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A Molecular Model for Light Signal Perception and Interdomain Crosstalk in Phytochrome Photoreceptors

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Phytochromes are red and far-red light absorbing photoreceptors for photomorphogenesis in plants. The red/far-red wavelength reversible biliproteins are made up of two structural domains. The light-perceiving function of the photoreceptor resides in the N-terminal domain, whereas the signal transducing regulatory function is located within the C-terminal domain. The characteristic role of the phytochromes as photosensory molecular switches is derived from the phototransformation between two distinct spectral forms, the red light absorbing Pr and the far-red light absorbing Pfr forms. The photoinduced Pr $\rightleftarrows$ Pfr phototransformation accompanies subtle conformational changes throughout the phytochrome molecule. The conformational signals are subsequently transmitted to the C-terminal domain through various inter-domain crosstalks and induce the interaction of the activated C-terminal domain with phytochrome interacting factors. Thus the inter-domain crosstalks play critical roles in the photoactivation of the phytochromes. Posttranslational modifications, such as the phosphorylation of Ser-598, are also involved in this process through conformational changes and by modulating inter-domain signaling.

key words: chromophore / conformational changes / inter-domain signal transmission / phosphorylation / phototransformation / phytochromes

INTRODUCTION

Phytochromes are light receptors that mediate a variety of photomorphogenic processes in plants [1,2]. They function as a photosensory light switch, turned on and off by the photochromic transformation between the two spectral forms, the 670 nm-red light absorbing Pr and the 730 nm-far-red light absorbing Pfr forms [3,4]. The photoactivated Pfr then elicits downstream signaling events, finally regulating the expression of genes involved in photomorphogenesis, such as seed germination, leaf and stem growth, phototropism, photoperiod, shade avoidance, and flowering [4] .The phytochrome molecule can be described as having the globular N-terminal chromophore-binding domain (70 kDa) and the conformationally extended C-terminal domain (55 kDa). These two domains are connected via a flexible hinge region (Figure 1A) [5,6]. The structural architecture of the phytochrome molecule also well coincides with the functional organization. The N-terminal domain perceives light signals in the forms of red/far-red light intensity and its ratio, photoperiodic duration, and direction. The photosensory specificities among different phytochromes (phytochromes A, B, C, D, E in *Arabidopsis*) reside in the N-terminal domain. The C-terminal domain recognizes the downstream phytochrome interacting factors (PIFs), such as the recently identified PIF3 [7,8], nucleoside diphosphate kinase 2 (NDPK2) [9], and phytochrome kinase substrate (PKS1) [10]. Several conserved subdomains/motifs also have been identified in the C-terminal domain, such as those for regulatory function and for Ser/Thr kinase activity (Figure 1A). It also contains a pair of the *Per-Arnt-Sim* (PAS) motifs around the regulatory core region (Quail box), which have been implicated for protein-protein interactions and inter-domain communications in some sensory proteins (Figure 1B) [11,12]. It now appears that prokaryotic phytochromes, such as Synechocystis Cph1 [13,14] and bacteriophytochromes [15], also share similar structural and functional organizations, although some structural components are missing or replaced with other components.

In spite of the fact that the three dimensional structure of a phytochrome molecule is yet to be elucidated, the molecular and photochemical properties of the phytochromes have been well characterized. However, the molecular mechanisms of how the light signals are perceived and transmitted intramolecularly within the phytochrome molecules are just now beginning to emerge. According to a working model we proposed, the photoinduced isomerization of the chromophore modulates apoprotein:chromophore interactions, which subsequently trigger conformational changes through-

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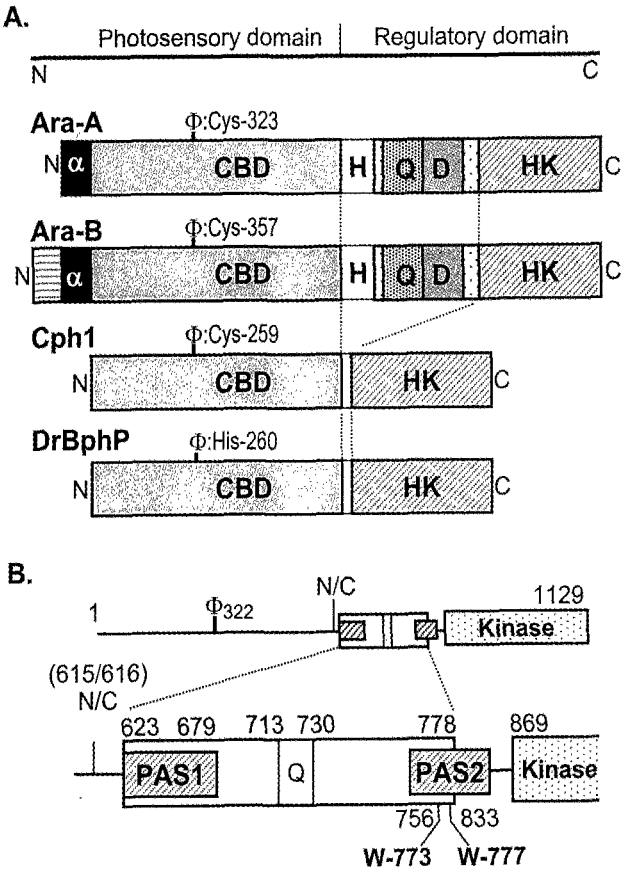

Figure 1. Domain structures of the phytochromes. (A) Structural and functional domains. Phytochromes have a two-domain structure, the photosensory N-terminal domain and the regulatory C-terminal domain. Structural and functional domains of the *Arabidopsis* phytochromes A and B, the *Synechocystis* Cph1, and an eubacterial DrBphP bacteriophytochrome are compared, including the N-terminal chromophore binding domain (CBD) and the C-terminal regulatory domain that are connected via the hinge region (H). The C-terminal regulatory domain includes the regulatory core sequence (Quail box, Q) and the dimerization motif (D). The histidine kinase motif (HK) is located in the C-terminal 250-residue region. Note that the Cph1 and DrBphP phytochromes do not have the N-terminal α-helix forming peptide region (α) and the regulatory/dimerization motif (Q/D). The phytochrome B has a 38-residue extension at the N-terminus (horizontal lined) that is not present in other phytochromes and may participate in a phytochrome B-specific inter-domain interaction. The DrBphP phytochrome does not have the conserved cysteine residue in the chromophore binding domain. The His-260, rather than a Cys residue, is the chromophore binding site in the DrBphP [15]. (B) Conserved subdomains/motifs in the C-terminal domain. The Per-Arnt-Sim (PAS) motifs overlap with the Quail box. The Trp-773 and Trp-777 residues reside near the PAS2 motif, and this peptide region undergoes remarkable conformational changes in the Pr ↔ Pfr phototransformation. Numbers are amino acid positions in the oat phytochrome A and indicate the N- and C-terminal residues of each structural domain or motif. N/C; the boundary between the N- and C-terminal domains (615/616), Φ; chromophore binding cysteine or histidine residues, N and C; N- and C-termini of each phytochrome protein. [Adopted from Park, C. -M., Bhoo, S. H., and Song, P. -S. (2000) *Seminars in Cell and Developmental Biology* 11, 449-456].

out the whole phytochrome molecule via inter-domain crosstalks [5,16], in a way similar to that in the visual pigment rhodopsin in animals [17].

CROSSTALK BETWEEN THE N- AND C-TERMINAL DOMAINS

The higher plant and prokaryotic phytochromes share basic structural and functional molecular architectures, with some differences as shown in Figure 1A [14,15,18-20]. The N-terminal domains carry determinants for the phytochrome individuality among different members of the phytochrome gene family [6]. However, the C-terminal domains are functionally inter-changeable, suggesting that the C-terminal domain provides a common structural motif for phytochrome:protein interactions for downstream signaling pathways. Interestingly, the PIFs identified so far exhibit diverse structural and functional properties [7-10].

A PIF may interact with different structural motifs in the C-terminal domain. For example, the PKS1 interacts with the Ser/Thr kinase motif [10]. The PIF3 and NDPK2 interact with the Quail box [7,9]. However, the structural motif that directly interacts with the PIF3 seems to be slightly different from that for the NDPK2. The PIF3 has a PAS motif in the N-terminal half, and the PIF3:phytochrome interaction appears to be mediated through the PAS domain [7]. The C-terminal domain may also take a different conformation and/or surface topography in concert with the N-terminal domain through specific inter-domain crosstalks, depending on light signals perceived by the phytochrome.

VOCABULARIES OF CROSSTALKING: CONFORMATIONAL CHANGES

The chromophore conformation and topography as well as the secondary and tertiary structures of the phytochromes significantly change through apoprotein:chromophore and inter-domain interactions [21-27]. The chromophore becomes more exposed in the Pfr form than that in the Pr form. However, the preferential exposure of the former is modulated by the α-helix forming N-terminal 6 kDa-peptide (Figure 1A) [25,28,29]. The 6 kDa-peptide seems to directly interact with the chromophore and possibly with other structural motifs, causing a series of conformational changes. The N-terminal domain is more exposed in the Pr form than in the Pfr form [30-32]. The hinge region is preferentially exposed in the Pfr form. However, these structural differences between the Pr and Pfr forms are not observed in the *Synechocystis* Cph1 phytochrome, primarily due to sequence truncations in the latter [19]. The Cph1 does not have the N-terminal 6 kDa-peptide chain and the C-terminal 250-residue segment that

contains the Quail box and the PAS motifs (Figure 1A and 1B) [5,14]. These characteristics indicate that subtle conformational changes occur in these peptide segments during the phototransformation of plant phytochromes.

The Pr and Pfr phytochromes also exhibit differential environment/exposure of tryptophan residues [25,33]. Subtle conformational changes are detected in the region around Trp-569 and Trp-572 [34,35], which are located close to the Ser-598 residue (see below). The Ser-598 residue is preferentially phosphorylated in the Pfr form *in vivo* (Figures 2 and 3) [10]. In addition, the Trp-773 and Trp-777 are preferentially modified by hydrogen bromide only in the Pfr form [23,36,37], indicating that the peptide region containing these two Trp residues also undergoes significant rearrangements and changes of surface topography during the phototransformation. The two Trp residues are located within the PAS2 motif (Figure 1B) and would be directly involved in the inter-domain interactions and/or in the protein-protein interactions. The light induced conformational changes and the resultant surface topographic alterations are prerequisites for the inter-domain crosstalks in the phytochrome photoactivation.

MODE OF CROSSTALKS: INTER-DOMAIN INTERACTIONS

The NDPK2 binds to the Quail box preferentially in the Pfr form, as revealed by the yeast two-hybrid analysis of the phytochrome C-terminal domain as the bait [9]. Without the N-terminal domain, the Quail box and PAS domains of the C-terminal domain as the bait for the yeast two-hybrid screening are fully exposed and therefore resemble the "photoactivated" phytochrome, as discussed later. This is also consistent with the preferential exposure of the peptide region containing the Trp-773 and Trp-777 in the Pfr form [36,37]. The PIF3 also binds to the Quail box preferentially in the Pfr form. However, the PIF3 appears to require both the N-terminal and the C-terminal domains for its binding (see later). [7,8] 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 accessible in both spectral forms. [10] However, the PKS1 phosphorylation and the phytochrome autophosphorylation are stimulated by a factor of 2 to 2.5 in the Pfr form, compared to the Pr form. The protein phosphorylation is an important regulatory factor for the phytochrome-PKS1 interaction. These observations suggest that differential inter-domain interactions activate a specific motif in the C-terminal domain for recognition by different PIFs.

The light signals perceived by the phytochromes can be differentiated at the structural/conformational level. For example, the α-helix forming motif of the N-terminal chain plays a critical role in the apoprotein:chromophore interactions [16]. Phytochrome B has an N-terminal 38-residue extension, in addition to the characteristic α-helix forming motif (Figure 1A). The 38-residue extension may directly participate in the apoprotein:chromophore interactions and induce a phytochrome B-specific inter-domain crosstalks with the C-terminal domain.

The light-induced conformational "signals" can be communicated to the C-terminal domain via intramolecular inter-domain interactions (Figure 2) [5]. The domain:domain crosstalk and/or the Pfr activity may be modulated by phytochrome phosphorylation/dephosphorylation at Ser-598 in the hinge region (unpublished results). The PIF3 was originally isolated in yeast two-hybrid screens using the C-terminal half as bait [7]. However, it also binds to the N-terminal domain and more strongly to the full-size phytochrome, indicating that the associations of PIF3 to the N-terminal and C-terminal domains are synergistic [8]. Alternatively, the enhanced binding of the PIF3 to the full length phytochrome may have resulted from its reduced binding to the C-terminal bait as the latter tends to aggregate extensively, thereby

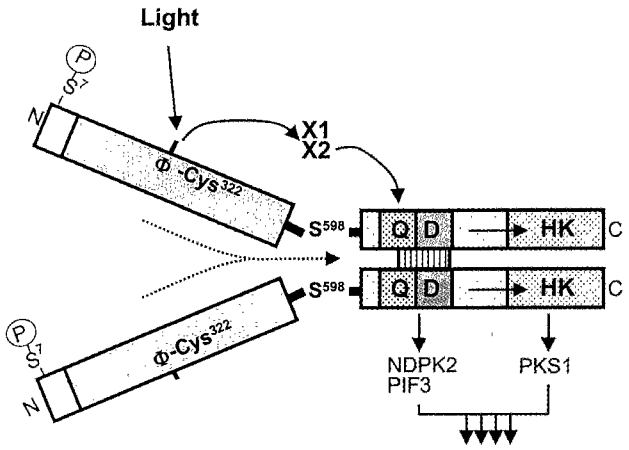

Figure 2. Inter-domain signal transmission in a dimeric oat phytochrome A. The Ser-598 is located in the hinge region and phosphorylated preferentially in the Pfr form. The Pfr-dependent conformational signals are generated through the chromophore photoisomerization by absorbing red light (R) in the N-terminal domain and trigger subtle conformational changes through the whole phytochrome molecule. The conformational signals are subsequently transmitted to the regulatory/dimerization domain (Q/D) either intramolecularly (bold dot arrows) or intermolecularly via the hypothetical signal transducers (bold solid arrows, X1 and X2). The photoactivated or uncovered Quail box (see Figure 3) interacts directly with NDPK2 and PIF3. The PKS1 seems to associate with the kinase motif. Ser-7 is phosphorylated *in vivo*. The N-terminal serine residue is *N*-acetylated and may stabilize the α-helical conformation in the 6 kDa-peptide region [76]. N and C; N- and C-terminal ends of the oat phytochrome. Numbers are the positions of amino acid residues in the oat phytochrome A. [Adopted from Park, C. -M., Bhoo, S. H., and Song, P. -S. (2000) *Seminars in Cell and Developmental Biology* 11, 449-456].

minimizing its PIF3 binding capacity. Whether or not direct inter-domain interactions are required for the phytochrome:PIF3 association remains to be further investigated.

The intermolecular signal transmission via putative signal transmitter proteins (for example, X1 and X2 in Figure 2) has not been explored [5]. The suppressor of phyA-105 (SPA1) protein may serve this role as it is activated specifically by phytochrome A [38]. However, these mechanisms are oversimplified. Both the intermolecular and intramolecular pathways could be integrated to generate differential Pfr or conformational signals and to modulate the inter-domain crosstalks [39].

The Pfr phytochrome is thought to be the physiologically active form in most cases in higher plants [2]. On the contrary, the Pr form of the *Synechocystis* Cph1 exhibits autophosphorylation activity [14]. In addition, the phytochrome B in the Pr form has been suggested to have a regulatory activity in seed germination [40-42]. This active Pr signaling could be explained either by the intramolecular or by the intermolecular pathway (Figure 2). The PIF3 and NDPK2 preferentially bind to the Pfr and positively regulate phytochrome signaling [7-9], but the PKS1 equally binds to both the Pr and Pfr phytochromes, and is suggested to be a negative regulator [10]. It is possible that the photoactivated Pfr signals induce the release of a putative Pr-specific factor from the Quail box [39]. Alternatively, the intermolecular pathway may have distinct signal mediators for the Pr and Pfr forms (X1 or X2 in Figure 2).

The inter-domain interactions, direct or indirect, are also important for the spectral integrity [43,44]. Our chemical crosslinking experiments detected red and far-red light-dependent interactions between the N-terminal peptide and the distal C-terminal peptide [45]. Both the Pr and Pfr phytochromes were treated with the thiol reactive dibromobimane and the resulting crosslinks between the N- and C-domains were analyzed after Glu- and Arg-specific endoprotease hydrolysis. The resultant peptide pool was resolved on SDS-PAGE and transferred onto a PVDF membrane. The blot was then probed with N- or C-terminal domain specific antibodies. The immunoblot analysis showed that the far N-terminal and the distal C-terminal peptides cross-linked only in the Pr form. 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. Work is currently under way to precisely map the interacting peptides using a hetero-bifunctional cross-linker, p-maleimidophenyl isocyanate, and a thiol specific cross-linker, 1,4-bis-maleimidyl-2,3-dihydroxybutane. Photo-affinity cross-linking [46,47], fluorescence resonance energy transfer [22], and ESR spin labels [22,48] will also be useful to gain more insight into the conformational dynamics accompanying the inter-domain crosstalks in the phytochrome molecule.

PHOSPHORYLATION: A SWITCH FOR INTER-DOMAIN CROSSTALK?

The phytochrome kinase hypothesis has been controversial for years [49-52]. However, reexamination of the phytochrome kinase activity, retriggered by the finding that the *Synechocystis* Cph1 phytochrome has histidine kinase activity [14], confirmed that plant phytochromes are serine/threonine kinases [10,53-56]. Phytochromes are also autophosphorylated [57,58,59-63]. The Ser-7 of the phytochrome A is phosphorylated *in vivo* in both the Pr and Pfr forms [61,63] . The Ser-17 is phosphorylated by protein kinase A *in vitro* only in the Pr form [59,63]. The Ser-598 is preferentially phosphorylated in the Pfr form *in vivo* [61,63]. Phosphorylation at a single residue can induce a profound conformational change in a protein [64-66]. CD analysis and proteolysis indicate that the phosphorylation of Ser-598, Ser-7, and Ser-17 induces subtle conformational changes in the phytochrome molecule. This, together with the finding that the Ser-598 phosphorylation is important for the light regulation of autophosphorylation and phosphotransfer activity [10], suggests that the Ser-598 phosphorylation could be a molecular modulator of the inter-domain interaction between the N- and C-terminal domains. However, when an oat phytochrome A mutant with the Ser-598 to Ala substitution was expressed in *Arabidopsis A*$^-$ plant, the transgenic plant exhibited hypersensitivity to far-red light. This suggests that the Ser-598 phosphorylation may have a negative regulatory role in photomorphogenesis (unpublished result), possibly by desensitizing the Pfr activity (see below, Figure 3).

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 [10], regulation of subcellular locations [7,8], or through regulation of the complex formation with other components [7]. The phosphorelay between the Cph1 and Rcp1 in Synechocystis has been well characterized *in vitro* [51]. The photoreversible PKS1 phosphorylation by the phytochrome kinase also resembles the Cph1-Rcp1 histidine kinase/phosphotransferase-mediated phosphorelay [10]. The photoactivated phytochromes are translocated into the nucleus, probably in a complex with unidentified cognate partner(s) and interact with the PIF3 in nucleus. The NDPK2 forms hexamers [7] and may form a complex with other factors either directly or indirectly through the phytochrome (unpublished result, Choi *et al.*). The complex seems to interact with auxin signaling cascades and exhibits pleiotropic effects on plant growth and development. These indicate that the conformational signals and the accompanying inter-domain crosstalks are further diversified by protein phosphorylation and amplified through complex interactions with downstream signaling components.

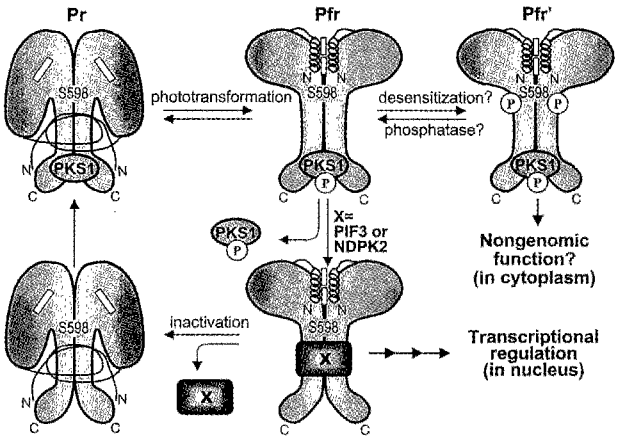

Figure 3. Conformational changes and inter-domain interactions in the phytochrome photoactivation. The photoactivation of the phytochrome includes changes in the domain conformation and in the surface topography triggered by apoprotein:chromophore interactions. The N-terminal 6 kDa-peptide region forms an ?-helical conformation in the Pfr but a random coil conformation in the Pr. The proximity between the N- and the C-termini in the Pr form is to depict the covered Quail box and shields the hinge region. Upon photoactivation, the hinge region is exposed, and the Ser-598 may be phosphorylated. The Pfr phytochrome autophosphorylates serine residues, including Ser-598, and phosphorylates the PKS1. The phospho-PKS1 is subsequently released from the photoactivated phytochrome, which can associate with the PIF3 or NDPK2. The Ser-598 phosphorylation is proposed as a mechanism to desensitize the Pfr activity. The desensitized Pfr (Pfr') does not associate with PIFs in this working model. Alternatively, it may have a nongenomic regulatory function in the cytoplasm. An unidentified serine/threonine protein phosphatase is postulated in the resensitization step. Open boxes; chromophores. X; phytochrome interacting factors (PIFs), such PIF3 or NDPK2. This diagram is modified from Smith (1999) [39], and the desensitization step is additionally included. [Adopted from Park, C. -M., Bhoo, S. H., and Song, P. -S. (2000) *Seminars in Cell and Developmental Biology* 11, 449-456].

A MODEL FOR THE INTER-DOMAIN CROSSTALKS

The conformational changes and inter-domain interactions involved in the phytochrome photoactivation are schematically depicted in Figure 3. The preferential exposure of the Pfr chromophore and of the hinge region as well as the specific phosphorylation of the Ser-598 in the Pfr phytochrome suggest that the phytochrome molecule in general exhibits a more exposed conformation in the C-terminal domain of the Pfr form than of the Pr form (Figure 3). This is also consistent with the higher susceptibility of the Pfr form to protease attacks than of the Pr form. The peptide region containing the Trp-773 and Trp-777 exhibits differential surface topographies between the Pr and Pfr forms. Considering their close proximity to the junction between the Quail box and the kinase motif (Figure 1B), this peptide region may have an important role for the phytochrome kinase activity, such as the PKS1 phosphorylation by the phytochrome.

In conclusion, the key consequence of the crosstalk between the N- and C-terminal domains is closing (in Pr) and opening (in Pfr) of the Quail box and the hinge region. In the Pfr form, the two regions are exposed, and the Ser-598 is phosphorylated. In our proposed working model, the Ser-598 of the Pfr phytochrome is either phosphorylated or unphosphorylated. The phosphorylation of Ser-598 desensitizes the Pfr phytochrome, and the desensitized Pfr does not associate with PIFs. Only the unphosphorylated Pfr will have the PIF binding capability. Thus the Ser-598 phosphorylation could be a desensitizing mechanism for the Pfr activity, as has been well characterized with the rhodopsin photoreceptor [67-69]. The model proposed (Figure 3) is consistent with the fact that only the C-terminal domain was used as bait to obtain the results from yeast two-hybrid screens [7-9]. The C-terminal peptide region, without the chromophore-bearing N-terminus, is like a 'photoactivated' Pfr form. The bait also does not contain the Ser-598 but can associate with the PIF3 and NDPK2. It is also in good agreement with our preliminary observation that the Ser598Ala mutant phytochrome A is hyperactive in *Arabidopsis* A$^-$ mutant. The transgenic *Arabidopsis* plant exhibited hypersensitivity to far-red light, suggesting that the Ser-598 phosphorylation has a negative regulatory role in photomorphogenesis. The desensitized Pfr phytochrome may be dephosphorylated by an unidentified serine/threonine protein phosphatase and recover the PIF binding capacity. Alternatively, the desensitized Pfr phytochrome with the phospho-Ser-598 could perform some nongenomic function in cytoplasm. In this view, the Ser-598 phosphorylation is a molecular modulator to further control the Pfr signals.

The preferred mode of crosstalking between the N- and C-terminal domains appears to be the Pr- and Pfr-dependent closing/opening of the Quail box for its PIF binding capacity. Obviously, the proposed model remains to be further refined. The molecular mechanisms for the photoactivated conformational changes and inter-domain crosstalks could be elucidated in more detail with 3-D structural information of the phytochrome molecule. Several research groups, including our own, have been working on the 3-D structural determination but without success so far. This is mainly due to the intrinsic properties of the phytochrome proteins, such as the tendency to aggregate [70,71] and structural and genetic heterogeneity [63]. Most of these problems are expected to be overcome in near future by employing recombinant expression systems and advanced molecular biological and biochemical methods. With detailed structural and functional information, it would be possible to develop the modified phytochrome molecules with improved inter-domain interacting activities, such as decreased shade avoidance [72,73] and red shifted absorbance maxima [74,75], that can be applied for agricultural crop plants.

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