PCP
PLANT & CELL PHYSIOLOGY

Evolution of Three LOV Blue Light Receptor Families in Green Plants and Photosynthetic Stramenopiles: Phototropin, ZTL/FKF1/LKP2 and Aureochrome

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(Received October 21, 2012; Accepted November 22, 2012)

Many organisms, including bacteria, fungi, animal, plants and algae, utilize blue light to adapt to a fluctuating light environment. Plants and algae, and photosynthetic stramenopiles in particular, require light energy for photosynthesis and have thus evolved a range of sophisticated light-sensing systems to utilize light information efficiently for growth, development and physiological responses. LOV (light, oxygen or voltage) domain photoreceptors are widely distributed among prokaryotic and eukaryotic organisms, and the number of specific LOV photoreceptors are increased in certain taxa. In this review, we summarize the molecular basis and physiological functions of three different families of LOV blue light receptors specific to green plants and photosynthetic stramenopiles: phototropin, ZEITLUPE/FLAVIN-BINDING, KELCH REPEAT, F-BOX 1/LOV KELCH PROTEIN 2 (ZTL/FKF1/LKP2) and aureochrome.

Keywords: *Arabidopsis* • aureochrome • blue light • LOV • phototropin • ZTL/FKF1/LKP2.

Abbreviations: ASK, Arabidopsis SKP1-like protein; AUREO, AUREOCHROME; bZIP, basic region/leucine zipper; CCA1, CIRCADIAN CLOCK ASSOCIATED 1; CDF, CYCLING DOF FACTOR; ChIP, chromatin immunoprecipitation; CO, CONSTANS; COP1, CONSTITUTIVE PHOTOMORPHOGENIC 1; cry, cryptochrome; CUL1, CULLIN1; dsRNA, double-stranded RNA; ELF3, EARLY FLOWERING 3; FKF1, FLAVIN-BINDING, KELCH REPEAT, F-BOX 1; *FT*, *FLOWERING LOCUS T*; GFP, green fluorescent protein; GI, GIGANTEA; LD, long day; LHY, LATE ELONGATED HYPOCOTYL; LKP2, LOV KELCH PROTEIN 2; LLP, LOV/LOV PROTEIN; LOV, light, oxygen or voltage; RNAi, RNA interference; phot, phototropin; phy, phytochrome; PRR5, PSEUDO RESPONSE REGULATOR 5; TOC1, TIMING OF CAB EXPRESSION 1; ZTL, ZEITLUPE.

Introduction

Light is one of the most important environmental signals for organisms to adapt to fluctuating natural conditions. In particular, photosynthetic organisms such as land plants and algae have aquired many light-sensing systems throughout the course of their evolution because they require light energy to perform photosynthesis and consequently grow and develop. For example, land plants have evolved many different classes of photoreceptors: the red/far-red light receptor phytochrome (phy) (Chen and Chory 2011), various blue light receptors such as cryptochrome (cry) (Liu et al. 2011a) and phototropin (phot) (Christie 2007), and the UV-B photoreceptor UV RESISTANCE LOCUS 8 (Heijde and Ulm 2012). Although phys and crys were first identified in plants, these photoreceptors were subsequently identified in various bacteria, fungi and animals (Rockwell et al. 2006, Chaves et al. 2011). In contrast, phots have been identified only in the green plant lineage, from green algae to flowering plants (Christie 2007). phots possess a serine/threonine kinase domain at the C-terminus and two 107 amino acid repeats at the N-terminus of the protein (Huala et al. 1997, Christie 2007) (**Fig. 1**). Similar motifs have been found in several proteins that bind a flavin prosthetic cofactor and mediate light, oxygen or voltage sensing and were thus named LOV domains (Huala et al. 1997) (**Fig. 2**). Each phot LOV domain (LOV1 and LOV2) binds FMN non-covalently at a 1 : 1 ratio and exhibits absorbance and fluorescence properties that overlap with the action spectrum for phototropism (Christie et al. 1999). Several proteins from archea, eubacteria and eukaryotes have been identified as having one LOV domain that is highly similar to those present in plant phots and thus are expected to bind a flavin cofactor and function as blue light receptors (Krauss et al. 2009, Herrou and Crosson. 2011). Indeed, the LOV domain has been identified as a ubiquitous modular unit that can be linked to diverse output domains (Krauss et al. 2009, Herrou and Crosson 2011).

In this review article, we discuss research progress regarding three families of LOV blue light receptors in green plants and photosynthetic stramenopiles: phototropin, ZEITLUPE/FLAVIN-BINDING, KELCH REPEAT, F-BOX 1/LOV KELCH PROTEIN 2 (ZTL/FKF1/LKP2) and aureochrome (**Fig. 1**). Although phot is specific to green plants (land plants and

Plant Cell Physiol. 54(1): 8–23 (2013) doi:10.1093/pcp/pcs165, available online at www.pcp.oxfordjournals.org

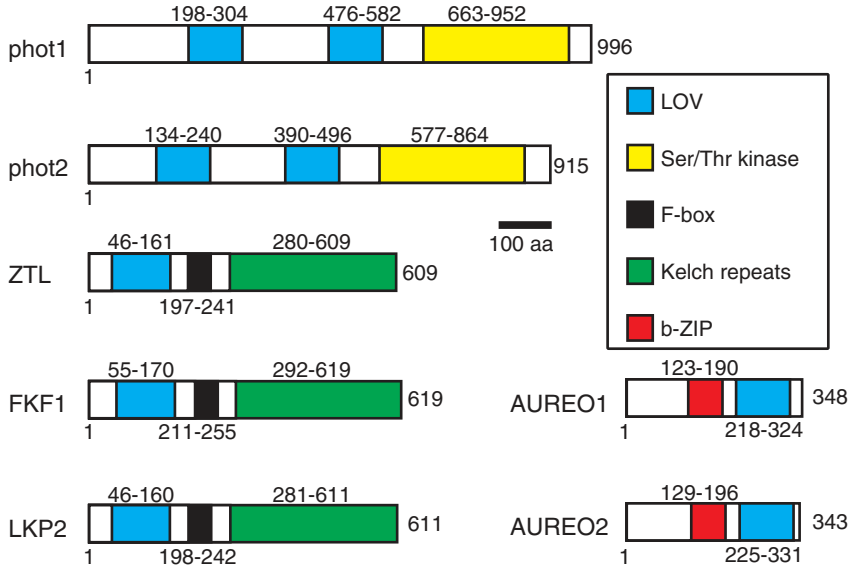

Fig. 1 Schematic alignment of the LOV blue light receptor protein families, phototropin, ZTL/FKF1/LKP2 and aureochrome, in green plants and photosynthetic stramenopiles. Phototropins (phot1 and phot2) and ZTL/FKF1/LKP2 (ZTL, FKF1 and LKP2) family members are *Arabidopsis thaliana* proteins, and two aureochromes (AUREO1 and AUREO2) are *Vaucheria frigida* proteins. Domains are indicated in the right-hand box. The first and the last amino acid numbers of each domain are indicated. The numbers on the right indicate the protein length. The AGI numbers and accession numbers are as follows: *PHOT1*, At3g45780; *PHOT2*, At5g58140, *ZTL*, At5g57360; *FKF1*, At1g68050; *LKP2*, At2g18915; *AUREO1*, AB252504; *AUREO2*, AB252505.

green algae), ZTL/FKF1/LKP2 is specific to land plants. Aureochrome is found only in photosynthetic stramenopiles. Land plants have another LOV blue light receptor family, LOV/LOV PROTEIN (LLP), which consists of only two tandem LOV domains (Ogura et al. 2008, Kasahara et al. 2010). However, LLP is not reviewed here because its physiological function is presently unknown (Ogura et al. 2008, Kasahara et al. 2010). First, we review recent structure–function analyses of phot blue light receptors. For more detailed information on their photochemical properties and physiological responses mediated by phots, readers should refer to previous comprehensive reviews on the appropriate topics: overall phot research (Christie 2007); photochemical and biochemical properties of phot (Tokutomi et al. 2008, Terazima 2011); phototropism (Iino 2001, Sakai and Haga 2012, Christie and Murphy 2013); chloroplast photorelocation movement (Suetsugu and Wada 2007, Banaś et al. 2012, Suetsugu and Wada 2012); and stomatal opening (Shimazaki et al. 2007, Inoue et al. 2010).

Recent Structure–Function Characterization of Phototropin Photoreceptor Kinases

Phototropin (phot) is a blue light receptor for various photomovement responses in green plants such as phototropism, chloroplast photorelocation movement, stomatal opening and leaf photomorphogenesis (Christie 2007, Kozuka et al. 2011) (**Fig. 3**). Many organisms of the green plant lineage from green algae to flowering plants possess two or more *PHOT* genes; for example, the model flowering plant *Arabidopsis thaliana* has two *PHOT* genes, *PHOT1* and *PHOT2* (Christie, 2007). More than 20 years have passed since Winslow Briggs' group published the first biochemical detection of phot as a 120 kDa plasma membrane-localized phosphoprotein (Gallagher et al. 1988). His group subsequently revealed that the 120 kDa phosphoprotein was the blue light receptor for phototropism in *A. thaliana* and exhibited blue-light-dependent autophosphorylation (Huala et al. 1997, Christie et al. 1998); the protein was thus named 'phototropin'.

Since its discovery, a great deal of information on phot has been obtained, primarily from molecular genetic research using Arabidopsis. phot1 and phot2 redundantly mediate phototropism, chloroplast photorelocation movement, stomatal opening, leaf flattening, palisade cell elongation and the growth inhibition of seedlings (Christie 2007, Kozuka et al. 2011) (**Fig. 3**). phot1 functions over a wide range of blue light fluence rates, while phot2 function is prominent under high blue light fluence rates (Kagawa and Wada 2000, Kinoshita et al. 2001, Sakai et al. 2001, Inoue et al. 2008b) (**Fig. 3**). These findings indicate that phot1 exhibits a higher degree of blue light sensitivity for all phot-mediated responses. Therefore, phot1 and phot2 may possess intrinsically different properties. However, the underlying basis for these functional differences between phot1 and phot2 still remains to be determined. However, as discussed below, the different photosensitivities between phot1 and phot2 appear to relate to differences within their N-terminal photosensory regions. Despite the functional redundancy between phot1 and phot2 for many responses, the chloroplast

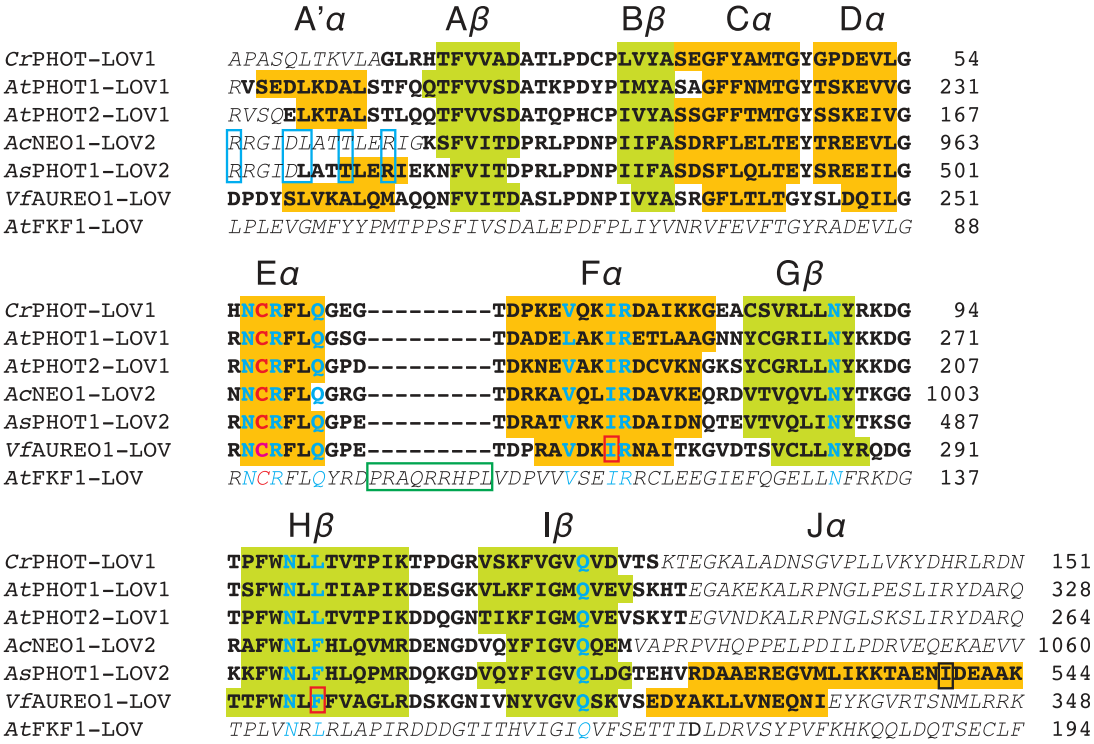

Fig. 2 Multiple sequence alignment of the LOV domains from five phot family members, AUREO1 and FKF1. Alignment is constructed based on previous studies (Nakasako et al. 2008, Mitra et al. 2012). For clarity, alignment was not constructed in the Jα-helix region. In the LOV domains whose crystal structures are resolved, the residues forming α-helices and β-sheets have been highlighted with orange and light green backgrounds, respectively. The residues in the region in which the secondary structures have not yet been determined are italicized. The cysteine residues essential for FMN–cysteinyl adduct formation are highlighted in red. Other FMN-interacting residues are highlighted in blue. The residues essential for the repression of the kinase domain in the *Chlamydomonas reinhardtii* phot LOV2 domain are surrounded by blue lines. The ZTL/FKF1/LKP2-specific amino acid insertion is surrounded by green lines. AUREO1 residues that are different from those in AUREO2 are surrounded by red lines. The isoleucine residue associated with the Jα-helix involved in the repression of phot1 kinase activity in darkness is surrounded by black lines. *Cr*, *Chlamydomonas reinhardtii*; *At*, *Arabidopsis thaliana*; *Ac*, *Adiantum capillus-veneris*; *As*, *Avena sativa*, *Vf*, *Vaucheria frigida*. AGI numbers and accession numbers are as follows: *CrPHOT*, AJ416557; *AtPHOT1*, At3g45780; *AtPHOT2*, At5g58140; *AcNEO1*, AB012082; *AsPHOT1*, AF033097; *AtFKF1*, At1g68050; *VfAUREO1*, AB252504.

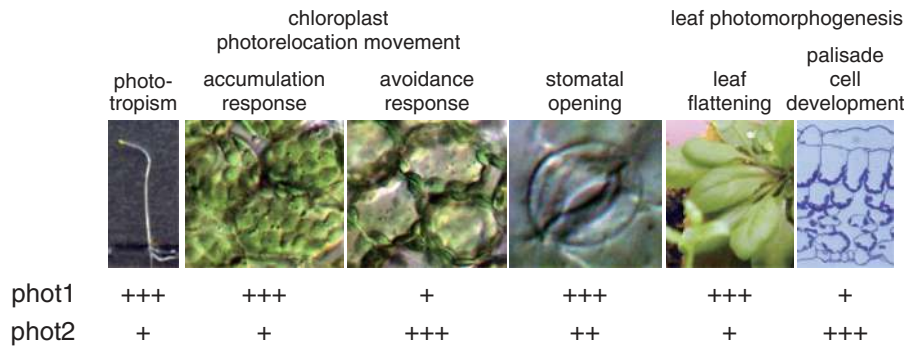

Fig. 3 Different light sensitivities of phot1 and phot2 in various physiological responses. phot1 is the main photoreceptor for phototropism, the chloroplast accumulation response and leaf flattening (indicated by +++), because *phot2* single mutants showed no defects in these responses under the light conditions examined (Sakai et al. 2001, Inoue et al. 2008b). However, phot1 shows negligible contributions to the chloroplast avoidance response and palisade cell development only in the *phot2* single mutant background (indicated by +) (Luesse et al. 2010, Kozuka et al. 2011). Conversely, phot2 is the main photoreceptor for the chloroplast avoidance response and palisade cell development (indicated by +++) (Jarillo et al. 2001b, Kagawa et al. 2001, Kozuka et al. 2011). However, the contributions of phot2 to phototropism, the chloroplast accumulation response and leaf flattening are only detectable under high light conditions in the *phot1* single mutant background (indicated by +) (Kagawa and Wada 2000, Sakai et al. 2001, Inoue et al. 2008b). Both *phot1* and *phot2* single mutants showed decreased stomatal opening under the low light conditions. However, the stomatal opening defect is more pronounced in the *phot1* mutant compared with the *phot2* mutant (Kinoshita et al. 2001), indicating that phot1 contributes more to this response (indicated by +++ vs. ++).

avoidance response is mediated solely by phot2 (Jarillo et al. 2001b, Kagawa et al. 2001, Luesse et al. 2010) (**Fig. 3**).

Difference in the expression pattern between phot1 and phot2

The protein level of phot1 is much higher than that of phot2 in dark-grown (etiolated) seedlings (Sakamoto and Briggs 2002, Kong et al. 2006, Aihara et al. 2008, Kang et al. 2008), in accordance with the dominant role of phot1 in phototropism (Huala et al. 1997, Sakai et al. 2001). Therefore, the main role of phot1 in phototropism most probably results from its abundance in etiolated seedlings. However, phot1 and phot2 functions have recently been assessed in more detail by placing their expression under the control of the same promoter. When functional phot–green fluorescent protein (GFP) fusion proteins (P1G for phot1 or P2G for phot2) were expressed at a similar level in the *phot1phot2* double mutant under the control of the *PHOT2* promoter (i.e. P2–P1G for phot1 or P2–P2G for phot2), only P1G could restore both the phototropic and chloroplast accumulation response under low blue light conditions (Aihara et al. 2008). These results therefore indicate that the higher light sensitivity of phot1, rather than its higher expression level relative to that of phot2, accounts for its contribution to low light-induced responses. Furthermore, domain swapping analysis of the N-terminal light-sensing region and the C-terminal kinase domain between phot1 and phot2 (i.e. P2–P1n/P2cG and P2–P2n/P1cG) showed that P2–P1n/P2cG but not P2–P2n/P1cG exhibited a light sensitivity similar to that of P2–P1G (**Fig. 4B**), indicating that the N-terminal photosensory region, including the LOV domains, is essential for the higher light sensitivity of phot1 over phot2 (Aihara et al. 2008). A recent study suggests that this difference in light sensitivity between phot1 and phot2 results from the difference in the lifetime of the FMN-C(4a)–cysteinyl adduct of LOV2 (Okajima et al. 2012).

Difference in blue-light-induced relocalization pattern between phot1 and phot2

Although phot1 and phot2 are localized to the plasma membrane (Sakamoto and Briggs 2002, Harada et al. 2003, Kong et al. 2006), their blue-light-induced relocalization pattern from the plasma membrane is quite different. Blue light partly induces P1G relocalization from the plasma membrane to the cytosol, possibly via clathrin-mediated endocytosis (Sakamoto and Briggs 2002, Aihara et al. 2008, Han et al. 2008, Wan et al. 2008, Kaiserli et al. 2009). Indeed, a higher amount of phot1 was detected by immunoblotting in the cytosolic protein fraction in blue-light-irradiated etiolated seedlings vs. seedlings maintained in darkness (Sakamoto and Briggs 2002, Han et al. 2008). Although blue light also induces P2G internalization from the plasma membrane, some fraction of P2G is associated specifically with the Golgi apparatus (Kong et al. 2006). The C-terminal half, including the entire kinase domain, is necessary and sufficient for plasma membrane localization, the association with the Golgi apparatus and to elicit specific phot-mediated responses (Kong et al. 2007, Aihara et al. 2008). The extreme C-terminal 42 amino acids of phot2 following the kinase core domain are necessary for Golgi association and for efficient plasma membrane localization (Kong et al. 2013). This short region is also necessary for the chloroplast avoidance response and partially for the accumulation response, but is dispensable for both phototropism and leaf flattening (Kagawa et al. 2004, Kong et al. 2013). These results imply that the blue-light-induced Golgi association of phot2 might be essential for the regulation of the chloroplast avoidance response. However, the domain swap protein P2n/P1cG harboring the N-terminus of phot2 and the C-terminus of phot1 is reported to retain the ability to induce the chloroplast avoidance response (**Fig. 4B**) despite showing no blue-light-induced Golgi association similar to P1G (Aihara et al. 2008) (**Fig. 4B**). Hence, light-induced Golgi association does not appear to explain why the chloroplast avoidance response is mediated exclusively by phot2.

Kinase activity and autophosphorylation of phototropins

The substitution of an aspartate residue for an asparagine (D806N for phot1 and D720N for phot2) (**Fig. 4A**) completely abrogates auto- and transphosphorylation activity in vitro and in vivo (Christie et al. 2002, Matsuoka and Tokutomi 2005, Cho et al. 2007, Inoue et al. 2008a, Kaiserli et al. 2009, Inoue et al. 2011) and disrupts the ability of phots to mediate all phot-mediated responses in vivo (Christie et al. 2002, Kong et al. 2006, Kong et al. 2007, Inoue et al. 2008a, Inoue et al. 2011). Therefore, the kinase activity of phot is essential for its function. Although ATP-BINDING CASSETTE B19 (Christie et al. 2011) and PHYTOCHROME KINASE SUBSTRATE 4 (Demarsy et al. 2012) were identified recently as potential substrates for phot kinase, how their phosphorylation contributes to specific phot-mediated responses requires further investigation and experimentation. Other studies have centered on dissecting the importance of receptor autophosphorylation in controlling phot-mediated responses. Recent mass spectrometry analyses of native phot1 immunoprecipitated from etiolated Arabidopsis seedlings using an anti-phot1 antibody revealed nine blue-light-induced autophosphorylation sites located within the N-terminus (S58, S170 and S185), the hinge region between LOV1 and LOV2 (S350, S376 and S410), the kinase activation loop (S849 and S851) and the extreme C-terminus (T933) (Inoue et al. 2008a) (**Fig. 4A**). Four of these in vivo phosphorylation sites (S58, S185, S350 and S410) were also identified by an independent mass spectrometry study (Sullivan et al. 2008). The serine residues of *Avena sativa* phot1a (*As*phot1a), corresponding to Arabidopsis phot1 S185 and S376, were found to be phosphorylated in vitro by protein kinase A (Salomon et al. 2003). Notably, S376 of phot1 corresponds to S344 of *Vicia faba* phot1b (*Vf*phot1b), the phosphorylation of which is necessary for binding to 14-3-3 proteins

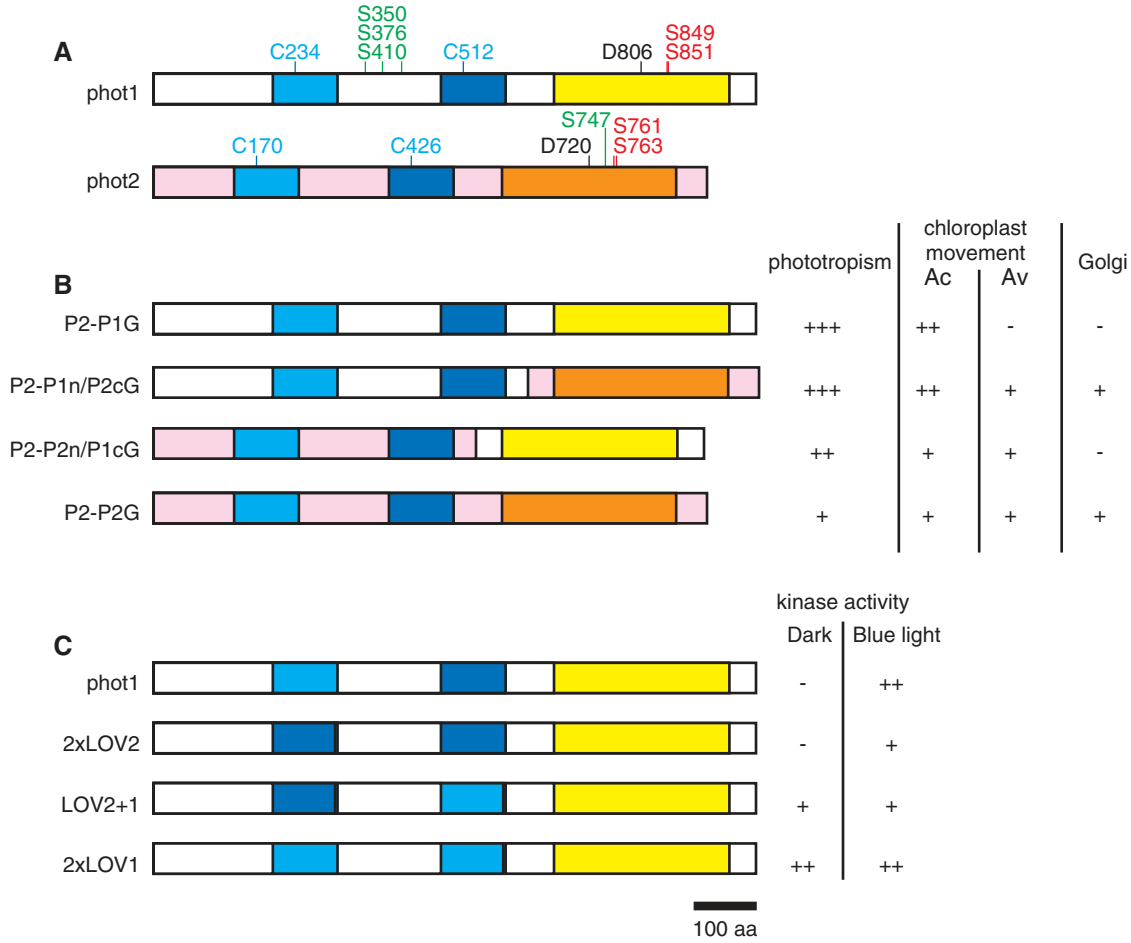

Fig. 4 Structure–function analyses of phot1 and phot2. LOV1 and LOV2 domains are indicated by sky blue and blue boxes, respectively. phot2 is colored in pink. The kinase domains are colored in yellow for phot1 and orange for phot2. (A) The position of amino acids that were examined by the mutational analyses. Cysteine residues in the LOV domains are indicated in sky blue. The aspartate residues essential for kinase activity are indicated in black. The 14-3-3-binding sites are indicated in green. Two autophosphorylation sites within the kinase activation loop are indicated in red. (B) Summary of the domain swapping analyses between phot1 and phot2 (Aihara et al. 2008). P1n/P2cG-expressing lines (P2–P1n/P2cG) show strong phototropism (indicated by +++) and chloroplast accumulation responses (Ac) (indicated by ++). P2n/P1cG-expressing lines (P2–P2n/P1cG) showed weak chloroplast accumulation responses (indicated by +) but slightly stronger phototropism (indicated by ++) compared with P2G-expressing lines (P2–P2G) (indicated by +). Although P1G-expressing lines (P2–P1G) did not exhibit a chloroplast avoidance response (Av) at the fluence rate examined (indicated by –), other lines were found to show the avoidance response (indicated by +). Both P2G and P1n/P2cG show blue-light-induced Golgi association (indicated by +), unlike P1G and P2n/P1cG (indicated by –). (C) Summary of the domain swapping analyses between LOV1 and LOV2 of phot1 (Kaiserli et al. 2009). Kinase activity of wild-type phot1 is repressed in darkness (indicated by –) and is activated by blue light (++). Replacement of LOV1 with LOV2 (2×LOV2) results in weak blue-light-induced kinase activation (indicated by +). Inversion of LOV1 and LOV2 (LOV2+1) results in constitutive but weak kinase activity. Replacement of LOV2 with LOV1 (2×LOV1) results in constitutive kinase activity (indicated by ++).

(Kinoshita et al. 2003). 14-3-3 binding is also implicated in phot-mediated activation of the plasma membrane H^+-ATPase as a primary mechanism of blue-light-induced stomatal opening (Kinoshita and Shimazaki 1999, Kinoshita et al. 2003). phot1 carrying simultaneous substitutions of S376 and S350 with alanines (S350A/S376A) impairs 14-3-3 binding activity (Inoue et al. 2008a, Sullivan et al. 2009), but has no impact on phot1 function in vivo (Inoue et al. 2008a). Furthermore, phot1 lacking its N-terminal 1–447 amino acid region, including the 14-3-3-binding region, is still functional when expressed in the *phot1phot2* double mutant (Sullivan et al. 2008). Hence, these findings indicate that blue-light-induced 14-3-3 binding to autophosphorylated phot1 is dispensable for phot1 function. More recently, Tseng et al. (2012) reported that S747 within the kinase domain of phot2 is essential for binding the 14-3-3λ isoform in yeast cells (Tseng et al. 2012) (**Fig. 4A**). The authors proposed that S747 of phot2 is autophosphorylated in vivo and binds 14-3-3λ to mediate stomatal opening specifically by phot2. However, another study has shown by far-Western blotting that phot1 and not phot2 specifically binds members of

the non-epsilon 14-3-3 family members (including 14-3-3λ and the closely related 14-3-3κ), and that mutation of the phot1 hinge phosphorylation sites (i.e., S350A/S371A/S410A) results in a loss of binding to these 14-3-3 isoforms (Sullivan et al. 2009). Confirmation of in vivo phosphorylation of S747 and its 14-3-3 binding activity thus warrants further examination to clarify its importance in regulating stomatal opening by phot2.

More importantly, among the nine phot1 autophosphorylation sites identified, only S849 and S851 partially abrogated phot1 functions when these residues were mutated to alanine (Inoue et al. 2008a). In particular, *phot1phot2* transgenic lines expressing the phot1 S851A substitution exhibited strong defects in phototropism, chloroplast accumulation response, stomatal opening and leaf flattening (Inoue et al. 2008a). Substitution of S851 with alanine also impacted the blue-light-induced internalization of phot1 in tobacco epidermal cells (Kaiserli et al. 2009). *phot1phot2* transgenic lines expressing the phot1 S849A/S851A double mutant showed more impaired stomatal opening and leaf flattening compared with lines expressing the phot1 S851A single mutant (Inoue et al. 2008a), suggesting that S849 also contributes to phot1 function. Although mass spectrometry analyses of phot2 immunoprecipitated from Arabidopsis could not detect any equivalent autophosphorylation sites within the kinase activation loop (i.e. S761 and S763) (**Fig. 4A**), mutation of S761 and/or S763 affected phot2 function in a manner similar to that found for the mutational analyses of phot1 (Inoue et al. 2011). In each of these studies, it was noted that the transgenic lines expressing phot1 S849A/S851A and phot2 S761A/S763A still retained a small degree of functionality, as well as blue-light-induced autophosphorytion activity (Inoue et al. 2008a, Inoue et al. 2011). These findings suggest that some other unidentified autophosphorylation sites contribute to regulating phot activity and function. Moreover, *phot1phot2* transgenic lines expressing phosphomimetic mutants of phot1 or phot2 (i.e. phot1 S849D/S851D or phot2 S761D/S763D, respectively) displayed nearly normal responsiveness for all phot-mediated responses examined and showed no phenotype indicative of constitutive activation (Inoue et al. 2008a, Inoue et al. 2011). These findings indicate that phosphorylation of these serine residues is insufficient to activate phot in the absence of light. Interestingly, two *phot2* null mutant alleles, *phot2-2* and *phot2-6*, carry T767I and E769K mutations within the phot2 kinase activation loop, respectively (Kagawa et al. 2001, Suetsugu et al. 2013). Detailed analyses of these mutations may help to clarify the role of the activation loop in autophosphorylation, stomatal opening and 14-3-3 binding by phot2.

Molecular mechanism of blue-light-dependent regulation of kinase activation by the LOV domains

The kinase domains of phots alone without the N-terminal light-sensing region display constitutive activity both in vitro and in vivo (Matsuoka and Tokutomi 2005, Kong et al. 2007), indicating that the N-terminal light-sensing region functions to repress phot kinase in darkness and that this mode of repression is relieved following blue light excitation. Truncated forms of the receptors comprising only the LOV2-kinase region have been shown to retain blue-light-dependent kinase activation in vitro (Matsuoka and Tokutomi 2005, Okajima et al. 2011). Indeed, phot1 LOV2-kinase has been reported to be able to mediate phot1-mediated responses when expressed in the *phot1phot2* double mutant, albeit with weaker activity compared with full-length phot1 (Sullivan et al. 2008, Kaiserli et al. 2009), suggesting that the N-terminal region including LOV1 is not essential for phot function. Domain swapping of the LOV domains has also provided further support for a model of kinase repression by LOV2. An engineered form of phot1 whose LOV1 and LOV2 domains were inverted (LOV2+1) exhibited constitutive weak kinase activity in the absence or presence of light (Kaiserli et al. 2009) (**Fig. 4C**). Replacement of LOV1 with LOV2 (2×LOV2) was found to maintain blue-light-induced kinase activation but with weak activity. Conversely, the reverse replacement (2×LOV1) resulted in constitutive kinase activity in the absence or presence of light (Kaiserli et al. 2009) (**Fig. 4C**). Thus, the intrinsic repression activity of LOV2 and its relative position in relation to the kinase domain appear to be necessary for light regulation by the LOV2-kinase module.

Nuclear magnetic resonance spectroscopy and solution structure analysis of the *Asphot1a* LOV2 domain identified an amphipathic α-helix consisting of approximately 20 residues C-terminal to the LOV2 domain (Harper et al. 2003). This helix, designated Jα, was also detected through crystallographic analysis of the *Asphot1a* LOV2 domain (Halavaty and Moffat 2007) (**Fig. 2**). The Jα-helix interacts with the β-scaffold of the LOV2 core in darkness. Following blue light exposure, a conformational change is induced in which the JA-helix–LOV2 interaction is disrupted (Harper et al. 2003). A mutation disrupting the Jα-helix–LOV2 interaction, I608E in Arabidopsis phot1, renders the kinase constitutively active (Harper et al. 2004, Jones et al. 2007). P1G-I608E has also been observed to be constitutively internalized from the plasma membrane in tobacco epidermal cells regardless of the light condition (Kaiserli et al. 2009) (**Fig. 2**; a black-boxed residue), demonstrating again that autophosphorylation impacts phot subcellular localization. A recent study using *Chlamydomonas* phot has found that a small region N-terminal to the LOV2 domain includes an amphipathic α-helix (Aα-helix) that, in addition to the Jα-helix, contributes to kinase repression in darkness (Aihara et al. 2012) (**Fig. 2**; blue-boxed residues). This helical region contributes to the formation of a hydrophobic core by facing the Aβ strand and Bβ strand of the LOV2 core in combination with the Jα-helix (Halavaty and Moffat 2007). Thus, unfolding of this hydrophobic core appeared to represent a key mechanism underlying the blue light induction of phot kinase activity.

The FMN-C(4a)–cysteinyl adduct formed within the LOV domain (C234 for phot1 LOV1, C512 for phot1 LOV2, C170

for phot2 LOV1 and C426 for phot2 LOV2) following blue light excitation is the primary mechanism by which these photosensors sense light (Salomon et al. 2000, Christie 2007) (**Figs. 2**, **4A**). Substitution of this highly conserved cysteine residue by alanine (C234A and C512A for phot1, and C170A and C426A for phot2) abrogates the photochemical reaction of each LOV domain [i.e. FMN-C(4a)–cysteinyl adduct formation] without affecting FMN binding activity and early photoproduct generation (Salomon et al. 2000, Swartz et al. 2001). Introduction of the C–A substitution into LOV2, but not LOV1, impairs light-induced auto- and transphosphorylation activity in vivo and in vitro (Christie et al. 2002, Matsuoka and Tokutomi 2005, Cho et al. 2007, Jones et al. 2007, Okajima et al. 2011). When analyzed in a *phot1-5phot2-1* 'null' mutant background (Suetsugu et al. 2013), the phot1 C512A mutant, but not the phot1 C234A mutant, exhibited phototropic and chloroplast accumulation responses similar to the *phot1phot2* mutant (Christie et al. 2002, Cho et al. 2007, Han et al. 2013), indicating that the photochemical reactivity of LOV2 is essential for phot1-mediated responses. However, equivalent mutations introduced into phot2 (phot2 C170A and phot2 C426A) resulted in phototropic responses in the *phot1phot2* mutant background that were similar to the *phot1* single mutant (Cho et al. 2007). The authors proposed that the leaky nature of the *phot2-1* allele combined with the potential of the residual protein to transphosphorylate the C–A inactivated forms of phot2 might account for this discrepancy. This, however, remains to be clarified since two separate reports indicated that the *phot2-1* mutant does not exhibit any residual expression of full-length phot2 that is detectable by immunoblotting (Sullivan et al. 2008, Suetsugu et al. 2013). Curiously, transgenic lines expressing phot1 C234A/C512A and phot2 C170A/C426A double mutant proteins were found to retain attenuated phototropic responses under high fluence rates of blue light (Cho et al. 2007). FMN binding activity and early photoproduct generation in LOV C–A mutants (Salomon et al. 2000, Swartz et al. 2001) might be responsible for initiating these phototropic responses. More recently, the *phot2* mutant allele, *phot2-10*, was identified which carries the R427Q mutation that disrupts FMN binding in LOV2 (Suetsugu et al. 2013). The *phot1-5phot2-10* double mutants exhibit no chloroplast avoidance response but retain subtle light-induced accumulation movement in addition to an attenuated phototropic response (Suetsugu et al. 2013). These recent findings provide further support for the conclusion that FMN binding by LOV2, and thus its photochemical activity, accounts for the majority of phot2 activity, whereas LOV1 has some residual photochemical function with respect to chloroplast accumulation and phototropic responsiveness. Kaiserli et al. (2009) have also proposed that photoactivation of LOV1 may act to arrest chloroplast accumulation movement (Kaiserli et al. 2009). Chloroplast accumulation in transgenic lines expressing phot1 in the *phot1-phot2* double mutant is inhibited at high fluence rates of blue light. This phenomenon was not observed in transgenic lines where the LOV1 domain was removed through truncation or inactivated by the C234A mutation. Further experimentation is now needed to reveal the in vivo function of LOV1.

Although several studies have demonstrated an oligomerization activity for LOV1 (mainly dimerization) (Salomon et al. 2004, Kagawa and Suetsugu 2007, Nakasako et al. 2008, Kaiserli et al. 2009, Katsura et al. 2009), the importance of oligomerization for phot function remains to be determined. Crystallographic analyses of LOV1 dimers from both phot1 and phot2 have revealed several critical residues for potential dimerization (Nakasako et al. 2008). Therefore, it is now possible to generate transgenic lines expressing phots that are potentially defective in dimerization to assess the functional significance of receptor oligomerization.

The ZTL/FKF1/LKP2 Protein Family Regulates Biological Timing Through Interacting with and/or Degrading Multiple Proteins

Regulated degradation of key components is essential for timekeeping mechanisms in a wide range of eukaryotes, and the specific F-box-containing E3 ubiquitin ligases (hereafter called simply 'F-box proteins') are involved in proteasome-dependent degradation (Mehra et al. 2009, Ito et al. 2012). Although photoreceptors and F-box proteins are physically separated in fungal and animal clock systems (Mehra et al. 2009), the land plant-specific LOV photoreceptor family, ZEITLUPE/FLAVIN-BINDING, KELCH REPEAT, F-BOX 1/LOV KELCH PROTEIN 2 (ZTL/FKF1/LKP2), has both photoreceptor and F-box protein activities combined within a single protein (Ito et al. 2012). ZTL/FKF1/LKP2 proteins consist of an N-terminal LOV domain, an F-box and the C-terminus with six Kelch repeats (**Fig. 1**). Recombinant LOV domains of Arabidopsis ZTL, FKF1 and LKP2 display an absorption spectrum similar to those of phototropin LOV domains, and the LOV domain of FKF1 has been shown to bind FMN (Imaizumi et al. 2003). However, the half-life rate for dark decay from the photoexcited state [FMN-C(4a)–cysteinyl adduct] of the FKF1 LOV domain is approximately 62.5 h (Zikihara et al. 2006), which is much longer than the decay rates reported for the LOV1 and LOV2 domains of plant and algal phots (typically several tens of seconds: Kasahara et al. 2002); a nine amino acid insertion specific to the ZTL/FKF1/LKP2 LOV domain in the loop between Eα and Fα (**Fig. 2**; green-boxed region) is involved in this slow decay property (Zikihara et al. 2006). Consequently, ZTL/FKF1/LKP2 proteins should not be able to revert fully to their dark state under a 24 h day–night cycle. However, because ZTL and FKF1 fluctuate in a circadian phase- and photoperiod dependent-manner (Imaizumi et al. 2003, Kim et al. 2003, Sawa et al. 2007) and are quite unstable (Kim et al. 2003, Kim et al. 2007, Fornara et al. 2009, Kim et al. 2011), a high turnover rate of ZTL/FKF1/LKP2 would be expected to promote recycling of these photoreceptors.

The F-box domain of ZTL/FKF1/LKP2 proteins interacts with several Arabidopsis SKP1-like proteins (ASKs) (Han et al. 2004,

Yasuhara et al. 2004, Kevei et al. 2006) and are necessary for their activity in vivo (Han et al. 2004, Kevei et al. 2006), indicating that ZTL/FKF1/LKP2 proteins function as part of the SKP(ASK)–Cullin–Rbx–F-box complex. Indeed, ASK1, CULLIN1 (CUL1) and *At*RBX1 have been shown to co-immunoprecipitate with ZTL (Han et al. 2004). Furthermore, *AtRBX1* RNA interference (RNAi) lines (Han et al. 2004) and weak *cul1* mutant plants (Harmon et al. 2008) are observed to phenocopy the long period phenotype characteristic of *ztl* mutants. The Kelch repeats of ZTL/FKF1/LKP2 serve as protein–protein interaction domains and FKF1–ZTL/LKP2 heterodimerization domains (Takase et al. 2011).

Functional divergence between ZTL/LKP2 and FKF1

The lycophyte *Selaginella moellendorffii* contains one ZTL/FKF1/LKP2 gene (Banks et al. 2011). The monocot and dicot *ZTL/FKF1/LKP2* genes are phylogenetically classified into two groups, *ZTL/LKP2* and *FKF1* (Boxall et al. 2005, Taylor et al. 2010), suggesting that *ZTL/LKP2* and *FKF1* genes have different functions. Indeed, Arabidopsis *ztl* mutants are primarily defective in circadian clock regulation and slightly defective in flowering time (Somers et al. 2000, Jarillo et al. 2001a, Somers et al. 2004, Takase et al. 2011), whereas *fkf1* mutants are defective in the photoperiodic control of flowering but show only subtle defects in circadian clock regulation (Nelson et al. 2000, Imaizumi et al. 2003, Baudry et al. 2010). Single *lkp2* mutants show nearly normal circadian and flowering phenotypes (Imaizumi et al. 2005, Baudry et al. 2010, Takase et al. 2011), resulting from much lower expression levels of *LKP2* compared with *ZTL* and *FKF1* (approximately 4% of *ZTL* levels) (Baudry et al. 2010). When *LKP2* or *FKF1* was expressed in *ztl* mutants under the control of the *ZTL* promoter, the expression of LKP2 but not FKF1 could rescue the circadian defects in *ztl* mutants (Baudry et al. 2010). In addition, the constitutive expression of *ZTL* and *LKP2* genes was observed to lead to arrhythmicity and late flowering (Kiyosue and Wada 2000, Schultz et al. 2001, Imaizumi et al. 2003, Somers et al. 2004, Takase et al. 2011), whereas constitutive expression of the *FKF1* gene had no influence on the circadian clock and flowering time (Imaizumi et al. 2003, Sawa et al. 2007). Collectively, these results indicate that ZTL/LKP2 primarily regulates the circadian clock and indirectly regulates flowering time through circadian clock regulation, and that FKF1 predominantly mediates photoperiodic flowering control. However, *ztl fkf1*, and more strongly *ztl fkf1 lkp2*, but not *ztl lkp2*, showed longer period defects than *ztl* (Baudry et al. 2010). Under long-day (LD) conditions, flowering time in *ztl fkf1 lkp2* plants was slightly delayed compared with *fkf1* plants (Fornara et al. 2009). Curiously, *ztl lkp2* double mutants displayed an early flowering phenotype under short-day conditions, and introgression of the *fkf1* mutation into *ztl lkp2* (i.e. *ztl fkf1 lkp2*) completely suppressed this phenotype (Takase et al. 2011). Thus, these photoreceptors have partial overlapping functions with respect to the regulation of the circadian clock and flowering time (Fornara et al. 2009, Baudry et al. 2010, Takase et al. 2011).

ZTL controls the circadian clock via the degradation of key clock proteins

In addition to ASK proteins, several interacting proteins of ZTL/FKF1/LKP2 have been identified or verified through various methods such as yeast two-hybrid analysis and pull-down assays (Más et al. 2003, Yasuhara et al. 2004, Fukamatsu et al. 2005, Imaizumi et al. 2005, Kiba et al. 2007, Kim et al. 2007, Sawa et al. 2007, Fujiwara et al. 2008, Johansson et al. 2011, Kim et al. 2011, Takase et al. 2011, Song et al. 2012). ZTL specifically interacts with TIMING OF CAB EXPRESSION 1 (TOC1) and PSEUDO RESPONSE REGULATOR 5 (PRR5) among five PRR proteins (TOC1, PRR3, PRR5, PRR7 and PRR9) through its LOV domain (Más et al. 2003, Yasuhara et al. 2004, Kiba et al. 2007, Fujiwara et al. 2008, Baudry et al. 2010). TOC1 is one of the core components of an autoregulatory negative feedback loop that consists of two Myb transcription factors: CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) (Nagel and Kay 2012). PRR5 is also essential for robust oscillator function (Nagel and Kay 2012). TOC1 and PRR5 proteins are rhythmically expressed: their protein levels decrease rapidly in darkness (Más et al. 2003, Kiba et al. 2007, Fujiwara et al. 2008,), and blue light has been shown to increase the stability of PRR5 (Kiba et al. 2007, Fujiwara et al. 2008) (**Fig. 5A**). TOC1 and PRR5 proteins accumulate constitutively at higher levels in the *ztl* mutant and are conversely reduced in *ZTL*-overexpressing lines (Más et al. 2003, Kiba et al. 2007, Fujiwara et al. 2008). These results indicate that ZTL mediates the proteolytic degradation of TOC1 and PRR5 and that blue light inhibits ZTL-mediated degradation of these proteins (**Fig. 5A**). Although FKF1 and LKP2 interact with TOC1 and PRR5 (Más et al. 2003, Yasuhara et al. 2004, Baudry et al. 2010), these proteins play only minor roles in TOC1 and PRR5 proteolysis compared with ZTL (Kiba et al. 2007, Baudry et al. 2010).

FKF1 controls photoperiodic flowering through both degradation and stabilization of transcription factors

FKF1 mediates photoperiodic flowering by activating the expression of the florigen *FLOWERING LOCUS T* (*FT*). *FT* expression under LD conditions is both dependent on and independent of a key photoperiod pathway transcriptional regulator known as CONSTANS (CO) (Imaizumi et al. 2003, Imaizumi et al. 2005, Sawa et al. 2007, Fornara et al. 2009, Salazar et al. 2009, Sawa and Kay 2011, Song et al. 2012). FKF1 associates with *CO* and *FT* promoters (Sawa et al. 2007, Sawa and Kay, 2011, Song et al. 2012) and regulates the activation of these genes in an LD-dependent manner (Imaizumi et al. 2003, Imaizumi et al. 2003, Imaizumi et al. 2005, Sawa et al. 2007, Fornara et al. 2009, Song et al. 2012). Three Dof transcription factors, CYCLING DOF FACTOR 1 (CDF1), CDF2 and CDF3, were identified

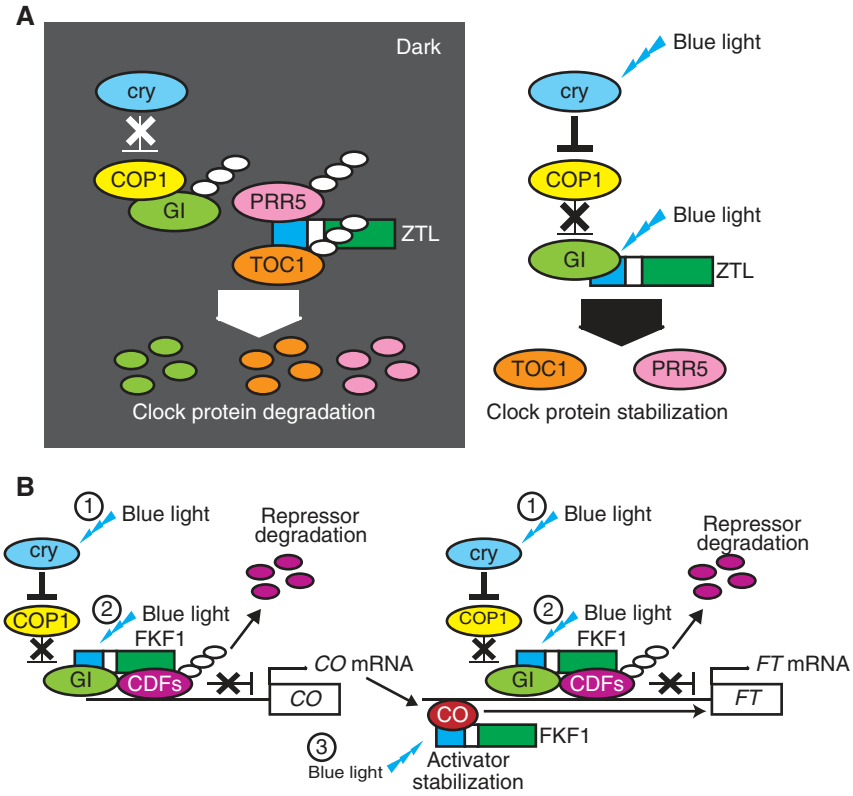

Fig. 5 Light-regulated degradation of key factors in the regulation of the circadian clock and photoperiodic flowering by ZTL/FKF1/LKP2. Figures are constructed based on previous studies (Yu et al. 2008, Ito et al. 2012, Song et al. 2012). For clarity, the adaptor function of ELF3 in the GI–COP1 interaction (Yu et al. 2008), the role of HSP90 in ZTL stabilization (Kim et al. 2011) and minor functions of FKF1/LKP2 or ZTL/LKP2 in the regulation of the circadian clock or photoperiodic flowering, respectively (Fornara et al. 2009, Baudry et al. 2010), are omitted. (A) Blue light regulation of TOC1 and PRR5 degradation by ZTL. In darkness, cryptochromes (crys) cannot inhibit COP1-mediated ubiquitin ligation (white ovals) and the subsequent degradation by the 26S proteasome (not indicated) of GI, resulting in low levels of GI. Furthermore, the ZTL LOV–GI interaction is weak in darkness. Consequently, TOC1 and PRR5 strongly interact with the ZTL LOV domain and are subsequently degraded through ZTL-mediated ubiquitin ligation. Under blue light, crys inhibit COP1-mediated GI degradation, resulting in the accumulation of GI. Blue light enhances ZTL–GI interaction via the ZTL LOV domain, and thus the interaction of TOC1 or PRR5 with the ZTL LOV domain is suppressed. Consequently, TOC1 and PRR5 accumulate in the light. (B) Multistep regulation by blue light in FKF1-mediated regulation of photoperiodic flowering. (1) cry increases GI abundance by suppressing COP1 (circle number 1 in **Fig. 4B**). (2) Blue light enhances FKF1–GI interaction via the FKF1 LOV domain (circle number 2 in **Fig. 4B**). FKF1 also interacts with CDFs, the negative regulators of *CO* and *FT* transcription, via the Kelch repeats (green) and promote the ubiquitin-mediated degradation of CDFs. (3) Blue light enhances the interaction of CO with the FKF1 LOV domain and stabilizes CO proteins (circle number 3 in **Fig. 4B**), resulting in the activation of *FT* transcription.

as proteins that interact with Kelch repeats of FKF1 (Imaizumi et al. 2005). These proteins also interact with LKP2, but not with the Kelch repeats of ZTL, at least in yeast cells (Imaizumi et al. 2005). Overexpression of *CDF* genes [*CDF1*, *CDF2*, *CDF3* and related genes (*CDF4*, *CDF5* and *COG1*)] induced a late flowering phenotype resulting from the suppression of the expression of both *CO* and *FT* (Imaizumi et al. 2005, Fornara et al. 2009). *cdf1 cdf2 cdf3 cdf5* quadruple mutants displayed a photoperiod-insensitive early flowering phenotype as well as elevated expression of both *CO* and *FT* (Fornara et al. 2009, Song et al. 2012). CDF1 associates with the *CO* and *FT* promoters (Imaizumi et al. 2005, Sawa et al. 2007, Song et al. 2012). Therefore, CDFs function as repressors of *CO* and *FT* transcription. Under LD conditions, CDF1 and CDF2 protein levels rapidly decline in the late afternoon (Imaizumi et al. 2005, Fornara et al. 2009), when FKF1 activates *CO* transcription (Imaizumi et al. 2003). CDF1 and CDF2 proteins accumulate constitutively at higher levels in *fkf1* mutants, and CDF2 protein levels exhibit greater stability in the *ztl fkf1 lkp2* triple mutant compared with the *fkf1* single mutant (Imaizumi et al. 2005, Fornara et al. 2009), indicating that FKF1 (and minimally ZTL and LKP2) mediates photoperiodic flowering induction through proteolytic degradation of CDFs, negative regulators of *CO* and *FT* (**Fig. 4B**). Interestingly, FKF1 not only degrades negative regulators (i.e. CDFs) but also functions to stabilize the key flowering regulator CO (Song et al. 2012). It was shown previously that CO accumulates in late afternoon under LD conditions and that blue light stabilizes CO proteins (Valverde et al. 2004). Recently, Song et al. (2012) have shown that FKF1 interacts with CO through its LOV domain and that blue light enhances the FKF1–CO interaction to stabilize CO (Song et al. 2012) (**Fig. 5B**). Previously, it was reported that CO and several

CO-like proteins (COLs) interact with FKF1, ZTL and LKP2 in yeast cells (Fukamatsu et al. 2005), implying that FKF1 and also ZTL/LKP2 might regulate CO and COL protein stability in a similar manner. Collectively, these findings indicate that FKF1 mediates FT-dependent induction of photoperiodic flowering in two ways: (i) FKF1 degrades CDFs in a proteasome-dependent manner to activate *CO* and *FT* transcription; and (ii) FKF1 stabilizes CO proteins to activate *FT* expression (Song et al. 2012) (**Fig. 5B**).

GIGANTEA is essential for ZTL and FKF1 functions

ZTL and FKF1 are unstable in darkness but are stabilized by blue light irradiation (Kim et al. 2003, Kim et al. 2007, Fujiwara et al. 2008, Fornara et al. 2009, Kim et al. 2011). It has been shown that the stability of ZTL is controlled by the proteasome (Kim et al. 2003) and HSP90 (Kim et al. 2011), and that ZTL and LKP2 are involved in the degradation of FKF1 (Takase et al. 2011). GIGANTEA (GI), another important factor in both circadian clock regulation and the photoperiodic flowering pathway, plays a pivotal role in blue-light-dependent stabilization of ZTL/FKF1 (Kim et al. 2007, Sawa et al. 2007, Fornara et al. 2009). ZTL and FKF1 (and also LKP2) interact with GI through their LOV domains both in vitro and in vivo, and blue light enhances this interaction (Kim et al. 2007, Sawa et al. 2007) (**Fig. 5**). Indeed, ZTL/FKF1 protein levels and stability are decreased in the *gi* mutant and increased in *GI*-overexpressing lines (Kim et al. 2007, Sawa et al. 2007, Fornara et al. 2009). As a result of reduced ZTL and FKF1 levels, TOC1, CDF1 and CDF2 are constitutively accumulated at higher levels in *gi* mutant plants similar to in the *ztl* mutant or *fkf1* mutant (Kim et al. 2007, Sawa et al. 2007, Fornara et al. 2009). Conversely, CDF2 proteins accumulated at a lower level in *GI*-overexpressing lines (Fornara et al. 2009). Interestingly, GI stability is controlled by an E3 ligase, CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), and the clock regulator EARLY FLOWERING 3 (ELF3) (Yu et al. 2008). Because cryptochromes repress COP1 activity under blue light conditions (Lian et al. 2011, Liu et al. 2011b, Zuo et al. 2011), photoactivated cryptochromes probably stabilize GI through COP1 inactivation (**Fig. 5**). Therefore, blue light stabilizes ZTL and FKF1 through the following mechanisms: (i) CRY-mediated GI accumulation by COP1 inactivation; and (ii) the enhancement of ZTL/FKF1–GI interaction (**Fig. 5**).

For a more detailed description of *ztl*/*fkf1*/*lkp2* mutant phenotypes and ZTL/FKF1/LKP2 functions in circadian clock and photoperiodic flowering regulation, readers should refer to the cited original papers and a recent comprehensive review (Ito et al. 2012).

Aureochrome: A LOV Photoreceptor Functioning as a Blue-Light-Regulated Transcription Factor in Stramenopiles

Photosynthetic stramenopiles are secondary endosymbionts and play an important role in aquatic primary productivity on the earth. Photosynthetic stramenopiles such as yellow-green algae (Xanthophyceae), brown algae (Phaeophyceae) and diatoms (Bacillariophyceae) utilize blue light to regulate growth and development and organelle movement. For example, in the xanthophycean algal genus *Vaucheria*, various responses such as phototropism (Kataoka 1975a, Kataoka 1975b), side branch formation (Takahashi et al. 2001) and chloroplast movement (Blatt and Briggs 1980) are regulated by blue light. Recently, Hironao Kataoka's group cloned four LOV domain-encoding cDNA fragments from *Vaucheria frigida* using LOV domain-specific degenerate PCR and obtained the full-length cDNA of two LOV photoreceptor genes (Takahashi et al. 2007). Proteins encoded by these two genes consist of a putative basic region/leucine zipper (bZIP) domain in the middle region of the protein and a LOV domain at the C-terminus (**Fig. 1**). These bZIP-LOV photoreceptors were named AUREOCHROMEs (AUREOs) after the typical golden-yellow color of many stramenopile species (Takahashi et al. 2007). *AUREO* genes were also found in other photosynthetic stramenopiles, such as *Aureococcus anophagefferens* (Pelagophyceae), *Thalassinosira pseudonana* and *Phaeodactylum tricornutum* (Bacillariophyceae), *Chattonella antiqua* (Raphidophyceae), *Ochromonas danica* (Chrysophyceae), *Fucus distichus* ssp. *Evanescens* and *Ectocarpus siliculosus* (Phaeophyceae) (Armbrust et al. 2004, Takahashi et al. 2007, Bowler et al. 2008, Ishikawa et al. 2009, Cock et al. 2010, Rayko et al. 2010). However, *AUREO* genes are not found in non-photosynthetic stramenopiles, which may have lost plastids secondarily after the divergence from a photosynthetic ancestor of extant stramenopiles (Janouškovec et al. 2010), implying that AUREOs have arisen as a result of secondary endosymbiosis but have been lost in non-photosynthetic stramenopiles.

Characteristics of AUREO LOV domains

Recombinant LOV domains of *Vaucheria* AUREO1 (*Vf*AUREO1) display an absorption spectrum similar to that of other LOV domains and bind FMN as a chromophore (Takahashi et al. 2007). The half-life rate for dark decay from the photoexcited state of the LOV domain from *Vf*AUREO1 is approximately 5 min at room temperature (Takahashi et al. 2007). This rate is slower than those of phot LOV domains (typically several tens of seconds: Kasahara et al. 2002). However, no FMN binding was detected for the recombinant LOV domains of *Vf*AUREO2 (Takahashi et al. 2007). Of the 11 residues essential for FMN binding within the LOV domain (Crosson and Moffat 2001), Ile270 and Phe298 present in *Vf*AUREO1 are changed to Leu277 and Val305, respectively, in *Vf*AUREO2 (Takahashi et al. 2007, Ishikawa et al. 2009) (**Fig. 2**; two red-boxed residues). Single I270L and F298V LOV mutations within the LOV domain of *Vf*AUREO1 did not impair FMN binding and result in several fold increases in light state lifetimes relative to the wild type (Mitra et al. 2012), suggesting that the absence of FMN binding in the LOV domain of *Vf*AUREO2 may be attributable to a combined effect of

Leu277 and Val305. A comparison between dark and light state crystal structures of *Vf*AUREO1 LOV showed light-induced FMN–cysteinyl adduct formation and conformational changes in Phe298, reinforcing the importance of Phe298 in AUREO LOV photochemistry (Mitra et al. 2012).

In the crystal structure of *Vf*AUREO1 LOV, the six monomers of *Vf*AUREO1 LOV are arranged as a trimer of antiparallel dimers, and light induces conformational changes in the quaternary structure of the dimer (Mitra et al. 2012). Laser-induced transient grating and size exclusion chromatography analyses have also revealed that the LOV domain of *Vf*AUREO1 exists as the monomer in darkness and undergoes blue-light-induced dimerization in solution (Toyooka et al. 2011). However, when inherently fused to its bZIP domain, the LOV domain exists as a dimer irrespective of the light conditions (Toyooka et al. 2011). The bZIP domain is known to function as a dimerization domain (Jakoby et al. 2002). Hence, this region appears to contribute to the constitutive dimerization status observed fot *Vf*AUREO1 (Toyooka et al. 2011).

The function of AUREO1 as a transcription factor

In addition to dimerization, bZIP domains have the ability to bind to DNA sequences with an ATGC core such as C-boxes (GACGTC) and G-boxes (CACGTG) (Jakoby et al. 2002). A random oligonucleotide binding assay showed that the N-terminal region of *Vf*AUREO1, including the bZIP domain, preferentially binds to the sequence TGACGT (Takahashi et al. 2007). Binding of *Vf*AUREO1 to the TGACGT sequence was enhanced by blue light and subsequently attenuated by prolonged periods of dark incubation (for 25 min) following blue light irradiation, indicating that *Vf*AUREO1 is a blue-light-regulated transcription factor (Takahashi et al. 2007). Consistent with this conclusion, GFP fusion proteins of *Vf*AUREO1 and *Vf*AUREO2 are localized in the nuclei when heterologously expressed in onion epidermal cells (Takahashi et al. 2007). However, analysis of *Vf*AUREO localization in *Vaucheria* is now required since stramenopiles exhibit different cell structures from seed plants; for example, *Vaucheria* is a multinucleate algae.

Roles of AUREO1 and AUREO2 in photomorphogenesis in *Vaucheria*

The body of *Vaucheria* is tubular, with some branches, and coenocytic (multinucleate without septa) (Takahashi et al. 2001). At the apical part of each branch, active tip growth occurs. Blue light controls the positions of growth points (phototropism) (Kataoka 1975b) and induces new growth points (side branch formation) (Takahashi et al. 2001). The functions of *AUREO* genes in *Vaucheria* have been analyzed using RNAi induced by double-stranded RNA (dsRNA) injection (Takahashi et al. 2007). Most *AUREO1*-silenced cells (called *aureo1*) display a retarded tip growth and abnormal tube morphology with constrictions and kinks (Takahashi et al. 2007). Furthermore, blue-light-induced side branch formation is strongly attenuated in *aureo1* lines (Takahashi et al. 2007). These results indicate that AUREO1 is a blue light receptor for the control and emergence of growth points in *Vaucheria* (**Fig. 6**). In contrast to *aureo1* lines, many immature sex organs were observed to emerge several weeks after silencing of *AUREO2*, indicating that AUREO2 is a negative regulator of sex organ formation (Takahashi et al. 2007) (**Fig. 6**). Double knockdown of both *AUREO1* and *AUREO2* induced abnormal tube morphology and attenuated blue-light-induced side branch formation but not premature sex organ formation, similar to *aureo1* cells, indicating that sex organ formation requires *AUREO1* function. Chloroplast and nuclear accumulation in the blue-light-irradiated area is a prerequisite for side branch emergence (Takahashi et al. 2001). Importantly, blue-light-induced chloroplast movement was normal in *AUREO* knockdown cells, indicating that other blue light receptors are responsible for this response in *Vaucheria* (**Fig. 6**). It is possible that two other uncharacterized LOV photoreceptors identified in *Vaucheria* may regulate chloroplast movement (Takahashi et al. 2007),

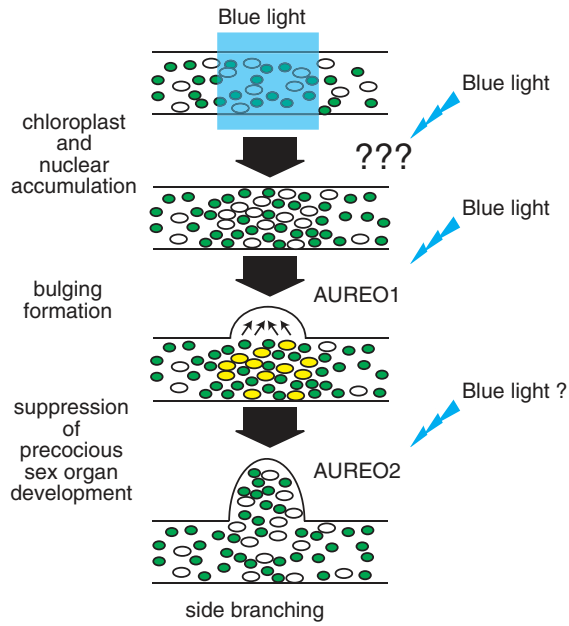

Fig. 6 A schematic model of blue-light-induced side branch formation mediated by multiple photoreceptors in *Vaucheria frigida*. This figure is constructed based on a previous study (Takahashi et al. 2007). The body of *Vaucheria* possesses multiple nuclei (white ovals) and chloroplasts (green ovals). In the area irradiated with blue light (for clarity, the irradiated area is indicated only in the first panel), nuclear and chloroplast accumulation is induced by an unidentified blue light receptor (???). Blue light enhances the DNA binding activity of AUREO1 in the blue-light-irradiated nuclei (yellow ovals), and consequently AUREO1 induces gene expression required for bulge formation. AUREO2 suppresses precocious sex organ development. However, the involvement of blue light absorption and DNA binding activities of AUREO2 in this suppression mechanism remain to be determined because the photoactivity of AUREO2 has not yet been demonstrated in vitro.

especially since no *PHOT*-encoding genes have been found in the genomes of stramenopiles examined to date (Armbrust et al. 2004, Takahashi et al. 2007, Bowler et al. 2008, Ishikawa et al. 2009, Cock et al. 2010).

The availability of genome sequences for some photosynthetic stramenopiles has enabled the identification of AUREO-regulated genes. Because the AUREO1 target *cis*-element has already been identified (Takahashi et al. 2007), the search for putative AUREO1 ortholog-binding sites in the promoter regions of whole genomes in sequenced stramenopiles is now possible. Furthermore, chromatin immunoprecipitation (ChIP) with next-generation sequencing or ChIP-on-chip analyses for AUREO target genes should increase our understanding of the transcriptional network underlying blue-light-induced photomorphogenesis in photosynthetic stramenopiles.

Concluding Remarks

For phots and ZTL/FKF1/LKP2, many studies have been performed using Arabidopsis, and much information regarding their mechanism of action and physiological function has been acquired. However, research on phots from non-flowering plants has not only shown that phot-mediated responses are conserved throughout the green plant leaneage (Kagawa et al. 2004, Kasahara et al. 2004) but has also uncovered new functions for phot blue light receptors. These include discoveries such as neochrome, a novel chimeric red/blue light receptor comprising the cromophore-binding region of phy fused to phot (Nozue et al. 1998, Kawai et al. 2003, Suetsugu et al. 2005), the involvement of phot in phy-mediated responses possibly via a direct interaction between phy and phot (Kasahara et al. 2004, Jaedicke et al. 2012) and a role for phot in the sex life cycle of *Chlamydomonas reinhardtii* (Huang and Beck 2003). By comparison, it will be important to study the function of LOV photoreceptors such as ZTL/FKF1/LKP2 in land plant species other than Arabidopsis. Furthermore, because many photosynthetic stramenopiles are different from *Vaucheria* in various aspects, the role of AUREO in other stramenopiles such as diatoms and brown algae should also be examined.

Recently, functional knowledge of the LOV2 domain of plant phots has been exploited to construct new artificial light-activated molecules (Strickland et al. 2008, Wu et al. 2009) in addition to a photoreversible fluorescent protein known as iLOV (Chapman et al. 2008, Christie et al. 2012). Furthermore, the light-dependent interaction between FKF1 and GI has been applied to develop a new light-activated dimerization technology (Yazawa et al. 2009). These technologies are likely to have wide application since their utility has been demonstrated in several cell types including bacterial, plant and animal cells. The recent determination of the structure of AUREO blue light receptors should also prove useful in the design and development of synthetic, photoactivatable transcription factors (Mitra et al. 2012). Thus, further studies of plant and algal LOV photoreceptors will clearly contribute to a broad range of research areas, as have many other aspects of plants and algal biology (Jones et al. 2008).

Funding

This work was supported by the Japanese Ministry of Education, Sports, Science, and Technology [17084006 to M.W.]; the Japan Society for the Promotion of Science [20227001, 22657016 and 23120523 to M.W., 20870030 to N.S.].

Acknowledgments

We thank Dr. John Christie (University of Glasgow) for critical reading of the manuscript. We are grateful to Dr. Fumio Takahashi (Tohoku University) and Dr. Takato Imaizumi (University of Washington) for helpful comments and discussion.

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