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Overexpression of Arabidopsis COP1 Results in Partial Suppression of Light-Mediated Development: Evidence for a Light-Inactivable Repressor of Photomorphogenesis

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Arabidopsis seedlings are genetically endowed with the capability to follow two distinct developmental programs: photomorphogenesis in the light and skotomorphogenesis in darkness. The regulatory protein CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1) has been postulated to act as a repressor of photomorphogenesis in the dark because loss-of-function mutations of COP1 result in dark-grown seedlings phenocopying the light-grown wild-type seedlings. In this study, we tested this working model by overexpressing COP1 in the plant and examining its inhibitory effects on photomorphogenic development. Stable transgenic Arabidopsis lines overexpressing COP1 were generated through Agrobacterium-mediated transformation. Overexpression was achieved using either the strong cauliflower mosaic virus 35S RNA promoter or additional copies of the wild-type gene. Analysis of these transgenic lines demonstrated that higher levels of COP1 can inhibit aspects of photomorphogenic seedling development mediated by either phytochromes or a blue light receptor, and the extent of inhibition correlated quantitatively with the in vivo COP1 levels. This result provides direct evidence that COP1 acts as a molecular repressor of photomorphogenic development and that multiple photoreceptors can independently mediate the light inactivation of COP1. It also suggests that a controlled inactivation of COP1 may provide a basis for the ability of plants to respond quantitatively to changing light signals, such as fluence rate and photoperiod.

INTRODUCTION

Light is one of the most important environmental factors influencing plant growth and development (Kendrick and Kronenberg, 1993). This can be best illustrated by light control of Arabidopsis seedling development (Wei et al., 1994a). Light-grown Arabidopsis seedlings develop short hypocotyls as well as open and expanded cotyledons and leaves, and they possess photosynthetically active chloroplasts. Many light-inducible genes, such as the chlorophyll *a/b* binding protein gene and the gene for the small subunit of ribulose bisphosphate carboxylase/oxygenase, are expressed at high levels. This developmental pattern is known as photomorphogenesis. In the absence of light, the photomorphogenic developmental pathway is repressed, and dark-grown seedlings have a very different developmental pattern, which is known as skotomorphogenesis. These seedlings have long hypocotyls and closed and unexpanded cotyledons, and they possess nonphotosynthetic etioplasts rather than chloroplasts. The light-inducible genes, which are expressed at high levels in light-grown seedlings, are expressed at very low levels in dark-grown seedlings. The choice between these two developmental patterns is flexible. For example, dark-grown seedlings can switch rapidly to photomorphogenic development when exposed to light. The molecular mechanisms controlling the switch between the two contrasting developmental fates are still not well understood.

At least three families of photoreceptors, namely the phytochromes, which absorb mainly red and far-red light (Quail, 1991; Furuya, 1993; Vierstra, 1993), the blue/UV-A photoreceptors (Ahmad and Cashmore, 1993; Kaufman, 1993; Kendrick and Kronenberg, 1993), and the UV-B photoreceptors (Mohr, 1986), are involved in monitoring ambient light conditions and regulating plant development. It has been documented that mutations affecting phytochrome A (*phyA*, Dehesh et al., 1993; Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993), phytochrome B (*phyB*, Reed et al., 1993; Wester et al., 1994), and a putative blue light photoreceptor (*hy4*, Ahmad and Cashmore, 1993) result in reduced responsiveness of plants to specific spectral regions of light signals, e.g., far-red, red, and blue light, and cause increased hypocotyl elongation. However, relatively little is known about how light signals absorbed by the photoreceptors are transduced to the nucleus, or how these signals trigger the various light-regulated developmental, physiological, and molecular responses. Recent biochemical studies using agonists or antagonists of possible

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signal transduction elements have suggested that trimeric G-proteins, cGMP, and calcium/calmodulin act as intermediates in phytochrome signal transduction (Romero et al., 1991; Neuhaus et al., 1993; Romero and Lam, 1993; Bowler et al., 1994). Trimeric G-proteins may also be involved in the blue light regulation of gene expression (Warpeha et al., 1991).

The isolation of Arabidopsis mutants defective in light-regulated seedling morphogenesis has led to the identification of many key players mediating light control of development (reviewed by Chory, 1993, and Deng, 1994; Wei et al., 1994a). Among these, *deetiolated1* (*det1*), *constitutive photomorphogenic1* (*cop1*), *cop8*, *cop9*, *cop10*, and *cop11* display the most pleiotropic phenotypes, with the dark-grown mutants phenocopying light-grown wild-type seedlings. All six pleiotropic *DET/COP* loci have also been independently defined as *FUSCA* (*FUS*) loci for the purple seed color and adult lethality caused by their severe mutations (Castle and Meinke, 1994; McNellis et al., 1994; Miséra et al., 1994). The pleiotropic nature of the mutant phenotypes implies that their gene products act early in the pathway before any major branch points of the regulatory cascades controlling specific aspects of light-regulated processes, such as cellular differentiation, plastid development, or hypocotyl elongation. Recently, molecular cloning of four pleiotropic *COP* loci, *COP1* (Deng et al., 1992; McNellis et al., 1994), *COP9* (Wei et al., 1994b), *COP11/FUS6* (Castle and Meinke, 1994), and *DET1* (Pepper et al., 1994), has been accomplished. Arabidopsis COP1 possesses a zinc binding motif, a putative coiled-coil domain, and multiple WD-40 repeats characteristic of the β subunits of trimeric G-proteins (Deng et al., 1992), and it bears significant homology to the $TAF_{II}80$ subunit of Drosophila TFIID (Dynlacht et al., 1993). The *COP9*, *COP11/FUS6*, and *DET1* genes encode novel proteins. Interestingly, the COP9 protein was found to be a component of a large (>560 kD) protein complex, which is responsive to light stimuli and requires COP8 and COP11 for its complex formation or stability (Wei et al., 1994b).

The recessive nature of the mutations in the *DET1*, *COP1*, *COP8*, *COP9*, *COP10*, and *COP11* loci implies that their wild-type gene products suppress photomorphogenic seedling development in darkness and that light acts to abrogate their repressive function. However, no direct evidence has been reported for any of the genes to substantiate this genetic model. We reasoned that if a member of the pleiotropic *COP/DET* gene products can act alone to repress photomorphogenic development, then an elevated level of this gene product might slow down the light inactivation process and lead to dark developmental characteristics in light-grown plants. To test this hypothesis, we generated transgenic Arabidopsis lines overexpressing COP1 and analyzed their phenotypes with respect to photomorphogenic seedling development under different light conditions. By employing specific spectral qualities of light, we were able to assess the contributions of distinct photoreceptors in mediating light inactivation of COP1. For example, photomorphogenic seedling development of Arabidopsis under continuous far-red light depends entirely on phytochrome A, because mutation of phytochrome A alone is equally as effective as a deficiency in all phytochromes for causing elongated hypocotyls and other skotomorphogenic characteristics of the continuous far-red light-grown seedlings (Dehesh et al., 1993; Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993). Therefore, if COP1 can act alone to repress photomorphogenic development, we anticipated that COP1 overexpression might cause a phenotype similar to the one observed for the photoreceptor mutants under specific light conditions.

RESULTS

Construction of Stable Transgenic Arabidopsis Lines Overexpressing COP1

To test whether an elevated level of COP1 can slow down the light inactivation process and lead to dark developmental characteristics in a light-grown plant, two gene cassettes, as shown in Figure 1, were constructed to achieve overexpression of *COP1* after stable transformation. The 35S–COP1 cassette (Figure 1A) utilized the strong cauliflower mosaic virus 35S

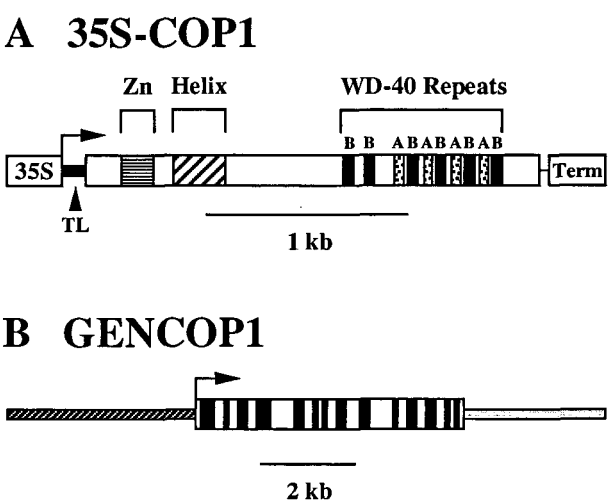


Figure 1. The *COP1* Expression Cassettes.

(A) The 35S–COP1 cassette consists of the cauliflower mosaic virus 35S RNA promoter (35S), a viral translational leader sequence (TL), the *COP1* cDNA containing the entire open reading frame, and the 35S polyadenylation signal (Term). The locations of three COP1 structural motifs, which are the zinc binding domain (Zn), the putative coiled-coil domain (Helix), and the WD-40 repeats (consisting of the A and B subrepeats), are indicated.

(B) The GENCOP1 cassette is a 12-kb *COP1* genomic clone consisting of the entire transcribed region of *COP1* as well as ~4 and 3 kb of 5′ and 3′ untranscribed DNA. In the transcribed region, exons and introns are indicated in black or white, respectively.

The transcriptional start sites in both constructs are indicated by the arrows.

promoter and was expected to achieve constitutive high-level expression of COP1. The GENCOP1 cassette (Figure 1B) was essentially a genomic copy of the wild-type *COP1* gene and was intended to produce a high level of COP1 by simply increasing the *COP1* gene copy number. By using the GENCOP1 cassette, we reasoned that the additional COP1 should be produced according to the wild-type expression pattern, which controls for any effects that might have been caused by ectopic expression using the 35S promoter.

Four 35S–COP1 transgenic lines with single insertion loci were established and designated as lines S1, S2, S3, and S4. Five transgenic lines containing two or more GENCOP1 insertion loci per haploid genome and most likely multiple copies per locus were also created. Three of these were in the wild-type background and were designated as G1, G2, and G3. The other two lines (M1 and M2) were in the *cop1-4* mutant background and had originally been produced for complementation of the *cop1-4* mutation (Deng et al., 1992). Homozygous 35S–COP1 and GENCOP1 lines were produced, and COP1 levels were quantified in seedling protein extracts using both protein blot and ELISA assays (see Methods). As shown in Figure 2, all the transgenic lines displayed a moderate, 1.7- to 4.2-fold increase in COP1 protein (at 76 kD) accumulation.

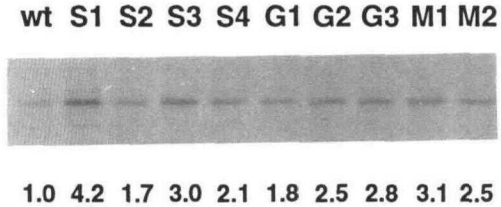


Figure 2. COP1 Levels in Wild-Type and Transgenic Arabidopsis Lines Overexpressing COP1.

The COP1 levels were quantified with two independent immunochemical assays. For protein gel blot analysis, 15 μg of soluble protein from 6-day-old light-grown seedlings was used. COP1 levels in the same protein extracts were also quantified by indirect ELISA, and the abundance of COP1 in each line relative to the wild type (wt), as determined by ELISA, is shown below the immunoblot, with the wild-type (No-O) level set as 1.0 unit. For more details, see Methods.

Arabidopsis Lines Overexpressing COP1 Show Reduced Responsiveness to Far-Red Light

We chose to examine the seedling development of the COP1-overexpressing lines in response to light, because Arabidopsis seedling morphogenesis is well characterized, dramatically regulated by light, and amenable to quantitative analysis. By examining seedling development under specific spectral qualities of light, we tested whether COP1 might be inactivated independently by different photoreceptors.

When grown under continuous far-red light, seedlings from all but one (S4) of the nine transgenic COP1-overexpressing lines had elongated hypocotyls in comparison to wild-type control seedlings, as shown in Figures 3A and 4A. The degree of hypocotyl elongation among the COP1-overexpressing lines roughly correlated with the level of detectable COP1 (Figure 4B). Line S1, which had the highest COP1 level, displayed a phenotype very similar to a null mutant of PHYA, which mediates inhibition of hypocotyl elongation by far-red light (Whitelam et al., 1993). The far-red light inhibition of hypocotyl elongation under our experimental conditions was mediated specifically by phytochrome A and not by phytochrome B, for a *phyB* mutant displayed wild-type–like hypocotyl length inhibition by far-red light (Figure 4A). An increase in the COP1 level quantitatively disrupted light signaling by phytochrome A, supporting the conclusion that phytochrome A–mediated far-red light inhibition of hypocotyl elongation is achieved through inactivation of COP1. However, none of the COP1-overexpressing lines were significantly affected with respect to hook opening and cotyledon development in far-red light (Figure 3A and data not shown), contrasting to the *phyA* mutants.

Overexpression of COP1 Also Reduces Arabidopsis Seedling Responsiveness to Blue Light

Under continuous blue light, the same eight COP1-overexpressing lines with a phenotype in far-red light displayed significant hypocotyl elongation relative to wild-type control plants, as shown in Figures 3B and 5A. Again, the degree of hypocotyl elongation correlated well with the detectable COP1 levels (Figure 5B), with the highly overexpressing line (S1) closely resembling the loss-of-function blue light photoreceptor mutant *long hypocotyl 4* (*hy4*, Koornneef et al., 1980; Ahmad and Cashmore, 1993). To demonstrate that the long hypocotyl phenotype caused by COP1 overexpression under our blue light conditions was specifically attributable to a defect in a blue light receptor signaling pathway rather than inhibition of signals from the phytochromes, which also absorb blue light (Kendrick and Kronenberg, 1993), the *hy1* mutant was analyzed under identical conditions. Although the *hy1* mutant does not accumulate detectable active phytochromes, probably because of a chromophore synthesis defect (Parks and Quail, 1991), it showed blue light inhibition of hypocotyl elongation similar to wild-type plants (Figure 5A).

Figures 3C and 6A show that the COP1-overexpressing lines, similar to the *hy4* mutants, also exhibited clearly reduced cotyledon expansion relative to wild-type plants upon extended growth (10 days) under continuous blue light, indicating a defect in blue light–stimulated cotyledon expansion. However, this effect on cotyledon expansion was not noticeable until 5 days (Figure 3B) and only became obvious after 6 or 7 days of growth under continuous blue light. Similar to the long hypocotyl phenotype, the degree of inhibition of cotyledon expansion among the overexpressing lines correlated with the amount of accumulated COP1 (Figure 6B). Thus, our results indicate that an increase in COP1 content can quantitatively block blue light receptor–mediated photomorphogenic development, consistent with the notion that the light signals perceived by at least one blue light photoreceptor are transduced to inactivate COP1.

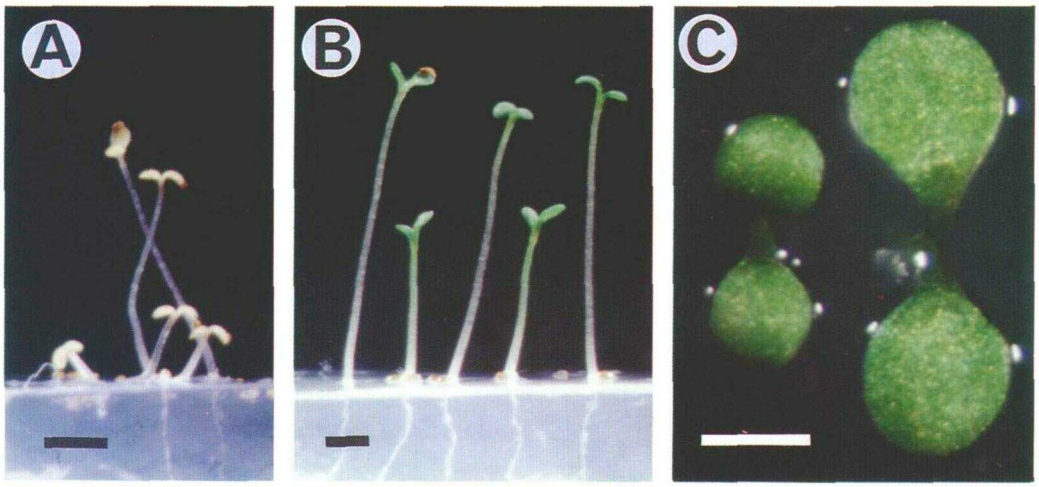


Figure 3. Morphogenetic Comparison of Wild-Type and Representative COP1-Overexpressing S1 Seedlings Grown under Monochromatic Light Conditions.

(A) Five-day-old Arabidopsis seedlings grown under continuous far-red light.
(B) Five-day-old Arabidopsis seedlings grown under continuous blue light.
(C) The cotyledons of 10-day-old Arabidopsis seedlings grown under blue light.
In both **(A)** and **(B)**, the short seedlings are wild type and the tall seedlings are COP1-overexpressers. In **(C)**, cotyledons of 10-day-old wild-type (right) and COP1-overexpressing (line S1, left) seedlings grown in continuous blue light are shown. Bar in **(A)** = 3 mm; bars in **(B)** and **(C)** = 1 mm.

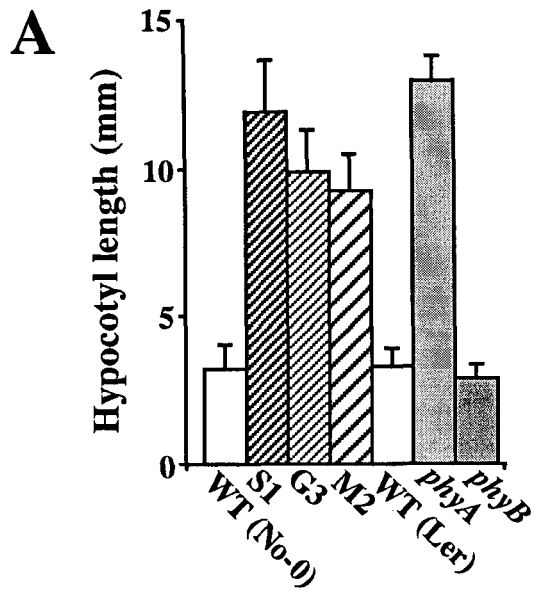
The Phenotype of Arabidopsis Transgenic Lines Is Gene Dosage Dependent

The correlation between COP1 protein levels and the strength of the phenotypes in the COP1-overexpressing lines implied a dosage dependence of COP1 action. To further substantiate this observation, we chose to analyze the seedling development of progeny from selfed heterozygous transgenic lines carrying the 35S–COP1 cassette at a single locus. In the progeny, three populations of seedlings with none, one, or two copies of the introduced transgene should segregate in a 1:2:1 ratio. If the phenotype caused by COP1 overexpression is dosage dependent, we predicted the presence of three corresponding phenotypic populations in the progeny. Figure 7 summarizes the results from the analysis of a representative transgenic line (S1). Under both continuous far-red (Figure 7A) and blue (Figure 7B) light conditions, three distinct populations with different degrees of hypocotyl elongation were evident in the segregating progeny, with an approximate ratio of 1:2:1 in the order of short, intermediate, and long hypocotyl populations. Evidently, the seedlings with short hypocotyls should represent the wild type without the transgene; the seedlings with intermediate and long hypocotyls should represent individuals heterozygous and homozygous for the transgene, respectively. To verify the predicted genotypes of different populations, we examined the presence of the transgene by nopaline assay because the transgene also carries a nopaline synthase gene (Deng et al., 1991). Examination of 20 individual seedlings randomly selected from short and long hypocotyl populations demonstrated that none of the seedlings with a short hypocotyl were nopaline positive, whereas all seedlings from the long hypocotyl population were. This result is consistent with the predicted genotypes of those populations and indicates a copy number–dependent effect of the *COP1* transgene.

COP1-Overexpressing Lines Exhibit Reduced Responsiveness to White Light

Although the COP1-overexpressing lines clearly exhibited partial etiolated seedling phenotypes under both continuous far-red and blue light conditions, we noted that all appeared indistinguishable from wild type when grown under continuous white light conditions (50 to 200 μmol m^{-2} sec^{-1}) at both seedling and adult stages. Although the reason is not clear, one possible explanation is that simultaneous activation of multiple photoreceptors under white light leads to a synergistic effect in inactivating COP1 and therefore requires a higher COP1 level to cause a detectable phenotypic defect.

To reveal any possible phenotypes that might be caused by COP1 overexpression in white light, seedling development of the COP1-overexpressing line S1 was examined under different photoperiod conditions, which are presumably less stringent white light treatments. Figure 8 summarizes results from analyses of hypocotyl length under both long-day (16-hr light/8-hr dark) and short-day (8-hr light/16-hr dark) photoperiods. Although less pronounced than under continuous red or blue light conditions, the COP1-overexpressing seedlings exhibited



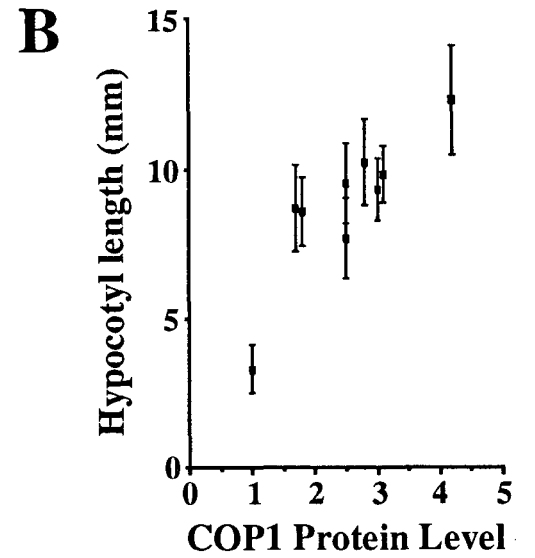
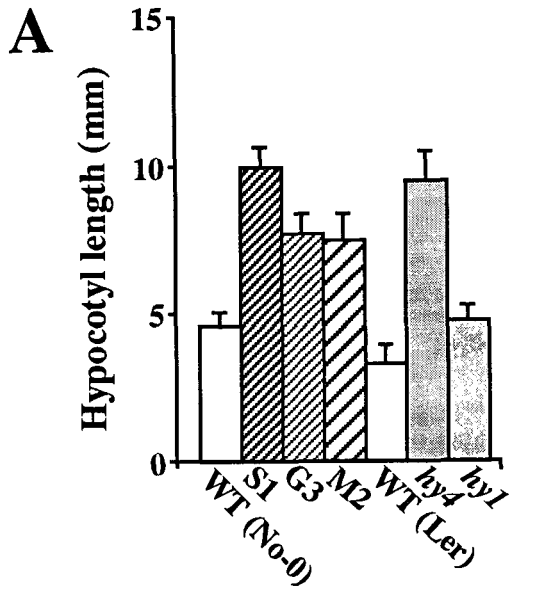


Figure 4. Effects of COP1-Overexpression on Arabidopsis Hypocotyl Length under Continuous Far-Red Light.

(A) Hypocotyl lengths of wild-type (WT), three representative COP1-overexpressing, and phytochrome photoreceptor mutant seedlings after 5 days of growth in continuous far-red light. *phyA* is a phytochrome A null mutant (Whitelam et al., 1993), and *phyB* is a phytochrome B mutant (Reed et al., 1993). Both No-O and Landsberg *erecta* (Ler) ecotypes of the wild type were included as controls for variations of phenotypic characteristics as a result of ecotype backgrounds, because the phytochrome mutants were in the Landsberg *erecta* ecotype and the transgenic lines were in the No-O ecotype.

(B) The COP1 protein levels in wild-type (No-O) and eight COP1-overexpressing lines (Figure 2, ELISA assay) were plotted against the hypocotyl lengths of 5-day-old seedlings grown under continuous far-red light.

Approximately 30 to 50 seedlings from each line were measured for each experiment. The error bars indicate standard deviations from the mean.



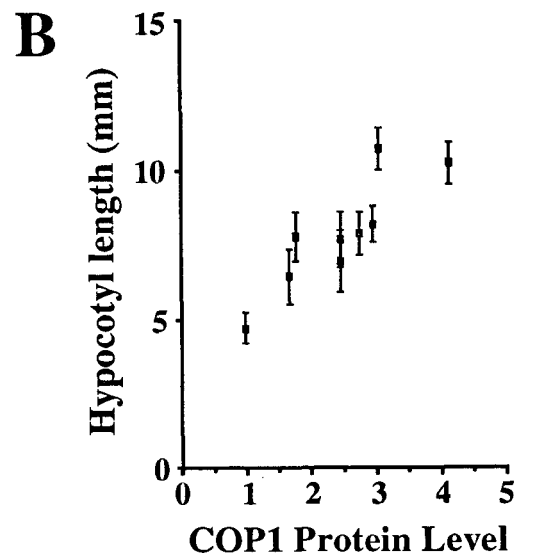


Figure 5. Effects of COP1-Overexpression on Arabidopsis Hypocotyl Length under Continuous Blue Light.

(A) Hypocotyl lengths of wild-type (WT), three representative COP1-overexpressing, and phytochrome photoreceptor mutant seedlings after 5 days of growth in continuous blue light. *hy4* is a putative blue light photoreceptor mutant, and *hy1* is a phytochrome chromophoreless mutant (Koornneef et al., 1980). Both No-O and Landsberg *erecta* (Ler) ecotypes of the wild type were included as controls for variations of phenotypic characteristics as a result of ecotype backgrounds, because the photoreceptor mutants were in the Landsberg *erecta* ecotype and the transgenic lines were in the No-O ecotype.

(B) The COP1 protein levels in wild-type and eight COP1-overexpressing lines (Figure 2) were plotted against the hypocotyl lengths of 5-day-old seedlings grown under continuous blue light.

Approximately 30 to 50 seedlings from each line were analyzed for each experiment. The error bars indicate standard deviations from the mean.

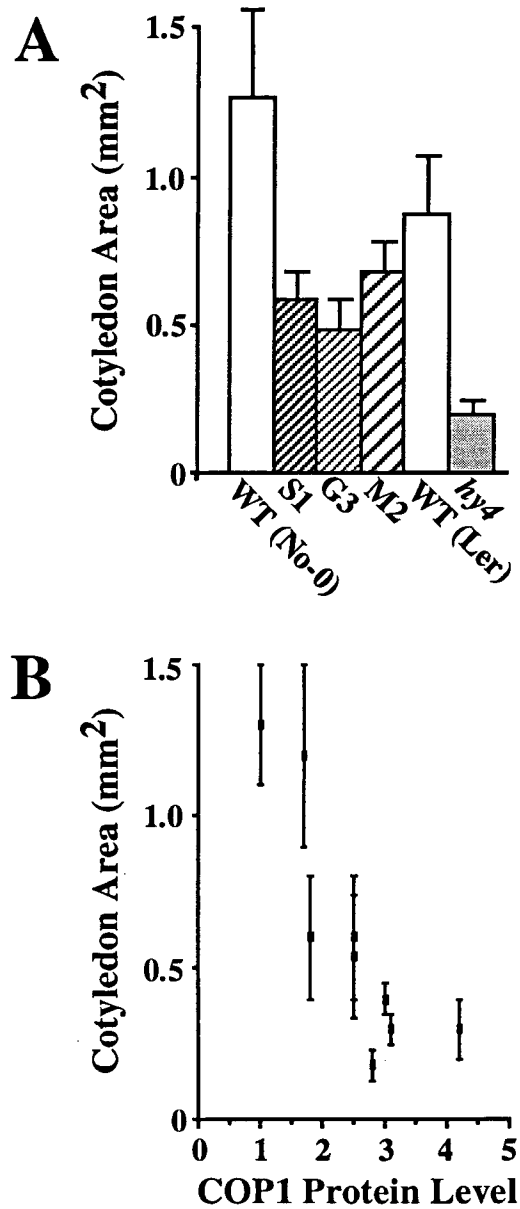


Figure 6. Effects of COP1 Overexpression on Arabidopsis Cotyledon Expansion under Continuous Blue Light.

(A) Cotyledon sizes of wild-type (WT), the representative COP1-overexpressing, and *hy4* mutant seedlings after 10 days of growth under continuous blue light. Both No-O and Landsberg *erecta* (Ler) ecotypes of the wild type were included as controls for variations of phenotypic characteristics as a result of ecotype backgrounds, because the *hy4* mutant was in the Landsberg *erecta* ecotype and the transgenic lines were in the No-O ecotype.

(B) The COP1 protein levels in wild-type (No-O) and eight COP1-overexpressing lines (Figure 2) were plotted against cotyledon sizes of 10-day-old seedlings grown under continuous blue light.

Cotyledon sizes of ~30 to 50 seedlings from each line were measured for each experiment according to a published procedure (Deng and Quail, 1992). The error bars indicate standard deviations from the mean.

statistically significantly longer hypocotyls than the wild-type seedlings under both the long-day and the short-day photoperiod conditions. However, no significant difference in the cotyledon development between the wild-type and the overexpression lines was observed under these conditions.

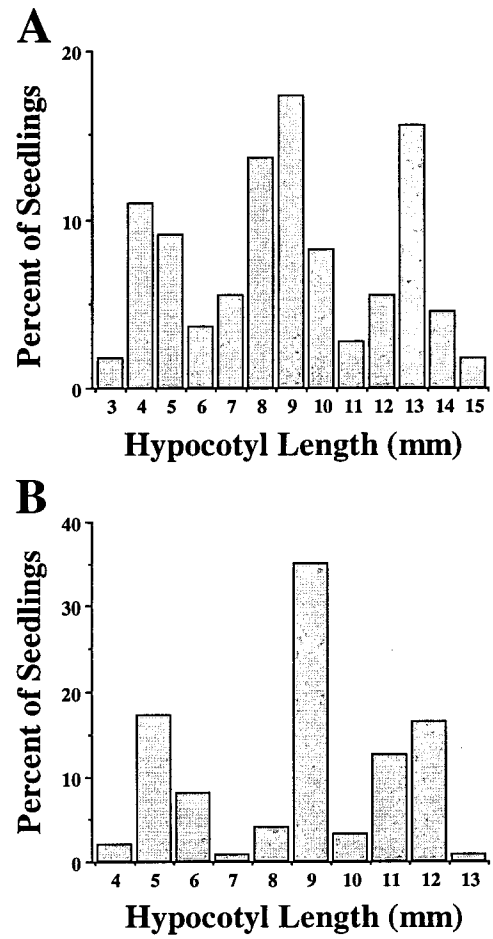


Figure 7. Phenotypic Segregation of a Transgenic COP1-Overexpressing Arabidopsis Line (S1) Heterozygous for the 35S–COP1 Transgene at a Single Genomic Locus.

(A) Six-day-old seedlings grown under continuous far-red light. A total of 110 seedlings were analyzed. The distribution of seedlings in the three populations with different degrees of hypocotyl elongation is as follows: short hypocotyl (length 3 to 6 mm), 28; intermediate hypocotyl (length 7 to 11 mm), 52; and long hypocotyl (length 12 to 15 mm), 30.

(B) Six-day-old seedlings grown under continuous blue light. A total of 122 seedlings were analyzed. The distribution of seedlings in the three populations with different degrees of hypocotyl elongation is as follows: short hypocotyl (length 4 to 6 mm), 33; intermediate hypocotyl (length 7 to 10 mm), 53; and long hypocotyl (length 11 to 13 mm), 36.

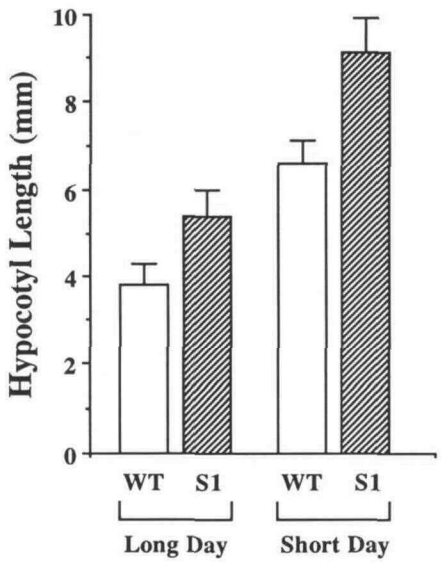


Figure 8. Effects of COP1 Overexpression on Hypocotyl Length under White Light Conditions.

Hypocotyl lengths of wild-type (WT; No-O) and the representative COP1-overexpressing (line S1) seedlings after 6 days of growth under either *long-day (16-hr light/8-hr dark) or short-day (8-hr light/16-hr dark)* photoperiods. During the light period, a 50 μmol m^{-2} sec^{-1} continuous white light was provided by cool fluorescent tubes (McNellis et al., 1994). At least 30 seedlings from each line were analyzed in each experiment, and the error bars indicate standard deviations from the mean.

Moderate *COP1 Overexpression* Does Not Alter the Light Responsiveness of the Light-Inducible Genes

To determine whether the partially etiolated phenotype of the COP1-overexpressing seedlings could be observed at the level of gene transcription, we compared the light responsiveness of the light-inducible genes in etiolated wild-type and COP1-overexpressing seedlings. The chlorophyll *a/b* binding protein genes were chosen for analysis because of the high sensitivity of these genes to light stimuli. In this experiment, 6-day-old dark-grown wild-type and COP1-overexpressing Arabidopsis seedlings were exposed to a 10-sec pulse of red light (100 μmol m^{-2} sec^{-1}) and then returned to darkness. Total RNA was isolated at 0, 1, 2, and 4 hr after the light pulse, and the level of the chlorophyll *a/b* binding protein gene transcripts was determined by quantitative RNA gel blot hybridization analysis. Figure 9 shows an RNA gel blot illustrating the accumulation of the chlorophyll *a/b* binding protein gene transcripts after a red light pulse in wild-type and COP1-overexpressing S1 seedlings. Both the wild-type and the COP1-overexpressing seedlings accumulated the chlorophyll *a/b* binding protein gene transcripts in response to a red light pulse. The time courses and levels of transcript accumulation appeared to be quite similar. Thus, under these experimental conditions, overexpression of COP1 did not inhibit or delay the light responsiveness of the chlorophyll *a/b* binding protein genes.

DISCUSSION

In this report, we summarized our studies using COP1-overexpressing lines to determine the role of COP1 in mediating light control of seedling development. This study provides novel evidence for COP1 as a repressor of photomorphogenesis. Our work substantiates and extends the working models based on previous observations made with loss-of-function mutational analyses.

COP1 Acts as an Autonomous and Light-Inactivable Repressor of Photomorphogenic Development

Two observations confirmed that overexpressing COP1 can indeed lead to partially etiolated phenotypes under a variety of light conditions. First, eight of nine transgenic lines overexpressing *COP1 exhibited variable degrees of etiolated* phenotypes. These phenotypes have not been observed in any of our transgenic lines carrying transgenes unrelated to COP1 (data not shown). Second, the phenotype of the transgenic COP1-overexpressing lines strictly cosegregated with the T-DNA containing the COP1-overexpressing cassettes (Figure 7 and data not shown).

The strength of the phenotypes in the transgenic lines correlated with the COP1 level (Figures 4, 5, and 6) and was independent of the expression cassettes, suggesting that the observed phenotype is unlikely to be caused by ectopic expression of COP1. Therefore, the phenotypes observed in the

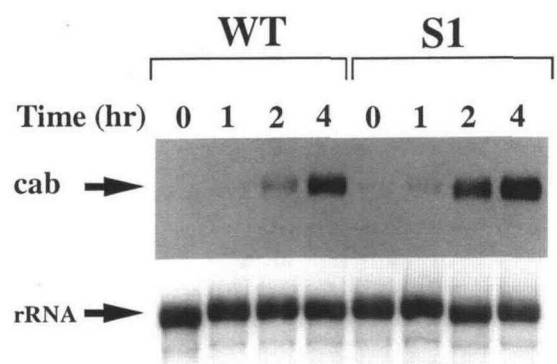


Figure 9. Induction of Accumulation of the Chlorophyll *a/b* Binding Protein Gene Transcripts in Etiolated Wild-Type and COP1-Overexpressing S1 Seedlings after a Red Light Pulse.

The level of chlorophyll *a/b* binding protein (*cab*) gene transcripts in 6-day-old etiolated wild-type and COP1-overexpressing Arabidopsis seedlings was assayed 0, 1, 2, and 4 hr after a red light pulse (10 sec, 100 μmol m^{-2} sec^{-1}) using RNA gel blot hybridization analysis. Equal amounts of RNA (5 μg) were loaded in each lane, and the 18S rRNA was used as a control for equal loading. For details, see Methods.

transgenic lines are likely a result of higher cellular levels of COP1 protein. Overexpression of COP1 alone was sufficient to repress aspects of photomorphogenic seedling development, suggesting that COP1 acts autonomously. Light signals, perceived by photoreceptors, are transduced to inactivate COP1 and thus to abrogate its suppressive action. COP1 can achieve the suppression of photomorphogenic seedling development by at least two means. COP1 may function in a rate-limiting step in the light-signaling cascade controlling seedling development. Alternatively, it may constitute a light-controlled transcriptional regulator directly responsible for suppressing the photomorphogenic developmental program.

Severe (possible null) mutations of all pleiotropic *COP/DET* loci, including *COP1*, are lethal, causing developmental arrest at the seedling stage and excessive anthocyanin accumulation, a phenotype known as *fusca* (Müller, 1963; Castle and Meinke, 1994; Miséra et al., 1994). It has been suggested that COP1 and its partners may function mainly during embryogenesis prior to germination to prepare the plant for adult growth (Castle and Meinke, 1994). As such, they might define a basic cellular environment for the light signal transduction network to function properly, but light signaling per se may not rely on COP1. The fact that moderate COP1 overexpression causes inhibition of photomorphogenic seedling development under specific light conditions and that no embryonic or other developmental phenotypes were observed suggests that the direct repression of photomorphogenic development is a primary role of COP1. However, our results do not exclude the possibility that COP1 is also involved in other developmental processes, including embryogenesis.

It is important to point out that none of the COP1-overexpressing lines displayed a complete suppression of photomorphogenic development under any light conditions. The cotyledon development of COP1-overexpressing lines was very similar to that of wild-type seedlings under white and far-red light, and reduced expansion was only observed after extended growth under blue light. The chlorophyll accumulation of the transgenic seedlings under different light conditions and the greening ability of the dark-grown transgenic seedlings after transfer to light were also similar to that of wild-type siblings. Therefore, chloroplast development did not appear to be significantly affected by the moderate overexpression of COP1. Further, the light responsiveness of light-regulated genes in the COP1-overexpressing lines is similar to that of the wild type (Figure 9). The incomplete suppression of photomorphogenic development in our transgenic lines has at least two possible implications for COP1 function. First, the sensitivity of different photomorphogenic responses to the cellular COP1 level may differ. Under far-red and blue light, for example, hypocotyl length in COP1-overexpressing lines approaches the length observed in the *phyA* photoreceptor or *hy4* mutants, whereas cotyledon size, apparently less sensitive to the COP1 level, does not. Second, some light-mediated responses, such as light induction of gene expression, may not be directly regulated by COP1, or at least not autonomously.

Multiple Photoreceptors Can Independently Inactivate COP1

By analyzing loss-of-function mutations, it became clear that phytochrome A (Dehesh et al., 1993; Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993), phytochrome B (Reed et al., 1993; Wester et al., 1994), and blue light photoreceptors (Liscum and Hangarter, 1991; Ahmad and Cashmore, 1993) are responsible for mediating the inhibition of hypocotyl elongation by continuous far-red, red, and blue light, respectively. Our studies demonstrated that COP1 overexpression caused long hypocotyl phenotypes under far-red and blue light conditions, which were designed to specifically elicit inhibition of hypocotyl elongation mediated by phytochrome A and blue light receptors, respectively. Furthermore, the phenotype of the overexpressing line with the highest level of COP1 almost mimics the phenotypes of the phytochrome A and HY4-deficient mutants under these specific spectral quality light conditions. Previous analyses of epistatic relationships have suggested that *COP1* is genetically downstream of the *PHYA* and *HY4* loci, which encode red/far-red and blue light photoreceptors, respectively (Ang and Deng, 1994). Our results strongly imply that phytochrome A and the blue light receptor HY4 can independently alleviate the suppression of photomorphogenic development mediated by COP1 when they are stimulated by light.

Under our red light conditions (60 μmol m^{-2} sec^{-1}), the No-O wild-type seedlings had equally long hypocotyls as when grown in darkness (data not shown). This indicated that the hypocotyl elongation response of the No-O wild type was not inhibited under our red light growth conditions. Therefore, we were unable to demonstrate whether COP1 overexpression could cause a long hypocotyl phenotype under our red light. However, our previous epistatic studies suggested that COP1 acts downstream of phytochrome B in mediating red light inhibition of hypocotyl elongation (Ang and Deng, 1994). Therefore, it is quite possible that phytochrome B can also relieve the suppression of photomorphogenic development mediated by COP1.

Implication of Dosage-Dependent Action of COP1: A Possible Role in Mediating Quantitative Responses to Light Signals

We have previously reported that the severity of *cop1* mutant phenotypes is correlated to the residual level of COP1 (Deng and Quail, 1992; McNellis et al., 1994). Based on this observation, we proposed that the light signals, once perceived by photoreceptors, are transduced to quantitatively reduce COP1 activity, which in turn dictates the extent of the plant responses (Deng and Quail, 1992). The data reported here confirmed our previous observation. The marked effects of moderate COP1 overexpression on Arabidopsis photomorphogenic development in the transgenic lines are well correlated with COP1

levels (Figures 4B, 5B, 6B, and 7), further suggesting the feasibility of quantitatively modulating the COP1 level (or activity) to achieve variable degrees of inhibition of photomorphogenic development. Therefore, the controlled inactivation of COP1 may provide a basis for the ability of plants to respond quantitatively to patterns of light signals, which are defined by fluence rate and photoperiod.

METHODS

Plant Materials and Growth Conditions

The *hy* mutant alleles including *hy1* (21.84N), *phyB* (*hy3*-Bo64), and *hy4* (2.23N) are in the Landsberg *erecta* ecotype and have been described by Koornneef et al. (1980). The *phyA* mutant was a phytochrome A null mutant allele in the Landsberg *erecta* ecotype known as *fhy2-1* (Whitelam et al., 1993). All transgenic lines produced were in the No-O background. Therefore, both No-O and Landsberg *erecta* wild-type strains were used in our physiological experiments to control for ecotype-specific variations. Plant germination and growth conditions in darkness and white light were exactly as described previously (Hou et al., 1993; McNellis et al., 1994).

For specific spectral quality light treatments, seeds were vernalized for 3 days, exposed to white light (50 μmol m^{-2} sec^{-1}) for 30 min, and then grown in either continuous far-red light (40 μmol m^{-2} sec^{-1}), or continuous red light (60 μmol m^{-2} sec^{-1}), or continuous blue light (50 μmol m^{-2} sec^{-1}) for 5 to 10 days at 22°C. Far-red light was provided by far-red enhanced fluorescent tubes (FL20S·FR74; Toshiba, Tokyo, Japan) through a far-red Plexiglas (FRS700; Westlake Plastics, Lenni, PA). Blue light was provided by blue light (peak 450 nm) enhanced fluorescent tubes (F15T8/247; GTE Sylvania, Danvers, MA) wrapped with one layer of a blue plastic filter (Lee Filters HT120; Lee Colortran Inc., Totona, NJ). Red light was provided by red light (peak 660 nm) enhanced fluorescent tubes (F15T8/2364; GTE Sylvania) wrapped with one layer of a red plastic filter (Lee Filter No. 106).

Construction of *CONSTITUTIVE PHOTOMORPHOGENIC1* Expression Cassettes and Stable Transformation

For the 35S–COP1 cassette (Figure 1A), the start codon of the *CONSTITUTIVE PHOTOMORPHOGENIC1* (*COP1*) cDNA sequence (McNellis et al., 1994) was mutated first to create a NcoI site. The entire *COP1* cDNA coding region was excised as a NcoI-BglII fragment and used to replace the β-glucuronidase gene of the pRTL2-GUS plasmid (Restrepo et al., 1990), which was excised with NcoI and BamHI. The resulting 35S–COP1 cassette consists of the cauliflower mosaic virus 35S RNA promoter, a viral translational enhancer sequence, the *COP1* cDNA containing the entire open reading frame, and the 35S polyadenylation signal. The GENCOP1 construct is a 12-kb *COP1* genomic clone consisting of the entire transcribed region of *COP1* as well as ~4 and 3 kb of 5′ and 3′ untranscribed DNA (Deng et al., 1992).

The 35S–COP1 and GENCOP1 expression cassettes were ligated into the binary transformation vectors pCIT20 and pCIT30 (Yanofsky et al., 1990; Deng et al., 1992), respectively, and transferred into *Agrobacterium tumefaciens* strain ASE by triparental mating (Valvekens et al., 1988). Root transformations of wild-type and *cop1-4* mutant strains in the No-O background were performed according to a published procedure (Valvekens et al., 1988; Deng et al., 1992), and transformants were selected by resistance to hygromycin and the presence of nopaline. The number of T-DNA loci in transgenic lines and the homozygosity were determined by segregation analysis (nopaline assay or hygromycin resistance) of the progeny after allowing the plant of interest to self. The homozygosity of the selected lines was further confirmed by the uniformity of the photomorphogenic seedling phenotypes among the selfed progenies.

COP1 Protein Analysis

Two independent immunochemical assays were used to quantify the COP1 levels. For protein gel blot analysis, 15 μg of total soluble protein from 6-day-old seedlings grown under far-red light, white light, or in darkness was electrophoresed, immunoblotted, and probed with rabbit polyclonal anti-COP1 antiserum as described previously (McNellis et al., 1994). The relative COP1 levels were similar whether the lines were grown in the light or darkness, and only results from light-grown seedlings are shown. The same protein extracts were also used to quantify COP1 levels by indirect ELISA with alkaline phosphatase essentially as described by Clark et al. (1986). The primary antibody was 1 μg/mL of affinity-purified rabbit polyclonal antiserum, and the secondary antibody was 0.1 μg/mL of a mouse monoclonal anti-COP1 antibody (A.G. von Arnim and X.-W. Deng, unpublished data).

RNA Gel Blot Analysis

For the light pulse experiments, wild-type and COP1-overexpressing seedlings were grown in darkness for 6 days as described previously (Hou et al., 1993; McNellis et al., 1994). The seedlings were then exposed to a 10-sec pulse of red light (100 μmol m^{-2} sec^{-1}), and total RNA was isolated 0, 1, 2, and 4 hr after the light pulse. Equal amounts of RNA (5 μg per lane) were subjected to RNA gel blot hybridization analysis as described previously (Deng et al., 1992). The RNA blots were hybridized either with a probe for the chlorophyll *a/b* binding protein gene transcript or with a probe for the 18S rRNA, which served as an equal loading control.

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