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Expanding Roles of PIFs in Signal Integration from Multiple Processes

Inyup Paik[1,3], Praveen Kumar Kathare[1,3], Jeong-Il Kim[1,2] and Enamul Huq[1,*]

[1]Department of Molecular Biosciences and The Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX 78712, USA

[2]Department of Biotechnology and Kumho Life Science Laboratory, Chonnam National University, Gwangju 61186, Korea

[3]These authors contributed equally to this article.

***Correspondence: Enamul Huq (huq@austin.utexas.edu)**

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ABSTRACT

PHYTOCHROME-INTERACTING FACTORs (PIFs) are members of the basic helix-loop-helix (bHLH) family of transcription factors in *Arabidopsis*. Since their discovery in phytochrome-mediated light signaling pathways, recent studies have unraveled new functions of PIFs in integrating multiple signaling pathways not only through their role as transcription factors directly targeting gene expression but also by interacting with diverse groups of factors to optimize plant growth and development. These include endogenous (e.g., hormonal) as well as abiotic (light, circadian, and elevated temperature) and biotic (defense responses) pathways. PIFs interact with key factors in each of these pathways and tailor the outcome of the signal integration among these pathways. This review discusses the roles of PIFs as pivotal signal integrators in regulating plant growth and development.

Key words: circadian clock, growth–defense tradeoff, hormone signaling, phytochrome-interacting factor, thermomorphogenesis, signal integration

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INTRODUCTION

Plants continually adapt to natural light environments to optimize their growth and development. The information of surrounding light conditions is monitored and perceived by several classes of photoreceptors, and the light signals are eventually transduced to the transcriptional network that drives multiple facets of photomorphogenesis (Bae and Choi, 2008). Among the transcription factors that regulate light signaling pathways, phytochrome-interacting factors (PIFs) are well characterized. PIFs are basic helix-loop-helix (bHLH) transcription factors, belonging to the 15 members of subgroup 15 among 162 members of the bHLH protein family in *Arabidopsis thaliana* (Bailey et al., 2003; Toledo-Ortiz et al., 2003; Khanna et al., 2004). To date, eight of the 15 members in the subgroup have been shown to interact with at least one of the phytochromes (phy), known as PIFs, including PIF1 (also referred to PIL5, At2g20180), PIF2 (PIL1, At2g46970), PIF3 (At1g09530), PIF4 (At2g43010), PIF5 (PIL6, At3g59060), PIF6 (PIL2, At3g62090), PIF7 (At5g61270), and PIF8 (At4g00050), whereas other members do not have any phytochrome binding motif (see the references in a recent review [Lee and Choi, 2017]).

Since the discovery of PIF3, the founding member that negatively regulates phytochrome-mediated light signaling (Ni et al., 1998; Kim et al., 2003; Monte et al., 2004), PIFs have been described as central players in transducing light signals perceived by phytochromes (Castillon et al., 2007; Leivar and Quail, 2011). However, further studies have shown that PIFs are also involved in many other signaling pathways, such as thermal-induced responses, circadian clock or hormonal signaling, developmental and sugar-derived signaling, and responses to biotic and abiotic stresses. Tremendous progress has been made recently in dissecting the phytochrome–PIF signaling interface by demonstrating new evidence that phytochrome acts as a kinase, identifying new kinases for PIFs, and understanding mechanistic details on PIF stability and their DNA binding ability. This review primarily focuses on the expanding roles of PIFs as signal integrators in plant growth and development by highlighting and discussing recent advances on the function of PIFs in regulating multiple processes. For learning the details on the roles of PIFs in phytochrome signaling, several recent research and review articles might have presented better analyses (Leivar and Quail, 2011; Leivar and Monte, 2014; Xu et al., 2015; Shin et al., 2016; Kim et al., 2016a, 2016b; Lee and Choi, 2017; Ni et al., 2017).

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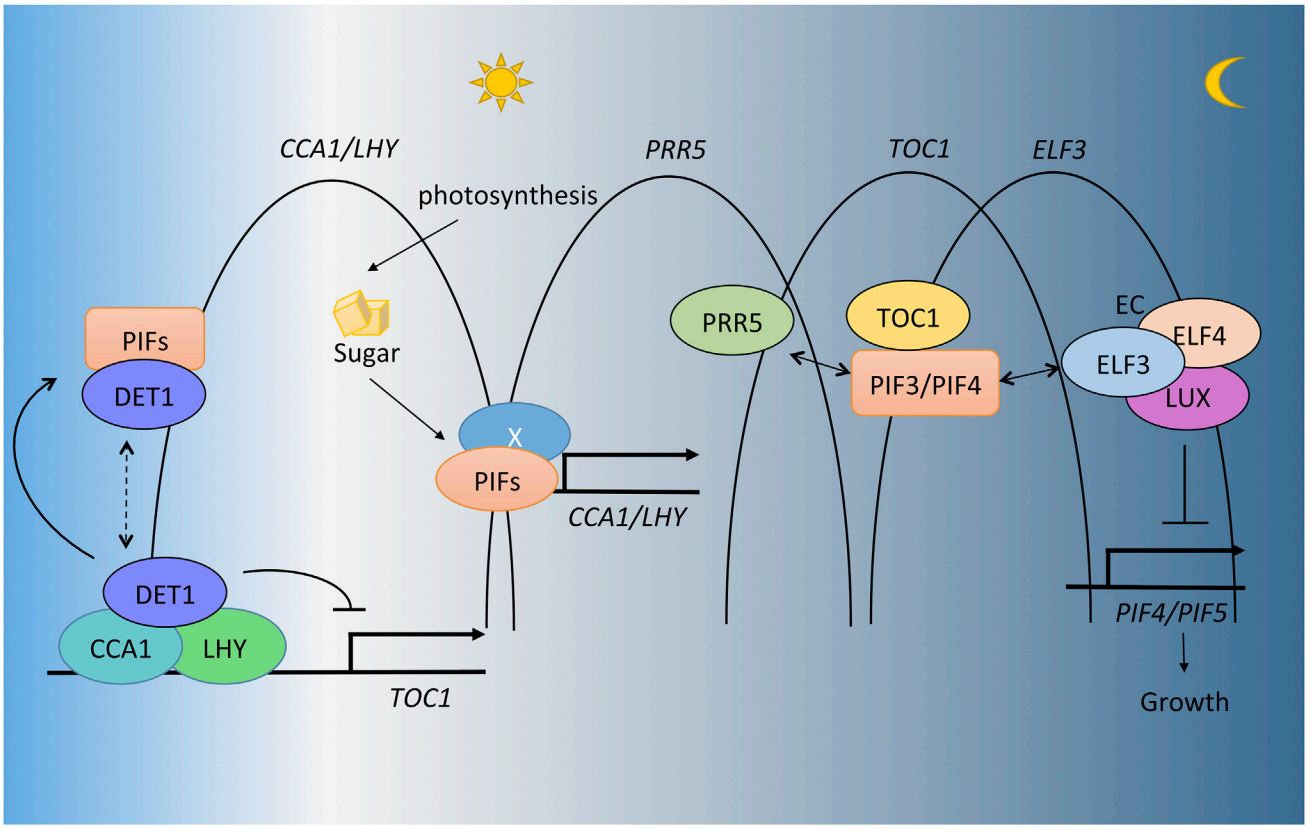

Figure 1. Dynamic Involvement of PIFs in Regulating Circadian Clock and Diurnal Growth in *Arabidopsis*.
PIFs have been shown to participate in both the input and output pathways of circadian clock. (Left) DET1 forms a complex with CCA1/LHY to repress *TOC1* expression in the morning. DET1 also directly interacts with and stabilizes PIFs in the dark. PIFs might form a complex with DET1-CCA1-LHY to form a transcription complex as indicated by a dotted line. (Middle) PIF1, PIF3, PIF4, and PIF5 are necessary to mediate metabolic signaling to the clock by directly binding to the CCA1/LHY promoters in response to sugar. X indicates an unknown factor or sugar-induced modification necessary for enhanced PIF binding to the CCA1/LHY promoters. (Right) Sequential expression of *PRR5* and *TOC1* gates the growth by inhibiting PIF functions, while ELF3-ELF4-LUX forms an evening complex (EC) that represses *PIF4*/*PIF5* expression to repress growth during early evening.

PIFs FUNCTION IN CIRCADIAN CLOCK INPUT AND OUTPUT PATHWAYS

Light is known as a crucial input to the circadian clock of living organisms. Light is perceived by a series of photoreceptors in plants, thereby making plants fit to changing environmental conditions. Since photoreceptors are responding at the forefront to the incoming light, it is reasonable that light input to the circadian clock is relayed by these photoreceptors. For example, phytochromes and cryptochromes (CRY) are responsible for the light entrainment of the circadian clock in *A. thaliana*, resulting in long-period phenotypes in these photoreceptor mutants (Somers et al., 1998). As many studies continue to report the function of PIFs in photomorphogenesis and other signaling pathways, it is feasible that PIFs are also involved in regulation of the circadian clock. Central clock components CCA1/LHY, MYB transcription factors, contain G-box in their promoters, which is known as the *cis*-element for binding of PIFs. Recent reports showed a direct *in vivo* and *in vitro* association of PIFs with the G-box element on CCA1/LHY promoters by chromatin immunoprecipitation (ChIP) and gel-shift assays (Martinez-Garcia et al., 2000; Oh et al., 2012). This indicates that PIFs might also have a role in the direct input to the clock. However, a role for PIFs in the *Arabidopsis* circadian clock has been elusive mainly because *pif* single and double mutants did not show any changes in the circadian clock (Viczian et al., 2005; Nusinow et al., 2011).

Strikingly, a recent study showed that *pifQ* (*pif1*, *pif3*, *pif4*, *pif5* quadruple) mutant displays longer period compared with the wild type in the presence of sucrose (Shor et al., 2017). This result appears to be inconsistent with the general roles of PIFs as negative regulators of the light signaling pathways, given that phytochrome mutants exhibit long-period phenotype due to a lack of red light entrainment to the circadian clock (Somers et al., 1998). Thus, if PIFs are to relay photo-signal to the circadian clock, a short-period phenotype is expected in the *pifQ* mutant. Therefore, the long-period phenotype shown in *pifQ* suggests a lack of direct relationship between PIFs and the light input to the circadian clock. Instead, this study showed that metabolic input to the clock was impaired in the *pifQ* mutant and the longer-period phenotype was suppressed by the inhibition of photosynthesis or lack of exogenously supplied sugar. More interestingly, the addition of sucrose significantly enhanced the binding of PIFs to the *CCA1/LHY* promoters (Figure 1). The photosynthesis-derived sugar can entrain the *Arabidopsis* circadian clock by suppressing a morning gene, *PRR7* (Haydon et al., 2013). However, PIFs do not bind directly to the promoter of *PRR7* but bind to the promoters of *CCA1/LHY* (Figure 1). These

data suggest that PIFs mediate metabolic input signal to the clock independent of *PRR7* expression, highlighting a unique role of PIFs in the sugar-mediated entrainment of the *Arabidopsis* circadian clock. However, the detailed mechanisms by which sugar enhances the binding of PIFs on the target promoters remain to be answered.

Recently, a number of reports suggested a role for PIFs in the diurnal regulation of growth in association with circadian clock factors. For example, the central circadian clock component TOC1 (TIMING OF CAB EXPRESSION 1) that suppresses the expression of *CCA1* (*CIRCADIAN CLOCK-ASSOCIATED 1*) and *LHY* (*LATE ELONGATED HYPOCOTYL*) was shown to interact with PIF3 in yeast (Yamashino et al., 2003). This interaction has been recently confirmed and analyzed *in planta* (Soy et al., 2016). These authors showed that the physical interaction between PIFs and TOC1 results in ''coincident co-binding'' to the promoters of dawn-phased genes under diurnal condition to optimize their expression (Figure 1). TOC1 inhibits the transcriptional activity of PIF3; thus TOC1–PIF3 interaction establishes a growth inhibition at early dusk and gates growth to predawn. The same group further analyzed genome-wide gene expression under short-day conditions (Martin et al., 2016). They identified a total of 349 PIFs- and short-day co-regulated genes, among which 55% were induced and 42% were repressed by both short days and PIFs. These data suggest that PIF3 and possibly other PIFs are tightly interconnected with the circadian clock output through direct interaction with TOC1 to co-regulate the expression of target genes. In addition, a recent paper also reported direct interactions between TOC1/PRR5 (PSEUDO-RESPONSE REGULATOR 5) and PIF4, which results in TOC1/PRR5-mediated suppression of PIF4 transcription activity (Figure 1) (Zhu et al., 2016). These data again suggest a direct association of the circadian clock components with PIFs in *Arabidopsis*. Furthermore, the expression of *PIF4* and *PIF5* is regulated by the circadian clock (Yamashino et al., 2003; Nozue et al., 2007). The transcription of *PIF4* and *PIF5* is repressed by the evening complex, ELF3 (EARLY FLOWERING 3)-ELF4 (EARLY FLOWERING 4)-LUX (LUX ARRHYTHMO, also known as PHYTOCLOCK 1), thus promoting cell elongation in a time-dependent manner late at night (Figure 1) (Nusinow et al., 2011).

It is notable that DET1 (DE-ETIOLOATED 1) possesses a transcriptional repression activity and directly associates with CCA1/LHY *in vivo*, thereby regulating *TOC1* expression (Figure 1) (Lau et al., 2011). In a separate study, DET1 has been shown to directly interact with PIFs and stabilize them in the dark by unknown mechanisms (Dong et al., 2014). Thus, it is possible that PIFs are located in the same complex with CCA/LHY/DET1. However, further studies are necessary to examine any possible role of PIFs on the CCA1/LHY/DET1 transcriptional complex. Taken together, the circadian clock and PIFs have a mutual regulatory relationship to optimize growth and development of plants.

PIFs PLAY A CENTRAL ROLE IN THERMAL-INDUCED MORPHOGENESIS

Temperature is a major environmental cue that influences the distribution and seasonal responses of plants. The recent trend in increasing global temperature is likely to have an adverse effect on plant growth and development, resulting in decreased crop yields (Battisti and Naylor, 2009; Lobell and Gourdji, 2012; Quint et al., 2016). Thus more attention has recently been given to thermal-induced morphogenesis, termed thermomorphogenesis, which is characterized by elongated hypocotyls and petioles, narrow leaves, and accelerated flowering (Quint et al., 2016). Among PIFs, PIF4 has been reported as a key regulator of thermomorphogenesis in plants (Koini et al., 2009; Franklin et al., 2011; Sun et al., 2012). PIF4 was initially identified as a bHLH factor that negatively regulates phytochrome B (phyB) signaling (Huq and Quail, 2002). Later, it was found that PIF4 is also responsible for the hypocotyl elongation in response to high ambient temperature, in which high temperature regulates the transcription and post-translational stabilization of PIF4. In addition, PIF4 promotes flowering at high temperature by directly binding to the promoter of the florigen, *FT* (*Flowering Locus T*) (Kumar et al., 2012).

The phenotypic similarities between thermomorphogenesis and shade avoidance response are striking. Both responses are characterized by a rapid and dramatic increase in the extension growth of stems and petioles at the expense of leaf growth and reproductive development (Quint et al., 2016; Legris et al., 2017). Although plants continue to monitor and respond to the changing environmental cues, especially light signals, light always coincides with the heat radiation emitted by the sun in Nature. Therefore, it is not surprising that changes in plant morphology in response to high ambient temperature and by vegetation shade are very similar. Consistent with this, a light receptor, phyB, has been identified as a plant thermosensor, as it undergoes thermal reversion, i.e., thermal reversion of the active Pfr form to an inactive Pr form (Figure 2) (Jung et al., 2016; Legris et al., 2016). This phenomenon is also more commonly referred to as ''dark reversion,'' although ''thermal reversion'' can occur under both dark and light conditions. As the active form of phyB inhibits PIF4 activity and also triggers rapid phosphorylation and degradation of PIF4 through the ubiquitin–proteasome system (Leivar and Quail, 2011), it is possible that the high-temperature-mediated thermal reversion of phyB results in enhanced PIF4 stability and activity to trigger thermomorphogenic responses. Similar to phyB, the blue light receptor CRY1 (Cryptochrome 1) has been shown to regulate PIF4 activity (Ma et al., 2016) (Figure 2). CRY1 directly interacts with PIF4 in a blue light-dependent manner and inactivates PIF4 transcription activity. The blue light, therefore, is able to suppress thermomorphogenesis by suppressing PIF4 activity.

Multiple factors have been identified that modulate the function of PIF4 in the context of thermomorphogenesis. For example, ELF3, as part of a component in the evening complex of circadian clock, has been shown to suppress the transcription of *PIF4* and *PIF5* (Nusinow et al., 2011). In addition, ELF3 also directly binds to PIF4 (Nieto et al., 2015), which prevents PIF4 from activating its transcriptional targets (Figure 2). Interestingly, ELF3 itself was shown to be necessary for temperature-mediated control of plant growth by targeting multiple loci in a temperature-dependent manner (Box et al., 2015). FCA (FLOWERING TIME CONTROL PROTEIN) was found to be another PIF4 regulator in thermomorphogenesis (Figure 2) (Lee et al., 2014). FCA is an RNA binding protein that functions in autonomous flowering

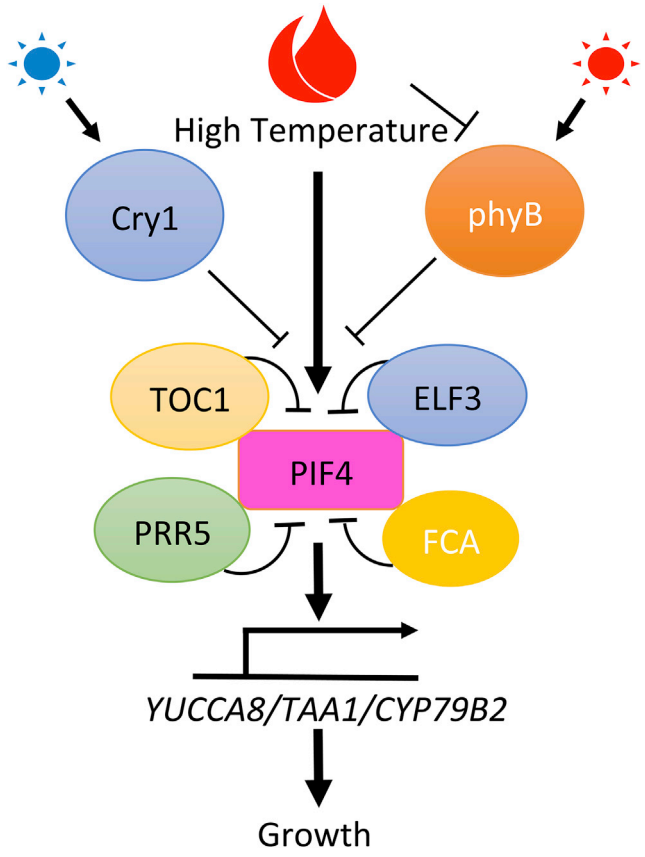

Figure 2. PIF4 Plays a Central Role in Integrating Light and High-Temperature Signaling to Promote Growth.
High ambient temperature induces *PIF4* expression and also stabilizes PIF4 protein. Multiple regulators have been shown to directly interact with PIF4 and regulate its function by inhibiting DNA binding, transcription activity, and protein stability. While two photoreceptors, phyB and Cry1, function to inhibit PIF4 activity in response to red and blue light signals, respectively, only phyB has been shown to act as a thermosensor in *Arabidopsis*. PIF4 directly activates the expression of *YUC/TAA1/CYP79B2* genes to promote growth.

pathway to suppress *FLC* (*FLOWERING LOCUS C*) expression (Macknight et al., 1997). Notably, it was shown that FCA also mediates high ambient temperature-driven early flowering in *Arabidopsis* (Blázquez et al., 2003; Lee et al., 2014). In response to high temperature, FCA binds to PIF4, which dissociates PIF4 from its direct target gene *YUC8* (*YUCCA8*) to suppress hypocotyl elongation. Moreover, a recent study has identified TOC1/PRR5 as PIF4 inhibitors (Figures 1 and 2) (Zhu et al., 2016). Given that TOC1/PRR5 peaks in a sequential manner during the light/dark cycle, these authors hypothesize that the inhibition by TOC1/PRR5 controls circadian gating of PIF4 in thermomorphogenesis. It is notable that the circadian gating of thermomorphogenesis enables plants to respond to external temperatures differently at different times of the day. TOC1 peaks in the evening so that it can prevent overgrowth of plants by high temperature during evening hours. PRR5 peaks in the afternoon so that the sequential expression of PRR5/TOC1 ensures tight gating of plant growth in response to high ambient temperature (Figures 1 and 2). Taken together, PIF4 plays a central role as a key regulator in controlling thermomorphogenesis, and its activity is fine-tuned by multiple factors in a time-dependent manner.

Despite these extensive reports, there are still many important questions yet to be answered. What is/are the upstream receptor(s) that perceive high ambient temperature and ultimately induce *PIF4* expression? Recently, phyB has been shown to be one of the thermosensors. However, additional thermosensor(s) must be present, as phyB alone cannot explain all the phenotypic changes associated with thermomorphogenesis. In addition, what makes PIF4 function uniquely at high temperature, and what are the differences between PIF4 and other PIFs that uniquely position PIF4 as a central thermal transcription factor? Further studies are necessary to answer these questions.

PIFs INTEGRATE LIGHT AND HORMONAL SIGNALING PATHWAYS

Plant hormones play pivotal roles in modulation of plant growth and development, as well as plant responses to external factors, including biotic and abiotic stresses. Plant hormones are essentially small chemical compounds derived from tightly regulated metabolic pathways. The biosynthetic and signaling pathways of several plant hormones have been extensively characterized through biochemical and forward genetics approaches (Santner and Estelle, 2009). However, in recent years much of the research on hormone signaling has been focused on the interconnections that exist among different hormones, and also between hormones and external environmental signals, including light and temperature (Jaillais and Chory, 2010). These studies highlight the importance of signaling integrators that connect light signaling with those of hormonal pathways, although it is far from complete (Lau and Deng, 2010; de Wit et al., 2016).

PIFs play key roles in signal integration between light and hormone signaling pathways (see the discussions below and Figure 3). In response to light quality and quantity, and developmental or external cues, PIFs coordinate light and hormone signaling by regulating the hormone biosynthesis and/or the expression of key components in hormone signal transduction, and/or by directly interacting with components of the hormone signaling pathways (Lucas and Prat, 2014; de Wit et al., 2016).

PIFs Mediate Light and Gibberellic Acid Pathways

PIFs and gibberellic acid (GA; a positive regulator of germination) regulate each other's signaling pathways at multiple levels throughout the plant life cycle. PIF1, a strong inhibitor of seed germination in the dark, suppresses GA signaling both directly and indirectly. PIF1 directly binds to the promoters of two *DELLA* genes, *RGA1* (*REPRESSOR OF GA1-3*) and *GAI* (*GIBBERELLIC ACID INSENSITIVE*), and activates the expression of these genes that act as suppressors of GA signaling (Figure 3A) (Oh et al., 2007). Moreover, PIF1 also regulates the biosynthesis of GA, but rather indirectly by activating the expression of a number of genes, including *DAG1* (*DOF AFFECTING GERMINATION 1*), *SOM* (*SOMNUS*) and *GA2ox2* (GIBBERELLIN 2-OXIDASE 2). DAG1 and SOM repress the expression of key GA biosynthetic genes *GA3ox1* and *GA3ox2*, while activating the GA catabolic

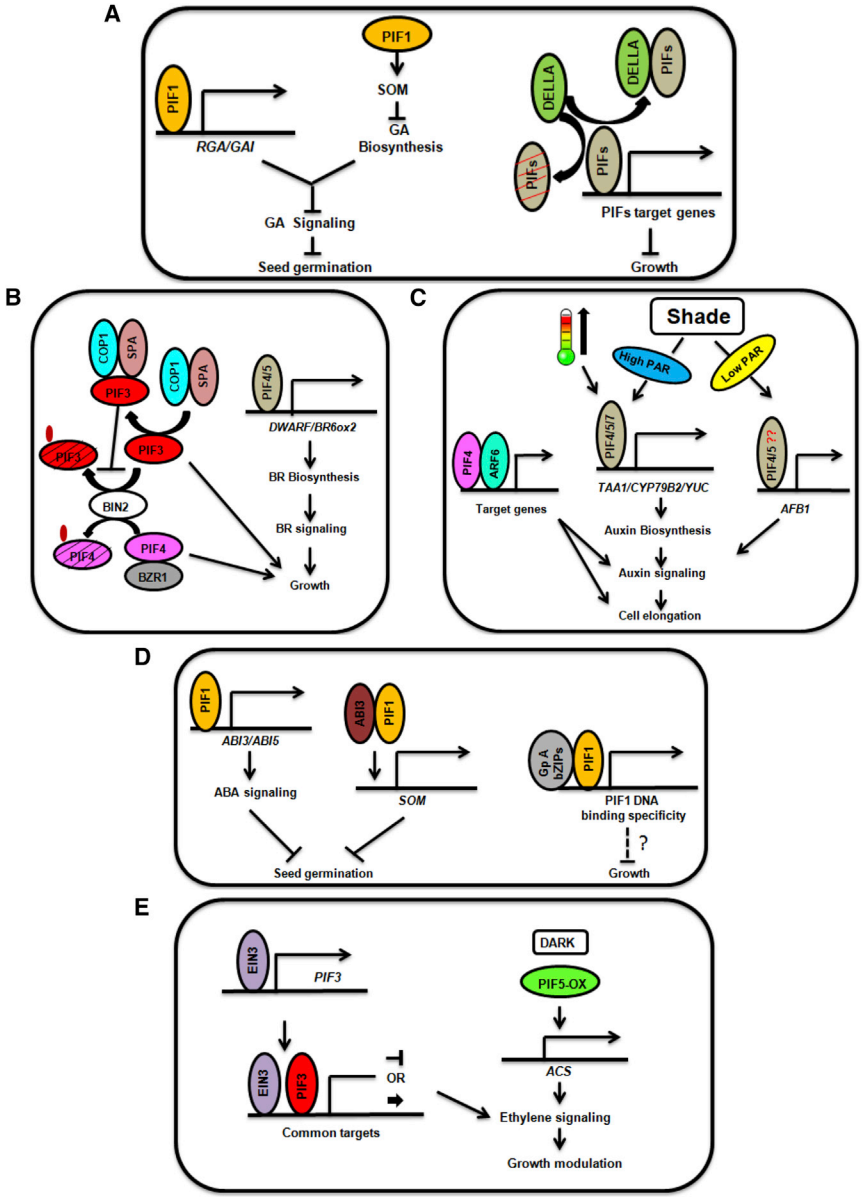

Figure 3. PIFs Integrate Light and Hormone Signaling Pathways to Modulate Growth in *Arabidopsis*.

(A) (Left) PIF1 has an exclusive role in regulating seed germination by directly activating *RGA* and *GAI* expression that inhibit GA signaling. PIF1 also inhibits GA biosynthesis indirectly by activating *SOM* expression, resulting in inhibition of seed germination in the dark. Light-induced degradation of PIF1 promotes seed germination. (Right) PIFs physically interact with DELLA proteins and this interaction results in inhibition of DNA binding activities of PIFs. DELLA proteins also induce degradation of PIFs in darkness and inhibit subsequent growth.

(B) (Left) PIF4 interacts with BZR1 and regulates growth in response to BR and light signal. BIN2 phosphorylates PIF3 and PIF4 independent of light and promotes their degradation in darkness. However, COP1/SPA1 interact with PIF3 and prevent BIN2-mediated phosphorylation and degradation in the dark. (Right) PIF4 and PIF5 directly activate BR biosynthetic pathway genes to promote growth.

(C) (Left) PIF4 forms a complex with ARF6 and promotes growth in response to light and auxin signaling. (Middle) In response to high ambient temperature and shade conditions, PIF4/PIF5/PIF7 promote auxin biosynthesis to promote cell elongation. (Right) PIF4/PIF5 and possibly other PIFs also activate the expression of auxin receptor *AFB5* in response to low-PAR shade conditions to promote auxin signaling and subsequent growth.

(D) (Left) PIF1 directly activates the expression of the ABA signaling components (*ABI3* and *ABI5*). PIF1 also interacts with ABI3 and the PIF1–ABI3 complex directly inhibits the expression of *SOM*, which in turn inhibits GA biosynthesis to suppress seed germination. (Right) PIF1 and possibly other PIFs directly interact with group A bZIP proteins (e.g., ABI5). This interaction regulates the DNA binding specificity and target gene selection of PIF1 and possibly other PIFs.

(E) (Left) Ethylene signaling factor EIN3 directly activates the expression of *PIF3*, which in turn binds to DNA along with EIN3 to regulate ethylene signaling as well as chlorophyll biosynthesis and growth. (Right) Overexpression of *PIF5* activates the expression of *ACC Synthase* (*ACS*) genes, which results in increased ethylene biosynthesis and signaling to modulate growth.

gene *GA2ox2* (Kim et al., 2008; Gabriele et al., 2010). The SOM branch has been further elucidated by showing that SOM directly represses the expression of two Jumonji C (JmjC) domain-containing histone arginine demethylases encoded by *JUMONJI 20* (*JMJ20*) and *JMJ22* (Cho et al., 2012). The light-induced degradation of PIF1 results in a decreased amount of SOM, which in turn increases the amount of JMJ20 and JMJ22. Removal of methyl groups from the arginine residues of histone H4 on the promoters of *GA3ox1* and *GA3ox2* results in higher expression of these biosynthetic enzymes, promoting GA production and concomitant seed germination.

Reciprocally, DELLAs also regulate the stability and activity of PIFs to regulate photomorphogenesis. Several independent studies demonstrated that the DELLA proteins physically interact with PIFs and sequester them into an inactive complex to restrain PIFs from binding to their targets (Feng et al., 2008; de Lucas et al., 2008; Gallego-Bartolomé et al., 2010). Recently it has also been shown that DELLAs not only sequester PIFs but also induce their degradation (Figure 3A) and, more importantly, DELLA-mediated degradation of PIFs functions independently of well-established phyB/LRBs pathways (Li et al., 2016). Thus, crosstalks between light and GA signaling pathways play an important role in fine-tuning germination and photomorphogenesis for optimal growth and development of plants.

Role of PIFs in Light and Brassinosteroid Pathways

Brassinosteroids (BRs) are steroid hormones that play diverse roles in plant growth and responses to external stresses, as well as acting in a concert with light signaling pathway principally through PIFs (Lozano-Durán and Zipfel, 2015; Saini et al., 2015).

In a study aimed at the identification of components of the light signaling pathway that interact with BZR1, PIF1 and PIF4 were identified as interacting partners of BZR1 (BRASSINAZOLE-RESISTANT 1) (Oh et al., 2012). The BZR1 is a transcription factor that selectively binds to BR response element (CGTG(T/C)G), and functions as both activator and repressor of distinct target genes. It was established that the functional PIF4–BZR1 complex co-regulates the expression of both light- and BR-responsive genes, including *PRE*s (*PACLOBUTRAZOL RESISTANCE*), which function in growth promotion over immunity (see below). In addition, BIN2 (BRASSINOSTEROID-INSENSITIVE 2), a glycogen synthase kinase 3 (GSK3) family of protein kinase that phosphorylates BZR1 in the BR signaling pathway, has been shown to phosphorylate PIF3 and PIF4. BIN2-mediated phosphorylation of PIF4 leads to the degradation of PIF4 through the ubiquitin–proteasome system, and this process is required for diurnal growth of hypocotyls (Figure 3B) (Bernardo-García et al., 2014). Strikingly, COP1/SPA complex interacts with PIF3 and prevents BIN2-mediated phosphorylation of PIF3 in a non-proteolytic manner (Ling et al., 2017). In addition, very recently PIFs were also shown to modulate BR signaling by regulating BR biosynthesis (Wei et al., 2017). PIF4 and PIF5 bind to the promoter regions and induce the expression of *DWF4* (*DWARF4*) and *BR6ox2* (*BRASSINOSTEROID-6-OXIDASE 2*), two key enzymes involved in BR biosynthesis (Figure 3B). Thus, PIFs regulate both BR biosynthesis and signaling pathways to optimize plant growth in response to light and BR.

PIFs Function in Light and Auxin Signaling Pathways

Auxin is a group of small organic molecules that regulate almost every aspect of plant growth and development essentially by modulating the cell division and cell elongation (Santner and Estelle, 2009). To regulate growth, plants often modulate either the biosynthesis and/or the sensitivity of auxin signaling (de Wit et al., 2016). *Arabidopsis* seedlings grown in either shade or high-temperature conditions display longer hypocotyls and petioles. However, mutants defective in auxin responses or in light signaling such as *pif* mutants do not show such phenotypes (Gray et al., 1998; Tao et al., 2008; Hornitschek et al., 2012). In accordance with the aforementioned phenotypic differences, pharmacological, molecular, and biochemical assays have demonstrated that some of the PIFs (PIF4, PIF5, and PIF7) bind to promoter regions and induce the expression of *TAA1* (*TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS 1*), *CYP79B2* (*CYTOCHROME P450, FAMILY 79, SUBFAMILY B, POLYPEPTIDE 2*), and several *YUC* (*YUCCA*) genes selectively under shade and/or high-temperature conditions (Figure 3C) (Franklin et al., 2011; Li et al., 2012; Sun et al., 2012). Similarly, several auxin signaling genes including *AFB1* (*AUXIN SIGNALING F-BOX1*), *IAA19* (*INDOLE-3-ACETIC ACID INDUCIBLE 19*), and *IAA29* were also found to be either direct or indirect targets of PIF4 and PIF5 (Hornitschek et al., 2012; Hersch et al., 2014). Interestingly, alternating roles of PIF4 and PIF5 as modulators of auxin sensitivity or biosynthesis have been observed under low red/far-red ratio (R:FR) of different PAR (photosynthetically active radiation) levels (Hersch et al., 2014). In this study, it was shown that PIF4 and PIF5 under low R:FR of high PAR increase the auxin biosynthesis by directly upregulating the expression of several auxin biosynthetic genes. However, under low R:FR and low PAR, PIF4 and PIF5 do not upregulate the auxin biosynthesis; rather they collectively enhance the auxin sensitivity, tentatively due to the increased expression of *AFB1* auxin co-receptor (Figure 3C).

Adding to the complexity of already known interactions between light and auxin signaling, it was found that PIF4 strongly interacts with *ARF6* (*AUXIN RESPONSE FACTOR 6*) to form a functional complex (similar to PIF4–BZR1) to cooperatively activate several genes in light and auxin signaling pathways (Figure 3C) (Oh et al., 2014). Thus, PIFs regulate both the biosynthesis and sensitivity of auxin signaling to optimize growth in response to light quality, quantity, and duration. However, it is not clear how the PIF4–ARF6 transcription complex selectively binds to target promoters and regulates their expression.

During seedling de-etiolation, light signal inhibits hypocotyl elongation while it promotes the cotyledon expansion. One of the long-standing questions in the field is how the light signaling achieves this contrasting growth pattern in these two organs. In a recent study, it was shown that light oppositely regulates the organ-specific expression of a group of *SAUR* (*Small Auxin Upregulated RNA*) genes, named *lirSAUR* (*l*ight-*i*nduced in cotyledons and/or *r*epressed in hypocotyl *SAUR*s) (Sun et al., 2016). These genes are direct targets of PIFs: PIFs activate the expression of these genes in the hypocotyls to promote hypocotyl elongation, while inhibiting their expression in the cotyledons to repress cotyledon expansion. Although PIFs are equally degraded in both hypocotyls and cotyledons, light-induced degradation of PIFs results in reduced expression of *SAUR*s in the hypocotyls but increased expression in the cotyledons, driving the contrasting growth pattern in an organ-specific manner. However, it is still unknown how PIFs activate gene expression in one organ while inhibiting the gene expression in another organ. As the authors suggested, the promoters of these genes might be epigenetically regulated in different organs. Alternatively or in addition to epigenetic changes, organ-specific specificity factors might be necessary to establish these contrasting expression patterns as recently shown for PIF1 (see below) (Kim et al., 2016a).

PIFs Mediate Light and Abscisic Acid Signaling Pathways

Similar to GA, BRs, and auxin, the light signaling pathway is also closely associated with the abscisic acid (ABA) pathway. Unlike complex interactions between light signaling and GA, BR, and auxin signaling pathways, molecular interactions with ABA are relatively less complex (de Wit et al., 2016; Yu and Huang, 2017). In imbibed seeds incubated in darkness, PIF1 binds to the promoter regions and activates the transcription of ABA signaling factors, including *ABI3* (*ABA-INSENSITIVE 3*) and *ABI5* (Figure 3D). These transcription factors not only enhance the ABA biosynthesis and signaling but also repress GA signaling to inhibit the germination (Oh et al., 2009; Park et al., 2011). In addition, PIF1 physically interacts with ABI3 in imbibed seeds to cooperatively regulate the expression of *SOM*, a negative regulator of seed germination (Park et al., 2011). Moreover, the group A bZIP proteins, including ABI5, directly interact with PIF1 and modulate the DNA binding specificity of PIF1 (Kim et al., 2016a). However, this study does not demonstrate the biological significance of the modulation of

DNA binding specificity of PIF1. Thus, although PIF1 in cooperation with components of ABA signaling controls the developmentally crucial phase of seed germination in response to endogenous and external cues, further studies are necessary to understand how the group A bZIP transcription factors, including the ABA signaling factors, modulate the DNA binding specificity of PIFs to regulate seed germination and seedling establishment.

PIFs Function in Light and Ethylene Signal Integration

The interactions between light and ethylene pathways has been extensively reviewed recently (Yu and Huang, 2017). Similarly to other hormones, PIFs mediate interactions between light and ethylene pathway by regulating both biosynthesis and signaling of ethylene responses. For ethylene biosynthesis, overexpression of *PIF5*, but not other *PIFs*, has been shown to upregulate the expression of members of the *ACS* (*1-aminocyclopropane-1-carboxylic acid* [*ACC*] *SYNTHASE*) genes (Figure 3E), resulting in an increased production of ethylene in the dark (Khanna et al., 2007). Therefore, *PIF5* overexpression lines display triple responses, characteristic of either ethylene overproduction or constitutively active ethylene signaling. However, this study could not confirm whether the endogenous PIF5 regulates ethylene biosynthesis as *pif5* mutant neither displayed defects in *ACS* gene expression nor ethylene-related phenotypes. This is a typical complicacy with overexpression studies for transcription factors that are able to homo- and heterodimerize. It is still possible that PIF5 might regulate ethylene biosynthesis in a tissue- or cell-type-specific manner, which might have been masked in this study due to the use of whole seedlings. Alternatively, PIF5 might interact with other bHLH proteins that regulate ethylene biosynthesis. Thus, overexpression of PIF5 may simply show a dominant negative phenotype due to the titration of other bHLH proteins.

For interaction with ethylene signaling, PIFs have been shown to genetically interact with ethylene signaling factors. For example, ethylene signaling stabilizes the downstream transcription factors EIN3/EIL1 (ETHYLENE INSENSITIVE 3 and EIN3-like 1) that induce the expression of *PIF3* in the cotyledon of growing seedlings (Figure 3E) (Zhong et al., 2014). EIN3/EIL1 also activates *ERF1* (*ETHYLENE RESPONSE FACTOR 1*) in the hypocotyls to inhibit hypocotyl elongation. Thus, EIN3/EIL1 coordinately regulate the expression of *PIF3* in the cotyledons and *ERF1* in the hypocotyls to prevent photo-oxidative damage of seedlings emerging from subterranean darkness. Recently, PIFs and EIN3 have been shown to mediate transcriptional co-regulation of common target genes. In this study, PIFs and EIN3 did not physically interact with each other. However, they bind to several common target promoters and regulate their expression in the same direction either interdependently or additively (Figure 3E) (Jeong et al., 2016). The authors performed ChIP assays to examine interdependency between PIF4 and EIN3. However, instead of using the mutant backgrounds, the authors used dark versus light (assuming PIFs will be degraded under light) or presence of exogenously added Ag^+ or ACC (mimicking ±ethylene). Under these conditions, they did not observe any interdependent DNA binding *in vivo*. Thus, these data are inconclusive as to whether PIFs and EIN3 can bind to DNA interdependently *in vivo*. Further studies are necessary to understand how PIF3 and EIN3 target downstream common targets.

INVOLVEMENT OF PIFs IN GROWTH–DEFENSE TRADEOFFS

Plant growth is plastic throughout its life cycle and displays remarkable adaptation in response to biotic and abiotic threats. Plants growing under less external stress show a robust growth with relatively high accumulation of biomass before they enter into the reproductive stage. On the contrary, under suboptimal conditions plants not only slow down the rate of growth but also accelerate the developmental transition from vegetative to reproductive growth resulting, in significantly lower biomass accumulation and production of fewer seeds (Ballaré, 2014; Zust and Agrawal, 2017).

In recent years, PIFs have been shown to govern both growth and defense responses in plants (Leivar and Monte, 2014). Recent studies have shown that the *loss-of-function* mutation or ectopic expression of one or more of the eight closely related PIFs severely alters the fine balance between growth–defense tradeoffs in plants (Yang et al., 2012; Campos et al., 2016; Gangappa et al., 2017). Therefore, plants carefully regulate the level of active PIFs through multiple mechanisms including transcriptional regulation and post-transcriptional regulation via ubiquitin–proteasome-mediated degradation, heterodimerization-mediated protein sequestration, and probably other as yet unknown mechanisms (Lucas and Prat, 2014; Xu et al., 2015, 2017; Zhu et al., 2015; Yu and Huang, 2017). One of the classical examples of growth–defense tradeoffs in plants occurs during the pathogen attack. To spread the disease rapidly, pathogens typically promote the plant growth by altering their signaling networks, while plants tend to reduce growth by repressing several growth-promoting genes, including PIFs, to limit the spread of the disease (Figure 4A) (Windram et al., 2012).

Jasmonic acid (JA) regulates plant growth and development as well as plant responses to biotic and abiotic stresses. JA elicits the defense signaling by promoting the degradation of JAZ (JASMONATE-ZIM DOMAIN) proteins, i.e., repressors of JA-responsive genes involved in defense signaling (Campos et al., 2016). A recent study demonstrated the molecular link through which JA participates in plant growth–defense tradeoff (Yang et al., 2012). JAZ9, one of the JAZ proteins, inhibits the interaction between RGA (a DELLA repressor protein) and PIF3. This study suggests that, in plants growing under near-optimal growth conditions, JAZ9 levels are high due to low amounts of JA, which releases PIF3 from RGA–PIF3 complex, resulting in growth promotion. On the contrary, in response to pathogen attacks plants activate JA signaling, leading to enhanced degradation of JAZ9, which in turn stabilizes RGA–PIF3 interaction and sequesters PIF3 away from target genes (Figure 4B). Consistently, JA treatment activates defense responses and represses the growth, while overexpression of *PIF3* partially suppresses JA-induced inhibition of growth. Therefore, JA signaling indirectly regulates the growth–defense tradeoff via PIF3.

It is interesting to note that PIF3's primary interacting partner phyB is also involved in the growth–defense tradeoff

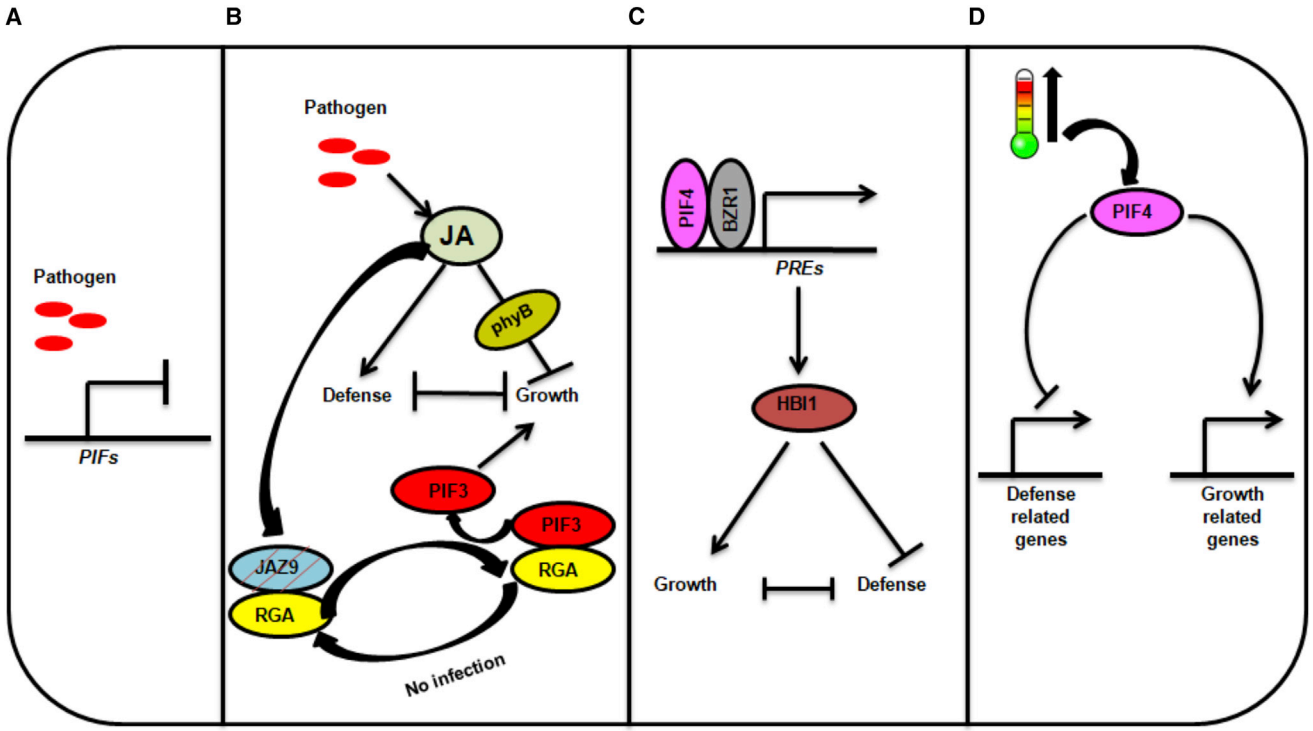

Figure 4. PIFs Regulate Growth–Defense Tradeoffs in *Arabidopsis*.
Plants use multiple mechanisms involving PIFs as core factors to fine-tune growth in response to pathogen attacks.
(A) In response to pathogen attack, plants downregulate the expression of *PIFs* to suppress growth.
(B) To counter the pathogen attacks, plants activate the JA signaling which promotes defense responses, while suppressing growth by modulating the functions of light signaling components. Activated JA signaling promotes the degradation of JAZ9, releasing RGA from JAZ9-RGA complex, which in turn forms another complex with PIF3 to inactivate PIF3 activity. Secondly, activated JA signaling genetically interacts with phyB to repress plant growth.
(C) PIF4 interacts with BZR1 to form a complex and collectively activates the expression of *PREs*. PREs in turn indirectly activate HBI1. HBI1 promotes growth, while suppressing defense signaling in the absence of pathogen challenge.
(D) High-temperature-activated PIF4 actively promotes growth by upregulating the growth-related genes and simultaneously represses the defense responses by suppressing the expression of genes involved in plant defense.

(Figure 4B). It is widely accepted that the defense responses against pathogen attacks and plant growth are coupled processes, occurring primarily due to the partitioning of photoassimilates (Zust and Agrawal, 2017). Accordingly, healthy plants are believed to proportionate nutrients and energy requirements to balance the active growth and basal level of immunity for possible pathogen attacks. Nevertheless, under the pathogen attacks, plants divert most of the nutrients and energy from growth toward the defense, leading to relatively stunted growth (Huot et al., 2014). However, this assumption was shown to be untrue, at least partially, in a recent study. It has been demonstrated that the JA-mediated growth–defense tradeoff is rather uncoupled in *jazQ phyB* (*jaz1/3/4/9/10 phyB*) mutant (Campos et al., 2016), as it shows physiological traits of constitutively heightened defense responses as well as an active growth. Such an enhanced defense and an active growth was absent from either *jazQ* (enhanced resistance but stunted growth) or *phyB* (active growth but diminished resistance), concluding that at least the JA-mediated signaling network, which is activated in response to pathogen attack, attenuates the growth signaling. It is possible that the elevated level of PIFs in the *jazQ phyB* mutant might have contributed to the uncoupling of the growth–defense tradeoff in this background.

Seedlings growing under darkness exhibit rapid growth and low level of immunity against plant pathogens (Roden and Ingle, 2009). PIFs, which are relatively stable in the dark, activate the transcription of growth-promoting genes. Nevertheless, experimental evidence gathered in recent studies suggests that PIF4 regulates growth–defense tradeoff in the dark indirectly by increasing the activity of HBI1 (HOMOLOG OF BEE2 INTERACTING WITH IBH1) (Lozano-Durán and Zipfel, 2015). HBI1 is a bHLH protein that suppresses defense signaling while promoting an active growth (Fan et al., 2014). In darkness, PIF4 interacts with BZR1 and the PIF4–BZR1 complex cooperatively activates the expression of *PRE*s (*PACLOBUTRAZOL RESISTANCE*) (Zhang et al., 2009; Oh et al., 2012). PREs interact with IBH1 (ILI1 BINDING BHLH 1; a protein that inactivates HBI1 upon interaction) and inhibit the activity of IBH1, thus promoting the activity of HBI1 resulting in reduced defense response (Figure 4C).

Plants growing at higher than ambient temperature display rapid growth of multiple organs, albeit at the cost of relatively diminished immunity against pathogens (Gangappa et al., 2017). As discussed above, a number of studies in the past decade have demonstrated the involvement of PIF4 as one of the crucial components in high-temperature-mediated growth (Koini et al.,

2009; Franklin et al., 2011; Sun et al., 2012). However, only very recently it was shown that PIF4 signaling not only modulates growth but also controls the plant defense responses under elevated temperature (Gangappa et al., 2017). Nucleotide binding and leucine-rich repeat (NB-LRR) proteins such as SNC1 (suppressor of *npr1-1*, constitutive 1) are important modulators of temperature-sensitive plant defense responses. The *snc1-1* mutant displays constitutive activation of immune responses and severe growth defects under ambient temperature, but not at higher temperature (Zhu et al., 2010). The recent study showed that the temperature-induced suppression of defense responses in *snc1-1* is nullified in *snc1-1 pif4* double-mutant background (Gangappa et al., 2017). They also showed that the expression of a number of defense-related genes is significantly increased in the *pif4* mutant, while expression of growth-related genes decreased significantly. On the contrary, overexpression of *PIF4* behaves oppositely, indicating that PIF4 indeed modulates growth–defense tradeoff under higher temperature (Figure 4D). Interestingly, they also found that overexpression of *PIF4* lacking the basic domain (PIF4Δb) functions as a dominant negative, as it significantly downregulates the expression of growth-associated genes while upregulating defense-responsive genes. However, the major drawback of the study is the use of PIF4Δb itself. It is well known that PIF4 forms heterodimers with other PIFs and HLH proteins through its HLH domain. Hence, overexpression of PIF4Δb might result in excessive sequestration of other PIFs and HLH proteins through heterodimerization, which in turn might have caused some of the genetic and physiological responses they observed in PIF4Δb transgenic plants. Therefore, further studies are necessary to understand how PIF4 and/or other PIFs regulate these responses.

CONCLUSIONS AND FUTURE PERSPECTIVES

PIFs have expanded their horizon from central phytochrome signaling components to crucial signal integrators of multiple signaling pathways regulating plant growth and development. They do so by expanding their repertoire of genetic and physical interactions with components of multiple pathways. Although a considerable amount of information about signal integration by PIFs has been learned in recent years, we are still at an early stage of our understanding of the molecular details. A comprehensive analysis of PIF-interactome might uncover new roles of PIFs in signal integration from other pathways. Our knowledge about the known physical interactions of PIFs is also rudimentary. Apart from DELLA-mediated inhibition of PIF-DNA binding, the significance of PIF's interactions with ARF6 and BZR1 is only at a genetic level. How do these transcription factors directly interact with DNA? The fact that PIF-DNA binding ability is facilitated by the bZIP and other families of transcription factor suggests a cooperative DNA binding. Thus, PIF4 and BZR1 might form a complex and bind to DNA as heterotetramer. However, PIF4-BZR1-ARF6 might form a complex on the DNA with neighboring DNA binding sites, facilitating each other's binding. PIFs also function as an activator in one organ while being a repressor in another organ. It is still unknown whether PIFs achieve this bifunctional transcriptional regulation by interacting with other transcriptional co-regulators and/or by interacting with chromatin-modifying enzyme complexes to open up chromatin for activation while closing chromatin for repression.

In recent years, PIFs have been discovered from *Physcomitrella* to higher plants including a variety of crop plants. A major future challenge will be to transfer the knowledge gained from model plants to crops to produce genetically tailored plants that can grow under adverse climate (e.g., elevated temperature) with increased biomass and yield. In summary, due to their extensive networking capabilities, PIFs hold great potential for future biotechnological applications.

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