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Research Article

FAR-RED INSENSITIVE 219 and phytochrome B corepress shade avoidance via modulating nuclear speckle formation

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Abstract

Plants can sense the shade from neighboring plants by detecting a reduction of the red:far-red light (R:FR) ratio. Phytochrome B (phyB) is the primary photoreceptor that perceives shade light and regulates jasmonic acid (JA) signaling. However, the molecular mechanisms underlying phyB and JA signaling integration in shade responses remain largely unknown. Here, we show the interaction of phyB and FAR-RED INSENSITIVE 219 (FIN219)/JASMONATE RESISTANT1 (JAR1) in a functional demand manner in Arabidopsis (*Arabidopsis thaliana*) seedling development. Genetic evidence and interaction studies indicated that phyB and FIN219 synergistically and negatively regulate shade-induced hypocotyl elongation. Moreover, phyB interacted with various isoforms of FIN219 under high and low R:FR light. Methyl jasmonate (MeJA) treatment, *FIN219* mutation, and *PHYBOE digalactosyldiacylglycerol synthase1-1* (*dgd1-1*) plants, which show increased levels of JA, altered the patterns of phyB-associated nuclear speckles under the same conditions. Surprisingly, *PHYBOE dgd1-1* showed a shorter hypocotyl phenotype than its parental mutants under shade conditions. Microarray assays using *PHYBOE* and *PHYBOE fin219-2* indicated that *PHYB* overexpression substantially affects defense response-related genes under shade light and coregulates expression of auxin-responsive genes with FIN219. Thus, our findings reveal that phyB substantially crosstalks with JA signaling through FIN219 to modulate seedling development under shade light.

Introduction

Plants can adjust their growth patterns by sensing changes in light environments, such as the relative ratio of red (R) and far-red (FR) light. When plants encounter shade light with an R:FR ratio <1, they start to alter growth by the so-called shade avoidance syndrome, including elongated hypocotyl, stem, and petiole, a reduction of chlorophylls as well as leaf size, and early flowering to survive under shade condition (Casal 2012). Emerging evidence indicates that photoreceptors and phytohormones, including jasmonic acids (JAs), participate in the shade avoidance signaling pathway (Djakovic-Petrovic et al. 2007; Keuskamp et al. 2010). However, the crosstalk between them upon exposure to shade remains elusive.

The phytochrome B (phyB) is a primary photoreceptor to perceive shade light. Photoactivated phyB caused by high R:FR light enters the nucleus, forming nuclear speckles (NS) and associates with transcription factors such as

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phytochrome interacting factors PIF4 and PIF5, which results in degradation of PIF proteins and a photomorphogenic development of seedlings (Lorrain et al. 2008). However, under a low R:FR light ratio (shade condition), phyB becomes inactive and may remain in the cytoplasm or the nucleus without the NS formation, which leads to PIF4/5 binding to the promoters of target genes, thus giving rise to shade signaling and responses (Hornitschek et al. 2012). Further studies reveal that PIF7 is vital in regulating shade responses and dephosphorylated upon shade light exposure (Li et al. 2012). Recent evidence indicates that circadian clock components link with PIF proteins to repress PIF-mediated transcriptional activation of target genes (Zhang et al. 2020). Further, phyA upregulates the circadian clock components to suppress shade avoidance in canopy shade conditions (Fraser et al. 2021). Therefore, shade avoidance signaling involves integrated networks to trigger shade responses.

The integrated networks in shade signaling include the crosstalk between shade light and various phytohormones. Shade-induced elongation growth substantially involves auxin from auxin biosynthesis and transport to signaling (Casal 2012), suggesting that auxin is vital in shade-mediated responses. Besides, gibberellins (GAs) are closely linked with shade-mediated responses through the DELLA-PIFs pathway. Shade light increases bioactive GAs, triggering degradation of negative regulators DELLA proteins. The resulting PIF proteins, such as PIF4 and PIF5, can bind to the promoters of target genes, which contributes to shade-induced growth (Djakovic-Petrovic et al. 2007; Keuskamp et al. 2010). Other hormones such as ethylene, salicylic acid, and abscissa acid also play roles in the modulation of shade-mediated growth responses (Casal 2012; Huot et al. 2014). In addition, recent evidence reveals that jasmonates (JAs) are involved in the regulation of shade responses and become a vital issue in the integration of plant growth and defense responses (Huot et al. 2014). Moreover, JAs are involved in plant defense and the regulation of plant growth and development, suggesting that JAs may be an excellent candidate to elucidate the tradeoffs between plant growth and defense response under shading conditions. FAR-RED INSENSITIVE 219 (FIN219)/JASMONATE RESISTANT1 (JAR1) is a JA-conjugating enzyme responsible for the formation of a physiologically active JA-isoleucine (JA-Ile) and participates in phyA-mediated FR light signaling (Hsieh et al. 2000; Staswick et al. 2002). Previous studies indicated that FIN219/JAR1 was involved in shade light signaling (Swain et al. 2017). Further, FIN219/JAR1 differentially affects the expression of shade signaling bHLH factors PIF5 and PAR1, leading to increased expression of auxin-responsive genes such as *IAA29* and *SAUR68* in response to shade light. Moreover, co-immunoprecipitation (Co-IP) studies reveal that FIN219 interacts with phyA and COP1 under shading conditions (Swain et al. 2017). This association may result in a release of shade-induced growth. The regulatory relationships among phyA, FIN219, and COP1 in response to shade light remain elusive.

The phyB perceives the shade light to initiate shade avoidance responses, such as enhanced stem and petiole elongation. Shade light reduces phyB activity by abolishing the NS that are a typical feature of phyB under high R:FR conditions (Trupkin et al. 2014). NS patterns consist of various sizes and numbers of particles and are a dynamic process. The components involved in NS formation remain largely unknown. Arabidopsis (*Arabidopsis thaliana*) phyB photoactivated by red light migrates to the nucleus and forms NS (Van Buskirk et al. 2012). *PHYB* overexpression lines (*PHYBOE-GFP*) exhibit 2–3 substantial NS. Chen et al. (2010) identified HEMERA (HMR) by using *PHYBOE-GFP* as a target for screening candidates affecting NS formation patterns and showed that HMR affects the size and the number of NS. Its mutant *hmr-1* almost abolishes NS formation, resulting in substantially increased stability of PHYA, PIF1, and PIF3. Further evidence indicates that HMR directly interacts with photoactivated phyA and phyB to increase their accumulation, leading to NS formation and PIF degradation (Galvao et al. 2012). The physiological functions of NS remain unclear, although some data suggest that NS is implicated in protein degradation or splicing events (Van Buskirk et al. 2012; Feng et al. 2020).

FIN219/JAR1 participates in shade light signaling (Robson et al. 2010; Swain et al. 2017). The phyB is a primary photoreceptor for perceiving shade light and triggers shade-mediated responses. Moreover, both proteins function as negative regulators in modulating shade avoidance responses. It will be interesting to examine whether they have a regulatory relationship in response to shade light. Thus, we apply molecular genetics, cell biology, and molecular biology approaches to reveal their functions in shade signaling. Here, we report that FIN219 exists in phosphorylated and dephosphorylated forms and becomes dephosphorylated upon exposure to shade light. Co-IP studies reveal that phyB interacts with phosphorylated FIN219 under high R:FR light and with dephosphorylated one under shade light. Moreover, FIN219 alters phyB-associated NS patterns under high R:FR light. Taken together, phyB and FIN219 may fine-tune with each other in response to shade light, leading to modulation of seedling development.

Results

fin219-2 mutant shows defects in hypocotyl and petiole elongation under shade light

FIN219/JAR1 is a JA-conjugating enzyme (Staswick et al. 2002) and regulates shade-mediated responses such as hypocotyl elongation (Swain et al. 2017). To further elucidate the functional roles of FIN219 involved in shade light signaling, we examine phenotypic responses of *fin219-2* mutant, a null allele, under low R:FR (shade) light. As expected, the *fin219-2* mutant exhibits a longer hypocotyl than wild-type Columbia-0 (Col-0) under shade light (Fig. 1, A and B). Besides, *fin219-2* also shows a hyponastic response of leaves

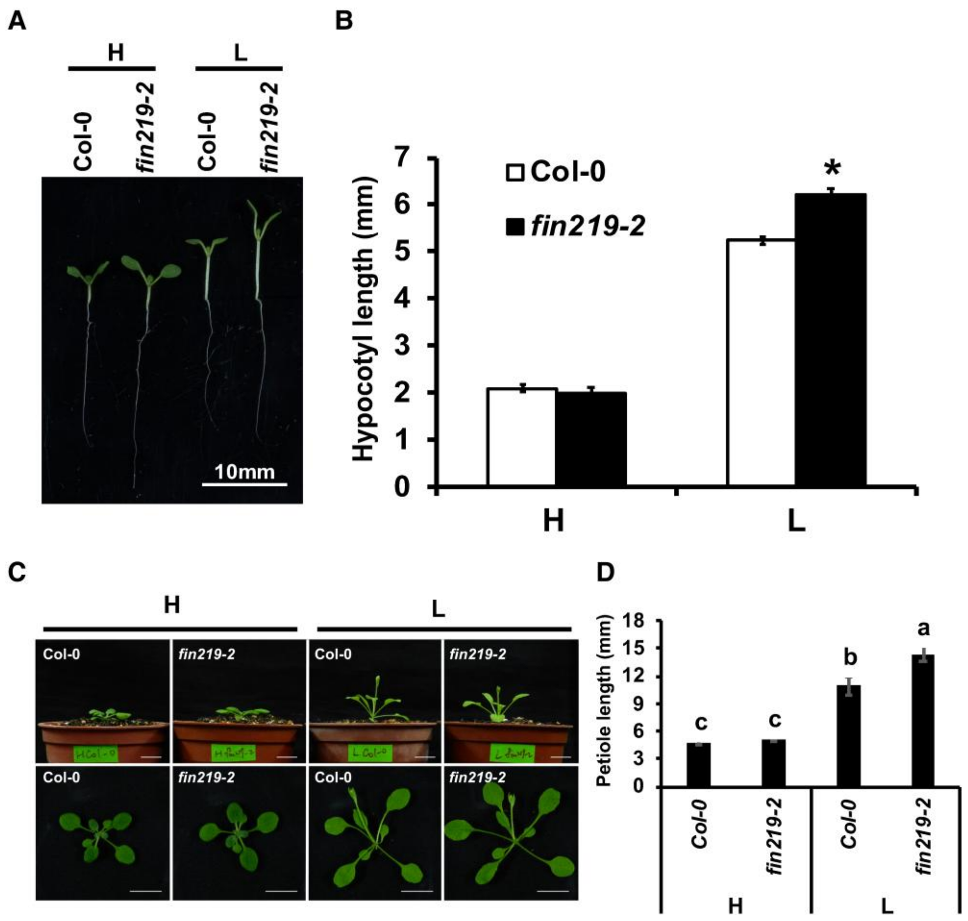

Figure 1. FIN219 is a negative regulator of shade light-triggered hypocotyl and petiole elongation. **A)** The 6-d-old wild-type Col-0 and *fin219-2* mutant phenotype under high (H) and low (L) R:FR ratio light conditions. Scale bar: 10 mm. **B)** The quantitative measurement of the hypocotyl lengths of the seedlings shown in (A). Data are presented as mean ± SD ($n \geq 25$). Asterisks indicate significant differences in the hypocotyl lengths between Col-0 and *fin219-2* seedlings with $P < 0.05$ by Student's *t*-test. **C)** Images of Col-0 and *fin219-2* seedlings grown under high (H) and low (L) R:FR ratio light conditions for 15 d. Images of the side view in Col-0 and *fin219-2* mutant are shown in the upper panel, and pictures of the top view in the lower forum. Scale bar: 10 mm. **D)** The quantitative measurement of the petiole lengths of the seedlings shown in (C). Data are presented as mean ± SD ($n \geq 25$). Different lowercase letters represent significant differences in the hypocotyl lengths of Col-0 and *fin219-2* (1-way ANOVA, Tukey's HSD, $P < 0.05$).

compared to Col-0 under shade light (Fig. 1, A and C). Further examination indicates that *fin219-2* has a longer petiole than Col-0 under shade light rather than high R:FR light (Fig. 1D). Previous studies reported that petiole hyponasty was an ethylene-driven, adaptive response (Polko et al. 2011). Moreover, JA and ethylene integrate to regulate plant growth and defense responses. How JA and ethylene are involved in controlling petiole elongation in response to shade light remains elusive.

FIN219/JAR1 becomes dephosphorylated upon exposure to shade light

PhyB acts as the primary photoreceptor for the perception of the shade light, and phyB and FIN219 are negative regulators in shade light signaling. Whether both have a regulatory relationship in regulating shade responses will be worth studying further. Thus, we examine the expression levels of *PHYB* and *FIN219* genes in respective mutants under high and low R:FR light conditions. Surprisingly, the *PHYB* transcript level substantially decreases in *fin219-2* compared to that in Col-0 under both high and low R:FR light conditions. However, the *FIN219* transcript level in *phyB-1* is comparable with wild-type Landsberg *erecta* (L*er*) under the same conditions (Supplemental Fig. S1A). The monoclonal antibody is raised against PHYB and specifically detects the full-length and the C-terminal region of PHYB protein. PHYB protein slightly decreases in *phyA-201* mutant compared to L*er* (Supplemental Fig. S2, A and B).

Further, PHYB protein level in *fin219-2* slightly decreases under high R:FR light and is comparable with Col-0 under low R:FR light. However, the FIN219 protein level in *phyB-1* is similar to wild-type L*er* under high R:FR light and slightly increases under low R:FR light (Supplemental Fig. S1B).

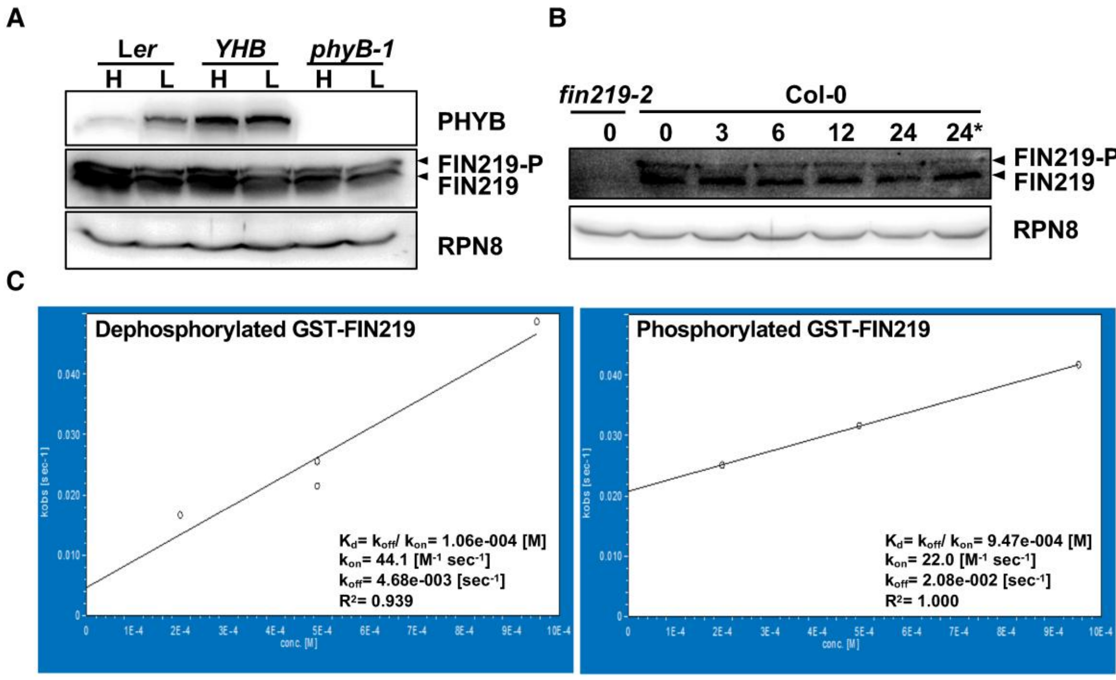

Figure 2. FIN219 is a phosphoprotein that prefers a dephosphorylated one in response to shading conditions. **A)** Immunoblot analysis of FIN219 and PHYB proteins in wild-type L*er*, *YHB*, and *phyB-1* mutant plants under high and low R:FR conditions. The seedlings of L*er*, *YHB*, and *phyB-1* mutants grown under high (H) and low R:FR (L) conditions for 6 d were harvested and subjected to analyses of the phosphorylation status of FIN219 protein by SDS-PAGE. The upper and lower arrows indicate the phosphorylated and dephosphorylated forms of FIN219, respectively. **B)** Determining the phosphorylation status of FIN219 protein in wild-type Col-0 in the light transition from high to low R:FR light. Seedlings are collected at 0, 3, 6, 12, and 24 h during the light transition. The lane labeled with 24* indicates that the protein extracts at 24 h were treated with the alkaline phosphatase (CIP, NEB) for 30 min. The upper and lower bands show the phosphorylated and the dephosphorylated forms of FIN219, respectively. Regulatory particle NON-ATPASE SUBUNIT 8A (RPN8) was used as a loading control. **C)** Binding affinity of FIN219 to JA and isoleucine (Ile) analyzed with QCM analyzer. The dephosphorylated recombinant FIN219 protein (glutathione S-transferase-fused FIN219, GST-FIN219) was purified from the *E. coli* expression system. The phosphorylated FIN219 was prepared by coincubating the GST-FIN219 recombinant protein and casein kinase II (CKII, NEB) at 30°C for 30 min. The dephosphorylated FIN219 has a dissociation constant (K_d) of 1.06e−004[M], and phosphorylated FIN219 has a K_d of 9.47e−004[M] for conjugating JA-Ile with JA, Ile, and ATP. k_{on}, k_{off}, and K_{obs} are indicated as association rate constant, dissociation rate constant, and observed rate constant, respectively.

These data suggest that phyB and FIN219 may regulate each other at the transcript or the protein level in response to light conditions. To further verify their regulatory relationship, we determine PHYB protein levels and FIN219 phosphorylation state in L*er*, *YHB* line, a constitutively active *PHYB* mutant, and *phyB-1* mutant (Su and Lagarias 2007; Hu et al. 2009). Intriguingly, FIN219 appears to exist in 2 bands, phosphorylated and dephosphorylated, in wild-type L*er* under high R:FR light and minor in phosphorylated one under low R:FR light. However, PHYB protein in L*er* accumulates substantially under low R:FR light (Fig. 2A). PHYB level in *YHB* is abundant under high R:FR light and slightly decreased under low R:FR light. In contrast, FIN219 level is reduced in the dephosphorylated band and phosphorylated one under low R:FR light. Both isoforms of FIN219 in *phyB-1* are substantially decreased under high and low R:FR (Fig. 2A). To verify the existence of FIN219 isoforms in response to shade light, we further perform light transition studies by transferring Col-0 from high to low R:FR light for different periods. Protein gel blot assays indicate that FIN219 indeed exists in phosphorylated and dephosphorylated bands, and then becomes more dephosphorylated at 24 h exposure to shade light (Fig. 2B), which is confirmed with the increased dephosphorylated band by the addition of alkaline phosphatase (CIP, NEB) treatment at 24 h shade light exposure (labeled with 24*) (Fig. 2B), suggesting that FIN219 exists in phosphorylated and dephosphorylated isoforms. In contrast, PHYB protein levels in Col-0 gradually increase in the light transition from high to low R:FR conditions (Supplemental Fig. S3). Further enzymatic activity assays by quartz crystal microbalance (QCM) analyzer indicate that the dephosphorylated GST-FIN219 has a higher affinity to JA with K_d 1.06×10^{-4} for the production of JA-isoleucine than the phosphorylated one with K_d 9.47×10^{-4} (Fig. 2C).

The *phyB-1* and *fin219-2* mutants show a genetic regulatory relationship under shade light condition

Further, we generate the *PHYB* overexpression line (*PHYB-GFP* overexpression in *phyB-1*; *PHYBOE-G*; Supplemental Fig. S4),

and *PHYBOE-G fin219-2*, *YHB fin219-2*, and the double mutant *phyB-1 fin219-2* by crossing to understand the regulatory relationships between phyB and FIN219 under shade light. Both *phyB-1* and *fin219-2* mutants show a more prolonged hypocotyl phenotype than their respective wild-type backgrounds under shading conditions, suggesting their negative roles in regulating shade-induced hypocotyl growth. In contrast, *PHYBOE-G* exhibits a comparable hypocotyl phenotype with wild-type L*er* under shade light (Fig. 3, A and B). Intriguingly, *PHYBOE-G fin219-2* shows an even shorter hypocotyl than its parental *PHYBOE-G* and *fin219-2* under shade light (Fig. 3, A and B), which implies that phyB and FIN219 may mutually regulate each other via a negative manner. Similarly, *YHB fin219-2* shows a substantial reduction of hypocotyl length compared to *fin219-2* under shade light (Fig. 3, A and B). The double mutant *phyB-1 fin219-2* displays a hypocotyl phenotype close to *phyB-1* under high and low R:FR light (Fig. 3, A and B), suggesting that FIN219 functions in shade-mediated hypocotyl growth may depend on phyB. In addition, since the *fin219-2* mutant also enhances the shade-induced petiole elongation (Fig. 1D), we examine the petiole lengths in these genetic materials shown above. Surprisingly, *PHYBOE-G fin219-2* shows a significant increase in the petiole length compared to *PHYBOE-G*. Still, it is similar to *fin219-2* under shade light (Supplemental Fig. S5), suggesting that phyB involved in regulating shade-mediated petiole elongation depends on FIN219. The *phyB-1 fin219-2* shows a much shorter petiole than *phyB-1* and *fin219-2* under shade light conditions (Supplemental Fig. S5), implying that FIN219 and phyB may apply different mechanisms in regulating petiole elongation as compared to those in the control of hypocotyl elongation under shading conditions.

phyB interacts with phosphorylated FIN219/JAR1 under high R:FR light and with dephosphorylated FIN219/JAR1 under low R:FR light

Since both phyB and FIN219 show a genetic regulation in shade-mediated responses, we wonder if they can interact with each other. Thus, we utilize yeast 2-hybrid, bimolecular fluorescence complementation (BiFC), and Co-IP approaches to examine their possible interaction. We generated the full-length, N-, and C-terminal regions of PHYB ($PHYB^{FL}$, $PHYB^{N}$, and $PHYB^{C}$, respectively) and $FIN219^{FL}$, $FIN219^{N}$, and $FIN219^{C}$ for interaction studies (Fig. 3C). Due to the self-activation of $PHYB^{FL}$-BD, PHYB fused with the activation domain (PHYB-AD) is used for yeast 2-hybrid assays. The results indicate that $PHYB^{FL}$-AD and $PHYB^{N}$-AD interact with $FIN219^{FL}$-BD under high and low R:FR light (Fig. 3D). Further BiFC studies in *Nicotiana benthamiana* leaves reveal that PHYB interacts with FIN219 under high and low R:FR light conditions (Fig. 3E). FIN219 also interacts with itself under the same conditions (Fig. 3E). Moreover, we examined the subcellular localization and cellular fractionation of FIN219 and phyB in *N. benthamiana* leaves under high and low R:FR conditions. Results indicate that FIN219 and phyB do co-localize in the cytosol under both high and low R:FR conditions (Supplemental Figs. S6 and S7). To substantiate phyB and FIN219 interaction *in planta*, we carried out Co-IP studies using the *PHYB* overexpression line (*PHYBOE-G*). Surprisingly, phyB interacted with phosphorylated FIN219 under high R:FR light and with dephosphorylated FIN219 under low R:FR light (Fig. 3F). It seems that phyB has a higher affinity with phosphorylated FIN219 than with dephosphorylated one (Fig. 3F). FIN219 activity assays with the QCM analyzer reveal that dephosphorylated FIN219 has a higher enzymatic activity than phosphorylated FIN219 in binding JA with isoleucine (Fig. 2C). It is possible that phyB binding with the dephosphorylated FIN219 might interfere with the regulatory role or enzymatic activity of FIN219, leading to a reduction of JA signal transduction, which contributes to shade-induced growth (Fig. 3, A and B). Accordingly, the *phyB-1* mutant released the dephosphorylated FIN219 and enhanced expression of JA-responsive genes such as *VSP1* under shade light (Fig. 3G).

Exogenous MeJA treatment enhances the formation of phyB-associated NS under shading conditions

Photoactivated phyB enters the nucleus and leads to the NS formation under high R:FR light. However, shade light abolishes the formation of phyB-associated NS (Hornitschek et al. 2012). Since FIN219 interacts with phyB under high and low R:FR light conditions, we wonder if FIN219-mediated JA levels affect the patterns of phyB-associated NS. Firstly, we use the other *PHYB* overexpression line $35S_{pro}$:*PHYB-YFP-HIS* (*PHYBOE-Y*) for this purpose because the *PHYBOE-Y* line gives rise to a stronger fluorescent signal than *PHYBOE-G*. Thus, *PHYBOE-Y* will be used for the rest of the phyB-associated NS studies. The *PHYBOE-Y* is treated with MeJA under high (ambient) and low R:FR light (shade) conditions and subjected to the examination of NS patterns. As expected, PHYB-YFP-associated NS are detected under ambient light without MeJA treatment (Mock) and minor with an increased number in the nucleus with MeJA treatment (Fig. 4A, top panel). Surprisingly, PHYB-YFP-mediated NS under shade light with MeJA treatment are detected and similar to those in the ambient light with the mock treatment, but not in the mock control under shade light (Fig. 4A, bottom panel). These results suggest exogenous JA levels may harm phyB functions in response to shade light.

FIN219 and phyB show a synergistic relationship in response to MeJA under high and low R:FR light conditions

To substantiate MeJA effects on phyB-associated NS under shade light, we examine hypocotyl phenotypes of phyB-related mutants in the presence of MeJA. The *fin219-2* mutant exhibits a long-hypocotyl phenotype compared to Col-0 under shade and shows less sensitivity to MeJA-mediated inhibition of

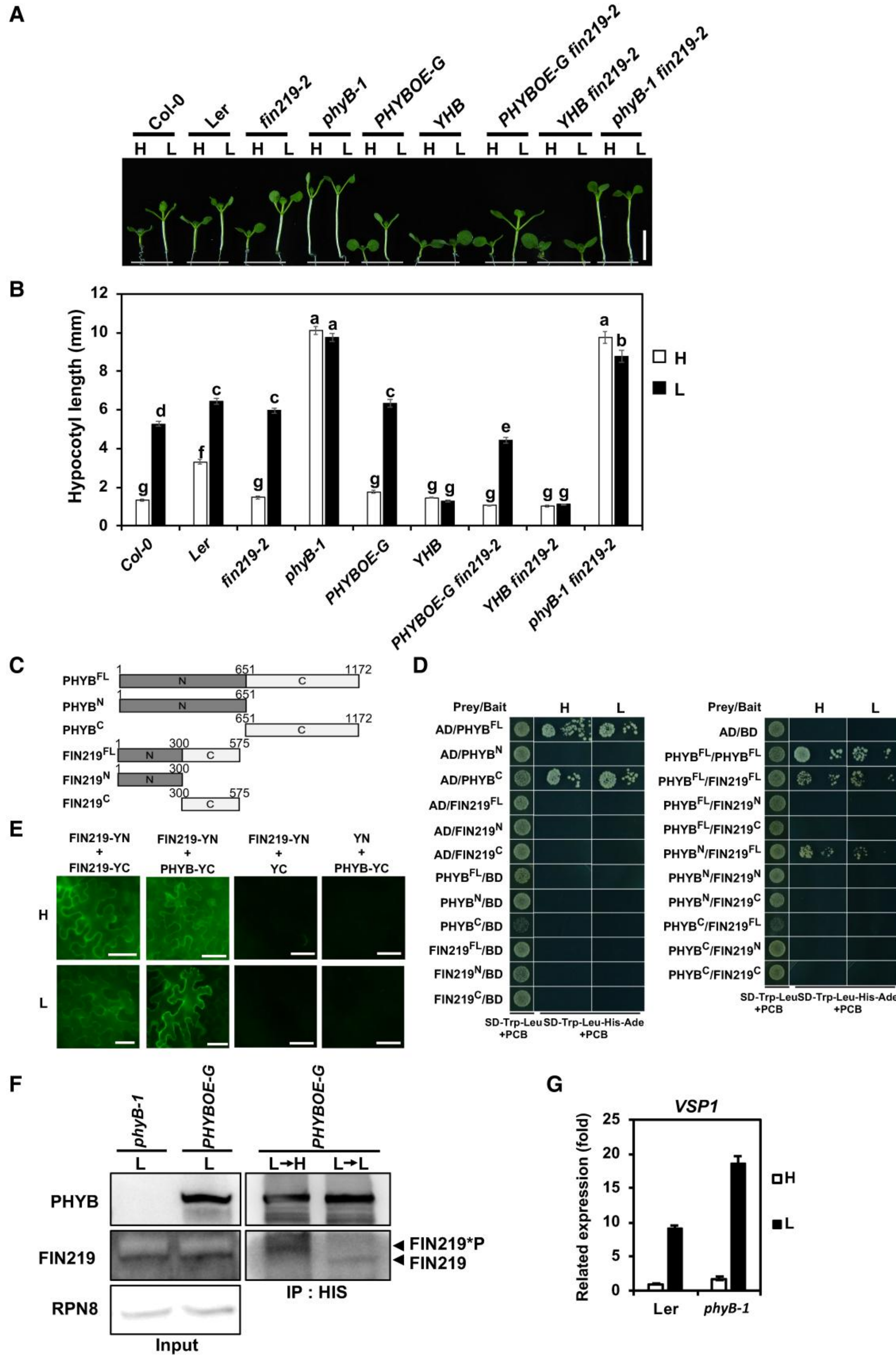

Figure 3. phyB interacts with FIN219 in the cytosol, possibly suppressing FIN219 activity under shading conditions. **A)** The phenotype of wild-type Col-0 and L*er* and different mutants grown under high and low R:FR light conditions. The seedlings of Col-0, L*er*, *fin219-2*, *phyB-1*, *PHYBOE-G*, *YHB*, *PHYBOE-G fin219-2*, *YHB fin219-2*, and *phyB-1 fin219-2* were grown under high R:FR for 2 d, then transferred to high (H) or low (L) R:FR light for

(continued)

hypocotyl elongation under shade light (Fig. 4, B and C). In contrast, *phyB-1* mutant showed a remarkable reduction of hypocotyl growth by MeJA under shade light. However, *YHB* and *YHBfin219-2* exhibit a very short hypocotyl phenotype under high and low R:FR light conditions with or without MeJA treatment (Fig. 4, B and C). Interestingly, the double mutant *phyB-1 fin219-2* shows an even longer hypocotyl than *phyB-1* and *fin219-2* in the presence of MeJA under both high and low R:FR light conditions (Fig. 4, B and C). This result suggests that phyB and FIN219 synergistically regulate MeJA-modulated hypocotyl growth under light conditions, likely through direct interaction.

Increased endogenous MeJA level enhances phyB-associated NS formation under shade light

In addition, we crossed *PHYBOE-Y* with a *dgd1-1* mutant containing high levels of JA to generate *PHYBOE-Y dgd1-1* plants for further examination of phyB-associated NS formation. PHYB-YFP-mediated NS in *PHYBOE-Y dgd1-1* plants could be detected under ambient and even shade light conditions (Fig. 5A), which is consistent with MeJA-induced formation of PHYB-associated NS under shade light, as shown in Fig. 4A. *PHYBOE-Y* line shows PHYB-mediated NS under red light and high R:FR light conditions (Fig. 5B). Similarly, *PHYBOE-Y dgd1-1* plants exhibit more PHYB-mediated NS under shading conditions in addition to red and high R:FR light (Fig. 5B). Moreover, *PHYBOE-Y fin219-2* plants show a substantial number of smaller PHYB-associated NS than *PHYBOE-Y* and *PHYBOE-Y dgd1-1* under high R:FR light conditions (Fig. 5, A–C) and have no NS formation under shade light (Fig. 5, B and C). However, *PHYBOE-G fin219-2* showed a hypocotyl length similar to *PHYBOE-G* under high R:FR light and a shorter hypocotyl than *PHYBOE-G* under shade light (Fig. 3B), which suggests that FIN219 may harm phyB functions under shade light. Interestingly, *PHYBOE-Y dgd1-1* showed a synergistic effect on hypocotyl growth under shade light, leading to a much shorter hypocotyl than *PHYBOE-Y* and *dgd1-1* mutant only under shading conditions (Fig. 5, D and E), rather than other light conditions (Supplemental Fig. S8). This result suggests that phyB and MeJA-mediated signaling synergistically trigger adverse effects on shade-induced hypocotyl growth. Therefore, enhanced formation of PHYB-associated NS under shade light results in inhibition of shade-induced hypocotyl growth.

PHYB overexpression substantially affects defense response-related genes under shade light and coregulates expression of auxin response-related genes with FIN219

Current data indicate that phyB and FIN219 work together to regulate shade-mediated hypocotyl growth negatively. To further understand their effects on genome-wide gene expression profiles under shade light versus high R:FR light, we apply microarray assays using *PHYBOE-G* and *PHYBOE-G fin219-2* lines to examine gene expression profiles. The results indicate that the *PHYBOE* line affects 256 genes regulated by shade light. GO term analyses reveal that *PHYB* overexpression affects defense response and stress-related genes (Fig. 6A and Supplemental Table S1). In addition, *fin219* mutation influences 611 genes under shade light conditions. *PHYB* and *FIN219* coregulate 105 genes, consisting of auxin-responsive genes, including *ATHB-2*, *SAUR7/15/27*, *IAA6/19/29*, and *WES1*, and flower development as well as far-red light-responsive genes such as *ATHB-2*, *IAA29*, and *HFR1* (Fig. 6, B and C). *ATHB-2* and *HFR1* participate in shade signaling. Expression levels of *HFR1* and *IAA29* genes are remarkably upregulated in *fin219-2* and substantially increased in the double mutant *phyB-1 fin219-2* under shade light conditions as compared to those in the respective single mutant (Fig. 6D), which suggests that phyB and FIN219 synergistically and negatively regulate the expression of shade-responsive genes under shade light conditions.

Figure 3. (Continued)
another 4 d and subjected for phenotypic examination. Scale bar: 5 mm. **B)** Quantitative measurement of hypocotyl lengths of the seedlings shown in (A). Data are presented as mean ± SE ($n \geq 25$). Different lowercase letters represent a statistically significant difference in hypocotyl lengths by 1-way ANOVA, Tukey's HSD, $P < 0.05$. **C)** Schematic diagram of the full-length and different truncated forms of PHYB and FIN219 used in yeast 2-hybrid assay. $PHYB^{FL}$, the full-length of PHYB; $PHYB^{N}$, the N-terminal 651 amino acid region of PHYB; $PHYB^{C}$, the C-terminal 651 to 1,172 amino acid part of PHYB. $FIN219^{FL}$, the full-length of FIN219; $FIN219^{N}$, the N-terminal 300 amino acid region of FIN219; $FIN219^{C}$, the C-terminal 301 to 575 amino acid part of FIN219. The numbers on the box indicate the position of amino acid residues. **D)** Yeast 2-hybrid assays of the interaction between FIN219, phyB, and their specific domains. Yeast cells were grown on SD/-Trp/-Leu and SD/-Trp/-Leu/-His/-Ade selection medium with 25 μM PCB under high (H) or low (L) R:FR light conditions for 4 d. Yeast cells were 10-fold diluted and shown on the right side of the nondiluted one. **E)** The interaction of FIN219 and phyB by BiFC analysis. FIN219 fused with YN (FIN219-YN), and PHYB fused with YC (PHYB-YC) are used for BiFC assays and introduced in *N. benthamiana* leaves. Interaction of FIN219-YN and FIN219-YC is performed as a positive control. Transformed leaves were incubated in high (H) or low (L) R:FR conditions for 16 h. Scale bar: 50 μm. **F)** The interaction of FIN219 and phyB in *PHYB* overexpression line *PHYBOE-G* by Co-IP assay. The *$35S_{pro}$:PHYB-GFP-HIS (PHYBOE-G)* transgenic plants were used for the interaction studies of phyB and FIN219. The *phyB-1* mutant is used as a negative control. Total proteins were extracted from the seedlings grown under low R:FR light for 3 d. Then the PHYB-GFP-HIS fusion protein was immunoprecipitated by using an anti-HIS tag antibody with 2 h incubation under high (L > H) or low R:FR (L > L) light conditions. RPN8 is a loading control. Arrows indicate the phosphorylated and dephosphorylated FIN219. **G)** Relative expression of JA responsive genes such as *VSP1* in L*er* and *phyB-1* mutant under high and low R:FR light conditions. The seedlings were grown under high and low R:FR light conditions and subjected to the examination of *VSP1* gene expression by RT-qPCR. Data were presented as means of 3 biological replications ± SD and 30 seedlings were used for each experiment. The expression level of *VSP1* is normalized by *UBQ10*.

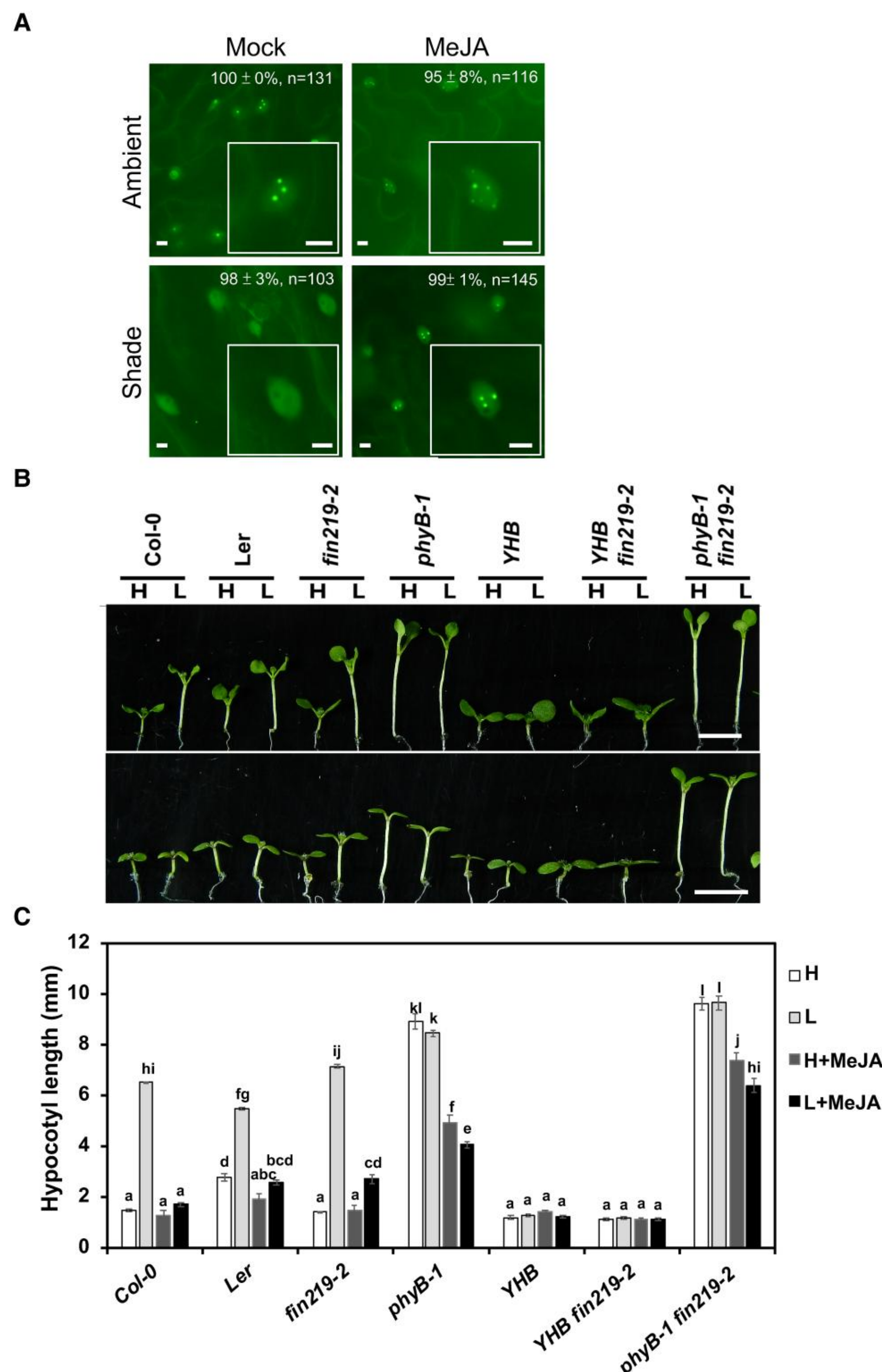

Figure 4. MeJA treatment promotes the formation of phyB NS under shade light, and phyB and FIN219 show a synergistic relationship in response to JA-regulated hypocotyl elongation under light conditions. **A)** PHYB-associated NS formation enhanced by MeJA under shade light. The *PHYBOE-Y* transgenic plants were grown under long-day conditions (16 h light, 8 h dark) for 3 wk and then transferred to ambient (high R:FR) or shade (low R:FR) light conditions in the absence (Mock) or in the presence of MeJA treatments. The percentage of the nucleus showing NS in each treatment is shown at the top of the image. Data were presented as percentage ± SD. Scale bar: 5 μm. **B)** The seedling phenotype of Col-0, L*er*, and various mutants under high and low R:FR light with MeJA (lower panel) or without MeJA (upper panel) treatment. All plant materials were grown under high R:FR light for 2 d and then transferred to high R:FR (H) or low R:FR (L) light for 4 d in the presence or absence of MeJA treatments in the growth medium. Scale bar: 5 mm. **C)** Quantitative measurement of hypocotyl lengths of the seedlings shown in (B). Data are presented as mean ± SE ($n \geq 25$). Different lowercase letters represent a statistically significant difference in hypocotyl lengths by 1-way ANOVA, Tukey's HSD, $P < 0.05$.

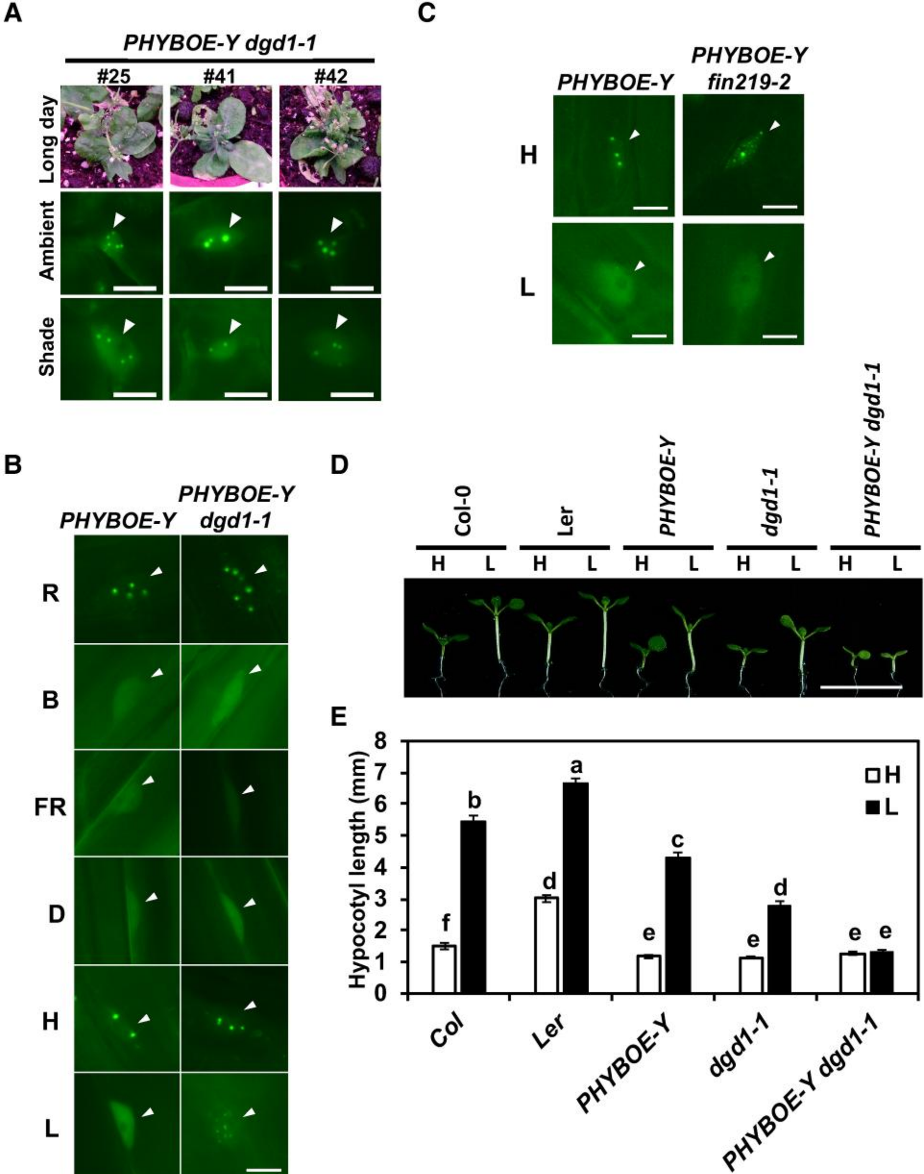

Figure 5. A high level of endogenous JA in *PHYBOE-Y dgd1-1* promotes the formation of phyB NS under shade light conditions, and *PHYBOE-Y dgd1-1* exhibits an even shorter hypocotyl than its parental lines under shade light. **A)** phyB-associated NS patterns in *PHYBOE-Y dgd1-1* in response to ambient and shade light under long-day conditions. The *PHYBOE-Y dgd1-1* was grown under long-day conditions (16 h light/8 h dark) for 20 d and then transferred to high R:FR (Ambient) or low R:FR (Shade) light conditions for 16 h. White arrowheads indicate the location of the nucleus. Scale bar: 10 μm. Three independent lines of *PHYBOE-Y dgd1-1*, *#25*, *#41*, and *#42* are used in the study. **B)** phyB-associated NS patterns in *PHYBOE-Y* and *PHYBOE-Y dgd1-1* under different light conditions. The phyB NS in *PHYBOE-Y* and *PHYBOE-Y dgd1-1* seedlings were observed under indicated light conditions for 3 d. R: red light, 5 μmol m^{-2} s^{-1}; FR: far-red light, 1.6 μmol m^{-2} s^{-1}); B: blue light, 2.3 μmol m^{-2} s^{-1}; D: darkness; H: high R:FR light; L: low R:FR light. White arrowheads indicate the nucleus. Scale bar: 10 μm. **C)** phyB-associated NS patterns in *PHYBOE-Y* and *PHYBOE-Y fin219-2* under high (H) or low (L) R:FR light conditions. White arrowheads indicate the location of the nucleus. Scale bar: 10 μm. **D)** The hypocotyl phenotype of Col-0, L*er*, *PHYBOE-Y*, *dgd1-1*, and *PHYBOE-Y dgd1-1* seedlings grown under high (H) or low R:FR (L) light conditions. All seedlings were grown under high R:FR for 2 d and then transferred to high R:FR (H) or low R:FR (L) light for another 4 d. Scale bar: 5 mm. **E)** Quantitative measurement of hypocotyl lengths of the seedlings shown in (D). Data are presented as mean ± SE ($n \geq 25$). Different lowercase letters represent a statistically significant difference in hypocotyl lengths by 1-way ANOVA, Tukey's HSD, $P < 0.05$.

In summary, our study reveals that the photoactivated phyB, PfrB, interacts with phosphorylated FIN219 under high R:FR light (ambient light), allowing dephosphorylated FIN219 to generate more active forms of JA-Ile, which in turn enters the nucleus to induce phyB-associated NS formation, leading to a photomorphogenic development of seedlings. In contrast, under low R:FR light (shade light), the Pr form phyB, PrB, associates with dephosphorylated FIN219

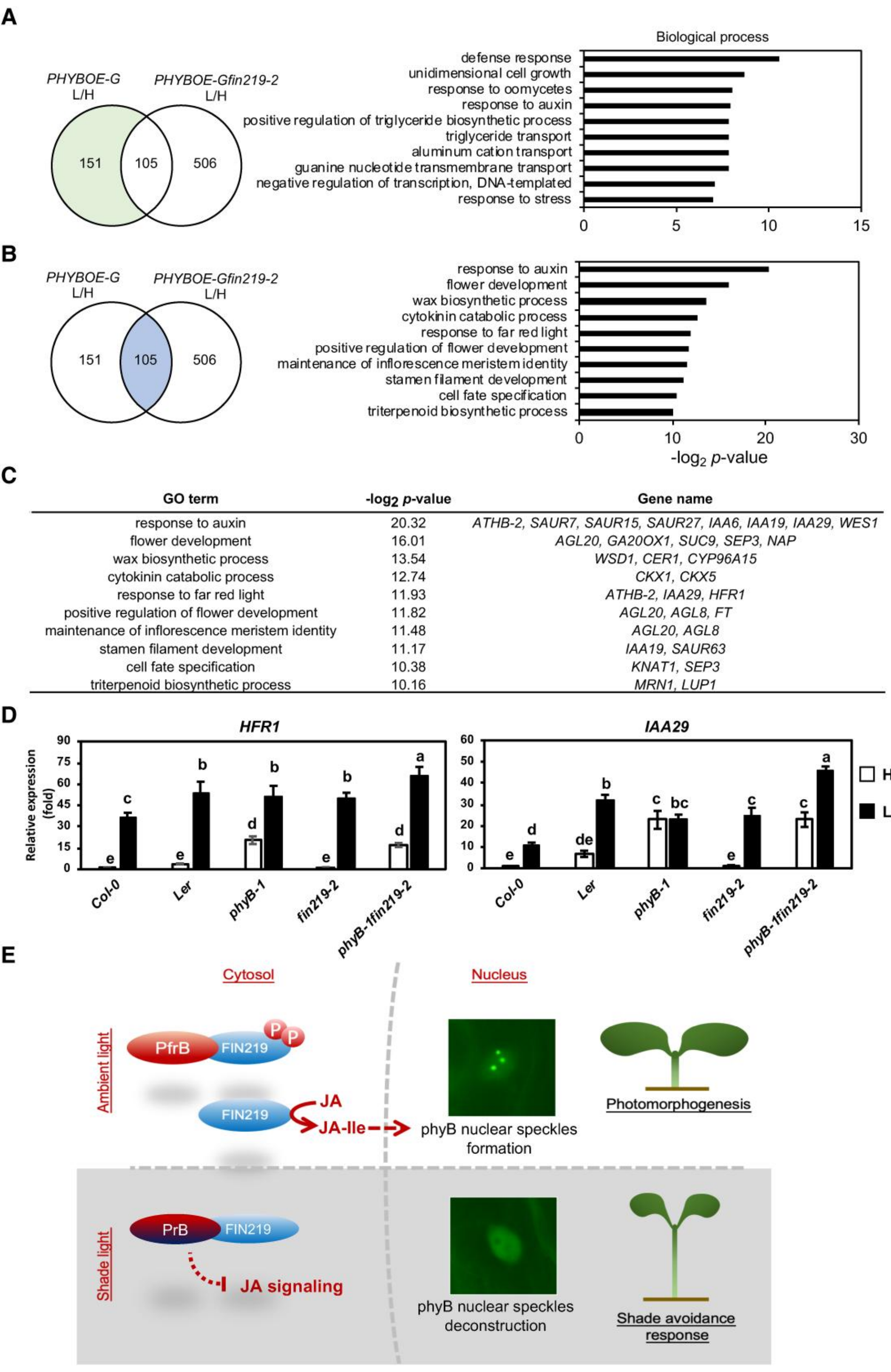

Figure 6. phyB and FIN219 synergistically suppress the expression of shade-responsive genes. **A)** Differentially expressed genes (DEGs) shown by Venn's diagram in *PHYBOE-G* and *PHYBOE-G fin219-2* seedlings under high and low R:FR light conditions. The seedlings were grown under high (H) and low (L) R:FR light conditions and then subjected to microarray assays. Two biological replicates are applied in the assays. The common

(continued)

to possibly suppress JA-mediated signaling, which abolishes the formation of phyB-mediated NS, giving rise to shade-induced hypocotyl growth (Fig. 6E). Therefore, phyB and FIN219 work together to fine-tune the seedling development in response to light.

Discussion

Previous studies reported that shade light could suppress JA-mediated defense responses (Izaguirre et al. 2006; Moreno et al. 2009; Chico et al. 2014). The photoreceptor phyB is critical in regulating growth-defense tradeoffs (Chico et al. 2014; Havko et al. 2016). The molecular mechanisms underlying the crosstalk between phyB and JA-mediated responses remain largely unclear. Here, we report that phyB and FIN219 regulate each other at different levels, including transcripts, proteins, and NS formation, primarily through physically direct interaction to modulate shade-induced seedling growth. In particular, FIN219-mediated JA-Ile levels substantially regulate phyB functions in fine-tuning hypocotyl elongation of Arabidopsis seedlings.

Both FIN219 and phyB play opposing roles in shade avoidance response

The widely accepted model indicates that phyB-mediated light signaling and JA signaling pathways repress the gene expressions involved in shade avoidance responses (Chico et al. 2014; Havko et al. 2016). Conformational changes of phyB from PfrB to PrB and decomposition of NS attenuate the phyB-mediated suppression of plant growth under shading conditions (Van Buskirk et al., 2014). However, the molecular mechanisms underlying shade-mediated suppression of JA signaling remain largely unknown. Previous studies revealed phyB suppressed the JAZs under high R:FR conditions. Accordingly, JAZs downregulate the JA signaling pathway by the inactivation of phyB when plants are exposed to low R:FR conditions (Chico et al. 2014). Our study demonstrates that FIN219 plays a negative role under shade light conditions, based on shade-related elongated phenotypes and upregulated expression of shade avoidance responsive genes in *fin219-2* mutant (Figs. 1 and 6D). However, we propose that the function of FIN219 is initially inactivated in response to shading conditions, in which the defect in FIN219 will not lead to severe phenotypes of shade avoidance responses compared to the wild-type plants. Based on this speculation, the elongated phenotype of *fin219-2* under shading conditions implies that FIN219 may have a specific function that partially suppresses the shade avoidance response under low R:FR conditions.

There are 2 possible ways that FIN219 suppresses the shade avoidance response. The first one is a JA signaling pathway-dependent manner. FIN219 might trigger the synthesis of JA-Ile, which partially represses plant growth under shading conditions. The other one is a JA signaling pathway-independent manner. FIN219 might impact the functions of specific components that positively trigger shade avoidance responses through physical interaction or in different ways. Our studies focus on the JA-dependent manner, which nicely fits our observations. The JA signaling pathway is slightly activated when plants expose to a shade light environment. Previous studies revealed similar results: specific JA responsive marker genes such as *VSP1*, *PDF1.2*, *MYC2*, and *COI1* show higher expression levels under shade light than ambient light conditions (Moreno et al. 2009). The expression patterns of these genes demonstrate that partial JA responses are slightly activated when plants are exposed to environments without biotic or abiotic stresses under shade light conditions than the ambient one.

Nevertheless, consistent with the general theory of growth-defense tradeoffs, the shade light treatment would attenuate the induction of some JA responsive genes such as *PDF1.2* and *COI1* after JA treatment and inhibit plant defense responses. Unexpectedly, *VSP1* and *MYC2* expression levels are still increasing along with the JA levels. Thus, we

Figure 6. (Continued)
genes affected by 2 replicates in the *PHYBOE-G* and *PHYBOE-G fin219-2* are included in DEGs assays. The DEGs with $\log_2$(fold change) ≥ 1 were selected. The DEGs shown in the left part of the venn's diagram (light green region) are specifically affected by *PHYBOE-G* in low R:FR light versus high R:FR light and are used for further analysis of biological process GO-terms shown in the right panel. **B)** DEGs shown by Venn's diagram in *PHYBOE-G* and *PHYBOE-G fin219-2* seedlings grown under high and low R:FR light conditions. The DEGs shown in the intersection of the venn's diagram (light blue region) are co-affected by *PHYBOE-G* and *FIN219* in low R:FR light versus high R:FR light and are used for further analysis of biological process GO-terms shown in the right panel. **C)** The overlapping of Venn's diagram shown in the light blue in (B) indicates the common genes affected by *PHYBOE-G* and *PHYBOE-G fin219-2*. The top 10 GO terms in the biological process were presented with $-\log_2$(P-value). Gene names identified were shown in the list. **D)** Expression of the represented shade responsive genes in wild type, *phyB-1*, *fin219-2*, and *phyB-1 fin219-2* mutants under high or low R:FR light conditions. The seedlings were grown under high (H) and low R:FR (L) light conditions and subjected to RNA extraction and further expression studies by RT-qPCR. *HFR1* and *IAA29* are identified in (B) and (C). Data were presented as means of 3 biological replication ± SD and 30 seedlings were used for each experiment. Different letters above the bars indicate statistically significant differences in hypocotyl lengths with $P < 0.05$ according to 1-way ANOVA with post hoc Tukey's test. **E)** A model illustrates that phyB and FIN219 participate in the regulation of shade-mediated hypocotyl growth. In the ambient light, photoactivated phyB, PfrB, interacts with phosphorylated FIN219 under high R:FR light, leading to more active forms of JA-Ile production by dephosphorylated FIN219, which in turn enters the nucleus to induce phyB-associated NS formation and a photomorphogenic development of seedlings (top panel). However, under shade light, the Pr form of phyB, PrB, interacts with dephosphorylated FIN219 to suppress JA-mediated signal transduction, leading to the abolishment of phyB-mediated NS formation, and enhancement of shade-induced hypocotyl growth. JA, jasmonic acid; JA-Ile, jasmonic acid-isoleucine.

propose that the shading condition mainly downregulates defense responses by desensitizing JA, while FIN219 still partially suppresses the phenotype of shade avoidance responses under shade conditions. Thus, specific components might inhibit FIN219 activity under shading conditions since FIN219 protein levels decrease upon exposure to simulated shade light conditions.

Light regulates the phosphorylation status and enzymatic activity of FIN219

The growth-defense tradeoffs result from resource restriction (Huot et al. 2014). Suppression of the defense responses under shade conditions is a generally accepted phenomenon. However, the phosphorylation status and enzymatic activity assays of FIN219 indicate that FIN219 is active under shade light (Fig. 2), suggesting that JA signaling may still work simultaneously. However, shade-responsive regulators may suppress FIN219-mediated JA signaling to trigger shade-induced growth. The photoreceptor phyB, containing a serine/threonine kinase activity, might be responsible for the suppression of FIN219 in 2 ways because of the changes in the phosphorylation status of FIN219 during the light transition from high R:FR to low R:FR light (Fig. 2B). Firstly, phyB may directly phosphorylate FIN219, leading to a reduction of FIN219 activity. Secondly, phyB suppresses the enzymatic activity of FIN219 through direct interaction. Here, we found that *phyB-1* shows a lower level of phosphorylated FIN219 compared to wild-type L*er* under high R:FR condition (Fig. 2A). The active phyB might promote the phosphorylation of FIN219 directly or indirectly. However, currently, no evidence supports that phyB acts as a functional kinase in Arabidopsis, and phyB may cooperate with PPKs to regulate the phosphorylation of PIFs (Ni et al. 2017). Recent evidence also indicates that SPA1 functions as a serine/threonine kinase, and phyB interacts with SPA1 through its C-terminal domain to recruit PIF1 for phosphorylation-mediated protein degradation (Paik et al. 2019). Previous studies even showed that the HKRD domain in the C terminus of PHYB is dispensable via the functional assays of the truncated phyB (Krall and Reed 2000). Here, we demonstrate that the physical interaction between phyB and FIN219 was found in the cytosol by yeast 2-hybrid, BiFC, and Co-IP assays under both high and low R:FR conditions (Fig. 3, C–F). Interestingly, phyB interacts with the dephosphorylated FIN219 specifically under low R:FR, rather than high R:FR (Fig. 3F), leading to possible suppression of FIN219 activity through direct interaction under shade light. The output results in shade-induced hypocotyl elongation. The regulatory relationship between phyB and FIN219 under shade conditions reflects a substantial increase of a JA-responsive *VSP1* gene expression in *phyB-1* mutant compared to wild-type L*er* under shade light (Fig. 3G). This result implies that phyB plays a negative role in regulating JA-responsive gene expression under shade conditions via attenuating the regulatory role of FIN219 in JA signal transduction, but we still need more solid evidence to support this speculation.

FIN219 and phyB mutually regulate each other in a negative way

Genetic studies of the *phyB-1 fin219-2* double mutant indicate that FIN219 functions in shade-induced hypocotyl elongation depend on phyB (Fig. 3, A and B). Moreover, extremely short hypocotyl in *YHB* and *YHB fin219-2* is consistent with this speculation. Besides, the light sensitivity of phyB in *PHYBOE-G* and *PHYBOE-G fin219-2* is still responding to shade light, but the *YHB* mutant is defective in light sensing (Fig. 3, A and B). Therefore, *PHYBOE-G* and *PHYBOE-G fin219-2* overexpression lines show elongated hypocotyls under shade. In contrast, *YHB* and *YHB fin219-2* barely respond to shade light. However, *PHYBOE-G fin219-2* show less long hypocotyl than *PHYBOE-G* under shade conditions, suggesting that FIN219 regulates phyB negatively under shade-induced growth. Previous studies showed that FIN219 suppressed COP1 even in the dark by retaining it in the cytosol via physical interaction, leading to a short-hypocotyl phenotype in darkness (Hsieh et al. 2000; Wang et al. 2011). Therefore, the interaction of FIN219 and phyB may have a similar effect on phyB by confining it in the cytosol so that phyB fails to get into the nucleus to trigger photomorphogenesis.

The JA signaling pathway represses plant growth by triggering phyB-contained NS formation

Our results demonstrate that the PHYB-YFP signal appears in the nucleus under high R:FR and low R:FR conditions in the presence of MeJA (Figs 4, A and 5, A–C). The most distinctive pattern of the PHYB-YFP signal is specifically detected with NS formation under high R:FR rather than low R:FR light conditions. It appears that the shuttling between the cytosol and the nucleus does not directly affect photomorphogenesis and shade avoidance responses but the formation of phyB-mediated NS. Therefore, this result indicates that phyB NS plays a crucial role in triggering the photomorphogenic development of seedlings. Moreover, both exogenous and endogenous JA triggers the formation of phyB NS directly under the shade (Figs 4, A and 5, A and B). The accumulation of phyB NS has served as a sign of photomorphogenesis. This result provides additional perspective on the crosstalk between growth and defense signaling pathways. PhyB is not the receptor of JA, so there should be other components that might respond to JA levels, leading to the formation of phyB-associated NS. Previous studies revealed that the appearance of phyB NS required *HEMERA (HMR)*, *PHOTOPERIODIC CONTROL OF HYPOCOTYL 1 (PCH1)*, and *PCHL-LIKE (PCHL)* (Chen et al. 2010; Huang et al. 2016; Cheng et al. 2020). However, our microarray data indicate that high R:FR light or JA treatments do not substantially alter the expression levels of these genes. But JA, ABA, and specific abiotic or biotic stresses slightly downregulate *HMR*

expression (Kilian et al. 2007; Pandey et al. 2010; Bohmer and Schroeder 2011; Chen et al. 2015; Waese et al. 2017). HMR protein is also substantially degraded under heat treatment and abolishing NS (Legris et al. 2017; Qiu et al. 2021). Thus, shade light and JA signaling pathways may regulate HMR by directly modulating its protein stability rather than its RNA expression level.

In addition, the analyses of the NS formation under different monochromatic lights indicate the specific regulation of phyB NS by red light and high R:FR light, as well as high levels of endogenous JA in *PHYBOE-Y dgd1-1* under low R:FR light (Fig. 5B). The Pfr form of phyB might likely be the critical component in this regulation. Moreover, phyB NS formation depends on the percentage of its PfrB conformer (Chen et al. 2003). JA may stabilize the PfrB in the NS directly or indirectly and slows down the conversion from PfrB to PrB so that it can constantly promote photomorphogenesis. Alternatively, the emerging evidence indicates that the formation of the NS is due to the so-called liquid–liquid phase separation (LLPS), in which collections of mRNAs associate with proteins to form droplets (Riback et al. 2017; Cuevas-Velazquez and Dinneny 2018; Langdon and Gladfelter 2018). These droplets are liquid-like membraneless structures and rapidly respond to environmental and endogenous signals by reversibly initiating the onset of assembly and disassembly in the process, leading to control of gene expression in response to developmental programs and stress signals (Van Buskirk et al. 2012; Zhu et al. 2022). Our studies reveal that FIN219/JAR1, responsible for the formation of physiologically active JA-Ile, positively regulates the expression of *PHYB* transcripts under high and low R:FR light conditions (Supplemental Fig. S1A). Moreover, JA acts as a stress hormone and is involved in stress responses. It is possible that stress signals, such as light and heat, induce JA production, leading to the induction of phyB-associated NS formation, which rapidly causes changes in gene expression in fine-tuning plant growth and development and stress responses. Interestingly, the LLPS-mediated phyB NS formation has been reported recently (Chen et al. 2022). The NTE motif located at the N terminus of phyB is crucial in the light- and thermos-sensing module and the C terminus of phyB plays a role in self-oligomerization, which is the essential domain for the NS formation. Whether LLPS regulates JA-triggered NS formation will be an exciting topic and remains elusive.

In summary, we provide evidence showing that a new mechanism plays a vital role in the crosstalk between growth and defense through mutually antagonistic regulation of phyB and FIN219/JAR1 under high or low R:FR light conditions. Although we demonstrate the regulatory relationship between them and MeJA can promote the formation of phyB NS, the critical components induced by JAs, leading to the NS formation, remain unclear. Further investigations are necessary to clarify precisely the mechanisms of JA-triggered NS formation. Isolating the NS complex and analyzing the complex with proteomic strategies will be ideal for uncovering the mechanisms of growth-defense tradeoffs.

Materials and methods

Plant materials and growth conditions

Wild-type Arabidopsis (*A. thaliana*) used in this study is Col-0 or L*er* ecotypes. The mutant *fin219-2* is in the Col-0 background (Hsieh et al. 2000). The *phyB-1* and *YHB* are in L*er* genetic backgrounds as described previously (Hirschfeld et al. 1998; Hu et al. 2009). The double mutant *phyB-1 fin219-2* and *YHB fin219-2* were generated by crossing, and homozygous lines were confirmed by genotyping. Seeds were stratified at 4°C in the dark for 4 d and then grown on 1/2 Murashige and Skoog (MS) medium containing 0.3% (w/v) sucrose and 0.9% (w/v) agar at 22°C growth chamber under continuous white light. To generate $35S_{pro}$:*PHYB-GFP-HIS/phyB-1* (*PHYBOE-G*) and $35S_{pro}$:*PHYB-YFP-HIS/phyB-1* (*PHYBOE-Y*) transgenic plants, the full-length coding sequence of *PHYB* was firstly cloned into *pEarleygate 103* and *pEarleygate 101*, respectively, by using Gateway system (Invitrogen). The resulting constructs were transformed into *Agrobacterium tumefaciens* strain GV3101. Then, Col-0 and *phyB-1* mutant were transformed by floral dipping. We further generated *PHYBOE-Y dgd1-1*, *PHYBOE-Y fin219-2*, and *PHYBOE-G fin219-2* transgenic plants by crossing $35S_{pro}$:*PHYB-YFP/phyB-1* with *dgd1-1* (Lin et al. 2016) and *fin219-2*, and *PHYBOE-G* with *fin219-2*, respectively.

Measurement of hypocotyl and petiole lengths

Wild type and mutants were grown under high R:FR (ratio = 7) for 2 d and then transferred to high R:FR or low R:FR (ratio = 0.4) for another 4 d at 22°C. Light intensity was approximately 60 μmol m^{-2} s^{-1}. The hypocotyl lengths of seedlings ($n \geq 25$) were measured by using ImageJ software. Besides, we measured the petiole lengths of adult plants ($n = 8$).

Protein extraction and western blotting

Seedlings were ground to powder with liquid nitrogen, and soluble proteins were extracted with the buffer (50 mM Tris–HCl, pH7.5, 150 mM NaCl, 10 mM $MgCl_2$, 0.1% NP-40 [v/v], 1 mM PMSF, 1xProtease inhibitor cocktail [Sigma-Aldrich]). Total 100–150 μg of proteins were separated by SDS-PAGE and transferred onto the PVDF membrane. After blotting, FIN219 and PHYB proteins were detected by specific monoclonal antibodies FIN219 and PHYB, respectively, with 1:5,000 dilution. We used KETA CL to visualize the chemiluminescence signal.

Yeast 2-hybrid assays

The full-length (1 to 1,172 aa), N terminus (1 to 651 aa), and C terminus (652 to 1,172 aa) of PHYB, and the full-length (1 to 575 aa), N terminus (1 to 300 aa) as well as C terminus (301 to 575 aa) of FIN219 were cloned into both pGADT7 and pGBKT7 vectors, respectively. AH109 yeast strain was used as a host for transformation. We incubated the transformed yeast cells at 28°C for 4 d. Cotransformation of the plasmids was confirmed with the SD/-Trp/-Leu selection medium. Protein–protein interaction assays were approved by

SD/-Trp/-Leu/-Ade/-His selection medium under high or low R:FR light conditions. All selection mediums contain 25 μM phycocyanobilin (PCB), so that PHYB recombinant protein was able to respond to light treatment.

Interaction assays by BiFC

For BiFC analysis, *35S$_{pro}$:PHYB-YC* and *35S$_{pro}$:FIN219-YN* on *pEarleygate 201* and *pEarleygate 202* vectors were transiently expressed in 3-wek-old N. *benthamiana* by Agro-infiltration with GV3101 strain. After 3-d incubation under white light conditions, plants were transferred to high or low R:FR light treatment for 16 h at 22°C. Then we examined the YFP fluorescence signal in the epidermal cells of *N. benthamiana* leaves with a Nikon H600L microscope.

Co-IP

We ground the seedlings of *PHYBOE-G* transgenic plants and *phyB-1* mutant harvested from low R:FR light conditions with liquid nitrogen and extracted soluble proteins in the buffer (50 mM Tris–HCl, pH7.5, 150 mM NaCl, 10 mM $MgCl_2$, 0.1% NP-40 [v/v], 1 mM PMSF, 1× protease inhibitor cocktail [Sigma-Aldrich]). Then, we used around 1.5 mg of protein extracts for Co-IP. The antibody against His-tag was incubated with protein G magnetic beads (Protein G Mag Sepharose Xtra, GE Healthcare) in the TBS buffer for 15 min and then washed with TBS buffer. The antibody-coating beads were coincubated with each protein extract for 1 h at 22°C under high or low R:FR conditions. After discarding the supernatant, we washed the beads 3 times with protein extraction buffer, as mentioned above. Finally, the immunoprecipitated proteins were analyzed with SDS-PAGE and proceeded with western blot by PHYB and FIN219 monoclonal antibodies.

RNA extraction, RT-PCR, and quantitative real-time PCR (RT-qPCR) analysis

The RNA extraction procedures followed RNA Plus mini kit (LabPrep). The total RNA 1 μg was converted to the first-strand cDNA pool by adding oligo-dT primers, RNasin (Promega), and reverse transcriptase (Applied Biosystems). After reverse transcription, we used 1μL aliquot of 2-fold diluted cDNA in RT-qPCR and RT-PCR for gene expression analysis. Gene-specific primers used in the experiment were as follows: UBQ10, 5′-TCCGGATCAGCAGAGGCTTA-3′, and 5′-TCAGAACTCTCCACCT CAAG-3′; FIN219, 5′-AAAACGC TGTGCTGAAGTAGCT-3′, and 5′-ATGTTGGAGAAGGTTG AAACTTTC-3′; PHYB, 5′-GATGATTCACCTAATCTTC- 3′, and 5′-CGTCGTTAGACACACAAACACTC-3′; HFR1, 5′-TTC AGTTACTCGAAAAGGTTCCA-3′, and 5′- CGAAACCTTGT CCGTCTTG-3′; IAA29, 5′-CTTCCAAGGGAAAGAGGGTGA-3′, and 5′-TTCCGCAAAGATCTTCCATGTAAC-3; VSP1, 5′-GTTGATGGATTTGGAGTTGGAAG-3′, and 5′-TCTGAG CTGTTCTTGCGTATAG-3′. We performed semiquantitative RT-PCR reactions with the following conditions: 95°C 5 min, 28–35 cycles of denaturation (95°C 30 s), annealing (52–60°C 30 s), and elongation (72°C 30 s). The final elongation step was performed at 72°C for 10 min, and completed reactions were maintained at 16°C. We further performed quantification RT-qPCR with the Bio-Rad CFX384 instrument. Samples were analyzed with 1XiQ SYBR Green supermix (Bio-rad), and a PCR program was used: 50°C 10 min, 95°C 1 min, 40 cycles of denaturation 95°C 1 s, annealing and elongation 60°C 30 s.

Assays of PHYB NS formation

PHYBOE-Y and *PHYBOE-Y dgd1-1* plants were grown under long-day conditions (16 h light and 8 h dark) for 3 wk after germination. Isolated leaves of plants were treated with 100 μM MeJA or ethanol (control) for 16 h under high and low R:FR light conditions and used to examine the PHYB-YFP-associated NS with a Nikon H600L microscope.

Determination of the phosphorylation status of FIN219 in Arabidopsis

Plants materials were grown under high R:FR (ratio = 7) conditions for 6 d and then transferred to low R:FR (ratio = 0.4) light for another 24 h. We collected the seedlings at indicated time points (0, 3, 6, 12, and 24 h) during the light transition process. Protein extractions of samples were incubated with protein sample buffer at 65°C for 10 min and separated with SDS-PAGE. Respective-specific antibodies detected FIN219, PHYB, and RPN8 proteins.

Microarray assays

PHYBOE-G and *PHYBOE-G fin219-2* seedlings were grown under high and low R:FR light conditions described in the Materials and methods and then subjected to microarray assays. We extracted total RNAs with RNA Plus mini kit (LapPrep). The assays consist of 2 biological replicates, and each replicate contains at least 30 seedlings. We analyzed the RNA expression levels with Affymetrix GeneChip (Arabidopsis Genome ATH1 Array) and normalized microarray data by scaling normalization with CLC Genomics Workbench (QIAGEN). The following criteria selected the genes with significant changes: $\log_2|\text{fold change}| \geq 1$ and *P*-value of *t*-test <0.05.

Analysis of differential expression genes (DEGs)

We conducted Gene Ontology enrichment analysis to clarify the DEGs at the aspects of cellular components, molecular function, and biological process levels. We also referred to functional annotation as the method reported by Klopfenstein et al. (2018), and $\log_2(P\text{-value}) < -7$ was considered a statistically significant difference.

Preparation of cytosol and nuclei fractionation

We transformed *N. benthamiana* leaves with *35S$_{pro}$:FIN219-GFP-HIS/pEarleygate103* and *35S$_{pro}$:PHYB-YFP-HA/pEarleygate101* constructs by agro-infiltration. After the transformation, the plant materials were incubated under high R:FR light conditions for 2 d and then transferred to

high or low R:FR light conditions for another day. *Nicotiana benthamiana* leaves were harvested and used for the cytosol and nuclei fractionation according to the method reported by Estavillo et al. (2014) with slight modification. In brief, the leaves were homogenized gently by a tissue-grinding pestle to prevent the nuclear membrane from breaking. We extracted the cytosolic protein fraction with the extraction buffer (0.4 M sucrose, 50 mM Tris–HCl pH7.5, 3 mM EDTA, 1× protease inhibitor cocktail [Sigma-Aldrich]) and then centrifuged with 16,000 × *g* to precipitate the noncytosolic fraction. The purified cytosolic proteins were subjected to Co-IP assays.

Statistical analysis

We performed statistical data analyses using 1-way ANOVA with SPSS software or Student's *t*-test with Excel software to evaluate significant differences between samples. The difference is significant at the *P*-value <0.05.

Constructs

The information about the constructs used in this study is shown in Supplemental Table S2.

Accession numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: *PHYB* (AT2G18790), *FIN219* (AT2G46370), *DGD1* (AT3G11670), *HFR1* (AT1G02340), *VSP1* (AT5G24780), *IAA29* (AT4G32280), and *UBQ10* (AT4G05320).

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Supplemental data

The following materials are available in the online version of this article.

Supplemental Figure S1. PHYB and FIN219 modulate each other at the transcript and protein levels.

Supplemental Figure S2. PHYB monoclonal antibody specifically detects the C-terminus of phyB protein in Arabidopsis.

Supplemental Figure S3. PHYB protein increases in response to low R:FR light.

Supplemental Figure S4. *PHYB* overexpression can rescue *phyB-1* mutant, resulting in a hypersensitive short-hypocotyl phenotype under red light.

Supplemental Figure S5. phyB and FIN219 show a mutually antagonistic relationship regulating shade-induced petiole elongation.

Supplemental Figure S6. Subcellular localization assays indicate that phyB and FIN219 colocalize in the cytosol and the nucleus, and only phyB associates with the nuclear speckles under high R:FR light.

Supplemental Figure S7. Coimmunoprecipitation assay reveals that the cytosolic phyB interacts with FIN219 in *Nicotiana benthamiana* leaves under high and low R:FR light conditions.

Supplemental Figure S8. Endogenous high JA levels in *PHYBOE-Y dgd1-1* significantly suppress shade-induced hypocotyl elongation.

Supplemental Table S1. Gene names identified in Fig. 6A.

Supplemental Table S2. The information about the constructs used in the studies.

Conflict of interest statement. The authors declare no competing interest.

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