Phytochrome A Enhances the Promotion of Hypocotyl Growth Caused by Reductions in Levels of Phytochrome B in Its Far-Red-Light-Absorbing Form in Light-Grown Arabidopsis thaliana

Jorge José Casal*
Departamento de Ecología, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martin 4453, 1417-Buenos Aires, Argentina

We sought to determine if phytochrome B (phyB)-mediated responses to the red light (R)/far-red light (FR) ratio are affected by phytochrome A (phyA) activity in light-grown seedlings of Arabidopsis thaliana. Pulses of FR delayed into the dark period were less effective than end-of-day (EOD) FR in promoting hypocotyl growth over a given period in darkness. White light minus blue light interposed instead of darkness between the end of the white-light photoperiod and the FR pulse was sufficient to maintain responsivity to the red light (R)/far-red light (FR) ratio. In sunlight, phyA seedlings of Arabidopsis responded to a range of high EOD R/FR ratios, whereas the phyA mutant required stronger reductions in the EOD R/FR ratio. In etiolated Arabidopsis seedlings responding to a single EOD R+FR pulse in WT Arabidopsis, cucumber, mustard, sunflower, tobacco, and tomato, but not in phyA Arabidopsis or in the aurea mutant of tomato. WT seedlings of Arabidopsis showed no response to the "early warning" signals of neighboring vegetation, and hypocotyl-growth promotion occurred at higher plant densities than in the WT. Thus, under a series of light conditions, the sensitivity or responsivity to reductions in the R/FR ratio were larger in WT than in phyA seedlings. A product of phyA is therefore proposed to enhance the hypocotyl-growth response to decreases in phyB in FR-light-absorbing form in light-grown seedlings.

A strong co-action between phyA and phyB can be observed during de-etiolation (Casal, 1995). Hypocotyl growth in etiolated Arabidopsis seedlings is unaffected by a single R or FR pulse predicted to establish divergent levels of phyB Pfr. Seedlings pretreated with continuous FR respond to a subsequent R but not to a FR pulse. The continuous FR pretreatment is perceived by phyA, and the terminal R pulse is perceived by phyB. Thus, the final response depends on the interdependent co-action of phyA and phyB (Casal, 1995). Responsivity amplification toward phyB Pfr can also be achieved with blue-light pretreatments perceived by the putative photoreceptor coded by the HY4 gene (Casal and Boccalandro, 1995). In etiolated seedlings emerging from the soil, the requirement for prolonged exposure to light absorbed by phyA to couple phyB to the control of hypocotyl growth could avoid large responses to transient gaps in the soil surface.

phyA and phyB may have some interaction not only in etiolated but also in light-grown seedlings. Neither phyA nor phyB mutant seedlings of Arabidopsis show hypocotyl-growth response to "early-warning" signals of neighboring vegetation (Yanovsky et al., 1995). Overexpression of phyA increases the sensitivity to small drops in EOD R/FR (Casal et al., 1995). The aurea mutant of tomato, which is not a phyA mutant but has spectrally active phyB and no spectrally active phyA (Sharma et al., 1993), shows impaired sensitivity to small reductions in the EOD R/FR ratio (Casal and Kendrick, 1993). The purpose of this work was to investigate the hypothesis that phyA activity enhances hypocotyl-growth responses to R/FR ratios perceived by phyB in light-grown seedlings. Two predictions based on this hypothesis were tested. First, the response to lowering phyB Pfr levels should decay after a white-light-to-dark transition, because in darkness the level of phyA Pfr is expected to decrease (due to destruction without phototransformation of newly synthesized phyA Pfr) and the cycling of phyA ceases. Second, the response to changes in the R/FR ratio should be impaired in the phyA mutant. Different R/FR treatments were provided largely as pulses.

Five phytochrome apoprotein genes are present in Arabidopsis thaliana (L.) Heyn. (Sharrock and Quail, 1989; Clack et al., 1994). De-etiolation under very dense canopies, a FR-rich environment, depends on phyA (Yanovsky et al., 1995), which contributes to hypocotyl growth inhibition even in seedlings grown under high R/FR ratios (Johnson et al., 1994; Yanovsky et al., 1995). phyB is involved in the perception of R/FR signals of neighboring vegetation in Arabidopsis and cucumber (Ballaré et al., 1991; Whitelam and Smith, 1991; Yanovsky et al., 1995). We sought to determine if phyB-mediated responses to the R/FR ratio are modulated by phyA activity in light-grown seedlings.

Abbreviations: E, putative product of phyA activity; EOD, end of day; FR, far-red light; Pfr/P, proportion of phytochrome in its far-red light-absorbing form; phyA, phytochrome A; phyB, phytochrome B; R, red light; WT, wild type.

* E-mail jjcasal@criba.edu.ar; fax 541-521-1384.

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followed by darkness, avoiding the use of supplementary FR during daytime. Supplementary FR may cause a stronger promotion of hypocotyl growth in phyA mutant seedlings than in the WT, probably due to a high-irradiance reaction of phyA in the WT, partially counteracting the promotion of hypocotyl growth caused by lowering phyB Pfr levels (Johnson et al., 1994; Yanovsky et al., 1995).

**MATERIALS AND METHODS**

**Protocol 1**

Two basic protocols were used for growth-room experiments; one involved growth measurements over a single night, and the other integrated the effects of light treatments over several days. For protocol 1, seeds of WT Arabidopsis thaliana (L.) Heynh. cv Landsberg erecta, or of the phyA-201 (Nagatani et al., 1993), phyB-1, or phyB-5 mutants (Koornneef et al., 1980; Somers et al., 1991; Reed et al., 1993) were sown in plastic pots (2.5 cm diameter, 3.5 cm height) filled with silty clay soil watered to field capacity. The pots were sown in pots (2.5 cm diameter, 3.5 cm height) filled with silty clay soil. The length of the internodes was measured from the cotyledonary node to the uppermost leaf insertion of hypocotyl growth after a white-light-to-dark transition was calculated as the difference of hypocotyl length measured with a ruler to the nearest 0.5 mm, or under a magnifying glass to the nearest 0.25 mm. The 10 tallest seedlings were averaged per box (i.e. per replicate).

**Greenhouse Experiments**

To investigate the response to natural changes in the R/FR ratio, seeds of WT or phyA-201 Arabidopsis were sown in pots as indicated for protocol 1. The number of seeds per pet was varied to modify plant density or to include small “fences” of WT seeds at one side of the tagged seed. After incubation in darkness at 7°C (3 d), the pots were exposed to a R pulse, incubated in darkness at 25°C (24 h), and transferred to the greenhouse. Final hypocotyl length was measured 10 d later.

**Light Sources**

White light (250 μmol m⁻² s⁻¹) was provided by high-pressure sodium lamps (Philips SON, Buenos Aires, Argentina). White light minus blue light (190 μmol m⁻² s⁻¹) was provided by the white-light source in combination with one orange and one yellow acetate filter (La Casa de 1 Acetato, Buenos Aires, Argentina). Calculated phytochrome photoequilibria (Pfr/P) (Casal, 1995) were 85 and 86%, respectively, for orange and yellow. Scans are shown by Casal and Boccalandro (1995) as orange plus blue light and orange light. Sources for R, FR, or R+FR pulses (calculated Pfr/P = 87, 3, and 61 or 33, respectively) were as described previously (Casal, 1995). Fluence rates were 35 to 50 μmol m⁻² s⁻¹. The duration of the EOD pulse was 10 min, and that of the hourly pulses was 3 min.

**RESULTS**

**The Ability to Respond to a Pulse of FR Is Lost in Darkness**

As a prerequisite, the involvement of phyB in the control of hypocotyl growth after a white-light-to-dark transition (see Nagatani et al., 1991; López-Juez et al., 1992) was confirmed for the two basic protocols used for growth-room experiments with Arabidopsis. For protocol 1, hypocotyl-length increment (mm): WT, EOD R = 0.7 ± 0.2, EOD FR = 1.3 ± 0.2; phyB-1, EOD R = 1.4 ± 0.3, EOD FR = 1.5 ± 0.2. For protocol 2, hypocotyl length (mm): WT, EOD R = 3.5 ± 0.2, EOD FR = 5.7 ± 0.1; phyB-5, EOD R = 6.2 ± 0.2, EOD FR = 5.9 ± 0.1. Any direct contribution to EOD responses by phytochromes other than phyB was below the detection level.

If phyA activity were necessary for full phyB-mediated responses, the latter should be (a) impaired in WT seedlings not exposed to light absorbed by phyA, and (b) recovered after exposure to this light in WT but not in phyA-mutant seedlings. Light-grown plants of Arabidopsis, cucumber, and mustard were given either a R or a FR EOD pulse and transferred to darkness. Over the first 24 in darkness, the rate of stem extension growth (in mm) was higher in FR- than in R-treated plants (Arabidopsis, R = 0.7 ± 0.1, FR = 1.4 ± 0.1; cucumber, R = 5.5 ± 0.4, FR =
12.3 ± 1.3; mustard, R = 2.4 ± 0.3, FR = 5.1 ± 0.5). After incubation in darkness for 24 h, the seedlings were exposed to a second R or FR pulse (18–25 mmol m⁻² s⁻¹) in factorial combination with the EOD light pulse (Fig. 1A, top). Between 24 and 48 h after the beginning of darkness, stem growth continued responding to the first pulse but was not obviously affected by the second light pulse (Fig. 1A). The rate of hypocotyl growth in darkness was set by the EOD light treatments, but was not reset by another light pulse 24 h later. The lack of detectable response to a R pulse after EOD FR may be due in part to the persistent promotion of stem growth caused by incubations with low Pfr levels (Casal and Smith, 1988a). The response to a FR pulse delayed into the dark period was calculated as the proportion of the effect of an EOD R pulse compared with an EOD FR pulse over the same period (i.e. length increment was measured between 6 and 30 h, 12 and 36 h, etc., after the transition to darkness). The promotion induced by FR was partial after 6 h in darkness and null after 12 or 18 h (Fig. 1B), as well as after 24 h (see Fig. 1A).

In other experiments, WT and phyA-201 Arabidopsis seedlings were exposed to three 7-h photoperiods, each followed by EOD R, EOD FR, or EOD R plus a subsequent FR pulse delayed 3, 6, or 9 h into the dark period. In both genotypes, the response to a FR pulse decreased gradually when the light treatment was delayed into the dark period (Fig. 2). A FR pulse given after 9 h in darkness was ineffective, despite the fact that this treatment established low proportions of phyB Pfr during the subsequent 8 h of darkness. The proportion of the maximal response (i.e. EOD FR versus EOD R = 100%) was calculated for each independent repetition of the experiment (four trials), averaged, and used for statistics. The decrease in response to a FR pulse was faster in seedlings lacking phyA (compare percentages in Fig. 2, top and bottom).

**phyA Activity Maintains the Ability to Respond to a FR Pulse**

Arabidopsis seedlings were exposed to three 7-h photoperiods, each followed either by EOD R with or without a FR pulse delayed 9 h into the dark period (Fig. 3A), or by 9 h of phytochrome-absorbable radiation (i.e. white light minus blue light, providing a calculated Pfr/P similar to that of EOD R) with or without a final FR pulse (Fig. 3B). No response to FR delayed 9 h into the dark period was observed in WT or phyA seedlings (Fig. 3A). In contrast, when WL was followed by white light minus blue light, a significant response to the subsequent FR pulse occurred in the WT, but not in the phyA mutant (Fig. 3B). As expected, the response to a FR pulse after white light minus blue light was also absent in phyB mutant seedlings (hypocotyl length in mm: no FR = 5.0 ± 0.1, FR = 5.1 ± 0.1). Taken together, these observations indicate that 8 h of low phyB Pfr levels is sufficient to induce a response if a light signal precedes the drop in phyB Pfr, and that phyA is involved in the perception of this light signal.

The latter phenomenon was investigated in further detail in seedlings grown for two photoperiods under white light and exposed during the time corresponding to the third

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**Figure 1.** Reduced effect of FR pulses delayed into the dark period compared with those given at the EOD. Seedlings of Arabidopsis, cucumber, and mustard were grown in pots under photoperiods of 16 h for 3, 6, or 14 d (respectively), and given a R or a FR pulse at the end of the last photoperiod (basic protocol 1). A, Stem-length increment after R or FR pulses given 24 h after the beginning of darkness in factorial combination with the EOD light pulses. B, Effect of FR delayed into the dark period relative to the effect of EOD FR. Length-increment measurements were always carried out during 24 h in darkness. Data are means ± SE of 22 to 25 (Arabidopsis), 19 to 21 (cucumber), or 7 (mustard) replicate plants.
photoperiod (16 h) to (a) darkness (Fig. 4A), (b) white light minus blue light (Fig. 4B), or (c) white light (Fig. 4C). At the end of this period the seedlings received a R or a FR pulse (Fig. 4, top). There was no response to FR compared with R in dark-adapted seedlings (Fig. 4A). White light minus blue light (i.e. phytochrome-absorbable radiation) was enough to re-establish the ability to respond to FR in the WT but not in the phyA mutant (Fig. 4B). When white light was given during the third photoperiod both genotypes responded normally (Fig. 4C). The bizarre observations that hypocotyl growth can actually be promoted by phyA (compare WT and phyA-201 in Fig. 4B) and by exposure to supplementary blue light (compare phyA-201 in Fig. 4, B and C) were confirmed using a different allele, pkyA-1 (Whitelam et al., 1993) (data not shown). It must be noted that the ability to respond to EOD FR was already present in seedlings exposed to white light for 2 d (hypocotyl-length increment in mm: WT = 0.7 ± 0.2; FR = 1.3 ± 0.2; phyA-201, R = 1.0 ± 0.2, FR = 1.6 ± 0.2). This ability was lost if neither phyA nor a blue-light photoreceptor were active during the 3rd d. To investigate whether the phyA mutation affects extension growth in darkness in a phyB background, phyB and phyA/phyB mutants were grown under white-light photoperiods, exposed over the third photoperiod to white light minus blue light (as in Fig. 4B), and transferred to darkness after the EOD R pulse. Hypocotyl growth in darkness was not reduced by the phyA mutation (length increment in mm: WT = 0.5 ± 0.1; phyB-1 = 1.0 ± 0.1; phyB-5 = 1.4 ± 0.1; phyA-201 phyB-1 = 2.1 ± 0.1; phyA-201 phyB-5 = 1.9 ± 0.2).

Effectiveness of EOD versus Hourly Light Pulses

Arabidopsis seedlings were grown for 3 d under photoperiods of 16 h and subsequently exposed to EOD R (Pfr/P = 87%) followed by 24 h of darkness, EOD R+FR (Pfr/P = 61%) followed by 24 h of darkness, or EOD R+FR followed by a 3-min R+FR pulse every hour throughout the night. Hourly pulses of a Pfr/P = 61% were expected to establish a level of phyB Pfr lower than EOD R and similar to EOD Pfr/P = 61%. In contrast to EOD R+FR treatment, in which phyA Pfr was destroyed in darkness, hourly pulses of R+FR maintained a certain level of phyA Pfr throughout the night as a result of a balance between destruction and phototransformation of newly synthesized phyA Pfr. Compared with EOD R, hourly pulses of R+FR caused a stronger promotion of hypocotyl growth than EOD R+FR in WT, but not in phyA Arabidopsis seedlings (Fig. 5A). The stronger effect of hourly light pulses was not noted in younger seedlings (data not shown), suggesting that a certain degree of de-etiolation was critical for this...
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Effect to occur. No significant differences between EOD and hourly R+FR were observed in the phyB-5 mutant (hypocotyl length increment in mm: 2.4 ± 0.3 and 2.0 ± 0.3, respectively).

The above protocol provides a tool with which to investigate whether phyA (or other phytochrome showing dark decay) is likely to have similar effects on the response to "light-stable" phytochrome(s) in light-grown plants of other species. A higher promotion of stem growth by hourly compared with EOD R+FR pulses was observed in WT cucumber, mustard, sunflower, tobacco, and tomato (Fig. 5A). The aurea mutant of tomato, which has spectrally active phyB but no spectrally active phyA (Sharma et al., 1993), showed no additional promotion by repeated pulses. Hourly R or FR pulses had the same effect as EOD pulses (Fig. 5B).

Figure 4. Response of WT and phyA-201 seedlings to a R versus a FR pulse as affected by darkness (A), white light minus blue light (B), or white light (C) during the time corresponding to the third photoperiod. After two white-light photoperiods the seedlings were transferred to the different light/dark conditions and then given a R or a FR pulse. Hypocotyl-length increment was measured during the subsequent 24 h in darkness (basic protocol 1). Data are means ± se of 7 to 8 (A) or 12 to 14 (B and C) replicate plants.

Figure 5. Response of WT and phyA-201 seedlings of Arabidopsis and seedlings of different species to EOD versus hourly pulses provided throughout the night. At the end of the last photoperiod the light-grown seedlings were given EOD light pulses (10 min) and transferred either to darkness or to hourly light pulses (3 min) for 24 h. Stem-length increment was calculated for this 24-h period (basic protocol 1). A, Data are means ± se of 10 (Arabidopsis), 24 (cucumber), 14 (mustard), 19 (sunflower, hypocotyl stage), 7 (sunflower, first internode stage), 9 (tobacco), or 18 (tomato) replicate plants. i, Internode growth; h, hypocotyl growth (younger seedlings). B, The number of replicates is 6 to 7 (Arabidopsis, cucumber) or 19 to 22 (mustard).
Impaired Sensitivity to EOD Reductions of the R/FR Ratio in the phyA Mutant

To investigate the sensitivity to EOD R/FR ratio signals, WT and phyA seedlings were exposed for 3 d to photoperiods of white light (7 h), each one terminated with an EOD light pulse. The increase in hypocotyl length caused by a FR, compared with a R, EOD pulse was similar for WT and phyA-mutant seedlings (Fig. 6A; see also Dehesh et al., 1993). The shape was compared by plotting the response induced by intermediate Pfr/P relative to the R versus FR response (i.e. the maximum response) against calculated Pfr/P. WT seedlings responded mainly in the range of high Pfr/P, whereas phyA-mutant seedlings responded largely in the range of low Pfr/P (Fig. 6A). A similar experiment was conducted with either WT tobacco seedlings or those transformed with the oat PHYA gene under the control of a constitutive promoter. The extent of response to EOD R versus FR was similar in WT and phyA-overexpressor seedlings (Fig. 6B). Overexpression of phyA exaggerated the response in the range of high Pfr/P (Fig. 6B; see also Casal et al., 1995).

Reduced Sensitivity to Neighbor Signals in the phyA Mutant

Pots were sown with different densities of either WT or phyA-mutant seeds. One-day-old seedlings were transferred from darkness to a greenhouse and exposed to natural photoperiods. The final length of the hypocotyl is shown in Table I. Compared with low-density controls (<0.4 plants/cm²), relatively small increments of plant density promoted hypocotyl growth in WT seedlings. The range of response was shifted toward higher densities in the phyA mutant. Thus, the absence of phyA reduced sensitivity to plant density signals.

In another experimental setting, one WT or phyA mutant seed was sown at the center of each pot. A dense row of WT seeds was placed approximately 2 mm to the south of tagged WT or phyA seeds in half of the pots. One-day-old seedlings were transferred to the greenhouse. The presence of a green fence placed to the south of the plants increased hypocotyl length in WT seedlings (see also Ballaré et al., 1987; Casal and Kendrick, 1993), but had no significant effects in the phyA mutant (Table II; see also Yanovsky et al., 1995).

DISCUSSION

phyA Activity Affects Stem Growth Promotion Caused by Lowering phyB Pfr

Low EOD R/FR ratios release hypocotyl growth from the inhibition imposed by phyB Pfr (Nagatani et al., 1991; López Juez et al., 1992). The presence of active phyA is necessary for maximum sensitivity or responsivity toward reductions in phyB Pfr, as indicated by the effects of light compared with darkness on the subsequent response to a FR pulse in WT seedlings, and the higher responsivity or sensitivity in WT compared with phyA seedlings.

In darkness, destroyed phyA Pfr is not replaced by phototransformation of newly synthesized phyA Pr, and no phyA cycling occurs. Thus, the putative active components of phyA are predicted to decrease after the end of the photoperiod. In WT seedlings of Arabidopsis, cucumber, and mustard a dark period interposed between the EOD R
Table I. Hypocotyl length in WT and phyA-201 seedlings of Arabidopsis grown at different densities

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<th>Plant Density</th>
<th>Hypocotyl Length</th>
<th>phyA-201</th>
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<tr>
<td>cm⁻²</td>
<td>WT mm</td>
<td>phyA-201</td>
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<td>&lt;0.4</td>
<td>3.4 ± 0.2</td>
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<td>0.4–1.6</td>
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<td>1.6–3.2</td>
<td>4.9 ± 0.2</td>
<td>4.8 ± 0.2</td>
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<td>&gt;3.2</td>
<td>4.8 ± 0.3</td>
<td>6.1 ± 0.3</td>
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The impaired growth responses to reduced phyB Pfr are not the simple consequence of higher background growth rates in the phyA mutant.

In some experimental conditions (Figs. 2, 3, 5, and 6; Table I) the lack of normal responses to lowering phyB Pfr approximately counteracted the already higher rates of hypocotyl growth in the phyA mutant. On this basis one might speculate that the higher background growth rates and the lack of normal responses to lowering phyB Pfr in the phyA mutant (i.e. the two differences between it and WT, mentioned above) could be manifestations of the same phenomenon. For instance, a certain degree of hypocotyl growth inhibition could depend on the co-action between phyA activity and phyB Pfr, as was observed in etiolated seedlings (Casal, 1995). However, this model is only partially consistent with available data. The bizarre observation that hypocotyl-growth rate can actually be lower in the phyA mutant than in the WT after EOD FR (Fig. 4B; J.J. Casal, unpublished observations with the phyA-1 mutant) cannot be accounted for by this scenario. In addition, according to this model the decrease in promotion of hypocotyl growth observed in WT seedlings when a FR pulse is delayed into the dark period should be accompanied by

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<th>Condition</th>
<th>Hypocotyl Length</th>
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<td>WT mm</td>
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<tr>
<td>Isolated control</td>
<td>2.2 ± 0.2</td>
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<td>Neighbors added</td>
<td>3.7 ± 0.1</td>
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Whetherell, 1968), Fuschia hybrida (Vince-Prue, 1977), Phaseolus vulgaris, and Glycine max (Buck and Vince-Prue, 1985) prolonged R+FR provided during the night was also more effective in promoting stem growth than an EOD R+FR pulse.

When Arabidopsis seedlings were exposed to a series of EOD R/FR ratios (and calculated Pfr/P), hypocotyl-growth promotion was less sensitive in the phyA mutant than in the WT compared with the control exposed to EOD R (Fig. 6A). The aurea mutant of tomato is also less sensitive to reductions in EOD R/FR than WT seedlings (Casal and Kendrick, 1993). Transgenic tobacco and tomato seedlings overexpressing Avena phyA are more sensitive to reductions in EOD R/FR ratio than their corresponding WT (Casal et al., 1995) (Fig. 6B). Thus, a direct correlation between phyA levels and sensitivity toward reductions in EOD R/FR ratio is observed across different species. Similarly, the phyA-1 (Yanovsky et al., 1995) and phyA-201 (Tables I and II) mutants of Arabidopsis and the aurea mutant of tomato (Casal and Kendrick, 1993) show reduced sensitivity to neighboring signals.
increasing rates of stem growth in darkness. In other words, there would be no promotion by FR because the promotion already occurred due to the lack of phyA activity. This was not the case, however; after EOD R, a FR pulse delayed 24 h in darkness had no effect on extension growth between 24 and 48 h after the white-light-to-dark transition, but during the same period seedlings exposed to EOD R grew less than during the first 24 h in darkness (see "Results") and less than the seedlings exposed to EOD FR (Fig. 1A) (see also Casal and Smith [1989] for detailed extension-growth kinetics in darkness in fully de-etiolated mustard). In summary, it is not clear whether phyA-mediated responsiveness amplification is necessary only once, or if it is still required after de-etiolation is completed. In either case, responsiveness amplification toward phyB Pfr cannot account for the novel phenomenon described here.

Evidence That phyA Activity Enhances the Promotion Caused by Reductions in phyB Pfr

The observations presented here can be accounted for by an alternative model, in which E enhances the promotion of stem growth in response to reductions in phyB Pfr. A blue-light photoreceptor should be able to yield a product with a similar function, since some consequences of the absence of phyA are obvious only in the absence of blue light as well (e.g. Fig. 4) (see also Casal and Smith, 1988b). According to this model, E should be produced during the day and decay during the night (Figs. 1 and 2). Thus, after prolonged darkness, when neither phyA nor a blue-light photoreceptor produce E, FR is unable to promote growth (Figs. 1, 2, 3A, and 4A). E appears more necessary for the initiation of the release of hypocotyl growth from inhibition than for the continuation of the elevated growth rates: a FR pulse given after 24 h in darkness is unable to promote growth between 24 and 48 h, but after EOD FR the stem growth rate remains high between 24 and 48 h in darkness (Fig. 1A). The phyA/phyB double mutant of Arabidopsis grows tall in darkness, even after white light minus blue light, again suggesting that E participates particularly in the release from inhibition by phyB (which has not been established in the double mutant). The release from growth inhibition might require more than the mere reversal of the steps necessary to establish the inhibition.

Under the present conditions, the maximum promotion of hypocotyl growth was achieved by different combinations of intensity of the reduction in phyB Pfr/P and the level of putative factor E produced by phyA and the blue-light photoreceptor(s). When the level of phyB Pfr was reduced to a minimum by EOD FR, the absence of phyA had no significant effect on the extent of response (Figs. 2, 4C, and 6, A and C) (see also Dehesh et al., 1993), provided that blue light was present immediately before the FR pulse (Figs. 3B and 4, B and C). When the reduction of phyB Pfr was more modest the lack of phyA impaired the promotion of hypocotyl growth, even after white light was administered (Fig. 6, B and D). When phyA was activated by hourly pulses during the night (Fig. 5A), the extent of promotion caused by small drops in phyB Pfr (e.g. from Pfr/P = 87 to 61%) was as large as the promotion caused by EOD FR (Pfr/P = 3%) (Fig. 5B).

phyA does not appear to control the levels of E by setting an endogenous circadian rhythm. Compared with EOD FR, the effect of a FR pulse delayed 6 h into the dark period was intermediate and no promotion by a FR pulse given 12, 18, or 24 h after a white-light-to-dark transition was observed (Fig. 1). Furthermore, in darkness the response to FR decreased with different intensity but similar shape in WT and phyA seedlings (Fig. 2).

Several physiological phenomena are mediated by phyA: (a) very-low-fluence responses, independent of phyB (Casal, 1995; Botto et al., 1996); (b) high-irradiance responses independent of phyB (McCormac et al., 1993; Dehesh et al., 1993; Nagatani et al., 1993; Whiteman et al., 1993); (c) responsivity amplification (via a high-irradiance response) toward phyB Pfr (Casal, 1995; Casal and Boccalandro, 1995); and (d) enhancement of the response to reductions in phyB Pfr (this paper). This information provides a basis for further studies (e.g. investigation of the loci of perception, analysis of the effects using molecular markers, isolation of specific mutants) to investigate whether phyA operates via single or multiple transduction chains.

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