PCP
PLANT & CELL PHYSIOLOGY

Mini Review

Cytoplasmic Phytochrome Action

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(Received May 12, 2010; Accepted June 21, 2010)

Phytochrome photoperception is a common mechanism for the detection of red and far-red light in bacteria, cyanobacteria, fungi and plants. However, the responses following phytochrome activation appear to be quite diverse between species. Lower plants, such as mosses, show phytochrome-mediated directional responses, namely phototropism and polarotropism. These cannot be explained by nuclear gene regulation and are thought to be triggered by phytochromes in the cytoplasm or at the plasma membrane. In higher plants, similar directional responses are mediated via phototropin, a blue light receptor, with phytochromes mainly controlling morphogenetic responses through gene regulation. However, cytoplasmic phytochrome responses exist in higher plants too, which appear to be intertwined with directional blue light perception. By summarizing the respective findings, a possible conservation of cytoplasmic phytochrome function in higher and lower plants is addressed here.

Keywords: Cytoplasmic phytochrome • Higher and lower plants • Localization • Photoreceptor evolution • Signaling.

Abbreviations: B light, blue light; bHLH/PIF, basic helix–loop–helix/phytochrome-interacting factor; FR light, far red light; GAF, cGMP phosphodiesterase/adenylyl cyclase/FhlA; GFP, green fluorescent protein; GUS, β-glucoronidase; NEO, neochrome; NLS, nuclear localization signal; PAS, PER/Arnt/Sim; PHY, phytochrome-specific domain/phytochrome; phyA, phytochrome A; phyB, phytochrome B; Pr, phytochrome R light-absorbing form; Pfr, phytochrome FR light-absorbing form; R light, red light; R_{pol}, polarized red light; SAP, sequestered areas of phytochrome; YFP, yellow fluorescent protein.

Phytochromes

Phytochromes are red (R)/far-red (FR) light photochromic receptors, initially identified in plants. As phytochromes also exist in organisms such as bacteria (Davis et al. 1999), cyanobacteria (Hughes et al. 1997, Yeh et al. 1997) and fungi (Montgomery and Lagarias 2002, Blumenstein et al. 2005), it appears that they belong to an ancient photosensory mechanism that regulates development. Phytochromes can adopt two interchangeable conformations depending on the light quality of their surroundings. After post-translational autocatalytic assembly with an open chain tetrapyrrol, they take on their R light-sensing conformation (Pr), which converts upon absorption of R photons into the FR light-sensing conformation (Pfr; Borthwick et al. 1952). The reaction is reversible, thus establishing a phytochrome Pr/Pfr photoequilibrium, reflecting the light conditions of the environment. This becomes particularly relevant for plants under a leaf canopy, where Chl absorbs R but not FR light (Holmes and Smith 1975). However, non-photosynthetic organisms such as bacteria and fungi also possess phytochromes, differing slightly in their structure and spectral properties from those of plants (Davis et al. 1999). Phytochromes contain a conserved photosensory module, consisting of a PAS (PER/Arnt/Sim), a GAF (cGMP phosphodiesterase/adenylyl cyclase/FhlA) and a so-called PHY (phytochrome-specific domain/phytochrome) domain. The PHY domain—initially thought to be unique for phytochromes—was recently shown to be structurally a GAF domain, exhibiting an additional tongue-like protrusion (Essen et al. 2008). The protrusion creates, together with the other GAF and PAS domains, the tripartite chromophore-binding pocket (Essen et al. 2008). The chromophore attaches covalently to a conserved cysteine residue within the first GAF domain in the case of cyanobacteria and plant phytochromes (Lagarias and Rapoport 1980, Hahn et al. 2006) or to a cysteine residue in the N-terminal extension in the case of bacteria and fungi (Lamparter et al. 2004). This structural difference divides both groups phylogenetically. However, the basic photochemistry appears to be the same—the covalently attached bilin chromophore isomerizes upon absorption of light, leading to the different spectral properties of Pr and Pfr (Rüdiger et al. 1983, Davis et al. 1999).

The C-terminal half of canonical phytochromes shows homology to histidine kinases, implying that phosphotransfer plays a role in downstream signaling. Unlike prokaryotic or fungal phytochromes, for which this type of two-component signaling has been shown (Yeh et al. 1997, Blumenstein et al. 2005), plant phytochromes appear to have developed a more complex signal transduction. Plant phytochromes lack the conserved histidine which is required for histidine kinase activity (Schneider-Poetsch et al. 1991); instead they exhibit Ser/Thr

Editor-in-Chief's choice

Plant Cell Physiol. 51(8): 1248–1254 (2010) doi:10.1093/pcp/pcq091, available FREE online at www.pcp.oxfordjournals.org

kinase activity (Yeh and Lagarias 1998). Additionally, plant phytochromes acquired two further PAS domains located between the N-terminal sensory module and the C-terminal histidine kinase-related domain. As point mutations in the double PAS region often exhibit impaired signaling, this domain was proposed to play a role as the signaling output domain of plant phytochromes (Quail et al. 1995). In order to elucidate the molecular mechanism(s) of phytochrome-mediated responses, the intracellular localization of the phytochrome molecule becomes relevant.

Localization

The amino acid sequence of phytochromes is not conclusive in determining their subcellular localization, as no specific localization motifs can be detected or predicted for any of the phytochromes, which also do not contain any of the hydrophobic cores or patches found in membrane proteins. Accordingly, immunolocalization of plant phytochromes in tissue sections revealed a cytoplasmic distribution in darkness (Pratt and Coleman 1971, Coleman and Pratt 1974). Upon irradiation with R light, rapid formation of cytosolic aggregates that were termed sequestered areas of phytochrome (SAPs) has been observed (Mackenzie et al. 1975). Detailed analyses of SAP localization and biochemical purifications suggested membrane or organelle association (Galston 1968, Marme 1974, Mackenzie et al. 1975). However, due to methodological limitations, phytochrome membrane attachment has never been unequivocally demonstrated (Nagatani et al. 1988) nor has it been shown to be a functional necessity in higher plants (Quail 1983). Nevertheless, the possibility that such an arrangement exists cannot be excluded, leaving phytochrome membrane association a matter still to be resolved.

Observations of phytochrome reporter gene fusions revealed that phytochrome B (phyB) and phytochrome A (phyA) partially translocate into the nucleus after activation through irradiation (Kircher et al. 1999, Yamaguchi et al. 1999). PhyA:GFP (green fluorescent protein) rapidly accumulates (~20 min) after irradiation with pulses of R or continous FR light in the nucleus, conditions under which phyA also induces de-etiolation (Kircher et al. 1999, Kim et al. 2000). PhyB:GFP does not migrate into the nucleus under FR light conditions, but rather requires continous R light to do so, reflecting the physiological functions of the molecule (Kircher et al. 1999). Both phyA and phyB localize into nuclear subcompartments after irradiation, often referred to as speckles or nuclear bodies (Kircher et al. 1999, Chen et al. 2003). The kinetics of phyB:GFP nuclear import are much slower than those of phyA, suggesting a different import mechanism or regulation. Indeed, the N-terminal half of phyB fused to GFP localizes to the cytoplasm (Matsushita et al. 2003), whereas the C-terminus has the capacity to localize β-glucuronidase (GUS) or GFP into the nucleus and even compartmentalize into nuclear bodies independently of light (Sakamoto and Nagatani 1996). Truncation studies of the phyB C-terminus showed that the PAS repeat (amino acids 594–917) is necessary and sufficient for nuclear localization, suggesting a putative cryptic nuclear localization signal (NLS) in this region (Sakamoto and Nagatani 1996, Chen et al. 2005). A point mutant in the PAS repeat region (G767R) fails to localize into the nucleus (Matsushita et al. 2003). The photosensory GAF–PHY domains interact in a light-dependent manner with the PAS repeat in a yeast two-hybrid assay. This could represent a means of unmasking a cryptic NLS, in turn leading to the observed light-dependent nuclear accumulation of phyB (Chen et al. 2005). An alternative way to explain phyB nuclear import is the light-dependent interaction with signaling partners, mainly members of the nuclear-targeted bHLH/PIF (basic helix–loop–helix/phytochrome-interacting factor) family, which might contribute to nuclear translocation of phyB (Ferenc Nagy, personal communication).

Unlike in the case of phyB, the components facilitating nuclear import of phyA have been identified to be two small proteins, FHY1 (far-red elongated hypocotyl) and FHL (FHY1-like) (Desnos et al. 2001, Zeidler et al. 2001, Zhou et al. 2005). Although an *fhl* mutant only displays a subtle phenotype, overexpression of FHL is able to complement the *fhy1* mutant fully, suggesting overlapping functionality with FHY1 (Zhou et al. 2005). Indeed, the nuclear accumulation of phyA in *fhy1* is slower but not completely suppressed unless FHL is also absent (Hiltbrunner et al. 2005, Hiltbrunner et al. 2006, Rösler et al. 2007). In the *fhl*/*fhy1* double mutant, nuclear import of phyA:GFP is undetectable even in FR or R light, but can be completely restored by adding FHY1 transgenically (Rösler et al. 2007). Interaction of phyA with FHY1 alone appears to be insufficient for nuclear translocation, since *phyA402*, a mismatch mutation allele, is still capable of interacting with FHY1 but does not translocate into the nucleus in R light (Müller et al. 2009). A candidate trigger signal for nuclear translocation and interaction of both proteins could be phosphorylation of FHY1 (Shen et al. 2009). Complex formation of phyA/FHY1 and FHL also displays light dependency, since the binding affinity of FHY1 for phyA changes with light quality (Hiltbrunner et al. 2005, Shen et al. 2009). Assessment of the longevity and binding affinity of such a phyA/FHY1 complex holds much promise for the understanding of the high irradiance response and the very low fluence response.

Similar localization studies in protoplasts of the moss *Physcomitrella* using yellow fluorescent protein (YFP) fusions of four phytochromes (PpPHY1-PpPHY4) revealed cytoplasmic localization of the photoreceptor, in darkness and R light (Uenaka and Kadota 2007). Cytoplasmic localization of phytochromes has also been suggested for ferns (Wada 1988) and algae (Nagata 1979). Although light conditions are crucial for either phytochrome expression (Sharrock and Quail 1989), stability or degradation (Clough et al. 1999, Hennig et al. 1999) in both higher and lower plants (Zeidler et al. 1998, Mittmann et al. 2004), they do not seem to affect phytochrome localization in lower plants. Nuclear translocation of the R light-activated phytochrome seems to be specific to higher

plant phytochromes. Nevertheless, all plant phytochromes can be considered to be at least temporarily cytoplasmic and, as part of the photoreceptor molecules appear to remain localized to the cytoplasm regardless of the light conditions (Hisada et al. 2000), it is worth exploring their function.

Phytochrome cytoplasmic functions

Following nuclear translocation of the activated photoreceptor, phytochrome responses are associated with massive changes in gene expression (Tepperman et al. 2001). However, some phenomena cannot be explained through the synthesis of a gene product that would subsequently be responsible for the observed response (Quail 1983). Thus, the existence of such cytoplasmic phytochrome responses has been a catalyst for discussions on whether gene regulation or a biochemical reaction is the primary action mechanism of phytochromes, two not mutually exclusive concepts.

Certain phytochrome responses are simply too fast for a transcription/translation-based action mechanism. Flower induction in *Pharbitis* can be induced through an R light pulse during the night, but cannot be reversed through FR light after 4 min (Fredericq et al. 1964). R/FR light reversible changes of the surface potential of *Hordeum* coleoptiles occur as rapidly as 30 s (Tanada 1968). The closure of *Albizzia* pinnules after R light treatment was demonstrated in the presence of a protein synthesis inhibitor (actinomycin), pointing to a response pathway independent of protein synthesis (Jaffe and Galston 1967). Indeed, similar leaf movements in *Salmanea* are mediated through ion fluxes in the pulvini, detectable within 2 min (Racusen and Satter 1975). The fastest phytochrome response described so far is the cytoplasmic streaming of *Vallisneria*, which can be locally stimulated through Pfr formation and becomes measurable within 2.5 s (Takagi et al. 2003). The addition of actin inhibitors (latrunculin or cytochalasin) prior to the R light pulse depleted cytoplasmic motility, suggesting dependence on actin filament rearrangement, a mechanism that is also necessary to execute phytochrome responses in mosses (Meske and Hartmann 1995, Meske et al. 1996).

Photoreversibility, effective fluence rate and occurrence in mature plants taken together suggest that most of these fast responses are mediated by phyB-type phytochromes (Racusen and Satter 1975, Takagi et al. 2003). Cytoplasmic responses regulated by phyA have been described using the *fhl/fhy1* double mutant, which is impaired in phyA nuclear translocation (Rösler et al. 2007). Here, cytoplasmic phyA is sufficient to sensitize phototropism by R light given prior to unilateral blue (B) light to stimulate the response (Parks et al. 1996, Rösler et al. 2007). Furthermore, *fhl/fhy1* seedlings germinating in unilateral B light are able to abrogate gravitropism and grow straight towards B light (**Fig. 1**), a response depending on phyA (Lariguet and Fankhauser 2004). It is noteworthy that both cytoplasmic responses occur in B light rather than R light and modulate a directional response. The recent observation of phyA coordinating the localization and distribution of PHOT1 proposes a possible mechanism for this (Han et al. 2008). A brief pulse of R light retains PHOT1 on the plasma membrane, thereby increasing the sensitivity to a subsequent lateral phototropic stimulus by B light. The response has an escape time of ~25 min which would suffice to induce the transcription of hitherto unknown factors (Han et al. 2008). If this were to be the case, a phyA signaling route for transcriptional control independent of nuclear translocation would have to be implied. Possible candidates for this would be the previously discussed second messengers calcium/calmodulin or cGMP (Bowler et al. 1994).

While in higher plants directional responses, such as phototropism and chloroplast relocation, are B light mediated and sensed by phototropins (Christie et al. 1998, Kagawa et al. 2001), mosses appear to use both R and B light, with phototropins playing a clearly inferior role to phytochromes (Kasahara et al. 2004). Tip cells of protonema filaments of the mosses *Ceratodon* and *Physcomitrella* bend towards unilateral R light (**Fig. 1**). This effect is FR light reversible and R light fluence rate

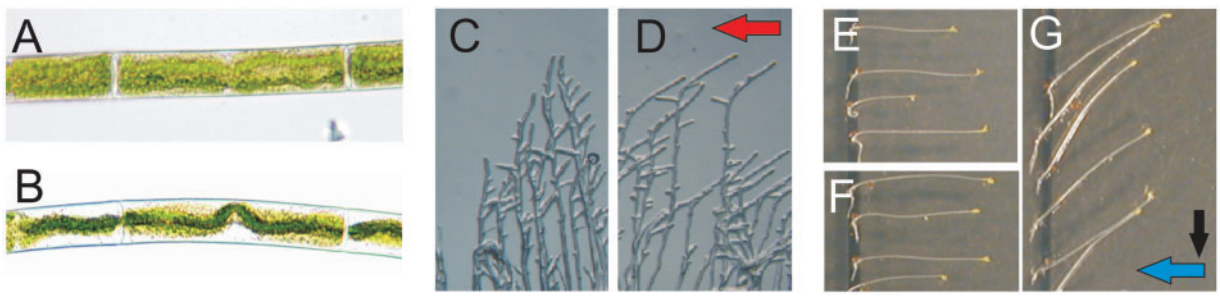

Fig. 1 Examples of cytoplasmic responses. Whereas in low light conditions the giant chloroplast of *Mougeotia* exposes its wide side (A), it rotates in high light conditions to minimize its light-exposed surface (B). This effect is R/FR light reversible and supposedly NEO mediated. The moss *Physcomitrella* grows negatively gravitropically in darkness (C) and shows phy-mediated positive phototropism towards R light coming from the right side (D). Arabidopsis wild type (WT) abrogates phyA-mediated gravitropism under B light and only grows towards the light (E), the *fhl/fhy1* double mutant which is disturbed in phyA nuclear translocation behaves like the WT (F) while the *phyA* mutant, unable to abrogate gravitropism, reacts to both stimuli, light (blue arrow for E–G) and gravity (black arrow for E–G) indicating that this response is mediated by cytoplasmic phyA.

dependent—quantitatively but, more surprisingly, also qualitatively (Cove et al. 1978, Jenkins and Cove 1983a, Jenkins and Cove 1983b, Jenkins and Cove 1983c). Low and high fluence rates of R light (<0.5 and >5 μmol m^{-2} s^{-1}) lead to negative phototropism, whereas medium fluence rates induced positive phototropism (Mittmann et al. 2004). Detailed analyses of knockout lines of four phytochromes of the moss *Physcomitrella* revealed PHY4 as the photoreceptor involved in R light-mediated positive phototropism (Mittmann et al. 2004). *Phy4* null mutant tip cells retain negative phototropism in low fluence rates of R light, but are hyposensitive in higher fluence rates. Furthermore, protonema cells of *phy4* show a defect in chloroplast relocation responses and polarotropism (Mittmann et al. 2004). If wild-type protonema filaments are exposed to polarized R light (R_{pol}) they will grow perpendicularly to the electrical vector of R_{pol} (Nebel 1969, Esch et al. 1999, Mittmann et al. 2004). Similarly, spore germination is sensitive to both unidirectional and polarized light, with filament outgrowth occurring in the direction of light or perpendicular to the electrical vector of R_{pol}, respectively (Burgess and Linstead 1981, Cove et al. 1996). The same holds true for fern protonemata (Wada et al. 1981, Kadota and Wada 1999).

Since phototropism and polarotropism integrate both directional and vectorial information of the incident light, a soluble, free-floating photoreceptor can hardly integrate and/or transmit this information via gene regulation in the growth direction. According to the Jaffe/Etzhold/Haupt model, such directional/vectorial responses derive from phytochrome molecules that are anisotropically fixed in or close to the plasma membrane (Jaffe and Etzold 1965, Haupt et al. 1969). Experiments with tip cells of *Ceratodon* demonstrated phytochrome-dependent re-orientation of actin filaments prior to the phototropic response (Meske et al. 1996). *Physcomitrella* knockout lines lacking subunits of the ARP2/3 complex are deficient in actin polymerization and show severe defects in protoplast regeneration and polar tip growth (Perroud and Quatrano 2006). These mutants are also compromised in their response towards polarized white light. A direct connection to disturbed localization of the photoreceptor could not be proven, and it hence remains unclear whether moss phytochromes are attached to actin or whether actin reorientation is a secondary effect (Harries et al. 2005, Perroud and Quatrano 2006). Moss phytochromes are similar to higher plant B-type phytochromes at the amino acid sequence level and also lack a clear localization or anchoring motif (Zeidler et al. 1998). As no moss phytochrome-interacting proteins have been identified so far, which could exhibit a role as scaffold protein, the paradox of directional sensing by a soluble photoreceptor remains unsolved.

Functional conservation of cytoplasmic signaling

A vectorial phytochrome response has never been reported for the more complex tissue of higher plants, which scatters light. Nevertheless, the possibility of a similar arrangement and pathway to that observed in mosses cannot be excluded. The interplay between PHOT1 and phyA in Arabidopsis (Lariguet et al. 2006, Rösler et al. 2007, Han et al. 2008) implies a close connection between these photoreceptors, but the predominant functionality of phytochrome has shifted during evolution from direction sensing to a modulating role in directional responses. A light-dependent relocalization of PHOT1 and an influence of phyA on the kinetics of this effect has been shown (Han et al. 2008). Although a functional dependency of phytochromes on membranes was proposed, leading to the formulation of the membrane hypothesis, it could never be compellingly shown. The discovery that PKS1, a plasma membrane-associated protein, directly interacts with both phyA and PHOT1 (Lariguet et al. 2006) may be the key to the understanding of partial or temporal attachment of phytochromes to membranes.

In this context, the evolution of the chimeric photoreceptor neochrome (NEO)—which detects R and B light, regulating chloroplast positioning—in the fern *Adiantum* and the green algae *Mougeotia* (**Table 1**) is relevant (Nozue et al. 1998, Kawai et al. 2003, Suetsugu et al. 2005). NEO comprises the N-terminal sensory module of phytochrome fused to a full-length phototropin (Nozue et al. 1998, Suetsugu et al. 2005). The phototropin-part is membrane anchored through an as yet unknown mechanism, which could explain a phytochrome response from fixed molecules. However, a NEO homolog cannot be found either in the *Physcomitrella* (Rensing et al. 2008) or in the Arabidopsis genome sequence (The Arabidopsis Genome Initiative 2000).

At first sight, phytochrome signal transduction appears not to be conserved between higher and lower plants. Homologs of the most prominent and well characterized components of Arabidopsis downstream signaling, such as the PIF or PKS family members, cannot be directly detected in the *Physcomitrella* genome sequence. This is not very surprising, as these factors are involved in the regulation of gene expression or in execution of very low fluence responses (Lariguet et al. 2003) and such phytochrome responses are not well known in *Physcomitrella*. It is also rather euphemistic to assume that all signaling components have been identified, particularly since no specific screen for cytoplasmic components has been carried out so far. Nevertheless, phototropin signaling appears to be conserved between lower and higher plants, as the fern neochrome *Ac*NEO1 can restore B light phototropism in the Arabidopsis *phot1*/*phot2* background (Kanegae et al. 2006). Homologs of the few components already identified, such as NPH3 or CHUP1, can be found in both *Physcomitrella* and Arabidopsis. As NEO also executes R light phototropism when transformed into *phot1*/*phot2*-deficient Arabidopsis plants (Kanegae et al. 2006), either the PHY part of the molecule can activate the PHOT part, which executes the response, or alternatively the more ancient PHY part can still feed straight into the directional response pathway.

Table 1 Summary of likely cytoplasmic responses of PHY and PHOT in different plant taxa

Phyla	Response	Photoreceptor	References
Chlorophyta	Chloroplast movement[a]	NEO	Suetsugu et al. (2005)
Bryophyta	Phototropism	PHY	Lamparter et al. (1997); Mittmann et al. (2004); Kadota et al. (2000); Sato et al. (2001)
	Polarotropism		
	Chloroplast movement		
	Chloroplast movement	PHOT	Kadota et al. (2000); Sato et al. (2001); Kasahara et al. (2004)
Pteridophyta	Chloroplast movement	PHOT	Kawai et al. (2003); Kagawa et al. (2004)
	Phototropism		
	Chloroplast movement	NEO	Nozue et al. (1998); Kawai et al. (2003)
	Phototropism		
Spermatophyta	Abrogation of gravitropism	PHY	Rösler et al. (2007)
	Enhancement of phototropism		
	Phototropism	PHOT	Huala et al. (1997); Kagawa et al. (2001)
	Chloroplast movement		

[a] Suggested, based on complementation studies in fern.

The parallel evolution of neochrome shows that algae and ferns probably had to face the same environmental challenges, which led to a link of PHY and PHOT signaling, resulting in the neochrome chimera. As for mosses, the challenge was solved by a functional fusion of the involved photoreceptors and their downstream signaling, rather than by genetic fusion. A functional dependence of PHY and PHOT still exists in higher plants but, as they had to adap further to an increasingly complex environment, the responses may have diversified.

Funding

This work was supported by the Deutsche Forschungsgemeinschaft [Hu702/5 to K.J., and Ze485/2 to J.R. and M.Z.].

Acknowledgments

We thank Paula Duque for critically reading the manuscript and Jon Hughes for helpful discussions. We thank Andrea Weisert for the *Mougeotia* images.

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