Diverse Responses to Blue Light via LOV Photoreceptors

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Plants utilize light not only as an energy source for photosynthesis, but also as an environmental cue to direct numerous developmental and physiological processes. For this purpose, plants have evolved two main types of photoreceptors: the phytochromes (Franklin and Quail 2010), which sense red/far-red light, and the ultraviolet (UV)-A/blue light (BL)-sensing photoreceptors, which include cryptochrome (Liu et al. 2011), phototropin (Christie 2007) and other BL receptors described below. In addition, a third class of UV-B receptor, UVR8, was recently identified (Rizzini et al. 2011).

The long history of research on BL receptors dates back to the initial observation made by the famous Charles Darwin, in which he found that plants were able to move towards light (Darwin and Darwin 1881). For a long time, the nature of the chromophore in BL receptors was much disputed as to whether it was a flavin or a carotenoid (Senger 1980). This issue was partially resolved through the identification of the first BL receptor, which corresponded to a flavin protein named cryptochrome (Ahmad and Cashmore 1993). Shortly after this discovery, the Briggs group demonstrated that the disrupted gene in Arabidopsis non-phototropic hypocotyl 1 (nph1) mutants encoded a BL receptor required for phototropic response (Huala et al. 1997, Christie et al. 1998). A homolog of NPH1, NON-PHOTOTROPIC HYPOCOTYL-LIKE 1 (NPL1), was soon identified (Kagawa et al. 2001); thereafter, NPH1 and NPL1 were renamed phototropin 1 (phot1) and phot2, respectively.

Phototropins mediate diverse plant responses, such as chloroplast relocation movements (Kagawa et al. 2001, Sakai et al. 2001), stomatal opening (Kinoshita et al. 2001), early hypocotyl growth inhibition (Folta and Spaulding 2001), leaf flattening (Sakamoto and Briggs 2002), leaf positioning (Inoue et al. 2005), nuclear positioning (Iwabuchi et al. 2007, Tsuboi et al. 2007), sun tracking (Inoue et al. 2008b) and leaf photomorphogenesis (Kozuka et al. 2011), in which phot1 acts over a broad range of light intensities, whereas phot2 acts as a high-light sensor. These physiological responses contribute towards enhancing photosynthesis in the plant by increasing the absorption of light and CO2 (Takemiya et al. 2005).

In addition to the two phototropins, a BL receptor family of proteins characteristically containing one light, oxygen or voltage (LOV) domain, an F-box and a Kelch repeat has been identified in Arabidopsis. These proteins include ZEITLUPE (ZTL), FLAVIN-BINDING KELCH REPEAT F-BOX 1 (FKF1) and LOV KELCH PROTEIN 2 (LKP2), and are proposed to regulate the circadian clock and photoperiodic flowering by controlling BL-dependent protein degradation (Ito et al. 2012).

A third type of LOV protein, aureochrome (AUREO), was identified in the stramenopile alga, Vaucheria frigida (Takahashi et al. 2007). AUREO has a basic region/leucine zipper (bZIP) domain as well as a LOV domain, and is thought to act as a BL-regulated transcription factor. Thus, the LOV BL receptor family in plants has divergent members who have a variety of physiological functions triggered by the photochemical reactions of FMN in the LOV domain.

In This Issue: All You Need is LOV

In this Special Focus Issue, we present papers contributed by various experts in the field of LOV photoreceptor research, which describe updates and new research on photoreception, signal transduction and physiological functions of LOV-containing phototropins and aureochromes.

As mentioned previously, several families of LOV domain-containing BL receptors have been identified in land plants and photosynthetic stramenopiles. On pages 8–23, Suetsugu and Wada (2013) review recent research progress with special reference to the structure–function relationships of these photoreceptors, and their physiological roles in planta. Specifically, the authors discuss the roles of phototropins as autophosphorylating protein kinases, the latest research in land plant-specific ZTL/FKF1/LKP2 proteins and on AUREO proteins, and provide new insights for future investigations.

The phototropins are thought to act as BL-regulated protein kinases through autophosphorylation (Inoue et al. 2008a) to exert the above-mentioned physiological responses, and contain two N-terminal LOV photoreceptive domains named LOV1 and LOV2 (Huala et al. 1997) and a serine/threonine (Ser/Thr) kinase domain in the C-terminus. Out of the two LOV domains, LOV2, plays a major switching role in the
phototropic response in vivo (Christie et al. 2002, Cho et al. 2007, Aihara et al. 2008, Kaiserli et al. 2009) and in BL regulation of the kinase activity in vitro (Matsuoka and Tokutomi 2005, Okajima et al. 2012). In this issue, Han et al. (2013) investigated the role of the phot1 LOV domains in chloroplast movement and leaf positioning in response to BL using mutant plants developed from a previous study, which harbor light-inactive LOV1 or/and LOV2 domain(s) (Cho et al. 2007). The authors demonstrate that the LOV2 domain also plays a major role in the BL regulation of these responses. Furthermore, the authors found that red light enhanced BL-induced chloroplast movement, while phytochrome A (phyA), but not phytochrome B (phyB), mediated this enhanced response. This phenomenon parallels both the red light enhancement of phototropism via phyB, mediated this enhanced response. This phenomenon parallels both the red light enhancement of phototropism via phyA and the red-light-induced retention of phototropins at the plasma membrane of leaf mesophyll cells.

Unlike the other major phytochrome and cryptochrome photoreceptors, phototropins characteristically localize to the plasma membrane (Christie 2007). While the C-terminal kinase domain is important for this localization, the exact moiety responsible for this positioning and its physiological role in phototropin-mediated responses remains unknown. On pages 57–68, Kong et al. (2013a) narrowed down the membrane association domain to a small part of the C-terminus of phot2, and investigated its physiological role. In wild-type plants, BL causes the release of phot2 from the plasma membrane (Sakamoto and Briggs 2002) to the Golgi apparatus (Kong et al. 2006). However, Kong et al. (2013a) found that C-terminal deletion mutants of phot2 failed to associate with Golgi membranes and were incapable of mediating the chloroplast avoidance response. This suggests that the discrete C-terminal portion is necessary for both the membrane association and phot2-dependent chloroplast avoidance response. Interestingly, phot2 C-terminal deletion mutants were still capable of other phot2-mediated responses including chloroplast accumulation, phototropism and leaf flattening.

The chloroplast avoidance response mediated by phot2 under strong BL (Kagawa et al. 2001) is crucial for reducing photodamage to chloroplasts under ever-changing light environments (Kasahara et al. 2002). However, the mechanism(s) by which phot2—rather than phot1—mediates this response under strong light remains unknown, although potential clues could be sought through defining the subcellular localization of phot2. On pages 80–92, Kong et al. (2013b) demonstrated the presence of both phot1 and phot2 on the outer chloroplast membrane, with phot2 accumulating at greater levels. Interestingly, the authors showed that deletion of the phot2 C-terminal region affected both the localization of phot2 and the avoidance response, whereas reduced phot2 levels in the cytoplasm did not impair the avoidance response. These findings indicate important roles for the phot2 C-terminal region in directing localization to the chloroplast envelope and in mediating the avoidance response.

It has been established that phototropins enhance leaf flattening via NON-PHOTOTROPIC HYPOCOTYL 3 (NPH3) in the presence of BL (Sakamoto and Briggs 2002, Inoue et al. 2008b). However, the mechanism(s) by which phototropins mediate this response remains unknown. On pages 69–79, Kozuka et al. (2013) uncovered an unexpected role for phyB in this process. The phototropin-deficient double mutant phot1phot2 displayed downward curled leaves, whereas addition of the phyB mutation in the triple mutant restored the flattened leaves. The results suggest that phot1/2 and phyB act antagonistically to regulate leaf curling. In accordance with this finding, leaf flattening is also promoted by end-of-day far-red light treatment, which eliminates the active Pfr phytochrome. Collectively, these results indicate that phototropins promote leaf flattening through the suppression of phyB activity. Kozuka et al. (2013) further report that NPH3 was involved in promoting leaf flattening in phyB mutants, and suggest that NPH3 functions downstream of phyB. These results not only provide a new dimension to our understanding of the molecular mechanisms of leaf flattening, but also imply a cross-talk between phototropins and phyB signaling mechanisms.

The signal transducer ROOT PHOTOTROPISM 2 (RPT2) was identified as being another phototropin signaling component (Sakai et al. 2000), which functions in phot1-specific pathways to regulate phototropism (Inada et al. 2004). However, it is unknown whether RPT2 also mediates leaf flattening and leaf positioning. On pages 36–47, Harada et al. (2013) show that rpt2 mutants exhibit phenotypes similar to those of phot1phot2 double mutants, while disruption of the Phot1 gene in rpt2 mutants surprisingly restored the wild-type phenotype. In contrast, phot2 rpt2 double mutants did not display signs of recovery, thus suggesting that phot2 probably functions in the absence of phot1. These results suggest that RPT2 mediates leaf flattening and positioning via the phot1-specific pathway, and that phot1 may inhibit a component(s) of the phot2 signaling pathway. Moreover, the authors also found that RPT2 enhanced plant growth under weak to strong light conditions.

Protein phosphatase 1 (PP1) mediates signaling from phototropins to H+–ATPase in guard cells (Kinosita et al. 2001, Takemiya et al. 2006, Shimazaki et al. 2007). PP1 is a Ser/Thr protein phosphatase comprising a catalytic subunit (PP1c) and a regulatory subunit that is thought to control catalytic activity, subcellular localization and substrate specificity of PP1c. On pages 24–35, Takemiya et al. (2013) identified and functionally characterized PP1 regulatory subunit 2-like protein 1 (PRSL1)—a regulatory subunit of PP1. Specifically, a T-DNA insertion in PRSL1 caused impaired stomatal opening, H+ pumping and H+–ATPase phosphorylation in guard cells in response to BL, but did not affect other phototropin-mediated responses. Further, co-expression of PP1c with PRSL1 enhanced PP1c localization to the cytoplasm of guard cells, but sole expression of PP1c caused preferential localization of PP1c to the nucleus. These findings suggest that PRSL1 probably functions as a regulatory subunit of PP1c to stimulate the localization of PP1c to the cytoplasm and also mediates phototropin signaling in guard cells.

The yellow-green algae Vaucheria contains two AUREO homologs named aureochrome-1 (AUREO1) and aureochrome-2...
Both AUREO1 and 2 contain a LOV domain and an upstream bZIP domain. On pages 93–106, Hisatomi et al. (2013) prepared three recombinant AUREO1 proteins: a full-length protein (FL), an N-terminal truncated protein containing bZIP and LOV (ZL), and a LOV-only-containing protein. The recombinant proteins were all capable of the cyclic reaction as observed with the phototropin LOV domains upon BL excitation. FL and ZL recombinant proteins bound to DNA in a sequence-specific manner; however, the LOV-only protein did not, confirming that the bZIP domain is necessary for DNA binding. ZL formed a homodimer, possibly through formation of disulfide bonds between the two cysteine residues in the bZIP domain and the linker region adjacent to the LOV domain, respectively, which were thought to stabilize the structure and facilitate DNA binding. Finally, BL induced α-helical unfolding in the LOV domain but folding in other regions of the protein. Based on these results, the authors proposed a model for BL-induced conformational changes and DNA binding of AUREO1.

Final Remarks: A Bright Future for LOV Proteins

LOV domain-containing proteins are the most recently discovered photoreceptors in plants, and dramatic progress in understanding the functional role of these proteins has subsequently been made. However, the mechanisms by which these photoreceptors generate the many diverse physiological responses remain unknown. In this issue, investigations on the chloroplast avoidance response, stomatal opening, leaf flattening and positioning were reported, but no common component responsible for these signaling pathways has so far been found, except for in phototropin signaling. Such diverse responses are likely to be brought about by both tissue-specific expression and the subcellular localization of phototropins and their substrate proteins (Kong et al. 2013b), such as ATP-BINDING CASSETTE B19 (Christie et al. 2011) and PHYTOCHROME KINASE SUBSTRATE 4 (Demarsy et al. 2012).

Further, LOV proteins represent an invaluable practical resource for researchers across fields. For instance, they have recently attracted much attention from researchers of optogenetics due to their potential use as fluorescent probes (Christie et al. 2011). ATP-BINDING CASSETTE B19 (Christie et al. 2011) and PHYTOCHROME KINASE SUBSTRATE 4 (Demarsy et al. 2012)

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References


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