Photoreceptors as Light Sensors in Plants

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Abstract **- Photoreceptors are the proteins that are specially designed to perceive light and signal certain biological effects in the plant. Phytochromes are a class of photoreceptor in plants, bacteria and fungi used to detect light. They are sensitive to light in the red and far-red region of the visible spectrum. Cryptochromes are a class of flavoproteins found in plants and animals that are sensitive to blue light. They are involved in the circadian rhythms and the sensing of magnetic fields in a number of species. Phototropins are plant-specific blue light receptors for phototropism, chloroplast movement, leaf expansion, and stomatal opening. All these responses are thought to optimize photosynthesis by helping to capture light energy efficiently, reduce photodamage, and acquire CO2. ZEITLUPE (ZTL), a photoreceptor with E3 ubiquitin ligase activity, communicates end-of-day light conditions to the plant circadian clock.**

Index Terms **- Cryptochrome, phototropin, phytochrome, UVR8, ZEITLUPE.**

INTRODUCTION

The receptor molecules used by plants to detect sunlight are termed photoreceptors. Photoreceptors absorb a photon of a particular wavelength and utilize this energy, thus initiating a photoresponse (Galvão and Fankhauser, 2015). All the known photoreceptors (except UVR8) consist of proteins bound to non-protein light-absorbing prosthetic groups (chromophores). Protein structures of the different photoreceptors vary and are involved in regulation of downstream signaling. Other common aspects of photoreceptors include sensitivity to light (quantity, quality, and intensity) and the time duration of photoperiod. Photoreceptors help plants regulate developmental processes over their lifetime by sensitizing them to incident light. They also initiate protective processes in response to harmful radiations.

Plant photoreceptors fall in various categories (Briggs and Olney, 2001):

- Phytochromes
- Cryptochromes
- Phototropins
- UVR8
- ZEITLUPE (ZTL) family

The action spectra of light-regulated responses demonstrate that plants are highly sensitive to UV-B radiation (280-320 nm), UV-A radiation (320-380 nm), blue light (380-500 nm), red light (620-700 nm), and far-red light (700-800 nm). UV-B radiation is detected by the UV RESISTANCE LOCUS 8 (UVR8) protein, UV-A and blue light by cryptochromes, phototropins (Christie and Briggs, 2001) and red and far-red light by phytochromes. ZEITLUPES, blue light absorbing forms, are light-responsive F-box proteins that target proteins functioning in the circadian rhythms and the photoperiodic regulation of flowering. Phytochrome and cryptochrome are soluble receptors that function primarily in the nucleus to regulate the activity of transcription factors. These two classes collectively regulate developmental responses to light intensity and also mediate responses to variations in the spectral quality of light that indicate shading by other plants.

Phytochromes

The discovery of a ubiquitous plant pigment, the phytochrome , and the evidence that it regulates almost every aspect of a plant's response to light was a path-breaking discovery for plant science. The discovery team at the United States Department of Agriculture (USDA), Beltsville Agricultural Research Center (BARC) in Beltsville Maryland, USA got motivated by the experimental work of Borthwick and Parker in the 1930s, which quantified phenomenon of photoperiodism and alongwith Hendricks, a renowned soil chemist, searched for a chemical basis for non-photosynthetic processes of plants to light. In 1940-1960, they speculated the presence of a photoreversible pigment regulating photoperiodism in flowering, seed germination, and several other photomorphogenic responses in plants. It was also

noticed that the red light responses were reversed by far-red light. In 1959, the biophysicist Butler and biochemist Siegelman, using a specialized spectrophotometer, finally isolated the phytochrome pigment from dark-grown 'corn' seedlings. In 1964, Siegelman and Firir extracted phytochrome from oat seedlings in a highly purified state and also analyzed its structure.

Quail and Lagarias purified the intact phytochrome molecule in 1983 and subsequently, in 1985, Hershey and Quail published its first gene sequence. By 1989, molecular genetics studies revealed the existence of more than one type of phytochrome, e.g., the pea plant possessed two phytochromes (type I and type II predominant in etiolated seedlings and green plants respectively). Recently, technique of genome sequencing has confirmed that Arabidopsis has five phytochrome genes (PHY A,B,C,D,E), the rice plant has only three (PHY A,B,C), and maize has six phytochromes (PHY A1, A2; PHY B1,B2; PHY C1,C2). All these phytochromes use considerably different protein parts but possess common phytochromobilin as their light-harvesting chromophore. Phytochrome A is quickly destroyed in the Pfr form compared to other members of the family. In the late 1980s, it was established that Phytochrome A is destroyed by the ubiquitin system, a major intracellular protein degradation system identified in eukaryotes.

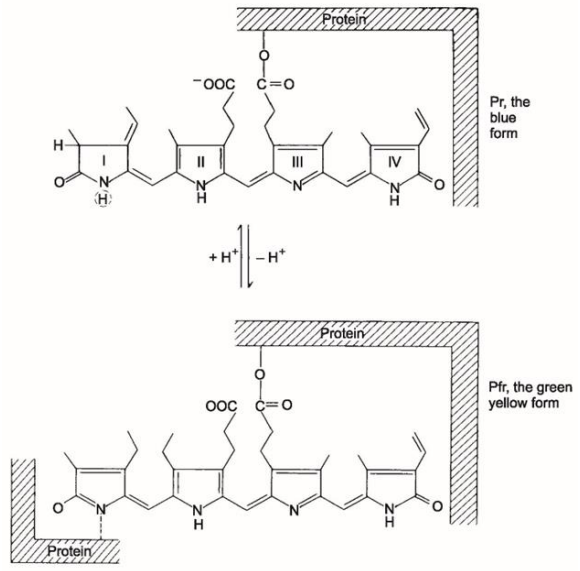

Fig1. The chemical structure of a plant phytochrome molecule .

Phytochrome is a large dimeric protein made up of two equivalent subunits. The monomer has a molecular mass of 124,000 daltons with a high proportion of polar amino acids and is water-soluble . It is believed to be a blue-green biliprotein and is covalently bound to an open chain tetrapyrrole chromophore molecule as shown in Fig 1and 2 (Rockwell et al., 2006). It was established by Borthwick and Hendricks that phytochrome exists in two forms - Pr (red light absorbing form) and Pfr (far-red light absorbing form) as shown in Fig. 3. Initial studies in short-day plants in late 1940s suggested that red light was most effective as a light – break, with a maximum effectiveness near 660 nm. Borthwick and his colleagues also observed that red light inhibition of flowering in Xanthium could be reversed with far-red. At about same time, photoreversibility of seed germination was noticed. Thus, red/far-red photoreversibility of the light-break indicates the role of phytochrome in the control of flowering in short-day and long-day plants. Studies on phytochrome mutants in Arabidopsis have suggested that PHY A is essential to induce flowering of long-day plants under certain conditions, whereas PHY B seems to inhibit flowering. It has been confirmed now Pfr form induces flowering in long-day plants and Pr form in short-day plants (Fig 4).

A wide variety of developmental responses are controlled by phytochrome:

- Leaf expansion and stem elongation
- Germination of many seeds
- Straightening of the hook-shaped shoot of many seedling (unfolding of plumular hook)
- Phototropism
- Geotropism
- Nyctinastic movements (day-night movements of leaves)
- Orientation response of chloroplasts in certain algae (Mougeotia), i.e., the chloroplasts remain flat under low light intensities but rotate within the cell through an angle of 90°, or at higher intensities the chloroplast 'edge-on' to the light.
- Regulate many biochemical phenomenon, e.g., formation of protochlorophyll as well as carotenoids in the leaves, and of anthocyanin and flavonoid pigments in flowers, stems and fruits.
- Photoperiodic responses.
- The cytosolic Ca2+ concentration

- Fern spore germination.
- Sex expression
- Regulation of nitrate reductase activity
- Pollen germination
- Differentiation of stomata and tracheary elements
- Tanada effect

Mode of action: Phytochrome-mediated responses are linked to considerable changes in gene expression (Fig 5).

(i) Phytochrome- interacting factors (PIF) found in the nucleus inhibit the expression of phytochrome-dependent genes. PIF protein acts as a negative regulator and perhaps supresses transcription via attaching to the promotor site of a photoresponsive gene. Most of the PIF proteins remain intact in the darkness, but are rapidly degraded in the light.

(ii) Phytochrome accumulates and remains in the cytosol as Pr (inactive) form in the dark. Upon irradiation with red light, Pr form is transformed to Pfr (active) form, which is then imported into the nucleus.

(iii) Pfr binds to PIF protein, which undergoes degradation by 26S-proteasome system, thereby activating transcription of phytochrome-responsive genes.

(iv) Far-red light will change the Pfr back to Pr.

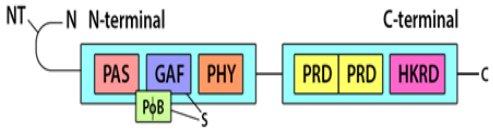

FIG 2. Schematic representation of a plant phytochrome domains. The N-terminal moiety of phytochrome contains the PAS-GAF-PHY domains, which comprise the chromophore-binding, photosensory region of phytochrome. The C-terminal moiety of phytochrome contains two PRD domains and a HKRD domain. A hinge region separates the N-terminal and C-terminal moieties of the molecule. The chromophore phytochromobilin (PØB) is attached to cysteine residues in the proteins by a thioester link (-S-).

PAS domain (Per-Arnt-Sim domain) ; GAF domain (domain named after the proteins it is found in :- cGMP-specific phosphodiesterases, adenyl cyclases and FhlA); NT (N-terminal extension); PRD (PAS-related domain); HKRD (Histidine kinase-related domain).

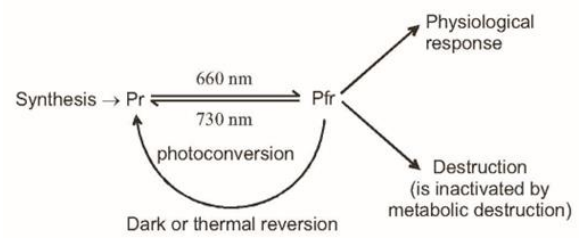

Fig 3. Two interconvertible forms of phytochrome, Pr and Pfr.

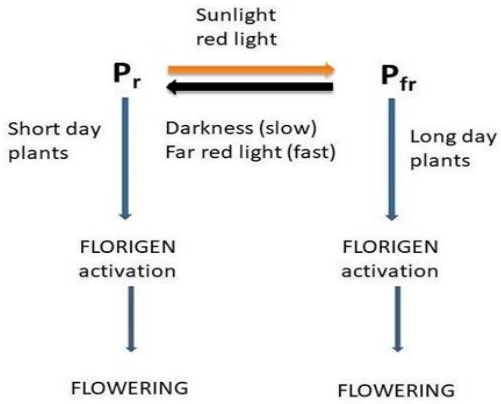

Fig 4. Phytochrome is produced in Pr form during dark period. In dark-grown plants, phytochrome is found in a red light absorbing form known as Pr. Thus red light (660 nm) would convert the pigment to a far-red absorbing form known as Pfr (730 nm) and the conversion was fully reversible. Pfr form promotes flowering in long-day plants and Pr form in short-day plants.

Phytochrome-regulated responses are categorized into three groups based on their energy demands- very low fluence responses (VLFRs), low-fluence responses (LFRs) and high-irradiance responses (HIRs).

VLFRs (e.g. Red-light induced germination of Arabidopsis seeds, red-light stimulated growth of coleoptile in etiolated oat seedlings, red-light induced inhibition of growth of mesocotyl in etiolated oat seedlings)

- These phytochrome responses can be induced by fluence as low as 0.0001µmol m-2. (Amount of light required to induce the responses is known as fluence. Total fluence = fluence rate x length of time of irradiation.)
- They become saturated at about 0.05 µmol m-2
- Non-photoreversible
- Obey the law of reciprocity (reciprocal relationship between fluence rate and time duration)

LFRs (e.g. Promotion of lettuce seed germination, inhibition of hypocotyl elongation, regulation of leaf movements, promotion of de-etiolation, seed germination)

- These phytochrome responses cannot be induced until the fluence reaches 1.0 µmol m-2
- They become saturated at about 1000 µmol m-2
- Photoreversible
- Obey the law of reciprocity

HIRs (e.g. Synthesis of flavonoids in various dicot seedlings, inhibition of hypocotyl elongation in mustard and lettuce seedlings, induction of flowering in Hyoscyamus, production of ethylene in Sorghum)

- These require continuous exposure to light of relatively high irradiance. Response is directly proportional to the irradiance until the response saturates and additional light has no further effect.
- HIRs saturate at much higher fluences as compared to LFRs-at least 100 times more.
- Non-photoreversible and time-dependent
- Don't obey the law of reciprocity

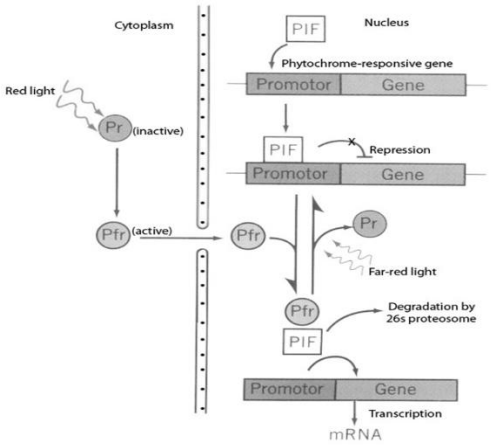

Fig 5. A general model for phytochrome action

Cryptochromes

Cryptochromes are photoreceptors of blue-light that regulate several blue-light responses, including suppression of elongation of hypocotyls, promotion of cotyledonary expansion, membrane depolarization, inhibition of petiole elongation, stomatal opening and closing, anthocyanin production, and circadian clock entrainment (Chentao and Takeshi, 2005). Cryptochrome1 (CRY1) was originally identified in Arabidopsis in 1993 (Ahmad and Cashmore, 1993) and later discovered in many organisms, including cyanobacteria, ferns, algae, fruit, flies, mice, and humans (Thompson and Sancar, 2004).

The photoreceptor is believed to be chromoprotein, made up of two parts: a chromophore, or light absorbing moiety, and a protein, called apoprotein. The general consensus is that cryptochrome is a flavin pigment. The three most commonly occurring flavins are riboflavin and its two nucleotide derivatives-flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The flavin may be present in free state or associated with proteins- flavoprotein, and are currently the most favoured candidates for cryptochrome. They represent a very small part of the vast pool, thus making it difficult to isolate and unequivocally establish its physiological role. Cryptochrome possesses two chromophores - FAD and a pterin, resembling with microbial DNA repair enzyme, photolyase. .

Plant cryptochrome can be considered like a molecular light switch where absorption of blue photons at the N-terminal photosensory site results in protein conformational changes at the C-terminus, which , in turn, initiates signaling by binding to specific partner proteins. Cryptochromes bind a flavin adenine dinucleotide (FAD) and the pterin 5,10-methyltetrahydrofolate (MTHF) as chromophores (Fig 6). Blue light absorption alters the redox status of the bound FAD chromophore, thus triggering photoreceptor activation. Activation mechanism involves a conformational change in cryptochrome, enabling it to bind to other protein partners. Dimerization of cryptochromes, mediated by the photolyase-like domain, may be important for their signaling. Cryptochromes also act as key regulators of a range of plant stress responses, such as drought, salinity, heat, and high radiation. The coaction of cryptochrome, phytochrome and phototropins affects the developmental functions such as stem elongation inhibition, and regulation of flowering as well as the circadian clock.

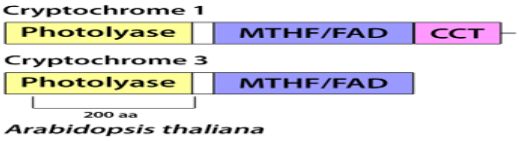

Fig 6. Cryptochromes (1and 3) from Arabidopsis thaliana depicting the photolyase like domain, FAD-binding domain, and the cryptochrome C-terminal domain (CCT).The light-capturing cofactor 5,10-methyltetrahydrofolate (MTHF) and the catalytic cofactor flavin adenine dinucleotide (FAD) are linked to the protein via non-covalent association.

Arabidopsis contains three cryptochrome genes : Cry1, Cry2, and Cry3. Cryptochrome homologs 1, 2, and 3 have different developmental impacts, and are strictly localized unlike phytochromes. While Cry1 and Cry2 are generally located in the nucleus, while Cry3 is confined to chloroplasts and mitochondria. Cry2 protein is preferably damaged under blue light, whereas Cry1 is much more stable. Unlike Cry1, Cry2 does not play any important role in stem elongation inhibition. In addition, Cry1, and to a lesser extent Cry2, is associated with the setting of the circadian clock in Arabidopsis, whereas Cry2 has a key role in the initiation of flowering. Nuclear cryptochromes inhibit COP1-induced protein degradation. Blue light-induced phosphorylation of cryptochrome appears to be important in modulating its activity i.e. maintaining the C terminus of Cry1 in an active conformation and in the case of Cry2, promoting its degradation.In addition to controlling the levels of transcription factors, cryptochrome can also directly attach to and modulate the functioning of specific DNA-binding proteins

Mode of action: During dark, gene CONSTITUTIVE PHOTOMORPHOGENESIS1 (COP1) in association with SUPPRESSOR OF PHYA 1(SPA1) and other factors, acts to damage transcription factors i.e. HY5, which induce the expression of genes required for photomorphogenesis (Müller and Bouly, 2015). Upon activation by blue light, the nuclear cryptochrome 1(cry1) forms a complex with SPA1 and COP1 that prevents them from acting, thus terminating the breakdown of HY5 and other transcription factors that promote photomorphogenesis (Fig 7).

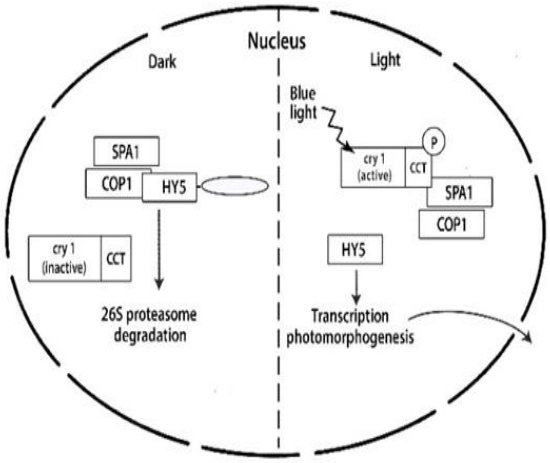

FIG 7. Regulation of photomorphogenesis by interaction of cry1 with COP1/SPA1 complex. The cryptochrome1 (cry1) is inactive in the dark and COP1/SPA1 complex degrades HY5, the transcription factor required for photomorphogenesis. Blue light activates cry1 and the activated cry1 forms a complex with COP1/SPA1, thereby preventing them from degrading HY5 protein and promoting photomorphogenic development.

Phototropins

Phototropins were first discovered by the American biologist Winslow R. Briggs (1928-2019). Phototropins are plasma membrane-associated protein kinases responsible for regulating phototropism, light-induced chloroplast movement, control of stomatal aperture and regulation of hypocotyls and leaf expansion. Angiosperms contain two phototropin genes, PHOT1 and PHOT2. PHOT1, the primary phototropic receptor in Arabidopsis, regulates phototropism both in response to low and high fluence rates of blue light whereas PHOT2 mediates phototropism under high light intensities.

Each phototropin possesses two flavin mononucleotide (FMN) chromophores that can induce conformational changes. Phototropin consists of two light-sensing Light-Oxygen-Voltage (LOV) domains, LOV1 and LOV2, each bound to a chromophore flavin mononucleotide (Fig 8). LOV2 domain, in particular, is essentially required for blue light-induced kinase activation and autophosphorylation of the phototropin photoreceptor. The function of LOV1 domain might be in receptor dimerization. Blue light absorption by phototropins induces a conformational change that uncages their kinase domain, causing their autophosphorylation and induces signal transduction pathways that led to the redistribution of PIN auxin efflux carriers necessary for directional growth and to the changes in plasma membrane ion fluxes that regulate guard cell turgor. Blue light, sensed by phototropins, triggers the activation of H+-ATPase located in plasma membrane which ultimatey regulates opening of the stomata (Fig 9). Phototropins mediate chloroplast accumulation and avoidance responses to weak and strong light via F-actin filament assembly. However, chloroplasts move to the bottom of the cell in darkness (Fig 10).

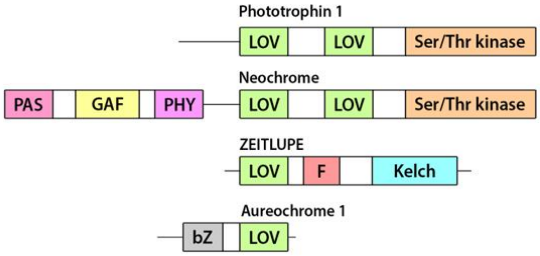

FIG 8. The domain compositions of phototropin and related LOV-domain phototropins such as, neochrome, ZEITLUPE, and aureochrome. Photoreceptors 1 and 3 belong to Arabidopsis, 2 and 4 to Adiantum and Vaucheria respectively.

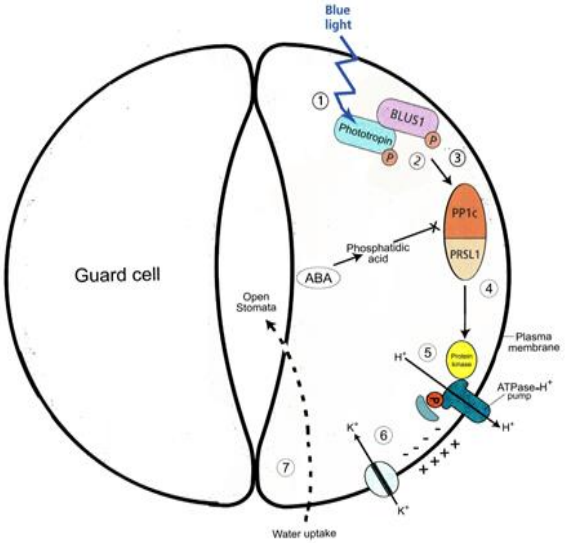

Fig 9. Phototropin signal transduction pathway (1) and (2) Phototropins get autophosphorylated on absorption of blue light and in turn, phosphorylate BLUE LIGHT SIGNALING 1(BLUS1).(3) and (4) BLUS1 controls a protein phosphatase1, PP1c (subunit PRSL1) , which in turn controls the activity of protein kinase . (5) The protein kinase promotes the binding of a regulatory protein (14-3-3) to the H+-ATPase pump confined to the plasma membrane. (6) and (7) Membrane hyperpolarization drives K+ uptake leading to water absorption and finally opening of stomata. ABA triggers the production of phosphatidic acid,which blocks PP1 activity.

PROTEIN PHOSPHATASE1 (PP1) is a serine/threonine protein phosphatase composed of a catalytic subunit (PP1c) and a regulatory subunit, PP1 REGULATORY SUBUNIT2-LIKE PROTEIN1(PRSL1)

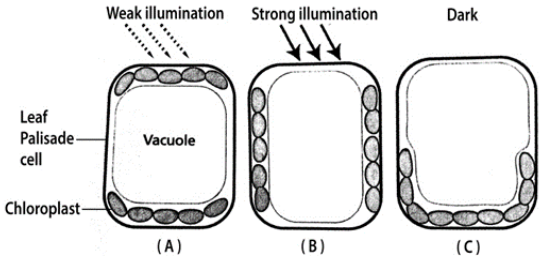

FIG 10. Distribution patterns of chloroplasts within palisade cells of Arabidopsis leaf in view of the changing illumination conditions (A) Under weak illumination, chloroplasts accumulate near the upper and lower sides of cells. (B) In strong illumination, the chloroplasts migrate to the lateral walls of cells. (C) In the dark, chloroplasts shift to the bottom surface of the cells. Redistribution of chloroplasts within the cells modulates light absorption and prevents photodamage.

UV RESISTANCE LOCUS 8 (UVR8)

Unlike phytochrome, cryptochrome, and phototropin, UVR8 lacks a prosthetic chromophore. UVR8 monomer is a seven-bladed β-propeller protein which, in the absence of UV-B radiations, forms functionally inactive homodimers.The two identical subunits of UVR8 are joined in the dimer by a meshwork of salt bridges created between tryptophan molecules, which function like primary UV-B sensors, and closeby arginine residues. On absorption of UV-B radiations, the tryptophan residues undergo structural changes that help in breaking the salt bridges, leading to the dissociation of the two functionally active monomers. Monomers interact with nuclear proteins, COP1-SPA1 complex, to activate the transcription of the transcription factor HY5, which is associated with the regulation of UV-B induced genes expression (Fig 11). Photomorphogenic responses to UV-B include increased photosynthetic efficiency, entrainment of circadian clock, stomatal density, leaf cell expansion, reduced hypocotyl elongation, flavonoid biosynthesis, endoreduplication in epidermal cells etc. Genes for RUP proteins are activated, which facilitate dimerization of UVR8 monomers, thereby inactivating them. The regenerated dimer then again gets ready for photoreception.

ZEITLUPE

ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA in blue light. ZEITLUPE recently discovered photoreceptor, senses blue light fluence to

mediate circadian timing in Arabidopsis thaliana (Brian and Takato, 2014). Further, it has been observed in Arabidopsis and Populus that ZEITLUPE also promotes ABA-induced stomatal closure.

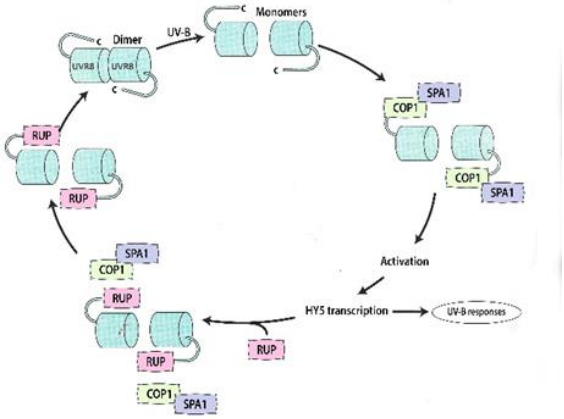

Fig 11. The UVR8 signaling pathway. The photoreceptor UVR8 (dimer) absorbs UV-B radiations and forms monomers. The monomers then interact with the COP1/SPA1 complex to activate the transcription of UV-B responses. RUP proteins get induced and facilitate dimerization of UVR8 monomers as well as dissociation of COP1/SPA1 complex. The regenerated dimer is ready for photoreception.

RUP proteins (REPRESSOR OF UV-B PHOTOMORPHOGENESIS proteins)

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