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Genetic and Transgenic Evidence That Phytochromes A and B Act to Modulate the Gravitropic Orientation of *Arabidopsis thaliana* Hypocotyls

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Hypocotyls of *Arabidopsis thaliana* exhibit negative gravitropism in the dark, growing against the gravity vector. The direction of growth is randomized in red light (R). In single mutants lacking either phytochrome A or B randomization of hypocotyl orientation in R is retained. However, a double mutant lacks this response, indicating that either phytochrome A or B is capable of inducing randomization and phytochrome A and B are the only phytochromes involved in this process. The induction of randomization was confirmed using lines that express to different levels *PHYA* and *PHYB* cDNAs. Overexpression of *PHYA* cDNAs induced randomization of hypocotyl orientation in the dark. Dark randomization was also seen in the *phyB-1* mutant but not in two other *phyB* alleles, suggesting that dark randomization in the *phyB-1* line may be due to a second mutation. When germination was induced by gibberellin, rather than exposure to brief white light, randomization in the dark associated with phytochrome A overproduction was not observed but was retained in the *phyB-1* mutant. Overexpression of *PHYB* cDNAs induced a light-dependent randomization of hypocotyl orientation that responded to R:far-red light ratio. We conclude that the default situation in Arabidopsis hypocotyls is, therefore, negative gravitropism, and either phytochrome A or phytochrome B can mediate randomization.

The phytochromes are a family of photoreceptors having multiple functions in the regulation of plant processes in response to the light environment (Smith and Whitelam, 1990; Smith, 1994a). Elucidation of these functions has been greatly helped by the selection of mutants deficient in the action of a particular phytochrome. phyA functions primarily in regulating the FR high-irradiance response, whereby continuous FR inhibits hypocotyl elongation as a function of fluence rate (Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993). Other processes in which a role for phyA has been shown include the regulation of germination (Shinomura et al., 1994) and the promotion of flowering by end-of-day FR treatment (Johnson et al., 1994). phyB functions in a number of different processes, including seed germination; gravitropism; cotyledon unfolding; hypocotyl, stem, and petiole elongation; the transition from vegetative to reproductive growth; and responses of light-grown seedlings to the relative amounts of R and FR (Smith and Whitelam, 1990; Goto et al., 1991; Whitelam and Smith, 1991; Liscum and Hangarter, 1993; Reed et al., 1993; Robson et al., 1993; Neff and Van Volkenburgh 1994; Shinomura et al., 1994). In the light-grown plant, many of the processes mediated by phyB contribute to the shade-avoidance syndrome, in which plants detect FR reflected from neighbors, resulting in growth changes appropriate to the competition for light (Smith, 1994a, 1995).

Arabidopsis hypocotyls exhibit negative gravitropism when grown in the dark, growing in the opposite direction to the gravity vector; however, the direction of growth is highly randomized when seedlings are exposed to continuous R. Liscum and Hangarter (1993) showed that the *phyB-1* mutant of Arabidopsis exhibits randomized growth under both continuous R and dark growth conditions and concluded that the photocontrol of negative gravitropism acts through phyB. The *hy2* mutant expresses wild-type levels of phytochrome apoproteins but lacks photoreversible holoproteins due to a presumed deficiency in the biosynthesis of the phytochrome chromophore (Parks and Quail, 1991). This mutant was shown to exhibit negative gravitropism in both the dark and R (Liscum and Hangarter, 1993). Phytochrome molecules exist in one of two forms: one that absorbs R, Pr, and one that absorbs FR, Pfr. The two forms interconvert on absorption of appropriate wavelengths of light. It has been demonstrated at least for phyA that the apoprotein is synthesized in the R-absorbing form, PrA (Parks et al., 1987). It is assumed that phytochromes synthesized in the *hy2* mutant are present in a form similar to Pr but lack a chromophore and cannot photoconvert to Pfr. These observations allowed the conclusion that it is PrB that induces negative gravitropism and that in WT it is the depletion of PrB by photoconversion to PfrB, rather than the production of PfrB per se, that attenuates hypocotyl gravitropism resulting in highly randomized growth (Liscum and Hangarter, 1993).

Liscum and Hangarter (1993) did not investigate the gravitropic responses of mutants simultaneously deficient in both phyA and phyB. In this paper we show that *phyA/phyB* double mutants completely lack the R-mediated randomization of growth orientation. In addition to null mutants, transgenic lines overexpressing *PHYA* or *PHYB*

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Abbreviations: FR, far-red light; LED, light-emitting diode; phyA, phytochrome A; phyB, phytochrome B; R, red light; VLFR, very low fluence response; W, white light; WT, wild-type. Nomenclature for phytochrome genes and products is per Quail et al. (1994).

cDNAs have proved useful in determining the functions of individual phytochromes (Boylan and Quail, 1991; Wagner et al., 1991; Smith, 1994b). In this paper we also examine the effect of overexpressing *PHYA* and *PHYB* cDNAs on the gravitropic response. If PrB induces negative gravitropism, as proposed by Liscum and Hangarter (1993), then high levels of phyB expressed by the transgene should maintain negative gravitropism under light treatments that result in randomization of growth in WT because the cellular concentration of PrB would be high under such light conditions. We also used lines expressing introduced *PHYA* cDNAs to probe the role of phyA in the induction of gravitropic randomization. The use of several lines expressing cDNAs at different levels allows a comparison of response measured with level of phytochrome expression and also excludes the possibility that an observed phenotype is due to insertional inactivation by the transgene.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of *Arabidopsis thaliana* (L.) Heynh. were obtained from the WT and five homozygous lines derived from the ecotype Nossen. The transgenic lines designated Z15, ABO, and D2 had been transformed with an Arabidopsis *phyB* cDNA fused to the cauliflower mosaic virus 35S promoter. Immunoblot quantification indicated that the *PHYB* transgenic lines contained phyB at 15-fold (Z15), 30-fold (ABO), and 60-fold (D2) the light-grown wild-type level of phyB (D. Wagner and P. H. Quail, personal communication). The *PHYA* transgenic lines, designated 13K7 and 21K15, had been transformed with oat *PHYA* fused to the cauliflower mosaic virus 35S promoter. Quantification was less precise in the *PHYA* lines, but the sequence of expression level was WT < 13K7 < 21K15 (M.T. Boylan and P.H. Quail, personal communication). Phytochrome mutants in the Landsberg *erecta* ecotype were *phyA-2*, *phyB-1*, and *phyB-7*; the double mutant was *phyA-2/phyB-1*. Phytochrome mutants in the RLD ecotype were *phyA-101* and *phyB-6*; the double mutant was *phyA-101/phyB-6*. Seeds were incubated for 4 d at 4°C on BG11 (Stanier et al. 1971) agar medium in Petri plates and then exposed to fluorescent white light for 30 min to induce uniform germination. The plates were vertically oriented to allow the seedlings to grow along the surface of the agar and were incubated in darkness at 25°C for 1 d before transfer to one of the treatments described below. After 3 d plates were photographed, and the orientation of the hypocotyl was measured. When the 30-min W pretreatment was replaced by GA pretreatment, seeds were soaked in a solution of 500 μg/mL GA in the dark for 1 h, washed twice with water before immediate plating onto vertically oriented Petri plates, and transferred to dark growth conditions.

Light Sources

R was obtained from LEDs with an emission maximum at 665 nm and a half-bandwidth of 27 nm and provided a fluence rate of 22 to 24 μmol m^{-2} s^{-1} at the seed surface; R was given either continuously or as two treatments of 2 h per 24-h period. A range of R:FR ratios was obtained using a mixed array of both R and FR LEDs (Quantum Devices, Barneveld, WI). The R LEDs were as above. The emission maximum of the FR LEDs was 730 nm and a half-bandwidth of 29 nm. The mixed array provided a fluence rate of 20 to 22 μmol m^{-2} s^{-1} at wavelengths between 400 and 800 nm and was given as two 1-h periods per 24-h period. FR was obtained from the mixed array with the R LEDs switched off, and the resulting FR light was filtered through black Plexiglas. The resulting light was provided continuously and at a fluence rate of 11 to 13 μmol m^{-2} s^{-1}. Fluence rates at the level of the seedlings were measured with an Analytical Spectral Devices personal spectrometer model PS2 000A (Analytical Spectral Devices, Boulder, CO). The phytochrome photoequilibrium set up by the R:FR source was measured as a bioassay using a sample of isolated oat phyA in an Aminco dual-wavelength spectrophotometer model DW2A (American Instrument Co., Silver Spring, MD).

Measurement of Growth Orientation and Statistical Methods

After treatment the plates were photographed, and the image was projected and traced along with a horizontal reference onto paper, from which angle measurements were taken. The angle of orientation was expressed according to the method of Liscum and Hangarter (1993), who found that in darkness and in R values were normally distributed around 0° (vertical). Angles to the left of vertical were assigned a negative value; those to the right were assigned a positive value. The SD was used as a measure of randomization around the vertical (0°).

RESULTS AND DISCUSSION

The Effects of phyA and phyB Deficiency on Gravitropism

Results similar to those obtained by Liscum and Hangarter (1993) for single null mutants are shown in Tables I and II. Lack of phyA had little effect compared to WT, with strong negative gravitropism under dark growth condi-

Table I. *Hypocotyl orientation of phytochrome mutant and wild-type Arabidopsis seedlings in ecotype Landsberg erecta grown in the dark or exposed to continuous R*

Seedlings and data were handled as described in "Materials and Methods." The orientation of hypocotyl angle was measured as the SD around the vertical 0°. Values represent the pooled SD for a minimum of 150 seedlings from at least three replicate experiments. High SDs indicate randomization of hypocotyl growth.

Genotype	Treatment	SD
Landsberg *erecta*	Dark	20.0
Landsberg *erecta*	Continuous R	58.3
phyA-2	Dark	16.6
phyA-2	Continuous R	56.3
phyB-1	Dark	79.4
phyB-1	Continuous R	92.8
phyB-7	Dark	22.1
phyB-7	Continuous R	76.6
phyA-2/phyB-1	Dark	23.4
phyA-2/phyB-1	Continuous R	27.1

Table II. *Hypocotyl orientation of phytochrome mutant and wild-type Arabidopsis seedlings in ecotype RLD grown in the dark or exposed to continuous R*

Seedlings and data were handled as described in "Materials and Methods." The orientation of hypocotyl angle was measured as the SD around the vertical 0°. Values represent the pooled SD for a minimum of 150 seedlings from at least three replicate experiments. High SDs indicate randomization of hypocotyl growth.

Genotype	Treatment	SD
RLD	Dark	24.4
RLD	Continuous R	83.4
phyA-101	Dark	9.9
phyA-101	Continuous R	78.8
phyB-6	Dark	22.1
phyB-6	Continuous R	58.1
phyA-101/phyB-6	Dark	7.9
phyA-101/phyB-6	Continuous R	13.22

tions and randomization under continuous R. In phyB-deficient mutants, randomization of hypocotyl angle under continuous R was seen. Under dark growth conditions there was a difference between the Landsberg *erecta* and the RLD ecotypes, with dark randomization occurring in the *phyB-1* allele of the former but not the *phyB-6* allele of the latter. Table I shows that the *phyB-7* allele of Landsberg *erecta* is consistent with the *phyB-6* mutant, being negatively gravitropic in the dark. We conclude, therefore, that randomization in the dark is caused by a second mutation in the *phyB-1* mutant and that a mutant that is deficient in phyB alone will show negative gravitropism in the dark and randomization in continuous R.

In the absence of both phytochromes (i.e. in the *phyA/phyB* double mutant) no randomization of growth was seen under continuous R or in dark growth conditions. The behavior of the double mutant is crucially important for interpretation. Because there is no randomization by continuous R, it is clear that no other phytochrome than phyA or phyB is required for this response. This means that the interpretation reasonably reached by Liscum and Hangarter (1993) concerning the data available to them, namely that PrB imposes negative gravitropism in the WT, is untenable. If PrB were necessary for negative gravitropism, then the growth orientation of the *phyA/phyB* double mutants would be randomized. We conclude, therefore, that either phyA or phyB is capable of randomizing growth orientation and that negative gravitropism is the default situation in the absence of both phytochromes. To probe the relative importance of phyA and phyB in hypocotyl growth orientation, we investigated the responses of transgenic seedlings that produce increased levels of either phyA or phyB.

The Effects of phyB on Gravitropism

Arabidopsis lines that overexpress *PHYB* cDNAs were used to test two alternative hypotheses. First, according to the hypothesis of Liscum and Hangarter (1993), in which PrB is proposed to impose negative gravitropism, increasing levels of phyB would be predicted to lead to negative gravitropism under radiation conditions that induce randomization in WT. In other words, with high levels of phyB, higher levels of PrB would be present at all photoequilibria, indicating that R would lead to lower levels of randomization than are achieved in WT plants. The alternative hypothesis, suggested by the results of the single and double null mutants in Tables I and II, is that PfrB induces randomization. In this scenario, higher levels of phyB might be expected to lead to increases in randomization of hypocotyl angle upon treatment with R, because of a higher cellular concentration of PfrB.

Figure 1 shows the effect of two pulses of R of 2 h duration per 24-h period on the distribution of hypocotyl angle in three transgenic lines expressing different levels of phyB (Z15, ABO, D2) and their isogenic WT (Nossen). This low-level R treatment was chosen because we had previously determined that it provided intermediate, nonsaturating responses. Contrary to the result predicted by the Liscum and Hangarter hypothesis, the values for hypocotyl angle in seedlings expressing lower levels of phyB (Nossen and Z15) are clustered around the vertical, displaying negative gravitropism, whereas seedlings expressing higher

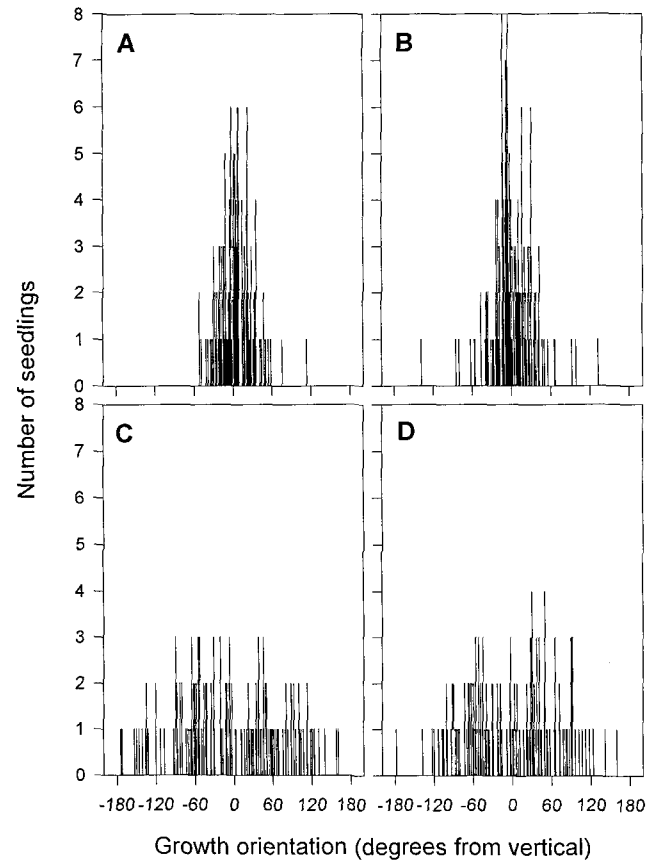

Figure 1. Frequency histogram showing hypocotyl orientation of WT and transgenic lines expressing different levels of phyB after exposure to two periods of 2 h of R per 24-h period, between which seedlings were maintained in darkness. Seedlings and data were handled as described in "Materials and Methods." Hypocotyl orientation of lines expressing low levels of phyB, WT (A) and Z15 (B), was centered near vertical 0°, whereas lines expressing higher levels of phyB, ABO (C) and D2 (D), hypocotyl orientation were greatly randomized.

levels of phyB (ABO and D2) exhibit greater randomization of hypocotyl angle, or loss of negative gravitropism. This indicates that high levels of phyB do not induce negative gravitropism through PrB. Table III shows the SDs calculated from the angles of hypocotyls grown in the dark, under continuous R, and under continuous FR. The four strains do not appear to be different from one another in any growth regime, indicating that overexpression of a *phyB* cDNA does not affect the wild-type response, i.e. negative gravitropism in the dark, and that continuous R was sufficient to cause complete randomization in all strains. Under continuous FR the level of randomization was greater than that seen in the dark but less than that seen in continuous R, suggesting that PfrB is responsible for randomizing hypocotyl orientation.

To test whether randomization is a function of Pfr concentration, we studied the effect of different phytochrome photoequilibria, set up by varying the R:FR ratio of the incident light, on the SDs of hypocotyl growth around 0° (vertical) (Fig. 2). Under the highest R:FR ratio the SDs of hypocotyl angle are as follows: in Nossen, 23.3; in Z15, 33.2; in ABO, 71.6; and in D2, 65.6. Under the lowest R:FR ratios the SDs decrease to 13.8, 17.8, 35.9, and 36.4, respectively. These data indicate that decreasing the phytochrome photoequilibrium by decreasing the R:FR ratio causes a decrease in the SDs of hypocotyl orientation. The decrease is more pronounced in lines with high levels of phyB (ABO and D2); however, all lines are responsive to the R:FR ratio. Higher levels of expression of the introduced *PHYB* gene result in larger SDs, or greater randomization, under all R:FR ratios.

These results indicate that the removal of PrB is not the mechanism responsible for the loss of negative gravitropism in R but, conversely, that PfrB induces randomization. This randomization, when phyB is present at high levels, is sufficient to overcome the default mechanism that imposes negative gravitropism in the absence of both phyA and phyB.

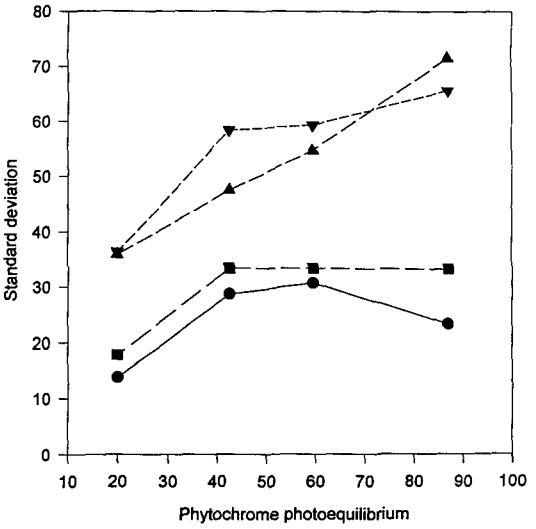

Figure 2. The SDs of hypocotyl orientation around the vertical of WT and transgenic lines expressing different levels of phyB under different R:FR ratios. Light was provided as two pulses of 1 h per 24-h period, between which seedlings were maintained in darkness. The phytochrome photoequilibrium was measured as described in "Materials and Methods." Seedlings and data were handled as described in "Materials and Methods." The SDs of hypocotyl orientation of lines expressing low levels of phyB, WT (●) and Z15 (■), were low, indicating that orientation was clustered around the vertical. The SDs of hypocotyl orientation of lines expressing high levels of phyB, ABO (▲) and D2 (▼), were high, indicating randomization and lack of negative gravitropism. All strains appear to respond to the R:FR ratio, showing decreased SDs of hypocotyl angles under low R:FR ratio compared to high R:FR ratio.

Table III. *Effect of dark, continuous R, and continuous FR on growth orientation of wild-type and transgenic lines expressing different levels of phyB*

Seedlings and data were handled as described in "Materials and Methods." The orientation of hypocotyl angle was measured as the SD around the vertical 0°. Values represent the pooled SD for a minimum of 150 seedlings from at least three replicate experiments. High SDs indicate randomization of hypocotyl growth. The genotypes are as described in "Materials and Methods." The approximate relative levels of expressed phyB are: Nossen, 1; Z15, 15; ABO, 30; and D2, 60.

Genotype	Treatment	SD
Nossen	Dark	24.5
Nossen	Continuous R	52.3
Nossen	Continuous FR	25.4
Z15	Dark	18.57
Z15	Continuous R	65.6
Z15	Continuous FR	22.1
ABO	Dark	19.3
ABO	Continuous R	71.9
ABO	Continuous FR	40.3
D2	Dark	19.8
D2	Continuous R	55.8
D2	Continuous FR	48.2

The Effects of phyA on Gravitropism

Comparison of the results for the *phyB* single mutants and the *phyA/phyB* double mutants in Tables I and II indicates that phyA is also capable of inducing randomization. In the RLD ecotype the SD of hypocotyl angles in the *phyB* mutant increases from 22.1 to 58.1, comparing dark and continuous R growth conditions. Even in the presence of the second-site mutation in the *phyB-1* mutant of Landsberg *erecta*, an increase in randomization is seen in R from 79.4 to 92.8, which must be due to the action of phyA.

Table IV shows the SD of hypocotyl orientation of WT and transgenic Arabidopsis expressing increased levels of phyA. Overexpression of *PHYA* cDNAs leads to randomization of hypocotyl orientation. Unlike overexpression of *PHYB* cDNAs, this randomization is seen in the dark and is largely unaffected by growth under continuous R or FR, although higher levels of phyA are associated with higher degrees of randomization under all growth conditions. Thus, as the level of phyA relative to that of phyB increases, there is an increased tendency to randomization in the dark, or under continuous FR, so that in the highest expressers (21K15), randomization in the dark is as great as that under continuous R. All treatments involved a pre-

Table IV. *Effect of dark, continuous R, and continuous FR on growth orientation of wild-type and transgenic lines expressing different levels of phyA*

Seedlings and data were handled as described in "Materials and Methods." The orientation of hypocotyl angle was measured as the SD around the vertical 0°. Values represent the pooled standard deviation for a minimum of 150 seedlings from at least three replicate experiments. High SDs indicate randomization of hypocotyl growth. The genotypes are as described in "Materials and Methods." The levels of expressed phyA are Nossen < 13K7 < 21K15.

Genotype	Treatment	SD
Nossen	Dark	24.5
Nossen	Continuous R	52.3
Nossen	Continuous FR	25.4
13K7	Dark	44.5
13K7	Continuous R	61.6
13K7	Continuous FR	51
21K15	Dark	67.4
21K15	Continuous R	62.8
21K15	Continuous FR	57.1

treatment with W to stimulate germination. When this was replaced by treatment with GA (Table V), dark randomization was not seen, suggesting that the randomization observed in the dark treatments was due to a phyA-mediated VLFR induced by the W pretreatment. This conclusion is supported by studies of the mutants, particularly the RLD ecotype in which the absence of phyA in the single and double mutants is associated with less randomization in dark growth conditions (Table II) and may reflect the absence of a phyA-mediated VLFR stimulated by the W pretreatment. Dark randomization in lines overexpressing *PHYA* cDNAs is not analogous to that seen in the *phyB-1* mutant, which when pretreated with GA retains dark randomization (Table V). These data suggest that the predominant activity associated with the action of phyA in hypocotyl gravitropism is randomization induced by a VLFR.

When one interprets data from lines overexpressing cDNAs, an account must be taken of the possibility that an aberrant phenotype being displayed is due to ectopic expression of the cDNA. At present there are no examples of problems due to ectopic expression involving phytochrome genes, and there are several experiments that suggest that phytochromes behave normally when overexpressed. First, phyA expressed by a transgene in Arabidopsis maintains the ability to display a FR high-irradiance response and is light labile (Boylan and Quail, 1991). Second, phyB expressed by a transgene has been shown to be spectrally active in transgenic Arabidopsis, undergoing R:FR-dependent conformational changes and to be stable in the light (Wagner et al., 1991). Third, the dominant negative suppression of Arabidopsis photoresponses by mutant sequences of *PHYA* genes was not due to aberrant expression patterns for either the introduced or endogenous Arabidopsis phytochromes (Boylan et al., 1994). Other possible mechanisms, such as co-suppression and competition for a limiting amount of chromophore, were also effectively discounted.

In this study overexpressed phyB remains responsive to R:FR ratio in regulating the gravitropic response of hypocotyls. Such a response to R:FR ratio is characteristic of the action of phyB. Overexpression of the *PHYB* cDNA does not cause negative gravitropism in the dark, which means that overexpressed PrB is not affecting the response seen in the WT. Additionally, Z15 behaves similarly to WT in all experiments despite the fact that the *PHYB* cDNA will be expressed ectopically in this line. Consequently, the effects of overexpressing the *PHYB* cDNA in the lines ABO and D2 are most likely due to the level of expression rather than its ectopic nature.

Interpretation

The data presented here clearly demonstrate that either phyA or phyB is capable of causing randomization of hypocotyl growth orientation. The data in Tables I and II concerning mutants demonstrate that in the absence of phyA R causes randomization through the action of phyB, which would be classically interpreted as being due to the conversion of PrB to PfrB. In the *phyA* mutants of both ecotypes the degree of randomization in continuous R is similar to that in the corresponding WT, indicating that phyB is able to substitute for the action of phyA. It is interesting that the converse is not true in the RLD ecotype, in which less randomization is seen in continuous R in the absence of phyB, suggesting that phyA cannot fully substitute for phyB.

Overproduction of phyB leads to increased sensitivity to R in the randomization of growth orientation; furthermore, growth orientation is a function of the photoequilibrium established by exposure to mixtures of continuous R and FR. Liscum and Hangarter (1993) reached the conclusion, logically correct on the basis of the data available to them, that the Pr form of phyB imposed negative gravitropism and that it was the removal of PrB by light, rather than the production of PfrB, that led to randomization. This conclusion was strongly dependent on the finding that the *hy2* mutant, which presumably contains only phytochrome apoproteins, is negatively gravitropic in both light and dark. According to the Liscum and Hangarter hypothesis, it is proposed that the PhyB apoprotein has the action putatively assigned to PrB in imposing negative gravitropism. Our data show that negative gravitropism occurs in the absence of phyB and therefore does not require PrB. Moreover, the activity associated with the action of phyB as shown by the mutants and overexpressing lines is randomization of hypocotyl orientation.

Table V. *Hypocotyl orientation of phyB-1, phyA overexpresser, and wild type when induced to germinate using GA and grown in complete darkness*

Seedlings and data were handled as described in "Materials and Methods." The orientation of hypocotyl angle was measured as the SD around the vertical 0°. Values represent the pooled SD for a minimum of 150 seedlings from at least three replicate experiments. High SDs indicate randomization of hypocotyl growth.

Genotype	SD
Landsberg *erecta*	14.8
phyB-1	65.3
Nossen	10.2
21K15	11.3

The highly randomized growth seen in the *phyB-1* allele in Landsberg *erecta* is not consistent with at least two other *phyB* alleles; *phyB-6* in RLD and *phyB-7* in Landsberg *erecta*. Consequently, it seems likely that *phyB-1* contains a second-site mutation that affects negative gravitropism. When the *phyB-1* mutant was germinated via a GA pretreatment in the complete absence of light, randomization persisted, confirming that this action was not associated with the action of phytochrome.

Because of the presence of a second-site mutation in the *phyB-1* mutant that results in high levels of randomization, it was important to determine the action of phyA in the absence of this mutation. In the RLD ecotype, phyA is clearly able to induce randomization of hypocotyl orientation in continuous R in the absence of phyB. In the phyA overproducers, dark randomization is seen, which is greater in the higher level expressers but is absent when the W pretreatment is replaced by GA pretreatment, indicating that "dark randomization" is actually a VLFR.

In conclusion, therefore, we propose that negative gravitropism is the default situation in Arabidopsis hypocotyls and that either PfrA or PfrB, produced by photoconversion of PrA or PrB, can cause randomization. No other phytochrome is apparently capable of this action. In this respect, the induction of randomization is a normal, phytochrome-mediated response, which, like most others in Arabidopsis, requires continuous irradiation for maximum effect. There is no requirement to invoke action of the Pr form of either phyA or phyB in this response. The dark randomization of hypocotyl orientation observed in dark treatments in the *phyB-1* mutant and in lines overexpressing *PHYA* cDNAs were induced by different mechanisms. In the former, randomization is probably due to a second-site mutation; in the latter, randomization is due to a phyA-mediated VLFR.

It is now possible to speculate that the function of phyA and phyB in the developing seedling with respect to gravitropism is to switch off negative gravitropism in the presence of light, thereby allowing other phototropic stimuli to determine the orientation of growth. The randomized state may simply occur because of the lack of phototropic stimuli to replace gravitropism, i.e. as a consequence of using monochromatic light, rather than represent a desired response induced in a developing seedling.

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