The Plant Cell, Vol. 8, 1951–1959, November 1996

det1, *cop1*, and *cop9* Mutations Cause Inappropriate Expression of Several Gene Sets

Raphael Mayer,[1] Dora Raventos,[2] and Nam-Hai Chua[3]

Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, New York 10021-6399

Genetic studies using Arabidopsis offer a promising approach to investigate the mechanisms of light signal transduction during seedling development. Several mutants, called *det/cop*, have been isolated based on their deetiolated/constitutive photomorphogenic phenotypes in the dark. This study examines the specificity of the *det/cop* mutations with respect to their effects on genes regulated by other signal transduction pathways. Steady state mRNA levels of a number of differently regulated gene sets were compared between mutants and the wild type. We found that *det2*, *cop2*, *cop3*, and *cop4* mutants displayed a gene expression pattern similar to that of the wild type. By contrast, *det1*, *cop1*, and *cop9* mutations exhibited pleiotropic effects. In addition to light-responsive genes, genes normally inducible by plant pathogens, hypoxia, and developmental programs were inappropriately expressed in these mutants. Our data provide evidence that DET1, COP1, and COP9 most likely act as negative regulators of several sets of genes, not just those involved in light-regulated seedling development.

INTRODUCTION

The expression of many developmentally regulated genes is known to be controlled at the transcriptional level, but little is known about the signal transduction pathways that regulate gene transcription. In plants, the source of the signal controlling gene expression may be hormones, gases, light, and pathogens. Photoreceptors are among the few plant receptors that have been unequivocally identified. In Arabidopsis, five photoreceptor genes for red light and far-red light and two photoreceptor genes for blue light have been characterized (reviewed in Millar et al., 1994). Recently, there has been increasing interest in identifying components that act downstream of photoreceptors in the light signal transduction pathway. Biochemical approaches have implicated the involvement of cGMP, calcium, and calmodulin in transducing phytochrome A signals (Neuhaus et al., 1993; Bowler et al., 1994a, 1994b). Several genetic screens designed to isolate mutants deficient in light signal transduction components have also been developed. One strategy was to isolate mutants with a light-grown morphology when grown in the dark, based on the assumption that mutants constitutively active in the light signaling pathway should have such a phenotype. Several such mutants have been isolated and are termed *det* (for deetiolated) or *cop* (for constitutive photomorphogenesis) (Chory et al., 1989, 1991; Deng et al., 1991). In a different genetic screen, Misera et al. (1994) identified nine *fusca* (*fus*) mutants that accumulate high levels of anthocyanin in the seed and also show light-independent seedling development. Genetic analyses showed that some *det/cop* mutants are allelic to some *fus* mutants (Castle and Meinke, 1994; Pepper et al., 1994).

In contrast to dark-grown wild-type seedlings, which possess elongated hypocotyls and unexpanded and hooked cotyledons, dark-grown *det1*, *cop1*, and *cop9* mutants and most of the other *det/cop* mutants have short hypocotyls and expanded cotyledons. Most show elevated expression of photoregulated genes and increased anthocyanin content in the cotyledons. In addition, most dark-grown *det/cop* mutants have differentiated plastids as opposed to etioplasts in etiolated wild-type seedlings. There is, however, some variation in the phenotypes among the *det/cop* mutants. For example, dark-grown *cop2*, *cop3*, and *cop4* mutants have expanded and unhooked cotyledons, but, unlike other *cop* mutants, they have long hypocotyls (Hou et al., 1993). Whereas dark-grown *cop4* mutants exhibit higher expression of photoregulated genes encoding the chlorophyll *a/b* binding protein (*CAB*), ribulose-1,5-bisphosphate carboxylase (*RBCS*), and ferredoxin type A, *cop2*, and *cop3* mutants express these genes at a level similar to that of the wild type (Hou et al., 1993).

DET1, *COP1*, and *COP9* genes have recently been cloned, and their roles in light signaling are being characterized (Deng et al., 1992; Pepper et al., 1994; Wei et al., 1994). Chimeric proteins of both DET1 and COP1 fused to the β-glucuronidase (GUS) reporter protein were shown to be nuclear localized (Pepper et al., 1994; von Arnim and Deng, 1994). More detailed studies on the localization of the COP1–GUS fusion protein

[1] Current address: Vitality Biotechnologies, Einstein Building, Carmel Industries Park, Tirat Hacarmel, Israel 39101.
[2] Current address: Department of Plant Physiology, Copenhagen University, Oster Farimagsgade 2A, 1353 Copenhagen K, Denmark.
[3] To whom correspondence should be addressed.

revealed that in the upper part of the hypocotyl, COP1 is present in the nucleus in the dark but not in the light. It was concluded that in the dark, COP1 functions in the nucleus to suppress photomorphogenesis and to promote skotomorphogenesis, and that in the light, it is relocalized to the cytoplasm, thus allowing photomorphogenesis to proceed (von Arnim and Deng, 1994).

Pepper et al. (1994) have undertaken a detailed morphological and molecular analysis of an allelic series of *det1*. It was found that the mutant phenotypes are contributed by two classes of overlapping effects. Whereas all mutant alleles expressed *CAB* and *RBCS* genes in the dark, the most severe allele, *det1-6*, caused the accumulation of high levels of anthocyanin and loss of tissue-specific expression of the chalcone synthase gene (*CHS*) and *CAB*, with the *det1-6* mutants exhibiting defects in general growth and development. Their results indicate that DET1 activity is needed for correct temporal and spatial expression of light-regulated genes.

Genetic analyses designed to determine the hierarchy of *det/cop/fus* and the photoreceptor mutants have shown that *det/cop/fus* mutants are epistatic to the latter (Chory, 1992; Ang and Deng, 1994; Misera et al., 1994). The genetic relationship of *cop1* and other *det/cop/fus* mutants to phytochrome and the light-dependent nuclear localization of COP1 suggest that *cop1* and other *det/cop/fus* mutants may function as repressors of photomorphogenesis in light signal transduction downstream of phytochrome. Consistent with its proposed role as a repressor of gene expression, COP1 was found to contain WD-40 repeats. A very well characterized WD-40–containing repressor is the yeast TUP1 protein (Ronne, 1995). This protein was first thought to specifically mediate glucose repression, a glucose-induced repression of genes required for glucose metabolism. Subsequent investigations, however, have revealed that it also has a more general function, that of mediating the repression of other sets of genes (e.g., for heme production).

Several other results suggest that in addition to photoregulated seedling development, the *det/cop* genes may play a role in other aspects of plant development. (1) Physiological and developmental studies by Castle and Meinke (1994) showed that the *fus6* (*cop11*) mutation has pleiotropic effects, interfering with normal responses to sugars, hormones, and developmental signals, in addition to light. Because *fus6* itself is induced by light, however, it could be argued that it acts differently from, and perhaps downstream of, *det/cop* genes. (2) In contrast to the general notion that *det/cop* mutants are phytochrome independent, recent studies have demonstrated that seed germination in *det1*, *cop1*, *cop8*, *cop10*, and *cop11* are in fact dependent on phytochromes (Shinomura et al., 1994; Wei et al., 1994). (3) The aberrant morphology of light-grown *det/cop* mutants suggests a general defect in developmental programming. For example, a mutant carrying a strong allele dies after developing only a few leaves, regardless of the growth medium. This is difficult to explain on the basis of a specific derepression of light-regulated development only.

The considerations mentioned above prompted us to test the range of signal transduction pathways that are affected by several *det/cop* mutants. Using RNA gel blot hybridizations, we analyzed the steady state mRNA levels of a subset of genes regulated by light, pathogens, hypoxia, temperature, and developmental signals. We show that the *det1*, *cop1*, and *cop9* mutations affect not only light-regulated genes but also a diverse group of genes controlled by other signal transduction pathways.

RESULTS

det/cop Mutations Affect Expression Levels of Light-Regulated Genes in the Light

The role played by the *DET/COP* gene products in the repression of light-inducible genes in etiolated seedlings has been established previously (reviewed in Deng, 1994). We compared the expression of four nuclear-encoded, light-responsive genes, *CAB*, *RBCS*, *CHS*, and nitrate reductase (*NR*), in light-grown seedlings of the wild type and of *det* and *cop* mutants. To this end, wild-type seedlings and mutants were germinated on Murashige and Skoog medium (Murashige and Skoog, 1962) plus 3% sucrose in the dark for 6 days and then transferred to 16-hr-light/8-hr-dark cycles for 7 additional days. Growth in the dark allowed us to separate homozygous mutants from heterozygotes and wild-type seedlings. Steady state mRNA levels were examined by RNA gel blot hybridizations, and the results were quantified by using a PhosphorImager.

Figure 1 shows that the expression of *CAB*, *RBCS*, and *CHS* in light-grown wild-type seedlings and *det2* and *cop2*, *cop3*, and *cop4* mutants was similar. By contrast, the expression levels of these genes in *det1-1*, *cop1-5*, and *cop9-1* were significantly different from those of the wild-type. The *CAB* gene was underexpressed in *det1-1*, *cop1-1*, *cop1-5*, and *cop9-1* (16, 75, 6, and 52%, respectively) relative to the wild-type expression level, whereas *RBCS* was underexpressed only in *cop1-5*. Although we noted a more marked reduction in expression levels, these results generally confirm previous observations (Chory et al., 1989; Deng et al., 1992; Wei and Deng, 1992; Hou et al., 1993), suggesting that the developmental stage of the mutants used in our analyses was comparable to that used in previous studies. An opposite expression pattern was observed for *CHS*, which was overexpressed in *det1-1*, *cop1-1*, and *cop9-1* (three-, 20-, and eightfold, respectively), relative to the wild-type level; for the lethal *cop1-5* allele, *CHS* expression level was 200-fold above the wild-type level. Under the same conditions, the expression level of *NR* was similar in the wild type and the mutants.

A stronger effect on light-responsive gene expression was obtained with *cop1-5* and *cop9-1* as compared with *cop1-1* and *cop9-2*, respectively; the latter alleles are weaker than the former (Figure 1). This observation applies to genes that are either negatively (*CAB* and *RBCS*) or positively (*CHS*) affected by the mutations. Therefore, our data suggest a correlation between the effect of a *det/cop* mutation on gene expression

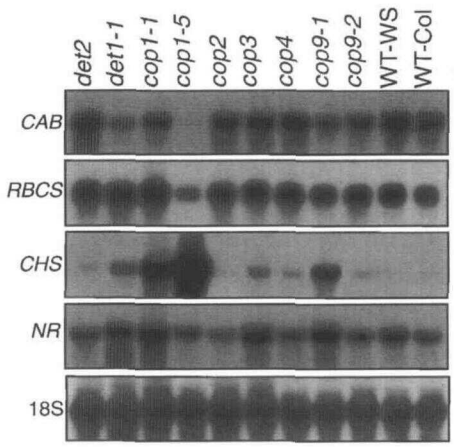

Figure 1. Gel Blot Analysis of Steady State mRNA Levels of Light-Regulated Genes in *det/cop* Mutants and Wild-Type Seedlings.

Germinated seedlings were grown in darkness for 6 days. The mutants were scored and then transferred to 16-hr-light/8-hr-dark cycles for an additional 7 days. Each lane contains 10 μg of RNA. After hybridizations, membranes were exposed to x-ray film for different amounts of time to obtain suitable exposures for each transcript. WT-WS and WT-Col, wild-type Arabidopsis ecotypes Wassilewskija and Columbia, respectively.

and the severity of the mutation, as previously determined by the seedling morphology of the mutants. Pepper et al. (1994) had reported previously that certain light-responsive promoters are very sensitive to the levels of DET1 activity. Together, all of these results are consistent with a role for *det/cop* genes in the regulation of light-responsive genes.

Defense Genes Are Activated in *det/cop* Mutants in the Absence of a Pathogen

The increase of *CHS* expression in *det1-1*, *cop1-1*, *cop1-5*, and *cop9-1* could have been due to a specific effect of the mutations on light signal transduction or to additional effects on the defense signal transduction pathway thus far not determined. Whereas it is unclear whether *CHS* is induced in Arabidopsis in response to pathogens, genes encoding lipoxygenase (*LOX*), glutathione *S*-transferase (*GST*), and phenylalanine ammonia-lyase (*PAL*) are known to be induced during pathogenesis as well as by salicylic acid. To examine the activity of the stress/defense signaling pathway, we determined the expression level of the three defense-regulated genes, *PAL*, *LOX*, and *GST*. Figure 2A shows that wild-type seedlings and *det2*, *cop2*, *cop4*, and *cop9-2* seedlings expressed very low levels of *PAL*, *LOX*, and *GST* mRNAs, and that *PAL* and *GST* mRNA levels were only slightly higher in *cop3* seedlings. By contrast, an increase in the *PAL* mRNA level was observed in *det1-1*, *cop1-1*, and *cop1-5*; a significant increase in the *LOX* mRNA level was observed in *cop1-5* and *cop9-1*; and *GST* was overexpressed by ~20-fold in all four mutants (*det1*, *cop1-1*, *cop1-5*, and *cop9-1*).

To extend our analysis of the defense signaling cascade, we also determined the expression levels of three pathogenesis-related (*PR*) genes, *PR-1*, *PR-4*, and *PR-5*. In addition to being involved in a plant's response to pathogens, these genes are also induced by salicylic acid, and the induction requires new protein synthesis (Uknes et al., 1992; Qin et al., 1994). Figure 2B shows that the three *PR* genes were expressed at very low levels in wild-type, *det2*, *cop2*, and *cop4* seedlings. By contrast, *PR-1* was overexpressed in *det1-1*, *cop1-1*, *cop9-1*, and *cop9-2* by ~63-, 10-, 250-, and 26-fold, respectively, relative to the wild-type level. *PR-5* was induced exclusively in *cop9-1*, which expressed the highest level of *PR-1*. Less marked effects were observed for *PR-4*, which was also overinduced in *det1-1*, *cop1-1*, *cop1-5*, *cop9-1*, and *cop9-2*. Taken together, these results demonstrate that mutations in *det1*, *cop1*, and *cop9* lead either directly or indirectly to constitutive activity of the defense signaling pathway.

It is noteworthy that *PR-1* was overexpressed in *cop1-1* (a weak allele) rather than *cop1-5* (a strong allele), whereas the

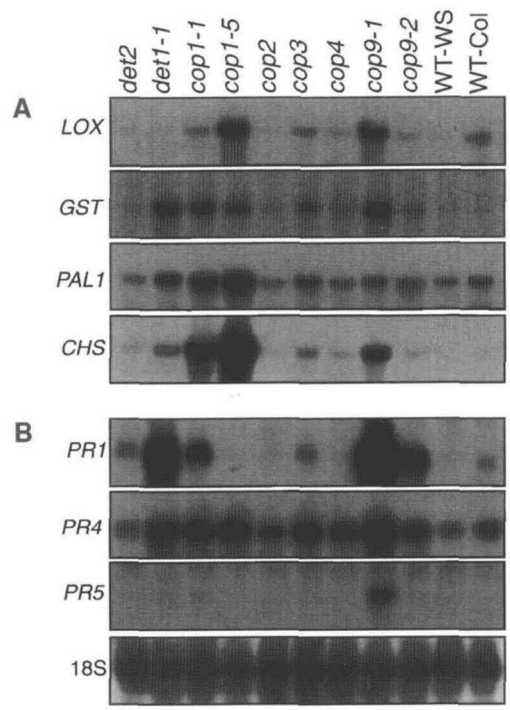

Figure 2. Expression of Defense-Related Genes in *det/cop* Mutants.

Seedling growth conditions and RNA extraction and analysis are as described in the legend to Figure 1. Each lane contains 10 μg of RNA. WT-WS and WT-Col are as given in the legend to Figure 1.

(A) Early-induced defense genes.

(B) Late-induced defense genes.

expression of *PAL1* and *LOX* in these mutants was correlated with allele severity. On the other hand, the *GST* mRNA expression level was independent of the allele strength of *cop1*. Because the strengths of the different *cop1* alleles were scored on the basis of photomorphogenetic phenotypes, it is not surprising to find a lack of correlation between the severity of the mutant allele and the induction of defense-related genes.

Inappropriate Expression of Hypoxia-Induced and Developmentally Regulated Genes

The constitutive expression of defense-related genes in *det/cop* mutants prompted us to examine the expression of other regulated genes in these mutants. Figure 3 shows that the alcohol dehydrogenase (*ADH*) gene, which is induced by hypoxia (Dolferus et al., 1994), and the malate synthase (*MAS*) gene, which encodes a developmentally regulated enzyme, were not expressed in wild-type, *det1-1*, *det2*, *cop2*, *cop3*, and *cop4* seedlings but were expressed in *cop1* seedlings and, to a lesser extent, in *cop9* seedlings. The *MAS* gene is known to be developmentally upregulated during the early stages of seedling germination (Graham et al., 1990). We therefore examined the mRNA level of the albumin (*ALB*) 2S gene, which is normally expressed in seeds of wild-type plants (Guerche et al., 1990). To our surprise, we found that *ALB* 2S was expressed in vegetative tissues of both *cop1-1* and *cop9-1* seedlings, with the expression level being much higher in the latter mutant. A low level of expression of *ALB* 2S was also seen in *det2* and *cop1-5* seedlings. Taken together, the expression patterns of *ADH*, *MAS*, and *ALB* 2S suggest that among the *det/cop* mutants studied, only *cop1-1*, *cop1-5*, *cop9-1*, and *cop9-2* are defective in hypoxia and/or developmental regulation.

As a control, we examined the expression of a gene induced by heat stress (*HSP81-2*) and cytoskeletal genes encoding actin and profilin in the *det/cop* mutants. Figure 3 shows that the expression levels of these genes in the mutants are not significantly different from those in the wild type.

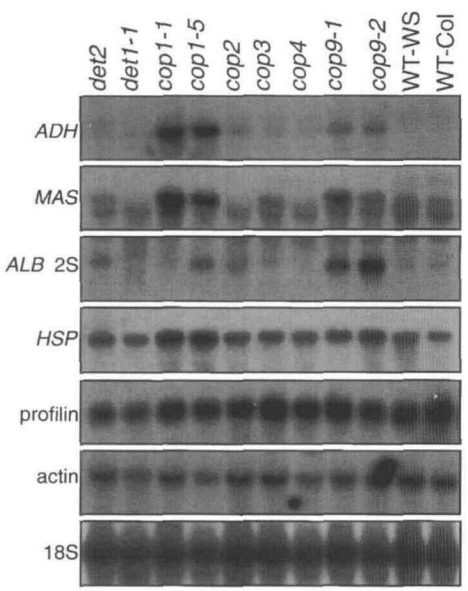

Figure 3. Expression of Hypoxia- and Developmentally Regulated Genes in *det/cop* Mutants.

Seedling growth conditions and RNA extraction and analysis are as described in the legend to Figure 1. Each lane contains 10 μg of RNA. WT-WS and WT-Col are as given in the legend to Figure 1.

Tissue Specificity of Genes Inappropriately Expressed in *det1-1*, *cop1-1*, and *cop9-1*

So far, we have shown by RNA gel blot hybridizations that *det1*, *cop1*, and *cop9* constitutively express a wide range of genes that are normally induced by external signals. Because whole seedlings were used in these analyses, it was not clear whether the genes concerned were expressed in the same cell types as light-responsive genes, for example, *CAB*. To address this issue, we performed RNA in situ hybridization analysis using selective gene probes.

We analyzed the expression pattern of *ADH* in *cop1-1* seedlings and that of *PR-1* in *det1-1* and *cop9-1* seedlings; both are examples of non-light-responsive genes. In confirmation of our RNA gel blot results, wild-type seedlings did not express *ADH* or *PR-1* when analyzed with in situ hybridization (data not shown). Figures 4A, 4B, 5A, 5B, 6A, and 6B show that very little or no staining was observed in the mutants hybridized with the sense RNA probes; however, a strong signal was observed when similar mutants were hybridized with antisense RNA probes. *ADH* expression was especially strong in the mesophyll cells of cotyledons and leaves of *cop1-1*. Some *cop1-1* seedlings showed intense staining in the cotyledons (Figures 4E and 4G), whereas other seedlings from the same population showed leaf staining (Figures 4D and 4F). This could reflect developmental differences due to heterogenous germination times of the seedlings among the population. Thus, in younger seedlings, *ADH* RNA was detected primarily in the cotyledons; however, in slightly older seedlings, it was found in the leaves. In addition, ~60% of the *cop1-1* seedlings showed the presence of *ADH* RNA in xylem tissues adjacent to the root tip (Figure 4C). Control experiments with the *ADH* sense RNA probe demonstrated that the background signal was significantly less intense. Overall, this pattern of tissue specificity for *ADH* expression agrees with previously published results that under hypoxia, *ADH* is expressed primarily in leaves (Dolferus et al., 1994). These results are consistent with the notion that *ADH* overexpression in *cop1* mutants is the result of constitutive activation of the hypoxia signaling pathway found predominantly in mesophyll cells.

Hybridization with the antisense RNA probe demonstrated

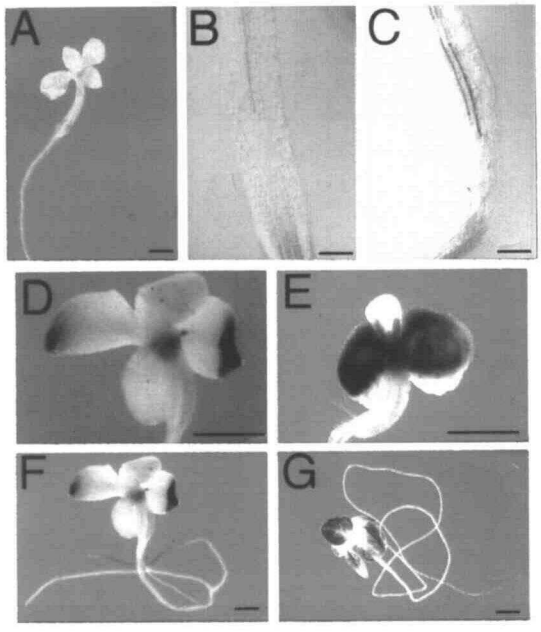

Figure 4. Localization of *ADH* mRNA in *cop1-1* by in Situ Hybridization.

Seedling growth conditions are as described in the legend to Figure 1 and Methods. Seedlings were analyzed for RNA expression by hybridization with sense and antisense RNA probes. Hybridized RNA was stained according to the instructions in the alkaline phosphatase–based DIG RNA detection kit (Boehringer Mannheim) and as described in Methods. Images in **(A)** and **(D)** to **(G)** were taken using a Nikon SMZ-U dissecting microscope (Natick, MA). Images in **(B)** and **(C)** were taken using a Zeiss Axiovert 135 microscope (Thornwood, NY).
(A) and **(B)** Control hybridization with the *ADH* sense RNA probe.
(C) to **(G)** Hybridization with the *ADH* antisense mRNA probe.
Bars in **(A)** and **(D)** to **(G)** = 1 mm. Bars in **(B)** and **(C)** = 0.1 mm.

that *PR-1* is expressed in mesophyll cells and leaf trichomes of *det1-1* (Figure 5) and in cotyledons and leaves of *cop9-1* (Figure 6). In *cop9-1* seedlings, the hybridization pattern was patched and, in some cases, limited to individual mesophyll cells or patches of cells (Figures 6C to 6H). A different hybridization pattern was observed in *det1-1* seedlings. In these seedlings, most of the *PR-1* expression was detected in large areas of the mesophyll tissue and cotyledons and in both mesophyll cells and leaf trichomes (Figures 5C to 5E). The different *PR-1* expression pattern in *det1-1* and *cop9-1* seedlings is surprising and may suggest a different mechanism for *PR-1* activation in the two mutants.

DISCUSSION

Specificity of *det/cop* Mutants

det/cop mutants have been isolated on the basis of their deetiolated/constitutive photomorphogenic phenotypes in the dark and have been characterized as light signal transduction mutants acting downstream of photoreceptors (Chory, 1992; Deng, 1994). The specificity of the *det/cop* mutations with respect to gene expression, however, has not been rigorously examined thus far. For example, it is not known whether these mutations will affect genes other than those regulated by light. Based on their gene expression pattern, we show here that *det/cop* mutants can be roughly classified into two groups, although there are some differences in the expression profile within a group. The first group, which includes *det2*, *cop2*, *cop3*, and *cop4*, did not display any misexpression of genes responsive to pathogens, hypoxia, and developmental signals. Within this group, only *det2* (Chory et al., 1991) and *cop4* (Hou et al., 1993) showed a higher dark expression level of *RBCS*, *CAB*, and *CHS* as compared with the wild type; nonetheless, the three genes remained light responsive in the mutants. Although *cop2* and *cop3* showed a partial photomorphogenic phenotype in the dark (Hou et al., 1993), this can be explained by a partial activation of a non-light-related transduction pathway, such as the ethylene signal transduction pathway. Genetic epistasis experiments have placed the *det2* mutation downstream of photoreceptors (Chory, 1992). Recent characterization of the *DET2* gene, however, showed that it encodes an enzyme in the brassinolide biosynthetic pathway (Li et al., 1996).

The *det1*, *cop1*, and *cop9* mutants, which belong to the second group, showed an altered expression pattern of genes inducible by light and pathogens. In addition, *cop1* and *cop9* mutations also cause ectopic expression of genes normally regulated by hypoxia and developmental signals, respectively. These results clearly demonstrate that the effects of these mutations on gene expression are pleiotropic and not just restricted to target genes of light signal transduction pathways.

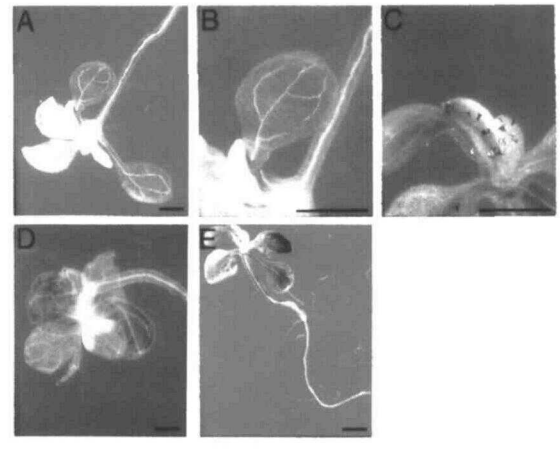

Figure 5. Expression of *PR-1* mRNA in *det1-1*.

Seedling growth conditions and in situ hybridizations and analysis are as described in the legend to Figure 4.
(A) and **(B)** Control hybridization with the *PR-1* sense RNA probe.
(C) to **(E)** Hybridization with the *PR-1* antisense mRNA probe.
Bars = 1 mm.

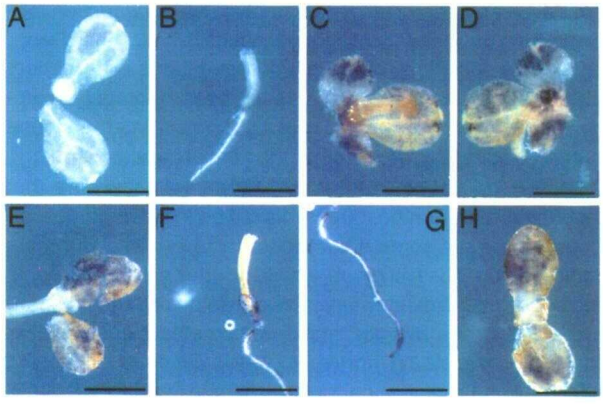

Figure 6. Expression of *PR-1* mRNA in *cop9-1*.

Seedling growth conditions and in situ hybridizations and analysis are as described in the legend to Figure 4.
(A) and **(B)** Control hybridization with the *PR-1* sense RNA probe.
(C) to **(H)** Hybridization with the *PR-1* antisense mRNA probe.
Bars = 1 mm.

Our gene expression studies confirm and extend previous physiological and developmental results on *fus6* (Castle and Meinke, 1994).

DET1, COP1, and COP9 Are Negative Regulators of Several Signaling Pathways

Because of the developmental abnormalities of the mutant seedlings, we cannot rule out completely the possibility that the constitutive expression of defense-regulated genes, such as *PR*, *LOX*, and *GST*, in *det1/cop1/cop9* is due to a secondary response brought about by stress. We consider this possibility to be unlikely for two reasons. (1) Whereas the expression level of the defense-regulated gene correlated with *cop9* allele severity, this was not the case for the *cop1* alleles. Moreover, it should be emphasized that *cop1-5*, carrying a null allele, did not constitutively express *PR-1* at all (Figure 2). (2) Genes controlled by hypoxia and a developmental program were also ectopically expressed in the *cop1/cop9* mutants (Figure 3). Therefore, the ectopic expression of defense-, hypoxia-, and developmentally regulated genes in *cop1 and cop9* is likely a direct consequence of the mutations. Our results provide evidence that DET1, COP1, and COP9 function as negative regulators of several gene sets to repress the expression of not only light-responsive genes but also genes activated by other signaling pathways.

There appears to be a linkage between certain stress conditions and the deetiolation process. For example, periodic exposure of etiolated seedlings to high temperatures (Kloppstech et al., 1991) and shaking of etiolated seedlings in liquid cultures (Araki and Komeda, 1993) can trigger both plastid development and light-responsive gene expression in the dark.

Cloning and characterization experiments revealed that COP1 contains a zinc-binding motif as well as four WD-40 repeats at its C terminus (Deng et al., 1992). DET1, on the other hand, is a novel protein with no recognizable motif to date (Pepper et al., 1994). Both proteins are known to be localized in the nucleus, which is consistent with their proposed role as repressors. The pleiotropic effects of DET1/COP1 are reminiscent of two eukaryotic repressors, TUP1 (see Ronne, 1995; Roth, 1995) and EXTRA SEX COMB (ESC) (see Orlando and Paro, 1995). TUP1 was first proposed to specifically mediate glucose repression in yeast, but subsequent investigations showed that it also represses genes regulated by oxygen, cell type, and DNA damage (see Ronne, 1995; Roth, 1995). ESC, a member of the Drosophila Polycomb-group (Pc-G) genes, is needed for repression of homeotic genes at ~6 hr from the beginning of embryo development (Gutjahr et al., 1995). It is remarkable that, like COP1, ESC and TUP1 contain six and seven WD-40 repeats, respectively. These two repressors are thought to interfere with the basal transcription machinery and also to promote nucleosome assembly leading to a repressed chromatin structure (Orlando and Paro, 1995; Roth, 1995). TAFII80, a transcription coactivator of the TFIID complex, also contains seven copies of the WD-40 repeat at its C terminus (Dynlacht et al., 1993). The common structural motif has prompted the proposal that the repressor activity of ESC and TUP1 may be executed through competition with TAFII80 for binding to the TFIID complex (Gutjahr et al., 1995). A similar mechanism of action may be envisaged for COP1. A role of DET/COP proteins in repressing basal transcription would be consistent with the observation that whereas several *det/cop* mutants expressed photoregulated genes in the dark, the expression of these genes continued to be inducible by light.

Like DET1 and COP1, COP9 is also a nuclear protein that appears to exist in a large complex (Wei et al., 1994). It has been claimed that light may induce changes in this complex, whose formation or stability may require COP8 and COP11. In this connection, the *cop11* mutation was previously isolated as *fus6* by Castle and Meinke (1994), who clearly demonstrated that the mutation is pleiotropic. Although we did not investigate the gene expression pattern of *fus6* (*cop11*) in this work, our results with *cop9* showed that the mutation also affects developmental signaling and defense gene regulation. These results, together with those of Castle and Meinke (1994), suggest that the putative COP9/COP8/COP11 complex is involved in the negative regulation of not one but several pathways. It would be interesting to investigate the effects of pathogens, salicylic acid, and hypoxia on the stability of the COP9/COP8/COP11 complex as well as the intracellular trafficking of COP1.

Conclusions

There are precedents in other eukaryotic systems in which a gene first thought to regulate only one pathway was later discovered to have a wider mode of action. Examples are the *TUP1*

gene of yeast (Roth, 1995) and the *Notch* gene of Drosophila (Artavanis-Tsakonas et al., 1995). We believe that the *det/cop* genes of Arabidopsis also belong to this class. The characterization of the *det/cop* mutants have already contributed to our understanding of the molecular mechanisms involved in deetiolation. We believe that further investigations into the effects of these mutations on other signaling pathways will help to elucidate the broader roles of DET/COP proteins in other aspects of plant development.

With the benefit of hindsight, it is perhaps not surprising that the *det/cop* mutations are not specific to the light signal transduction pathway, because they were isolated on the basis of their deetiolated phenotype in the dark, and light was not used at all in the screen. As discussed in detail elsewhere (Millar et al., 1994), this deetiolated phenotype could also be phenocopied by the exogenous application of cytokinin (Chory et al., 1994), by periodic heat shock (Kloppstech et al., 1991), or by shaking Arabidopsis seedlings in liquid cultures (Araki and Komeda, 1993). These treatments were all conducted in the dark. A more stringent screen, such as the use of light-regulated promoter–reporter gene fusions in transgenic plants, may yield mutants of greater specificity (Millar et al., 1992; Li et al., 1995). Finally, our studies reemphasize the need to test thoroughly for a range of effects in any mutation to establish its specificity for a particular signal transduction pathway.

METHODS

Plant Materials and Growth Conditions

The *cop1-1*, *cop2*, *cop3*, *cop4*, *cop9-2*, *det1*, and *det2* mutations are in the *Arabidopsis thaliana* Columbia ecotype, whereas the *cop1-5* and *cop9-1* mutations are in the Wassilewskija ecotype. *cop1-1*, *cop4-1*, *cop1-5*, and *cop9-1* were grown as heterozygotes. For germination, seeds were surface sterilized and then plated on minimal Murashige and Skoog medium (Murashige and Skoog, 1962) supplemented with 90 mM sucrose. After a cold treatment for 4 days in the dark, seeds were exposed to 1 hr of white light (30 $\mu E\ m^{-2}\ sec^{-1}$) to promote germination. After germination and growth in darkness at 22°C for 6 days, the mutants were scored and the seedlings were transferred to diurnal cycles of 16 hr of light (30 $\mu E\ m^{-2}\ sec^{-1}$)/8 hr of dark for 7 additional days. In all cases, seedlings were harvested at 4.5 hr into the light phase.

RNA Gel Blot Analysis

Seedlings were harvested and frozen immediately in liquid nitrogen. Total RNA isolation, electrophoresis of RNA, gel blotting, and filter hybridization were as previously published (Kuhlemeier et al., 1988; Lam et al., 1989). The cDNA probes used in this study are as follows: *CAB*, *RBCS*, *CHS*, and *NR*, as described by Barnes et al. (1996); *LOX1*, a 1.4-kb EcoRI fragment encoding Arabidopsis lipoxygenase (Melan et al., 1993); *GST*, a 0.9-kb BamHI fragment containing the Arabidopsis *GST* coding region; *PAL1*, a SalI-NotI 0.8-kb fragment of the Arabidopsis expressed sequence tag (EST) clone 82B7T7; *PR-1* and *PR-4*, EcoRI-XhoI 0.72-kb fragments containing the coding region of *PR-1* and *PR-4* (Uknes et al., 1992); *PR-5*, a BamHI-KpnI 0.98-kb fragment containing the Arabidopsis *PR-5* coding region (Uknes et al., 1992); profilin, a 0.4-kb fragment containing the Arabidopsis profilin1 coding region (Christensen et al., 1996); actin, a 0.75-kb HindIII-EcoRI fragment of pAAc-1 (Nairn et al., 1988); *HSP81-2*, a 1.1-kb EcoRI fragment containing the Arabidopsis *HSP81-2* coding region (Takahashi et al., 1992); *ADH*, a HindIII-PstI fragment of the kAt3011 plasmid (Chang and Meyerowitz, 1986); *MAS*, a SalI-NotI 1.8-kb fragment of the Arabidopsis EST clone 34f10t7; *ALB* 2S, a SalI-NotI 1.8-kb fragment of the Arabidopsis EST clone 65g5t7; and 18S DNA, an EcoRI 1.2-kb fragment containing the coding region of the Arabidopsis 18S DNA (Takahashi et al., 1992). Expression levels were quantified with a PhosphorImager (model 4a00E; Molecular Dynamics, Sunnyvale, CA).

In Situ Hybridization

In situ hybridization was conducted by hybridization of RNA from dehydrated, proteolized, and fixed wild-type and mutant seedlings with digoxigenin (DIG)-labeled RNA probes (1 to 3 μg/mL). After washes and hydrolysis of single-stranded RNA using RNase A, DIG-labeled, double-stranded RNA was detected by using an anti-DIG alkaline phosphatase–conjugated antibody. The procedure was essentially as described by de Almeida-Engler et al. (1994), except that before the proteolysis step, the sample was treated with formaldehyde for 30 min, washed (three times) with PBS containing 0.1% Tween 20 (PBT) for 1 min, and then treated under vacuum conditions with PBT containing 2% Driselase (Sigma) for 15 min. Using DIG labeling kits (Boehringer Mannheim) and oligonucleotides, we generated DIG-labeled probes that were designed to contain the T3 promoter sequence on the upper strand and T7 promoter sequence on the bottom strand. The T3 promoter sequence is 5′-AAATTAACCCTCACTAAAGGG-3′; T7 promoter sequence, 5′-TAATACGACTCACTATAGGG-3′; *ADH* gene, upper strand of T3 (position 1582), 5′-AAATTAACCCTCACTAAAGGGGGAGTGACTGATCTTCAGCC-3′, and bottom strand of T7 (position 2013), 5′-TAATACGACTCACTATAGGGCCGATGATCCTAGAAGCACC-3′. The probe for the *PR-1* gene was synthesized directly from the plasmid pBluescript SK−, using the plasmid T3 and T7 promoters.

ACKNOWLEDGMENTS

We thank Diane Shevell, Simon Barnes, and Diana Horvath for useful discussions and critical reading of this manuscript; Linda Castle, David Meinke, Joanne Chory, and Xing-Wang Deng for seeds of *det/cop* mutants; T. Kaye Peterman for the AtLOX1 plasmid; Frederick Ausubel for the pGST plasmids; and Leslie Friedrich and John Ryals for the *PR* plasmids. We also thank Vered Raz for her assistance in establishing the in situ hybridization protocol. R.M. was supported by a BARD Postdoctoral Fellowship (No. FI0144-92) and a Winston Fellowship. This work was supported in part by a National Institutes of Health grant (No. GM44640) to N.-H.C.

Received July 17, 1996; accepted September 9, 1996.

REFERENCES

Ang, L.-H., and Deng, X.-W. (1994). Regulatory hierarchy of photomorphogenic loci: Allele-specific and light-dependent interaction between the *HY5* and *COP1* loci. Plant Cell **6,** 613–628.

Araki, T., and Komeda, Y. (1993). Flowering in darkness in *Arabidopsis thaliana.* Plant J. **4,** 801–811.

Artavanis-Tsakonas, S., Matsuno, K., and Fortini, M.E. (1995). Notch signaling. Science **268,** 225–232.

Barnes, S.A., Quaggio, R.B., Whitelam, G.C., and Chua, N.-H. (1996). *FHY1* defines a branch point in *phyA* signal transduction pathways for gene expression. Plant J., in press.

Bowler, C., Neuhaus, G., Yamagata, H., and Chua, N.-H. (1994a). Cyclic GMP and calcium mediate phytochrome phototransduction. Cell **77,** 73–81.

Bowler, C., Yamagata, H., Neuhaus, G., and Chua, N.-H. (1994b). Phytochrome signal transduction pathways are regulated by reciprocal control mechanisms. Genes Dev. **8,** 2188–2202.

Castle, L.A., and Meinke, D.W. (1994). A *FUSCA* gene of Arabidopsis encodes a novel protein essential for plant development. Plant Cell **6,** 25–41.

Chang, C., and Meyerowitz, E.M. (1986). Molecular cloning and DNA sequence of the *Arabidopsis thaliana* alcohol dehydrogenase gene. Proc. Natl. Acad. Sci. USA **83,** 1408–1412.

Chory, J. (1992). A genetic model for light-regulated seedling development in *Arabidopsis.* Development **115,** 337–354.

Chory, J., Peto, C., Feinbaum, R., Pratt, L., and Ausubel, F. (1989). *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. Cell **58,** 991–999.

Chory, J., Nagpal, P., and Peto, C.A. (1991). Phenotypic and genetic analysis of *det2,* a new mutant that affects light-regulated seedling development in *Arabidopsis.* Plant Cell **3,** 445–459.

Chory, J., Reinecke, D., Sim, S., Washburn, T., and Brenner, M. (1994). A role of cytokinins in de-etiolation in Arabidopsis. *det* mutants have an altered response to cytokinins. Plant Physiol. **104,** 339–347.

Christensen, H.E.M., Ramachandran, S., Tan, C.-T., Surana, U., Dong, C.-H., and Chua, N.-H. (1996). *Arabidopsis* profilins are functionally similar to yeast profilins: Identification of a vascular bundle-specific profilin. Plant J. **10,** 269–279.

de Almeida-Engler, J., van Montagu, M., and Engler, G. (1994). Hybridization in situ of whole-mount messenger RNA in plants. Plant Mol. Biol. Rep. **12,** 321–331.

Deng, X.-W. (1994). Fresh view of light signal transduction in plants. Cell **76,** 423–426.

Deng, X.-W., Caspar, T., and Quail, P.H. (1991). *cop1*: A regulatory locus involved in light-controlled development and gene expression in *Arabidopsis.* Genes Dev. **5,** 1172–1182.

Deng, X.-W., Matsui, M., Wei, N., Wagner, D., Chu, A.M., Feldmann, K.A., and Quail, P.H. (1992). *COP1,* an Arabidopsis regulatory gene, encodes a protein with both a zinc-binding motif and a Gβ homologous domain. Cell **71,** 791–801.

Dolferus, R., Jacobs, M., Peacock, W.J., and Dennis, E.S. (1994). Differential interactions of promoter elements in stress responses of the *Arabidopsis ADH* gene. Plant Physiol. **105,** 1075–1087.

Dynlacht, B.D., Weinzierl, R.O., Admon, A., and Tjian, R. (1993). The dTAFII80 subunit of Drosophila TFIID contains β-transducing repeats. Nature **363,** 176–179.

Graham, I.A., Smith, L.M., Leaver, C.J., and Smith, S.M. (1990). Developmental regulation of expression of the malate synthase gene in transgenic plants. Plant Mol. Biol. **15,** 539–549.

Guerche, P., de Almeida, E.R., Schwarztein, M.A., Gander, E., Krebbers, E., and Pelletier, G. (1990). Expression of the 2S albumin from *Bertholletia excelsa in Brassica napus.* Mol. Gen. Genet. **221,** 306–314.

Gutjahr, T., Frei, E., Spicer, C., Baumgartner, S., White, R.A.H., and Noll, M. (1995). The polycomb-group gene, *extra sex combs,* encodes a nuclear member of the WD-40 repeat family. EMBO J. **14,** 4296–4306.

Hou, Y., von Arnim, A.G., and Deng, X.-W. (1993). A new class of Arabidopsis constitutive photomorphogenic genes involved in regulating cotyledon development. Plant Cell **5,** 329–339.

Kloppstech, K., Otto, B., and Sierralta, W. (1991). Cyclic temperature treatments of dark-grown pea seedlings induce a rise in specific transcript levels of light-regulated genes related to photomorphogenesis. Mol. Gen. Genet. **225,** 468–473.

Kuhlemeier, C., Cuozzo, M., Green, P.J., Goyvaerts, E., Ward, K., and Chua, N.-H. (1988). Localization and conditional redundancy of regulatory elements in *rbcS-3A,* a pea gene encoding the small subunit of ribulose-bisphosphate carboxylase. Proc. Natl. Acad. Sci. USA **85,** 4662–4666.

Lam, E., Benedyk, M., and Chua, N.-H. (1989). Characterization of phytochrome-regulated gene expression in a photoautotrophic cell suspension: Possible role for calmodulin. Mol. Cell. Biol. **9,** 4819–4823.

Li, H.-M., Culligan, K., Dixon, R.A., and Chory, J. (1995). *CUE1*: A mesophyll cell-specific positive regulator of light-controlled gene expression in Arabidopsis. Plant Cell 7, 1599–1610.

Li, J., Nagpal, P., Vitart, V., McMorris, T.C., and Chory, J. (1996). A role for brassinosteroids in light-dependent development of *Arabidopsis.* Science **272,** 398–401.

Melan, M.A., Dong, X., Endara, M.E., Davis, K.R., Ausubel, F.M., and Peterman, T.K. (1993). An *Arabidopsis thaliana* lipoxygenase gene can be induced by pathogens, abscisic acid, and methyl jasmonate. Plant Physiol. **101,** 441–450.

Millar, A.J., Short, S.R., Hiratsuka, K., Chua, N.-H., and Kay, S.A. (1992). Firefly luciferase as a reporter of regulated gene expression in higher plants. Plant Mol. Biol. Rep. **10,** 324–337.

Millar, A.J., McGrath, R.B., and Chua, N.-H. (1994). Phytochrome phototransduction pathways. Annu. Rev. Genet. **28,** 325–349.

Misera, S., Muller, A.J., Weiland-Heidecker, U., and Jurgens, G. (1994). The *FUSCA* genes of *Arabidopsis*: Negative regulators of light responses. Mol. Gen. Genet. **244,** 242–252.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco and cultures. Physiol. Plant. **15,** 473–497.

Nairn, C.J., Winesett, L., and Ferl, R.J. (1988). Nucleotide sequence of an actin gene from *Arabidopsis thaliana.* Gene **65,** 247–257.

Neuhaus, G., Bowler, C., Kern, R., and Chua, N.-H. (1993). Calcium/calmodulin–dependent and independent phytochrome signal transduction pathways. Cell **73,** 937–952.

Orlando, V., and Paro, R. (1995). Chromatin multiprotein complexes involved in the maintenance of transcription patterns. Curr. Opin. Genet. Dev. **5,** 174–179.

Pepper, A., Delaney, T., Washburn, T., Poole, D., and Chory, J. (1994). *DET1*, a negative regulator of light-mediated development and gene expression in Arabidopsis, encodes a novel nuclear-localized protein. Cell **78,** 109–116.

Qin, X.-F., Holuigue, L., Horvath, D.M., and Chua, N.-H. (1994). Immediate early transcription activation by salicylic acid via the cauliflower mosaic virus *as-1* element. Plant Cell **6,** 863–874.

Ronne, H. (1995). Glucose repression in fungi. Trends Genet. **11,** 12–17.

Roth, S.Y. (1995). Chromatin-mediated transcriptional repression in yeast. Curr. Opin. Genet. Dev. **5,** 168–173.

Shinomura, T., Nagatani, A., Chory, J., and Furuya, M. (1994). The induction of seed germination in *Arabidopsis thaliana* is regulated principally by phytochrome B and secondarily by phytochrome A. Plant Physiol. **104,** 363–371.

Takahashi, T., Naito, S., and Komeda, Y. (1992). Isolation and analysis of the expression of two genes for the 81-kilodalton heat-shock proteins from Arabidopsis. Plant Physiol. **99,** 383–390.

Uknes, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E., and Ryals, J. (1992). Acquired resistance in Arabidopsis. Plant Cell **4,** 645–656.

von Arnim, A.G., and Deng, X.-W. (1994). Light inactivation of Arabidopsis photomorphogenic repressor COP1 involves a cell-specific regulation of its nucleocytoplasmic partitioning. Cell **79,** 1035–1045.

Wei, N., and Deng, X.W. (1992). COP9: A new genetic locus involved in light-regulated development and gene expression in Arabidopsis. Plant Cell **4,** 1507–1518.

Wei, N., Chamovitz, D.A., and Deng, X.-W. (1994). Arabidopsis COP9 is a component of a novel signaling complex mediating light control of development. Cell **78,** 117–124.