INTRODUCTION TO PHOTOMORPHOGENESIS

Many hands make light work

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Introduction

The responses of plants to the light environment have fascinated biologists for well over 100 years (Briggs, 2006). Early studies in photomorphogenesis focused necessarily on morphological aspects of plant responses; germination, seedling establishment, plant architecture, and flowering time are all regulated by light. More recently, and particularly with the advent of the genetic model Arabidopsis thaliana, the focus has shifted to understanding plant responses at the molecular level. Understanding and genetically manipulating these processes may provide the subtle control of plant growth that will permit successful alteration of these traits for agricultural benefit. This Focus Section, based on the Photomorphogenesis session at this year’s Society for Experimental Biology annual conference, contains reviews directed at both the progress in understanding the molecular basis of light-signalling pathways and how to translate this information for agricultural gain.

Plant photomorphogenesis: light work

Photomorphogenic responses confer a fitness advantage allowing an individual to maximize the potential of the light environment for photosynthesis (Scha¨fer and Nagy, 2006). In many species, breaking of seed dormancy is only possible under light conditions that are favourable to growth. Following germination, most seedlings undergo a light-dependent de-etiolation whereby the seedling becomes established: hypocotyl elongation ceases, cotyledons open (in plants that have them) and the photosynthetic machinery is assembled. Without light at this point, seedlings remain etiolated: the cotyledons remain closed and the hypocotyl is hooked to protect the shoot meristem as it undergoes rapid elongation to push upwards through covering soil or leaf litter to reach the light. All energy is diverted to elongation and, consequently, de-etiolation does not occur until light is detected. This response displays a very clear fluence dependency: the greater the quantity of light received the greater the inhibition of elongation growth (Schäfer and Nagy, 2006). Directional responses can also be clearly observed at this stage. The elongating hypocotyl will bend towards the direction of greatest light intensity, the seedlings exhibiting a classic phototropic response. This response can be observed throughout the life history of the plant and it plainly confers another important advantage in the quest to maximize light capture. A similar reaction can be observed when plants experience vegetative shade. However, this shade-avoidance response has the subtle distinction that plants grow away from shade rather than towards light. The shade-avoidance response is not observed in all species, but instead is specific to plants adapted to grow in open areas that will need to compete strongly with their neighbours to remain in direct sunlight. Changes in the quality of light reflected from neighbouring plants triggers this shade-avoidance response (or shade-avoidance syndrome) including an inhibition of branching and a promotion of elongation growth as plants anticipate shading by competitors (Franklin and Whitelam, 2005). Finally, timing of flowering in many species is governed by light. In this case, duration of light is the important factor. Lengthening days (or more strictly shortening nights) signal the approach of spring while shortening days (lengthening nights) signal the approach of winter. This regulation of flowering by light is mediated through interaction with an internal 24 h timekeeper known as the circadian clock to ensure that the flowering process is receptive to light during the evening. When plants receive a light input at this time it either triggers flowering in long-day plants or inhibits flowering in short-day plants (Yanovsky and Kay, 2003). Flowering, though, can also be triggered under prolonged unfavourable light conditions such as heavy shading by neighbouring plants (Franklin and Whitelam, 2005). This ‘last resort’ ensures survival into the next generation, producing seed as quickly as possible.

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Plant photoreceptors: the many hands

The diverse array of responses described above, not surprisingly, require an equally diverse array of photoreceptors to control them (Fig. 1). These photoreceptors include the red and far-red-absorbing phytochromes and the blue and UV-A-absorbing cryptochromes, phototropins, and ZTL/ADO family of proteins, though evidence now strongly predicts the existence of additional, unknown photoreceptors absorbing in UV-B and in green regions of the electromagnetic spectrum.

The phytochromes are reversibly photochromic proteins encoded in plants by a small nuclear gene family of 3–5 members (phyA–phyC in rice and phyA–phyE in Arabidopsis). They exist as red and far-red absorbing forms, Pr and Pfr, with absorption of red light by Pr triggering a conversion to the Pfr form and absorption of far-red light converting Pfr back to the Pr form (Rockwell et al., 2006). This photoconversion is mediated by the covalently-bound, linear tetrapyrrole chromophore, phytochromobilin, which is assembled in darkness to give the inactive Pr form of phytochrome. Only following conversion to the active Pfr form are responses such as promotion of germination and de-etiolation triggered (Schäfer and Nagy, 2006). In addition to enabling the detection of light, the photoreversibility of phytochrome is the key to phytochrome’s role in shade avoidance. Light reflected from a plant is depleted in red and blue wavelengths, but is rich in far-red light. As a consequence, the majority of the phytochrome pool is converted into the inactive Pr form. The loss of Pfr removes an inhibitor of elongation growth and triggers shade avoidance. The degree of shading is accurately reflected by the red:far-red ratio and this in turn determines the position of the Pr/Pfr equilibrium and the degree of elongation (Franklin and Whitelam, 2005).

Another class of photoreceptors is the cryptochromes that absorb light in the blue to UV-A wavelength range via their non-covalently bound flavin adenine dinucleotide and pterin chromophores. There are two well-characterized cryptochromes, cry1 and cry2, that play important roles in the regulation of de-etiolation. A third, recently identified cryptochrome (cry3), localizes to the chloroplast where it may regulate gene expression (Banerjee and Batschauer, 2005). Thus, the cryptochromes work together with the phytochromes to ensure de-etiolation over a broad light spectrum. Both cryptochrome and phytochrome regulate flowering time, measuring the duration of light and ensuring that the circadian clock is correctly set at dawn to keep the whole process exquisitely fine-tuned (Somers et al., 1998).

![Fig. 1. Our molecular understanding of photomorphogenic responses has been greatly enhanced by the use of the model plant Arabidopsis. As described in the articles in this Focus Section, a number of photoreceptor families, the phytochromes (phys), the cryptochromes (crys), the phototropins (phot), the zeitlupe family (ZTL), and unknown green and UV receptors regulate growth responses in Arabidopsis. The best characterized are the phys which localize to the nucleus where they interact with bHLH transcription factors (phytochrome-interacting factors or PIFs) to regulate development. The phys, the crys and ZTL also mediate light input to photoperiodic pathways regulating flowering time. While much progress has been made in elucidating these light-signalling pathways, the challenge now is to determine to what extent this knowledge can be transferred to important crop species such as maize and rice.](image-url)
A third class of photoreceptors, the phototropins, also absorb in the blue/UV-A. Phototropins are, like cryptochromes, flavoprotein photoreceptors but are structurally unrelated. In contrast to the cryptochromes, phototropins bind two molecules of flavin mononucleotide as chromophores within specialized photosensory modules known as LOV domains (Christie, 2007). LOV domains exhibit homology to motifs found in a diverse range of eukaryotic and prokaryotic proteins involved in sensing Light, Oxygen, or Voltage, hence the acronym LOV. As their name suggests, phototropin 1 (phot1) and phototropin 2 (phot2) are the main photoreceptors governing phototropic curvature and, in general, operate to control a range of processes that optimize the photosynthetic efficiency of plants and promote growth (Christie, 2007). They also regulate chloroplast movement in Arabidopsis. In high light intensities, chloroplasts are arranged along the anticlinal wall of the cell to prevent photo-damage, whereas in low light intensities, chloroplasts are arranged on the upper periclinal wall in order to maximize light absorption. Phototropins also function to regulate leaf positioning and expansion, stomatal opening, and the rapid but transient growth inhibition of young seedlings upon their emergence from the soil.

The fourth class is a second group of LOV-domain-containing proteins known as the ZTL/ADO family (Banerjee and Batschauer, 2005). This recently-discovered family consists of three members: Zeitlupe (ZTL, also known as Adagio, ADO), Flavin-binding, Repeat F-box 1 (FKF1), and LOV Kelch Protein 2 (LKP2). These proteins share three characteristics: a LOV domain (for blue/UV-A detection), an F-box (to elicit degradation of protein targets), and a C-terminal Kelch domain (to mediate protein–protein interactions). Members of the ZTL/ADO family play roles in the targeted degradation of components associated with regulating circadian clock function and flowering. ZTL plays a relatively limited role that appears to be specific to light input to the circadian clock (Kim et al., 2007). As yet, no specific photoreceptor roles have been assigned to LKP2. However, FKF1 exhibits photoreceptor activity in detecting long days and activating the photoperiodic flowering pathway in Arabidopsis (Imaizumi et al., 2003).

**Importance in agriculture: making it work for you**

Photomorphogenesis is particularly important in agriculture where maximizing yield is of paramount importance. However, light responses are not always desirable in an agricultural setting. The shade-avoidance syndrome confers an important advantage for inter-species competition, but becomes deleterious in a uniform field of crops. As planting density is increased, overall yield is decreased as resources are allocated into elongation rather than harvestable produce (Robson et al., 1996). Although shade-avoidance responses in crops have been severely reduced through intensive selection, they still limit the number of plants per square metre that can be sown, despite an excess of water, nutrients, and sunlight. Indeed, this is still readily apparent in an agricultural setting. As shown in Fig. 2, maize plants at the edge of the field show a clear inhibition of elongation as the bulk of the crop undergoes a competitive shade-avoidance response. In this issue, Kebrom and Brutnell (2007) discuss recent progress in understanding the molecular mechanisms of shade avoidance in the grasses, including many of the world’s major crops. Taking their cue from recent developments in identifying components of shade-avoidance signalling in Arabidopsis they discuss phytochrome regulation of shade avoidance in maize, rice, and sorghum. One aspect that is particularly strongly affected by shade in the grasses is the production of axillary branches, which are inhibited in shade in favour of increased apical dominance. In this case, many of the regulatory genes have been identified first in maize and rice and the major challenge will be linking these genes to the light-signalling pathways controlling axillary meristem production. To date, this has only been done for the bHLH transcription factor, TB1, which has been shown to repress axillary meristem development in maize and rice and shows increased expression in the apically dominant phyB mutant of sorghum (Kebrom et al., 2006). These developments in understanding the molecular mechanisms underlying shade avoidance build on breeding programmes in cereal...
grasses. However, as pointed out by Kebrom and Brutnell (2007), recent interest in lignocellulosic-based biofuels might turn this requirement on its head. Instead of increasing grain yield at the expense of biomass, breeders and genetic engineers may be spending their time reversing this priority.

Another photomorphogenic response that is extremely important in agriculture is the regulation of flowering time. The latitudinal range of many crops is limited by the daylength during the growing season. For example, crops adapted to short days encountered at the equator are often unable to flower in more polar regions where daylength is much longer during the summer. In this issue, Izawa (2007) discusses what is known about the control of flowering time. The latitudinal range of many crops is limited by the daylength during the growing season. For example, crops adapted to short days encountered at the equator are often unable to flower in more polar regions where daylength is much longer during the summer. In this issue, Izawa (2007) discusses what is known about the control of flowering time in rice and contrasts this to the situation in Arabidopsis. He goes on to propose a possible explanation for the adaptation of rice from its natural habitat in the tropics to the more northern latitudes where it is currently grown (Fig. 2). This explanation is based on the antagonistic action during long days of the evolutionary conserved Hdl pathway and the unique Ehd1 pathway. An understanding of different mechanisms regulating flowering time may be important in adapting a wide range of crop species to different latitudes in response to long-term trends in environmental change.

A green revolution?

As discussed above the effects of light on plant growth and development are largely driven by red/far-red (600–800 nm) and UV-A/blue (320–500 nm) wavelengths of the electromagnetic spectrum. By comparison, the effects of green/yellow light (500–600 nm) on plant development have received very little attention to date. The rationale behind this is relatively simple: plants, because they are green, reflect these wavelengths, rendering them inconsequential with respect to controlling plant growth. Indeed, green light has been used routinely as a ‘safe light’ when harvesting dark-grown or light-treated tissue for photobiological analysis. As discussed by Folta and Maruhnich (2007) in this issue, the use of green light may not be as ‘safe’ as one may have predicted. Phytochromes and cryptochromes, albeit red/far-red and blue light receptors respectively, do absorb green wavelengths to a certain degree. This has made characterization of green light responses in plants difficult to interpret. Only recently has a detailed analysis of the effects of green light been made possible owing to the availability of photoreceptor mutants in Arabidopsis. The advent of high power narrow bandwidth light sources has also increased the specificity of photobiological analysis in that defined regions of the electromagnetic spectrum can be examined. Consequently, recent photochemical and photophysiological studies have been able to indicate clearly that green light does have a regulatory influence on various plant responses. For instance, cryptochrome activity is reversed by green light (Bouly et al., 2006), resulting from the nature of the photochemical reactivity associated with the flavin chromophore of the photoreceptor. Given the known role of cryptochromes in regulating light-induced stomatal opening, it will now be important to establish whether this unique mode of photochemistry accounts for the blue-green reversibility observed for this response (Frechilla et al., 2000). Green light has also been shown to promote hypocotyl elongation in dark-grown Arabidopsis seedlings in a dose-dependent manner (Folta, 2004) that coincides with a down-regulation of plastid transcripts (Dhingra et al., 2006). In addition, supplementary green light irradiation has been reported to increase plant biomass (Sommer et al., 2001), yet the photoreceptor(s) responsible remains to be identified.

So what could be the molecular identity of such an elusive green light receptor in plants? Potential chromophores for such a photoreceptor include flavin, retinal, and possibly haem. However, the nature of the chromophore as well as the apoprotein it binds to awaits identification and further studies in this area of research are clearly needed. In the meantime, plant photobiologists are now faced with an obvious concern. Working under a green safe light was bad enough, but will night vision, infrared-goggles be a necessary accessory for the dark room?

Phytochrome signalling: making light work

In contrast to these recently described responses to green light, red and far-red responses mediated by the phytochrome photoreceptors were some of the earliest to be characterized (see Sage, 1992, for a colourful history of phytochrome research). Phytochrome was also the first photoreceptor to be partially purified from plants (Butler et al., 1959) and remains the most studied of the photoreceptors even now. Perhaps unsurprisingly then, the elucidation of the initial events in phytochrome signal transduction has been something of a holy grail for plant photobiology research. Only in the last 10 years or so has there been significant progress in understanding the processes involved in mediating phytochrome signalling.

The phytochromes are synthesized and assembled in the cytosol and, it was long thought that they functioned there as well. The realization that phytochrome could be translocated to the nucleus (Sakamoto and Nagatani, 1996) was therefore a key discovery and set phytochrome research in a new direction. It has subsequently been demonstrated that all phytochromes undergo light-dependent nuclear localization (Fig. 1) (Kircher et al., 2002) and that, at least for phytochrome B, nuclear localization is required for (at least the majority of) its biological activity (Huq et al., 2003; Matsushita et al., 2003). Our current
understanding of the mechanisms of light-dependent entry of phytochrome to the nucleus is reviewed in this issue by Kevei et al. (2007), a combined contribution from two of the pioneer laboratories in the field. One of the key points noted by the authors is that for phyA and phyB, the kinetics of localization is similar to the kinetics of the responses mediated by these two phytochromes. Such an observation is strongly suggestive of a crucial role for nuclear localization in the signal transduction process. phyA shows a rapid localization in response to a pulse of light of any wavelength, typical of the, so-called, very-low fluence response while phyA nuclear accumulation continues to increase for up to 2 h if maintained in continuous far-red light, typical of a phyA-mediated far-red high irradiance response. In contrast, phyB shows a red/far-red reversible nuclear localization, as expected for phyB-mediated low-fluence responses. Once in the nucleus the phytochromes localize in discrete foci known as nuclear bodies, but how do they get there in the first place? Kevei et al. (2007) also discuss this process. Phytochromes are comprised of an N-terminal photosensory domain and a C-terminal domain that is thought be have a more regulatory role. Current evidence suggests that it is the N-terminal region of the phyB molecule that confers the light dependency of nuclear localization, while the C-terminal domain contains the main signal sequence necessary for the physical translocation (Matsushita et al., 2003). For phyA, nuclear entry requires FHY1 and FHL, both positive regulators of phyA signalling (Zhou et al., 2005). These proteins, both of which contain nuclear localization signals, interact with the N-terminal domain of phyA in a light-specific manner (Hiltbrunner et al., 2006). Thus the C-terminal region of phyA seems less important in the nuclear localization process. However, whereas a nuclear-localized phyB N-terminal domain is able partially to rescue a phyB mutant (Matsushita et al., 2003), a nuclear-localized N-terminal phyA is not able to rescue a phyA mutant (Mateos et al., 2006), indicating that the C-terminal domain of phyA instead contains important signalling components.

So what exactly is phytochrome doing in the nucleus? The molecular properties of the phytochrome protein itself have long been thought to hold the key to its function. As mentioned above, plant phytochromes consist of a photosensory core containing the chromophore with a regulatory C-terminus that consists of two PAS domains that are commonly involved in protein–protein interaction and a kinase domain (Rockwell et al., 2006). It was somewhat of a surprise then that targeting of the photosensory domain alone to the nucleus was sufficient for biological activity of phyB (Matsushita et al., 2003), although the nature of the primary signalling mechanism remains unclear. The exact role of the C-terminal domain is also unresolved although some things are known. For example, it is the region flanked by the two PAS domains that is critical for proper nuclear localization and also for dimerization (Kevei et al., 2007). Also, there now seems to be broad agreement that phytochrome is a kinase, an issue that had been somewhat controversial (Yeh and Lagarias, 1998). However, the role of the kinase domain in phytochrome signalling is still unknown. Phytochrome is phosphorylated at numerous sites and it is clear that dephosphorylation via overexpression of phosphatases (Ryu et al., 2005) leads to enhancement of signalling activity. Some, but not all, of these phosphorylation sites are targets for autophosphorylation and it is possible that light-dependent phytochrome kinase activity acts as a desensitizer of phytochrome signalling rather than a direct mediator of it. However, a number of other proteins have been identified as potential targets for phytochrome kinase activity including the CRY photoreceptors and the cytoplasmic protein PKS1 (Chen et al., 2004).

Perhaps the most exciting discovery in this area though relates to a group of basic helix–loop–helix transcription factors known as the phytochrome-interacting factors (PIFs), reviewed in this issue by Monte et al. (2007). The first of these proteins to be identified, PIF3, is found constitutively bound to G-box sequences common in the promoters of light-regulated genes where it can interact with the Pfr form of phyB following nuclear entry (Fig. 1) (Martinez-Garcia et al., 2000). PIF3 is actually one of a 15-member subfamily of the bHLH superfamily, five of which, PIF1, PIF3, PIF4, PIF5, and PIF6, have now been demonstrated to interact with phytochrome (Duck and Fankhauser, 2005). Of these, physiological analyses of mutants have demonstrated that PIF4 and PIF5 act as negative regulators of phyB signalling, while PIF1 and PIF3 have a negative role in both phyA and phyB signalling (Duck and Fankhauser, 2005; Monte et al., 2007). Monte et al. (2007) carefully review the most recent findings in this area. PIF1 and PIF3 have both been shown to be degraded to low steady-state levels upon light treatment (Bauer et al., 2004; Shen et al., 2005) suggesting that, at least in part, it is removal of these negative regulators that permits phytochrome signalling upon transfer to light. phyA and phyB also act to phosphorylate PIF3 prior to its degradation (Al-Sady et al., 2006) suggesting that this may be a key part of the signalling process. However, as the authors explain, the view of PIFs as general negative regulators appears oversimplified. PIF3 affects only a small number of phytochrome-regulated genes and appears to have a positive role in some early responses to light (Monte et al., 2004), suggesting that the PIFs may actually exert different effects on different pathways. Furthermore, in etiolated seedlings, PIF3 mostly affects early phyA-regulated gene expression changes in response to light in the period before PIF3 is degraded (Tepperman et al., 2006). There would, therefore, appear to be a distinct mechanism for
the negative regulation of the later physiological responses mediated by phyB that were initially described as defective in pif3 mutants. Monte et al. (2007) describe recent evidence that leads them to propose that the regulation of phyB signalling may involve modulation of the availability or activity of the phyB photoreceptor itself and go on to suggest that PIFs may even titrate out the photoreceptor in some way, preventing its action.

In summary, these five focus papers illustrate the complexity of light signalling and demonstrate just how much we still do not know. This is never truer than when comparing light signalling in Arabidopsis to signalling pathways in our major crop plants (Fig. 1). Although common photodetection systems are apparent between monocots and dicots, whether the hands of light (and time) operate in a similar manner between these species is not fully understood. There is no doubt though that the knowledge gained from a lowly weed such as Arabidopsis will have an important role to play in engineering our crops of the future.

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