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An emerging molecular map of the phytochromes

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ABSTRACT

Molecular mapping studies and sequence comparisons are providing provocative new insights into regions of the phytochrome polypeptide important to the functional activities of the photoreceptor. The NH_2-terminal structural domain contains the determinants for photoperception, and for the differences in photosensory specificity and photolability between phyA and phyB. However, a contiguous COOH-terminal domain is also required for the transfer of perceived informational signals downstream to transduction pathway components and for PfrA-specific degradation to proceed. The COOH-terminal domains of phyA and phyB are functionally interchangeable in these processes and a core sequence at the proximal end of this domain contains determinants necessary for signal transfer from both phyA and phyB, suggesting a common biochemical mechanism of signal transfer for the two photoreceptors. Striking sequence similarity between the NH_2-terminal domain of a *Synechocystis* protein, ORF SLR0473, and the phytochromes indicates that the cyanobacteria contain phytochrome-related photoreceptors. The COOH-terminal domains of ORF SLR0473 and the phytochromes are also related to one another and both show sequence similarities to the sensor histidine kinases. These data raise the possibility that the cyanobacteria have a functional photoregulated histidine kinase signalling system and that the plant phytochromes represent remnants of that system.

Key-words: functional domains; histidine kinase; photosensory perception; phytochromes; signal transduction; structural domains; structure–function relationships; two-component systems.

INTRODUCTION

In the absence of definitive evidence establishing the biochemical mechanism of phytochrome (phy) action, a major strategy followed by many researchers over the years has been to map molecular domains associated with various of the measurable activities of the photoreceptor family. This process has been greatly facilitated in recent times by the use of genetic and molecular approaches. The major findings from these studies are compiled in Fig. 1 and discussed briefly below.

The coordinates in Fig. 1 (referred to as 'position' numbers throughout the paper) are those of the consensus sequence developed by Mathews, Lavin & Sharrock (1995) in aligning the amino acid sequences of the five-membered phy family from Arabidopsis, and other available full-length sequences. The generic phytochrome corresponding to this consensus sequence is depicted diagrammatically at the top of the figure. The extremities of this consensus correspond to the Arabidopsis phyB sequence, the longest family member, and the indents at each end depict the extent and location of the shorter Arabidopsis phyA sequence within the alignment. At the bottom of Fig. 1 is a diagrammatic alignment of phyA through phyE of Arabidopsis and a plot of the degree of amino acid sequence identity among the five sequences across the length of the aligned polypeptides from Clack, Mathews & Sharrock (1994). The locations of the various molecular activities that have been mapped within the photoreceptor are depicted by the solid black horizontal bars.

STRUCTURAL DOMAINS

Evidence from biochemical studies on phyA indicated some years ago that the polypeptide folds into two major structural domains: a globular NH_2-terminal domain cradling the covalently attached chromophore in a hydrophobic pocket, and a more extended COOH-terminal domain, with a short proteolytically vulnerable linker segment connecting the two (see Quail 1991). The behaviour of the NH_2-terminal domains of phyA and phyB when expressed alone in transgenic plants is consistent with these data (Cherry *et al.* 1993; Boylan, Douglas & Quail 1994; Wagner, Koloszvari & Quail 1996a). Electron microscopic images (Jones & Erickson 1989) and low angle X-ray analysis (Tokutomi *et al.* 1989) are also consistent with these data although differing on interpretation of the shape of the NH_2-terminal domain (see Quail 1991). The molecular and biochemical data indicate that the junction between the two domains is around position 673 (Fig. 1).

SPECTRAL ACTIVITY

Chromophore lyase

The seminal demonstration by Lagarias and coworkers that the PHYA apoprotein can autocatalytically attach the

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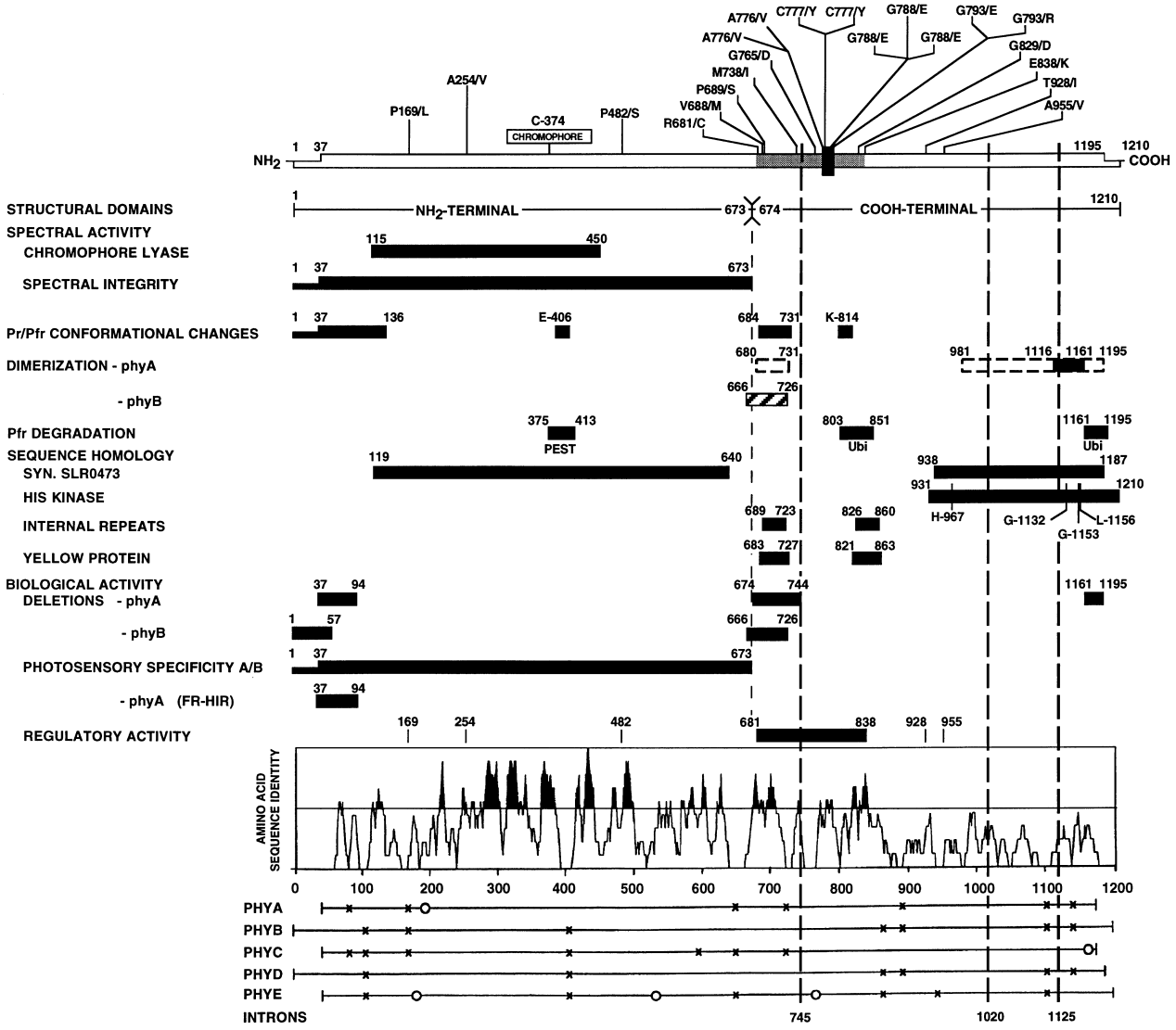

Figure 1. Molecular map of phytochrome. A schematic diagram of a 'consensus' phytochrome molecule derived from the alignment of multiple full-length phytochrome polypeptide sequences by Mathews *et al.* (1995) is shown at the top. The coordinates for this figure and throughout the paper are those for the consensus line of this alignment and are referred to as 'positions' within the consensus sequence. The extremities of the schematic (positions 1 and 1210) correspond to the NH_2- and COOH-termini, respectively, of Arabidopsis phyB, the longest sequence in the alignment. The indented positions at each end (positions 37 and 1195) correspond to the NH_2- and COOH-termini, respectively, of Arabidopsis phyA. The location of the tetrapyrrole chromophore covalently linked to a cysteine is indicated at position 374. The residue substitutions indicated at various positions along the consensus molecule are a composite of phyA and phyB missense mutations causing loss of regulatory activity (see Quail *et al.* 1995). The stippled area demarcates a region containing a high density of such mutations (the 'core' region) and the small black box, a short segment containing multiple substitutions of individual residues. The horizontal bars in the body of the figure show the locations within the polypeptide of the activity, property, or feature of the molecule shown to the left (discussed in detail in the text). At the bottom is a plot of the distribution of amino acid sequence identity across the aligned polypeptide sequences of the five Arabidopsis phytochromes PHYA, B, C, D and E, shown below (from Clack *et al.* 1994). The light vertical dashed line at position 673/674 represents the junction of the two major structural domains. The heavy vertical dashed lines represent the positions of the introns within the structural *PHY* genes of higher plants (except *PHYC* which lacks the intron at position 1020; Cowl *et al.* 1994).

chromophore *in vitro* (Lagarias & Lagarias 1989; Wahleithner, Li, & Lagarias 1991; Cornejo *et al.* 1992) as well as in living yeast (Li & Lagarias 1994) has been followed by similar demonstrations *in vitro* for phyB (Kunkel *et al.* 1993) and phyC (B. Parks & P. Quail, unpublished results). Analysis of deletion derivatives of phyA and phyB expressed in yeast or transgenic plants has mapped the chromophore lyase activity sufficient for attachment to within the NH_2-terminal segment between positions 115 and 450 (Fig. 1) (Deforce *et al.* 1991; Boylan & Quail 1991; Cherry *et al.* 1993; Vierstra 1993; Cherry & Vierstra 1994; Wagner *et al.* 1996a). In a more detailed site-directed mutational analysis of five conserved residues (positions 360, 369, 372, 375 and 377) surrounding the

chromophore attachment residue (the cysteine at position 374), Song and colleagues have obtained evidence that none is essential for chromophore lyase activity, suggesting a structural rather than a catalytic role (Deforce, Furuya & Song 1993; Song, Park & Furuya 1997).

Spectral integrity

Sequences necessary to sustain complete spectral integrity equivalent to that of the full-length molecule extend in both directions beyond that for chromophore lyase activity to encompass most, if not all, of the NH_2-terminal domain (positions 1–673, Fig. 1). Spectral integrity here refers to maintenance of photoreversibility and absorption spectra in both Pr and Pfr forms unaltered from that obtained with the native photoreceptor. Analysis of deletion derivatives of phyA in transgenic seedlings has shown that the full NH_2-terminal structural domain (positions 1–673) is sufficient for spectral integrity (Boylan & Quail 1991; Cherry *et al.* 1993; Boylan *et al.* 1994; Cherry & Vierstra 1994). A series of deletions covering a variety of segments within the first 115 positions at the NH_2-terminus (residues 1–69 of the phyA sequence) has revealed subdomains necessary for stability and spectral integrity of the Pfr form alone (positions 51–94) or of both the Pr and Pfr forms (positions 51–115) (Cherry *et al.* 1992, 1993; Boylan *et al.* 1994; Cherry & Vierstra 1994; Jordan *et al.* 1995). These data are consistent with earlier proteolytic studies on native phyA suggesting interaction of this NH_2-terminal segment with the chromophore, especially in the Pfr form (see Jones *et al.* 1985; Vierstra & Quail 1986). Similarly, determinants between positions 524 and 673 at the COOH-terminal end of the NH_2-terminal structural domain have been shown to be necessary for spectral normalcy (Boylan & Quail 1991; Cherry *et al.* 1993; Boylan *et al.* 1994). Preliminary spectral analysis of some comparable deletion derivatives of phyB suggests a similar pattern to that of phyA (Wagner *et al.* 1996a).

Pr/Pfr CONFORMATIONAL CHANGES

Conformational changes in the protein moeity induced by photoconversion between the Pr and Pfr forms have been mapped in phyA to the NH_2-terminal segment (within positions 37–136), to a segment around position 406 near the chromophore attachment site, to a segment in or near the linker region (positions 684–731), and to a region around position 814 (Fig. 1). Most of these data have come from older biochemical, immunochemical or physicochemical studies on extracted or purified, non-recombinant phytochrome (see Lagarias & Mercurio 1985; Wong *et al.* 1986; Grimm *et al.* 1988; McMichael & Lagarias 1990a,b; Quail 1991; Song 1993; Furuya & Song 1994).

DIMERIZATION

Proteolytic fragmentation coupled with the use of monoclonal antibodies against mapped epitopes provided evidence some years ago that the COOH-terminal structural domain of phyA carries the determinants for dimerization, whereas the NH_2-terminal domain is released as a monomer upon cleavage, indicating no stable intra- or intersubunit interactions (see Jones & Quail 1986; Quail *et al.* 1987; Quail 1991). Transgene-encoded NH_2-terminal domains of both phyA and phyB behave as globular monomers, confirming this latter conclusion (Cherry *et al.* 1993; Boylan *et al.* 1994; Wagner *et al.* 1996a), and the transgene-encoded COOH-terminal domain of phyB has been shown to associate into homodimers in Arabidopsis (Wagner *et al.* 1996a).

Using a conceptually elegant prokaryotic assay system, Edgerton & Jones (1992) presented data interpreted to indicate that two regions of the phyA COOH-terminal domain (positions 680–731 and 1116–1195, respectively) are capable of mediating dimerization (Fig. 1). However, subsequent analysis of a COOH-terminal deletion series by Cherry *et al.* (1993) showed that simply removal of residues COOH-terminal of position 981 was sufficient to cause loss of dimerization capacity. This result indicates that neither the initial (positions 680–731) nor revised (positions 652–741) central segments (Edgerton & Jones 1992, 1993) are sufficient to mediate dimerization in the context of the native molecule. Moreover, since internal deletion of a similar region of phyB (positions 666–726) has been shown not to affect dimerization when expressed in transgenic Arabidopsis (Wagner *et al.* 1996a), it would appear that this region is neither necessary nor sufficient for dimerization in the context of the native molecule. By contrast, the COOH-terminal deletion analysis by Cherry *et al.* (1993) indicates that residues between positions 981 and 1161 are necessary for dimerization of native phyA in transgenic plants. Combined with the proposal from the prokaryotic assay that positions 1116–1195 are necessary for dimerization (Edgerton & Jones 1992), these transgenic plant data might suggest that critical residues for dimerization lie between positions 1116 and 1161 (Fig. 1).

PfrA DEGRADATION

The rapid intracellular degradation of phyA induced upon its photoconversion to the Pfr form (representing a 100-fold greater turnover rate for PfrA than PrA) is one of the earliest molecular properties of the photoreceptor recorded in the literature (Vierstra 1994). The other phytochromes (phyB, C, D and E) do not display this dramatic isoform-specific difference in degradation rate and are therefore considered to be more ‘light stable’ (Somers *et al.* 1991; J. Tepperman, R. Sharrock & P. Quail, unpublished results). Recent domain swapping experiments using transgenic Arabidopsis have provided evidence that the determinants specifying recognition of phyA for degradation in the Pfr form reside in the NH_2-terminal structural domain (Wagner *et al.* 1996b). However, these determinants are alone insufficient for degradation because the NH_2-terminal domain alone of phyA is light stable *in vivo* (Boylan *et al.* 1994), requiring a contiguous COOH-terminal domain for degradation to proceed (Wagner *et al.* 1996b). Indeed, deletion of the extreme COOH-terminus of the phyA polypeptide has been reported to stabilize the molecule as

Pfr (Vierstra 1993, 1996), localizing one determinant necessary for degradation to a small stretch of amino acids at the extremity of this domain (Fig. 1). Because the full COOH-terminal domain from either phyA or phyB supports degradation when fused to the phyA NH_2-terminal domain, the necessary determinants are apparently common to both family members (Wagner *et al.* 1996b).

Proposed mechanisms of selective PfrA degradation have focused on the possible involvement of the ubiquitin-mediated proteolytic pathway and/or the hypothesized PEST-sequence mechanism (see Vierstra 1994; Clough & Vierstra 1997). There is evidence that phyA is rapidly ubiquinated *in vivo* upon conversion to Pfr and kinetic data are at least partially consistent with subsequent degradation ensuing via the ubiquitin pathway (Vierstra 1994, 1996). The proposed location of the ubiquitin target site (one or more of the three lysine residues between positions 803 and 851) in the COOH-terminal domain (Vierstra 1993, 1994, 1996), as well as the conservation of these three lysine residues between phyA and phyB sequences (Mathews *et al.* 1995) would be consistent both with the need for a contiguous COOH-terminal domain for degradation (Boylan *et al.* 1994; Wagner *et al.* 1996b) and with the interchangeability of the phyA and phyB COOH-terminal domains in this process (Wagner *et al.* 1996b). Unfortunately, site-directed substitution of arginines for the three lysines apparently did not affect the rate of PfrA degradation (Vierstra 1996), indicating that if ubiquitination does occur at these sites it is not necessary for proteolysis. Thus, although still clearly a viable possibility a direct link between ubiquitination of PfrA and degradation remains to be demonstrated.

The presence of a PEST sequence (Rogers, Wells & Rechsteiner 1986) and its associated cluster of acidic residues in phyA, but not phyB, C, D or E sequences (Quail *et al.* 1991; Mathews *et al.* 1995), as well as its location adjacent to the chromophore (positions 375–413) in the amino terminal domain (Fig. 1), and the photoconversion-induced exposure of this region in the Pfr form (Grimm *et al.* 1998), are all consistent both with the selective degradation of phyA following Pfr formation and the location of the determinants for this selectivity in the NH_2-terminal domain (Wagner *et al.* 1996b). One possibility would be that the PEST sequence provides specific recognition of PfrA by the degradative machinery which then initiates degradation via the ubiquitin pathway involving determinants in the COOH-terminal domain. Unfortunately, no direct experimental test of the relevance of the PEST sequence to PfrA degradation has been reported to date and the importance of this sequence to the degradation of several other PEST-containing proteins has been questioned (Rechsteiner 1991; Vierstra 1994).

SEQUENCE HOMOLOGY

Schneider-Poetsch and colleagues provided the first provocative evidence from computerized database searches that the distal half of the COOH-terminal domain of the phytochromes (positions 931–1210) exhibits a degree of sequence similarity to the sensor histidine kinase module of bacterial two-component signalling systems (Schneider-Poetsch & Braun 1991; Schneider-Poetsch *et al.* 1991; Schneider-Poetsch 1992), thereby raising the possibility that the photoreceptors function as a family of photoregulated histidine kinases. Subsequently, three considerations tended to detract from the likelihood that this simple extrapolation from the prokaryotic systems is accurate. First, the histidine residue that is autophosphorylated in the majority of the bacterial kinases is conserved at that site (position 967) only in the monocot phyAs and Arabidopsis phyC, and not any of the other reported phy sequences (Mathews *et al.* 1995). Secondly, attempts to detect autophosphorylation of histidine residues in preparations of purified phyA were unsuccessful (D. Dailey & P. Quail, unpublished results). Thirdly, site-directed mutagenesis of four single residues [positions 967 (histidine), 1132 (glycine), 1153 (glycine), 1156 (leucine); see Fig. 1] in oat phyA (the latter three residues conserved among all phytochromes and the majority of histidine kinases) failed to abrogate the activity of the molecule when tested in transgenic Arabidopsis (Boylan & Quail 1996).

However, the recent sequencing of the entire genome of the cyanobacterium *Synechocystis* sp. strain PCC6803 (Kaneko *et al.* 1996) has strongly revitalized this proposal. One of the open reading frames (ORFs) in the *Synechocystis* genome (Cyanobase database), designated ORF SLR0473, has regions of striking sequence similarity both to the phytochromes and to the sensor histidine kinases (see Quail 1997). ORF SLR0473, encoding 748 amino acids, consists of an NH_2-terminal domain with 36% identity (60% similarity) to the NH_2-terminal domain of phyE between positions 119 and 640, and an immediately adjacent COOH-terminal domain with 20% identity (52% similarity) to the COOH-terminal domain of phyE between positions 938 and 1187 (Fig. 1; see Quail 1997). This latter region of phyE is within the proposed histidine kinase-like domain of the phytochromes. In turn, the COOH-terminal domain of ORF SLR0473 shows convincing sequence similarity to the histidine kinase module of established bacterial two-component systems (e.g. 31% identity and 55% similarity to *B. subtilis* KinA), including the presence of the conserved signature motifs, designated H, N, G1, F and G2 (Parkinson & Kofoid 1992). These data suggest that the *Synechocystis* SLR0473 protein may be a photoregulated histidine kinase and that the plant phytochromes may be evolutionary remnants of these prokaryotic molecules (see Schneider-Poetsch & Braun 1991; Schneider-Poetsch *et al.* 1991). Strong support for at least part of this notion has been provided recently by Lagarias and coworkers who have shown that the SLR0473 protein can attach tetrapyrrole chromophores, including phytochromobilin, with the formation of a chromoprotein that is fully photoreversible by red and far red light analogous to plant phytochromes (K. C. Yeh & J. C. Lagarias, University of California, Davis, USA, personal communication). In addition, a genetically defined locus for chromatic adaptation in the cyanobacterium *Fremyella* has also

recently been shown to encode a protein with some sequence similarity to the phytochromes in its NH_2-terminal domain and to the histidine kinases in its COOH-terminal domain (Kehoe & Grossman 1996).

Two other related observations arising from sequence comparisons are that the COOH-terminal domain of the phytochromes contains two repeats of a sequence (positions 689–723 and 826–860, respectively) (Jones & Edgerton 1994), and that these repeats have weak sequence similarity to a segment of photoactive yellow protein from the purple bacterium *Ectothiorhodospira* (Lagarias, Wu & Lagarias 1995) (Fig. 1). The significance of these findings is yet to be determined.

BIOLOGICAL ACTIVITY

Deletion analysis

A number of investigations have used overexpression of various deletion derivatives of phyA or phyB in transgenic plants to identify regions of the photoreceptors necessary for biological activity (Boylan & Quail 1991; Cherry *et al.* 1992, 1993; Boylan *et al.* 1994; Cherry & Vierstra 1994; Wagner *et al.* 1996a). Although subtleties exist between the effects of some individual constructs and plant systems, the basic conclusion from these combined studies is that deletion of relatively short segments at either terminus of the polypeptide either eliminates or modifies detectable biological activity. The results indicate that determinants within the first 60 residues or less at the NH_2-terminus of phyA (positions 37–94) (Boylan *et al.* 1994; Cherry & Vierstra 1994; Jordan *et al.* 1995) or phyB (positions 1–57) (Wagner *et al.* 1996a), and within as few as 35 residues at the COOH-terminus of phyA (positions 1161–1195) (Cherry *et al.* 1993; Cherry & Vierstra 1994) are necessary for normal biological activity (Fig. 1). In addition, internal deletions near the linker region at the proximal end of the COOH-terminal domain of phyA (positions 674–744) (Boylan *et al.* 1994) or phyB (positions 666–726) (Wagner *et al.* 1996a) also eliminate normal activity, indicating the necessity of this region. The limitation of such data is that it is unknown whether these regions are directly involved in photoreceptor activity or the deletions indirectly perturb the function of another critical part of the molecule. One interesting 'gain-of-function' effect is that either deletion of the serine-rich region between residues 6 and 12 of phyA (positions 42–51) (Jordan *et al.* 1995) or site-directed substitution of alanines for all the serines in this region (Stockhaus *et al.* 1992) result in enhanced biological activity of the transgene-encoded phyA. This result suggests that the serines in this region may mediate attenuation of phyA activity.

Photosensory specificity

Like other receptors, the phytochromes can be considered to exhibit dual molecular functions: a sensory function involving perception and interpretation of the incoming light signals, and a regulatory function involving biochemical transfer of the perceived information to downstream transduction chain components (Fig. 2). The phytochrome system is well known to monitor multiple parameters of the light environment (wavelength, fluence rate, etc.), to do so in multiple sensory 'modes' (LFR, VLFR, HIR, etc.), and to regulate multiple facets of growth and development throughout the plant life cycle via modulated gene expression (Kendrick & Kronenberg 1994). Studies with photoreceptor mutants of Arabidopsis have shown that some of this sensory complexity, at least in early seedling development, is attributable to the fact that phyA is predominantly, if not exclusively, responsible for FRc perception in the FR-HIR, whereas phyB is predominantly responsible for Rc perception in the R-HIR, and that the two phytochromes transduce mutually antagonistic signals in response to Rc or FRc enrichment (see Quail *et al.* 1995; Smith, Xu & Quail 1997).

To begin to map the determinants involved in this photosensory specificity, reciprocal domain-swap experiments have been performed in which the NH_2-terminal domain of phyA fused to the COOH-terminal domain of phyB at position 673 (phyA/B) and the converse fusion protein (phyB/A) have been overexpressed in transgenic Arabidopsis (Wagner *et al.* 1996b). The data show that the wavelength-dependent seedling responses to Rc or FRc mediated by the phyA/B fusion resemble those of phyA, whereas the responses mediated by the phyB/A fusion mimic those of phyB (Fig. 3). The evidence therefore indicates that the determinants for the photosensory specificity of phyA and phyB to FRc and Rc, respectively, reside in the NH_2-terminal domains. Because deletion of the NH_2-terminal 52 residues of phyA (positions 37–94) produces a transgene-encoded molecule defective in FRc perception, but retaining Rc responsiveness, it is possible that one or more of these determinants of phyA photosensory specificity reside in this short-terminal segment (Boylan *et al.* 1994).

On the other hand, the NH_2-terminal domain of neither phyA (Boylan *et al.* 1994) nor phyB (Wagner *et al.* 1996a) when expressed alone exhibits normal biological activity (Fig. 3) despite being fully photoactive and having apparently normal spectral properties. This result indicates that a contiguous COOH-terminal domain provides determinants necessary for transmission of perceived photosignals. Because the COOH-terminal domains of phyA and phyB appear to be fully interchangeable in this function, the critical COOH-terminal determinants appear to be common to both phytochromes (Wagner *et al.* 1996b). From these data alone, it cannot be determined whether this domain plays a purely structural role or is directly involved in signal transfer. However, at least for phyB, the COOH-terminal domain is not capable of autonomously performing phyB function (Fig. 3; Wagner *et al.* 1996a). This result indicates that the phytochromes appear to be unlike some biological molecules, such as receptor kinases, in which removal of the ligand-binding (receptor) domain results in constitutive activation of the signalling (catalytic) domain (Struhl, Fitzgerald & Greenwald 1993).

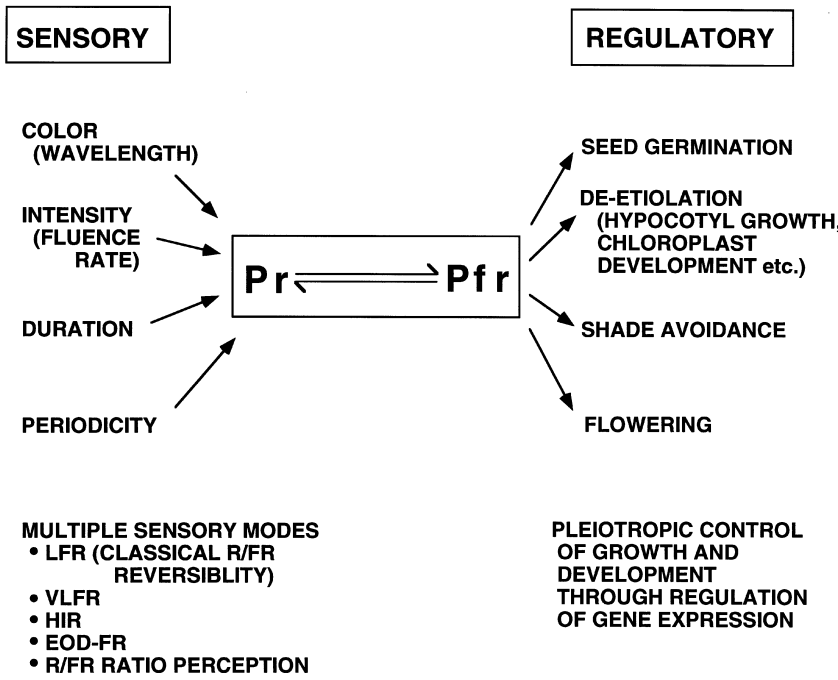

Figure 2. Dual molecular functions of the phytochromes. The photoreceptor molecule can be considered to have two sequential functions: sensory, defined as perception and interpretation of signals from the light environment, and regulatory, defined molecularly as biochemical transfer of the perceived signals to transduction pathway components that ultimately regulate morphogenesis through altered gene expression. The principal parameters in the light environment known to be monitored by the phytochrome system are shown to the left, as are the multiple sensory 'modes' displayed by the photoreceptor: LFR = low fluence response; VLFR = very low fluence response; HIR = high irradiance response; EOD-FR = end-of-day far red; R/FR ratio = the ratio of the fluence rates of red to far red light (the parameter used for vegetative shade or proximity detection). Major facets of plant growth and development controlled by the phytochromes are indicated to the right.

Regulatory activity

One strategy for identifying determinants in the phytochrome protein potentially involved in downstream signal transfer, as distinct from signal perception, is to screen for missense mutations within the polypeptide that do not affect expression level, spectral properties, or gross structural properties (dimer formation, apparent molecular cross-section), but do disrupt biological activity. Such mutant molecules can be considered to be competent in photoperception but defective in regulatory activity. The mutated resides detected in a series of such screens involving phyA and phyB are compiled at the top of Fig. 1 (see Quail *et al.* 1995; Wagner & Quail 1995; Xu *et al.* 1995). A majority of these mutations (76%) cluster at the proximal end of the COOH-terminal domain (positions 681–838), designated the 'core' region, with multiple substitutions occurring at four positions within an 18 residue sub-segment (positions 776–793). The data indicate therefore that this restricted 'core' region of the polypeptide contains determinants necessary for effective communication of perceived light signals to the cellular transduction circuitry. Because the COOH-terminal domains of phyA and phyB are functionally interchangeable (Fig. 3) and because the regulatory missense mutations cluster in the same region for both phyA and phyB (Quail *et al.* 1995; Wagner & Quail 1995; Xu *et al.* 1995), it would appear that the same function is disrupted in both molecules by these mutations. If the region between positions 681 and 838 is directly involved in the transfer of signalling information from photoreceptor to recipient transduction component, the biochemical mechanism of this transfer would appear to be the same for phyA and phyB.

How could the specificity of signal perception determined by the NH_2-terminal domains of phyA and phyB be reconciled with a putative common biochemical mechanism of downstream signal transfer specified by the 'core' region? One possible formal model is depicted in Fig. 4 (Wagner, Hoecker & Quail 1997). In this model, the NH_2-terminal domains of phyA and phyB carry different determinants that allow each to recognize (bind to) its own cognate reaction partner(s) (target selection) upon appropriate signal perception. The biochemical modification of the bound reaction partner involving the 'core' COOH-terminal region would then be identical in all cases (e.g. phosphorylation of a residue in the reaction partner). Subsequent steps in the signal cascade could then either converge immediately or remain independent to the point where the common cellular functions controlling the growth and developmental responses to phyA and phyB are affected. This model is analogous to that of many other families of receptor molecules, especially the receptor kinases.

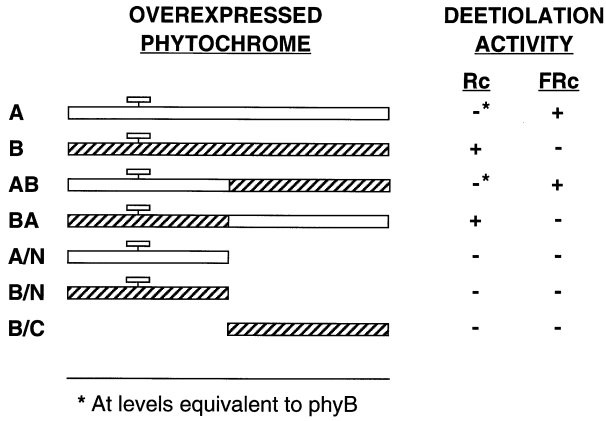

Figure 3. Activity of transgene-encoded phytochrome derivatives in enhancing the deetiolation process when overexpressed in Arabidopsis. Constructs: A, oat phyA; B, rice phyB; AB, chimeric protein with NH_2-terminal domain of oat phyA (positions 37–673) fused to COOH-terminal domain of rice phyB (positions 673–1210); BA, chimeric protein with NH_2-terminal domain of rice phyB (positions 1–673) fused to COOH-terminal domain of oat phyA (positions 673–1195); A/N, NH_2-terminal domain of oat phyA only (positions 37–673); B/N, NH_2-terminal domain of rice phyB only (positions 1–666); B/C, COOH-terminal domain of rice phyB only (positions 647–1210). Deetiolation activity = capacity of transgene encoded phytochrome to enhance deetiolation (suppress hypocotyl elongation) of transgenic Arabidopsis seedlings in Rc or FRc. + = enhanced deetiolation relative to non-transgenic Arabidopsis; – = no enhancement of deetiolation relative to non-transgenic Arabidopsis; -* = quantitatively less effective than rice phyB in enhancement of deetiolation in Rc on a per mole basis of overexpressed phytochrome. Data compiled from Boylan & Quail (1991), Wagner *et al.* (1991), McCormac *et al.* (1993), Boylan *et al.* (1994) and Wagner *et al.* (1996a,b).

An integrated picture

Several interesting insights emerge from considering the various features or activities that have been defined for a given region of the phytochrome molecule.

(1) The locations of the introns in the higher plant *PHY* genes do not appear to correlate in any obvious way with the structural domains or various functional activities described (Fig. 1). In addition, the algal phytochromes have larger numbers of introns than their higher plant counterparts (Lagarias *et al.* 1995; Winands & Wagner 1996), and Arabidopsis *PHYC* lacks the intron at position 1020 (Cowl *et al.* 1994). The functional significance, if any, of these observations is unknown.

(2) The region of the higher plant phytochromes that has the highest sequence similarity to the *Synechocystis* SLR0473 ORF (positions 119–640) corresponds extremely well to the region most highly conserved among the higher plant phytochromes themselves (Fig. 1). Indeed 67% of the invariant residues in this region of the plant phytochromes are also invariant in the *Synechocystis* sequence (data not shown). This observation is consistent with the demonstrated functional homology between the cyanobacterial and plant sequences in chromophore attachment and photoreversibility.

(3) Each of the regions that undergo photoinduced conformational changes overlaps with other activities. The NH_2-terminus (positions 1–136) and the region surrounding position 406 are each within the larger domain defined as determining the differences in the photosensory specificity and Pfr-specific degradation between phyA and phyB (Fig. 1). As each of these functions requires phototransformation, each of these conformational changes is potentially involved in either or both functions. Similarly, the regions from positions 684–731 and surrounding position 814 both lie within the relatively conserved 'core' region, defined functionally as being necessary for downstream signal transmission (Fig. 1). This correlation would be consistent with the notion that photoconversion induced conformational changes expose surfaces in this region necessary for biochemical transfer of perceived informational signals to phytochrome reaction partners (Fig. 4).

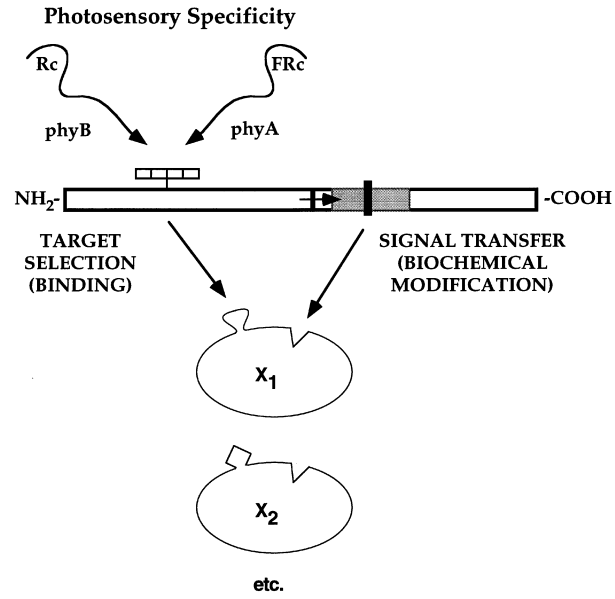

Figure 4. Two-point contact model of phytochrome action. NH_2-terminal domains of phyA and phyB are postulated to contain distinct determinants which recognize specific cognate determinants (protruding triangle or diamond) on separate reaction partners (X1 and X2, respectively) in response to photosignal perception (induced target selection). Biochemical transfer of the perceived signal to the reaction partner is postulated to be functionally identical for the two photoreceptors and to involve interaction of the 'core' region (stippled area) of the COOH-terminal domain of each with a common determinant (indented triangle) present on all reaction partners (X1, X2, etc.). Thus, the specificity in signal perception exhibited by phyA and phyB involving the capacity to discriminate between Rc and FRc signals is postulated to be transduced at the first step via selection of different cognate molecular targets that undergo the same local biochemical modification in the process of signal transfer from the photoreceptor. (From Wagner, Hoecker & Quail 1997.)

(4) It should be noted that, despite the attractiveness of the possibility that the phytochromes may function as photoregulated histidine kinases, none of the mutations affecting regulatory activity thus far reported falls within the postulated kinase domain, and site-directed mutations targeted at conserved residues within this domain failed to disrupt phytochrome activity *in vivo* (Fig. 1). Although there are many possible explanations for these observations, caution is still needed in attempting to transpose the bacterial two-component model directly to the phytochrome system.

CONCLUSIONS

Our contemporary view of the phytochrome molecule integrates evidence from earlier biochemical, immunochemical, and spectroscopic studies with that from more recent molecular and genetic studies. Many of the recent advances have come from the capacity afforded by molecular and genetic approaches to perform functional assays on mutagenized molecules in living plants, and to search for similar sequences or structural motifs that have identified functional activities in the rapidly expanding sequence databases. Regions of the molecule potentially involved in reaction partner recognition and in signal transfer have been identified by functional assay, and a domain with tantalizing sequence similarity to the prokaryotic sensor histidine kinases remains the best clue currently available to a potential biochemical mechanism of signal transfer. Despite these advances, however, the picture we have of the photoreceptor molecule remains relatively crude, and the molecular mechanism of phytochrome action remains to be identified. The continued use of the combined power of quantitative photobiological, molecular-genetic and biochemical approaches holds the promise of significant progress on these problems in the near future.

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