Dissecting the phytochrome A-dependent signaling network in higher plants

Haiyang Wang[1] and Xing Wang Deng[2]

[1]Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY 14853, USA
[2]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA

Plants monitor their ambient light environment using a network of photoreceptors. In *Arabidopsis*, phytochrome A (phyA) is the primary photoreceptor responsible for perceiving and mediating various responses to far-red light. Several breakthroughs in understanding the signaling network mediating phyA-activated responses have been made in recent years. Here, we highlight several key advances: the demonstration that light regulates nuclear translocation of phyA and its associated kinase activity; the revelation of a transcriptional cascade controlling phyA-regulated gene expression; the detection of a direct interaction between phyA and a transcription factor; and the identification and characterization of many phyA-specific signaling intermediates, some of them suggesting the involvement of the ubiquitin–proteasome pathway.

Sessile plants have adopted a high degree of developmental plasticity to optimize their growth and reproduction in response to their ambient environments. Light is one of the major environmental signals that influence plant growth and development. Not only is light the primary energy source for plants but also it provides them with positional information to modulate their developmental processes, including seed germination, seedling de-etiolation, gravitropism, phototropism, chloroplast movement, shade avoidance, circadian rhythms and flowering time. Plants can detect almost all facets of light, including its direction, duration, quantity and wavelength, using three major classes of photoreceptors. Phytochromes predominantly absorb the red and far-red wavelengths (600–750 nm), whereas cryptochromes and phototropins perceive blue and ultraviolet A (UV-A) wavelengths (320–500 nm), and unidentified UV-B photoreceptors absorb UV-B (282–320 nm) [1,2]. These photoreceptors perceive and interpret ambient light signals and transduce them to modulate plant growth and development. The light control of seedling development has been conveniently used as a model for dissecting the signaling mechanisms of these photoreceptors [3–7]. In this article, we focus on recent advances in our understanding of the signaling network of phytochrome A (phyA). We attempt to outline the recent conceptual breakthroughs, the standing issues and possible future directions in phyA-signaling-mechanism research.

Corresponding author: Xing Wang Deng (xingwang.deng@yale.edu).

Molecular properties and functional roles of phyA

Historically, phyA was the first phytochrome discovered in higher plants [1] and all higher plants have a distinct phyA among phytochrome families of variable sizes. All known phyAs from higher plants are abundant in dark-grown plants and are rapidly degraded upon light exposure. They are therefore classified as a light-labile phytochrome. The purified phyA molecule is a soluble, dimeric chromoprotein that consists of two ~125-kDa polypeptides with a single covalently attached tetrapyrrole chromophore, phytochromobilin. The photosensory activity of the molecule results from its capacity to undergo a light-induced, reversible switch between two conformers: the red-light-absorbing Pr form and the far-red-light-absorbing Pfr form. Phytochrome is synthesized in the Pr form in dark-grown seedlings. Upon exposure to red light, the Pr form is converted to the Pfr form, and exposure to far-red light reverts the Pfr form to the Pr form [8].

There are five distinct phytochromes in *Arabidopsis*, designated phyA to phyE. These photoreceptors have unique, sometimes partially redundant or antagonistic, roles in different photomorphogenic responses [9] (Box 1). PhyB to phyE predominantly regulate light responses under continuous red and white light. Most of their known responses can be grouped into the classical red–far-red photoreversible phytochrome responses, the so-called low-fluence responses (LFRs, fluence requirement 1–1000 μmol m^2). The classical example of a phytochrome-mediated LFR is red-light-induced germination of lettuce seeds. This induction can be inhibited by subsequent far-red light treatment. The seeds can be repeatedly treated by sequential red or far-red light, and the ultimate germination response depends only on the last light treatment. Thus, photoreversibility is one characteristic feature of LFR responses. Another distinguishing feature of LFR is that it conforms to the law of reciprocity – a response depends on the total number of photons received irrespective of the duration of exposure [7,8].

PhyA is unique among all phytochromes because it is solely responsible for the very-low-fluence response (VLFR) and for the far-red-light-dependent high-irradiance

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Box 1. Photomorphogenesis and skotomorphogenesis

Photomorphogenesis

Photomorphogenesis is broadly defined as the control of plant form by the ambient light conditions. Light control of plant growth and development occurs throughout the life cycle, from seed germination and de-etiolation through vegetative architecture (e.g. internode elongation and leaf expansion) to flowering induction. All these events are mediated primarily by changes in the expression levels of light-regulated genes. In a narrow sense, photomorphogenesis is usually referred to as the seedling 'de-etiolation' process. Light-grown seedlings are characterized by short hypocotyls, open, expanded cotyledons, and the development of the proplastids into mature green chloroplast [1,78].

Skotomorphogensis

Skotomorphogensis (or etiolation) is the developmental pathway undertaken by seedlings grown in the dark and is characterized by long hypocotyls, closed cotyledons and apical hooks, and the development of the proplastids into etioplasts [1,78].

response (HIR). The VLFR includes light effects on the expression of some genes, seed germination and the gravitropic control of hypocotyl growth, and it can be induced with extremely low photon fluences of 0.001–1.000 μmol m^2 of either red or far-red light pulses. Although the VLFR is not photoreversible, it does obey the law of reciprocity (i.e. the VLFR senses photon fluences). The HIR requires relatively high photon fluence rates and a long duration of irradiation, and it is fluence rate and not total fluence that defines this type of responses. Typical HIRs include inhibition of hypocotyl elongation, opening of the apical hook, expansion of the cotyledons, accumulation of anthocyanin and a far-red light preconditioned block of greening during seedling development [7,8] (Fig. 1). The central dogma for phytochrome action (that Pfr is the biologically active form) applies to the LFRs mediated by phyB–phyE and the VLFRs mediated by phyA. However, for phyA-mediated far-red-light HIRs, a short-lived intermediate generated during Pfr-to-Pr photoconversion was suggested to be the physiologically active form [10].

PhyA control of gene expression

In response to the far-red light signal, it is thought that phyA leads to photomorphogenic development largely by controlling far-red-light-responsive nuclear gene expression. Traditional approaches have revealed several dozen individual genes whose expression is regulated by light [11–13]. Recently, DNA-microarray technology has been used to investigate genome-wide gene-expression profiles controlled by phyA [14,15]. These studies provided substantial evidence for the notion that phyA is the photoreceptor responsible for far-red-light-induced genome expression [14]. Furthermore, many cellular metabolic and regulatory pathways are coordinately regulated by light. Some (including all photosynthetic genes, glycolysis and the tricarboxylic acid cycle) are activated by light, whereas others [such as cell-wall-loosening enzymes and water-channel proteins (aquaporins)] are repressed by light [14].

By measuring the time-dependent global gene-expression profiles, a large proportion of the early phyA-regulated genes have been found to be known or putative transcriptional regulators, whereas most structural and metabolic genes belong to the late-response class. The data suggest that the massive change in gene expression induced by phyA activation is probably a result of a transcriptional cascade [15].

Light-induced nuclear import of phyA

Because phytochromes are synthesized in the cytoplasm, we must address the question of how the photoactivated phytochromes control light-responsive gene expression in the nucleus. Early studies using an immunohistological approach and cell fractionation assays supported the notion that phytochromes are predominantly localized outside the nucleus [1]. Recently, it was shown that upon photoconversion of Pr to Pfr, all phytochrome species tagged with green fluorescent protein (GFP) can translocate from the cytoplasm into the nucleus, where they form intranuclear speckles [16,17]. For phyA, brief irradiation

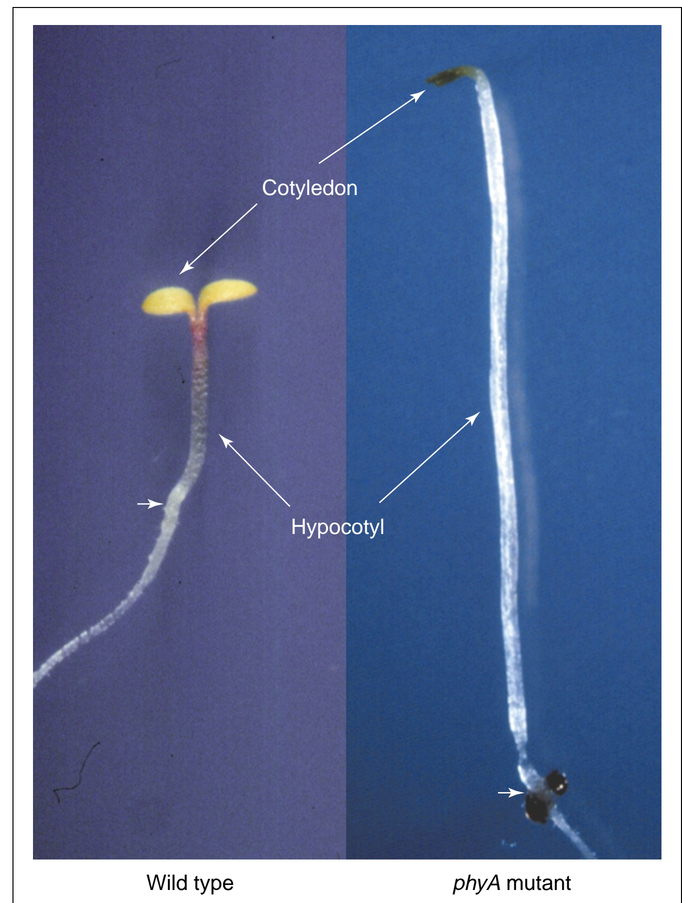

Fig. 1. The contrasting phenotypes of wild-type and *phyA* mutant *Arabidopsis* seedlings (5 days old) grown under far-red light. Wild-type seedlings undergo photomorphogenesis and are characterized by short hypocotyls and open, expanded green cotyledons. By contrast, *phyA* mutant seedlings are blind to far-red light and exhibit skotomorphogenic development or etiolation (Box 1), which is characterized by elongated hypocotyls, closed cotyledons and apical hooks. Short arrows indicate the junctions of hypocotyl and root.

with red or far-red light can induce rapid (a few minutes) nuclear import, and intranuclear speckle formation of phyA–GFP is preceded by even faster cytosolic-spot formation by the fusion protein when irradiated with red (but not far-red) light, a phenomenon reminiscent of SAP (sequestered area of phytochrome) formation. Additionally, nuclear translocation of phyA is also induced by continuous far-red but not by continuous red light treatment. Thus, the nuclear translocation of phyA is mediated by both the VLFR and the far-red HIR, indicating that phyA regulates its own nuclear import. Although the exact physical nature of the nuclear speckles is currently unknown, a recent study demonstrated that they are required for phyA function [18].

At the current stage, little is known about the molecular machinery and factors modulating the nucleocytoplasmic partitioning of phytochromes. It has been speculated that the Pr form of phyA is anchored or retained in the cytosol, whereas the Pfr form does not interact with the anchoring proteins and is thus subject to nuclear import. Identification of the phyA nuclear localization signal and nuclear export signal should help us to understand how light-regulated changes in phyA localization are brought about.

PhyA as a light-regulated kinase

How does photoactivated phyA transfer the light signal to its downstream targets? Does phyA have enzymatic activity? Recent studies using recombinant DNA technology have added much convincing evidence to the long-standing view of phytochromes as light-regulated kinases [19,20]. Furthermore, the recent discovery of phytochrome-like photoreceptors in bacteria has also provided strong evolutionary evidence in support of such a view [21]. Bacteriophytochromes can perceive light signals and relay information via a His-kinase signaling cascade, probably through a response regulator [22]. Higher-plant phytochromes and bacteriophytochromes share sequence similarity in their C-termini [23] and it has been demonstrated that, unlike their bacterial counterparts, higher plant phytochromes autophosphorylate on Ser/Thr rather than His/Asp [20].

The claim that higher-plant phytochromes are Ser/Thr kinases has gained support from studies that have mapped putative phosphorylation sites for oat phyA, and targeted mutagenesis studies have implicated them in regulating phyA signaling [24]. In addition, several phyA-interacting factors or putative *in vivo* substrates of phyA kinase activity have been identified in recent years. For example, phytochrome-interacting factor 3 (PIF3), phytochrome kinase substrate 1 (PKS1), nucleoside diphosphate kinase 2 (NDPK2), cryptochromes (both CRY1 and CRY2) and the AUX/IAA proteins were found to be able to interact with phyA; in some cases, their phosphorylation was enhanced by the conversion of phyA to the active Pfr form [25–29]. However, it should be realized that, even if it has been documented, no physiological relevance of the phosphorylation of these molecules has been demonstrated *in vivo*. Therefore, in spite of the compelling evidence for the kinase activity of phyA, the connection between phyA kinase activity and a phyA photosignal transfer event still remains to be established.

It is known that reversible protein phosphorylation is essential for controlling the flow of many signaling processes. Thus, it is anticipated that protein phosphatases might also participate in the regulation of phyA signaling. A recent study has identified such a phosphatase, flower-specific, phytochrome-associated protein phosphatase (FYPP), which dephosphorylates phyA in a light-dependent manner and modulates phyA-mediated light signals in the control of flowering time [30].

Genetically identified phyA-specific signaling intermediates

Detailed genetic analyses have suggested that phyA signal transduction has at least two branches, which correspond to VLFR and HIR signaling. The Columbia (Col) ecotype does not respond to very low light fluences [31]. Two quantitative trait loci, *VLF1* and *VLF2*, are responsible for the expression of VLFR [31] but their molecular nature is currently unknown. Mutants for several components affected in the HIR branch of phyA signaling pathway have been identified (Fig. 2), including FHY1, FHY3 [32], FIN2 [33], SPA1 [34], FAR1 [35], FIN219 [36], PAT1 [37], EID1 [38], HFR1/RSF1/REP1 [39–41], LAF1 [42], LAF6 [43] and FHY4 [44]. The *fhy1*, *fhy3*, *fhy4*, *fin2*, *fin219*, *far1*, *laf1*, *laf6* and *hfr1*/*rep1*/*rsf1* mutants show less sensitivity in continuous far-red light, indicating that their genes encode positive regulators of the phyA signaling pathway. Among the identified positive regulators of phyA signaling, the loss-of-function mutants (mostly null

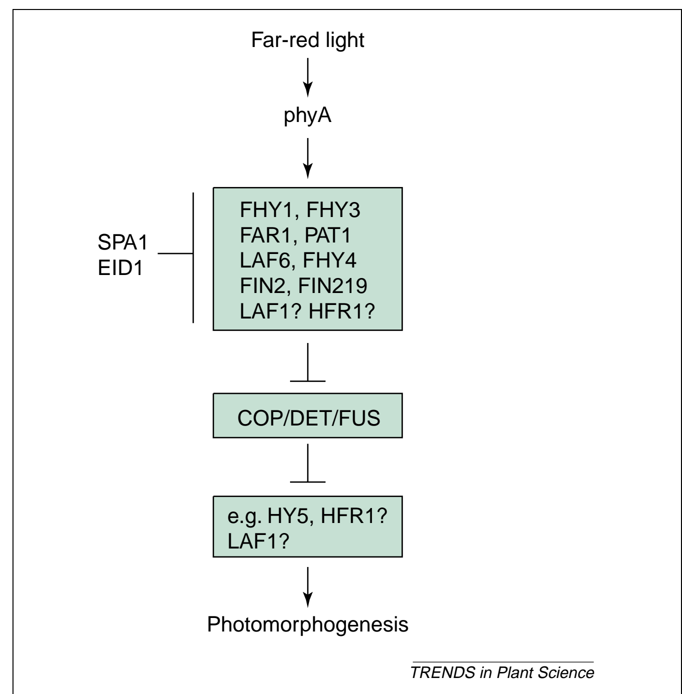

Fig. 2. A simplified genetic model for the phyA-mediated signaling pathway (adapted from Ref. [7]). Several phytochrome A (phyA)-specific signaling molecules were identified by their involvement in far-red-light high-irradiance responses. These components presumably act upstream of the *COP/DET/FUS* genes, thus controlling the accumulation of crucial transcription factors (e.g. HY5 and potentially LAF1 and HFR1) and the degree of photomorphogenic development. The genes encoding all these components except FIN2 and FHY4 have been cloned. Arrows indicate a positive action, whereas the bars indicate a repressive effect. A '?' next to a component indicates its unverified placement in the signaling network model.

alleles) exhibit only partial defects of various degrees in phyA signaling under far-red light. This suggests that phyA signaling involves multiple branches or parallel pathways controlling overlapping yet distinctive sets of far-red-light-mediated HIRs [7]. It has been shown that at least FHY1 is also involved in mediating the VLFR [31].

Several phyA signaling intermediates have been characterized at the molecular level. For example, LAF6 is a plastid-localized ATP-binding-cassette protein involved in coordinating intercompartmental communication between plastids and the nucleus [43]. PAT1 is a new member of the GRAS (*GAI*, *RGA*, *SCR*) family [37], whereas FIN219 is a GH3-like protein whose expression is rapidly induced by auxin [36]. Both PAT1 and FIN219 are cytoplasmic proteins, whereas FAR1, FHY3, SPA1, HFR1, LAF1 and EID1 are all nuclear. Interestingly, *FAR1* and *FHY3* encode two closely related proteins that constitute one branch of a small gene family [35,45]. FHY1 is a novel protein [46], whereas HFR1 is an atypical basic helix–loop–helix (bHLH) transcription factor closely related to PIF3 [39], and LAF1 is a Myb-type transcription activator [42].

It is worth mentioning that the jasmonate response locus *JAR1* maps to the same locus as *FIN219*. Intriguingly, multiple loss-of-function alleles of *jar1* mutants respond normally to far-red light (measured by hypocotyl growth) and, unlike the *jar1* alleles, *fin219* mutants show no obvious change in responses to jasmonate [47]. This discrepancy might be explained by the fact that the mutation in *fin219* is epigenetic, involving aberrant promoter methylation [36]. Biochemical analysis indicated that JAR1 (FIN219) belongs to the acyl adenylate-forming firefly luciferase superfamily, and it displays adenylation activity specific for jasmonic acid, suggesting that JAR1 could activate the carboxyl groups of certain substrates for their subsequent biochemical modification [47].

In contrast to the many mutants with reduced phyA responses, only two mutants (*spa1* and *eid1*) show enhanced phyA-specific light responses [34,38]. Genetic and physiological results indicate that the encoded proteins function as negatively acting components of phyA signaling and are involved in different but interacting phyA-dependent signal-transduction chains. SPA1 functions as a negative effector in both the VLFR and the HIR, whereas EID1 is only involved in signaling cascades regulating the HIR [48]. SPA1 contains WD-40 repeats similar to COP1 and exhibits some weak homology with protein kinases [49], whereas EID1 is an F-box protein and a likely component of so-called SCF (Skp1/Cdc53/F-box protein) complexes [50].

The biochemical function of most known phyA signaling molecules (such as PAT1, FAR1, FHY3 and SPA1) remains largely unknown and is likely to be a primary focus of future research. In addition, earlier microinjection and pharmacological studies have implicated the cytosolic heterotrimeric G proteins, Ca^{2+}–calmodulin and cGMP as candidate second messengers in phyA signaling [51,52]. We still do not know how these cytosolic signaling components work together with the defined nuclear factors to mediate phyA signaling.

PhyA can directly target light signals to light-responsive promoters

The finding that PIF3, a member of the bHLH superfamily of transcriptional regulators, can interact directly with both phyA and phyB suggests that phytochromes might regulate nuclear gene expression via direct interaction with transcription factors [53,54]. In support of this notion, phyB can bind specifically and photoreversibly to PIF3 that is already bound to its cognate DNA-binding site (the light-responsive G-box DNA sequence CACGTG). Furthermore, the expression of *CCA1* and *LHY1*, which have G-box motifs in their promoters and encode Myb-like transcription factors known to be involved in regulating light- and clock-related responses, is reduced in transgenic *Arabidopsis* seedlings with reduced PIF3 levels [55]. These and other data are consistent with a model in which light induces the phytochrome molecule to translocate into the nucleus, where it binds in its active Pfr form to promoter-bound PIF3 and facilitates transcriptional activation of specific target genes (Fig. 3).

Furthermore, *HFR1*, a genetically identified positive regulator specific to phyA signaling, encodes a bHLH protein closely related to PIF3 that can form a homodimer as well as a heterodimer with PIF3 [39]. These findings suggest that phyA signaling might directly regulate a diverse set of genes through an integrated transcriptional

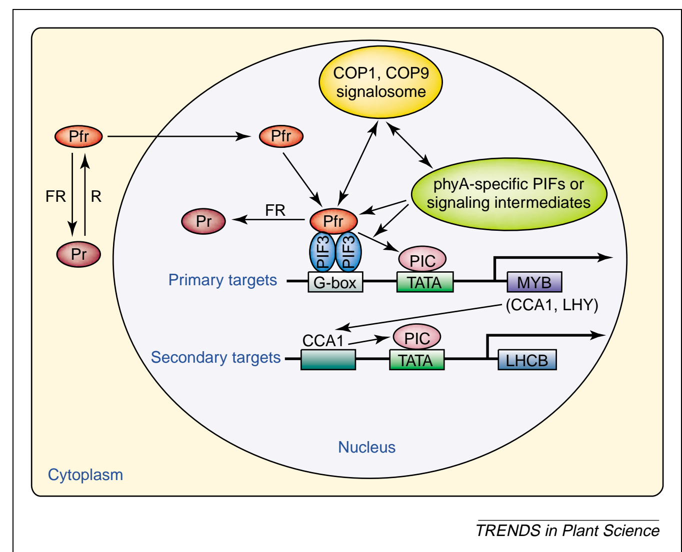

Fig. 3. A molecular model depicting phytochrome control of gene expression (adapted from Ref. [4] and reproduced, with permission, from Ref. [7]). Upon photoconversion to the Pfr form, phytochrome A (phyA) translocates into the nucleus, where it interacts with the G-box-bound PIF3 and activates the expression of the primary target genes (such as the Myb-class transcription factors CCA1 and LHY). The encoded primary target gene products (many of which are transcriptional regulators) are, in turn, responsible for orchestrating the expression of the secondary target genes (e.g. the induction of *LHCB* expression by CCA1), thus generating a transcriptional network controlling different aspects of phyA physiology. Other phyA-interacting factors (PIFs; e.g. PKS1, NDPK2, cryptochromes, auxins/indole-acetic acids) and their signaling intermediates might function as modifiers to participate in such a direct light-signaling pathway. Moreover, COP1 and the COP9 signalosome could also interact (directly or indirectly) with the transcriptional machinery, PIFs or other signaling intermediates, and regulate their abundance through a light-regulated proteolytic process. Notice that the far-red-light-absorbing Pfr could be the physiologically active form (for very-low-fluence responses) or a short-lived intermediate could be generated during the Pfr to Pr photoconversion (for far-red-light-dependent high-irradiance responses) to activate gene expression. Abbreviations: FR, far-red light; Pfr, far-red-light-absorbing conformer of phyA; PIC, pre-initiation complex; Pr, red-light-absorbing conformer of phyA; R, red light; TATA, TATA box.

network orchestrated by an array of interacting bHLH proteins. There are ~135 bHLH proteins in *Arabidopsis* [56] and it is known that bHLH proteins can form homodimers and/or heterodimers. Future studies are needed to explore the possible combinations of these bHLH proteins and to sort out their downstream target genes.

It should be mentioned that some phytochrome-mediated responses occur quickly (within minutes; e.g. the unrolling of the primary leaf wrapped within oat coleoptiles), posing the question of whether nuclear gene expression regulation would be sufficiently quick. Such quick responses are possibly best explained by assuming rapid alterations in inter- or intracellular ion balances; presumably, phytochromes can mediate ionic concentrations within cell compartments that modulate extension growth [6,7]. However, electrophysiological evidence for the involvement of ion fluxes in these responses is still lacking.

Regulated proteolysis in phyA signaling

There is accumulating evidence that regulated proteolysis plays a crucial role in controlling the timing and amplitude of phyA signaling processes. First, phyA itself is rapidly degraded upon photoconversion to the Pfr form and this degradation involves the ubiquitin–proteasome pathway [57]. This downregulation of the intracellular phyA levels in response to light probably provides an adaptation mechanism to the ambient light environment.

Far-red-light activation of phyA also regulates proteolysis of downstream transcription factors such as HY5 and HYH, bZIP transcription factors that act to promote photomorphogenesis [58,59]. For example, HY5 levels are ~20 times higher in light-grown seedlings than in dark-grown seedlings, and HY5 levels quantitatively affect the degree of photomorphogenesis [60]. The degradation of HY5 in the dark involves a protein named CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), a negative regulator of photomorphogenesis. COP1 is a RING-finger protein with WD-40 repeats [61]. Studies with a functional β-glucuronidase–COP1 fusion protein indicate that COP1 acts within the nucleus to suppress photomorphogenic development in darkness, and that inactivation of COP1 by light was accompanied by reduced COP1 abundance in the nucleus [62]. In the dark, COP1 directly interacts with and targets HY5 for degradation via a 26S proteasome-mediated process. Thus, COP1 presumably functions as a ubiquitin ligase [60].

The finding that SPA1, a negative regulator of phyA signaling, has a WD-40 repeat domain (which is frequently used in E3 complexes for recruiting substrates [63]) with homology to that of COP1 suggests that SPA1 might be also involved in regulated proteolysis of various phyA signaling intermediates. In support of this possibility, a direct physical interaction has been reported between COP1 and SPA1 [64]. This observation raises the possibility that these two proteins function together in targeting substrate proteins (e.g. HY5, LAF1 and HFR1) for degradation by the proteasome, with SPA1 as a cofactor specific to the phyA signaling pathway to modulate COP1 function. The recent report that EID1, another negative regulator of phyA signaling, encodes an F-box protein and a likely component of a SCF^{EID1} ubiquitin-ligase complex [50] underscores a possible role for targeted protein degradation in phyA signaling.

In addition to COP1, genetic screens have identified nine other pleiotropic *COP/DET/FUS* loci whose gene products act as negative regulators of photomorphogenesis and function downstream of multiple photoreceptors including phyA [65]. Among these loci, six (CSN1–CSN4, CSN7 and CSN8) encode six of the eight subunits of the COP9 signalosome protein complex [66,67]. Strikingly, the COP9 signalosome shares subunit-by-subunit similarity to the lid subcomplex of the 26S proteasome and is also involved in regulated proteolysis [68,69]. This notion is further supported by the recent finding that COP10 is an E2 ubiquitin-conjugating enzyme variant [70]. Further characterization of the functional relationships between the various phyA signaling molecules and the COP/DET/FUS proteins is likely to enhance our understanding of the integration of phyA-specific signaling events with this group of general repressors of photomorphogenesis.

How are the COP proteins inactivated?

It is generally assumed that light-induced photomorphogenic development requires the inactivation of these COP/DET/FUS proteins. However, little is known about how the light-activated photoreceptors regulate the activities of those downstream COP/DET/FUS proteins to bring about the physiological responses. Although it has been shown that nuclear abundance of COP1 is regulated by light acting through these photoreceptors [71], the mechanisms governing this process are largely unknown. Recent studies have shown that photoactivated cryptochromes can interact physically with COP1 and inhibit COP1 E3 ubiquitin-ligase activity, leading to HY5 accumulation and photomorphogenic development [72,73], but the same mechanism might not apply to phyA. In addition, it has been proposed that these protein–protein interactions might be responsible for the rapid, initial inactivation of COP1 activity, but that longer-term inactivation of COP1 is achieved by subsequent depletion of the molecule from the nucleus.

Conclusions and prospects

Dramatic progress has been made during the past decade in understanding phyA signaling mechanism, largely owing to the use of molecular genetics in the model plant *Arabidopsis*. However, it has also become clear that our information about the nature and extent of the phyA-mediated signaling network is still limited and fragmented. Much remains to be learned to build a connected phyA signaling network with an understanding of how the various nodes of the network interact to transmit the light signals.

With the completion of the *Arabidopsis* genome project, new technologies have emerged that allow global and systematic approaches to dissecting signaling pathways. For example, a recent study attempted to analyze the genetic relationships among the various genetically defined phyA signaling mutants using cDNA microarray technology [74]. Clustering analysis of the genome expression profiles supports the notion that phyA signaling might entail a

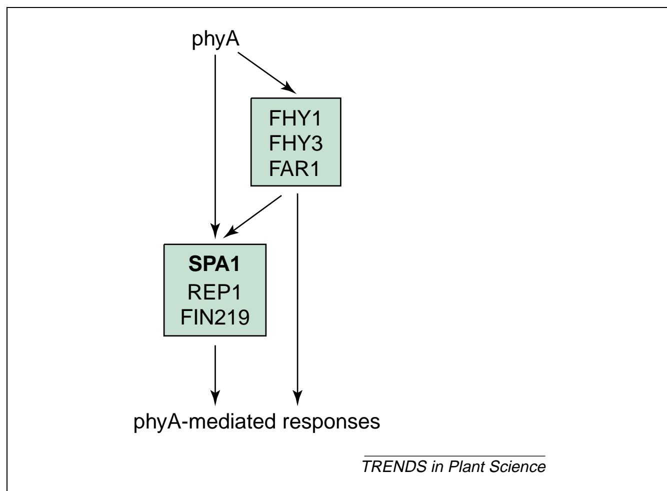

Fig. 4. A genetic model of the possible relationships between the various phytochrome A (phyA) signaling molecules. FHY1, FHY3 and FAR1 probably act upstream in the phyA signaling network, whereas SPA1, REP1 and FIN219 probably act further downstream in the network. All these components except SPA1 are positive regulators; SPA1 (in bold) is a negative regulator of the signaling pathway. These components could directly or indirectly regulate overlapping and distinct sets of gene expression and phyA-mediated photomorphogenic responses.

network with multiple paths controlling overlapping yet distinct sets of gene expression. FHY1, FAR1 and FHY3 probably act upstream in the phyA signaling network, close to the phyA photoreceptor itself. FIN219, SPA1 and REP1 probably act somewhere more downstream in the network and control the expression of smaller sets of genes [74] (Fig. 4). Although such microarray-based expression profiling has been valuable in dissecting cellular responses and identifying potential downstream targets of the various signaling components, it has its limitations. First, for a particular signaling component, not all downstream target genes' expression is affected by its activity. Second, changes in some gene expression could be secondary effects of the changed developmental processes. Future application of the recently developed chromatin immunoprecipitation chip technology [75], together with expression profiling, will help to identify the direct target genes of various transcription regulators involved in phyA signaling.

Most gene function is largely manifested by the activity of its encoded protein. Thus, analysis of the activities and interactions of proteins will be essential for understanding the phyA signaling network. Several innovative proteomic approaches have recently emerged and are likely to make their way into phyA signaling research. For example, affinity-capture-based techniques (e.g. tandem affinity purification) in conjunction with enhanced mass spectrometry for characterizing *in vivo* multiprotein complexes [76], and high-throughput protein microarray [77] could be valuable tools in dissecting the phyA signaling network.

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