Plants Possess Multiple Photoreceptors

Because plants are photo-auxotropic they are particularly sensitive to their light environment. To fine-tune their development according to light intensity, direction, spectral quality, and periodicity they possess a multiplicity of light sensors (1). In Arabidopsis there are eight identified photoreceptors, but this list is still incomplete. It includes three UV-A/blue light receptors (phototropin, a photoreceptor to sense light direction, and two cryptochromes that are light-stable (11). Numerous recent reviews cover phy-mediated responses, shade avoidance, and the regulation of flowering time by day length (20).

Photoreceptors

Multiple Phytochromes Have Overlapping and Distinct Functions

Phytochromes were originally defined as the receptors responsible for red, far-red reversible, plant responses (7–9). Photobiological experiments led to the proposal that phy exists in two spectral forms: the inactive Pr form (red light absorbing) phototransforms into the active Pfr form (far-red light absorbing) upon absorption of red light. This reaction can be reversed when Pfr is converted to Pr upon absorption of far-red light. Purification of phy from plants confirmed the existence of those two spectrally interconvertible forms (10). phy are classified into two groups; type I (phyA in Arabidopsis) is light-labile and type II (phyB–phyE in Arabidopsis) is light-stable (11). Numerous recent reviews cover phy-mediated photomorphogenesis in detail (12–19).

Photobiological and genetic studies have revealed that this small gene family plays important roles in seed germination, seedling de-etiolation, neighbor perception and avoidance, and the transition from vegetative to reproductive growth (induction of flowering). At the molecular and cellular level phy responses include: development of the chloroplast, inhibition or promotion of cell growth (depending on the organ), ion fluxes at the plasma membrane, and gene expression responses (1). Genetic screens to identify loci implicated in phy responses have yielded four apoprotein mutants (phyA, phyB, phyD, and phyE), two chromophore mutants (hy1 and hy2), and numerous mutants implicated in phy-mediated signaling. The analysis of these mutants highlighted the role of phytochromes in sensing light quality, intensity, and the duration of the light cycle and revealed that type I and type II phy have distinct modes of photoreception (14, 15).

Light-stable phy are responsible for the classical red/far-red reversible phy responses. In Arabidopsis phyB plays the most prominent role; it is the major red light receptor for seedling de-etiolation, and it affects many light-regulated cell elongation responses, shade avoidance, and the regulation of flowering time by day length (20). phyD and phyE mutants have more subtle phenotypes that are only revealed in double or triple mutant combinations (21–23). Because certain phytohormone mutants also display similar phenotypes, a subset of phy responses might be mediated by light-regulated hormonal signaling (14, 24–26).

phyA, the only type I phy in Arabidopsis, plays a major role in gene expression and germination in response to very low fluences of broad spectrum light as well as in sensing day-length extension (27–30). phyA is also essential for de-etiolation in far-red enriched light (31–33). Such conditions are found when a young seedling develops under a dense canopy of plants. This is a particularly interesting phy function because, contrary to most phy responses, it is induced by far-red light and inhibited by red light (see above) (34). This high irradiance response to far-red light identifies a novel form of active phy, Pr, that has been cycling through Pfr, which will be referred to as Pr*. Pr* has acquired novel properties that are distinct from Pr and Pfr, but the molecular nature of the distinction between Pr* and Pr is unknown (34). As illustrated above, type I and type II phy play distinct roles; however, it must be pointed out that depending on the response their role can be overlapping, coordinated, or even antagonistic (35–39).

Molecular Properties of Phytochromes and Bacteriophytochromes

Phytochromes bind phycobilinobilin (PdB) via a thioether linkage to a cysteine residue in the most conserved domain among phy (Fig. 1). The first committed step in chromophore biosynthesis is the cleavage of the tetrapyrrole ring of heme (Fig. 1A). This reaction is catalyzed by a heme oxygenase encoded by the HY1 gene in Arabidopsis (40, 41). hy2 mutants are most probably defective in the PdB synthase enzyme; this step is followed by an isomerization in the C-3 double bond of PdB (42). The nature of the PdB isomer is still unclear, but phy itself is capable of catalyzing this reaction (42). phy chromophore mutants can be mimicked by over-expression of a mammalian biliverdin reductase (43). phy apoprotein binds to the 3E-PdB in the cytoplasm to yield the Pr form of the photoreceptor. This reaction requires the bilin lyase domain (BLD) of the photoreceptor. Absorption of red light triggers a “Z” to “E” isomerization in the C-15 double bond between the C and D rings of the linear tetrapyrrole, resulting in the far-red light-absorbing form Pfr (44) (Fig. 1A). Conformational changes in the protein backbone are required to maintain this high energy state of the photoreceptor (45). Pfr can be converted to Pr either by a slow non-photoinduced reaction (dark reversion) or much faster upon absorption of far-red light. It is generally assumed that all phy have the same chromophore. Because of the very low levels of type II phy this has not been verified in vivo. Analysis of reconstituted recombinant phyA, phyB, phyC, and phyE reveals that they have similar but not identical spectral properties (46–48).

Phytochromes are soluble homodimers composed of two functional domains: an N-terminal light-sensing domain and a C-terminal signaling domain (Fig. 1). The N-terminal portion is necessary and sufficient for photoreception and possesses the bilin lyase activity allowing attachment of the chromophore to the apoprotein (42). The minimal BLD is actually less than 200 amino acids long (49). The first 70 amino acids of the protein are dispensable for chromophore binding; they constitute the N-terminal extension (ATE). The ATE is poorly conserved, possibly accounting
Deinococcus radiodurans regulator of chromatic adaptation E from Fremyella diplosiphon absorption spectra of Pr and Pfr are indicated. BVR, indicates the site of chromophore attachment to a cysteine in the bilin arrow the C-15 double bond between the C and D rings (indicated in lyase domain. Absorption of red light triggers a “Z” to “E” isomerization in chrome-related from Rhodospirillum centenum.

The importance of the C-terminal half of plant phytochromes is surprising; however, deletion of the HKRD domain of phyB has a surprisingly, however, deletion of the HKRD domain of phyB has a half-life similar to the one of PfrA. Ubiquitination of PrA* might be implicated in stabilization of the Pfr form of phyB (4, 47, 56, 57). Interestingly a mutation in the BLD domain of phyB has the opposite effect, leading to a phyB protein locked in the Pr conformation (58).

The HRKD domain of plant phytochromes is only distantly related to bacterial histidine kinases, and several residues essential for kinase activity are absent in plant phy (66). In fact, work over the past 20 years has indicated that oat phyA might be a Ser/Thr kinase, and this has been confirmed convincingly quite recently (19, 54). Recombinant oat phyA is a light and chromophore-modulated protein kinase with Pfr being a more active form than Pr (Fig. 2C) (54). Oat phyA is a phosphoprotein in vivo, two phosphorylation sites have been mapped, and interestingly they correspond to residues phosphorylated after in vitro kinase assays (67). Ser-7 is constitutively phosphorylated, and mutagenesis studies suggest that phosphorylation of this residue is implicated in down-regulation of phyA signaling (50). Ser-599 phosphorylation is only observed in phyA extracted from light-treated plants (67). Phosphorylation of this residue might therefore be a molecular tag distinguishing between different forms of phy. The importance of this residue has been demonstrated in vitro because a S599K mutant loses light-regulated kinase activity (68). The crypto- chromes and PKS1 (phytochrome kinase substrate 1) are also substrates of phyA as a protein kinase, but the role of phosphorylation during phy-mediated light signaling remains to be determined in vivo (68, 69). Based on reverse genetic studies it has been proposed that PKS1 acts as a negative regulator of phyB and phyA signaling (68).2 The phyA-cryptochrome interaction might be the molecular basis for the co-action between those photoreceptors (69). These studies suggest a role for phy-mediated phosphorylation; quite surprisingly, however, deletion of the HRKD domain of phyB has a milder phenotype than certain point mutations in the HRKD (52).

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Fig. 1. Structural domains and spectral properties of phytochromes. A, biosynthetic pathway of the phytochrome chromophore. Enzymes are indicated in red, and the Arabidopsis genes are indicated. Note that chromophore binding is autocatalytic; it requires the bilin lyase domain of the phytochrome; phytochromes also possess Pab isomerase activity. The arrow indicates the site of chromophore attachment to a cysteine in the bilin lyase domain. Absorption of red light triggers a “Z” to “E” isomerization in the C-15 double bond between the C and D rings (indicated in bold). The absorption spectra of Pr and Pfr are indicated. BVR, biliverdin reductase. B, structural domains of prokaryotic and plant phytochromes. PAS is the acronym from the founding members of this protein domain (Per/Arndt/Sim). Cph1, cyanobacterial phytochrome 1 from Synechocystis sp. PCC6803; RocA, regulator of chromatic adaptation E from Fremyella diplosiphon; BphP, bacteriophototyochromes 1 from Deinobacter radiodurans; Ppr, PYP-phytochrome-related from Rhodospirillum centenum.

for some functional differences among phy. Structure function analysis has revealed that in phyA, the ATE is composed of two subdomains (50, 51). The ATE might be implicated in stabilization of the Pfr form of the photoreceptor, which is particularly interesting in view of the large structural changes observed in this part of the protein upon Pr to Pfr phototransformation (45).

The importance of the C-terminal half of plant phytochromes is highlighted by the numerous missense mutations affecting this portion of the protein (4, 52). This signaling domain is composed of a PAS (Per/Arndt/Sim)-related domain (PRD) and a histidine ki-

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1 J. Casal, J. Chory, and C. Fankhauser, unpublished data.
Subcellular localization is probably a major level of regulation for plant phytochrome action. Both phyA and phyB are cytoplasmic in the dark, and appropriate light treatments trigger their translocation into the nucleus (Fig. 2B) (12). This relocation takes several hours for phyB in contrast with the more rapid translocation of phyA. This differential behavior correlates well with the sequential action of those phy during light-induced inhibition of hypocotyl growth (39). The slow nuclear translocation of phyB implies that PfrB will be present in the cytoplasm where it could also play a role. Several rapid phy effects, such as ion fluxes at the plasma membrane, have been reported; they could be induced by the cytoplasmic pool of PfrB (76).

**Signalining, What Happens after Photoperception?**

Pharmacological studies using microinjection of a tomato phy mutant have identified heterotrimeric G proteins, cGMP and Ca\(^{2+}\), as second messengers in phy signaling (77). Genetic screens have identified two classes of signaling components, those acting downstream of a single photoreceptor and those acting downstream of multiple photoreceptors (Fig. 3). This presumably reflects the fact that light signals perceived by different photoreceptors must be integrated (14, 15). The latter class (light signal integration, see Fig. 3) includes both positively acting factors (i.e. HY5) and a large group of negative regulators of photomorphogenesis (DET/COP/FUS) (16, 17). Mutants with phenotypes under specific light conditions (i.e. only red light) are considered as acting early in the cascade. The study of such mutants and of phy interacting proteins reveal a complex signaling web (Fig. 3) (14, 15, 26, 78–82). These loci can be classified into three groups: those acting specifically downstream of phyA, downstream of phyB, or downstream of both.
Interestingly both nuclear and cytoplasmic factors have been identified, and these signaling branches include positive and negative regulation (Fig. 3). Most of the cloned genes code for proteins with poorly defined biochemical functions. It is currently quite hard to propose a model that integrates all those factors, particularly because the relative position of these elements in the chain of events is still largely unknown.

Paradoxically, the best understood branch of phy signaling appears to be rather simple (Fig. 2B). PrfB is selectively imported into the nucleus where it interacts with PIF3, a bHLH transcription factor. This interaction occurs specifically with PrfB but not PrfB and irrespective of DNA binding by PIF3 (Fig. 2B) (83). How interaction with phyB affects PIF3 activity remains to be solved. RSF1/HRF1/RE1P1 is another bHLH transcription factor that is quite related to PIF3 and also plays an important role in phy signaling (82, 84–86). RSF1/HRF1/RE1P1 is, however, implicated in phy-A- and not phy-B-mediated signaling (82, 84–86). There is currently no data indicating a direct interaction between RSF1/HRF1/RE1P1 and phyA (85). The presence of bHLH transcription factors in both phyA and phyB signaling is particularly noteworthy in view of the recently uncovered convergence of multiple phy signaling pathways on a single promoter (87). Genetic studies for both phy3 and RSF1/HRF1/RE1P1 have revealed that they play important roles in phy signaling, but they also indicate that these transcription factors only account for part of the response initiated by the phy (82, 83, 85, 86). These recent studies illustrate the potentially very large scope of signaling initiated by the phytochrome, but we should keep in mind that much remains to be done to have a global view of the multiple events initiated by those photoreceptors.

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