Plant Physiol. (1997) 114: 637–641

Antagonistic but Complementary Actions of Phytochromes A and B Allow Optimum Seedling De-Etiolation[1]

Harry Smith*, Yong Xu[2], and Peter H. Quail

Department of Botany, University of Leicester, Leicester, LE1 7RH United Kingdom (H.S.); United States Department of Agriculture, Plant Gene Expression Center, Albany, California 94710 (Y.X., P.H.Q.); and Department of Plant Biology, University of California, Berkeley, California 94720 (P.H.Q.)

Using dichromatic radiation, we show that the actions of phytochromes A and B (phyA and phyB) in *Arabidopsis thaliana* are antagonistic in mediating red and far-red radiation effects on seedling de-etiolation and yet act in a complementary manner to regulate de-etiolation, irrespective of spectral composition. At low phytochrome photoequilibria inhibition of hypocotyl extension was strong, because of the action of a far-red high-irradiance response mediated by phyA. At high phytochrome photoequilibria inhibition of hypocotyl extension was also strong, because of the action of phyB. At intermediate photoequilibria hypocotyl inhibition was less strong. In their natural environment, this dual action will strongly retard hypocotyl growth and promote cotyledon opening and expansion both in open daylight and under dense vegetation. Overlapping action by phyA and phyB will substantially promote de-etiolation in sparse vegetation. The antagonistic and complementary actions of phyA and phyB, therefore, allow the optimum regulation of seedling growth after emergence from the soil.

Seedlings of most higher plants, after germination under the soil, display an etiolated pattern of growth and development. Etiolation may be regarded as an adaptation to life in the absence of radiant energy, in which stem extension is favored at the expense of leaf development. Etiolation increases the probability that the leaves may be elevated above the soil surface and become photoautotrophic before the resources of the seed are depleted. De-etiolation and the successful establishment of photoautotrophy require the sensitive detection of signals from the light environment, so that stem elongation may be inhibited, leaf development stimulated, and the formation of effective photosynthetic machinery initiated. Several photoreceptors are involved in the overall process of de-etiolation, including the blue/UV-light-absorbing cryptochromes, the R- and FR-absorbing phytochromes, and intermediates in the biosynthetic pathway of the chlorophylls. Understanding the individual roles of these photoreceptors is important toward realizing a general elucidation of the processes contributing to successful seedling establishment under natural conditions.

The signal-perception roles of the phytochromes have become a fertile area of research in recent years, particularly following the isolation and characterization of mutants lacking individual members of the phytochrome gene family (Koornneef et al., 1980) and the characterization of the genes encoding the family members (Sharrock and Quail, 1989). In Arabidopsis studies of the *phyA* and *phyB* mutants have led to hypotheses on the physiological functions of these members of the phytochrome family (Whitelam and Harberd, 1994; Smith, 1995; Furuya and Schäfer, 1996). phyA is proposed to be responsible for the response of plants to continuous FR radiation, a response known as the FR-HIR. It is also proposed that phyA is responsible for the responses of dark-imbibed seeds and dark-grown seedlings to extremely small amounts of light, the so-called VLFRs (Botto et al., 1996; Parks et al., 1996; Shinomura et al., 1996; Mazella et al., 1997). On the other hand, phyB is responsible for the FR-reversible responses of etiolated seedlings to pulses of low-fluence R (the so-called low-fluence responses; Furuya and Schäfer, 1996), or to continuous R radiation (Quail et al., 1995), and is one of the phytochromes responsible in established plants for the perception of FR radiation reflected from neighbors, and the consequent induction of shade-avoidance responses (Smith, 1995). Both phyA and phyB, therefore, have roles in de-etiolation.

Recently, it has become acknowledged that phyA and phyB act antagonistically in regulating hypocotyl extension growth (Quail et al., 1995). In a *phyA* mutant continuous R absorbed by phyB initiates de-etiolation and inhibits hypocotyl elongation, but this response is inhibited by simultaneous irradiation with continuous FR, which is also absorbed by phyB. In contrast, in a *phyB* mutant continuous FR absorbed by phyA inhibits hypocotyl extension, and this response is reduced by continuous R, which is also absorbed by phyA. This latter response was

[1] H.S. was the recipient of a University of California Miller Foundation Visiting Professorship during the course of this work. This work was supported by the Department of Energy, Office of Basic Energy Sciences grant no. FG03-96ER13742, National Institutes of Health grant no. GM47475, and U.S. Department of Agriculture Current Research Information System grant no. 5335-21000-006-00D.

[2] Present address: Qiagen, Inc., 9600 De Soto Avenue, Chatsworth, CA 91311.

* Corresponding author; e-mail has@le.ac.uk; fax 44–116–252–2791.

Abbreviations: FR, far-red light; FR-HIR, far-red-light high-irradiance response; HIR, high-irradiance response; Pfr/P, phytochrome photoequilibrium; phyA, phyB, phytochrome A and B, respectively; R, red light; R:FR, ratio of photon irradiance in the R and FR radiation bands; VLFR, very-low-fluence response.

classically demonstrated by the dichromatic irradiation experiments of Hartmann (1966), when he showed that hypocotyl extension in lettuce was regulated in such a manner that maximum inhibition occurred under mixed R and FR radiation that established an optimum, low concentration of Pfr.

The possible ecological function of the phyA-mediated FR-HIR has remained a mystery since Hartmann (1966) first described its essential characteristics, and it became even more difficult to understand when Arabidopsis *phyA* mutants were shown to be virtually indistinguishable from wild-type seedlings when grown in white light (Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993; Johnson et al., 1994). Recently, a putative role for phyA has been suggested by Yanofsky et al. (1995), who showed that the *phyA* mutant was less adaptable than wild-type seedlings when de-etiolating under dense canopy conditions. The evidence points to a role for phyA in de-etiolation, but its function should not be considered in isolation from that of phyB. In this paper we examine the functional antagonism between phyA and phyB in Arabidopsis and propose that this antagonism allows the two photoreceptors to act in a complementary manner to provide optimum de-etiolation responses to the light environment, almost irrespective of the relative amounts of R and FR in the actinic radiation.

MATERIALS AND METHODS

Plant Materials and Growth Regime

Seeds of the RLD ecotype of *Arabidopsis thaliana* were used as follows: wild-type (RLD); a phyA-null mutant (*phyA-101*) originally designated *hy8–1* (Parks and Quail, 1993) and referred to here as *phyA*; a phyB-null mutant originally designated as *hy3-Bo64* (Koornneef et al., 1980), later designated *phyB-1* and introgressed into RLD by T. Short, and referred to here as *phyB*; and the corresponding double mutant *phyA/phyB* (for *phy* mutant designations, see Quail et al. [1994]).

Seeds of each strain were sown in sectors in 9-cm Petri dishes on ½× Murashige and Skoog medium and allowed to imbibe for 4 d at 4°C in total darkness. Germination was stimulated by exposure to white light at approximately 100 μmol m^{-2} s^{-1} for 3 h and the dishes were returned to total darkness at 20°C for 2 d. The light treatments were then applied for a further 2 d, after which the seedlings were laid out for photography. Hypocotyl lengths were measured from the projected photographic images using a digitizing tablet and computer-aided analysis.

Light Treatments

Seedlings were exposed to continuous radiation either from a FR fluorescent bank or from an R-emitting diode source (LED; Quantum Devices, Inc., Barneveldt, WI) or simultaneously from both sources. The spectra of the radiation emitted by the two sources are shown in Figure 1. Spectra were monitored using a spectroradiometer (L1800, Li-Cor, Lincoln, NE). Control batches were held under the FR source, but were covered with aluminum foil. The dishes were oriented so that they were exposed to FR from above, and/or R from below, and radiation levels were measured with a radiometer/photometer (model 550–1, EG&G Gamma Scientific, San Diego, CA).

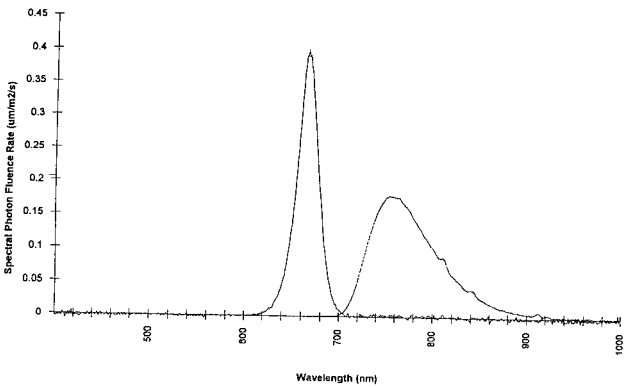

Figure 1. Spectral photon distributions of the bank of FR fluorescent lamps and the R-emitting diode assembly.

Pfr/P Measurements

Pfr/P, established by the single and mixed light sources, were measured using a crude phytochrome preparation from etiolated oats following the procedure of Parks and Quail (1993). The preparation was exposed to the light sources for 15 min in an ice bath, placed in a dark container, and returned to the laboratory where spectral reversibility measurements were made using a computerized dual-wavelength photometer with absorption maxima set at 660 and 730 nm (Pratt et al., 1985).

RESULTS

The experimental rationale of these investigations was simple: etiolated seedlings were exposed to monochromatic R or FR, or to dichromatic mixtures of R plus FR, and the resultant effects on hypocotyl extension were measured. In all cases the FR irradiance was kept constant and the R irradiance varied. In this way, seedlings were exposed to a range of mixed R and FR, establishing a corresponding range of Pfr/P. Samples of the raw data are shown in Figure 2, giving data for treatments with proportions of R and FR of 10:1, 0.1:1, and 0.02:1.

The behavior of all genotypes was predictable, based on current knowledge of the actions of phyA and phyB. The wild-type RLD showed inhibition of hypocotyl elongation under all the R, FR, and R plus FR treatments. Hypocotyl elongation in the *phyA* mutant was uninhibited by the monochromatic FR radiation, and showed similar responses to the RLD under R. However, under the mixed R plus FR source, the degree of inhibition of hypocotyl elongation was severely reduced as the ratio of R:FR decreased. Hypocotyl extension in the *phyA/phyB* double mutants was not inhibited under any of the monochromatic or dichromatic radiation sources. These data show that phyB operates to inhibit hypocotyl extension as a function of the photoequilibrium established, high Pfr/P causing inhibi-

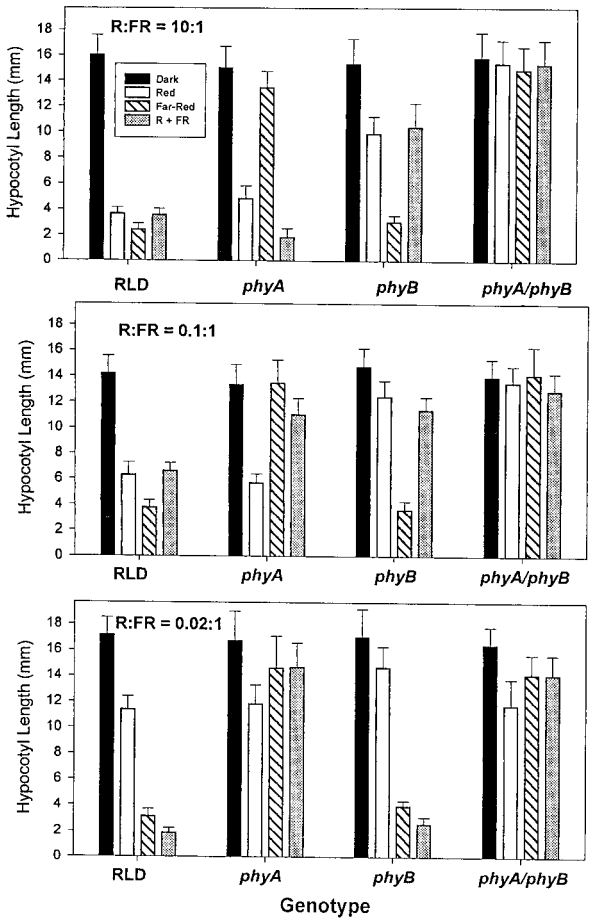

Figure 2. Hypocotyl elongation of wild-type Arabidopsis seedlings (RLD), and of the *phyA*, *phyB*, and *phyA/phyB* mutants under the indicated light treatments. Seedlings were exposed either to R, FR, or to R plus FR mixtures for 2 d; controls were kept in darkness. In all treatments the fluence rate of the FR irradiation treatment was identical (4 μmol m^{-2} s^{-1}). The fluence rate of the R treatment varied; in the top, the R fluence rate was 40 μmol m^{-2} s^{-1}; in the middle , it was 0.4 μmol m^{-2} s^{-1}; and in the bottom it was 0.08 μmol m^{-2} s^{-1}. These settings provided R:FR mixtures of 10:1, 0.1:1, and 0.02:1, respectively.

tion and low Pfr/P being ineffective; this is characteristic of a low-fluence response, and of the perception of the R:FR ratio and induction of shade avoidance.

The *phyB* mutant exhibited complementary responses to those of the *phyA* mutant, with extension growth strongly inhibited by FR, and by mixtures of R plus FR that have a high component of FR. This inhibition of extension growth has the characteristics of a FR-HIR. Increasing proportions of R combined with FR reduced the inhibition of extension growth. However, the absence of phyB did not completely suppress the growth response to R; this phenomenon is presumably a manifestation of a VLFR mediated by phyA (Mazella et al., 1997).

To relate these data to the proportions of phytochrome established in the Pr and Pfr form, the Pfr/P established by the mixed radiation sources in a partially purified preparation of *Avena sativa* phytochrome exposed to the experimental sources, was measured by dual-wavelength photometry. Figure 3 shows the relationship between Pfr/P and hypocotyl extension in the four genotypes (data derived from Fig. 2 and additional data). The hypocotyl lengths are normalized to unity for the dark treatments in all cases. This figure shows that hypocotyl extension in the RLD wild type is substantially inhibited at all Pfr/P established by the mixed R and FR sources. Extreme photoequilibria are most inhibitory, but intermediate photoequilibria cause more than 50% inhibition of extension growth. The two single mutants indicate that hypocotyl inhibition at low photoequilibrium is a phyA-mediated response, whereas that at high photoequilibrium is predominantly mediated by phyB. Part of the inhibition at high photoequilibria (and presumably also at other photoequilibria) appears to be due to the VLFR mediated by phyA, since this inhibition is absent in the *phyA/phyB* double mutants. The dual and overlapping actions of phyA and phyB, therefore, result in strong inhibition of hypocotyl extension at all ratios of R to FR radiation. Over the whole of the Pfr/P range studied, the responses mediated by phyA and by phyB appear to be almost exactly additive.

DISCUSSION

De-etiolation is a crucial step in seedling establishment, since the transition from heterotrophic dependence on stored reserves to photoautotrophic independence must be rapid so that the plant may effectively compete with neighbors. The significance of this transition is attested to by the multiplicity of photoreceptors involved. The results presented here provide new detail on the roles of phyA and phyB and demonstrate that the distinct characteristics of these two members of the phytochrome family allow for optimum de-etiolation, irrespective of the spectral quality of the radiation to which the emerging seedling is exposed.

Upon emergence, the seedling may be faced with immediate competition from neighbors in dense plant stands. Under such conditions, the spectral quality of the radiation will be high in FR reflected from and scattered through

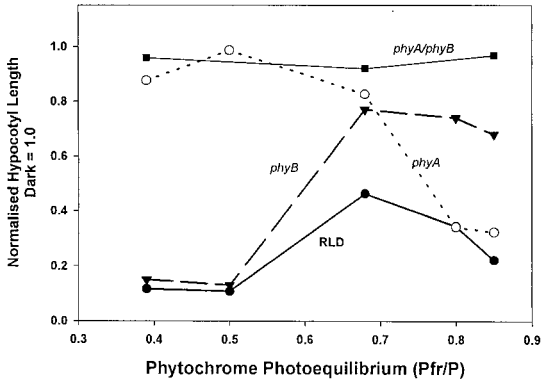

Figure 3. Hypocotyl length as a function of Pfr/P established by different R:FR ratios. The data of Figure 3 and additional data are plotted as a function of Pfr/P. Data were normalized to a value of unity for the dark controls in each experiment. Pfr/P were determined spectrophotometrically with phytochrome-containing tissue extracts exposed to the different R:FR light treatments.

neighboring vegetation. These reflection signals will initiate the characteristic shade-avoidance response, mediated by phyB and other phytochromes, in which resources are preferentially allocated to elongation growth at the expense of leaf development (Smith, 1982, 1995). Shade avoidance is an extremely important competitive strategy that confers a strong, adaptive advantage (Schmitt et al., 1995), but for seedlings with little reserves can be counterproductive. It has been well established that shade-avoidance reactions are only effective when the plant receives sufficient PAR to provide the resources for the accelerated growth (Smith and Hayward, 1985; Casal and Smith, 1989). This may seem somewhat of a paradox, since it suggests that shade avoidance is only effective when the plants are not shaded! However, shade-avoidance reactions are most effective before actual shading occurs, since plants detect their near neighbors by perceiving reflected FR and, thereby, anticipating impending competition (Ballaré et al., 1987). For establishing seedlings, however, reserves are limiting and shade avoidance would result in futile and fatal over-extension. It may be argued, admittedly on somewhat teleological grounds, that emerging seedlings need a mechanism through which early shade-avoidance reactions are suppressed, at least until reserves are built up. The data presented here indicate that the FR-HIR mediated by phyA provides such a mechanism.

Recently, Yanofsky et al. (1995) demonstrated that the Arabidopsis *phyA* mutant was incapable of effective de-etiolation when grown in natural shade, leading to high seedling mortality, and they concluded that the phyA-mediated FR-HIR was required for seedling survival under such conditions. Our data are consistent with this conclusion, but in addition point to complementary actions of phyA and phyB in coordinating de-etiolation under all natural radiation conditions. On this model, the phyA-mediated FR inhibition of extension growth prevents emerging seedlings from exhibiting an inappropriate shade-avoidance response mediated principally by phyB. This complementarity is short-lived, however, because phyA is lost rapidly in the light through Pfr-specific degradation, and because phyA synthesis is down-regulated by Pfr. Thus, within 24 to 48 h, the inhibitory action of FR mediated by phyA is lost, allowing shade-avoidance reactions to become evident. This behavior has already been observed in Arabidopsis and in other seedlings by measuring the temporal changes in responses of seedlings to FR added to continuous background white fluorescent light (McCormac et al., 1992). In these experiments hypocotyl extension was initially inhibited by the supplementary FR, but inhibition was replaced by growth stimulation after 1 to 2 d. Thus, the special characteristics of phyA—its accumulation in seedlings grown in the dark and its rapid loss after transition to the light—allow it to act in the proposed complementary manner with phyB during the early stages of seedling establishment. The complementarity between the actions of phyA and phyB require that FR inhibits extension growth via phyA, whereas it stimulates extension growth via phyB; thus, complementarity requires antagonism.

In the natural environment, of course, other photoreceptors and other photochemical reactions are important in de-etiolation, and these investigations do not address the roles of the cryptochromes, of protochlorophyllide reduction, or of the recently characterized FR-mediated blockage of subsequent greening (Barnes et al., 1996) in seedling establishment in dense canopies. Nevertheless, there is a prima facie case to consider the principal function of the phyA-mediated FR-HIR in preventing inappropriate shade-avoidance reactions in emergent seedlings. Fifteen years ago, Holmes et al. (1982) showed that hypocotyl growth of etiolated mustard seedlings was inhibited by light, irrespective of the Pfr/P established, but after a period of de-etiolation in continuous white light inhibition became strongly dependent on Pfr/P. The data presented here for Arabidopsis explain and extend the earlier mustard data by demonstrating that the photoequilibrium-independent growth inhibition observed in dark-grown seedlings is a result of the complementary actions of two phytochromes, one of which (phyA) is lost during de-etiolation.

Finally, these data are somewhat surprising with respect to the photoequilibrium at which the FR-HIR appears to be active. The action spectrum of the FR-HIR in Arabidopsis has not been published, but action spectra for other species indicate a sharp maximum between 710 and 720 nm, indicating that maximum action should occur only at very low Pfr/P. Indeed, Hartmann (1996) showed that maximum action in lettuce occurred at a photoequilibrium (Pfr/P) of approximately 0.03. In Figure 3 maximum inhibition of hypocotyl extension in both the RLD and the *phyB* mutant was evident up to a photoequilibrium of approximately 0.5. In terms of the natural environment, such a photoequilibrium will be established in rather light shade so that, in consequence, relatively little competition from neighboring plants would be sufficient to elicit FR-mediated extension growth inhibition in young seedlings.

Recently, much interest has been shown in the role of the phyA-mediated VLFR in initiating photomorphogenic responses at extremely low fluences (for review, see Furuya and Schäfer [1996]), with the hypothesis that phyA acts as an antenna that detects the emergence of seedlings from the soil. This may be a valid idea, but the extent of the VLFR inhibition of hypocotyl extension in Arabidopsis is quite small compared with the inhibition exerted by the phyA-mediated FR-HIR or the phyB-mediated inhibition caused by continuous R. In Figure 3 it is proposed that the inhibition seen in the *phyB* mutant at photoequilibria above 0.7 is due to the phyA-mediated VLFR, because this inhibition is absent in the *phyA/phyB* double mutant. If this is so, the VLFR only contributes at the maximum about 20% of the inhibition seen in the wild-type plants. It is questionable whether such a response can have significant ecological importance, even though it is saturated at extremely low fluences and, therefore, represents a highly sensitive perception mechanism.

Received December 13, 1996; accepted March 6, 1997.

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