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Review article

Cryptochrome photoreceptors in green algae: Unexpected versatility of mechanisms and functions☆

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ABSTRACT

Green algae have a highly complex and diverse set of cryptochrome photoreceptor candidates including members of the following subfamilies: plant, plant-like, animal-like, DASH and cryptochrome photolyase family 1 (CPF1). While some green algae encode most or all of them, others lack certain members. Here we present an overview about functional analyses of so far investigated cryptochrome photoreceptors from the green algae *Chlamydomonas reinhardtii* (plant and animal-like cryptochromes) and *Ostreococcus tauri* (CPF1) with regard to their biological significance and spectroscopic properties. Cryptochromes of both algae have been demonstrated recently to be involved to various extents in circadian clock regulation and in Chlamydomonas additionally in life cycle control. Moreover, CPF1 even performs light-driven DNA repair. The plant cryptochrome and CPF1 are UVA/blue light receptors, whereas the animal-like cryptochrome responds to almost the whole visible spectrum including red light. Accordingly, plant cryptochrome, animal-like cryptochrome and CPF1 differ fundamentally in their structural response to light as revealed by their visible and infrared spectroscopic signatures, and in the role of the flavin neutral radical acting as dark form or signaling state.

1. Introduction

Light has major impacts on photosynthetic microorganisms (microalgae) serving either as energy source for photosynthesis or providing information about the environment. Light quality and intensity trigger behavioral and developmental responses of algae under the control of sensory photoreceptors and entrain their circadian clocks by light-dark cycles (Hegemann, 2008). In the past years an increasing number of algal genomes has become available (Tirichine and Bowler, 2011) and allowed to search for and to compare different algal photoreceptors. The variety of photoreceptors in algal systems is unexpectedly high and complex (Mittag et al., 2005; Hegemann, 2008; Kianianmomeni and Hallmann, 2014; Tilbrook et al., 2016). It may reflect the natural habitats of algae, including frequent changes in light conditions. Algae live in freshwater, in moist soil, or in the marine environment. There, they are being exposed to different light qualities and intensities throughout the water column (Kirk, 1994) as well as throughout the day-night cycles of the different seasons.

The photoreceptors in algae include those found in land plants (Losi and Gärtner, 2012; Galvao and Fankhauser, 2015) such as phytochrome absorbing red and far-red light (found also in the form of the hybrid neochrome), UV resistant locus 8 (UVR8) capturing UV-B light, and phototropin as well as plant cryptochrome being sensitive to UV/A and blue light (Kianianmomeni and Hallmann, 2014; Fortunato et al., 2016; Tilbrook et al., 2016). These are complemented by a fascinating variety of phytochromes and cryptochromes covering the whole visible spectral region (Beel et al., 2012; Rockwell et al., 2014). In addition, other types of receptors have been identified for the first time in algae and have been partially characterized in the meantime. These comprise microbial rhodopsins such as cation/anion channelrhodopsins and histidine kinase rhodopsins, light-oxygen-voltage (LOV) proteins such as aureochromes, and finally blue light sensor using flavin (BLUF) proteins (Hegemann, 2008; Schellenberger Costa et al., 2013; Huysman et al., 2013; Kianianmomeni and Hallmann, 2014; Govorunova et al., 2015; Banerjee et al., 2016). Most of the research on algal photoreceptors has been performed in a few species tractable to transformation and genetic manipulation with a main focus on the green freshwater alga *Chlamydomonas reinhardtii*. Recently, the receptors of the marine green

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Abbreviations: aCRY, animal-like cryptochrome; BLUF, blue light sensor using flavin; *C. reinhardtii*, *Chlamydomonas reinhardtii*; CPD, cyclobutane pyrimidine dimer; CPF1, Cryptochrome Photolyase Family 1; CRY, cryptochrome; LOV, light-oxygen-voltage; *O. tauri*, *Ostreococcus tauri*; pCRY, plant cryptochrome from *C. reinhardtii*

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picoalga *Ostreococcus tauri* have gained attention fueled by the finding of a LOV-histidine kinase similar to bacterial photoreceptors (Krauss et al., 2009; Djouani-Tahri et al., 2011). Another recent model organism for photoreception with a very distinct evolutionary origin (secondary endosymbiont) is the marine diatom *Phaeodactylum tricornutum* (Depauw et al., 2012; Fortunato et al., 2016; see also Essen et al., König et al. and Kroth et al., this issue).

The biflagellate *C. reinhardtii* lives in fresh water and moist soil. This organism has served as a model for investigating fundamental biological processes such as photosynthesis, the structure and function of flagella as well as photoperception leading to the development of optogenetics (Merchant et al., 2007; Hegemann, 2008; Kianianmomeni and Hallmann, 2014; Govorunova et al., 2017). Several biological processes/metabolic pathways are controlled by light in *C. reinhardtii.* Examples are photosynthesis (Eberhard et al., 2008; Petroutsos et al., 2016), transcript levels of proteins for chlorophyll and carotenoid biosynthesis (Im et al., 2006; Beel et al., 2012), the cell cycle (Oldenhof et al., 2004) or nitrogen metabolism (Chen and Silflow, 1996).

Light is also essential for the Chlamydomonas sexual cycle. It controls the steps of (i) gamete formation, (ii) maintenance of gamete mating competence and (iii) zygote germination (Pan et al., 1997; Huang and Beck, 2003; Goodenough et al., 2007; Zou et al., 2017). Vegetative Chlamydomonas cells become pregametes in the dark when deprived from a nitrogen source. Their switch to gametes along with the restoration of dark-inactivated gametes is triggered mainly by blue, but to a small extent also by red light (Weissig and Beck, 1991; Pan et al., 1997). Gametes of both mating types form zygotes, which need again light (mainly blue and to a lesser degree red light) as well as the presence of a nitrogen source in the medium for their germination (Huang and Beck, 2003; Goodenough et al., 2007; Zou et al., 2017).

Moreover, light is important for photoorientation of *C. reinhardtii* (Hegemann, 2008). The swimming of cells toward or away from a light source is called phototaxis. Action spectra for phototaxis showed a maximum sensitivity at around 500 nm (Foster et al., 1984). The origin of photoorientation is the pigmented eyespot, which acts as a directional antenna to allow the unicellular organism to detect light direction and intensity (Schmidt et al., 2006 and references therein). The photomobility responses were shown to be mediated by depolarizing the algal membrane upon illumination, thereby initiating a signal cascade that finally affects the flagellar beating (summarized in Govorunova et al., 2017).

Light is also important for the circadian clock of Chlamydomonas, which is entrained by light-dark cycles. Several processes are controlled by the circadian clock in *C. reinhardtii* including chemotaxis, cell division, UV sensitivity, adherence to glass, and starch metabolism (summarized in Schulze et al., 2010). Components affecting phase, period and/or amplitude of the circadian clock have been identified (Schulze et al., 2010; Matsuo and Ishiura, 2011; Zou et al., 2017). Moreover, a clock-dependent rhythmic accumulation of cells in the light was described as rhythm of photoaccumulation or rhythm of phototaxis (Bruce, 1970; Mergenhagen, 1984; Forbes-Stovall et al., 2014) and it was shown that red, green and blue light can all reset the phase of this circadian rhythm (Johnson et al., 1991; Kondo et al., 1991; Forbes-Stovall et al., 2014).

For all light-driven processes mentioned above, photoreceptors that respond specifically to the different wavelengths of the visible spectrum are required. Chlamydomonas has a large variety of photoreceptors, and while the mechanisms of some of them have been biophysically well studied, there is still limited information on their biological and potentially overlapping functions. The channelrhodopsins (ChRs) 1 and 2 are light-gated ion channels located in the region of the plasma membrane associated with the eyespot; these photoreceptors mainly absorb green and blue light (Nagel et al., 2002, 2003). The mechanism of light activation and ion transport of ChRs has been investigated extensively in the absence of the long C-terminal extension, revealing an ion conduction pathway within the 7-transmembrane helix architecture (Kato et al., 2012; Ernst et al., 2014). Both ChRs are critical for photoorientation as well as the photophobic response (Sineshchekov et al., 2002; Govorunova et al., 2004). The blue light photoreceptor phototropin (PHOT) comprises two sensory light-oxygen-voltage (LOV) domains and a serine-threonine kinase. PHOT is involved in the sexual cycle, controlling gametogenesis and germination (Huang and Beck, 2003), the expression of certain genes (Im et al., 2006), and the regulation of phototactic behavior (Trippens et al., 2012). Moreover, it is directly linked to photosynthesis and photoprotection (Petroutsos et al., 2016). Full-length PHOT has served as a compact biophysical model for higher plant phototropins. The investigations uncovered the structural reorganization of the kinase and of the relative domain orientations by activation of the LOV domains (Pfeifer et al., 2010; Okajima et al., 2014). In addition, several histidine kinase rhodopsins (HKRs) are encoded on the Chlamydomonas genome (Kateriya et al., 2004). HKR1 of Chlamydomonas was found to act as a bimodal switch responsive to ultraviolet and blue light (Luck et al., 2012; see also Luck and Hegemann this issue) but its biological function is not known.

Analyses of the full genome sequence of Chlamydomonas showed that this alga has not only a plant cryptochrome but also an animal-like cryptochrome. However, it lacks the red light photoreceptor phytochrome (Mittag et al., 2005; Merchant et al., 2007), although it is clear that red light is effective in clock phase resetting and other processes (Johnson et al., 1991; Kondo et al., 1991; Pan et al., 1997; Im et al., 2006; Alizadeh and Cohen, 2010; Zou et al., 2017). First members of the red light signaling pathway, which reset the phase of the circadian clock, have been recently characterized (Niwa et al., 2013; Kinoshita et al., 2017). Unexpectedly, one of the cryptochromes of *C. reinhardtii* has been demonstrated to be involved in red light sensing (Beel et al., 2012; Oldemeyer et al., 2016). In this review, we will focus on the current knowledge of biological functions (see Sections 2–4) and mechanisms (see sections 5–8) of cryptochromes in green algae, especially cryptochrome members from *C. reinhardtii* and *O. tauri*.

2. What are cryptochromes and which kinds of cryptochromes have been identified in green algae?

Cryptochromes (CRYs) are known as blue light receptors in bacteria, fungi, plants and insects and as components of the circadian oscillator, the latter mainly in mammals (Sancar, 2003; Chaves et al., 2011). All of the CRY proteins share a conserved photolyase homology region (PHR) of about 500 amino acids and a C-terminal extension of various lengths. In the PHR domain, flavin adenine dinucleotide (FAD) is bound as a chromophore. Additionally, some CRYs may also bind an antenna pigment such as 5,10-methenyltetrahydrofolate. CRYs are derived from the photolyases that catalyze the blue-light dependent repair of UV light-induced damages. Photolyases are either specific for cyclobutane pyrimidine dimers (CPD) such as PHR2 in Chlamydomonas (Petersen and Small, 2001) or for (6-4) photoproducts.

While it was thought for a long time that CRYs have lost photolyase activity, it turned out recently that some algal CRYs, the cryptochrome photolyase family 1 (CPF1) members, have at least dual functions. They have kept photolyase activity and additionally act as blue light receptors and/or clock components (Coesel et al., 2009; Heijde et al., 2010). The CPF1 group is phylogenetically close to (6-4) photolyases, animal CRYs and animal-like CRYs (aCRY). The latter small subfamily comprises a member from *C. reinhardtii* (*Cr*aCRY) that is responsive to yellow and red light in addition to blue light (Beel et al., 2012). The C-terminal extension of CPF1 and *Cr*aCRY is relatively short, in contrast to the plant CRYs, where it is significantly longer. All so far studied plant CRYs act as blue light receptors in plants and forward information to entrain the circadian clock among other functions (Chaves et al., 2011). Besides, there are also the so-called CRY-DASH (*Drosophila*, *Arabidopsis*, *Synechocystis*, *Homo*)-type proteins as a separate subfamily of cryptochromes (Brudler et al., 2003), identified in organisms from bacteria to vertebrates (Fortunato et al., 2015). CRY-DASHs display DNA repair