A green light for cryptochrome research

A photoreceptor protein that mediates plant developmental responses to blue light has been identified; the molecular properties of this 'cryptochrome', CRY1, suggest a novel mechanism of initiating signal transduction.

A feature common to all living organisms is the acquisition of information about their surroundings, through the perception of environmental signals. As a consequence of their sessile nature, plants need to be especially plastic in their development, and so show a vast array of adaptive responses to environmental cues. Plants have evolved exquisite sensory systems for monitoring their environment and initiating appropriate developmental responses. As plants are photoautotrophic and depend upon photosynthesis for their survival, they are especially sensitive to alterations in their light environment. Plants monitor properties of incident light, such as its intensity, quality, direction and duration, and use this information to modulate developmental responses, to control their architecture and to determine the onset of flowering. The developmental responses of plants to properties of the light environment are collectively referred to as photomorphogenesis.

Photomorphogenetic responses of plants involve the action of several distinct classes of photoreceptors that are sensitive to different regions of the light spectrum, including the ultraviolet (UV), blue and red/far-red regions. Specific blue-light sensing systems, which are found in bacteria, fungi and animals as well as plants, have been studied for more than a century. Perhaps the most prominent blue-light responses of plants are the phototropisms (the bending responses of plant organs in relation to the direction of illumination), the inhibition of axis (hypocotyl, for example) elongation, the opening of stomata, the rearrangement of chloroplasts and the blue-light dependent regulation of expression of several genes.

From physiological studies of these and other phenomena, and, more recently, from the analysis of mutant strains, compelling evidence for the existence of multiple blue-light photoreceptors has been obtained. But despite the wealth of blue-light responses and more than a century of scientific study, the chemical nature of the blue-light photoreceptors remained elusive. Several candidate blue-light photoreceptors have been proposed, including flavins and carotenoids, based on the similarity between their absorption spectra and the action spectra for blue-light responses. As a pun on the cryptic nature of the photoreceptors and the prevalence of blue-light responses in cryptogamic (lower) plants, the term cryptochrome was coined for these receptors.

Anthony Cashmore and colleagues [1–3] have recently reported the identification and initial molecular analysis of one of the higher plant cryptochromes. The breakthrough came with the cloning of the *HY4* gene of *Arabidopsis thaliana* [1]. The *hy4* (*hy* for long hypocotyl) mutant of *Arabidopsis*, isolated more than decade ago by Maarten Koornneef and colleagues [4], fails to show the inhibition of hypocotyl elongation that is the normal response to seedling irradiation with blue light. In Cashmore's laboratory [1], a *hy4* mutant was identified among a population of 5 000 lines of *Arabidopsis* seedlings containing random genomic insertions of T-DNA as a result of *Agrobacterium*-mediated transformation. The *hy4* phenotype was found to co-segregate with resistance to kanamycin, a phenotypic marker of T-DNA insertion, indicating that the *HY4* gene had been tagged. Sequences adjacent to the T-DNA insertion were cloned and used to screen an *Arabidopsis* cDNA library, from which a clone containing an open reading frame encoding a predicted 681 amino-acid (75 kDa) protein was isolated. Additional *hy4* alleles were sequenced, confirming that the cloned cDNA corresponded to the *HY4* gene.

The first ~ 500 residues of HY4 protein — now called CRY1 for cryptochrome 1 — are strikingly similar in sequence to the apoproteins of type I microbial DNA photolyases, a class of flavoenzymes that catalyze light-dependent repair of cyclobutane-type pyrimidine dimers in UV-light-damaged DNA. Typically, photolyases contain two blue-light absorbing chromophores: a light-harvesting chromophore, usually methenyltetrahydrofolate (a pterin) or a deazaflavin, bound at the amino terminus of the enzyme, and reduced flavin adenine dinucleotide ($FADH_2$), involved in catalysis and bound at the carboxyl terminus of the enzyme. The pterin or deazaflavin chromophore absorbs a photon of light and transfers the energy to $FADH_2$, which in turn transfers an electron to a bound pyrimidine dimer, initiating the bond rearrangement that splits the cyclobutane ring to restore the pyrimidines. Although CRY1 shows a high degree of sequence similarity to regions of photolyases around the chromophore-binding domains, it differs significantly from them in that it possesses an extended sequence of about 200 residues, carboxy-terminal to the flavin-binding domain (Fig. 1). This extension shows some sequence similarity to smooth muscle tropomyosin A and, by analogy, may be involved in the interaction between CRY1 and other proteins.

In a follow up to the determination of the *HY4* coding sequence, the molecular properties of CRY1 have been analyzed [2]. For this, the *HY4* coding sequence was

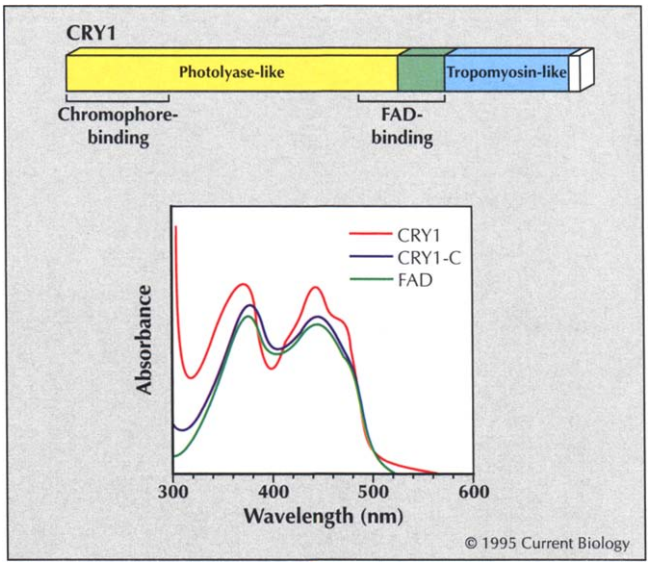

Fig. 1. Structural and spectral features of CRY1. The CRY1 protein has domains that show sequence similarity to both microbial photolyase and tropomyosin A. The locations of the putative chromophore-binding sites are indicated, and the green shaded area represents a 40 amino-acid overlap between the photolyase-like and tropomyosin-like domains. Recombinant CRY protein purified from insect cells has an absorption spectrum typical of a flavoprotein. The chromophore released from CRY1 by acid treatment (CRY1-C) has absorption characteristics identical to those of flavin adenine dinucleotide (FAD).

incorporated into a baculovirus expression vector, which was used to transfect cultured (Sf9) insect cells. Soluble CRY1 was produced in the insect cells and, after isolation and purification to near homogeneity, it was found to be a yellow protein with an absorption spectrum resembling that of a flavoprotein (Fig. 1). Despite its similarity to photolyases, CRY1 purified from Sf9 cells shows no photolyase activity. Heat or acid treatment was found to release the non-covalently bound CRY1 chromophore, which was shown to be fully oxidized FAD. The redox state of the CRY1 flavin chromophore contrasts with that typical of isolated photolyases, in which the flavin is in a more reduced state. Furthermore, whereas isolated bacterial photolyase containing oxidized FAD and lacking the second chromophore can be fully photoreduced to the $FADH_2$ state without intermediates, photoreduction of CRY1 under the same conditions yielded a redox intermediate that absorbed green light. This intermediate was shown to be the neutral radical flavosemiquinone (FADH•), a semi-reduced flavin. Determination of the redox potential of purified CRY1 protein under equilibrium conditions indicated that the bound flavin was predominantly FADH•.

As it is possible that the CRY1 flavin could exist as FADH• *in vivo*, one might suppose that CRY1 would be able to mediate plant responses to green light as well as to blue and near-UV (UV-A) light. Indeed, it turns out that green light can cause inhibition of *Arabidopsis* hypocotyl elongation, and that *hy4* mutants are less sensitive to green light. That CRY1 action *in vivo* can confer sensitivity to green light, in addition to blue and UV-A light, has been confirmed by Cashmore and colleagues [3], who have generated transgenic tobacco plants that express the *HY4* gene under the control of a strong promoter. Compared with control seedlings, transgenic seedlings expressing the *HY4* transgene were found to display a marked short-hypocotyl phenotype under blue, UV-A and green light conditions. The transgenic seedlings showed no alteration in their sensitivity to red and far-red light, wavebands that activate the phytochrome photoreceptor system.

These experiments provide direct experimental evidence that CRY1 really is a photoreceptor. The hypersensitivity of transgenic tobacco seedlings to blue, UV-A and green light indicates that the cellular machinery for transducing CRY1 signals is conserved among different plant species. Furthermore, the extent of the hypocotyl-inhibition response in transgenic seedlings was found to be dependent upon the level of expression of CRY1, suggesting that the cellular concentration of the photoreceptor determines photosensitivity. This suggestion is consistent with the observation that the *Arabidopsis hy4* mutation is semi-dominant; the one functional copy of the *HY4* gene in *HY4/hy4* heterozygotes is insufficient to confer a wild-type sensitivity to blue light [4].

The CRY1 protein purified from Sf9 cells was found to contain no chromophore other than FAD. If this were the sole chromophore responsible for the activity of the photoreceptor, and if it could oscillate between redox states (FAD and FADH•, for example), then one could speculate that the redox status of the plant cell may influence the relative sensitivity of CRY1 to UV-A, blue and green light. However, evidence for the presence of a second CRY1 chromophore has come from the laboratory of Aziz Sancar [5]. In this study, a fusion protein consisting of the photolyase-like region of CRY1 linked to maltose binding protein was expressed in *Escherichia coli*. The isolated fusion protein was yellow and found to contain two chromophores, identified as FAD and methenyltetrahydrofolate. The fusion protein showed no photolyase activity.

An identical chromophore composition was also found for the protein SA-phr1, from the mustard *Sinapis alba*, expressed in *E. coli*. The sequence of this protein shows high degree of similarity to CRY1 in the photolyase-like region, and it was initially thought to be a photolyase [6]. However, recombinant SA-phr1 protein from *E. coli* does not show photolyase activity, and its expression cannot complement a photolyase-deficient phenotype in an *E. coli* strain lacking the enzyme. Although SA-phr1 lacks the tropomyosin-like extension present at the carboxyl terminus of CRY1, it is possible that this protein is another higher plant blue-light photoreceptor.

A gene encoding a third possible blue-light photoreceptor has recently been cloned [7]. The *CPH1* gene of *Chlamydomonas reinhardtii*, a unicellular green alga, encodes an 867 residue protein, the first ~500 amino

acids of which are highly similar in sequence to CRY1 and photolyases. Like CRY1, the CPH1 protein differs from photolyases in having a carboxy-terminal extension. The extension in CPH1 is larger than in CRY1 — 367 as opposed to 200 residues — and there is little similarity between them. The *CPH1* gene is not linked to the *phr* mutation of *Chlamydomonas*, which confers a deficiency in photolyase activity.

The isolation of the *HY4* gene, and the subsequent characterization of the CRY1 protein, have heralded a new era in cryptochrome research. The search for other blue-light photoreceptors is now on. That the *hy4* mutant of *Arabidopsis* retains some responsivity to blue light, most notably blue-light-induced phototropic curvature, indicates that there must be other photoreceptors active in this part of the spectrum. Cashmore and colleagues have already isolated an *Arabidopsis* gene encoding CRY2, a protein similar to CRY1 in the photolyase-like domain but with an unrelated carboxy-terminal extension. It remains to be seen if CRY2, SA-phr1 and CPH1 proteins really are photoreceptors, but the demonstration that CRY1 expression in transgenic plants confers a light-conditional phenotype shows how it may be possible to test the hypothesis. Finally, the similarity between CRY1 and photolyases suggests that CRY1 initiates signal transduction by mediating an electron transfer reaction: if confirmed, this would be an entirely novel signal transduction mechanism.

References

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