Light modulates the gravitropic responses through organ-specific PIFs and HY5 regulation of *LAZY4* expression in *Arabidopsis*

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Light and gravity are two key environmental factors that control plant growth and architecture. However, the molecular basis of the coordination of light and gravity signaling in plants remains obscure. Here, we report that two classes of transcription factors, PHYTOCHROME INTERACTING FACTORS (PIFs) and ELONGATED HYPOCOTYL5 (HY5), can directly bind and activate the expression of *LAZY4*, a positive regulator of gravitropism in both shoots and roots in *Arabidopsis*. In hypocotyls, light promotes degradation of PIFs to reduce *LAZY4* expression, which inhibits the negative gravitropism of hypocotyls. *LAZY4* overexpression can partially rescue the negative gravitropic phenotype of *pifq* in the dark without affecting amyloplast development. Our identification of the PIFs-LAZY4 regulatory module suggests the presence of another role for PIF proteins in gravitropism, in addition to a previous report demonstrating that PIFs positively regulate amyloplast development to promote negative gravitropism in hypocotyls. In roots, light promotes accumulation of HY5 proteins to activate expression of *LAZY4*, which promotes positive gravitropism in roots. Together, our data indicate that light exerts opposite regulation of *LAZY4* expression in shoots and roots by mediating the protein levels of PIFs and HY5, respectively, to inhibit the negative gravitropism of shoots and promote positive gravitropism of roots in *Arabidopsis*.

light | gravitropism | PIFs | HY5 | LAZY4

Light and gravity are two critically important environmental signals for plant growth. Light regulates the development of plants throughout their life cycle from seed germination to flowering (1). Gravity controls the growth orientation and architecture of plants to ensure that shoots grow upward for efficient photosynthesis and reproduction and that roots grow downward for improved water absorption, nutrient acquisition, and anchorage (2–5). However, little is known about the molecular basis of how light and gravity coordinately regulate plant growth.

Upon illumination, the *Arabidopsis* transcriptome undergoes massive changes that are mediated by many transcription factors (6). PHYTOCHROME INTERACTING FACTORs (PIFs), a set of basic helix–loop–helix transcription factors that include PIF1, PIF3, PIF4, and PIF5, play important roles in phytochrome-signaling pathways (7). PIFs are negative regulators of photomorphogenesis, and the quadruple *pif* mutant (*pifq*) exhibits a photomorphogenic phenotype in darkness (8, 9). Upon interaction of photoactivated phytochromes with PIFs, PIF proteins are phosphorylated, ubiquitinated, and degraded, resulting in inhibition of PIF function and thus transcriptional reprogramming (10–14). All four PIFs (PIF1, PIF3, PIF4, and PIF5) were reported to preferentially bind to a core G-box DNA-sequence motif (CACGTG) and/or a PBE-box (CACATG) motif in the target promoters (15–18). In addition to their central role in photomorphogenesis, PIFs were also found to be involved in light inhibition of negative gravitropism of hypocotyls, possibly through targeting of REPRESSOR OF PHOTOSYNTHETIC GENES1

(RPGE1) to suppress conversion of endodermal gravity-sensing starch-filled amyloplasts into other plastids in darkness (19, 20).

ELONGATED HYPOCOTYL 5 (HY5), a well-known basic leucine zipper (bZIP) family transcription factor (21), promotes plant photomorphogenesis under diverse light conditions mainly through transcriptional regulation (22–25). Light stabilizes HY5 protein, and HY5 directly binds to ACGT-containing elements (ACEs) in the promoters of target genes to regulate expression of light-responsive genes (22, 23, 26, 27). HY5 was also reported to be involved in primary and lateral root formation and root gravitropism (22, 28, 29). Light signals perceived by the shoot also regulate root development (30–32). Under natural conditions, aboveground light can be stem-piped to roots to activate root phyB, which triggers HY5 accumulation to modulate root growth and gravitropism (31). Light-stabilized HY5 protein in shoots moves directly to roots to activate expression of *HY5* and the nitrate transporter *NRT2.1*, which thereby regulate root growth and nitrate uptake (30).

Gravity provides a continuous directional environmental signal to plants. Plants are able to perceive the gravity vector mainly through specialized sensing cells called "statocytes," which are located in shoot endodermal cells and root columella cells in *Arabidopsis thaliana* (3, 4, 33, 34). Statocytes contain high-density, starch-accumulating plastids known as amyloplasts that, under steady-state conditions, settle to the bottom of cells.

Significance

Light and gravity are two key environmental factors that co-ordinately regulate plant gravitropism, but the underlying molecular mechanisms that drive this regulation are poorly understood. Here, we reveal that light represses expression of *LAZY4*, a positive regulator of gravitropism, by promoting degradation of PIF proteins to inhibit negative gravitropism of hypocotyls in *Arabidopsis*. Meanwhile, light induces *LAZY4* expression by promoting HY5 accumulation to enhance positive gravitropism in roots. Our findings provide key insights regarding the mechanism by which light mediates organ-specific gravitropism in plants.

Once the growth direction of the plant changes, amyloplasts sediment to the new cell bottom, which may convert the physical vector signal to the biochemical signal. Then, auxin is transported in a polar manner to form a gradient across the organ, which leads to the asymmetric growth of plant organs (35, 36).

LAZY family genes are a group of key factors that function in gravity signaling in several plant species (37–40). The rice *LAZY1* gene was the first family member to be identified and was mapped using a rice cultivar line exhibiting a prostrate shoot phenotype (37, 38, 41, 42). A previous study identified a family of six *LAZY1*-like genes in *Arabidopsis*, with *AtLAZY1* exhibiting the most similar sequence to *OsLAZY1*. The lateral shoots of *lazy1* plants show a larger growth angle compared to the wild type (39). Moreover, loss of *LAZY2* and *LAZY4* function enhances the *lazy1* gravitropic phenotype (39, 43, 44). Several studies have reported redundant function of *LAZY2/LZY2/NGR1/DRO3*, *LAZY3/LZY4/NGR3/DRO2*, and *LAZY4/LZY3/NGR2/DRO1* in root gravitropism in *Arabidopsis* (40, 43, 44). Among these *LAZY* genes, the *lazy4 (dro1)* single mutant exhibits larger lateral root growth angles (43–45). Consistent with these findings, *OsDRO1/OsLAZY4* controls rice root system architecture through positive regulation of the crown root angles (46). Recent studies showed that gravity-triggered polarly distributed LAZYs (LZYs) may recruit RLD family proteins to regulate asymmetric PIN3 distribution in the lateral root cap columella, which directs polar auxin transport to control bending growth in gravitropism (43, 47).

Here, we report that PIFs activate expression of *LAZY4*, a representative *LAZY* family member, in hypocotyls by directly binding to the G-box in the *LAZY4* promoter in darkness. Light suppresses *LAZY4* expression in hypocotyls by promoting degradation of PIFs, thus inhibiting negative gravitropism of hypocotyls. Increased *LAZY4* expression in the *pifq* mutant is able to partially rescue the interrupted hypocotyl gravitropism phenotype of dark-grown *pifq* but not the development of endodermal amyloplasts. These data suggest the presence of another mechanism for regulation of hypocotyl negative gravitropism that is distinct from the previously reported PIFs-amyloplast pathway. We also demonstrate that HY5 is stabilized by light and binds to the *LAZY4* promoter through ACEs, which results in induction of *LAZY4* expression in roots and promotion of root positive gravitropism. Our study reveals a molecular basis for the coordination of light and gravity signaling in plants.

Results

PIFs Bind Directly to the *LAZY4* Promoter In Vivo.
Previous studies proposed that light may inhibit the negative gravitropism of hypocotyls by promoting degradation of PIFs (9, 19). To test this model in our experimental condition, we grew seedlings on vertical plates in the dark or under red-light conditions. We observed that hypocotyls of dark-grown wild-type (Col) seedlings grew upward, whereas the hypocotyls of red-light–grown Col and dark-grown *pifq* exhibited interrupted negative gravitropism (Fig. 1*A*). A previous study suggested that PIFs maintain amyloplast development in the dark to promote negative gravitropism of *Arabidopsis* hypocotyls in the dark (19). To uncover specific molecular connections between the light signal and gravitropism, we searched for gravitropism-related genes in PIF targets by analyzing previously reported PIFs chromatin immunoprecipitation sequencing (ChIP-seq) data (15, 17, 18). We found that all four PIFs (PIF1, -3, -4, -5) bind to the *LAZY4* promoter through the G-box located ∼2,000 bp upstream of the ATG start codon (*SI Appendix*, Figs. S1*A* and S2). As recent studies demonstrated that *LAZY4* plays important roles in the gravitropism of both shoots and roots (40, 43–45), we speculated that *LAZY4* may function downstream of PIFs, thus serving as a key node in light modulation of gravitropism. To confirm the ChIP-seq data described above, we performed chromatin immunoprecipitation (ChIP) assays using 4-d-old dark-grown Col

and *PIF1/3/4/5-Myc* seedlings. Our analysis showed that all four of the PIF-Myc factors bound the G-box region of the *LAZY4* promoter (Fig. 1 *B–E*).

Light Inhibits *LAZY4* Expression in Hypocotyls by Promoting PIF Degradation.
In order to detect whether PIFs regulate *LAZY4* transcript levels in hypocotyls, we compared the expression levels of *LAZY4* in wild-type and *pifq* hypocotyls. Our analyses of seedlings grown in the dark for various time periods revealed that levels of *LAZY4* transcripts were notably decreased in *pifq* mutant hypocotyls, indicating that PIFs positively regulate *LAZY4* expression in *Arabidopsis* hypocotyls (Fig. 1*F*). As PIF proteins are rapidly degraded following light exposure, we next tested whether light inhibits *LAZY4* transcription in hypocotyls. The expression of *LAZY4* was dramatically decreased in the hypocotyls of wild type after light exposure, whereas the reduction of *LAZY4* transcripts in the hypocotyls of the *pifq* mutant was not as obvious (Fig. 1*G*). Taken together, these results demonstrate that PIFs are the major transcriptional activators of *LAZY4* in hypocotyls and that light-promoted PIF degradation leads to a dramatic decrease of *LAZY4* expression in hypocotyls.

Overexpression of *LAZY4* Partially Rescues the Gravitropic Phenotype of *pifq* without Affecting Amyloplast Development.
As previously reported, *LAZY4* is a positive regulator of gravitropism in both shoots and roots (40, 43–45). To perform genetic analysis of the relationship between PIFs and *LAZY4*, we introduced a LAZY4-GFP fusion protein under the control of the native *LAZY4* promoter into the *pifq* background and obtained three independent *LAZY4p:LAZY4-GFP/pifq* lines. In these three lines, the levels of *LAZY4* transcripts were found to be higher than in the wild-type controls (*SI Appendix*, Fig. S3), indicating that these plants are *LAZY4* overexpression lines. These results suggest the existence of other uncharacterized transcription factors that may activate *LAZY4* expression in the dark. All three *LAZY4p:LAZY4-GFP/pifq* lines displayed improved negative gravitropism of their hypocotyls in the dark when compared with *pifq* (Fig. 2*A*). From these data, we concluded that *LAZY4* acts downstream of PIFs and that the interrupted negative gravitropic phenotype of the *pifq* mutant can be partially rescued by increasing *LAZY4* expression levels.

A previous study reported that, in the dark, PIFs suppress the conversion of endodermal starch-filled amyloplasts into plastids with small starch granules, thereby enabling negative gravitropism of hypocotyls (19). Here, we found that PIFs positively regulate *LAZY4* expression through direct binding of the *LAZY4* promoter and ultimately promote hypocotyl negative gravitropism in the dark. Therefore, we investigated whether the PIFs-LAZY4 module also mediates amyloplast development. We examined and compared the endodermal amyloplasts in Col, *pifq*, and *LAZY4:LAZY4-GFP/pifq* lines by I$_2$-KI staining. Amyloplast development is similar in the columella cells of all of the root caps. In contrast, hypocotyls of *pifq* exhibited fewer amyloplasts compared to wild-type hypocotyls, whereas LAZY4-GFP expression in the *LAZY4:LAZY4-GFP/pifq* lines did not rescue this reduced amyloplast development (Fig. 2*B*). Taken together, our data demonstrate that increasing *LAZY4* transcript levels in the *pifq* mutant partially rescues hypocotyl negative gravitropism without altering endodermal amyloplast development. Thus, the PIFs-LAZY4 regulatory module represents another mechanism by which PIFs function in the coordination of light and gravity signaling, and this mechanism is independent from the previously reported PIF-amyloplast pathway (19).

HY5 Directly Binds to and Promotes Expression of *LAZY4* in *Arabidopsis* Roots.
The investigation described above revealed a mechanism by which light inhibits the negative gravitropism of hypocotyls. We next asked whether light regulates the positive
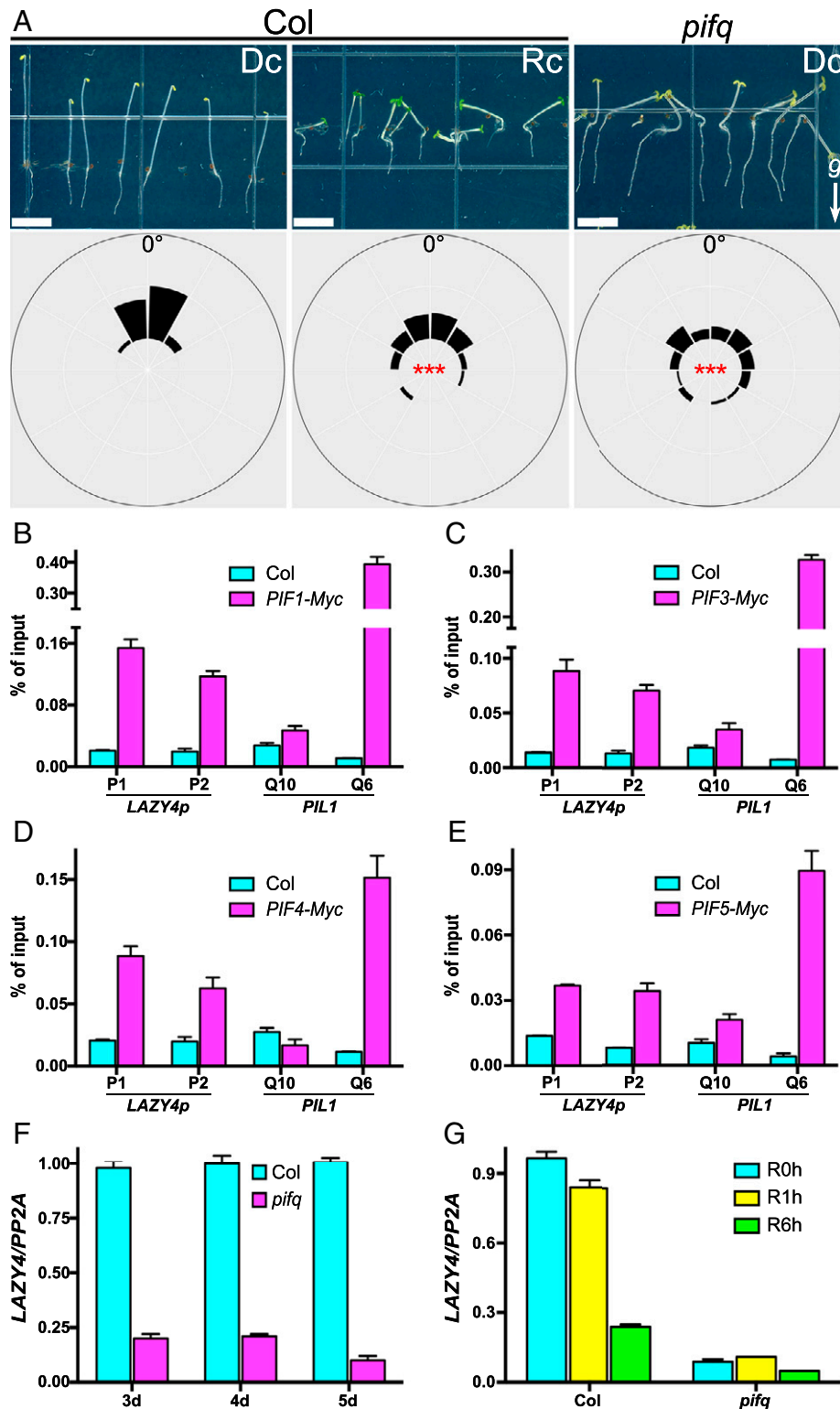
Fig. 1. PIFs directly activate *LAZY4* expression in *Arabidopsis* hypocotyls. (*A*) Orientation of wild-type (Col) and *pifq* hypocotyls in red light or dark conditions. Seedlings were grown on the surface of vertical agar plates in dark or red light for 4 d (*Top*), and statistical analyses of the hypocotyls' growth orientations are shown (*Bottom*). Frequencies of hypocotyl growth directions are shown in the corresponding angular position around a circle at intervals of 30° (*n* = 50 to 90). For statistical analyses, means of the absolute value of the angles between the growth direction and the upward vertical axis were compared. Asterisks indicate significant differences compared to Col in the dark, as determined by one-way ANOVA (***P < 0.001). The arrow labeled with a "g" represents the gravity direction (Scale bars, 5 mm.) (*B–E*) PIFs bind to the *LAZY4* promoter in ChIP assays. Seedlings of 4-d-old dark-grown Col and *PIF1-Myc* (*B*), *PIF3-Myc* (*C*), *PIF4-Myc* (*D*), and *PIF5-Myc* (*E*) were collected for ChIP analyses using anti-Myc beads. *LAZY4p-P1* and *LAZY4p-P2* are two G-box–containing fragments within the *LAZY4* promoter (see *SI Appendix*, Fig. S2, for their detailed locations). *PIL1-Q10* and *PIL1-Q6* were used as a negative and positive controls, respectively. (*F* and *G*) *LAZY4* expression levels in Col and *pifq* hypocotyls. Total RNA was extracted from the hypocotyls of seedlings grown in the dark for 3, 4, or 5 d (*F*) or grown in the dark for 4 d and then transferred to red light for 1 or 6 h (*G*). Then, the levels of *LAZY4* transcripts were examined by qRT-PCR. (*B–G*) Error bars represent the SD of three technical replicates. All experiments were repeated three times, and a representative result is shown.
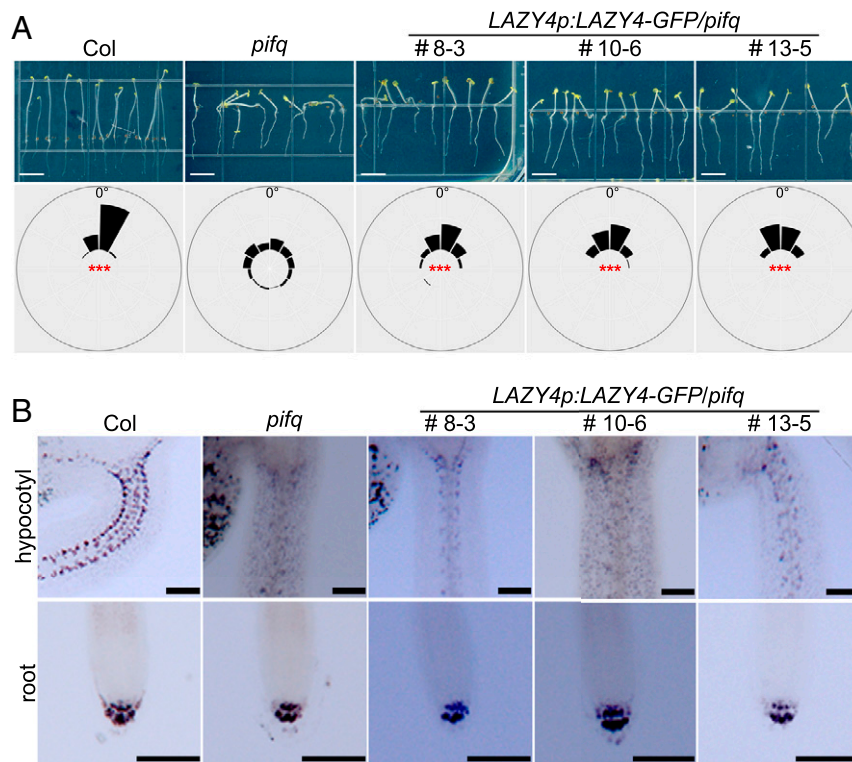
Fig. 2. Expression of LAZY4-GFP partially rescues hypocotyl negative gravitropism but not amyloplast development in the *pifq* mutant. (*A*) Overexpression of *LAZY4-GFP* partially rescued the negative gravitropism of *pifq* hypocotyls. Seedlings of Col, *pifq*, and three *LAZY4p:LAZY4-GFP/pifq* transgenic lines were grown vertically in the dark for 4 d (*Top*). Frequencies of hypocotyl growth directions are shown in the corresponding angular position around a circle at intervals of 30° (*n* = 50 to 90; *Bottom*). For statistical analyses, means of the absolute value of the angles between the growth direction and upward vertical axis were compared. Asterisks indicate significant differences compared to *pifq*, as determined by one-way ANOVA (***$P < 0.001$). The arrow labeled with a "g" represents the gravity direction (Scale bars, 5 mm.) (*B*) Overexpression of *LAZY4-GFP* did not rescue amyloplast development in *pifq* hypocotyls. Amyloplasts in the hypocotyls (*Top*) and roots (*Bottom*) of 4-d-old dark-grown Col, *pifq*, and three *LAZY4p:LAZY4-GFP/pifq* transgenic lines were detected by I_2-KI staining (Scale bars, 200 μm.)

gravitropism of roots. Previous research reported that a mutation in HY5, a core positive transcription factor in photomorphogenesis, weakened gravitropic responses in both primary and lateral roots (22, 28, 29). We first examined the primary root gravitropism of the *hy5* mutant and found it to be defective compared to the wild type in a gravistimulation test (Fig. 3*A*). The lateral root angles of the *hy5-215* mutant were also found to be significantly larger when compared to those of the wild type (Fig. 3*B*). Taken together, these data suggest that HY5 plays roles in regulating the gravitropism of roots, and we next pursued study of the underlying molecular basis of the role of HY5 in this regulation.

As HY5 is an extensively studied bZIP transcription factor, we searched for gravitropism-related genes in the targets of HY5. Analysis of existing HY5 ChIP-chip data (26, 27) revealed that HY5 can also bind to the *LAZY4* promoter (*SI Appendix*, Fig. S1*B*). In order to confirm the binding of HY5 to the *LAZY4* promoter and identify its binding region, we divided the *LAZY4* promoter into three overlapping fragments, designated a, b, and c (*SI Appendix*, Fig. S2). Using a yeast one-hybrid assay, we found that HY5 was able to bind the c fragment of the *LAZY4* promoter (Fig. 3*C*). Further mutagenesis analysis revealed that HY5 primarily binds to the *ACE8* and/or *ACE9* regions in the c fragment of the *LAZY4* promoter (Fig. 3*D* and *SI Appendix*, Fig. S2). We next performed an electrophoretic mobility shift assay (EMSA), which further demonstrated the direct binding of HY5 to the *ACE8* and *ACE9* regions within the c fragment of the *LAZY4* promoter (Fig. 3*E*). In addition, HY5 was shown to bind the *LAZY4* promoter in vivo in our ChIP-qPCR analyses (Fig. 3*F*).

Taken together, our data demonstrate that HY5 directly binds to the *ACE8* and/or *ACE9* regions in the c fragment of the *LAZY4* promoter in vitro and in vivo.

To investigate the role of HY5 in regulating *LAZY4* expression and root gravitropism, we compared the expression of *LAZY4* in roots from the wild type and *hy5* mutant using qRT-PCR analyses. Levels of *LAZY4* transcripts decreased in *hy5* mutant roots at all time points examined, suggesting that HY5 induces *LAZY4* transcription in *Arabidopsis* roots (Fig. 3*G*). A previous study showed that *LAZY4* expresses in both columella and endodermal cells of roots, but only the expression of *LAZY4* in root columella cells plays a role in root gravitropic responses (43). Columella cells locate in the root tips, and we found that the expression of *LAZY4* was actually reduced in the root tips when *HY5* was mutated (*SI Appendix*, Fig. S4*A*). Since light promotes the accumulation of HY5 protein by repressing COP1 function (24, 48), we speculated that light may promote *LAZY4* accumulation in roots, which we confirmed (Fig. 3*H*). Importantly, light-induced expression of *LAZY4* was completely abolished in the *hy5* mutant (Fig. 3*H*). Taken together, our data demonstrate that, in roots, HY5 positively regulates *LAZY4* expression through direct binding to the *LAZY4* promoter and that light increases accumulation of HY5 protein, which promotes *LAZY4* expression.

The Defective Root Gravitropism of the *hy5* Mutant Is Largely Rescued by Increased *LAZY4* Expression. As light is capable of inducing *LAZY4* expression via HY5 accumulation and *LAZY4* plays positive roles in root gravitropism (40, 43–45), we speculated that light promotes positive gravitropism of roots. Our
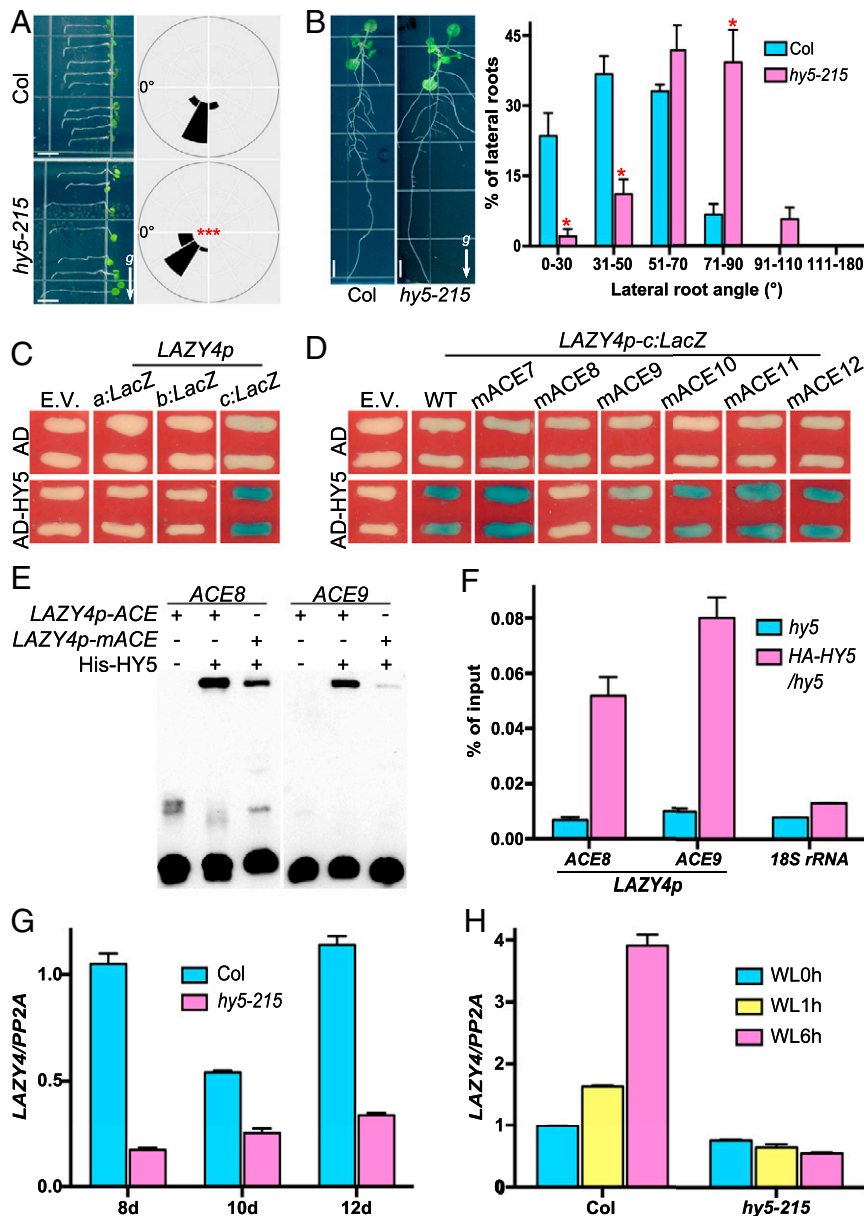
Fig. 3. HY5 directly activates *LAZY4* expression in roots. (*A* and *B*) Mutation of HY5 disrupts *Arabidopsis* root gravitropism under light. (*A*) Seedlings of Col and *hy5-215* were grown vertically in white light for 3 d and then rotated 90° for 12 h to induce gravistimulation. The angles between main root directions and the left horizontal axis were calculated. The frequencies of main root angles are shown in the corresponding angular position around a circle at intervals of 30° (*n* = 54). (*B*) Seedlings of Col and *hy5-215* were grown vertically in white light for 12 d, and the distributions of lateral root angles were analyzed. Individual lateral root angle values were sorted into six categories, and percentages of each category were calculated. Bars represent SEM (*n* = 4 experiments; 40 to 50 lateral roots per experiment). In *A* and *B*, asterisks indicate significant differences, as determined by Student's *t* test (***$P < 0.001$, *$P < 0.05$). Arrows with a "g" represent the gravity direction (Scale bars, 5 mm.) (*C* and *D*) HY5 binds to the *LAZY4* promoter in the yeast one-hybrid assay. (*C*) The letters a, b, and c indicate three fragments of the *LAZY4* promoter, as shown in *SI Appendix*, Fig. S2. Empty vector (E.V.) expressing the AD domain alone served as the negative control. (*D*) mACE7 to mACE12 represent fragment c with mutations (ACGT to gggg) in six different regions. (*E*) His-HY5 binds to *LAZY4p-ACE8* and *LAZY4p-ACE9* probes in EMSA. Recombinant His-HY5 was purified from *Escherichia coli*. *LAZY4p-ACE* and *LAZY4p-mACE* are wild-type and mutated probes, respectively. (*F*) HY5 binds to the *LAZY4* promoter in ChIP assays. Seedlings of 4-d-old white light-grown Col and *HA-HY5/hy5* were collected for ChIP analysis. *LAZY4p-ACE8* and *LAZY4p-ACE9* are two small fragments within fragment c of the *LAZY4* promoter and were detected by qPCR. The *18S rRNA* was used as a negative control. (*G* and *H*) HY5 positively regulates *LAZY4* expression in *Arabidopsis* roots. (*G*) Seedlings of Col and *hy5-215* were grown in white light for 8, 10, or 12 d or (*H*) in the dark for 4 d and then transferred to white light for 1 and 6 h, respectively. WL, white light. In *G* and *H*, total RNA was extracted from the roots, and the expression of *LAZY4* was detected by qRT-PCR. *PP2A* was used as an internal control. (*F–H*) Error bars represent the SD of three technical replicates. These experiments were repeated three times, and a representative result is shown.

gravistimulation experiment indeed confirmed this expectation (Fig. 4*A*). This result is consistent with previous reports (49–52).

To perform a genetic analysis of the relationship between HY5 and *LAZY4*, we introduced a LAZY4-GFP fusion protein driven by the native *LAZY4* promoter into the *hy5* mutant and two independent *LAZY4p:LAZY4-GFP/hy5-215* lines that were

used for root gravitropism assays. The *LAZY4* expression levels in these two lines were clearly higher than those in the *hy5* mutant but less than that in the wild type (*SI Appendix*, Fig. S4*B*). We first examined the primary root gravitropism of the transgenic lines. *LAZY4* expression driven by the native *LAZY4* promoter is able to partially rescue the gravitropic phenotype of
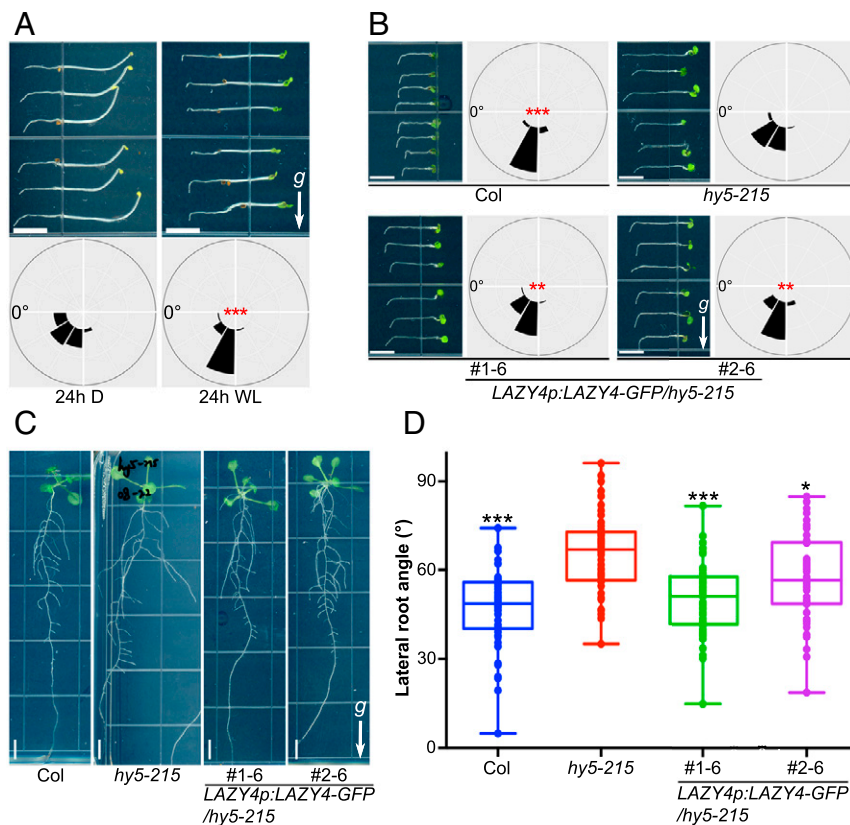
Fig. 4. Expression of LAZY4-GFP largely rescues the positive gravitropism of *hy5-215* roots. (*A*) Light promotes the gravitropism of *Arabidopsis* roots. Three-day-old dark-grown seedlings were either maintained in the dark or transferred to white light followed by a 90° rotation for 24 h to induce grav-istimulation. Frequencies of root angles are shown in the corresponding angular position around a circle at intervals of 30° (*n* = 54). Asterisks indicate significant differences, as determined by Student's *t* test (***P < 0.001). The arrow with a "g" represents the gravity direction (Scale bars, 5 mm.) (*B*) Expression of *LAZY4-GFP* partially rescued the gravitropism of the *hy5* main roots. Seedlings of Col, *hy5-215*, and two *LAZY4p:LAZY4-GFP/hy5-215* transgenic lines were grown in white light for 3 d and then rotated 90° for 12 h to induce gravistimulation. Frequencies of root angles are shown in the corresponding angular position around a circle at intervals of 30° (*n* = 54). Asterisks indicate significant differences compared to *hy5-215*, as determined by one-way ANOVA (***P < 0.001, **P < 0.01). The arrow with a "g" represents the gravity direction (Scale bars, 5 mm.) (*C* and *D*) Expression of *LAZY4-GFP* largely rescued the gravitropism of *hy5* lateral roots. Seedlings of Col, *hy5-215*, and two *LAZY4p:LAZY4-GFP/hy5-215* transgenic lines were grown in white light for 12 d (*C*), and the distribution of lateral root angles were analyzed (*D*, *n* = 40 to 50 for each genotype). Asterisks indicate significant differences compared to *hy5-215*, as determined by one-way ANOVA (***P < 0.001, *P < 0.05) (Scale bars, 5 mm.)

hy5 roots (Fig. 4*B*). The lateral roots of *hy5-215* plants exhibit a larger growth angle, as previously reported (22, 28, 29), whereas the lateral root angles of the two transgenic lines are similar to that of wild-type plants (Fig. 4 *C* and *D*). Taken together, our data suggest that *LAZY4* acts downstream of HY5 and that the abnormal gravitropism of *hy5* roots can be largely rescued by increasing *LAZY4* expression.

PIFs and HY5 Control *LAZY4* Expression in Hypocotyls and Roots, Respectively, to Regulate Gravitropism. The study described above revealed that both PIFs and HY5 positively regulate the *LAZY4* transcript levels. However, PIFs play an important role in regulation hypocotyl gravitropism, whereas HY5 functions mainly in root gravitropism. To uncover the specificity of these two groups of light-regulated transcription factors in gravitropism, we compared *LAZY4* expression in hypocotyls and roots of Col, *pifq*, and *hy5-215* in parallel during the dark-light transition. Although HY5 showed some positive regulation on *LAYZ4* expression, PIFs predominantly mediated *LAZY4* expression in hypocotyls (Fig. 5*A*). In contrast, HY5 predominantly mediated *LAZY4* expression whereas PIFs did not show any regulation in roots (Fig. 5*B*). These expression patterns explain the observed organ-specific roles of PIFs and HY5 in gravitropism well.

From our findings, we propose a model to describe how light differentially mediates the expression of *LAZY4* via PIFs and HY5 to regulate the gravitropism of hypocotyls and roots, respectively, in *Arabidopsis* (Fig. 5*C*). In hypocotyls, PIFs are stabilized in the dark, which activates *LAZY4* expression and promotes the negative gravitropism of hypocotyls. Upon light exposure, phytochromes inhibit PIFs by targeting them for protein degradation, which results in reduced *LAZY4* expression and inhibition of hypocotyl negative gravitropism. In roots, HY5 is degraded in darkness through the 26S proteasome pathway, leading to reduced *LAZY4* expression in roots and relatively weak gravitropism. Upon light exposure, HY5 is stabilized to activate *LAZY4* transcription in roots, which promotes root positive gravitropism (Fig. 5*C*).

Discussion

Light and gravity are constant environmental factors that coordinate to control plant development, but the mechanisms that underlie this coordinated regulation remain elusive. Red light inhibits the negative gravitropism of *Arabidopsis* hypocotyls, and the loss of phytochrome A and B function suppresses red-light inhibition of hypocotyl negative gravitropism (53, 54). Phytochromes promote the conversion of gravity-sensing amyloplasts
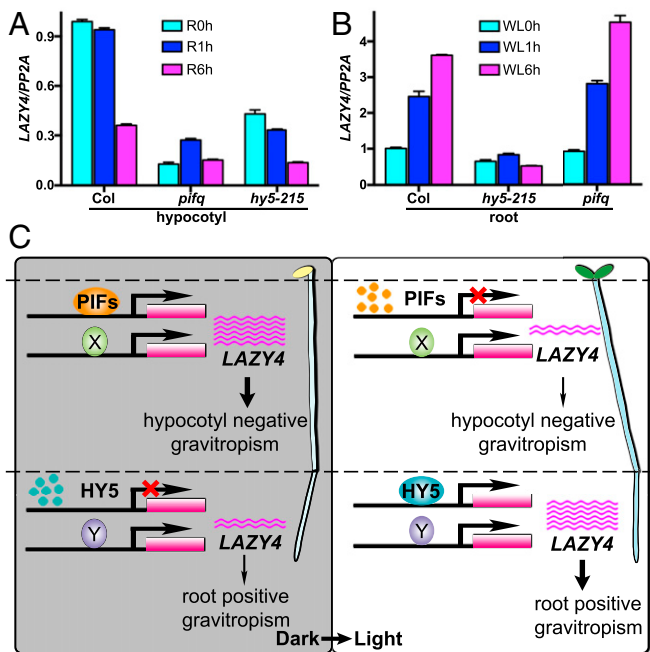
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Fig. 5. PIFs and HY5 control the expression of *LAZY4* in hypocotyls and roots, respectively, to regulate gravitropism. (*A* and *B*) *LAZY4* expression levels in Col, *pifq*, and *hy5-215* hypocotyls transferred from dark to red light (*A*) and in roots transferred from dark to white light (*B*). Total RNA was extracted from hypocotyls or roots from seedlings growing in the dark for 4 d and then transferred to light for 1 and 6 h, respectively. Transcripts were detected by qRT-PCR. Error bars represent the SD of three technical replicates. These experiments were repeated three times, and a representative result is shown. R, red light. WL, white light. (*C*) A model describing the roles of PIFs and HY5 in regulation of hypocotyl and root gravitropism via regulation of *LAZY4* expression. In the dark, high accumulation of PIF proteins activates *LAZY4* expression in hypocotyls to maintain their negative gravitropism. After light exposure, PIF proteins degrade quickly, and *LAZY4* expression is dramatically reduced, which leads to inhibition of the negative gravitropism of hypocotyls. In roots, light exposure promotes accumulation of HY5 proteins, and *LAZY4* expression increases, which promotes the positive gravitropism of roots. X and Y indicate speculated uncharacterized factors in the hypocotyls and roots, respectively.

to other plastids by mediating PIF degradation, which reduces the plant's gravity-sensing capability and leads to inhibition of hypocotyl negative gravitropism (19). Subsequent studies revealed that endodermal PIF1 directly targets REPRESSOR OF PHOTOSYNTHETIC GENES1 (RPGE1) to inhibit conversion of amyloplasts to plastids with high expression of photosynthetic genes (20). Here, we revealed that *LAZY4*, a key factor in gravity signaling, is directly activated by PIFs in darkness to promote negative gravitropism of hypocotyls (Fig. 1). Previous studies showed that PIFs could function as either transcriptional activators or repressors (17, 18, 55–59), and PIFs clearly work as transcriptional activators in the regulation of *LAZY4* in this study. Importantly, we demonstrated that this PIFs-*LAZY4* module regulates gravitropism using a mechanism distinct from mediation of amyloplast development (Fig. 2). Together, these studies show that PIFs, the key transcriptional factors that function in light signaling, promote negative gravitropism of hypocotyls through at least two independent pathways: maintenance of amyloplast development and activation of *LAZY4* gene expression. Since the *lazy4* single mutant did not display reduced hypocotyl negative gravitropism (43, 44), and the possibility of the down-regulation of all of the *LAZY* family members simultaneously in the *pifq* mutant was excluded (*SI*

Appendix, Fig. S5), how reduction of *LAZY4* expression contributes to interrupted gravitropic response of *pifq* hypocotyls is unclear and needs further investigations in the future. PHYTOCHROME KINASE SUBSTRATE 4 (PKS4) and GRAVITROPIC IN THE LIGHT 1 (GIL1) have also been identified as negative regulators of light-inhibited negative gravitropism of *Arabidopsis* hypocotyls (60, 61). In the future, our understanding of the underlying molecular mechanism of light inhibition of hypocotyl negative gravitropism may be improved by studying the possible connections between these factors.

Light clearly promotes the gravitropic growth of roots. For example, maize roots and rice crown roots grew almost horizontally in the dark, and light exposure resulted in angled root growth (49–51). The roots of *Arabidopsis phyA phyB* double mutants exhibited a reduced gravitropic curvature (52). Mutation of HY5, a key transcription factor and positive regulator in photomorphogenesis, led to defective gravitropic responses in both primary and lateral roots (22, 28, 29). However, we still do not understand the molecular mechanisms that explain how these light-signaling factors regulate gravitropism. Our study shows that light-stabilized HY5 directly binds to the *LAZY4* promoter and induces *LAZY4* expression to promote root positive gravitropism (Figs. 3 and 4), revealing a molecular mechanism by which a key light-signaling factor regulates root gravitropism.

As a key environmental factor, light regulates many developmental processes of plants, and light-signaling pathways have been extensively studied. However, light-mediated processes differ in various plant organs. This phenomenon has been recognized for several decades, but the mechanisms underlying these differences have just begun to be revealed in recent years. Previously, we found that light promotes cotyledon expansion while inhibiting hypocotyl elongation by differentially mediating the expression of *SAUR* genes in *Arabidopsis* (62, 63). In this study, we demonstrated that light inhibits the gravitropism of hypocotyls but promotes the gravitropism of roots via differential regulation of *LAZY4* gene expression to reveal a paradigm of organ-specific light response in plants. Although PIFs and HY5 are expressed in both hypocotyls and roots (22, 28, 30, 31, 64), they predominantly promote the expression of *LAZY4* in hypocotyls and roots, respectively, in regulating gravitropic responses (Fig. 5). We speculate that unknown cofactors specifically expressed in hypocotyls or roots may work together with PIFs and HY5 to mediate the organ-specific regulation of *LAZY4* expression, a hypothesis that will be addressed in future experiments.

Materials and Methods

Plant Materials and Growth Conditions. The wild-type *A. thaliana* line used in this study was Columbia-0. The mutants and transgenic lines used in this study were described previously: *hy5-215* (22); *pif1 pif3 pif4 pif5* (*pifq*) (8); *PIF1-Myc*, *PIF3-Myc*, *PIF4-Myc*, and *PIF5-Myc* (65); and *hy5* (SALK_096651) (66).

For all experiments, *Arabidopsis* seeds were surface-sterilized with 15% NaClO for 5 min followed by rinsing with sterile H$_2$O three times. Seeds were sown onto Murashige and Skoog medium (MS, 1% sucrose and 0.8% agar) and cold-treated at 4 °C for 2 to 3 d in the dark. Subsequently, the seeds were irradiated with white light (100 μmol·m^{-2}·s^{-1}) for 3 h to induce germination and then grown under various light conditions.

Plasmid Construction. The *LAZY4* genomic sequence was fused with GFP and introduced into pJim19 (Bar) to generate the *LAZY4p:LAZY4-GFP* (Bar) construct. The *LAZY4p:LAZY4-GFP* (Bar) plasmid was digested with SbfI and EcoRI, and the *LAZY4p:LAZY4-GFP* cassette was inserted into the pCambia1300 plasmid to generate *LAZY4p:LAZY4-GFP* (Hyg). The *LAZY4p:LAZY4-GFP* (Bar) and *LAZY4p:LAZY4-GFP* (Hyg) plasmids were then introduced into *Agrobacterium tumefaciens* (strain GV3101) and transformed into the *hy5-215* and *pifq* mutants via the floral dip method.

To generate the *LAZY4p-a:LacZ*, *LAZY4p-b:LacZ*, and *LAZY4p-c:LacZ* reporter constructs, the promoter fragments were amplified from the *LAZY4p:LAZY4-GFP* construct by PCR and cloned into the EcoRI-SalI site of pLacZi2μ (67). *LacZ* reporter genes driven by the various c fragments of the *LAZY4* promoter with mutations in ACEs were amplified by overlap extension PCR

using the *LAZY4p-c:LacZ* construct as the template. The AD-HY5 construct was described previously (68).

All primers used in this study are summarized in *SI Appendix*, Table S1.

Quantitative Real-Time PCR. Total RNA from hypocotyls or roots was extracted using the Takara MiniBEST Plant RNA Extraction Kit (Takara). Reverse transcription was performed using ReverTra Ace qPCR RT Master Mix (TOYOBO) according to the manufacturer's instructions. SYBR Premix ExTaq (Takara), complementary DNA templates, and primer sets were combined, and real-time qPCR analyses were performed on an Applied Biosystems 7500 Fast Real-Time PCR detection system (ABI). Each experiment was repeated with three independent samples, and qRT-PCR reactions were performed with three technical replicates for each sample. *PP2A* was used as an internal control. The primers used for qRT-PCR are listed in *SI Appendix*, Table S1.

Yeast One-Hybrid Assays. The AD-fusion plasmid was cotransformed with the *LacZ* reporter genes under the control of various *LAZY4* promoter fragments into the yeast strain EGY48. Transformants were selected and grown on proper dropout plates and then transferred to proper dropout medium containing 5-bromo-4-chloro-3-indolyl-β-ᴅ-galactopyranoside for interaction tests. Yeast transformation was conducted as described in the Yeast Protocols Handbook (Clontech).

EMSA. EMSAs were performed using biotin-labeled probes and the Lightshift Chemiluminescent EMSA kit (Thermo Fisher). Briefly, 1 μg of His-HY5 fusion protein was incubated together with biotin-labeled wild type or mutated probes in 20-mL reaction mixtures containing 10 mM Tris-HCl, 150 mM KCl, 1 mM dithiothreitol, 50 ng/μL poly(dI-dC), 2.5% glycerol, 0.05% Nonidet P-40, 100 μM MgCl$_2$, and 0.5 mg/mL bovine serum albumin for 20 min at room temperature and separated on 6% native polyacrylamide gels in Tris-glycine buffer. The labeled probes were detected according to the instructions provided with the EMSA kit. The sequences of the oligonucleotides used to generate the biotin-labeled probes are shown in *SI Appendix*, Table S1.

ChIP. ChIP assays were performed as previously described (26). Seedlings were grown in the dark or in white light for 4 d, and then samples were harvested and treated with 1% formaldehyde under vacuum to cross-link the protein and DNA. Samples were ground into powder in liquid nitrogen, sonicated, and then incubated with anti-Myc agarose beads (Invitrogen) or anti-HA (Cell Signaling Technology) antibody to isolate chromatin complexes. Protein A magnetic beads (Invitrogen) were used to precipitate chromatin complexes from samples incubated with the anti-HA antibody. The precipitated DNA was recovered and analyzed by real-time PCR using the primers listed in *SI Appendix*, Table S1.

Gravitropism Analyses. To investigate light-regulated gravitropic responses of hypocotyls, seeds were first irradiated with white light (100 μmol·m^{-2}·s^{-1}) for 3 h to induce germination and then incubated vertically in the dark or in red light (5 μmol·m^{-2}·s^{-1}) for 4 d. Growth orientations of hypocotyls were measured by degrees from the vertical axis.

For gravistimulation analyses of main roots, seedlings were grown vertically on MS plates for 3 d in darkness or white light. Then, seedlings of similar lengths were selected and transferred to new MS plates, with their roots vertically downward, and incubated for another 3 h. Next, the plates were rotated by 90° for gravitropic stimulation. Dim green light was used during manipulation of the seedlings in the dark. The curvature of the roots was measured from the digital images using ImageJ software (https://imagej.nih.gov/ij/).

To observe the lateral root angles, plants were grown vertically under constant white light for 12 d. The lateral root angles relative to the direction of gravity vector were measured using ImageJ at the position of 2 mm from the base of the lateral roots.

Staining of Amyloplasts. Amyloplasts were stained with iodine as described previously (19). Four-day-old dark-grown seedlings were fixed with FAA (5% formaldehyde, 45% ethanol, 5% acetic acid) solution at 4 °C for 24 h. Then, the fixed seedlings were rinsed once in 50% (vol/vol) ethanol and stained in I$_2$-KI solution (2% [wt/vol] iodine, 5% [wt/vol] potassium iodine, and 20% [wt/vol] chloral hydrate) for 1 to 2 min. Finally, the seedlings were de-stained in a 1:1:1 trichloroacetic acid:phenol:lactic acid solution for 5 min. Light microscopy was used to observe the amyloplasts.

Data Availability. All data are included in the paper and *SI Appendix*. The materials described in the manuscript are freely available to readers upon request.

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