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Minireview

Phytochrome-Interacting Factors Have Both Shared and Distinct Biological Roles

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Phytochromes are plant photoreceptors that perceive red and far-red light. Upon the perception of light in Arabidopsis, light-activated phytochromes enter the nucleus and act on a set of interacting proteins, modulating their activities and thereby altering the expression levels of ~10% of the organism's entire gene complement. Phytochrome-interacting factors (PIFs) belonging to Arabidopsis basic helix-loop-helix (bHLH) subgroup 15 are key interacting proteins that play negative roles in light responses. Their activities are post-translationally countered by light-activated phytochromes, which promote the degradation of PIFs and directly or indirectly inhibit their binding to DNA. The PIFs share a high degree of similarity, but examinations of *pif* single and multiple mutants have indicated that they have shared and distinct functions in various developmental and physiological processes. These are believed to stem from differences in both intrinsic protein properties and their gene expression patterns. In an effort to clarify the basis of these shared and distinct functions, we compared recently published genome-wide ChIP data, developmental gene expression maps, and responses to various stimuli for the various *PIF*s. Based on our observations, we propose that the biological roles of PIFs stem from their shared and distinct DNA binding targets and specific gene expression patterns.

The Phytochrome-Interacting Factors (PIFs) are bHLH Transcription Factors that Mainly Act to Repress Light Responses

The PIFs are bHLH transcription factors belonging to Arabidopsis bHLH subgroup 15, which consists of seven PIFs and eight other members (Toledo-Ortiz et al., 2003). Studies have shown that PIFs and some other members mediate light signaling to regulate various developmental and physiological processes (Bae and Choi, 2008; Casal, 2013; Chen and Chory, 2011; Kami et al., 2010; Leivar and Quail, 2011; Li et al., 2011). PIF3, the first identified PIF, was found by a yeast two-hybrid screen that used a C-terminal domain of Arabidopsis phytochrome B (phyB) as bait, and was further shown to interact with the C-terminal domains of both Arabidopsis phytochrome A (phyA) and rice phyB (Ni et al., 1998). Furthermore, although PIF3 was initially identified using the C-terminal domain of phyB, it was also shown to interact with the N-terminal domain of phyB, suggesting that PIF3 interacts with both the N- and C-terminal domains of phytochromes (Ni et al., 1998). Thereafter, additional PIFs were identified by similar yeast two-hybrid screenings and their amino acid similarities to PIF3 (Leivar and Quail, 2011).

Not all members of bHLH subgroup 15 bind to phytochromes; seven members [PIF1 (also known as PIL5, PIF3-LIKE 5), PIF3, PIF4, PIF5 (also known as PIL6), PIF6 (also known as PIL2), PIF7, and PIF8] bind to the light-activated form of phytochromes (the Pfr form), whereas the remaining eight members [PIL1, SPT (SPATULA), ALC (ALCATRAZ), HFR1 (LONG HYPOCOTYL IN FAR-RED 1), bHLH23, bHLH56, bHLH119, bHLH127] either do not bind to phytochromes or their bindings have not yet been shown experimentally (Leivar and Quail, 2011). Rice, a model monocot plant that diverged from Arabidopsis 130 to 200 million years ago, contains six PIF-like bHLH transcription factors (OsPIL11 to OsPIL16) that are characterized by the presence of motifs similar to the Arabidopsis phyB binding motif (APB), along with two other bHLH transcription factors that are similar to Arabidopsis HFR1 and SPT (OsHFR and OsSPT) (Nakamura et al., 2007; The Rice Annotation Project Database for OsHFR and OsSPT). Thus, PIF family members are present in both dicot and monocot plants. Furthermore, the ever-expanding pool of plant genome sequence data has revealed that PIF-like bHLHs are not restricted to flowering plants; they are also present in the genomes of non-flowering plants, such as *Selaginella moellendorffii* (e.g., NCBI Gene ID 9657644) and *Physcomitrella patens* (e.g., NCBI Gene ID 5927830). The ubiquity of PIF-like bHLHs in land plants suggests that these transcription factors play important roles in shaping the life of land plants.

Arabidopsis PIFs play largely negative roles in phytochrome-mediated red light signaling, as can be inferred by the exaggerated photomorphogenic phenotypes of dark- or light-grown single and multiple loss-of-function mutants, and the exaggerated skotomorphogenic phenotypes of light-grown transgenic plants overexpressing PIFs (Huq and Quail, 2002; Kim et al., 2003; Leivar et al., 2008a; Nozue et al., 2007; Oh et al., 2004; Shin et al., 2009). The negative role of PIFs is best exemplified

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by the phenotypes of *pif* quadruple mutants lacking *PIF1*, *PIF3*, *PIF4* and *PIF5* (*pifq*) (Leivar et al., 2008a; Shin et al., 2009). Dark-grown *pifq* mutants display constitutive photomorphogenic phenotypes including short hypocotyls, open cotyledons, de-etiolated plastids with accumulated chlorophyll precursors, and the loss of hypocotyl negative gravitropism, all of which are characteristics of light-grown wild-type seedlings. Dark-grown *pifq* mutants also resemble red light-grown wild-type seedling in their gene expression patterns, which are characterized by high expression of chloroplast- and photosynthesis-related genes (Leivar et al., 2009; Shin et al., 2009). The overall correlation coefficient for gene expression between dark-grown *pifq* and red light-grown wild-type seedlings is 0.72, indicating a close similarity (Shin et al., 2009). However, not all PIFs play negative roles in phytochrome-mediated light signaling. For example, overexpressed PIF6 inhibits hypocotyl elongation under red light, indicating that it can play a positive role in phy-mediated light signaling (Penfield et al., 2010). Among the other bHLH members, PIL1 and HFR1 are positive signaling components capable of inhibiting hypocotyl elongation (Fairchild et al., 2000; Salter et al., 2003; Sessa et al., 2005), whereas SPT promotes hypocotyl elongation (Penfield et al., 2005). Thus, it seems that the morphological and physiological light responses are determined by the sum of the positive and negative impacts of PIFs and the other bHLHs.

Phytochromes Post-Translationally Inhibit PIFs in Arabidopsis

The negative roles of the major PIF proteins are countered by light-activated phytochromes in at least three different ways (Fig. 1). First, the light-activated phytochromes counter PIFs by promoting the sequential phosphorylation, ubiquitylation, and degradation of PIFs (PIF1, PIF3, PIF4, and PIF5) through the 26S proteasome (Al-Sady et al., 2006; Lorrain et al., 2008; Nozue et al., 2007; Oh et al., 2006; Park et al., 2004; Shen et al., 2005). More detailed descriptions of this process can be found in other reviews (Bu et al., 2011; Casal, 2013; Leivar and Quail, 2011). Second, light-activated phytochromes counter PIFs by inhibiting the DNA binding of PIF1, PIF3, and PIF7, independent of their protein degradations. Three lines of evidence support this conclusion: (a) ChIP assays showed that PIF1, PIF3, and PIF7 do not bind to their target promoters in the presence of red light-activated phytochromes, but bind efficiently to promoters if phytochromes are inactivated by a far-red light (Li et al., 2012b; Park et al., 2012); (b) *in vitro* binding assays showed that the Pfr of recombinant phyB inhibits the bindings of recombinant PIF1 and PIF3 to their target promoter fragments (Park et al., 2012); and (c) the Pfr of the N-terminal domain of phyB, which is capable of inducing light responses (Matsushita et al., 2003; Oka et al., 2004), also inhibits the DNA binding of PIFs both *in vitro* and *in vivo* (Park et al., 2012). Interestingly, the Pfr of the N-terminal domain of phyB does not promote the degradation of PIF3 *in vivo*, supporting that this Pfr counters the negative roles of PIFs by inhibiting their DNA binding without promoting their protein degradation. Third, light-activated phytochromes indirectly inhibit the DNA binding of PIFs through other proteins, such as HFR1 and DELLAs, which are transcriptional regulators responsible for repressing GA responses. HFR1 has been shown to heterodimerize with PIFs to inhibit their DNA binding (Hornitschek et al., 2009), whereas DELLAs inhibit the DNA binding of PIFs by interacting with them (de Lucas et al., 2008; Feng et al., 2008). When phytochromes are activated by red light, HFR1 and DELLA proteins are stabilized *via* the inhibition of COP1 (Yang et al., 2001; 2005) and the reductions in GA levels (Achard et al., 2007), respectively. More detailed descriptions of how DELLAs inhibit the DNA binding of PIFs can be found in other reviews (Daviere et al., 2008; Hartweck, 2008).

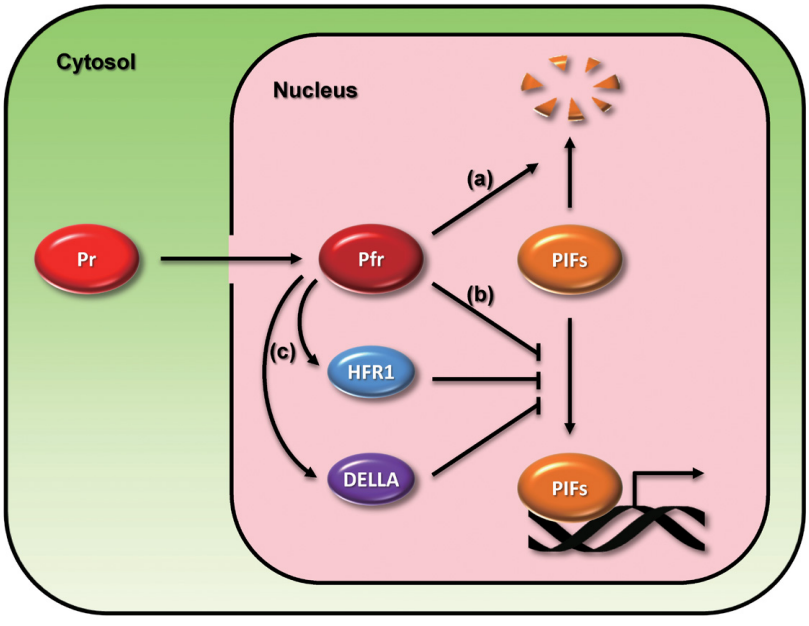

Fig. 1. Inhibition of PIF proteins by light-activated phytochromes. Light-activated phytochromes (Pfr) inhibit PIF proteins in three different ways: (a) Pfr induces phosphorylation, ubiquitilation and degradation of PIFs; (b) Pfr directly inhibits the DNA binding of PIFs independent of PIF degradation; and (c) Pfr indirectly inhibits the DNA binding of PIFs by stabilizing HFR1 and DELLAs, which interact with PIFs and inhibit their DNA binding.

Table 1. Biological roles of PIFs and related genes in Arabidopsis and rice

Gene symbol	Accession code	Biological roles
PIF1	AT2G20180	Seed germination (Oh et al., 2004), skotomorphogenesis[a]
PIF3	AT1G09530	Ethylene-induced hypocotyl elongation (Zhong et al., 2012), skotomorphogenesis[a]
PIF4	AT2G43010	High temperature-induced hypocotyl elongation and flowering (Franklin et al., 2011; Koini et al., 2009; Kumar et al., 2012), stomatal development (Casson et al., 2009), cold acclimation (Lee and Thomashow, 2012), shade avoidance (Lorrain et al., 2008), skotomorphogenesis[a]
PIF5	AT3G59060	Shade avoidance (Lorrain et al., 2008), skotomorphogenesis[a]
PIF6	AT3G62090	Seed dormancy by splice variant and the inhibition of hypocotyl elongation (Penfield et al., 2010)
PIF7	AT5G61270	Shade avoidance (Li et al., 2012b), cold acclimation (Kidokoro et al., 2009; Lee and Thomashow, 2012)
PIF8	AT4G00050	
PIL1	AT2G46970	Circadian gating of shade avoidance (Salter et al., 2003)
HFR1	AT1G02340	Inhibition of PIFs (Hornitschek et al., 2009; Lorrain et al., 2009)
SPT	AT4G36930	Carpel development (Heisler et al., 2001), seed germination and hypocotyl elongation (Penfield et al., 2005), root growth (Makkena and Lamb, 2013)
ALC	AT5G67110	Fruit dehiscence (Rajani and Sundaresan, 2001)
bHLH23	AT4G28790	
bHLH56	AT4G28800	
bHLH119	AT4G28811	
bHLH127	AT4G28815	
OsPIL11	LOC_Os12g41650	Hypocotyl elongation in Arabidopsis (Nakamura et al., 2007), hypocotyl elongation inhibition in Tobacco (Li et al., 2012a)
OsPIL12	LOC_Os03g43810	Hypocotyl elongation in Arabidopsis (Nakamura et al., 2007)
OsPIL13	LOC_Os03g56950	Drought-induced internode growth Inhibition in Rice (Todaka et al., 2012), hypocotyl elongation in Arabidopsis (Nakamura et al., 2007)
OsPIL14	LOC_Os07g05010	Hypocotyl elongation in Arabidopsis (Nakamura et al., 2007)
OsPIL15	LOC_Os01g18290	Hypocotyl elongation in Arabidopsis (Nakamura et al., 2007)
OsPIL16	LOC_Os05g04740	
OsHFR	LOC_Os04g52770	
OsSPT	LOC_Os06g06900	

[a]Skotomorphogenesis includes hypocotyl elongation, negative gravitropic growth of hypocotyl, apical hook formation, and the inhibition of cotyledon opening and chlorophyll synthesis (Leivar et al., 2008a; Shin et al., 2009).

PIFs Play Shared and Distinct Roles in Arabidopsis

Phenotypic analyses of *pif* single and multiple mutants have indicated that different PIFs have shared and distinct roles in Arabidopsis (Table 1). Some responses are regulated mainly by a single PIF. For example, PIF1 is the major regulator that inhibits seed germination and hook and cotyledon opening in the dark (Leivar et al., 2012; Oh et al., 2004; Shin et al., 2009); PIF3 is the major regulator that promotes hypocotyl elongation in response to ethylene (Zhong et al., 2012); and PIF4 is the major regulator that mediates the ability of high temperature to promote hypocotyl elongation and early flowering (Franklin et al., 2011; Koini et al., 2009; Kumar et al., 2012). Other responses are regulated by two or more PIFs. For example, PIF1 and PIF3 are the major regulators that inhibit the expression of chlorophyll biosynthetic genes and promote hypocotyl negative gravitropism (Shin et al., 2009; Stephenson et al., 2009); PIF4 and PIF7 are the major regulators that diurnally repress *CBF* gene expressions (Lee and Thomashow, 2012); and PIF3, PIF4, PIF5, and PIF7 are the major regulators that promote hypocotyl elongation and shade avoidance responses (Leivar et al., 2008b; Li et al., 2012b; Lorrain et al., 2008). Similar shared and distinct roles have also been seen for other bHLHs. For example, HFR1 inhibits hypocotyl elongation under far-red light (Fairchild et al., 2000; Fankhauser and Chory, 2000; Soh et al., 2000), whereas ALC and SPT redundantly regulate the development of the valve margin and the dehiscence zone during gynoecium development (Groszmann et al., 2011). Quantitative analyses of various *pif* single and multiple mutants have demonstrated that different PIFs exert different degrees of regulatory power on the mRNA expression levels of the various target genes (Zhang et al., 2013). It has been speculated that these shared and distinct roles of PIFs could be due to differences in their intrinsic properties or mRNA expression patterns.

PIFs Differ in Their Intrinsic Protein Properties and mRNA Expression Patterns

Multiple lines of evidence indicate that PIF proteins intrinsically differ in their activities. For example, phyA and phyB bind to different PIFs with different affinities *in vitro*. Among the characterized PIFs, phyA binds strongly to PIF1 but weakly to PIF3,

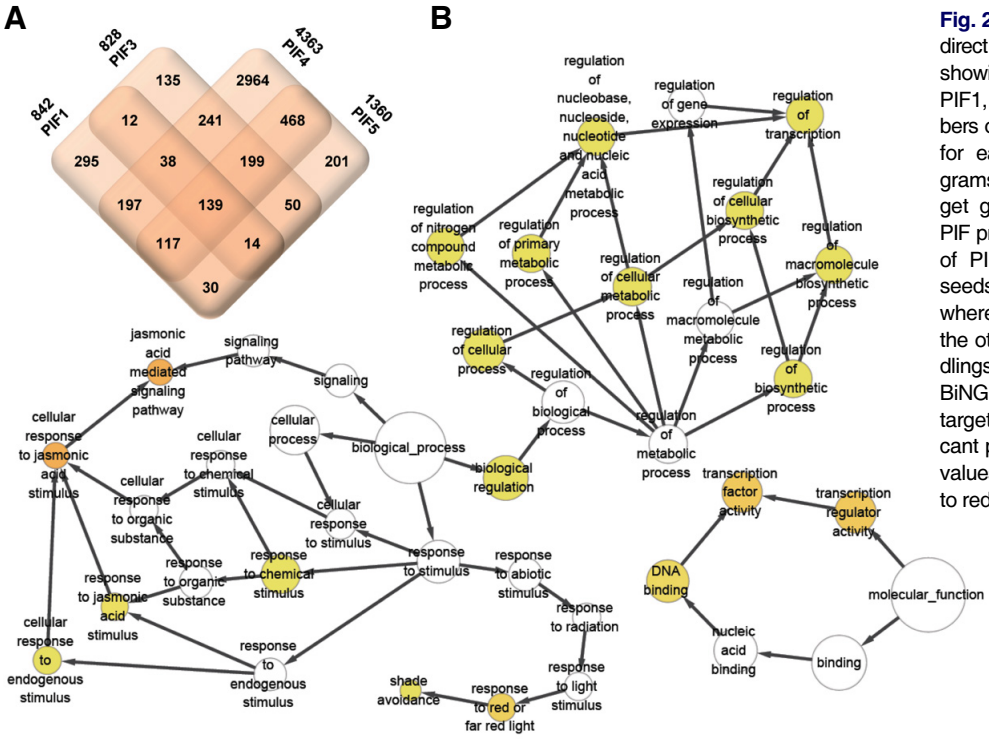

Fig. 2. PIFs have shared and distinct direct target genes. (A) Venn diagram showing the direct target genes of PIF1, PIF3, PIF4, and PIF5. The numbers of shared or unique target genes for each PIF are indicated in diagrams while the numbers of total target genes are indicated above the PIF protein names. Direct target genes of PIF1 were identified in imbibed seeds using a ChIP-Chip method, whereas the direct target genes of the other PIFs were identified in seedlings using ChIP-seq methods. (B) BiNGO analysis of 139 shared PIF target genes. Only statistically significant processes are indicated, with p-values color-coded from yellow (high) to red (low).

whereas phyB binds strongly to PIF1 and PIF3, but only moderately to PIF4 (Huq and Quail, 2002; Huq et al., 2004). PhyA does not bind to PIF5 or PIF7, whereas phyB binds to both of these PIFs (Leivar et al., 2008b; Shen et al., 2007). While we do not yet know if these different binding affinities lead to functional differences among PIFs *in vivo*, these observations show that PIFs have intrinsic differences in their affinities to bind different phytochromes. In addition, genome-wide DNA binding site analyses have indicated that PIFs also have shared and distinct intrinsic properties in promoter binding. ChIP-Chip and ChIP-seq analyses have identified the genome-wide DNA binding sites for PIF1, PIF3, PIF4, and PIF5 (Fig. 2), which reportedly have 842, 828, 4,363, and 1,360 direct target genes, respectively (Hornitschek et al., 2012; Oh et al., 2009; 2012; Zhang et al., 2013). This wide range of target gene numbers could reflect differences in the intrinsic binding properties of the various PIFs, differences in the experimental conditions (e.g., the use of seeds vs. seedlings), or differences in analytical methods (e.g., the stringency of the criteria used for peak identification). Because of the heterogeneities in the reported analyses, it is difficult to make a conclusive comparison. Nevertheless, it is noteworthy that 139 of the identified target sites are shared by all four PIFs, suggesting that these PIFs have shared properties that allow them to choose common target sites. Meanwhile, even when the comparison includes the numerous PIF4 target genes (4,363), specific target sites are found for PIF1 (295), PIF3 (135), PIF4 (2,964), and PIF5 (201), indicating that these four PIFs have distinct binding targets. BiNGO analysis of the 139 shared target genes indicates that they are enriched for processes such as transcription regulator activity, hormone signaling pathways, and response to red and far-red light (Maere et al., 2005). These findings are consistent with the notion that PIFs coordinate various hormone signals as master transcription factors, thereby regulating various physiological and developmental processes. Most of these genome-wide binding analyses were performed with transgenic plants expressing PIFs under the control of the constitutively active CaMV *35S* promoter (In the case of PIF4 ChIP-seq, *PIF4* promoter was used). Thus, the identification of both shared and distinct direct target sites indicate that the four PIF proteins have both shared and distinct intrinsic properties for DNA binding, which may be at least partly responsible for their shared and distinct roles.

The different expression patterns of *PIF* mRNAs are also partly responsible for the shared and distinct roles of PIFs in Arabidopsis. PIF1 is a good example of how the mRNA expression pattern dictates the biological role of a PIF. The *pif1* mutants germinate even in the absence of light-activated phytochromes but have hypocotyl lengths similar to those of wild-type plants under red light, indicating that PIF1 is a major regulator of seed germination but not hypocotyl elongation (Oh et al., 2004). However, overexpressed PIF1 is capable of promoting hypocotyl elongation (Oh et al., 2004). This phenotypic discrepancy between the mutants and overexpression lines reflects that the *PIF1* mRNA is strongly expressed in imbibed seeds but not in seedlings. PIF3, PIF4, and PIF5 provide additional examples of mRNA expression patterns that specify the roles of PIFs in plants. PIF3 is the major regulator of ethylene-induced hypocotyl elongation under light conditions (Zhong et al., 2012); PIF4 is the major regulator of high temperature-induced hypocotyl elongation and early flowering (Franklin et al., 2011; Koini et al., 2009; Kumar et al., 2012); and PIF4 and PIF5 are major regulators of rhythmic growth during hypocotyl elongation (Nozue et al., 2007). Examination of their mRNA expression patterns has revealed that *PIF3*, *PIF4*, and *PIF5* are the major *PIF*s whose mRNAs expression levels are induced by the specific conditions under which their actions are seen: the *PIF3* mRNA is induced by ethylene (Zhong et al., 2012); the *PIF4*

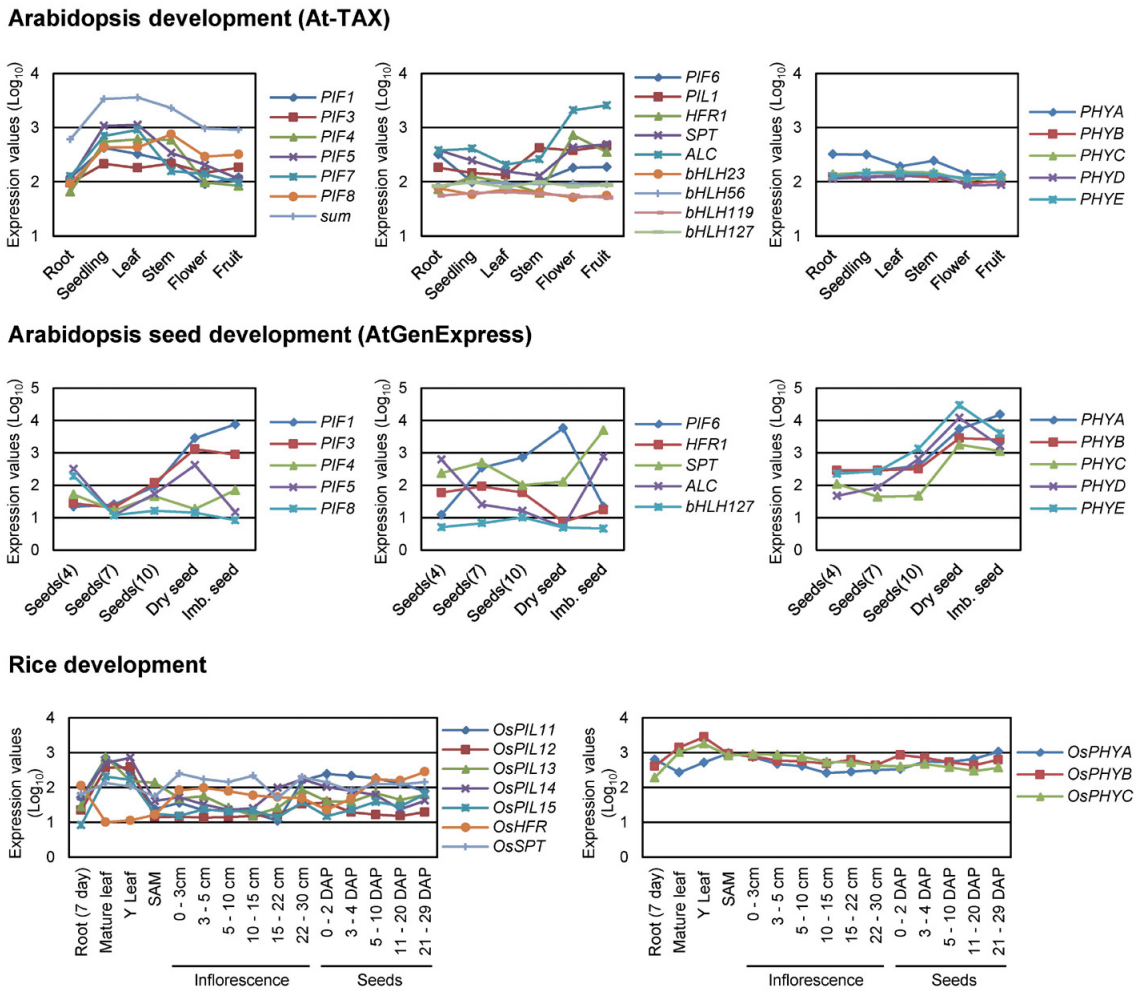

Fig. 3. PIFs have shared and distinct expression patterns throughout the life cycle of plants. Pre-processed expression values were obtained from tileviz (http://jsp.weigelworld.org/tileviz, At-TAX) and AVT (http://jsp.weigelworld.org/expviz, AtGenExpress) for Arabidopsis, and from rice PLEXdb (http://www.plexdb.org/plex.php?database=Rice, Gene expression atlas, OS5) for rice. Numbers in parentheses following seeds in the seed development indicate the developmental stages of maturing seeds. Seeds sample of stage 4 contains the silique tissue while other seeds do not. Gene expression data for dry and 24-h-imbibed (imb.) seeds was obtained from 'AtGE hormones' in AVT.

mRNA is induced by high temperature (Franklin et al., 2011; Koini et al., 2009; Kumar et al., 2012); and the *PIF4* and *PIF5* mRNAs are expressed during the dawn phase of short days (Nozue et al., 2007). Other examples include ALC and SPT. Phenotypic analyses of *alc* and *spt* single mutants have suggested that ALC is necessary for the development of the dehiscence zone (Rajani and Sundaresan, 2001), while SPT is necessary for the development of carpel margin tissues (Heisler et al., 2001). The *alc spt* double mutant further aggravates these mutant phenotypes, indicating that the proteins redundantly regulate these processes (Groszmann et al., 2011). Furthermore, when overexpressed under control of the *35S* promoter, *ALC* and *SPT* can partly complement the *spt* and *alc* mutants, respectively, supporting the notion that the mRNA expression patterns of *ALC* and *SPT* are partly responsible for their shared and distinct roles in Arabidopsis (Groszmann et al., 2011).

The Expression Levels of *PIF*s are Developmentally Regulated Throughout the Plant Life Cycle

The mRNA expression patterns of *PIF*s have not yet been systematically reviewed. To provide an overview of how *PIF*s are expressed throughout the life cycle of plants, we extracted publically deposited expression data and analyzed the expression patterns of *PIF* mRNAs. Arabidopsis data were obtained from At-TAX (Arabidopsis thaliana Tiling Array Express), which is based on whole-genome tiling arrays that cover all Arabidopsis genes (Laubinger et al., 2008), and AtGenExpress, which is based on Affymetrix gene chips lacking probes for five genes (*PIL1*, *PIF7*, *bHLH23*, *bHLH56*, and *bHLH119*) (Schmid et al., 2005). Rice data were obtained from rice PLEXdb (Plant Expression Database), which is based on Affymetrix 57k Rice GeneChips lacking a probe for *OsPIL16* (Dash et al., 2012).

*PIF*s are expressed differentially during development (Fig. 3). Among the Arabidopsis *PIF*s, six 'shoot' *PIF*s (*PIF1*, *PIF3*, *PIF4*,

PIF5, *PIF7*, and *PIF8*) show similar expression patterns characterized by higher expression in the seedling and leaf compared to the root, flower or fruit. In contrast, five 'fruit' *PIF*s (*PIL1*, *PIF6*, *HFR1*, *SPT*, and *ALC*) show similar expression patterns characterized by higher expression in the flower and fruit compared to the seedling and leaf. The remaining *bHLH*s (*bHLH23*, *bHLH56*, *bHLH119*, and *bHLH127*) are expressed at relatively low levels throughout the plant's life cycle. Among the shoot *PIF*s, *PIF5* shows the highest expression, followed by *PIF7*, *PIF4*, *PIF8*, *PIF1*, and *PIF3*. *PIF3* is expressed at similarly low levels among the different organs, whereas *PIF8* (whose role has not yet been characterized) is expressed at relatively high levels in all of the aboveground organs. When the expression levels of all shoot *PIF*s are combined, the summed *PIF* expression is about 5-fold higher in seedlings and leaves compared to roots, providing additional evidence that the shoot PIFs function mainly in the seedlings and leaves. Consistent with their predicted stages and sites of function, PIF3, PIF4, PIF5, and PIF7 have been shown to regulate hypocotyl elongation, shade-avoidance responses, and leaf development (Casson et al., 2009; Leivar et al., 2008b; Li et al., 2012b; Lorrain et al., 2008). *PIF8* has not yet been characterized, but its expression pattern suggests that it may also play roles in seedling and leaf development. Among the fruit *bHLH*s, *ALC* shows the highest expression, followed by *SPT*, *PIL1*, *HFR1*, and *PIF6*. The high expression levels of *ALC* and *SPT* are consistent with their identified roles in fruit development (Groszmann et al., 2011), where they are necessary for the development of the valve margin and the dehiscence zone during gynoecium development (Heisler et al., 2001; Rajani and Sundaresan, 2001). *SPT* is also expressed at a relatively high level in the root where it regulates the size of root meristem and primary root growth (Makkena and Lamb, 2013). The roles of the other fruit bHLHs have not yet been deciphered. An interesting deviation between the identified roles and expression patterns is seen for *HFR1*. Although it is more highly expressed in the flower and fruit compared to the seedling and leaf, it is known to be a key regulator of hypocotyl elongation and shade avoidance responses (Fairchild et al., 2000; Fankhauser and Chory, 2000; Sessa et al., 2005; Soh et al., 2000). To date, no report has shown that HFR1 plays a role in flowers or fruits. Although this seeming discrepancy can be accounted for by the strong induction of *HFR1* mRNA under far-red light (Soh et al., 2000) or shade in seedlings (Sessa et al., 2005), it would be interesting to carefully examine the involvement of HFR1 in flower and fruit development.

*PIF*s are also differentially expressed during seed development (Fig. 3), as assessed using AtGenExpress data. In siliques (seed stage 4), *PIF5*, *PIF8*, *ALC*, and *SPT* are expressed at relatively high levels (expression value > 100), whereas *PIF1*, *PIF3*, *PIF4*, *PIF6*, *HFR1*, and *bHLH127* are expressed at relatively low levels (expression value < 100). The high-level expression of *ALC* and *SPT* is consistent with their roles in gynoecium development (Groszmann et al., 2011), whereas the functional significance of PIF5 and PIF8 in silique is not known. In developing seeds from stage 7 to stage 10, the expression of *PIF1*, *PIF3*, *PIF5*, and *PIF6* increases as the seeds mature, whereas the expression levels of the other genes either decrease slightly (*HFR1*, *SPT*, and *ALC*) or remain the same (*PIF4*, *PIF8*, and *bHLH127*). Interestingly, the expression levels of *PHYD* and *PHYE* also increase during seed maturation. The functional implication of this simultaneous increase in phytochrome and *PIF* mRNAs is not yet known. When we compared the expression levels in dry seeds, we observed that three genes (*PIF1*, *PIF3*, and *PIF6*) are expressed at very high levels (expression value > 1,000), two genes (*PIF5* and *SPT*) are expressed at relatively high levels (100 < expression value < 1000), and the remaining five genes (*PIF4*, *PIF8*, *HFR1*, *ALC*, and *bHLH127*) are expressed at relatively low levels. During the seed imbibition, the expression levels of *PIF5* and *PIF6* decrease more than 10-fold, whereas those of *SPT* and *ALC* increase more than 10-fold. In imbibed seeds, *PIF1* and *SPT* show expression values higher than 4,000, while those of *PIF3* and *ALC* are lower than 1,000, and those of the remaining six genes are lower than 100. The expression patterns of *PIF*s are consistent with some of their identified roles in seeds. In agreement with the high-level expression of *PIF6* mRNA during seed development and in dry seeds, mutation of *PIF6* was shown to increase seed dormancy, while overexpression of an alternatively spliced *PIF6* decreased seed dormancy (Penfield et al., 2010). The ability of PIF1 and SPT to inhibit seed germination is also consistent with their high expression levels in imbibed seeds (Oh et al., 2004; Penfield et al., 2005). The roles of the other PIFs during seed maturation and germination remain to be further elucidated.

Rice *PIL*s (*OsPIL11* to *OsPIL15*) also show differential expression during development (Fig. 3). Similar to the expression patterns of the Arabidopsis shoot *PIF*s, the expression levels of all *OsPIL*s are more than 10-fold higher in the leaf than in the root or inflorescence. This high-level expression of *OsPIL*s in leaves suggests that (similar to the Arabidopsis shoot *PIF*s) they might function in leaves. Some *OsPIL*s are also expressed during seed development: *OsPIL11* is highly expressed (expression value > 100) in developing seeds; *OsPIL13*, *OsPIL14*, and *OsPIL15* are moderately expressed in developing seeds; and *OsPIL12* shows a relatively low expression level in seeds. When overexpressed in Arabidopsis, OsPIL11 to OsPIL15 were found to promote hypocotyl elongation, indicating that they are also similar to the Arabidopsis shoot PIFs in their abilities to promote hypocotyl elongation (Nakamura et al., 2007). The overexpression of *OsPIL13* promoted internode elongation in rice, whereas the overexpression of repression domain-fused *OsPIL13* inhibited this elongation, further supporting the notion that some OsPILs may promote cell elongation in their native plants (Todaka et al., 2012). Unlike the shoot PIFs, however, OsPIL13 did not interact with rice phyB in a yeast two-hybrid assay (Todaka et al., 2012). Further studies are needed to determine if any of the OsPILs interact with phytochromes and regulate light responses in rice.

The Expression Levels of *PIF*s are Regulated by Various Internal and External Stimuli

Dynamic regulation of *PIF* mRNAs could be found in response to various stimuli such as diurnal cycle, circadian clock, phytohormones, and abiotic stress. We exploited published microarray data from DIURNAL project (Filichkin et al., 2011; Mockler et al., 2007), AtGenExpress (Schmid et al., 2005), At-TAX (Zeller et al., 2009), and rice PLEXdb (Dash et al., 2012) which are based on either affymetrix gene chip or genome tiling array. In DIURNAL, oscillating transcripts are identified using the best matching oscillation model with a specific correlation cutoff (Mockler et al., 2007).

Both Arabidopsis *PIF*s and rice *PIL*s are mostly expressed rhythmically in L12/D12 diurnal cycles (correlation coefficient > 0.8, Fig. 4). Two notable exceptions are *OsPIL14/15* and *OsHFR*, which do not display diurnal expression patterns. When the plants entrained in L12/D12 are subjected to continuous light condition, a few *PIF*s and their related genes are still expressed

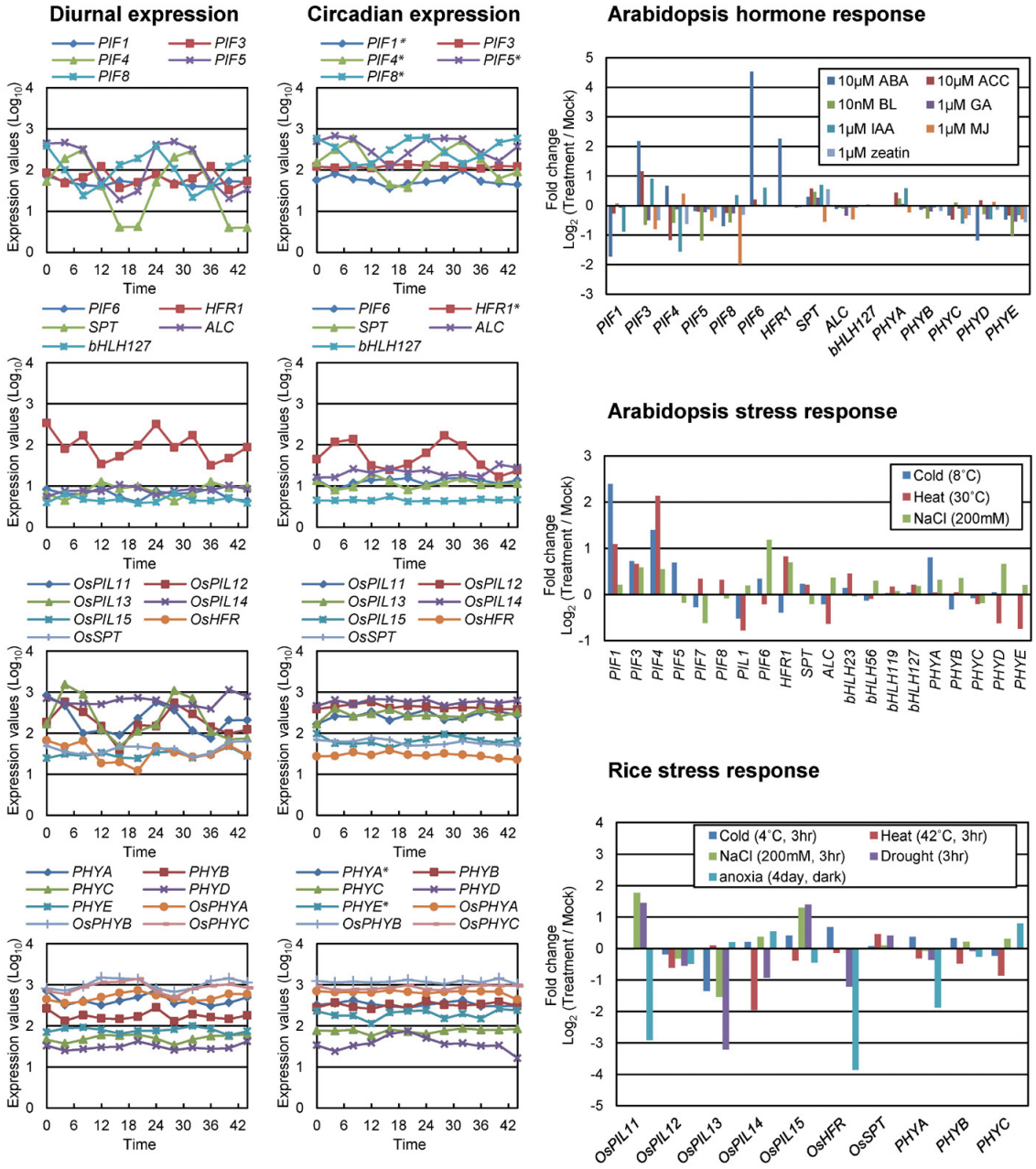

Fig. 4. PIFs have shared and distinct expression patterns in response to diurnal cycles, hormones, and abiotic stress. Diurnal and circadian expression data were obtained from COL LDHH (Arabidopsis diurnal), LL12_LDHH (Arabidopsis circadian), LDHH (rice diurnal), and LLHH_LDHH (rice circadian) in DIURNAL project version 2.0 (http://diurnal.mocklerlab.org). Oscillating genes by circadian clock are indicated with asterisks. Expression data for hormone and abiotic stress treatments were obtained from tileviz, AVT and rice PLEXdb, and expression patterns are presented as fold changes between hormone/stress treatment and mock treatment. For Arabidopsis, 7-day-old continuous light-grown seedlings were used to assess hormone responses (3 h), and 10-day-old continuous light-grown seedlings were used to assess abiotic stress responses (1 h). For rice abiotic stress responses, the fold changes between stress treatment and control treatment were derived from the OS10 (cold, salt and drought), OS14 (anoxia), and OS25 (heat) modules of rice PLEXdb.

rhythmically. These circadian-regulated genes include *PIF1* (peak at ZT5), *PIF4* (ZT7), *PIF5* (ZT5), *PIF8* (ZT22), and *HFR1* (ZT6). Among these genes, *PIF4* and *PIF5* are previously known to be regulated by internal circadian clock (Nozue et al., 2007; Yamashino et al., 2003). Interestingly, the *HFR1* mRNA shows a similar oscillation pattern with the *PIF4* and *PIF5* mRNAs. Since *HFR1* gene is a direct target of PIFs encoding atypical bHLH transcription factor that inhibits PIF activity through heterodimerization (Hornitschek et al., 2009), this expression pattern may reflect a feedback regulatory circuit be-

tween PIFs and HFR1. Unlike Arabidopsis *PIF*s, none of *OsPIL*s display a circadian-regulated mRNA pattern.

*PIF*s are also differentially expressed in response to hormones and abiotic stress (Fig. 4). Among hormones, abscisic acid (ABA) activates the expression of *PIF3*, *PIF6*, and *HFR1*, but represses *PIF1*; ACC (ethylene precursor) activates *PIF3* but represses *PIF4*; brassinolide (BL), auxin (IAA), and methyl jasmonate (MeJA) repress *PIF5*, *PIF4*, and *PIF8*, respectively, indicating that the shoot *PIF*s differentially respond to different hormones. The activation of *PIF3* mRNA expression by ACC was shown to be important for its role in ethylene-induced hypocotyl elongation in Arabidopsis (Zhong et al., 2012). The functional significance of other *PIF* expression patterns in response to hormones has not been determined. Abiotic stresses also regulate the expression of *PIF*s. Among the examined abiotic stresses, cold treatment activates the expression of *PIF1*, *PIF4*, and *PIF7*, but represses *OsPIL13*; heat activates *PIF1* and *PIF4* but represses *OsPIL14*; salt activates *PIF6*, *OsPIL11*, and *OsPIL15*, but represses *OsPIL13*; drought activates *OsPIL11* and *OsPIL15*, but represses *OsPIL13* and *OsHFR*; Anoxia represses *OsPIL11* and *OsHFR*. The activations of *PIF4* expression by heat was shown to be important for its role in heat-induced hypocotyl elongation in Arabidopsis (Franklin et al., 2011; Koini et al., 2009; Kumar et al., 2012), while the repression of *OsPIL13* mRNA by drought was also shown to be important for drought-induced repression of stem elongation in rice (Todaka et al., 2012). The functional significance of other expression patterns are not fully understood, but these expression patterns might give clues on their biological roles. For example, anoxia strongly represses the *OsHFR* mRNA. Since anoxia promotes the elongation of rice coleoptiles, it will be interesting to determine if the repression of *OsHFR* by anoxia contributes the elongation of coleoptiles by anoxia.

CONCLUSION

Our brief survey indicates that PIFs and related genes have shared and distinct biological roles arising from their shared and distinct intrinsic protein properties and gene expression patterns. Genome-wide ChIP data indicate that different PIFs bind to shared and distinct target sites. Since PIFs are transcription factors, the binding to a specific promoter is likely to alter the expression of a target gene, supporting the notion that the shared and distinct roles of PIFs partly stem from the nature of their target sites. Furthermore, expression map analysis indicates that PIFs are expressed in shared and distinct developmental stages and organs, indicating that the shared and distinct roles of PIFs also partly stem from their expression patterns.

Most Arabidopsis *PIF*s and rice *OsPIL*s are mainly expressed in seedlings and leaves and show expression patterns consistent with their identified roles, which include promoting cell elongation, inhibiting chlorophyll biosynthesis, and promoting shade-avoidance responses. However, the expression patterns of *PIF*s are not monolithic; rather, different *PIF*s show wide variations in their developmental expression patterns and expression levels in each organ. The gene expression data also indicate that different *PIF*s respond either similarly or distinctively to the diurnal cycle, plant hormones, and abiotic stress. Combined with the dynamic post-translational regulations of PIF proteins, the wide variations in their mRNA expression patterns place the PIFs among the most dynamic plant transcription factors making connection points for developmental and environmental signals to merge in shaping the plant life cycle.

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