A Competitive Peptide Inhibitor KIDARI Negatively Regulates HFR1 by Forming Nonfunctional Heterodimers in *Arabidopsis* Photomorphogenesis

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Dynamic dimer formation is an elaborate means of modulating transcription factor activities in diverse cellular processes. The basic helix-loop-helix (bHLH) transcription factor LONG HYPOCOTYL IN FAR-RED 1 (HFR1), for example, plays a role in plant photomorphogenesis by forming non-DNA binding heterodimers with PHYTOCHROME-INTERACTING FACTORS (PIFs). Recent studies have shown that a small HLH protein KIDARI (KDR) negatively regulates the HFR1 activity in the process. However, molecular mechanisms underlying the KDR control of the HFR1 activity are unknown. Here, we demonstrate that KDR attenuates the HFR1 activity by competitively forming nonfunctional heterodimers, causing liberation of PIF4 from the transcriptionally inactive HFR1-PIF4 complex. Accordingly, the photomorphogenic hypocotyl growth of the *HFR1*-overexpres-sing plants can be suppressed by KDR coexpression, as observed in the *HFR1*-deficient *hfr1-201* mutant. These results indicate that the PIF4 activity is modulated through a double layer of competitive inhibition by HFR1 and KDR, which could in turn ensure fine-tuning of the PIF4 activity under fluctuating light conditions.

INTRODUCTION

Light is the most prominent environmental factor that influences plant physiology and development throughout the life cycle. It is the sole energy source for photosynthesis and acts as a signaling cue to regulate diverse aspects of plant growth and developmental processes, such as seed germination, hypocotyl growth, tropic responses, shade avoidance response, root development, and floral transition (Deng and Quail, 1999; Kami et al., 2010).

Light signals are perceived by multiple photoreceptors in plants. The photoreceptors are classified into 5 major families according to their protein structures and light wavelengths that they perceive: phytochromes (phys), cryptochromes (crys), phototropins (phots), UV RESISTANCE LOCUS 8 (UVR8), and ZEI-TLUPE (ZTL)/FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1)/LOV KELCH PROTEIN 2 (LKP2) F-box proteins (Kami et al., 2010). Whereas phys (phyA to phyE) are responsible for perception of red and far-red lights, crys (cry1 and cry2), phots (Phot1 and Phot2), and ZTL/FKF1/LKP2 respond to blue and UV-A lights (Briggs and Olney, 2001; Chen et al., 2004; Demarsy and Fankhauser, 2009; Lin, 2002). The UVR8 protein has recently been characterized as an UV-B receptor (Christie et al., 2012; Kai-serli and Jenkins, 2007). Upon stimulation by diverse light wavelengths, responsible photoreceptors are activated through conformational changes and regulate downstream events by modulating activities of diverse transcription factors (Kevei et al., 2007; Kimura and Kagawa, 2006; Liu et al., 2011; Quail, 2000). Therefore, understanding gene expression regulation is critical for studies on light signal transduction path-ways.

Activities of transcription factors are regulated at multiple levels: transcription, translation, posttranscriptional RNA metabolism, and posttranslational modification. Dynamic formation of homodimers and heterodimers also plays an important role in regulating transcription factor activities by modulating their functional specificities and diversities (Baxevanis and Vinson, 1993; Izawa et al., 1993; Vinson et al., 1993). Dimer formation is also involved in dominant-negative regulation of transcription factors. A distinct regulatory mechanism, which involves small interfering peptides (siPEPs), has recently been established as a way of inhibiting transcription factor activities (Seo et al., 2011). The siPEPs possess protein-protein interaction domains but lack other functional domains, such as transcriptional regulation domains and/or DNA-binding domains. Although they have no biochemical activities per se., the siPEPs are able to competitively form heterodimers with target transcription factors and efficiently attenuate their activities (Seo et al., 2011).

An intriguing example of competitive inhibition of transcription factors is HFR1 regulation of PIF4 and PIF5, both of which are

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bHLH transcription factors involved in hypocotyl elongation and shade avoidance response (Hornitschek et al., 2009). Upon exposure to lights, the phy photoreceptors are translocated into the nucleus, where they interact with PIF transcription factors (Kevei et al., 2007) and induce PIF degradation (Quail, 2000). In contrast, the PIFs are stabilized in the shade and function during the shade avoidance response. HFR1, an atypical bHLH transcription factor consisting of 292 residues, is also induced in the shade. HFR1 inhibits the PIF4 activity by forming non-DNA-binding heterodimers (Hornitschek et al., 2009), which signifies a fine-tuning mechanism of shade avoidance response in plants.

Another atypical HLH protein, KDR, consists of 94 residues and has been identified as a negative regulator of HFR1 (Hyun and Lee, 2006). It has been suggested that KDR might interfere with the HFR1 function possibly through physical interactions. This current study provides not only biochemical evidence supporting that KDR inhibits the HFR1 function by competitively forming non-functional heterodimers and thus derepresses the PIF4 activity but also provides a versatile scheme for the transcriptional control of plant photomorphogenesis.

MATERIALS AND METHODS

Plant materials and growth conditions
All *Arabidopsis thaliana* lines used were in Columbia background (Col-0). Plants were grown in a controlled culture room set at 22°C with a relative humidity of 55% under long day conditions (LDs, 16-h light/8-h dark). White light illumination (120 μmol photons/m^{2}sec) was provided by fluorescent FLR40D/A tubes (Osram, Korea).

To produce transgenic plants overexpressing either *KDR* or *HFR1* gene, full-size cDNAs were subcloned into the binary pB2GW7 vector under the control of the Cauliflower Mosaic Virus (CaMV) 35S promoter (Invitrogen, USA). *Agrobacterium*-mediated transformation of *Arabidopsis* plants was performed according to a modified floral dip method (Clough and Bent, 1998). The activation-tagged *kdr-D* mutant has been described previously (Hyun and Lee, 2006). The *hfr1-201* loss-of-function mutant was isolated from an *Arabidopsis* mutant pool of T-DNA insertion lines deposited in the *Arabidopsis* Biological Resource Center (ABRC, Ohio State University). Genetic crosses were carried out as previously described (Mayer et al., 1993).

For treatments with different light wavelengths, seedlings were grown in darkness or under red, far-red, or blue light (12, 8, and 15 μmol m^{-2}s^{-1}, respectively) in a VS-940L-DUAL incubator (Vision, Korea) equipped with red, far-red, or blue light-emitting diodes. For each treatment, hypocotyl lengths of 30 seedlings grown for 5 days were measured and averaged. Statistical significance of the measurements was determined using the Student's *t*-test or one-way ANOVA with Fisher's *post hoc* test.

Coimmunoprecipitation (Co-IP) assays
A MYC-coding sequence was fused in-frame to the 5′ end of the *HFR1* gene, and the gene fusion was subcloned under the CaMV 35S promoter in the modified pBA002 vector (Kim et al., 2006). The expression construct was transformed into Col-0 plants. A green fluorescent protein (GFP)-coding sequence was fused in-frame to the 3′ end of the *KDR* gene, and the *KDR-GFP* gene fusion was overexpressed under the control of the CaMV 35S promoter in *MYC*-ox and *MYC-HFR1*-ox transgenic plants. Total protein extracts were prepared from 2-week-old *MYC*-ox X *KDR-GFP*-ox and *MYC-HFR1*-ox X *KDR-GFP*-ox plants grown on MS-agar plates at 22°C under LDs. The total

protein extracts were mixed with an anti-MYC antibody coupled to agarose beads (Millipore, USA) in extraction buffer (20 mM Tris, pH 7.4, 100 mM NaCl, 0.5% Nonidet P-40, 0.5 mM EDTA, 0.5 mM PMSF, and protease inhibitor cocktail) and incubated for 2 h at 4°C. The beads were recovered by centrifugation (5,000 × *g*, 4°C, 1 min) and washed five times with fresh extraction buffer, each time for 1 min. The bound proteins were eluted with 1× SDS-PAGE loading buffer by boiling for 5 min and subject to SDS-PAGE. Immunological analysis was performed using anti-GFP and anti-MYC antibodies.

Yeast two-hybrid assays
Yeast two-hybrid assays were carried out using the BD Matchmaker system (Clontech, USA). The pGADT7 vector was used for GAL4 AD (activation domain) fusion, and the pGBKT7 vector was used for GAL4 BD (DNA-binding domain) fusion. Yeast strain AH109, which has chromosomally integrated reporter genes *lacZ* and *HIS* under the control of the GAL1 promoter, was used for transformation. The *KDR* and *HFR1* cDNA sequences were subcloned into the pGBKT7 and pGADT7 vectors. Transformation of vector constructs into AH109 cells was performed according to the manufacturer's instruction. Colonies obtained were streaked on selective medium without histidine (His), adenine (Ade), leucine (Leu), and tryptophan (Trp).

The pBridge vector (Clontech) was used for yeast three-hybrid screening. The *HFR1*, *PIF4*, and *KDR* cDNAs amplified by RT-PCR were subcloned into the pBridge vector. The *KDR* cDNA was subcloned into the NotI-digested pBridge vector so that its expression was controlled by the methionine-repressible pMET25 promoter. The expression constructs were cotransformed into AH109 cells. The colonies were streaked on media without Leu, Trp, and His in the presence or absence of methionine.

Subcellular localization
For detection by fluorescence microscopy, the GFP-coding sequence was fused in-frame to the 5' ends of the *KDR*, *HFR1*, *PIF3*, *PIF4*, and *PIF5* genes in the pB7FWG2 vector. The expression constructs were transformed into *Arabidopsis* protoplasts by polyethylene glycol-calcium transfection (Yoo et al., 2007). The subcellular distribution of the proteins was visualized by differential interference contrast microscopy (DIC) and fluorescence microscopy. The GFP fusion proteins were excited at 488 nm, and the green and red fluorescence signals were filtered with HQ515/30. The autofluorescence of the chlorophylls was excited at 568 nm and emitted with the E600LP filter. The merged signals were obtained using a Confocal Assistant 4.02 (Todd Clark Brelje, Freeware).

Bimolecular fluorescence complementation (BiFC) assays
BiFC assays were carried out as described previously (Hong et al., 2011). The *PIF4* cDNA was fused in-frame to the 3′ end of a gene sequence encoding the C-terminal half of an enhanced yellow fluorescent protein (EYFP) in the pSATN-cEYFP-C1 vector (E3082). The *HFR1* cDNA was fused in-frame to the 3′ end of a gene sequence encoding the N-terminal half of EYFP in the pSATN-nEYFP-C1 vector (E3081). The nYFP-HFR1 and cYFP-PIF4 vectors were cotransformed into *Arabidopsis* mesophyll protoplasts by polyethylene glycol-calcium transfection (Yoo et al., 2007). At 16 h after transfection, reconstitution of YFP fluorescence was monitored by fluorescence microscopy using a Zeiss LSM510 confocal microscope (Carl Zeiss, Germany) with the following YFP filter set up: excitation 515 nm, 458/514 dichroic, and emission 560-615 BP filter.
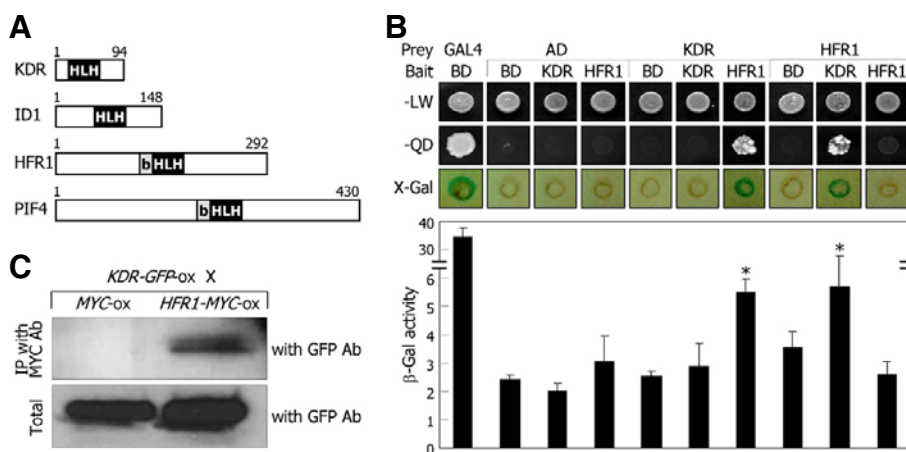
Fig. 1. Interaction of KDR with HFR1. (A) Protein domain structures of KDR and bHLH transcription factors. bHLH, basic helix-loop-helix motif. Numbers indicate residue positions. KDR lacks the basic region required for DNA binding. (B, C) Interactions of KDR and HFR1 in yeast cells (B) and *in planta* (C). In (B), growth of yeast transformants on selective media lacking Leu, Trp, His, and Ade (-QD) and the blue-colored product in the presence of X-gal indicate positive interactions (upper panel). -LW indicates Leu and Trp drop-out plates. AD and BD, GAL4 activation and DNA-binding domains, respectively. Three independent measurements of β-Gal activities were averaged and statistically treated using the Student's *t*-test (*P < 0.01) (lower panel). Bars indicate standard error of the mean. In (C), total protein extracts were prepared from 2-week-old whole plants grown on MS-agar plates at 23°C under LDs. They were then immunoprecipitated (IP) using an anti-MYC antibody coupled to agarose beads and subject to Western blot assays using anti-MYC (upper panel) and anti-GFP (middle panel) antibodies. Total protein extracts were also subject to Western blot assays using an anti-GFP antibody (lower panel).

Transcriptional activation activity assays

For transient expression in *Arabidopsis* protoplasts, several reporter and effector plasmids were constructed. The reporter plasmid contains 4 copies of the GAL4 upstream activation sequence (UAS) and the β-glucuronidase gene (GUS). To construct the p35S:KDR/HFR1/PIF4 effector plasmids, the genes were fused to the GAL4 DNA-binding domain and inserted into an expression vector containing the CaMV 35S promoter. The reporter and effector plasmids were cotransformed into *Arabidopsis* protoplasts by polyethylene glycol-mediated transformation method (Yoo et al., 2007). GUS activities were measured by the fluorometric method as described previously (Jefferson et al., 1987). A CaMV 35S promoter-luciferase (LUC) construct was also cotransformed as an internal control. The luciferase assay was carried out using the Luciferase Assay System Kit (Promega, USA).

RESULTS

KDR interacts with HFR1

bHLH transcription factors achieve regulatory specificities and diversities by forming extensive sets of homo- and heterodimers (Littlewood and Evan, 1995). HFR1 and PIFs are representative bHLH transcription factors involved in light signaling (Fig. 1A). HFR1 has been shown to interact with PIFs to form comprehensive interaction networks (Hornitschek et al., 2009). A small HLH protein KDR shares limited sequence homology with HFR1 (Hyun and Lee, 2006). We were interested in investigating how KDR regulates HFR1 and PIFs in plant photomorphogenesis.

Cell growth assays on selective media and β-galactosidase (β-Gal) activity assays both indicated that KDR interacts with HFR1 in yeast cells (Fig. 1B). Co-IP assays were also carried out to examine whether the KDR-HFR1 interaction occurs in *planta*. The *KDR-GFP* and *HFR1-MYC* gene fusions were transformed into Col-0 plants, resulting in the *KDR-GFP*-ox and *HFR1-MYC*-ox transgenic plants. The *KDR-GFP*-ox/*HFR1-MYC*-ox plants were also generated by a genetic cross. The results showed that KDR-GFP proteins can be pulled down with HFR1-

MYC proteins (Fig. 1C), thereby supporting the direct interactions between KDR and HFR1 *in vivo*.

### KDR inhibits nuclear localization of HFR1

HFR1 and PIF transcription factors are primarily localized in the nucleus (Jang et al., 2005; 2010). To investigate the subcellular localization of KDR, a *KDR-GFP* gene fusion, in which a GFP-coding sequence was fused in-frame to the 3′ end of the *KDR* gene, was expressed transiently in *Arabidopsis* protoplasts. KDR was found in both the nucleus and cytoplasm, whereas HFR1 and PIFs were localized predominantly in the nucleus (Fig. 2A).

Based on the subcellular distribution of KDR and its interaction with HFR1, it was proposed that KDR influences the subcellular localization of HFR1. To examine this hypothesis, the *HFR1-GFP* gene fusion along with the *KDR* gene was transiently coexpressed in *Arabidopsis* protoplasts. GFP signals were detected in both the nucleus and cytoplasm (Fig. 2B), indicating that KDR moderately inhibits the nuclear localization of HFR1.

KDR interferes with the formation of HFR1-PIF4 heterodimers

Because HFR1 is known to interact with PIF4 and PIF5 (Hornitschek et al., 2009), the experiments were designed to determine whether KDR affects the HFR1-PIF4 interaction. Yeast three-hybrid assays were used, in which the *KDR* gene was expressed under the control of a methionine-suppressible promoter (pMET25) in yeast cells expressing the BD-PIF4 and AD-HFR1 fusions (Fig. 3A). Cell growth assays on selective media and β-Gal activity assays showed that induction of the *KDR* expression substantially repressed the formation of HFR1-PIF4 heterodimers (Figs. 3B and 3C).

The KDR-mediated competitive inhibition of HFR1-PIF4 heterodimer formation was also examined by BiFC assays. The HFR1-nYFP and PIF4-cYFP expression constructs were transiently expressed in *Arabidopsis* protoplasts. The results showed that HFR1-PIF4 heterodimer formation was considerably suppressed when KDR was present (Fig. 3D), further supporting

A

B
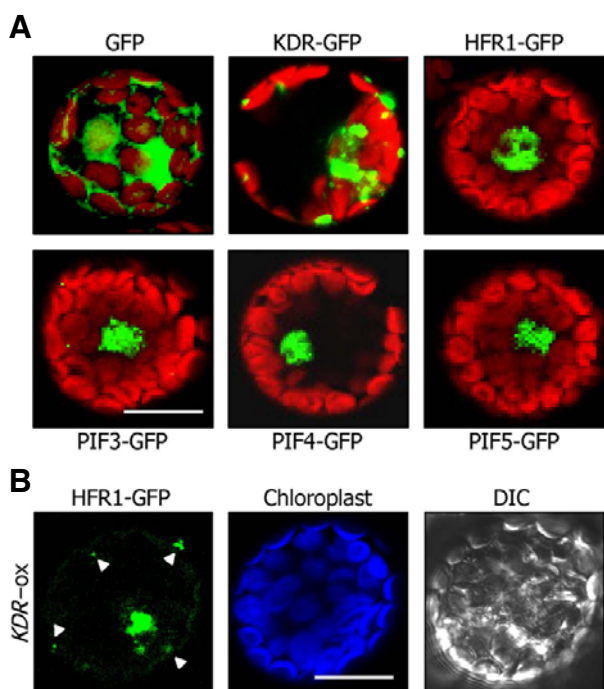
Fig. 2. Subcellular localization of KDR and bHLH transcription factors in *Arabidopsis* protoplasts. (A, B) A GFP-coding sequence was fused in-frame to the 3′ ends of *KDR* and bHLH transcription factor genes. The gene fusions were expressed transiently in *Arabidopsis* protoplasts and subject to fluorescent microscopy (A). In (B), the 35S:*KDR* construct was coexpressed with the 35S:*HFR1-GFP* construct in *Arabidopsis* protoplasts. The photograph is a representative of protoplasts (n > 30) that exhibit similar patterns of fluorescence signals. Arrowheads indicate cytoplasmic localization of HFR1-GFP. Scale bar = 10 μm.

the competitive inhibition of HFR1-PIF4 interaction by KDR.

### KDR modulates PIF4 activity *via* HFR1

HFR1 suppresses the transcriptional regulation activities of PIF4 and PIF5 by forming non-DNA-binding heterodimers (Hornitschek et al., 2009). Since KDR was found to regulate the HFR1-PIF4 interaction, a question was raised as to whether KDR affects the transcriptional regulation activity of PIF4.

To address this, we measured transcriptional regulation activities of PIF4, HFR1, and KDR in *Arabidopsis* protoplasts either with or without HFR1 and KDR coexpression (Fig. 4A). KDR had no discernible transcriptional regulation activity, while GUS reporter activities were increased by expression of either *PIF4* or *HFR1* gene (Fig. 4B). The transcriptional activation activity of PIF4 was more prominent than that of HFR1. Notably, *KDR* coexpression significantly reduced the transcriptional activation activity of HFR1, thereby indicating the negative regulation of HFR1 by KDR.
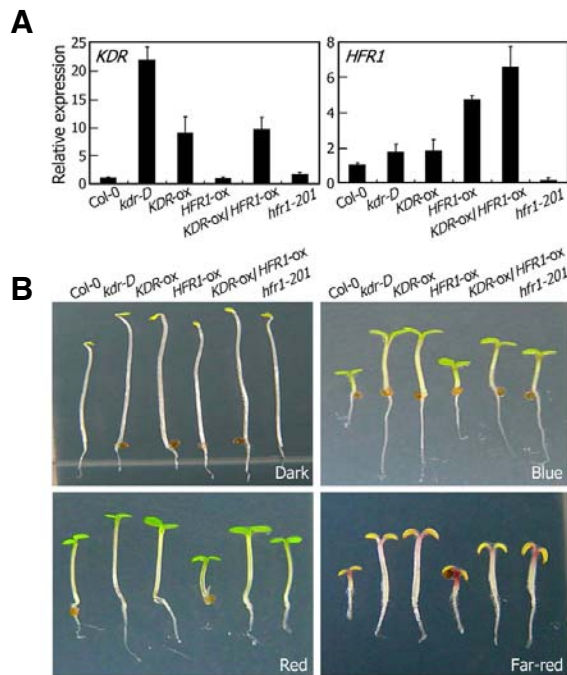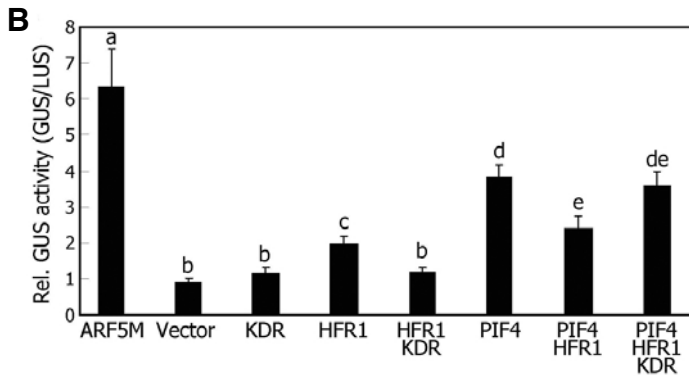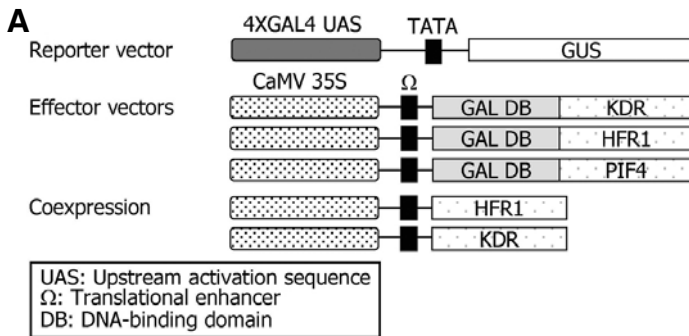
The PIF4 activity was detectably repressed by HFR1, which has been reported previously (Hornitschek et al., 2009). The inhibitory effects of HFR1 on the PIF4 activity were substantially suppressed by KDR coexpression. These observations indicated that KDR inhibits the HFR1 action in negatively regulating the PIF4 activity, probably liberating PIF4 from the HFR1-PIF4 complex.

Photomorphogenic hypocotyl growth of *HFR1*-ox plants is suppressed by KDR

Our biochemical approaches demonstrated that KDR competitively interacts with HFR1 and thus attenuates its biological activity in photomorphogenesis. To further examine the dominant-negative regulation of HFR1 by KDR, we genetically crossed the *KDR*-ox plants with the *HFR1*-ox plants to generate the *KDR*-ox/*HFR1*-ox plants. The high-level expression of *KDR* and *HFR1* genes in the *KDR*-ox and *HFR1*-ox transgenic plants was well maintained in the *KDR*-ox/*HFR1*-ox plants (Fig. 5A).

A

B

C

D
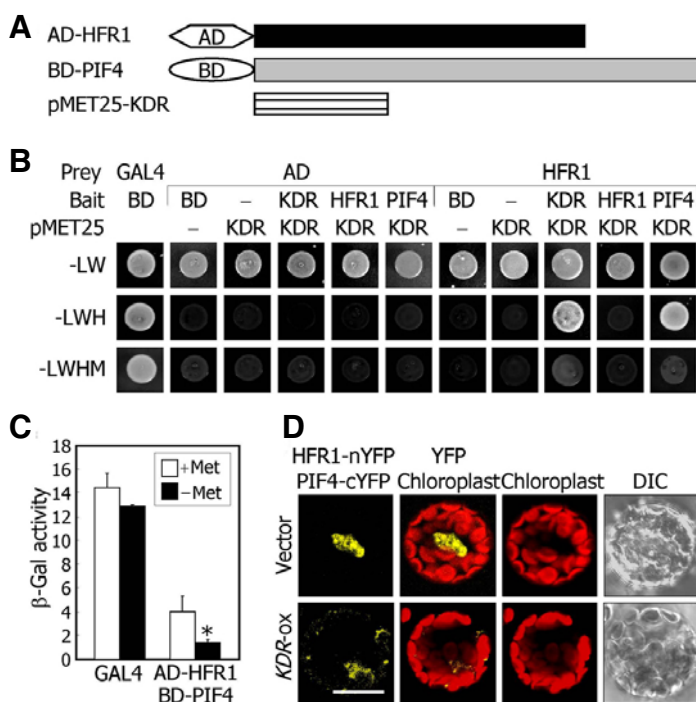
Fig. 3. KDR-mediated inhibition of HFR1-PIF4 heterodimer formation. (A) Expression constructs for yeast three-hybrid assays. The *KDR* gene was expressed under the control of the methionine (Met)-suppressible promoter (pMET25-KDR). AD, activation domain; BD, DNA-binding domain. (B, C) Inhibition of HFR1-PIF4 interactions by KDR in yeast cells. Yeast three-hybrid assays were carried out, and growth of yeast cells on selective media (-LWHM) (B) and β-Gal activities (C) were analyzed. The *KDR* gene is not expressed on -LWH plates but is expressed on -LWHM plates. In (C), β-Gal activities were measured in either the presence or absence of Met. Three independent measurements were averaged and statistically treated using the Student's *t*-test (*P < 0.01). Bars indicate the standard error of the mean. (D) Inhibition of HFR1-PIF4 interactions by KDR in *Arabidopsis* protoplasts. Partial YFP fusions containing either HFR1 or PIF4 were coexpressed transiently in *Arabidopsis* protoplasts and visualized by differential interference contrast microscopy (DIC) and fluorescence microscopy. The photograph is a representative of protoplasts (n > 20) that exhibit similar patterns of fluorescence signals. Scale bar = 10 μm.

A

Reporter vector

4XGAL4 UAS   TATA
GUS

Effector vectors

CaMV 35S   Ω
GAL DB   KDR
GAL DB   HFR1
GAL DB   PIF4

Coexpression

HFR1
KDR

UAS: Upstream activation sequence
Ω: Translational enhancer
DB: DNA-binding domain

B

Fig. 4. Derepression of transcriptional regulatory activity of PIF4 by KDR. (A) Expression vectors for transient expression assays in *Arabidopsis* protoplasts. GAL4 transient expression assays were performed as described previously (Miura et al., 2007). The *Renilla* luciferase gene was employed as an internal control for normalization of the values in individual assays. (B) Transcriptional activation activity assays. Five independent measurements were averaged and statistically treated. Different letters represent significant differences at P < 0.05 (one-way ANOVA with Fisher's *post hoc* test). Bars indicate standard error of the mean.

A

B

C

D

Fig. 5. Suppression of the HFR1 activity by KDR. (A) Levels of the *HFR1* and *KDR* transcripts in the *HFR1*- and *KDR*-overexpressing plants. Two-week-old plants grown on MS-agar plates were used for the extraction of total RNA. Transcript levels were determined by qRT-PCR. Biological triplicates were averaged and statistically treated using the Student's *t*-test (*P < 0.01). Bars indicate standard error of the mean. (B, C) Suppression of *HFR1*-ox phenotypes by *KDR* coexpression. Seeds sown on MS-agar plates were placed at 4°C in the dark. Seeds were exposed to white light for 6 h to induce germination and then transferred to growth chambers with different light wavelengths (B). The *KDR*-overexpressing *kdr-D* plants and *HFR1*-deficient *hfr1-201* mutant were also included in the assays. The lengths of approximately 20 hypocotyls were averaged for each plant genotype and statistically treated using the Student's *t*-test (*P < 0.05) (C). Bars indicate standard error of the mean. (D) Expression of the *DREB2A* gene in the *KDR*-ox/*HFR1*-ox plants. Seedlings grown on MS-agar plates for 5 days in darkness were exposed to either blue light or darkness for 1 h, and whole plants were used for the extraction of total RNA. Transcript levels were determined by qRT-PCR. Biological triplicates were averaged and statistically treated using a student's *t*-test (*P < 0.01). Bars indicate standard error of the mean.

The gain-of-function *kdr-D* mutant and *KDR*-ox plants both showed long hypocotyls especially under blue and far-red lights, whereas *HFR1*-ox plants showed relatively short hypocotyls (Figs. 5B and 5C). Notably, the hypocotyl phenotypes of the *HFR1*-ox plants were suppressed by *KDR* coexpression under different light conditions, as observed in the loss-of-function *hfr1-201* mutant (Figs. 5B and 5C). This finding indicated that the non-DNA-binding KDR protein inhibits the HFR1 activity in hypocotyl elongation.

To determine the genetic relationship between *KDR* and *HFR1*, we analyzed the expression patterns of blue light-responsive gene *DEHYDRATION-RESPONSIVE ELEMENT BINDING PROTEIN 2A* (*DREB2A*) in wild-type, *KDR*-ox, *HFR1*- ox, and *KDR*-ox/*HFR1*-ox plants after exposure to blue light for 1 h. The expression level of the *DREB2A* gene was increased in response to blue light treatment in wild-type plants. However, the degree of the induction in the *KDR*-ox transgenic plants was reduced compared to those in the wild-type plants (Fig. 5D). Notably, the higher gene expression in the *HFR1*-ox plants was compromised in the *KDR*-ox/*HFR1*-ox plants, in which the gene expression was comparable to that in the *KDR*-ox transgenic plants (Fig. 5D). These observations unequivocally indicated that the phenotypes of the *KDR*-ox/*HFR1*-ox plants were not caused by simple additive interactions but through the inhibition of HFR1 activity by KDR.

DISCUSSION

The basic helix-loop-helix domains in bHLH transcription factors consist of approximately 60 residues. The N-terminal basic sequence of the domain is responsible for DNA binding, and the C-terminal HLH region mediates protein-protein interactions. Interestingly, among the 162 bHLH members in *Arabidopsis*, 27 have atypical or disrupted basic sequences (Littlewood and Evan, 1995). These atypical members presumably may act as negative regulators of typical bHLH transcription factors by forming non-DNA-binding heterodimers.

The PIF transcription factors are the major regulators of plant growth and development. The PIFs integrate environmental signals, including light and temperature signals with internal cues (e.g., gibberellin and circadian clock-mediated signals) (Castillon et al., 2007; Leivar and Quail, 2011; Yoo et al., 2011). Because of their biological importance in plants, the PIF activities are elaborately regulated. In addition to transcriptional control, they are regulated through protein phosphorylation and ubiquitination-mediated protein degradation (Castillon et al., 2007). Recent studies have also shown that PIFs are regulated by competitive inhibition mechanisms. For instance, competitive interaction of PIF4 with DELLA proteins results in decreased DNA binding of PIF4 (Schwechheimer and Willige, 2009). HFR1 has also been reported to attenuate the PIF activities by competitive heterodimer formation (Hornitschek et al., 2009).

Remarkably, the HLH protein KDR, which lacks the functional DNA-binding domain, inhibits the HFR1 functions in a similar manner, derepressing the PIFs factors. These observations reveal that multiple layers of competitive inhibition modulate the PIF activities in photomorphogenesis such as shade avoidance response, as evidenced in this work.

In addition, the KDR-HFR1 interaction could also be relevant in far-red and blue light signaling (Fairchild et al., 2000; Fankhauser and Chory, 2000). A previous study has shown that PIF4 and PIF5 are required for de-etiolation of seedlings grown under far-red light (Lorrain et al., 2009). In fact, physiological relevance of the KDR-HFR1 interaction in far-red light signaling

has been suggested before (Hyun and Lee, 2006). Moreover, previous studies and our own data also demonstrate the HFR1 inactivation by KDR under blue light (Duek and Fankhauser, 2003), further supporting the biological importance of the KDR-mediated competitive inhibition under fluctuating light conditions.

In this study, we provide the mechanistic explanation as to how KDR regulates light signaling. KDR forms heterodimers with HFR1, which induces, at least in part, sequestration of HFR1 in the cytoplasm and leads to the liberation of PIFs from their negative regulator HFR1. Consistently, the *KDR*-ox transgenic plants are phenotypically similar to the *hfr1* loss-of-function mutant and transgenic plants overexpressing *PIF3/4/5* genes (Nusinow et al., 2011; Soy et al., 2012; Yang et al., 2005). Genetic analysis also supports the epistasis of *KDR* to *HFR1*. Our data also show that KDR may be important for the regulation of HFR1 itself because KDR can suppress the transcriptional activation activity of HFR1. Overall, our observations have contributed to elucidation of the as-yet unidentified biological relevance of the HFR1 transcription factor in photomorphogenesis and seedling development in plants.

KDR and its gene homologues, such as *BANQUO* (*BNQ*) genes, encode atypical HLH proteins. It has been found that BNQ1/bHLH136, BNQ2/bHLH134, and BNQ3/bHLH161 proteins also physically interact with HFR1 (Mara et al., 2010). However, the BNQ proteins seem to have somewhat distinct physiological roles. The *BNQ1*, *BNQ2*, and *BNQ3* genes are regulated specifically by floral homeotic proteins APETALA3 (AP3) and PISTILLATA (PI) in flower organogenesis (Mara et al., 2010). It is likely that HFR1 serves as an integrator of diverse input signals mediated by the atypical HLH proteins. Therefore, dynamic interactions and competitions among the KDR and BNQ proteins may constitute the regulatory roles played by HFR1 in plant photomorphogenesis. Examination of temporal and spatial expression patterns of the *HLH* genes and the search for novel interacting partners of the HLH proteins will be helpful for further understanding the role of HFR1 and the functional specificity of the KDR proteins in light and developmental signal transduction pathways.

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