Photomorphogenesis: **Light receptor kinases in plants!**

Christian Fankhauser* and Joanne Chory*†

Plants must adapt to a capricious light environment, but the mechanism by which light signals are transmitted to cause changes in development has long eluded us. The search might be over, however, as two photoreceptors, phytochrome and NPH1, have been shown to autophosphorylate in a light-dependent fashion.

Addresses: *Plant Biology Laboratory and †Howard Hughes Medical Institute, Salk Institute, 10010 North Torrey Pines Road, La Jolla, California 92037, USA.

Current Biology 1999, 9:R123–R126
http://biomednet.com/elecref/09609822009R0123

 ISSN 0960-9822

Because plants use light energy in photosynthesis, they are extremely sensitive to their light environment. Light affects plants throughout their life cycle, during processes such as seed germination, seedling and vegetative development and the transition to flowering [1]. Plants are sensitive to the quality, quantity, duration and direction of light [1], and to achieve this sensitivity they are armed with a battery of photoreceptors. For example, photoreceptors that detect UV-B light — not yet molecularly characterized — are important for seedling development in response to low doses of UV-B light [2]. Two classes of photoreceptors detect UV-A and blue light: cryptochromes (CRY1 and CRY2) and a photoreceptor known by its gene name as 'non-phototropic hypocotyl 1' (NPH1). The former play major roles during seedling development and the transition to flowering, whereas the latter is required for directional growth towards a light source (phototropism) [3,4]. Phytochromes are a class of red/far-red photoreceptors — five types, phytochromes A to E, have been defined in *Arabidopsis* — that affect all aspects of plant development; they also play accessory roles in UV-B and blue-light sensing [2,5].

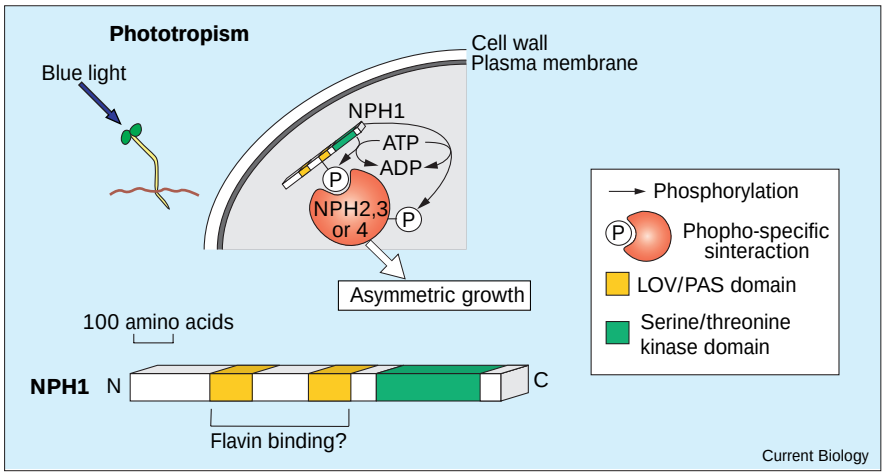
In *Arabidopsis*, genetic analysis has demonstrated that, for most developmental transitions, there is a large degree of redundancy among, and multiple interactions between, different photoreceptors [5,6]. Phototropism, by contrast, appears primarily to use a single photoreceptor, NPH1, with some influence from cryptochromes and phytochromes [7–9]. Phototropism has long been suspected to require protein kinase activity, because it is correlated with blue-light-induced phosphorylation of a 120 kDa membrane protein [10]. This light-dependent phosphorylation can be mimicked *in vitro* when membrane fractions prepared from dark-grown tissue are subsequently irradiated with blue light [10]. Interestingly, *nph1* mutant plants fail to exhibit phototropism and also lack this 120 kDa protein, and cloning of the *NPH1* locus showed that it encodes a putative 120 kDa protein kinase [4]. NPH1 is conserved in numerous plant species. The protein has a carboxy-terminal domain with all the signatures of a serine/threonine protein kinase, and the amino terminus has two repeats of about 110 amino acids — known as LOV domains — that are related to motifs present in a large group of sensor proteins. Interestingly, the LOV domains are also related to the better-known PAS domains, found in a number of regulatory proteins including phytochromes (see below) [11].

Briggs and colleagues [7] have now convincingly shown that NPH1 is the photoreceptor that mediates phototropism. They have demonstrated that recombinant NPH1 is a chromoprotein that binds non-covalently to flavin mononucleotide (FMN), with spectral properties very similar to the action spectrum for phototropism in *Arabidopsis*. It is therefore very likely that NPH1 binds to this same chromophore in plants. Briggs and colleagues [7] propose that the NPH1's LOV/PAS domains mediate binding of the chromophore; this is a distinct possibility, as LOV/PAS domains serve as protein–protein interaction domains in some cases, and bind a prosthetic group in other proteins [11]. Mutations in a similar domain of the *Neurospora* WC-1 blue-light photoreceptor result in blind strains, further emphasizing the importance of the LOV domain in blue-light sensing [12].

The biochemical characterization of recombinant NPH1 also identifies a mechanism by which the environmental signal it detects is transmitted further in the plant. Recombinant NPH1 autophosphorylates poorly in the dark, and blue-light treatments greatly increase the protein's autophosphorylation. The blue-light fluence response and kinetics of this reaction are very similar for both recombinant and plant-derived NPH1 [7]. Taken together, the results clearly demonstrate that NPH1 is the photoreceptor that mediates phototropism in *Arabidopsis*, and suggest that phosphorylation is the biochemical mechanism initiating this signaling cascade (Figure 1).

It will now be interesting to see how the different phosphorylation states of NPH1 modulate phototropism. Mapping the phosphorylation sites of NPH1, manipulating the cloned *NPH1* gene to code for mutant forms of the protein with altered phosphorylation sites and then reintroducing the mutant genes into *Arabidopsis* should give us the beginning of an answer. Finding other substrates of the NPH1 kinase activity will be another important step. Three other loci that specifically affect

Figure 1



Directional growth towards an asymmetrical light source is known as phototropism. In *Arabidopsis* seedlings, this is a blue light response and is mediated by NPH1. NPH1 is localized at the plasma membrane and autophosphorylates in a blue-light-dependent manner. This could mediate phospho-specific interactions with downstream factors, such as NPH2, NPH3 or NPH4. Alternatively, NPH1 might phosphorylate NPH2, NPH3 or NPH4. These two models are not mutually exclusive and are hypothesized to initiate a signaling cascade that ultimately results in asymmetric growth.

phototropism have been identified in *Arabidopsis* — *NPH2*, *NPH3* and *NPH4* [13]. The molecular cloning of these genes will identify potential NPH1 substrates that are clearly implicated in the process of phototropism. *In vivo*, NPH1 protein is associated with membrane fractions; its amino-acid sequence does not, however, show any hydrophobic domains that could serve as a membrane anchor, and the recombinant protein is soluble. It will be interesting to see whether NPH1 associates with the membrane through binding of another protein, such as NPH2, NPH3 or NPH4.

Turning to the phytochromes, although these were the first plant photoreceptors to be molecularly characterized, it has taken a long time to determine a signaling mechanism for red/far-red light sensing [14]. Phytochromes photoconvert between red-light (Pr) and far-red-light (Pfr) absorbing forms. Most physiological responses correlate with the presence of the Pfr form, and they can be inhibited by irradiation with far-red light (which converts Pfr back to Pr); it is therefore generally accepted that Pfr is the active form of the protein. Both forms are soluble dimeric proteins with two major domains; the amino-terminal portion is necessary and sufficient for the protein's native spectral properties. The chromophore is a linear tetrapyrrole covalently bound to an invariant cysteine residue; light-induced isomerization between rings C and D of the chromophore accounts for the interchangeable spectral properties of phytochrome. This Pr-to-Pfr transition is accompanied by rearrangements of the protein backbone [14].

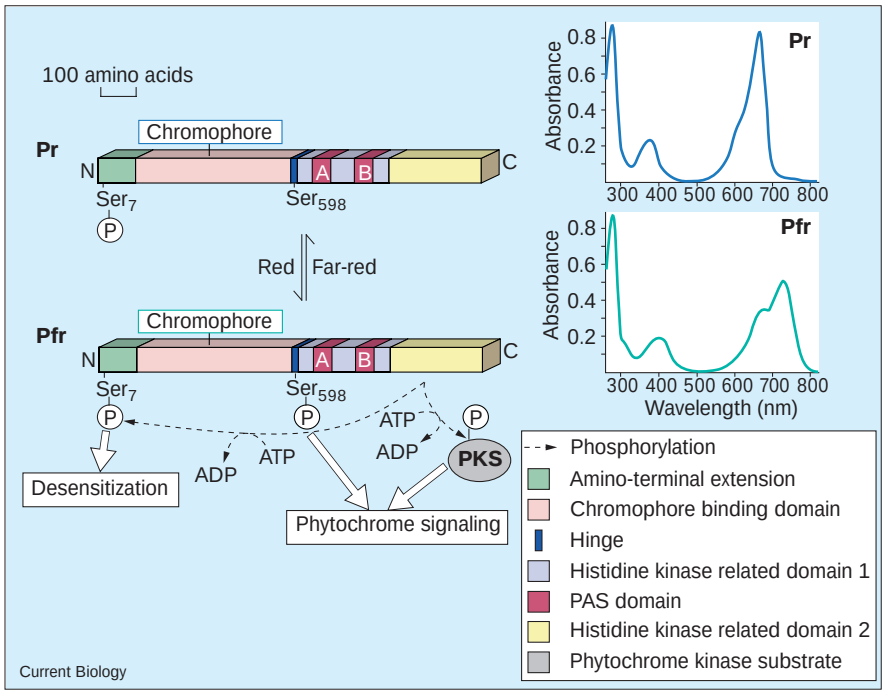
In 1992, Schneider-Poetsch [15] recognized that, near their carboxyl termini, phytochromes show a modest but significant similarity to the histidine kinases of bacterial 'two-component' sensors. But the critical histidine residues for kinase activity in most bacterial sensors are not conserved in all phytochromes. By the late 1980s, there was already biochemical evidence that phytochromes might be protein kinases. This was the subject of intense debate, summarized in two recent reviews [14,16], but the issue remained unresolved for 10 years.

Two years ago, the idea that phytochrome might be a protein kinase resurfaced with the discovery of cyanobacterial phytochromes. Cloning the photoreceptor for chromatic adaptation in the cyanobacterium *Fremyella* identified a histidine kinase with an amino terminus significantly similar to the chromophore binding domain of phytochrome [17]. Until then, it was believed that phytochromes are unique to plants. Around the same time, the genome-sequencing project for the cyanobacterium *Synechocyctis* PCC6803 revealed open reading frames with even more striking similarities to plant phytochromes. *Synechocystis* phytochrome — dubbed cyanobacterial phytochrome 1 (Cph1) — was found to have spectral properties very similar to those of their plant relatives [18]. Cph1's output domain has all the hallmarks of a bacterial histidine kinase, and Lagarias and co-workers [19] demonstrated that it is indeed a histidine kinase and, more importantly, that this activity is modulated by light. These results strongly suggest that plant phytochromes have histidine kinase ancestry, but are they histidine kinases?

The tenacity of Lagarias and co-workers has finally paid off. In a recent paper [20] they report that recombinant oat and algal phytochromes purified from two different sources have light- and chromophore-regulated autophosphorylation activity. Recombinant oat phytochrome A was shown to exhibit kinase activity with similar biochemical properties to those observed with the plant-purified protein; it is stimulated by polycations, inhibited by pyrophosphates, and shows differential phosphorylation according to the Pr/Pfr ratio. Moreover, the kinase activity is independent of

Figure 2

Phytochromes exist in either red-light absorbing (Pr) or far-red-light absorbing (Pfr) forms. The absorption spectra of both forms are depicted on the right hand side. A schematic representation of Pr and Pfr is on the left hand side. It is hypothesized that the red-light stimulated phytochrome kinase activity initiates light signaling by phosphorylating other substrates (PKSs) and/or by phospho-specific interactions with downstream elements of the signaling cascade. Phosphorylation of the serine-rich amino-terminal region of phytochrome might downregulate the response.



phytochrome concentration, consistent with an intramolecular phosphotransfer reaction. Unlike their prokaryotic relatives, however, plant phytochromes phosphorylate serine and threonine, rather than histidine or aspartate, residues. It should be noted that this is not the first example of a eukaryotic serine kinase that is clearly related to histidine kinases [21]. Phospho-serine and phospho-threonine are the end products of the reaction; the existence of less stable intermediates, such as phospho-aspartate and phospho-histidine, has not been ruled out.

Phytochromes are the only proteins that have been shown to have intramolecular kinase activity regulated by light and chromophore. What is the physiological relevance of this autophosphorylation? Phytochrome A is a phosphoprotein *in vivo*, and two of the phosphorylation sites, serine 7 and serine 598, have been mapped [22]. Interestingly, substituting serine residues in a serine-rich region near the protein's amino terminus by alanines, or their deletion, creates a hyperactive phytochrome [16]. This suggests that phosphorylation near the amino terminus — which may or may not be autophosphorylation — plays a role in downregulating the phytochrome signal. Serine 598 is preferentially phosphorylated in the Pfr form, which might create phospho-specific interfaces for interaction with downstream elements of the signaling cascade in a light-dependent fashion (Figure 2) [22]. Phytochrome B undergoes light-dependent translocation from the cytoplasm to the nucleus [23], so it is possible that phosphorylation plays a role in phytochrome subcellular localization.

Much remains to be done in terms of characterizing the kinase activity of phytochromes. For example, what consensus sequence do they phosphorylate preferentially? Do different phytochromes, which play distinct roles *in vivo*, phosphorylate different substrates? What constitutes a phytochrome kinase domain? The carboxy-terminal region of phytochrome is composed of two domains: a proximal half containing two PAS repeats, and a distal part that was originally shown to be similar to bacterial histidine kinases. Upon closer examination, Yeh and Lagarias [20] concluded that both of these domains are similarly related to histidine kinases. The plant phytochrome output domain might therefore have arisen by duplication of a bacterial histidine kinase domain. Which one of these has the kinase activity? Is a combination of both required? Is more than this carboxy-terminal region required to recreate an active kinase domain?

Phytochrome can phosphorylate a number of substrates in addition to itself, but none of them is differentially phosphorylated in response to variations in the Pr/Pfr ratio [20,24]. A number of factors acting downstream of phytochrome have been identified genetically, and one protein has recently been cloned on the basis of it interaction with phytochrome [25]. It is now important to test whether these proteins are substrates of phytochrome's kinase activity, and determine if they are preferentially phosphorylated by the Pfr form of phytochrome. Ultimately, whether phosphorylation of such a factor is connected to phytochrome signaling will have to be tested *in vivo*.

How many substrates do phytochromes have? A number of features, such as the multitude of phytochrome responses, all suggest that phytochrome signaling requires an elaborate web of interactions. One possible model could be that phytochromes are the 'workhorses' of photomorphogenesis that phosphorylate a multitude of substrates. An additional level of complexity comes from the well documented interactions between phytochromes and blue light photoreceptors [6]. A recent study suggests [24] that phosphorylation could be a link between phytochrome and cryptochrome signaling. By determining a possible biochemical mechanism for light signaling, the recent studies [7,20] give us a new tool to decipher light signal transduction.

Acknowledgements

We wish to thank Leslie Barden for her help with artwork. We are grateful to Tedd Elich, Manfred Gahrtz, Julin Maloof and Michael Neff for helpful comments on this manuscript. C.F. is supported by a post-doctoral fellowship from the Swiss National Science Foundation. Our research on phytochrome is funded by a grant from the National Institute of Health (GM52413) to J.C.

References

1. Chory J: Light modulation of vegetative development. *Plant Cell* 1997, 9:1225-1234.
2. Kim BC, Tennessen DJ, Last RL: UV-B-induced photomorphogenesis in ***Arabidopsis thaliana***. *Plant J* 1998, 15:667-674.
3. Suarez-Lopez P, Coupland G: Plants see the blue light. *Science* 1997, 279:1323-1324.
4. Huala E, Oeller PW, Liscum E, Han IS, Larsen E, Briggs WR: Arabidopsis NPH1: a protein kinase with a putative redox-sensing domain. *Science* 1997, 278:2120-2123.
5. Whitelam GC, Devlin PF: Roles of different phytochromes in ***Arabidopsis*** photomorphogenesis. *Plant Cell Environ* 1997, 20:752-758.
6. Mohr H: Coaction between pigment systems. In *Photomorphogenesis in Plants.* Edited by Kronenberg REKGH. Netherlands: Martinus Nijhoff; 1986:547-564.
7. Christie JM, Reymond P, Powell GK, Bernasconi P, Raibekas AA, Liscum E, Briggs WR: ***Arabidopsis*** NPH1: a flavoprotein with the properties of a photoreceptor for phototropism. *Science* 1998, 282:1698-1701.
8. Janoudi AK, Gordon WR, Wagner D, Quail P, Poff KL: Multiple phytochromes are involved in red-light-induced enhancement of first-positive phototropism in ***Arabidopsis thaliana***. *Plant Physiol* 1997, 113:975-979.
9. Ahmad M, Jarillo JA, Smirnova O, Cashmore AR: Cryptochrome blue-light photoreceptors of ***Arabidopsis*** implicated in phototropism. *Nature* 1998, 392:720-723.
10. Short TW, Briggs WR: The transduction of blue light signals in higher plants. *Annu Rev Plant Physiol Plant Mol Biol* 1994, 45:143-171.
11. Zhulin IB, Taylor BL, Dixon R: PAS domain S-boxes in Archaea, Bacteria and sensors for oxygen and redox. *Trends Biochem Sci* 1997, 22:331-333.
12. Ballario P, Talora C, Galli D, Linden H, Macino G: Roles in dimerization and blue light photoresponse of the PAS and LOV domains of Neurospora crassa white collar proteins. *Mol Microbiol* 1998, 29:719-729.
13. Liscum E, Briggs WR: Mutations of arabidopsis in potential transduction and response components of the phototropic signaling pathway. *Plant Physiol* 1996, 112:291-296.
14. Quail PH: An emerging molecular map of the phytochromes. *Plant Cell Environ* 1997, 20:657-665.
15. Schneider-Poetsch HAW: Signal transduction by phytochrome: phytochromes have a module related to the transmitter modules of bacterial sensor proteins. *Photochem Photobiol* 1992, 56:839-846.
16. Elich TD, Chory J: Phytochrome: if it looks and smells like a histidine kinase, is it a histidine kinase? *Cell* 1997, 91:713-716.
17. Kehoe DM, Grossman AR: Sensor of chromatic adaptation is similar to phytochrome and ethylene receptors. *Science* 1996, 273:1409-1412.
18. Hughes J, Lamparter T, Mittman F, Hartmann E, Gärtner W, Wilde A, Börner T: A prokaryotic phytochrome. *Nature* 1997, 386:663.
19. Yeh KC, Wu SH, Murphy JT, Lagarias JC: A cyanobacterial phytochrome two-component light sensory system. *Science* 1997, 277:1505-1508.
20. Yeh KC, Lagarias JC: Eukaryotic phytochromes: light-regulated serine/threonine protein kinases with histidine kinase ancestry. *Proc Natl Acad Sci USA* 1998, 95:13976-13981.
21. Harris RA, Hawes JW, Popov KM, Zhao Y, Shimomura Y, Sato J, Jaskiewicz J, Hurley TD: Studies on the regulation of the mitochondrial alpha-ketoacid dehydrogenase complexes and their kinases. *Adv Enzyme Regul* 1997, 37:271-293.
22. Lapko VN, Jiang X-Y, Smith DL, Song P-S: Mass spectrometric characterization of oat phytochrome A: isoforms and posttranslational modifications. *Prot Sci* 1999, in press.
23. Sakamoto K, Nagatani A: Nuclear localization activity of phytochrome B. *Plant J* 1996, 10:859-868.
24. Ahmad M, Jarillo JA, Smirnova O, Cashmore AR: The CRY1 blue light photoreceptor of ***Arabidopsis*** interacts with phytochrome A ***in vitro***. *Mol Cell* 1998, 1:939-948.
25. Ni M, Tepperman JM, Quail PH: PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix–loop–helix protein. *Cell* 1998, 95:657-667.