

Promotion of photomorphogenesis by COP1

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Abstract

CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) represses photomorphogenesis in darkness by targeting nuclear-localized transcription factors to proteasome-mediated degradation. Upon light exposure, COP1 migrates to the cytosol allowing photomorphogenesis to proceed but the residual nuclear pool down-regulates light signaling mediated by phytochrome A. Here we show that weak alleles of *cop1* exhibit reverse photomorphogenic responses, i.e. reduced rather than enhanced cotyledon unfolding, under red light compared to darkness. Conversely, *COP1* overexpressors, which de-etiolate poorly under blue or far-red light, showed enhanced photomorphogenesis under red light. The positive relationship between COP1 and photomorphogenic response required phytochrome B. Thus, genetic manipulation of COP1 levels differentially affects phytochrome A- compared to phytochrome B-mediated responses. We hypothesize that COP1 could be involved in degradation of negative regulators of photomorphogenesis or in transcriptional activation, as observed for some E3 ligases in mammalian development.

Abbreviations: COP1, CONSTITUTIVE PHOTOMORPHOGENIC 1, *COP1* OX, *COP1* overexpressor; phyA, phytochrome A; phyB, phytochrome B; WT, wild type

Introduction

When seeds germinate in the darkness of the soil, the stem of the seedlings grows fast, the cotyledons remain folded and the photosynthetic machinery is rudimentary. Upon reaching the light, the developmental pattern undergoes a shift from skoto to photomorphogenesis via de-etiolation responses that include stem growth arrest, cotyledon unfolding and building of photosynthetic capacity. The light signal is perceived mainly (but not exclusively) by phytochrome A (phyA), phytochrome B (phyB) and cryptochromes 1 and 2, which show maximum activity under far-red, red and blue light, respectively (Liscum *et al.*, 2003; Parks, 2003).

CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) is a regulatory protein that represses photomorphogenesis in darkness (Deng *et al.*, 1991; Deng *et al.*, 1992; Ang and Deng, 1994). *cop1* mutant seedlings de-etiolate in the dark (McNellis *et al.*, 1994a) and show a transcriptome pattern in the dark that resembles that of the wild type (WT) in the light (Ma *et al.*, 2002). Seedlings overexpressing *COP1* or *COP1* fused to *GUS* show poor de-etiolation under white, blue or far-red light (McNellis *et al.*, 1994b). Structurally, COP1 possesses a zinc-binding RING-finger motif; a coiled-coil domain and a domain of WD-40 repeats (Deng *et al.*, 1992). Among the weak *cop1* alleles, *cop1-6* is affected in the nuclear

localization signal (Stacey *et al.*, 2000), *cop1-4* lacks a C-terminal fragment, including the nuclear localization signal and the WD-40 domain (McNellis *et al.*, 1994a), and *cop1^{eid6}* is affected in the RING domain (Dieterle *et al.*, 2003). In darkness, both *cop1-4* and *cop1-6* seedlings are de-etiolated whereas *cop1^{eid6}* seedlings are etiolated (McNellis *et al.*, 1994a; Dieterle *et al.*, 2003). COP1 acts as E3 ubiquitin ligase within the nucleus, conferring substrate specificity by direct recognition of the substrate proteins (Saijo *et al.*, 2003; Seo *et al.*, 2004). The proteins produced by *cop1-4* and *cop1-6* alleles are predicted to localize to the cytosol. COP1 repression of photomorphogenesis in darkness results from direct interaction with positive regulators of photomorphogenesis such as HY5, which as a result of this interaction become targeted to degradation via the 26S proteasome (Osterlund *et al.*, 2000). The *hy5* mutation suppresses the *cop1* phenotype in darkness (Ang and Deng, 1994). After light perception, multiple photoreceptors induce COP1 re-localization to the cytosol (Osterlund and Deng, 1998), thus increasing the pool of photomorphogenic transcription factors (e.g. HY5) in the nucleus.

COP1 retains a role in the light as *cop1* mutants show exaggerated photomorphogenesis in the light at the seedling and adult-plant stages (McNellis *et al.*, 1994a). HYH is a HY5 homologue that physically interacts with COP1 and requires COP1 for degradation (Holm *et al.*, 2002). The *HYH* gene is highly expressed under red or blue light and the *hyh* mutation reduces photomorphogenesis under blue light but it does not suppress the *cop1* phenotype in darkness (Holm *et al.*, 2002). Under far-red light, the low residual nuclear levels of COP1 interact with SPA1 and ubiquitylate both HY5 (Saijo *et al.*, 2003) and LAF1 (Seo *et al.*, 2003), a MYB transcription factor that promotes phyA-mediated photomorphogenesis (Ballesteros *et al.*, 2001). The phenotype of *spa1* is observed under far-red or red light, not in the dark (Hoecker *et al.*, 1998). COP1 acts as an E3 ligase to regulate phyA signaling by targeting elimination of the phyA photoreceptor itself (Seo *et al.*, 2004). In addition, the *cop1^{eid6}* retains an apparently normal etiolated phenotype in the dark but it shows enhanced sensitivity to far-red light for the control of hypocotyl length inhibition and anthocyanin accumulation (Dieterle *et al.*, 2003). Thus,

COP1 represses phyA-mediated signaling under far-red light. The latter action could be independent from the repressor function in the dark because the weak *cop1-4* mutant fails to repress de-etiolation in the dark and retains apparently normal sensitivity to far-red light. The aim of this work is to investigate the role of COP1 in phyB-mediated photomorphogenesis.

Materials and methods

Plant material and light sources

The two weak *cop1* alleles used here (*cop1-4* and *cop1-6*) are in the Columbia background (McNellis *et al.*, 1994a) and were compared to the Columbia WT of *Arabidopsis thaliana*. We used two transgenic lines overexpressing *COP1* (*COP1 OX*). One of these lines bears the *COP1* gene under the control of the 35S promoter in the Nossen background (McNellis *et al.*, 1994b). The other line (identified as *GUS:COP1 OX* in the figures) bears a *GUS:COP1* fusion under the control of the 35S promoter (von Arnim and Deng, 1994). The *GUS-COP1* fusion transgene is fully functional in complementing *cop1-5* (von Arnim *et al.*, 1997).

The *phyA-211* (Nagatani *et al.*, 1993) and *phyB-9* (Reed *et al.*, 1993) mutants, both in the Columbia background, were included in some experiments. The *phyA-211 cop1-6* double mutant was obtained by isolating seedlings of the F2 generation showing intermediate de-etiolation in far-red light. The *phyA-211* genotype was confirmed by PCR using the primers 5'-TTATCCACAGGGTTACAGGG-3' and 5'-GCATTCTCCT TGCATCATCC-3', which produced a 1136-bp fragment only in the WT. A control pair of primers was also used to check DNA presence and integrity in both genotypes. The *cop1-6* mutation was confirmed by the de-etiolated phenotype in darkness. The *phyB-9 cop1-4* and the *phyB-9 cop1-6* double mutants were obtained by testing the presence of both mutations in successive generations. The *phyB-9* mutation was detected by using dCAPS (Neff and Chory, 1998). The de-etiolated phenotype in darkness indicated the presence of *cop1-6* or *cop1-4* mutation. *COP1 OX* lines in the *phyA-211* or *phyB-9* backgrounds were obtained by testing the photoreceptor mutations as indicated above and the transgene by kanamycin resistance after 10 days under white

light in MS medium supplemented with 1% of sucrose and kanamycin (50 $\mu\text{g/ml}$).

For physiological experiments, twelve seeds of each genotype were sown in clear plastic boxes (40 \times 33 mm \times 15 mm height), containing 3 ml of agar 0.8 % (w/v). These boxes were stored 3 d in darkness at 5 $^{\circ}\text{C}$, exposed to a saturating red light pulse (30 min) and incubated in darkness for 24 h at 25 $^{\circ}\text{C}$ before transfer to the different light treatments for 3 d. In kinetics experiments the seedlings were grown in darkness for 2 d before transfer to red light. Red light was provided by red fluorescent tubes (40/15, Philips). Far-red light was provided by incandescent lamps in combination with blue acrylic filters (Paolini 2031), a red acetate filter and water filter. Blue light was provided by fluorescent tubes in combination with a blue acetate.

Isolation of RNA and RNA gels

Total RNA was isolated by RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total RNA was size-fractionated on a 1.5% agarose MOPS-formaldehyde gel and transferred to a nylon membrane filter (Hybond, Amersham, USA) by capillary action in 20 \times SSC (Carrari *et al.*, 2001). The membrane was reversibly stained with methylene blue 0.04% (w/v), scanned and total RNA of each lane was quantified with an imager system (Fluor-S Multimager, Bio-Rad, Hercules, USA). *CABI* probe (pAB140, ABRC, Ohio) labeling was performed with the Prime-a-gene kit, (Promega, Madison, USA) with ^{32}P dCTP. Hybridization was carried out at 42 $^{\circ}\text{C}$ for 16 h in a solution containing 6 ml of ULTRAhyb (Ambion, Austin, USA) and the labeled probe. Treatment of the photographic films was as described by Carrari *et al.* (2001). To correct subtle differences in RNA loading among lanes, we calculated band intensity (corresponding to *CABI* transcript) relative to total RNA quantified for each lane.

Western blot analysis

Protein extraction and Western blot analysis were essentially performed as described previously (Saijo *et al.*, 2003).

Plant observations, chlorophyll determination and statistics

Hypocotyl length was measured to the nearest 0.5 mm with a ruler and the angle between the cotyledons was measured with a protractor. Average values were calculated for each box of seedling (i.e., one replicate) and used for statistical analysis. For measurements of chlorophyll, 30 seedlings were harvested and placed in 1 ml of N-N'-dimethyl formamide kept in vials wrapped in aluminum foil. The samples were stored in darkness at 4 $^{\circ}\text{C}$ for 3 d before absorbance measurements to calculate chlorophyll levels according to Inskeep and Bloom, (1985). Each experiment was conducted on 3 to 5 independent occasions; the data (replicate boxes) were pooled for the analysis.

Results

Weak cop1 mutants under red light

To investigate the effects of COP1 on the magnitude of light responses, seedlings of the WT (Columbia) and of the *cop1-6* and *cop1-4* mutants (two weak alleles of *cop1*) were grown in darkness or under hourly pulses of red light, a light condition where de-etiolation is mediated mainly by phyB (Yanovsky *et al.*, 1997). As expected, both *cop1-6* and *cop1-4* showed reduced hypocotyl growth and enhanced cotyledon unfolding in darkness (Figure 1A). The reduction of hypocotyl length caused by red light compared to darkness was smaller in the *cop1* mutants than in the WT but this could be a consequence of the already short hypocotyl of the mutants in darkness (Figure 1B). Noteworthy, while red light unfolded the cotyledons in the WT, the same treatment reduced cotyledon angle in *cop1-6* and *cop1-4*; i.e., these mutants showed a reverse cotyledon-angle response to red light (Figure 1C). The analysis of cotyledon unfolding under different fluence rates of continuous red light showed similar thresholds of sensitivity for the positive effect observed in the WT and the negative effect observed in *cop1-6* and *cop1-4* (Figure 1D).

To investigate whether reduced cotyledon unfolding is a normal response to exaggerated light signaling we used two experimental approaches. A transgenic line overexpressing *PHYB*

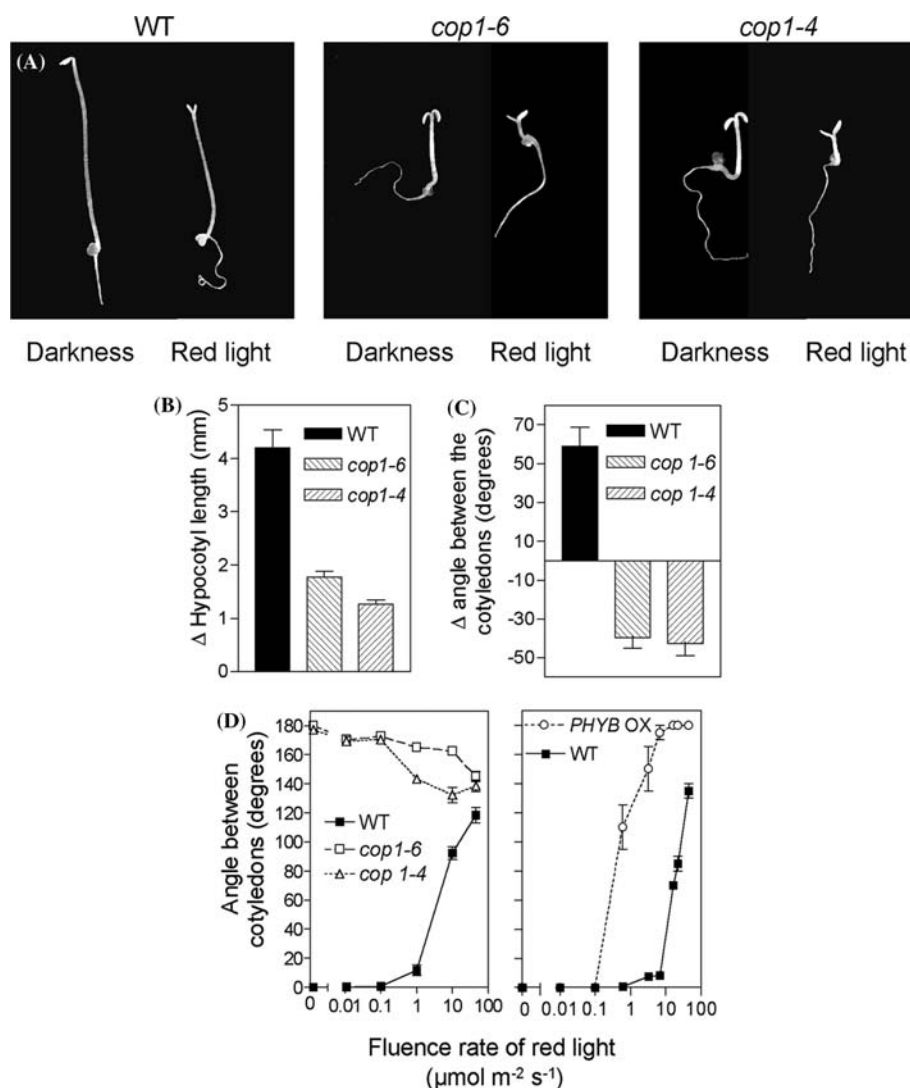


Figure 1. Reverse cotyledon-unfolding response to red light in *cop1-4* and *cop1-6* mutants. Seedlings of the WT (Columbia) and of the *cop1-4* and *cop1-6* mutants grown under hourly pulses of red light (3 min, $20 \mu\text{mol m}^{-2} \text{s}^{-1}$) or in darkness (A). Δ Hypocotyl length (B) was calculated as hypocotyl length of seedlings grown in darkness minus hypocotyl length of seedlings grown under red-light pulses. Δ Cotyledon opening (C) was calculated as angle between cotyledons of seedlings grown under hourly red-light pulses minus angle between the cotyledons of seedlings grown in darkness. Seedlings of the *cop1* mutants, of a *PHYB* overexpressor line (*PHYB OX*) and of the WT (Columbia and Nossen, respectively) were also exposed to different fluence rates of continuous red light (D). Data are means and SE of 5 independent experiments (one Δ value per experiment) (B, C) and at least 3 replicate boxes (D).

(Wagner *et al.*, 1991) showed maximum unfolding under $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ of continuous red light and retained full unfolding at $45 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 1D). This pattern contrasts with the response of the *cop1* mutants, which retained maximum unfolding at $0.01 \mu\text{mol m}^{-2} \text{s}^{-1}$ and reduced angle at $45 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 1D). Under $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ of white light, WT (Columbia or

Nossen) seedlings show maximal cotyledon unfolding and increasing fluence rate up to $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ does not reduce the angle between cotyledons (data not shown). Thus, the reverse cotyledon unfolding response observed in the *cop1* mutants is not phenocopied by the WT or *PHYB* overexpressors under the high irradiances tested here.

phyB is required for the reverse cotyledon-angle response in *cop1*

When the *cop1-6* and *cop1-4* mutants were grown under hourly pulses of far-red light (3 min, $60 \mu\text{mol m}^{-2} \text{s}^{-1}$), hypocotyl-growth inhibition showed WT levels (Δ hypocotyl length compared to dark controls, mm, mean \pm SE, WT = 1.2 ± 0.1 ; *cop1-4* = 1.1 ± 0.1 ; *cop1-6* = 1.6 ± 0.2). In addition, no reverse cotyledon unfolding response was statistically significant under hourly pulses of far-red light (Δ angle between cotyledons compared to dark controls, degrees, mean \pm SE, WT = 10 ± 5 ; *cop1-4* = -9 ± 6 ; *cop1-6* = -5 ± 3). Since the effects of far-red light compared to darkness are mediated by *phyA* while those of red light are mediated largely by *phyB*, we speculated that the reverse response could be photoreceptor-specific. To investigate this issue we produced double mutants of *cop1* with *phyB* and *phyA* in the same genetic background and the seedlings were exposed to hourly pulses of red light or continuous far-red light. Although in the WT continuous far-red perceived by *phyA* has a much larger effect than hourly red light (Figure 2, WT), in the *cop1* mutants it caused a weaker reverse cotyledon-angle response (Figure 2, *cop1-6* and *cop1-4*) again arguing in favor of a

selective effect. The *phyB-9* mutation was epistatic to *cop1-6* and *cop1-4* for the reverse cotyledon-angle response under red light or far-red light (Figure 2, *cop1-4 phyB* and *cop1-6 phyB*). The *phyA-211* mutation had little effect under red light but it was epistatic under far-red light (Figure 2, *cop1-4 phyA*). We conclude that the reverse cotyledon-angle response is mediated primarily by *phyB* and secondarily by *phyA*.

Enhanced photomorphogenesis of COP1 OX under red light

To further characterize the role of COP1 under light, we investigated photomorphogenic responses of two *COP1* OX lines. In agreement with previous studies (McNellis *et al.*, 1994b; Osterlund and Deng, 1998), both lines showed reduced hypocotyl growth inhibition and cotyledon opening under continuous far-red or blue light and no phenotype in darkness (Figure 3A and B). However, under red light *COP1* OX showed enhanced rather than reduced photomorphogenesis (Figure 3A and B). This observation admits two interpretations. One, consistent with the behavior of *cop1* mutants, is that COP1 can promote *phyB*-mediated photomorphogenesis. The other is that the *COP1* transgene causes light-quality dependent co-suppression of the endogenous *COP1* gene. The immunological analysis of

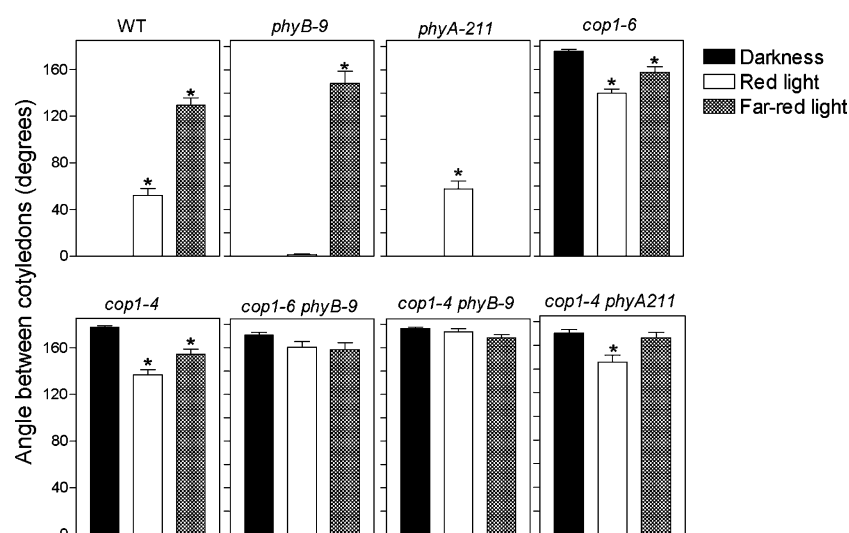


Figure 2. *phyB* is required for the reverse cotyledon unfolding response to red light in *cop1-4* and *cop1-6* mutants. Seedlings of the WT, *phyB-9*, *phyA-211*, *cop1-6*, *cop1-4*, *cop1-6 phyB-9*, *cop1-4 phyB-9*, *cop1-6 phyA-211* were grown under hourly pulses of red light (3 min, $20 \mu\text{mol m}^{-2} \text{s}^{-1}$), continuous far-red light ($10 \mu\text{mol m}^{-2} \text{s}^{-1}$) or in darkness. Data are means and SE of at least 8 replicate boxes. Asterisks indicate significant differences ($p < 0.05$) with dark controls.

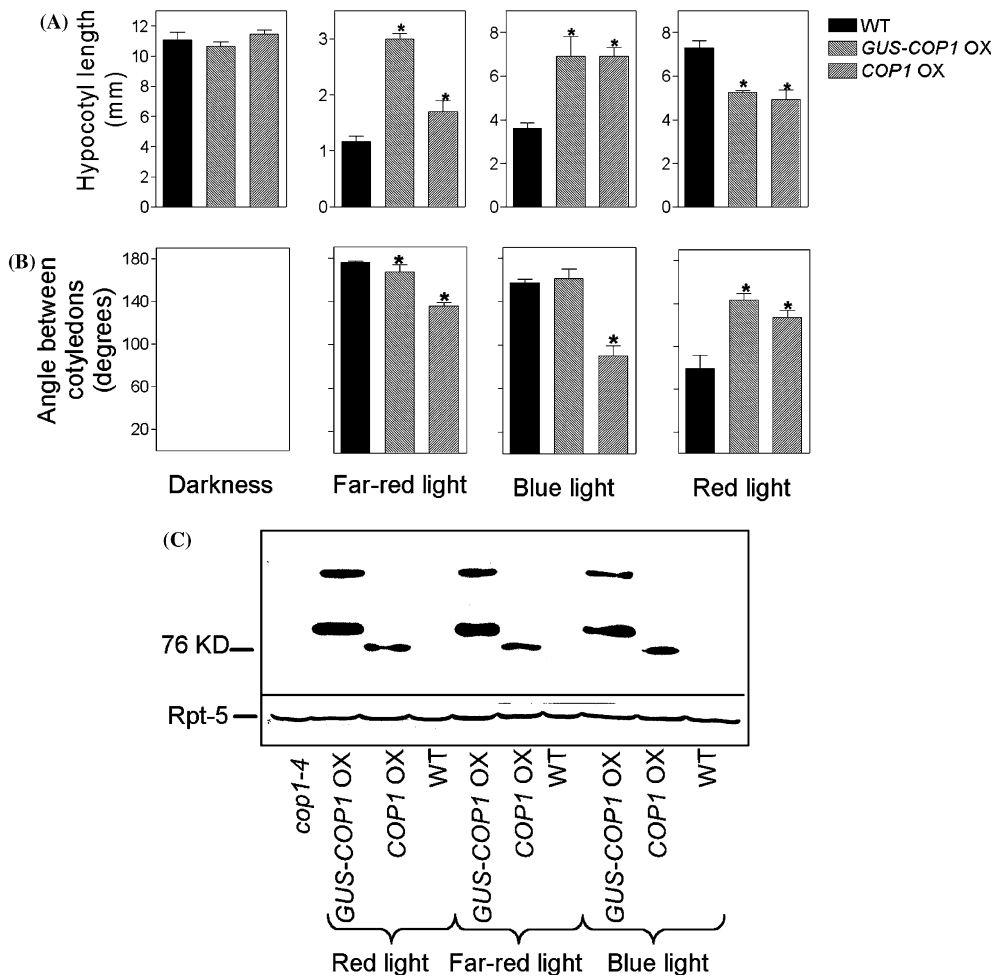


Figure 3. COP1 overexpression enhances photomorphogenesis under red light and represses photomorphogenesis under blue or far-red light. Hypocotyl length (A) and cotyledon angle (B) in seedlings of the WT (Nossen) and *COP1 OX* grown under continuous far-red ($25 \mu\text{mol m}^{-2} \text{s}^{-1}$), blue ($20 \mu\text{mol m}^{-2} \text{s}^{-1}$) or red light ($17 \mu\text{mol m}^{-2} \text{s}^{-1}$), or in darkness. Data are means and SE of at least 7 replicate boxes. COP1 protein levels in WT and *COP1 OX* seedlings grown for 5 d under continuous red, far-red and blue light (C). *cop1-4* seedlings grown under white light were included as negative controls (left lane). The three bands revealed by the antibody against COP1 correspond to GUS-COP1, putative truncated GUS-COP1 (both only observed in *GUS-COP1 OX*) and endogenous COP1. Membranes were blotted with an antibody against Rpt-5 as control of even loading.

COP1 protein levels showed no differences under red compared to blue or far-red light, providing no support for the latter contention (Figure 3C).

To characterize the response in further detail we investigated the fluence-rate dependency of the enhanced cotyledon unfolding in *COP1 OX* lines under red light. The transgenic lines showed enhanced sensitivity and a higher maximum response than the WT (Figure 4A). To investigate the kinetics of the enhanced cotyledon unfolding in *COP1 OX* the seedlings were grown in darkness

for 2 d and then transferred to continuous red light. The angle between cotyledons was already larger in *COP1 OX* lines after 12h of red light (Figure 4 B, C).

Following a similar time protocol we observed enhanced chlorophyll accumulation in the *COP1 OX* (Figure 5A) and higher *CAB1* transcript level in *COP1 OX* compared to WT (Figure 5B). Thus, overexpression of *COP1* enhanced not only morphological responses under red light but also the assembly of the photosynthetic machinery.

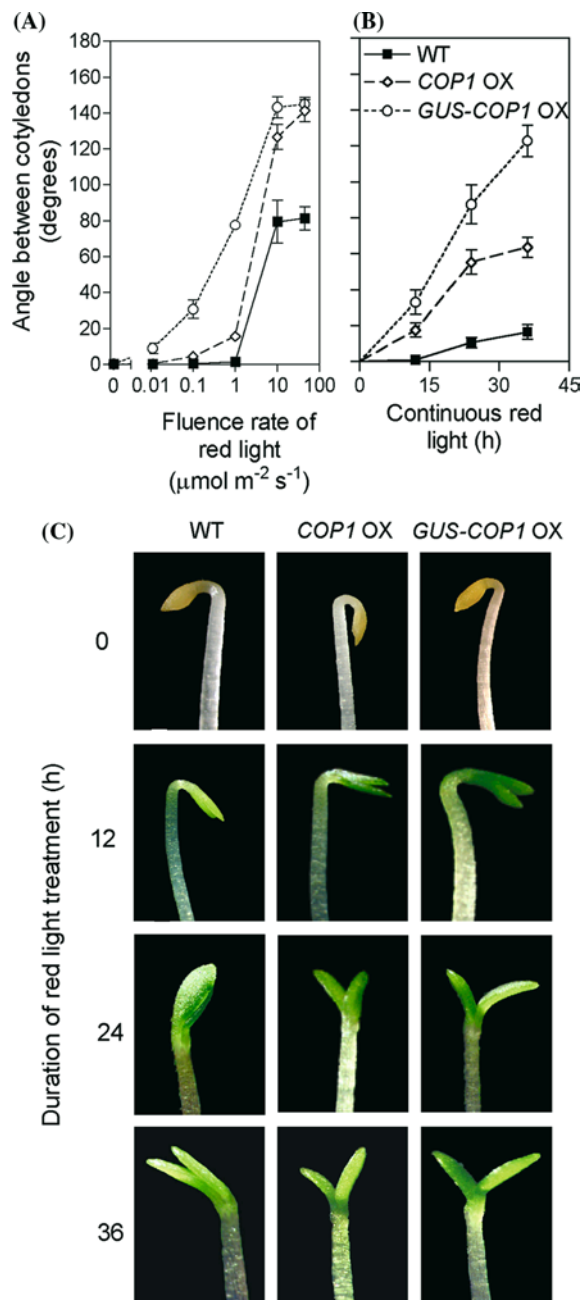


Figure 4. Cotyledon unfolding in WT and *COP1 OX* lines under red light. Cotyledon angle in seedlings exposed for 3 days to the indicated fluence rates of red light (A). For kinetic studies, two-day-old etiolated seedlings of the WT (Nossen) and *COP1 OX* lines were shifted to continuous red light ($17 \mu\text{mol m}^{-2} \text{s}^{-1}$) for the time indicated in abscissas (B). Data are means and SE of at least 12 replicate boxes. A detail of the cotyledons of representative seedlings is shown in C.

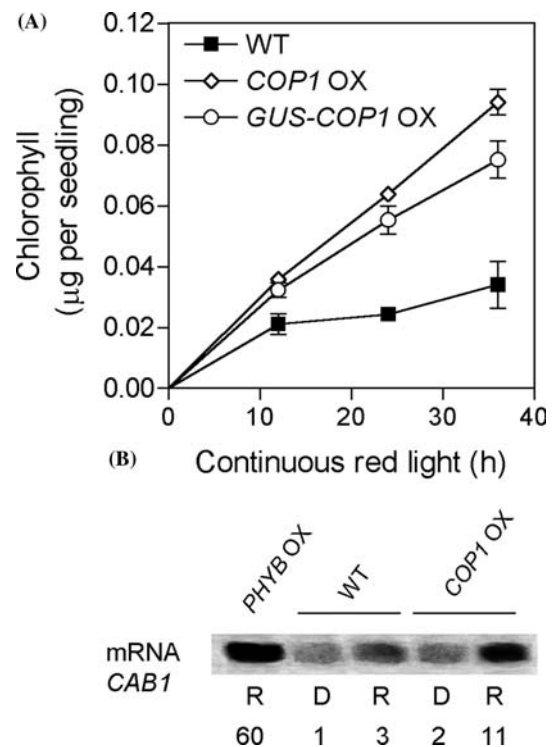


Figure 5. *COP1* overexpression enhances *CAB1* expression and chlorophyll accumulation under red light. Two-day-old etiolated seedlings of the WT (Nossen) and *COP1 OX* were exposed to continuous red light ($17 \mu\text{mol m}^{-2} \text{s}^{-1}$) for the time indicated in abscissas and harvested for chlorophyll determination (A). Data are means and SE of at least 4 replicate determinations using 30 seedlings per replicate. Two-day-old etiolated seedlings of the WT and *COP1 OX* were exposed to 6 h of red light ($17 \mu\text{mol m}^{-2} \text{s}^{-1}$) or remained in darkness before harvest for the analysis of *CAB1* expression (B). The *PHYB* overexpresser line ABO (Wagner *et al.*, 1991) was also included as control. Numbers below the bands correspond to the transcript level of *CAB1* relative to total RNA quantified for each lane in the membrane.

phyB is required for the enhanced photomorphogenesis of *COP1 OX* under red light

In the *phyB* mutant background, overexpression of *COP1* failed to enhance cotyledon unfolding in response to red light (Figure 6). The *phyA* mutation had no significant effects under red light. Under continuous far-red, *COP1 OX* showed reduced cotyledon angle (WT = 170 ± 4 degrees; *COP1 OX* = 137 ± 4 degrees) and in the *COP1 OX* background the *phyB* mutation caused a further reduction (*COP1 OX phyB*: 116 ± 4 degrees) suggesting that the performance of *COP1 OX* under this light condition could result from a

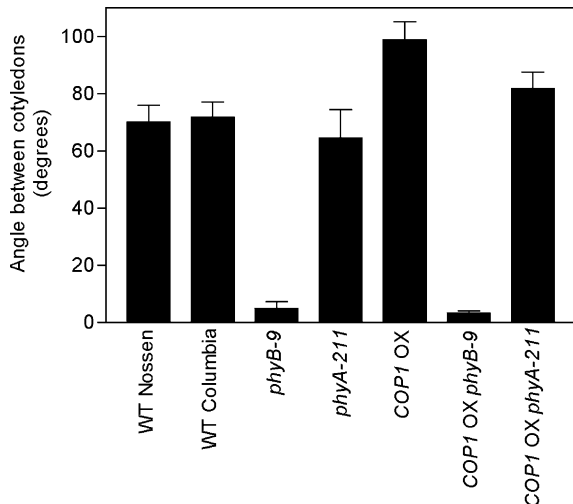


Figure 6. *phyB* is required for the enhanced photomorphogenesis of *COP1 OX* transgenics under red light. One-day-old seedlings of the WT (Nossen and Columbia), *phyB-9*, *phyA-211*, *COP1 OX*, *COP1 OX phyB-9*, *COP1 OX phyA-211* were grown under hourly pulses of red light (3 min, $20 \mu\text{mol m}^{-2} \text{s}^{-1}$). Data are means and SE of at least 10 replicate boxes. No cotyledon opening was observed in darkness for any genotype.

balance between repression of the *phyA*-mediated pathway and promotion of the *phyB*-mediated pathway.

Discussion

This paper describes a positive correlation between *COP1* levels and Arabidopsis responses to red light. The role of *COP1* as a repressor of photomorphogenesis in darkness is solidly established on the basis of several lines of evidence. The *cop1* mutants show photomorphogenesis in darkness (Deng *et al.*, 1991) and under the latter conditions its transcriptome resembles that of WT seedlings grown in the light (Ma *et al.*, 2002). The molecular mechanisms of this function as repressor involves the action of *COP1* as E3 ligase, targeting transcription factors involved in the photomorphogenic responses to degradation in the 26-S proteasome (Osterlund *et al.*, 2000; Holm *et al.*, 2002; Saijo *et al.*, 2003). The *COP1 OX* transgenics show reduced photomorphogenesis under blue or far-red light (McNellis *et al.*, 1994b; Osterlund and Deng, 1998; Figure 3 A, B) a phenotype that could result from a limited ability to translocate the exaggerated amount of *COP1* from

its place of action in the nucleus and/or the ability of *COP1* to down regulate light signaling. Recently, the occurrence of interaction between *COP1* and *SPA1* (Saijo *et al.*, 2003), and the characterization of the novel *cop1^{eid6}* allele, which bears an amino acid transition in a conserved histidine residue of the RING finger domain (Dieterle *et al.*, 2003), have revealed that *COP1* is actually a negative regulator of *phyA* signaling. The latter function of *COP1* appears to be different from that as repressor of photomorphogenesis in darkness because *cop1^{eid6}* shows enhanced sensitivity to light but no photomorphogenesis in darkness, while the *cop1-4* allele, which eliminates the WD40 domain of the protein, shows photomorphogenesis in darkness but an apparently normal range of sensitivity to light (Dieterle *et al.*, 2003).

The positive correlation between *COP1* levels and the response to red light comes from the analysis of both weak mutants and overexpressors. The *cop1-4* and *cop1-6* mutants showed fully unfolded cotyledons in darkness. Unexpectedly, red light, which enhances cotyledon unfolding in the WT, reduced cotyledon unfolding in these mutants (Figure 1). Cao *et al.* (2000) also observed reduced expression of photosynthetic genes in response to red light in *cop1-4* and *cop1-6*. The effect reported here is not the unspecific result of “exaggerated light signaling” in the *cop1* mutants exposed to light because far-red light, which is significantly more effective than red light to unfold the cotyledons in the WT, had a weaker negative effect than red light. Furthermore, the *cop1-4 phyB* and *cop1-6 phyB* double mutants showed no negative photomorphogenesis under red or even far-red light (Fig 2), despite the fact that *phyB* is not required for photomorphogenesis under far-red light in the WT. In addition, in *cop1* mutants the negative photomorphogenic effect required a minimum fluence rate of red light similar to that necessary to induce photomorphogenesis in the WT (Figure 1D) and cotyledon unfolding was not reduced by high irradiances of red or white light even in transgenic seedlings overexpressing *PHYB*.

In accordance with previous results (McNellis *et al.*, 1994b; Osterlund and Deng, 1998), *COP1 OX* lines showed no phenotype in darkness and reduced photomorphogenesis under far-red or blue light. However, the opposite was true under red light. Inhibition of hypocotyl growth,

cotyledon unfolding, chlorophyll synthesis and *CAB1* gene expression were all enhanced in transgenic lines overexpressing *COP1* under red light (Figure 3, 4, 5). Again, this effect was entirely dependent on the presence of phyB as revealed by the fully epistatic effect of the *phyB* mutation (Figure 6). The *COP1 OX* lines showed similar *COP1* levels under red, blue or far-red light, indicating that the differential effect under red light compared to the other wavebands cannot be accounted for by light-quality dependent co-suppression of *COP1* expression (Figure 3C). The consistent effects of *COP1* overexpression and *cop1* mutations argue against a predominant role of ectopic expression in the generation of the phenotype of *COP1 OX* lines.

Two observations favor the idea of a positive correlation between responses to red light and the nuclear-localized pool of COP1. First, the effects are relatively rapid (less than 6 h) compared to the time required to produce a detectable migration of COP1 to the cytosol (at least 24h of light, von Arnim *et al.*, 1997). Second, the *cop1-6* allele is impaired due to the inability of the mutated protein to localize to the nucleus but when the seedlings are grown under permissive temperatures normal localization and dark repression of photomorphogenesis are observed (Ma *et al.*, 2002). The apparently functional COP1-6 protein in the cytoplasm was unable to promote photomorphogenesis under red light, as the *cop1-6* and the truncated *cop1-4* alleles showed a quantitatively similar negative cotyledon unfolding at high fluence rates of red light.

The positive effect of COP1 levels on the response to red light could reflect a physiological role of COP1 as promoter of photomorphogenesis under red light, in addition to its well-known role as repressor in darkness, far-red and blue light. The opposite effect of COP1 on phyA and phyB-mediated responses suggests that COP1 could regulate the relative signaling current via each photoreceptor and therefore determine the most effective light signal. At least two alternative working models could account for a positive regulation of photomorphogenesis by COP1. First, since COP1 is involved in the degradation of positive regulators of photomorphogenesis like HY5 and LAF1 (Osterlund *et al.*, 2000; Seo *et al.*, 2003), a tentative model could be based on COP1-mediated targeting to degradation of a light-induced

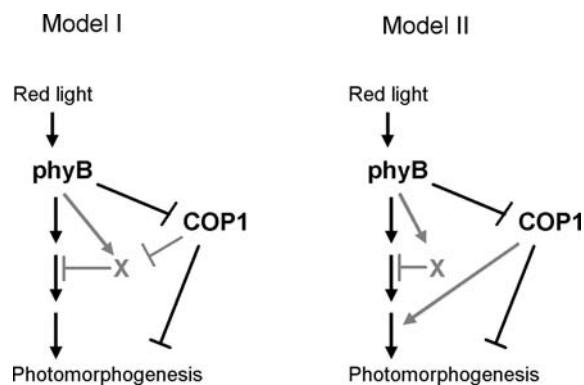


Figure 7. Working models that account for the positive correlation between COP1 activity and the response to red light.

negative regulator of phyB signaling (model I in Figure 7). According to this model, when the *cop1* mutants are grown in the dark full cotyledon unfolding is achieved but under red light phyB would induce a repressor of its own signaling network (there are various examples of such regulatory loops in signaling circuits, Casal *et al.*, 2004), which in the absence of functional COP1 would negatively regulate the photomorphogenic response. The second model (model II in Figure 7) is based on recent observations in mammalian cells. Two F box/WD-40-containing factors, which act as E3 ligases genetically linked to ubiquitin-dependent degradation, are unexpectedly required for gene activation by DNA-binding proteins (Perissi *et al.*, 2004). The latter involves recruitment of ubiquitin/19S proteasome complexes but not necessarily their action via proteolytic functions (Perissi *et al.*, 2004). Following the analogy, COP1 could activate phyB-mediated transcription. The positive correlation between COP1 levels and the response to red light is not easy to account for on the basis of COP1-enhanced phyB stability or activity. In fact, the negative response of cotyledon unfolding in *cop1* mutants showed the same range of sensitivity than the positive response in the WT (Figure 1D) and was eliminated, rather than enhanced, in the *phyB* background (Figure 2, *cop1-4 phyB* and *cop1-6 phyB*). Although the findings reported here are unexpected, the occurrence of bifunctional molecules (promoter/repressor) is not necessarily rare. Even a basal transcription factor has been shown to activate or repress transcription in *Drosophila* (Willy *et al.*, 2000). *det1* also promotes the expression of light-induced genes in the

dark and inhibits their expression in the light (Maxwell *et al.*, 2003). Perhaps bifunctional proteins are more frequent in plants than previously predicted, but one of the functions remains hidden by the other, making it difficult to find unless specific conditions are identified.

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