Mini-Review

Inter-Domain Signal Transmission within the Phytochromes

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Phytochromes (with gene family members phyA, B, C, D, and E) are a wavelength-dependent light sensor or switch for gene regulation that underscore a number of photoresponsive developmental and morphogenic processes in plants. Recently, phytochrome-like pigment proteins have also been discovered in prokaryotes, possibly functioning as an auto-phosphorylating/phosphate-relaying two-component signaling system (Yeh et al., 1997). Phytochromes are photochromically convertible between the light sensing Pr and regulatory active Pfr forms. Red light converts Pr to Pfr, the latter having a "switch-on" conformation. The Pfr form triggers signal transduction pathways to the downstream responses including the expression of photosynthetic and other growth-regulating genes. The components involved in and the molecular mechanisms of the light signal transduction pathways are largely unknown, although G-proteins, protein kinases, and secondary messengers such as Ca^{2+} ions and cGMP are implicated. The 124–127 kDa phytochromes form homodimeric structures. The N-terminal half contains the tetrapyrrolic phytochromobilin for red/far-red light absorption. The C-terminal half includes both a dimerization motif and regulatory box where the red light signal perceived by the chromophore-domain is recognized and transduced to initiate the signal transduction cascade. A working model for the inter-domain signal communication within the phytochrome molecule is proposed in this Review.

INTRODUCTION

The red- and far-red reversible light switch molecules, phytochromes, mediate a number of growth and developmental processes in plants and in certain microorganisms. [For a previous mini-review, see Song, 1993; for phytochrome-related articles in this journal, Hahn & Song, 1987; Kim & Song, 1987; Kim & Chae, 1987; Kim et al., 1991; Park & Chae, 1992; Han & Chae, 1993; Park et al., 1996; 1998.] The light responsive growth and developmental responses are referred to as photomorphogenesis. To control photomorphogenesis, phytochromes mediate regulation of the expression of light-responsive genes, either directly or indirectly. The molecular light switch can be turned on by red light and turned off by far-red light. The red light activated phytochrome (Pfr) acts as a regulatory transducer to initiate the downstream signal cascade, according to the photochromic scheme, Fig. 1.

We have been studying the light-sensing and on/off-switching mechanisms of phytochrome A, specifically
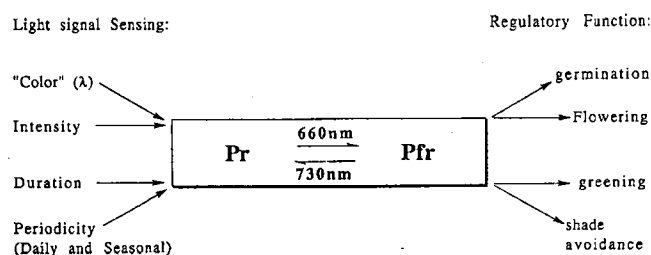
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Fig. 1. Phytochromes as light switch/sensor and regulatory transducer (modified from Quail, 1997).

focusing on the chromophore:apophytochrome interactions and post-translational modification, i.e. phosphorylation, involved in the photochromic function of phytochrome A. There are five members of the phytochrome gene family (phytochromes A, B, C, D, E; Hershey *et al.*, 1985; reviewed by Quail, 1998 and Whitelam, 1998). Of these, phytochromes A and B are best characterized and appear to play predominant sensory roles in etiolated (dark-grown; responsive under very low fluence rates) and green (light-grown; responsive to high fluence rates) plants, respectively. The photochromically-induced conformational changes are involved in intra-molecular inter-domain signaling within the phytochrome A and B molecules. In this review, we will relate the photochromic structural properties to the functional role of phytochrome's two domains, the amino- and carboxyl-terminal domains.

Phytochromes exist as homodimers in solution. Low resolution electron microscopy suggests oat phyA to be of an approximate Y-shape (Jones and Erickson, 1989). A similar structure was proposed for pea phyA on the basis of small angle X-ray scattering data (Nakasako *et al.*, 1990). It is generally accepted that the C-terminal domain provides dimerization motif(s) for both phyA and phyB. However, specific dimerization motifs remain unresolved; thus, residues 599–683 (Edgerton and Jones, 1992; 1993), 730–821 (Romanowski and Song, 1992), 772–807 (Partis and Grimm, 1990), and 919–1094 (Cherry *et al.*, 1993) have been proposed as dimerization motifs.

To resolve this, we expressed a series of C-terminal truncation, internal deletion, and site-specific pea phyA mutants (S.H. Bhoo, Ph.D. dissertation). These mutants were then cloned into pAS2-1 and pACT2 vectors for yeast two-hybrid analysis (hereafter, y2h) and dimerization assays. Results indicated that region 730–821 serves as a homodimerization interface comprised of two surface antiparallel β-strands at 735–750 and 780–800. Site-specific mutants further revealed that the latter residues serve as an electrostatically stable dimerization motif. While the Edgerton and Jones motif overlaps with the Quail box, the Romanowski and Song motif leaves the box open and "unengaged". The latter model is consistent with the functional role of the Quail box. However, it remains to be seen if the y2h results are borne out at the protein level, ultimately by 3-D structure determination.

Photochromic Characteristics of the Phytochromes

Phytochromes as light sensors and switches

Phytochromes are a molecular light switch for photomorphogenesis in the plant's growth and development which are controlled by red and far-red wavelength light. The photomorphogenic responses of plants to red/far-red light include germination, stem growth, chloroplast development, biosynthesis of

chlorophylls and other pigments, flowering, and circadian rhythm ("biological clock"). These light responses are regulated by the expression of gene(s) specific for each of the photomorphogenic processes (recent reviews include Song, 1993; Furuya and Song, 1994; Quail *et al.*, 1995; Quail, 1998).

Phytochromes are biosynthesized in the dark-grown seedling in its Pr, red-absorbing form. Upon absorbing red light of 660 nm, Pr is transformed to the physiologically active Pfr, the far-red absorbing form. The latter is revertible to the Pr form by far-red light of 730 nm. The signal transduction pathway from the red light signal perceived by Pr in cytosol to the Pfr-regulation of gene expression in the nucleus is largely unknown. Figure 2 schematically illustrates the light signaling role of phytochromes in the plant cell. Intense efforts are beginning to reveal the biochemical and genetic bases of the transduction mechanisms (discussed at a recent European Symposium on Photomorphogenesis, Berlin, March 21–26, 1999).

The pigment moiety of phytochrome is phytochromobilin, an open tetrapyrrole, which is covalently
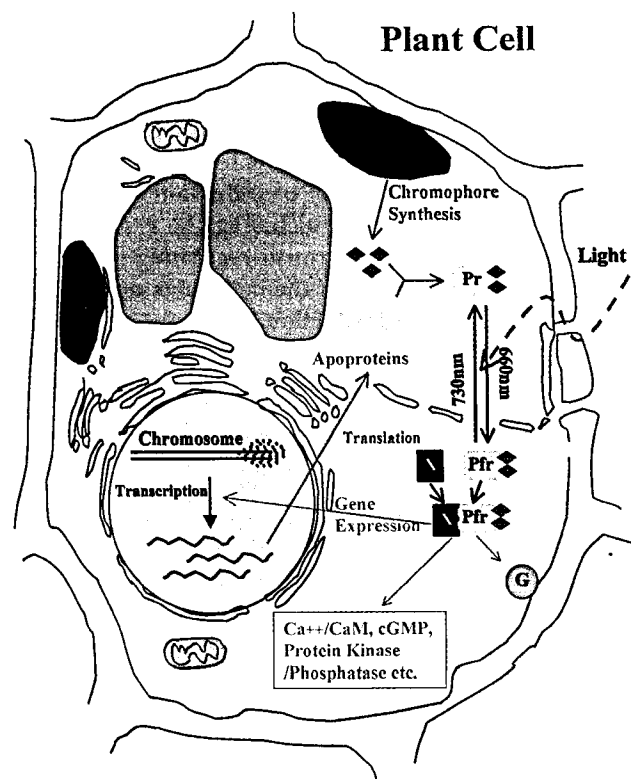
Fig. 2. Light signal transduction pathway mediated by phytochromes in plant cell. X, putative receptor/transducer. Phytochromobilin is synthesized within the chloroplast. Mitochondria and vacuoles are also shown. Recent studies have indicated that phytochromes localize into the nucleus in a light-dependent manner (see the Abstracts, Berlin meeting, op. cit.).
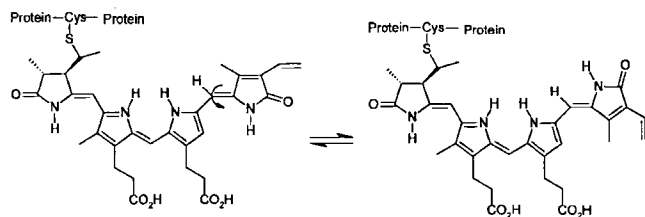
Fig. 3. The photoisomerization of phytochromobilin in the Pr ⇌ Pfr photochromic transformation of phytochromes.

linked to the apophytochrome via a thioether bond (Lagarias and Rapoport, 1980). The photochromic Pr → Pfr transformation involves isomerization of the chromophore at the 15,16C=C bond (Farrens et al., 1989; Rospendowski et al., 1989). Figure 3 shows the photoisomerization of the chromophore, as deduced by resonance Raman spectroscopy (Fodor et al., 1990; reviewed by Braslavsky et al., 1997).

Photochromism probed by site-specific mutagenesis We have been interested in the mechanism of the phytochrome photochromism by identifying the "catalytic" residues involved in the chromophore:apophytochrome interactions. Results from deletions, site-specific mutations, and esterification of the propionate side chain gave us at least a partial picture of the chromophore:apophytochrome interactions in pea phytochrome A. Thus, the phytochrome photochromism can be described in terms of only a small number of specific amino acid residues and at least the essential amphiphilic α-helix segments centered around Gln-391 and upstream (residues 88–250) of the chromophore binding site (Bhoo et al., 1997; Bhoo & Song, unpublished data). In addition, the N-terminal α-helix is induced in the Pfr form. We previously identified the histidine-324 next to the chromophore-linked cysteine-323 playing a critical role for conferring photochromism to the phytochromobilin chromophore in native phytochromes. We further investigated the mechanism of the light-switching function of the phytochromes by means of additional mutation and biophysical measurements (Deforce et al., 1991; 1993; Chen et al., 1997). Results suggest that histidine-324 is required to stabilize the Pfr form ("switch on" form) of phytochromes. In addition, isoleucine-80 is absolutely essential for the phototransformation of phytochromes. To position the phytochromobilin properly and to catalyze its photochromic transformation, both propionate side chains are electrostatically essential. When one of the carboxylate groups is methylated, the photochromism is abolished, although chromophore ligation is unaffected. When both groups are esterified, the chromophore ligation too is inhibited, suggesting that the chromophore: apophytochrome interactions include an essential

electrostatic contribution of the propionate side chains. (The amino acid numbering system used throughout, unless noted otherwise, is based on Cys-323 being the chromophore linkage site.)

In spite of intense efforts (including our own) to crystallize various phytochrome proteins from different sources and recombinant constructs for structure determination over the past two decades, the 3D-structures of phytochromes are not yet available. The information from these studies could be used for molecular modeling of the phytochrome chromophore pocket, as discussed in the Supplementary Information appended to the *J. Am. Chem. Soc.* paper (Bhoo et al., 1997). Some of the site-specific mutants obtained from the photochromism study exhibit further spectral separation of the Pr and Pfr absorbance maxima. These mutant phytochromes are interesting photochemically, as the spectral separation minimizes the overlap and thus the photoequilibrium between the Pr and Pfr species.

Inter-Domain Signal Transmission in Phytochromes

Signal perception: Conformational and topographic changes Quasi-elastic light scattering measurements indicated that the gross tertiary structures of the phyA Pr and Pfr forms are similar (Sarkar et al., 1984). However, Pr → Pfr phototransformation is accompanied by a subtle conformational change in the apoprotein moiety, detectable by proteolytic mapping, CD and fluorescence, and other methods (reviewed in Furuya and Song, 1994). Considering the published results from our laboratory, a working model for the conformational changes is cartoon represented in Fig. 4. This cartoon summarizes our
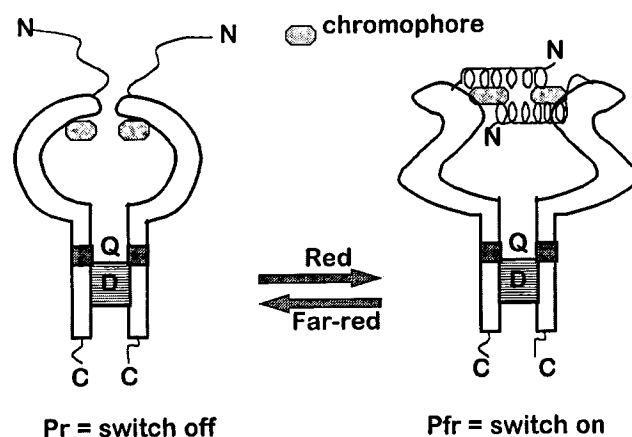

Fig. 4. A cartoon representation of the light-induced conformational changes in phytochrome A existing as a homodimer. The phytochromobilin chromophore is only partially inserted (rings C and D) between the amphiphilic N-terminal α-helices (Wells et al., 1996b)

previous results and in part illustrates the main theme of the present review, namely, inter-domain signaling between the N-terminal chromophore and the C-terminal regulatory domains. The N-terminal peptide chain, corresponding to approximately 60 amino acid residues, assumes a random coil conformation in the Pr form. Upon Pr → Pfr phototransformation, the N-terminal chain "captures" the Pfr-chromophore as it isomerized and moved out of its hydrophobic binding pocket. The chromophore:chain interaction then induces amphiphilic α-helix, as detected by steady state (Sommer and Song, 1990) and time-resolved CD spectroscopic methods (Chen et al., 1993). The Pfr-induced α-helix apparently results from its stabilization by the phytochromobilin chromophore (Vierstra et al., 1987; Sommer and Song, 1990). In analogy to the heme:α-helical peptide sandwich "maquette" (Robertson et al., 1994; Sharp et al., 1998), we proposed a similar tetrapyrrole:amphiphilic peptide sandwich, as schematically shown in the cartoon, Fig. 4 (Wells et al., 1996a; 1996b; Song, 1998). The propensity of phytochrome for binding exogenous tetrapyrroles may also involve interactions with the N-terminal chain (Singh and Song, 1989). However, the schematic model (Fig. 4) clearly is inadequate to elucidate the structural basis of inter-domain signaling within the phytochrome molecule.

Among the five phytochrome gene family members (A,B,C,D,E), only phytochromes A and B are reasonably well established for their specific functions. Phytochrome A is abundant and functionally active in dark-grown plants, whereas phytochrome B functions in light-grown green plants. Phytochrome A induces germination of *Arabidopsis thaliana* in response to a very low fluence rate in a red/far-red irreversible manner, whereas phyB induces germination in response to a high fluence rate in a photoreversible manner (Shinomura et al., 1996). From genetic mutant studies, it has been established that the N-terminal chromophore domain determines the phyA vs phyB differential light perceptions, whereas the regulatory sequence (714–731; "Quail box") appears to function as a transducer element in a phyA- and phyB-independent manner. The different light signals perceived by phyA and phyB are transmitted to the Quail box via the Pfr form, where downstream signaling events begin. What is the nature of this inter-domain signal transmission? Our hypothesis is that the light-induced amphiphilic helix of the N-terminal peptide is involved in the inter-domain molecular recognition of the Quail box. The different size and amphiphilicity of the N-terminal chain α-helix may be recognized as phyA- and phyB-specific signals by the Quail box.

Light-induced conformational changes and surface topography From a fluorescence quenching study, we found that two Trp residues, near the Quail box of oat phyA, became preferentially exposed in the Pfr form (Wells et al., 1994). We speculated the possibility that the preferential exposure of the Trp residues arose from unmasking of the Quail box in the Pfr form. We set out to explore such a cross-talk between the N- and C-terminal domains by probing the surface exposures of all 22 cysteine residues with iodoacetamide as a SH-specific reagent. Of the 11 Cys residues in the N-terminal domain, only one reactive Cys-311 showed a significant dependence of its surface exposure on the Pr → Pfr phototransformation. Remarkably, virtually all Cys residues in the C-domain reacted with iodoacetamide in a phototransformation-independent manner. Although we were unable to detect Pr → Pfr-dependent preferential exposure of Cys residues in and around the Quail box, as had been anticipated from the Trp exposure, we found the high reactivity of Cys-715 within the Quail box particularly noteworthy. This residue is conserved among all members of the phytochrome family. The highly exposed surface of the box sequence may indicate direct involvement of the Cys residue as a part of the molecular recognition module for putative regulator components. Clusters of high sequence homology around another highly reactive Cys-774 and -809, conserved among all phytochrome members, also suggest the functional importance of this region. An apparently fully exposed and open conformation of the C-terminal half of the phytochrome protein appears to be an essential feature of the molecule for its inter-domain signaling. As shown in Fig. 5, the N-terminal domain is compact and globular, judging from the buried Cys residues.

The Cys topography shown in Fig. 5 had been obtained from the observed reactivities of Cys residues in the presence of a 5000-fold excess of iodoacetamide for 1 h at 20°C. Iodoacetamide is a relatively small polar reagent, so under these conditions the Pr- vs Pfr-dependent preferential or differential exposures of Cys residues in the C-terminal domain might have been difficult to detect. Thus, it is desirable to follow the iodoacetamide reaction kinetically and at lower molar excess over phytochrome than previously used.

Inter-domain signal transmission As stated earlier, the phytochrome protein can be described as having two structural domains, a globular chromophore-containing N-terminal domain and an extended C-terminal domain having a common regulatory motif ("Quail box", Fig. 6). Determinants for the differential light sensory specificities of phyA vs B apparently reside within the N-terminal domain, whereas the Quail box in the C-terminal domain is common for both types of phytochromes (Quail et al., 1995). This means that the Quail box recognizes a specific "Pfr signal" or conformation independent of phyA vs phyB.

This molecular recognition/communication can be described in terms of interactions with inter-molecular
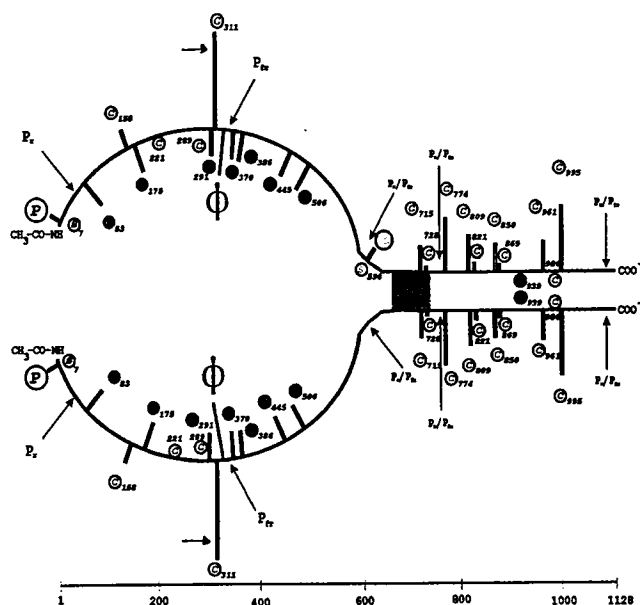
Fig. 5. A schematic drawing for the molecular surface topography of Cys residues in the phytochrome A dimer. Exposed Cys residues are shown by open rectangles, where the height of the rectangles correlates with the relative reactivity of the Cys residues. The increase of the reactivity of Cys-311 in Pfr (as compared to Pr and indicated by a horizontal arrow) is shown by solid black rectangles. Buried Cys residues are shown in solid circles with black bars to the inside. Partially buried, slowly reacting Cys residues are shown in open circles. Note that most of the Cys residues in the N-domain are buried, reflecting the globular fold of each chromophore (ϕ)-containing subunit. In contrast, those in the C-domain are exposed, indicating an open/extended conformation. The encircled P indicates phosphorylation of Ser-7 in vivo and Ser-17 by protein kinase A, respectively (Lapko et al., 1996; 1997). Preferential proteolytic sites in Pr and Pfr are also indicated by arrows. The bar with numbers at the bottom of the figure represents amino acid positions in nonlinear scale for oat phytochrome A (Lapko et al., 1998).
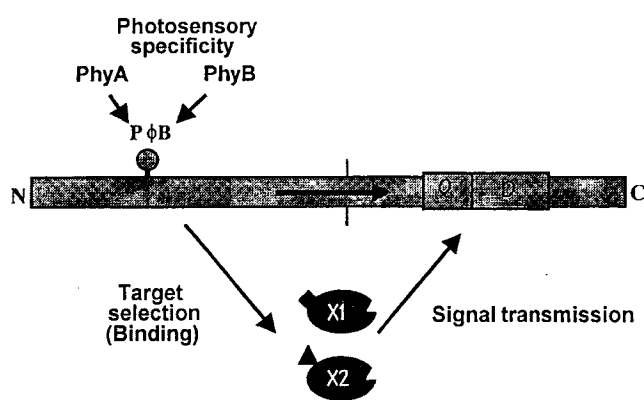

Fig. 6. Inter-domain signal transmission through intramolecular and inter-molecular routes. X1, X2, hypothetical receptors for the inter-molecular signaling: D, dimerization motif; PϕB, phytochromobilin; Q, Quail box.

phytochrome:signal transmitter protein(s), for example, the SPA1 protein (Hoecker et al., 1998; 1999), and/or via intra-molecular inter-domain interaction (between the chromophore domain and the Quail box). To explore the possible inter-domain interaction, we examined the effect of sequentially deleting the C-terminal sequences including Quail box and dimerization motifs on the spectra and photochemical properties of phytochromes. The results are summarized here.

The absorbance maximum of the Pfr form of phyA is blue shifted by up to 17 nm (341 cm^{-1}) upon C-terminus deletion, while less significantly affecting the Pr absorbance maximum, as shown in Table 1 (Song et al., 1997; Song, 1998). Specifically, the blue shift of the Pfr absorbance maximum gets steeper as the deletion gets closer to the Quail box. Further deletion to the hinge region does not cause an additional blue shift. It therefore appears that the quaternary structure of the phytochrome A molecule involves some interactions with the chromophore domain. If so, the light signal perceived in the N-terminal chromophore domain is transmitted to the C-terminal regulatory ("Quail box") domain through an intramolecular interaction between the two halves of the phytochrome molecule.

In a preliminary study (unpublished results by Y.K. Kwon, S.H. Bhoo, and P.-S. Song), we showed that the bifunctional thiol cross-linker dibromobimane, having a short spacer arm length of less than 0.8 nm (Kosower et al., 1979; 1987), cross-linked the C- and D-domains in the Pr form of phyA, but not Pfr. We used N- and C-domain specific antibodies for the detection of the cross-linked products. We confirmed similar cross-linking of the two domains under different conditions, suggesting that it is the Pr form whose N-domain interacts with or close to the C-domain. Considering the preliminary observation, a systematic cross-linking study is warranted.

Table 1. Spectral shifts of the C-terminal deletion mutants relative to full-length recombinant pea phytochrome A assembled with the phycocyanobilin chromophore analog.

Mutant	Blue shift in nm relative to native phyA	
	Pr	Pfr
WT, full-length (1–1124)	0	0
Deletion 1050–1124	0	−4
Deletion 772–1124	−2	−8
Deletion 731–1124	−2	−14
Deletion 653–1124	−4	−16
Deletion 601–1124	−6	−17
Deletion 494–1124	−6	−17
Deletion 247–1124	NA	NA

NA; not applicable due to the deletion of the chromophore binding Cys-323.

Phytochrome phosphorylation What is the switch that turns on the inter-domain signal communication between the photosensory signal (N-terminal) and the regulatory transducer (C-terminal) domains? One intriguing possibility is the post-translation modification, specifically phosphorylation. A number of observations and indirect lines of evidence for the possible role of protein phosphorylation in the downstream of the phytochrome-mediated light signal transduction pathway have been reported (reviewed by Singh and Song, 1990). We observed that phosphorylation/dephosphorylation of nuclear proteins is modulated by red/far-red light and phytochrome (Romero et al., 1991). Most recently, Lagarias' laboratory (Yeh et al., 1997; Yeh and Lagarias, 1998) has shown that a cyanobacterial phytochrome exhibits a "two-component" light sensory system in which the prokaryotic phytochrome homolog Cph1 behaves like a light-regulated histidine kinase in vitro (reviewed by Quail, 1997a; 1997b; Elich and Chory, 1997; Fankhauser and Chory, 1999).

To understand the possible functional role of phytochrome phosphorylation, we investigated phytochrome A as a protein kinase-A substrate, determined the phosphorylation sites, and analyzed the phosphorylation-induced conformational changes in oat phytochrome. We used bovine heart protein-kinase A for this study. The phosphorylated phytochromes were characterized by proteolytic mapping, circular dichroism, and time-resolved fluorescence quenching of Trp residues. There were two specific phosphorylation sites for oat phytochrome A by protein kinase A. Serine-17 was phosphorylated only in the Pr form of phytochrome. However, no significant conformational change was induced by the in vitro phosphorylation. The fact that the Ser-17 site was inaccessible to protein kinase-A for phosphorylation in the Pfr form of the phytochrome suggests that the Pr → Pfr phototransformation induced a conformational change along the N-terminal chain, consistent with α-helical folding there in the Pfr form. However, the Ser-17 phosphorylation of the Pr form did not affect Pr → Pfr phototransformation and the phototransformation-inducible -helical folding of the N-terminal chain. On the other hand, Ser-598 in the hinge region between the N-terminal chromophore and the C-terminal domains was phosphorylated equally in both Pr and Pfr species. Ser-598 phosphorylation contributed to a detectable conformational change in the hinge region, exposing the Lys-536-Asn-537 bond to tryptic attack.

To address the functional implications of phytochrome phosphorylation, we examined the phosphorylation patterns of oat phytochrome A under different conditions. By using proteolytic mapping and HPLC-mass spectrometry, we identified the phosphorylation site of phytochrome, serine-7, when phytochrome was isolated in the Pfr form (Lapko et al., 1997). However, this serine was

also phosphorylated in the Pr form in vivo. Determining the specific Ser site for phosphorylation was analytically challenging. There are 10 phosphorylatable serines in clusters along the amino terminal chain of phytochromes, yet only Ser-7 is phosphorylated specifically when phytochrome was isolated in the Pfr form (Fig. 7). Initially, we thought the phosphorylation of Ser-7 was specific for Pfr (i.e. tissue and isolation under red light), and that the switch-on conformation of phytochrome involved a specific phosphorylation along the amino terminal chain of phytochrome. However, the same Ser site was found to be phosphorylated when phytochrome was isolated in the Pr form (Lapko et al., 1999; unpublished results).

We recently showed that Ser-598 is phosphorylated in vivo in a Pr → Pfr, i.e. red/far-red light dependent manner. Thus, this Ser residue is specifically phosphorylated in Pfr, but not in the Pr form. This contrasts the protein kinase-A catalyzed phosphorylation of the same Ser residue which is equally well phosphorylated in Pr and Pfr forms in vitro. We propose that the phosphorylation at the hinge region is functionally relevant for phytochrome action. We further hypothesize that the phosphorylation/dephosphorylation of Ser-598 serves as a switch for inter-domain signaling between the N-terminal photosensing domain and the C-terminal regulatory domain of phytochromes. Figure 8 summarizes the current status of phytochrome phosphorylation.

Not indicated in Fig. 8 is the possible tyrosine phosphorylation of oat phytochrome A. In a preliminary study, we investigated the possibility that tyrosine kinases and/or phosphatases are involved in phytochrome-mediated light signal transduction. We found that a 124-kDa protein in crude extracts of the dark-grown oat seedlings tyrosine-phosphorylated, as determined by Western blotting with a phosphotyrosine-specific monoclonal antibody. The 124-kDa protein also immunoprecipitated with the anti-phytochrome antibody, and it cross-reacted with the anti-phosphotyrosine antibody. The apparent level of tyrosine phosphorylation in the 124-kDa protein decreased with red light treatment of the crude extract. These results seem to suggest that either

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           1        5              10                    20
Oat A3    S S S R P A - - S S S S S R N R Q S S Q A R V L A Q
Oat A4    S S S R P A - - S S S S S R N R Q S S R A R V L A Q
Rice A    S S S R P T Q C S S S S S R T R Q S S R A R I L A Q
Maize A1  S S S R P A H S S S S S S R T R Q S S R A R I L A Q
Ara A     S G S R P T Q S S E G S R R S R H S - - A R I I A Q
Pea A     S T T R P S Q S S N N S G R S R N S - - A R I I A Q
Zea A     S G S R P T Q S S S N S G R S R H S - - T R I I A Q
                            ↑
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Fig. 7. Serine clusters along the N-terminal sequences of different plant phytochrome A. The site of phosphorylation is Ser-7 (Lapko et al., 1997).
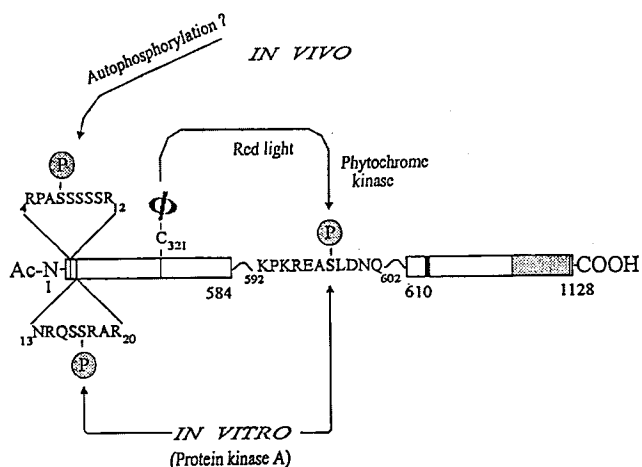
Fig. 8. Oat phytochrome phosphorylation. The N-terminal (residues 1–584) and C-terminal domains (residues 610–1128), which have a significant homology among different phytochromes, are shown in rectangles. The proteolytically vulnerable hinge region with the phosphorylation site at Ser-598 is shown by the curved line ϕ, chromophore thioether-bonded to Cys-321 (oat phyA numbering system). The bold face line upstream of the C-domain is the Q-box. The stippled region has sequence homology to the histidine kinase module of bacterial two-component signaling systems (Schneider-Poetsch, 1992). The encircled P indicates phosphorylation sites. *In vitro* protein kinase A-catalyzed phosphorylation sites, at Ser-17 and Ser-598. *In vivo* phosphorylation sites, Ser-7 and Ser-598, by "phytochrome kinase", a phytochrome-associated kinase activity (Lapko et al., 1999).

phytochrome is tyrosine phosphorylated or it co-immunoprecipitated with a phosphotyrosine-containing protein of the same molecular weight (Sommer et al., 1996). It remains to be seen if the apparent tyrosine phosphorylation of phytochrome, especially in its Pr form, implicates a signaling event, such as the activation of phospholipase C.

Is phytochrome phosphorylation a signaling switch? For possible implications in phytochrome-mediated light signalings, protein phosphorylation and dephosphorylation has been suggested to play an important role in photomorphogenesis. In a seminal study, Lagarias and his coworkers showed that phytochrome A isolated from dark-grown oat seedlings autophosphorylated in the presence of polycations such as poly-L-lysine (Wong et al., 1986). This extremely interesting report was subsequently challenged by Rudiger, Song, and Kim (Grimm et al., 1989; Kim et al., 1989; Park et al., 1998) by observing that the protein kinase activity of phytochrome preparations can be chromatographically separated from the purified phytochrome proteins. Others also noted that most phytochromes do not contain typical canonical sequences characteristic of protein kinases (for example, Boylan and

Quail, 1996). However, Lagarias and his coworkers continued to obtain several lines of evidence consistent with the notion that phytochrome as the red/far-red photoreceptor is also an enzyme with protein phosphorylating activities. (For a recent review, see Elich and Chory, 1998.) The most interesting result along this line was published by Lagarias' group (Yeh et al., 1997). In this paper, they described a prokaryotic phytochrome-like protein (Cph1) from *Synechocystis* bacterium exhibiting both autophosphorylating and phosphoryl transferring activities *in vitro*, in a manner analogous to the bacterial 2-component signaling system (Schneider-Poetsch, 1992). Similar phosphoryl transfer activities have not been demonstrated with plant phytochromes. The question of whether or not phytochrome is an intrinsic protein kinase remains to be definitively answered. However, the latest observation raises many interesting questions as to the potential functional modes of phytochromes, besides being photoreceptors. There is no doubt that phytochrome is specifically phosphorylated *in vivo*, in a red/far-red light dependent manner (Lapko et al., 1999).

Is Ser-598 phosphorylation the switch? As shown in Fig. 8, Ser-598 is phosphorylated in a Pr → Pfr phototransformation-dependent manner (Lapko et al., 1999). It is intriguing that this phosphorylation may serve as a switch for the inter-domain signaling. We are currently generating a Ser598Ala mutant to examine the possible switching role of Ser-598 phosphorylation and dephosphorylation and its phenotype *in vivo*.

Photosensory specificity: Phytochrome A vs phytochrome B As mentioned previously, the photosensory specificities of phyA and phyB are determined by the N-terminal chromophore domain. However, spectroscopic and photochemical properties of phyA and phyB *in vitro* are essentially indistinguishable. Only the kinetics of Pfr → Pr dark reversion appears to be significantly different. The Pfr form is energetically less stable than the Pr form. The Pfr form of certain phytochrome species, especially those from dicot plants, reverts to the Pr form in the dark. From phyAB/BA chimera studies, the dark reversion appears to be determined by the N-terminal domain (Eichenberg et al., 1999). Native 124-kDa oat phyA exhibits virtually no dark reversion, but truncation of a 6-kDa N-terminal chain (resulting in 118-kDa phyA species) accelerates the dark reversion (Hahn and Song, 1981). Since the N-terminus is the most prominent distinguishing feature of phyB, compared to phyA (Fig. 9), we suggest that amphiphilic α-helix of the N-terminal chain induced by Pr → Pfr phototransformation determines the phyA- and phyB-specific conformations for their photosensory signaling specificities.
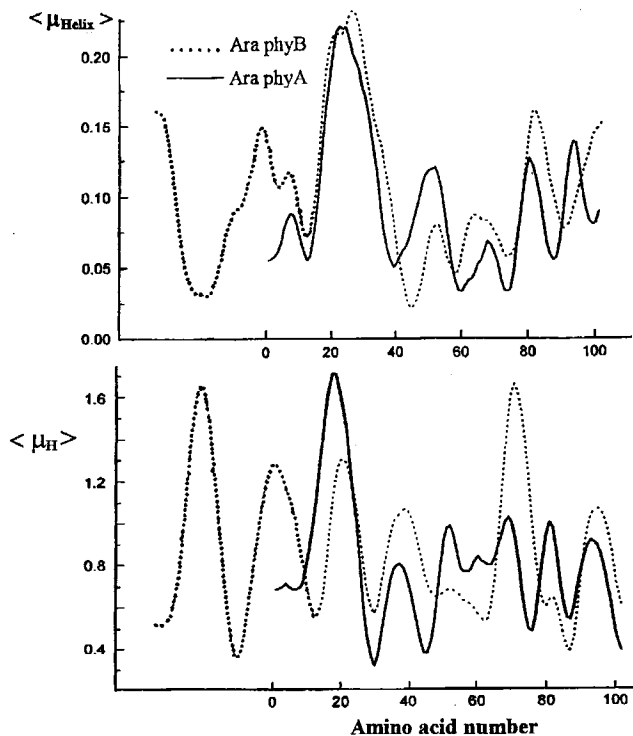
Fig. 9. Helix moment and hydrophobic moment profiles for *Arabidopsis thaliana* phyA and phyB. Note that phyB has an extended N-terminal chain (Parker and Song, 1990; Furuya and Song, 1994).
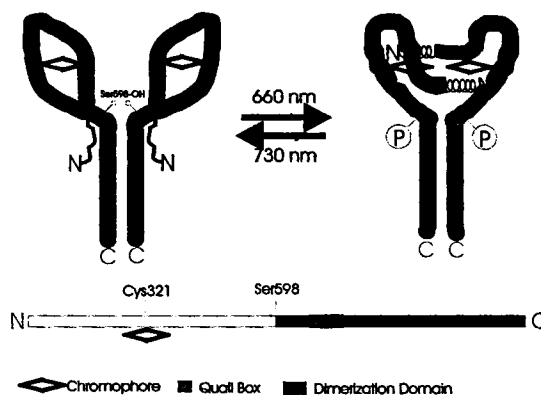

Fig. 10. A hypothetical schematic model for the molecular switching mechanism for the inter-domain signal communication in phytochromes. Protein phosphorylation at Ser-598 in oat phyA and its equivalent Ser/Thr residue in the hinge region of other phytochrome species is proposed to serve as the switch for the Pr ↔ Pfr dependent conformational change around the Quail box.

The Pr → Pfr-induced conformational changes in phyA and phyB will be analyzed and compared from steady state CD data, as described previously (Sommer and Song, 1990; Deforce *et al.*, 1994). The light-induced conformational change is localized along the 6-kDa N-terminal chain in phyA. With the 6-kDa chain truncated, the oat 118-kDa phytochrome A shows no CD detectable conformational change (Vierstra *et al.*, 1987). It is recalled that the N-terminal halves determine the *in vivo* photosensory activities of activities of phyAB and phyBA chimeras in which the entire N- and C-domains are swapped (Quail *et al.*, 1995). To elucidate the α-helix folding along the N-terminal chain, we have generated several phyA and phyB N-terminal chimera (unpublished data). In addition to CD studies, it will be interesting to do cross-linking experiments with phyB in comparison with phyA and phyAB/BA chimeras. It will be useful to find out if the phyB N-terminal extension also α-helically folds on Pr → Pfr phototransformation. If it does, we will be able to answer the question of how the the extended N-terminal chain serves to provide a phyB-specific signal. We will then be able to assess the role of the N-terminal conformations of the phyAB/BA chimeras in modulating the inter-domain signal communication in phytochromes A and B.

A Working Model for Inter-Domain Signaling

We have just begun exploring the structural and conformational basis of the inter-domain signaling in phytochromes. The available information is clearly insufficient to propose a realistic model for the intra-molecular inter-domain signal communication. Nevertheless, we propose a working model to accommodate the currently available data and to serve as an initial hypothesis for further testing and refinement. Figure 10 presents such a hypothetical model. According to this model, the Quail box is "covered" by the N-terminal peptide chain in the Pr form, whereas the box is opened in the Pfr form of phytochromes, thus exposing the critical regulatory motif for recognition by its receptor or transducer proteins such as PIF3 (Ni *et al.*, 1998) and NDPK2 (Yi *et al.*, 1998; Choi *et al.*, 1999).

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