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FAR-RED INSENSITIVE 219 modulates CONSTITUTIVE PHOTOMORPHOGENIC 1 activity via physical interaction to regulate hypocotyl elongation in Arabidopsis

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ABSTRACT

FAR-RED INSENSITIVE 219 (FIN219) in Arabidopsis is involved in phytochrome A-mediated far-red (FR) light signaling. Previous genetic studies revealed that FIN219 acts as an extragenic suppressor of CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1). However, the molecular mechanism underlying the suppression of COP1 remains unknown. Here, we used a transgenic approach to study the regulation of COP1 by FIN219. Transgenic seedlings containing ectopic expression of the FIN219 N-terminal domain in wild-type Columbia (named NCox for the expression of the N-terminal coiled-coil domain and NTox for the N-terminal 300 amino-acid region) exhibited a dominant-negative long-hypocotyl phenotype under FR, reflected as reduced photomorphogenic responses and altered levels of COP1 and HY5. Yeast two-hybrid, pull-down, and bimolecular fluorescence complementation assays revealed that FIN219 could interact with the WD-40 domain of COP1 and with its N-terminal coiled-coil domain through its C-terminal domain. Further in vivo coimmunoprecipitation study confirms that FIN219 interacts with COP1 under continuous FR light. Studies of the double mutant fin219-2/cop1-6 indicated that HY5 stability requires FIN219 under darkness and FR light. Moreover, FIN219 levels positively regulated by phytochrome A can modulate the subcellular location of COP1 and are differentially regulated by various fluence rates of FR light. We concluded that the dominant-negative long-hypocotyl phenotype conferred by NCox and NTox in a wild-type background was caused by the misregulation of COP1 binding with the C terminus of FIN219. Our data provide a critical mechanism controlling the key repressor COP1 in response to FR light.
**INTRODUCTION**

Light affects almost every aspect of plant development from seed germination to flowering, especially seedling development. In the absence of light, seedlings undergo so-called skotomorphogenesis and show long hypocotyls, unexpanded cotyledons, undeveloped chloroplasts and blocked expression of light-regulated genes. In the presence of light, they undergo photomorphogenesis and show short hypocotyls, expanded cotyledons, well-developed chloroplasts and expression of light-responsive genes, which give plants the ability to perceive light energy and signals to survive in environments (Kendrick and Kronenberg 1994).

Plants contain multiple photoreceptors to sense light environments, including light quality, quantity, duration and direction. Genetic and molecular studies of Arabidopsis have identified numerous light-signaling intermediates, including positive and negative regulators (Ni et al., 1998; Soh et al., 1998; Hoecker et al., 1999; Hudson et al., 1999; Bolle et al., 2000; Fairchild et al., 2000; Fankhauser and Chory, 2000; Hsieh et al., 2000; Soh et al., 2000; Ballesteros et al., 2001; Desnos et al., 2001; Dieterle et al., 2001; Zeidler et al., 2001; Wang and Deng, 2002). Some regulators are located in the nuclei (Ni et al., 1998; Hoecker et al., 1999; Hudson et al., 1999; Fairchild et al., 2000; Fankhauser and Chory 2000; Soh et al., 2000; Ballesteros et al., 2001; Wang and Deng 2002); others are in the cytoplasm (Bolle et al., 2000; Hsieh et al., 2000) or in both subcellular locations (Desnos et al., 2001; Zeidler et al., 2001). A group of components downstream of light signaling, named COP/DET/FUS as repressors of photomorphogenesis in darkness, play central roles in the integration of far-red (FR), red and blue light signaling (Kwok et al., 1996). Especially, COP1, composed of 3 recognizable structural domains -- an N-terminal RING-finger domain, a coiled-coil domain, and a C-terminal WD-40 repeat domain -- can partition between the nucleus and cytosol in response to darkness or light (Deng et al., 1992; von Arnim and Deng, 1994). COP1 has E3 ligase activities to target positive regulators -- ELONGATED HYPOCOTYL 5 (HY5), LONG AFTER FAR-RED LIGHT 1 (LAF1), phyA and LONG HYPOCOTYL IN FAR-RED LIGHT 1 (HFR1) -- for ubiquitin-mediated protein degradation by the 26S proteasome to repress photomorphogenic development (Osterlund et al., 2000b; Saijo et al., 2003; Seo et al., 2003; Duk et al., 2004; Jang et al., 2005; Yang et al., 2005). SUPPRESSOR OF PHYA 1 (SPA1) can interact with COP1 to negatively regulate the accumulation of HY5 for repressing light signaling.
Moreover, SPA1 downregulates HY5 protein levels via direct interaction without changes in COP1 levels under continuous FR (cFR) light (Saijo et al., 2003). However, the molecular mechanisms that regulate negative key players such as SPA1 and COP1 in photomorphogenesis are largely unknown.

A molecular genetic approach to isolate genetic modifiers of COP1 revealed 2 genes, *HY5* and *FAR-RED INSENSITIVE 219 (FIN219)*, as extragenic suppressors of COP1; these have been characterized at the molecular level (Ang and Deng, 1994; Ang et al., 1998; Hsieh et al., 2000). HY5, a basic leucine zipper transcription factor, functions as a positive regulator downstream of COP1 and is regulated by multiple photoreceptors to control various light responses (Osterlund et al., 2000a), including gravitropism, wavy root growth, and hypocotyl elongation (Oyama et al., 1997), as well as the integration of light signals and various phytohormones to regulate germination and seedling development (Vandenbussche et al., 2007; Alabadi et al., 2008; Chen et al., 2008). HY5 interacting with COP1 and SPA1 leads to its degradation to trigger skotomorphogenic development (Saijo et al., 2003). In contrast, its accumulation at high levels leads to photomorphogenic development. Thus, HY5 also plays a key role in light signaling.

FIN219 was cloned by a map-based method and found to encode a 575 amino-acid GH3-like protein; its transcripts are rapidly induced by auxin. The *fin219* mutant (renamed *fin219-1* in this study) exhibits a long-hypocotyl phenotype only under cFR light and was thought to be an epiallele containing changes of methylation status in the promoter region (Hsieh et al., 2000; unpublished data). Transgenic seedlings overexpressing *FIN219* display a hypersensitive phenotype under cFR light (Hsieh et al., 2000). Thus, FIN219 might play a role in the phyA-mediated FR signaling pathway. Staswick et al. (2002) reported that *JASMONATE RESISTANT 1 (JAR1)*, isolated by positional cloning from the *jar1-1* mutant, with the substitution of Phe for a conserved Ser-101 in its open reading frame, is in the same locus as *FIN219* and belongs to the firefly luciferase family of adenylate-forming enzymes. Further studies indicated that JAR1 is actually a jasmonic acid (JA)-amino synthetase and mediates the conjugation of JA with various amino acids, especially isoleucine (Staswick and Tiryaki, 2004). Interestingly, the JA–isoleucine (JA-Ile) conjugate can complement the JA insensitivity of *jar1-1*, which has been shown to have no active JAR1 enzyme (Staswick and Tiryaki, 2004; Suza and Staswick, 2008). Recently, we showed that *jar1-1* has a long-hypocotyl phenotype under weak FR light and a phenotype
comparable to that of fin219-1 (Chen et al., 2007).

To further understand the FIN219 function in the integration of light and JA signaling pathways and the molecular mechanism underlying the suppression of COP1 by FIN219 in modulating hypocotyl elongation of Arabidopsis seedlings, we characterized transgenic seedlings with ectopic expression of the N-terminal region of FIN219/JAR1 and a putative coiled-coil domain. Our data reveal that FIN219/JAR1 negatively regulates COP1 levels via physical interaction and is required for HY5 stability to modulate photomorphogenic responses of Arabidopsis seedlings.

RESULTS

Transgenic Seedlings in Wild Type Expressing the FIN219 N Terminus Exhibit a Dominant-Negative Long-Hypocotyl Phenotype Specifically under cFR Light

The fin219-1 mutant displays a long-hypocotyl phenotype specifically under cFR light (Hsieh et al., 2000). FIN219 isolated by a map-based method encodes a GH3-like protein containing 2 putative coiled-coil domains, one in the N terminus and the other in the C terminus (Fig. 1A), which are believed to be involved in protein–protein interaction. To further understand the functional roles of these domains in phyA-mediated FR light signaling, we generated β-glucuronidase (GUS)-fused constructs containing the N-terminal 53 (35S::GUS-FIN219-NC) or 300 amino acid residues (35S::GUS-FIN219-NT) of FIN219 that harbor a putative coiled-coil domain at the very end of the N terminus of FIN219 (named NCox for the expression of the N-terminal coiled-coil domain and NTox for the N-terminal 300 amino-acid region) (Fig. 1A) and ectopically expressed them in wild-type Columbia (Col) and fin219 mutants. NCox and NTox seedlings in Col displayed a longer hypocotyl phenotype than did the wild type specifically under cFR light but not other light conditions, including darkness (Fig. 1, B and C). In addition, we included a null mutant of FIN219 (fin219-2, SALK_059774) obtained from the Arabidopsis Biological Research Center that has no FIN219 transcripts or proteins (Chen et al., 2007; also see Fig. 1-3). The fin219-2 mutant exhibited a hypocotyl length similar to that of fin219-1 under cFR light, especially weak FR light (Fig. 1, B and C). Further examination under different fluence rates of FR light revealed NCox and NTox transgenic seedlings in Col with hypocotyl lengths similar to that of fin219-1 at 1–2 µmol m⁻²s⁻¹ low-fluence rate of FR and intermediate lengths between that of fin219-1 and the wild type
under 20 µmol m⁻² s⁻¹ FR light (Fig. 1D). The fin219-2 mutant displayed hypocotyl lengths similar to that of NCox and NTox transgenic seedlings in Col under the same conditions (Fig. 1D). However, NCox and NTox in a fin219-2 or fin219-1 mutant background did not show hypocotyls longer than that of fin219-2 or fin219-1 (data not shown), which suggests that the dominant-negative phenotype conferred by ectopic expression of the FIN219 N terminus in Col requires the presence of functional FIN219.

Ectopic Expression of the FIN219 N Terminus in Wild Type was Reflected by Reduced Photomorphogenic Responses and Changes in Expression of Key Regulators in Light Signaling

To further understand whether the reduced photomorphogenic development resulting from ectopic expression of the FIN219 N terminus in the wild type under cFR light affects phyA-mediated responses, we examined anthocyanin levels of NCox and NTox transgenic seedlings in Col grown under cFR light for 3 days. NCox and NTox transgenic seedlings in Col showed substantially lower anthocyanin levels than did the wild type under the same conditions (Fig. 1E), which is consistent with significantly lower expression of the gene chalcone synthase (CHS) than in the wild type under the same conditions (Supplemental Fig. S1A); moreover, anthocyanin levels in fin219-2 were comparable to that of fin219-1 (Fig. 1E). In addition, NCox and NTox transgenic seedlings in Col displayed a defect in phytochrome A-mediated FR blockage of greening response, but NTox transgenic seedlings in Col were more prominent in this aspect (Fig. 1E). Interestingly, fin219-2 showed a degree of defect similar to that of fin219-1 in the FR-mediated blockage of greening (Fig. 1E), which suggests that FIN219 may play a role in the process of FR blockage of greening response. However, how various deletions or mutations of FIN219 result in different effects of greening after FR irradiation remains to be elucidated. In addition, the fin219-1 mutant displayed a slightly delayed flowering phenotype under long-day conditions (Hsieh et al., 2000); we further found that the delayed flowering phenotype of both NCox and NTox transgenic plants in Col was even enhanced in terms of number of rosette leaves and days to flowering under long-day conditions (Supplemental Fig. S1B). The fin219-2 mutant showed a trend similar to that of NCox and NTox in Col in the control of flowering (Supplemental Fig. S1B).
Since FIN219/JAR1 has been shown to be a JA-conjugating enzyme (Staswick and Tiryaki, 2004), NCox and NTox transgenic seedlings in Col were examined to see whether they show altered responses to exogenous JA. The response of NCox and NTox in Col to methyl jasmonate (MeJA) or coronatine was similar to that of the wild type (Supplemental Fig. S2). Furthermore, we examined the level of the JA-Ile in NCox and NTox transgenic seedlings in Col under FR light by GC/MSD method. There is no significant difference between wild type and NCox and NTox transgenic seedlings, but the fin219-2 mutant contains only small amount of JA-Ile (Table 1), which implies that the dominant-negative phenotype of NCox and NTox in Col under FR light is not due to changes in the level of JA-Ile and their responses to MeJA or coronatine. Therefore, the ectopic expression of the FIN219 N terminus in Col results in pleiotropic effects on Arabidopsis development.

Furthermore, to understand the molecular mechanisms underlying the long-hypocotyl phenotype of NCox and NTox in Col under cFR light, we found GUS-FIN219-NC and GUS-FIN219-NT transcript overexpression in NCox and NTox transgenic seedlings in Col, respectively (Supplemental Fig. S1A). The transcripts of two key modulators, the repressor of photomorphogenesis COP1 and positive regulator of light signaling HY5, in NCox and NTox transgenic seedlings in Col remained largely the same as in the wild type (Supplemental Fig. S1A). We further examined the protein expression in these seedlings grown under the same conditions. GUS-FIN219 fusion proteins were detected by FIN219 polyclonal antibodies raised against the N terminus of FIN219, and their level was substantially lower than that of their respective transcripts (Fig. 1F; Supplemental Fig. S1A), which implies that the expression of the transgenes may involve posttranscriptional regulation. Endogenous FIN219 level was largely unaffected in the NCox and NTox transgenic seedlings in Col grown under cFR light (Fig. 1F), whereas the FIN219 level was abolished in fin219-2. In addition, COP1 protein levels were increased in NTox and fin219-2 and especially fin219-1 and NCox as compared with the wild type (Fig. 1F). HY5 protein level was substantially reduced in fin219-1, NCox, NTox and fin219-2 transgenic seedlings. Intriguingly, fin219-1 and fin219-2 showed a significantly reduced level of the lower-band, unphosphorylated form of HY5 (Fig. 1F), shown to be the active form in light signaling (Hardtke et al., 2000).

Because NCox and NTox transgenic seedlings in Col exhibited a hypophotomorphogenic phenotype under cFR light but not under other light
conditions (Fig. 1), we wondered whether reduced HY5 protein in NCox and NTox transgenic seedlings in a wild-type background occurred specifically under cFR light. Thus, we determined the level of HY5 protein in seedlings grown in white light (Supplemental Fig. S3A). The result supported the notion that HY5 level remains constant in fin219-1, NCox, NTox and fin219-2 as well as the wild type. The levels of COP1 were largely equal among these seedlings (Supplemental Fig. S3A). In contrast, COP1 levels were slightly reduced in all fin219 mutants and NTox transgenic lines, except for NCox under continuous red light (Supplemental Fig. S3B). HY5 levels were slightly increased in all the samples examined, but the lower-band, unphosphorylated form of HY5 was slightly lower in level as compared with the wild type (Supplemental Fig. S3B). Under blue light, the expression of COP1 was slightly reduced as well in all samples, and that of HY5 was largely the same as compared with the wild type (Supplemental Fig. S3C). In the dark, the expression of FIN219 remained the same in all samples examined as compared with the wild type (Supplemental Fig. S3D); however, COP1 level in fin219-1, NCox, NTox and fin219-2 remained largely the same as in the wild type, thus leading to barely detectable differences in level of HY5 (Supplemental Fig. S3D). Thus, FIN219 may differentially regulate both COP1 and HY5 under various light conditions, with substantial effect in cFR light.

**FIN219 Modulates COP1 Levels in Response to FR Light to Regulate Hypocotyl Elongation**

To further confirm the authenticity of the regulation between FIN219 and COP1, we examined COP1 levels in FIN219-overexpressing transgenic seedlings (FIN219ox) under cFR. Transgenic FIN219ox seedlings exhibited a hypersensitive hypocotyl phenotype as compared with the wild type under cFR light (Fig. 2, A and B). In contrast, fin219-2 and hy5-1 seedlings displayed a hyposensitive long hypocotyl; cop1-4 seedlings containing expression of the N-terminal 282 amino acids of COP1 proteins showed a markedly short hypocotyl under the same condition (Fig. 2, A and B; McNellis et al., 1996). Further examination revealed that FIN219 protein was highly expressed in FIN219ox; COP1 level was increased in fin219-2 and substantially reduced in FIN219ox (Fig. 2C). Intriguingly, COP1 level was increased in hy5-1, which is consistent with a previous report of the hy5 mutant isolated as an
extragenic suppressor of cop1-6 (Ang and Deng, 1994; Ang et al., 1998). By contrast, HY5 levels were greatly reduced in fin219-2 and increased in FIN219ox (Fig. 2C).

**Analysis of the Double Mutant fin219-2/cop1-6 Reveals that HY5 Stability Requires Functional FIN219 in Darkness and cFR Light**

To further understand whether COP1 serves as a direct target of FIN219, we generated the double mutant for fin219-2 and cop1-4 or cop1-6, a weak allele of COP1 with a mutation at the splicing junction of its intron 4, which leads to detectable COP1 proteins of wild-type size and abundance (McNellis et al., 1994). Surprisingly, the double mutants fin219-2/cop1-4 and fin219-2/cop1-6 exhibited a short-hypocotyl phenotype similar to respective single mutants cop1-4 and cop1-6 under both darkness and cFR light conditions (Fig. 3, A and B), which is distinct from previous results showing that the original double mutant fin219-1/cop1-6 exhibited a long-hypocotyl phenotype in the dark (Hsieh et al., 2000). Thus, suppression of cop1-6 by the fin219 mutation might require physical interaction of FIN219 and COP1. Further examination revealed the FIN219 protein level reduced in the cop1-4 mutant as compared to Col in the dark but not in cFR light (Fig. 3C), which implies that COP1 in the dark might directly affect FIN219 stability, or influence the stability of another factor that regulates FIN219 expression. However, studies of the double mutants fin219-2/cop1-4 and fin219-2/cop1-6 also revealed that the effect of fin219 mutation on COP1 suppression was allele-specific such as with the fin219-2 and cop1-6 combination but not with cop1-4, which is consistent with previous results showing allele-specific genetic interaction between fin219-1 and cop1-6, instead of cop1-5, a null mutant of cop1 (Hsieh et al., 2000). This allele-specific regulation was substantiated with significant reduction of HY5 in fin219-2/cop1-6 but not fin219-2/cop1-4 under darkness and cFR light conditions (Fig. 3C), which suggests that FIN219 can protect HY5 against protein degradation by COP1. Thus, FIN219 may function as a positive regulator of hypocotyl elongation under FR by negatively regulating COP1 level, thus leading to an accordingly increased HY5 level.

**FIN219 may Exist as a Dimer via Intermolecular Interactions Between its N-Terminal and C-Terminal Domains to Regulate Photomorphogenic Development**
Ectopic expression of the N terminus of FIN219 results in a hypophotomorphogenic phenotype under cFR light, with increased level of COP1 and reduced level of HY5 (Fig. 1). To further understand the mechanisms causing the changes in levels of the vital regulators COP1 and HY5 leading to a long-hypocotyl phenotype of NCox and NTox seedlings in a wild-type background specifically under cFR light, we performed interaction studies by yeast two-hybrid and pull-down assays to determine whether the N-terminal coiled-coil domain of FIN219 can bind to the endogenous full-length FIN219. The β-galactosidase activities in yeast two-hybrid assays revealed that the full-length FIN219 could interact with itself and both the N-terminal and C-terminal coiled-coil domains of FIN219 but with higher affinity to the N-terminal coiled-coil domain (Fig. 4A). Moreover, the recombinant MBP-FIN219-NC, GST-FIN219-NC, and GST (or MBP)-FIN219-CC containing the C-terminal coiled-coil domain with 47 amino acids of FIN219 and GST-full-length FIN219 (GST-FIN219FL) fusion proteins were purified to be homogeneous and used for binding assays. The binding affinity of MBP-FIN219-NC was strong with GST-FIN219-CC, weak with GST-FIN219FL and absent with GST-FIN219-NC or GST alone (Fig. 4B). Further tests demonstrated that MBP-FIN219-CC could bind with GST-FIN219FL and GST-FIN219-NC (Fig. 4C).

To further confirm the interaction between the full-length FIN219 and itself or its N-terminal coiled-coil domain, we performed bimolecular fluorescence complementation (BiFC) assay using the mesophyll protoplasts isolated from fin219-2. The full-length FIN219 interacted with itself in the cytoplasm under both darkness and cFR light (Fig. 4D, top panel). The control with the combination of FIN219-YN and YC-FIN219 showed no fluorescence signal (Fig. 4D, bottom panel). Other combinations such as YN-FIN219 and YC-FIN219 also showed interaction in the cytoplasm in both darkness and cFR (Supplemental Fig. S4). Further BiFC assays indicated that the full-length FIN219 could interact with the N-terminal coiled-coil domain (FIN219-NC) in the dark and cFR light (Fig. 4E, top panel). Thus, the full-length FIN219 can interact with the N-terminal coiled-coil domain of FIN219 through its C-terminal coiled-coil domain.

To further understand whether this binding involves intermolecular or intramolecular interaction, we introduced constructs harboring the C-terminal 171 or 275 amino-acid residues of FIN219 fused with GUS (FIN219-C171ox or FIN219-C275ox) into NCox and NTox transgenic plants in Col. The resulting transgenic
seedlings did not show even longer hypocotyls than their parental background under cFR light (Supplemental Fig. S5), which suggests that the binding of the N-terminal coiled-coil domain of FIN219 with its C terminus may occur in an inter- not intramolecular manner.

**FIN219 can Interact with COP1 in the Cytoplasm under Darkness and cFR Light**

Here, we demonstrate that FIN219 negatively regulates COP1 levels, which raises the possibility that the suppression of COP1 by FIN219 may directly involve physical interaction, which might contribute to the dominant-negative phenotype conferred by ectopic expression of the FIN219 N terminus. Yeast two-hybrid assay revealed that FIN219 could interact with the C-terminal WD-40 domain of COP1 via its coiled-coil domain in the N or C terminus (Fig. 5, A and B). The zinc-finger domain and the coiled-coil domain of COP1 seem to fine-tune the binding affinity between FIN219 and COP1 (Fig. 5A). The coiled-coil domain in the C terminus of FIN219 appears to have higher affinity with COP1 than the one in the N terminus (Fig. 5B), which implies that the N terminus may negatively regulate interacting activities of the C-terminal domain binding with COP1. Since HY5 stability requires functional FIN219, determining whether both proteins interact with each other was of interest. However, the 2 proteins did not interact by yeast two-hybrid assay (data not shown). Further, in pull-down assays, various GST-FIN219 fusion proteins and MBP-COP1 fusion proteins (MBP-COP1FL and MBP-COP1-WD-40) were used to confirm the respective interacting domain. GST-FIN219 full-length fusion (GST-FIN219FL) and the C-terminal 275 amino-acid fusion (GST-FIN219-CT) were able to bind with the MBP-COP1 full-length fusion (MBP-COP1FL) (Fig. 5C); other fusions, including the N terminus (GST-FIN219-NT), N-terminal coiled-coil domain (GST-FIN219-NC), and C-terminal coiled-coil domain (GST-FIN219-CC), were not able to bind with MBP-COP1FL (Fig. 5C). GST-FIN219-CC might not be able to bind with MBP-COP1FL because of the structural mask of MBP-COP1FL fusions. This speculation was supported by results showing that GST-FIN219FL, GST-FIN219-CT, and GST-FIN219-CC were able to bind with the MBP-WD-40 domain of COP1 fusion (MBP-COP1-WD-40) (Fig. 5D).

Moreover, BiFC assay with protoplasts isolated from fin219-2 further confirmed the
interaction of FIN219 and COP1 (Fig. 5E). Co-immunoprecipitation (Co-IP) studies using wild-type Col and fin219-2 seedlings grown in cFR for 3 days showed that FIN219 did interact with COP1 (Fig. 5F). This interaction occurred in the cytoplasm under both darkness and cFR light (Fig. 5E). Intriguingly, the interaction of FIN219 and COP1 was shown with two or more specific spots that appear to have similar features with the formation of nuclear speckles due to COP1 and HY5 interaction in the nucleus (Jang et al., 2005). In fact, we observed a high ratio of similar cytoplasmic speckle structure when both FIN219 and COP1 were co-transfected into protoplasts in darkness and far-red light (data not shown). We hypothesized that FIN219 levels might modulate the subcellular location of COP1. Thus, transgenic plants harboring a glucocorticoid-inducible FIN219 construct were generated. Homozygous FIN219-inducible transgenic seedlings treated with 1 μM dexamethasone (Dex) in darkness and cFR light for 3 days showed a markedly short hypocotyl phenotype as compared with the wild type under the same conditions (Supplemental Fig. S6, A and C), in which FIN219 levels were highly induced by Dex up to a 10-fold increase (Supplemental Fig. S6, B and D). Moreover, when we introduced GFP-COP1 into FIN219 protoplasts, followed by Dex-induction in darkness, most of the GFP-COP1 was excluded out of the nuclei and become cytoplasm-localized (Fig. 6, A and B). Further examination indicated that FIN219 was highly induced in the protoplasts treated with Dex, and COP1 protein level remained largely the same in both control and Dex-treated samples, whereas HY5 level was significantly increased in Dex-treated protoplasts (Fig. 6C), which suggests that induction of FIN219 protein levels can mediate COP1 transition from the nucleus to the cytoplasm, thus resulting in an increase of nuclear HY5 levels even in the dark and a photomorphogenic response.

**FIN219 Protein Levels Show a FR Fluence-Dependent Regulation**

Because the original fin219-1 mutant showed a substantial long-hypocotyl phenotype with both low and high fluence rates of FR and the fin219-2 exhibited a significant phenotype only in the low fluence FR (Fig. 1D), we wondered whether this discrepancy was due to a change in COP1 or HY5 levels. Further examination of fin219-1 and fin219-2 mutants under low and high fluence rates of FR (LF and HF, respectively) indicated consistent phenotypic responses similar to those shown in Fig. 1D (Fig. 7, A and B), which was also reflected in high accumulation of anthocyanin in
wild-type Col under HF, an intermediate level in fin219-2 and a lesser level in fin219-1 (Fig. 7A, bottom panel; Fig. 7C), whereas the phyA-211 mutant showed an insensitivity of hypocotyl elongation and no anthocyanin accumulation in both conditions (Fig. 7, A–C). Interestingly, FIN219 levels in Col and fin219-1 were slightly reduced under HF as compared with under LF. Moreover, FIN219 level in the phyA-211 mutant was reduced under LF and HF conditions as compared with Col in respective fluences. In contrast, COP1 level in Col did not show a significant difference between LF and HF; it increased greatly in both fin219-1 and fin219-2 but with less increase in fin219-2 under HF and showed a comparative level in phyA-211. Surprisingly, HY5 level in fin219-2 under HF was significantly increased up to the wild-type level under LF (Fig. 7D), which is consistent with the short-hypocotyl phenotype for the fin219-2 under the HF condition. However, the level of HY5 in fin219-2 under LF was greatly reduced to low levels similar to those in fin219-1 under both LF and HF conditions (Fig. 7D), which might be why the fin219-1 mutant still showed a substantial long-hypocotyl phenotype under HF. In addition, the level of HY5 was much reduced in phyA-211 under both LF and HF conditions as compared with Col under the same conditions (Fig. 7D). Thus, these data suggest that FIN219 may have a differential role in the regulation of HY5 in LF and HF to modulate hypocotyl elongation.

DISCUSSION

COP1 functions as a repressor of photomorphogenesis in darkness and has an E3 ligase activity involved in the protein degradation of light signaling players, including the photoreceptors phytochromes and Cryptochromes (Seo et al., 2004; Jang et al., 2010; Wang et al., 2001; Yang et al., 2001); however, the information on the regulation of COP1 activities is limited. Here, we demonstrate that FIN219/JAR1 negatively regulates COP1 levels through physical interaction under cFR light and can modulate the subcellular location of COP1 even in the dark. Moreover, we also provide evidence showing that HY5 protein stability requires FIN219. Thus, our data reveal a new finding that FIN219/JAR1 functions as a positive regulator of phyA-mediated FR light signaling by controlling the levels of COP1 and HY5 to modulate photomorphogenic development in Arabidopsis.

Our recent data demonstrated that jar1-1 showed a long-hypocotyl phenotype under
a FR-light fluence rate below 20 µmol m\(^{-2}\) s\(^{-1}\) (Chen et al., 2007). Especially, it exhibited a hypocotyl length similar to that of fin219-1 at 1.47 µmol m\(^{-2}\) s\(^{-1}\) FR light. Moreover, wild-type Columbia seedlings overexpressing FIN219 displayed a hypersensitive phenotype under a low-fluence rate of FR (LF) light (Hsieh et al., 2000), which implies that FIN219 may play a vital role under LF light. Here, we further show that a fin219-2 allele and NCox, as well as NTox, with ectopic expression of the FIN219 N-terminal domain in wild type, produce a hyposensitive long-hypocotyl phenotype under cFR light (Fig. 1), which is consistent with features of reduced photomorphogenic development, including decreased expression of CHS and reduced anthocyanin accumulation in various fin219 mutant seedlings grown under the same conditions (Supplemental Fig. S1A; Fig. 1). In addition, the phyA-null mutant, phyA-211, resulted in reduced levels of FIN219 under LF and HF conditions (Fig. 7D). All these data strongly indicate that FIN219/JAR1 participates in phyA-mediated FR signaling.

To further illustrate the molecular mechanism underlying the long-hypocotyl phenotype of NCox and NTox in wild type background specifically under cFR light, we found that the expression of COP1, a repressor of photomorphogenesis in the dark (Deng et al., 1992), was differentially increased in level in fin219, NCox and NTox seedlings in a wild-type background grown under cFR light (Fig. 1F); however, it was much reduced in the FIN219-overexpressed transgenic line FIN219ox (Fig. 2C), which implies that FIN219 negatively regulates COP1 level under cFR light. Moreover, HY5, a positive regulator of light signaling existing in both phosphorylated and unphosphorylated forms in Arabidopsis (Hardtke et al., 2000), was substantially decreased in level in fin219-1, NCox, NTox and fin219-2 mainly under cFR light but not other light conditions (Fig. 1F; Supplemental Fig. S3). This specificity of HY5 reduction is consistent with the specific phenotype that fin219-1, NCox, NTox and fin219-2 conferred. Intriguingly, fin219-1 and fin219-2 showed greatly reduced levels of the unphosphorylated form of HY5 (Fig. 1F), previously shown to be active and unstable in light signaling (Hardtke et al., 2000), which implies that FIN219 may affect HY5 stability. In addition, COP1 works synergistically with SPA1, Arabidopsis suppressor of phyA-105, to negatively control HY5 abundance under cFR light (Saijo et al., 2003; Yang and Wang, 2006). Here, we found that the double mutant fin219-2/cop1-6 showed greatly reduced HY5 level in the dark and cFR light (Fig. 3C) as compared to that in the parental cop1-6 under the same conditions, which indicates
that the presence of FIN219 somehow stabilizes HY5 level. The cop1-6 mutant contains the wild-type size and abundance of COP1 (McNellis et al., 1996); the double mutant fin219-2/cop1-6 might increase functional COP1 level, thus leading to HY5 degradation in cFR light and darkness. Moreover, when FIN219 is induced at high levels by Dex treatment and can move COP1 to the cytoplasm even in darkness (Fig. 5E; Fig. 6), whereas in the absence of FIN219, the wild-type size of COP1 in cop1-6 might stay in the nucleus, thus resulting in increased degradation of HY5 in both darkness and cFR light (Fig. 3C). In addition, SPA1 was shown to enhance COP1 E3 ligase activity to negatively influence HY5 levels under cFR light (Saijo et al., 2003). Our preliminary data also showed upregulated expression of SPA1 transcripts in the fin219-1 mutant under cFR light (Supplemental Fig. S7). The double mutant fin219-2/cop1-6 likely contains increased levels of SPA1, thus giving rise to enhanced COP1 E3 ligase activity, which leads to increased HY5 degradation. However, we still do not have a good explanation for the comparable level of COP1 in the double mutant fin219-2/cop1-6 and the wild type (Fig. 3C), which might involve feedback regulation. Therefore, FIN219 plays an important role in phyA-mediated FR light signaling to negatively regulate COP1 and positively regulate HY5 levels for photomorphogenic development of Arabidopsis seedlings.

Furthermore, FIN219 was shown to interact with the WD-40 domain of COP1 through its coiled-coil domain in the C terminus (Fig. 5), where the N-terminal coiled-coil domain of FIN219 bound in a manner of intermolecular interaction (Fig. 4). The intermolecular interaction of FIN219-NC/-NT and FIN219 was further implicated with the results showing that the introduction of the C-terminal domains of FIN219 (FIN219-C171ox or FIN219-C275ox) into NCox or NTox transgenic plants in a wild-type background did not result in even longer hypocotyl phenotype than NCox or NTox (Supplemental Fig. S5). These results suggest that the dominant-negative phenotype conferred by NCox or NTox involves intermolecular binding of the N terminus of FIN219/JAR1 and the endogenous full-length FIN219/JAR1, instead of intramolecular interaction of its N and C termini. However, we still could not rule out the possibility of intramolecular interaction, with misregulation of normal FIN219/JAR1 complex function or failure of forming accurate structure of FIN219/JAR1 complex between the NCox or NTox construct and the FIN19 C-terminal construct FIN219-C171 or FIN219-C275, resulting in a hypophotomorphogenic phenotype in cFR light. Therefore, ectopic expression of the
N terminus of FIN219 in wild type likely competes with endogenous COP1 to bind with the C-terminal coiled-coil region of endogenous FIN219, thus leading to a reduction in HY5 level and a long-hypocotyl phenotype in cFR light. However, in the wild type under cFR, FIN219 exists as a homodimer or heterodimer with COP1 in the cytoplasm, which leads to an increase of HY5 level in the nucleus and further photomorphogenic development. This notion was substantiated by the ectopic expression of the C terminus of FIN219, which entailed COP1 in the cytoplasm, thus stabilizing HY5 in the nucleus, which leads to a hypersensitive short-hypocotyl phenotype in cFR light (Supplemental Fig. S8).

Our previous studies indicated that the fin219-1 mutation can suppress the short-hypocotyl phenotype of cop1-6 in darkness, that is, the double mutant fin219-1/cop1-6 exhibited a long-hypocotyl phenotype in darkness, but the double mutant fin219-1/cop1-5 still showed the cop1-5 mutant phenotype with short hypocotyls, which indicates an allele-specific suppression by FIN219 (Hsieh et al., 2000). In contrast, our double mutant studies revealed that the fin219-2 mutation did not suppress cop1-6 or cop1-4 in darkness and cFR light; moreover, the fin219-2/cop1-6 but not fin219-2/cop1-4 resulted in significant reduction of HY5 protein level in darkness and cFR. This allele-specific effect may involve interaction of the FIN219 in fin219-1 and the WD-40 domain of COP1. The fin219-2/cop1-6 does not contain FIN219 protein, which leads to COP1 or partial COP1 activity action in the nuclei that results in HY5 level reduction. However, the fin219-2/cop1-4 does not have FIN219, and cop1-4 only contains the N-terminal 282 amino acids without the WD-40 domain; thus HY5 is not degraded. Taken together, these data indicate that the interaction of FIN219 and COP1 is critical to modulate the level and stability of HY5 for photomorphogenic development.

JAR1 has been well documented to play a role in plant defense responses (Staswick et al., 1998; Ferrari et al., 2003; Kazan and Manners, 2008) and acts as a JA-amino acid synthetase with a preferred generation of JA-isoleucine (Guranowski et al., 2007; Suza and Staswick, 2008). A recent report showed that the JA-isoleucine (JA-Ile) conjugate but not other JA-derivatives such as JA or MeJA could enhance the physical interaction between COI1 and JASMONATE ZIM-DOMAIN PROTEIN 1 (JAZ1), thus leading to JAZ1 degradation and JA signaling (Chini et al., 2007; Thines et al., 2007); therefore, the JA-Ile conjugate acts as an active form of JA to fulfill physiological functions in plant defense responses and development. Here, we
demonstrated that ectopic expression of the N terminus of FIN219/JAR1 resulted in a dominant-negative long-hypocotyl phenotype under cFR light, which did not alter endogenous levels of JA-Ile as well as the root and hypocotyl responses to exogenous MeJA or coronatine (Table 1; Supplemental Fig. S2); therefore, coronatine, a phytotoxin with a structure mimicking that of JA-Ile, can initiate jasmonate responses once it is perceived by the jasmonate receptor COI1, even in fin219-2 or jar1-1 mutants. Interestingly, the coi1-16 mutant as a control in this study exhibited a long-hypocotyl phenotype in cFR light in the absence of coronatine and remained with an unchanged phenotype even in the presence of coronatine (Supplemental Fig. S2, E and F). Therefore, jasmonate perception contributes to FR-mediated inhibition of hypocotyl elongation, which is consistent with the results of Robson et al. (2010) showing that COI1 can modulate responses to light, including photomorphogenic responses to FR light, flowering and shade responses. Here, we further found that FIN219 exhibits a FR fluence rate-dependent expression pattern with more abundant level in LF and a reduction in level in HF, leading to a high level of HY5 in HF (Fig. 7D). It appears that FIN219 in wild-type Col under LF and HF can negatively regulate COP1 and entrap it in the cytoplasm through physical interaction; however, the FIN219 in fin219-1 was unable to suppress COP1 level by interaction, leading to COP1 in the nuclei and accordingly HY5 degradation under LF and HF. In contrast, in fin219-2 under LF, COP1 stayed in the nuclei, giving rise to HY5 degradation, but under HF, some of COP1 proteins moved to the cytoplasm, thus leading to an increase of HY5 level. HY5 increase in level in fin219-2 under HF may involve another factor responsible for COP1 reduction and increase in HY5 level (Fig. 7D), whereas, in the dark, FIN219 mainly affects COP1 partition, but not COP1 levels, between the nucleus and the cytoplasm (Fig. 6C; Supplemental Fig. S3D). Thus, FIN219 levels responding to different fluence rates of FR may provide a molecular mechanism to modulate shade responses for production of JA-Ile in response to the ratio of red and FR light.

Recent evidence revealed that the Arabidopsis phytochrome chromophore mutants hy1 and hy2 show elevated levels of JA and constant activation of COI1-dependent JA responses; moreover, JA inhibits the expression of a group of light-inducible photosynthetic genes (Zhai et al., 2007). Thus, phytochrome chromophore-mediated light signaling and the JA signaling pathway may have a mutual antagonistic relation. As well, phytochrome inactivation by FR can strongly reduce plant sensitivity to
jasmonates (Moreno et al., 2009) and a recent report indicated that phyA is required for JA- and wound-mediated JAZ1 degradation (Robson et al., 2010). Moreover, phyA in rice requires jasmonate for photodestruction (Riemann et al., 2009). Hence, phyA- and jasmonate-mediated signalings are mutually regulated. Here, we showed that phyA positively regulates FIN219/JAR1 level under LF and HF (Fig. 7D), which may have a good link to negatively control JAZ1 level. In addition, FIN219/JAR1 also shows a gradual downregulated expression pattern in the dark–FR light transition (data not shown), possibly giving rise to reduced levels of JA-Ile, which allows for rapid induction of photosynthesis-related genes such as \textit{RbcS} and \textit{Cab} to undergo photomorphogenic development. This raises an interesting question: How does FIN219/JAR1, a jasmonate-conjugating enzyme, work in the integration of FR light and JA signaling? We propose that FIN219/JAR1 may have a dual function, one with JA conjugating enzyme activity and the other as a component of phyA-mediated FR signaling. This dual function of FIN219/JAR1 might have a synergistic or independent effect. For instance, FIN219/JAR1 as a JA conjugating enzyme may trigger COP1 out of the nuclei as shown in Fig. 6, and then serve as a component of FR signaling to bind with the WD-40 domain of COP1 in the cytoplasm, thus resulting in an increase of HY5 levels in the nuclei; however, the molecular mechanism underlying FIN219/JAR1 dual functions remains to be elucidated.

Intriguingly, several other members such as GH3.2, GH3.5, GH3.6 and GH3.10 of the \textit{GH3} gene family in Arabidopsis have been reported to participate in light signaling (Takase et al., 2004; Tanaka et al., 2002; Nakazawa et al., 2001; Takase et al., 2003) and categorized as Group II or Group I (GH3.10) based on sequence homology and enzymatic activity (Staswick et al., 2002; Staswick and Tiryaki, 2004). These GH3 members with functional roles in light signaling are either IAA-amido synthetase (Group II) or JA-amido synthetase (Group I), and contain a coiled-coil domain either in the N terminus or C terminus. FIN219/JAR1 has two coiled-coil domains, one in the N terminus and the other in the C terminus that has been shown to interact with FIP1 (Chen et al., 2007). Here, we showed that the C-terminal coiled-coil domain of FIN219 is able to interact with COP1. It will be worthy to see how the binding of FIP1 with FIN219 affects FIN219-COP1 interaction. Therefore, these GH3 members may act in a similar manner as FIN219 with a dual function, that is, they may have enzymatic activities and nonenzymatic function via protein-protein interacting manner. The characteristic of FIN219/JAR1 with a dual function is implicated by a
recent paper showing that a 2C-type protein phosphatase, PP2CA, can directly interact with a protein kinase OST1 leading to inhibition of the kinase independently of its phosphatase activity in response to low levels of ABA, which results in the activation of the SLAC1 anion channel with basal activity (Lee et al., 2009). Thus, FIN219/JAR1 can serve as a good candidate to understand the integration between FR light and JA signaling pathways.

**MATERIALS AND METHODS**

**Plant Materials and Growth Conditions**

The mutants, fin219-1 (Hsieh et al., 2000), jar1-1 (Staswick et al., 2002), fin219-null (SALK_059774 from ABRC; named fin219-2 in this study), cop1 alleles (cop1-4 and cop1-6) and two transgenic plants, NCox and NTox, are in the *Arabidopsis thaliana* Columbia ecotype; hy5-1 is in the Landsberg ecotype background. *Arabidopsis* seedlings were grown in an E30LEDL3 growth chamber (Percival Scientific Inc.) for phenotype analysis. The wavelengths of FR, red, and blue LED light sources (Percival Scientific Inc.) were 739 ± 31 nm, 669 ± 25 nm, and 470 ± 25 nm, respectively. Light fluence rates were measured by the LI-200SA pyranometer sensor (LI-COR). The light fluence rates used for FR, blue, red and white light were 1.47, 5.08, 6.34 and 5.88 µmol m⁻² s⁻¹, respectively. *Arabidopsis* seedlings were grown on GM agar plates containing 0.3% sucrose for phenotype and molecular analysis as described previously (Hsieh et al., 2000).

**Gene Constructs and Transgenic Plants**

To construct β-glucuronidase (GUS)-FIN219-N53 and GUS-FIN219-N300 plasmids, the cDNA sequences corresponding to the N-terminal 53 and 300 amino acids (NC and NT, respectively) of FIN219 were amplified with primers (see supplementary methods) and the full-length of FIN219 cDNA clone #11-2 as a template. Both PCR products were subcloned into the BglII site of the pRTL2-GUS plasmid. The PstI DNA fragments were released from pRTL2-GUS-FIN219-NC and pRTL2-GUS-FIN219-NT constructs, subcloned into the binary vector pPZP221, and then introduced into plants by the floral dip method (Clough and Bent, 1998). To clone the coiled-coil domain and the 275 amino acids in the C-terminal region of FIN219 (FIN219-CC and FIN219-CT, respectively), the cDNA clone #11-2 was used for PCR amplification. For recombinant protein constructs, various BglII FIN219 fragments
were cloned into pGEX-4T-1 (Amersham) and pMAL-C2X (New England Biolabs).

**Generation of Double Mutants of fin219-2 and cop1-4 or cop1-6**

For double mutant production, *cop1-4* or *cop1-6* as male was crossed with *fin219-2* as female. The resulting F₂ generation progenies were used for PCR-based genotyping by using the combinations of T-DNA primer (LBa1: 5’-TGGTTCACGTAGGGCCCATCG-3’) and FIN219-specific primer (FIN219N299_R: 5’-CTTTGCCTGGGAAGAGGC-3’) for the *FIN219* locus, mutant specific primer (cop1-4-Rv-1669m: 5’-CAGTTGACTGATTCAAACTTA-3’) and wild-type COP1-specific primer (cop1-4-Fw-326: 5’-CCCTGTTGTAGCCACACCTCACC-3’) for the *cop1-4* mutant locus, and mutant specific primer (cop1-6-Rv-2295m: 5’-ACATTCTTGAAATCATTGAACC-3’) and wild-type COP1-specific primer (cop1-6-Fw-1274: 5’-ACGTATAACCCCTGATGTT-3’) for the *cop1-6* mutant locus. For identification of homozygous double mutants, the following wild-type specific primers were used for genomic genotyping; FIN219: FIN219N299_R and FIN219F_XhoI: 5’-CAGCTCGAGATGTTGGAGAAGGTTGA-3’, cop1-4: cop1-4-Fw-326 and cop1-4-Rv-1669: 5’-CAGTTGACTGATTCAAACTTG-3’, cop1-6: cop1-6-Fw-1274 and cop1-6-Rv-2295: 5’-ACATTCTTGAAATCATTGAAC-3’. One candidate was identified as homozygous for the *fin219-2* mutant locus but heterozygous for the *cop1-4* or *cop1-6* mutant locus. This line was further subjected to phenotyping in the dark, thus leading to a ratio of 3 long to 1 short hypocotyl phenotype. Thus, the short hypocotyl seedlings were transferred to white light for the next generation and further genotyping until homozygous loci for the *fin219-2* and *cop1-4* or *cop1-6* mutations were identified.

**Analysis of Pigments**

Seedlings of wild-type Columbia, *fin219/jar1*, *NCox* and *NTox* transgenic plants were grown in cFR light for 3 days for investigation of anthocyanin accumulation or followed by another 1-day exposure of continuous white light for analysis of chlorophyll content. The measurements of chlorophyll and anthocyanin were described previously (Hsieh et al., 2000).

**Protein Extraction and Protein Gel Blot Analysis**
Seedlings of wild-type Columbia, fin219/jar1, NCox and NTox transgenic plants grown in cFR light at indicated fluence rate for 3 days were harvested and frozen immediately for protein extraction. Total protein extraction was described previously (Hsieh et al., 2000). In total, 100 µg total protein was separated on 12% SDS-polyacrylamide gel and blotted onto an Immobilon-P membrane (Millipore). Protein gel blot analysis involved standard methods (Sambrook and Russelle, 2001) and detection was with use of anti-FIN219, anti-COP1 (Hsieh et al., 2000), anti-HY5 (Osterlund et al., 2000a), and anti-RPN8 (Yang et al., 2004) specific antibodies. Protein expression data are the mean of at least 2 independent replicates. Data were analyzed by ANOVA with Scheffe’s test. The differences were considered significant at $P<0.05$.

**Yeast Two-Hybrid Assays**

Yeast two-hybrid assays involved use of the LexA-based two-hybrid system. Various COP1 fragments were cloned into pJG4-5 as described previously (Ang et al., 1998). Full-length and different deletions of FIN219 were generated by PCR and cloned into pEG202. All constructs were transformed into the yeast strain EGY48 with pSH18-34 encoding a LacZ reporter gene, and yeast cells were grown on minimal medium/-His/-Trp according to the manufacturer’s instructions (Clontech). Transformed colonies were plated onto minimal medium/-His/-Trp/-Leu/+Galactose/+Raffinose/X-gal. Yeast cell selection and β-galactosidase activity assay were essentially as standard methods (Clontech).

**Protein Interactions by Pull-Down Assays**

Various MBP and GST fusion proteins were purified from *Escherichia coli* as described previously (Saijo et al., 2003; Chen et al., 2007). Five micrograms of recombinant protein was mixed with 1 ml of binding buffer (50 mM Tris at pH 7.5, 100 mM NaCl, 0.2% glycerol, 0.6% Triton X-100, 0.5 mM β-mercaptoethanol). After incubation at 4°C for 1 h, the reaction mixture was further incubated with glutathione sepharose 4B beads (Amersham) at 4°C for another 1 h. The beads were washed 5 times with washing buffer (50 mM Tris, pH 7.5, 100 mM NaCl, 0.6% Triton X-100), then the pellet was boiled with 2x sample buffer and underwent SDS-PAGE. Western blot analysis was performed by standard methods (Chen et al., 2007).
Protoplast Transfection and Bimolecular Fluorescence Complementation (BiFC) Analysis

Arabidopsis mesophyll protoplast isolation and transfection were as described (Yoo et al., 2007). The 4-week-old well-expanded leaves were cut into 1-mm pieces and incubated in enzyme solution (20 mM MES, 1.5% cellulase R10, 0.4% macerozyme R10, 0.4 M mannitol and 20 mM KCl, 10 mM CaCl2, 5 mM β-mercaptoethanol, 0.1% bovine serum albumin, pH 5.7) for 3 h. Protoplasts were collected by centrifugation at 100g and washed twice with W5 solution (2 mM MES, 154 mM NaCl, 125 mM CaCl2 and 5 mM KCl, pH 5.7). Protoplasts were re-suspended in W5 solution and incubated on ice for at least 30 min, then washed with MMG solution (4 mM MES, 0.4 M mannitol, 15 mM MgCl2, pH 5.7) and re-suspended at 2x 10^5 cell/ml in MMG solution. For protoplast transfection, 200 μl protoplasts was mixed with 20 μl DNA and 220 μl PEG solution (40% PEG 4000, 0.2 M mannitol, 100 mM CaCl2), then transfected protoplasts were washed twice, re-suspended in W5 solution, and incubated under experimental conditions. We constructed BiFC plasmids as described (Walter et al., 2004). Various DNA fragments of FIN219 and COP1 were cloned into 35p-YFP-N155/pRTL2 and 35p-YFP-C84/pRTL2, respectively. The nuclei of protoplasts were stained with 4',6-diamidino-2-phenylindole (DAPI). All fluorescent images were obtained by use of a BX51 fluorescent microscope with a DP-72 digital camera (Olympus) and processed by use of Adobe Photoshop.

Protein-Protein Interactions by Coimmunoprecipitation Analysis

Coimmunoprecipitation analysis was performed as described (Chen et al., 2007). The seedlings grown in cFR for 3 days were ground with grinding buffer (50 mM Tris-HCl, pH 5.7, 150 mM NaCl, 10 mM MgCl2, 0.1% BSA, 0.1% Nonidet P-40, 1 mM Phenylmethanesulfonyl fluoride, 1 X Protease Inhibitor Cocktail (Sigma), 40 μM MG132). Total 2 mg proteins were mixed with beads, and incubated at 4 °C for 4 hr and then washed 3 times with grinding buffer without BSA. Pellets were analyzed by standard SDS-PAGE and subjected for Western blotting analysis.

Generation of the Glucocorticoid-Inducible FIN219 Construct and Dexamethasone (Dex) Treatment
The full-length fragment of FIN219 was subcloned into the XhoI site of the pTA7002 plasmid that contains a glucocorticoid-inducible promoter as described previously (Aoyama and Chua, 1997). For phenotype and protein gel blot analyses, homozygous T4 transgenic seeds were grown on 0.3% GM plates containing EtOH or 1 μM Dex under darkness and cFR for 3 days. For protoplast analysis, the transfected protoplasts were cultured in W5 solution containing EtOH or 1 μM Dex in the dark.

**Production of FIN219 Polyclonal Antibodies**

A cDNA fragment corresponding to the N-terminal 300 amino acids of FIN219 was amplified by PCR with the primers containing the BamHI site (see supplementary method) and the cDNA clone #11-2 as a template, then cloned into the expression vector pCAL-n for protein expression in the *E. coli* host BL21(DE3). The resulting recombinant proteins were purified by gel electroelution and then sent to the animal center for raising the polyclonal antibodies from rabbits.

**Measurement of JA-Ile levels in Arabidopsis seedlings**

The isolation and quantification of JA-Ile was performed as described (Staswick and Tiryaki, 2004; Suza and Staswick, 2008). About 1.5-2 gram of 3-day-old far-red light-grown seedlings were ground in liquid nitrogen and extracted with 10 ml 80% methanol containing 10 nM dihydrojasmonic acid (DHJA, Tokyo Chemical Industry co., as internal control), and then centrifuged for 10 min at 3000 xg. The samples were proceeding through DEAE Sephadex A-25 resins (GE Healthcare) and the SiOH column (MACHEREY-NAGEL). The sample was eluted with 1 column volume of 0.2% acetic acid in ethylacetate and dried in a stream of nitrogen, and then dissolved in the isoctane, which was analyzed by an Agilent Technologies 6890N GC and 5975 MSD with a DB-1 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific), and the column gradient was 60-300 °C in 25.4 min. The standard curve of JA-Ile was obtained from the integrated peak area of the serial concentrations of synthetic JA-Ile and compared with the internal standard. The level of JA-Ile in the samples was then determined by comparing with the standard curve.

**Supplemental Data**

The following materials are available in the online version of this article.
Supplemental Figure S1. Investigation of light responsive genes and flowering time for FIN219 N-terminal transgenic plants.

Supplemental Figure S2. Physiological responses of the FIN219 N-terminal transgenic seedlings in the presence of methyl jasmonate (MeJA) or coronatine.

Supplemental Figure S3. Protein gel blot analyses of FIN219, COP1, and HY5 levels in various fin219 mutants and FIN219 N-terminal transgenic seedlings under different light conditions.

Supplemental Figure S4. The full-length FIN219 interacts itself by BiFC.

Supplemental Figure S5. Hypocotyl lengths of transgenic seedlings harboring ectopic expression of the C terminus of FIN219/JAR1 in NCox or NTox genetic background under continuous far-red (cFR) light condition.

Supplemental Figure S6. Phenotype and protein gel blot analysis of FIN219 inducible transgenic seedlings under cFR light.

Supplemental Figure S7. RNA gel blot analysis of SPA1 transcript levels in wild-type and the fin219-1 mutant seedlings.

Supplemental Figure S8. The phenotype and FIN219 expression in the FIN219 C-terminal transgenic seedlings under cFR condition.

Supplemental Table S1. A list of oligonucleotide sequences used in this study.

Supplemental Methods. Gene constructs used in this study; Quantification of Northern and Western blots.

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**Figure legends**

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|        | Col  | fin219-1 | NCox  | NTox  | fin219-2 |
|--------|------|----------|-------|-------|----------|
| Values | 10.4 ± 2.1\(^a\) | 10.7 ± 3.2\(^a\) | 11.1 ± 2.3\(^a\) | 11.0 ± 3.0\(^a\) | 1.3 ± 0.4\(^b\) |

Values (pmol/g fresh weight) shown here are the mean ± SD of three biological replicates of 3-day-old seedlings under cFR condition (1.47 µmol m\(^{-2}\) s\(^{-1}\)). Different lowercase letter represents significant difference by ANOVA at *P*<0.05.