CSIR NET Life Science Unit 6

Phytochromes

Phytochrome is a photomorphogenic pigment that absorbs red and far-red light and causes photomorphogenesis. It also absorbs blue light. Phytochromes have Been found in most plants where It regulates many growths and developmental processes such as photoperiodic Induction of flowering, chloroplast development (not including chlorophyll synthesis), leaf senescence, leaf abscission, seed germination, stem elongation, etc. Phytochrome is a family of chromo proteins with small covalently bound pigment molecules. Phytochrome proteins occur as a dimer of two ~125 kDa polypeptides, each with a covalently attached pigment molecule. The pigment is called the *chromophore*. It is a linear tetrapyrrole termed **phytochromobilin** and similar in structure to mammalian bile pigments, bilirubin. Phytochromobilin is synthesized in the plastids and its precursor is δ-aminolaevulinic acid. Together, the apoprotein (polypeptide chain) and its chromophore make up the holoprotein. Assembly of a proprotein with its chromophore is autocatalytic and occurs spontaneously.

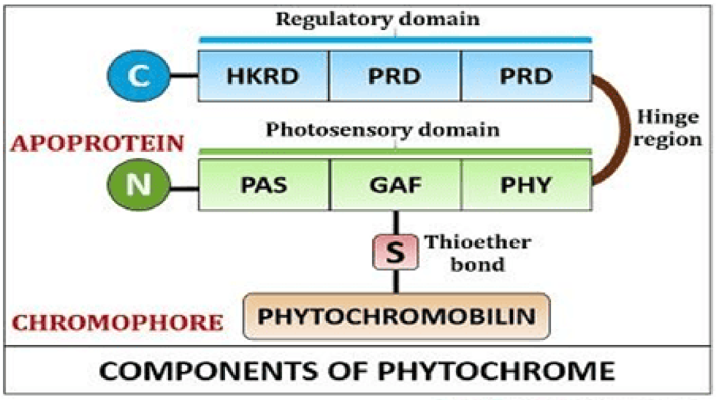
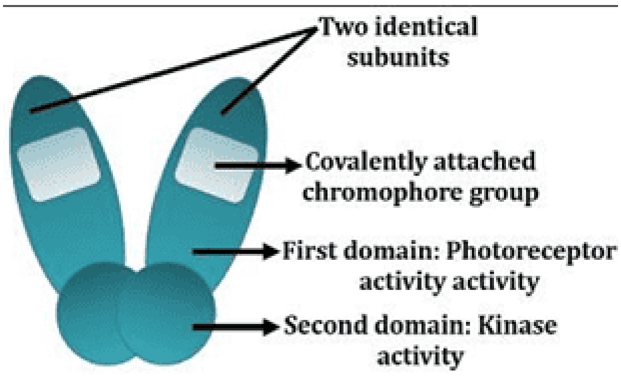


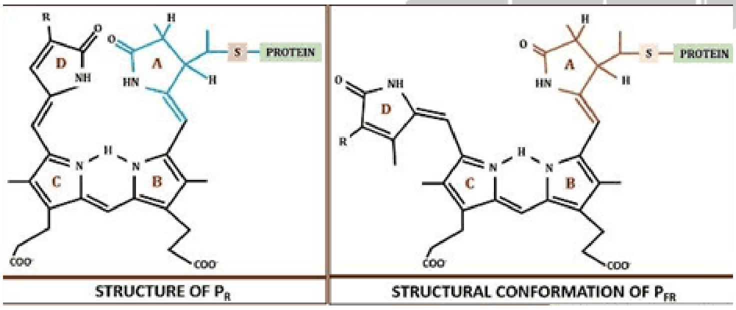
Fig: A structural domain is a phytochrome. The N-terminal half contains a PAS domain, GAF domain, and PHY domain. The PAS-GAF-PHY domains comprise the *photosensory region* of phytochrome. The C-terminal half contains two PAS-related domains (PRD) that mediate phytochrome dimerization and a histidine kinase-related domain (HKRD). B. In the structure of phytochrome, the regulatory domain contains the dimerization domain and the histidine kinase-related domain.



Structure of Phytochrome

The phytochrome pigment is found to be present in two photo reversible forms:

1. Red light-absorbing (666 nm) Pr form
2. Far-red light-absorbing (730- nm) Pfr form



The ***Pr form absorbs red light (at a peak of 660 nm*** bright blue in color and inactive form. When it absorbs red light it is converted to the ***Pr form***. The ***Pfr form absorbs far-red light (maximum at 730 nm),*** green (olive) in color, and active form. When it absorbs far-red light, it is converted to the Pr form. Pfr can also spontaneously revert to the Pr form in the dark over time (dark reversion).

$$P \underset{Far-red\ light}{\overset{Red\ light}{\rightleftarrows}} P_{fr} \rightarrow \textbf{Numerous responses}$$

- When the chromophore absorbs light, there is a slight change in its structure. This causes a change in the conformation of the protein portion to the form that initiates a response. The action spectrum of light needed for phytochrome-mediated responses shows a peak in the red at about 666nm. These responses can be reversed by an application of far red-light (peak at 730 nm) soon after the red treatment.
- The change in absorbance is caused by the conversion of the photoreceptor from one structural form to another. The red-absorbing

form changes to the far-red absorbing form when it absorbs red light (666 nm) and back again when it absorbs far-red light (730 nm). Upon absorption of light, the Pr chromophore undergoes a cis-trans isomerization in the C-15 and C-16 double bonds. Very dim light will work if the irradiation time is enough. This reciprocal between fluence rate and time is known as the law of reciprocity. Both VLFRs and LFRs obey the law of reciprocity.

- The **HIRs** require prolonged exposure to the light of a relatively high photon family. The HIR does not show red and far-red Photoreversibility and does not obey the law of reciprocity.

	VLFR	**LFR**	**HIR**
Photoreversibility	***No***	***Yes***	***No***
Reciprocity	***Yes***	***Yes***	***No***
Fluence requirement	***<1 m mol m^{-2}***	***1-1000 mmol m^{-2}***	***>1000 mmol m^{-2}***
Photoreceptor	***PHYA***	***PHYB, PHYD and PHYE***	***Dark grown -phy A; Light brown – phy B***
Active wavelength	***Red and Blue***	***Red and Fag-red***	***Dark Brown -far-red and blu Light grown-red***

Between C and D rings of the linear tetrapyrrole. However, a recent NMR analysis showed that the A pyrrole ring around C4-C5 double bond rotates during photoconversion.

- ***Phytochrome in all plants is synthesized in dark entirely as Pr. Absorption of red light by Pr converts it to Pfr and absorption of far-red light by Pfr converts it to Pr.*** However, the absorption spectra of the two forms overlap in the red region of the spectrum.

- When Pr molecules are exposed to red light, most of them absorb it and are converted to Pfr, but some of the Pfr also absorb the red light and are converted back to Pr because both Pr and Pfr absorb red light.
- Thus, the proportion of phytochrome in the Pfr form after saturating irradiation by red light is only about 85%.
- Similarly, a very small amount of far-red light also absorbed by Pr makes it impossible to convert Pfr entirely to Pr by broad-spectrum far-red light. Instead, an equilibrium of 97% Pr and 3% Pfr is achieved.
- ***This equilibrium is termed the photostationary state. Both Pr and Pfr forms also absorb blue light. Hence phytochrome effects can also be mediated by blue light.***
- There are two different cases of phytochrome with distinct properties.

These have been termed Type I and Type II phytochromes.

Phytochromes proteins are enclosed by the phytochrome gene family termed PHY.

Its five members are PHYA, PHYB, PHYC, PHYD, and PHYE.

- **Type I phytochrome, which is encoded by the PHYA gene, is abundant in dark-grown plants (etiolated plants).** Its concentration decreases rapidly upon exposure to light as a result of transcription inhibition, mRNA degradation, and proteolysis.

- **Type II phytochrome (encoded by the PHYB, PHYC and PHYD, and PHYE genes) is light-stable and present in both light-grown and dark-grown plants**. In light-grown plants, PHYB is the most abundant phytochrome, **whereas PHYC-PHYE is less abundant.**

Phytochrome response

Phytochrome responses can be distinguished by the amount of light required. The number of photons impinging on the unit surface area is termed fluence. Its unit is mol m^{-1} Fluence rate, that is fluence per unit time, is termed as irradiance. Its unit is mol m^{-2} $sec^{-1.}$ Different phytochrome's responses occur at different light furnaces. These responses (LFRs) and high-irradiance responses (HIRs).

- **VLFR** can be initiated by fluences below I mmol m^{-} Because of the amount of light that is needed to induce a response in a minute, a very small amount (less than 0.02%) of the total phytochrome is converted to Pfr. Thus far-red light cannot reverse VLFRs.

- **LFRs** are initiated by fluences of 1 m mom-2 and they are saturated at 1000 mmol-2.
- The classical red and far-red photoreversible responses of phytochromes which were discovered by Hendricks and Borthwick are examples of LRFs.
- These responses show phytochromes which were discovered by **Hendricks and Borthwick** are examples of LFRs. These responses show photo reversibility because the far-red light reverses the responses.
- Both VLFRs and LFRs can be induced by low fluence. The total fluence is a function of two factors: the fluence rate and the irradiation time. Thus a brief pulse of red light will induce a response.