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Combinatorial interaction of light-responsive elements plays a critical role in determining the response characteristics of light-regulated promoters in *Arabidopsis*

Sudip Chattopadhyay, Pilar Puente, Xing-Wang Deng and Ning Wei*
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA

Summary

We have studied the roles of PhyA, PhyB and CRY1 photoreceptors and the downstream light-signaling components, COP1 and DET1, in mediating high-irradiance light-controlled activity of promoters containing synthetic light-responsive elements (LRE). Promoters with paired LREs were able to respond to a wide spectrum of light through multiple photoreceptors, while the light-inducible single LRE promoters primarily responded to a specific wavelength of light. In addition, our results indicate that Cry1 is involved in PhyB-mediated red-light induction of the *G-GATA/NOS101* promoter, and that both Cry1 and PhyB are required for effective repression of the *GT1/NOS101* promoter by red or blue light. An interaction between PhyA and PhyB in mediating *GT1-GATA/NOS101* promoter light activation was also observed. Furthermore, our data indicate that COP1 and DET1 exert negative control in the dark only on paired LRE promoters but not single LRE promoters. From these results, we conclude that the combinatorial interaction of LREs is essential in determining the ability of light-responsive promoters to be modulated by crucial cellular regulators and to respond to diverse light environments.

Introduction

Light influences the developmental processes of higher plants throughout their entire life cycle. Most light-regulated developmental processes are triggered by alterations in gene expression through the regulation of transcription of specific genes (Terzaghi and Cashmore, 1995; Tobin and Kehoe, 1994). Some of these genes, such as *CAB* and *RBCS* (encoding chlorophyll *a*/*b* binding proteins of the photosystem II light-harvesting complex and the small subunit of the ribulose bisphosphate carboxylase, respectively) are expressed at high levels upon exposure to light, whereas other genes, such as *PHYA* and the genes encoding NADPH-protochlorophyllide reductase and asparagine synthetase, are negatively regulated by light (Gilmartin *et al.*, 1990; Quail, 1991; Silverthorne and Tobin, 1987).

In higher plants, at least three photoreceptor systems are present to sense light signals: phytochromes, blue light receptors (also known as cryptochromes) and UV light receptors (Ahmad and Cashmore, 1993; Furuya, 1993; Quail *et al.*, 1995. *Arabidopsis thaliana* contains five genes (*PHYA, B, C, D and E*) that encode phytochrome apoproteins (Clack *et al.*, 1994). By analyzing the hypocotyl elongation under continous light conditions, a high-irradiance light response, it has been determined that PhyA is responsible for perceiving far-red light, whereas PhyB plays a primary role in perceiving red light (Quail *et al.*, 1995). Recently, it has been shown that PhyD plays a minor role in mediating leaf expansion and stem elongation in conjunction with PhyB (Aukerman *et al.*, 1997). Cry1 is a well defined blue-light receptor, mutations in which (*hy4* mutants) cause a decrease in sensitivity to high-irradiance blue light-mediated inhibition of hypocotyl elongation (Ahmad and Cashmore, 1993; Lin *et al.*, 1995).

Biochemical and cell biological studies have suggested that G proteins, cGMP and calcium/calmodulin play early roles in processing the phytochrome transmitted light signals and in mediating gene expression (reviewed by Mustilli and Bowler, 1997). Additionally, a number of genetic loci, *HY5*, *FHY1*, *FHY3*, *PRC1, RED1* and *PEFs*, have been reported that are involved in multiple or specific phytochrome-mediated signal transduction pathways (Ahmad and Cashmore, 1996; Desnos *et al.*, 1996; Koornneef *et al.*, 1980; Wagner *et al.*, 1997; Whitelam *et al.*, 1993). Moreover, genetic studies suggested that the pleiotropic *COP/DET/FUS* genes act as repressors of photomorphogenic development and light-induced gene expression, and that light signals from multiple photoreceptors inactivate the repressive action of these gene products (Chory, 1993; McNellis and Deng, 1995; Wei and Deng, 1996).

Studies on light control of transcription by deletion and mutagenesis analysis of the light-regulated promoters, such as *CAB, RBCS* and *CHS* (chalcone synthase) promoters, has led to identification of a number of light-responsive elements (LREs) (Anderson *et al.*, 1994; Gilmartin *et al.*, 1990; Ha and An, 1988; Kehoe *et al.*, 1994). These LREs, for example the G, GATA (or I) and GT1 motifs, are frequently found in various minimal light-responsive

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*For correspondence (fax +1 203 432 3854;
e-mail nwei@peaplant.biology.yale.edu).

promoters derived from different genes. However, some of the LREs are also present in promoters that are not light-regulated; and no single LRE has been found in all of the light-regulated promoters (Arguello-Astorga and Herrera-Estrella, 1996; Terzaghi and Cashmore, 1995). Recent studies have demonstrated that the combinatorial interaction of distinct LREs is required for the proper light responsiveness of promoters (Degenhardt and Tobin, 1996; Feldbrugge *et al.*, 1997; Puente *et al.*, 1996). These multiple LRE-containing promoters not only possess the ability to confer higher activities in light-grown plants over dark-grown plants, but also are capable of responding to phytochrome-mediated low-fluence light pulses and to developmental signals such as tissue specificity and chloroplast development. Although promoters with a single LRE alone, such as the G box or GATA motif, can respond to continuous high-irradiance light and chloroplast differentiation, they are unable to respond to phytochrome-mediated low-fluence light, and their function is dependent on the promoter context (Puente *et al.*, 1996).

Despite revelations of the multiple LREs and the light-signaling components acting at different levels in the signal transduction pathways, our understanding of the signal transduction processes that couple photoperception to the regulation of transcription remains fragmented. The goal of this study was to define the light-responsive characteristics of representative LREs when present alone or in pairs in a basal promoter context. Specifically, we examined these synthetic promoters in *phyA, phyB* and *cry1* mutant backgrounds in response to various wavelengths of light. We also analyzed the role of downstream signaling components, COP1 and DET1, in mediating light signals to various synthetic promoters containing single or paired LREs.

Results

Experimental design

We focused on three well studied LRE motifs, G, GT1 and GATA (Figure 1) to determine their light-responsive characteristics. These LREs were synthesized as tetrameric repeats and were placed, alone or in paired combinations, upstream of the basal non-light-inducible promoter, NOS101, and the GUS (β-glucoronidase) reporter gene (Puente *et al.*, 1996). Stable transgenic *Arabidopsis* carrying each of the five synthetic and one native promoter reporter constructs (Puente *et al.*, 1996) were used: *G-GATA/NOS101* and *GT1-GATA/NOS101* as paired element constructs; *G/NOS101*, *GATA/NOS101* and *GT1/NOS101* as single element constructs; and *CAB1* promoter as a native promoter construct (Ha and An, 1988). All these constructs were individually introduced by genetic crosses into different photoreceptor null mutants (*phyA-1, phyB-B064* and *cry1/hy4–2.23N*) and into weak alleles of *cop1–4* and *det1–1* mutants.

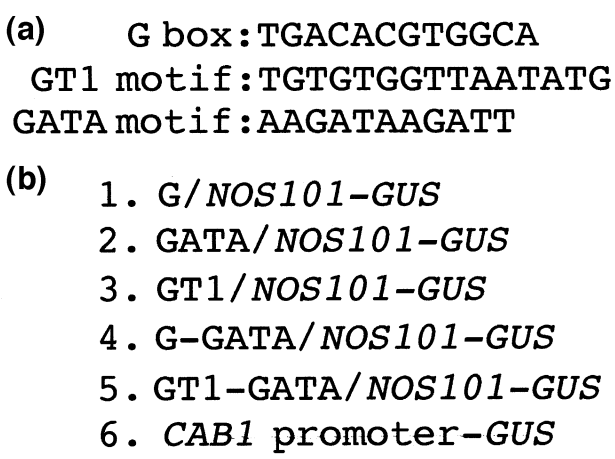

Figure 1. Synthetic and native promoter::GUS fusion constructs used in this study.
The three light-responsive elements (LREs) G, GATA and GT1 represent consensus sequences derived from well-characterized light-regulated promoters (panel (a), Puente *et al.*, 1996). Each LRE was present as a tetrameric repeat in the constucts shown in panel (b). The *NOS101* is the non-light-inducible basal promoter (–101 to +4) of the nopaline synthase gene (Mitra and An, 1989; Puente *et al.*, 1996).

We chose to focus on the high-irradiance response under continuous light of various wavelengths. This strategy was motivated by the fact that only under these high-irradiance conditions has the wavelength specificity of PhyA, PhyB and Cry1 been established (Quail *et al.*, 1995). Further, the high-irradiance response was observed by all synthetic promoters available, while the low-fluence response was only found among the paired LRE synthetic promoters (Puente *et al.*, 1996).

Promoters with paired elements are able to respond to a broad spectrum of light signals

We first analyzed the responsiveness of *G-GATA/NOS101* and *GT1-GATA/NOS101* paired LRE promoters to 48 h of constant far-red light (FR), red light (RL), blue light (BL) or white light (WL). The *G-GATA/NOS101* promoter was induced by all wavelengths of light tested, and, depending on the specific wavelengths of light, conferred about two- to fourfold higher expression of the transgene compared with the dark-grown siblings (Figure 2a). Similarly, the *GT1-GATA/NOS101* promoter showed about three- to sixfold induction at various wavelengths of light (Figure 3a). Both of the paired LRE promoters displayed similar levels of induction at various wavelengths of light, with BL and WL exhibiting the highest levels of induction, while RL and FR inductions were comparatively low (Figures 2a and 3a).

To further define the involvement of the specific photoreceptors in mediating light inductions, we examined the

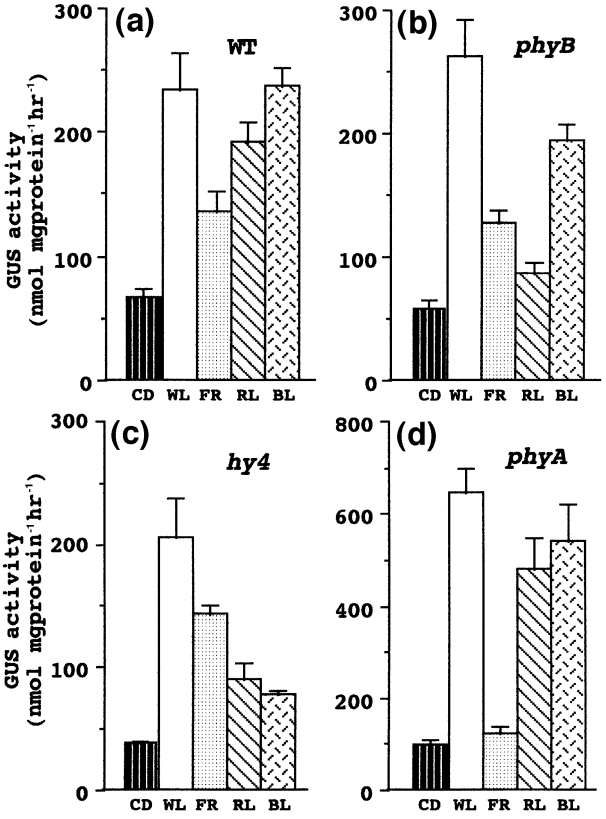

Figure 2. Light induction of the *G-GATA/NOS101* promoter and the involvement of photoreceptors in mediating light signals.
Four-day-old dark-grown seedlings (CD) containing the *G-GATA/NOS101*::GUS transgene were exposed to WL, FR, RL and BL for 48 h and the GUS activities were measured. The levels of induction at different wavelengths of light are shown for the wild-type (a), *phyB* mutant (b), *hy4* mutant (c) and *phyA* mutant (d) seedlings. The error bars indicate the standard deviation from the means of four independent experiments.

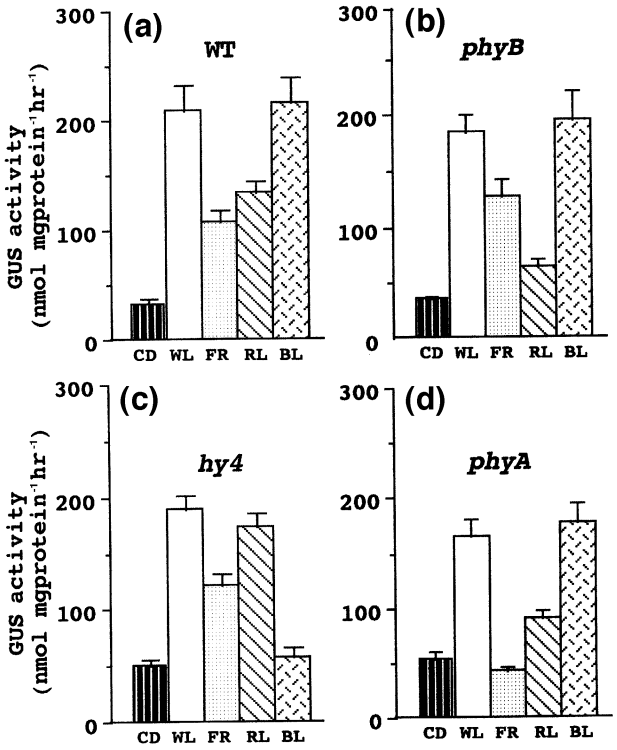

Figure 3. Light induction of the *GT1-GATA/NOS101* promoter and the involvement of photoreceptors in mediating light signals.
For experimental details, see the legend to Figure 2.

activities of these two promoters in different photoreceptor mutant backgrounds. The mutation in the *phyB* locus specifically reduced the RL induction of *G-GATA/NOS101* (Figure 2b), indicating that PhyB is primarily involved in the RL induction of this promoter. In *hy4* mutants, the BL induction was significantly compromised. Additionally, the RL induction was also affected in this mutant background (Figure 2c). These results suggest that although Cry1 is primarily involved in BL induction, it is also required for optimal RL induction in addition to the PhyB photoreceptor. There was very little induction, if any, of the *G-GATA/NOS101* promoter by FR in the *phyA* mutant, whereas the induction at other wavelengths of light was not affected (Figure 2d). This suggested that PhyA was mainly responsible for the FR induction.

The induction of the *GT1-GATA/NOS101* promoter by RL was significantly reduced in *phyB* mutants without major changes of induction at other wavelengths of light (Figure 3b). In *hy4 and phyA* mutants, there was almost no induction of *GT1-GATA/NOS101* promoter by BL and FR, respectively (Figure 3c,d). These results suggest that PhyB, Cry1 and PhyA are mainly responsible for mediating RL, BL and FR induction of this promoter, respectively. However, we noted a substantial reduction in the RL induction of the *GT1-GATA/NOS101* promoter in *phyA* mutants, indicating that functional PhyA is also required for RL induction of this promoter.

The WL inductions of both promoters were not notably affected by any of the specific photoreceptor mutants, which suggests that the three photoreceptors (PhyA, PhyB and Cry1) act redundantly in their contributions to the observed WL induction (Figures 2 and 3). Taken together, these results indicate that both paired LRE-containing promoters are able to respond to a broad spectrum of light through the three photoreceptors tested. The data also suggest that Cry1 is required for optimal RL induction of *G-GATA/NOS101* (Figure 2c), and PhyA is required for full RL induction of *GT1-GATA/NOS101* (Figure 3d).

Light-inducible promoters with a single element are only responsive to a limited spectral region of light

The promoters with G or GATA motifs alone were previously found to have significantly higher activities in constant WL than in darkness (Puente *et al.*, 1996). Here we asked whether these single element-containing promoters

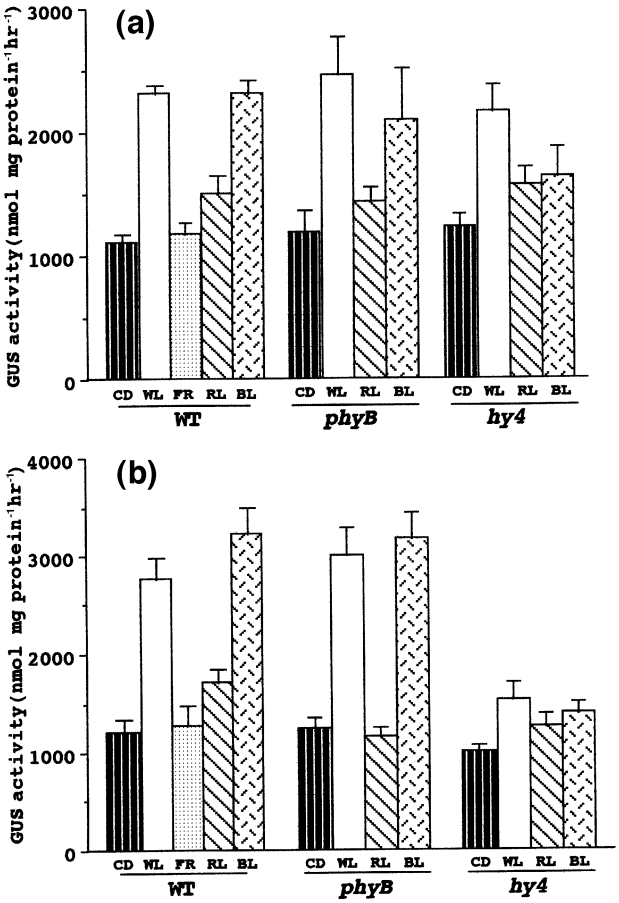

Figure 4. Light induction of *G/NOS101* and *GATA/NOS101* promoters and the involvement of photoreceptors in mediating light signals.
Four-day-old dark-grown seedlings (CD) containing the *G/NOS101*::GUS (a) or *GATA/NOS101*::GUS (b) transgenes were exposed to WL, FR, RL and BL for 48 h and the GUS activities were measured. The levels of induction of the promoters at different wavelengths of light are shown for the wild-type seedlings (left panels), *phyB* mutant seedlings (middle panels) and *hy4* mutant seedlings (right panels). The error bars indicate the standard deviation from the means of four independent experiments.

were also capable of responding to various wavelengths of light. As shown in Figure 4, both promoters were most responsive to BL (two- to threefold induction) and WL (about twofold induction), and very little induction of these promoters was observed in RL and FR. These results suggest that the single element-containing promoters tested are only able to respond to a limited wavelength region of light, in contrast to the paired LRE-containing promoters.

The induction patterns of these two promoters in the photoreceptor mutants were different. For the *GATA/NOS101* promoter (Figure 4b), the weak induction by RL was completely abolished in the *phyB* mutant, suggesting that PhyB is responsible for the weak RL induction. In addition, the BL induction and the WL induction were decreased in *hy4* mutants, suggesting that Cry1 is responsible for mediating the BL induction, and that Cry1 also plays a major role in WL induction of the *GATA/NOS101* promoter. On the other hand, in the case of the *G/NOS101* promoter (Figure 4a), the *phyB* mutation did not affect the weak RL induction, suggesting that PhyB was not essential in mediating this RL induction of the *G/NOS101* promoter. In the *hy4* mutant, the BL induction was substantially reduced, but the WL induction was not. This result suggests that although Cry1 plays an important role in mediating BL induction of *G/NOS101* promoter, it may not be the critical photoreceptor responsible for the elevated promoter activity in WL. It is possible that other photoreceptors besides PhyB and Cry1 are involved in the light induction of the *G/NOS101* promoter.

The activity of the GT1-containing promoter is repressed by a wide spectrum of light signals

It has been shown that the *GT1/NOS101* promoter is induced in darkness or repressed by light (Puente *et al.*, 1996). Experiments were conducted to determine the effect of different wavelengths of light on this promoter during the transitions from total darkness to various light conditions. All wavelengths of light tested caused lower *GT1/NOS101* promoter activity, with WL being the most effective and FR the least (Figure 5a). In the *phyB* mutant background, both RL and BL repression were significantly reduced, resulting in a higher GUS activity in RL and BL (Figure 5b). Similarly, in the *hy4* mutants, BL and RL repressions were both minimized, with the maximum effect being in BL (Figure 5c). In addition, the WL repression became less effective in *phyB* and *hy4* mutants. These results suggest that both PhyB and Cry1 are required for maximum light repression of *GT1/NOS101* promoter activity, and that the two photoreceptors appear to act cooperatively.

Since the moderate repression of FR was not affected in *phyA* or the other two photoreceptor mutants (Figure 5), further experiments were conducted to delineate the FR light-mediated repression of the *GT1/NOS101* promoter. As shown in Figure 6, FR repression of the *GT1/NOS101* promoter was notable in constant FR-grown seedlings, although the repression was quite moderate relative to the constant WL-grown seedlings. However, the FR repression was completely absent in light transfer experiments. The transition of WL-grown seedlings into FR led to a significant de-repression in the promoter activity, identical to that of WL-grown seedlings transferred to darkness (Figure 6). The mild FR repression of the *GT1/NOS101* promoter was only detectable when the dark-grown seedlings were transferred to FR. It is possible therefore that FR-mediated weak repression of the *GT1/NOS101* promoter may represent an indirect effect of the FR-mediated high-irradiance response.

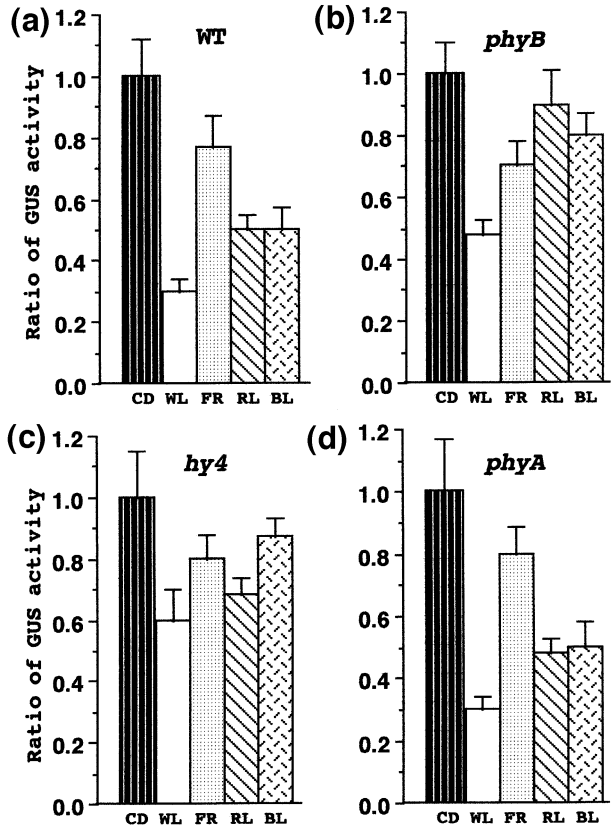

Figure 5. Inhibition of the *GT1/NOS101* promoter by different wavelengths of light.
Four-day-old dark-grown seedlings (CD) containing the *GT1/NOS101*::GUS transgene were exposed to WL, FR, RL and BL for 48 h and the GUS activities were measured. The ratios of GUS activity in 4-day-old dark-grown plus 48 h light-grown versus 6-day-old dark-grown are shown in each case. The levels of repression at different wavelengths of light are shown for the wild-type (a), *phyB* mutant (b), *hy4* mutant (c) and *phyA* mutant (d) seedlings. The error bars indicate the standard deviation from the means of four independent experiments.

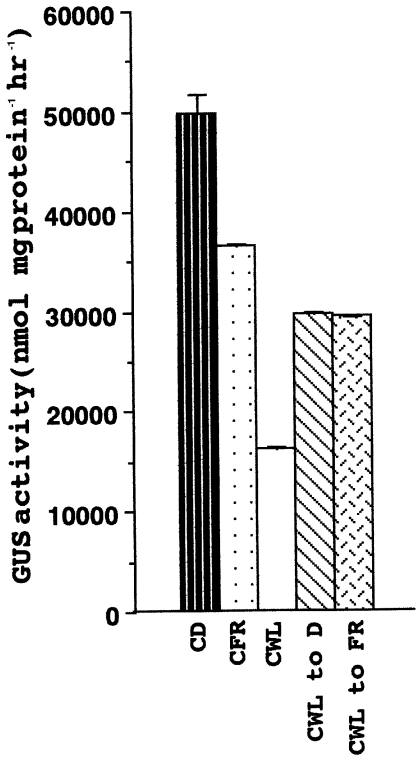

Figure 6. Comparison of GUS activities of 6-day-old wild-type seedlings containing the *GT1/NOS101*::GUS transgene grown under FR light (CFR), darkness (CD) or white light (CWL) and 4-day-old WL-grown seedlings transferred to far-red (CWL to FR) or darkness (CWL to D) for 2 days. The error bars indicate the standard deviation from the means of four independent experiments.

Promoters with paired elements are de-repressed in cop1 *and* det1 *mutants in the dark*

It has been shown that in the pleiotropic *cop/det/fus* mutants, many normally light-activated promoters become active in the darkness (reviewed by Wei and Deng, 1996). To examine how the synthetic promoters relate to the regulatory pathways defined by those mutations, the promoter–reporter transgenes were crossed into *cop1–4 and det1–1* mutant backgrounds and their expression patterns were analyzed. Since all transgenes in the *det1–1* background exhibited almost identical GUS staining patterns to those in the *cop1–4* background, only *cop1–4* GUS staining results are shown (Figure 7).

For transgenes with single LRE-containing promoters, i.e. *G/NOS101* and *GATA/NOS101*, activity was found in all the organs including cotyledons, hypocotyls and roots. The *cop1* and *det1* mutations did not alter the tissue specificity of the promoter activities in the dark. In the light, although the tissue-specific expression patterns remained unchanged, the expression levels of these two transgenes were drastically reduced in *cop1–4* and *det1–1* mutants as compared to wild-type (Figure 7f,g). Quantitative GUS activity measurements indicated that the activities of the single element-containing promoters in the light-grown *cop1–4* and *det1–1* mutants were essentially similar to those of dark-grown wild-type and mutant seedlings (Figure 8a). Therefore, the light-dependent induction of *G/NOS101* and *GATA/NOS101* single LRE-containing promoters was completely abolished by *cop1* and *det1* mutations.

The effects of *cop1* and *det1* mutations on paired LRE-containing promoters were rather different. In the dark-grown *cop1–4 and det1–1* mutants, *G-GATA/NOS101::*GUS, *GT1-GATA/NOS101::*GUS and *CAB1::*GUS transgenes were highly expressed specifically in the cotyledons in contrast to the low expression levels in the etiolated wild-type siblings (Figure 7c,d,e). This cotyledon-specific expression pattern observed in the dark-grown mutants resembled the light-grown wild-type seedlings expressing the corresponding transgenes (Figure 7h and Puente *et al.*, 1996). A quantitative analysis indicated that the dark expression

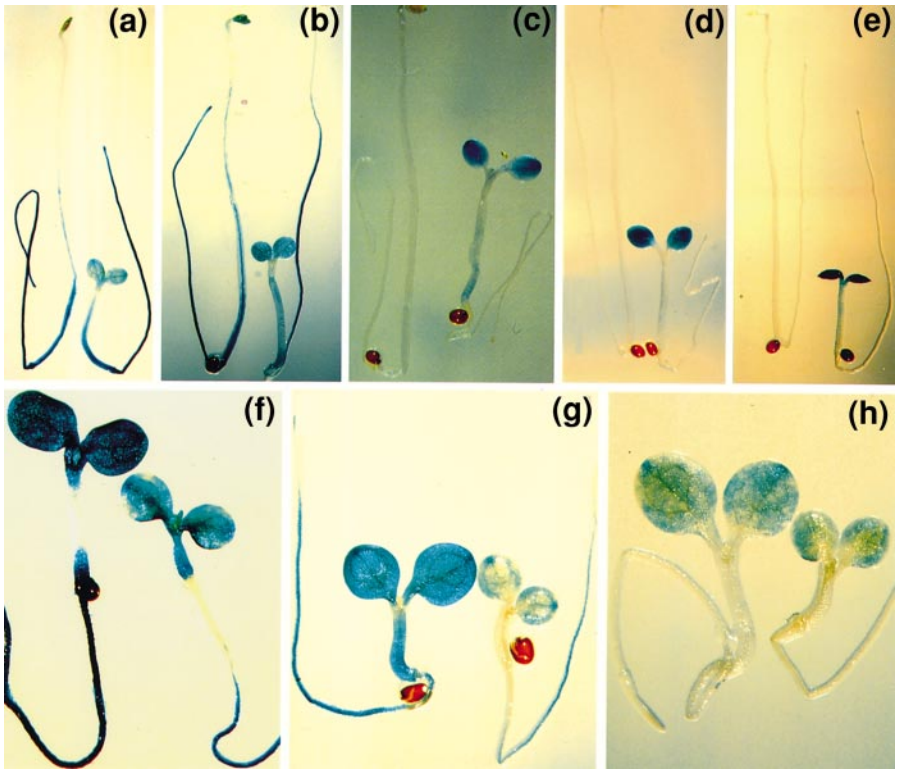

Figure 7. Tissue-specific expression of different transgenes (as measured by GUS staining) in wild-type or *cop1–4* mutant seedlings grown for 6 day in the light or in darkness.
Seedlings containing *G/NOS101*::GUS (a), *GATA/NOS101*::GUS (b), *G-GATA/NOS101*::GUS (c), *GT1-GATA/NOS101*::GUS (d) or *CAB1*::GUS (e) transgenes were grown in constant darkness for GUS staining. Seedlings containing *G/NOS101*::GUS (f), *GATA/NOS101*::GUS (g) or *GT1-GATA/NOS101*::GUS (h) transgenes were also grown in constant light for GUS staining; the staining patterns of the *G-GATA/NOS101*::GUS and *CAB1*::GUS transgenes were very similar to that of the *GT1-GATA/NOS101*::GUS transgene under constant light. Wild-type seedlings are shown on the left and *cop1–4* mutant seedlings are on the right in each panel. Almost identical staining patterns for the transgenes were obtained in the *det1–1* mutant background as in the *cop1–4* mutant background. Seedlings containing the same transgenes were stained for exactly the same length of time.

levels of these transgenes in *cop1–4* and *det1–1* mutants increased by more than threefold relative to the wild-type seedlings and were almost at the same levels as the light-grown mutant siblings (Figure 8b). In the light, the promoter activities of these three transgenes in the mutants were similar or slightly decreased when compared to wild-type (Figure 8b), and the tissue-specific expression patterns of the mutants were indistinguishable from the wild-type counterparts (Figure 7h and data not shown). Therefore, the mutations in *cop1* and *det1* essentially mimic light signals in mediating the tissue- specific induction of the paired LRE synthetic promoters even in the absence of light. These data suggest that the synthetic promoters with paired LREs resembles numerous native light- activated promoters not only in terms of their light-responsive characteristics and developmental control, but also in their responses to the negative regulatory components COP1 and DET1.

Discussion

Several recent studies have reported the fluence- and wavelength-dependent light induction of gene expression for specific native promoters (Batschauer *et al.*, 1996; Hamazato *et al.*, 1997), but the roles of individual LREs within the promoter in mediating such light induction were still unclear. In this study, we systematically investigated how the high-irradiance signals of various wavelengths of light are integrated at the individual LREs in light-responsive promoters. Our results with a series of synthetic promoters, which represent the simplest promoters that exhibit light responsiveness, provide new insights into the features of transcriptional regulation in response to light.

Interaction of photoreceptors in mediating light regulation of promoter activities

In this study, we confirmed that PhyA, PhyB and Cry1 are primarily responsible for mediating the high-irradiance signals from far-red, red and blue wavelengths of light to the promoter LREs. These three photoreceptors act redundantly in the WL activation of *G-GATA/NOS101* and *GT1-GATA/NOS101* paired-LRE promoters. More importantly, our results revealed the overlaps between these photoreceptor-mediated signaling pathways. By studying hypocotyl elongation and anthocyanin accumulation, it has

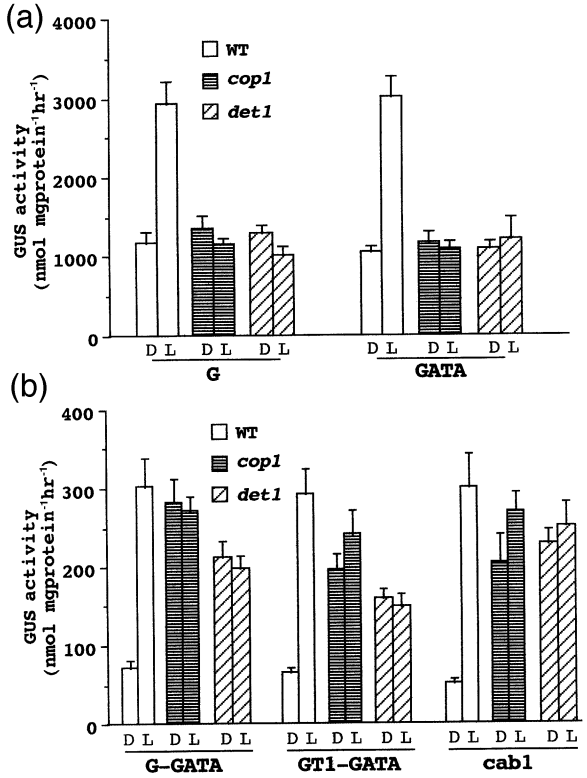

Figure 8. Comparison of GUS activities of different transgenes in wild-type, *cop1–4* and *det1–1* mutant backgrounds.
Seedlings were grown for six days in constant WL or in constant darkness before the GUS activities were measured.
(a) Relative GUS activities of the light-inducible single element-containing promoters (*G/NOS101* and *GATA/NOS101*) in wild-type and mutant backgrounds.
(b) Relative GUS activities of the paired element-containing promoters (*G-GATA/NOS101* and *GT1-GATA/NOS101*) as well as the *CAB1* promoter in wild-type and mutant backgrounds.

been noted that BL responses mediated by Cry1 require functional phytochromes (Ahmad and Cashmore, 1997; Casal and Boccalandro, 1995). In our gene expression studies, we observed the reverse dependence. For example, the optimal RL induction of *G-GATA/NOS101* promoter, which was primarily mediated by PhyB (Figure 2b), required functional Cry1 (Figure 2c). This suggested that Cry1 or a Cry1-mediated pathway may participate in the phytochrome pathways in some circumstances. The reciprocal dependence of PhyB and Cry1 was also manifested in the light repression of the *GT1/NOS101* promoter. In this case, repression by RL required functional PhyB as well as Cry1, and repression in BL required Cry1 as well as PhyB (Figure 5b,c). Thus, it appears that signaling pathways initiated by phytochromes and the Cry1 BL receptor interact to mediate wide range of events including hypocotyl elongation, anthocyanin accumulation (Ahmad and Cashmore, 1997; Casal and Boccalandro, 1995) and gene expression.

Phytochrome A and B are known to have distinct and overlapping functions (Quail, 1995; Reed *et al.*, 1994). Not only do they act redundantly and antagonistically, but here we showed that they also act interdependently in the light induction of the *GT1-GATA/NOS101* promoter. The *phyA* mutant not only completely abolished FR induction, but also substantially reduced the RL induction of the *GT1-GATA/NOS101* promoter. Thus PhyA is required for the optimal RL induction of this promoter. Interestingly, the interactions observed between PhyA and PhyB and between PhyB and Cry1 seem to be highly dependent on the promoter context or specific LRE combinations. This would argue against a generalized simple mechanism for these interactions. Obviously, further studies are necessary to understand these interactions at the molecular level.

Light regulation of synthetic promoters containing paired LREs or single LREs is fundamentally different

It is a common theme in eukaryotic transcriptional regulation that a single promoter *cis*-element can represent the genomic target for a particular stimulus, and the combination of different *cis*-elements in a promoter allows it to respond to multiple signals, sometimes in a synergistic manner (Hill and Treisman, 1995). However, in the case of light-regulated gene expression in plant cells, increasing lines of evidence have shown that a minimum of two different LREs in specific combinations are required to confer proper light induction (Degenhardt and Tobin, 1996; Feldbrugge *et al.*, 1997; Puente *et al.*, 1996). In a previous study, we have shown that only those promoters with paired LREs are able to respond to phytochrome-mediated low-fluence light pulses and are able to be activated in a tissue-specific manner (Puente *et al.*, 1996). Here we present additional evidence to further augment the conclusion that the light-responsive promoters with paired LREs are fundamentally different from the promoters with single LREs.

First, promoters with paired LREs (*G-GATA/NOS101* and *GT1-GATA/NOS101*) are able to respond to a wide spectrum of light signals involving multiple photoreceptors including PhyA, PhyB and Cry1. The single-LRE promoters are only able to respond to a particular wavelength of light. For example, although both the *G/NOS101* and the *GATA/NOS101* promoters were non-responsive to FR, the double element promoter *G-GATA/NOS101* could be effectively induced by FR through PhyA (Figure 2).

Second, promoters with paired LREs or a single LRE respond differently to the photomorphogenic repressor COP1 and DET1. The *cop1* and *det1* mutations lead to activation of many light-induced promoters in the dark (Chory *et al.*, 1989; Deng *et al.*, 1992). Not surprisingly, *cop1–4* and *det1–1* mutations led to light-independent expression of the *CAB1* promoter as well as light-inducible synthetic promoters (Figure 8). However, such light inde-

pendence was achieved in different ways for single- and paired-LRE promoters. For *CAB1* and the paired-LRE promoters (*G-GATA/NOS101* and *GT1-GATA/NOS101*), *cop1–4 and det1–1* mutations activated the promoters in the dark. However, in the case of the single-LRE promoters (*G/NOS101* and *GT1/NOS101*), the *cop1–4* and *det1–1* mutations diminished the promoter activities in the light. This observation implies that, in the absence of light, COP1 and DET1 are able to impose a negative control only on the paired-LRE promoters, but not on the single-LRE promoters.

The mechanism for the reduced activity of the single-LRE promoters in the light-grown *cop1–4 and det1–1* mutants is not clear. One speculation is that these two repressors of photomorphogenesis, COP1 and DET1, might also function as activators for certain types of promoters. In support of this possibility, it has been shown that COP1 is required for the activation of *PHYA* gene expression in the dark. *PHYA* gene expression is normally activated by darkness, but the dark induction is abolished in the *cop1* mutant, resulting in a low level of expression in both light and dark conditions (Deng *et al.*, 1991). Thus, COP1 can act as a positive regulator under certain circumstances.

Taken together, the distinct behaviors of the promoters with paired LREs and with single LREs cannot be explained by the additive action of the two elements. It appears that direct light-mediated transcriptional regulation requires the corporate interaction of at least two different *cis*-elements in a promoter. Accordingly, at least two different types of transcription factor are necessary to mediate light-regulated gene expression for a given promoter, and that a single transcription factor alone is insufficient to bring about proper light responsiveness. Therefore, the interaction and coordination of different sequence-specific transcription factors are critical for promoter activation in response to the signals from multiple photoreceptors as well as for promoter repression through a mechanism mediated by the *COP/DET* genes.

Experimental procedures

Plant materials and growth conditions

The photoreceptor mutant alleles used were *phyA-1* (Whitelam *et al.*, 1993), *phyB-B064* (Koornneef *et al.*, 1980), and *hy4–2.23N* (Ahmad and Cashmore, 1993; Koornneef *et al.*, 1980). The *cop1–4* (Deng and Quail, 1992; McNellis *et al.*, 1994a) and *det1–1* (Chory *et al.*, 1989; Pepper *et al.*, 1994) mutations used for this study have been described previously as indicated. All the promoter::GUS fusion constructs have been described by Puente *et al.* (1996). These stable transgenes were introduced into photoreceptor mutants (*phyA, phyB, hy4*) as well as *cop* and *det* mutants by crossing with the wild-type transgenic lines. Hygromycin-resistant seedlings (Puente *et al.*, 1996) were allowed to self. The homozygous mutant lines with the transgene were selected in the F_2 generation and allowed to self. Finally, the mutant lines homozygous for each transgene were obtained from the F_3 generation for further studies. For the dark versus light experiments with *cop1* and *det1* mutants, *Arabidopsis* seedlings were grown in constant white light (110 mmol m^{-2} sec^{-1}) or in constant darkness for 6 days. For the light induction experiments, 4-day-old dark-grown seedlings were exposed to white light (110 mmol m^{-2} sec^{-1}), standard far-red light (McNellis *et al.*, 1994b), red light (104 mmol m^{-2} sec^{-1}) and blue light (68 mmol m^{-2} sec^{-1}) for defined time periods before the measurement of GUS activities.

GUS assays

GUS enzyme activity in transgenic *Arabidopsis* seedlings was measured following the method of Puente *et al.* (1996). The protein concentration was determined by Lowry assay kit according to the manufacturer's instructions (Sigma, St Louis, Missouri, USA). Histochemical staining was performed by fixing the seedlings in 2% paraformaldehyde in sodium phosphate buffer pH 7.0 for 10 min under vacuum. The samples were washed in phosphate buffer twice, then the staining solution (Jefferson *et al.*, 1987) was added. The samples in the staining solution were vacuumed for 2 min and were incubated further at 37°C overnight.

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