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Phytochrome Interacting Factors: central players in phytochrome-mediated light signaling networks☆

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To adapt to the surrounding environment, plants constantly monitor and respond to changes in the red and far-red regions of the light spectrum through the phytochrome family of photoreceptors. Extensive efforts using genetic, molecular and photobiological techniques have led to the identification of a group of basic helix–loop–helix transcription factors called the Phytochrome Interacting Factors, PIFs, which directly bind to the photoactivated phytochromes. Members of the PIF family have been shown to control light-regulated gene expression directly and indirectly. PIF1, PIF3, PIF4 and PIF5 are degraded in response to light signals, and physical interaction of PIF3 with phytochromes is necessary for the light-induced phosphorylation and degradation of PIF3. PIFs constitute an excellent model for the investigation of the biochemical mechanisms of signal transfer from photoactivated phytochromes and the light-regulation of gene expression that controls photomorphogenesis in plants.

Phytochrome-mediated light signaling

Light is a key environmental factor that regulates plant growth and development. It is involved in controlling multiple responses in the plant life cycle, including seed germination, seedling de-etiolation, phototropism, shade avoidance, circadian rhythms and flowering time (collectively termed as photomorphogenesis). Plants have evolved mechanisms to detect the presence or absence of light in addition to the duration, wavelength and intensity of incident light. They detect light through an array of photoreceptors, each responding to specific regions of the light spectrum. The phytochrome (phy) family perceives and responds to the red and far-red regions, whereas phototrophins and cryptochromes perceive and respond to the UVA and blue regions [1]. Furthermore, there is also evidence for the existence of an unidentified UVB-light receptor(s) and an additional UVA and blue-light receptor(s) [2–4]. The integration of the light signals captured by these photoreceptors modulates plant development.

The phytochrome family in *Arabidopsis thaliana* is composed of five members (phyA, phyB, phyC, phyD and phyE) with unique and overlapping photosensory characteristics and biological functions [5,6]. Phytochromes are present as homodimers and heterodimers *in vivo* [7], and each subunit consists of a ~125 kDa polypeptide covalently linked to an open-chain tetrapyrrol chromophore, phytochromobilin [8]. They exist in two reversible conformations that have different spectroscopic and functional characteristics: the red-light-absorbing Pr form (biologically inactive), and the far-red-light-absorbing Pfr form (biologically active). Red light induces a Pr to Pfr conformational shift that exposes a nuclear localization signal and promotes translocation of the Pfr form into the nucleus [9,10]. It has been demonstrated that nuclear translocation is necessary for the majority of the biological functions of phyA and phyB [11–14]. However, phyA also shows distinct cytosolic functions, such as controlling negative gravitropism in blue-light- and red-light-enhanced phototropism [14]. In the nucleus, phytochromes localize to speckles or nuclear bodies [15] and trigger a transcription cascade that leads to the regulation of light-responsive genes. Approximately 2500 genes in *Arabidopsis thaliana* (10% of the genome) are regulated by phytochromes under prolonged light exposure, whereas ~250 genes are regulated at least two-fold under continuous red light within 1 h [16,17]. How phytochromes can mediate this large response to light has been the subject of intensive research. Genetic, biochemical and molecular studies have identified both positively and negatively acting factors in the phytochrome signaling pathways. Recent evidence suggests that one mode of phytochrome signaling is initiated by the direct interaction of the biologically active forms of phytochromes with members of the basic helix–loop–helix (bHLH) transcription factor superfamily called the Phytochrome Interacting Factors (PIFs). This review will focus on the PIF family of phytochrome signaling factors. For a detailed review on phytochrome signaling in plants, readers are directed to several recently published articles [8,17,18].

Identification and characteristics of PIFs

The founding member of the PIF family is PIF3. PIF3 was isolated from a yeast two-hybrid (Y2H) screen using the C-terminal domain of phyB as bait [19]. Subsequently, it was shown, using Y2H and *in vitro* coimmunoprecipitation (co-IP) assays that PIF3 interacts with the C-terminal domains of both phyA and phyB from *A. thaliana* and rice. Moreover, using elegant *in vitro* co-IP and yeast two-hybrid

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assays, it was shown that PIF3 selectively interacts with the full-length biologically active Pfr forms of both phyA and phyB [20–22]. PIF3 showed higher affinity to the Pfr form of the N-terminal domain (amino acid residues 1–645) than the non-photoactive C-terminal domain (amino acid residues 645–1211) of phyB [20,22]. Both N- and C-terminal domains of phyB contribute to the strong Pfr-specific interaction of PIF3 with full-length phyB [20]. It is still unclear which regions of phyA are involved in the interaction with PIF3. Taken together, these results established PIF3 as the first phytochrome-interacting signaling factor positioned to receive the light signal from photoactivated phytochrome molecules.

The second member of the PIF family, PIF4, was isolated by the convergence of both genetic and reverse-genetic approaches [23]. With the advent of genome sequencing, several other PIFs were also identified by sequence homology to PIF3, and named PIF1, PIF5 and PIF6 [24,25]. Owing to sequence similarities to PIF3, several of these factors were also named as PIF3-like factors (PILs). For example, PIF1, PIF5 and PIF6 were also called PIL5, PIL6 and PIL2, respectively (Table 1) [26]. All the PIFs identified so far are members of the *A. thaliana* bHLH subfamily 15 [27,28]. However, not all bHLHs in subfamily 15 interact with phytochromes and, therefore, are not PIFs. PIF1, PIF3 and PIF6 have a stronger affinity for the Pfr form of phyB compared to PIF4 and PIF5 (Figure 1a). Moreover, only PIF1 and PIF3 interact with the Pfr form of phyA, and PIF1 showed a much stronger affinity for phyA than PIF3. These data suggest that PIFs might interact with multiple phytochromes with differential affinities and might transduce light signals with varying efficiency to control gene expression.

PIFs, being members of the bHLH superfamily, have a signature bHLH domain consisting of two distinct regions: an ~15 amino acid basic region involved in binding to the target DNA, and an ~60 amino acid HLH region involved in dimerization. Through their basic regions, bHLH factors bind a *cis*-acting regulatory element found in the promoter region of target genes. The majority of the bHLHs bind to a *cis*-element called an E-box (5′-CANNTG-3′). There are different types of E-boxes depending on the central two nucleotides. PIF1, PIF3 and PIF4 bind specifically to a subtype of the E-box called the G-box (5′-CACGTG-3′) (Figure 1b) [23,24,29]. However, sequence divergence outside of the G-box might confer further binding specificity for these bHLH factors as observed for animal bHLHs [30,31].

The HLH region allows the formation of homodimers and/or heterodimers. Some factors can form heterodimers with multiple partners, increasing the diversity of these regulatory proteins. PIF3 can homodimerize, but can also heterodimerize with PIF4 (Figure 1b) [27]. Both the PIF3–PIF3 homodimer and the PIF3–PIF4 heterodimer can bind to the G-box DNA sequence elements [27]. PIFs can also heterodimerize with other non-PIF bHLHs. PIF3 heterodimerizes with HFR1, an atypical bHLH factor, functioning positively in far-red- and blue-light signaling pathways [32,33]. However, the functional significance of these heterodimerizations is not clear.

Sequence alignments showed that PIFs share in common a conserved sequence motif at their N-terminal region, designated as the active phytochrome-binding (APB) motif [25]. Site-directed mutagenesis showed that four invariant amino acid residues (ELxxxxGQ) common in all PIFs are critical determinants of the APB motif. This motif is both necessary and sufficient for binding to the biologically active Pfr form of phyB [25]. Moreover, bHLH23, another member of subfamily 15 closely related to PIFs, has a natural mutation in a key residue (G to S) of the APB motif, and does not bind phyB, suggesting that the conserved sequence at the APB motif is important for phytochrome interaction. However, despite the presence of the invariant residues in the APB-like motif, several other closely related bHLH proteins in subfamily 15 (e.g. PIL1, bHLH56, bHLH72, bHLH16 and bHLH127) did not show interaction with phyB in *in vitro* co-IP assays [25]. It is possible that these factors have relatively weak affinities for phyB and, therefore, fail to show interaction in the *in vitro* assay. Alternatively, sequences within and around the APB motif can also play significant roles in determining the affinity for phyB, and these factors might lack additional appropriate residues for interaction with phyB. Further characterization of crucial amino acid residues using site-directed mutagenesis is necessary to determine the sequence requirement in the APB motif for the Pfr-specific interaction with phyB.

Among the PIFs, only two members, PIF1 and PIF3, bind to the Pfr form of phyA in *in vitro* co-IP and

Table 1. Light specificity and biological functions of the PIF family members

PIF#	AGI#	AtbHLH #	APB/APA domain	Light specificity[a]	Biological functions	Refs
PIF1/PIL5	At2g20180	bHLH015	+/+	R/FR	Hypocotyl elongation Cotyledon expansion Hypocotyl gravitropism in the dark Seed germination Chlorophyll biosynthesis	[24,37]
PIF3	At1g09530	bHLH008	+/+	R	Hypocotyl elongation Cotyledon expansion Hypocotyl gravitropism in the dark Chlorophyll biosynthesis Anthocyanin biosynthesis	[19,37–39,44]
PIF4	At2g43010	bHLH009	+/−	R	Hypocotyl elongation Cotyledon expansion	[23]
PIF5/PIL6	At3g59060	bHLH065	+/−	R	Hypocotyl elongation Cotyledon expansion	[25,26,40]
PIF6/PIL2	At3g62090	bHLH132	+/−	Unknown	Unknown	[25,26]

[a]FR, far-red light; R, red light.

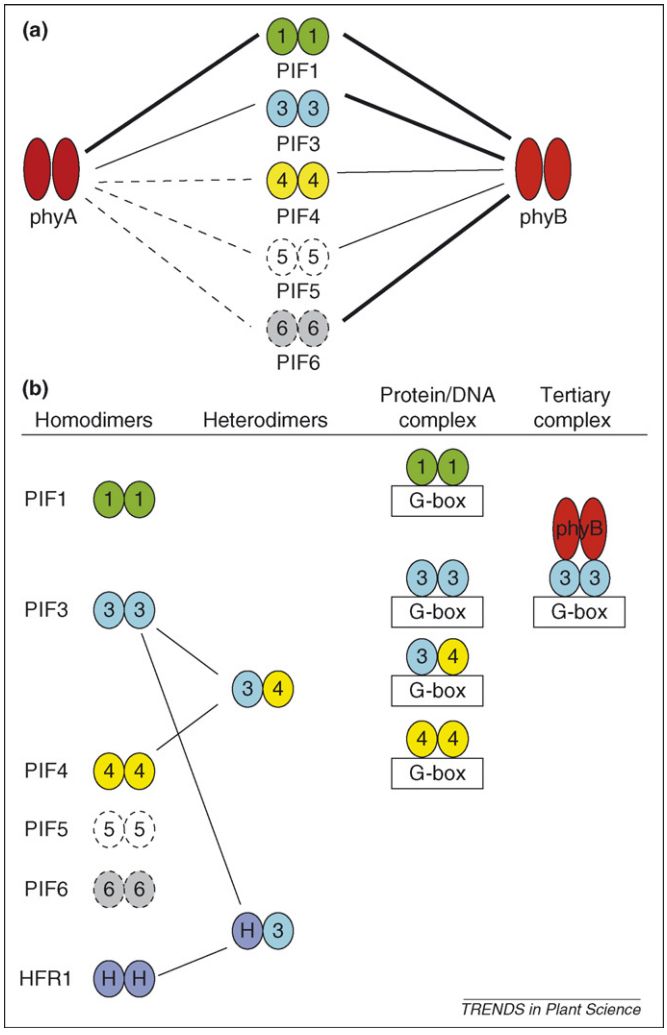

Figure 1. Physical interactions of PIFs with phytochromes and with other PIFs. **(a)** PIFs selectively interact with the biologically active forms of phyA and phyB with differential affinity. The strengths of the interactions are represented by the relative thickness of the connecting lines [24,25]. Thicker connecting lines indicate stronger interactions than thinner connecting lines. Dashed lines indicate very weak affinity. **(b)** Homodimerization and heterodimerization among PIFs and with a non-PIF bHLH factor involved in light signaling pathways. Homodimers and heterodimers of PIFs also bind G-box DNA-sequence elements. Only PIF3 binds the G-box and the Pfr form of phyB simultaneously. Homodimerization of PIF5 and PIF6 has not been confirmed.

light-dependent Y2H assays [21,22,24] (E. Huq, unpublished). PIF1 showed much stronger affinity for phyA than PIF3. However, the sequence motif necessary for phyA interaction is not as conserved as the APB motif. A putative active-phyA (APA)-binding motif is necessary for the interaction between PIF3 and phyA in a light-dependent Y2H assay [34]. Moreover, this domain is necessary for the red-light-induced degradation of PIF3 *in vivo*. Two phenylalanine residues (F203 and F209 in PIF3) are important determinants in the interaction between PIF3 and phyA, as site-directed mutagenesis of these two phenylalanine residues to alanine residues abolished this interaction. Although a similar motif is present in PIF1, site-directed mutagenesis of two similar phenylalanine residues in PIF1 did not reduce interaction between PIF1 and phyA in *in vitro* co-IP assays (E. Huq, unpublished), suggesting that the APA motif is not highly conserved in PIFs. phyA has been shown to interact with other proteins (e.g. PKS1 and NDPK2) in a light-independent or weakly light-dependent manner [35,36]. These proteins do not share any sequence homology to PIFs. A sequence alignment of all the phyA-interacting proteins also failed to identify any conserved motif (E. Huq, unpublished). Therefore, it appears that the sequence of the APA motif might be more diverse than that of the APB motif. Isolation and characterization of additional factors that selectively bind to the Pfr form of phyA is necessary to identify a conserved APA motif.

Biological functions of PIFs

Although PIFs are highly similar in sequence and overall motif structure, the monogenic *pif* mutants show unique as well as common morphological phenotypes in light signaling pathways. This suggests that they do not act redundantly, as is the case for many gene families, and that they have overlapping as well as distinct biological functions [23,24,37–40].

PIF1 is involved in a variety of biological processes ranging from the repression of light-induced seed germination, the light-induced inhibition of hypocotyl elongation, the hypocotyl negative gravitropism in the dark to chlorophyll accumulation in light. *pif1* mutants germinate after far-red-light exposure, indicating a disruption in the maintenance of dormancy [37]. *pif1* mutants also exhibit slightly shorter hypocotyl length under alternating far-red-light and dark cycles and reduced hypocotyl negative gravitropism in the dark compared to wild type [37]. *pif1* seedlings exhibit photooxidative damage (bleaching) and fail to green when dark-grown seedlings are transferred to light [24]. This phenotype is more severe if the seedlings are kept in darkness for longer times before being transferred to light. The *pif1* bleaching phenotype is the result of the accumulation of four- to sixfold more protochlorophyllide, a phototoxic chlorophyll precursor, over wild-type levels. Furthermore, *pif1* mutants accumulate higher levels of chlorophyll when young dark-grown seedlings are transferred to light, suggesting that PIF1 acts as a negative regulator of chlorophyll biosynthesis [24].

PIF3 functions to control morphological phenotypes and biochemical pathways in response to light. The initial characterization of PIF3 involved antisense lines that showed a hyposensitive phenotype under continuous red light, suggesting that PIF3 functions positively in controlling photomorphogenesis [19]. However, several independently isolated *pif3* alleles (obtained by T-DNA insertion and fast-neutron-induced deletion) have shorter hypocotyls and more expanded cotyledons than wild-type seedlings under continuous red light, suggesting that PIF3 functions as a negative regulator of morphological phenotypes under red light [38,39]. By contrast, PIF3 functions positively in chloroplast development and greening processes during the initial hours of de-etiolation, because *pif3* seedlings have chlorophyll levels lower than those of wild type [38,39]. PIF3 also acts positively in the light-induced accumulation of anthocyanin [39]. However, because PIF3 is so closely related to PIF1, it is possible that *pif3* seedlings also undergo photooxidative damage under light conditions, reducing the ability of these seedlings to synthesize chlorophylls. Further experiments are necessary to distinguish whether the reduced chlorophyll content of the

pif3 mutant is due to positive role of PIF3 in these pathways or to the phototoxicity effects under these light conditions.

PIF4 functions negatively in the phyB-mediated inhibition of hypocotyl elongation. Under continuous red light, *pif4*/*srl2* seedlings have shorter hypocotyls and expanded cotyledons compared to wild-type seedlings [23]. When exposed to continuous red light, *pif5* seedlings also have a hypersensitive phenotype similar to that of *pif4* seedlings, suggesting that PIF5 also functions as a negative regulator of phyB signaling [40]. The biological function of PIF6 is unknown (Table 1). *pif4 and pif5* seedlings do not display any phenotype under far-red light (phyA response) or in the dark. Therefore, it appears that the major biological function of these factors is to negatively regulate light-induced photomorphogenic development.

All PIFs have been shown to interact with the central clock component APRR1/TOC1 using Y2H assays [26]. Moreover, both *PIF4* and *PIF5* mRNA and protein levels are strongly regulated by the circadian clock, and this regulation is involved in the control of the rhythmic growth pattern of *Arabidopsis* seedlings under day–night cycles [26,40,41]. These data suggest that PIF4, PIF5 and possibly other PIFs function in the circadian clock. The circadian clock defects for other *pif* mutants have not yet been demonstrated.

Identification and light-regulation of direct target genes of PIFs

Because PIFs are transcription factors capable of binding directly to both potential target gene promoters and photoactivated phytochromes, they are ideal to investigate the mechanisms of light-regulated gene expression. The interaction of photoactivated phyB with DNA-bound PIF3 suggested a provocative model for the direct regulation of gene expression by phytochromes in response to light [29,42]. However, conclusive evidence in favor of this or any other model is still absent owing in part to the lack of known direct target genes of PIFs. With the recent optimization of the chromatin immunoprecipitation (ChIP) assay, direct target genes of PIF1 and PIF3 have been identified. Using ChIP and *in vitro* gel-shift assays, it was shown that PIF1 directly activates two genes in the dark, *GAI* and *RGA*, which encode DELLA proteins involved in GA-mediated regulation of seed germination (Figure 2a) [43]. ChIP assays also showed that, *in vivo*, PIF3 binds the promoters of six genes that are involved in controlling anthocyanin biosynthesis [44]. Both PIF1 and PIF3 control other pathways involved in photomorphogenesis, in addition to seed germination and anthocyanin biosynthesis. Moreover, there are no known targets of PIF4, PIF5 and PIF6. Therefore, the identification and characterization of genomic targets of PIFs using the ChIP-chip technique is necessary to understand fully how these factors control photomorphogenesis [45,46].

The identification of PIF target genes allowed further understanding of the role of PIFs in the light regulation of these genes. The expression of *GAI* and *RGA*, two direct target genes of PIF1, is downregulated by light, which is consistent with the light-induced degradation of PIF1 (Figure 2b) [43,47,48]. Similarly, the direct target genes of PIF3 (e.g. *CHS, CHI, F3H, DFR* and *LDOX*) are downregulated by far-red light [44]. However, the PIF3 protein level is not reduced by light under these

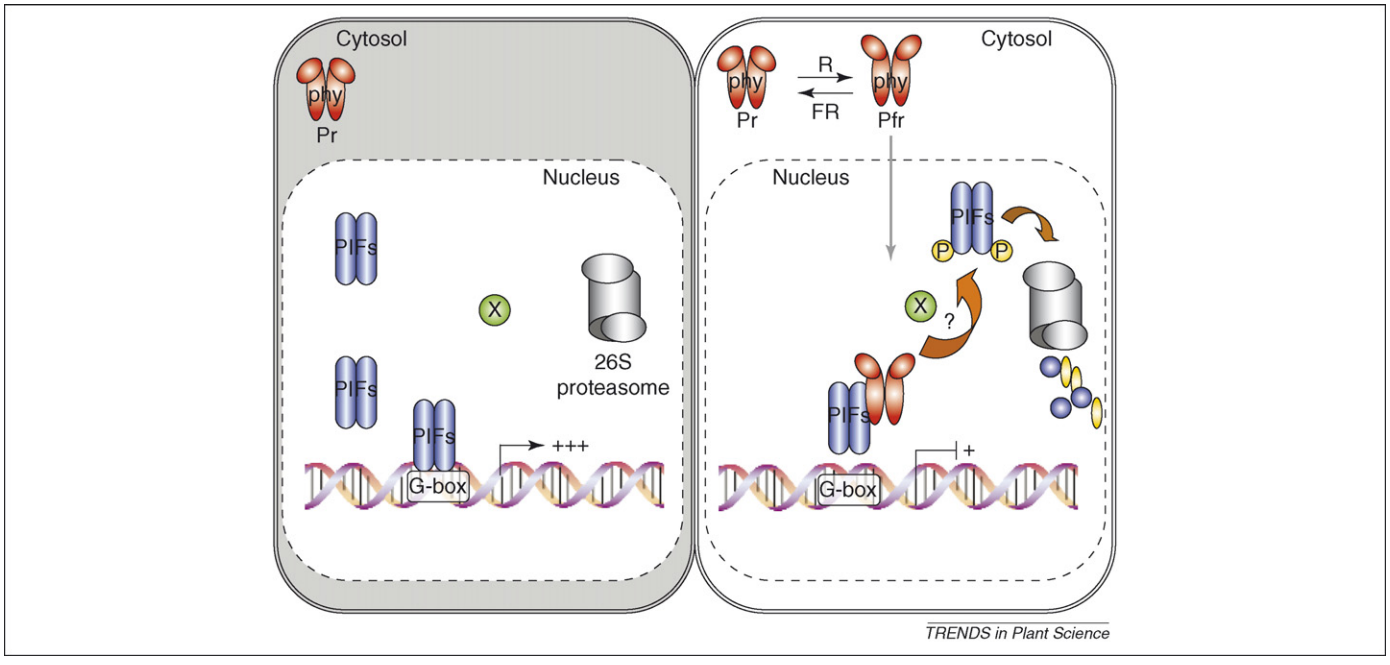

Figure 2. Model of PIF function in phytochrome signaling pathways. **(a)** In the dark, PIFs are constitutively localized to the nucleus, whereas phytochromes are localized to the cytosol. PIFs negatively regulate photomorphogenesis by activating gene expression (e.g. *GAI* and *RGA* for inhibiting seed germination in the dark [43]). **(b)** Light signals induce photoconvertion of phytochromes to the active Pfr forms before nuclear migration. In the nucleus, phytochromes physically interact with PIFs, which results in phosphorylation of PIF3 and possibly other PIFs either directly or indirectly. The phosphorylated forms of PIFs are recognized by an ubiquitin ligase and are subsequently degraded by the 26S proteasome. The light-induced proteolytic removal of PIFs results in relieving the negative regulation of photomorphogenesis (e.g. reduction in *GAI* and *RGA* expression to promote seed germination in light [43]). X, indicates an unknown factor that might be involved in the light-induced phosphorylation of PIFs. Abbreviations: FR, far-red light; P, phosphorylated; Pfr, far-red-absorbing form of phytochrome; Pr, red-absorbing form of phytochrome, R, red light.

conditions [44]. PIF3 binding to these promoters is also not regulated by light. Furthermore, the differences in expression of the above genes have only been shown under continuous far-red light without any dark control, which prevents the evaluation of the relative effect of light on the expression of these genes. These results are not consistent with previous reports that PIF3 is degraded under both red- and far-red-light conditions [38,44,49]. Therefore, it is still not clear how PIF3 directly controls these genes to promote anthocyanin biosynthesis in response to light.

PIF3 has also been implicated in rapid gene expression in response to light. Microarray analysis showed that, within one hour of red light exposure, the expression of several genes is compromised in the *pif3* mutant compared to wild type [38]. Of the genes that showed strong PIF3-dependent regulation, several are photosynthesis- or chloroplast-related, zinc-finger transcription factors and RNA polymerase sigma factor E, which might regulate the chloroplast genome. However, the expression of these genes might be indirectly regulated by PIF3, as direct binding of PIF3 to these promoters has not been shown. It appears that PIFs can activate gene expression in the dark and that the light-induced degradation of PIFs might reduce expression of certain target genes. However, PIFs might also be involved in the regulation of gene expression in response to light. Further investigations are necessary to determine whether PIFs are involved in light-regulated gene expression.

Regulation of PIF function

Although PIFs are transcription factors capable of activating and/or repressing gene expression, only PIF1 has been shown to have transcriptional activation activity *in vivo* [24]. Strikingly, this activity is reduced in light in a phytochrome-dependent manner. Furthermore, PIF1, PIF3, PIF4 and PIF5 proteins are rapidly degraded in response to light signals [38,41,47–50]. Treatment with proteasomal inhibitors prevent degradation, providing evidence that PIFs are degraded by the ubiquitin/26S proteasomal pathway. These results are consistent with a recent report that mutation in CUL1 (*cul1-6* allele), a core subunit of the *Arabidopsis* SCF E3 ligase complex, results in a reduced sensitivity to red light [51]. The half-life for PIF1 and PIF3 is ~10–15 min under red light, suggesting that these factors might function transiently during the dark to light transition [47,50]. It has been determined that phyA, phyB and phyD are necessary for the light-induced degradation of PIF3, whreas COP1, another negative regulator of light signaling, is necessary for the stability of PIF3 in the dark [50]. Because the major biological function of PIFs is the negative regulation of photomorphogenesis, it is not surprising that light negatively regulates PIFs function through phytochromes to promote photomorphogenesis [52].

Although the rapid degradation of PIF3 led to the conclusion that this protein functions transiently in light signaling pathways [50], subsequent studies have shown that PIF1, PIF3, PIF4 and PIF5 re-accumulate in the dark during recurring light–dark cycles [38,41,47]. The recurring expression of PIF4 and PIF5 has been shown to control rhythmic growth pattern of *Arabidopsis* seedlings under day–night cycles [41]. Therefore, PIFs might fine-tune photomorphogenic development throughout the plant life cycle.

Although the mechanism of light-induced PIF degradation is still unknown, a recent pivotal article has shed some light on the initial steps by showing that PIF3 is phosphorylated in response to light signals in a phytochrome-dependent manner and that the phosphorylated form is rapidly degraded in light [34]. Strikingly, direct physical interactions between PIF3–phyA and PIF3–phyB contribute to the light-induced phosphorylation and subsequent degradation of PIF3. Missense mutations in both the APA and APB domains of PIF3 eliminated direct physical interactions with phyA and phyB, respectively. This PIF3 missense mutant, which does not interact with phytochromes, is not phosphorylated and is therefore stable under light conditions. These data suggest that the first step in the light-induced degradation of PIF3 is the phosphorylation of PIF3 after direct physical interaction with phytochromes (Figure 2). Work in other systems has shown that many substrates of the ubiquitin/26S proteasome pathway are phosphorylated before degradation [53]. Therefore, PIFs might be phosphorylated in response to light signals in a phytochrome-dependent manner and the phosphorylated forms are degraded by the ubiquitin/26S proteasome pathway to remove the negative regulation of photomorphogenesis (Figure 2).

PIFs involvement in hormone signaling

There is mounting evidence suggesting that light signals coordinate with hormone signaling pathways to control photomorphogenesis [54]. The most direct known link between PIFs and hormone signaling is the involvement of PIF1 in gibberellin (GA)-mediated seed germination. PIF1 inhibits seed germination by repressing GA biosynthetic genes and by activating GA catabolic genes, resulting in a reduced level of bioactive GA in wild-type seeds [48]. In addition to the regulation of GA metabolism, PIF1 controls GA sensitivity by directly activating the expression of *GAI* and *RGA*, two key DELLA protein-encoding genes that function as repressors of GA signaling [43]. Moreover, PIF1 activates ABA biosynthesis to promote seed dormancy. Light signals perceived by phytochromes present in the seed (mainly phyB) induce the degradation of PIF1 to promote GA biosynthesis, increase GA sensitivity and decrease ABA biosynthesis to promote seed germination. Involvement of other PIFs in hormone signaling has not been shown. However, the expression of *PIF* genes is regulated not only at the tissue-specific (Figure 3a) and developmental stage-dependent manner (different stages of plant life cycle) (Figure 3b), but also by multiple hormones, biotic and abiotic stress conditions (Figure 3c), suggesting that PIFs might function in multiple hormone and stress signaling pathways in different organs.

Future perspectives

Although it is well established that PIFs are central players in phytochrome signaling networks, several key questions regarding PIFs still remain unanswered. What

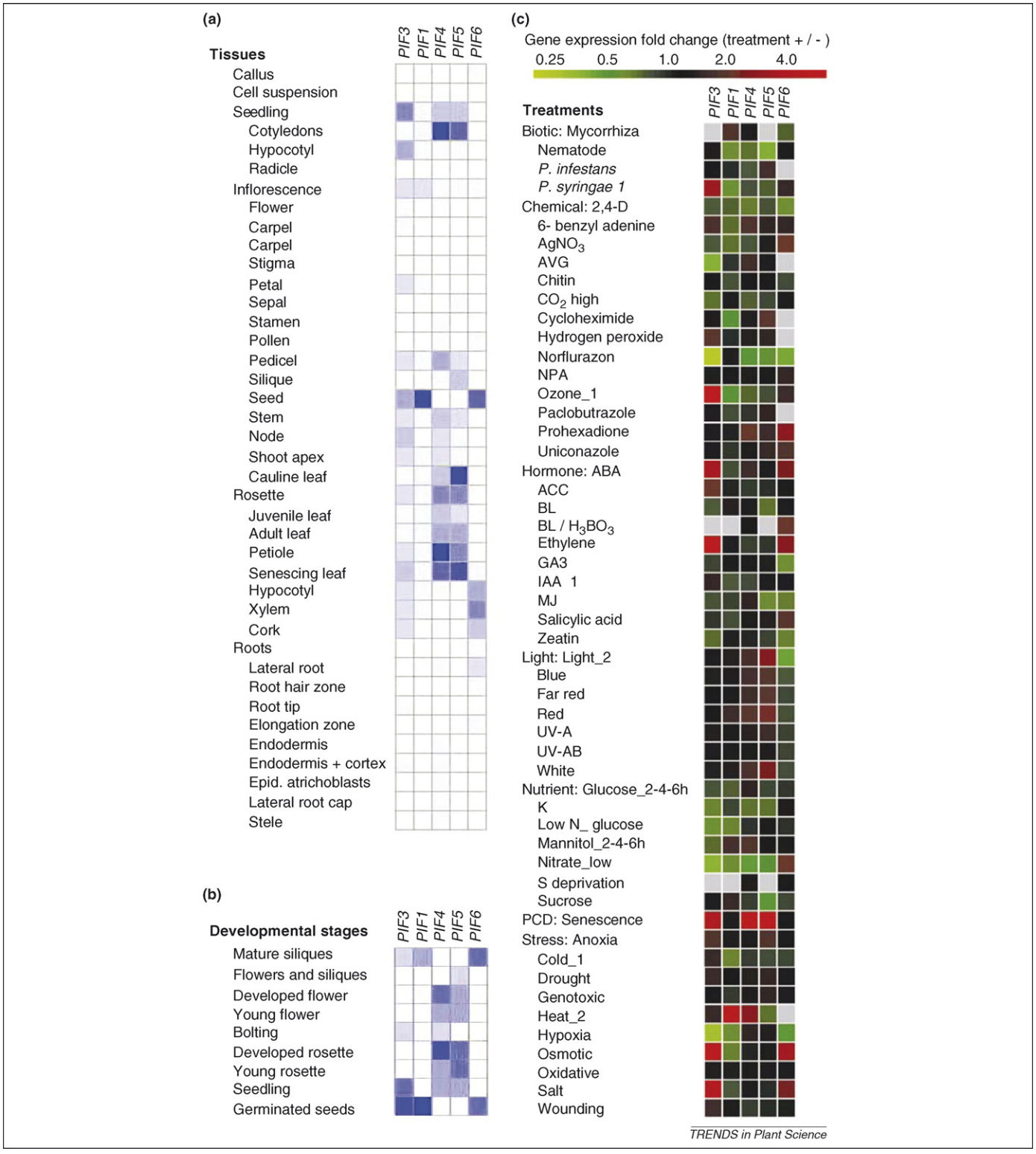

Figure 3. Expressions of *Arabidopsis PIF* genes are regulated by diverse stimuli and in a tissue and developmental stage-specific manner. **(a)** Tissue-specific expression patterns of selected *PIF* genes. **(b)** Developmental stage-specific expression patterns of selected *PIF* genes. **(c)** Regulation of *PIF* genes by various hormones, chemicals, biotic and abiotic stress conditions. Expression data were obtained from Genevestigator [59].

is the kinase that phosphorylates PIF3 and possibly other PIFs in response to light? What are the factors responsible for the recognition and subsequent ubiquitination of the phosphorylated forms of PIFs leading to their degradation under light? The phytochromes are excellent candidates for the kinase that phosphorylates PIFs; phyA has been shown to function as a non-conventional serine/threonine kinase [55]. Moreover, the direct physical interactions of PIFs with photoactive phytochromes are necessary for the light-induced phosphorylation and degradation of PIF3 *in vivo* [34]. However, the putative kinase domain of phytochromes, located at their C-terminal domains, is not necessary for at least one phytochrome (e.g. phyB) signaling pathway [12,56], suggesting that phytochromes

might not directly function as a kinase. Therefore, the kinase that phosphorylates PIF3 and possibly other PIFs, either directly or indirectly in association with phytochromes, remains to be identified. Moreover, the identification of factors (e.g. E3 ligase) that recognize the phosphorylated forms of PIFs for subsequent ubiquitination will provide more insight into the mechanism of the light-induced degradation of PIFs.

Phosphorylation and ubiquitination of transcription factors are common post-translational modifications [57,58]. Often transcription factors are phosphorylated and/or ubiquitinated at the transcription initiation complex to activate their transcription activation activity and to tag them for subsequent degradation by the ubiquitin/26S proteasome pathway [57,58]. This allows cells to respond proportionately to the stimuli that activate the transcription factor. Given that PIF3 is necessary for light-induced gene expression, does light control homo- and heterodimerization, DNA binding and/or the transcriptional activation activity of PIFs? The combined power of biochemical, molecular genetic and photobiological techniques will help answer these questions.

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