Phytochrome Signaling Is Mediated by PHYTOCHROME INTERACTING FACTOR in the Liverwort Marchantia polymorpha

Keisuke Inoue,^a Ryuichi Nishihama,^a Hideo Kataoka,^a Masashi Hosaka,^a Ryo Manabe,^a Mika Nomoto,^b Yasuomi Tada,^c Kimitsuken Ishizaki,^a,d and Takayuki Kohchia,1
^a Graduate School of Biostudies, Kyoto University, Kyoto 606-8502, Japan
^b Division of Biological Science, Graduate School of Science, Nagoya University, Chikusa, Nagoya 464-8602, Japan
^c Center for Gene Research, Nagoya University, Chikusa, Nagoya 464-8602, Japan
^d Graduate School of Science, Kobe University, Kobe 657-8501, Japan

ORCID ID: 0000-0003-0504-8196 (K. Ishizaki)

Phytochromes are red light (R) and far-red light (FR) receptors that play important roles in many aspects of plant growth and development. Phytochromes mainly function in the nucleus and regulate sets of genes by inhibiting negatively acting basic helix-loop-helix transcription factors named PHYTOCHROME INTERACTING FACTORS (PIFs) in Arabidopsis thaliana. Although R/FR photoreversible responses and phytochrome genes are well documented in diverse lineages of plants, the extent to which phytochrome signaling is mediated by gene regulation beyond angiosperms remains largely unclear. Here, we show that the liverwort Marchantia polymorpha, an emerging model basal land plant, has only one phytochrome gene, Mp-PHY, and only one PIF gene, Mp-PIF. These genes mediate typical low fluence responses, which are reversibly elicited by R and FR, and regulate gene expression. Mp-phy is light-stable and translocates into the nucleus upon irradiation with either R or FR, demonstrating that the single phytochrome Mp-phy exhibits combined biochemical and cell-biological characteristics of type I and type II phytochromes. Mp-phy photoreversibly regulates gemma germination and downstream gene expression by interacting with Mp-PIF and targeting it for degradation in an R-dependent manner. Our findings suggest that the molecular mechanisms for light-dependent transcriptional regulation mediated by PIF transcription factors were established early in land plant evolution.

INTRODUCTION

Light is important for sessile plants not only as a source of energy for photosynthesis but also as a major source of environmental information that triggers adaptations in their growth and development. Land plants have acquired multiple photoreceptors, such as phytochromes, cryptochromes, phototropins, ZEITLUPE/FLAVIN BINDING, KELCH REPEAT, F-BOX1/LOV KELCH PROTEIN2, and UV RESISTANCE LOCUS8, to perceive the light environment precisely (Kami et al., 2010; Ito et al., 2012; Jenkins, 2014; Christie et al., 2015). Among these photoreceptors, phytochromes are the sole receptors for red light (R) and far-red light (FR). Phytochromes exist in two different forms, the R-absorbing Pr form and the FR-absorbing Pfr form. R triggers activation of phytochromes by converting the Pr form to the Pfr form, whereas FR inactivates them by converting the Pfr form back to the Pr form.

Phytochromes are grouped into two types, type I and type II, which have distinct biochemical and physiological properties in Arabidopsis thaliana. The type I phytochrome A (phyA) is highly abundant in etiolated seedlings and is rapidly degraded upon the conversion to the Pfr form, whereas the type II phytochromes phyB to phyE are relatively light-stable (Sharrock and Clack, 2002). Monocots such as rice (Oryza sativa) and maize (Zea mays) also have light-labile phyA and light-stable phyB (Reddy and Sharma, 1998; Xie et al., 2014). The rapid decline of light-labile phytochromes is observed within 2 to 4 h after R irradiation, although the degradation rate depends on the plant species. In Arabidopsis, both types of phytochromes translocate from the cytosol into the nucleus in response to light and mainly function in the nucleus (Kircher et al., 1999; Yamaguchi et al., 1999; Huq et al., 2003; Klose et al., 2015). Hence, light-dependent nuclear translocation is especially important for phytochrome signaling in Arabidopsis.

The molecular mechanism for translocation is also different between type I and type II phytochromes. Nuclear translocation of phyA is triggered by either R or FR and requires binding to FARR-RED ELONGATED HYPOCOTYL1 (FHY1) or FHY1-LIKE (FHL), which transports phyA to the nucleus (Hiltbrunner et al., 2006; Genoud et al., 2008; Rausenberger et al., 2011). By contrast, phyB efficiently translocates into the nucleus only in response to R (Yamaguchi et al., 1999). phyB does not require FHY1 or FHL for nuclear translocation and is thought to enter the nucleus via its own nuclear localization signal or by binding to transcription factors involved in phytochrome signaling (Chen et al., 2005; Pfeiffer et al., 2012).

Physiological responses mediated by phytochromes are mainly classified into three modes of action, namely, low fluence response (LFR), very low fluence response (VLFR), and high irradiance response (HIR), according to fluence requirements, photoreversibility,
and reciprocity (Mancinelli, 1994; Casal et al., 1998). Among these responses, LFR is a well-documented response, which shows reciprocity and photoreversibility: It is induced by R and reversibly inhibited by FR. VLFR is induced by very-low-intensity visible light of any wavelength and does not show photoreversibility, as a very low ratio of the Pfr form of phytochrome is sufficient to trigger the response (Shinomura et al., 1996). HIR requires prolonged exposure to relatively high-intensity light and shows neither reciprocity nor R/FR photoreversibility. Under carefully designed experimental conditions, however, FR-HIR shows reciprocity and FR/R (not R/FR) photoreversibility (Shinomura et al., 2000). In Arabidopsis, phyA contributes to VLFR and FR-HIR (Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993; Shinomura et al., 1996), while phyB mainly regulates LFR and R-HIR (Mancinelli, 1994; Shinomura et al., 1996).

Since the phytochromes in Arabidopsis mainly function in the nucleus as described above, most of the phytochrome responses result from regulation of gene expression via transcription factors. Collectively, numerous studies have demonstrated that there are two main pathways in phytochrome signaling. In the first pathway, PHYTOCHROME INTERACTING FACTORS (PIFs), belonging to the basic helix-loop-helix (bHLH) transcription factor family, were the first transcription factors to be identified and implicated in phytochrome signaling (Ni et al., 1998; Leivar and Monte, 2014). PIFs act as negative regulators of phytochrome signaling by modulating the expression of a large set of genes in the dark. Light-activated phytochromes interact with PIFs and trigger phosphorylation and subsequent proteasomal degradation of PIFs in a light-dependent manner. The changes in protein abundance of PIFs result in various physiological responses, such as seed germination, seedling deetiolation, inhibition of hypocotyl elongation, and shade avoidance responses. In the second pathway, CONSTITUTIVE PHOTOMORPHOGENIC1 (COP1) and SUPPRESSOR OF PHYA-105 (SPA) family proteins represent central negative regulators of light signaling (Huang et al., 2014). In the dark, a COP1/SPA complex targets a set of photomorphogenesis-promoting factors, such as ELONGATED HYPOCOTYL5, for proteasomal degradation to inhibit photomorphogenesis. Recent analysis has shown that light-activated phytochromes interact with the SPA family in a light-dependent manner and promote the dissociation of the COP1/SPA complex (Lu et al., 2015; Sheerin et al., 2015). Phytochrome-dependent regulation of gene expression has also been reported in several angiosperms (Sawers et al., 2002; Takano et al., 2005; Kebrom et al., 2006; Takano et al., 2009; Kebrom et al., 2010) and gymnosperms (Alosi and Neale, 1992; Christensen et al., 2002). However, the molecular mechanisms of R-mediated transcriptional regulation in these species are still poorly understood.

In contrast to the phytochromes in Arabidopsis, the phytochromes in the fern Adiantum capillus-veneris and the moss Physcomitrella patens reportedly function largely in the cytosol or at the plasma membrane and regulate phototropism and chloroplast photorelocation movement, which are mainly regulated by the phototropins in Arabidopsis (Rösl et al., 2010; Jaedicke et al., 2012). Recently, nuclear functions of phytochromes in Ad. capillus-veneris and P. patens have been suggested. In Ad. capillus-veneris, microbeam irradiation in the nucleus to selectively activate nucleus-localized phytochrome was able to induce spore germination (Tsuboi et al., 2012). In P. patens, PHY1 and PHY3 translocate from the cytosol into the nucleus via identical mechanisms to those in seed plants, and they regulate HIR-like responses under continuous FR (Possart and Hillbrunner, 2013). However, whether phytochromes regulate gene expression outside of angiosperms has not been directly confirmed.

The liverwort Marchantia polymorpha is an emerging model plant among the basal land plants, owing to several advantages for genetic studies: a low-genetic redundancy, a haploid-dominant life cycle, and tractable genetic tools, such as high-efficiency transformation methods and genetic technologies (Ishizaki et al., 2008, 2013, 2016; Kubota et al., 2013; Sugano et al., 2014; Eklund et al., 2015; Kato et al., 2015; Flores-Sandoval et al., 2016; Nishihama et al., 2016). Since liverworts represent an early divergent lineage of extant land plants (Qiu et al., 2006), investigations using liverworts are of particular interest in terms of land plant evolution. In addition, genome and transcriptome projects are ongoing by the Community Sequencing Program in the U.S. Department of Energy Joint Genome Institute.

Several studies of R responses in M. polymorpha have been reported (Ninnemann and Halbsguth, 1965; Fredericq and De Greef, 1966; De Greef et al., 1971; Otto and Halbsguth, 1976; Hartmann and Jenkins, 1984). A pulse of FR at the end of the photoperiod induces vertical upward growth of thalli and a decrease in the amount of chlorophyll, and the effect of R is cancelled by FR followed by a pulse of R, suggesting that a phytochrome is involved in these responses (Fredericq and De Greef, 1966; Hartmann and Jenkins, 1984). Experiments using pulses of R and FR demonstrate that senescence of thalli and germination of gemmae may be regulated by phytochromes (Ninnemann and Halbsguth, 1965; De Greef et al., 1971; Otto and Halbsguth, 1976). Recently, a phytochrome in M. polymorpha has been shown to regulate cell cycle reentry of differentiated cells in thallus explants, as well as cell shapes in newly regenerating tissues (Nishihama et al., 2015). In this study, we discovered the lone, single-copy genes for phytochrome and PIF in M. polymorpha. We examined the biochemical and cell biological characteristics of the single phytochrome and the involvement of the single PIF in R signaling. Our data show that M. polymorpha has a light-dependent transcriptional regulatory mechanism mediated by a module consisting of the phytochrome and PIF transcription factor.

RESULTS

Mp-PHY, the Only Phytochrome Gene in M. polymorpha

To identify the R and FR receptor phytochrome in M. polymorpha, we screened cDNA and genomic libraries and cloned a phytochrome gene (see Methods). BLAST searches against M. polymorpha transcriptome and genome databases revealed that this gene, named Mp-PHY, is the only copy resembling a phytochrome gene in the genome, which is consistent with previous reports showing the existence of one phytochrome gene in other liverwort species (Suzuki et al., 2001; Mathews, 2006; Li et al., 2015). The amino acid sequence of Mp-phy differs by only six residues from that of M. paleacea phytochrome, which has been reported to reside in a clade sister to that of phytochromes in seed plants (Supplemental Figure 1; Li et al., 2015). The deduced amino acid sequence of Mp-PHY included the typical domains of the
canonical phytochromes in land plants (Supplemental Figure 2). The genomic DNA sequence corresponding to Mp-PHY revealed that all of the three intron-insertion sites in the coding sequence were identical to those of most phytochrome genes in land plants (Supplemental Figure 2). To examine the spectral properties of Mp-phy, we expressed the N-terminal photosensory module of Mp-phy protein with phytochromobilin (PΦB) chromophore in *Escherichia coli*. The recombinant Mp-phy protein exhibited a typical R/FR photoreversibility (Supplemental Figures 3A and 3B), much the same as is observed in the phytochromes in *Arabidopsis* (Mukougawa et al., 2006), suggesting that Mp-PHY encodes a functional and canonical phytochrome.

### Mp-phy Protein Is Light-Stable in Vivo

We next examined whether Mp-phy more closely resembled typical type I or type II phytochromes in terms of light stability. Ten-day-old wild-type plants grown under continuous white light were transferred to the dark for 48 h and then irradiated with either R or FR for different time periods before harvesting. Immunoblot analysis revealed that the abundance of Mp-phy was slightly increased during a prolonged incubation in the dark (Figure 1A). The level of Mp-phy was not changed within 8 h after irradiation with R or 24 h after FR, though irradiation with R for 24 h caused a slight reduction of Mp-phy (Figures 1B and 1C). Given that phyA in *Arabidopsis*, rice, and maize show strong downregulation by R within 2 to 4 h (Reddy and Sharma, 1998; Sharrock and Clack, 2002; Xie et al., 2014), these results indicate that Mp-phy is light-stable in vivo, which is comparable to the type II phytochromes in *Arabidopsis* and grasses.

### Nuclear Translocation of Mp-phy Is Induced by Both R and FR

In *Arabidopsis*, phyA is rapidly translocated from the cytosol into the nucleus under either R or FR, while the nuclear accumulation of phyB is observed only under R (Kircher et al., 1999; Yamaguchi et al., 1999). To investigate the subcellular localization of Mp-phy, we generated transgenic plants expressing Mp-phy as a fusion protein with the fluorescent reporter Citrine at the C terminus under the control of the cauliflower mosaic virus 35S (35S) promoter. One-day-old gemmalings grown under continuous white light were transferred to the dark for 6 d and then irradiated with either R or FR for 10 min before observation. The phy-Citrine fusion protein in the dark-adapted gemmalings was mainly located at the cell periphery, although we have been unable to determine whether this signal was derived from the cytosol or plasma membrane. Upon the exposure to either R or FR, phy-Citrine accumulated within 10 min in a single large organelle of the cell that was presumed to be the nucleus (Figure 2A). A mutated Mp-phy containing an amino acid substitution of the conserved Tyr-241 residue to His (Mp-phyY241H) was reported to be constitutively active in vivo (Nishihama et al., 2015), similar to phyBY276H, the corresponding mutant protein in *Arabidopsis* (Su and Lagarias, 2007; Nishihama et al., 2015). The recombinant Mp-phyY241H protein completely lacked R/FR photoreversibility, as was observed with phyBY276H (Supplemental Figures 3C and 3D). We also generated transgenic plants expressing PHYY241H-Citrine under the control of the 35S promoter and found that the phyY241H-Citrine fusion protein localized in the nucleus without any exposure to light (Supplemental Figure 4). These results suggest that the active Pfr form of Mp-phy is translocated into the nucleus. To confirm the light-dependent nuclear translocation of Mp-phy, we also performed subcellular fractionation experiments. Ten-day-old wild-type plants grown under continuous white light were transferred to the dark for 48 h and then irradiated with either R or FR for 60 min before harvesting. Consistent with the fluorescence imaging, immunoblot analysis revealed that the amount of Mp-phy protein in the nucleus-enriched fraction increased within 10 min in response to either R or FR (Figures 2B and 2C). Although light induced rapid nuclear import of Mp-phy, subcellular fractionation results showed that the majority of Mp-phy was still retained in the cytosol (Figures 2B and 2C). Furthermore, a band with lower mobility was also observed in the nucleus-enriched fraction under FR, suggesting that the Mp-phy protein in the nucleus undergoes some type of posttranslational modification under FR (Figure 2C). Contrary to the results obtained with the fluorescence reporter assay, subcellular fractionation revealed a small amount of Mp-phy in the nucleus before light irradiation (Figures 2B and 2C). A similar observation was reported for At-phyA in dark-grown seedlings, which was presumed to be generated by brief light exposure before seedling germination.

![Figure 1. Light Stability of Mp-phy Protein.](image-url)
Taken together, these results suggest that the subcellular distribution pattern of Mp-phy is similar to that of the type I phytochrome in Arabidopsis.

Mp-phy Photoreversibly Regulates Gemma Germination

To investigate physiological functions of Mp-phy, we prepared transgenic plants expressing the Mp-PHY cDNA of the wild type (proEF1:PHY) and the putative constitutively active allele (proEF1:PHYY241H) driven by the M. polymorpha ELONGATION FACTOR1α (Mp-EF1) promoter (Althoff et al., 2014). These transgenic plants did not show any obvious growth defects under continuous white light (Supplemental Figure 5). Forty years ago, it was suggested that gemma germination in M. polymorpha might be regulated by phytochrome (Otto and Halbsguth, 1976). We therefore directly examined whether Mp-phy regulates gemma germination using proEF1:PHY and proEF1:PHYY241H.

Consistent with the original report (Otto and Halbsguth, 1976), the germination rate of wild-type gemmae was increased by a pulse of R, and the effect of R was cancelled by a subsequent pulse of FR (Figure 3A). The R-induced germination was increased in a fluence-dependent manner (Supplemental Figure 6). In addition, irradiation by either a pulse of FR or continuous FR was not effective for gemma germination (Figure 3A), suggesting that the mode of action of gemma germination in M. polymorpha can be classified as a typical LFR.

proEF1:PHY gemmae exhibited a slightly higher germination rate than wild-type gemmae in response to the pulse of R, while the inhibitory effect of FR still remained (Figure 3A). In contrast, virtually all the proEF1:PHYY241H gemmae germinated even in the dark, and the inhibitory effect by FR was not observed (Figure 3A). qRT-PCR analysis showed that the expression levels of Mp-PHYY241H were almost the same as those of proEF1:PHY (Supplemental Figure 7), indicating that the phenotype observed in proEF1:PHYY241H was not due to an elevated expression level of Mp-PHYY241H. These results strongly support the notion that Mp-phyY241H is constitutively active in vivo, consistent with our previous observation (Nishihama et al., 2015).

Mp-phy Regulates Gene Expression in a Light-Dependent Manner

Phytochromes in Arabidopsis regulate the expression of a large set of genes to adapt to changing light environments (Quail, 2010).
We asked whether Mp-phy can similarly regulate gene expression in response to R and FR. Ten-day-old plants grown under continuous white light were transferred to the dark for 48 h and then irradiated with a pulse of R with or without a subsequent pulse of FR before incubation in the dark for various periods. In wild-type and proEF1-PHY plants, the expression levels of a LIGHT-HARVESTING CHLOROPHYLL A/B BINDING PROTEIN (LHCB) and PROTOCHLOROPHYLLIDE OXIDOREDUCTASE (POR) homologs increased after the exposure to R, and these inductions were inhibited by a subsequent pulse of FR (Figure 3B). In contrast, proEF1-PHY showed robust expression of the LHCB and POR genes even in the dark and no response to FR (Figure 3B). Consistent with the pattern of transcript-level changes, the promoter activity of the LHCB gene also showed photo-reversibility (Supplemental Figure 8 and Supplemental Methods). These results imply that Mp-phy can regulate light-responsive gene expression reversibly via R and FR at the transcriptional level.

**M. polymorpha Has a Single PIF Transcription Factor**

Since phytochrome-mediated transcriptional regulation was observed in *M. polymorpha*, we next looked for a transcription factor that could be involved in Mp-phy signaling. PIF family members belong to the subfamily VII(a+b) of bHLH transcription factors (Pires and Dolan, 2010). BLAST searches against the *M. polymorpha* genome database (Phytozome v11) and phylogenetic analysis revealed that there is only one homologous gene (Mapoly0039s0059), which belongs to a clade including the PIF family in Arabidopsis and rice (Figure 4). This gene, named Mp-PIF, was predicted to encode a protein with a highly conserved bHLH domain at the C terminus and a putative Active Phytochrome A binding (APA) motif in the middle, which in Arabidopsis is important for the interaction with the Pfr form of phyA (Supplemental Figure 9). However, Mp-PIF does not contain an obvious Active Phytochrome B binding (APB) motif, which is required for binding with the Pfr form of phyB and is present in all PIF members in Arabidopsis (Supplemental Figures 9 and 10). PIF-like genes in *P. patens* also lack the APB motif but contain a putative APA motif (Supplemental Figure 10), suggesting that the APB motif might have been acquired during the course of land plant evolution. The *M. polymorpha* protein most similar to Mp-PIF, Mapoly0502s0001, was placed in another clade, subfamily XI, supporting the notion that *M. polymorpha* has only a single PIF transcription factor.

**Mp-PIF Acts as a Negative Regulator in Mp-phy Signaling**

To investigate whether Mp-PIF is involved in Mp-phy signaling, we generated knockout mutants of Mp-PIF (pPIF) using homologous recombination-based gene targeting (Ishizaki et al., 2013) (Supplemental Figure 11). pPIF plants did not show any obvious growth defects under continuous white light (Supplemental Figure 5). We examined the Mp-phy-regulated gemma germination in these mutants, pPIF gemmae germinated irrespective of light conditions (Figure 5A), as observed for proEF1-PHY gemmae (see Figure 3A). The loss of light dependency in gemma germination
in pifKO was recovered by introducing a genomic fragment of the Mp-PIF locus (Figure 5A), clearly showing that Mp-PIF is the gene responsible for the altered light responsiveness of the gemma germination in pifKO.

We then investigated the role of Mp-PIF in the Mp-phy-regulated expression of LHCb and POR. In pifKO plants, the expression levels of LHCb and POR were constitutively higher than in wild-type plants under all the light conditions tested (Figure 5B), although the expression levels gradually decreased during incubation in the dark. The complemented lines showed normal expression patterns of LHCb and POR as seen in wild-type plants. Overall, these results suggest that Mp-PIF negatively regulates gemma germination and the expression of light-responsive genes in the Mp-phy signaling pathway.

**Mp-phy Regulates Mp-PIF Protein Abundance in a Light-Dependent Manner**

Since Mp-PIF appears to be involved in transcriptional regulation in Mp-phy signaling, we examined the molecular mechanism for light-dependent regulation of gene expression. In Arabidopsis, the Pfr form of phytochromes interacts with PIFs and targets PIFs for proteasomal degradation (Al-Sady et al., 2006; Shen et al., 2007, 2008). We therefore examined the light-dependent interaction between Mp-phy and Mp-PIF and the degradation of Mp-PIF. Ten-day-old plants grown under continuous white light were transferred to the dark for 48 h and then irradiated with either R or FR. Mp-PIF was almost undetectable in continuous white light, but Mp-PIF accumulated within 24 h after the transition to the dark (Figure 6A). Additionally, a pulse of FR before the transition to the dark accelerated the accumulation of Mp-PIF in the dark (Figure 6B), probably reflecting the faster FR-mediated photoconversion of Mp-phy to the Pr form compared with its relatively slow conversion in the dark. Mp-PIF that had accumulated in the dark-adapted thalli was rapidly degraded within 30 min by R but not by FR (Figures 6C and 6D). Transcript levels of Mp-PIF were not reduced under R (Supplemental Figure 12), suggesting that the rapid reduction of Mp-PIF levels is probably due to post-translational regulation. Furthermore, treatment with MG132, an inhibitor of the 26S proteasome, reduced the degradation of
Mp-PIF by R irradiation (Figure 6E). These results suggest that the ubiquitin-proteasome system is involved in the turnover of Mp-PIF under R. Moreover, in the proEF1:PHY241H line, Mp-PIF did not accumulate even after prolonged incubation in the dark (Supplemental Figure 13), suggesting that the rapid degradation of Mp-PIF under R is triggered by the Pfr form of Mp-phy.

To investigate the interaction between light-activated Mp-phy and Mp-PIF, we performed in vitro pull-down assays using an E. coli-expressed chitin binding domain-tagged N-terminal fragment of Mp-PHY with or without PFB and in vitro-synthesized FLAG-tagged Mp-PIF. The Pfr form of Mp-phy readily interacted with FLAG-PIF, whereas neither the Mp-PHY apoprotein nor the Pr form of Mp-phy could bind to FLAG-PIF (Figure 7A). We next examined whether the putative APA motif is necessary for binding to Mp-phy. Point mutations of two Phe residues within this motif completely eliminated the interaction between Mp-phy and Mp-PIF (Figure 7B), suggesting that the motif is required for the interaction with Mp-phy.

To confirm the light-dependent interaction between Mp-phy and Mp-PIF in vivo, communoprecipitation analysis was performed using transgenic plants expressing 3×FLAG-tagged Mp-PIF under the control of the Mp-PIF promoter in pifKO plants. Ten-day-old plants grown under continuous white light were transferred to the dark for 48 h and then irradiated with R or with R followed by FR before harvesting. The interaction between Mp-phy and Mp-PIF was observed under R and inhibited by the subsequent FR (Figure 7C), suggesting that Mp-PIF interacts preferentially with the Pfr form of Mp-phy.

DISCUSSION

Phytochromes are found in many photosynthetic eukaryotes. Recent phylogenetic analyses demonstrate that plant phytochromes were acquired in the last common ancestor of Archaeplastida and that the canonical plant phytochromes originated in an ancestor of streptophytes (Duanmu et al., 2014; Li et al., 2015). Although R/FR photoreversible responses and the distribution of phytochrome genes have been reported in several angiosperms (Mancinelli, 1994), gymnosperms (Alosi and Neale, 1992; Christensen et al., 2002; Mølmann et al., 2006), ferns (Haupt, 1985; Tsuoi et al., 2012), bryophytes (Hartmann and Jenkins, 1984), and charophytes (Weisenseel and Ruppert, 1977; Sokol and Stross, 1992), little is known about the molecular mechanisms of phytochrome signaling apart from those of Arabidopsis. In this study, we found that phytochrome signaling in a liverwort is mediated by a single PIF transcription factor.

Light Stability and Subcellular Distribution of Mp-phy

In several angiosperms such as Arabidopsis, rice, and maize, the phytochromes are grouped into type I and type II. P. patens also has multiple phytochromes, which are split into two branches and are suggested to have distinct functions. Their gene families resulted from lineage-specific gene duplication (Mittmann et al., 2004; Li et al., 2015). Unlike these plants, the liverwort M. polymorpha has only the single phytochrome Mp-PHY in its genome, as in other liverwort species reported by Li et al. (2015). It would be of interest to know whether Mp-phy shares biochemical characteristics with type I or type II. To address this question, we analyzed two criteria, the light stability and light quality dependency of nuclear translocation.

We first demonstrated that Mp-phy is light-stable in vivo as is the case with At-phyB (Figure 1). Interestingly, Mp-phy possesses
a Lys residue corresponding to Lys-206 of At-phyA, which is the main ubiquitination site for its proteasomal degradation (Rattanapisit et al., 2016). The type II-like photostability of Mp-phy suggests that the mechanism for rapid degradation via the ubiquitination of Lys-206 of At-phyA could have been acquired during the course of functional differentiation of phytochromes following gene duplication.

We also demonstrated that Mp-phy rapidly translocates into the nucleus in response to either R or FR (Figure 2). Although At-phyA translocates into the nucleus under both R and FR, it accumulates only under FR due to its instability under R (Oka et al., 2012). However, Mp-phy is comparatively stable (Figure 1B), which may therefore permit its nuclear accumulation under R as well as FR. FHY1 and FHL are known to play an important role in the nuclear accumulation of At-phyA under FR (Hiltbrunner et al., 2006; Genoud et al., 2008; Rausenberger et al., 2011). Recently, a functional homolog of FHY1 in P. patens was shown to be similarly involved in the regulation of the FR-induced nuclear translocation of phytochromes (Possart and Hiltbrunner, 2013). These findings suggest that the M. polymorpha FHY1 homolog might regulate the nuclear translocation of Mp-phy under FR. Taken together, Mp-phy possesses combined properties of type I and type II phytochromes.

**Mp-phy-Mediated Responses**

Mp-phy regulated gemma germination and light-responsive gene expression in an R-dependent manner, and the inductive effect of R was clearly cancelled by a subsequent pulse of FR (Figure 3), indicating that Mp-phy is responsible for the LFR, as are type II phytochromes in other land plants. Interestingly, neither a pulse of FR nor continuous FR was effective for gemma germination and the induction of LHCb expression, although the nuclear accumulation of Mp-phy was observed under FR (Figures 2 and 3), suggesting that these responses are not simply regulated by the subcellular distribution of Mp-phy. In Arabidopsis, the nucleus-localized phyA regulates VLFR and FR-HIR under FR (Shinomura et al., 1996, 2000). FR-induced nuclear accumulation of phytochrome and HIR-like responses are observed in P. patens (Possart and Hiltbrunner, 2013). In this study, we could not elucidate the significance of the FR-induced nuclear accumulation of Mp-phy. In our previous observation, however, we found that FR is required for the growth phase transition of M. polymorpha (Chiyoda et al., 2008; Kubota et al., 2014). The nucleus-localized Mp-phy under

---

**Figure 6. R-Dependent Degradation of Mp-PIF.**

Protein accumulation levels of Mp-PIF were examined by anti-Mp-PIF immunoblot analyses using nucleus-enriched fractions from wild-type and pifKO plants. (A) to (D) Plants were grown under continuous white light for 10 d and transferred to either of the following light conditions: (A) darkness for the indicated periods (h); (B) darkness for the indicated periods (h) with and without preirradiation of a FR pulse (9000 μmol photons m⁻² s⁻¹; pFR); (C) and (D) darkness for 48 h followed by irradiation with R (30 μmol photons m⁻² s⁻¹; [C]) or FR (30 μmol photons m⁻² s⁻¹; [D]) for the indicated periods (min).

(E) Plants were grown under continuous white light for 10 d and transferred to the dark for 48 h (D) and then irradiated with R (30 μmol photons m⁻² s⁻¹) for 30 min (R). Plants were treated with 50 μM MG132 or DMSO (solvent control) for 30 min before irradiation with R. Immunoblotting with anti-Histone H3 is shown as a loading control.
The proteins bound to the chitin resin were probed with anti-Mp-PHY or anti-FLAG antibodies.

We detected a slower migrating band of the Mp-phy protein in proPIF:PIF-3:FLAG/pifKO lines (Figure 2), Mp-PIF is not degraded under FR (Figure 6). These findings indicate that liverworts, an early divergent lineage of land plants, had already acquired the transcriptional regulation system mediated by the phytochrome-bHLH interaction and that the light-dependent degradation of the bHLH would be the underlying mechanism of R signaling in land plants. In Arabidopsis, PIFs undergo phosphorylation before their degradation (Al-Sady et al., 2006; Shen et al., 2007, 2008). The stimulus-induced phosphorylation and subsequent degradation of substrates by SCF complexes has been observed in many eukaryotes (Skaar et al., 2013). Further analysis will clarify whetherMp-PIF undergoes phosphorylation before its degradation.

Our findings highlight the similarities and the differences in PIF-mediated phytochrome signaling in M. polymorpha and Arabidopsis (Figure 8). Under R, Mp-phy translocates into the nucleus (Figure 2) and interacts with Mp-PIF via the APA motif (Figure 7). Mp-PIF is rapidly degraded via the 26S proteasome, probably following the interaction with the Pfr form of Mp-phy (Figures 6 and 7), suggesting that Mp-PIF is inhibited by light-activated Mp-phy through a molecular mechanism that is also found in Arabidopsis. These findings indicate that liverworts, an early divergent lineage of land plants, had already acquired the transcriptional regulation system mediated by the phytochrome-bHLH interaction and that the light-dependent degradation of the bHLH would be the underlying mechanism of R signaling in land plants. In Arabidopsis, PIFs undergo phosphorylation before their degradation (Al-Sady et al., 2006; Shen et al., 2007, 2008). The stimulus-induced phosphorylation and subsequent degradation of substrates by SCF complexes has been observed in many eukaryotes (Skaar et al., 2013). Further analysis will clarify whetherMp-PIF undergoes phosphorylation before its degradation.

We demonstrated that the only PIF transcription factor in M. polymorpha, Mp-PIF, functions in phytochrome signaling and is involved in the negative regulation of the LFR, affecting gemma germination probably via light-dependent gene expression (Figures 4 and 5). At-PIFs accumulate in the dark and are degraded upon irradiation with R. In contrast to Arabidopsis, Os-PIL1 has been shown not to interact with Os-phyB and has been suggested to be light-stable in rice (Todaka et al., 2012). Mp-PIF accumulated in the dark and was degraded selectively in response to R possibly via the interaction with the Pfr form of Mp-phy (Figures 6 and 7), suggesting that Mp-PIF is inhibited by light-activated Mp-phy through a molecular mechanism that is also found in Arabidopsis. These findings indicate that liverworts, an early divergent lineage of land plants, had already acquired the transcriptional regulation system mediated by the phytochrome-bHLH interaction and that the light-dependent degradation of the bHLH would be the underlying mechanism of R signaling in land plants. In Arabidopsis, PIFs undergo phosphorylation before their degradation (Al-Sady et al., 2006; Shen et al., 2007, 2008). The stimulus-induced phosphorylation and subsequent degradation of substrates by SCF complexes has been observed in many eukaryotes (Skaar et al., 2013). Further analysis will clarify whetherMp-PIF undergoes phosphorylation before its degradation.

FR might be involved in the regulation of the growth phase transition in M. polymorpha.

We detected a slower migrating band of the Mp-phy protein in the nucleus under FR, but not under R (Figures 2B and 2C). It has been reported that nucleus-localized At-phyA is phosphorylated specifically under FR and that this phosphorylation may enhance the association with COP1 (Saijo et al., 2008), although the functional importance of this phosphorylation still remains unknown. Mp-phy might undergo a similar regulation via post-translational modification under FR as observed in At-phyA.

**Phytochrome-PIF-Mediated Transcriptional Regulation for R Signaling**

In Arabidopsis, PIFs have been shown to play an essential role in phytochrome signaling (Leivar and Monte, 2014). However, reports on PIF functions in other plant species are very limited. Here, we demonstrated that the only PIF transcription factor in M. polymorpha, Mp-PIF, functions in phytochrome signaling and is involved in the negative regulation of the LFR, affecting gemma germination probably via light-dependent gene expression (Figures 4 and 5). At-PIFs accumulate in the dark and are degraded upon irradiation with R. In contrast to Arabidopsis, Os-PIL1 has been shown not to interact with Os-phyB and has been suggested to be light-stable in rice (Todaka et al., 2012). Mp-PIF accumulated in the dark and was degraded selectively in response to R possibly via the interaction with the Pfr form of Mp-phy (Figures 6 and 7), suggesting that Mp-PIF is inhibited by light-activated Mp-phy through a molecular mechanism that is also found in Arabidopsis. These findings indicate that liverworts, an early divergent lineage of land plants, had already acquired the transcriptional regulation system mediated by the phytochrome-bHLH interaction and that the light-dependent degradation of the bHLH would be the underlying mechanism of R signaling in land plants. In Arabidopsis, PIFs undergo phosphorylation before their degradation (Al-Sady et al., 2006; Shen et al., 2007, 2008). The stimulus-induced phosphorylation and subsequent degradation of substrates by SCF complexes has been observed in many eukaryotes (Skaar et al., 2013). Further analysis will clarify whetherMp-PIF undergoes phosphorylation before its degradation.
therefore, the physiological response and gene expression do not occur (Figure 5). By contrast, in Arabidopsis, an extremely low ratio of the Pfr form of phyA can induce the degradation of PIF1 and PIF3 under FR (Park et al., 2004; Al-Sady et al., 2006; Shen et al., 2008). This strong light sensitivity of PIF degradation is considered to be responsible for seed germination under VLFR condition (Oh et al., 2004; Shen et al., 2008). Given that FR-induced VLFR is not observed in M. polymorpha (Figures 3 and 5), high light sensitivity of PIF degradation may be required for the VLFR. PIF4 and PIF5 are not degraded under FR, consistent with the fact that they do not have the APA motif (Lorrain et al., 2008; Leivar and Monte, 2014). PIF4 and PIF5 regulate the shade avoidance response under a low R-to-FR ratio (Lorrain et al., 2008). The acquisition of two types of phytochromes and functionally diverged PIFs may have allowed complex responses to various R to FR ratios during land plant evolution.

Light regulation of phytochrome subcellular localization has been reported in the prasinophyte green alga Micromonas pusilla, which suggests that light-dependent nuclear translocation of phytochrome predates the divergence of streptophytes and prasinophytes (Duanmu et al., 2014). However, the signaling mechanisms might be different. Phytochromes in prasinophytes contain an intact histidine kinase domain with light-dependent kinase activity, which could mediate phytochrome signaling (Duanmu et al., 2014). In contrast, all streptophytes examined so far have phytochromes that lack a His residue critical for phosphorelay, suggesting the establishment of distinct signaling mechanisms in streptophytes. PIF-mediated transcriptional regulation could represent one such mechanism, and it may have contributed to the acquisition of diversity in phytochrome signaling. Our results show that the PIF-mediated regulatory mechanism of R signaling had already been established in a liverwort and perhaps in the common ancestor of land plants. It would be of interest to find out whether acquisition of the phytochrome-PIF system contributed to adaptation to a terrestrial environment.

Figure 8. A Model of Phytochrome Signaling through PIFs in M. polymorpha and Arabidopsis.

Model of phytochrome signaling under R (A) and FR (B), Schematic illustration for M. polymorpha (left) and Arabidopsis (right). Blue and red circles indicate the APA motif and the APB motif, respectively. Dotted lines indicate degradation. Dashed lines indicate the nucleus.
METHODS

Plant Materials and Growth Conditions

Female and male Marchantia polymorpha accessions, Takaraga-ke-2 (Tak-2) and Takaraga-ke-1 (Tak-1), respectively (Ishizaki et al., 2008), were cultured aseptically on half-strength Gamborg’s B5 medium (Gamborg et al., 1968) containing 1% sucrose and 1.2% agar under 50 to 60°C with a cold cathode fluorescent lamp (OPT-40C-N-L; Optrom) at 22°C. F1 spores were obtained by crossing Tak-2 and Tak-1.

Light Sources

R was given by a red LED illuminator (ML-R18, SANYO Electric; peak emission at 657 nm, half-bandwidth of 12 nm). FR was given by a far-red LED illuminator (ML-IF18, SANYO Electric; peak emission at 734 nm, half-bandwidth of 13 nm). The light intensity was measured using an LI-250A light meter (LI-COR Biosciences) equipped with an SKR 110 red/far-red sensor (Skye instruments).

Cloning of Mp-PHY

Partial phytochrome gene fragments were obtained by sequencing the M. polymorpha EST clone rlwb02n17, which showed significant similarity to the 3’-half of the phytochrome coding sequence in Marchantia polymorpha (Suzuki et al., 2001) (GenBank accession number BAB39687). The sequence of the 5’-terminal end was obtained by 5′ RACE. For 5′ RACE, total RNA was isolated from ~100 mg of M. polymorpha thallus tissue using an RNaseasy plant mini kit (Qiagen). The first-strand cDNA was synthesized using SuperScript III (Thermo Fisher Scientific) and the gene-specific primer 5′-ACCTCCAGTGGTCACTG-3′. The first-strand cDNA was tagged with poly dC by terminal deoxynucleotidyl transferase (TaKaRa). 5′-RACE-PCR of dC-tailed cDNA was performed using the nested gene-specific primer 5′-CGAGGCGGATACTGCTGAG-3′ and abridged anchor primer (Thermo Fisher Scientific). Another round of nested PCR was performed with the nested gene-specific primer 5′-AGGAATTCAGGGCTGACG-3′ and abridged universal amplification primer (Thermo Fisher Scientific). Genomic DNA fragments corresponding to Mp-PHY cDNA were isolated by screening a PAC genomic library using the primer pair 5′-CGAGT-TACGAGAGTTTGGG-3′ and 5′-TTCTCCTGATGGTCACTG-3′, as described previously (Okada et al., 2000). After Spel digestion of the PAC clone, pMM24-1366, 7-kb fragments were subcloned and sequenced.

Phylogenetic Analysis

For alignments of amino acid sequences, we used the Muscle program (Edgar, 2004) implemented in Geneious software (version 8.1.3; Biomatters; http://www.geneious.com/) with default parameters. For phylogenetic analyses, we used the bHLH domain of PIFs and other bHLH transcription factors. The alignment is available as Supplemental Data Set 1. The phylogenetic tree was constructed using the PhyML program version 3.2.0 (Guindon et al., 2010) using the JTT model and four categories of rate substitution. Tree topology, branch length, and substitution rates were optimized, and the tree topology was searched using the nearest-neighbor interchange and subtree pruning and regrafting methods. Bootstrap proportions were computed from 1008 trials. The Chlorella vulgaris and Chlamydomonas reinhardtii bHLH sequences reported by Pires and Dolan (2010) were used as the outgroup.

Generation of Transgenic Lines

To obtain the overexpression lines of Mp-PHY, the coding sequence (CDS) of Mp-PHY was amplified by RT-PCR from wild-type mRNA with the primer pair 5′-CACCATGTCGACACCAAGGTCAC-3′ and 5′-TTATTCTCACTGGCGAGCAT-3′ and cloned into pENTR/TOPO vector (Thermo Fisher Scientific). The cloned sequence was then transferred to the pMPGW103 vector (Ishizaki et al., 2015) to generate pMH01. To obtain the overexpression lines of Mp-PHY241H, the plasmid carrying Mp-PHY cDNA was mutagenized using a Phusion site-directed mutagenesis kit (New England Biolabs) with the primer pair 5′-CACCAAATTCATGAGCTG-3′ and 5′-CCATAACCGCTGCTAACCT-3′. The cloned sequence was then transferred to the pMPGW103 vector to generate pMH02. To obtain the overexpression lines of phy-Citrine and phyY241H-Citrine fusion proteins under the control of the 35S promoter, the CDSs of Mp-PHY and Mp-PHY241H without the stop codon were amplified by RT-PCR from wild-type cDNA with the primer pair 5′-CACCATGTCGACACCAAGGTCAC-3′ and 5′-TTTCACACTCCAGCAGCAT-3′ and cloned into pENTR/D-TOPO vector. The cloned sequences were then transferred to the pMPGW106 vector (Ishizaki et al., 2015) to generate pMH06 and pMH07. pMH01, pMH03, pMH06, and pMH07 were introduced into F1 sporulations using Agrobacterium tumefaciens C58C1 GV2290 as previously described (Ishizaki et al., 2008).

To obtain the knockout lines of Mp-PIF, the 5′- and 3′-homologous arms were amplified from the wild-type genomic DNA by PCR with the primer pairs 5′-CTAAGTAGCGTCTTGAATGCGTACTCG-TACTG-3′/5′-CCGGCGAGCTTACCTTGCAAGCTATG-3′ and 5′-AACACTAGTGCCGACACCTGCAAGAACGAG-3′/5′-TTATCCCTAGGCGGCCAGAGTCTCGTG-3′, respectively. The amplified 5′- and 3′-homologous arms were cloned into the PacI and Ascl sites, respectively, of the pPHY-Tmp1 vector (Ishizaki et al., 2013) with an In-Fusion HD cloning kit (Clontech) to generate pIN005. pIN005 was introduced into F1 sporulations. Screening of targeted lines was performed as described previously (Ishizaki et al., 2013). Gene-specific primers used in the screening are diagrammed in Supplemental Figure 11. To obtain the complementation lines of pifKO, the promoter and CDS of Mp-PIF were amplified from wild-type genomic DNA by PCR with the primer pairs 5′-CACCTTACGAGGGCTGACG-3′ and 5′-TGAGATGTCAGACTG-3′, as described previously (Okada et al., 2000). After Spel digestion of the PAC clone, pMM24-1366, 7-kb fragments were subcloned and sequenced.

Gemma Germination Assay

Gemmae were plated on half-strength Gamborg’s B5 medium containing 1% sucrose and 1.2% agar under green light and then irradiated with FR (30 μmol photons m⁻² s⁻¹) for 15 min to inactivate M. phy. Gemmae imbibed in the dark for 1 d were kept under various light conditions and scored for germination in 6 d.

RNA Extraction and RT-PCR

Frozen plants were ground into a fine powder in liquid nitrogen by shaking for 1 min with a metal cone in a collection tube using a Multi-Beads Shocker (Yasu Kikai). Total RNA was extracted from whole plants using TRIzol reagent (Thermo Fisher Scientific) and treated with RNase-Free DNase (Qiagen) following the manufacturers’ instructions. Reverse transcription was performed with ~1 μg of RNA with an oligo(dT) primer using ReverTra
Ace (Toyobo) following the manufacturer’s instructions. Quantitative RT-PCR was performed with a CFX36 real-time PCR detection system (Bio-Rad) using SYBR Green I Nucleic Acid Gel Stain (Lonza) to monitor double-stranded DNA synthesis. Mp-EF1 and Mp-APT were used as internal controls for normalization of the PCR (Saint-Marcoux et al., 2015). The following thermal cycling protocol was used for all PCRs: 95°C for 30 s, 40 cycles of 95°C for 5 s, and 60°C for 30 s. Primer pairs used for each gene were as follows: for Mp-PHY, 5'-GGGCTGGTAGAATC-CAAGG-3' and 5'-CCCTGAACTCATAAAGCAACG-3'; for Mpf-PF, 5'-CAGGGATCATGATGGAATGCG-3' and 5'-AGATGATGGAGCGAATGCTG-3'; for LHB, 5'-GGAGATTCTGGATTGATGATTG-3' and 5'-GCTGCGTTCAATCTCGGC-3'; for POR, 5'-GTCGATGAAGCGAGACGACAACC-3' and 5'-GACGGAATCTAGGGAACACC-3'; for Mp-EF1, 5'-AAACCGGTGAAATGAGGAGG-3' and 5'-TCTAGAGTCGTCGCCGT-3'; for Mp-APT, 5'-CGCTGGTTGATGGAATGCG-3' and 5'-TTTCTGCTTGCATTAGG-3'. All samples were amplified in triplicate, and the relative expression levels of the target genes were calculated as described (Vandesompele et al., 2002).

Production of Anti-Mp-PHY and Anti-Mp-PIF Antibodies

For the production of the Mp-PHY antibody, we used an N-terminal fragment of Mp-PHY spanning amino acids 1 to 612 fused to the chitin binding domain (CBD) at the C terminus, and after transformation of Escherichia coli strain ER2566, the fusion protein was purified following a previously described procedure (Mukougawa et al., 2006). For the production of the Mp-PF antibody, a nonconserved fragment of the Mp-PF protein including amino acids 1 to 257 with a 6×His affinity tag at the N terminus was expressed in E. coli strain Rosetta2(DE3). The E. coli cells expressing 6×His-PF(N257) were grown at 37°C to log phase and then induced with 1 mM IPTG for 24 h at 15°C. Cells were collected and resuspended in lysis buffer (20 mM Tris-HCl, pH 8.0, 25% [v/v] glycerol, 10 mM MgCl₂, 250 mM sucrose, 5 mM DTT, 1 mM PMSF, 20 μM MG132, 1× Complete EDTA-Free Protease Inhibitor). The homogenate was centrifuged at 16,000 g for 10 min at room temperature. The supernatants were subjected to immunoblot analysis. For primary antibodies, anti-Mp-PHY and anti-Histone H3 were used as described above. Anti-Mp-PF was diluted 1:2000.

In Vitro Pull-Down Assay

For bait proteins, an N-terminal fragment of Mp-PHY fused to the CBD at the C terminus with or without PAB was expressed in E. coli as described above. The recombinant protein was incubated with chitin resin (New England Biolabs) at 4°C for 1 h and washed three times with binding buffer (20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% [v/v] Triton X-100, and 0.01% [v/v] BSA). The protein concentrations of affinity-bound Mp-PHY apoprotein and holoprotein were estimated by immunoblot analysis. For prey proteins, FLAG-tagged Mp-PF and Mp-PF(F297A,F300A) were synthesized by an in vitro transcription and wheat germ cell-free translation system (BioSieg) according to the manufacturer’s instructions. The reaction mixture was centrifuged at 15,000 g for 10 min, and the supernatant was used for the pull-down assays after estimation of the product yield by immunoblot analysis. The bait phy(N612)-CBD holoprotein was irradiated with a pulse of R (9000 μmol photons m⁻² s⁻¹), or a pulse of R followed by FR (10,800 μmol photons m⁻² s⁻¹) before use. Ten microliters of the affinity-bound phy(N612)-CBD apoprotein or holoprotein (Pfr and Pr) and 10 μL of the in vitrotranslated FLAG-PF or FLAG-PF(F297A,F300A) were mixed with 80 μL of binding buffer and incubated at 4°C for 1 h in the dark. After washing the resin three times with 1 mL of binding buffer under green light, the bound proteins were subjected to immunoblot analysis with anti-Mp-PHY antibody (used as described above) and anti-FLAG M2 antibody (1:2000 dilution).

Coimmunoprecipitation Assay

The following procedure was performed under green light. Plants (2.0 g) were harvested and ground into a fine powder in liquid nitrogen and mixed with 6 mL of lysis buffer as described in the subcellular fractionation procedures. After subcellular fractionation as described above, the pellet containing nuclei was resuspended with 1 mL of extraction buffer (20 mM Tris-HCl, pH 8.0, 5 mM EDTA, 150 mM NaCl, 1% [v/v] Triton X-100, 0.5% [v/v] sodium deoxycholate, 0.01% [v/v] SDS, 5 mM DTT, 1 mM PMSF, 20 μM MG132, and 1× Complete Protease Inhibitor) and incubated on ice for 10 min. The lysate was centrifuged at 16,000 g for 15 min at 4°C, and the supernatant was subjected to immunoprecipitation. Coimmunoprecipitation was performed using the μMACS DYKDDDDK isolation kit (Miltenyi Biotec) according to the manufacturer’s instructions. The immunoprecipitates were subjected to immunoblot analysis with anti-Mp-PHY and anti-FLAG M2 antibodies as described above.

Fluorescence Microscopy

Gemmae were grown under continuous white light for 1 d, transferred to the dark for 6 d, and then irradiated with either R or FR for 10 min. Fluorescence derived from Citrine was detected with an Olympus FV1000 confocal laser.
scanning microscope equipped with a GaAsP detector using a 515-nm laser for excitation, and a detection window in the range of 535 to 565 nm.

Accession Numbers

The sequences of the Mp-PHY and Mp-PIF genes are available in DDBJ under the following accession numbers: Mp-PHY (LC093264) and Mp-PIF (LC093265). The accession numbers of the analyzed proteins in Figure 4 are as follows: PIF1 (AEC06979), PIF3 (AEE28458), PIF4 (AEC10198), PIF5 (AEE79871), PIF6 (AEE80307), PIF7 (AEE97445), PIF8 (ABG25060), SPT (ABH04366), ALC (ABI49493), bHLH23 (AEE85454), bHLH56 (AEE85454), bHLH119 (AEE85547), bHLH127 (AEE85548), and LRL1 (AEC07551) from Arabidopsis; Os01g0236100 (BAF04689), Os03g0639300 (ABF97814), Os03g0782500 (ABF99196), Os05g0193100 (BAS92179), Os07g0143200 (BAF20790), Os12g0610200 (ABA98363), and Os02g0795800 (XP_015627343) from rice; and Pp001G155100 (Phpat.001G155100), Pp002G038300 (BAF20790), Os01g0236100 (BAF04689), Os03g0639300 (ABF97814), Os03g0782500 (ABF99196), Os05g0193100 (BAS92179), Os07g0143200 (BAF20790), Os12g0610200 (ABA98363), and Os02g0795800 (XP_015627343) from rice; and Pp001G155100 (Phpat.001G155100), Pp002G038300 (Phpat.002G038300), Pp014d091800 (Phpat.014d091800), Pp017G085300 (Phpat.017G085300), and Pp017G062100 (Phpat.017G062100) from Physcomitrella patens.

Supplemental Data

Supplemental Figure 1. Alignment of amino acid sequences of phytochromes from M. polymorpha and M. paecaece.
Supplemental Figure 2. Alignment of amino acid sequences of phytochromes from M. polymorpha and Arabidopsis.
Supplemental Figure 3. Spectral properties of recombinant Mp-phy and Mp-phyY241H.
Supplemental Figure 4. Light-independent nuclear localization of phyY241H-Citrine.
Supplemental Figure 5. Phenotypes of wild-type, proEF1-PHY, proEF1: PHYY241H, and pifKO plants.
Supplemental Figure 6. Fluence-response relationship for gemma germination.
Supplemental Figure 7. Transcript levels of Mp-PHY in transgenic lines.
Supplemental Figure 8. Photoreversible promoter activity of the LHCb gene.
Supplemental Figure 9. Alignment of amino acid sequences of Mp-PiF and PiFs from Arabidopsis.
Supplemental Figure 10. Sequence alignments of the APA and APB motifs in PiFs.
Supplemental Figure 11. Generation of pflKo plants by homologous recombination.
Supplemental Figure 12. Transcript levels of Mp-PiF during the transition from darkness to R.
Supplemental Figure 13. Levels of Mp-PiF protein in transgenic lines.
Supplemental Data Set 1. Text file of the alignment used for the phylogenetic analysis in Figure 4.
Supplemental Methods.
Supplemental References.

ACKNOWLEDGMENTS

We thank Tokitaka Oyama and Tomaaki Muranaka (Kyoto University) for assistance of luciferase bioluminesence assay and James A. Hejna (Kyoto University) for critical reading of the manuscript. This work was supported by KAKENHI Grant-in-Aids for Scientific Research on Innovative Area (Nos. 23120516 and 25113009 to T.K.), for Scientific Research (B) (No. 26291059 to T.K.), for Scientific Research (C) (No. 24570048 to R.N.), for Young Scientists (B) (No. 22770035 to K. Ishizaki), and Research Fellowships for Young Scientists (24-7049 to K. Inoue) from the Japan Society for the Promotion of Science.

AUTHOR CONTRIBUTIONS


Received December 29, 2015; revised May 18, 2016; accepted May 30, 2016; published June 1, 2016.

REFERENCES


Phytochrome Signaling Is Mediated by PHYTOCHROME INTERACTING FACTOR in the Liverwort *Marchantia polymorpha*

Keisuke Inoue, Ryuichi Nishihama, Hideo Kataoka, Masashi Hosaka, Ryo Manabe, Mika Nomoto, Yasuomi Tada, Kimitsune Ishizaki and Takayuki Kohchi

*Plant Cell* 2016;28;1406-1421; originally published online June 1, 2016;
DOI 10.1105/tpc.15.01063

This information is current as of December 30, 2017