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*cop*1: a regulatory locus involved in light-controlled development and gene expression in *Arabidopsis*

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Light signals from the environment are perceived by specific regulatory photoreceptors in plants and are transduced by unknown mechanisms to genes that control growth and development. We have identified a genetic locus in *Arabidopsis thaliana*, which appears to play a central role in this transduction process. Mutations in this locus, designated *cop*1 (constitutively photomorphogenic), result in dark-grown seedlings with the morphology of wild-type seedlings grown in the light. In addition, these mutations lead to constitutive expression of an array of normally light-regulated genes in dark-grown seedlings and in light-grown adult plants placed in darkness. Promoter–reporter fusion constructs of some of these genes are constitutively expressed in dark-grown transgenic *cop*1 seedlings, indicating that the aberrant behavior of these genes results primarily from aberrant modulation of their promoter activities in the mutant. In contrast, light control of seed germination and diurnal control of *cab* gene expression is normal in the *cop*1 mutants. Because these mutations are recessive, we conclude that in seedlings and adult plants, the wild-type *cop*1 gene product normally acts in darkness to repress the expression of genes involved in the photomorphogenic developmental pathway and to promote the expression of genes involved in the dark-adaptive developmental pathway, and that regulatory photoreceptors act to reverse this action upon exposure to light. However, photocontrol of seed germination and diurnal rhythms is apparently exerted via one or more separate pathways not involving the *cop*1 product.

[*Key Words*: *Arabidopsis*; gene expression; light regulation; photomorphogenesis]

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Light is arguably the single most important environmental factor influencing plant growth and development. Plants not only use incident radiation as an energy source but also as an informational signal to control development in a process termed photomorphogenesis (for review, see Kendrick and Kronenberg 1986). At least three photoreceptors, phytochrome, a blue/UV-A light receptor (also called cryptochrome), and a UV-B light receptor, are utilized to mediate such light-regulated events. Many plant responses mediated by the different photoreceptors are well characterized; and, in some cases, the response has been shown to involve regulation of transcription of specific genes (Tobin and Silverthorne 1985; Kuhlemeier et al. 1987; Kendrick and Kronenberg 1988; Gilmartin et al. 1990). Some genes, many of which are involved in photosynthesis, are induced to high levels upon exposure to light, whereas others, such as those encoding type-A phytochrome, are repressed upon exposure to light (Lissemore and Quail 1988; Sharrock and Quail 1989). In some cases, the promoter elements involved in light regulation have been delineated (Giuliano et al. 1988b; Manzara and Gruissem 1988; Bruce et al. 1989; Bruce and Quail 1990; Donald and Cashmore 1990; Gilmartin et al. 1990), and protein factors that interact with these elements have been characterized and their mRNAs cloned (Dehesh et al. 1990; Gilmartin et al. 1990). However, little is known about what comprises photoreceptor signal transduction pathways and how they control plant growth and developmental responses through regulated gene expression (Kendrick and Kronenberg 1986; Gilmartin et al. 1990).

Seedling development in *Arabidopsis thaliana* provides a useful model system for the study of photomorphogenesis of higher plants. First, this plant has well-documented advantages for genetic and molecular manipulations (for review, see Meyerowitz 1989). In addition, *Arabidopsis* seedlings, like other higher plants, are genetically endowed with the ability to follow two different strategies of development, skotomorphogenesis and photomorphogenesis, depending on the ambient light conditions (Mohr and Shropshire 1983). Skotomorphogenesis is the developmental strategy followed in darkness. Similar to other dicotyledonous plants, dark-grown *Arabidopsis* seedlings have long hypocotyls, unopened apical hooks, and undeveloped (small and unopened) cotyledons, with etioplasts and retarded cell-

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type differentiation (e.g., no stomata). In contrast, photomorphogenesis is the developmental strategy followed in the light. Light-grown *Arabidopsis* seedlings have short hypocotyls, no apical hooks, and open and enlarged cotyledons, with developed chloroplasts and differentiated cell types. There is also a dramatic change in the pattern of gene expression. The light signals perceived by all three known photoreceptor systems apparently contribute to the decision to use either the skotomorphogenic or photomorphogenic pathway (Mohr and Shropshire 1983; Kendrick and Kronenberg 1986).

Two different genetic screening procedures have been employed to identify genes involved in *Arabidopsis* photomorphogenesis. One approach has been to isolate mutants that show dark-grown morphology when germinated in the light (Koornneef et al. 1980; Adamse et al. 1989; Chory et al. 1989b). Six loci which, when mutated, result in long hypocotyls in the light (*hy* mutations), have been identified. Three of these loci (*hy*1, *hy*2, *hy*6) result in a deficiency of functional phytochrome (Parks et al. 1989; Chory et al. 1989b). The other approach has been to isolate mutants that show light-grown morphology when germinated in the dark (Chory et al. 1989a). Mutations in the *det*1 locus result in a variety of light-grown characteristics in dark-germinated seedlings, including the accumulation of mRNAs for genes that are normally only induced by light. However, mutations in the *det*1 locus have little effect on the response of light-regulated genes to dark adaptation of light-grown adult plants (Chory et al. 1989a). This raises the question of whether different sets of genes are involved in the control of different light-regulated developmental processes.

To address the molecular mechanisms underlying signal transduction in *Arabidopsis* photomorphogenesis, we have chosen to identify genes whose products may play key regulatory roles in this process. We have initiated systematic genetic screening to isolate mutants that show constitutive photomorphogenic (*cop*) phenotypes, that is, that develop in darkness as wild-type seedlings do in the light. Although the recently identified *det*1 mutants also fit these criteria (Chory et al. 1989b), we reasoned that multiple genetic loci are likely to be involved in the complex regulatory circuitry that controls photomorphogenesis and that a full understanding of this circuitry will require characterization of these multiple components. Furthermore, we are also interested in determining whether genes are involved in the control of photomorphogenesis in both seedlings and adult plants or whether separate sets of genes are involved at different developmental stages. Here, we report the identification and characterization of a gene (*cop*1) that appears to play a regulatory role both in the photomorphogenesis of seedlings and in the dark-adaptation response of adult *Arabidopsis* plants. We have analyzed the characteristics of the *cop*1 mutants at the morphologic, cellular, subcellular, and gene expression levels. The question of whether the effects of *cop*1 mutations on gene expression are exerted via the promoters of light-responsive genes has been addressed using promoter–reporter gene fusions in transgenic *cop*1 plants.

Results

Isolation of mutants that show constitutive photomorphogenic phenotypes

We have isolated four independent *Arabidopsis* lines carrying recessive mutant alleles at a locus, designated *cop*1. Details of the mutant isolation procedures and identification of these multiple alleles (*cop*1-1 through *cop*1-4) will be presented elsewhere (X.-W. Deng and P.H. Quail, in prep.). Seedlings with mutations in the *cop*1 locus develop in darkness as wild-type seedlings do in the light (Fig. 1). This behavior is similar to that reported previously for the *det*1 and *det*2 mutants of *Arabidopsis* (Chory et al. 1989a). Dark-grown *cop*1 seedlings exhibit short hypocotyls and open and enlarged cotyledons, and accumulate high levels of anthocyanin. However, they lack chlorophyll, because chlorophyll biosynthesis requires light. Mutations in the *cop*1 locus also have an effect on light-grown plants, resulting in severe dwarfism and low fertility (X.-W. Deng and P.H. Quail, in prep.). Complementation tests of *cop*1 with *det*1 and *det*2 mutants (Table 1) indicate that the *cop*1 mutation defines a genetic locus that is different from *det*1 and *det*2. Because the *cop*1-1 allele is the first one isolated and the most advanced in backcrossing, it has been used for most of the characterizations reported here.

Dark-grown cop1 *seedlings exhibit light-grown characteristics at cellular and subcellular levels*

The constitutive photomorphogenic characteristics of the dark-grown *cop*1 mutant are also evident at the cellular level. Unlike dark-grown, wild-type seedlings that have very elongated hypocotyl cells, the hypocotyl cells of dark-grown *cop*1 seedlings are short and are even slightly shorter than those of light-grown, wild-type seedlings (data not shown). Cross sections of cotyledons show that the morphologies of dark- and light-grown (Fig. 2A and B, respectively) *cop*1 cotyledons are almost identical to each other and very similar to light-grown, wild-type seedlings (Fig. 2D), except that the mutant has less air space between the mesophyll cells. The cotyledons of dark-grown *cop*1 seedlings clearly develop stomatal structures in the epidermis and various other differentiated cell types within the organ (Fig. 2A), whereas dark-grown, wild-type seedlings lack both features (Fig. 2C). The cellular basis for cotyledon enlargement in dark-grown *cop*1 seedlings as compared with that of wild type is due to both the cell expansion and cell division (Fig. 2A and C).

Plastid morphology in dark- and light-grown *cop*1 seedlings was examined by electron microscopy (Fig. 3). Typical plastids in cotyledons of dark- and light-grown wild-type seedlings are shown in Figure 3, A and B, respectively. The morphologies of these plastids are similar to those of other plant species (Kirk and Tilney-Bassett 1978). Plastids from dark-grown seedlings (etioplasts) contain a central paracrystalline assembly of tubules, termed the prolamellar body. Plastids from light-grown seedlings (chloroplasts) have typical thyla-

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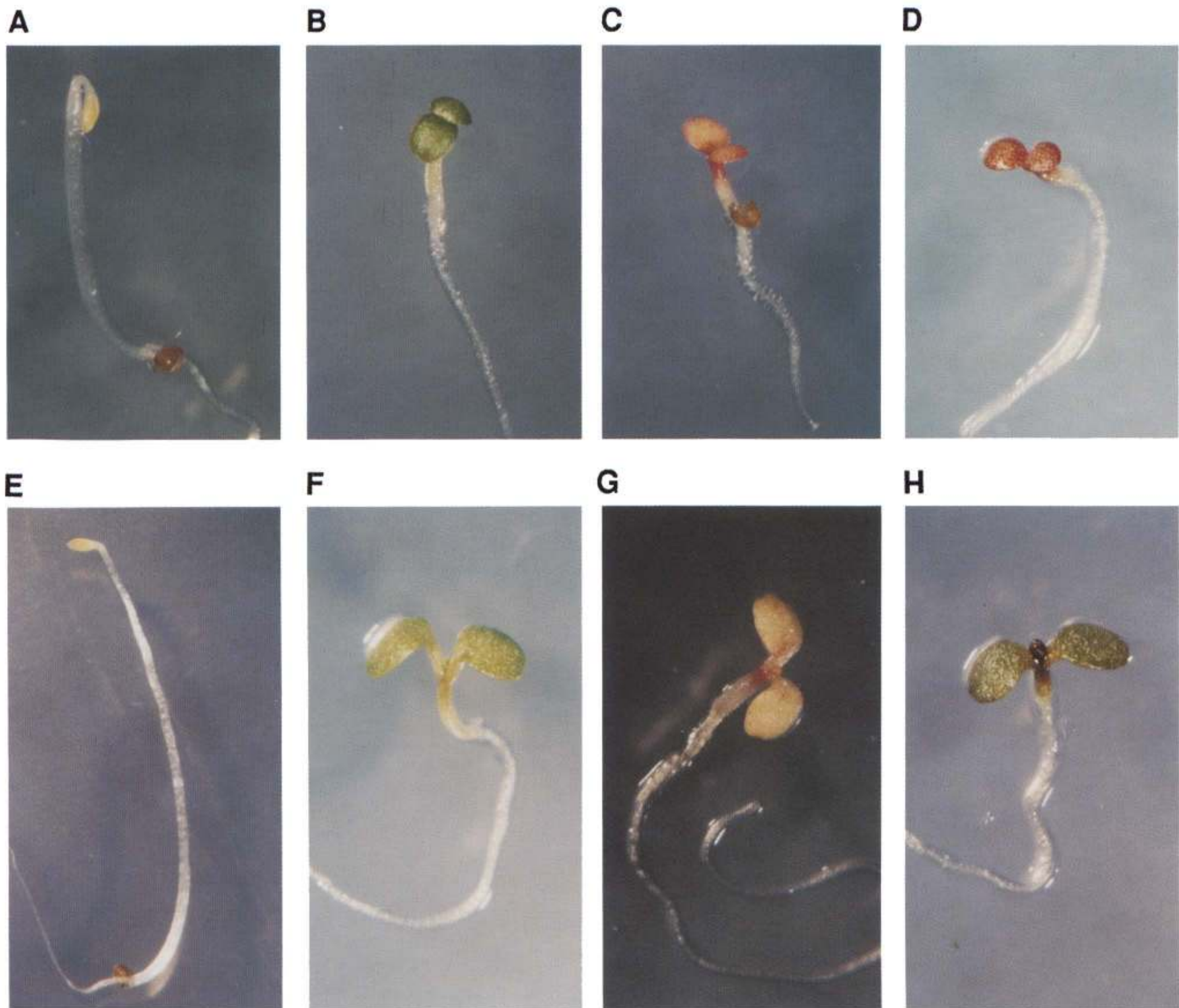

Figure 1. Phenotypes of dark- and light-grown *cop*1 and wild-type *Arabidopsis* seedlings. Seedlings were grown for 3 (*A–D*) or 6 (*E–H*) days from germination. (*A* and *E*) Dark-grown, wild-type seedlings; (*B* and *F*) light-grown, wild-type seedlings; (*C* and *G*) dark-grown *cop*1-1 seedlings; (*D* and *H*) light-grown *cop*1-1 seedlings. The same magnification was used for all panels except *E*, which has two-thirds of the magnification used for the others.

koid membrane structure, composed of stacked and unstacked regions. In Figure 3, C and D, two representative plastids from dark-grown *cop*1-1 and *cop*1-2 seedlings are shown. In contrast with etioplasts of dark-grown, wild-type seedlings, the plastids from dark-grown *cop*1 seedlings clearly show signs of chloroplast development, as evidenced by the lack of prolamellar bodies and the formation of some layer two-stacked thylakoid membrane structures. Plastid morphology in cotyledons of light-grown *cop*1 seedlings is identical to that of light-grown, wild-type seedlings (data not shown).

Mutations in the cop1 *locus lead to constitutive expression of light-regulated genes in seedlings and mature plants*

The observation that dark-grown *cop*1 seedlings appear to have many, if not all, of the physiologic and morphologic characteristics associated with light-grown, wild-type seedlings prompted us to examine whether the expression of light-regulated genes is also altered. We examined, by Northern blot analysis, mRNA levels of eight representative nuclear- and plastid-encoded genes whose expression normally is subjected to light control in both seedlings and adult plants (Fig. 4). Equal amounts of 18S cytoplasmic rRNA detected in the different lanes (Fig. 4J) confirms that the same amount of total RNA was used for each sample. The changes in mRNA levels observed in wild-type plants can be divided into three groups. mRNA levels for the five nuclear-encoded, light-inducible genes (*rbc*S, *cab*, *fed*A, *chs*, *nia*2) were very low but detectable in dark-grown seedlings, increased substantially in light-grown seedlings and adult plants, and re-

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Table 1. *Phenotypic segregation in the F_1 progeny of crosses between* cop1 *and* det *mutations*

Parental genotype	Germination rate (%)	Number of seedlings: wild type	Number of seedlings: mutant
det1/det1 × *cop1-1/cop1-1*	96	23	0
det2/det2 × *cop1-1/cop1-1*	82	24	0

Seedling phenotypes were examined after growth for 6 days in the dark.

turned to low levels after dark adaptation (Fig. 4A–E). These results are consistent with previous observations for these genes (Chory et al. 1989a; T. Caspar et al., in prep.). Conversely, *phy*A mRNA levels were high in dark-grown seedlings, decreased in light-grown seedlings and adult plants as reported (Sharrock and Quail 1989), and returned to high levels again after dark adaptation (Fig. 4F). The two plastid-encoded genes (*rbc*L and *psb*A) form their own group (Fig. 4G and H). Like nuclear-encoded, light-inducible genes, the mRNA levels for *rbc*L and *psb*A were low in dark-grown seedlings and high in light-grown seedlings and adult plants. However, these mRNA levels decreased relatively little, if any, in dark-adapted adult plants (two- to threefold for *rbc*L mRNA). Hybridization signals for plastid 16S rRNA indicate that these observed differences are not primarily due to major differences in the contribution of total plastid RNA to the total RNA sample (Fig. 4I).

Comparison of the mRNA levels for the light-regulated genes at the same developmental stages in *cop1* and wild-type plants shows that expression in the *cop1* mutant is more nearly constitutive. *phy*A gene expression is constitutively repressed in dark-grown *cop1* seedlings and in dark-adapted adult *cop1* plants, as it is in wild-type plants in the light (Fig. 4F). Conversely, the mRNA levels of light-inducible nuclear genes are already high in dark-grown seedlings, and, with the exception of *chs*, do not decrease upon dark adaptation in adult *cop1* plants (Fig. 4A–E). Although the level of *chs* mRNA in dark-adapted *cop1* adult plants is lower than before dark adaptation, it is close to that of light-grown, wild-type seedlings and adult plants (Fig. 4D). In contrast, *chs* mRNA levels in dark-adapted adult wild-type plants are undetectable, even after longer exposures of Northern blots, under which conditions the *chs* mRNA in dark-grown, wild-type seedlings is clearly detectable (data not shown). mRNA levels of the two plastid-encoded genes, *rbc*L and *psb*A, in dark-grown *cop1* seedlings are almost as high as those of light-grown seedlings and adult *cop1* plants (Fig. 4G and H). These increases in plastid gene mRNA levels in dark-grown *cop1* seedlings are primarily due to gene-specific modulation, but with some possible contribution from the apparent 50% increase in total plastid RNA level (Fig. 4I).

It is not clear why the absolute levels of *cab* and *fed*A mRNAs are consistently lower in adult *cop1* plants than those of wild-type adult plants (Fig. 4B and C). On the other hand, high *chs* mRNA levels in both dark- and light-grown *cop1* seedlings (Fig. 4D) correlate well with the high levels of anthocyanin in these seedlings (see Fig. 1). The low level of anthocyanin in light-grown adult *cop1* plants (data not shown) as compared with *cop1* seedlings also correlates well with *chs* mRNA levels (Fig. 4D).

Mutations in the cop1 *gene activate light-inducible promoters in the absence of light*

The constitutive accumulation of mRNA for light-regu-

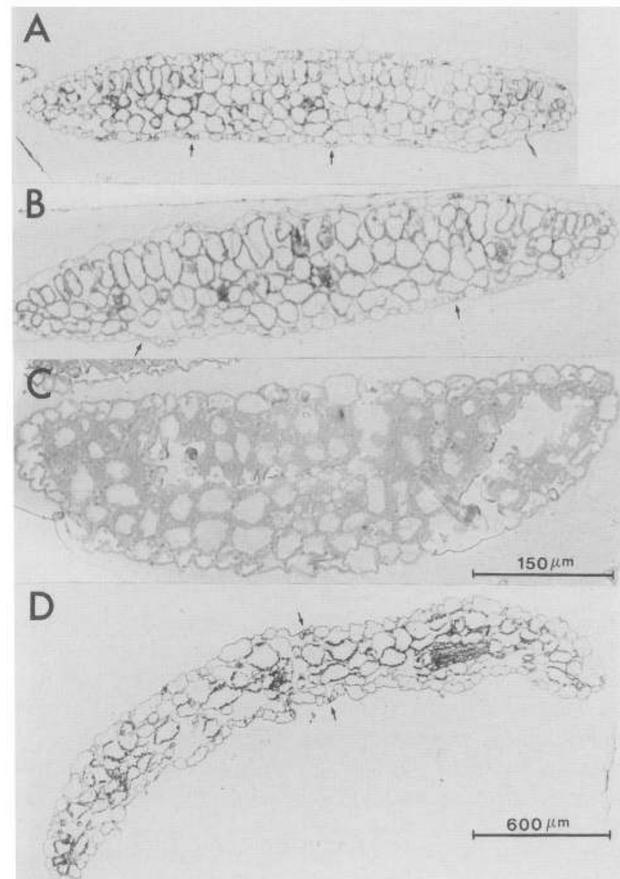

Figure 2. Morphogenetic comparison of cotyledon cross sections from 6-day-old *cop1-1* seedlings grown in the dark (*A*) or light (*B*) and 6-day-old wild-type seedlings grown in the dark (*C*) or light (*D*). Light micrographs were taken of 1-µm sections of the fixed and embedded seedlings (see Materials and methods). The same magnification was used for all panels (scale shown in *D*), except *C*, which was magnified fourfold greater than the others. Examples of stomata are indicated by arrows in *A*, *B*, and *D*. Note that there are at least twice as many cells per cotyledon in dark-grown *cop1* seedlings (*A*) as in dark-grown, wild-type seedlings (*C*) when cross sections from similar positions are compared.

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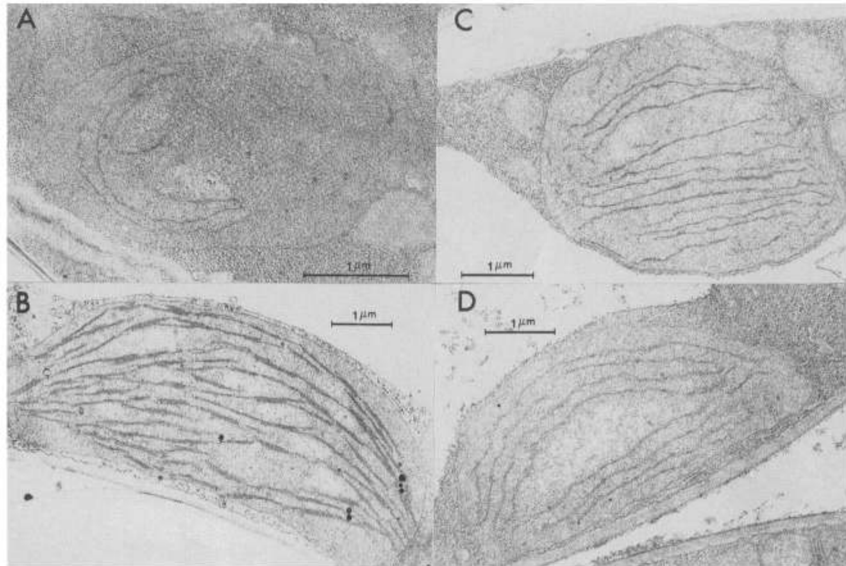

Figure 3. Morphogenetic changes in plastids of dark-grown seedlings caused by *cop*1 mutations. Plastids in cotyledons of 6-day-old dark-grown (*A*) and light-grown (*B*) wild-type seedlings, or of 6-day-old *cop*1-1 (*C*) and *cop*1-2 (*D*) dark-grown mutant seedlings are shown. Typical etioplasts (*A*) and chloroplasts (*B*) are present in dark- and light-grown, wild-type seedlings, respectively, whereas the plastids in cotyledons of dark-grown mutants (*C* and *D*) show similarity to chloroplasts. The magnification scale is indicated for each panel.

lated genes in dark-grown *cop*1 seedlings could result from transcriptional activation of the promoters of these genes. To test this possibility, promoter–β-glucoronidase (GUS) reporter fusions were constructed (Fig. 5; T. Caspar et al., in prep.), transformed into wild-type plants, and introduced into *cop*1 mutant plants by conventional genetic crossing (see Materials and methods). At least five transgenic lines, whose GUS expression patterns paralleled those of the endogenous genes, were generated for each construct.

Qualitatively similar results were obtained from different transgenic lines that contained the same promoter–GUS construct. The results obtained from a single representative transgenic line of each construct for both wild-type and mutant plants (see Materials and methods) are shown in Figure 5. The control 35S promoter shows slightly higher activities in dark-grown seedlings than in light-grown seedlings in both wild type and mutant (Fig. 5A). However, this difference is considerably less than for the other promoters. The transcriptional activities of both *cab*1 and *fed*A full-length promoters were light inducible in wild-type plants (Fig. 5B and C; T. Caspar et al., in prep.). The *cab*1 promoter activity was induced 20-fold by light (compared with >100-fold induction at the mRNA level; Fig. 4B), whereas the *fed*A promoter was consistently induced 2- to 5-fold by light (compared with 8-fold induction at the mRNA level; Fig. 4C; T. Caspar et al., in prep.). The *fed*A promoter activity in dark-grown *cop*1 seedlings is higher than that of light-grown *cop*1 seedlings (Fig. 5B). This result indicates that the *fed*A promoter is fully active in dark-grown mutant seedlings, which is consistent with the result for mRNA levels (Fig. 4C). Qualitatively, *cab*1 promoter activity also correlates with mRNA accumulation (Fig. 4B). That is, *cab*1 promoter activity in dark-grown *cop*1 seedlings is higher than that in dark-grown, wild-type seedlings and lower than that in light-grown *cop*1 seedlings. Also similar to the *cab* mRNA level changes (Fig.4B), the *cab*1 promoter activity in light-grown *cop*1 seedlings is lower than that of light-grown, wild-type seedlings (Fig. 5C). However, it is difficult to quantitatively correlate the extent of GUS activity induced by light in transgenic plants with endogenous mRNA level changes, because GUS expression may be affected by the chromosomal location of the introduced DNA, the background level of promoter activity under noninductive conditions, and the long half-life of the GUS protein. Because the *cab* mRNA level shown in Figure 4 is the sum of three *cab* genes (*cab*1–*cab*3), it is also possible that the total *cab* mRNA level may not necessarily reflect the changes of *cab*1 mRNA (Karlin-Neumann et al. 1988). However, quantification of individual *cab* mRNAs in *cop*1 plants by S1 protection analysis indicates that all three mRNAs are likely to be affected by the *cop*1 mutation to the same degree (data not shown).

To begin to identify sequences in light-regulated promoters potentially involved in dark activation in the *cop*1 mutant, a *cab*1 promoter deletion mutant was fused to GUS and introduced into wild-type and mutant plants (Fig. 5D). This promoter fragment (from −200 to +67) has been shown in transgenic tobacco plants to be the minimum sequence required for full light inducibility and tissue-specific expression (Ha and An 1988). Our results confirm that this fragment does confer correct light inducibility (>10-fold induction) and tissue specificity (data not shown) in wild-type *Arabidopsis* plants. However, the absolute activity of this construct was much reduced in both dark- and light-grown seedlings compared with the full-length promoter. Nevertheless, the activity of this fragment is modulated in *cop*1 seedlings in a manner parallel with that of the full-length

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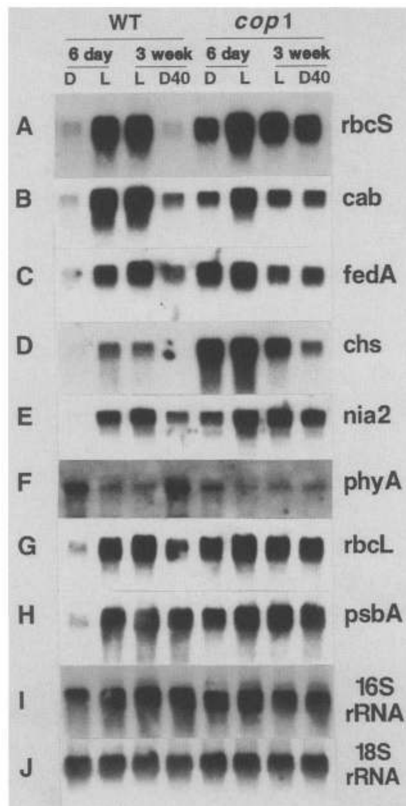

Figure 4. Northern blot analysis of steady-state RNA levels of nuclear- and plastid-encoded genes. RNA levels from wild-type (WT) and *cop1*-1 mutant plants were analyzed. Total RNA was isolated from seedlings grown for 6 days in the dark (D) or light (L); from adult plants grown for 3 weeks in continuous light (L); and from adult plants grown for 3 weeks in continuous light and then returned to darkness for 40 hr before harvest (D40). RNA isolation, hybridization probe preparation, and Northern hybridization are described in Materials and methods. Equal amounts of total RNA from the different plant samples were used, and 10 identical blots were hybridized with labeled gene-specific probes for 10 different genes: (*rbcS*) Gene insert for small subunit (SSU) of ribulose-1,5-bisphosphate carboxylase (Krebbers et al. 1990); (*cab*) gene for chlorophyll a- and b-binding proteins of photosynthetic light-harvesting complexes (Leutwiler et al. 1986); (*fedA*) ferredoxin type A gene (Somers et al. 1990); (*chs*) chalcone synthase gene (Feinbaum and Ausubel 1988); (*nia2*) nitrate reductase gene 2 (Crawford et al. 1988); (*phyA*) type-A phytochrome gene (Sharrock and Quail 1989); (*rbcL*) plastid gene encoding the large subunit (LSU) of ribulose-1,5-bisphosphate carboxylase (Zarawski et al. 1981); (*psbA*) plastid gene encoding the 32-kD protein of photosystem II (Zarawski et al. 1982); and genes for plastid (16S rRNA) (Deng and Gruissem 1987) and cytoplasmic (18S rRNA) small rRNAs (Jorgensen et al. 1987). Northern blots were exposed to X-ray film for different periods of time to obtain suitable exposures for each transcript.

promoter (Fig. 5D). These results indicate that this mutant promoter contains sufficient sequence for the dark activation of the *cab1* promoter in *cop1* mutant plants.

The cop1 *mutant does not affect phytochrome control of seed germination*

The initial germination process (as distinct from subsequent seedling growth and development) is clearly under phytochrome control in wild-type *Arabidopsis* seeds (Chory et al. 1989a; X.-W. Deng et al., unpubl.). We therefore examined whether the *cop1* mutation affects phytochrome control of seed germination in addition to seedling development. Seeds from homozygous *cop1* plants were pretreated with different light regimes before germination in darkness (Table 2). Germination rates are high in the green light, red light, and far-red followed by red light treatments. In contrast, germination rates after far-red alone, and red followed by far-red light treatments show a decrease of at least 10-fold relative to the above-mentioned treatments. These results are very similar to those for wild-type seeds (data not shown; Chory et al. 1989a). The inhibition of germination by far-red light and the reversibility of red and far-red treatments unambiguously show that the *cop1* mutation does not affect phytochrome control of seed germination. The high germination rates of unirradiated seeds or seeds treated with green safelight and the reduction in this rate by far-red light alone implies that the amount of active phytochrome in the untreated seeds was sufficient to promote germination without further treatment. These results indicate that separable mechanisms are responsible for induction of seed germination and photomorphogenesis of seedlings, although light and phytochrome are involved in both processes.

The cop1 *mutation does not change diurnal fluctuations in* cab *mRNA levels*

It is well documented that the transcriptional activity of several plant genes is subjected to diurnal control (Giuliano et al. 1988a; Nagy et al. 1988). The best-studied example is the *cab* genes. *cab* mRNA levels and transcriptional activity fluctuate during the diurnal cycle, reaching the lowest point in the middle of the night and the highest level in early afternoon (Giuliano et al. 1988a; Nagy et al. 1988). This periodic fluctuation is apparently controlled by an endogenous circadian clock, whose phase can be entrained by light, possibly through the phytochrome system (Giuliano et al. 1988a; Nagy et al. 1988; Lam and Chua 1989). We examined whether the *cop1* mutation affects diurnal control of *cab* mRNA levels. Figure 6 shows that *cab* mRNA levels in the *cop1* mutant fluctuate with the same periodicity as those in wild-type plants, albeit with a lower amplitude. These results indicate that the wild-type *cop1* gene product appears to be involved only in light regulation of *cab* gene expression and not in diurnal control of its expression. It should be noted that the adult wild-type and *cop1* plants used for the dark-adaptation experiments shown in Figure 4 were grown under continuous light (24 hr/day) to suppress the diurnal fluctuations in mRNA levels for simplification of the dark-adaptation studies.

Discussion

Here, we report the characterization of a genetic locus, designated *cop1*, which plays a regulatory role in the

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	Construct	wt Dark	wt Light	*cop1* Dark	*cop1* Light
A	-340 35S +6 GUS nos 3'	1469	1335	1120	880
B	-1850 fedA +77 GUS nos 3'	7251	15053	23157	14793
C	-1281 cab1 +67 GUS nos 3'	563	11563	931	2221
D	-200 cab1 +67 GUS nos 3'	36	338	80	194

Figure 5. Modulation of promoter activity by light and by the *cop1* mutation. The promoter–GUS fusion constructs used for genetic transformations are shown at *left*, and the GUS activities (nmoles 4-methylumbelliferone/hr per mg of protein) of transgenic wild-type (wt) or mutant seedlings containing these constructs grown either in dark or light are shown at *right*. The four promoters used are a 346-bp CaMV 35S promoter fragment (Rogers et al. 1986) (*A*), a 1.9-kb promoter fragment from the *Arabidopsis fedA* gene (Sommers et al. 1990; T. Caspar et al. in prep.) (*B*), a 1.35-kb promoter fragment from the *Arabidopsis cab1* promoter (Ha and An 1988) (*C*), and a 267-bp minimal promoter fragment from the *cab1* gene (*D*). The details of promoter–GUS fusion construction, genetic transformation, and GUS activity assay are described in Materials and methods. In each promoter–GUS construct, the end positions (base pairs) of the promoter fragment are marked relative to the transcription start site (+1). GUS activities are the average of three independent measurements, among which the variation was <5%.

control of photomorphogenesis in *Arabidopsis*, both in seedlings and adult plants. Dark-grown *cop1* seedlings exhibit seedling morphology, cell and organelle differentiation, and gene expression—that are normally observed only in light-grown, wild-type seedlings (Kendrick and Kronenberg 1986; Kuhlemeier et al. 1987). The recessive nature of the four mutant alleles obtained implies that a loss of function leads to the constitutive photomorphogenic phenotype. These observations suggest that the wild-type *cop1* gene is an early regulatory gene in the cascade of changes that control photomorphogenesis in *Arabidopsis*. The encoded product (COP1) of the wild-type *cop1* locus appears to have a critical role in coupling light perception to morphogenesis and, therefore, in switching between skotomorphogenic and photomorphogenic pathways during seedling development. On the other hand, the severe defects observed in adult light-grown *cop1* plants (X.-W. Deng and P.H. Quail, in prep.) suggest that the *cop1* locus also plays a role in the development of light-grown plants.

The morphology of dark-grown *cop1* seedlings is similar to that reported recently for *det1* mutants, which also have a light-grown phenotype when grown in the dark (Chory et al. 1989a). However, the two mutations are at different genetic loci (Table 1) on two different chromosomes (X.-W. Deng and P.H. Quail, in prep.) and there are two distinct regulatory differences between these two classes of mutants. First, *cop1* does not affect normal phytochrome control of seed germination (Table 2), whereas *det1* seeds germinate regardless of the presence or absence of active phytochrome (Chory et al. 1989a). Second, adult *cop1* mutant plants do not display a decrease or increase in the mRNA levels of light-regulated genes upon dark adaptation (Fig. 4) in contrast with wild-type and *det1* plants (Chory et al. 1989a). The *det1* gene product is apparently involved in light regulation of germination and seedling development but not in dark adaptation, whereas the *cop1* product is involved in light regulation in seedling development and dark adaptation of adult plants, but not in seed germination. Thus, different regulatory circuitry may mediate the light regulation of seed germination, seedling development, and dark-adaptation processes. On the other hand, a given regulatory component (such as *det1* and *cop1*) could be

Table 2. *Germination rates for* cop1 *mutant seed under different light conditions*

Light treatment	Seeds	Seedlings	Germination rate (%)
Dark	123	66	54
Green safelight	111	59	53
Red	125	84	67
Far-red	115	6	5
Red/far-red	100	5	5
Far-red/red	112	63	56

The light treatments were green safelight, 2 min; red, 5 s saturating pulse; far-red, 10 s saturating pulse. In the case of two different light treatments, the second light exposure immediately followed the first exposure.

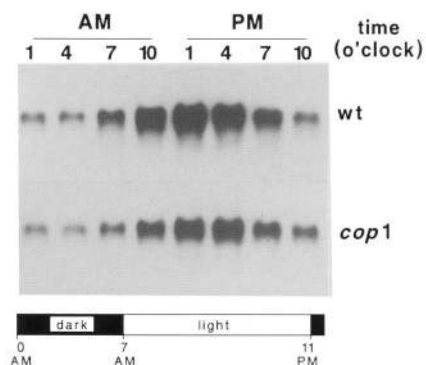

Figure 6. Diurnal fluctuation of *cab* mRNA levels in wild-type (wt) and *cop1* mutant plants. Wild-type and *cop1* mutant plants were grown under a 16-hr light/8-hr dark (11 p.m. to 7 a.m.) photoperiod for 21 days. The plants were then harvested at 3-hr intervals for 24 hr as shown. Total RNA was isolated from these plants, and 5 μg from each sample was used for Northern analysis of *cab* mRNA (see Materials and methods).

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involved in more than one of the regulatory circuitries. It will be interesting to know how *det1* and *cop1* relate to each other and how they regulate photomorphogenesis.

The *cop1* mutation affects plant responses that are mediated by all three photoreceptor systems. These responses include inhibition of hypocotyl elongation (Koornneef et al. 1980; Kendrick and Kronenberg 1986) and modulation of gene expression (*cab*, Karlin-Neumann et al. 1988; *fedA* and *nia2*, T. Caspar et al., in prep.; *chs*, Chory et al. 1989a). Therefore, the function of COP1 does not appear to be restricted to the signal transduction pathway of a single photoreceptor, implying that the photoreceptors or their transduction pathways merge at some point to modulate the activity of COP1.

The fact that the germination rate of *cop1* mutant seeds is still fully under phytochrome control (Table 2) suggests that both the relevant phytochrome and the transduction pathway leading to this response are unaffected by the *cop1* mutation. It also suggests that germination and photomorphogenesis during seedling development involve two independent developmental commitments. Although phytochrome modulates both commitments, the data indicate either that there is a branchpoint in the phytochrome signal transduction pathway, after which different components are involved in controlling the two processes, or that two separate types of phytochrome with independent signal transduction pathways operate in parallel to control the two processes (see Smith and Whitelam 1990). Similarly, the normal periodicity in the diurnal fluctuation of *cab* mRNA levels in *cop1* mutants suggests that COP1 is not involved in the diurnal control of *cab* gene expression.

The expression of a variety of plant nuclear genes is either positively or negatively regulated by the presence of light, the effects of which are mediated by either phytochrome (such as *cab*, Karlin-Neumann et al. 1988; *phyA*, Lissemore and Quail 1988; *fedA* and *nia2*, T. Caspar et al., in prep.) or the blue-light receptor (such as *chs*, Chory et al. 1989a). It has been demonstrated for most of these genes that light modulates transcriptional activity through their promoter sequences (Lissemore and Quail 1988; Bruce et al. 1989; Gilmartin et al. 1990; T. Caspar et al., in prep.). On the basis of our analysis of mRNA levels and promoter–GUS constructs in transgenic plants (Figs. 4 and 5), we propose that in wild-type plants, COP1 is involved in the modulation of promoter activity of light-regulated genes. To achieve this, COP1 must, in darkness, concomitantly suppress the transcriptional activity of light-inducible promoters (by activation of a repressor or inhibition of an activator) and activate or derepress the transcriptional activity of light-repressible promoters. Light is thus postulated to repress COP1 function via photoreceptor activation, as do mutations in the *cop1* gene.

Overall, our experimental results and current knowledge of photomorphogenesis (Kendrick and Kronenberg 1986; Adamse et al. 1988; Gilmartin et al. 1990) are consistent with the following concept of light-regulated development in *Arabidopsis*. Seed germination and seedling development involve two distinct decisions: the first involving germination; and the second involving a choice between two developmental strategies—skotomorphogenesis and photomorphogenesis (Mohr and Shropshire 1983). A set of control genes (such as *det1* and *cop1*) are involved in making these decisions. Light signals are perceived by regulatory photoreceptors and are transduced by still unknown transduction pathways to modulate the activities of the products of these control genes and thereby affect these developmental decisions. In the case of *cop1*, the fact that the mutations are recessive indicates that loss of COP1 function is responsible for the switch from skotomorphogenesis to photomorphogenesis in the mutants in darkness. Perception and transduction of light signals in some way abrogates COP1 activity leading to a phenocopy in wild-type plants of the *cop1* mutation.

Materials and methods

Plant materials and growth conditions

A. thaliana ecotype Columbia was the parental strain used for the isolation of photomorphogenic mutants. Wild-type seeds and some M2 seed batches mutagenized with ethylmethanesulfonate (EMS) were purchased from Lehle Seeds (Tucson, AZ). Our own EMS-treated M2 seeds were produced according to Sommerville and Ogren (1982).

Unless specified otherwise, *Arabidopsis* seeds were routinely surface-sterilized for ~15 min in 30% bleach (Clorox), rinsed at least five times, and plated in petri plates (150 × 25 mm) containing growth medium (GM; Valvekens et al. 1988). After cold treatment at 4°C for 2–4 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. For the dark-adaptation experiment, *Arabidopsis* plants were grown in continuous light (24 hr/day) for 3 weeks. *Arabidopsis* plants in petri dishes were either harvested for experiments or transferred to soil to grow to maturity for genetic manipulations or seed set. For all reported experiments, except the seed germination study, seeds (mixture of mutant and wild type) from plants heterozygous for *cop1* mutations were used. Wild-type and mutant plants were harvested individually and pooled when necessary to assure the same growth conditions for wild-type and *cop1* mutants. The light source for *Arabidopsis* plant growth was a combination of fluorescent and incandescent lights, ranging from 100 to 300 μEinstein/m^2 per sec. For mutant screening, ~2000 seeds were placed in each plate and incubated in the dark at 22°C for 7 days after cold treatment. To study the effect of light on seed germination, seeds from plants homozygous for the *cop1* mutation were used. Sources of specific wavelengths of lights (red, far-red, green safelight) were identical to those published (Bruce et al. 1989).

Analysis of anthocyanin and chlorophyll pigments

Anthocyanin content in *Arabidopsis* seedlings was measured by the procedure of Schmidt and Mohr (1981), using 0.5 ml of extraction media for 20–40 light-grown seedlings or 60–120 dark-grown seedlings. For chlorophyll measurement, 20–60 *Arabidopsis* seedlings were harvested in a microcentrifuge tube containing 0.5 ml of 80% acetone. The seedlings were ground with a ground-glass rod until the chlorophyll was completely extracted. The extract was then centrifuged at maximum speed in a microcentrifuge for 10 min, and the supernatant was used to measure chlorophyll a and chlorophyll b (Arnon 1949).

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Light and electron microscopy

The fixation and embedding of *Arabidopsis* seedlings was performed according to a published procedure (Deng and Gruissem 1987). Seedlings grown on petri plates for 6 days in the dark or light were harvested and immediately subjected to fixation. The embedded cotyledons were sectioned using an ultramicrotome (model MT 6000-XL from RMC, Inc.). Thick sections (1 μm) were stained with toluidine blue for light microscopy examinations. Thin sections (0.1–0.2 μm) were poststained with 5% (wt/vol) uranyl acetate in water for 20 min and counterstained with 3% (wt/vol) lead citrate for 10 min. After the sections were dried, the plastids in these sections were examined and photographed using a transmission electron microscope.

RNA analysis

For 6-day-old dark- and light-grown *Arabidopsis* seedlings, wild-type or *cop*1-1 mutant individuals were harvested from petri plates and frozen immediately in liquid nitrogen. For 3-week-old mature plants, wild-type and mutant individuals that had been separated onto different petri plates, when 5 days old, were pooled and frozen in liquid nitrogen at the specified time points. Dark-grown and dark-adapted plants were harvested under dim green safelight. Isolation of total RNA, electrophoresis and blotting of RNA, and hybridization with radioactively labeled DNA probes were as described previously (Sharrock and Quail 1989). The DNA probes used were a 0.55-kb DNA fragment [generated by polymerase chain reaction (PCR)] corresponding to the entire open reading frame of the *Arabidopsis* small subunit of ribulose-1,5-bisphosphate carboxylase gene (*rbc*S; Krebbers et al. 1988); a 0.5-kb *Bam*HI–*Sst*I DNA fragment corresponding to the coding region of the *Arabidopsis cab*3 gene (Leutwiler et al. 1986); a 1.5-kb *Hin*cII–*Eco*RI DNA fragment containing the *Arabidopsis fed*A gene (Somers et al. 1990); a 0.95-kb cDNA coding for the *Arabidopsis* chalcone synthase gene (*chs*, Feinbaum and Ausubel 1988); a 0.9-kb DNA fragment (generated by PCR) encoding the first 300 amino acids of an *Arabidopsis* nitrate reductase gene (*nia*2, Crawford et al. 1988); a 0.2-kb DNA fragment derived from the *Arabidopsis* phytochrome A (*phy*A) gene 3′-noncoding sequence (Sharrock and Quail 1989); a 1.25-kb *Pst*I–*Eco*RI DNA fragment containing most of the coding region of a spinach chloroplast *rbc*L gene (Zurawski et al. 1981); a 1.2-kb *Bgl*II–*Xba*I DNA fragment containing most of the coding region of a spinach chloroplast *psb*A gene (Zurawski et al. 1982); a 3.0-kb *Bam*HI DNA fragment from the spinach chloroplast genome containing the entire 16S rRNA gene (Deng and Gruissem 1988); and a 1.0-kb *Eco*RI–*Bam*HI DNA fragment containing the coding region of the pea nuclear 18S rRNA gene (Jorgensen et al. 1987). The identity of cloned DNA fragments produced by PCR (*rbc*S, *nia*2) was confirmed by sequencing. For all probes except *phy*A, ~200 ng of each purified DNA fragment was labeled to high specific activity (~2×10^5 to 3×10^5cpm/ng of DNA) by random oligomer priming, denatured by boiling for 5 min, and used for hybridization. For the *phy*A probe, a single-stranded DNA probe was prepared by using a single-stranded DNA template of an M13mp18 clone shown to contain sequence specific for the *phy*A gene (Sharrock and Quail 1989).

Promoter–GUS constructs and genetic transformation

A 2.3-kb fragment containing the GUS-coding sequence and the 3′ sequence from the nopoline synthase (NOS) gene (Jefferson et al. 1987) was inserted into the filled-in *Hin*dIII site of the pcit20 vector (a derivative of pMON754; Rogers et al. 1986; obtained from Dr. E. Meyerowitz, California Institute of Technology), to create plasmid CGUSA (T. Caspar et al., in prep.). Promoter DNA fragments were inserted upstream of the GUS-coding sequence of CGUSA (see Fig. 5). These included a fragment from −340 to + 6 bp from the 35S cauliflower mosaic virus (CaMV) promoter (Rogers et al. 1986), a fragment from −1850 to +77 bp from the *Arabidopsis fed*A promoter (Somers et al. 1990; T. Caspar et al., in prep.), a fragment from −1281 to +67 bp from the *Arabidopsis cab*1 gene (Ha and An 1988; Karlin-Neumann et al. 1988), and a minimal (from −200 to +67 bp) *Arabidopsis cab*1 promoter. For each promoter, the position of the transcriptional initiation site is designated +1. These constructs were introduced into *Arabidopsis* (ecotype No-O) using *Agrobacterium*-mediated transformation of root explants according to Valvekens et al. (1988). At least five independent transgenic *Arabidopsis* plants were obtained for each construct. Two representative lines for each promoter–GUS fusion construct that produced the expression pattern expected of the endogenous genes were chosen to cross with *cop*1 plants. The F_2 seeds produced by selfing the F_1 plants, which consisted of one-quarter mutant and three-quarters wild type, were germinated and grown for 6 days under various light conditions, and 20–100 seedlings (both mutants and wild type) were collected, pooled, and assayed for GUS activity. Because both mutant and wild-type seedling pools were randomly collected from the same F2 population, any variation that might be contributed by the genetic backgrounds of the respective parents should have been minimized. With the exception of one line that was transformed with a 35S–GUS construct, the T-DNA insert sites appeared to be unlinked to the *cop*1 locus in the transgenic lines chosen.

GUS activity assay

Tissue-specific expression of introduced promoter–GUS constructs was monitored by histochemical staining. Briefly, *Arabidopsis* seedlings were harvested and immersed in 50 mM phosphate buffer containing 1 mM 5-bromo-4-chloro-3-indolyl-β-glucuronide (X-Gluc), frozen at −80°C, thawed at room temperature, and incubated at 37°C for 2–24 hr, depending on the amount of GUS activity present in the seedlings. GUS enzyme activity in transgenic *Arabidopsis* seedlings was quantified according to Jefferson (1987) by measuring the fluorescence of methylumbelliferone (MU) produced by GUS cleavage of methylumbelliferyl-β-D-glucuronide. Protein concentration was determined by the Bio-Rad protein assay (Bio-Rad) according to the manufacturer's suggested procedure.

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