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Review Article

Phytochrome-mediated light signaling in plants: emerging trends

Laju K. Paul and Jitendra P. Khurana

Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi - 110 021

ABSTRACT

Phytochromes maximally absorb in the red and far-red region of the solar spectrum and play a key role in regulating plant growth and development. Our understanding of the phytochrome-mediated light perception and signal transduction has improved dramatically during the past decade. However, some recent findings challenge a few of the well-accepted earlier models regarding phytochrome structure and function. Identification of a serine/threonine specific protein phosphatase 2A (FyPP) and a type 5 protein phosphatases (PAPP5), and the phytochrome-mediated phosphorylation of phytochrome interacting factor 3 (PIF3), auxin inducible genes (Aux/IAA) and cryptochromes have opened new vistas in phytochrome biology. Importantly, the significance of proteolysis and chromatin-remodeling pathways in phytochrome signaling is becoming more apparent. The emerging concept of phytochrome as a master regulator in orchestrating downstream signaling components has become more convincing with the advent of global expression profiling of genes. Upcoming data also provide fresh insights into the nuclear localization, speckle formation, nucleo-cytoplasmic partitioning and organ-specificity aspects of phytochromes. This article highlights recent advances in phytochrome biology with emphasis on the elucidation of novel components of light signal transduction. **[Physiol. Mol. Biol. Plants 2008; 14(1&2) : 9-22]** *E-mail : khuranaj@genomeindia.org*

Key words : *Phytochromes, phosphorylation, kinase, nuclear-cytoplasmic partitioning, proteolysis, chromatin-remodeling, organ-specific responses, phytochrome-interacting factors.*

Abbreviations : *phy-phytochrome, PKS1-Phytochrome Kinase Substrate 1, NDPK2-Nucleoside Diphosphate Kinase 2, COP1-Constitutive Photomorphogenesis 1, HY5-Long Hypocotyl 5, PIF3-Phytochrome Interacting Factor 3, LAF1-Long After Far-red light 1, SPA1-Suppressor of Phytochrome A 105 1, DET1-De-etiolated 1*

Like other living organisms, plant development is also determined genetically but is modulated dramatically by diverse environmental signals. Among these, light plays a profound role and regulates virtually all aspects of plant life cycle, starting from seed germination through to senescence. Plants perceive changes in the ambient light environment by distinct sensory photoreceptors. The conventional photoreceptors include three major classes in plants, viz. the red/far-red (R/FR) light-sensing phytochromes and UV-A/blue light-perceiving cryptochromes and phototropins (Jiao *et al.*, 2007). However, the molecular nature of the UV-B (280-320 nm) photoreceptor(s) is still elusive. Recently, additional blue light photoreceptors called ZEITLUPE have been characterized (Somers *et al.*, 2000; Imaizumi *et al.*, 2003). In lower organisms like *Adiantum*, a fern, and the alga *Mougeotia*, a unique chimeric photoreceptor, neochrome, has been identified, which can perceive light both in the red/far-red as well as UV-A/blue region to regulate chloroplast relocation and other plant responses (Suetsugu *et al.*, 2005; Sato *et al.*, 2007; Suetsugu and Wada, 2007). There are also reports substantiating many green-light (GL)-mediated responses in plants and, consequently, there are speculations for the occurrence of even a zeaxanthin-based compound as a green light receptor (Frechilla *et al.*, 2000; Talbott *et al.*, 2003; Folta, 2004; Dhingra *et al.*, 2006; Folta and Maruhnich, 2007).

Ever since the principal photoreceptor, phytochrome, was detected in oats spectroscopically (Butler *et al.*, 1959), special attention has been paid by the scientific community to unravel its structure, function and role in light signaling. As a result, great wealth of data have accumulated during the past few decades that have tremendously helped us to fill the major gaps in our understanding of the molecular mechanisms underlying phytochrome-mediated signaling and its role in major developmental pathways of germination, de-etiolation,

Correspondence and Reprint requests : Professor Jitendra P. Khurana, Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi 110021, India. Phone : 24115126 (O), Fax : 24115270

shade avoidance and flowering in plants (Khurana *et al.*, 1996, 1998, 2004; Quail, 2002a,b; Casal and Yanovsky, 2005; Chu *et al.*, 2005; Franklin *et al.*, 2005; Wang, 2005; Mathews, 2006; Rockwell and Lagarias, 2006; Rockwell *et al.*, 2006; Jiao *et al.*, 2007). Phytochrome-mediated responses are also interconnected with other signaling networks, including those derived from environmental cues, hormonal pathways, and circadian clock, adding a further level of complexity to the scenario. However, in the present review, only the more recent breakthrough findings that advance our knowledge of phytochrome biology and help define the role of novel components in light signaling are addressed. Since most of the pivotal experiments conducted in the field of phytochrome research have centred around the model plant *Arabidopsis thaliana*, this briefing will center on *Arabidopsis* unless specified otherwise.

Distribution of phytochromes

Phytochromes are widely distributed among flowering plants, moss, fern, green alga, fungi and prokaryotes (Montgomery and Lagarias, 2002). Till date, more than 120 phytochromes and phytochrome-related protein sequences are reported from diverse organisms (Rockwell *et al.*, 2006). In plant kingdom, phytochromes are encoded by a small gene family. The numbers of phytochromes vary between plant species. In general, considering the diploid genome, there are three forms of phytochromes in monocots (e.g. rice) and five in dicots (e.g. *Arabidopsis thaliana*). Some of the phytochrome genes sequenced/characterized include at least one in cucurbits, cuscuta (phyA); two in oat (phyA1 and A2), soybean, tobacco, pea, potato (phyA and phyB), wheat (phyA and phyC); three in rice and sorghum (phyA-C); five in *Arabidopsis* (phyA-E), tomato (phyA, phyB1, phyB2, phyE and phyF) and three pairs in maize (phyA1, A2, B1, B2, C1 and C2) {for details, see Rockwell *et al.*, 2006}. In early years of phytochrome research (from 1950s to mid-1990s), it was thought to be exclusively present in higher plants. However, since the discovery of the cyanobacterial chromatic adaptation sensor RcaE (Kehoe and Grossman, 1996), phytochromes have now been characterized outside plant kingdom, such as cyanobacteria (Cph1/CphA, Cph2 and CphB/BphP), nonphotosynthetic bacteria (BphPs) and fungi (Fphs) (Blumenstein *et al.*, 2005; Froehlich *et al.*, 2005; Rockwell *et al.*, 2006), largely due to the availability of sequences of large number of microbial genomes. It indeed has been quite useful in not only establishing the ancestry of higher plant phytochromes but also providing unequivocal evidence for their biological role as a photoactivated kinase.

Phytochrome-mediated responses — energy dependence

Phytochromes exist in two photo-interconvertible forms, the biologically inactive red-absorbing Pr (λ_{max} 666nm) and the active far-red-absorbing Pfr (λ_{max} 730nm) forms that act as an on/off switch to trigger the downstream signaling components, leading eventually to the regulation of the gene expression and consequently photomorphogenesis (Quail, 2002a,b; Khurana *et al.*, 2004; Jiao *et al.* 2007). Besides their sensitivity to red and far-red light for photoconversion, different species of phytochromes exhibit differential sensitivity (stability or lability) to light. Based on their sensitivity to light, phytochromes have been classified into the light-labile type I and the light-stable type II species (Quail, 1997a; Sharrock and Clack, 2002). Among different phytochrome species known, phyA (although abundant in dark) is considered the light labile (Type I) species and the other forms (phyB, phyC, phyD and phyE), although less abundant, are considered light stable (Type II) species (Sharrock and Quail, 1989; Clack *et al.*, 1994).

Like other sensory photoreceptors, phytochromes not only sense quality and quantity of light but also its duration. Depending upon the energy of light required, phytochrome responses have been classified into low-fluence responses ($LFR_{s,}$ saturated at 10^{-6}-10^{-3} mol m^{-2} ; these are reversible), very-low-fluence responses ($VLFR_s$, saturated at 10^{-12}-10^{-7} mol m^{-2}; these are irreversible), and high irradiation responses ($HIR_{s,}$ require continuous high frequency long-term illumination and are wavelength dependent) (Smith and Whitelam, 1990; Nagy and Schafer, 2002; Chen *et al.*, 2004). Among the various phytochromes, phyA mediates FR-HIR and VLFR, whereas phyB regulates R-HIR and LFR during photomorphogenesis in *Arabidopsis* (Nagy and Schafer, 2002; Quail, 2002a).

Some of the phytochrome-mediated responses that have been studied extensively include onset of seed germination, cotyledon expansion, cessation of hypocotyls/stem growth, chloroplast differentiation, shade avoidance, anthocyanin accumulation, floral transition and, of course, changes in gene expression. In many cases the light-mediated responses are controlled by the coordinated action of different photoreceptors. For instance, responses like seed germination and shade-avoidance are controlled solely by phytochromes, whereas cotyledon expansion, stem growth, entrainment of circadian clock and floral induction are controlled by both phytochromes and cryptochromes (Hennig *et al.*, 1999; Mas *et al.*, 2000; Mazzella *et al.*, 2001).

Structure of phytochromes

Plant phytochromes are homodimers where each monomer is ca 125 kDa polypeptide, depending upon the species, harbouring a bilin chromophore (phytochromobilin). All phytochromes presumably harbour the same chromophore, which is covalently attached to the apoprotein via a thioether linkage between a Cys residue (in the N-terminal half) and the bilin A-ring. The phytochrome apoprotein has two main domains, an N-terminal photosensory signal input and a C-terminal signal output domain with regulatory roles. However, the N-terminal domain isolated from phyB, when dimerized and localized in the nucleus, triggered full phyB responses with much higher photosensitivity than the full-length phyB, indicating that the C-terminal domain might be attenuating the activity of phyB rather than positively transducing the signal (Matsushita *et al.*, 2003). The two domains are connected via a flexible hinge region. The N-terminal domain is further subdivided into four subdomains: P1 (N-terminal extension, NTE), P2 (PAS domain), P3 (bilin lyase domain, BLD/GAF domain) and P4 (phytochrome domain, PHY) (see Figure 1). The nature of chromophore varies with phytochrome subfamilies. Plants use phytochromobilin, whereas cyanophyceae Cph1s and Cph2s use phycocyanobilin as chromophore, linked covalently to a conserved Cys residue in P3 domain (Wu and Lagarias, 2000; Lamparter *et al.*, 2001). In case of Bph1s and Fphs, biliverdin functions as chromophore bound to the P2 domain at N-terminal region (Lamparter *et al.*, 2004; Wagner *et al.*, 2005). On the other hand, higher plant phytochrome C-terminal domain consists of a PAS-related domain containing (PRD), two PAS domains (PAS-A and PAS-B) and a histidine kinase-related domain (HKRD), which in fact is a serine threonine kinase domain (Figure 1), whereas its ancestors invariably harbour histidine kinase domain. All phytochromes except Cph2 share P2 domain, however, only plant phytochromes possess two PAS domains in the C-terminal region. Fungal Fphs have additional C-terminal response regulator domain (RR/REC) as compared to Cph1 and BphP families (see Figure 1; Montgomery and Lagarias, 2002; Wang, 2005; Rockwell and Lagarias, 2006; Rockwell *et al.*, 2006). Recent unveiling of the crystal structure of the conserved photosensory core of bacteriophytochrome DrBphP holoprotein from *Deinococcus radiodurans* has provided new insights to phytochrome biology (Wagner *et al.*, 2005). These findings for the first time provided direct evidence for the interactions between the PAS, GAF and PHY domains in phytochrome. Strikingly, a deep trefoil knot has been identified in the interface between PAS and GAF domains, believed to result in a much more rigid structure than expected for phytochrome and thus facilitating the photoconversion process (for more details, see Wagner *et al.*, 2005; Rockwell and Lagarias, 2006, Rockwell *et al.* 2006).

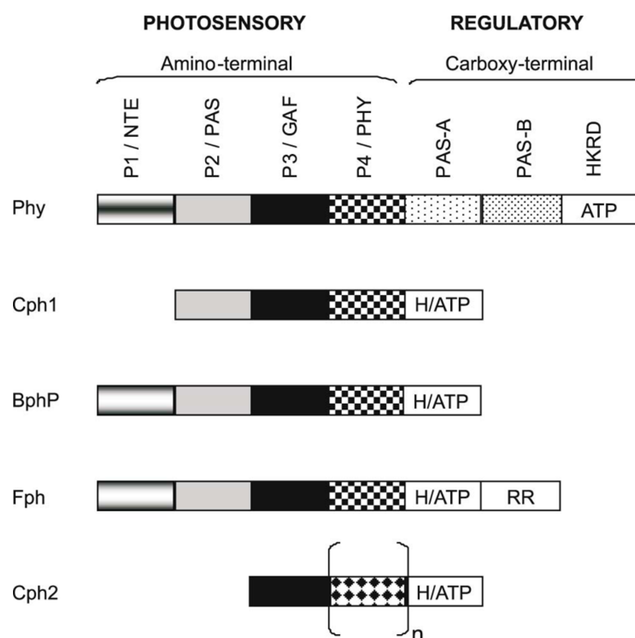

Fig. 1. The domain structure of phytochrome family. The phytochromes depicted here are representative members from plants (Phy), cyanobacteria (Cph1 and Cph2), non-photosynthetic bacteria (BphP) and fungi (Fph). For details, refer text.

Phytochromes: novel insights into their mechanism of action

Extensive progress has been made in recent years towards understanding the structure, function and signaling mechanisms of phytochromes. Phytochrome molecule has evolved gradually from a simple light-sensing moiety to a phosphoprotein, a kinase and a master regulator in the modulation of several downstream genes involved in various developmental pathways. Besides being a key modulator in light signaling, phytochromes are also interlinked with chromatin modulation and ubiquitin-mediated proteolysis. On the other hand, more recent advances also challenge many traditional views held on phytochrome signaling, especially the molecular functions of the structural domains. In the following pages, a brief overview of some of these novel and emerging themes in phytochrome signaling is provided.

Phosphorylation and kinase activity

The presence of a histidine kinase-related domain

(HKRD) in the C-terminus of higher plant phytochromes suggested that they may phosphorylate specific target proteins and behave as a light-regulated protein kinase. The first evidence for the kinase activity of phytochrome came essentially from the observation that the purified preparations of phyA autophosphorylate (Wong *et al.*, 1989) and that plant phytochrome and prokaryotic protein kinase sequences resembled significantly (McMichael and Lagarias, 1990). However, the key conserved residues present within a typical histidine kinase domain (HKD) were absent in phytochrome HKRD and thus these observations demonstrating the kinase activity of phytochrome were considered equivocal (Boylan and Quail, 1996; Elich and Chory, 1997; Quail, 1997b; Cashmore, 1998). However, when autophosphorylation/histidine kinase activity of cyanobacterial phytochrome Cph1 was finally established (Hughes *et al.*, 1997; Yeh *et al.*, 1997), the idea that plant phytochromes do indeed behave as a kinase acquired credibility. Yeh and Lagarias (1998) did indeed provide unflinching experimental evidence that purified recombinant plant phytochromes exhibit serine/threonine kinase activity. It was thus concluded that the eukaryotic phytochromes are in fact histidine kinase paralogs (of bacterial phytochromes) with serine/threonine substituting for histidine residues. Furthermore, phytochromes phosphorylate substrates like cryptochromes (Ahmad *et al.*, 1998), Phytochrome Kinase Substrate 1 (PKS1) (Fankhauser *et al.*, 1999), and Aux/IAA proteins (Colon-Carmona *et al.*, 2000). Although, the Ser/Thr kinase activity of phytochromes is now generally accepted, the exact kinase domain awaits identification.

Like traditional kinases, phytochrome has been demonstrated to function as a phosphoprotein (McMichael and Lagarias, 1990; Lapko *et al.*, 1997). The *in vivo* studies conducted on oat phyA showed phosphorylation at Serine-7 (irrespective of Pr/Pfr) and Serine-598 (Pfr specific) residues, whereas *in vitro* phosphorylation has been established at Serine-17 (Pr specific) and Serine-598 (Pfr specific) sites, respectively (Lapko *et al.*, 1997, 1999). Besides its autophosphorylating property, very few proteins have been shown to phosphorylate phytochromes. A phytochrome-associated kinase that specifically phosphorylates Serine-598, and another kinase, CM1 K, that phosphorylates Serine-7 of oat phyA do exist (Kim *et al.*, 2005). To regulate the phosphorylation status of phytochrome, one can presume the occurrence of phosphatases too. Two such phytochrome-specific phosphatases were characterized from *Arabidopsis*, a serine/threonine specific protein phosphatase 2A (FyPP) (Kim *et al.*, 2002), and a type 5 protein phosphatases (PAPP5) (Ryu *et al.*, 2005). Interestingly, their activity is of contrasting nature since FyPP negatively regulates phytochrome signaling whereas PAPP5 positively influences phytochrome stability. Moreover, PAPP5 is nuclear localized, whereas FyPP is cytoplasmic in localization. Here, it is important to note that PAPP5-mediated dephosphorylation enhanced the binding affinity of phytochromes towards its downstream signaling component, NDPK2 (Ryu *et al.*, 2005), identified earlier by yeast two-hybrid assay. Despite these studies on phytochrome phosphorylation, their functional significance in regulating plant development is still not clearly defined. Nevertheless, the substitution of N-terminal Serine-7 and Serine-17 by Alanine caused an increase in biological activity of phyA, suggesting that phytochrome-mediated responses are desensitized by photoreceptor phosphorylation (Stockhaus *et al.*, 1992).

The phosphorylation status of phytochromes has been found to control protein-protein interaction between phytochromes and downstream signaling components. For example, phosphorylation of Serine-598 did not affect phytochrome stability, but affected the interaction with signaling components NDPK2 and PIF3 (Kim *et al.*, 2004). Since phytochromes also phosphorylate other sensory photoreceptors, i.e. cryptochromes, and also Aux/IAA proteins which negatively regulate auxin action, the role of phytochrome kinase activity in the cross-talks between light and other signals are becoming more apparent. These studies indicate unambiguously that reversible phosphorylation of phytochromes is a key biochemical mechanism in early light signaling in plants. The phosphorylation blocks the interaction with its signal transducers and destabilizes phytochromes, while the dephosphorylation enhances the interaction and increases the phytochromes stability (see Figure 2). However, the precise mechanisms behind the phosphorylation of substrate proteins by phytochromes remain to be unravelled.

Phytochromes and cytoplasmic signaling

In earlier studies, the focus was on the cytoplasmic factors that interact with phytochrome and serve as early steps in light signaling. Such studies provided evidence that phytochrome phototransduction involves activation of G proteins coupled with either calcium-calmodulin pathway or cGMP cascade (or both) in regulating expression of light-responsive genes (Neuhaus *et al.*, 1993; Bowler *et al.*, 1994; Mustilli and Bowler, 1997). However, a direct role for the heterotrimeric G protein complex in red and far-red light signal transduction is now being questioned (Jones *et al.*, 2003). But, the

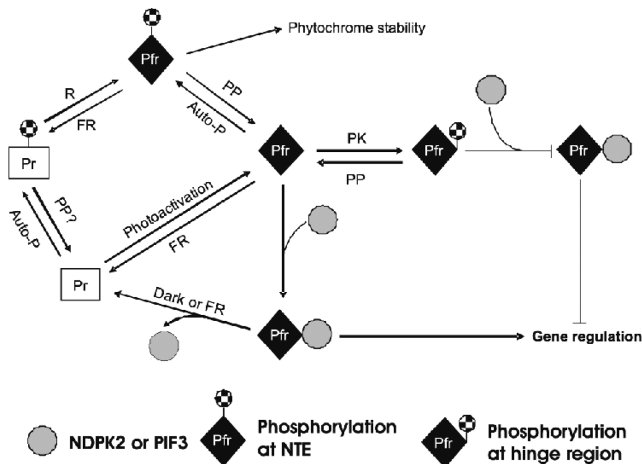

Fig. 2. Hypothetical model depicting the phosphorylation and kinase activity of phytochromes. Autophosphorylation occurs at the NTE of both Pr and Pfr forms (at Ser-7 or Ser-17 in oat phyA). Pfr autophosphorylation (Auto-P) regulates phytochrome stability. Phosphorylation at the hinge region by protein kinase (PK) at Ser-598 of Pfr form prevents interaction with downstream signal transducers like NDPK2 and PIF3. However, dephosphorylation by protein phosphatases (PP), such as FyPP and PAPP5, promotes the interaction with downstream signal transducers.

identification of a cytoplasmic-localized calcium-binding SUB1 protein that negatively regulates cryptochrome and phyA responses strengthens the role of calcium in cytoplasmic light signaling (Guo *et al*., 2001). Consistent with these results, phyA phosphorylated the constitutively cytoplasmic PKS1 protein, and *pks1* mutant was hypersensitive to red light, providing additional evidence for a phytochrome-associated cytoplasmic signaling mechanism (Fankhauser *et al*., 1999; Quail, 2002a). What happens to PKS1 after phosphorylation in cytoplasm still remains obscure. One assumption is that PKS1 might be negatively regulating phytochrome nuclear translocation by preventing the photoreceptor's movement from cytoplasm to nucleus by either remaining attached to it or by an as yet unknown mechanism. Thus, despite the fact that most of the recent data emphasize phytochrome functions in the nucleus, it does indeed interact with some cytoplasmic proteins too for its biological activity.

Nuclear localization of phytochromes

Phytochrome was believed to be a cytoplasmic protein for a fairly long time since its identification. The availability of the nucleotide sequence of phytochromes made it possible to analyze various functional domains. Although no clear nuclear localization signal (NLS) could be identified in the C-terminal domain of phytochrome, using transgenic *Arabidopsis* plants expressing fusion protein of GUS or GFP with C-terminal fragment of phyB, Akira Nagatani's group showed that phytochrome translocates from cytoplasm to the nucleus in presence of light (Sakamoto and Nagatani, 1996; Yamaguchi *et al*., 1999). On the other hand, the N-terminal domain fused to GFP or GUS was confined to the cytoplasm, regardless of the light conditions (Matsushita *et al*., 2003). Subsequently, light-dependent nuclear translocation of other phy proteins was also reported (Kircher *et al*., 1999, 2002; Hisada *et al*., 2000; Kim *et al*., 2000). In conditions of darkness, both phyA and phyB localize mainly in the cytoplasm (Yamaguchi *et al*., 1999; Hisada *et al*., 2000; Kim *et al*., 2000; Matsushita *et al*., 2003). Recently, a factor responsible specifically for the light-regulated nuclear accumulation of phyA has been identified as FHY1 (Hiltbrunner *et al*., 2005). Subsequently, FHL, a close homolog of FHY, has also has been ascribed a role in nuclear-accumulation of phyA. The *fhy1 fhl* double mutant is virtually blind to far-red light and nuclear accumulation of phyA is completely inhibited in an FHY1 FHL RNAi knock-down line (Hiltbrunner *et al*., 2006).

Phytochromes and nuclear speckle formation

During localization studies, phytochrome was found to be associated with speckles inside the nucleus (also called foci or nuclear bodies) (Kircher *et al*., 1999). In presence of light, the phyB–GFP fusion protein translocates into the nucleus and forms speckles in 2 h whereas phyA–GFP molecules are transported into the nucleus within 15 min in *Arabidopsis*, indicating that kinetics of nuclear localization and speckle formation vary with the type of phytochromes (Kircher *et al*., 1999; Kim *et al*., 2000). The nuclear accumulation and speckle formation of phyA–GFP were equally effective upon red, far-red and blue-light irradiation, whereas the phyB-GFP protein formed the speckles only under red-light (Gil *et al*., 2000; Kim *et al*., 2000). Phytochromes carrying missense mutations in the C-terminal PAS domain (and not in the HKRD domain) failed to form speckles inside the nucleus, indicating the importance of PAS domain region for speckle formation (Kircher *et al*., 2002; Chen *et al*., 2003). However, it was not necessary that intact PAS domain is essential for nuclear localization of phytochrome. For instance, *phyA-302* alleles carrying missense mutation at amino acid 777 (Glu to Lys) in the PAS2 motif of the C-terminal domain, showed normal translocation to the nucleus under continuous far-red light, but failed to produce nuclear speckles (Yanovsky *et al*., 2002). Interestingly, some mutations in the N-terminal domains and at the hinge region of

phytochromes affected the speckle formation, but it is now believed to be an indirect effect of altered spectral properties of the photoreceptor (Casal *et al*., 2002; Chen *et al*., 2003). Importantly, factors that interact with phytochromes such as cryptochrome 2 (cry2), Constitutive Photomorphogenesis 1 (COP1) and Phytochrome Interacting Factor 3 (PIF3) were shown to co-localize with phytochrome in the nuclear speckles (Mas *et al*., 2000; Bauer *et al*., 2004; Seo *et al*., 2004). In addition, mutant phytochrome alleles that fail to form speckles show reduced biological activities, suggesting that speckle formation and phytochrome-mediated light signaling are directly linked (Chen *et al*., 2003). An apparent paradox emerges when the N-terminal fragments of phyB exhibit increased signaling activity even without forming speckles in the nucleus (Matsushita *et al*., 2003; Oka *et al*., 2004). Thus, the exact biological significance of nuclear speckles remains to be enigmatic.

Phytochrome interacting factors (PIFs)

Since the enunciation of the concept that phytochrome acts as a protein kinase, there has been intensive research activity to identify phytochrome-interacting partner(s) that could trigger light signaling. The first phytochrome-interacting protein, PIF3 (Phytochrome Interacting Factor 3), a nuclear-localized bHLH transcription factor, was identified by Peter Quail's group, using yeast two-hybrid system to screen *Arabidopsis* cDNA library with the C-terminal domain of phyB as a bait (Ni *et al*., 1998). Later, other PIF3-related bHLH proteins like PIF1/PIL5, PIF4, PIF5/PIL6 and PIF6/PIL2 were also found to preferentially interact with phytochromes in the Pfr conformation (Huq and Quail, 2002; Khanna *et al*., 2004; Oh *et al*., 2004). HFR1 is another bHLH protein that dimerizes with PIF3 in yeast although it does not bind directly to phytochrome (Fairchild *et al*., 2000). Although, PIF interaction domain in phytochromes was initially thought to be the PAS-related domain at the C-terminal region, later studies indicated that both the C-terminal and the N-terminal halves of phyB are capable of interacting with PIF3 (Zhu *et al*., 2000). However, PIF3 binds to phyA less preferentially as compared to phyB (Ni *et al*., 1999). The PIF3 antisense lines showed reduced light sensitivity and alterations in the regulation of several photoresponsive genes (Ni *et al*., 1998). Additionally, red-light hypersensitivity (and not to far-red) due to enhanced PIF3 transcript levels were observed in the *Arabidopsis* mutant *poc1* (*photocurrent1*), which contains a T-DNA insertion in *PIF3* promoter region (Halliday *et al*., 1999). It is worth to mention here that Bauer *et al*. (2004) later identified that, although *poc1* has increased *PIF3* transcript levels, the level of PIF3 protein was undetectable in these mutants. Subsequently, PIF3 and phyB complexes were shown to bind *in vitro* to the light-responsive G-box elements (that are present on many light-regulated genes) {Martinez-Garcia *et al*., 2000; Duek and Fankhauser, 2005}. At the same time, PIF3 acts as a positive regulator of anthocyanin and chlorophyll accumulation (Kim *et al*., 2003; Monte *et al*., 2004). Recently, it has been demonstrated that PIF3 in concert with HY5, binds to separate sequence elements in the same gene promoters, to positively regulate anthocyanin biosynthesis (Shin *et al*., 2007). Taken together, although these results indicate that PIF3 may act positively in phyB signal transduction, however, several other recent publications contradict such a speculation. The first objection was raised when Kim *et al*. (2003) observed that PIF3 negatively regulates phyB-mediated hypocotyl elongation and both phyB- and phyA-mediated cotyledon opening and expansion. Based on these contradicting results, it is reasonable to suspect that PIF3 either activates negative or positive regulators of downstream gene expression or, alternately, acts as an activator or a repressor of downstream gene expression, depending on specific promoter elements and/or interactions with other factors.

Ubiquitin-mediated protein degradation and phytochrome signaling

Light-regulated ubiquitin-mediated proteolysis has become another emerging aspect of phytochrome signaling (Hoecker, 2005). The mechanism of proteolysis involves the covalent attachment of ubiquitin protein to the substrate, involving the sequential activities of an ubiquitin-activating enzyme (E1), an ubiquitin-conjugating enzyme (E2) and an ubiquitin ligase (E3), and finally the degradation of the substrate by 26S proteasome. The E3 ubiquitin ligase enzyme determines the specificity of substrate to be ubiquitinated. Interestingly, in *Arabidopsis* more than 1300 putative E3 ubiquitin ligases are reported (Smalle and Vierstra, 2004); recently, a detailed survey and analysis of ubiquitin-ligase family and F-box protein genes (encoding a component of E3 ligase complex) have been carried out (Gingerich *et al*., 2007; Jain *et al*., 2007). Among them, COP1, the key repressor of photomorphogenesis, is the most widely investigated E3 ligase in the plant kingdom. COP1 contains a RING-finger zinc-binding domain, a coiled-coil domain and a WD-40 repeat motif (Hardtke and Deng, 2000). Considering the fact that COP1 regulates more than 20% of genes in *Arabidopsis* genome in dark, and out of which approximately 20% are transcription factors, it is reasonable to speculate that

COP1-mediated protein degradation in plants might target several downstream signaling components (Ma *et al*., 2002). Accordingly, many transcription factors, such as Long Hypocotyl 5 (HY5), HY5 homolog (HYH), Long after Far-red Light 1 (LAF1) and Long Hypocotyl in Far-red1 (HFR1), that are involved in the positive regulation of light signaling were found to be regulated by COP1 in the dark (Osterlund *et al*., 2000; Holm *et al*., 2002; Seo *et al*., 2003; Jang *et al*., 2005). Additionally, COP1 regulates the degradation of the photoreceptor phyA in light, and surprisingly stabilizes PIF3 in the dark by an unknown mechanism (Bauer *et al*., 2004; Seo *et al*., 2004). The role of proteolysis in the regulation of other PIF proteins is also becoming more relevant. For instance, PIF1, a repressor of photomorphogenesis is degraded through the ubiquitin-26S proteasome pathway (Shen *et al*., 2005). In this respect, it is worth noting that photoactivated phytochrome induces rapid PIF3 phosphorylation prior to its proteasome-mediated degradation (see Figure 3; Al-Sady *et al*., 2006).

One of the negative regulator of phyA signaling, SPA1 (Suppressor of Phytochrome A105 1) has also been shown to interact with COP1 and together they suppress photomorphogenesis (see Figure 4, Laubinger *et al*., 2004). Moreover, the coiled-coil domain of SPA1 enhances the *in vitro* ubiquitination of LAF1 by COP1 at lower concentration, whereas full-length SPA1 reduces the E3 ubiquitin ligase activity of COP1 towards HY5 (Hoecker and Quail, 2001; Saijo *et al*., 2003; Seo *et al*., 2003). The SPA1-related proteins, SPA3 and SPA4, also interact with COP1 and act as negative regulators for far-red, red and blue light responses (Laubinger and Hoecker, 2003). In addition, two F-box proteins (a part of SCF class of E3 ubiquitin ligase), EID1 and AFR1, are involved in phyA signaling, further highlighting the importance of ubiquitin-regulated proteolysis in light signaling (Dieterle *et al*., 2001; Harmon and Kay, 2003; Marrocco *et al*., 2006).

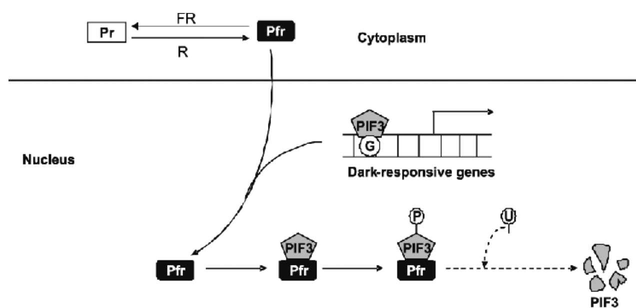

Fig. 3. Model depicting the fate of PIF3 inside the nucleus. In dark, PIF3 activates the genes responsible for skotomorphogenesis and as a result inhibits photomorphogenic responses. In presence of light, Pfr form of phytochrome migrates into the nucleus and interacts with PIF3, followed by phosphorylation, ubiquitination and the degradation of PIF3 by 26S prtoteasome.

In *Arabidopsis*, other members of the *COP/DET/FUS* class of genetic loci are also involved in ubiquitin-mediated proteasome pathway. Interestingly, the COP9 signalosome (CSN) which resembles the lid sub-complex of the 19S regulatory particle of the 26S proteasome was initially identified as a repressor of photomorphogenesis (Hardtke and Deng, 2000). In *Arabidopsis*, six of the COP/DET/FUS loci encode subunits of the CSN complex. The substrates targeted by COP9 signalosome for degradation include positive regulators of photomorphogenesis in the dark. For instance, null mutations within COP9 signalosome components prevent degradation of HY5, the major positive regulator of photomorphogenesis (Hardtke and Deng, 2000). Similarly, COP10, an ubiquitin-conjugating E2 enzyme (though not a component of CSN) regulates protein degradation

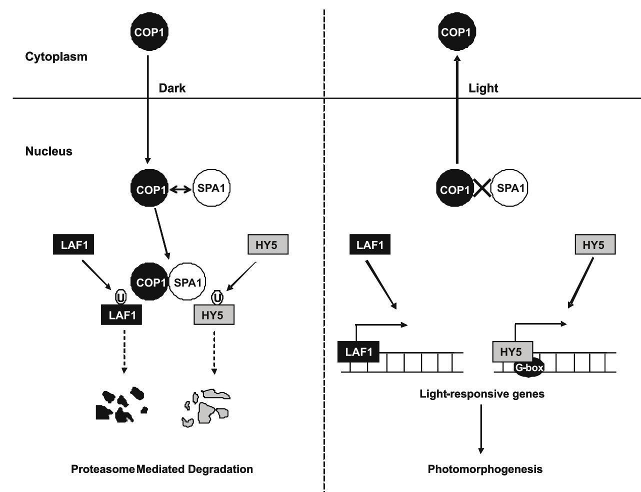

Fig. 4. Role of ubiquitin-mediated proteasome in the regulation of LAF1 and HY5. In the dark-grown seedlings, COP1, a repressor of photomorphogenesis, is more abundant in the nulceus and forms a complex with SPA1. The COP1-SPA1 complex in turn ubiquitinates the positive transcriptional regulators, LAF1 and HY5, which ultimately leads to their degradation by 26S proteasome, thus repressing the expression of light responsive genes in dark. However, in presence of light, COP1 migrates to the cytoplasm, and consequently LAF1 and HY5 are prevented from proteasome-mediated degradation, thus causing an increase in their abundance in the nucleus. As a result, LAF1 and HY5 bind to the light responsive elements (LREs) in the promoters of light-responsive genes and enhance their expression, leading eventually to photomorphogenesis.

through the help of COP1 and CSN (Hellmann and Estelle, 2002; Suzuki *et al*., 2002). COP10 may also be involved in HY5 degradation (Suzuki *et al*., 2002). Together these results implicate that light signaling and ubiquitin-mediated pathways are interconnected in a larger dimension than earlier anticipated. However, the molecular mechanisms that control proteolysis under different light conditions and the role of other photoreceptors involved still remains obscure.

Chromatin-remodeling and phytochromes

Chromatin is supramolecular structure that the eukaryotic genome is packaged into, composed of DNA and proteins, most of which are histones. The term "chromatin remodeling" describes a broad range processes that alter chromatin structure and changes its accessibility to a variety of protein factors that target DNA during replication, recombination and transcription (Hsieh and Fischer, 2005). Recent studies provide new insights into the role of chromatin remodeling in the light regulated expression of genes. A correlation between the increased acetylation of histones H3 and H4 in the promoter region of pea plastocyanin gene and its light-induced transcription gave the initial clue for a light-mediated alteration in nucleosome accessibility (Chua *et al*., 2001, 2003). Likewise, evidences obtained from the genetic analysis of *Arabidopsis* mutants for histone acetyltransferases, like HAF2 and GCN5, revealed repression of photomorphogenesis, whereas mutation in the histone deacetylase, HD1/HDA19 locus, activated light-mediated responses, suggesting that chromatin remodeling may be a prerequisite for light-regulated transcription (Bertrand *et al*., 2005; Benhamed *et al*., 2006). It is worth noting that, HAF2 and GCN5 are required for histones H3 and H4 acetylation of several light-responsive genes, whereas HD1 has opposite effects on the same promoters (Bertrand *et al*., 2005; Benhamed *et al*., 2006).

Another major light-signaling component that has also turned out to be a key player in chromatin-remodeling is the DET1 (de-etiolated) protein. Similar to *cop/det/fus* mutants, plants defective in DET1 display constitutive de-etiolation in darkness (Chory and Peto, 1990). However, unlike COP proteins, DET1 does not participate in proteasome pathway, but forms a complex with Damaged DNA-Binding 1 (DDB1), a protein implicated in the recruitment of histone acetyltransferases and COP10 in modifying chromatin molecules (Schroeder *et al*., 2002; Yanagawa *et al*., 2004). DET1 binds to the non-acetylated amino-terminal tail of histone H2B in nucleosome core particles (Benvenuto *et al*., 2002). Based on the available clues, it has been speculated that, in dark, DET1 binds to H2B and DDB1 to repress transcription, whereas in light, the DET1/DDB1 complex recruits histone acetyltransferase, causing acetylation of H2B and thus resulting in the activation of transcription (Benvenuto *et al*., 2002; Schroeder *et al*., 2002). Summing up, DET1 might regulate chromatin conformation, leading to the regulation of many genes involved in photomorphogenesis. However, whether light-regulated chromatin modifications are specific to the quality of light and type of photoreceptors involved is still not known.

Phytochrome-regulated gene transcription

Most of the early information on light-induced changes in gene expression in plants has largely come from the work on light up-regulated *CAB* and *RBCS*, and light down-regulated *PHYA* and *PCR* (Terzaghi and Cashmore, 1995; Tyagi and Gaur, 2003). The molecular genetic analyses of *Arabidopsis* mutants have also revealed that the expression of several genes is regulated by light. An overall picture of the whole genome expression related to phytochrome-mediated signaling became apparent with the emergence of microarray technology. For instance, using a cDNA microarray containing 9216 *Arabidopsis* ESTs (representing ~6120 unique genes, i.e. nearly 30% of the genome) in seedlings grown under white, red, far-red and blue light conditions, Ma *et al.* (2001) demonstrated that approximately one-third (32 %) of the ESTs were regulated 2-fold or more by light, and at least 26 cellular pathways were differentially regulated during photomorphogenesis. Furthermore, Jiao *et al*. (2005) utilized the microarray technology (using 70-mer oligonucleotide microarrays representing 36,926 rice and 25,676 *Arabidopsis* genes, respectively) to compare the genome expression changes during light-regulated seedling development in rice and *Arabidopsis*, respectively. They observed that ~20 % of the genes in both rice and *Arabidopsis* seedlings are regulated by white light and that the genome expression profile of photomorphogenesis is more conserved than skotomorphogenesis. The microarray analysis in other studies revealed the enrichment of many transcription factors regulating light-responsive genes during photomorphogenesis (Tepperman *et al*., 2001, 2004). Importantly, Tepperman *et al*. (2001) observed that 10 % of the genes represented in a high-density oligonucleotide array (for 8,200 different *Arabidopsis* genes) are regulated by phyA, in response to continuous far-red light, and out of which 44% of the genes responding to the signal within 1 h are transcription factors. Strikingly, phyA controls the transcription of

early responsive genes under both far-red and red-light, although phyB is the major red light receptor (Tepperman *et al*., 2006). Given that phytochrome coordinates the transcription of a master-set of regulatory proteins that ultimately trigger the expression of downstream genes, it has been experimentally validated that phytochrome signaling revolves around a transcriptional cascade, which culminates in the light-modulated transcription of about 2500 genes in *Arabidopsis* (Gyula *et al*., 2003). However, it is rather well established that the LREs invariably work in a combinatorial fashion to sense and respond to monochromatic lights (see Terzaghi and Cashmore, 1995). Interestingly, promoters of many of these genes contain light-responsive elements (LREs) such as G-box, SORLIP, and SORLREP. Although G-box acts as a DNA binding motif for many of the phytochrome interacting bHLH and bZIP factors, it is still unclear how they orchestrate the expression of several downstream genes (Tepperman *et al*., 2006).

In recent past, the whole genome expression analysis has also been carried out to understand phytochrome-mediated responses at the organ-specific level. Each organ in a plant exhibits distinct developmental responses to light, although they share common light perception and signaling systems (Quail, 2002b). For example, light triggers cotyledon expansion and leaf development, but at the same time inhibits hypocotyl growth in *Arabidopsis* (Neff *et al*., 2000). Similarly, the early red-light-responsive gene regulation is mediated mainly by phyA, whereas the inhibition of hypocotyl elongation by red-light is under the strong control of phyB, and the red-light responsive cotyledon expansion and hook opening are mediated by other phytochromes (Tepperman *et al*., 2004, 2006). The genome expression profiles of light-grown rice and *Arabidopsis* organs (cotyledons, hypocotyls and roots) with their dark-grown counterparts showed a significant overlap in light- and dark-grown organ pairs (~90 % for rice and ~70 % for *Arabidopsis*, respectively) {Jiao *et al*., 2005}. However, *Arabidopsis* roots appeared to have more specific light-regulated genes than cotyledons, whereas rice roots have even more light-regulated genes than shoots. Moreover, the overlaps among light-regulated genes are less than 1 % of all light-regulated genes, and were differentially regulated by light in all three tissues, thus it is likely that light signaling cascades vary in different organs and cell types (Jiao *et al*., 2005; Ma *et al*., 2005). Alternatively, using light-mediated inhibition of hypocotyl growth and stimulation of cotyledon expansion as criteria, Khanna *et al*. (2006) studied the impact of targeted mutations in 32 representative genes on the phy-induced seedling de-etiolation process. Based on this analysis they identified 63 % of the lines (20) displaying distinct aberrant photoresponsiveness in hypocotyls and cotyledons, suggesting the immediate divergence of phytochrome signaling at organ level.

FUTURE PERSPECTIVES

Tremendous progress has been made during the past decade in understanding phytochrome signaling mechanisms, largely due to the use of extensive molecular and genetic studies in the model plant *Arabidopsis*. As a result, several genes involved in phytochrome-responsive light signal transduction have been identified. Many phytochrome-mediated responses are also regulated by other photoreceptors like cryptochromes and phototropins, indicating combinatoral interaction of these sensory photoreceptors perceiving different light signals. In addition, it has now been well established that various plant hormone and light signaling components crosstalk to regulate various developmental responses. However, the exact molecular mechanisms behind such cross-talks remain elusive, making the scenario more complicated than earlier anticipated. In a similar fashion, direct downstream targets of transcription factors in the light-regulated transcription networks are barely been explored. Much remains to be learned about the role of photoreceptors outside nucleus and the molecular basis for organ-specific light responses and their coordination between organs. To resolve some of these intricacies, one requires to address these problems using a combined strategy integrating conventional genetic and advanced molecular approaches.

Our current understanding of the structure and function of phytochromes is mainly derived from the genetic and molecular studies on photoreceptor loss-of-function *Arabidopsis* mutants. Despite the painstaking efforts to identify constitutive phytochrome mutants and identifying the loci involved, it is striking to note that although *cop/det/fus* mutants are constitutively photomorphogenic, they still depend on light for function. The attempts made to explain this unusual observation failed miserably for a long period. A breakthrough concept of light-independent signaling has emerged very recently when Lagarias's group isolated and characterized the first class of phytochrome gain-of-function mutants in *Arabidopsis*, wherein a Tyrosine residue in the conserved GAF domain is mutated to Histidine (phyA^{Y242H} and phyB^{Y276H}, respectively) {Su and Lagarias, 2007}. Surprisingly, the transgenic plants expressing phyA^{Y242H} and phyB^{Y276H} complemented *phyB* mutants and displayed constitutive

photomorphogenic responses, indicating that these dominant negative mutations bypassed the prerequisite photoconversion that otherwise is essential for phytochrome-mediated light signaling (Su and Lagarias, 2007). In order to understand the intricacies associated with phytochrome signaling, several novel molecular approaches have proved to be a powerful tool. For instance, the integration of genome-wide microarray analysis with chromatin immunoprecipitation (ChIP-on-chip assays) has been recently used to identify direct targets of light-responsive genes (Lee *et al*., 2007). Other innovative approaches such as affinity-capture-based proteomic techniques in conjunction with high-throughput global gene expression profiling will definitely be of much value in future, for dissecting and identifying the as yet unknown signaling components involved in the phytochrome-mediated pathway.

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