An Introduction to Phytochromes

Varsha Gupta, Department of Biotechnology, Chhatrapati Shahu Ji Maharaj University, Kanpur.
Baishnab Charan Tripathy, School of Life Sciences, Jawaharlal Nehru University, New Delhi. E.mail: varsha5@yahoo.co.uk

Abstract:

Light is a crucial environmental cue for plants, affecting their growth, development, and physiology. Light is perceived by specialized photoreceptor proteins, including phytochromes, which are involved in a wide range of light-mediated processes. Phytochromes are a family of photoreceptor proteins sensitive to red and far-red light. They are found in plants, algae, and some bacteria and play a key role in regulating plant growth and development, including seed germination, stem elongation, leaf expansion, and flowering. Phytochromes exist in two interconvertible forms: Pr and Pfr. The Pr form absorbs red light and is converted into the Pfr form, which absorbs far-red light and is then converted back into the Pr form by red light. The ratio of Pr to Pfr determines the plant's physiological response to light, with different responses occurring at different ratios. Phytochromes are involved in many different signaling pathways in plants.

Introduction:

Plants respond in many ways to their environment. Plants are adapted to live in extremely different light conditions, i.e., on forest floors, in the deep ocean, or in open fields of the tropics. There are substantial differences in the spectral distribution of light in these habitats. Plants tend to adapt the structure of photosynthetic apparatus and pigment composition to light quality and quantity. Light regulates Chl biosynthesis. Chl itself or Chl biosynthetic intermediates are involved in regulating chloroplast biogenesis. Through photosynthesis, light provides the energy source for plants and, ultimately, for all living organisms. In response to the fluctuating environment, nonmotile plants must be able to sense varying light signals and optimize growth and development. Higher plants possess sophisticated photosensory and signal transduction systems to monitor the light signal's direction, quantity, and quality and adjust their growth and development through regulated gene expression at every stage of their life cycle as germination, seedling development, and flowering. These light-regulated developmental processes are known as photomorphogenesis.

Light-dependent development of plants involves the combined action of several necessary photoreceptors, including the red/far-red light-absorbing phytochromes (Quail., 1995), the blue/UV-A light-absorbing cryptochromes (Ahmad and Cashmore, 1996), and distinct UV-A (Young et al., 1992) and UV-B (Beggs and Wellman, 1985; Christie and Jenkins, 1996) light photoreceptors. Cryptochromes and phytochromes mediate similar physiological responses. Plants respond to a broad light spectrum, ranging from UV-B to far-red light. Many physiological, photobiological, and molecular genetic studies have demonstrated that plants possess distinct photoreceptors sensing UV-B, UV-A, blue, green, red, and far-red light (Kendrick and Kronenberg, 1994). Christie et al. (1999) identified a hybrid photoreceptor from the fern *Adiantum capillus-veneris*. Its N-terminal 566 amino acids show high homology to phytochrome. Moreover, recombinant protein, expressed in *E. coli* and reconstituted with a phycocyanobilin chromophore, shows phytochrome's red-, far-red-reversibility characteristic. However, downstream of a linking domain, the protein shows remarkable similarity to phototropin, containing two LOV domains and a Ser/Thr kinase domain. Hence this single chromoprotein has phytochrome- and phototropin-like properties, referred to as "superchrome" (Briggs and Olney. 2001).

Phytochrome

Phytochromes are soluble pigmented proteins of approximately 125 kDa. The prototypical phytochrome is a homodimer, each subunit containing a covalently linked linear tetrapyrrole chromophore (Vierstra, 1993; Jones and Edgerton, 1994). Phytochrome interacting factors are a family of basic-helix-loop-helix interacting with red and far-red photoreceptors (Pham et al. 2018). The traditional view of phytochrome is that it mediates responses to red and far-red light through its ability to photo interconvert between two stable isomers, a red light absorbing form, termed Pr (lmax= 660nm) and far-red light absorbing form, termed Pfr (l max= 730nm) (Kendrick and

Kronenberg, 1994). Based on physiological, genetic, and biochemical studies, Pfr is an active form, although Pr may play a role in some light conditions (Reed et al., 1994; Shinomura et al., 1994).

All higher plants examined and many lower plants and algae have multiple genes for phytochromes (Pratt, 1995; Quail et al., 1995). For example, in *Arabidopsis*, the apoprotein component of phytochrome is encoded by five genes, termed PHYA through PHYE (Sharrock and Quail, 1989; Clack et al., 1994). PHYA is a light labile phytochrome. It is proteolyzed after photoconversion to its Pfr form (Somers et al., 1991). By contrast, PHYB and PHYC appear light-stable phytochromes (Somers et al., 1991). They constitute two distinct pools, denoted Type I and Type II. Type I phytochrome is more abundant in etiolated tissue but is light labile due to the rapid degradation and/or sequestration of Pfr.

Conversely, Type II is present in much lower amounts in dark-grown tissue but is light-stable. Consequently, type I phytochrome is more critical for the initial de-etiolation process. Type II responses predominate in mature plants (Quail et al., 1995; Smith, 1995). PhyA is a Type I phytochrome, synthesized in the dark and removed rapidly in the light. In contrast, PhyB-E is light stable and probably constitutes the Type II pool (Clack et al., 1994). PhyA is localized in the cytoplasm and is a homodimer (Furuya and Scafer, 1996). PhyB is also dimeric and localized in the cytoplasm. However, evidence has indicated that it may translocate to the nucleus in the light (Sakamoto and Nagatani, 1996).

The higher plant phytochrome molecule can be divided into two globular domains (Jones and Edgerton, 1994; Quail et al., 1995). A highly conserved N-terminal domain of approximately 600 amino acids binds the chromophore. It retains the ability of native phytochrome to photo interconvert between Pr and Pfr. The less well-conserved C-terminus is involved in the homodimerization of two monomers and the transduction of the light signal (Edgerton and Jones, 1992, 1993; Cherry et al., 1993). Electron microscopy of pure phytochrome homodimers has revealed a tripartite arrangement comprising two globular N-terminal domains tethered to a central core composed of the two C-terminal domains (Jones and Edgerton, 1994).

A protease-sensitive hinge region separates these domains:

Two types of studies have been performed on the photochemically active N-terminal chromophore binding domain- overexpression in transgenic plants and in vitro assembly of truncated or mutant recombinant phytochromes in yeast (Deforce et al., 1991; Wahleithner et al., 1991; Cherry et al., 1993; Gartner et al., 1996). Earlier studies had established that Cys-322 of oat PHYA was responsible for chromophore binding and that the 70kDa N-terminal region was sufficient for photosensory function (Lagarias and Rapoport, 1980; Boylan et al., 1994).

40-kDa region flanking Cys-322 is sufficient for chromophore attachment and photo reversibility (Cherry et al., 1993; Gartner et al., 1996). A blue shift of the Pfr absorption maximum and increased nonphotochemical reversion of Pfr to Pr when a 6-10-kDa region at the N-terminus is removed, suggesting that this region may be involved in establishing the proper chromophore-protein environment (Cherry et al., 1992).

The ability of overexpressed deletion derivatives of phytochrome to enhance hypocotyl growth inhibition has been used as an assay for phytochrome function. This assay has implicated both the N- and C-termini in signaling (Cherry et al., 1992, 1993; Boylan et al., 1994; Emmler et al., 1995). Deletion analysis of *Oat* PHYA suggests that the N-terminal 52 amino acids are required for far-red light signaling in Arabidopsis. However, they are not prominent in white or red light signaling (Boylan et al., 1994).

Additional evidence for the functional importance of the phytochrome N-terminus comes from experiments showing that multiple Ser-to-Ala substitutions at the N-terminus of PHYA, or deletion of this region results in enhanced activity in the hypocotyl growth inhibition assay (Stockhaus et al., 1992; Jordan et al., 1996). Although the roles of these Ser residues in the biological activity of

phytochrome are unknown, it has been established that this Ser-rich region is phosphorylated by an endogenous kinase activity that copurifies with PHYA (Wong et al., 1996).
It has been hypothesized that the Ser residues are involved in the desensitization of stimulated phytochrome. Thus, mutating or deleting them would result in a phytochrome whose signal could not be dampened.
A region at the N-terminus of the C-terminal domain of PHYA (amino acids 617 to 686) appears to be important in phytochrome's regulatory function because deletion of this region results in a photoactive dimer with no biological activity in the transgenic plant assay (Boylan et al., 1994). As was found in the PHYA studies, both N- and C-terminal domains of PHYB are required for its function. One surprising result from the PHYB studies was that overexpression of full-length *Arabidopsis* PHYB and either the N- or C-terminal domain of PHYB interferes with endogenous PHYA activity in LF far-red light (Wagner et al., 1996b). No interference was seen in red light, suggesting that overexpression of these domains does not interfere with PHYB activity. Wagner et al. (1996b) suggest that overexpression of PHYB interferes with endogenous PHYA activity because of competition for a common signal transduction component. The Arabidopsis phyB, phyD, and phyE phytochromes regulate plant developmental and growth responses to continuous red light and the ratio of red to far-red light. They are also more highly related in sequence to each other and more recently derived evolutionarily than phyA and phyC. Since the complementation analysis using phyB-1 null mutant with PhyB promoter driving expression of the PHYB, PHYD, or PHYE coding sequences indicated that only PB-phyB transgene complements red light hypocotyl growth phenotype ultimately demonstrates that Arabidopsis phyB-related apoproteins differ significantly in their capacities to signal in various seedling and adult plant phytochrome responses.

Under pulses of R, phyB is very active in inducing a dwarf hypocotyl phenotype, whereas phyD and phyE are inactive. Under high-fluence continuous R, phyD shows a gain in activity, whereas phyE does not. These demonstrate significant differences in the inherent regulatory activities of these receptor apoproteins. To localize the sequence determinants of these functional differences, chimeric proteins were constructed by shuffling amino-terminal, central, and carboxy-terminal regions of phyB and phyD. Overexpression analysis of the phyB/D chimeras shows that the central region of these proteins is most critical in determining their respective activities (Goosey et al., 2003b).

Functions of Phytochrome:

Plants use these photoreceptors to accurately sense and respond to light intensities that vary over seven to eight orders of magnitude. Phytochromes regulate gene expression in photosynthesis, photomorphogenesis, and the circadian clock. Phytochromes also play a role in plant defense against pathogens and regulating plant interactions with other organisms, such as insects and fungi. In addition to their physiological roles, phytochromes are also used in agriculture and horticulture to control plant growth and development. For example, phytochromes are used to manipulate flowering time in crops and control ornamental plants' elongation. Phytochromes are essential photoreceptor proteins in plants, allowing them to respond and adapt to changing light conditions. They have critical applications in agriculture and horticulture. Plants sense many aspects of light in their environment, including its wavelength, duration, intensity, and direction. Light significantly affects seedlings' morphogenesis during the transition phase of plants from heterotrophic to photoautotrophic growth.

Some light responses, such as the induction of nuclear-encoded genes encoding the light-harvesting chlorophyll proteins of photosystem II (LHCB), can be initiated by fluences as low as 0.1 nmol m^{-2}. These are classified as very-low fluence responses (VLF) (e.g., transcription of specific photosynthetic genes). Other responses (e.g., Lettuce seed germination), which cannot be initiated until the fluence reaches one mmol m^{-2}, are referred to as low-fluence (LF) responses.

Lastly, responses such as stem growth inhibition and floral induction, and inhibition of hypocotyl elongation, which are elicited by prolonged or continuous irradiation by fluences>10 mmol m^{-2}, are known as the high-irradiance response (HIR), phytochromes are responsible for VLF, LF, and HIR responses to red and far-red light (Mancinelli and Rabino, 1978; Kaufman et al., 1984). LF and high-fluence detection systems mediate blue light responses (Warpeha and Kaufman, 1990). The phototropin (phot1 and phot2) represent a new class of receptor kinases that appear exclusive to plants and absorb blue and UV-A light. Genetic analysis has shown that phot1 and phot2 exhibit partially overlapping functions in mediating phototropism, chloroplast migration, and stomatal opening in Arabidopsis (Briggs and Christie 2002).

Seedling development of higher plants best illustrates the effects of light on plant development—short photo exposure to the roots induced leaf opening. However, any injury to the primary root inhibited leaf formation (Mishra et al., 2001). Seedlings of dicotyledonous plants can follow two distinct strategies of development, skotomorphogenesis in darkness or photomorphogenesis in light. Dark-grown seedlings have long hypocotyl, unopened apical hooks, and underdeveloped (small and unopened) cotyledons that contain etioplasts. Once the seedling perceives sufficient light, it will de-etiolate, a developmental process that optimizes the body plan of the seedling for efficient photosynthetic growth. During de-etiolation, the apical hook opens, cotyledons expand, chloroplasts develop, and a new gene expression program is induced. The role of vascular tissue in conducting light was analyzed in Vessels, fibers (both xylem and phloem fibers), and tracheids in woody plants are shown to conduct light efficiently along the axial direction of both stems and roots via their lumina (vessels) or cell walls (fibers and tracheids). Other components, such as sieve tubes and parenchyma cells, are not efficient axial light conductors. This indicates that signals from the external light environment can enter the interior of stems above ground and are conducted by vascular tissue toward roots underground.

Conclusion:

Light availability is a crucial factor regulating appropriate responses to competition from the neighbor during vegetative growth. Later in vegetative development, light sensing allows plants to time the transition to reproductive growth properly. When the seedlings of flowering plants are grown in the dark (etiolated), they cannot make chlorophyll because the enzyme that reduces protochlorophyllide to chlorophyllide- protochlorophyllide oxidoreductase (POR) –requires light. The light conducted probably contributes directly to photomorphogenic activities within them. Of the various photoreceptors, the most intensively studied is a family of photoreversible red/far-red absorbing chromoproteins called phytochromes which mediates many important process in plants.

References:

Ahmad, M. and Cashmore, A.R. (1996) Seeing blue: The discovery of cryptochrome. Plant Mol. Biol. 30: 851-861.

Beggs, C.J. and Wellman, E. (1985) Analysis of light controlled anthocyanin formation in coleoptiles of Zea mays L. The role of UV-B, blue, red, and far-red light. Photochem. Photobiol. 41: 481-486.

Boylan, M., Douglas, N., and Quail, P.H. (1994) Dominant negative suppression of Arabidopsis photoresponses by mutant phytochrome A sequences identifies spatially discrete regulatory domains in the photoreceptor. Plant Cell 6: 449-460.

Briggs, W.R. and Christie, J.M. (2002) Phototropins 1 and 2: versatile plant blue-light receptors. Trends Plant Sci. 7: 204-10.

Briggs, W.R. and Olney, M.A. (2001) Photoreceptors in plant photomorphogenesis to date. five phytochromes, two cryptochromes, one phototropin, and one superchrome. Plant Physiol. 125: 85-8.

Cherry, J.R., Hondred, D., Walker, J.M. and Vierstra, R.D. (1992) Phytochrome requires the 6kDa-N terminal domain for full biological activity. Proc. Natl. Acad. Sci. USA 89: 5039-5043.

Cherry, J.R., Hondred, D., Walker, J.M., Keller, J.M., Hershey, H.P., and Vierstra, R.D. (1993) Carboxy-terminal deletion analysis of oat phytochrome A reveals the presence of separate domains required for structure and biological activity. Plant Cell. 5: 565-575.
Christie, J.M. and Jenkins, G.I. (1996) Distinct UV-B and UV-A/blue light signal transduction pathways induce chalcone synthase gene expression in Arabidopsis cells. Plant Cell 8: 1555-1567.
Christie, J.M., Salomon, M., Nozue, K., Wada, M. and Briggs, W.R. (1999) LOV (light, oxygen, or voltage) domains of the blue-light photoreceptor phototropin (nph1): binding sites for the chromophore flavin mononucleotide. Proc Natl Acad Sci U S A. 96: 8779-83.
Clack, T., Mathews, S. and Sharrock, R.A. (1994) The phytochrome apoprotein family in Arabidopsis is encoded by five genes: the sequence and expression of PHYD and PHYE. Plant. Mol. Boil. 25: 413-427.
Deforce, L., Tomizawa, K.-I., Ito, N., Farrens, D., Song,P.-S., and Furuya,M. (1991) In vitro assembly of apophytochrome and apophytochrome deletion mutants expressed in yeast and phycocyanobilin. Proc. Natl. Acad. Sci. USA 88: 10392-10396.
Edgerton, M.D. and Jones, A.M. (1993) Subunit interactions in the carboxy-terminal domain of phytochrome. Biochemistry 32: 8239-8245.
Edgerton, M.D., and Jones, A.M. (1992) Localization of protein-protein interactions between subunits of phytochrome. Plant Cell 4: 161-171.
Emmler, K., Stockhaus, J., Chua, N.-H. and Scafer, E. (1995) An amino-terminal deletion of rice phytochrome A results in a dominant negative suppression of tobacco phytochrome A activity in transgenic tobacco seedlings. Planta 197: 103-110.
Furuya, M. and Schafer, E. (1996) photoperception and signaling of induction reactions by different phytochromes. Trends Plant Sci. 1: 301-307.
Gartner, W., Hill, C., Worm, K., Braslavsky, S.E., and Scaffner, K. (1996) Influence of expression system on chromophore binding and preservation of spectral properties in recombinant phytochrome A. Eur. J. Biochem. 236: 978-983.
Jones, A.M., and Edgerton, M.D. (1994) The anatomy of phytochrome, a unique photoreceptor in plants. Semin. Cell. Biol. 5: 295-302.
Kaufman, L.S., Thompson, W.F. and Briggs, W.R. (1984) Different red light requirements for phytochromes-induced accumulation of cab and rbcS RNA. Science 226: 1447-1449.
Kendrick, R.E. and Kronenberg, G.H.M. (1994) Photomorphogenesis in Plants. Dordrecht, The Netherlands: Kluver. 2nd Edition.
Lagarias, J.C., and Rapoport, H. (1980) Chromopeptides from phytochrome. The structure and linkage of the Pr form of the phytochrome chromophore. J.Am. Chem. Soc. 102: 4821-4828.
Mancinelli, A.L. and Rabino, I. (1978) The high irradiance responses of plant photomorphogenesis. Bot. Rev. 44: 129-180.
Mishra, N.S., Mallick, B.N. and Sopory, S.K. (2001) Electrical signal from root to shoot in Sorghum bicolor: induction of leaf opening and evidence for fast extracellular propagation. Plant Sci. 160: 237-245.
Pham VN, Kathare PK, Huq E. Phytochromes and Phytochrome Interacting Factors. Plant Physiol. 2018 Feb;176(2):1025-1038. doi: 10.1104/pp.17.01384. Epub 2017 Nov 14. PMID: 29138351; PMCID: PMC5813575.
Pratt, L.H. (1995) Phytochromes-differential properties, expression patterns and molecular evolution. Photochem. Photobiol. 61: 10-21.
Quail, P.H., Boylan, M.T., Parks, B.M., Short, T.W., Xu, Y., and Wagner, D. (1995) Phytochromes: Photosensory perception and Signal Transduction. Science 268: 675-680.
Reed, J.W., Nagatani, A., Elich, T.D., Fagan,M., and Chory,J. (1994) Phytochrome A and phytochrome B have overlapping but distinct functions in Arabidopsis development. Plant Physiol. 104: 1139-1149.

Sakamoto. K. and Nagatani, A. (1996) Nuclear localization activity of phytochrome B. Plant J. 10: 859-868.
Sharrock, R.A. and Quail, P.H. (1989) Novel Phytochrome sequences in arabidopsis thaliana: structure, evolution, and differential expression of a plant regulatory photoreceptor family. Genes Dev. 3: 1745-1757.
Shinomura, T., Nagatani, A., Chory, J., and Furuya, M. (1994) The induction of seed germination in Arabidopsis thaliana is regulated principally by phytochrome B and secondarily by phytochrome A. Plant Physiol. 104: 363-371.
Smith, H. (1995) Physiological and ecological function within the phytochrome family. Annu. Rev. plant. Physiol. Plant Mol. Boil. 46: 289-315.
Somers, D.E., Sharrock, R.A., Tepperman, J.M., and Quail, P.H. (1991) The hy3 long hypocotyls mutant of Arabidopsis is deficient in phytochrome B. Plant Cell. 3: 1263-1274.
Vierstra, R.D. (1993) Illuminating phytochrome functions. Plant Physiol. 103: 679-684.
Wahleithner, J.A., Li, L.. and Lagarias, J.C. (1991) Expression and assembly of spectrally active recombinant holophytochrome. Proc. Natl. Acad. Sci. USA. 88: 10387-10391.
Warpheha, K.M.F. and Kaufman, L.S. (1990) Two distinct blue-light responses regulate epicotyl elongation in pea. Plant Physiol. 92: 495-499.
Wong, Y.-S., Cheng, H.-C., Walsh, D.A., and Lagarias, J.C. (1996) Phosphorylation of Avena phytochrome in vitro as a probe for light induced conformational changes. J. Biol. Chem. 261: 12089-12097.
Young, J.C., Liscum, E. and Hangarter, R.P. (1992) Spectral dependence of light-inhibited hypocotyls elongation in photomorphogenic mutants of Arabidopsis: evidence for a UV-A photosensor. Planta 188: 106-114.