark or light conditions than in wild-type seedlings. This result is consistent with the high level of anthocyanin accumulation observed in both dark- and light-grown *cop*9-1 seedlings (Figure 1), because chalcone synthase is the first committed enzyme in the anthocyanin biosynthetic pathway.

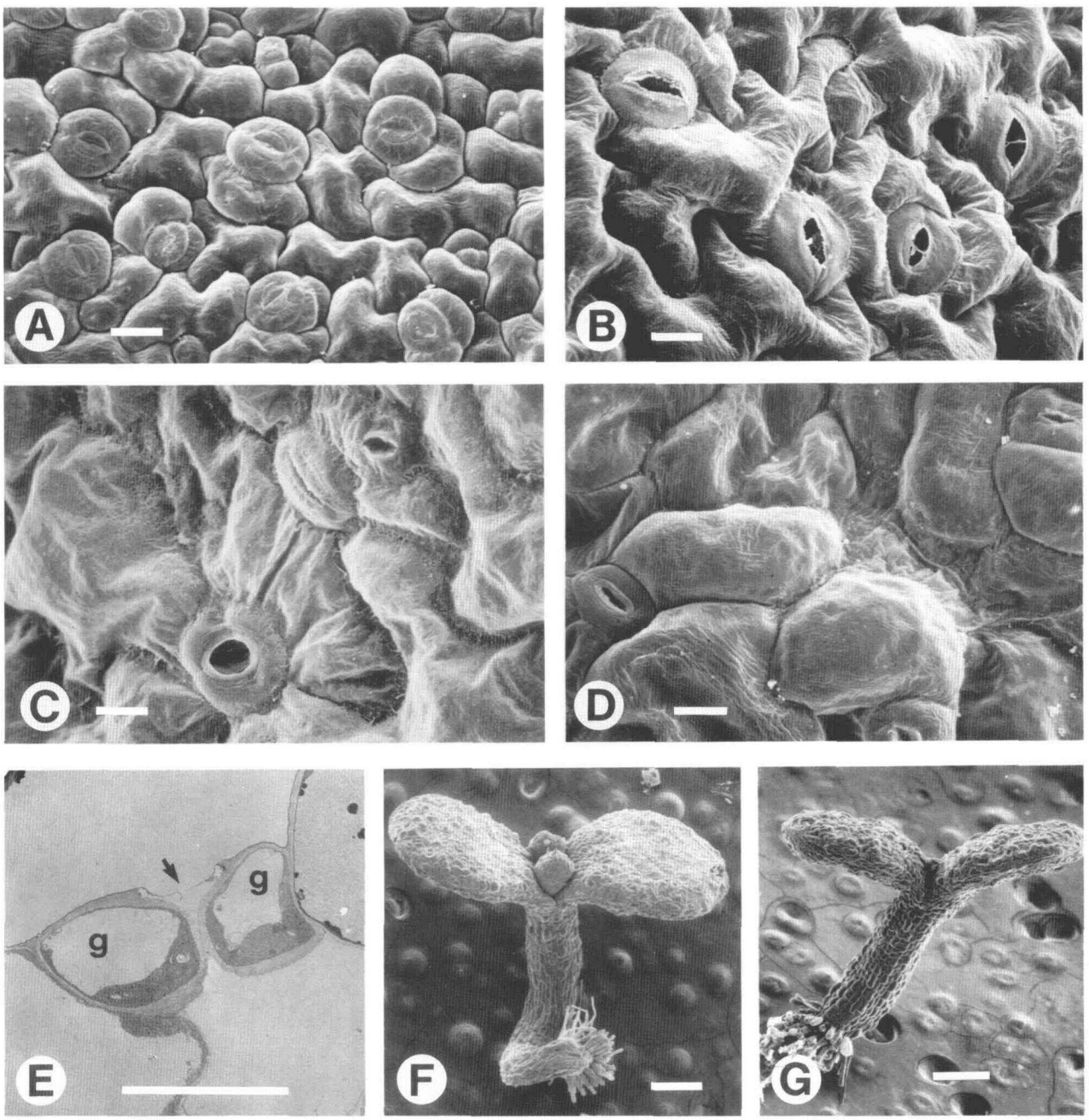

Figure 3. Epidermal Cell Morphology and Differentiation.

Epidermal cells were examined by scanning electron microscopy, as shown in **(A)**, **(B)**, **(C)**, **(D)**, **(F)**, and **(G)**, and by transmission electron microscopy, as shown in **(E)**.

(A) Cotyledon epidermal surface of a 10-day-old dark-grown wild-type seedling.

(B) Cotyledon epidermal surface of a 10-day-old light-grown wild-type seedling.

(C) Cotyledon epidermal surface of a 10-day-old dark-grown *cop*9-1 seedling.

(D) Cotyledon epidermal surface of a 10-day-old light-grown *cop*9-1 seedling.

(E) A cross-section view of an immature stomatal structure from a 6-day-old dark-grown *cop*9-1 seedling. The upper side of the micrograph corresponds to the cotyledon surface. The guard cells (g) and intercellular matrix (see arrow) are shown.

(F) Ten-day-old dark-grown *cop*9-1 seedling: morphology of the whole seedling and its surface cells.

(G) Ten-day-old light-grown *cop*9-1 seedling: morphology of the whole seedling and its surface cells.

Bars = 10 μm in **(A)** to **(E)** and 200 μm in **(F)** and **(G)**.

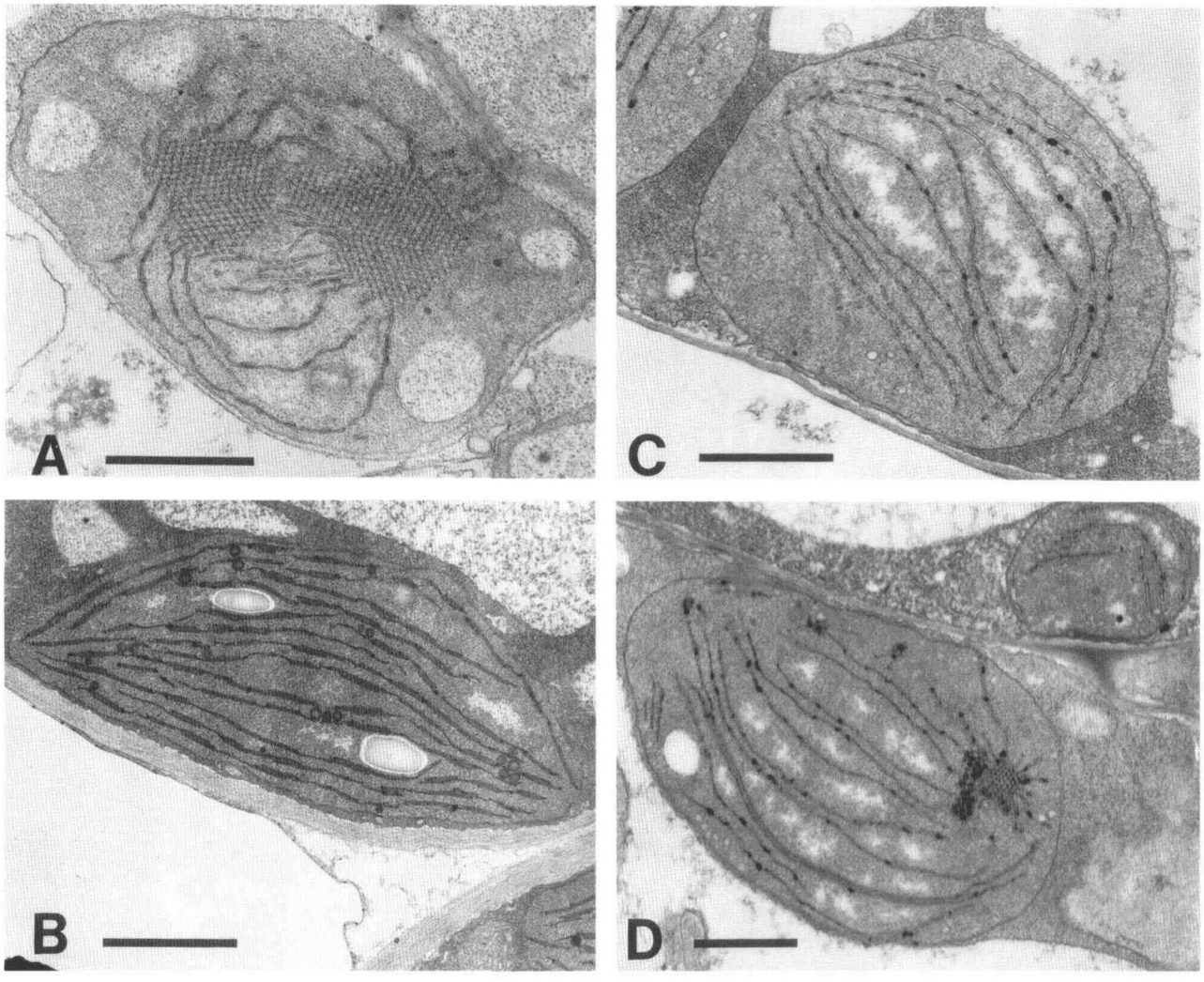

Figure 4. Morphogenetic Changes in Plastids of Dark-Grown Seedlings Caused by the *cop*9 Mutation.

(A) Etioplast from a 6-day-old dark-grown wild-type seedling.
(B) Chloroplast from a 10-day-old light-grown *cop*9-1 mutant seedling.
(C) and **(D)** Representative plastids from 10-day-old dark-grown *cop*9-1 mutant seedlings.
Bars = 1 μm.

Constitutive Expression of Light-Inducible Genes Is Modulated by Promoter Activation in the Dark-Grown *cop*9 Mutants

Light modulates the expression of most light-regulated genes at the level of transcriptional initiation (Kuhlemeier and Chua, 1987; Quail, 1991; Thompson and White, 1991). It is likely, therefore, that the high mRNA levels of these genes observed in dark-grown *cop*9 mutants result from increased transcriptional activity in the dark. To test this possibility, promoter–β-glucuronidase (GUS) reporter fusion constructs, which were carried in Arabidopsis transgenic lines (Deng et al., 1991), were introduced into the *cop*9-1 mutant by genetic crossing. Thus, the in vivo activities of the test promoters can be determined by measuring the corresponding GUS activity.

Two light-regulated promoters (*rbc*S-1A and *cab*1) and a control promoter (the cauliflower mosaic virus [CaMV] 35S promoter) were selected for our experiments, and the results are summarized in Figure 6. The GUS activities of dark-grown *cop*9 seedlings were comparable to (in the case of *cab*1 promoter, Figure 6B), or even slightly higher than (the *rbc*S promoter, Figure 6A), those of light-grown mutants. In contrast, wild-type seedlings showed a dark/light increase of approximately threefold or 10-fold for *rbc*S or *cab*1 promoters, respectively. The CaMV 35S promoter was more active in dark-grown than in light-grown plants, but the dark/light ratios of

the activity were almost the same in wild-type and mutant seedlings (Figure 6C). However, much lower absolute GUS activity of the CaMV 35S promoter–GUS fusion in mutant than in wild-type seedlings was consistently observed. Such lower overall GUS activities were also noticed for *cab* and *rbc*S promoter-fusion constructs in both dark- and light-grown mutant seedlings. It implies that the suppression of GUS expression in the mutant is a general property of the mutant plants rather than a gene-specific modulation. Qualitatively, the activities of the fused promoters correlate well with the mRNA level of corresponding genes shown in Figure 5. This suggests that the elevated expression of these light-regulated genes in dark-grown *cop*9-1 mutants results from increased transcriptional activity of their promoters.

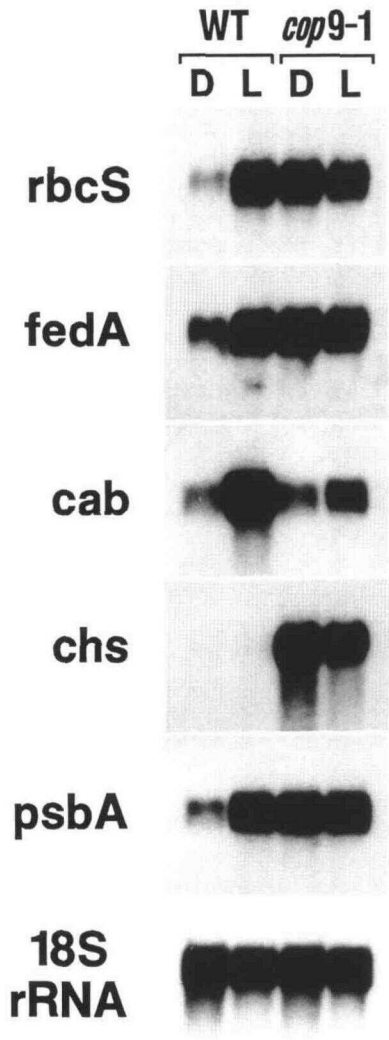

Figure 5. Gel Blot Analysis of Steady State RNA Levels of Nuclear- and Plastid-Encoded Genes.

Twelve-day-old wild-type (WT) or *cop*9-1 seedlings grown in the dark (D) and light (L) were sampled. The hybridization probes are the following: *rbc*S, gene for the small subunit of ribulose-1,5-bisphosphate carboxylase (Krebbers et al., 1988); *fed*A, ferredoxin type A gene (Somers et al., 1990); *cab*, gene for the chlorophyll *a/b* binding protein of photosynthetic light-harvesting complexes (Leutwiler et al., 1986); *chs*, chalcone synthase gene (Feinbaum and Ausubel, 1988); *psb*A, plastid gene encoding the 32-kD protein of photosystem II (Zurawski et al., 1982); 18S rRNA, gene for cytoplasmic 18S ribosomal RNAs (Jorgensen et al., 1987). Two micrograms of total RNA was used in each lane for hybridization with *rbc*S, *cab*, *psb*A, and the 18S rRNA, and 5 μg was used for *fed*A and *chs*.

The *cab*1 promoter–GUS transgenic line used in this experiment contained a minimal light-responsive promoter (−250 to +67) (Deng et al., 1991). This promoter fragment has been shown in both transgenic tobacco (Ha and An, 1988) and Arabidopsis (Deng et al., 1991) to be sufficient for full light-regulated and tissue-specific expression. We have previously shown that this small promoter fragment contains all necessary information for mediating the response to *cop*1 mutations (Deng et al., 1991). The result shown in Figure 6B suggests that the fragment also contains sufficient information for mediating the response to the *cop*9-1 mutation. It is therefore probable that both the *cop*1 and *cop*9 loci modulate *cab*1 expression through the same promoter element.

*COP*9 Locus Also Plays an Essential Role during Normal Arabidopsis Development under Light Conditions

The *cop*9-1 mutation not only leads to a constitutive photomorphogenic phenotype in dark-grown seedlings, but also has a severe effect on light-grown adult plants. Even under optimal light conditions, plants homozygous for the *cop*9-1 mutation develop at most four pairs of tiny rosette leaves (rosette diameter of less than 0.5 cm) and senesce before maturation and transition to reproductive development. We have so far not observed a single mutant plant (of hundreds of *cop*9-1 mutants) that had a life span of more than 5 weeks and/or initiated reproductive development, such as flower bud formation or bolting. Such an adult-lethal phenotype in the light-grown *cop*9 plants strongly indicates that the *COP*9 locus plays an essential role during normal growth and development of Arabidopsis under light conditions. It is worth noting that the T-DNA insertional mutant allele (*cop*1-5, presumably a null allele) of the *COP*1 locus also leads to a similar adult-lethal phenotype (Deng et al., 1992).

It has been reported that light-grown *det*1 and *cop*1 mutant plants develop chloroplasts instead of amyloplasts in the root cells, resulting in greenish roots (Chory and Peto, 1990; Deng and Quail, 1992). We have noticed that light-grown *cop*9-1 plants also develop greenish roots, which are distinct from the roots of dark-grown mutant or light-grown wild-type plants (data not shown). This observation suggests that the *COP*9 locus is also involved in the control of tissue-specific plastid differentiation.

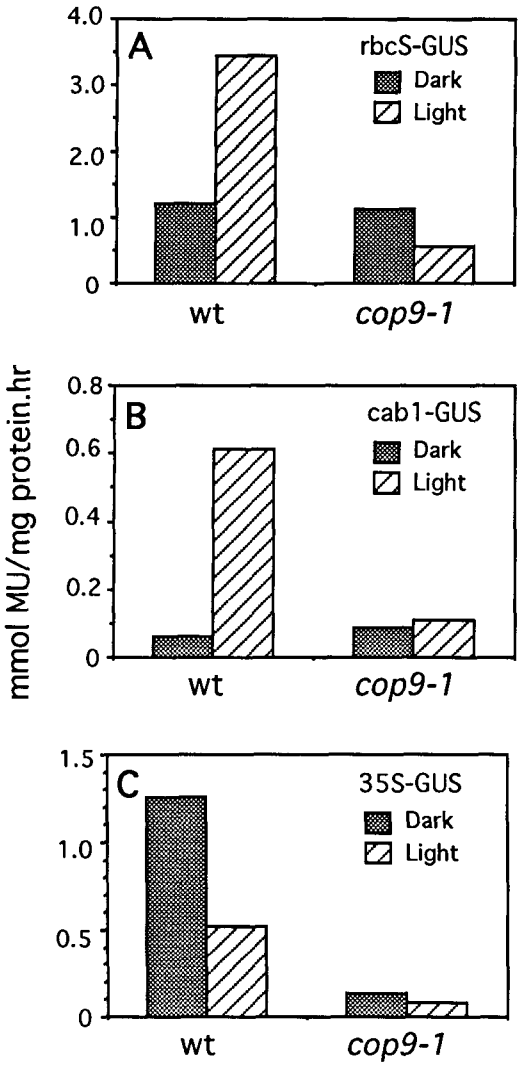

Figure 6. GUS Activity Assay of Promoter–GUS Fusions in Wild-Type and *cop*9-1 Mutant Seedlings Grown in the Dark or Light.

(A) The 1.7-kb fragment from the Arabidopsis *rbc*S-1A promoter (−1700 to +21) (Donald and Cashmore, 1990).

(B) The minimal promoter fragment (−250 to +67) from the Arabidopsis *cab*1 gene (Ha and An, 1988).

(C) The 346-bp CaMV 35S promoter fragment (Rogers et al., 1986). The promoter–GUS fusion constructs used have been described previously (*rbc*S-1A–GUS, Donald and Cashmore, 1990; *cab*1-GUS and 35S-GUS, Deng et al., 1991). The GUS activities shown (millimoles of 4-methylumbelliferone per milligram of protein per hour) are the average of three independent extractions and measurements from one particular experiment, and the variation between different trials was less than 5% of the values. These experiments were repeated two to three times for each construct. wt, wild type.

Phenotype of the *cop*9 Mutants Overlaps with That of the *cop*1 Mutants

All of the *cop*9 mutant characteristics examined thus far closely resemble those caused by the *cop*1 and *det*1 mutations. Because the *det*1 and *cop*1 mutations have different effects on two light-regulated processes, phytochrome control of seed germination and dark adaptation of light-grown plants, we further examined the effect of the *cop*9 mutation on these two developmental processes.

The initial seed germination process in Arabidopsis is thought to be under phytochrome control because far-red light treatment, which reduces active phytochrome, greatly reduces the germination rate of wild-type seeds and because red light, which converts the inactive phytochrome to the active form, reverses far-red light inhibition (Chory et al., 1989; Deng et al., 1991). To test the effect of the *cop*9-1 mutation on this phytochrome-controlled process, germination rates of *cop*9-1 seeds after different light treatments (Deng et al., 1991) were determined. The results showed that a 5-sec far-red light pulse reduced the rate of *cop*9-1 seed germination from 80 to 20%. A red light pulse following the far-red pulse reversed this effect (data not shown). Therefore, as it is for *cop*1 mutants, the germination of *cop*9-1 seeds is still under phytochrome control.

To examine whether the mutation in the *COP*9 locus also affects repression of light-inducible genes during the dark-adaptive process of light-grown plants, expression of representative light-regulated genes in light-grown and dark-adapted (for 2 days) wild-type and mutant plants was analyzed. As shown in Figure 7, the mRNA levels of all three genes (*rbc*S, *fed*A, and *cab*) examined decreased extensively upon 2-day dark adaptation in wild-type plants. In *cop*9-1 mutant plants, however, the expression of all three genes remained at a constant level after dark adaptation. This property of the *cop*9 mutants is also found in *cop*1 (Deng et al., 1991), but not *det*1 mutants (Chory et al., 1989). Note again that *cab* gene expression was severely suppressed in both light-grown and dark-adapted mutant plants (Figure 7, section cab).

Expression of the *COP*1 Gene in the *cop*9 Mutant

Collectively, our results establish that the *cop*9 and *cop*1 mutations have parallel phenotypes, and therefore their gene products may have closely related roles during light-regulated development of Arabidopsis. As a first step in analyzing possible interactions between *COP*9 and *COP*1 genes at the molecular level, we examined the effect of the *cop*9-1 mutation on the accumulation of *COP*1 mRNA in both dark- and light-grown seedlings. As illustrated in Figure 8, the *cop*9 mutation leads to approximately a threefold to fivefold increase in *COP*1 mRNA. However, as it is for wild-type plants, the difference in *COP*1 expression between light- and dark-grown mutant seedlings was very little. This suggests that the *COP*9 gene is involved in the suppression of *COP*1 gene expression in a

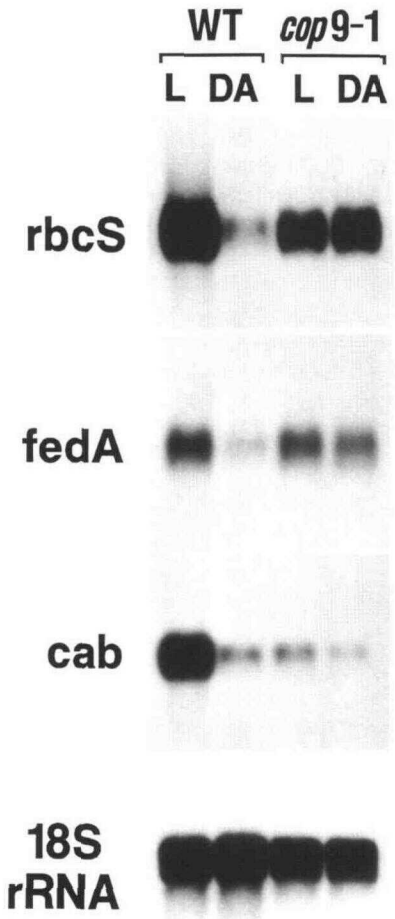

Figure 7. Gel Blot Analyses of Steady State RNA Levels of Light-Inducible Genes from Light-Grown and Dark-Adapted Plants.

Wild-type (WT) or *cop*9-1 mutant plants were grown in continuous light for 2 weeks (L), and plants grown in the same light conditions followed by 48 hr of dark adaptation (DA) were sampled. The hybridization probes are as given in the legend to Figure 5. Equal amounts of total RNA were loaded in each lane (2 μg for *rbc*S, *cab*, and the 18S rRNA; 5 μg for *fed*A).

light-independent manner, although further studies are necessary to understand its functional implication.

DISCUSSION

We report here the identification and characterization of a new Arabidopsis locus, *COP*9. A recessive mutation in the *COP*9 locus leads to a constitutive photomorphogenic phenotype in the absence of light. Specifically, the dark-grown *cop*9-1 seedlings exhibit a wide variety of traits normally associated with light-grown plants, including open and enlarged cotyledons, short hypocotyls, advanced cell-type differentiation, initiation of chloroplast differentiation, and high-level expression of light-inducible genes. This phenotype implies that the mutation in the *COP*9 locus uncouples the perception of light signals from the commitment to the photomorphogenic program in early seedling development of Arabidopsis. In addition, light-grown *cop*9 plants are unable to complete their life cycle and display many abnormalities including lack of dark/light regulation of photosynthetic gene expression during dark adaptation and chloroplast differentiation in root tissue. These characteristics indicate that COP9 has a fundamental role during adult plant development as well.

Mutations in three other Arabidopsis loci that lead to a similar phenotype have been described (*det*1 and *det*2, Chory et al., 1989a, 1991; *cop*1, Deng et al., 1991; Deng and Quail, 1992). Phenotypic comparison of *cop*9 with these mutants is summarized in Table 2. Although most of the morphological characteristics of dark-grown seedlings are similar among those mutants, there are some major differences. For instance, *cop*1 and *cop*9 do not affect the phytochrome-controlled seed germination process, whereas *det*1 does. This suggests that the relevant phytochrome and the transduction pathway

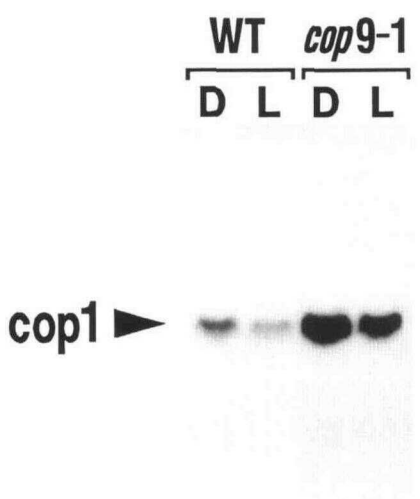

Figure 8. RNA Gel Blot Analysis of *COP*1 Expression in Wild-Type and *cop*9-1 Mutant Seedlings.

Twelve-day-old wild-type (WT) and *cop*9-1 mutant seedlings grown in the dark (D) or light (L) were used for RNA extraction. Total RNA of 30 μg was loaded in each lane. The blot was hybridized with the antisense RNA riboprobe as described in Methods.

leading to this response are not affected by *cop*1 or *cop*9 mutations. Therefore, seed germination and photomorphogenesis during seedling development may involve two independent developmental commitments. Moreover, *cop*9, *cop*1, and *det*2 mutants, but not *det*1, are defective in light/dark regulation of photosynthetic gene expression in dark-adapted adult plants. This suggests that distinct signaling pathways may be responsible for photomorphogenesis of seedling development and light-dependent processes in adult plants, although both processes share common components (such as *cop*1 and *cop*9). Further, the *det*2 mutant does not exhibit any chloroplast differentiation in dark-grown cotyledons and light-grown root tissue as do the three other mutants. This suggests that the control of plastid differentiation and other photomorphogenic traits requires distinct sets of regulatory components.

*cop*9 and *cop*1 are the only two photomorphogenic mutants sharing the same range of phenotypes so far examined (Table 2), suggesting that the gene products of *COP*9 and *COP*1 control the same range of physiological functions. It is therefore possible that the two gene products may be closely associated, physically as a complex or indirectly along the same signaling pathway, to control the developmental switch between photomorphogenesis and skotomorphogenesis. Several lines of evidence support this idea. First, the *cop*9-1 mutant not only has a phenotype identical to that of *cop*1 mutants (especially the T-DNA–tagged allele), but also modulates expression of the *cab*1 gene through the same promoter region. Second, the presence in the COP1 protein of a domain homologous to the β subunit of trimeric G-proteins in addition to a Zn binding motif (Deng et al., 1992) fits in well with the notion that COP1 may be an integral component of the molecular master switch that turns on or off the photomorphogenic pathway. Recent studies of Gβ domain–containing proteins from other systems (Frankel, 1991; Simon et al., 1991; Williams et al., 1991; Deng et al., 1992) all suggest the involvement of the G-protein domains in protein–protein interactions. It is reasonable to assume that the COP1 protein is also able to interact or complex with other protein components, and mutations in their genes would lead to the same phenotype as the *cop*1 mutants. The *COP*9 gene could encode such a COP1-interactive component that is essential for the COP1 function. Third, the increased mRNA level of the *COP*1 gene in the *cop*9-1 mutant plants would suggest that the *COP*9 gene product has an effect on the expression of the *COP*1 gene. Thus, an indirect interaction between *COP*1 and *COP*9 loci is implicated. To address the relationship between *COP*1 and *COP*9 at the molecular level, knowledge of the molecular identity of the *COP*9 gene will be required.

METHODS

Plant Materials and Growth Conditions

The wild-type and *cop*9 mutants of *Arabidopsis thaliana* are in an ecotype Wassilewskija (WS) background. The *cop*9-1 mutants were recovered from screening of the Du Pont T-DNA mutagenized lines (Feldmann, 1991). Seeds from plants heterozygous for *cop*9-1 mutations were used in all experiments. From this heterozygous population, wild-type and mutant plants were harvested individually and pooled separately to assure the same growth conditions. For germination, seeds were surface sterilized for ~15 min in 30% bleach (Clorox), rinsed at least five times, and plated in Petri plates (100 × 25 mm) containing growth medium (GM; Valvekens et al., 1988). After cold treatment at 4°C for 7 to 10 days in the dark, the plates were incubated in a growth chamber at 22°C in complete darkness or in a cycle of 16-hr light/8-hr darkness, or in continuous light for RNA and β-glucuronidase (GUS) activity analyses. The light source for Arabidopsis plant growth was a combination of fluorescent and incandescent lights, ranging from

Table 2. Phenotype Comparison of *cop*9, *cop*1, *det*1, and *det*2 Mutants[a]

	Wild Type	*cop*9	*cop*1	*det*1	*det*2
Morphology of dark-grown seedlings					
Cotyledons	Closed, unexpanded	Opened, enlarged	Opened, enlarged	Opened, enlarged	Opened, enlarged
cell-type differentiation	Retarded	Light-grown-like	Light-grown-like	Light-grown-like	NA[b]
Hypocotyl	Long	Short	Short	Short	Short
Chloroplast differentiation	No	Some	Some	Some	No
Increased expression level of light-inducible genes					
in dark-grown seedlings	No	Yes	Yes	Yes	Yes
in dark-adapted plants	No	Yes	Yes	No	Yes
Seed germination under phytochrome control	Yes	Yes	Yes	No	NA
Anthocyanin accumulation in the dark	No	Yes	Yes	Yes	Yes
Chloroplast differentiation in light-grown root	No	Yes	Yes	Yes	No

[a] The table is compiled from the following references: Chory et al. (1989a; *det*1), Chory and Peto (1990; *det*1), Chory et al. (1991; *det*2), Deng et al. (1991; *cop*1), Deng and Quail (1992; *cop*1), and this work (*cop*9).

[b] NA indicates data not available.

50 to 100 μE m^{-2} sec^{-1}. In the seed germination experiments, light sources of specific wavelengths (red, far-red, green safelight) were used as described previously (Deng et al., 1991).

Light and Electron Microscopy

The fixation and embedding of Arabidopsis seedlings for light and transmission electron microscopy were performed according to a published procedure (Deng and Gruissem, 1987). The embedded material was sectioned using an ultramicrotome. The staining procedure has also been described previously (Deng et al., 1991).

For scanning electron microscopy, seedlings were fixed in 4% glutaraldehyde in 100 mM sodium phosphate buffer, pH 6.8, at room temperature for 2 hr. The seedlings were then rinsed eight times in the same buffer without glutaraldehyde and dehydrated in a graded ethanol series. Dehydrated material was then critical point dried in liquid CO_2. Individual seedlings were mounted on the stubs and sputter-coated with gold-palladium in an SPI sputter coater. Specimens were examined in an ISI SS40 scanning electron microscope.

RNA Analysis

Wild-type or *cop*9 seedlings grown under dark or light conditions for 10 to 12 days were used for RNA analysis. The dark-grown seedlings and dark-adapted plants were harvested under a dim green safelight. Isolation of total RNA, electrophoresis and blotting, and hybridization with radioactively labeled DNA probes were performed as described by Sharrock and Quail (1989). Hybridization probes for *rbcS*, *fedA*, *cab*, *chs*, and the 18S rRNA were made from the purified DNA fragment as described by Deng et al. (1991). Probes were labeled with ^{32}P-dCTP using a random priming DNA labeling kit (U.S. Biochemicals). To detect the *COP*1 mRNA shown in Figure 8, an antisense riboprobe corresponding to an entire *COP*1 cDNA clone was used following a published protocol (Deng et al., 1992).

Transgenic Plants and GUS Activity Assay

Promoter–GUS fusion constructs and plant transformation were described by Deng et al. (1991). The transgenic lines were crossed with heterozygous *cop*9-1 plants. The F_1 plants heterozygous for both the *cop*9-1 and promoter–GUS fusion were self-pollinated. The F_2 seeds, which consisted of one-quarter mutant and three-quarters wild type, were used for the GUS assay. The ratio of plants carrying promoter–GUS constructs among F_2 wild-type and mutant populations was monitored by histochemical staining as described by Deng et al. (1991). GUS activity in transgenic Arabidopsis seedlings was determined according to Jefferson (1987) by measuring the fluorescence of 4-methylumbelliferone produced by GUS cleavage of 4-methylumbelliferyl β-D-glucuronide. Total protein concentration was determined by the Bio-Rad protein assay according to the manufacturer's suggested procedure.

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