Genetic and Developmental Control of Nuclear Accumulation of COP1, a Repressor of Photomorphogenesis in Arabidopsis

Albrecht G. von Arnim, Mark T. Osterlund, Shing F. Kwok, and Xing-Wang Deng*

Department of Biology (A.G.v.A., M.T.O., X.-W.D.), and Department of Genetics (S.F.K.), Yale University, New Haven, Connecticut 06520–8104

Using a β-glucuronidase (GUS) reporter-COP1 fusion transgene, it was shown previously that Arabidopsis COP1 acts within the nucleus as a repressor of seedling photomorphogenic development and that light inactivation of COP1 was accompanied by a reduction of COP1 nuclear abundance (A.G. von Arnim, X.-W. Deng [1994] Cell 79: 1035–1045). Here we report that the GUS-COP1 fusion transgene can completely rescue the defect of cop1 mutations and thus is fully functional during seedling development. The kinetics of GUS-COP1 relocalization in a cop1 null mutant background during dark/light transitions imply that the regulation of the functional nuclear COP1 level plays a role in stably maintaining a committed seedling’s developmental fate rather than in causing such a commitment. Analysis of GUS-COP1 cellular localization in mutant hypocotyls of all other pleiotropic COP/DET/FUS loci revealed that nuclear localization of GUS-COP1 was diminished under both dark and light conditions in all mutants tested, whereas nuclear localization was not affected in the less pleotropic cop4 mutant. Using both the brassinosteroid-deficient mutant det2 and brassinosteroid treatment of wild-type seedlings, we have demonstrated that brassinosteroid does not control the hypocotyl cell elongation through regulating nuclear localization of COP1. The growth regulator cytokinin, which also dramatically reduced hypocotyl cell elongation in the absence of light, did not prevent GUS-COP1 nuclear localization in dark-grown seedlings. Our results suggest that all of the previously characterized pleiotropic COP/DET/FUS loci are required for the proper nuclear localization of the COP1 protein in the dark, whereas the less pleiotropic COP/DET loci or plant regulators tested are likely to act either downstream of COP1 or by independent pathways.

Ambient light conditions affect plant development throughout all developmental stages (Kendrick and Kronenberg, 1994; Liscum and Hangarter, 1994; Smith, 1995). For instance, seedling development in the angiosperm Arabidopsis thaliana depends on light signals to choose between the photomorphogenic and the skotomorphogenic (etiolo-

gical) pathway (for review, see von Arnim and Deng [1996]). Seedlings grown under light conditions show open and expanded cotyledons, a short hypocotyl, chloroplast development, and a high expression level for light-inducible genes. Under dark conditions, however, the seedlings exhibit long hypocotyls, closed and undeveloped cotyledons with etioplasts instead of chloroplasts, and low or undetectable levels of light-inducible genes. A complex and still largely unknown signaling network that coordinates seedling development in response to light is being unraveled (for review, see Bowler and Chua [1994]; Millar et al., 1994; McNellis and Deng, 1995; Quail et al., 1995).

During recent years, genetic analysis has demonstrated that a set of regulatory genes functions in darkness to suppress the photomorphogenic pathway, which in turn allows etiolation to occur (Chory et al., 1989, 1991; Deng et al., 1991; Wei and Deng, 1992; Hou et al., 1993; Miséra et al., 1994; Wei et al., 1994b; Kim et al., 1996; Kwok et al., 1996). Among them, 10 pleiotropic det-etiolated (DET), constitutively photomorphogenic (COP), or fusca (FUS) loci have been identified by way of mutations that result in pleiotropic photomorphogenic seedling development in darkness (Chory et al., 1989; Deng et al., 1991; Wei and Deng, 1992; Castle and Meinke, 1994; Miséra et al., 1994; Kwok et al., 1996). This class of pleiotropic COP/DET/FUS genes is presumably involved in the primary switch between seedling developmental patterns in response to light. One common feature of the pleiotropic COP/DET/FUS genes is that their severe alleles invariably lead to lethality after seedling development (Castle and Meinke, 1994; Miséra et al., 1994; Kwok et al., 1996).

On the other hand, several loci that have mutations that resulted in less pleiotropic photomorphogenic developmental phenotypes in darkness have also been identified (such as cbb2 [Kaussmann et al., 1996]; cyp/cbb3 [Kaussmann et al., 1996; Szekeres et al., 1996]; dwf1, allelic to dim/cbb1 [Kaussmann et al., 1996; Takahashi et al., 1995]; det2 [Chory et al., 1991]; cop2/amp1/pt-1, hsl1/cop3 [Chaudhury et al., 1993; Hou et al., 1993; Lehmann et al., 1996]; and cop4 [Hou et al., 1993]; suppressors of hy2 or shy [Kim et al., 1996]). Their dark-grown seedlings have lost some of the characteristics of the dark developmental path-

1 This work was supported by National Institutes of Health (NIH) grant no. GM47850 to X.-W.D. and Department of Energy grant no. DE-FG02-96ER20223 to A.G.v.A. X.-W.D. is a National Science Foundation Presidential Faculty Fellow, and M.T.O. and S.F.K. were NIH predoctoral trainees.

2 Present address: Department of Botany, HBB 437, The University of Tennessee, Knoxville, TN 37996–1100.

* Corresponding author; e-mail xingwang.deng@yale.edu; fax 1–203–432–3854.

Abbreviations: BR, brassinolide; DAPI, 4,6-diamidino-2-phenylindole.
way and exhibit aspects of photomorphogenic development. It is interesting that phenotypic defects in three of the less pleiotropic genes are rescued by treatment with brassinosteroids, suggesting that this class of plant growth regulators is required for efficient hypocotyl cell elongation during the etiolation response (det2 [Li et al., 1996]; cpl/cbh3 and diefl/dim/cbl1 [Kauschmann et al., 1996; Szekeres et al., 1996]).

To date, the precise molecular function of the pleiotropic COP/DET/FUS gene products remains unknown. At least some of the COP/DET/FUS proteins, such as COP9 and COP11, form part of a light-modulated multisubunit protein complex (Wei et al., 1994a; Chamovitz et al., 1996; Staub et al., 1996). Both COP1 and DET1 were also reported to be nuclear regulators (Pepper et al., 1994; von Arnim and Deng, 1994). Overexpression analysis of COP1 has substantiated the genetic model that it acts as a repressor of photomorphogenic development (McNellis et al., 1994b, 1996). When expressed as a fusion protein with the GUS reporter, COPl is found in the nuclei of Arabidopsis hypocotyl cells when plants are grown in darkness. In contrast, under light conditions, the hypocotyl cell nuclei lack GUS-COP1 protein. Moreover, the light-dependent subcellular partitioning of COPl is tissue-type specific: In the cells of the root, light signals do not affect the subcellular localization of COPl, and the protein remains nuclear at all times (von Arnim and Deng, 1994). Thus, it was proposed that COPl acts within the nucleus to repress photomorphogenesis, whereas its activity is controlled at least in part by a light-modulated and tissue-specific regulation of its concentration in the nucleus. These studies have not only suggested an intriguing mechanism for the reversible inactivation of the nuclear regulator COP1 by light but have also provided a framework to test the functional relationship between COPl and other photomorphogenic genes. For example, mutations in genes that are directly involved in properly localizing COPl to the nucleus would result in a phenotype similar to copl mutations themselves, whereas other genes involved in more downstream or independent pathways may have no effect on COPl nuclear localization.

Here we report further analyses of the GUS-COP1 fusion transgene in Arabidopsis and show that it is fully functional during seedling development, demonstrating the suitability of the fusion protein as an indicator for COPl localization. By analyzing GUS-COP1 localization in a panel of mutant backgrounds and in the presence of plant growth regulators, we were able to address the genetic and developmental regulation of COPl nuclear abundance and thus the light control of plant development.

MATERIALS AND METHODS

The transgenic lines harboring GUS-COP1, GUS-Nla, and GUS transgenes have been described (von Arnim and Deng, 1994). Mutant alleles of the genes used in this work are det1–1 (Chory et al., 1989; Pepper et al., 1994), cop4–1 (Hou et al., 1993), det2–1 (Chory et al., 1991; Li et al., 1996), cop8–1 (Wei et al., 1994b), cop9–1 (Wei and Deng, 1992), cop10–1 (Wei et al., 1994b), cop11–1 (fus6, Wei et al., 1994b), fus4–1, fus5–1, fus 11–1, and fus12–1 (Miséra et al., 1994). Mutant plants carrying the desired transgene were generated by standard genetic crossing. Progeny F1 plants heterozygous for both the transgene and the mutation were selected based on the transgene selectable marker, kanamycin resistance, as well as on F2 segregation analysis. Homozygous mutant plants carrying the GUS-fusion transgene were examined in the F2 or the following F3 population.

Complementation Analysis

Arabidopsis lines carrying the indicated transgenes were crossed to homozygous cop1–4 or heterozygous cop1–5 mutant plants, and F1 plants heterozygous for both the cop1 allele and the transgene were identified. Segregation of the mutation and the transgene were analyzed in the selfed progeny (F2 population). In the absence of complementation, we expected to observe one-quarter mutant seedlings. In addition, both mutants and wild-type plants should show GUS expression. In the event of complementation, we expected an increase in the ratio of wild-type to mutant plants from 3:1 up to 15:1, and none of the mutants should stain for GUS. To confirm the result, we examined segregation of the cop1 allele and the transgene in F3 populations derived by selfing of homozygous mutant F2 plants that were heterozygous for GUS-COP1 (cop1/cop1; GUS-COP1/wt). These F2 plants lacked copies of the wild-type COPl allele and could be identified because the ratio of wild-type to mutant plants reverted to 3:1 in their progeny, with none of the mutants but all of the wild-type plants staining for GUS. Such cop1/cop1-mutant F3 populations were used for the kinetic analysis of GUS-COP1 relocalization.

Histochemical Localization of GUS Activity

Arabidopsis seedlings were harvested and stained in GUS assay buffer as described previously (von Arnim and Deng, 1994), except that the initial fixation step before GUS staining was usually omitted. Omitting the fixation did not alter the pattern of subcellular localization, but it increased the overall speed or intensity of GUS staining. For microscopic observation of GUS-COP1 localization, areas of tissue were chosen that showed intense cytoplasmic inclusion bodies but lacked diffuse blue staining, because the diffuse staining would otherwise have obscured the staining of the nucleus. For quantitative comparisons of nuclear staining intensity, care was taken to compare cells from areas of tissue with equally intense cytoplasmic inclusion bodies. Staining times varied between 3 and 6 h, with photomorphogenic seedlings usually requiring longer staining times than etiolated seedlings. Placing seedlings into the staining solution should interrupt protein-trafficking processes, and any effect of the staining time on GUS-COP1 relocalization can therefore be excluded.

For GUS-Nla and GUS alone, which do not form cytoplasmic inclusion bodies as a reference point, staining was allowed to proceed until the staining intensity in the root tip was approximately equal to that in corresponding GUS-
COP1 transgenic seedlings under the same experimental conditions. Stained seedlings were fixed in 10% acetic acid, 3.7% formaldehyde, and 50% ethanol or as described by von Arnim and Deng (1994) and destained through a graded ethanol series before mounting in water with 1 mg/L DAPI. Samples were examined on a microscope (Axiophot Zeiss) with epifluorescence optics and a DAPI filter set no. 02 (Zeiss). Micrographs were usually taken with a color CCD camera (model 72, Sony, Tokyo, Japan) to visualize DAPI staining. In some cases micrographs were taken on 35-mm slide film (400 ASA, Kodak), and slides were scanned using Polaroid (Cambridge, MA) or Nikon slide scanners. Micrograph were assembled using Adobe Photoshop software (Adobe Systems Inc., Mountain View, CA) for the Macintosh computer.

**Kinetics of GUS-COP1 Relocalization**

Wild-type and copl-5 seedlings carrying the GUS-COP1 transgene were germinated in continuous white light (120 μE m⁻² s⁻¹) for 5 d. They were kept under light conditions for 5 d or transferred to darkness for 12, 24, and 36 h toward the end of the 5-d period. Seedlings were harvested into wells of coded tissue culture dishes and stained for GUS activity. For each treatment we typically evaluated the percentage of cells showing nuclear staining by examining at least 20 cells in each of at least three individual hypocotyls. Before decoding the information concerning genotype and sample treatment, we graded stained samples according to their increasing nuclear localization of GUS-COP1. We investigated whether one genotype showed consistently more intense staining than the other and found that this was not the case.

**Growth Regulator Treatments**

BA was added to cooled autoclaved growth medium (Deng et al., 1991) at the indicated concentrations from a stock solution in DMSO. BR was added from a stock solution in 100% ethanol. BR treatment of light-grown seedlings (Fig. 3) was performed on seedlings growing under 30 μE m⁻² s⁻¹ white light. Under these conditions, the GUS-COP1 seedlings are slightly elongated compared with the controls lacking GUS-COP1, indicating that the light intensity used did not completely saturate the inhibition of hypocotyl elongation.

### Table 1. Complementation of the cop1 mutant seedling phenotype by GUS-COP1 transgene

<table>
<thead>
<tr>
<th>Cross</th>
<th>Generation</th>
<th>Total</th>
<th>Phenotype</th>
<th>Ratio Wild-Type/Mutant</th>
<th>GUS+ among cop1-Like</th>
</tr>
</thead>
<tbody>
<tr>
<td>cop1-4 × GUS-COP1</td>
<td>F₂</td>
<td>286</td>
<td>cop1</td>
<td>16/270</td>
<td>16.9</td>
</tr>
<tr>
<td></td>
<td>F₃</td>
<td>45</td>
<td>Wild</td>
<td>8/37</td>
<td>4.6</td>
</tr>
<tr>
<td>cop1-5 × GUS-COP1</td>
<td>F₂</td>
<td>171</td>
<td>null</td>
<td>8/163</td>
<td>20.4</td>
</tr>
<tr>
<td>cop1-5 × GUS</td>
<td>F₂</td>
<td>559</td>
<td>wild</td>
<td>121/438</td>
<td>3.6</td>
</tr>
</tbody>
</table>

* F₂ population from an F₁ individual with cop1/cop1; transgene/wild-type genotype. **n.d., Not determined.
change in the staining pattern was apparent after 24 h of light (Fig. 2B) or darkness (Fig. 2E) and after 36 h (Fig. 2, C and F) of treatment. However, for each treatment there was no difference between the kinetic behaviors of the nuclear level of GUS-COP1 in the wild-type or cop1-5 null backgrounds. We therefore conclude that the endogenous COP1 protein does not interfere significantly with the nuclear localization of GUS-COP1 and that the kinetics of relocation for the functional GUS-COP1 protein is a rather slow process. This conclusion implies that the repartitioning of GUS-COP1 in response to light is unlikely to be the cause for the concurrent developmental switch; rather, repartitioning must be a consequence of other, earlier, and as-yet undefined light-triggered events. However, it is possible that light-regulated GUS-COP1 accumulation or depletion within the nucleus plays a role in stabilizing the resulting developmental program under the prevailing light conditions, and other photomorphogenic genes may be involved in this light-regulated subcellular repartitioning process.

**GUS-COP1 Nuclear Localization in Darkness Is Defective in All Pleiotropic cop/det/fus Mutants**

If any of the pleiotropic COP/DET/FUS genes (COP8-COP10, DET1, FUS4-FUS6, FUS11, and FUS12, apart from COP1) were required for the nuclear accumulation of COP1, their loss-of-function mutations should prevent the nuclear accumulation of COP1 in the dark, which would result in the observed pleiotropic photomorphogenic phenotype. To test this hypothesis, the GUS-COP1 transgene was crossed into representative alleles of all available cop/det/fus mutant backgrounds other than cop1. The effect of the mutations on COP1 nuclear localization was examined by analyzing the subcellular GUS-staining patterns. Similar to the cop9 mutant (Chamovitz et al., 1996), each of the other eight pleiotropic mutants tested exhibited the same phenotype with respect to GUS-COP1 localization, and the data obtained with dark-grown cop10, fus12, and det1 seedlings are shown as examples in comparison with the wild type (Fig. 2, G-N). It is evident that no nuclear staining for GUS-COP1 was detected in the dark-grown Arabidopsis hypocotyls of any of the mutants analyzed. This absence resembles that of the GUS protein control (data not shown). Therefore, the wild-type gene products of all of the nine pleiotropic cop/det/fus genes are required for the nuclear accumulation of the GUS-COP1 protein under dark-grown conditions.

Several pieces of evidence indicate that the mutations do not, however, result in a general defect in nuclear uptake. First, as a positive control, we analyzed the ability of each mutation to affect the nuclear localization of the GUS-Nla fusion protein, a constitutively nuclear protein in which nuclear localization in transgenic Arabidopsis was not affected by the presence or absence of light signals (von Arnim and Deng, 1994). The GUS-Nla protein could localize to the nuclei of all photomorphogenic mutant hypocotyls under both light and dark conditions. Second, the root-tip cells of several cop/det/fus mutants showed nuclear accumulation of GUS-Nla.
Figure 2. A to F, Kinetics of GUS-COP1 relocalization between cytoplasm and nucleus in cop1-5 mutant seedlings. Seedlings were stained for GUS activity (brightfield images) and for the position of the nucleus (arrows) using DAPI and epifluorescence illumination (insets). Scale bars = 10 μm. A to C, Dark to light shift: Seedlings were kept in darkness for 5 d (A) or transferred to light for 24 h (B) or 36 h (C) toward the end of 5 d of germination. For the cell shown in A, the cytoplasmic fraction of GUS-COP1 is shown in the left inset. D to F, Light to dark shift: Seedlings were germinated in continuous white light and kept under light conditions for 5 d (D) or transferred to darkness for 24 h (E) or 36 h (F) toward the end of the 5-d period. G to N, Subcellular localization of GUS-COP1 in hypocotyls of severe cop, fus, and det gene mutants. G and H, cop10; I and J, fus12; K and L, det1; and M and N, wild type. Dark-grown (G, I, K, and M) and light-grown seedling hypocotyls (H, J, L, and N) are arranged in pairs.

We therefore conclude that mutations in the nine pleiotropic cop/det/fus loci specifically affect, directly or indirectly, the nuclear accumulation of GUS-COP1 in the hypocotyl cells of dark-grown seedlings. It should be noted that none of the mutant phenotypes were detectably affected by the presence of the GUS-COP1 transgene.

The Less Pleiotropic cop4 Mutation Has No Effect on Nuclear Accumulation of GUS-COP1

We also tested the effect of less pleiotropic photomorphogenic mutations on the nuclear accumulation of GUS-COP1. As an example. The dark-grown
cop4-1 mutant exhibits photomorphogenic cotyledon development, including cell differentiation and light-regulated gene expression, but has normal etioplast development (Hou et al., 1993). However, the dark-grown cop4-1 mutants have a regular long hypocotyl resembling that of the wild type, except for a deficiency in gravitropic orientation (Hou et al., 1993). As shown in Figure 3, C and F, the GUS-COP1 protein localized normally to the hypocotyl cell nuclei of dark-grown and light-grown cop4 seedlings just as in the wild type. This result indicates that the COP4 gene product is not required for nuclear accumulation of GUS-COP1 in darkness, which is consistent with the observed normal hypocotyl elongation in cop4-1 mutant seedlings.

**Cytokinin Inhibits Hypocotyl Elongation but Does Not Prevent GUS-COP1 Nuclear Accumulation in Dark-Grown Arabidopsis Seedlings**

Since all pleiotropic cop/det/fus mutants that lacked nuclear staining of GUS-COP1 in the dark-grown hypocotyl cells also exhibited a severe inhibition of hypocotyl cell elongation, it was necessary to test whether the lack of nuclear accumulation is not a consequence of the inhibition of hypocotyl cell elongation. To address this, we examined the effect of cytokinin, which severely inhibits hypocotyl cell elongation, on the nuclear accumulation of GUS-COP1 in the hypocotyl cells of dark-grown Arabidopsis seedlings.

**Figure 3.** A to F, Subcellular localization of GUS-COP1 in dark-grown (top row) and light-grown (second row) seedlings of the mild photomorphogenic mutants det2 (B and E) and cop4 (C and F). The wild type is shown in A and D for comparison. For details, see legend to Figure 2. G to J, Cytokinin treatment of dark-grown wild-type seedlings. Subcellular localization of GUS-COP1 in the presence (G and H) and absence (I and J) of 0.03 mM cytokinin. H and J show nuclear DAPI stains of the cells in G and I, respectively. K to P, BR treatment of seedlings grown for 5 d under constant white light: Subcellular localization of GUS-COP1 (K–N) and GUS-Nla (O and P) in untreated (K and O) or BR-treated (L, M, N, and P) seedlings. K, GUS-COP1, no BR; L, GUS-COP1, 10 nM BR; M, GUS-COP1, 1 μM BR; N, GUS-COP1, 1 mM BR; nuclear staining as seen in N is rare and occurs in approximately 2% of the hypocotyl cells; O, GUS-Nla, no BR; P, GUS-Nla, 100 nM BR. Nuclei are indicated by arrows and dashed oval regions.
lings. Wild-type Arabidopsis seedlings carrying the GUS-COP1 transgene were germinated in darkness on increasing concentrations of the cytokinin BA. Consistent with data previously reported (Chory et al., 1994; Su and Howell, 1995), increasing the cytokinin concentration resulted in dramatically shortened hypocotyls (Fig. 4) and a decrease in hypocotyl cell length (not shown). Note that the hypocotyl of det1–1 mutant seedlings, which lacks visible nuclear COP1 (Fig. 2), was longer than the hypocotyl of wild-type plants treated with 30 μM cytokinin. However, as shown in Figure 3G, GUS-COP1 was retained in the hypocotyl cell nuclei even under the highest concentration of cytokinin used (30 μM). A similar efficiency of nuclear accumulation was observed for the GUS-Nia control protein (not shown). These data suggest that inhibition of hypocotyl elongation does not itself detectably affect GUS-COP1 nuclear localization in the dark.

The Inhibition of Hypocotyl Elongation Caused by a Defect in the Brassinosteroid Biosynthetic Pathway Does Not Prevent Nuclear Accumulation of GUS-COP1

Arabidopsis det2 mutants show a reduced hypocotyl cell elongation, similar to det1 (Fig. 4), as well as a moderate derepression of light-inducible gene expression (Chory et al., 1991). Recently, the molecular cloning of DET2 has suggested that it encodes a reductase in the BR biosynthetic pathway, implying that brassinosteroids play an important role in light-regulated development of higher plants (Li et al., 1996). To test whether BR can achieve this role by regulating COP1 localization, nuclear GUS-COP1 staining was first examined in wild-type seedlings under light conditions with increasing levels of BR. BR increased the hypocotyl length (Fig. 5) and decreased the cotyledon size (not shown) of 5-d-old light-grown seedlings, suggesting that BR indeed counteracts two typical photomorphogenic growth responses (von Arnim and Deng, 1996). A limited number of hypocotyl cells (approximately 2%) from the seedlings elongated by BR treatment revealed a nuclear GUS-COP1 level that was clearly increased (Fig. 3N) in comparison with the untreated controls (Fig. 3K). However, the majority of hypocotyl cells still exhibited no nuclear staining (Fig. 3, L and M), similar to the untreated wild-type seedlings. Such a result is typical for a minimal increase in nuclear staining, close to the threshold of detection. We also observed this result at early times (12–24 h) after light to dark shifts of GUS-COP1 transgenic seedlings. We interpret this result as an indication that nuclear GUS-COP1 accumulation is stimulated slightly in hypocotyl cells under the influence of BR.

To further examine the extent to which brassinosteroid may stimulate the nuclear accumulation of GUS-COP1, we introduced GUS-COP1 into the severe allele det2–1, which is thought to carry a null mutation for BR biosynthesis (Li et al., 1996), and analyzed its nuclear localization pattern. Despite the partial de-etiolation in det2, GUS-COP1 remained localized to the nuclei in the dark-grown det2 hypocotyl cells (Fig. 3, B and E), and the intensity of nuclear staining appeared only slightly reduced as compared with that seen in dark-grown wild-type seedlings (Fig. 3, A and D). To properly interpret the level of nuclear staining, it is illustrative to compare the hypocotyl lengths of dark-grown det1 and det2 seedlings alongside their levels of nuclear GUS-COP1 protein. Although det1 and det2 seedlings have, in fact, hypocotyls of comparable lengths, the nuclear level of GUS-COP1 is dramatically lower in det1 (Fig. 2, K and L) than that in det2 seedlings (Fig. 3, B and E). Therefore, the role of DET1 in securing the nuclear accumulation of GUS-COP1 must be far more significant than
the role of DET2. These distinct effects of det1 and det2 mutations on the nuclear localization of GUS-COP1 are consistent with the notion that these two genes act in separate pathways, as reasoned previously (Li et al., 1996). The partial reduction in nuclear staining intensity seen in dark-grown det2 mutant seedlings is consistent with a partial response to BR.

DISCUSSION

Implications of Temporal Patterns of GUS-COP1 Relocalization

We have previously demonstrated that the activity of the Arabidopsis repressor of photomorphogenesis COP1 may be regulated by a light-mediated nucleocytoplasmic partitioning process (von Arnim and Deng, 1994). Here we analyzed the kinetics of relocalization of COP1 nucleocytoplasmic partitioning during light/dark transitions to clarify the role of this process in the light control of Arabidopsis seedling development. To this end, the GUS-COP1 transgene was first crossed into the cop1-5 null mutant background, which lacks detectable COP1 protein (Deng et al., 1992; McNellis et al., 1994a). This was possible because the GUS-COP1 gene completely reverted the lethal cop1-5 mutant phenotype to wild type. After re-examining the kinetics of GUS-COP1 relocalization in response to shifts from darkness to light and vice versa in the absence of endogenous COP1 protein, we reaffirmed that relocalization of GUS-COP1 proceeds relatively slowly, especially when compared with many known light-inducible developmental and physiological responses (Cosgrove, 1994; Adamska, 1995; Terzaghi and Cashmore, 1995). It is therefore likely that the switch from the etiolation pathway toward the photomorphogenic pathway and vice versa may be triggered by some rapid signaling event(s) other than the subcellular localization of COP1, and the light-regulated nuclear depletion of COP1 may serve to maintain the resulting developmental program. It is unlikely that our analysis severely underestimated the speed of relocalization of the endogenous COP1 protein, which might be expected if the cellular relocalization machinery became saturated by an excess of GUS-COP1 protein. Although GUS-COP1 is overexpressed approximately 10-fold compared with endogenous COP1 (von Arnim and Deng, 1994), the majority of this protein is in the insoluble cytoplasmic “inclusion body” and therefore should not affect the relocalization machinery, if the latter was indeed saturable. Although we cannot rule out that the larger GUS-COP1 fusion protein might have more difficulty interacting with the cellular machinery, the complete phenotypic rescue of GUS-COP1 to the cop1-5 null mutation would argue against it.

The slow kinetics of relocalization of the GUS-COP1 protein is in line with the assumption that, under photoperiods experienced by Arabidopsis thaliana during the growing season in its natural, temperate zone environment, 8 to 14 h of darkness during the night should be insufficient to reverse the commitment of a light-grown seedling from the photomorphogenic toward the etiolated pathway. Rather, once photomorphogenesis has been initiated, a minute level of nuclear COP1, close to the detection limit imposed by the sensitivity of the GUS reporter, may be all that is required to carry out the functions of COP1 during light-grown development (Deng et al., 1992; Miséra et al., 1994; von Arnim and Deng, 1994). A low level of nuclear COP1 would reflect the commitment to a photomorphogenic program under regular short- or long-day photoperiods, contrasting with the undetectable nuclear COP1 level under high intensity, continuous white light. In fact, such a low level of nuclear GUS-COP1 protein is observed when Arabidopsis seedlings are grown under a day/night cycle (von Arnim and Deng, 1994). The low level of nuclear COP1 may be sufficient to carry out the functions of the COP1 protein during normal light development (Deng et al., 1992) and throughout the vegetative stage of development (Miséra et al., 1994), whereas a higher level of nuclear COP1 is required for full repression of the photomorphogenic program in dark-grown seedlings. The need for a full reversion to the dark developmental pathway, although possible under conditions such as the sudden covering up of a seedling by falling leaves or debris, is probably rarely encountered. To convince the seedling that such a condition has arrived, light deprivation for longer than 14 h may be needed to accumulate a high level of nuclear COP1. In conclusion, our data fit the interpretation that the level and kinetics of nuclear GUS-COP1 are designed to retain the developmental commitment of the Arabidopsis seedling to a particular developmental pathway, and the relatively slow kinetics of relocalization may act as a built-in dampening factor to avoid rapid shifts between the seedling’s overall developmental pattern during the frequent fluctuations in ambient light levels over the day/night cycle.

Genetic Interactions Regulating the Nuclear Accumulation of COP1

Mutations in at least nine Arabidopsis genes result in a phenotype that is either indistinguishable or closely related to that of cop1 mutants. These genes have recently been designated as the pleiotropic COP/DET/FUS loci (Kwok et al., 1996), and their similar phenotypes are thought to indicate that the corresponding wild-type gene products interact closely in the repression of photomorphogenesis. We have therefore analyzed the requirement for each of eight of these nine loci on the nuclear localization of GUS-COP1 in Arabidopsis hypocotyl cells and found that each of them is required for the nuclear accumulation of GUS-COP1. An identical result was recently described for the ninth gene of the group, COP9 (Chamovitz et al., 1996). In contrast to the pleiotropic COP/DET/FUS loci, mutations in a less pleiotropic gene, COP4 (Hou et al., 1993), did not reveal an involvement in regulating the nuclear localization of COP1. This result is consistent with the placement of COP4 function in a pathway downstream from or independent of COP1.

Our result reconfirms the close mutual interaction of the 10 COP/DET/FUS gene products. It remains to be shown whether the function of the nine gene products is to retain GUS-COP1 in the nucleus after it has been imported, or
whether their function is to mediate nuclear import of GUS-COP1. The nuclear localization of DET1 (Pepper et al., 1994), COP9, (Chamovitz et al., 1996), and COP11/FUS6 (Staub et al., 1996) is consistent with either of these functions, retention in the nucleus or assistance in nuclear uptake. The protein complex formation among at least two of the COP/DET/FUS gene products, namely COP9 and COP11/FUS6, and possibly involving COP8 (Wei et al., 1996; Staub et al., 1996), is also consistent with our data. Indeed, if the COP9 complex is essential, one would predict that cop9 and cop11 mutations as well as cop8 have identical effects on GUS-COP1 localization, as has been observed. Additional molecular analysis is required to understand the role of other COP/DET/FUS gene products in retaining COP1 in the nucleus.

Effects of Plant Hormones and Growth Regulators

Apart from the effects of photomorphogenic mutations, a number of plant growth regulators and plant hormones have been shown to affect seedling development in ways that are reminiscent of the effects of light or dark signals (Chory et al., 1994; Su and Howell, 1995; Weatherwax et al., 1996). However, only very recently has it been realized that brassinosteroids can act as growth regulators to contribute to the skotomorphogenic development seen in dark-grown seedlings (Mandava, 1988; Kauschmann et al., 1996; Li et al., 1996; Szekeres et al., 1996). Their contribution is particularly intriguing because the application of brassinosteroids can partially ameliorate some aspects of the mutant phenotypes, cop1, for example, of the COP/DET/FUS class (Szekeres et al., 1996). Other growth regulators, such as cytokinins and auxins, have so far proven unable to rescue at least the cop1 mutant phenotype (Deng et al., 1991; Deng and Quail, 1992).

To test the hypothesis that brassinosteroids may mediate hypocotyl cell elongation by increasing the nuclear accumulation of GUS-COP1, we have made use of a mutation that is thought to disrupt the biosynthesis of brassinosteroids in Arabidopsis, namely det1 (Li et al., 1996). Apart from reduced fertility and slower senescence, det2 mutants show a mild defect in photomorphogenesis, having partially lost many morphological attributes of the etiolated seedling but retaining the arrest in chloroplast development from the wild-type phenotype (Chory et al., 1991). The DET2 gene product has similarity to mammalian steroid reductases and, importantly, the end product of the DET2-mediated biosynthetic pathway completely rescues the mutant phenotype of det2 but not of det1 (Li et al., 1996). It is thought that a functional brassinosteroid synthesis pathway is essential but not sufficient for the full complement of etiolated phenotypes under dark conditions. However, even in the absence of a functional DET2 product, GUS-COP1 protein was found in the nucleus of dark-grown hypocotyl cells (Fig. 3). Clearly, signaling by brassinosteroids is not essential for the nuclear accumulation of COP1. However, a quantitative contribution of brassinosteroid/DET2 signaling could not be excluded. Indeed, such a contribution was suggested by the observed stimulation of GUS-COP1 localization to the nucleus that was caused by treatment of light-grown seedlings with BR (Fig. 3).

While brassinosteroids can mediate cell elongation responses in the context of etiolation, cytokinins are thought to initiate many of the developmental responses that are also controlled by light signals. For example, treatment of dark-grown seedlings with the cytokinin BA or other cytokinins results in opening of the apical hook, partial unfolding and expansion of cotyledons, plastid development, derepression of light-inducible genes, and strong inhibition of hypocotyl cell elongation (Chory et al., 1994; Su and Howell, 1995). Two explanations for the role of cytokinin during seedling development have been put forward. On one hand, cytokinins may be part of the light signal transduction pathway (Chory et al., 1994), and on the other, they may act independently of light on the same morphogenic responses (Su and Howell, 1995). Unfortunately, viable mutations known to specifically reduce the concentration of cytokinins are not available in Arabidopsis. We have therefore examined the possible contribution of cytokinins to the exclusion of GUS-COP1 from the nuclear compartment by growing seedlings in darkness on increasing concentrations of the cytokinin BA.

Although this treatment resulted in a severe reduction in hypocotyl length, GUS-COP1 nuclear localization was not abolished. This was particularly significant in comparison to the complete absence of GUS-COP1 nuclear localization in the det1 mutant, in which hypocotyl cell length is similar to that after cytokinin treatment. Cytokinin may therefore act downstream or independently of GUS-COP1 localization when mimicking photomorphogenic responses in dark-grown seedlings. This conclusion is consistent with results obtained in a quantitative analysis of the interaction between cytokinin and light treatments on hypocotyl length in Arabidopsis (Su and Howell, 1995).

ACKNOWLEDGMENTS

We are grateful for the mutant lines det1 and det2 from Dr. J. Chory and for the mutant lines fus4, fus5, fus11, and fus12 from Dr. S. Miséra. In addition, the generous supply of BR from Dr. S. Fujioka is acknowledged. We thank L.H. Ang for assistance with a number of the genetic crosses.

Received November 25, 1996; accepted March 28, 1997.

Copyright Clearance Center: 0032-0889/97/114/0779/10.

LITERATURE CITED

Chaudhury AM, Letham S, Craig S, Dennis ES (1993) ampi, a mutant with high cytokinin levels and altered embryonic pat-
tern, faster vegetative growth, constitutive photomorphogenesis, and precocious flowering. Plant J 4: 907–916


