PLANT BIOLOGY

Stem-piped light activates phytochrome B to trigger light responses in *Arabidopsis thaliana* roots

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The roles of photoreceptors and their associated signaling mechanisms have been extensively studied in plant photomorphogenesis with a major focus on the photoresponses of the shoot system. Accumulating evidence indicates that light also influences root growth and development through the light-induced release of signaling molecules that travel from the shoot to the root. We explored whether aboveground light directly influences the root system of *Arabidopsis thaliana*. Light was efficiently conducted through the stems to the roots, where photoactivated phytochrome B (phyB) triggered expression of *ELONGATED HYPOCOTYL 5* (*HY5*) and accumulation of HY5 protein, a transcription factor that promotes root growth in response to light. Stimulation of HY5 in response to illumination of only the shoot was reduced when root tissues carried a loss-of-function mutation in *PHYB*, and *HY5* mutant roots exhibited alterations in root growth and gravitropism in response to shoot illumination. These findings demonstrate that the underground roots directly sense stem-piped light to monitor the aboveground light environment during plant environmental adaptation.

INTRODUCTION

Light regulates virtually all aspects of plant growth and developmental processes throughout the life cycle (*1*). Various photoreceptors perceive a wide range of light wavelengths, such as ultraviolet (UV), blue (B), red (R), and far-red (FR), to monitor the plant's environment. The roles of photoreceptors and their associated signaling mechanisms have been extensively investigated mostly in the light-induced changes (photomorphogenesis) in the aerial (aboveground) structures (*2*, *3*), but light also influences growth and development of the underground root system (*4*–*6*). For example, R and FR light-sensing phytochromes are present in the roots and mediate primary root elongation, gravitropism, and jasmonic acid responses (*6*, *7*). Cryptochromes and phototropins, both of which sense B light, regulate primary root growth and root phototropism, respectively (*8*, *9*). In addition, UV-B light is known to trigger root photomorphogenesis (*10*, *11*). These effects of light on root growth occur when roots are directly exposed to light in an experimental setting.

Light signals perceived by the shoot also regulate root development through the transfer of signaling molecules from the shoot to the root. Activation of phytochrome A (phyA) and phyB stimulates the shoot-to-root transport of the hormone auxin and promotes lateral root production (*12*). In phytochrome-deficient mutants, lateral root development is suppressed in both light- and dark-grown plants, showing that shoot phytochromes are required for this developmental process. It has been reported that phyB induces expression of *ELONGATED HYPOCOTYL 5* (*HY5*) and promotes stabilization of the HY5 protein (*2*). The accumulated HY5 protein moves from the shoots to the roots, where it activates a gene encoding nitrate transporter to enhance nitrate uptake (*13*), consistent with the notion that shoot-sensed light signals are transmitted to the roots.

It has been suggested that the roots directly sense light under natural soil conditions. Genes encoding photoreceptors are expressed in root cells (*12*, *14*) and can be activated by direct light stimulation (*8*–*11*). Aboveground light might be conducted through the soil to the roots (*15*, *16*). However, light only penetrates a few millimeters into the soil, and the penetration rate is highly variable, depending on soil composition and layering. Alternatively, light could be conducted through plant tissues, such as the vascular system, to the roots (*17*, *18*).

Here, we demonstrate that light was conducted from the shoots to the roots of the model plant *Arabidopsis thaliana* through the stem and that root phyB was directly activated by this stem-piped light. Stem-piped light promoted the nuclear import of activated phyB in the roots of soil-grown plants, and this promoted accumulation of HY5 in the roots, triggering gravitropic responses. Our findings are consistent with the hypothesis that the roots monitor the aboveground light environment by directly sensing stem-piped light under natural conditions.

RESULTS

Shoot light influences gene expression in the roots

In an initial effort to understand the signaling link between light and root growth, we used genome-wide gene expression profiling using *Arabidopsis* plants under assay conditions that mimicked the natural root growth environment, in which roots remain in the dark. Either the shoots or the roots of dark-pretreated, wild-type Columbia (Col-0) *Arabidopsis* plants were exposed to light, and the roots of the light-treated plants were harvested for RNA sequencing analysis (fig. S1). Compared to transcripts in the roots of dark-treated plants, 988 transcripts differentially accumulated in the roots of root-illuminated plants (801 increased and 187 decreased) and 690 transcripts were differentially expressed in the roots of shoot-illuminated plants (606 increased and 84 decreased) (Fig. 1A and tables S1 and S2).

Among the differentially expressed genes, 198 genes were regulated by both shoot-light and root-light conditions (Fig. 1B and table S3). Of these, 166 transcripts increased in abundance and 29 decreased under both conditions. The remaining three transcripts increased in abundance under

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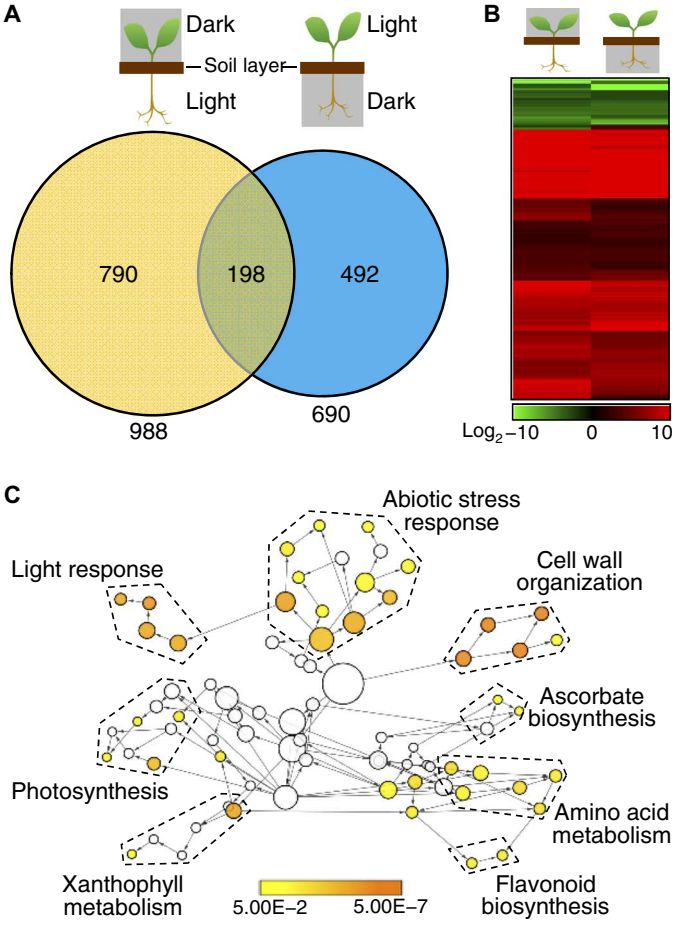

Fig. 1. Shoot light influences gene expression in the roots. Transcriptomic analysis of roots from dark-conditioned Col-0 plants in which either the shoots or the roots were exposed to light for 1 day. Biological triplicates were averaged. (**A**) Venn diagram of differentially expressed root genes. (**B**) Heat map of transcripts responsive to both light treatments. Scale bar indicates fold changes (log_2 values). (**C**) GO analysis of differentially expressed root genes under both shoot-light and root-light conditions. Colored nodes in the network diagram represent significantly overrepresented GO terms (Benjamini-Hochberg–corrected $P < 0.01$). Scale bar indicates P values.

shoot-light conditions but decreased under root-light conditions. These data suggest that aboveground light plays a role in regulating root gene expression, even when roots are not directly exposed to light. Gene Ontology (GO) analysis revealed that the co-regulated root genes include those involved in various metabolic pathways, abiotic stress responses, and light responses (Fig. 1C). We were interested in the co-regulated root genes that are involved in light responses, in particular the gene encoding positive photomorphogenic regulator HY5, which plays a central role in plant photomorphogenesis (*19*, *20*), and its direct target genes *CHALCONE SYNTHASE* (*CHS*) and *PHOTOLYASE 1* (*PHR1*) (table S4) (*20*, *21*).

Root phyB is required for shoot light–mediated gene regulation in roots

Photoreceptor genes are known to be expressed in the roots (*12*, *14*). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) assays revealed that the abundance of *PHYA*, *PHYB*, and *cryptochrome 2* (*CRY2*) transcripts in root tissues was comparable to that found in shoot tissues (fig. S2). The induction of *HY5* and its target genes in the root under shoot-light conditions was largely suppressed in *phyB-9* plants (Fig. 2A), indicating that phyB is primarily responsible for the induction of these genes in the root under these conditions.

Light-induced activation of phyB promotes HY5 activity at both the transcriptional and protein levels during shoot photomorphogenesis (*20*, *22*). Therefore, we asked whether the induction of *HY5* in the root under shoot-light conditions was mediated by shoot phyB or root phyB. We performed micrografting experiments using Col-0 and *phyB-9* plants and illuminated only the shoots of the grated plants. Grafting *phyB-9* mutant shoots onto Col-0 roots did not affect the expression of *HY5* and HY5 target genes in the root, whereas grafting Col-0 shoots onto *phyB-9* roots significantly suppressed the expression of *HY5* and HY5 target genes in the root (Fig. 2B). These results indicate that root phyB is important for shoot light–induced root gene expression. It has been reported that shoot HY5 protein is moved to the roots, where it induces its own transcription and modulates nitrogen uptake (*13*). It is apparent that HY5 function in the roots is mediated by two distinct pathways: one by shoot HY5 and the other by root phyB.

A constitutively active form of phyB containing a Tyr-to-Val substitution at amino acid position 303 (hereafter $phyB^{YVB}$) has been reported to exhibit light-independent signaling activity in transgenic plants (*23*). We performed grafting experiments with wild-type L*er* plants and $phyB^{YVB}$ transgenic plants. Grafted plants were grown in the dark before harvesting root samples. As expected, expression of *HY5* and HY5 target genes was greater in grafted plants with $phyB^{YVB}$ roots than in those with $phyB^{YVB}$ shoots (Fig. 2C). In addition, shoot light triggered the nuclear import of phyB fused to green fluorescent protein (phyB-GFP) in root cells (Fig. 2, D to F, and fig. S3). These observations indicate that root phyB mediates the induction of genes in the root in response to shoot light.

To examine whether the activation of root phyB occurs under physiological light-dark cycling, we grew plants in the dark for 16 hours and then exposed only the shoots to light. We found that the nuclear import of phyB-GFP in the root initiated within 2 hours of exposure to light, reaching a peak nuclear accumulation within 4 hours after illumination (Fig. 3A). Similarly, the expression of genes downstream of HY5 was rapidly induced within 4 hours under identical conditions (Fig. 3B), consistent with the hypothesis that root phyB is activated by shoot light. Meanwhile, the expression of *HY5* was not affected by shoot light under the assay conditions, suggesting that root HY5 protein is stabilized by root phyB-perceived light signals but not originated from the shoots during the early light response.

Root phyB mediates HY5 stabilization during root development

It is well known that phyB-mediated light signals stabilize the HY5 protein during plant photomorphogenesis (*22*). We therefore investigated whether root phyB contributes to the stabilization of HY5 in the roots of plants expressing HY5 fused to GFP (HY5-GFP). In the roots under shoot-light conditions, shoot light enhanced the stability of root HY5-GFP (Fig. 4A). Western blot analysis revealed that the abundance of root HY5 increased under shoot-light conditions (Fig. 4B). However, the abundance of HY5 did not increase in the *phyB-9* mutant under identical assay conditions, consistent with a role for phyB in the accumulation of HY5 in the root.

Shoot light activated root phyB to stabilize HY5 in root cells. HY5 is known to be involved in primary and lateral root formation and root gravitropism (*24*–*26*). A series of micrografting experiments between Col-0 plants and *hy5-221* plants revealed that primary root growth

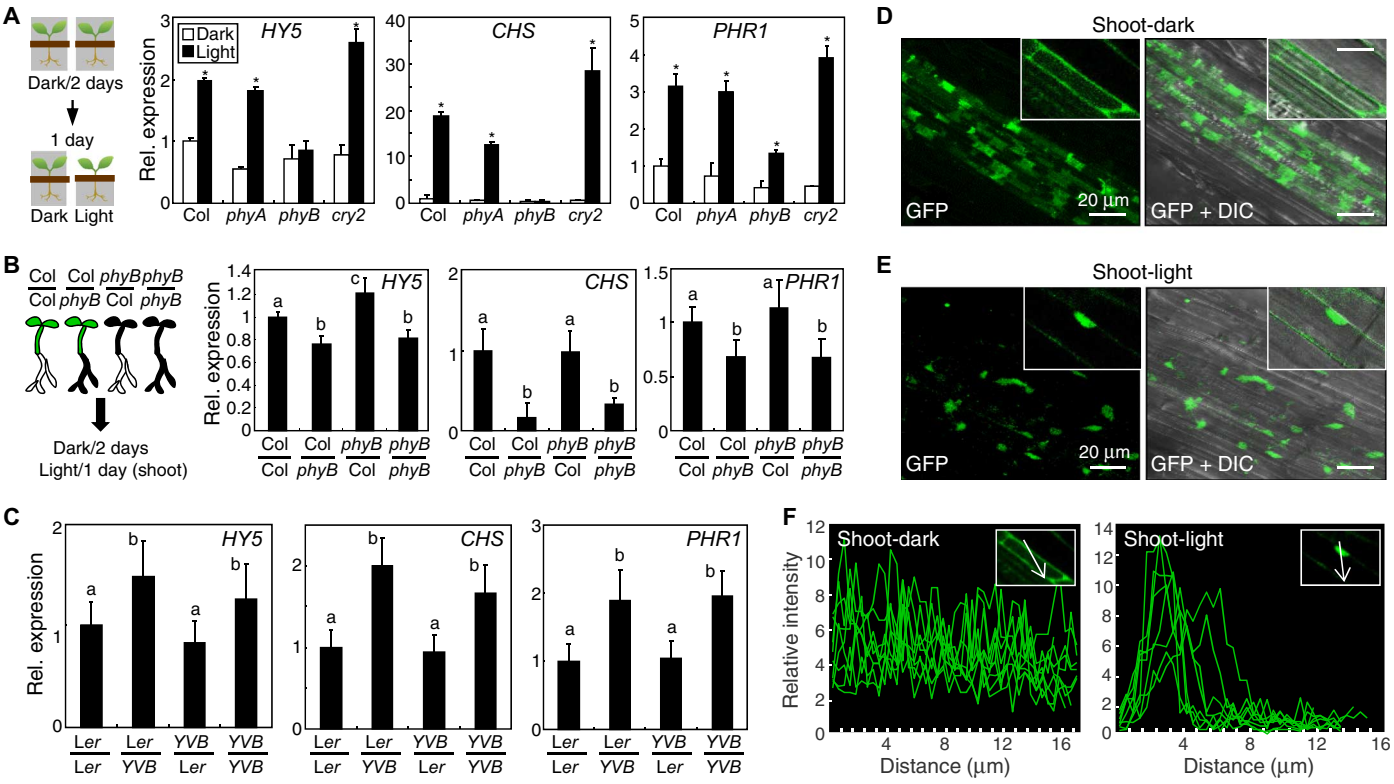

Fig. 2. Root phyB underlies the light-mediated induction of root genes. (**A** to **C**) qRT-PCR analysis of gene expression in roots. Gene expression was normalized using an internal control (*At3g13920*) for each reaction. Measurements from three independent experiments were averaged. Error bars indicate SE. (A) Expression of *HY5*, *CHS*, and *PHR1* in the roots of wild-type Col-0 plants and photoreceptor mutants *phyA-211*, *phyB-9*, and *cry2-1*. Two-week-old plants were exposed to light conditions as illustrated on the left before qRT-PCR. Statistical significance was determined by Student's *t* test (*$P < 0.01$). (B) Expression of *HY5*, *CHS*, and *PHR1* in the roots of grafted Col-0 and *phyB-9* plants after exposure of shoots to light. Different letters represent a significant difference ($P < 0.05$) determined by one-way analysis of variance (ANOVA) with post hoc Tukey test. (C) Expression of *HY5*, *CHS*, and *PHR1* in the roots of grafted *Ler* and *phyB*^{YVB} (*YVB*) plants grown in the dark for 3 days. Different letters represent a significant difference ($P < 0.05$) determined by one-way ANOVA with post hoc Tukey test. (**D** to **F**) Effects of shoot illumination on the nuclear localization of root phyB. Dark-conditioned 35S:*PHYB-GFP* plants were either left in the dark (D) or exposed to light (E) for 1 day before fluorescence microscopy of root cells. DIC, differential interference contrast. Insets are enlarged views of representative single cells. (F) Fluorescence intensities were measured across 10 single cells over the distance of ~16 μm (*43*), as indicated by arrows in insets. Fluorescence was dispersed in the shoot-dark roots (left panel) and clustered in the nuclear region in the shoot-light roots (right panel).

was impaired in grafted plants with *hy5-221* roots (Fig. 4, C and D). In addition, root gravitropism was also reduced in the grafted plants with *hy5-221* roots (Fig. 4E), whereas lateral root formation was not affected by the *hy5* mutation in the root under our assay conditions (fig. S4). These observations indicate that stabilization of HY5 by shoot light–activated phyB shapes root architecture.

We next analyzed the root morphology of *hy5-221* plants grown under shoot-light conditions, which mimicked the natural root growth environment. The mutant exhibited impaired root gravitropism (Fig. 4F). However, primary root growth of the mutant was normal under identical assay conditions (fig. S5). These observations indicate that the root phyB-HY5 module mediates shoot-sensed light signaling in root architecture with a primary role in root gravitropism among the root phenotypes examined.

Stem-piped light activates root phyB

A remaining question was how shoot light activated root phyB and stabilized HY5. We first investigated whether downstream mediators of light-induced signaling activate root phyB and HY5. Indole-3-acetic acid (an auxin), methyl jasmonic acid, and sucrose are among the molecules that are known to travel through plant tissues to mediate responses to light (*12*, *27*, *28*). Treating dark-grown plants with any of these compounds did not influence the nuclear import of phyB and the stability of HY5 in root cells (fig. S6), demonstrating that these mobile signals did not induce the photoactivation of phyB and HY5 in the root.

Another possibility was that shoot light was directly transduced through the stems to the roots (*17*, *18*). Using optical methods to investigate whether light is transduced through the plant body (Fig. 5A), we used white light source in the B to near-infrared (B–nearIR) spectral range (400 to 1000 nm) with a peak at 700 nm (fig. S7). We found that light in the R to near-infrared (R–nearIR) spectral range (670 to 1000 nm) was efficiently conducted through segments of tissue including both stem and root tissues with a peak conductance at 750 nm (Fig. 5, B and C, and fig. S7). Light in the green-to-red (G–R) light spectral range (500 to 670 nm) was also conducted through the stem, although much less efficiently than light of longer wavelengths. Light transmission assays using a source emitting a different spectrum of light showed that light in a G–R spectral range was efficiently transmitted through stem-root segments (fig. S8). Measurements of the fluence rates of light transmitted through stem-root segments with varying lengths revealed that the intensity of light wavelengths in a spectral range of 450 to 700 nm was more rapidly reduced compared to light of longer wavelengths (fig. S9), which is likely due to the absorption of B and R light by chlorophyll.

To determine whether activation of phyB in the roots of shoot-illuminated plants could be the result of light penetrating the soil to impinge on the roots, we completely blocked light transmission through the soil layer by covering the soil surface with aluminum foil and

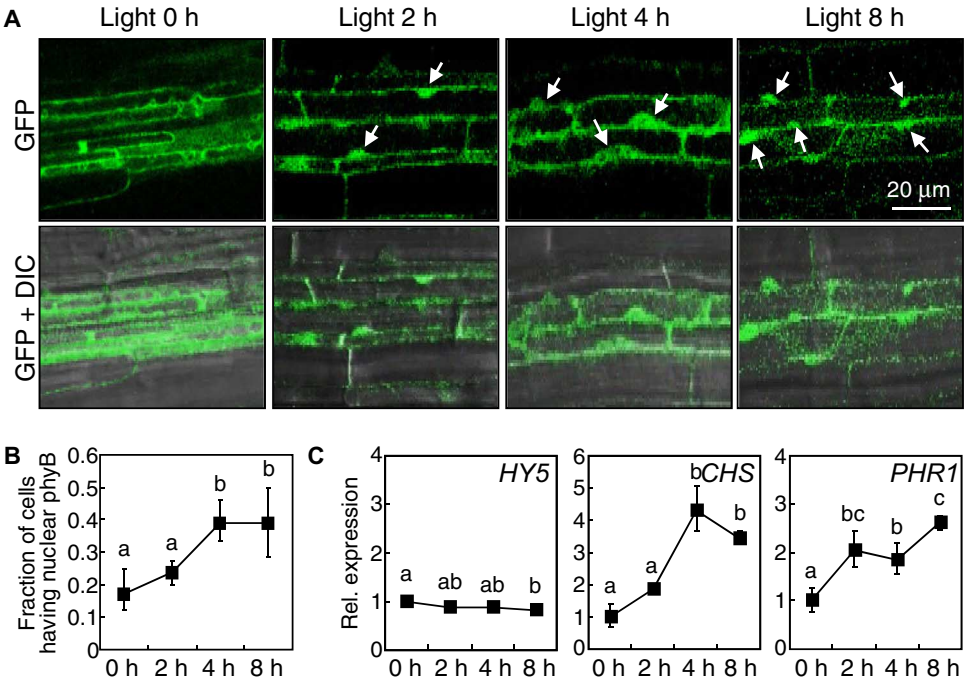

Fig. 3. Root phyB is activated by shoot light under 24-hour diurnal rhythms. (**A**) Nuclear localization of root phyB induced by shoot illumination. The shoots of dark-conditioned 35S:*PHYB-GFP* plants were exposed to light for up to 8 hours before fluorescence microscopy of root cells. Arrows indicate nuclei. (**B**) Fraction of root cells exhibiting nuclear phyB. Four measurements, each with 15 to 25 root cells, were averaged and statistically analyzed. Different letters represent a significant difference ($P < 0.05$) determined by one-way ANOVA with post hoc Tukey test. Error bars indicate SE. (**C**) Expression kinetics of *HY5* and the HY5 target genes *CHS* and *PHR1* in root cells. Col-0 plants were treated as described in (A), and root samples were harvested for total RNA extraction at the indicated times. Expression of genes was examined by qRT-PCR, using an internal control (*At3g13920*) in each reaction for normalization. Measurements from three independent experiments were averaged. Error bars indicate SE. Different letters represent a significant difference ($P < 0.05$) determined by one-way ANOVA with post hoc Tukey test.

covering the foil with fine soil particles up to 3 mm in depth (fig. S10). Root phyB-GFP was nuclearly localized in these plants, as observed in light-grown plants (Fig. 5D). The abundance of HY5-GFP protein in the root also increased in the soil-blocked plants (Fig. 5E) in response to shoot illumination, excluding the possibility that stabilization of HY5 is dependent upon light that penetrates the soil. Consistent with the activation of phyB and HY5 in the root by stem-piped light, the expression of *HY5* and HY5 target genes was increased in the soil-blocked plants, which is similar to what we observed in light-grown plants (Fig. 5F).

To verify that the observed light responses of the roots are mediated by stem-piped light, we removed the shoots of soil-grown plants and then subjected the remaining roots to one of three different treatments: We took the remaining roots out of the soil and exposed them to light, left the remaining roots in the soil in the dark, or covered the soil surface with an additional layer of fine soil particles to a depth of 3 mm and left the covered roots in the light (fig. S11A). Whereas the nuclear import of root phyB was stimulated when the amputated roots were exposed to light, it was not stimulated in roots maintained in the dark or in roots covered in soil after amputation of the shoot (fig. S11, B and D). Similarly, HY5 stability in the soil-covered roots was not increased as observed in dark-treated roots (fig. S11, C and E), indicating that there was no leak of light during our assays.

Aboveground light regulates root gravitropism through root phyB

Under low R-FR light conditions, the FR-absorbing form (Pfr) of phyB is converted to the physiologically inactive R-absorbing form (Pr) (*29*, *30*). It has also been reported that phyB is translocated to the nucleus to trigger light responses during hypocotyl growth under FR-rich light conditions (*31*). We found that the R/FR ratio of stem-piped light was relatively low (~0.01), raising the question as to whether or not the photon fluences and spectral compositions of the stem-piped light are sufficient to activate root phyB.

To directly test whether the spectrum of light that is piped through stems triggers light responses in the roots, we exposed roots to FR-rich light with an R/FR ratio of ~0.01, which is similar to the ratio observed in the stem-piped light (fig. S12). Because total fluence of light is also important for phytochrome photoactivation (*32*), we calculated the total fluence of the stem-piped light in the assays. Under the light illumination conditions used (120 μmol m^{-2} s^{-1}), total fluences of the stem-piped light for 24 hours through stem-root segments of 3.5, 2.5, and 1.5 cm at 730 nm were about 5.5×10^3, 1.7×10^5, and 2.5×10^5 μmol m^{-2}, respectively. To irradiate generate total fluences similar to these light conditions, we exposed the roots to 15 μmol m^{-2} s^{-1} FR-rich light for 6 min, 3 hours, and 4 hours, which corresponds to 5.4×10^3, 1.62×10^5, and 2.16×10^5 μmol m^{-2}. PhyB-GFP rapidly translocated into nuclei after the FR-rich light treatment (Fig. 6A). Whereas the nuclear import of phyB in roots of transgenic plants expressing phyB-GFP was not evident 6 min after light treatments, most phyB was detected in the nuclei after 3-hour light treatments. Accumulation of HY5 protein was also initiated rapidly (<6 min) after the FR-rich light exposure in Col-0 roots (Fig. 6B). In contrast, HY5 accumulation occurred more slowly in *phyB-9* roots. These observations indicate that stem-piped light with a relatively low R/FR ratio efficiently activates phyB to induce HY5 accumulation in the roots.

DISCUSSION

It is widely documented that light influences a broad spectrum of root growth and developmental processes, including primary root growth and lateral root formation and patterning (*4*, *5*), and root responses to external stimuli, such as phototropic and gravitropic growth (*5*, *6*). Photoreceptors and light-signaling molecules, such as auxin, jasmonic acid, and sucrose, have been proposed to play a role in the photoregulatory development of the root system (*12*, *27*, *28*). However, most previous studies have been conducted using plants grown on agar medium, in which the roots are exposed to light during root growth assays. Therefore, how aboveground light influences the underground root system under natural conditions has not been explored.

Here, we used a plant culture system that mimics nature conditions, in which the roots of photomorphogenic mutants and grafted plants remain in the dark during root growth assays. Our data show that in soil-grown *Arabidopsis* plants, aboveground light is efficiently transmitted

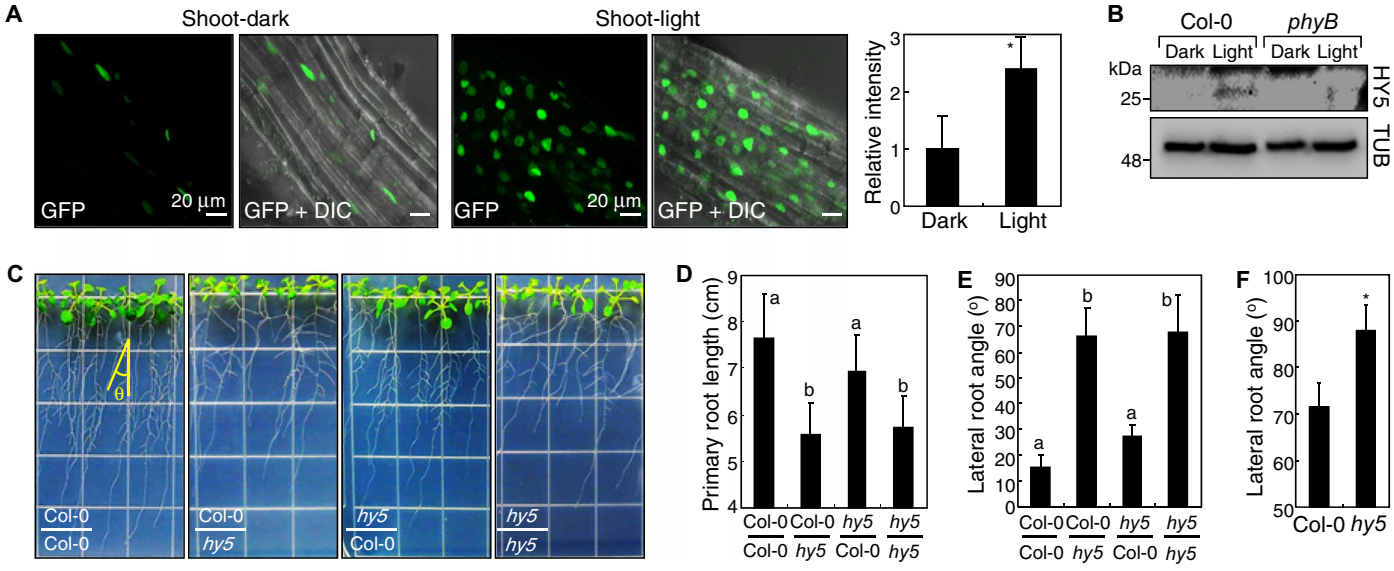

Fig. 4. Shoot light stabilizes root HY5 through phyB during root development. (**A**) Effects of shoot illumination on the stability of HY5 stability. The shoots of dark-conditioned transgenic plants expressing *HY5-GFP* were either left in the dark (left) or exposed to light (middle) for 1 day before fluorescence microscopy of root cells. Relative fluorescence intensities were averaged and subjected to Student's *t* test (*$P < 0.01$, $n = 10$) (right). (**B**) Effect of shoot illumination on stability of HY5 in roots of Col-0 and *phyB-9* plants that were placed in the dark for 2 days and then exposed to light for 1 day. Roots were harvested for protein extraction and Western blot assays using an antibody that recognizes HY5. Immunological detection of α-tubulin (TUB) was performed for protein quality control. (**C**) Root phenotypes of grafted Col-0 and *hy5-221* plants. The labels in each image indicate the genotype of the shoot (top) and root (bottom). Grafted plants were grown for 2 weeks on vertical plates containing 0.5% sucrose. θ, lateral root angle. (**D**) Primary root growth lengths of ~20 grafted plants were averaged. Different letters represent a significant difference ($P < 0.05$) determined by one-way ANOVA with post hoc Tukey test. Error bars indicate SE. (**E**) Lateral root angle (θ) values for the longest lateral roots of ~20 grafted plants were averaged. (**F**) Lateral root angle (θ) in *hy5-221* mutants grown for 2 weeks under conditions in which only the shoots were exposed to light. Roots of four to seven plants were measured and statistically analyzed (Student's *t* test, *$P < 0.01$).

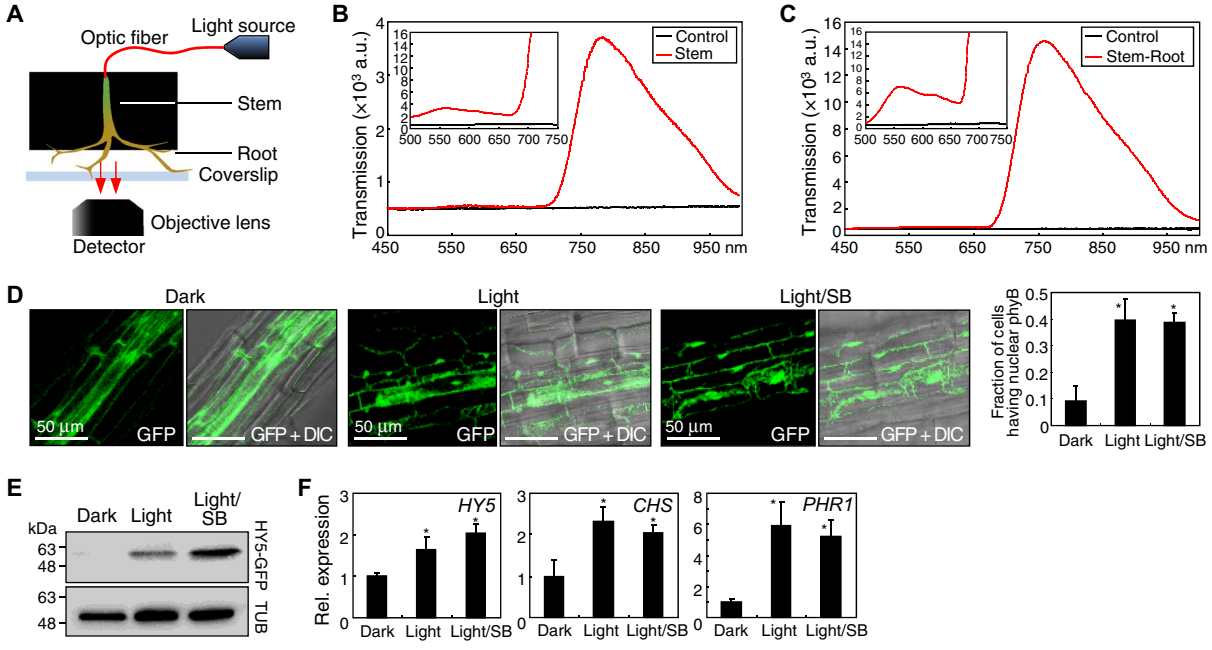

Fig. 5. Stem-piped light activates root phyB. (**A** to **C**) Stem or stem-root segments of ~3.5 cm were prepared from 6-week-old Col-0 plants. (A) Schematic diagram of optical experiments. Transmission of light through segments of stems (B) or stems plus roots (stem-root) (C). Insets show amplified light spectra from 500 to 750 nm. a.u., arbitrary unit. (**D**) Effect of stem-piped light on the nuclear localization of root phyB. Soil-grown 35S:*PHYB-GFP* plants were placed in the dark for 2 days and then kept in the dark, exposed to light, or exposed to light under soil light block (SB) conditions, in which the soil surface was covered with aluminum foil and fine soil particles, for 1 day before fluorescence microscopy. The fraction of root cells exhibiting nuclear phyB was measured (right). Four measurements were averaged and statistically analyzed (Student's *t* test, *$P < 0.01$). Error bars indicate SE. (**E**) Root HY5 stability. 35S:*HY5-GFP* plants were treated as described in (D). Total proteins were extracted from the roots for Western blot analysis using an antibody that recognizes GFP. (**F**) qRT-PCR analysis of the accumulation of *HY5* transcripts and the HY5 targets *CHS* and *PHR1* in root cells of Col-0 plants treated as described in (D). Biological triplicates were averaged and statistically analyzed (Student's *t* test, *$P < 0.01$).

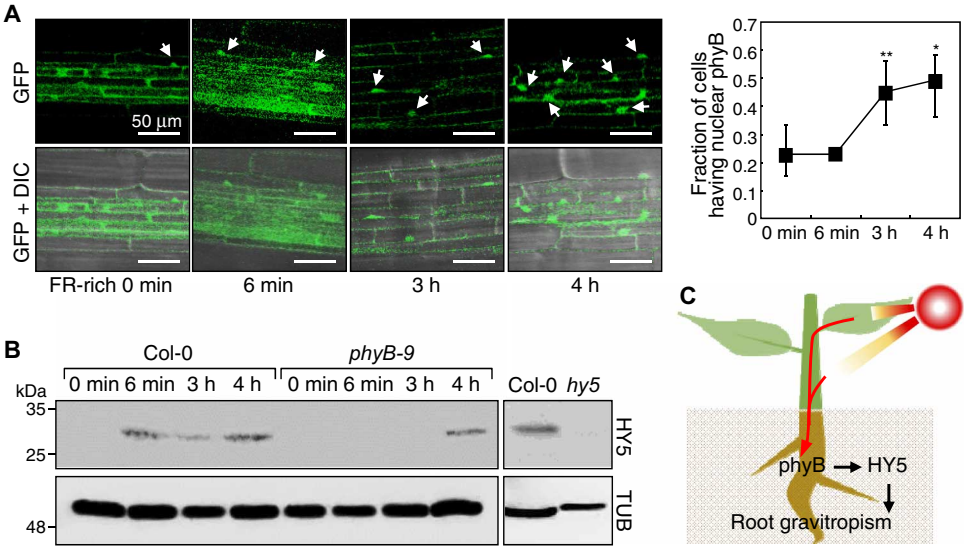

Fig. 6. FR-rich light induces light response in the roots. (**A**) Effects of FR-rich light on the nuclear localization of phyB in root cells. Roots of 35S:*PHYB-GFP* plants were exposed to FR-rich light for the indicated times before fluorescence microscopy. Fraction of root cells exhibiting nuclear phyB was measured (right). Four measurements, each with 15 to 25 root cells, were averaged and statistically analyzed (Student's *t* test, $*P < 0.01$, $**P < 0.05$). (**B**) Effects of FR-rich light on the stability of HY5 in the root. Roots of Col-0 and *phyB-9* plants were exposed to FR-rich light for the indicated times before protein extraction. Antibodies that recognize HY5 and α-tubulin (TUB) were used for Western blots. The *hy5-221* mutant (*hy5*) was used to verify that the detected bands represent HY5 protein. Blots are representative of three independent experiments. (**C**) Schematic diagram of stem-piped light signaling during root gravitropism.

through the stems to the roots and that this stem-piped light affects root architecture, and in root gravitropism in particular, through phyB-mediated stabilization of HY5 in the root. When grown on agar, the roots of a HY5-defective mutant exhibited alterations in primary root growth and root gravitropism. In contrast, when grown in soil, root gravitropism was perturbed, but primary root growth was normal in the mutant. From these results, we infer that the activation of root phyB and HY5 by stem-piped light primarily plays a role in root gravitropism.

Together with previous reports on the roles of various signaling molecules and photoreceptors that influence root architecture (*4*–*6*, *12*, *27*, *28*), our data suggest that multiple light-signaling pathways downstream of different photoreceptors are involved in distinct aspects of root photomorphogenesis. It is also possible that the roots can directly sense aboveground light that is transmitted through the surface soil layer (*33*). The phyB-sensed light signals are most likely not mediated entirely by HY5, because several other signaling molecules are known to mediate light signaling downstream of multiple photomorphogenic processes (*12*, *27*, *28*).

HY5 has been reported to be a mobile photomorphogenic regulator that is transported from the shoots to the roots, where it induces the expression of *HY5* and *NITRATE TRANSPORTER 2.1* to enhance nitrate uptake (*13*). It is thus likely that shoot light affects physiological and developmental processes in the roots through at least two distinct routes: one through the shoot-derived mobile HY5 and the other through the phyB-mediated stabilization of HY5 in response to stem-piped light. It will be interesting to examine whether these two light-signaling pathways modulate distinct sets of physiological and developmental processes in the roots or regulate root processes in a coordinated manner.

We found that light in the FR–nearIR range was efficiently transmitted through the stems and roots, consistent with previous reports that examined light transmission through stem or root segments (*17*, *18*). Using sensitive light-detecting methods, we found that light in the G–R range is also transmitted through the stem and root segments, which is in contrast to the previous reports, in which it was reported that only FR light was transmitted through stems and root segments (*17*, *18*). Despite its lower transmission efficiency compared to that of FR light, light in this G–R wavelength range would be sufficient to activate phyB and possibly other photoreceptors in the roots, given the extremely high light sensitivity of these receptors (*34*, *35*). In support of this, it has been observed that a small portion of phytochromes exists in the Pfr form even under low R-FR light conditions (*29*); this observation is consistent with the notion that at least a portion of root phyB is activated by stem-piped light of a relatively low R/FR ratio. The transmission of light in a broad wavelength range is also consistent with the notion that root architecture is modulated by multiple photoreceptors and light-signaling pathways.

Here, we demonstrated that stem-piped light activates root-localized phyB and its target HY5 to modulate the development of the root system, particularly root gravitropism (Fig. 6C). Sensing of stem-piped light by root phyB is part of the light adaptation mechanisms by which roots monitor fluctuations in the aboveground environment to optimize their growth and development under natural conditions. Although much more work is required to understand the molecular mechanisms underlying root photomorphogenesis, our findings provide a basis from which to continue the discovery of signaling molecules and pathways underlying root photomorphogenesis in plants.

MATERIALS AND METHODS

Plant materials and growth conditions

A. thaliana lines were in the Col-0 background, except for the *phyB*^{YVB} transgenic plants that express constitutively active phyB^{YVB}, which were in the Landsberg *erecta* (Ler) background (*23*). Sterilized *Arabidopsis* seeds were cold-treated at 4°C for 4 days in complete darkness to synchronize germination. Plants were grown on Murashige and Skoog (MS) agar plates or in soil under long days (LDs; 16-hour light and 8-hour dark) at 22°C with white light illumination (120 μmol m^{-2} s^{-1}) provided by fluorescent FLR40D/A tubes (Osram).

The *phyA-211*, *phyB-9*, *cry2-1*, and *hy5-221* mutants have been described previously (*36*, *37*). To generate 35S:*PHYB-GFP* and 35S:*HY5-GFP* transgenic plants, a GFP-coding sequence was fused in-frame to the 3′ ends of the phyB- or HY5-coding sequences, respectively, under the control of the cauliflower mosaic virus 35S promoter. The expression constructs were transformed into Col-0 plants by a modified floral dip method (*38*).

Analysis of gene expression

Expression of genes was quantified by qRT-PCR. For total RNA isolation, plant tissues were ground in liquid nitrogen. One milliliter of TRIzol reagent (Invitrogen) was added to each sample, and the mixture was centrifuged at 16,000*g* for 10 min at 4°C. Two hundred microliters of

chloroform was added to the supernatants, followed by centrifugation under the same conditions. The supernatant was transferred to a microcentrifuge tube containing 200 µl of isopropanol and 200 µl of high-salt solution (0.8 M trisodium citrate and 1.2 M sodium chloride), and this mixture was centrifuged at 16,000*g* for 10 min at 4°C. The RNA pellet was washed twice with 75% ethanol and dried before it was suspended in 50 µl of ribonuclease-free water.

qRT-PCRs were conducted according to guidelines that have been proposed to guarantee reproducible and accurate measurements (*39*). qRT-PCR runs were performed in 96-well blocks of the Applied Biosystems 7500 Real-Time PCR System with the SYBR Green I master mix in a volume of 20 µl. The two-step thermal cycling profile system we used was 15 s at 95°C for denaturation and 1 min at 60 to 65°C, depending on the calculated melting temperatures of PCR primers, for annealing and polymerization. The primers used are listed in table S5. As an internal control, primers specific for an *eIF4A* gene (*At3g13920*) was used in each PCR for normalization. All qRT-PCRs were performed in biological triplicates using total RNA samples extracted separately from three independent plant materials that were grown under identical conditions in the same experiment. The comparative $\Delta\Delta C_T$ method was used to evaluate relative quantities of each amplified product in the samples (*40*). The threshold cycle (C_T) was automatically determined for each reaction by the Applied Biosystems 7300 Real-Time PCR System (Thermo Fisher Scientific) set with default parameters.

RNA sequencing

Plants were grown on MS-Phytagel medium in plastic culture boxes at 22°C under LDs for 2 weeks. They were grown in the dark for 2 days to ensure complete phytochrome decay, and either the shoots or the roots were exposed to light or left in the dark for one additional day. The roots of the light-treated plants were harvested for total RNA extraction. Total RNA was extracted, as described above. The RNA samples were then subjected to RNA sequencing by ChunLab Inc. Genes with P value < 0.15 and fold change ≥ 4 were regarded as differentially expressed genes. GO analysis was performed using the Biological Networks Gene Ontology tool (BiNGO) with Benjamini-Hochberg–corrected $P < 0.01$. The network diagram shows significantly overrepresented GO terms.

Micrografting

Seedlings were grown on MS agar plates for 4 days under short days (8-hour light and 16-hour dark) at 22°C before grafting. Grafting was performed, as described previously (*41*). Grafted plants were grown on MS agar plates containing 0.5% sucrose for 1 week at 22°C under LDs before appropriate assays.

Fluorescence imaging

The 35S:*PHYB-GFP* and 35S:*HY5-GFP* transgenic plants were grown in soil for 2 to 5 weeks at 22°C under LDs. Root parts located about 4 cm below the soil surface were subjected to fluorescence imaging using an LSM 710 confocal microscope (Carl Zeiss).

To examine the effects of growth hormones and sucrose on the subcellular localization and relative accumulation of phyB-GFP and HY5-GFP in root cells, 3-week-old plants were grown for 2 days in the dark and transferred to liquid MS culture containing either 100 mM sucrose, 100 µM methyl jasmonic acid, or 100 µM indole-3-acetic acid in the dark for 1 day before fluorescence imaging.

To investigate whether root phyB is activated under physiological light-dark periods, plants were grown in the dark for 16 hours, and the shoots were exposed to light before fluorescence imaging of the root cells.

For FR-rich light illumination, the roots of 3-week-old plants grown in soil were grown in the dark for 48 hours and exposed to FR-rich light with an R/FR ratio of 0.01 and a fluence rate of 15 µmol m^{-2} s^{-1} provided by LED lamp (PARUS).

Immunoblot assays

Plants were grown in soil for 3 to 5 weeks. The root parts located about 4 cm below the soil surface were harvested for the extraction of total proteins. The root samples were ground in liquid nitrogen and mixed with protein extraction buffer [100 mM tris-Cl (pH 6.8), 4% SDS, 0.2% bromophenol blue, 20% glycerol, and 200 mM β-mercaptoethanol]. The mixtures were incubated at 90°C for 10 min and then centrifuged at 16,000*g* for 10 min. The supernatants were mixed with equal volumes of 2× SDS-PAGE (polyacrylamide gel electrophoresis) loading buffer and analyzed on 8% SDS-PAGE gel before transferring to polyvinylidene difluoride membrane. Antibodies recognizing the HY5 and GFP proteins (Santa Cruz Biotechnology) were used for the immunological detection of HY5 and HY5-GFP proteins, respectively.

Histochemical staining

For β-glucuronidase (GUS) histochemical staining, a GUS coding sequence was transcriptionally fused to the promoter sequences consisting of about 2 kilo–base pairs upstream of the translational start sites of *PHYA*, *PHYB*, and *CRY2* genes. The constructs were transformed into Col-0 plants. Transgenic plants were grown on MS agar plates at 22°C for 2 weeks under LDs. They were then incubated in X-Gluc solution for 16 hours at 37°C in the dark (*42*).

Light transmission assay

Six-week-old Col-0 plants grown at 22°C under LDs were used for light transmission assays. Light input was provided by fiber-coupled white light source (Thorlabs). Water-absorbed stem or stem-root segments were vertically oriented on coverslip. White light was applied into the upper part of the stem or stem-root segments using fiber optic cannula (Thorlabs). The stem-fiber junction was sealed with black paper tape to block light leakage. To obtain transmission spectra, transmitted light was measured with a confocal Raman spectrometer with a focal length of 50 cm (Andor Technology) equipped with a 1.4–numerical aperture 100× objective lens (Olympus), which was connected to an electron-multiplying charge-coupled device camera (Andor Technology). As a negative control, the stem or stem-root segments were sealed with black paper tape to block light transmission. To enhance the signal-to-noise ratio, 1000 spectra were sampled during light irradiation and averaged. Exposure time for each spectrum was 0.2 s.

SUPPLEMENTARY MATERIALS

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Fig. S1. Schematic diagram of plant sample preparation for RNA sequencing.
Fig. S2. Expression of photoreceptor genes in roots.
Fig. S3. Plant growth for fluorescence imaging of phyB and HY5 distribution in root tissues.
Fig. S4. Lateral root formation in grafted Col-0 and *hy5-221* plants.
Fig. S5. Primary root growth in soil-grown *hy5-221* mutants.
Fig. S6. Effects of growth hormones and sucrose on the nuclear import of phyB and HY5 stability in root cells.
Fig. S7. Normalized light transmission through stem and stem-root segments.
Fig. S8. Transmission of lights with different spectral compositions through stem-root segments.
Fig. S9. Fluence rates and spectral compositions of stem-piped light.
Fig. S10. Plant growth under soil light block conditions.
Fig. S11. Experimental evaluation of the soil cover treatments.
Fig. S12. Spectral composition of FR-rich light.

Table S1. Differentially expressed genes in the roots of plants having shoots in the dark and roots in the light.
Table S2. Differentially expressed genes in the roots of plants having shoots in the light and roots in the dark.
Table S3. Differentially expressed genes under both root-light and shoot-light conditions.
Table S4. Root genes that are differentially co-regulated by shoot light and root light.
Table S5. Primers used in this study.

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