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Independent Regulation of Flowering by Phytochrome B and Gibberellins in Arabidopsis[1]

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Phytochromes and gibberellins (GAs) coordinately regulate multiple aspects of Arabidopsis development. Phytochrome B (PHYB) promotes seed germination by increasing GA biosynthesis, but inhibits hypocotyl elongation by decreasing the responsiveness to GAs. Later in the life cycle of the plant, PHYB and GAs have opposite effects on flowering. PHYB delays flowering, while GAs promote flowering, particularly under noninductive photoperiods. To learn how PHYB and GAs interact in the control of flowering, we have analyzed the effect of a *phyB* mutation on flowering time and on the expression of the floral meristem-identity gene *LFY* (*LEAFY*). We show that the early flowering caused by *phyB* correlated with an increase in *LFY* expression, which complements our previous finding that GAs are required for activation of *LFY* under noninductive photoperiods (M.A. Blázquez, R. Green, O. Nilsson, M.R. Sussman, D. Weigel [1998] Plant Cell 10: 791–800). Since *phyB* did not change the GA responsiveness of the *LFY* promoter and suppressed the lack of flowering of severe GA-deficient mutants under short days, we propose that PHYB modulates flowering time at least partially through a GA-independent pathway. Interestingly, the effects of PHYB on flowering do not seem to be mediated by transcriptional up-regulation of genes such as *CO* (*CONSTANS*) and *FT* (*Flowering locus T*), which are known to mediate the effects of the photoperiod-dependent floral-induction pathway.

The control of plant development by light is exerted through the activity of photoreceptors. Among these, phytochromes mediate the responses to red and far-red light (Fankhauser and Chory, 1997). In Arabidopsis, phytochromes are encoded by five different genes, *PHYA* through *PHYE* (Sharrock and Quail, 1989; Quail et al., 1995). While each phytochrome seems to have specific roles, there is also considerable overlap in the function of individual phytochromes (Reed et al., 1994; Aukerman et al., 1997; Devlin et al., 1998). For example, PHYA seems to have a primary role in germination and in the regulation of seedling morphogenesis by far-red light (Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993). Later in development, PHYA is involved in sensing photoperiod, which is reflected in the insensitivity of *phyA* mutants to night breaks (Reed et al., 1994). PHYB, on the other hand, is an essential component of the shade-avoidance mechanism, and modulates the expression of genes in response to red light. Null mutations in *PHYB* cause increased elongation of hypocotyls, leaf petioles, and stems, as well as decreased chlorophyll accumulation and earlier flowering under both long and short photoperiods (Reed et al., 1993).

Phytochromes interact with plant hormones of the GA class to regulate certain aspects of plant development (Chory and Li, 1997). The phenotype of mutants defective in GA biosynthesis has confirmed that GAs regulate processes such as seed germination, cell expansion, and flowering, all of which are also under the control of phytochromes. For example, the Arabidopsis *spy* (*spindly*) mutant has a slender stature, is pale green, and flowers early, thus resembling *phyB* mutants, or wild-type plants treated with GA_3 (Jacobsen and Olszewski, 1993). An opposite phenotype is seen in GA-deficient mutants of Arabidopsis that are dark-green dwarves that flower late (Koornneef and van der Veen, 1980). This phenotype is particularly severe in the *ga1-3* mutant, which is blocked in a very early step of GA biosynthesis, and never flowers under short days (Wilson et al., 1992). Reduced expression of *LFY* (*LEAFY*), a floral meristem-identity gene, seems to be a main cause of the flowering defect in *ga1-3* mutants, since constitutive expression of *LFY* from a transgene is sufficient to restore flowering of *ga1-3* mutants under short days (Blázquez et al., 1998).

There are at least two possible mechanisms by which phytochromes and GAs may interact. The finding of elevated GA levels in the *phyB*-deficient mutants *ein* of *Brassica rapa* and ma_3^R of sorghum has suggested that PHYB regulates GA biosynthesis in certain species (Rood et al., 1990; Beall et al., 1991). However, the relationship between GA biosynthesis and PHYB activity in sorghum is complex, as GA biosynthesis follows a circadian regulation, and the ma_3^R mutant shows a phase shift in GA accumulation (Lee et al., 1998). An interaction between phytochromes and GA biosynthesis is also supported by the finding that GA-biosynthetic genes in Arabidopsis and spinach are regulated by light. In both species, GA 20-oxidase mRNA levels are higher under long than under short photoperiods (Xu et al., 1995; Wu et al., 1996). It has been suggested that GA

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Abbreviation: RT, reverse transcriptase.

20-oxidase activity is limiting for stem elongation in long days, and that long photoperiods raise the concentration of active GAs above a certain threshold (Talón et al., 1991).

Similarly, GA 3β-hydroxylation is promoted by pulses of far-red light in cowpea (Martínez-García and García-Martínez, 1992) and by red light in Arabidopsis seeds (Yamaguchi et al., 1998), indicating that the synthesis of active GA species is under phytochrome control. In different situations, however, phytochromes have been shown to affect the responsiveness to GAs, rather than their biosynthesis. Putative *phyB* mutants of pea and cucumber, *lv* and *lh*, as well as the *phyB* mutant of Arabidopsis have wild-type levels of endogenous GAs but show enhanced hypocotyl elongation in response to exogenous GAs (Weller et al., 1994; López-Juez et al., 1995; Reed et al., 1996). At least in Arabidopsis, PHYB thus appears to control GA-dependent hypocotyl elongation by modulating GA sensitivity as opposed to regulating GA biosynthesis.

In this study, we have addressed the question of whether PHYB and GAs interact in the regulation of flowering in a way similar to what is observed for other responses, such as hypocotyl elongation, in Arabidopsis. Using promoter activity of the floral regulator *LFY* as an indicator, we show that PHYB and GAs regulate *LFY* expression independently. This finding is corroborated by the observation that the loss of PHYB function allowed GA-deficient mutants to flower under short days.

MATERIALS AND METHODS

Plant Material

LFY::GUS lines (DW150-304 and 304G1) in the Landsberg *erecta* background of Arabidopsis have been previously described (Blázquez et al., 1997, 1998; Hempel et al., 1997). Lines 304B5 (*phyB-5 LFY::GUS*) and 304G1B5 (*ga1-3 phyB-5 LFY::GUS*) were constructed by crossing line 304G1 (*ga1-3 LFY::GUS*) to plants carrying the *phyB-5* mutations, a null allele in the Landsberg *erecta* background (Reed et al., 1993). Transgenic plants homozygous for *ga1-3* were initially identified by their short stature and dark-green color, and were then confirmed by PCR (Silverstone et al., 1997). The presence of the *phyB-5* allele was also monitored by PCR using a dCAPS marker (Neff et al., 1998). Lines homozygous for the transgene were identified by testing F_3 progeny.

Growth Conditions

For experiments on soil, seeds were stratified for 2 to 3 d at 4°C before sowing. Plants were grown at 23°C in long (16 h of light and 8 h of dark) or short days (9 h of light and 15 h of dark) under a mixture of 3:1 cool-white and Gro-Lux fluorescent lights (Osram Sylvania, Danvers, MA). The spectral quality of the light received by the plants under these conditions was determined with a portable spectroradiometer (model LI-1800, LI-COR) and is shown in Figure 1. *ga1-3* mutants required exogenous GAs to germinate (Koornneef and van der Veen, 1980) and were incubated with 50 μM GA_3 (Sigma) during stratification. Seeds were rinsed thoroughly with water before sowing. Vegetatively growing plants were sprayed twice weekly with a solution of 100 μM GA_3 and 0.02% (v/v) Tween 20 (Bio-Rad).

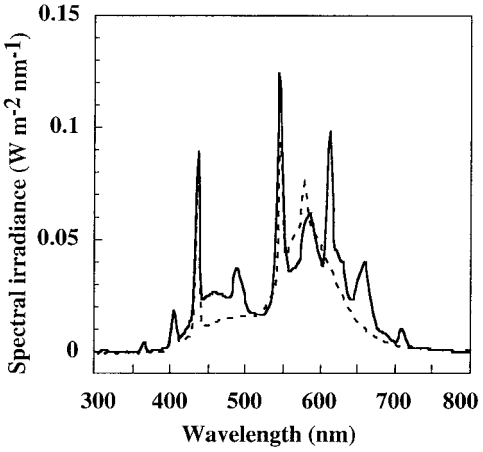

Figure 1. Light spectrum inside the growth chambers used in this work. The solid black line represents the spectrum provided by a 3:1 mixture of cool-white to fluorescent light, while the dotted line represents the spectrum provided by cool-white light alone.

The dose-response experiments with GA_3 or paclobutrazol (Zeneca Ag Products, Wilmington, DE) were performed with seedlings growing on MS plates (Murashige and Skoog, 1962) without Suc under the light conditions described above. The fluence rate was around 66 μmol m^{-2} s^{-1}. In experiments with soil-grown plants, paclobutrazol was applied by watering with a 37 mg/L solution.

Hypocotyl and GUS Activity Measurements

Hypocotyls were measured using a digitized image of 12 to 18 seedlings that had been placed between transparent acetate sheets (Neff and Chory, 1998). The image was analyzed with the public domain NIH Image program (developed at the United States National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image). For quantitative measurements of GUS activity using 4-methylumbelliferyl-β-D-glucopyranoside as a substrate, samples of plants grown on soil or MS plates were collected and treated as previously described (Blázquez et al., 1997).

RNA Extraction and Analysis

Total RNA was extracted with TRIzol reagent as indicated by the manufacturer (GIBCO-BRL). RT-PCR was conducted on 1 μg of total RNA. cDNA synthesis was performed with a reverse transcription kit (Promega). A fragment of the *CO* (*CONSTANS*) gene was amplified using oligonucleotides 5′-ACG CCA TCA GCG AGT TCC-3′ and 5′-AAA TGT ATG CGT TAT GGT TAA TGG-3′ as primers (P. Reeves and G. Coupland, personal communication). *FT* was amplified using 5′-ACT ATA TAG GCA TCA TCA CCG TTC GTT ACT CG-3′ and 5′-ACA ACT GGA ACA ACC TTT GGC AAT G-3′ (J.H. Ahn and D. Weigel, unpublished data). As a control we used oligos 5′-GAT CTT TGC

CGG AAA ACA ATT GGA GGA TGG T-3′ and 5′-CGA CTT GTC ATT AGA AAG AAA GAG ATA ACA GG-3′, which amplify two polyubiquitin gene fragments in the Landsberg *erecta* ecotype (Callis et al., 1995). The amplified fragments were separated on an agarose gel, blotted onto a membrane, and hybridized with radiolabeled *CO*, *FT*, and *UBQ10* probes. Signal intensities were determined with a phosphor imager (Molecular Dynamics), and the values in the exponential range of amplification were compared.

RESULTS

Enhanced *LFY* Up-Regulation in *phyB* Mutants

Mutations at the *PHYB* locus cause early flowering, especially under short photoperiods (Goto et al., 1991; Reed et al., 1993) (Table I). Since several other mutations that affect flowering time also change the expression level of *LFY* (Blázquez et al., 1998; Nilsson et al., 1998), we investigated the effect of the *phyB-5* null mutation on the activity of the *LFY* promoter using a fusion of the *LFY* promoter to the *GUS* reporter, which faithfully reflects endogenous *LFY* expression (Blázquez et al., 1997). Plants homozygous for the *phyB-5* mutation and a *LFY::GUS* transgene (304B5) and isogenic $PHYB^+$ plants (DW150-304) were grown on soil under long and short photoperiods, and GUS activity in the apices was determined at different ages during the vegetative phase. As shown in Figure 2, the *phyB-5* mutation caused an increase in the expression of *LFY::GUS* under both photoperiods, although this effect was more pronounced under short days. This result paralleled the acceleration of flowering observed in these plants (Table I), and indicates that PHYB represses *LFY* expression.

Application of GA_3 from germination on accelerated flowering of wild-type plants, but not of *phyB-5* mutants under short days (Table I). Although the number of rosette leaves was lower in GA_3-treated *phyB-5* plants than in untreated plants, the total number of leaves, including cauline leaves, was not significantly different between these populations (Table I). Consistent with the absence of an effect on flowering time, we observed no further increase in *LFY::GUS* expression when *phyB-5* plants were treated with GA_3 (results not shown).

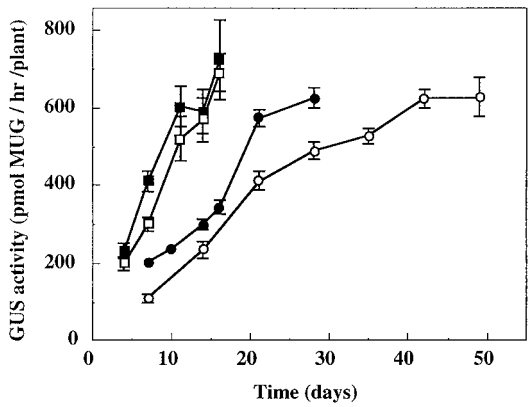

Figure 2. LFY::GUS expression during vegetative growth of *phyB-5* mutants. Plants homozygous for the *LFY::GUS* transgene either in a $PHYB^+$ (DW150-304; white symbols) or *phyB-5* background (307B5; black symbols) were grown in long days (squares) or short days (circles) until flower buds were visible to the naked eye. Values are expressed as means ± 2 SE (n = 12). Time represents days after sowing. Error bars that are not visible are contained within the symbol. MUG, 4-Methylumbelliferyl-β-D-glucopyranoside.

Suppression of the Flowering Defect of *ga1* Mutants by *phyB*

The increased expression level of *LFY::GUS* in *phyB-5* mutants resembles what is seen upon application of GAs to wild-type plants (Blázquez et al., 1997) or in mutants with enhanced GA-signaling, such as *spy* (Blázquez et al., 1998). Since several other effects of PHYB signaling, such as hypocotyl elongation and seed germination, appear to be mediated by GAs, we wanted to know whether the early flowering of *phyB* mutants depended on the activity of GAs. Therefore, we constructed *ga1-3 phyB-5* mutant plants and cultivated them under long and short photoperiods. Under long days, the double mutants were similar in size to *ga1-3* plants, and much smaller than *phyB-5* or wild-type plants, as previously described (Peng and Harberd, 1997). While flowering time of *ga1-3 phyB-5* mutants under long days was not different from that of *ga1-3* plants, the *phyB-5* mutation suppressed the flowering defect of *ga1-3* mutants under short days (Table I; Fig. 3). When plants were grown in a mixture of fluorescent and cool-white light, suppression was observed in over 90% of the double mutants after

Table I. *Flowering time of the ga1-3 and phyB-5 lines used in this study*

All lines are in the Landsberg *erecta* background and homozygous for a *LFY::GUS* transgene. RL, Rosette leaves; CL, cauline leaves; TL, total number of leaves. Values are the means ± 2 SE (i.e. with a 95% confidence interval). $n \geq 12$ plants.

Line	Genotype	Long Days			Short Days			Short Days + GA_3		
		RL	CL	TL	RL	CL	TL	RL	CL	TL
150-304	Wild type	8.8 ± 0.2	3.1 ± 0.4	11.9 ± 0.3	25.1 ± 0.9	7.5 ± 0.3	32.6 ± 0.8	16.2 ± 0.9	10.0 ± 0.5	26.2 ± 0.8
304B5	*phyB-5*	7.4 ± 0.5	4.9 ± 0.7	12.1 ± 0.8	9.1 ± 0.6	4.6 ± 0.4	13.7 ± 0.7	7.0 ± 0.3	5.6 ± 0.4	12.6 ± 0.8
304G1	*ga1-3*	–[a]	–[a]	15.3 ± 0.7	>58	–[b]	>58	18.0 ± 0.9	8.3 ± 0.8	26.3 ± 0.9
304G1B5	*ga1-3 phyB-5*	–[a]	–[a]	17 ± 1	–[a]	–[a]	26 ± 1	8.1 ± 0.6	5.2 ± 0.3	13.3 ± 0.7

[a] – indicates that these plants did not bolt in long days and that rosette and cauline leaves could not be distinguished. [b] – indicates that *ga1-3* plants did not flower in short days and that rosette and cauline leaves could not be distinguished.

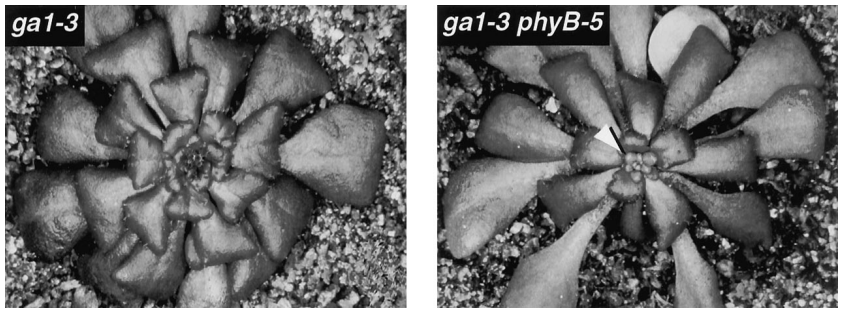

Figure 3. Suppression of the *ga1-3* flowering defect in short days by *phyB-5*. Representative plants were photographed 50 d after sowing. Arrowhead indicates flowers.

7 weeks. Among these plants, the number of leaves produced before flowering did not deviate much from the mean, and the value was intermediate between that of wild-type and *phyB-5* plants (Table I).

Decreased *LFY* expression appears to be a main cause of the late-flowering phenotype of *ga1* mutants under long days, and of the inability of *ga1-3* mutants to flower at all under short days (Blázquez et al., 1998). To determine whether *phyB* suppressed the *ga1* mutant flowering defect by restoring more normal levels of *LFY* promoter activity, we examined *LFY::GUS* expression in *ga1-3 phyB-5* double mutants. As shown in Figure 4A, *LFY::GUS* expression under long days followed a similar pattern in both *ga1-3* and *ga1-3 phyB-5* mutants. The expression levels were very low during the first 15 d of growth, and although *LFY::GUS* expression eventually increased, it never reached the levels seen in wild-type plants. Application of GA_3 restored the expression pattern seen in wild-type plants and *phyB-5* single mutants. In contrast to long days, *LFY::GUS* expression remained very low in short-day-grown *ga1-3* single mutants during the entire experimental period (Fig. 4B) (Blázquez et al., 1998). Although *LFY::GUS* expression in *ga1-3 phyB-5* double mutants increased shortly before flowering occurred, it was not different from the *ga1-3* single mutant during the first 4 weeks. Upon application of GA_3, *ga1-3* and *ga1-3 phyB-5* mutants flowered as early as wild-type and *phyB-5* plants treated with GA_3 (Table I), which was paralleled by a similar increase in *LFY::GUS* expression (Fig. 4B).

Interaction between PHYB and GAs

The suppression by *phyB-5* of the flowering defect of *ga1-3* mutants, along with the weak up-regulation of *LFY::GUS* expression in the *ga1-3 phyB-5* double mutants are compatible with the idea that PHYB modulates flowering and *LFY* expression independently of GAs. However, the *ga1-3* mutation does not completely abolish GA biosynthesis, and several GA species are still detectable in the *ga1-3* mutant (Zeevaart and Talón, 1992; A. Silverstone, and T.-P. Sun, personal communication). In addition, there is the possibility that physiologically relevant levels of exogenous GAs are carried over from the parental generation or from the seed treatment required for germination. Thus, PHYB could act by increasing the low levels of GA biosynthesis or by enhancing the responsiveness toward the small amount of GAs present in *ga1-3* mutants. An effect of *phyB* on GA biosynthesis is unlikely, since overall levels of several GA intermediates are unchanged in *phyB* mutants compared with the wild type (Reed et al., 1996). *phyB* mutants show enhanced responsiveness to GAs, as monitored by the dose response of hypocotyl elongation (Reed et al., 1996). We have found that continuous watering of plants with paclobutrazol, an inhibitor of the early steps of

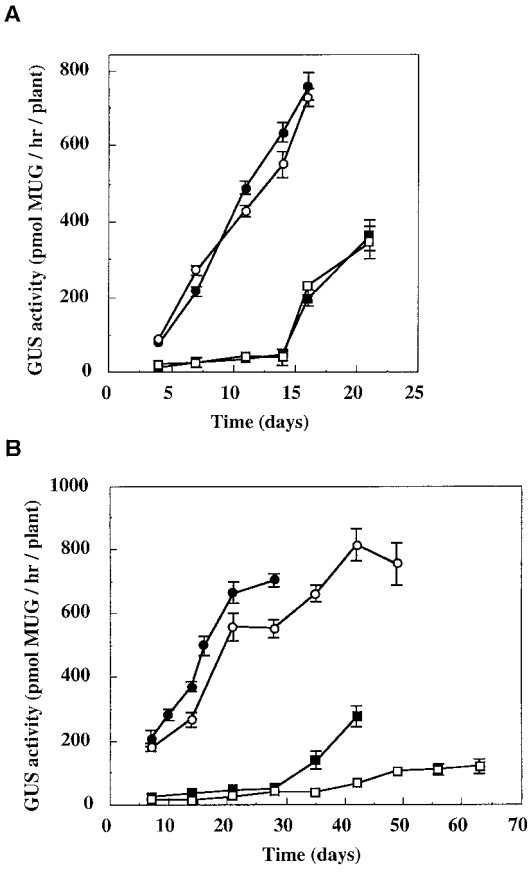

Figure 4. LFY::GUS expression during vegetative growth of *ga1-3* and *ga1-3 phyB-5* mutants. Plants homozygous for the *LFY::GUS* transgene were grown in long (A) or short (B) days until flower buds were visible to the naked eye, except in the case of the *ga1-3* mutant without GA_3 treatment under short days (□), which had not flowered at the end of the experiment. 304G1 (*ga1-3*, white symbols) and 304G1B5 (*ga1-3 phyB-5*, black symbols) were treated with GA_3 (circles) or left untreated (squares). Values are expressed as means ± 2 SE ($n = 12$). Time represents days after sowing. Error bars that are not visible are contained within the symbol. MUG, 4-Methylumbelliferyl-β-D-glucopyranoside.

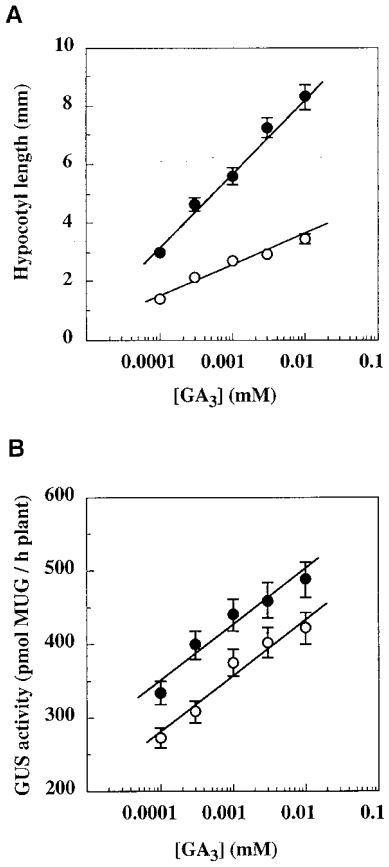

Figure 5. Responses of *ga1-3* and *ga1-3 phyB-5* seedlings to exogenous GA_3. Seeds of the lines 304G1 (*LFY::GUS ga1-3*, ○) and 304G1B5 (*LFY::GUS ga1-3 phyB-5*, ●) were sown on MS plates containing the indicated concentrations of GA_3. Hypocotyl length (A) and GUS activity (B) were determined 7 d after sowing. Values are expressed as means ± 2 SE (n = 12). Time represents days after sowing. Error bars that are not visible are contained within the symbol. MUG, 4-Methylumbelliferyl-β-D-glucopyranoside.

GA biosynthesis (Rademacher, 1991), did not prevent the flowering of *ga1 phyB* or *phyB* mutants under short days, while it abolished flowering of wild-type plants (results not shown).

To resolve the question of whether PHYB affects flowering by regulating GA biosynthesis or GA response, we determined whether increased responsiveness to GAs could account for the increased *LFY::GUS* expression in *phyB* mutants. *ga1-3* and *ga1-3 phyB-5* seedlings carrying a *LFY::GUS* transgene were grown on plates containing increasing concentrations of GA_3. The hypocotyl length of each seedling was determined before measuring LFY::GUS activity. As previously reported (Reed et al., 1996), the hypocotyl of *phyB* mutants was longer than that of $PHYB^+$ plants at all GA_3 concentrations. More importantly, *phyB* mutants were also more responsive to exogenous GA_3 than wild-type plants (Fig. 5A). In contrast, levels of LFY::GUS activity in *phyB* mutants did not show increased responsiveness to exogenous GA_3 over the range of concentrations tested (Fig. 5B). The results were the same under long or short days.

To confirm that the responsiveness to exogenous GA_3 reflects the behavior toward endogenous GAs, we analyzed both hypocotyl length and LFY::GUS activity in wild-type and *phyB-5* seedlings growing on plates with increasing concentrations of paclobutrazol. As expected, paclobutrazol reduced the elongation of wild-type and *phyB* hypocotyls starting at concentrations as low as 0.03 μM (Fig. 6A) (Reed et al., 1996). At higher concentrations, paclobutrazol inhibited hypocotyl elongation faster in *phyB-5* mutants than in wild-type plants. Although paclobutrazol reduced *LFY::GUS* expression in both wild-type and *phyB-5* plants, there was no difference in responsiveness between the two lines (Fig. 6B).

Effect of *phyB* Mutation on Expression of *CO* and *FT*

GAs act redundantly with the long-day-dependent pathway of floral induction, as GA deficiency has much weaker effects on flowering in long than in short days (Wilson et al., 1992). In addition, double mutants carrying both the *ga1-3* mutation and a mutation in *CO*, an essential element

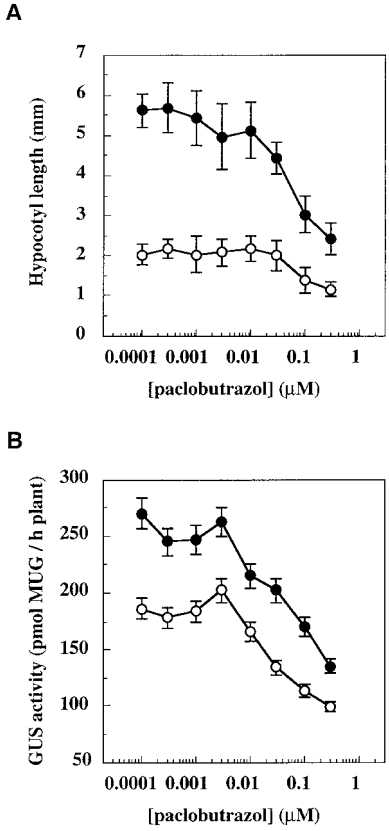

Figure 6. Responses of *ga1-3* and *ga1-3 phyB-5* seedlings to the GA-biosynthesis-inhibitor paclobutrazol. Seeds of the lines DW150-304 (*LFY::GUS*, ○) and 304B5 (*LFY::GUS phyB-5*, ●) were sown on MS plates containing the indicated concentrations of paclobutrazol. Hypocotyl length (A) and GUS activity (B) were determined 7 d after sowing. Values are expressed as means ± 2 SE (n = 12). Time represents days after sowing. Error bars that are not visible are contained within the symbol. MUG, 4-Methylumbelliferyl-β-D-glucopyranoside.

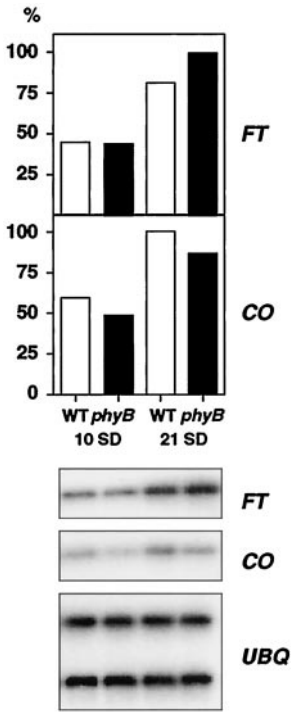

Figure 7. *CO* and *FT* RNA expression in *phyB* mutants. Seedlings of the lines DW150-304 (wild type [WT], white bars) and 304B5 (*phyB*-5, black bars) were grown on MS plates under short days (SD), and harvested during the 8th h of light on the indicated days. Expression was analyzed by RT-PCR (bottom panel) and the signals quantified and normalized using *UBQ* expression as a control (top panel, arbitrary units).

of the long-day pathway, often do not flower at all in long days (Putterill et al., 1995). Since all of our other data pointed to a GA-independent effect of PHYB on flowering, we wanted to determine whether the early flowering of *phyB* mutants was caused by an increase in the expression of genes known to be involved in the photoperiod-dependent pathway that promotes flowering. The expression of two genes in this pathway has been shown to be limiting for flowering, since their overexpression causes very early flowering under both long and short days. These genes are *CO* (Simon et al., 1996) and *FT* (I. Kardailsky and D. Weigel, unpublished data).

When we analyzed the expression of *CO* and *FT* by RT-PCR, we found that the expression levels of *CO* and *FT* did not differ dramatically between wild-type and *phyB*-5 plants (Fig. 7), suggesting that the early-flowering phenotype of *phyB* mutants under short days is not caused by overexpression of genes in the long-day pathway.

DISCUSSION

The level of *LFY* expression is an important determinant for the identity of the primordia that arise at the flanks of the shoot apical meristem during the transition to flowering (Blázquez et al., 1997). This idea has been corroborated by the observation that certain mutations that delay flowering, such as *co* or *gi* (*gigantea*), also reduce the level of *LFY* expression during the time that the transition to flowering occurs in wild type (Nilsson et al., 1998). In a similar way, the acceleration of flowering caused by mutations such as *spy* is paralleled by increased *LFY* expression (Blázquez et al., 1998). Hence, it is not surprising that PHYB, which represses flowering, functions as a negative regulator of the *LFY* promoter, although it has been difficult to assess whether *phyB* mutants flower early exclusively because of increased *LFY* expression, or also because of an increased response to *LFY* activity (Nilsson et al., 1998).

In our growth conditions (Fig. 1), PHYB affected flowering time mainly under short photoperiods. Although the total number of leaves produced was similar under long and short days (Table I), the number of days needed to produce the first flower was higher under short days, as previously reported (Koornneef et al., 1995). Accordingly, the increase of *LFY* promoter activity caused by the *phyB* mutation was more clearly observed under short than under long days. It has been previously observed that the contribution of different photoreceptors to the control of flowering time changes with photoperiod (Bagnall et al., 1995). For example, a specific role for the blue/UVA photoreceptor encoded by *CRY2* is the promotion of flowering under long days (Guo et al., 1998; Lin et al., 1998).

The observation that a *phyB* mutation has a more pronounced effect on *LFY* expression under short days suggests an interaction with GAs, since GAs are essential for flowering under noninductive conditions (Martínez-Zapater et al., 1994). However, we present evidence that PHYB and GAs regulate *LFY* expression through independent pathways, since a *phyB* mutation did not enhance the response of the *LFY* promoter to GAs. An independent action was confirmed by the observation that *phyB* suppressed the flowering defect of *ga1* mutants under short days, even when any *GA1*-independent synthesis of *ent*-kaurene was inhibited by paclobutrazol treatment. The simplest scenario for this suppression would be activation of the long-day-dependent pathway of flowering. However, the observation that the levels of RNA expression of *CO* and *FT*, the two genes believed to act downstream in the long-day pathway (Simon et al., 1996; Koornneef et al., 1998), are not dramatically changed in *phyB* mutants suggests that this activation would not occur at the transcriptional level. Alternatively, the suppression could take place through the *FCA*-dependent autonomous pathway (Macknight et al., 1997).

An important conclusion from our study and previous studies is that the relationship between PHYB and GAs is complex. For certain responses, such as germination, the GA-deficient *ga1* mutant is completely epistatic over *phyB*. However, most other characteristics of *ga1 phyB* double-mutant seedlings are intermediate between those observed for the single mutant parents (Peng and Harberd, 1997; this study). Finally, while PHYB regulates hypocotyl elongation by modulating responsiveness to GAs (Reed et al., 1996), PHYB acts independently of GAs in the control of flowering time (this study). That genes do not always interact in the same fashion, even when controlling the same targets, is not uncommon in development. For instance, PHYA and PHYB regulate common responses to

light, but they do so differently depending on the particular response. While both phytochromes have an inhibitory effect on hypocotyl elongation and promote seed germination, flowering is repressed by PHYB but promoted by PHYA.

It is not easy to imagine the molecular mechanism that integrates the different interactions between GAs and PHYB. Based on the effects of application of different GAs to ryegrass, it has been proposed that certain GA species possess low florigenic activity but promote stem elongation very efficiently and vice versa (Evans et al., 1990). For instance, 3β-hydroxylation is required for the promotion of stem elongation, but not of flowering in this plant (Evans et al., 1994). If these findings reflect the presence of different receptors for the various active GA species, PHYB might regulate receptors specific for GA species involved in hypocotyl and stem elongation but not the ones involved in flowering.

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