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Role of Cryptochromes in the plant growth and development

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Abstract:

Light regulates many aspects of plant growth and development, among which the conversion of etiolated leaves to photosynthetic competent green leaves (greening) is the most critical well-documented developmental process requiring light signals. Blue light responses are evolutionary among the most ancient and diverse light regulatory phenomena. Many such reactions in algal, bacterial, or fungal systems show action spectra consistent with a flavin chromophore, with activity peaks in the near UV (around 350nm) and blue (450-480nm) regions of the spectrum.

Cryptochromes:

Classically, responses to light have been grouped by the range of wavelengths that initiate the response and include UV-C (200-280nm), UV-B (280-320nm), UV-A (320-390nm), blue light responses (390-500nm) and red-far red responses (Batschauer, 1998; Khurana et al., 1998). Multiple blue /UV photoreceptors are known to be present in plants based on physiological evidence, such as the action spectrum and fluence dependence, and molecular evidence from cloning of several different photoreceptors with distinct functions assayed in corresponding mutants (Ahmad, 1999; Briggs and Liscum, 1997; Horwitz, 1994; Horwitz, and Berrocal, 1997; Jenkins, 1997; Jenkins et al., 1995; Lasceve et al., 1999).

Action spectra compiled from a number of such responses fit the general criteria of a flavin-type blue light photoreceptor; however, due in part to the difficulty in obtaining a good in vitro assay system for the flavin-type blue light-regulated phenomenon and in part to the numerous non-photoreceptor pigments absorbing in the blue (for instance, cytochromes, chlorophylls, carotenoids, and flavins bound to metabolic enzymes), there has until very recently been little progress towards identification of a receptor. In keeping with their elusive chemical identity, 'cryptochrome' has been proposed for such B-type receptors.

Cryptochrome Responses:

Specific blue light responses include phototropism, inhibition of hypocotyl elongation, and stomatal opening, which in many cases is thought to be mediated by flavin-type photoreceptors (Kaufman, 1993; Short and Briggs, 1994; Liscum and Hangarter, 1991). HY4 locus was identified in *Arabidopsis thaliana*. The hy4 mutant is one of several mutants which is specifically insensitive to blue light during the blue light-dependent inhibition of hypocotyl elongation response, which suggests that they lack the essential component of the cryptochrome-associated light-sensitive pathway. The HY4 gene was isolated by the method of gene tagging. HY4 encoded a protein that showed significant homology to the DNA photolyase of microbes.

Photolyases:

As photolyases are a rare class of flavoprotein, they catalyze blue light-dependent reactions (Sancar, G.B. 1990). The protein encoded by HY4 has a structure consistent with that of a flavin-type blue light photoreceptor. HY4 encoded a flavin-type photoreceptor mediating blue light-dependent inhibition of hypocotyl elongation in *Arabidopsis* (Ahmad and Cashmore, 1993).

It was referred to as CRY1, after cryptochrome, blue-UV-A photoreceptor. CRY1 was expressed as a soluble protein and purified to near homogeneity. Purified CRY1 was a yellow protein with an absorption spectrum resembling a flavoprotein. The chromophore was found to be noncovalently bound, completely released by heat or acid denaturation of CRY1. The absorption spectrum of the free chromophore was identical to that of fully oxidized flavin adenine dinucleotide (FAD). Despite the sequence homology between CRY1 and microbial DNA photolyases (Ahmad and Cashmore, 1993), CRY1 demonstrated no photolyase activity in vitro, and the expression of CRY1 could not rescue photolyase-deficient E.coli mutants. CRY1 is a non-photolyase, flavin-type photoreceptor (Ahmad and Cashmore, 1993).

The absorption spectrum of flavoproteins depends on the redox status of the bound flavin, which in turn is influenced by both the apoprotein to which the flavin is bound and the redox environment. CRY1 gene encoded a protein of 681 amino acids, of which the first 500 showed striking sequence homology with the microbial DNA photolyases. A distinction between CRY1 and the photolyases is the existence of a C-terminal extension of 200 amino acids beyond the homology region to the flavin-binding domain of photolyase, similar to rat smooth muscle tropomyosin. This domain can have a structural role or function in protein-protein interaction.

Photolyases harvest light energy employing a primary light-harvesting chromophore bound noncovalently at the amino-terminal of the molecule. This chromophore largely determines the action spectrum of photoreactivation; photolyases of the short wavelength type (absorbing maximally at 350-370nm), which have little activity in the blue, typically bind a pterin as the primary light-harvesting chromophore.

Photolyases of the long wavelength type, with absorption maximal around 450nm, typically bind 5-deazaflavin as the primary light-harvesting chromophore. Medium wavelength photolyases that absorb primarily in the blue (peak activity 410nm) but bind a pterin as primary light harvesting chromophores have been identified. In photolyases, trp (277) is highly conserved (utilized for substrate specificity), but this residue is not conserved in the case of cryptochromes, so they can not bind DNA.

The purified CRY1 receptor contrasted with photolyases, showing unexpected stability of a semiquinone redox intermediate that absorbed green light (500-600nm) (Lin et al., 1995).

Cryptochrome flavoproteins, which share sequence homology with light-dependent DNA repair photolyases, function as photoreceptors in plants and circadian clock components in animals. Coupled sequencing of an *Arabidopsis* cryptochrome gene with phylogenetic, structural, and functional analyses to identify a new cryptochrome class (cryptochrome DASH) in bacteria and plants, suggesting that cryptochromes evolved before the divergence of eukaryotes and prokaryotes. The cryptochrome crystallographic structure, reported for Synechocystis cryptochrome DASH, reveals commonalities with photolyases in DNA binding and redox-dependent function, despite distinct active-site and interaction surface features. Whole genome transcriptional profiling and experimental confirmation of DNA binding indicated that Synechocystis cryptochrome DASH functions as a transcriptional repressor.

CRY proteins:

The maximal activity of CRY1 is seen in the blue (400-500nm), with weaker responsivity to UV/A (300-400nm) and green light (500-600nm). CRY1 protein is found to be associated with the soluble fraction, but a small fraction may remain associated with the membrane pellet (Lin et al., 1995). CRY1 is expressed in all tissues (cotyledon, hypocotyl, roots) of seedlings and mature plants, and the expression does not appear light-regulated. During CRY1 function, rapid depolarization at the plasma membrane is observed during blue light-stimulated inhibition of hypocotyl elongation. Another CRY1-related sequence was detected in the *Arabidopsis* genome, and it was named CRY2. The amino acid sequence has extensive homology to CRY1 in the photolyase-like domain (57% identity) but a C-terminal extension of 100 amino acids with very little (15%) sequence relatedness to CRY1. CRY2 is almost identical (89%) to a presumed photolyase gene from mustard (*Sinapis alba*). However, it lacks the C-terminal extension of 100 amino acids in CRY2.

CRY1-related sequences have been identified in tomatoes, peas, and rice. Recently, a sequence with 49% amino acid sequence identity to CRY1 in the first 500 amino acids has been identified in *Chlamydomonas rheinhardtii*, mapping to a site on the genome distinct from that encoding photolyase activity (Small et al., 1995). The *Chlamydomonas* gene, named CPH1, has a 300 amino acid C-terminal extension with no homology to that of CRY1 or CRY2 and thereby almost certainly represents another cryptochrome photoreceptor of the CRY gene family.

The molecular mechanism of cryptochrome function in Arabidopsis is becoming increasingly evident. Studies show that CRY1 and CRY2 are localized in the nucleus and that CRY2 is regulated by blue light-dependent phosphorylation. Despite these advances, no positive cryptochrome signaling component has been identified. A novel Ser/Thr protein phosphatase (AtPP7) with high sequence similarity to the Drosophila retinal degeneration C protein phosphatase is an intermediate in blue light signaling. Transgenic Arabidopsis seedlings with reduced AtPP7 expression levels exhibit hypocotyl growth inhibition loss and limited cotyledon expansion in response to blue light irradiation. These effects are as striking as those seen in hy4 mutant seedlings deficient in CRY1.

AtPP7 transcript levels are not rated limiting, and AtPP7 probably acts downstream of cryptochrome in the nucleus, ensuring signal flux through the pathway. AtPP7 acts as a positive regulator of cryptochrome signaling in *Arabidopsis* (Moller et al., 2003).

The putative bHLH (basic Helix Loop Helix) transcription factor long hypocotyl in far-red (HFR1) is vital for a subset of phytochrome A (phyA)-mediated light responses. Interestingly, when grown in blue light, hfr1 alleles also have reduced de-etiolation responses, including hypocotyl growth, cotyledon opening, and anthocyanin accumulation. This phenotype is particularly apparent under high fluence rates. The analysis of double mutants between hfr1 and different blue light photoreceptor mutants demonstrates that, in addition to its role in phyA signaling, HFR1 is a component of cryptochrome 1 (cry1)-mediated light signaling. Moreover, HFR1 mRNA levels are high in blue and far-red light but low in red light. These results identify HFR1 as a positively acting component of cry1 signaling and indicate that HFR1 integrates light signals from phyA and cry1 (Duek and Fankhauser. 2003).

Coaction between different photoreceptors:

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. (Whitelam et al., 1997; Mohr, H. 1986). Studies have shown that other photoreceptors coordinate to mediate light-induced plant development, referred to as the coaction of photoreceptors (Sellaro et al., 2009; Su et al., 2017; Wang et al., 2018). This coaction may involve photo-regulatory protein kinases (Casas-Mollano et al., 2008; Wang et al., 2015b; Huang et al., 2016; Liu et al., 2017; Ni et al., 2017; Su et al., 2017b). Phototropism, by contrast, appears primarily to use a single photoreceptor, NPH1, with some influence from cryptochromes and phytochromes (Christie et al., 1998; Janoudi et al., 1997; Ahmad et al., 1998). Phototropism has long been suspected to require protein kinase activity because it is correlated with blue-light-induced phosphorylation of a 120 kDa protein (Short and Briggs. 1994).

NPH1 protein has a carboxy-terminal domain with all the serine/threonine protein kinase signatures. The amino terminus has two repeats of about 110 amino acids- known as LOV domains-that are related to motifs in a large group of sensor proteins. Interestingly, the LOV domains also relate to the better-known PAS domains found in several regulatory proteins, including phytochromes (Zhulin et al., 1997). Cristae et al. (1998) have shown that NPH1 is the photoreceptor that mediates phototropism. Recombinant NPH1 is a chromoprotein that binds noncovalently to flavin mononucleotide (FMN), with spectral properties very similar to the action spectrum for phototropism in *Arabidopsis*. Therefore, therefore likely that NPH1 binds to this same chromophore in plants. NPH1's LOV/PAS domains mediate the binding of the chromophore; this is a distinct possibility, as LOV/PAS domains sometimes serve as protein-protein interaction domains and bind a prosthetic group in other proteins (Zhulin et al., 1997). Mutations in a similar realm of the *Neurospora* WC-1 blue light photoreceptor result in blind strains, further emphasizing the importance of the LOV domain in blue light sensing (Ballario et al., 1998).

Conclusion: Cryptochromes are a class of blue light receptors found in plants that play essential roles in plant growth and development. Cryptochrome perform important functions in plants:

Photoperiodism: Cryptochromes regulate the circadian clock and photoperiodic responses in plants. They help plants sense changes in day length and coordinate their physiological processes accordingly, Photomorphogenesis: Cryptochromes also regulate plant growth and development in response to light. They help control various aspects of photomorphogenesis, such as the inhibition of stem elongation and the promotion of leaf expansion, Chloroplast movement: Cryptochromes regulate chloroplast movement in response to changes in light intensity. They help control the positioning of chloroplasts within the plant cell, which can affect the efficiency of photosynthesis, Blue-light-induced stomatal opening: Cryptochromes regulate stomatal opening in response to blue light. They help plants balance the need for carbon dioxide uptake for photosynthesis with the need to conserve water, Abiotic stress responses: Cryptochromes have been shown to play a role in plant responses to various abiotic stresses, such as cold and drought. They help regulate the expression of stress-responsive genes and contribute to the overall stress tolerance of plants.

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