Journal of Photoscience (2003), Vol. 10(1), pp. 157–164

Structure and Function of the Phytochromes: Light Regulation of Plant Growth and Development

Chung-Mo Park[1] and Pill-Soon Song[1,2]*
[1]Kumho Life & Environmental Science Laboratory, Kwangju 500-712, Korea
[2]University of Nebraska-Lincoln, NE 68588, USA

Light exerts two primary roles in plant growth and development. Plants acquire all biochemical energy required for growth and propagation solely from light energy via photosynthesis. In addition, light serves as a medium through which plants recognize environmental fluctuations, such as photoperiod and presence of neighboring animals and plants. Plants therefore constantly monitor the direction, intensity, duration, and wavelength of environmental light and integrate these light signals into the intrinsic regulatory programs to achieve an optimized growth in a given light condition. Although light regulates all aspects of plant growth and developmental aspects, the molecular mechanisms and signaling cascades involved have not been well established until recently. However, recent advances in genetic tools and plant transformation techniques greatly facilitated the elucidation of molecular events in plant photomorphogenesis. This mini-review summarizes the gist of recent findings in deetiolation and suppression of shade avoidance response as classic examples of the phytochrome-mediated photomorphogenesis.

key words: G-protein, growth hormone, light, photomorphogenesis, phytochrome, shade avoidance

INTRODUCTION

Light regulates virtually all aspects of plant growth and developmental processes throughout the whole life cycle, covering from seed germination to flowering, among which seedling growth and leaf development are most sensitive to light [1,2]. Plant growth and developmental processes regulated by light are collectively called photomorphogenesis. Plants therefore evolved a versatile set of photoreceptors, each with distinct roles, and various light signaling mediators.

Light signal transduction cascades that direct plant photomorphogenesis have been extensively studied by molecular biological and genetic analyses of various photomorphogenic mutants with altered light responses, mainly in model plants. A battery of signaling mediators have been identified and, their functional mechanisms have been elucidated in some detail. Interestingly, many signaling mediators that have been demonstrated in animals are also functional in plants. A generally accepted scheme for light signal transduction illustrates that light signals perceived by the photoreceptors are converted into biochemical or conformational signals, which are subsequently transmitted through down-stream signaling mediators, such as phytochrome-interacting factors (PIFs) [3-5], heterotrimeric G-proteins [6], Ras-like G-proteins [7], Ca^{2+}/calmodulin [8,9], and protein kinases/phosphatases [10], and finally regulate genes involved in growth and development (Figure 1).

The red and far-red light absorbing phytochromes and the blue

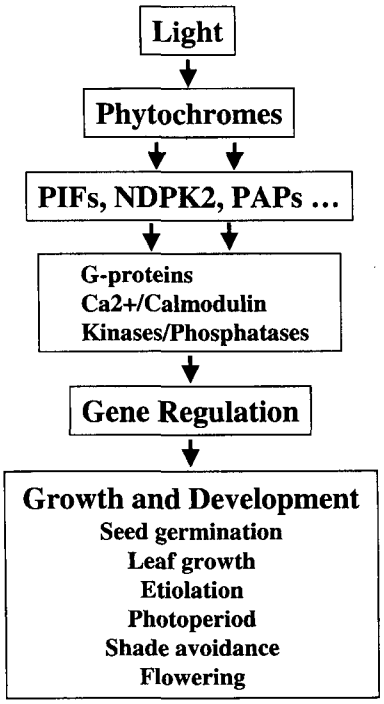

Figure 1. Phytochrome-mediated light signal transduction in plants. Light signals perceived by the phytochromes are transmitted through a series of signaling mediators and finally regulate gene expressions involved in growth and development.

*To whom correspondence should be addressed.
E-mail : pssong@kkpc.com
Received & Accepted; October 23, 2002

light absorbing cryptochromes are two principal photoreceptors that regulate most of the photomorphogenic processes in plants. The cryptochromes are phosphorylated by the phytochrome kinases [11]. In some cases, a photoreceptor is enough to trigger a specific light response. However, recent genetic studies on double and triple photoreceptor mutants have shown that the photoreceptors function in a mode of coactions [9,12-14]. The light responses mediated by the phytochromes are influenced by the cryptochromes and vice versa. Moreover, physical and functional interactions between the two photoreceptors have been confirmed biochemically and genetically, especially in flowering time control [11,15,16].

Most light responses in plants appear in forms of growth rate adjustments, entailing that growth regulators are critical components in light signal transduction. Studies on diverse photomorphogenic mutants have confirmed this notion. It is therefore generally accepted that light does not function independently but is integrated with endogenous growth regulators, such as growth hormones, for temporal and spatial regulation of plant photomorphogenesis [17]. For example, brassinosteroid (BR), auxin, and gibberellin (GA) are directly involved in the photomorphogenic processes, particularly in stem morphogenesis and leaf development [2,17]. Among them, the most widely studied is the interaction between light and BR. BR-deficient plants exhibit photomorphogenic development in the dark, such as development of primary leaves, apical hook and cotyledon opening, and thick short hypocotyls [18,19]. In the light, they show dwarfish stems and petioles, dark-green leaves, male sterility, and delayed senescence. However, the molecular mechanisms have not been fully elucidated at the molecular level yet.

A classic example of the phytochrome-mediated photomorphogenesis in higher plants is shade avoidance response. The shade avoidance response is a morphogenetic strategy for plants to seek light signal for their growth and development. It involves rapid stem growth toward the source of light as the result of cell elongation in the soil or in the canopy shade. In a recent study [7], we showed that cell elongation in the dark is induced by BR whose level is up- and down-regulated by dark and light, respectively, providing a first molecular basis for etiolation/deetiolation in plants. The rapidly growing etiolated seedling stops elongation of its hypocotyls once exposed to light. The same mechanism regulates the shade avoidance response. It is therefore evident that growth hormones play critical roles in the phytochrome-mediated light signal transduction.

In a previous review, the structure and function of the phytochrome photoreceptors has been discussed [20]. Here, we update recent advances in the phytochrome function, with a couple of illustrative examples of the phytochrome-mediated signal transduction pathway in plants, and provide some experimental clues that can be examined in future researches on light regulation of plant growth and development.

FUNCTIONAL CLUES FROM THE PHYTOCHROME STRUCTURE

The phytochrome molecule has a two-domain structure, the N-terminal chromophore-binding photosensory domain and the C-terminal regulatory domain that are connected by a flexible hinge region [21,22] (Figure 2). The C-terminal domain contains several structural motifs that directly interact with the phytochrome-interacting factors (PIFs). A conserved subdomain has been also identified in the C-terminal domain that is homologous to the prokaryotic histidine kinases. Although molecular biological and photochemical properties of the phytochromes have been well characterized, we are only beginning to understand as to how the phytochromes perceive light signals and how the light signals are converted into biochemical and conformational signals within the phytochrome molecule.

Conversion of light signals into conformational signals

One feature of the phytochromes is that the C-terminal domains are functionally interchangeable among different phytochromes, suggesting that they share common molecular mechanisms for the interaction with downstream signaling components. It is thus intriguing that PIFs identified so far exhibit diverse structural and functional properties [3-5,23], although they all interact with the C-terminal domain.

It has been well recorded that the chromophore topography and the secondary and tertiary structures of the phytochromes are significantly changed through apoprotein : chromophore and inter-domain interactions upon light perception [24-30]. The Pfr chromophore is more exposed than the Pr chromophore, which is modulated by the N-terminal α-helix forming 6 kDa-peptide [25,31,32]. The N-terminal domain is more exposed in the Pr form than in the Pfr form [33-35]. The hinge region is preferentially exposed in the Pfr form. These observations

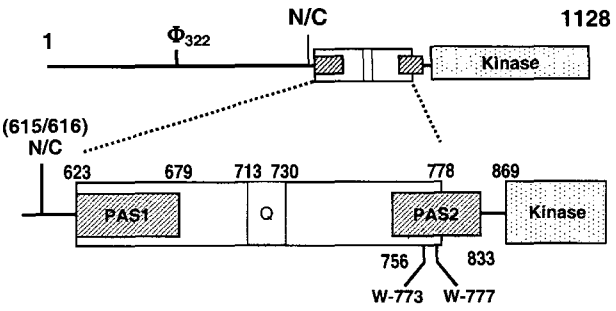

Figure 2. Schematic structural organization of monomeric phytochrome molecule. It consists of two structural domains connected by a hinge region (N/C). The N-terminal domain contains covalently bound chromophore at a distinct cysteine residue (Φ). The C-terminal domain contains several structural motifs that interact with various PIFs, including the PAS motif and Quail box (Q). The far C-terminal 200-residue region is highly homologous to the prokaryotic histidine kinase. Numbers indicate amino acid positions.

indicate that subtle conformational changes occur throughout the phytochrome molecule during the phototransformation. In this view, the phytochrome molecule is considered to be a molecular device that converts external light signals into internal conformational changes that are to be incorporated into subsequent biochemical events. It is therefore concluded that conformational signals as well as structural elements in the phytochrome molecule are important determinants that regulate the interactions with downstream signaling mediators, such as PIFs.

Inter-domain interactions in the phytochrome molecule

Another scheme to generate diverse conformational signals from the apparently simple structural organization of the phytochrome molecule is the inter-domain interactions. The NDPK2 binds to the Quail box preferentially in the Pfr phytochrome [3]. It has been suggested that both the N-terminal and C-terminal domains are required for full binding activity of the Pfr phytochrome to the PIF3 [23], although the Quail box also seems to play a major role. On the other hand, the PKS1 binds to the Ser/Thr kinase motif equally well in both the Pr and Pfr forms, indicating that this motif is exposed in both spectral forms [4]. However, the PKS1 phosphorylation and phytochrome autophosphorylation are stimulated by a factor of 2 to 2.5 in the Pfr form. It is thus probable that phosphorylation is an important regulatory factor for the phytochrome-PKS1 interaction. All these observations imply that differential inter-domain interactions activate a specific motif in the C-terminal domain to be recognized by different PIFs. The C-terminal domain has been proven to take various conformations and/or surface topologies in concert with conformational changes in the N-terminal domain through inter-domain crosstalks.

The light signals perceived by phytochromes are also differentiated at the molecular level. The N-terminal α-helix forming motif plays a critical role in the apoprotein : chromophore interaction [36]. It is thus notable that phytochrome B has an N-terminal 38-residue extension, in addition to the characteristic α-helix forming motif in the N-terminus. The 38-residue extension may directly participate in the apoprotein : chromophore interactions and induce a phytochrome B-specific inter-domain crosstalk with the C-terminal domain.

Molecular mechanisms for the inter-domain interactions

The inter-domain interactions in the phytochrome molecule can be regulated by two molecular mechanisms, intra-molecular and inter-molecular pathways depending on whether other signaling mediators are involved or not.

The photoinduced conformational signals could be further differentiated by inter-domain interactions through an intra-molecular pathway [21]. This pathway may be modulated by phytochrome phosphorylation/dephosphorylation at Ser-598 in the hinge region. The PIF3 bound strongly to the full-size Pfr phytochrome but moderately to the Pfr N-terminal domain and weakly to the C-terminal domain. Based on these observations, it has been suggested that the association of the PIF3 to the N-terminal and C-terminal domains are synergistic [23]. However this could be explained best by assuming that the C-terminal domain polypeptide is oligomerized so that the Quail box in this polypeptide becomes inaccessible to the PIF3. The full-length Pfr phytochrome keeps the protein in its native and dimeric state.

Also, an inter-molecular pathway via putative signal transmitter proteins could be involved [21]. For example, the SPA1 protein may serve this role as it is activated specifically by phytochrome A [37]. However, these mechanisms are oversimplified. Both the inter-molecular and intra-molecular pathways could be integrated to generate differential Pfr or conformational signals and to modulate the inter-domain crosstalks [39]. The inter-domain interactions, direct or indirect, are also important for the spectral integrity. Our chemical cross-linking experiments detected a R/FR-dependent interaction between the N-terminal peptide and the distal C-terminal peptide. The interaction between the N- and C-terminal peptides seems to shield the hinge region in the Pr form. However, the hinge region becomes exposed, and the Ser-598 is phosphorylated in the Pfr form.

Protein phosphorylation as an additional device to generate diverse conformational signals

The phytochrome photoreceptors are light-regulated serine/threonine-specific protein kinases [40,41]. Although it is still a matter of some debate, two recent observations strongly support the nature of the phytochrome kinase. A prokaryotic phytochrome Cph1, isolated from the cyanobacterium *Synechocystis sp. PCC6803,* has very similar structural and photochemical properties to those of the eukaryotic phytochromes and has a light-regulated histidine kinase activity [40]. The kinase activity of the higher plant phytochromes also has been demonstrated *in vitro* using highly purified native and recombinant proteins [40]. Besides the phytochrome itself that is autophosphorylated [42], additional phosphorylation substrates have been identified, including the PKS1 [10], the Aux/IAA [43], and the cryptochromes [11]. Furthermore, the phytochrome -cryptochrome coactions have been functionally confirmed, especially in flowering time control [9,13,15], suggesting that protein phosphorylation plays a critical role in plant photomorphogenesis.

Phytochromes are autophosphorylated [35,44-46]. The Ser-7 of the phytochrome A is phosphorylated *in vivo* in both the Pr and Pfr forms [42,46]. The Ser-17 is phosphorylated by protein kinase A *in vitro* only in the Pr form [42,45]. The Ser-598 is preferentially phosphorylated in the Pfr form *in vivo* [42,46]. Phosphorylation at a single residue can induce a profound conformational change in a protein [47-49]. CD analysis and proteolysis on the phytochromes have demonstrated that the phosphorylation of Ser-598, Ser-7, and Ser-17 induces subtle conformational changes in the phytochrome molecule.

Protein phosphorylation appears not only to regulate conformational changes but also to modulate the crosstalks between the N- and C-terminal domains. Activation of the PIFs by the phytochromes may be achieved through phosphorylation by the phytochrome kinase [4], regulation of subcellular locations [5,23], or through regulation of the complex formation with other components [40]. These indicate that conformational signals and accompanying inter-domain crosstalks are further diversified by protein phosphorylation and amplified through complex interactions with downstream signaling components.

Reversible protein phosphorylation in phytochrome signaling

Reversible protein phosphorylation, which is catalyzed by functionally coupled protein kinases and protein phosphatases, is a major signaling mechanism in eukaryotic cellular functions and exerts its role as a feedback control mechanism in various eukaryotic kinase signaling cascades [50-52]. Recent molecular biological and biochemical evidences indicate that coordinate interactions between coupled protein kinases and phosphatases also play important roles in plants, especially in cell cycle control in which a set of cyclin-dependent kinases and protein phosphatases are involved [53-54]. It also has been suggested that protein phosphorylation is an essential step in light signal transduction in plants and protein phosphatase(s) is possibly involved in this process [55-57].

The eukaryotic phytochromes are unique red/far-red light receptors in that they perceive environmental light through the N-terminal chromophore-binding domain and exert regulatory roles through the C-terminal domain. The C-terminal domain possesses structural elements required for interactions with down-stream signaling mediators [3-5]. It also contains a motif that is similar to the prokaryotic histidine kinases. It is now generally accepted that the phytochromes are serine/threonine-specific protein kinases that is regulated by light [58]. Furthermore, the phytochrome autophosphorylation at certain serine residues and phosphorylation of the phytochrome kinase substrates are essential for the phytochrome function [56,57]. Since the phytochromes are molecular light switches that regulate many photomorphogenic growth and developmental processes, the biochemical and physiological activities should be precisely controlled. One potential molecular means to achieve this would be to modulate the phosphorylation status of the phytochromes, demanding that the intrinsic phytochrome kinase activity is coupled with a protein phosphatase(s) as has been suggested (Figure 3). Recent identification of the phytochrome kinase substrates further supports this view.

The cryptochrome phosphorylation by the phytochrome kinases is regulated by light wavelength and required for the photoactivation of blue light responses [11]. Notably, the photoactivated cryptochromes down-regulates the COP1 activity via direct protein-protein interactions in blue light-mediated photomorphogenic responses [59]. It is therefore assumed that modulation of the phytochrome function by reversible phosphorylation is an essential event that influences diverse plant growth and developmental processes.

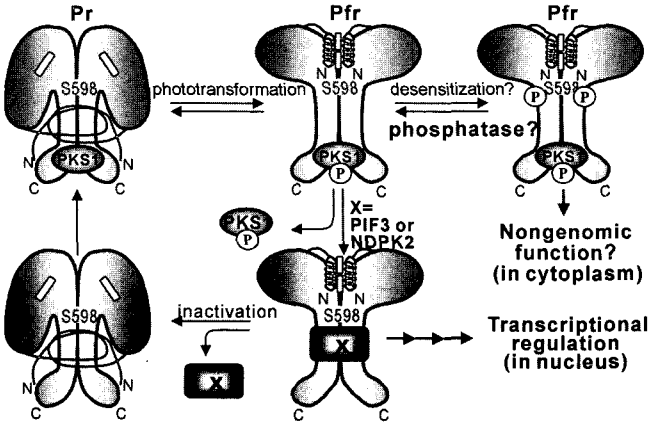

Figure 3. Phytochrome phototransformation and interactions with PIFs. The S598 in the Pr phytochrome is phosphorylated upon phototransformation into the Pfr phytochrome, which is designated as a desensitized Pfr phytochrome (Pfr') in a similar way as with the desensitized rhodopsin. However, the Pfr' phytochrome may not be physiologically functional. A protein phosphatase is proposed to dephosphorylate the phospho-S598, resulting in generation of the sensitized Pfr phytochrome that can interacts with PIFs. Adopted from Park *et al.* [20].

However, no such protein phosphatases have been identified, and no physiological roles of protein phosphorylation have been unequivocally elucidated at the molecular level yet. Considering the nature of the eukaryotic phytochrome kinase, light-regulated phytochrome autophosphorylation, the presence of phytochrome kinase substrates, and light wavelength-dependent phosphorylation of cellular proteins in plant photomorphogenesis, it is highly possible that phytochrome kinase-mediated light signals are further modulated by a phytochrome kinase-specific protein phosphatase (s) as previously suggested [41,57].

PHYTOCHROME FUNCTION IN DEETIOLATION AND SHADE AVOIDANCE

Seedling growth is a photomorphogenic process that is most responsive to light. Dark-grown seedlings are remarkably different from those grown in the light [2]. When seedlings are grown in the dark, the hypocotyls are abnormally extended at the expense of leaf and root growth. This light response, called etiolation, is an adaptive process for plants to quickly reach the light source so that they can efficiently perform photosynthesis. When plants reach the light source, they exhibit deetiolated development; hypocotyl elongation is suppressed, whereas leaf and root growth is accelerated. A similar light response that is regulated by an identical molecular mechanism is shade avoidance response.

We recently showed that a dark-induced Ras-like small G-protein, pea Pra2, plays a critical role in this process by

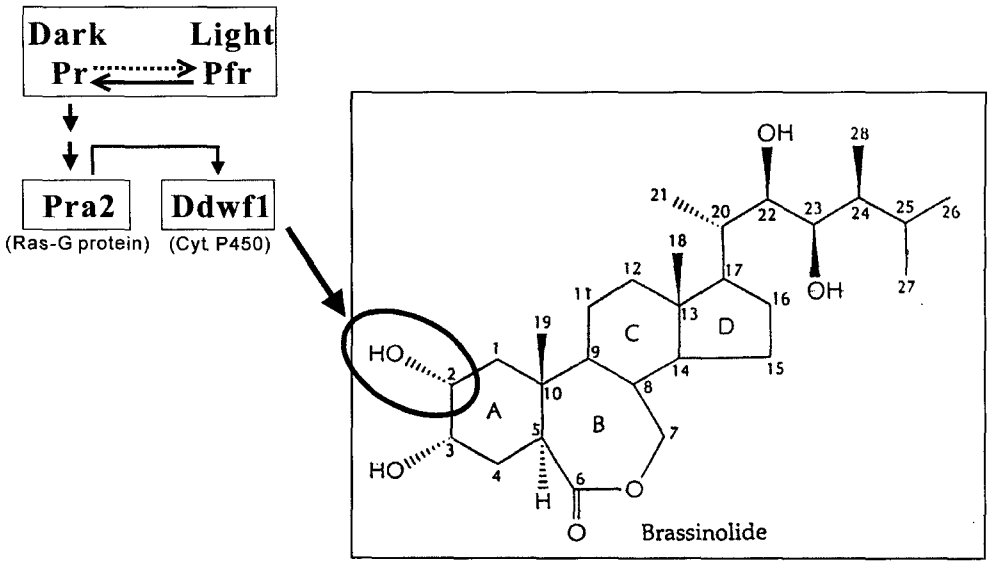

Figure 4. A proposed role of Pra2 in light regulation of BR biosynthesis during etiolation. The light-responsive Pra2 would either directly activate DDWF1 or catalyze the formation of a multi-component DDWF1 enzyme complex on ER membrane by recruiting other cofactor(s) as illustrated by X. The Pra2 and DDWF1 expressions are repressed by light, possibly through unidentified light-responsive transcription factors (TF1 and TF2). Adopted from Kang *et al.* [7].

activating a novel cytochrome P450 that catalyzes C-2 hydroxylation in the BR biosynthesis in *Arabidopsis thaliana* [7] (Figure 4). We demonstrated that Pra2 specifically regulates a novel cytochrome P450 hydroxylase that catalyzes C-2 hydroxylations in the BR biosynthetic pathway. The BR subsequently directs hypocotyl growth during the etiolation. Transgenic plants with reduced Pra2 exhibit dwarfish hypocotyls in the dark, which are completely restored by brassinolide (BL). Surprisingly, transgenic plants overexpressing the cytochrome P450 show elongated hypocotyls even in the light, which phenocopies the etiolated hypocotyl growth. These results indicate that Pra2 is a molecular knob that integrates light and BR signals in the hypocotyl elongation of etiolated seedlings, a first functional evidence for small G-proteins in plants.

Monomeric small G-proteins that belong to the Ras superfamily regulate numerous cellular processes in animals and plants, such as cell growth and differentiation, cell morphogenesis, and vesicle transport [60,61]. Previous experimental evidences have suggested that they also fulfill an important role in light signal transduction in plants [62,63]. However, studies on the roles of plant small G-proteins have not been far advanced yet, primarily because plants contain a big multi-gene family encoding various small G-proteins. For example, among the Rab subfamily alone, more than 30 genes have been identified in *Arabidopsis* [64]. The pea Pra2 small G-protein is exceptional in that the molecular mechanism for its light-regulated expression has been characterized in detail [65,66]. Its expression is dark-induced [67]. Notably, the 5' nontranscribing region of the *pra2* gene contains a dark-inducible element (DE1) that confers light down-regulation of a reporter gene [66]. Pra2 is expressed exclusively in the rapidly elongating upper region of etiolated pea epicotyls [65], where total phytochrome content is the richest [68]. In addition, this plant part is most responsive to BR [18]. Our experimental data as well as previous observations all support that Pra2 plays a crucial role in the integration of light with growth hormones, most probably BRs, during the etiolated seedling growth.

However, there should be more Ras-like small G-proteins involved in the light signal transduction and interactions between light signaling mediators and growth regulators in plants. First, several GTP-binding proteins have been differentially expressed under different light conditions, although their identities have not been determined. Secondly, there are at least several heterotrimeric G-proteins in animals that mediate different signals. On the contrary, only a single heterotrimeric G-protein is present in plants, raising a question how the diverse signals are mediated by the single heterotrimeric G-protein in plants. The answer would be provided by the fact that plants have more Ras-like small G-proteins than animals. For example, there are more than 120 genes encoding small G-proteins in *Arabidopsis*. It seems that each small G-protein mediates a distinct signal. Notably, we observed that a tobacco small G-protein gene, the *Nt-rab11d* whose gene product has a sequence identity (72%) to Pra2 [7], was drastically suppressed in the *pra2* transgenic plants, supporting this view.

A question of particular interest is how Pra2 mediates the phytochrome signals into BR signaling pathway. The *pra2* gene expression is induced predominantly in the dark but greatly suppressed by red light, suggesting that it is regulated by the phytochromes. A 12-bp light-responsive element (DE1) in the

promoter of the *pra2* gene is enough for red light response [66]. On the other hand, the phytochromes physically and functionally interact with the cryptochrome, especially in flowering time control. The cryptochromes are phosphorylated by the phytochrome kinases. Notably, the cryptochromes also interact with COP1, a photomorphogenic repressor with versatile roles in photomorphogenic growth and development, through protein-protein interactions [59]. Recently, transcription factors, DF1 and PLATZ1 from pea, that specifically bind the DE1 element have been identified [69,70]. It will be interesting to examine whether there are any functional interactions between COP1 and DF1 or PLATZ1.

INTERACTIONS AND INTEGRATIONS AMONG SIGNALING MEDIATORS

Structural and biochemical studies reveal that the phytochrome molecule contains various structural elements or motifs with distinct characteristics. In addition, light triggers complex conformational changes in conjunction with protein phosphorylation at distinct serine residues. These may work synergistically to generate diverse conformational changes within the phytochrome molecule, which may explain the multiple functions of the phytochromes in many photomorphogenic processes via interactions with multiple PIFs.

Signaling cascades downstream of the phytochromes are also complicated. Many morphological studies suggest that growth hormones are involved in the light-regulated plant growth and development [1,2]. For example, transgenic plants overexpressing phytochrome A exhibit a dwarfish appearance as observed in mutant plants that are deficient in BR or GA biosynthesis and/or perception. It is also well established that auxin exerts a critical role in phototropism, a pivotal light response that ensures optimal perception of sun light by leaves for efficient photosynthesis.

It is now evident that light signals perceived by plants are further modulated by intrinsic developmental factors as well as by other environmental cues. Flowering time control is another example in which multiple genetic pathways are linked. Light and GA signals are integrated via the so-called floral pathway integrators, such as LFY, FT, and AGL20 [71-73]. We therefore propose that light signal transduction mechanisms in plants should be considered as signaling networks rather than signaling cascades (or pathways). Furthermore, the interactions and integrations among the photoreceptors and various signaling mediators rather than the function of each light signaling mediator should be more seriously considered.

Acknowledgements – This paper is dedicated to Professor Sang Chul Shim (1936-2002). It is dedicated to Professor Masamitsu Wada in celebration of his 60th birthday. The work described here has been supported by grants from Kumho Petrochemical Co., Ltd., KRF, KISTEP, and BioGreen 21.

REFERENCES

1. Neff, M. M., Fankhauser, C., and Chory, J. (2000) Light: an indicator of time and place. *Genes Dev.* **14**, 257-271.
2. von Arnim, A. G., and Deng, X. -W. (1996) Light control of seedling development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 215-243.
3. Choi, G., Yi, H., Lee, J., Kwon, Y.-K., Soh, M. -S., Shin, B., Luka, Z., Hahn, T. -R., and Song, P. -S. (1999) Phytochrome signaling is mediated through nucleoside diphosphate kinase 2. *Nature* **401**, 610-613.
4. Fankhauser, C., Yeh, K. -C., Lagarias, J. C., Zhang, H., Elich, T. D., and Chory, J. (1999) PKS1, a substrate phosphorylated by phytochrome that modulates light signaling in *Arabidopsis*. *Science* **284**, 1539-1541.
5. Ni, M., Tepperman, J. M., and Quail, P. H. (1998) PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix-loop-helix protein. *Cell* **95**, 657-667.
6. Okamoto, H., Matsui, M., and Deng, X. W. (2001) Overexpression of the heterotrimeric G-protein alpha-subunit enhances phytochrome-mediated inhibition of hypocotyl elongation in *Arabidopsis*. *Plant Cell* **13**, 1639-1652.
7. Kang, J. -G., Ju, Y., Kim, D. -H., Chung, K.-S., Fujioka, S., Kim, J. -I., Dae, H. -W., Yoshida, S., Takatsuto, S., Song, P. -S., and Park, C. -M. (2001) Light and brassinosteroid signals are integrated via a dark-induced small G-protein in etiolated seedling growth. *Cell* **105**, 625-636.
8. Neuhaus, G., Bowler, C., Kern, R., and Chua, N. -H. (1993) Calcium/calmodulin-dependent and -independent phytochrome signal transduction pathways. *Cell* **73**, 937-952.
9. Guo, H., Mockler, T., Duong, H., and Lin, C. (2001) SUB1, an *Arabidopsis* Ca^{2+}-binding protein involved in cryptochrome and phytochrome coaction. *Science* **291**, 487-490.
10. Fankhauser, C. and Chory, J. (1999) Light receptor kinases in plants. *Curr. Biol.* **9**, R123-126.
11. Ahmad, M., Jarillo, J. A., Smirnova, O., and Cashmore, A. R. (1998) The CRY1 blue light receptor of *Arabidopsis* interacts with phytochrome A *in vitro*. *Mol. Cell* **1**, 939-948.
12. Neff, M. M. and Chory, J. (1998) Genetic interactions between phytochrome A, phytochrome B, and cryptochrome 1 during *Arabidopsis* development. *Plant Physiol.* **118**, 27-36.
13. Mockler, T. C., Guo, H., Yang, H., Duong, H., and Lin, C. (1999) Antagonistic actions of *Arabidopsis* cryptochromes and phytochrome B in the regulation of floral induction. *Development* **126**, 2073-2082.
14. Reeves, P. H. and Coupland, G. (2001) Analysis of flowering time control in *Arabidopsis* by comparison of double and triple mutants. *Plant Physiol.* **126**, 1085-1091.
15. Más, P., Devlin, P. F., Panda, S., and Kay, S. A. (2000) Functional interaction of phytochrome B and cryptochrome 2. *Nature* **408**, 207-211.
16. Martinez-Hernandez, A., Lopez-Ochoa, L., Arguello-Astorge,

G., and Herrera-Estrella, L. (2002) Functional properties and regulatory complexity of a minimal RBCS light-responsive unit activated by phytochrome, cryptochrome, and plastid signals. *Plant Physiol.* **128**, 1223-1233.

17. Li, J., Nagpal, P., Vitart, V., McMorris, T. C., and Chory, J. (1996) A role for brassinosteroids in light-dependent development of *Arabidopsis*. *Science* **272**, 398-401.
18. Neff, M. M., Nguyen, S. M., Malancharuvil, E. J., Fujioka, S., Noguchi, T., Seto, H., Tsubuki, M., Honda, T., Takatsuto, S., Yoshida, S., and Chory, J. (1999) *BAS1*: A gene regulating brassinosteroid levels and light responsiveness in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **96**, 15316-15323.
18. Clouse, S. D., and Sasse, J. M. (1998) Brassinosteroids: Essential regulators of plant growth and development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49**, 427-451.
19. Schumacher, K. and Chory, J. (2000) Brassinosteroid signal transduction: still casting the actors. *Curr. Opin. Plant Biol.* **3**, 79-84.
20. Park, C. -M., Bhoo, S. -H., and Song, P. -S. (2000) Inter-domain crosstalk in the phytochrome molecules. *Semin. Cell Dev. Biol.* **11**, 449-456.
21. Quail P. H. (1997) The phytochromes: A biochemical mechanism of signaling in sight? *Bioassays* **19**, 571-579.
22. Wagner, D, Fairchild, C. D., Kuhn, R. M., and Quail, P. H. (1996) Chromophore-bearing NH_2-terminal domains of phytochromes A and B determine their photosensory specificity and differential light lability. *Proc. Natl. Acad. Sci. USA* **93**, 4011-4015.
23. Ni, M, Tepperman, J. M., and Quail, P. H. (1999) Binding of phytochrome B to its nuclear signaling partner PIF3 is reversibly induced by light. *Nature* **400**, 781-783.
24. Lagarias, J. C. and Mercurio, F. M. (1985) Structure function studies on phytochrome: Identification of light-induced conformational changes in 124-kDa *Avena* phytochrome *in vitro*. *J. Biol. Chem.* **260**, 2415-2413.
25. Singh, B. R. and Song, P. -S. (1989) Interactions between native oat phytochrome and tetrapyrroles. *Biochim Biophys Acta* **996**, 62-69.
26. Farrens, D. L., Cordonnier, M. -M., Pratt, L. H., and Song, P. -S. (1992) The distance between the phytochrome chromophore and the N-terminal chain decreases during phototransformation: A novel fluorescence energy transfer method using labeled antibody fragments. *Photochem. Photobiol.* **56**, 725-733.
27. Parker, W., Partis, M., and Song, P. S. (1992) N-terminal domain of *Avena* phytochrome: Interactions with sodium dodecyl sulfate micelles and N-terminal chain truncated phytochrome. *Biochemistry* **31**, 9413-9420.
28. Deforce, L., Tokutomi, S., and Song, P. -S. (1994) Phototransformation of pea phytochrome A induces an increase in α-helical folding of the apoprotein: Comparison with a monocot phytochrome A and CD analysis by different methods. *Biochemistry* **33**, 4918-4922.
29. Wells, T. A., Nakazawa, M., Manabe, K., and Song, P. -S. (1994) A conformational change associated with the phototransformation of *Pisum* phytochrome A as probed by fluorescent quenching. *Biochemistry* **33**, 708-712.
30. Kneip, C., Hildebrandt, P., Schlamann, W., Braslavsky, S. E., Mark, F., and Schäffner, K. (1999) Protonation state and structural changes of the tetrapyrrole chromophore during the phototransformation of phytochrome: A resonance spectroscopic study. *Biochemistry* **38**, 15185-15192.
31. Vierstra, R. D., Quail, P. H., Hahn, T. R., and Song, P. -S. (1987) Comparison of the protein conformations between different forms (Pr vs. Pfr) of native (124 kDa) and degraded (118/114 kDa) phytochromes from *Avena*. *Photochem. Photobiol.* **45**, 429-432.
32. Song, P. -S. (1988) The molecular topography of phytochrome: Chromophore and apoprotein. *J. Photochem. Photobiol. B* **2**, 43-57.
33. Jones, A. M., Vierstra, R. D., Daniels, S. M., and Quail, P. H. (1985) The role of separate molecular domains in the structure of phytochrome from etiolated *Avena sativa L. Planta* **164**, 501-506.
34. Schendel, R. and Rüdiger, W. (1989) Electrophoresis and electrofocusing of phytochrome from etiolated *Avena sativa L. Z. Naturforsch* **44c**, 12-18.
35. Lapko, V. N., Jiang, X. -Y., Smith, D. L., and Song, P. -S. (1998) Surface topography of phytochrome A deduced from specific chemical modification with iodoacetamide. *Biochemistry* **37**, 12526-12535.
36. Song, P. -S., Sommer, D., Wells, T. A., Hahn, T. -R., Park, H. -J., and Bhoo, S. H. (1996) Light signal transduction mediated by phytochromes: Preliminary studies and possible approaches. *Ind. J. Biochem. Biophys.* **33**, 1-19.
37. Hoecker, U., Teppermann, J. M., and Quail, P. H. (1999) SPA1, a WD-repeat protein specific to phytochrome A signal transduction. *Science* **284**, 496-499.
39. Smith, H. (1999) Tripping the light fantastic. *Nature* **400**, 710-713.
40. Yeh, K. C. and Lagarias, J. C. (1998) Eukaryotic phytochromes: light-regulated serine/threonine protein kinases with histidine kinase ancestry. *Proc. Natl. Acad. Sci. USA* **95**, 13976-13981.
41. Fankhauser, C. (2000) Phytochromes as light-modulated protein kinases. *Semin. Cell Dev. Biol.* **11**, 467-473.
42. Lapko, V. N., Jiang, X. Y., Smith, D. L., and Song, P. -S. (1999) Mass spectrometric characterization of oat phytochrome A: isoforms and posttransformational modifications. *Protein Sci.* **8**, 1032-1044.
43. Colon-Carmona, A., Chen, D. L., Yeh, K. C., and Abel, S. (2000) Aux/IAA proteins are phosphorylated by phytochrome *in vitro*. *Plant Physiol.* **124**, 1728-1738.
44. Wong, Y. S., Cheng, H. C., Walsh, D. A., and Lagarias, J. C. (1986) Phosphorylation of *Avena* phytochrome *in vitro* as a probe of light-induced conformational changes. *J. Biol. Chem.* **261**, 12089-12097.
45. McMichael, R. W. Jr, and Lagarias, J. C. (1990) Phosphopeptide mapping of *Avena* phytochrome phosphorylated by protein kinases *in vitro*. *Biochemistry* **29**, 3872-3878.
46. Lapko, V. N., Jiang, X. Y., Smith, D. L., and Song, P. -S. (1997) Posttranslational modification of oat phytochrome A: Phosphorylation of a specific serine in a multiple serine cluster. *Biochemistry* **36**, 10595-10599.
47. Johnson, L. N. and Barford, D. (1993) The effects of

phosphorylation on the structure and function of proteins. *Annu Rev Biophys Biomol Struct.* **22**, 199-232.
48. Ago, T., Nunoi, H., Ito, T., and Sumimoto, H. (1999) Mechanism for phosphorylation-induced activation of the phagocyte NADPH oxidase protein p47(phox): Triple replacement of serines 303, 304, and 328 with aspartates disrupts the SH3 domain-mediated intra-molecular interaction in p47(phox), thereby activating the oxidase. *J. Biol. Chem.* **274**, 33644-33653.
49. Ramsey, A. J. and Fitzpatrick, P. F. (2000) Effects of phosphorylation on binding of catecholamines to tyrosine hydroxylase: Specificity and thermodynamics. *Biochemistry* **39**, 773-778.
50. Stone, J. M., Collinge, M. A., Smith, R. D., Horn, M. A., and Walker, J. C. (1994) Interaction of a protein phosphatase with an *Arabidopsis* serine-threonine receptor kinase. *Science* **266**, 793-795.
51. Westphal, R. S., Anderson, K. A., Means, A. R., and Wadzinski, B. E. (1998) A signaling complex of Ca^{2+}-calmodulin-dependent protein kinase IV and protein phosphatase 2A. *Science* **280**, 1258-1261.
52. Keyse, S. M. (2000) Protein phosphatases and the regulation of mitogen-activated protein kinase signaling. *Curr. Opin. Cell Biol.* **12**, 186-192.
53. Meszaros, T., Miskolczi, P., Ayaydin, F., Pettko-Szandtner, A., Peres, A., Magyar, Z., Horvath, G. V., Bako, L., Feher, A., and Dudits, D. (2000) Multiple cyclin-dependent kinase complexes and phosphatases control G2/M progression in alfalfa cells. *Plant Mol. Biol.* **43**, 595-605.
54. Boniotti, M. B. and Griffith, M. E. (2002) "Cross-talk" between cell division and development in plants. *Plant Cell* **14**, 11-16.
55. Sheen, J. (1993) Protein phosphatase activity is required for light-inducible gene expression in maize. *EMBO J.* **12**, 3497-3505.
56. Harter, K., Frohnmeyer, H., Kircher, S., Kunkel, T., Muhlbauer, S., and Schäfer, E. (1994) Light induces rapid changes of the phosphorylation pattern in cytosol of evacuolated parsley protoplasts. *Proc. Natl. Acad. Sci. USA* **91**, 5038-5042.
57. Chandok, M. R. and Sopory, S. K. (1996) Phosphorylation/dephosphorylation steps are key events in the phytochrome-mediated enhancement of nitrate reductase mRNA levels and enzyme activity in maize. *Mol. Gen. Genet.* **251**, 599-608.
58. Fankhauser, C. and Chory, J. (1997) Light control of plant development. *Annu. Rev. Cell Dev. Biol.* **13**, 203-229.
59. Wang, H., Ma, L.-G., Li, J. -M., Zhao, H. -Y., and Deng, X. W. (2001) Direct interaction of *Arabidopsis* cryptochromes with COP1 in light control development. *Science* **294**, 154-158.
60. Ma, H. (1994) GTP-binding proteins in plants: new members of an old family. *Plant Mol. Biol. 26,* 1611-1636.
61. Takai, Y., Sasaki, T., and Matozaki, T. (2001) Small GTP-binding proteins. *Physiol. Rev.* **81**, 153-208.
62. Nagano, Y., Okada, Y., Narita, H., Asaka, Y., and Sasaki, Y. (1995) Location of light-repressible, small GTP-binding protein of the YPT/rab family in the growing zone of etiolated pea stems. *Proc. Natl. Acad. Sci. USA* **92**, 6314-6318.
63. Romero, L. C., Sommer, D., Gotor, C., and Song, P. -S. (1991) G-proteins in etiolated *Avena* seedlings: Possible phytochrome regulation. *FEBS Lett.* **282**, 341-346.
64. Ueda, T., Matsuda, N., Uchimiya, H., and Nakano, A. (2000) Modes of interaction between the *Arabidopsis* Rab protein, Ara4, and its putative regulator molecules revealed by a yeast expression system. *Plant J.* **21**, 341-349.
65. Inaba, T., Nagano, Y., Sakakibara, T., and Sasaki, Y. (1999) Identification of *cis*-regulatory element involved in phytochrome down-regulated expression of the pea small G-protein gene *pra2*. *Plant Physiol.* **120**, 491-499.
66. Inaba, T., Nagano, Y., Reid, J. B., and Sasaki, Y. (2000) DE1, a 12-base pair *cis*-regulatory element sufficient to confer dark-inducible and light down-regulated expression to a minimal promoter in pea. *J. Biol. Chem.* **275**, 19723-19727.
67. Yoshida, K., Nagano, Y., Murai, N., and Sasaki, Y. (1993) Phytochrome-regulated expression of the genes encoding the small GTP-binding proteins in peas. *Proc. Natl. Acad. Sci. USA* **90**, 6636-6640.
68. Briggs, W. R. and Siegelman, H. W. (1965) Distribution of phytochrome in etiolated seedlings. *Plant Physiol.* **40**, 934-941.
69. Nagano, Y., Furuhashi, H., Inaba, T., and Sasaki, Y. (2001) A novel class of plant-specific zinc finger-dependent DNA-binding protein that binds to A/T-rich DNA sequences. *Nuc. Acid Res.* **29**, 4097-4105.
70. Nagano, Y., Inaba, T., Furuhashi, H., and Sasaki, Y. (2001) Trihelix DNA-binding protein with specificities for two distinct *cis*-elements: both important for light down-regulated and dark-inducible gene expression in higher plants. *J. Biol. Chem.* **276**, 22238-22243.
71. Kobayashi, Y., Kaya, H., Goto, K., Iwabuchi, M., and Araki, T. (1999) A pair of related genes with antagonistic roles in mediating flowering signals. *Science* **286,** 1960-1962.
72. Lee, H., Suh, S. -S., Park, E., Cho, E., Ahn, J. H., Kim, S. -G., Lee, J. S., Kwon, Y. M., and Lee, I. (2000) The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*. *Genes Dev.* **14**, 2366-2376.
73. Samach, A., Onouchi, H., Gold, S. E., Ditta, G. S., Schwarz-Sommer, Z., Yanofsky, M. F., and Coupland, G. (2000) Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis*. *Science* **288**, 1613-1616.