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Review

Illuminating Progress in Phytochrome-Mediated Light Signaling Pathways

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Light signals regulate a plethora of plant responses throughout their life cycle, especially the red and far-red regions of the light spectrum perceived by the phytochrome family of photoreceptors. However, the mechanisms by which phytochromes regulate gene expression and downstream responses remain elusive. Several recent studies have unraveled the details on how phytochromes regulate photomorphogenesis. These include the identification of E3 ligases that degrade PHYTOCHROME INTERACTING FACTOR (PIF) proteins, key negative regulators, in response to light, a better view of how phytochromes inhibit another key negative regulator, CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), and an understanding of why plants evolved multiple negative regulators to repress photomorphogenesis in darkness. These advances will surely fuel future research on many unanswered questions that have intrigued plant photobiologists for decades.

Trends

Two classes of repressors called COP/DET/FUS complex and PIFs synergistically repress photomorphogenesis in darkness.

Light signals perceived by phytochromes inhibit these repressors to promote photomorphogenesis.

$CUL3^{LRB}$ induces polyubiquitylation and subsequent co-degradation of PIF3 and PHYB through the 26S proteasome pathway.

$CUL4^{COP1–SPA}$ E3 ligase promotes rapid light-induced degradation of PIF1 to promote photomorphogenesis.

Phytochromes directly interact with SPA1 and reorganize the COP1–SPA interaction to inhibit COP1 activity.

Phytochrome-Mediated Light Signaling Pathways

Light is an essential commodity for photosynthetic energy production as well as an environmental cue for increasing awareness and fitness to the surrounding conditions. Plants employ two contrasting developmental programs to succeed in ambient light conditions: skotomorphogenesis and photomorphogenesis (Figure 1). Skotomorphogenesis is characterized by elongated hypocotyl, closed cotyledon, and an apical hook to allow young seedlings to grow rapidly in darkness using the reserve energy present in the seed. By contrast, photomorphogenesis is the process where light signals inhibit the rapid elongation of hypocotyl, expand the cotyledons, and promote greening to allow the seedling body to adjust for optimal light-harvesting capacity and autotrophic growth. To promote photomorphogenesis and actively suppress skotomorphogenic development, plants have evolved multiple photoreceptors to track a wide spectrum of light wavelengths in a local environment. These include the UVB-RESISTANCE 8 (UVR8 for UV-B light), cryptochromes (CRY), phototropins, and ZEITLUPE/FLAVIN-BINDING, KELCH REPEAT, F BOX 1/LOV KELCH PROTEIN 2 family of photoreceptors (ZTL/FKF1/LKP2) (for UV-A/blue light) and phytochromes (phy for red/far-red light) [1]. This review will focus on the phytochrome family of photoreceptors that are encoded by five genes in *Arabidopsis thaliana* (*PHYA–PHYE*) [2]. Phytochromes perceive the ambient red (R) and far-red (FR) light signals in the environment and promote gradual progression to photomorphogenic development by orchestrating an elaborate signaling mechanisms [3,4]. These include allosteric conformation change of phytochromes to a biologically active Pfr form from an inactive Pr form followed by nuclear translocation to inhibit two classes of repressors of photomorphogenesis called CONSTITUTIVELY PHOTOMORPHOGENIC/DEETIOLATED/FUSCA (COP/DET/FUS) complex and Phytochrome Interacting Factors (PIFs) (Figure 1) [4,5]. In darkness, these dual repressors are actively promoting skotomorphogenic development by suppressing photomorphogenesis, and

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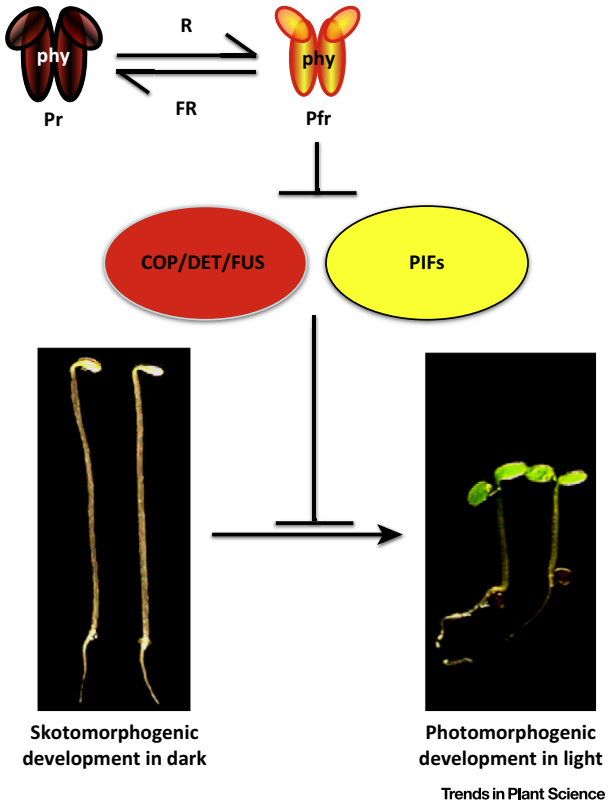

Figure 1. A Simplified View of the Phytochrome-Mediated Light Signaling Pathways. In the dark, phytochromes exist in the biologically inactive Pr form. The CONSTITUTIVELY PHOTOMORPHOGENIC/DEETIOLATED/FUSCA (COP/DET/FUS) complexes and PHYTOCHROME INTERACTING FACTOR (PIF) proteins function in the dark to repress photomorphogenic development. Seedlings grown in the dark show a long hypocotyl and closed cotyledons. Under light, phytochrome perceives red/far-red light signals and photoconverts from an inactive Pr form to an active Pfr form. The active Pfr form suppresses the functions of COP/DET/FUS complexes and PIFs. As a result, seedlings progress toward photomorphogenic development. Seedlings grown in light display short hypocotyl, open and expanded green cotyledons for optimal photosynthesis and autotrophic growth. Abbreviations: FR, far-red light; R, red light; Pfr, far-red light absorbing form of phytochrome; phy, phytochrome; Pr, red light absorbing form of phytochrome.

therefore inhibition of these repressors allows gradual progression to photomorphogenic development under light. This review will focus on recent progress on the mechanistic understanding of how phytochromes inhibit these repressors. For a detailed review on other aspects of photomorphogenesis, readers are directed to recent reports and reviews [6–13].

E3 Ligases for PIFs

PIFs belong to the basic helix-loop-helix (bHLH) family of transcription factors [14,15]. There are seven PIFs in *Arabidopsis* that function in a partially-differential to a largely-overlapping manner to regulate gene expression and ultimately photomorphogenesis [4,16–19]. All PIFs interact with the Pfr forms of phytochromes with differential affinities [4,20]. Phytochromes interact with PIFs through the APB (active phytochrome binding) or APA (active phytochrome A binding) domains present at the N termini of PIFs. Conversely, PIFs displayed higher affinity for the N terminus of phytochromes [21,22]. Direct physical interaction of PIFs with phytochromes leads to the light-induced phosphorylation followed by ubiquitylation and subsequent degradation of PIFs by the ubiquitin/26S proteasome system (UPS). In addition, light-induced phosphorylation is necessary for degradation of PIF3 [23]. The degradation kinetics of PIFs under different light qualities/quantities and early post-translational modifications have been extensively investigated [4]. A putative polyubiquitin binding factor called HEMERA is also necessary for degradation of PIF1

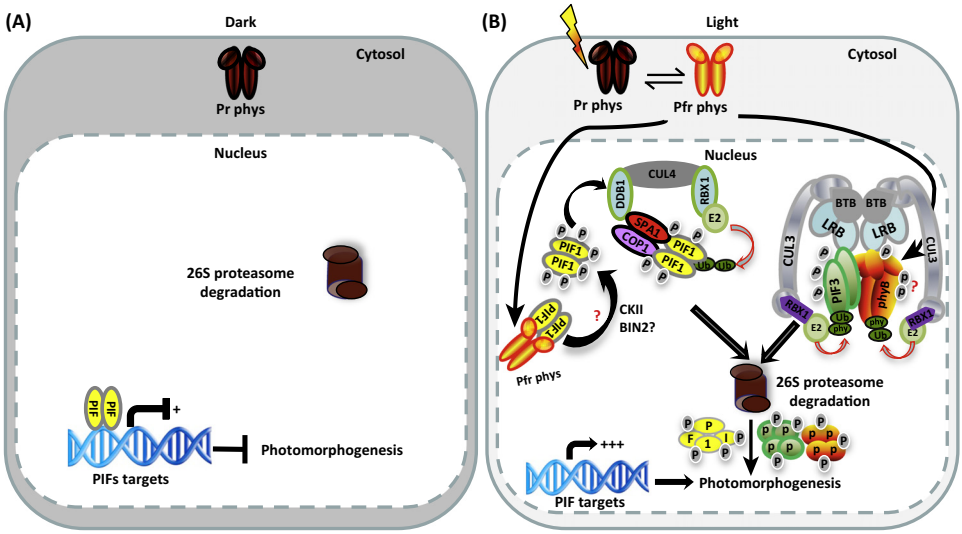

Figure 2. A Model Showing How Light Signals Induce Degradation of PHYTOCHROME INTERACTING FACTOR (PIF) proteins. (A) In the dark, the biologically inactive Pr form of phytochrome is localized in the cytosol. The nuclear localized PIFs homo- and heterodimers bind to the promoter region of light-regulated target genes and repress their expression to prevent photomorphogenesis. (B) Upon light exposure, the biologically active Pfr form of phytochrome translocates into nucleus. For PIF1, the interaction between the Pfr form of phytochromes and PIF1 triggers the rapid phosphorylation of PIF1 through an unknown kinase. The phosphorylated form of PIF1 is recruited to the CULLIN4$^{\text{CONSTITUTIVELY PHOTOMORPHOGENIC 1–SUPPRESSOR OF PHYA 1}}$ ($CUL4^{COP1–SPA1}$) complex for rapid ubiquitylation and subsequent degradation through the 26S proteasome pathway. For PIF3, phyB and PIF3 homodimers interact with each other to form a quaternary complex, which is phosphorylated by an unknown kinase. The phosphorylated PIF3 and phyB bimolecular tetramer is ubiquitylated by CULLIN3–RING BOX 1–LIGHT RESPONSE BTB (CRL3) E3 ubiquitin ligase complex. Subsequently, phyB and PIF3 are concurrently degraded by the 26S proteasome pathway. The destruction of the PIFs derepresses light-regulated gene expression and promotion of photomorphogenesis in response to light. Abbreviations: Pfr, far-red light absorbing form of phytochrome; phy, phytochrome; Pr, red light absorbing form of phytochrome; ub, ubiquitin.

and PIF3 under prolonged light [24,25]. However, the kinases that phosphorylate PIFs and the E3 ligases that ubiquitylate PIFs in response to light are under intense investigation. Two recent reports described the identification of E3 ligases for PIF degradation [26,27]. These studies highlight the complex mechanism of how PIFs are regulated to fine-tune photomorphogenesis.

A recent study described a CULLIN 3 (CUL3)-based E3 ligase for PIF3 degradation (Figure 2, right). The substrate adaptor component for this ligase is LRB (light response BTB) proteins [26]. LRBs belong to the BTB family (Bric-a-Brack/Tramtrack/Broad) and display strong affinity for the phosphorylated form of PIF3, which is consistent with the light-induced phosphorylation and subsequent degradation of PIFs. In addition, $CUL3^{LRB}$ can catalyze ubiquitylation of a phosphomimic form of PIF3 *in vitro*. Interestingly, LRBs recruit both PIF3 and phyB in the $CUL3^{LRB}$ complex for polyubiquitylation and subsequent co-degradation by the 26S proteasome pathway. Because LRBs interact with each other to dimerize, it is possible that the PIF3–phyB bimolecular tetramer is recognized by two $CUL3^{LRB}$ complexes for light-induced ubiquitylation [28,29]. This study also highlights the importance of receptor desensitization in many eukaryotic systems where the receptor is activated to transmit the incoming signal and then the receptor is either degraded or endocytosed to inactivate. This prevents the signaling pathways from overactivation under prolonged incoming signals [30]. However, the drawback of this study is the lack of any biological significance of PIF3 degradation. *lrb* double and triple mutants do display photomorphogenic phenotypes; however, these phenotypes are not consistent with PIF3 degradation, but are consistent with phyB degradation. In fact, LRBs have previously been shown to regulate phyB and phyD levels in response to light [29]. Not surprisingly, *lrb* double and

triple mutants are strongly hypersensitive to light as they have higher level of phyB and phyD under light. LRBs interact with phyB both *in vitro* and *in vivo* [26]. However, it is not clear whether LRBs have higher affinity for the phosphorylated form of phyB than the unphosphorylated form of phyB, although phyB is known to be phosphorylated [31]. Therefore, at the molecular level, LRBs function as *bona fide* E3 ligases for PIF3. However, the morphological phenotypes suggest that the main function of LRBs is to degrade phyB/D for receptor desensitization under prolonged light conditions, and the role of PIF3 is to enhance this process by helping the LRBs to recruit phyB/D. Because LRBs preferentially bind to the phosphorylated form of PIF3, and PIF3 binds to phyB/D, PIF3 might function as a cofactor for LRBs for enhanced substrate recruitment in this process. However, PIF3 is also degraded in this process without having any biological consequence for plants. Thus, it is likely that additional E3 ligase(s) are necessary for PIF3 and other PIFs degradation in response to light. In line with this prediction, a recent study described a well-established CUL4-based E3 ligase for PIF1 degradation in response to light [27]. In this case, COP1 and SPA proteins act as substrate adaptor components in recruiting preferentially the phosphorylated form of PIF1 in the $CUL4^{COP1-SPA}$ complex for light-induced ubiquitylation and subsequent degradation. The light-induced ubiquitylation followed by degradation of PIF1, but not the light-induced phosphorylation of PIF1, is defective in *cop1*, *spaQ*, and *cul4cs* backgrounds compared to wild type. This rapid degradation of PIF1 is mostly regulated by phyA, and phyA is not degraded under these conditions, suggesting that PIF1 and phyA may not be co-degraded as previously shown for PIF3–phyB co-degradation [26]. In addition, *cop1* and *spaQ* mutants display a strong hyposensitive phenotype in seed germination assays, consistent with the major role of PIF1 in this process. COP1 and SPA proteins as well as PIFs are well-established repressors of photomorphogenesis in the dark [4,5]. Strikingly, these repressors function synergistically to repress photomorphogenesis in the dark (see below) [32]. However, in response to light, one group of repressors (COP1/SPA) targets another group (PIFs) to promote their rapid degradation under low light conditions. This allows plants to prevent over repression of photomorphogenesis and gradually transition to photomorphogenic development. However, PIF1 is still degraded under prolonged light conditions in all the above mutants, suggesting additional E3 ligases are necessary for PIF1 and other PIF degradation. In addition, because PIF3 is unstable in *cop1* and *spa* mutants [19,33], it is not clear if CUL4-based E3 ligase also plays a role in the degradation of other PIF proteins.

Kinases for PIFs

One of the first post-translational modifications in PIFs before their degradation is light-induced phosphorylation [4]. Light-induced phosphorylation is a prerequisite for degradation of PIFs through the 26S proteasome system. As discussed above, both CUL3- and CUL4-based E3 ligases preferentially recruit the phosphorylated form of PIF3 and PIF1, respectively, to the E3 ligase complex for polyubiquitylation (Figure 2, right). Therefore, an intense search is underway to identify the kinases that phosphorylate PIFs in response to light. The first candidate considered for a PIF kinase was phytochrome itself because phytochrome has been shown to function as a serine/threonine kinase with a histidine kinase ancestry [34]. Direct physical interaction with phytochrome is necessary for the light-induced phosphorylation and degradation of PIFs. Plant phytochrome has been shown to phosphorylate other substrates including PKS1, FHY1, and IAA proteins [35–37]. In addition, bacterial phytochromes function as histidine kinases [38,39]. However, the drawback of all the above studies is that both the Pr and Pfr forms of phytochromes phosphorylated most of the substrates, despite the fact that only Pfr is the biologically active form of phytochrome. In addition, convincing *in vivo* evidence supporting the role of phytochrome as a kinase is still lacking because no kinase-inactive mutant form of phytochrome has been described that did not rescue the *phy* mutant phenotypes. Moreover, constitutively nuclear localized phytochromes do not induce photomorphogenesis in the absence of light, suggesting a Pfr-specific signaling mechanism [40–42]. In addition, a C-terminal single-nucleotide deletion mutant of phyB (*phyB-28*) expressing a truncated form without the histidine kinase-

related domain is still partially functional *in vivo*, suggesting that the putative kinase domain is not essential for phyB function [43]. However, several recent studies alleviate the concerns raised above. For example, the majority of the biological functions of phytochromes are located within the nucleus [40,41], although phyA displays roles in the cytosol [44,45]. The Pfr forms of all phytochromes translocate into the nucleus in response to light, while the Pr form is mostly in the cytosol [46], suggesting a physical separation of the substrates from the kinase. Moreover, phyB has been shown to sequester PIFs in response to light by direct physical interaction, and this sequestration contributes to the biological function of phyB [47]. Many targeted mutations in the putative kinase domain have been described previously that rescued the *phyA* mutant phenotypes [48]. However, these mutants might have rescued *phyA* mutant phenotypes due to phyA-mediated sequestration of PIFs similar to phyB, although sequestration of PIFs by phyA has not been demonstrated yet. Therefore, the rate-limiting steps might be at two levels: one at the nuclear translocation step of phytochromes to promote physical proximity to the substrates, and the other at the Pfr-specific interaction and phosphorylation of the substrates. The Pr-induced phosphorylation described previously might simply be forced phosphorylation due to the use of non-physiological amounts of the kinase and substrates in the *in vitro* experiments [34,36,37]. Thus, the hypothesis that kinase activity is one of the major biochemical functions of phytochromes might be worth revisiting.

Despite the above inconclusive hypothesis, targeted candidate gene approaches have identified two kinases recently that phosphorylate PIFs directly. These include casein kinase II (CK2) and BRASSINOSTEROID INSENSITIVE 2 (BIN2) (Figure 2, right) [49,50]. CK2 has been shown to phosphorylate seven serine/threonine (S/T) residues present in PIF1 *in vitro* [49]. Serine to alanine substitution mutations in six of these sites, especially the three consecutive S residues at the C-terminal end, drastically reduced the degradation of PIF1 in response to light *in vivo*. However, PIF1 was still phosphorylated in response to light, suggesting that CK2 is not the light-regulated kinase that phosphorylates PIF1 in response to light. BIN2 has been shown to phosphorylate PIF4 *in vitro*, and this phosphorylation alters the degradation kinetics of PIF4 in response to light and brassinosteroid (BR) [50]. However, it is still not clear whether CK2 and BIN2 phosphorylate PIFs in a light-dependent manner *in vivo*. Therefore, the light-regulated kinase that phosphorylates PIFs in response to light is still unknown [51].

Phytochrome-Mediated Inhibition of COP1 Activity

One of the long outstanding questions in light signaling pathways is how COP1 is inactivated by light to promote photomorphogenesis. Photobiological experiments demonstrated that both phytochromes and cryptochromes inactivate COP1 in response to red/far-red and blue light, respectively [52]. These photoreceptors employ dual mechanisms for this purpose. Under prolonged light as well as relatively shorter light exposure, COP1 is excluded from the nucleus [53,54]. However, COP1 is also rapidly inactivated by these photoreceptors to trigger light-induced gene expression and photomorphogenesis. CRY1 and CRY2 have been shown to directly interact with SPA1 to dissociate the COP1–SPA complex in response to blue light [55–57]. Nevertheless, how phytochromes inactivate COP1 was unknown until recently. Two recent studies showed that phytochromes also directly interact with SPA1 and reorganize the COP1–SPA complex in a light-dependent manner [58,59]. This reorganization leads to the separation of the physical contact between COP1 and SPA1, thereby reducing the ability of COP1 to degrade positively-acting transcription factors (e.g., HY5/HFR1/LAF1 and others) (Figure 3, right). The increased abundance of the positively-acting factors promotes photomorphogenesis in response to light. However, it is still not clear whether this separation only affects the SPA1-mediated enhancement of COP1 activity and/or directly inhibits the ability of COP1 to degrade the positively-acting transcription factors.

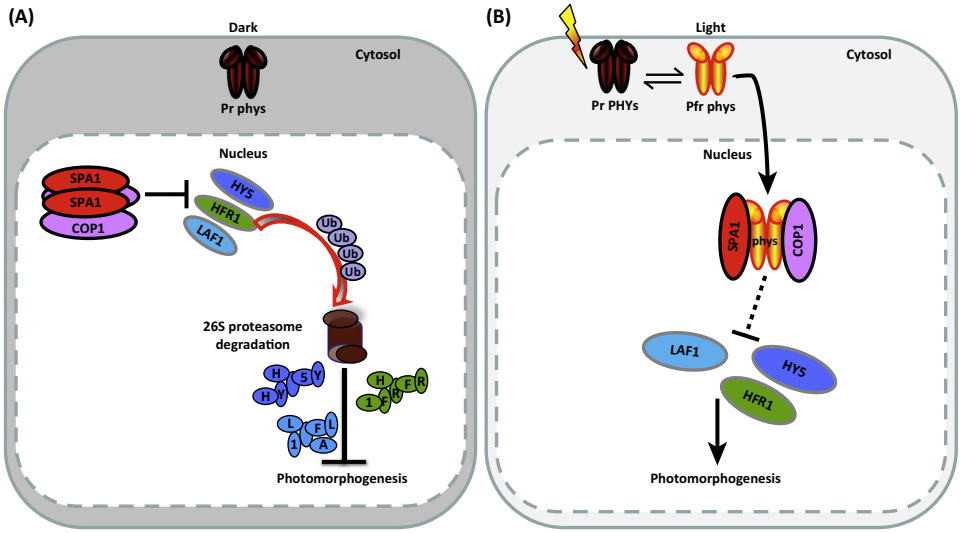

Figure 3. A Model Showing the Mechanisms of Inhibition of CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) Activity by Phytochromes in Response to Light. (A) In darkness, COP1–SUPPRESSOR OF PHYA (COP1–SPA) complexes repress photomorphogeneis by their E3 ligase activity. COP1 and SPA homodimers interact with each other to form a tetrameric complex. SPAs activate the COP1 E3 ligase activity to trigger the poly-ubiquitylation and proteasome-mediated degradation of positively-acting transcription factors [such as LONG HYPOCOTYL 5 (HY5), LONG HYPOCOTYL IN FAR-RED (HFR1), and LONG AFTER FAR-RED LIGHT 1 (LAF1)] that promote photomorphogenesis. (B) Upon illumination, the active Pfr forms of phytochromes translocate into the nucleus to interact with SPAs and disrupt the direct interaction between SPA1 and COP1. Without the activation of SPA, the positively-acting transcription factors (HY5, HFR1, and LAF1) accumulate in response to light. Increased abundance of the positively-acting transcription factors activates photomorphogenesis. Abbreviations: phy, phytochrome; Pfr, far-red light absorbing form of phytochrome; Pr, red light absorbing form of phytochrome; ub, ubiquitin.

Although these studies demonstrated a mechanism for rapid inactivation of COP1 by phytochromes, there are discrepancies between the two studies. For example, one study showed that the C terminus of SPA1 interacts with the N terminus of phyA [59]; while the second study showed the opposite interaction through the N-terminal kinase-like domain of SPA and the C terminus of phyA [58]. However, both groups showed that the full-length SPA1 interacts with the full-length phyB [58,59]. Surprisingly, CRY1 and CRY2 also interacted with SPA1 through different domains. The cryptochrome C-terminal extension (CCE) domain of CRY1 interacted with the C terminus of SPA1 [55,56]; however, the N-terminal photolyase-related (PHR) domain of CRY2 interacted with the N terminus of SPA1 [57]. Although these discrepancies need to be resolved in the future, both studies show a mechanism by which phytochromes can rapidly modulate COP1 activity to allow photomorphogenesis to proceed.

Nontranscriptional Roles of PIFs as Cofactors of E3 Ligase

As discussed above, photomorphogenesis is repressed by two distinct classes of proteins: one (COP/DET/FUS) complex involves ubiquitin-mediated degradation of the positively-acting factors (Figures 1 and 3, left) and the other encodes bHLH transcription factors (PIFs) (Figures 1 and 2, left) [4,5]. However, the relationship between these two groups of repressors was not clear until recently. Why have plants evolved two classes of repressors? Do they function additively or synergistically? Strikingly, a recent study demonstrated that these two groups of proteins function synergistically to repress photomorphogenesis [32]. Genetic analysis showed that *cop1pif* or *spa123pif1* combination mutants were more hypersensitive compared to their respective parents (Figure 4A). Biochemical analyses showed that HY5, the key target of the COP1/SPA complex, is much more abundant in the *cop1pif* or *spa123pif1* combination mutants

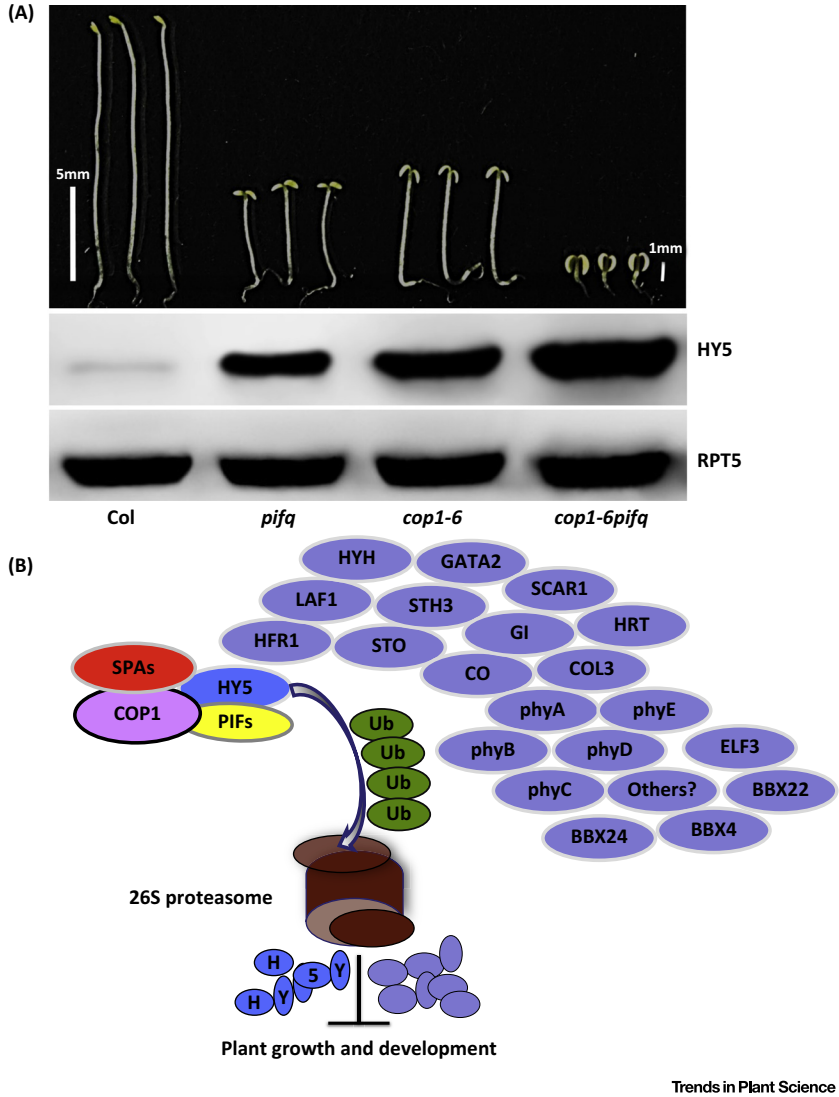

Figure 4. A Model of How COP1 and PIF Repressors Function Synergistically to Regulate Plant Growth and Development. (A) Top, Visible phenotypes of the wild-type, *pifq*, *cop1-6*, and *cop1-6pifq* seedlings. Seeds of various genotypes were grown on MS medium without sucrose for 5 days in the dark. Bottom, Western blot shows the level of the key positively-acting transcription factor HY5 in the four-day-old dark-grown seedlings in wild-type, *pifq*, *cop1-6*, and *cop1-6pifq* backgrounds. A REGULATORY PARTICLE 5 (RPT5) blot is shown as a loading control. (B) PIFs directly interact with COP1 and SPA1 as well as HY5. On the one hand, these interactions promote the recruitment of HY5 to the COP1–SPA complex. On the other, PIFs also promote the auto- and trans-ubiquitylation of COP1 E3 ligase activity to enhance the degradation of HY5 to repress photomorphogenesis. Several COP1 substrates are shown above. If PIFs interact with any of these substrates, PIFs might enhance their degradation through the COP1–SPA complex to regulate plant growth and development. Abbreviations: BBX22, B-BOX 22; BBX24, B-BOX 24; BBX4, B-BOX 4; CO, CONSTANS; COL3, CONSTANS-LIKE 3; COP1, CONSTITUTIVELY PHOTOMORPHOGENIC 1; ELF3, EARLY FLOWERING 3; GATA2, GATA TRANSCRIPTION FACTOR 2; GI, GIGANTEA; HFR1, LONG HYPOCOTYL IN FAR-RED; HRT, HYPERSENSITIVE RESPONSE TO TCV; HY5, LONG HYPOCOTYL 5; HYH, HY5-HOMOLOG; LAF1, LONG AFTER FAR-RED LIGHT 1; phyA–E, phytochromes A to E; PIF, PHYTOCHROME INTERACTING FACTOR; SCAR1, AtSCAR1; SPA1, SUPPRESSOR OF PHYA-105 1; STH3, SALT TOLERANCE HOMOLOG 3; STO, SALT TOLERANCE; ub, ubiquitin.

compared to the parental genotypes (Figure 4A). PIF1 physically interacted with COP1, SPA1, and HY5 both *in vitro* and *in vivo*. Moreover, PIF1 enhanced the substrate recruitment as well as auto- and trans-ubiquitylation activities of COP1 toward HY5 (Figure 4B). PIFs have been shown to function as transcriptional regulators controlling gene expression in various signaling

pathways including those regulated by light [7]. However, the above data suggest that PIFs have a pivotal non-transcriptional role in modulating signaling pathways in addition to transcriptional regulation. In fact, the results suggest that PIF1 functions as a cofactor for COP1 in this process. This is consistent with previous reports that PIFs promote COP1-mediated ubiquitylation of type II phytochromes (phyB–E) *in vitro* [60], in line with the increased level of phyB in higher-order *pif* mutants *in vivo* [4]. In addition, as discussed above, PIF3 also promotes degradation of phyB through $CUL3^{LRB}$ E3 ligase [26]. A B box-containing protein, BBX19, interacts with COP1 and ELF3 and promotes COP1-mediated degradation of ELF3 [61]. Very recently, DET1 has been shown to interact with PIFs and HFR1, and regulates HFR1 abundance post-translationally through $CUL4^{DET1–COP10}$ E3 ligase [62,63]. Apart from phytochromes, a host of diverse classes of factors directly interact with PIFs and regulate PIF functions. These include DELLA proteins (RGA/GAI), HLH proteins (HFR1/PAR/KIDARI), bZIP protein (HY5), transcriptional coregulators (BZR1/FHY1), histone-modifying enzyme (HDA15), and circadian clock regulators (PRR1/ELF3) [7,64–78]. In addition, COP1 and DET1 directly interact with multiple proteins to regulate various signaling pathways (Figure 4B) [5]. If PIFs interact with any of the COP1/DET1 substrates, PIFs might also regulate their abundance post-translationally, increasing the potential of synergistic regulation of multiple signaling and developmental pathways (Figure 4B). Thus it appears that the nontranscriptional roles of PIFs play an increasingly important if not equal role as transcriptional regulation by PIFs.

Outstanding Questions

What is the light-regulated kinase that phosphorylates PIFs in response to light?

HY5, the key positive regulator, is much more abundant in the *pifQ* mutant in the dark, potentially contributing to the *pifQ* phenotype in the dark. Because HY5 and PIFs bind to similar DNA sequence elements, are the PIF target genes also direct targets of HY5?

Most importantly, what is the biochemical function of phytochromes? Is phytochrome merely a scaffold protein to bring PIFs and the light-regulated kinase together? Alternatively, is phytochrome the light-regulated kinase as previously suggested?

Concluding Remarks and Future Directions

The discovery of multiple repressors functioning synergistically to suppress photomorphogenesis suggests that photomorphogenesis is the default pathway for plant development. Skotomorphogenesis is a repressed state of photomorphogenesis. Plants employ multiple layers of negative regulators to achieve a sufficiently repressed state in the dark. Light-activated phytochromes interact with PIFs to induce their phosphorylation by an as yet unknown kinase, and the phosphorylated form is ubiquitylated by various E3 ligases and degraded through the 26S proteasome pathway to initiate photomorphogenesis. The light-induced degradation of PIF1 by the $CUL4^{COP1–SPA}$ E3 ligase initiates gradual progression toward photomorphogenesis. Although we have a much better understanding of how light controls plant development, several key questions still remain unanswered (see Outstanding Questions Box). The answer to these questions awaits future research.

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