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REVIEW

Algal photoreceptors: in vivo functions and potential applications

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Abstract Many algae, particularly microalgae, possess a sophisticated light-sensing system including photoreceptors and light-modulated signaling pathways to sense environmental information and secure the survival in a rapidly changing environment. Over the last couple of years, the multifaceted world of algal photobiology has enriched our understanding of the light absorption mechanisms and in vivo function of photoreceptors. Moreover, specific light-sensitive modules have already paved the way for the development of optogenetic tools to generate light switches for precise and spatial control of signaling pathways in individual cells and even in complex biological systems.

Keywords LOV photoreceptors · Cryptochromes · Phytochromes · Rhodopsin-like photoreceptors · Synthetic biology

Introduction

Photosynthetic organisms, i.e., plants, most algae, and cyanobacteria, are able to harvest sunlight using photosynthetic pigments (e.g., chlorophyll) and to transform it into chemical energy. During evolution, these organisms also developed diverse light-sensitive proteins, so-called photoreceptors, and the corresponding signaling pathways to monitor light continuously and to adapt their physiological activities to environmental changes. In particular, many free swimming microalgae possess a sophisticated light-sensing system for the highly accurate monitoring of light (Foster and Smyth 1980). Changes in light intensity and wavelength are detected by photoreceptors, which induce a modified swimming behavior or trigger signal transduction cascades that generate physiological responses. In the last couple of years, a limited number of algal photoreceptors has been identified and partially characterized (Beel et al. 2012b; Coesel et al. 2009; Heijde et al. 2010; Kianianmomeni et al. 2009; Nagel et al. 2002, 2003), providing new insights into molecular mechanisms of the light-sensing systems and light-regulated cellular processes. Some of the algal photoreceptors display new properties, which are far from the classical picture of animal and plant photoreceptors, and have direct impact on our understanding of the evolution and function of light-sensitive proteins. The rich world of algal light reception contains classical photoreceptors such as phototropins (found in plants), cryptochromes (found in both plants and animals) and unusual rhodopsin-like photoreceptors that show light-dependent ion transport or enzyme activity (Huang and Beck 2003; Luck et al. 2012; Nagel et al. 2002, 2003; Reisdorph and Small 2004; Schröder-Lang et al. 2007). A couple of algal rhodopsins have already paved the way for development of optogenetic tools, a technology that provides a basis for precise spatiotemporal control of cell signaling (Mattis et al. 2012; Schröder-Lang et al. 2007; Zhang et al. 2010, 2011). Because of low-cost high-throughput sequencing technologies and because of the growing importance of algal model systems for biotechnological industry and basic research, genome information of numerous algae has become available during the last years. Some examples

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for algal model systems with available genome and transcriptome information are *Chlamydomonas reinhardtii* (*C. reinhardtii*) (Merchant et al. 2007), *Volvox carteri* (*V. carteri*) (Prochnik et al. 2010), *Phaeodactylum tricornutum* (Bowler et al. 2008), *Ostreococcus tauri* (Derelle et al. 2006), *Ostreococcus lucimarinus* (Palenik et al. 2007), *Thalassiosira pseudonana* (Armbrust et al. 2004), *Cyanidioschyzon merolae* (Matsuzaki et al. 2004), *Nannochloropsis* (Radakovits et al. 2012; Vieler et al. 2012), *Bathycoccus prasinos* (Moreau et al. 2012) and *Coccomyxa subellipsoidea* (Blanc et al. 2012). The production of additional genome and transcriptome data of the algae *Haematococcus pluvialis*, *Gonium pectorale*, *Eudorina elegans* and *Pleodorina starii* (Fig. 1) is currently in progress (http://www.volvocales.org; http://www.onekp.com) (Olson et al. 2011; Umen and Olson 2012).

In this review, we deal with new insights into the in vivo function of algal photoreceptor families, which have been gained during the last couple of years. These photoreceptor families include LOV-containing photoreceptors (phototropins, aureochromes and neochromes), cryptochromes, phytochromes and rhodopsins.

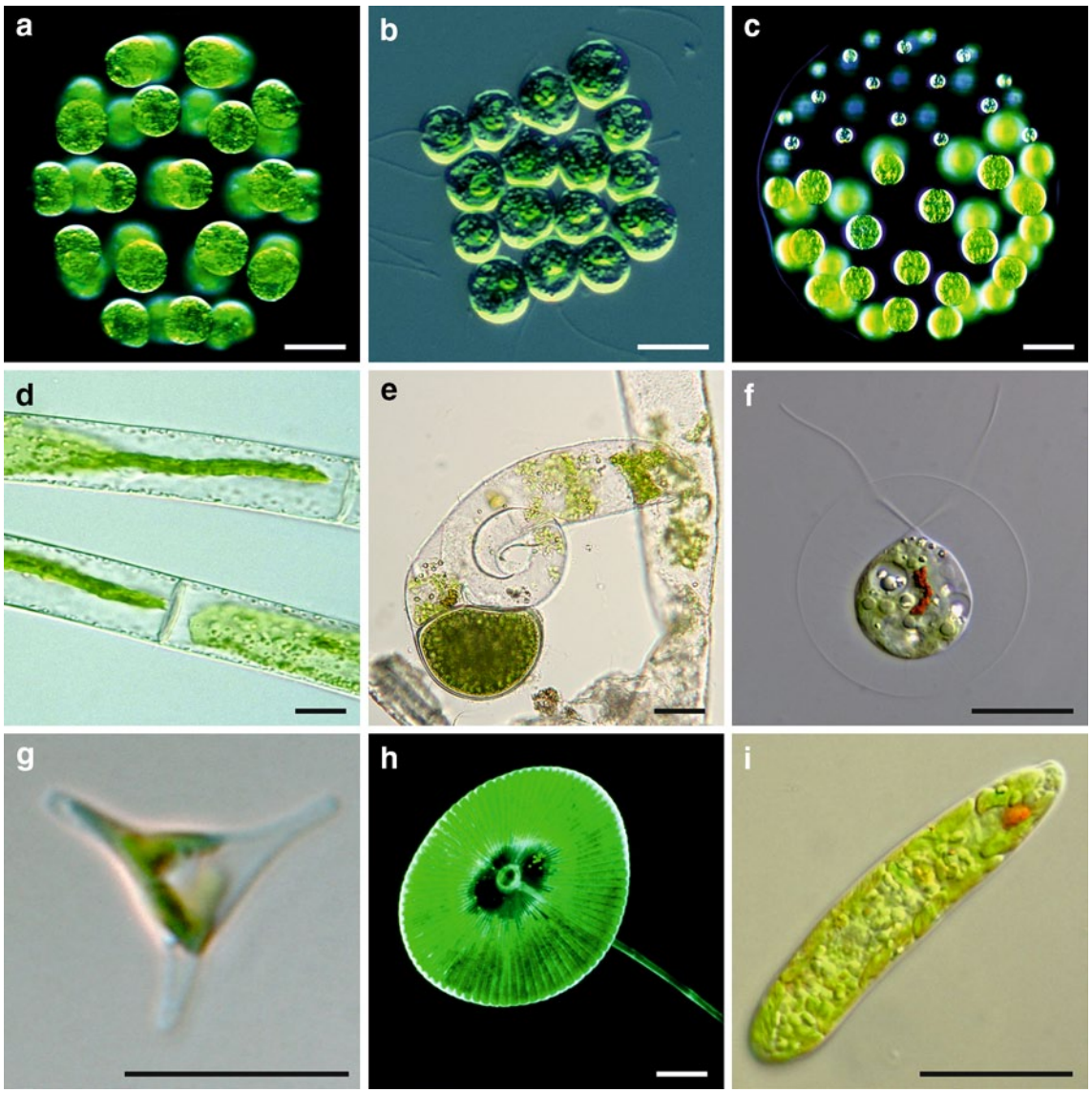

Fig. 1 Assortment of algal species with quite different phenotypes and organizational complexities. **a** *Eudorina* sp., **b** *Gonium* sp., **c** *Pleodorina* sp. (**a**–**c** kindly provided by Noriko Ueki), **d** *Mougeotia scalaris* (http://protist.i.hosei.ac.jp/PDB/Images), **e** *Vaucheria frigida* (courtesy of Malcolm Storey, http://www.bioimages.org.uk), **f** *Haematococcus pluvialis* (http://starcentral.mbl.edu/microscope/portal.php?pagetitle=assetfactsheet&imageid=20738), **g** *Phaeodactylum tricornutum* (courtesy of Ansgar Gruber, University of Konstanz), **h** *Acetabularia acetabulum* (http://paleopolis.rediris.es/cg/CG2006_BOOK_02/CG2006_B02_Fig_108.htm), **i** *Euglena gracilis* (kindly provided by Pavel Skaloud). *Scale bars* **a**–**g**, **i** 10 μm, **h** 1 mm

Much information about in vivo functions of algal photoreceptor comes from the unicellular green alga *C. reinhardtii* and its close multicellular relative *V. carteri*. Therefore, we will pay particular attention on photoreceptors of these two species. The unicellular alga *C. reinhardtii* is a widely recognized model organism for the investigation of biological processes including photosynthesis and light-dependent physiological and behavioral responses (Berthold et al. 2008; Foster et al. 1984; Huang and Beck 2003; Rochaix 2002). However, the confusing maze behind the individual activities of photoreceptors in the multicellular alga *V. carteri* will help us to understand the link between light and complex light-affected cellular processes such as cellular differentiation (Kirk and Kirk 1985), which have been required for evolutionary transition from unicellular organisms into a multicellular one (Kirk 2005).

In addition, we summarize potential applications of light-sensitive modules in synthetic biology and neuroscience for precise control of neural activities and signaling pathways.

In vivo function of algal photoreceptors

LOV-containing algal photoreceptors

Phototropins

The blue light receptor phototropin was originally identified as a 120-kDa membrane associated protein in *Arabidopsis* (Huala et al. 1997). Phototropins possess a photosensory region containing two light–oxygen–voltage (LOV) domains located at the N-terminal part and a C-terminal serine/threonine kinase domain (Fig. 2b) (Christie 2007; Huala et al. 1997). *Arabidopsis* has two phototropins, designated Phot1 and Phot2 (Briggs and Christie 2002), which are involved in a wide range of light-dependent responses like phototropism, chloroplast accumulation and avoidance response, stomata opening, nuclear positioning, leaf flattening, leaf positioning and rapid inhibition of hypocotyl growth (reviewed in Kami et al. 2010). Although both phototropins exhibit partially overlapping roles by mediating most of these responses, the chloroplast avoidance and nuclear positioning responses, which prevent photooxidative damage under high-light conditions, are mediated only by Phot2 (Jarillo et al. 2001; Kagawa et al. 2001; Kasahara et al. 2002). In the green alga *Mougeotia scalaris*, both phototropins, MsPHOTA and MsPHOTB, mediate the blue-light-induced chloroplast photoorientation (Suetsugu et al. 2005).

In contrast to the situation in *Arabidopsis* and *Mougeotia scalaris*, only a single phototropin was identified both in *V. carteri* and *C. reinhardtii* (VcPhot and CrPhot, respectively) (Fig. 2a) (Huang et al. 2002; Prochnik et al. 2010). Both algal proteins are smaller than higher plant phototropins, i.e., about 80 kDa in contrast to 120 kDa in higher plants, and show only 30–40 % identity with the plant phototropins (Huala et al. 1997; Huang et al. 2002; Prochnik et al. 2010). Despite this relatively low percentage of identity, typical phototropin responses were restored in an *Arabidopsis phot1–phot2* double mutant overexpressing the phototropin of *Chlamydomonas* (Onodera et al. 2005). This suggests that the basic signal transduction mechanism of phototropin is conserved between phototropins from algae and higher plants despite significant structural differences (Onodera et al. 2005). CrPhot seems to be stable in the light, which makes it likely to be involved in the light-induced developmental processes such as conversion of pregametes to gametes during gametogenesis in *C. reinhardtii* (Huang et al. 2002). *C. reinhardtii* knockdown strains with a reduced amount of phototropin were shown to be partially impaired in three light-dependent steps: (1) in the conversion of mating-incompetent pregametes to mature gametes, (2) in the restoration of the mating ability of gametes, which have been inactivated by incubation in the dark, and finally (3) in the germination of zygotes in response to light (Huang and Beck 2003). Moreover, CrPhot is also involved in controlling changes in the sensitivity of chemotaxis during sexual differentiation (Ermilova et al. 2004). In *C. reinhardtii*, vegetative cells and mating-incompetent pregametes are attracted to ammonium (Byrne et al. 1992; Sjoblad and Frederikse 1981). However, at the late phase of gamete formation, exposure to light results in gaining of mating competence by a loss of chemotactic sensitivity for ammonium (Byrne et al. 1992; Ermilova et al. 2003). The shut-off of chemotaxis during sexual development of *C. reinhardtii* is mediated by blue light and it is also partially controlled by phototropin (Ermilova et al. 2004). It was Huang and Beck (2003) who described that a low level of phototropin affects the light-induced changes in the transcript level of a cell-wall degrading enzyme, the gamete lytic enzyme (GLE); later, the involvement of phototropin in blue-light-mediated gene expression was analyzed in more detail; it was shown that phototropin is involved in the blue-light-mediated changes in transcript accumulation of genes encoding enzymes responsible for chlorophyll and carotenoid biosynthesis (Im et al. 2006). Finally, in situ immunofluorescence analyses showed that phototropin is not only located at the plasma membrane of the cell body, but also, to a little extent, in the flagella, where it is associated with the central core of the flagellum, the axoneme (Huang et al. 2002, 2004). Based on the analysis of a mutant defective in the *fla10* gene, in which intraflagellar transport is impaired, it was proposed that phototropin is a cargo for intraflagellar transport (Huang et al. 2004). Later, CrPhot has been identified in the

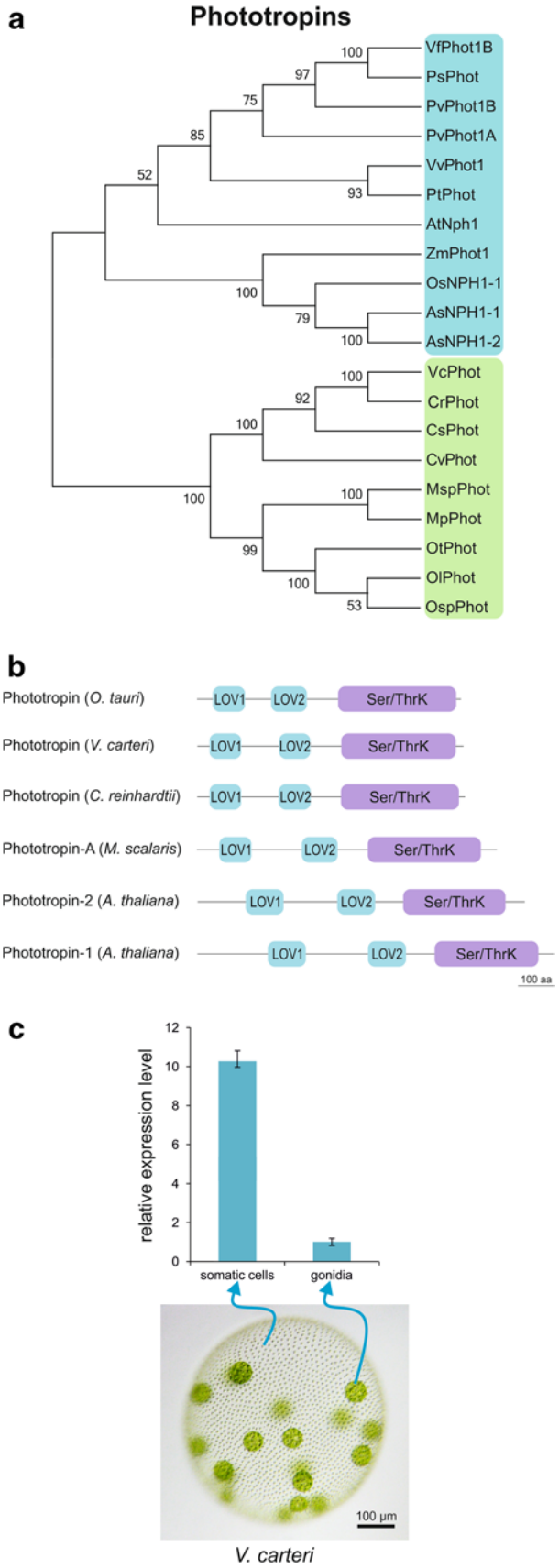

Fig. 2 The phototropins: phylogenetic tree of 20 phototropins, typical domain composition and expression characteristics of a selected phototropin, the phototropin of *V. carteri.* **a** Phylogenetic tree of phototropins. The tree was constructed with the maximum-likelihood (*ML*) method based on amino acid sequences using MEGA5 (Tamura et al. 2011). Bootstrap values for ML were calculated for 10,000 replications. Phototropins from plant and algae are highlighted in *blue* and *green* backgrounds, respectively. Source organisms: As, *Avena sativa*; At, *Arabidopsis thaliana*; Cr, *Chlamydomonas reinhardtii*; Cs, *Coccomyxa subellipsoidea* C-169; Cv, *Chlorella variabilis*; Mp, *Micromonas pusilla* CCMP1545; Msp, *Micromonas* sp. RCC299; Ol, *Ostreococcus lucimarinus*; Os, *Oryza sativa*; Osp, *Ostreococcus* sp. RCC809; Ot, *Ostreococcus tauri*; Ps, *Pisum sativum*; Pt, *Populus trichocarpa*; Pv, *Phaseolus vulgaris*; Vc, *Volvox carteri*; Vf, *Vicia faba*; Vv, *Vitis vinifera*; Zm, *Zea mays*. See Supplemental Table S1 for GenBank accession numbers. **b** Domain composition of phototropins. Domain composition of algal phototropins represented by phototropins of *O. tauri*, *V. carteri*, *C. reinhardtii* and *M. scalaris*, in comparison to Phototropin-1 and -2 of the land plant *A. thaliana*. *LOV* light, oxygen, or voltage domain, *Ser/ThrK* serine/threonine kinase domain. **c** The phototropin of *V. carteri*: relative cell type-specific transcript level of *V. carteri* phototropin in asexual reproductive cells (gonidia) versus somatic cells. The multicellular green alga *V. carteri* consists of 2,000–3,000 small, terminally differentiated, somatic cells at the surface and about 16 gonidia in the interior; all cells are embedded in a complex, but transparent extracellular matrix

eyespot proteome of *C. reinhardtii*; however, its function in phototaxis and eyespot development remained unclear at that time (Schmidt et al. 2006).

It should be stressed that most of the above-mentioned functions of *C. reinhardtii* phototropin were obtained based on the characterization of RNA interference strains that only result in a reduction of the level of wild-type product and, moreover, this partial knockdown is not stable over time (Boutros and Ahringer 2008; Schroda 2006). The knockout of *C. reinhardtii* phototropin using immotile *C. reinhardtii* target strains and single-stranded DNA (ssDNA) homologous recombination was reported by Zorin et al. (2009). In the knockout strain, $\Delta Phot^{G5}$, the eyespots were larger than in the parental strain (CW15-302). Moreover, the eyespot size increased in CW15-302 after the cultures were incubated several days in the dark, whereas the eyespot size of $\Delta Phot^{G5}$ remained unchanged (Trippens et al. 2012). When the $\Delta Phot^{G5}$ strain was rescued by complementation using the full-length phototropin gene, the eyespot size was again comparable to the eyespot size in the CW15-302 strain (Trippens et al. 2012); likewise, the eyespot size increased just as in CW15-302 when the rescued cells were grown in darkness. Complementation of the $\Delta Phot^{G5}$ strain with the phototropin kinase domain alone resulted in a light-independent reduction of the eyespot size (Trippens et al. 2012). In contrast, complementation with the photosensory LOV domains (LOV1 + LOV2) can trigger eyespot size reduction only in the light (Trippens et al. 2012).

Furthermore, it was observed that phototropin regulates expression of channelrhodopsin-1 (ChR1), a plasma-membrane localized, light-gated ion channel in the eyespot area (Berthold et al. 2008; Suzuki et al. 2003). More precisely, phototropin down regulates both the level of ChR1 at the onset of illumination and the ChR1 steady state level during the light period (Trippens et al. 2012). Because ChR1 is a primary photoreceptor for light-induced movements, phototropin seems to be involved in regulation of photoresponses in *C. reinhardtii*. The analysis of phototactic behavior of gametes from two strains overexpressing either the kinase domain or the LOV domains showed clear links between phototropin and phototactic responses. In wild-type gametes, blue light induces a positive phototactic movement, whereas UV light causes an avoidance response; this change in phototactic behavior was inverted in both overexpressing strains (Trippens et al. 2012). However, despite clear evidences, which show that CrPhot is an important component of different signaling pathways, no interacting proteins involved in the downstream signaling of CrPhot have been identified in *C. reinhardtii* so far.

Blue light is not only involved in eyespot size regulation but also in cell size regulation. *C. reinhardtii* cells, grown under blue light, shift the commitment point of the cell cycle to a later time point, which results in a larger cell size (Oldenhof et al. 2004a, b). Obviously, CrPhot also participates in mechanisms controlling cell size in the unicellular alga *Chlamydomonas* (Peter Hegemann, personal communication).

In the closely related multicellular green alga *V. carteri*, cell size is the only criterion that decides about the cell fate, i.e., cells with a diameter above ~8 μm will develop as reproductive cells, whereas cells with a diameter below ~8 μm will develop as somatic cells (Kirk et al. 1993). A preliminary analysis of cell type-specific gene expression indicated that the transcript level of *VcPhot* is higher in somatic cells than in asexual reproductive cells called gonidia (Fig. 2c) (Kianianmomeni and Hallmann, unpublished data). This result suggests a connection between *VcPhot* expression and the mechanism of cell size control during development of *V. carteri*.

Aside from the phototropins of *V. carteri*, *C. reinhardtii* and *M. scalaris*, quite a few other phototropins were identified in the last couple of years, but were not studied in detail. These phototropins come from *Chlorella variabilis* (Genbank accession number: EFN51280, Blanc et al. 2010), *Micromonas pusilla* CCMP1545 (Genbank accession number: XM_003063488, Worden et al. 2009), *Ostreococcus lucimarinus* CCE9901 (Genbank accession number: XP_001421797, Palenik et al. 2007), *Coccomyxa subellipsoidea* C-169 (Genbank accession number: EIE23763, Blanc et al. 2012) and *Ostreococcus tauri* (Genbank accession number: CAL58288).

The phototropin of the marine green alga *O. tauri* (OtPhot) (Fig. 2b) (Derelle et al. 2006; Veetil et al. 2011) shows spectral properties similar to those of higher plants (Veetil et al. 2011). However, the in vivo function of OtPhot remains to be clarified.

It should also be mentioned that members of the Zeitlupe protein family, which also contain LOV domains, have not been identified in the genomes of the green algae *V. carteri*, *C. reinhardtii* and *O. tauri* (Corellou et al. 2009; Merchant et al. 2007; Prochnik et al. 2010). The Zeitlupe proteins, i.e., ZEITLUPE (ZTL, GenBank Accession No. AF254413) (Somers et al. 2000), FLAVIN-BINDING KELCH REPEAT F-BOX1 (FKF1, GenBank Accession No. NM_105475) (Nelson et al. 2000) and LOV KELCH PROTEIN2 (LKP2, GenBank Accession No. NM_179652) (Schultz et al. 2001), regulate the circadian clock in *Arabidopsis*. It is unclear whether Zeitlupe-related proteins really do not occur in green algae or whether they have diverged greatly between green algae and higher plants like *Arabidopsis*.

Aureochromes

Aureochromes have been identified in the stramenopilic alga *Vaucheria frigida* (Takahashi et al. 2007). This photosynthetic green-yellow alga possesses two aureochrome genes, which encode two homologs, VfAureo1 and VfAureo2 (Fig. 3). Each aureochrome contains a LOV domain on the C-terminal half of the polypeptide and a basic-region/leucine-zipper (bZIP) domain on the N-terminal half (Takahashi et al. 2007) (Fig. 3b). The molecular mechanism of light absorption and conformational changes of aureochromes has been investigated in detail (Herman et al. 2013; Hisatomi et al. 2013; Toyooka et al. 2011). AfAureo1 binds FMN via its LOV domain and it shows an absorption spectrum similar to other proteins with LOV domains. The bZIP domain is capable of sequence-specific DNA binding, i.e., it binds to the sequence TGACGT. The binding of VfAureo1 to its target sequence was strongly enhanced by irradiation with blue light, implying that VfAureo1 is a blue-light-regulated transcription factor (Takahashi et al. 2007). The in vivo functions of VfAureo1 and VfAureo2 have been analyzed by gene knockdown with RNA interference (RNAi). The analysis of VfAureo1-knockdown cells indicated that VfAureo1 is a photoreceptor for blue-light-induced branching in *V. frigida*. In contrast, down regulation of VfAureo2 unexpectedly induced sex organ primordia instead of branches, suggesting that VfAureo2 may act as a sub-switch and cause the branch primordium to develop into a branch and not develop into a sex organ (Takahashi et al. 2007). Interestingly, double knockdown of both VfAureo1 and VfAureo2 leads to abnormal tube morphology and enhanced blue-light-induced branch

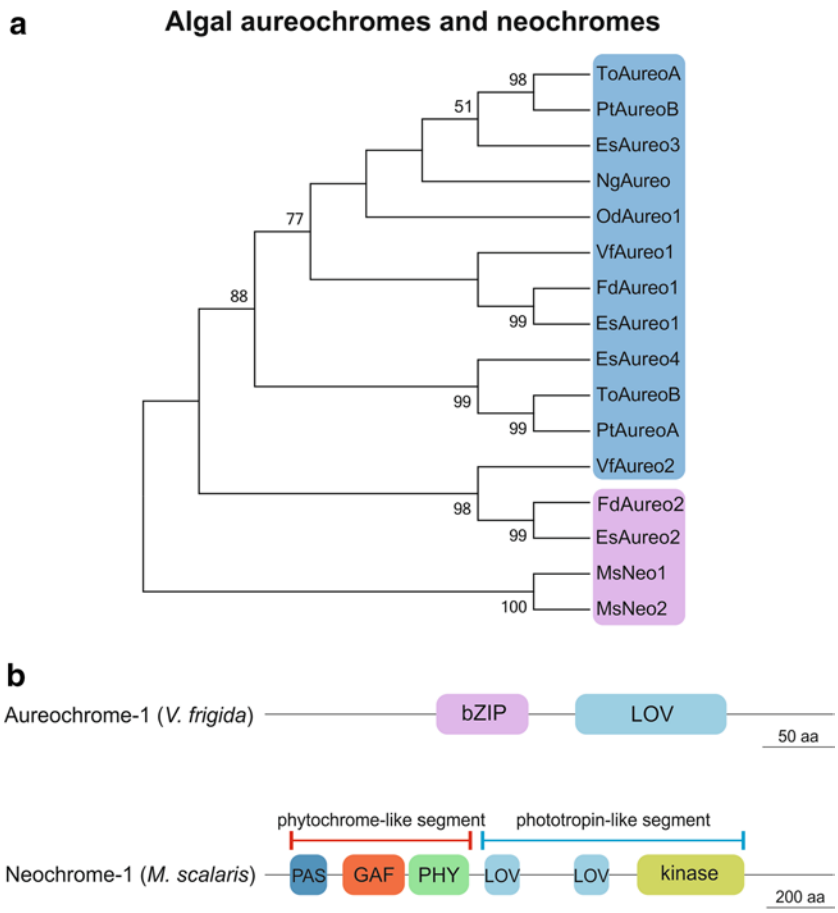

Fig. 3 The algal aureochromes and neochromes. **a** Phylogenetic tree of aureochromes (*blue* background) and neochromes (*purple* background). The trees were constructed as described in the legend of Fig. 2. Source organisms: Es, *Ectocarpus siliculosus*; Fd, *Fucus distichus* ssp. *Evanescens*; Ms, *Mougeotia scalaris*; Ng, *Nannochloropsis gaditana* CCMP526; Od, *Ochromonas danica*; Pt, *Phaeodactylum tricornutum*; To, *Thalassiosira oceanica*; Vf, *Vaucheria frigida*. **b** Domain compositions of aureochrome-1 from *Vaucheria frigida* and neochrome-1 from *Mougeotia scalaris*. *bZIP* basic-region/leucine-zipper, *LOV* light, oxygen, voltage sensor, *PAS* Per-ARNT-Sim domain, *GAF* GAF domain, *PHY* phytochrome

formation, but not premature sex organ formation. This result indicated that VfAureo1 is required for sex organ formation in *V. frigida* (Suetsugu and Wada 2013; Takahashi et al. 2007).

Several further aureochromes have been identified in the genomes of other algae such as *Ectocarpus siliculosus* (Phaeophyceae), *Fucus distichus* ssp. *Evanescens* (Phaeophyceae), *Chattonella antiqua* (Raphidophyceae), *Nannochloropsis gaditana* CCMP526 (Eustigmatophyceae), *Ochromonas danica* (Chrysophyceae), *Phaeodactylum tricornutum* (Bacillariophyceae), *Thalassiosira pseudonana* (Coscinodiscophyceae) and *Thalassiosira oceanica* (Coscinodiscophyceae) (Armbrust et al. 2004; Bowler et al. 2008; Cock et al. 2010; Ishikawa et al. 2009; Lommer et al. 2012; Radakovits et al. 2012; Rayko et al. 2010) (Fig. 3a).

Neochromes

In the filamentous green alga *Mougeotia scalaris*, photoorientation of the ribbon-shaped chloroplast has been shown to be regulated by red and blue light (Gabrys 1985; Gabrys et al. 1984; Haupt 1999). It was proposed that a chimeric photoreceptor for red and blue light is involved in the light-induced chloroplast orientation (Haupt 1999). Some time later, two genes resembling phytochrome 3 (*PHY3*) from *Adiantum capillus-veneris* have been identified in *M. scalaris* (Suetsugu et al. 2005). These genes encode hybrid proteins that contain a segment similar to the N-terminally located sensory module of phytochromes containing PAS, GAF and PHY domains and a phototropin-like segment containing two LOV domains and a serine/theronine kinase (Fig. 3b). These hybrid photoreceptors, which show typical bilin binding and red/far-red reversibility, have been called neochrome-1 and -2 (MsNeo1 and MsNeo2) (Suetsugu et al. 2005) (Fig. 3a). Spectral analysis of the photosensory region (i.e., the phytochrome-like segment and the two LOV domains) with phytochromobilin showed that the absorption spectra of MsNeo1 and MsNeo2 peak at 678 and 680 nm, respectively. The absorption spectra are similar to the action spectra for red-light-induced photoorientation of chloroplasts in *Mougestia scalaris* (Kagawa and Suetsugu 2007).

Transient expression of both MsNeo1 and MsNeo2 in an *Adiantum capillus-veneris* mutant (*rap2*) with a disruption of *neo* (*PHY3*) rescued the wild-type phenotype of *Adiantum*; without expression of MsNeo1 and MsNeo2 the *rap2* mutant is defective in red-light-induced phototropism and chloroplast relocation movement (Kadota and Wada

1999). Although both neochromes were able to rescue the defects of the *rap2* mutant (Suetsugu et al. 2005), their functions as blue light photoreceptors have been called into question. The main reasons are that the LOV domains of MsNeo1 and MsNeo2 cannot bind to FMN and they do not work as blue light photoreceptive domains (Suetsugu et al. 2005). Consequently, the missing features of neochromes led to believe that the two phototropins, MsPHOTA and MsPHOTB, identified in the genome of *M. scalaris*, are likely to be involved in blue-light-regulated chloroplast movement (Suetsugu et al. 2005).

Cryptochromes

Cryptochromes are flavoproteins with a highly conserved photolyase homology region (PHR) at their N-terminal domain including a flavin adenine dinucleotide (FAD)-binding domain, and a C-terminal extension of variable length (Fig. 4d–f) (Chaves et al. 2011). Functionally, cryptochromes are blue light receptors that are widely distributed among eubacteria, archaea and eukaryotes; they are divided into three major groups: (1) Plant cryptochromes: plant cryptochromes probably are derived from cyclobutane pyrimidine dimer (CPD) photolyases; they are involved in many cellular and physiological processes in plants including seedling growth, photoperiodic flowering and entrainment of the circadian clock (reviewed in Chaves et al. 2011; Kami et al. 2010). (2) Animal cryptochromes: animal cryptochromes, subsequently divided into types I and II, are closely related to (6–4) photolyases (reviewed in Chaves et al. 2011); they participate in the regulation of the circadian clock (Cashmore et al. 1999; Lin and Shalitin 2003) and are also known to mediate magnetoreception (Ceriani et al. 1999; Gegear et al. 2010; Kume et al. 1999; Ritz et al. 2000); (3) DASH (*Drosophila*, *Arabidopsis*, *Synechocystis* and Human) cryptochromes: DASH cryptochromes were identified first in cyanobacteria (Brudler et al. 2003) but later also in other eubacteria as well as in archaea, algae, plants, fungi and animals (Fig. 4a–c) (Chaves et al. 2011).

All three major groups of cryptochromes, i.e., plant-like, animal-like and DASH cyrptochromes, were identified in the nuclear genomes of the multicellular green alga *V. carteri* and the unicellular green alga *C. reinhardtii* (Fig. 4a–c) (Merchant et al. 2007; Prochnik et al. 2010). Each of both genomes contains one plant-like cryptochrome, one animal-like cryptochrome and two DASH cryptochromes.

Plant-like cryptochromes

The plant-like cryptochromes have been identified both in higher plants and algae. The presence of a single plant-like cryptochrome gene in both *V. carteri* (*VcCRYp* gene) and *C. reinhardtii* (*CrCRYp* gene) (Fig. 4a) was somewhat surprising because genomes of vascular plants contain between two (*Arabidopsis thaliana*) and five (in the fern *Adiantum capillus-veneris*) cryptochrome genes (Ahmad et al. 1998; Kanegae and Wada 1998; Lariguet and Dunand 2005; Lin et al. 1996). Therefore, (repeated) duplication of a precursor cryptochrome gene might play an important role in the evolution of different plant cryptochromes (Fig. 4a) (Lariguet and Dunand 2005). In synchronized *C. reinhardtii* cells, the CrCRYp cryptochrome accumulates in the dark and disappears rapidly when the light comes on (Reisdorph and Small 2004). The degradation of CrCRYp is mediated by the proteasome pathway in a light-dependent manner; the degradation is not only induced by blue light but also by red light (Reisdorph and Small 2004).

In the closely related multicellular alga *V. carteri*, the transcript level of the VcCRYp cryptochrome was found to be higher in somatic cells than in reproductive cells (Kianianmomeni and Hallmann, unpublished data). Somatic cells have a much lower photosynthetic capacity than reproductive cells (Choi et al. 1996) and undergo programmed cell death at the end of their lifespan (Pommerville and Kochert 1981a, b, 1982). In plants, cryptochromes are also involved in the light-dependent gene expression; the light dependence mainly affects genes involved in the response to biotic/abiotic stress and the regulation of photosynthesis (Danon et al. 2006; Lopez et al. 2011). The stronger expression of *VcCRYp* in somatic cells compared to reproductive cells suggests that this cryptochrome is an upstream or downstream regulatory component in cell type-specific regulation of photosynthesis and/or programmed cell death. Unfortunately, to our knowledge, no interaction partner of this plant-like cryptochrome or any other component of its signaling pathway has been identified so far in *Volvox* or in any related algae.

The photoreduction mechanism of green algal cryptochromes has been investigated in detail in the alga *Chlamydomonas* (Immeln et al. 2007). After blue light absorption by FAD, an electron transfer occurs from tryptophan to the excited FAD and leads to formation of a neutral radical form (Brautigam et al. 2004; Giovani et al. 2003; Langenbacher et al. 2009; Zeugner et al. 2005), which triggers conformational changes for further signal transduction (Giovani et al. 2003; Langenbacher et al. 2009; Yang et al. 2000). Based on findings in plants and animals, cryptochromes seem to have evolved from DNA photolyases, even though they lost their DNA repair activity during evolution (Cashmore et al. 1999; Sancar 2000). Photolyases are flavoproteins that mediate DNA repair in a light-dependent manner (Sancar 1994). They are activated by blue light and contain FAD as the catalytic chromophore and either methenyltetrahydrofolate (MTHF) or 8-hydroxy-7,8-didemethyl-5-deazariboflavin (8-HDF) as the second chromophore (Sancar 1994, 2003). During last

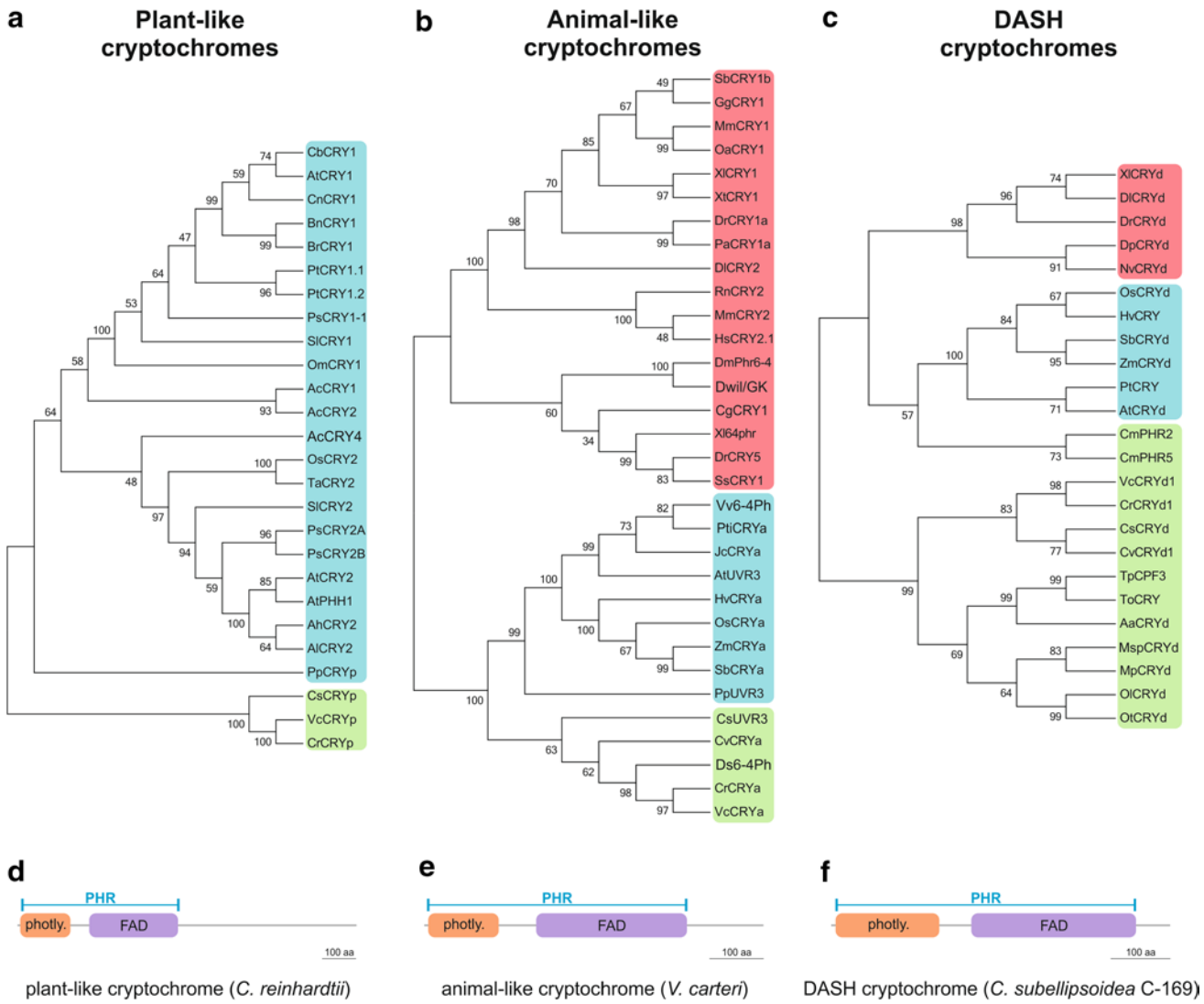

Fig. 4 The cryptochromes: phylogenetic trees of 26 plant-like, 32 animal-like and 24 DASH cryptochromes with typical domain compositions. **a** Phylogenetic tree of plant-like cryptochromes. **b** Phylogenetic tree of animal-like cryptochromes. **c** Phylogenetic tree of DASH cryptochromes. *Red background* cryptochromes and cryptochrome-like proteins from animals, *blue background* cryptochromes and cryptochrome-like proteins from plants, *green background* cryptochromes and cryptochrome-like proteins from algae. The trees were constructed as described in the legend of Fig. 2. Bootstrap values were calculated for 10,000 replications each. Source organisms: Aa, *Aureococcus anophagefferens*; Ac, *Adiantum capillus-veneris*; Ah, *Arabidopsis halleri* subsp. *gemmifera*; Al, *Arabidopsis lyrata* subsp. *Lyrata*; At, *Arabidopsis thaliana*; Bn, *Brassica napus*; Br, *Brassica rapa*; Cb, *Capsella bursa-pastoris*; Cg, *Crassostrea gigas*; Cm, *Cyanidioschyzon merolae*; Cn, *Cardamine nipponica*; Cr, *Chlamydomonas reinhardtii*; Cs, *Coccomyxa subellipsoidea* C-169; Cv, *Chlorella variabilis*; Dl, *Dicentrarchus labrax*; Dm, *Drosophila melanogaster*; Dp, *Daphnia pulex*; Dr, *Danio rerio*; Ds, *Dunaliella salina*; Dw, *Drosophila willistoni*; Gg, *Gallus gallus*; Hs, *Homo sapiens*; Hv, *Hordeum vulgare* subsp. *Vulgare*; Jc, *Jatropha curcas*; Mm, *Mus musculus*; Sb, *Sylvia borin*; Mp, *Micromonas pusilla* CCMP1545; Msp, *Micromonas* sp. RCC299; Nv, *Nematostella vectensis*; Oa, *Ovis aries*; Ol, *Ostreococcus lucimarinus*; Om, *Orobanche minor*; Os, *Oryza sativa*; Ot, *Ostreococcus tauri*; Pa, *Phreatichthys andruzzii*, Pp, *Physcomitrella patens*; Ps, *Pisum sativum*; Pt, *Populus tremula*; Pti, *Populus trichocarpa*; Rn, *Rattus norvegicus*; Sb, *Sorghum bicolour*; Sl, *Solanum lycopersicum*; Ss, *Salmo salar*; Ta, *Triticum aestivum*; Tp, *Thalassiosira pseudonana* CCMP1335; To, *Thalassiosira oceanica*; Xl, *Xenopus laevis*; Xt, *Xenopus tropicalis*; Zm, *Zea mays*; Vc, *Volvox carteri*; Vv, *Vitis vinifera*. Domain composition of cryptochromes: **d** plant-like cryptochromes, **e** animal-like cryptochromes, **f** DASH cryptochromes. *photly.* photolyase domain, *FAD* flavin adenine dinucleotide binding domain, *PHR* photolyase homology region

few years, additional antenna cofactors such as FMN, FAD and 6,7-dimethyl-8-ribityl-lumazine have been identified in photolayses (Geisselbrecht et al. 2012; Klar et al. 2006; Ueda et al. 2004).

Animal-like cryptochromes

Although the term "animal-like" tempts to think that the distribution of these cryptochromes is restricted to animals,

animal-like cryptochromes also have been found in higher plants and algae. Two algal animal-like cryptochromes, PtCPF1 of the diatom *Phaeodactylum tricornutum* and OtCPF1 of the green alga *Ostreococcus tauri*, are of special interest because their molecular activities blur the boundaries between cryptochromes and photolyases. The biochemical and functional characterization of PtCPF1 and OtCPF1 demonstrated that photolyases and cryptochromes can exhibit overlapping functionality (Beel et al. 2012a; Coesel et al. 2009; Heijde et al. 2010), which calls the strict functional distinction between photolyases and cryptochromes into question. PtCPF1 was able to repair (6–4) photoproduct damages in in-vivo DNA photorepair assays and it plays a major role in blue-light-regulated gene expression (Coesel et al. 2009). In transgenic *Phaeodactylum* strains overexpressing PtCPF1, the transcript levels of genes encoding components of the light-harvesting system (*LHCX* proteins), the carotenoid biosynthesis, the tetrapyrrole biosynthesis, the nitrogen metabolism and of other pathways were altered in comparison to wild-type strains (Coesel et al. 2009). A phenotype analysis of PtCPF1-knockdown mutants confirms that PtCPF1 of *Phaeodactylum* is involved in both repair of UV damage and photoprotection (De Riso et al. 2009).

In mammalians, cryptochromes act as integral components of a negative transcriptional feedback loop of the circadian clock (reviewed in Sancar 2004). The inhibition of Clock:BMAL1-activated transcriptions seems to be a common feature of vertebrate cryptochromes that has, remarkably enough, never been reported for any photolyase of vertebrates (Kobayashi et al. 2000; Yuan et al. 2007). The transcriptional repressor activity of vertebrate cryptochromes was analyzed in a heterologous mammalian system (i.e., monkey kidney cells) to specify the interaction of the algal animal-like cryptochrome PtCPF1, which is a blue/ultraviolet-A light photoreceptor, with the mammalian clock machinery. After heterologous expression of PtCPF1 in monkey kidney cells (COS7), PtCPF1 repressed the Clock:BMAL1-regulated expression of a reporter gene (Coesel et al. 2009). Thus, PtCPF1 is a dual-function blue light receptor with (6–4) photolyase activity that can influence the transcript levels of genes of different pathways (Coesel et al. 2009).

Shortly after the characterization of PtCPF1, OtCPF1 was identified in *Ostreococcus tauri*, the smallest free-living eukaryote (Heijde et al. 2010). Like PtCPF1, OtPCF1 is able to repress the Clock:BMAL1-regulated expression. In addition, OtCPF1 has the functional capability to strongly bind and repair (6–4) photoproducts. Moreover, the involvement of OtPCF1 in the control of the circadian rhythm of *Ostreococcus tauri* was reported (Heijde et al. 2010).

The algal proteins PtCPF1 and OtCPF1 are not the only proteins with both photolyase and light-sensing properties. Previously characterized fungal photolyases and photolyase-like proteins such as PHR1 from *Trichoderma atroviride* (Berrocal-Tito et al. 2007), CryA from *Aspergillus nidulans* (Bayram et al. 2008) and PHL1 from *Cercospora zeae-maydis* also display both DNA photorepair and gene expression regulatory activities (Bluhm and Dunkle 2008).

Recently, another cryptochrome of the alga *C. reinhardtii*, CrCRYa, was characterized (Beel et al. 2012b). CrCRYa is an animal-type cryptochrome that responds to both blue and red light. In red light, CrCRYa affects the transcript levels of the genes for the glutamate-1-semialdehyde aminotransferase (GSA), light-harvesting proteins (e.g., LHCBM6) and the psbA-binding protein (Beel et al. 2012b). So far no typical red light receptor, such as phytochrome, has been identified in *C. reinhardtii* or in the related alga *V. carteri* (Merchant et al. 2007; Mittag et al. 2005; Prochnik et al. 2010). Therefore, it was proposed that other light receptors, like CrCRYa, might be involved in the red-light signaling processes. Notably, CrCRYa not only affects genes involved in chlorophyll and carotenoid biosynthesis, synthesis of light-harvesting complexes, nitrogen metabolism and cell cycle control but it also participates in light-dependent transcript accumulation of genes encoding clock-relevant components (Beel et al. 2012b).

DASH cryptochromes

DASH cryptochromes (cry-DASH) can be found in cyanobacteria, eubacteria, archaea, algae, plants, fungi and animals (Chaves et al. 2011). These cryptochromes are able to repair photodamaged single-stranded, and loop-structured double-stranded DNA (Pokorny et al. 2008; Selby and Sancar 2006). Moreover, transcription repressor activity of DASH cryptochromes could be detected in *Synechocystis* by comparing expression profiles of a cry-DASH knockout mutant with a wild-type strain using microarray analysis (Brudler et al. 2003).

As mentioned above, the green algae *V. carteri* and *C. reinhardtii* have two DASH cryptochromes each (Merchant et al. 2007; Prochnik et al. 2010), but none of them has been characterized so far. Considering that cellular senescence is initiated in response to DNA damages (d'Adda di Fagagna 2008), characterization of the DASH cryptochromes of the multicellular alga *V. carteri* might reveal further insights into the function of these proteins during senescence and programmed cell death of the somatic cells (Pommerville and Kochert 1981a, 1982). The DASH cryptochromes might be down-regulated at the end of the life cycle and thus trigger a decrease in DNA repair capacity and accumulation of DNA damages, which in turn is a common mediator for cellular senescence (Chen et al. 2007).

In vitro characterization of the DASH cryptochrome OtCPF2 of the green alga *O. tauri* revealed both its specific binding to DNA damaged by cyclobutane pyrimidine dimers (CPD) and repair activity (Heijde et al. 2010). In the red alga *Cyanidioschyzon merolae*, three DASH cryptochromes (CmPHR2, CmPHR5 and CmPHR6) were recently identified and characterized to some extent (Asimgil and Kavakli 2012). Two of these DASH cryptochromes, CmPHR2 and CmPHR5, belong to the group of DASH cryptochromes from plants (Fig. 4c). Both CmPHR2 and CmPHR5 are able to repair CPD-damaged DNA. In contrast, no DNA repair activity was detected for CmPHR6. However, transgenic *E. coli* cells expressing CmPHR6 seem to be more resistant to UV light than wild-type cells, indicating that CmPHR6 might have an UV avoidance function to prevent UV-induced DNA damages. It was proposed that CmPHR6 is a cryptochrome that facilitated the transition from the DNA repair activity to the photoreceptor function (Asimgil and Kavakli 2012). Moreover, further components seem to be involved in the UV avoidance response in algae due to findings in *Arabidopsis* and subsequent identification of putative orthologs in algae. More precisely, the *Arabidopsis* protein UV RESISTANCE LOCUS 8 (UVR8) was shown to regulate changes in gene expression in response to UV-B irradiation (Brown et al. 2005; Kaiserli and Jenkins 2007; Rizzini et al. 2011) and putative orthologs of UVR8 have been identified in some algal genomes (Supplemental Figure S1). However, the actual in vivo function of these algal UV-B receptors remains to be determined.

Phytochromes

Phytochromes are red/far-red photoreceptors first discovered in plants; later they also have been found in bacteria. Phytochromes measure the changes in light quality in the red and far-red regions of the visible spectrum, allowing plants to assess the quantity of photosynthetically active light and they trigger shade avoidance responses (reviewed in Franklin 2008; Franklin and Whitelam 2005; Kami et al. 2010). Three domains (PAS–GAF–PHY) on the N-terminal half of the polypeptide form the photosensory core module that exhibits the main characteristic of a phytochrome: red/far-red photoreversibility (Fig. 5), i.e., it exists in two forms that are interchangeable and act like a light switch. Illumination with red light converts the red-light-absorbing form (biologically inactive form P_r, $\lambda_{max} = 670$ nm) to the far-red absorbing form (biologically active form P_{fr}, $\lambda_{max} = 730$ nm), while illumination with far-red converts P_{fr} to P_r (reviewed in Rockwell et al. 2006). Higher plants have several phytochromes, e.g., *Arabidopsis* possesses five phytochromes (phyA–phyE) (Clack et al. 1994). In contrast, no phytochrome genes could be identified so far in the genomes of both *V. carteri* and *C. reinhardtii*, even though red- and far-red-regulated gene expression has been observed in these algae (Alizadeh and Cohen 2010; Beel et al. 2012b; Mittag et al. 2005; Riano-Pachon et al. 2008) (Kianianmomeni and Hallmann, unpublished data). The absence of phytochromes might indicate that other photoreceptors such as animal-like cryptochromes, which absorb both blue and red light (Beel et al. 2012b), include the functions of red light photoreceptors in these free swimming green algae, whereas higher plants evolved specialized red light photoreceptors, the phytochromes.

Notably, phytochromes also have been found in charophyta, a division of green algae that includes the closest relatives of the embryophyte plants; charophytes are thought to be part of the evolutionary lineage that leads to vascular plants (Fig. 6). Phytochromes were identified in the charophytes: *Mougeotia scalaris* (MsPhy1, GenBank Accession No. AB206965 and S52048) (Winands and Wagner 1996; Winands et al. 1992) (Fig. 5), *Mesotaenium caldariorum* (McPhy1, GenBank Accession No. U31284) (Kidd and Lagarias 1990; Lagarias et al. 1995) and *Chara foetida* (CfPhy, GenBank Accession No. X80291) (Kolukisaoglu et al. 1995). As expected, the amino acid sequences of charophyte phytochromes are more related to plant phytochromes than to bacterial phytochromes. Nothing is known about the in vivo function of these phytochromes; only the light absorption properties of MsPhy1 and McPhy1 have been analyzed in some detail. The spectroscopic analysis of MsPhy1 displayed two phytochrome forms: a P_r form with an absorption maximum at 646 nm and a P_{fr} form with an absorption maximum at 720 nm (Jorissen et al. 2002). Similarly, McPhy1 showed absorption maxima at 650 and 722 nm for P_r and P_{fr}, respectively (Kidd and Lagarias 1990). In comparison to the plant phytochromes, which have absorption maxima at 670 nm for

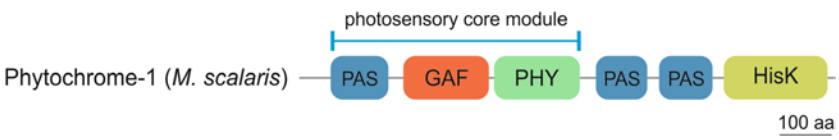

Fig. 5 The algal phytochromes. Domain composition of phytochrome-1 from *Mougeotia scalaris*. *PAS* Per-ARNT-Sim domain, *GAF* GAF domain, *PHY* phytochrome, *HisK* histidine kinase

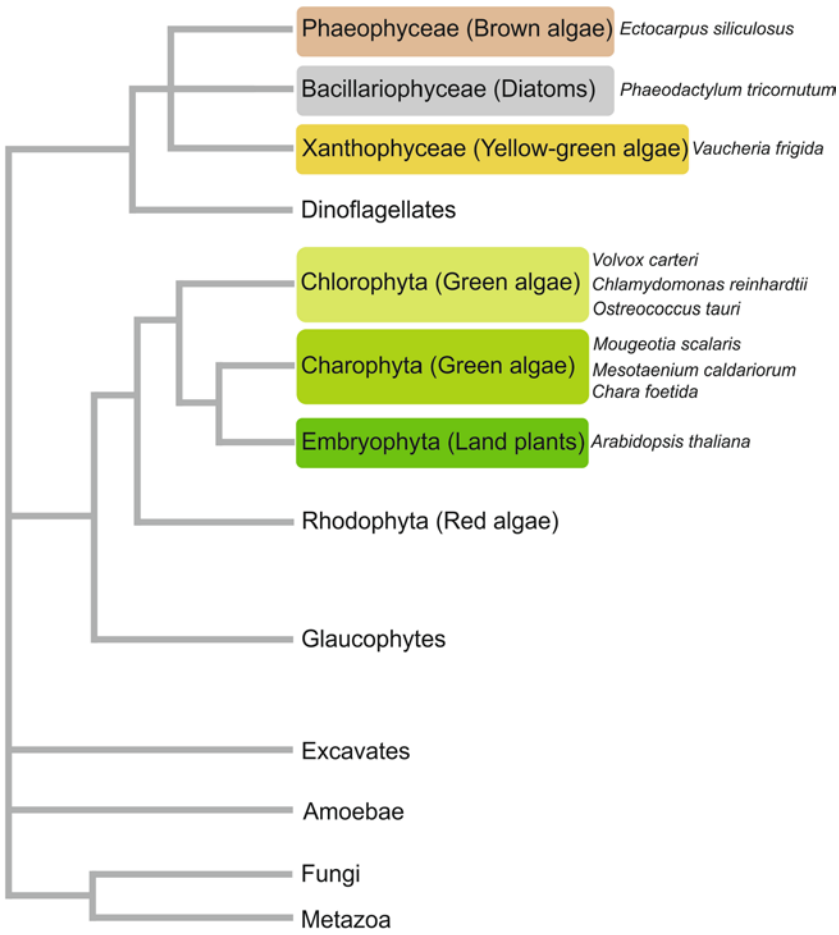

Fig. 6 Eukaryotic tree of life with emphasis on algae. Unrooted, schematic tree showing a simplified representation of phylogenetic relationships between major groups of eukaryotic algae and other eukaryotes. The names of both algal species and a reference land plant (*Arabidopsis thaliana*) discussed in this review are given on the right side of the group names. The tree was redrawn based on Kranz et al. (1995), Baldauf (2003) and Prochnik et al. (2010)

P_r and 730 nm for P_{fr} (Kami et al. 2010), the absorption spectra of the charophyte phytochromes are blue-shifted. This blue shift is an indication that plant and charophyte phytochromes use different kinds of chromophores, i.e., charophyte phytochromes utilize phycocyanobilin (PCB) whereas plant phytochromes utilize phytochromobilin (PϕB) as a chromophore (Jorissen et al. 2002; Wu et al. 1997).

Rhodopsin-like photoreceptors

Although light-induced locomotion of green flagellates, i.e., phototactic and photophobic responses, has been investigated for more than a century (Famintzin 1878; Holmes 1903), the molecular mechanisms underlying these light-dependent movements remained largely unclear until the last years, when the analysis of genome and transcriptome sequencing data allowed for the identification of many rhodopsin-like photoreceptors. However, the first evidence for the involvement of rhodopsin-like photoreceptors in light-induced locomotion of green flagellates came already in the 1980s from Foster et al. (1984). In their key experiment, they showed that the photobehavior responses of a retinal-deficient *Chlamydomonas* mutant can be restored solely by the addition of all-*trans* retinal, a photoreactive chromophore of the rhodopsins (Foster et al. 1984). Such photoreceptors were proposed to be localized in the eyespot apparatus (Foster and Smyth 1980), a primordial visual system found in many motile green algae and other photosynthetic microorganisms. Further experiments in Peter Hegemann's group led to the identification of the first rhodopsin gene (*Cop*); alternative splicing of the *Cop* pre-mRNA leads to two protein variants, CR1 (235 amino acid residues) and CR2 (243 amino acid residues). Although the amino acid sequences of CR1 and CR2 show a high degree of sequence identity (~91 %), both proteins have different hypothetical retinal-binding sites (Deininger et al. 1995; Fuhrmann et al. 2003). Both proteins are strongly enriched in the eyespot of *Chlamydomonas*; however, the concentration of CR2 is about 50-fold higher than the concentration of CR1 (Deininger et al. 1995; Fuhrmann et al. 2003). Although the abundant variant, CR2, was expected to be the responsible photoreceptor for light-induced movements in *Chlamydomonas*, no changes in phototaxis and photophobic responses were observed in transgenic strains with a reduced concentration of this protein (Fuhrmann

et al. 2001). Later, a rhodopsin homologous to CR2, volvoxrhodopsin-1 (VR1 or Vop), was identified in *V. carteri* (Fig. 7) (Ebnet et al. 1999), which shows 61 % sequence identity to CR2. Like CR1 and CR2, VR1 also belongs to the family of animal-type rhodopsins. It was proposed that VR1 is localized in the eyespot apparatus of somatic cells and serves as the light receptor in phototactic responses (Ebnet et al. 1999). But surprisingly, the transcript of VR1 was mainly expressed both in the eyeless reproductive cells and in embryos of *V. carteri*, whereas the parental somatic cells showed only minor expression of VR1 (Ebnet et al. 1999). The reason for these observations was unclear until Yuishiro Takahashi's group reported in 2009 that the homologous protein, CR2 from *Chlamydomonas*, forms a 1:1 complex with the chaperone Ycf4 and is likely to be involved in the assembly and biogenesis of photosystem I (Ozawa et al. 2009). The cell type-specific expression of VR1 in *Volvox* on the one hand and the involvement of the homologous CR2 in processes related to photosynthesis on the other hand suggest a function of VR1 as a sensory light receptor, which could regulate both the cell type-specific biosynthesis of chloroplast-related proteins and the activity of the photosynthetic process in *Volvox*.

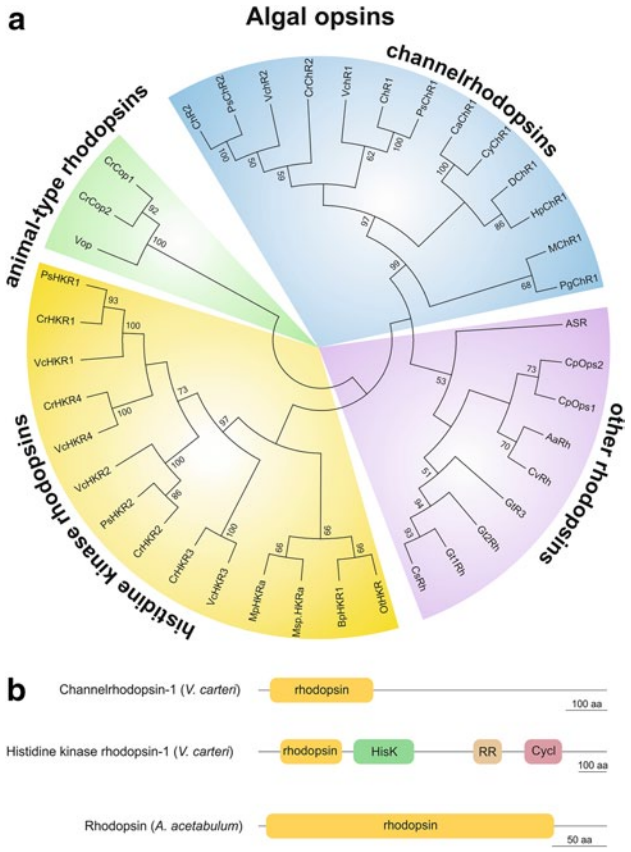

Fig. 7 The algal opsins: phylogenetic tree of 39 algal opsins with typical domain compositions. **a** The phylogenetic tree was constructed with the neighbor-joining method based on amino acid sequences of the corresponding rhodopsin domains as described in the legend of Fig. 2. Detailed information about the shown opsins is given in Supplemental Table S1. Source organisms: Aa, *Acetabularia acetabulum*; ASR, *Anabaena* sp.; Bp, *Bathycoccus prasinos*; Ca, *Chlamydomonas augustae*; Cp, *Cyanophora paradoxa*; Cr, *Chlamydomonas reinhardtii*; Cv, *Chlorella variabilis*; Cs, *Cryptomonas* sp. S2; Cy, *Chlamydomonas yellowstonensis*; D, *Dunaliella salina*; Hp, *Haematococcus pluvialis*; Gt, *Guillardia theta*; M, *Mesostigma viride*; Mp, *Micromonas pusilla*; Msp., *Micromonas* sp. RCC299; Ps, *Pleodorina starrii*; Vc, *Volvox carteri*. **b** Domain composition of four typical algal opsins. *HisK* histidine kinase domain, *RR* response regulator, *Cycl* adenylate/guanylate cyclase domain

Channelrhodopsins

A breakthrough toward the understanding of the role of rhodopsin-like light receptors in light perception and in light-induced movements came through the identification of two EST (expressed sequence tags) sequences, i.e., *ChR1* and Channelrhodopsin-2 (*ChR2*), in a *Chlamydomonas* cDNA database (http://est.kazusa.or.jp/en/plant/chlamy/EST/) by three research groups (Nagel et al. 2002, 2003; Sineshchekov et al. 2002; Suzuki et al. 2003). Both ChR1 and ChR2 are blue-light-activated ion channels with an absorption maximum at 500 and 470 nm, respectively (Nagel et al. 2002, 2003). These seven transmembrane-helix proteins are blue-light-gated cation channels that are covalently linked to their chromophore (all-*trans*-retinal). Channelrhodopsins belong to the class of microbial-type rhodopsins and show sequence similarity to bacteriorhodopsin (BR). Recently, the crystal structure of a channelrhodopsin at 2.3 Å resolution could show that the transmembrane domain and the position of the retinal are similar to the situation in BR (Kato et al. 2012). But in contrast to BR, channelrhodopsins have extended N-terminal and C-terminal domains. The N-terminal domain contributes to dimerization, which is another distinctive feature of channelrhodopsins, while BR assembles as a trimer (Kato et al. 2012). The dimer formation of channelrhodopsins has also been reported earlier by electron crystallography at 6 Å resolution (Müller et al. 2011). Both ChR1 and ChR2 are located at the eyespot apparatus of *Chlamydomonas* (Berthold et al. 2008; Boyd et al. 2011a; Mittelmeier et al. 2011; Suzuki et al. 2003; Wagner et al. 2008). The extended C-terminal domain of channelrhodopsins is possibly involved in the subcellular localization and in anchoring of these proteins at the eyespot apparatus (Kato et al. 2012; Kianianmomeni et al. 2009; Mittelmeier et al. 2011). The eyespot apparatus is a photoreceptive organelle found in many green flagellates; under the microscope the eyespot appears as an orange-red spot or stigma. The eyespot apparatus consists of carotenoid-filled pigment granules and rhodopsin-type photoreceptors. The pigment granules are arranged in several ordered layers in the stroma of the chloroplast, right beneath the chloroplast envelope membranes. Rhodopsin

photoreceptors, e.g., ChR1 and ChR2, are localized in the plasma membrane above the pigment granules (reviewed in Boyd et al. 2011b; Kreimer 2009). The eyespot apparatus is required for accurate light-monitoring and light-dependent movement responses to optimize the photosynthetic activities or to avoid photodamages. Gene silencing experiments provided evidence for the importance of ChR1 and ChR2 in light perception: when the mRNA levels of *ChR1* and *ChR2* were reduced, the cells showed a smaller photoreceptor current and a reduced light sensitivity (Sineshchekov et al. 2002). It was concluded that photoexcitation of ChR1 and ChR2 mediates phototactic orientation, i.e., changes in flagellar beat patterns through generation of their respective photoreceptor currents and, as a consequence, changes in the swimming direction (Sineshchekov et al. 2002). However, this hypothesis could only be confirmed for ChR1: the analysis of different RNAi-knockdown transformants with quite different ChR1 expression levels revealed a strong correlation between the amount of expressed ChR1 protein and the photocurrent amplitude (Berthold et al. 2008). Moreover, in contrast to wild-type gametes, no phototaxis or photophobic responses have been observed in the gametes of knockdown transformants expressing a reduced amount of ChR1 protein (Berthold et al. 2008). These results indicate that ChR1 is the main photoreceptor, which generates most of the photocurrent (Berthold et al. 2008; Govorunova et al. 2004). ChR2, in contrast, contributed only little to the photocurrent in gametes (Berthold et al. 2008). However, in vegetative *C. reinhardtii* cells, ChR2 contributes significantly to the photocurrent and to behavioral responses, especially after illumination with blue light (Berthold et al. 2008).

In contrast to the investigations in *Chlamydomonas*, functional analysis of photoreceptors in the multicellular alga *V. carteri* aims at the role of light-sensitive proteins in light-dependent developmental processes such as cellular differentiation. In *Volvox*, initiation of cell division and cellular differentiation are affected by light. In synchronized *V. carteri* cultures, the embryonic cleavage divisions of mature reproductive cells begin in the light period; later the divisions are completed in the dark (Desnitskiy 1984, 1985b). By turning off the light before initiation of division, reproductive cells of *V. carteri* will not initiate cleavage. However, so far both the required light quality and quantity are unknown; likewise, there is no information about which photoreceptors are involved in light-dependent initiation of cleavage divisions.

In other multicellular volvocine species like *V. tertius* and *V. aureus*, light is not only required for initiation of cleavage, but it is also required for the proceeding of the embryonic cleavage divisions (Desnitskiy 1985a). The difference in light dependence of cleavage between these *Volvox* species probably results from the fact that in *V. carteri* asexual reproductive cells first grow large and then divide rapidly, whereas reproductive cells of *V. tertius* and *V. aureus* are small when they begin to divide and they grow in size during cleavage divisions. In *V. carteri* a large pool of DNA precursors has to be built up in the large, mature reproductive cells before initiation of cleavage; this allows for a rapid series of cleavage divisions and the division even can be completed in the dark period, as postulated by Desnitskiy (Desnitskiy 1992). By contrast, in *V. tertius* and *V. aureus* the continuous growth in cell size during the divisions requires continuous photosynthetic activity to make the DNA necessary for each round of cleavage divisions (Desnitskiy 1992; Kirk 1998).

Another light-dependent process in *V. carteri* is the cellular differentiation. Under laboratory conditions, *V. carteri* algae can be grown synchronously in an 8-h-dark/16-h-light cycle with a life cycle of 48 h. After completion of cleavage divisions in the dark, the presumptive somatic and reproductive cells of juvenile spheroids remain undifferentiated until the light comes back on (Kirk and Kirk 1985). The pattern of proteins synthesized in both potential cell types is almost the same during the dark period and it changes rapidly when the light comes back on (Kirk and Kirk 1985). The action spectrum of this light-dependent protein synthesis is not the same as that for photosynthesis but it is shaped like the action spectrum of rhodopsin with a maximum around 510 nm (Kirk and Kirk 1985); it also correlates with the absorption maximum of VChR1 (500 nm at high pH, 540 nm at low pH) (Kianianmomeni et al. 2009; Zhang et al. 2008), a homolog of ChR1. Therefore, VChR1 might be responsible for light-dependent cellular differentiation or at least might be involved in this process. The involvement of VChR1 is also supported by the fact that the transcripts of *VChR1* accumulate during the dark period and reach their maximum level at the beginning of the light-dependent protein synthesis, right before the final cellular differentiation (Kianianmomeni et al. 2009). Besides, not only the action spectrum of the *Volvox* photocurrent and the absorption spectrum of VChR1 peak around 520 nm but also the action spectra of both negative and positive phototaxis (Sakaguchi and Iwasa 1979; Schletz 1976; Zhang et al. 2008); therefore, VChR1 has been assumed to be the main light receptor for *Volvox* phototaxis under vegetative conditions (Kianianmomeni et al. 2009).

An important characteristic of the *VChR1* gene is its cell type-specific transcription; it is highly expressed in somatic cells of *V. carteri* (Kianianmomeni et al. 2009). Recently, immunofluorescence microscopy studies revealed that ChR1, the homologous protein from *Chlamydomonas*, is localized in the plasma membrane directly above the eyespot pigment granule layers in the chloroplast. ChR1 is closely associated with the daughter four-membered microtubule rootlet (D4), a bundle of acetylated microtubules

extending from the daughter basal body toward the posterior of the cell. More precisely, ChR1 is localized near the end of the acetylated microtubules and along the D4 rootlet (Mittelmeier et al. 2011). Moreover, small spots of ChR1 were located near the basal bodies in both wild-type and flagella-less mutant cells (Mittelmeier et al. 2011). These spots were shaped like handlebars, which flank the basal bodies (Mittelmeier et al. 2011). In all volvocalean algae, basal bodies participate in various critical aspects of the cell division process (Ehler et al. 1995; Gaffal 1988; Johnson and Porter 1968; Kirk 1998). For *V. carteri*, it was suggested that proteins that are associated with basal bodies, especially those associated with the four-membered microtubule rootlets, prepare the cell to divide asymmetrically by changing the position of the basal body apparatus (Miller and Kirk 1999). The asymmetric cell division plays a key role in the development of *V. carteri*; asymmetric division produces large cells (asexual reproductive cells, sperm packets and eggs) and small cells (somatic cells) during asexual or sexual development (reviewed in Hallmann et al. 1998; Kirk 1997). Considering the localization of channelrhodopsin-1 and because *VChR1* is highly expressed in somatic cells, involvement in asymmetric cell division and cellular differentiation would be possible. No other genes, neither those involved in the flagellar apparatus, e.g., the flagellar α dynein (*dyhA*), nor those involved in germ-soma differentiation like the somatic regenerator (*regA*) show such cell type-specific transcript expression (Nematollahi et al. 2006).

In contrast to VChR1, the potential function of VChR2 is less conceivable. The absorption spectrum of VChR2 (λ_{max} = ~470 nm) shows a clear blue shift in comparison to the action spectrum of the light-dependent protein synthesis at the end of embryogenesis and also compared to the action spectrum of both the photocurrent and the phototaxis in *Volvox* (Kianianmomeni et al. 2009; Kirk and Kirk 1985; Sakaguchi and Iwasa 1979; Schletz 1976). Like *VChR1*, *VChR2* shows an extremely high transcript level in somatic cells in comparison to reproductive cells (Kianianmomeni et al. 2009). The fact that the mRNA expression of *VChR2* increases by 370 % after the switch to sexual development, suggests that VChR2 is involved in sexual development (Kianianmomeni et al. 2009). Interestingly, the blue light photoreceptor phototropin, which is involved in multiple steps in the sexual life cycle of *Chlamydomonas* (Huang and Beck 2003), did not show such an increase in the transcript level after the switch to sexual development in *V. carteri* (Kianianmomeni and Hallmann, unpublished data).

Histidine kinase rhodopsins

Another four rhodopsin-like photoreceptors, known as "enzymerhodopsins" or "histidine kinase rhodopsins" (*HKR1*, *HKR2*, *HKR3* and *HKR4*), have been identified in the genomes of both *V. carteri* and *C. reinhardtii* (Fig. 7) (Kateriya et al. 2004; Merchant et al. 2007; Prochnik et al. 2010). Histidine kinase rhodopsins normally consist of domains for a rhodopsin, a histidine kinase, a response regulator and an adenylyl or guanylyl cyclase (Fig. 7); however, HKR3 has no cyclase domain both in *C. reinhardtii* and in *V. carteri*. The four domains belong to a two-component signal transduction system (TCS), which is involved in a variety of signaling processes both in prokaryotes and eukaryotes (Hwang and Sheen 2001; Schaller et al. 2011; Stock et al. 2000). While bacterial TCSs use only the histidine kinase domain as a sensor, the eukaryotic TCSs of *Volvox* and *Chlamydomonas* also possess an N-terminal rhodopsin domain as an additional sensor for light. In the diatom *Thalassiosira pseudonana*, several two-component signaling proteins with other N-terminally located light-sensitive domains like Per-ARNT-Sim (PAS) and phytochrome have been identified (Bowler et al. 2008). These diatomal systems serve as a basic stimulus–response coupling mechanism to sense and respond to continuously changing environmental conditions (Stock et al. 2000). In prokaryotic systems, TCSs regulate global responses to stress stimuli, control the cell division or decide whether to continue with growth, to enter the stationary phase or to sporulate (Piggot and Hilbert 2004; Schaller et al. 2011). The environmental input signals cause autophosphorylation of the histidine kinase domain, which then transfers a high-energy phosphoryl group to the response regulator domain (Fig. 8). Next, the response regulator domain activates an effector domain, which triggers the corresponding cellular response (Stock et al. 2000).

The first identified member of the histidine kinase rhodopsin family was HKR1, which has been localized in the eyespot area of *C. reinhardtii* (Luck et al. 2012). This UVA-absorbing protein is bimodally switched by UV and blue light (Luck et al. 2012). It was assumed that HKR1 acts as an UV sensory rhodopsin in the eyespot of *Chlamydomonas*, which mediates general UV avoidance responses (Luck et al. 2012). The enzyme specificity of the cyclase in the response domain of HKR1 is not entirely clear; it is either an adenylyl or a guanylyl cyclase. In previous studies, the products of such cyclases, cyclic mononucleotides, were shown to be involved in cellular processes in *Chlamydomonas*. Accumulation of cyclic adenosine monophosphate (cAMP) has been observed in gametes undergoing agglutination (Goodenough 1989; Kooijman et al. 1990; Pasquale and Goodenough 1987). Moreover, Pasquale and Goodenough reported that exogenous dibutyryl-cAMP (a permeant analog of cAMP) induces all three agglutination-triggered responses: flagellar tip activation, loss of cell walls and mating structure activation (Pasquale and Goodenough 1987). Even

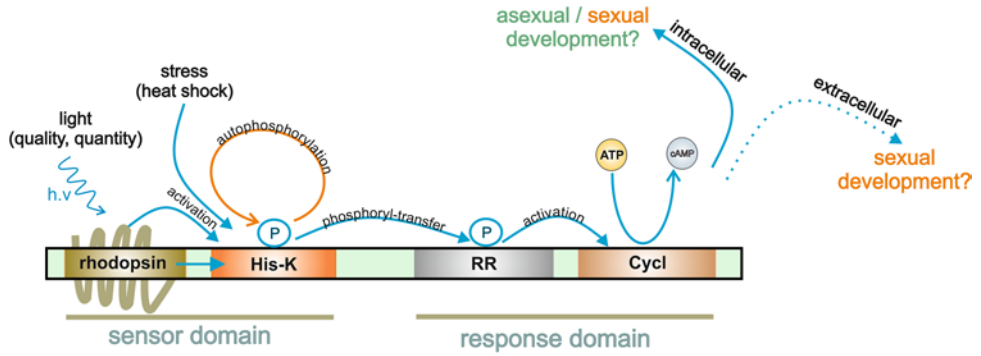

Fig. 8 Proposed model for the cascade function of *Volvox* histidine kinase rhodopsins and their potential involvement in asexual and sexual development. The histidine kinase domain of the photoreceptor is activated by environmental stimuli such as temperature stress or light; the light activation is mediated by the rhodopsin domain. Activation of the histidine kinase domain causes its autophosphorylation followed by the transfer of the phosphoryl group to the response regulator domain, which is activated in this way. The activated response regulator domain stimulates the cyclase domain, which produces cAMP from ATP. cAMP acts as a second messenger on intracellular (and maybe extracellular) targets, which finally trigger processes in asexual and sexual development. *HisK* histidine kinase domain, *RR* response regulator, *Cycl* adenylate/guanylate cyclase domain, *P* phosphoryl group

the mating function of non-agglutinating mutants can be rescued by treatment with dibutryl-cAMP. These results indicate that cAMP activates not only the known mating responses, but also other required responses (Goodenough 1989; Pasquale and Goodenough 1987). cAMP also was shown to be involved in the regulation of flagellar length in *Chlamydomonas* (Tuxhorn et al. 1998). In addition, cAMP-dependent kinase cascades and/or the gating of cAMP-gated ion channels were assumed to be involved in cAMP signal transduction in *Chlamydomonas* (Quarmby 1994; Quarmby and Hartzell 1994). Boonyareth et al. could show that an enhanced level of cAMP gets *Chlamydomonas* cells to swim toward green light and lower levels bias the cells to swim away from the light, implying a functional role of cAMP in regulation of phototaxis. The authors also speculated that an increased level of cAMP might be a result of rhodopsin activation (Boonyareth et al. 2009). As a consequence, it is also conceivable that histidine kinase rhodopsins with adenylyl cyclase activity might be involved in this signaling process.

In the multicellular alga *Volvox*, it was postulated that cAMP is an intracellular second messenger that is produced in response to the presence of the sex-inducer and triggers a signal cascade leading to sexual development (Kochert 1981). Moreover, it was reported that signal transduction with cAMP as a second messenger is mediated in *Volvox* by intracellular cAMP receptors called cAMP-binding proteins (Feldwisch et al. 1995). Furthermore, disintegration of sperm packets of sexual male *Volvox* algae has been observed after the addition of dibutyryl-cAMP (Waffenschmidt et al. 1990). It is known that sexual development of *Volvox* is not only induced by the sex-inducer but also in response to heat and oxidative stress (Kirk and Kirk 1986; Nedelcu 2005; Nedelcu and Michod 2003) and also light plays a critical role in the success of this sexual induction (Starr et al. 1980). In this regard, it is remarkable that cAMP has been shown to be involved in responses to heat and oxidative stress (Taminato et al. 2002; Wang et al. 2004). Provided that histidine kinase rhodopsins of *V. carteri* with adenyl cyclase activity can produce cAMP in a light-dependent manner in response to environmental signal inputs, it is likely that they participate in sexual development (Fig. 8). The produced intracellular cAMP/cGMP could also act as a regulator for activation of transcription factors required for development of the multicellular organism; at least, in many other eukaryotes it has been shown that activation of transcription factors is frequently the target of cAMP signaling (McDonough and Rodriguez 2012). Furthermore, some DNA-binding proteins mediate transcriptional responses to cAMP. These transcription factors are probably involved in the regulation of genes in certain cell types during development (Shaulsky and Huang 2005). Recently, it has been shown in *Ostreococcus* that a light-dependent change in the cAMP level controls the synthesis of CyclinA, which then interacts with the retinoblastoma protein (RB) to regulate the cell division pathway (Moulager et al. 2010).

Application of light-sensitive modules in synthetic biology and neurobiology

In living cells, biological processes show a great spatial and temporal variability. For example, regulation of gene expression, i.e., control of transcription and translation rates, typically occurs over tractable timescales of minutes to hours, while most post-translational regulatory mechanisms such as covalent protein modifications and binding

of interacting proteins occur within seconds (Olson and Tabor 2012).

Standard molecular biology techniques such as gene knockdown, gene up-regulation or gene overexpression are effective tools that aim at the functional characterization of proteins involved in various cellular processes; such techniques also allow for the identification of gene–phenotype relations using mutant strains. Moreover, the subcellular localization of target proteins can be determined by fusing genes of fluorescent proteins, such as the GFP gene, to the target genes. However, these tools are slow in timescale and lack the capacity to spatial control of protein activity and protein–protein interactions on the micron scale. In recent years, photo-sensitive modules from plants including algae have been used as tools to generate light switches for precise and specific control of diverse functions in living cells (Toettcher et al. 2011). These new tools can be used in two different ways to activate or modulate cellular activities: on one hand by light-induced manipulation of ion concentrations or levels of signaling molecules such as cAMP, and on the other hand, by linking light to protein activity and protein–protein interactions by light-switchable allostery, scaffolding and anchoring.

Light-induced manipulation of ion concentrations or levels of signaling molecules such as cAMP

As mentioned above, the channelrhodopsin ChR2 is a light-activated cation-specific ion channel of *Chlamydomonas* (Nagel et al. 2003). This ion channel can be expressed in mammalian neurons and then activated by blue light (~460 nm) (Fig. 9a), allowing reliable and precise control

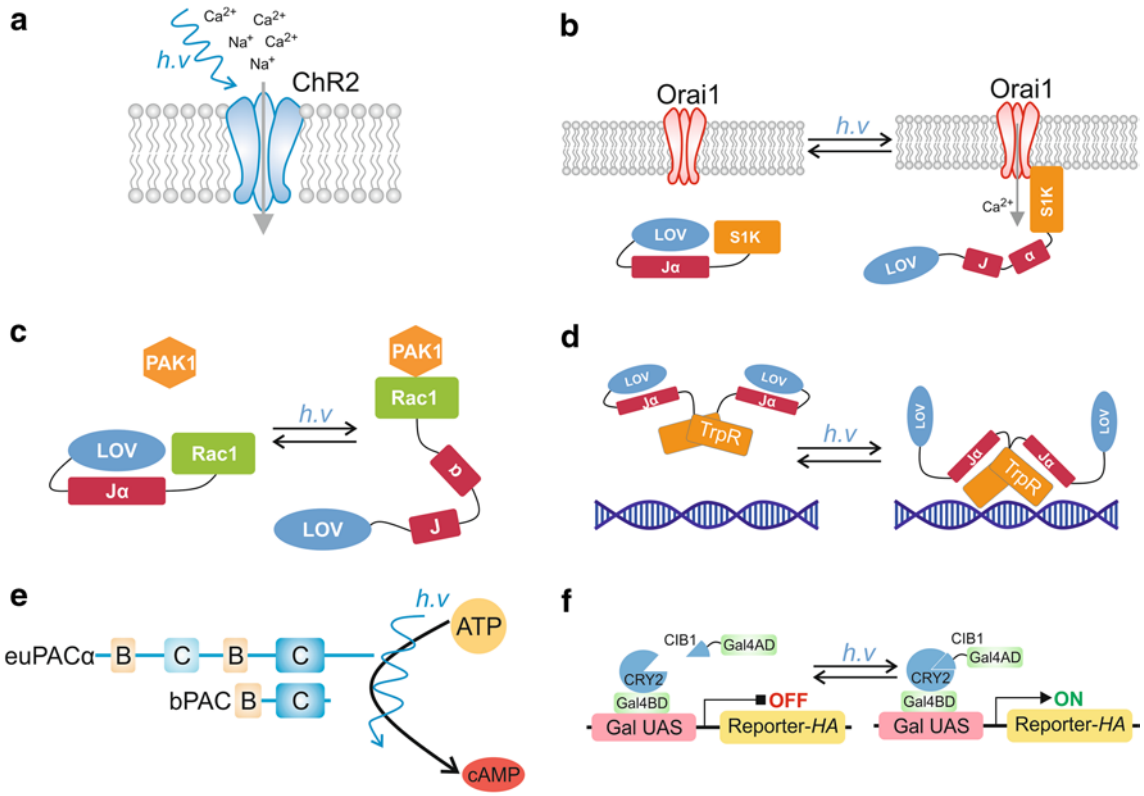

Fig. 9 Applications for light-sensitive modules in synthetic biology. **a** Generation of light-driven membrane depolarization using ChR2 to control neural spiking (Boyden et al. 2005). **b** Blue-light-activated LOV-Jα allows for translocation of the S1K domain to Orai1, a plasma membrane Ca^{2+} channel, which generates a local Ca^{2+} signal on the plasma membrane (Pham et al. 2011). In the dark, LOV blocks the interaction of S1K with Orai1. The blue light causes unfolding of Jα; once Jα is unfolded, LOV releases the S1K domain, which then interacts with Orai1. **c** Generation of a photoswitchable Rac1, a small signaling G protein, which normally binds PAK1 for downstream activations. However, in the dark the binding site of Rac1 for PAK1 is blocked by the LOV domain. Exposure to blue light causes unfolding of Jα, liberation of the PAK1-binding site and interaction of Rac1 with PAK1 (modified after Wu et al. 2009). **d** Light-regulation of DNA binding activity of the dimeric tryptophan repressor protein TrpR using LOV-Jα; in the dark, binding of LOV to a shared helix of TrpR populates an inactive conformation of the TrpR domain; unfolding of Jα allows for the release of the LOV domain and, thus, activation of the TrpR repressor (modified after Strickland et al. 2010). **e** Utilization of naturally occurring, light-sensitive adenylyl cyclases from the alga *Euglena gracilis* (euPAC) or the bacterium *Beggiatoa* (bPAC) to manipulate the cellular cAMP level by light (Ryu et al. 2010; Schröder-Lang et al. 2007; Stierl et al. 2011). **f** Construction of a light-activated transcription system based on the (rapid) interaction of the cryptochrome CRY2 with CIB1 upon blue light irradiation (modified after Kennedy et al. 2010; Liu et al. 2012)

of neuronal spiking (Boyden et al. 2005). In combination with a light-driven bacterial chloride pump (halorhodopsin, NpHR), multiple-color optical activation and silencing of neural circuitry were achieved on the millisecond timescale in living cells and even in freely moving animals (Han and Boyden 2007; Zhang et al. 2007). Such light-induced manipulations have been further developed by generating new channelrhodopsin variants (Gunaydin et al. 2010; Mattis et al. 2012; Prigge et al. 2012; Zhang et al. 2011), by identification of red-shifted channels in other algae (Govorunova et al. 2011; Kianianmomeni et al. 2009; Zhang et al. 2008) as well as through the improvement of molecular techniques, which allow functional expression in various living animals such as worms, fruit flies, mice and primates (Bi et al. 2006; Han et al. 2009; Honjo et al. 2012; Petreanu et al. 2007; Schroll et al. 2006; Zhang et al. 2007). Through these achievements, optogenetics technology has become widely used in neuroscience as a novel, revolutionary tool for fast neuronal control. Furthermore, the recent crystal structure of a channelrhodopsin is an important milestone for any future structure-based engineering of optimized channels with improved photocurrents, photosensitivity and kinetics (Kato et al. 2012).

Because the optogenetics technology allows to control the excitation and inhibition of specific circuit elements in mammalian neurons, even its application for restoration of visual functions and to treat psychiatric diseases in humans is currently under discussion (Deisseroth 2012). Preliminary experiments could already show that ChR2 can be used to restore a visual function in blind mice (Doroudchi et al. 2011), to investigate neural circuits underlying anxiety and fear related behaviors (Johansen et al. 2010; Tye et al. 2011) and to activate the primary visual cortex of primates with blue light stimuli causing eye movements (Jazayeri et al. 2012).

Photoswitchable proteins that change the level of specific signaling molecules, such as cAMP in a light-dependent manner, are also promising tools to manipulate animal behavior with external light stimuli. An example for such a light-sensitive protein is the light-activated adenylyl cyclase euPACα of the eukaryotic unicellular flagellate *Euglena gracilis*. euPACα was shown to be a blue light photoreceptor for photo avoidance (Iseki et al. 2002). euPACα possesses two subunits, each of which contains a light-sensitive, flavin-binding domain, designated as BLUF (sensors of blue-light using FAD) domain (Gomelsky and Klug 2002), followed by a catalytic adenylyl cyclase domain (Fig. 9e). Heterologous expression of euPACα allows for fine spatiotemporal control of the cAMP level in amphibian cells, i.e., *Xenopus laevis* oocytes, and in mammalian cells, i.e., human embryonic kidney (HEK293) cells (Schröder-Lang et al. 2007). Also insect cells have been modified: transgenic fruit flies expressing euPACα show changes in their behavior after blue light illumination (Schröder-Lang et al. 2007). In nematodes, i.e., cholinergic neurons from transgenic *Caenorhabditis elegans*, expression of euPACα allows manipulation of the behavior in a light-dependent manner. The cell type-specific expression of euPACα in cholinergic neurons was achieved using appropriate promoters. Hence, the light-induced amount of cAMP produced by euPACα in cholinergic neurons directly affected the intracellular signaling and caused changes in the swimming frequency and the speed of locomotion (Weissenberger et al. 2011). Furthermore, blue light stimulation of euPACα has been shown to increase the spike width in *Aplysia* sensory neurons (Nagahama et al. 2007). In *Drosophila* larvae that expressed euPACα in motor neurons, miniature excitatory junction potential (mEJP) frequency was highly increased after blue light irradiation (Bucher and Buchner 2009).

Recently, another photoactivated cyclase, bPAC (Fig. 9e), was characterized, which might to be even more favorable for analysis of signaling pathways in living organism compared to euPACα, because bPAC is smaller in size and shows a higher light-activated cyclase activity (Ryu et al. 2010; Stierl et al. 2011).

Linking light to protein activity and protein–protein interactions by light-switchable allostery, scaffolding and anchoring

In the blue light photoreceptor phototropin, the interaction between the photo-sensitive LOV domain and the amphipathic helix Jα plays a key role in the activation of the phototropin kinase domain. Although Jα associates with the LOV domain in the dark, illumination with blue light leads to Jα unfolding and dissociating from the LOV domain (Harper et al. 2003). This light-induced conformational change has been used to design LOV-based photoswitches, by insertion of LOV-Jα into a catalytic protein of interest. In its initial state (dark), the LOV domain sterically blocks the function of the protein of interest; the light-induced change in the conformation of LOV terminates the steric block and leads to protein activation (Fig. 9b–d).

In another experimental approach, both the LOV domain and the *E.coli* tryptophan repressor protein TrpR were used to create a series of DNA constructs for expression of the chimeric proteins (Strickland et al. 2008). One of these chimeric proteins showed about fivefold higher affinity to DNA in the illumination state compared to the dark state (Fig. 9d). The light-regulated affinity was even increased about 64-fold in subsequent rounds of rational mutagenesis (Strickland et al. 2010). In a similar way, also a fusion of the LOV domain with the *E. coli* dihydrofolate reductase (DHFR) led to a light-dependent dihydrofolate reductase activity (Lee et al. 2008).

After this pioneering work, Hahn et al. used LOV-Jα to control protein activity even in living cells (Wu et al. 2009). They fused LOV-Jα N-terminally to the small GTPase protein Rac1, a key regulator of actin dynamics and cell migration. In the dark state, the effector binding site of Rac1 is blocked by the LOV domain (Fig. 9c); after illumination, the light-induced conformational change of LOV-Jα results in Rac1 activity (Fig. 9c). Both the light activation and the light deactivation of Rac1 were sufficient to produce cell motility and control the direction of cell movement (Wu et al. 2009). Later, this photoactivatable form of Rac has also been used to control movement in zebrafish embryos (Walters et al. 2010; Yoo et al. 2010) and *Drosophila* ovary cells (Ramel et al. 2013; Wang et al. 2010). The photoactivatable Rac also allowed for light-dependent disassembling of vimentin intermediate filaments (VIF), which are a major cytoskeletal component in mouse embryo fibroblasts (Helfand et al. 2011).

Recently, LOV-Jα was even used to generate a chimeric protein that produces local or global signals by Ca^{2+} entry through the plasma membrane in response to light. For this purpose, Pham et al. fused the C-terminal fragment S1K of the calcium-sensor protein STIM1 to LOV-Jα (Fig. 9b) (Pham et al. 2011). In the dark state, the LOV domain prevents the interaction of S1K with the plasma membrane Ca^{2+} entry channel Orai1. Illumination with blue light changes the conformation of LOV-Jα and allows for translocation of S1K to the Orai1 channel, which opens and produces a local Ca^{2+} entry at the plasma membrane (Pham et al. 2011).

Further applications with LOV-Jα fusion proteins have been reported including a blue-light-regulated histidine kinase (Möglich et al. 2009), a photoactivated endonuclease (Schierling and Pingoud 2012), a light-activated dihydrofolate reductase and lipase (Krauss et al. 2010), and a photoactivated caspase-7 for rapid, light-induced stimulation of apoptosis (Mills et al. 2012). Even photocontrol of peptide activity was achieved, which allows for precise spatial and temporal control of cellular functions (Lungu et al. 2012). Likewise, light-dependent spatiotemporal control of gene activation, protein expression and purification was achieved with LOV-based techniques (Gawthorne et al. 2012; Polstein and Gersbach 2012; Wang et al. 2012).

Recently, Shu et al. produced an engineered, small fluorescent flavoprotein with only 106 amino acids in length (called MiniSOG), which was deduced from *Arabidopsis* phototropin-2. Fusions of MiniSOG with a target protein allow for correlated protein localization by fluorescence and electron microscopy on the same tissue sample (Shu et al. 2011).

Moreover, a LOV domain-based optogenetic tool was designed for control of protein degradation. This tool, the so-called generic photo-sensitive degron (psd) module, has been produced by combining the light-reactive LOV2 domain of *Arabidopsis* phot1 with the murine ornithine decarboxylase-like degradation sequence cODC1; this degron module can be used to control protein levels in biotechnological or biomedical applications (Renicke et al. 2013).

Additional strategies for the investigation of protein–protein interactions (anchoring, scaffolding) using light-sensitive modules are currently under development; these strategies also aim at the light-dependent induction and regulation of cell signaling. In another elegant experiment, the light-dependent interaction between the blue light receptor cryptochrome and a basic helix–loop–helix transcription factor CIB1 was already used to engineer a light-induced transcription system in living mammalian cells and in zebrafish embryos (Fig. 9f) (Kennedy et al. 2010; Liu et al. 2012). Moreover, this approach was used for blue-light-induced protein translocation (Kennedy et al. 2010) and for rapid and reversible protein oligomerization in living cells (Bugaj et al. 2013). Most recently, Zhang et al. developed a new set of tools for light-inducible transcriptional effectors (LITEs). The LITE system is composed of two parts: an "anchor" (a customizable TALE DNA-binding domain fused to the light-sensitive cryptochrome-2) and an "effector" domain (the interacting partner of cryptochrome-2, CIBI). This system enables optical control of gene transcription and it allows for reversible activation within minutes (Konermann et al. 2013). Fusion of an *Arabidopsis* cryptochrome with a DNA damage checkpoint protein even enabled the activation of the DNA damage signaling pathway in the absence of DNA damage (Ozkan-Dagliyan et al. 2013). Also the light-inducible interactions between phytochrome and the phytochrome interaction factor 3 (PIF3) (Leung et al. 2008; Tyszkiewicz and Muir 2008) or the LOV-containing F-box proteins FKF1 and its partner GIGANTEA (Yazawa et al. 2009) have been used to regulate various cellular activities. A light-switchable gene promoter system should also be mentioned, which was constructed using an *Arabidopsis* phytochrome; this system allows for reversible control of gene expression with red and far-red light (Shimizu-Sato et al. 2002). The far-red reversibility of phytochromes was also used for development of a light-switchable protein–protein interaction system for spatiotemporal control of cell signaling (Levskaya et al. 2009) and for the design of a red/far-red light-responsive bistable toggle switch to control molecular interventions in mammalian cells, tissues and organisms (Müller et al. 2013).

Conclusion

The light absorption system in eukaryotic (micro)algae includes highly sensitive photoreceptors, which change

their conformation in response to different light qualities on a subsecond time scale and induce physiological and behavioral responses. The confusing maze behind the different activities and characteristics of algal photoreceptors has changed our view of photoreceptor functions during the last couple of years.

Some of the light-sensitive modules are already used to engineer and design photoswitchable tools to control cellular and physiological activities in living organisms of different complexity. The capability of some algal photoreceptors to change the concentration of specific ions or signaling molecules such as cAMP makes light-sensitive proteins even more attractive for use in synthetic biology. Thus, a discussion about the in vivo use of light-sensing modules for manipulation and control of cell signaling pathways began not too long ago and the term "in vivo biochemistry" was coined for this strategy (Toettcher et al. 2011); currently, the development of techniques for light-dependent control of signaling pathways is under way.

The continuing increase in the number of sequenced algal genomes comes along with an increase in the number of identified genes coding for proteins with light-sensitive modules and, somewhat later, with the solution of their 3D structures. Thus, we assume that the diversity of light-sensitive modules will further grow and this will not only extend the source material for the generation of optogenetic tools but also foster the development of new light-based strategies in cell signaling research. Especially, new light-gated channels with an improved recovery time for neuronal firing at higher frequencies or light-sensing proteins with red-shifted action spectra will support any structure-based engineering and will help to expand the molecular toolbox with a variety of light-dependent tools in neuroscience. Algal species such as *Volvox barberi* and *Cryptomonas rostratiformis*, which show differences in swimming speeds (Solari et al. 2008) and action spectra of phototaxis (red-shifted) (Foster and Smyth 1980), might deliver channels with strongly shifted action spectra or different ion conductance, pH optima and detergent resistance.

Beside the search for new proteins with light-sensitive modules, researchers are also interested in the simplification of their molecular tools. In this regard, even smaller light-sensitive modules would be desirable, which would simplify the construction of hybrid genes. Smaller light-sensitive modules would also facilitate the generation of mutated and chimerized light-sensitive modules, which is useful to obtain artificial modules with completely new characteristics, such as modified light sensitivities, improved kinetic features and enhanced enzyme activities.

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