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Tuning in to the signals controlling photoregulated gene expression in plants

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Plants have developed flexible mechanisms to respond appropriately to environmental signals. These stimuli are transduced by largely unknown signalling pathways that are likely to be modulated by endogenous developmental signals to produce an integrated response that coordinately regulates gene expression. Light is a critical environmental signal that controls many aspects of plant development via a series of photoreceptors that are able to respond to different light wavelengths. Light is also the principal energy source for photosynthesis. The photosynthetic products are carbohydrates which are translocated in the form of sucrose from the photosynthetic (source) to non-photosynthetic (sink) organs. Consequently, the control of photoregulated genes must integrate developmental inputs with signals derived from the photoreceptors, from the photosynthetic apparatus and from metabolites such as sucrose.

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Introduction

Light is perceived in plants by a sophisticated system of photoreceptors (phytochromes, cryptochromes and UV-B receptors) which allow them to detect light wavelengths within a wide spectral range. The phytochromes are the best characterized of these photoreceptors. They exist in two different photoconvertible forms, the red light-absorbing form (Pr), which is generally inactive, and the far-red light-absorbing (Pfr) physiologically active form. In higher plants, the phytochromes are encoded by a gene family (e.g. *PHYA-E* in *Arabidopsis*; Sharrock and Quail, 1989; Clack *et al.*, 1994) and constitute two distinct pools, denoted Type I and Type II. Type I phytochrome is more abundant in etiolated tissue but is light labile due to the rapid degradation and/or sequestration of Pfr. Conversely, Type II is present in much lower amounts in dark-grown tissue but is light stable. As a consequence, Type I phytochrome is more important for the initial de-etiolation process, whilst Type II responses predominate in mature plants (Quail *et al.*, 1995; Smith, 1995). PhyA is a Type I phytochrome, as it is synthesized in the dark and removed rapidly in the light, whereas PhyB–E are light stable and probably constitute the Type II pool (Clack *et al.*, 1994).

PhyA has been shown to be localized in the cytoplasm and to exist as a homodimer (Furuya and Schäfer, 1996). PhyB is probably also dimeric and localized in the

cytoplasm, although recent evidence has indicated that it may translocate to the nucleus in the light (Sakamoto and Nagatani, 1996). If confirmed, this would suggest different modes of action of PhyA and PhyB, as there is no evidence that PhyA translocates to the nucleus. Activation of the phytochromes by light is mediated by a covalently bound linear tetrapyrrole, identified as phytochromobilin for PhyA. The other phytochromes are thought to bind the same chromophore, although this has not yet been proven because of the low levels of PhyB–E present in plant material and because of the heterogeneity of the Type II pool (Furuya and Schäfer, 1996).

Phytochrome response modes

The classical phytochrome-mediated response is activated by a single short pulse of red light and is reversed by a subsequent exposure to far-red light. Such responses are now denoted Low Fluence Response (LFR), and in addition Very Low Fluence Response (VLFR) and High Irradiance Response (HIR) can be defined (Table I). HIRs are further subdivided into red light- and far-red light-mediated HIR. Whilst HIRs require constant exposure to relatively high photon fluxes, VLFRs are activated by extremely low light intensities. In contrast to LFRs neither HIRs nor VLFRs are photoreversible. A further diagnostic feature used to categorize these responses is their conformity (or not) to the Bunsen–Roscoe Reciprocity Law (discussed more thoroughly in Mancinelli, 1994; Millar *et al.*, 1994; Smith, 1995). This photochemical law states that a response should be dependent only on the number of photons (fluence) causing the response and not on the rate at which the photons are delivered (fluence rate). Hence if the law is obeyed, a short bright pulse is equivalent to a longer period of dim irradiation, provided the same number of photons is delivered. For the phytochrome response modes, reciprocity is valid for VLFRs and LFRs, but not for HIRs. In addition to this series of distinguishing characteristics (Table I), each response is distinct in that it displays a clear fluence threshold at which the response saturates (e.g. Mandoli and Briggs, 1981). Taken together these observations would therefore tend to suggest that different signalling pathways are used to transduce each response.

The assignment of individual phytochromes to specific responses in VLFR, LFR and HIR conditions has been achieved in some cases, largely by physiological and molecular analysis of phytochrome-deficient mutants and by overexpression of individual phytochromes in mutant and wild-type backgrounds in *Arabidopsis* (Quail *et al.*, 1995; Quail, 1997). PhyA, for example, mediates hypocotyl growth inhibition in far-red light HIR, whereas PhyB is responsible for the same response in red light HIR. In other elegant studies, Shinomura *et al.* (1996) showed that

Table I. Diagnostic features of different phytochrome response modes

Response mode	Fluence requirement	Photoreversibility	Reciprocity valid
VLFR	100 pmol/m ² –100 nmol/m ²	no	yes
LFR	1–1000 µmol/m ²	yes	yes
HIR	>1000 µmol/m ²	no	no

Fluence requirements are based on Mancinelli, 1994 and references therein. LFR fluence requirements are for red light.

PhyA exclusively mediates seed germination in VLFR conditions, and that PhyB is exclusively responsible for this response in LFR conditions. Other responses, however, involve more than one phytochrome and in some cases they appear to interact additively and in other cases antagonistically (Reed *et al.*, 1994; Quail *et al.*, 1995; Smith, 1995). Hence, even though PhyA and PhyB have similar light absorption profiles, they mediate responses in different light conditions. This is a consequence of additional properties such as their relative abundance and PhyA light lability. In the natural environment, the ratio of red to far-red light is likely to be the principal determinant of the relative capabilities of PhyA, PhyB and the other phytochromes to mediate physiological responses (Smith, 1995).

Signal transduction pathways

The molecular mechanism of phytochrome activation of signal transduction is unknown. However, the recent cloning from Cyanobacteria of a phytochrome gene with strong similarities to bacterial two-component sensor kinases provides important information about phytochrome evolution as well as clues to its molecular action (Kehoe and Grossman, 1996; Quail, 1997). A model of phytochrome signalling initially proposed in 1985 (Van Der Woude, 1985) is currently receiving renewed attention (Furuya and Schäfer, 1996). In this model, PhyA is proposed to act as a heterodimer (i.e. PfrA:PrA) and PhyB as a homodimer (i.e. PfrB:PfrB). That PhyA functions as a heterodimer is indicated by the fact that extremely low levels of PfrA are generated in PhyA-mediated responses such as VLFR and far-red HIR, e.g. for seed germination in *Arabidopsis* the threshold fluence requirement for the PhyA-controlled VLFR is 10⁴-fold lower than for the PhyB-regulated LFR (Shinomura *et al.*, 1996), even though PhyA is <10²-fold more abundant than PhyB (Quail *et al.*, 1995). Furthermore, there is no evidence for cooperativity between the two phytochrome monomers (Furuya and Schäfer, 1996). These observations, coupled with the fact that PhyA is present at up to 10⁶ molecules per cell, indicate that no more than 10 PrA molecules would be converted to PfrA per cell in a typical VLFR (Furuya and Schäfer, 1996). Hence, assuming that PhyA-mediated responses are cell autonomous (Neuhaus *et al.*, 1993), a heterodimer model is, quite simply, the only way to reconcile the available data. PhyB responses, on the other hand, require considerably higher red light fluences, and a sufficient number of PfrB:PfrB homodimers can probably be generated to activate signal transduction.

If the homodimer:heterodimer model is correct, it would intuitively imply that distinct downstream effector proteins

are responsible for transducing PhyA heterodimer- and PhyB homodimer-activated signals. This may not be in agreement with results from domain-swapping experiments with oat PhyA and rice PhyB in transgenic *Arabidopsis* plants (Wagner *et al.*, 1996) and microinjection experiments in tomato (Bowler and Chua, 1994; Kunkel *et al.*, 1996). Phytochrome monomers consist of two major structural domains, an N-terminal domain that contains the chromophore-binding cysteine residue, and a C-terminal domain that carries the dimerization sites. In the experiments of Wagner *et al.* (1996), these domains were interchanged to generate PhyBA and PhyAB hybrid molecules. The phenotypes of plants containing these constructs indicate that the N-terminal domains contain the necessary determinants of photosensory specificity (far-red HIR for PhyA and red HIR for PhyB) and that the C-terminal domains are interchangeable and therefore functionally equivalent. Most simply, this would predict that both C-terminal domains interact with the same downstream effector protein. This is supported by the fact that the same small region within the C-terminal domain of both PhyA and PhyB has been identified by mutational analysis to be critical for regulatory activity (Xu *et al.*, 1995; Quail, 1997). However, possible complications in the experiments of Wagner *et al.* (1996) arise from the use of the constitutive 35S promoter to drive expression of the hybrid molecules and the use of wild-type *Arabidopsis* plants as hosts for these experiments (it is possible, for example, that the engineered phytochrome proteins interact with the native *Arabidopsis* phytochromes). Furthermore, the N-terminal domain can control regulatory as well as photosensory activity (e.g. the N-terminal serine residues in PhyA may be important for desensitization; Stockhaus *et al.*, 1992; Jordan *et al.*, 1995). Further studies with other PhyA/B hybrid proteins driven by PhyA and PhyB promoters in PhyA- and PhyB-deficient backgrounds should resolve these concerns.

In independent experiments, in the *au* tomato mutant (a chromophore biosynthetic mutant; Terry and Kendrick, 1996), microinjection-based approaches have indirectly identified heterotrimeric G proteins as the most upstream component of the PhyA signal transduction machinery (Figure 1; Neuhaus *et al.*, 1993; Bowler *et al.*, 1994a). Injection of purified oat PhyA into *au* hypocotyl cells, which normally lack anthocyanin and chloroplasts, results in cell-autonomous anthocyanin accumulation and chloroplast development. Light-regulated genes such as *CAB* (encoding chlorophyll a,b-binding proteins), *FNR* (encoding ferredoxin NADP⁺ oxidoreductase) and *CHS* (encoding chalcone synthase) are also activated by microinjected PhyA in a classical LFR red/far-red photoreversible response. Evidence of a role for heterotrimeric G proteins in PhyA responses comes from gain-of-function experiments in which GTPγS and cholera toxin (constitutive activators of heterotrimeric G proteins) were found to be able to reproduce PhyA responses in the light and to activate *CAB*, *FNR* and *CHS* gene expression in the dark, and from loss-of-function experiments in which GDPβS and pertussis toxin (inhibitors of heterotrimeric G protein function) could inhibit PhyA responses.

Utilizing the same microinjection system, three distinct signal transduction cascades involving cGMP and/or calcium were found to control PhyA responses downstream

Fig. 1. Signalling pathways controlling photoregulated gene expression in plants. A highly schematized model is shown in which different inputs controlling photoregulated gene expression (*CHS*, *FNR*, *ASI* and *CAB*) are integrated within the previously dissected PhyA-activated signal transduction pathways that are dependent upon cGMP and calcium (Bowler and Chua, 1994). PhyA pathways are indicated in red, PhyB in green, UV in blue and sucrose in grey. Negative regulation (denoted -ve) between the cGMP- and calcium-dependent pathways is indicated by dashed lines; whilst these pathways work in both directions for PhyA responses (reciprocal control; Bowler *et al.*, 1994b), in response to sucrose signals negative regulation is proposed to act in only one direction (from the cGMP pathway to the calcium pathways). Redox signals (denoted e^-) arising from plastoquinone are proposed to regulate the relative activities of the two calcium-dependent pathways. For further details see text.

of the G protein (Figure 1; Bowler *et al.*, 1994a). cGMP can stimulate *CHS* and anthocyanin biosynthesis, whilst calcium (and calmodulin) can activate *CAB* and partial chloroplast development. A third pathway, requiring both calcium and cGMP, is utilized to activate genes such as *FNR* and to produce fully mature chloroplasts. Furthermore, it is likely that this same pathway can mediate repression of the asparagine synthetase gene (*ASI*) in the light (Figure 1; Neuhaus *et al.*, 1997).

Microinjection experiments have also been performed in *au* with yeast-derived recombinant reconstituted PhyB (Kunkel *et al.*, 1996). Interestingly, although PhyB can activate both *CAB* and *FNR* genes, as well as stimulate chloroplast development, it can neither activate *CHS* nor promote anthocyanin accumulation. Unfortunately, experiments with modulators of G protein activity were not presented, so these results do not indicate whether PhyA and PhyB are both able to mediate responses via G proteins, calcium and cGMP. However, because the previously identified calcium/cGMP-dependent pathway controlling chloroplast development requires at least six times less cGMP than the pathway controlling anthocyanin biosynthesis (Bowler *et al.*, 1994b), an attractive hypothesis is that PhyA and PhyB both utilize the same general network of signalling pathways to regulate chloroplast biosynthesis, but that PhyB is not able to produce the higher levels of cGMP required for induction of the anthocyanin biosynthetic genes (Figure 1; Kunkel *et al.*, 1996). If this is the case, it may again suggest that PhyA and PhyB utilize the same effector protein to mediate these responses. However, interpretation of results from PhyA and PhyB microinjection experiments in *au* is complicated by the fact that *phyA* and *phyB* tomato mutants are not deficient in the predicted responses (van Tuinen

et al., 1995). Furthermore, experiments with these mutants indicate that PhyB can regulate anthocyanin biosynthesis (Kerckhoffs *et al.*, 1997b). These discrepancies may relate to different tissue specificities, as microinjection experiments have so far only been performed in sub-epidermal hypocotyl cells.

Due to the light conditions used in the microinjection experiments, it is probable that both the PhyA- and PhyB-mediated responses at the level of gene expression are LFRs and that sufficient PfrA can be generated to produce PfrA:PfrA homodimers. The results therefore suggest that PfrA homodimers can activate a response and that this response is different (because anthocyanin pigments are generated) from the PfrB homodimer-activated response. Consequently, the known differences in PhyA- and PhyB-mediated responses are not simply due to the different intracellular concentrations of these two phytochromes. Furthermore, the fact that in far-red HIR PhyA is able to generate anthocyanin pigment biosynthesis but not chloroplast development in *au* (Kunkel *et al.*, 1996) may indicate that PrA:PfrA heterodimers produce different responses to PfrA:PfrA homodimers. This hypothesis could therefore infer that the three phytochrome response modes (VLFR, LFR and HIR), each with its distinct threshold, utilize different signalling pathways, but that in LFR conditions PhyA and PhyB can utilize the same pathways (Figure 1). Independent support for this hypothesis could be obtained by the isolation of non-photoreceptor mutants specifically blocked in VLFR, LFR or HIR.

The phototransduction pathways utilized by cryptochrome and UV-B receptors are largely uncharacterized, although indirect pharmacological experiments indicate the use of pathways distinct from the phytochrome-activated pathways described above. In particular, a role for calcium and calmodulin in controlling the UV-mediated induction of *CHS* gene expression has been proposed (Figure 1; Christie and Jenkins, 1996; H.Frohn Meyer, C.Bowler, J.-K.Zhu, H.Yamagata, E.Schäfer and N.-H. Chua, in preparation).

Mutants affected in photomorphogenesis

As an alternative approach to identify signal transduction components controlling light-regulated responses, several genetic screens have been developed. The majority of photomorphogenic mutants have been characterized in *Arabidopsis* and can be classified as either insensitive or constitutive mutants (Chamovitz and Deng, 1996; Chory *et al.*, 1996). Insensitive mutants display a light-blind elongated phenotype in the light. Some of them are mutated in photoreceptor function [e.g. *hy3* (*phyB*), *hy8* (*phyA*), *hy4* (cryptochrome), *hy1* and *hy2* (chromophore biosynthetic mutants)], whilst others are presumed to encode positive regulators of light signal transduction pathways (e.g. *hy5*, which is insensitive to phytochrome- and cryptochrome-mediated responses, and *fhy1* and *fhy3*, which display light-insensitive phenotypes only in far-red HIR conditions; Whitelam and Harberd, 1994; Chamovitz and Deng, 1996; Chory *et al.*, 1996).

Conversely, constitutive de-etiolated mutants (e.g. *cop/det/fus/cpd*) display light-grown morphologies when grown in the dark, together, in some cases, with the

inappropriate expression of light-regulated genes such as *CAB* and *CHS* (Millar *et al.*, 1994). The recessive nature of these mutations suggests that they are loss-of-function and that the wild-type genes are repressors of photomorphogenesis in darkness. However, although epistasis tests with phytochrome-deficient mutants have indicated that they function downstream of phytochrome, they are not mutated specifically in phytochrome signal transduction because many have altered tissue specificities as well as other additional phenotypes not directly related to light (e.g. Chory and Peto, 1990; Millar *et al.*, 1994; Mayer *et al.*, 1996; Szekeres *et al.*, 1996). For example, light-grown *cop1*, *det1* and *cop9* mutants display elevated expression of genes involved in stress responses, such as those encoding glutathione transferases and pathogenesis-related PR proteins (Mayer *et al.*, 1996). Furthermore, a de-etiolated phenotype in the dark can be phenocopied by many different treatments, e.g. exogenous application of cytokinin (Chory *et al.*, 1994), periodic heat shock (Kloppstech *et al.*, 1991), shaking seedlings in liquid culture (Araki and Komeda, 1993) and growth on sugars (Jang *et al.*, 1997). These results therefore indicate the pleiotropic nature of these mutants and raise considerable difficulties for placing *COP*, *DET* and *FUS* gene products within phytochrome-regulated signal transduction pathways.

COP1 (FUS1), *COP9 (FUS7)*, *DET1 (FUS2)*, *DET2*, *FUS6 (COP11)* and *CPD* have been isolated recently by T-DNA tagging or map-based cloning (Deng *et al.*, 1992; Castle and Meinke, 1994; Pepper *et al.*, 1994; Wei *et al.*, 1994; Li *et al.*, 1996; Szekeres *et al.*, 1996). The products of these genes currently form two distinct groups, novel nuclear-localized proteins (*COP1*, *DET1*, *COP9* and *FUS6*) and brassinosteroid biosynthetic enzymes (*DET2* and *CPD*). Although none of the nuclear proteins appear to bind DNA directly, it has been proposed that they negatively regulate light-regulated (and other) genes in the dark by organizing repressive regions of chromatin. There are several indications that this may be the case, e.g. *COP1* shares some homology with yeast *TUP1*, which has been identified in several screens as a general transcriptional repressor (Roth, 1995). *TUP1* does not bind DNA directly, but is physically associated in a large protein complex which is thought to interact with sequence-specific DNA-binding proteins to repress specific chromatin domains (Roth, 1995; Kingston *et al.*, 1996). Although neither *COP1* nor *DET1* appears to be part of a complex, *COP9* is present in the nucleus as a large multiprotein complex which also contains the *FUS6* protein and whose formation depends upon the *COP8* gene product (Wei *et al.*, 1994; Staub *et al.*, 1996). Such complexes may be functionally equivalent to those found in yeast, and the pleiotropic phenotypes of the *cop/det/fus* mutants may be a result of destabilization of nucleosome structure. It is interesting to note that histone loss in yeast results in the spontaneous transcription of many genes (Han and Grunstein, 1988; Ptashne and Gann, 1997).

DET2 and *CPD* encode proteins involved in the biosynthesis of brassinosteroids (Li *et al.*, 1996; Szekeres *et al.*, 1996). These compounds are known to promote hypocotyl cell elongation, amongst other things (Mandava, 1988). To some extent, they can therefore be considered as negative regulators of photomorphogenesis, although they

are certainly not specific for light responses, as other mutants such as *axr1* and *etr1*, affected in auxin and ethylene responses respectively, are also differentially affected by brassinosteroids, as are other *cop/det/fus* mutants (Szekeres *et al.*, 1996). Furthermore, *dim* mutants (Takahashi *et al.*, 1995), which display reduced hypocotyl length in the dark but normal expression of photoinducible genes, are also likely to be blocked in brassinosteroid biosynthesis (Chory *et al.*, 1996; Szekeres *et al.*, 1996).

From the above discussion, it is clear that light is only one of many (exogenous and endogenous) factors that can modulate hypocotyl length. Therefore, hypocotyl length may not always be the best assay system for studying light signal transduction (Takahashi *et al.*, 1995), also because the role of gene regulation may be rather minimal in the response. A more targeted approach to identify components of signal transduction pathways specific for phytochrome might therefore be to isolate and characterize mutants with altered dynamics of light responses, such as light-hypersensitive mutants. Exaggerated photoresponse mutants have already been characterized in tomato (denoted *hp-1*, *hp-2*, *atv* and *lp*; Kendrick *et al.*, 1994; Kerckhoffs *et al.*, 1997a). In particular, *high pigment* mutants (*hp-1* and *hp-2*) are wild-type in darkness but in the light display higher anthocyanin levels, are shorter and darker than wild-type plants and have more deeply pigmented immature fruits. These phenotypes appear to be identical to those obtained by ectopic expression of *PHYA* in tomato (Boylan and Quail, 1989), indicating that the *hp* mutations may affect phytochrome responses fairly specifically.

Tomato is, in fact, an excellent model system for studying the light-regulated control of anthocyanin because anthocyanin production is strictly light-dependent, unlike in *Arabidopsis*. Furthermore, although cytokinin can reduce hypocotyl length in darkness in tomato, it does not result in the full de-etiolated phenotype (including open cotyledons and *CHS* gene expression) observed in *Arabidopsis* (Chory *et al.*, 1994; A.C.Mustilli, R.Ciliento and C.Bowler, unpublished observations). Dark-grown maize seedlings, however, display anthocyanin production in the presence of cytokinin, as in *Arabidopsis* (C.Tonelli, personal communication). It may be significant that despite years of photomorphogenesis research in tomato, a constitutive de-etiolated mutant has never been isolated (van Tuinen, 1996). This indicates either that (i) there is redundancy of gene function in tomato or (ii) that the signal transduction circuits regulating photomorphogenesis are wired differently in *Arabidopsis* and tomato. In our opinion, the above observations indicate that, whereas in *Arabidopsis* (and maize) there is a low-level flux through the signalling pathways in the dark, in tomato the pathways are totally inactive in the absence of light. This can explain why mutation of putative negative regulators of photomorphogenesis can be uncovered by screens for de-etiolated phenotypes in the dark in *Arabidopsis*, but not in tomato.

Feedback control from photosynthesis

Although it is clear that the role of photoreceptors is pivotal for seed germination and for the de-etiolation response, in mature plants the activity of the photosynthetic

apparatus is likely to exert a considerable feedback regulatory role on the expression of photosynthetic genes. Evidence for this is emerging from studies in green algae and photosynthetic bacteria (Allen *et al.*, 1995). In particular, it appears that redox signals arising from plastoquinone, in addition to modulating the light-harvesting capabilities of the two photosystems (PSI and PSII) via PSII CAB phosphorylation, are able to modify expression of nuclear-encoded photosynthetic genes (Escoubas *et al.*, 1995). Furthermore, in *Arabidopsis*, plastoquinone-derived redox signals have recently been found to modulate nuclear-encoded cytosolic antioxidant genes (Karpinski *et al.*, 1997), indicating the importance of such signals also in higher plants. Concerning phytochrome, redox signals would be most likely to interfere with the activity of the two signalling pathways controlling chloroplast functions, in order to modulate the relative activation of genes encoding PSI and PSII components (Figure 1).

Regulation by carbohydrates

An additional level of control of photoregulated gene expression derives from the products of photosynthesis, such as sucrose. Many photosynthetic genes have been found to be repressed by sucrose, whereas *CHS* and other genes encoding plant defence response components can be induced (Koch, 1996). Antisense and ectopic expression studies with a gene encoding hexokinase, the first enzyme in hexose assimilation, have indicated that this enzyme may be a key regulator of sugar responses (Jang *et al.*, 1997). It is now important to understand how these sugar-dependent signalling pathways interact with phytochrome-regulated pathways. Evidence for a specific interaction is that sucrose can inhibit de-etiolation in far-red light and can inhibit the PhyA-dependent far-red light-mediated inhibition of greening (Barnes *et al.*, 1996; Dijkwel *et al.*, 1997). Based on the known effects of sucrose on *CHS* and *CAB* gene expression, it is possible that sucrose interacts with a component of the reciprocal control mechanisms previously found to control cross-talk between the phytochrome-activated cGMP- and calcium-dependent pathways (Figure 1; Bowler *et al.*, 1994b). In particular, high concentrations of cGMP were found to inhibit photosynthetic gene expression but not to affect *CHS* gene expression. Consequently, sucrose may modulate photosynthetic gene expression by generating high concentrations of cGMP in the dark or by maintaining high levels in the light (Figure 1).

From this brief summary, it is clear that our knowledge of the signal transduction pathways controlling photoregulated gene expression remains sketchy, and some of the proposed interactions in Figure 1 are highly speculative. Nevertheless, research over the last years by many laboratories has provided a platform of information that will be built upon progressively to confirm or discount these current hypotheses. This, in turn, will allow a clearer understanding of the more complex interactions between the plant photoreceptors and endogenous signals derived, for example, from the circadian oscillator and from plant hormones.

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