A Small GTPase Activator Protein Interacts with Cytoplasmic Phytochromes in Regulating Root Development

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Phytochromes enable plants to sense light information and regulate developmental responses. Phytochromes interact with partner proteins to transmit light signals to downstream components for plant development. PIRF1 (phytochrome-interacting Rop guanine-nucleotide exchange factor (ROPGEF 1)) functions as a light-signaling switch regulating root development through the activation of ROPs (Rho-like GTPase of plant) in the cytoplasm. In vitro pulldown and yeast two-hybrid assays confirmed the interaction between PIRF1 and phytochromes. PIRF1 interacted with the N-terminal domain of phytochromes through its conserved PRONE (plant-specific ROP nucleotide exchanger) region. PIRF1 also interacted with ROPs and activated them in a phytochrome-dependent manner. The Pr form of phytochrome A enhanced the ROPGEF activity of PIRF1, whereas the Pfr form inhibited it. A bimolecular fluorescence complementation analysis demonstrated that PIRF1 was localized in the cytoplasm and bound to the phytochromes in darkness but not in light. PIRF1 loss of function mutants (pirf1) of Arabidopsis thaliana showed a longer root phenotype in the dark. In addition, both PIRF1 overexpression mutants (PIRF1-OX) and phytochrome-null mutants (phyA-211 and phyB-9) showed retarded root elongation and irregular root hair formation, suggesting that PIRF1 is a negative regulator of phytochrome-mediated primary root development. We propose that phytochrome and ROP signaling are interconnected through PIRF1 in regulating the root growth and development in Arabidopsis.

Light is the one of various environmental factors affecting plant root growth and development (1). Light-sensing photoreceptors such as phytochromes and cryptochromes regulate root growth and development, including lateral root growth (2), gravitropism (3), and root elongation (4). The root hair formation is particularly sensitive to light signals perceived by phytochromes (1). In phytochrome-mediated root developmental events such as lateral root growth and gravitropism, positive photomorphogenic factor HY5 plays a crucial regulatory role in Arabidopsis (5, 6). Phytochromes synthesized as inactive Pr forms in the dark change conformations into active far-red light-absorbing Pfr forms by red light. This photochromism of phytochromes enables plants to modulate red/far-red light signals to regulate developmental responses (7–10).

Current interest in phytochrome-mediated root development concerns root hair elongation, primary root formation, and photo- and gravitropisms (3, 4, 11–17). To discover the role of the phytochrome in roots, we studied the possible involvement of the phytochrome-interacting protein. In this regard, RopGEF (Rho of plants guanine nucleotide exchange factor) identified previously as a phytochrome-interacting protein (18) possibly participates in root development. ROPs play a signaling role in diverse developmental processes and regulate primary root elongation, lateral root formation, and root hair polarity in response to various environmental factors (19–25). Relevant to our study, RopGEF proteins play a critical role in ROP signaling through their ability to activate ROPs involved in the control processes of plant growth and development (22, 26–28). A previous study suggested the possible involvement of ROPs in the phytochrome signaling pathway, as the production of GTP by an NDPK2 enzyme was specifically activated by the Pfr form of phytochrome A (29).

In the present study, we suggest that RopGEF11 (PIRF1; phytochrome interacting RopGEF 1) activated an ROP in a phytochrome-dependent manner for root development in Arabidopsis. Interestingly, we found that cytoplasmic PIRF1 interacted with the N-terminal region of phytochromes A and B localized in the cytoplasm. Based on studies with PIRF1 knockout and overexpression mutant plants, we explore the functional role of the cytoplasmic phytochromes and PIRF1 in regulating root development through activation of an ROP.

EXPERIMENTAL PROCEDURES

Plant Materials and Growth Conditions—The Arabidopsis T-DNA insertion mutant (pirf1, Salk_126725) of PIRF1 and the phytochrome-null mutants (phyA-211 and phyB-9) were obtained from the Salk Institute and Arabidopsis Stock Center,
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respectively. A homozygote of pir1 was selected by genomic PCR, and the inhibition of PIRF1 gene expression in pir1 was confirmed by reverse transcription-PCR (RT-PCR) analysis using the gene-specific primers (see supplemental Table S1). The PIRF1 overexpression mutant (PIRF1-OX) was generated by transformation of the 3SS-myc-PIRF1 construct into wild type Arabidopsis (Col-0). The overexpression of myc-PIRF1 in PIRF1-OX was confirmed by RT-PCR and Western blot analysis with monoclonal anti-myc antibodies (Millipore, Billerica, MA) (data not shown). For seedling growth and light response experiments, Arabidopsis seeds were surface-sterilized and sown on plates containing one-half Murashige & Skoog medium, pH 5.7 and 0.8% Phyto agar (Duchefa Biochemie, Haarlem, The Netherlands). The plates were positioned vertically to allow root growth along the gel surface. Images of plant samples were captured using a Nikon D-70s digital camera, and the primary root lengths were measured using Image-Tool software (University of Texas Health Science Center, San Antonio, TX).

Light Conditions—Seedlings were irradiated with either continuous far-red (FRc; 730 nm, 23 μmol m⁻² s⁻¹) or red (Rc; 660 nm, 12 μmol m⁻² s⁻¹) light. Monochromatic red or far-red light was generated by light-emitting diodes (GFLE-102R, GoodFeeling, Gyeonggi-Do, Korea). Light intensities were measured using a Quantum-Photo Radiometer HD9021 (Delta OHM SRL, Selvazzano, Italy).

Yeast Two-hybrid Assay—For yeast two-hybrid analyses of PIRF1 and phytochromes, PIRF1 protein was fused to the GAL4 DNA binding domain. The N terminus (phyA-N or phyB-N) and C terminus (phyA-C or phyB-C) of phytochromes A (phyA) and B (phyB) were subcloned into the pGADT7 vector to express truncated proteins fused with the GAL4-activation domain. Yeast two hybrid assays were then performed according to the manufacturer’s recommended conditions (Matchmaker Two-hybrid System, Clontech, Mountain View, CA).

Co-immunoprecipitation Assay—The myc-tagged PIRF1-OX plants were grown in darkness for 4 days at 22 °C after germination. The seedlings were then harvested and homogenized with an extraction buffer (70 mM Tris-HCl, pH 8.3, 35% ethylene glycol, 98 mM (NH₄)₂SO₄, 7 mM EDTA, 14 mM sodium metabisulfite) containing protease inhibitor mixture (Roche Applied Science). Monoclonal anti-myc antibodies (Millipore) were incubated with the crude protein extracts at 4 °C overnight followed by incubation with protein G-agarose (Millipore) at 4 °C for 3 h. The beads were then washed six times with washing buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl₂, and 1% Nonidet P-40) and resuspended in 2× SDS-PAGE sample buffer. Proteins were separated on a 10% SDS-PAGE gel and transferred to polyvinylidene difluoride membrane (Invitrogen). Anti-myc (4A6, Millipore), anti-phyA (mAA for Arabidopsis phyA (30)) and anti-phyB (mBA for Arabidopsis phyB (30)) monoclonal antibodies were then used for detecting PIRF1 and two phytochromes, respectively. Horseradish peroxidase-conjugated anti-mouse IgG was used as the secondary antibody, and the signals were detected by chemiluminescence using an ECL reaction (GE Healthcare).

Histochemical GUS Assay—For histochemical β-glucuronidase (GUS) assays for PIRF1, ~0.9 kb of the promoter region of the PIRF1 gene was amplified by PCR and cloned into the pBI101 binary vector (Clontech). The resulting construct was then transformed into wild type Arabidopsis plants (Col-0), and transformants were selected on one-half mass spectrometry media containing 30 μg/ml kanamycin. Histochemical staining and microscopic analyses were performed as described previously (31).

GDP/GTP Exchange Assays—The GDP/GTP exchange activity of PIRF1 with ROP proteins was measured by the nitrocellulose filter binding method at room temperature essentially as described previously by Zheng et al. (32). Briefly, for the measurement of GDP binding, purified recombinant maltose binding protein tagging ROPs (MBP-ROPs) were rendered nucleotide-free by incubation for 5 min in a loading buffer containing 2 mM EDTA and 3 mM [³H]GDP, followed by the addition of 5 mM MgCl₂ and a further incubation for 20 min. Purified GST-PIRF1 was equilibrated for 15 min in an exchange buffer containing 1 mM GTP and then added to the ROP-containing solution. The reaction was then continued for the indicated periods. For the binding of [³⁵S]GTP (guanosine 5’-β-thio-triphosphate), 3 μM unlabeled GDP instead of [³H]GDP was used in the loading buffer and 5 μM [³⁵S]GTP instead of unlabeled GTP in the exchange assay buffer. Bound and free nucleotides were separated by filtration with nitrocellulose.

Transient Co-expression and Image Analysis of Arabidopsis Protoplast Cells—To confirm the subcellular co-localization and interaction between PIRF1 and phytochromes, we adopted a BiFC analysis using Arabidopsis protoplast cells. The cDNAs of phytochrome A and B were individually subcloned into BiFC vector (33) containing the N-terminal region of yellow fluorescent protein (YFP) and designated NE-PHYA and NE-PHYB, respectively. The cDNA of PIRF1 was also cloned into the BiFC vector containing the C-terminal region of YFP to yield CE-PIRF1. Protoplast preparations and transient transformations were performed according to the methods described by Yoo et al. (34). Protoplasts were isolated by incubating 3-week-old Arabidopsis leaves in enzyme solution containing cellulase and macerozyme, and the transformation of protoplasts was achieved by incubating plasmid DNA and protoplast in polyethylene glycol-calcium solution (40% PEG 4000, 0.2 M mannitol, 100 mM CaCl₂). Protoplasts co-transformed with CE-PIRF1 and NE-PHYA and NE-PHYB were incubated in darkness for 14 h, and the fluorescence emissions were measured. To examine the effects of light quality on the co-localization of PIRF1 and phytochromes, the transformed protoplasts were incubated in darkness for 14 h and kept in darkness or exposed subsequently to red or far-red light for 5 min and kept a further 30 min in darkness. Confocal imaging was then carried out using a LSM 510 META laser scanning microscope (Carl Zeiss, Thornwood, NY). Image analysis was carried out with LSC Image 5 Examination software (Carl Zeiss).

RESULTS
PIRF1 Is a Member of the RopGEF Family—In our previous study, we used the co-immunoprecipitation matrix-assisted laser desorption ionization time-of-flight/mass spectrometry
PIRF1 Interacts with N-terminal Region of Phytochromes—
To confirm the interaction between PIRF1 and phytochromes, we generated a recombinant GST-PIRF1 protein and performed in vitro pulldown assays with several recombinant oat phyAs. Full-length oat phytochrome A interacted with GST-PIRF1. In addition the N-terminal region (AN) of phyA was sufficient for the interaction with PIRF1 (see supplemental Fig. S2). The specific interactions between phytochromes and PIRF1 were further confirmed by yeast two-hybrid assays, showing that both phytochromes A and B interacted with PIRF1 through their N-terminal but not through C-terminal domains (Fig. 1A and supplemental Fig. S3). To further establish the interaction between PIRF1 and phytochromes in vivo, we generated myc-tagged PIRF1-overexpressing transgenic plants (PIRF1-OX). The myc-PIRF1 proteins were immunoprecipitated by myc antibodies from the crude protein extracts of PIRF1-OX seedlings. Western blotting analysis revealed that PIRF1 interacted with phyA and phyB in PIRF1-OX seedlings (Fig. 1, B and C).

Arabidopsis—RopGEF family proteins contain a conserved central PRONE domain with the variable N- and C-terminal regions. Their GEF activity toward ROPs reside in the PRONE domain (27). The variable regions of RopGEFs, however, are responsible for the regulation of PRONE function of RopGEF proteins (36). To better understand the structural requirement for the PIRF1 and phytochrome interactions, we generated various truncated GST-PIRF1 proteins and performed in vitro pulldown assays (Fig. 2). Phytochrome A bound with full length and PRONE domain-containing (NP and PD) recombinant PIRF1 proteins. However, neither the N terminus (NE) nor C terminus (CE) alone interacted with phytochrome A. These results indicate that the N-terminal domain of the phytochrome A and the PRONE domain of PIRF1 provide docking sites for PIRF1-phytochrome interactions.

PIRF1 Interacts with and Activates ROPs—In plants, ROPs are involved in diverse developmental processes through their activation by RopGEFs (22, 26). We examined the interaction and activation of PIRF1 over ROPs using in vitro pulldown assay and GDP/GTP exchange assay to verify the activity of PIRF1 as a functional RopGEF. Three typical ROPs (ROP2, ROP6, and ROP8) were selected from 11 ROPs for in vitro pulldown assay. Results revealed that PIRF1 directly interacted with three ROPs (Fig. 3A). We then tested the GEF activity of PIRF1 with one of the ROPs tested. The dissociation of [3H]GDP from MBP-ROP8 was accelerated with the addition of increasing concentrations of PIRF1 (Fig. 3B). The rates of both [3H]GDP dissociation and [35S]GTP binding to MBP-ROP8 were markedly enhanced by PIRF1 (Fig. 3C and supplemental Fig. S4). These results suggest that PIRF1 plays a functional role as a RopGEF protein in plants.

RopGEF Activity of PIRF1 Was Enhanced by a Pr-phytochrome A—ROP signaling is modulated by an interacting RopGEF proteins through regulation of their GEF activities. In tomato, pollen-specific receptor kinases LePRK1 and -2 interact with a tomato RopGEF homolog, LeKPP, and regulate its GEF activity (36, 37). Because PIRF1 interacts with phytochromes and ROPs, we tested whether phytochromes have any...
effect on PIRF1 activity. Surprisingly, PIRF1 activity was enhanced highly by the Pr form of phytochrome A, whereas the Pfr form decreased the activity (Fig. 4). These results suggest that the Pr form of phytochromes modulates ROP signaling by controlling the GEF activity of PIRF1 in Arabidopsis.

PIRF1 Interacts with Phytochromes in Cytoplasm—Another surprising finding in our study was that the interaction between PIRF1 and phytochromes occurred in the cytoplasm. The subcellular translocation of signaling molecules including photoreceptors plays a crucial role in the regulation of gene expression and protein degradation in plant photomorphogenesis (38). Light induces the translocation of phytochromes from the cytoplasm to the nucleus, where gene expression is directly modulated to control many developmental processes in plants (8, 9). Thus, many phytochrome signaling proteins such as PIF3 and PIF-like (PIL) are localized and interact with phytochromes in the nucleus (39, 40).

To explore the functional role of PIRF1, subcellular localization was analyzed using green fluorescent protein-tagged PIRF1 protein in Arabidopsis protoplast cells. PIRF1 was found to be localized in the cytoplasm of protoplast cells regardless of light exposure (see supplemental Fig. S5). To assess the possibility of the cytoplasmic interaction between PIRF1 and phytochromes at different light conditions, we used the BiFC method by co-transformation of PIRF1 (CE-PIRF1) and either phyA (NE-PHYA) or phyB (NE-PHYB). The interaction between PIRF1 and phytochromes in the transformed cells was observed in the dark (Fig. 5A) but absent in red light exposure (Fig. 5B), whereas the interaction between PIF3 and phytochromes in the nucleus was activated by the same light treatment (see supplemental Fig. S6). To confirm that PIRF1 interacts with phytochrome in cytoplasm, we monitored the changes of fluorescent cells as well as fluorescence transition from the cytoplasm to the nucleus by light illuminating the protoplast cells. Protoplasts were co-transformed with the NE-PHYA and CE-
PIRF1, CE-PIF3, or CE-PHYA. As expected, the dimerized phytochromes, NE-phyA and CE-phyA, were translocated from the cytoplasm to the nucleus with a red light pulse where the interaction was still maintained. In addition, translocated NE-phyA interacted with CE-PIF3 in the nucleus. However, NE-phyA and CE-PIRF1 interaction in the cytoplasm was markedly decreased by red light pulses (see supplemental Fig. S7). Therefore, PIRF1 is localized in the cytoplasm, where it probably binds the Pr form of phytochrome in the dark, whereas this association is abrogated by the red light induced translocation of phytochromes into the nucleus. Our results suggest that PIRF1 as a phytochrome partner protein may play a role in the cytoplasm.

**PIRF1 Negatively Regulates Root Development**—Having isolated and characterized the phytochrome-interacting protein PIRF1, we searched for its function in plant photomorphogen...
Light signaling (3, 6) as well as ROP signaling (19, 21) plays important roles in root development, including primary root elongation and root hair formation. First, we attempted to establish the phenotypic functional relationship between PIRF1 and phytochromes by generating T-DNA insertion knock-out (pirf1) (Salk_126725) (Fig. 8A) and overexpression mutants (PIRF1-OX) of A. thaliana. We analyzed the de-etiolation responses of these mutant seedlings, including hypocotyl growth, cotyledon opening, and greening under different light conditions. We could not observe any distinct difference in phenotype in pirf1 during early seedling development, but PIRF1-OX displayed inhibited root growth. We then looked at root phenotypes in these mutant seedlings under different light conditions. Primary roots of seedling in wild type plants were markedly elongated under white light, but the growth was retarded in the dark (seesupplemental Fig. S9). However, primary root elongation was markedly lower in PIRF1-OX seedlings under continuous white, red, and far-red lights than wild type and pirf1 seedlings (seesupplemental Fig. S9). The elongation of primary roots in the phytochrome null mutants, phyA-211 and phyB-9, also was inhibited under both continuous red (Rc) or far-red (FRc) light conditions suggesting the involvement of both phytochromes A and B (Fig. 8, B and C, and supplemental Fig. S9). In addition, pirf1 and phytochrome B null mutants showed elongated root growth in the dark. Inhibition of primary root growth by PIRF1 overexpression, and elongation of root in the pirf1 and phytochrome B null mutants in the dark, strongly suggest PIRF1 as a negative regulator by modulating its ROPs activity. This suggestion is consistent with the primary root elongation in transgenic ROP overexpressors (41). The lack of a distinct root growth phenotype in the pirf1 mutant is probably due to a functional redundancy among the RopGEF family members, similar to that among ROP families (42). On the other hand, the primary root lengths in PIRF1-OX and phy mutants were shorter than those of the wild type plants at fluence rates >0.1 mol−2 s−1, which suggest that PIRF1 func-
tions under high red or far-red light intensities (see supplemental Fig. S10).

Phytochrome null mutants showed defective root hairs in seedlings under light conditions (1). We also observed similar deficiency in root hair formation in phytochrome null mutants (phyA-211 and phyB-9) under continuous red or far-red light. A phenotype analysis of PIRF1-OX plants revealed that the over-expression of PIRF1 repressed a root hair formation (see supplemental Fig. S11). The similarity of root phenotypes in phytochrome null and PIRF1-OX mutants suggests that PIRF1 and phytochromes are on the same signaling pathway for the root development. In particular, we observed that the polarity of the root hairs also was disturbed markedly in the PIRF1-OX plants compared with the wild type, as was the case with phytochrome B null mutant. Relevantly, the disruption of root tip polarity also has been observed in transgenic ROP plants (19, 21, 41). Our results suggest that phytochromes and ROPs are required for root hair development and that PIRF1 serves as a signal connector between phytochrome and ROPs.

DISCUSSION

PIRF1 Is a Cytoplasmic Partner Protein of Pr-phytochrome—A number of potential signaling components that are specific to an individual phytochrome response appear to mostly interact with the C-terminal region of the phytochromes (7, 8, 38, 40, 43). However, the phytochrome N-terminal region has a regulatory function implicating that the N-terminal region could be a signaling domain for the photomorphogenesis in higher plants (9, 10, 44, 45). For instance, PAPP2C was found to interact with an N-terminal motif of the phytochromes in Arabidopsis and regulates the de-etiolation responses of seedlings by regulating the dephosphorylation of PIF3 (46). In the present study, we also demonstrate that PIRF1 functions as a partner protein through its interaction with the N-terminal regions of phytochromes (Fig. 1 and supplemental Figs. S2 and S3). Unlike most other phytochrome-interacting proteins, PIRF1 interacted specifically with an N-terminal domain of both phyA and phyB.

Phytochromes reside predominantly in the cytoplasm in dark grown seedlings and are translocated into the nucleus upon light irradiation to trigger diverse light responses (47, 48). Nuclear localization of phyA and phyB is a pivotal event to elicit the signaling pathway leading to photomorphogenesis in Arabidopsis (47-49). It also appears that cytoplasmic phytochromes play a role in phototropism and root development of plant seedlings even when the nuclear translocation of phyA or B was blocked (50, 51). Our findings show that PIRF1 localized and interacted with Pr-phytochromes in the cytoplasm in the dark, and it remained there even after Pr was converted to Pfr upon light exposure (Fig. 5 and supplemental Figs. S5 and S7).

PIRF1-Pr Complex Acts as a “Signal Switch” of ROP Signaling in Root Development—In most photomorphogenic events in higher plants, the Pfr form of phytochrome is the functionally active form (or “switch on” form) with the Pr form being silent (“switch off” form). Occasionally, the Pr form has been suggested as an active form (52, 53). Shinomura et al. (54) suggested the possibility that a Pr form (re-formed from the Pfr form) can play a role in the red/far-red pulse effects on the inhibition of hypocotyl elongation in seedling. Correll and Kiss (4) also suggested that root-localized phytochromes are able to regulate the root growth response to light through the inactive red-absorbing form (Pr) of phytochrome. Kang et al. (55) showed the induction of a small G protein and a brassinosteroid C2 hydroxylase (a cytochrome P450) in the dark grown Arabidopsis seedlings, implicating the possible role of the Pr form. Recent studies also have suggested that the cytosolic Pr form of phytochromes has a biological function during plant development (16, 50, 51). In addition, our results showed that the RopGEF activity of PIRF1 was enhanced by the Pr form of phytochromes but inhibited by the Pfr form (Fig. 4). Also, the Pr form of phytochromes interacted predominantly with the complexes of PIRF1 and ROP8 in vitro (Fig. 6), whereas no preference between Pr and Pfr forms was observed in the interaction of PIRF1 with phytochromes when ROP is excluded (see supplemental Fig. S8). Our results suggest that the Pr-phytochrome is nonsilent in the cytoplasm.

Phytochromes have been known to present abundantly in roots and root tip through spectrophotometric and immunocytochemical studies (56). Somer and Quail (31) also demonstrated that phytochrome genes were expressed highly in root through promoter analysis using GUS-tagging phytochromes. Various studies also showed that phytochromes are involved in various root development including root hair development (11), lateral root formation (5, 6), and tropism responses (3, 13). It was particularly interesting that light was able to penetrate through the interior of the stem and was conducted toward the roots (50). Although phyB and red light play a role in root development, no nuclear phyB-green fluorescent protein was detected in root cells that were 1 cm below the soil line (16). These findings suggest that light signals can directly propagate to the root system and trigger phytochrome activation through vascular conductance, but phyB activation for nuclear movement was not achieved via weak light signals. Salisbury et al. (16) proposed the possibility that cytosolic-localized Pr plays a role in controlling several responses during root development. This possibility was coincided with our results that Pr form of phytochrome might be involved in root development through interaction with PIRF1 and ROP in cytoplasm.

The previous reports (3, 4, 13, 16) and the results obtained from the present study strongly indicate that the phytochromes are able to detect light directly and play an important role in plant root development. Our results showed that the impairment of phytochrome signaling results in abnormalities in both root growth and root hair formation (Fig. 8 and supplemental Fig. S11). This observation is consistent with the report that phytochromes mediate the root development through regulating the expression of auxin response genes and auxin efflux genes (16); for example, hy5 mutation affects root development by regulating specific auxin-responsive genes (5, 6). Tian et al. (57) also showed that phyB regulates AUX/IAA activity by directly interacting with SHY2/IAA3. These observations are consistent with the phytochrome-mediated modulation of intercellular signalings, such as auxin transport and distribution, in controlling root development (16, 53). In addition, involvement of ROPs in auxin signaling is well known (26, 35). ROPs stimulate auxin-responsive gene expression (58) and
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functions as novel regulators for the proteolytic degradation of AUX/IAA, negative transcription regulator of auxin response gene expression (59). Also, our PIRF1-OX plants displayed abnormal root phenotypes similar to those of transgenic constitutively active ROP (CA-ROP) overexpressors (19, 21, 41), suggesting that root development is negatively regulated by ROPs. To probe the roles of PIRF1 in auxin-responsive gene expression, we examined the expression of several auxin response genes (IAA1, IAA3, and ARE) and efflux genes (PIN3, PIN4, and PIN7) in pirf1 and PIRF1-OX mutants. However, no variation in the gene expression of them was observed in those mutants (data not shown), suggesting that PIRF1 was not directly involved in the regulation of auxin-responsive gene expression.

As shown in our results (Fig. 8 and supplemental Fig. S10), phytochrome null mutants displayed severely retarded growth under red and far-red light. Overexpression of cry1 significantly increased the root elongation, whereas the knock-out mutant also showed reduced growth (60). Blue light signal through cry1 for root growth is transmitted via the auxin-signaling pathway. Phototropin also enhanced root growth efficiency and increased plant size and maturity (61). Therefore, root growth is the result of various light-signaling responses through different photoreceptors. In the present study, we found that the Pr-phytochromes interact with PIRF1 to modulate RopGEF activity in the cytoplasm, which possibly influences the root growth through the regulation by ROPs. These results establish the possible link between light and ROP signals in root growth and development. Thus, the Pr-PIRF1 pair appears to serve as a signaling switch for root growth inhibition. However, this switch is not the only switch for root growth and development because the knock-out mutants of phytochromes and pirf1 exhibited only minor effects in the dark. Nonetheless, the retardation of root growth in the PIRF1 overexpression mutant under light conditions strongly implicates a possible connection between phytochromes and ROPs via PIRF1 during root growth and development. Further studies are warranted to establish the mechanism of how phytochromes/light, PIRF1, and ROPs play a signaling role in root growth and development.

REFERENCES

1. De Simone, S., Oka, Y., and Inoue, Y. (2000) J. Plant Res. 113, 63–69
2. Kiss, J. Z., Miller, K. M., Ogden, L. A., and Roth, K. K. (2002) Plant Cell Physiol. 43, 35–43
3. Kiss, J. Z., Mullin, J. L., Correll, M. J., and Hangarter, R. P. (2003) Plant Physiol. 131, 1411–1417
4. Correll, M. J., and Kiss, J. Z. (2005) Plant Cell Physiol. 46, 317–323
5. Cluis, C. P., Mouchel, C. F., and Hardtke, C. S. (2004) Plant J. 38, 332–347
6. Sibout, R., Sukumar, P., Hettiaratchi, C., Holm, M., Muday, G. K., and Hardtke, C. S. (2006) PLoS Genet. 2, 1898–1911
7. Neff, M. M., Fankhauser, C., and Chory, J. (2000) Genes Dev. 14, 257–271
8. Quail, P. H. (2002) Nat. Rev. Mol. Cell Biol. 3, 85–93
9. Huq, E., and Quail, P. H. (2005) in Handbook of Photoreceptor Receptors. (Briggs, W. R., and Spudich, J. L., eds) pp. 151–170, Wiley-VCH Verlag GmbH, Weinheim, Germany
10. Kim, J. L., and Song, P. S. (2005) in Light Sensing in Plants (Wada, M., Shimazaki, K.I., and lino, M., eds) pp. 57–67, Springer-Verlag, Tokyo
11. Reed, J. W., Nagpal, P., Poole, D. S., Furuya, M., and Chory, J. (1993) Plant Cell 5, 147–157
12. Oyama, T., Shimura, Y., and Okada, K. (1997) Genes Dev. 11, 2983–2995
13. Ruppel, N. J., Hangarter, R. P., and Kiss, J. Z. (2001) Planta 212, 424–430
14. Hemm, M. R., Rider, S. D., Ogas, J., Murr, D. J., and Chapple, C. (2004) Plant J. 38, 765–778
15. Lin, R., and Wang, H. (2005) Plant Physiol. 138, 949–964
16. Salisbury, F. J., Hall, A., Griersen, C. S., and Halliday, K. J. (2007) Plant J. 50, 429–438
17. Boccalandro, H. E., De Simone, S. N., Bergmann-Honsberger, A., Schepens, I., Fankhauser, C., and Casal, J. J. (2008) Plant Physiol. 146, 108–115
18. Phee, B. K., Shin, D. H., Cho, J. H., Kim, S. H., Kim, J. I., Lee, Y. H., Jeon, J. S., Bho, S. H., and Hahn, T. R. (2006) Proteomics 6, 3671–3680
19. Li, H., Shen, J. J., Zheng, Z. L., Lin, Y., and Yang, Z. (2001) Plant Physiol. 126, 670–684
20. Jones, M. A., Shen, J. J., Fu, Y., Li, H., Yang, Z., and Grierson, C. S. (2002) Plant Cell 14, 763–776
21. Zheng, Z. L., Nafisi, M., Tam, A., Li, H., Crowell, D. N., Chary, S. N., Schroeder, J. I., Shen, J. J., and Yang, Z. (2003) Plant Cell 14, 2787–2797
22. Niibau, C., Wu, H. M., and Cheung, A. Y. (2006) Trends Plant Sci. 11, 309–315
23. Yang, Z., and Fu, Y. (2007) Curr. Opin. Plant Biol. 10, 490–494
24. Kost, B. (2008) Trends Cell Biol. 18, 119–127
25. Ishida, T., Kurata, T., Okada, K., and Wada, T. (2008) Annu. Rev. Plant Biol. 59, 365–386
26. Gu, Y., Wang, Z, and Yang, Z. (2004) Curr. Opin. Plant Biol. 7, 527–536
27. Gu, Y., Li, S., Lord, E. M., and Yang, Z. (2006) Plant Cell 18, 366–381
28. Shin, D. H., Kim, T. L., Kwon, Y. K., Cho, M. H., Yoo, J., Jeon, J. S., Hahn, T. R., and Bho, S. H. (2009) Plant Biotechnol. Rep. 3, 183–190
29. Shen, Y., Kim, J. I., and Song, P. S. (2005) J. Biol. Chem. 280, 5740–5749
30. Shimomura, T., Nagatani, A., Hanzawa, K., Kubota, M., Watanabe, M., and Furuya, M. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 8129–8133
31. Somers, D. E., and Quail, P. H. (1995) Plant J. 7, 413–427
32. Zheng, Y., Hart, M. J., and Cerione, R. A. (1995) Methods Enzymol. 256, 77–84
33. Bracha-Drori, K., Shichrur, K., Katz, A., Oliva, M., Angelovic, R., Volovsky, S., and Ohad, N. (2004) Plant J. 40, 419–427
34. Yoo, S. D., Cho, Y. H., and Sheen, J. (2007) Nat. Protoc. 2, 1565–1572
35. Berken, A. (2006) Cell Mol. Life Sci. 63, 2446–2459
36. Zhang, Y., and McCormick, S. (2007) Proc. Natl. Acad. Sci. U.S.A. 104, 18830–18835
37. Koo, P., Rhee, C.-H., Park, S., and Sheen, J. (2001) Curr. Opin. Plant Biol. 4, 182–189
38. Koo, P., Rhee, C.-H., Park, S., and Sheen, J. (2001) Curr. Opin. Plant Biol. 4, 182–189
39. Koo, P., Rhee, C.-H., Park, S., and Sheen, J. (2001) Curr. Opin. Plant Biol. 4, 182–189
40. Koo, P., Rhee, C.-H., Park, S., and Sheen, J. (2001) Curr. Opin. Plant Biol. 4, 182–189
55. Kang, J. G., Yun, J., Kim, D. H., Chung, K. S., Fujioka, S., Kim, J. I., Dae, H. W., Yoshida, S., Takatsu, S., Song, P. S., and Park, C. M. (2001) Cell 105, 625–636
56. Pratt, L. H. (1994) in Photomorphogenesis in Plants. 2nd Ed. (Kendrick, R. E and Kronenberg, G. H., eds) pp. 163–185, Kluwer Press, Dordrecht, Netherlands
57. Tian, Q., Nagpal, P., and Reed, J. W. (2003) Plant J. 36, 643–651
58. Tao, L. Z., Cheung, A. Y., and Wu, H. M. (2002) Plant Cell 14, 2745–2760
59. Tao, L. Z., Cheung, A. Y., Nibau, C., and Wu, H. M. (2005) Plant Cell 17, 2369–2383
60. Canamero, R. C., Bakrim, N., Bouly, J. P., Garay, A., Dudkin, E. E., Habricot, Y., and Ahmad, M. (2006) Planta 224, 995–1003
61. Galen, C., Rabenold, J. J., and Liscum, E. (2007) New Phytol. 173, 91–99