Journal of Experimental Botany, Vol. 58, No. 12, pp. 3125–3133, 2007
doi:10.1093/jxb/erm186 Advance Access publication 12 September, 2007

Journal of Experimental Botany
www.jxb.oxfordjournals.org

FOCUS PAPER

Out of the dark: how the PIFs are unmasking a dual temporal mechanism of phytochrome signalling

Elena Monte[1,*], Bassem Al-Sady[2], Pablo Leivar[3] and Peter H. Quail[3]

[1] *Departament de Genètica Molecular Vegetal, CSIC-IRTA. Barcelona, Spain*

[2] *Department of Biochemistry and Biophysics, UC San Francisco, San Francisco, CA, USA*

[3] *Department of Plant and Microbial Biology, UC Berkeley, CA and USDA/UCB Plant Gene Expression Center, Albany, NY, USA*

Received 12 April 2007; Revised 28 June 2007; Accepted 16 July 2007

Abstract

Following light-induced nuclear translocation, the phytochromes induce changes in gene expression to regulate plant development. PIF3 and other PIFs (phytochrome-interacting factors), members of the bHLH (basic helix–loop–helix) family of transcriptional regulators, interact specifically with the active Pfr conformer of the phytochrome molecule, suggesting that the PIFs are key components of phytochrome signal transduction. The mechanism by which the PIFs transduce phytochrome signals is not understood. After initial studies that suggested that PIF3 was a positive regulator of phytochrome signalling, mutant studies indicated that the PIFs primarily act as negative regulators in the pathway. Furthermore, in some cases they accumulate in the dark and are degraded upon illumination by the ubiquitin–26S proteasome system. At least for PIF3, the protein degradation depends on direct interaction with the phytochrome molecule and is preceded by protein phosphorylation. In this review, the current understanding of the role of the PIFs in phytochrome-mediated photomorphogenesis will be summarized, and recent findings suggesting an unanticipated dual mechanism of action of the PIFs will be discussed.

Key words: *Arabidopsis*, bHLH factors, phytochrome signalling, PIF3, protein degradation.

Introduction

Light is the most precious energy and informational resource for plants. As sessile organisms, plants need constantly to monitor their light environment to optimize their growth and development accordingly. Higher plants have at least four families of photoreceptors that sense the quantity, quality, direction, and duration of light: an elusive UV-B light receptor; the UV-A/blue light-absorbing cryptochromes; the UV-A/blue light-absorbing phototropins; and the red- (R) and far red- (FR) absorbing phytochromes (Schäfer and Nagy, 2006). These photoreceptors mediate different photomorphogenic responses throughout the life cycle of plants such as germination, seedling de-etiolation, shade avoidance, and flowering.

Phytochromes are soluble chromoproteins that have the capacity to interconvert reversibly between two conformers: the inactive R light-absorbing Pr form and the biologically active FR light-absorbing Pfr form (Tu and Lagarias, 2005). In *Arabidopsis*, the phytochromes are encoded by a small gene family of five members (*PHYA–PHYE*). Among them, phyA and phyB have the most prominent functions (Franklin and Whitelam, 2004), phyC has a complementary role to phyB in several responses (Franklin *et al*., 2003*a*; Monte *et al*., 2003) and has been recently shown to contribute to natural variation in flowering time (Balasubramanian *et al*., 2006), and phyD and phyE are often redundant to phyB but can have a more prominent role under specific environmental conditions (Franklin *et al*., 2003*b*; Halliday and Whitelam, 2003; Halliday *et al*., 2003).

Upon light activation, the phytochromes are translocated from the cytoplasm into the nucleus (Nagatani, 2004), where they induce rapid changes in gene

* To whom correspondence should be addressed. E-mail: emcgme@cid.csic.es

expression (Jiao *et al*., 2007). Once in the nucleus, the active phytochromes can interact with PIF3 (phytochrome-interacting factor 3) and related PIF members of the basic helix–loop–helix (bHLH) family of transcription factors (Duek and Fankhauser, 2005). Based on the initial studies with phyB and PIF3, it was proposed that the phytochrome–PIF interaction may provide a direct transcriptional mechanism that could bring the light signal directly to the promoters of light-regulated genes (Quail, 2002*a*). However, no evidence has been obtained to support this model, and the mechanism by which the PIFs regulate photomorphogenesis is still under intense research. Here a review is presented of how our understanding of the role of the PIFs has progressed since the discovery of the founding member PIF3, and recent data that suggest that the mechanism of action of the PIFs in phytochrome signalling is more complex than originally anticipated are discussed.

Phytochrome signalling components: PIF3 as a dream positive regulator candidate

The original phytochrome signalling candidates were identified by genetic screens for mutants defective in seedling de-etiolation under continuous long-term (4–5 d) FR (FRc) or R (Rc) irradiation. These screenings looked for defects in hypocotyl length and cotyledon area characteristic of photomorphogenic mutants affected in phyA or phyB signalling (Fankhauser and Casal, 2004). PhyA is the only phytochrome mediating seedling de-etiolation in FRc, and phyB is the main phytochrome species mediating the de-etiolation response to Rc, in particular the inhibition of hypocotyl length (Franklin *et al*., 2003*b*; Tepperman *et al*., 2004). Collectively, genetic evidence gathered through the identification of photomorphogenic mutants affected in seedling de-etiolation in FRc and/or Rc suggested a complex network of phytochrome signalling pathways: while some of the components were specific, some others were shared by both phyA and phyB. Also, positive and negative signalling components on each pathway were identified (Hudson, 2000). Some of them have been cloned and characterized (Wang and Deng, 2002; Hiltbrunner *et al*., 2005; Hiltbrunner *et al*., 2006). Progress on the function of these phytochrome signalling components is beyond the scope of this review.

A breakthrough in the phytochrome field came with the use of yeast two-hybrid screens carried out with the phytochrome molecule. This approach led to the exciting identification of the phytochrome-interacting PIF3 (Ni *et al*., 1998), a member of the bHLH superfamily of transcription factors (Toledo-Ortiz *et al*., 2003). Although the original yeast two-hybrid screen was carried out with the non-photoactive C-terminal domain of phyB, PIF3 was also found to interact with the photoactive N-terminal domain of phyB (Shimizu-Sato *et al*., 2002) as well as with the non-photoactive C-terminal domain of phyA (Ni *et al*., 1998), indicating that sufficient determinants for PIF3 binding are present in both phytochrome domains. *In vitro* pull-down assays with the full-length phytochrome molecule showed that PIF3 interaction is specific to the biologically active Pfr form of the phytochrome, with higher apparent affinity for phyB than phyA (Ni *et al*., 1999; Zhu *et al*., 2000). The identification of PIF3, together with the finding that the phytochrome rapidly translocates from the cytoplasm into the nucleus upon light activation (Sakamoto and Nagatani, 1996; Kircher *et al*., 1999; Yamaguchi *et al*., 1999), revolutionized the field of phytochrome signalling and created a new paradigm of phytochrome action where the light perceived by the phytochrome could be directly transduced onto pre-existing transcription factors such as PIF3 to modulate the expression of light-regulated genes (Quail, 2002*a*).

Two initial lines of evidence indicated that PIF3 was a central player in phytochrome signalling: (i) the Pfr-dependent binding was reduced in phytochrome missense mutant derivatives that were previously shown to be defective in signalling *in vivo* (Ni *et al*., 1999), thus providing correlative evidence that PIF3–phytochrome binding was important in the plant cell; and (ii) *Arabidopsis* antisense lines with reduced levels of PIF3 transcript showed strongly reduced sensitivity to Rc and FRc light during seedling de-etiolation (Ni *et al*., 1998). A second set of evidence led to the postulation of an initial model of phytochrome action through PIF3. First, PIF3 localizes constitutively into the nucleus (Ni *et al*., 1998) and is able to bind *in vitro* specifically to the G-box DNA sequence CACGTG, a DNA motif enriched in the promoters of light-responsive genes (Martínez-García *et al*., 2000). In addition, *in vitro* analysis also showed that phyB Pfr can specifically and photoreversibly form a ternary complex with the DNA-bound PIF3 (phyB Pfr–PIF3–G-box complex) (Martínez-García *et al*., 2000). Based on all the above, a model was proposed in which PIF3 would act as a central positive regulator of phytochrome necessary to induce light-regulated genes. In this model, phytochrome molecules would function as integral, light-switchable components of transcription regulator complexes at target promoters following photoconversion-induced translocation into the nucleus. The photoreceptor could then modify transcriptional activity directly by acting as a co-regulator or indirectly by biochemically modifying either PIF3 or other components of the transcriptional machinery (Quail, 2000, 2002*b*). This attractive model provided an explanation of how the phytochrome would transmit the light signal directly into the promoters of target genes, thus transmitting changes of light input into rapid modulation of gene expression through interaction with positive acting transcription factors such as PIF3.

However, later studies presented below challenged this initial working model.

PIF3 and other related PIFs as negative regulators of phytochrome signalling

Contrary to the initial work on PIF3, subsequent evidence did not support the early model of PIF3 acting as a positive regulator of phytochrome signalling. Instead, analyses of mutants deficient in PIF3 and other related bHLH proteins indicated that they mainly act as negative regulators in the pathway.

In *Arabidopsis*, the bHLH family of transcription factors has >160 members (Bailey *et al*., 2003; Heim *et al*., 2003; Toledo-Ortiz *et al*., 2003). Based on the evolutionary identity of the bHLH domain, it was established that PIF3 belongs to a phylogenetic subfamily of 15 members (Toledo-Ortiz *et al*., 2003). Six of these PIF3-like factors (PIF1/PIL5, PIF4, PIF5/PIL6, PIL1, HFR1, and SPT) have also been shown to be involved in phytochrome signalling (Duek and Fankhauser, 2005; Penfield *et al*., 2005). Of them, HFR1 and PIL1 do not interact directly with the phytochromes (Fairchild *et al*., 2000; Fankhauser and Chory, 2000; Soh *et al*., 2000; Khanna *et al*., 2004), whereas PIF1 (also known as PIL5 for phytochrome-interacting factor-3-like 5) (Huq *et al*., 2004; Oh *et al*., 2004), PIF4 (Huq and Quail, 2002), and PIF5 (PIL6) (Yamashino *et al*., 2003; Fujimori *et al*., 2004; Khanna *et al*., 2004) also interact preferentially with the Pfr form of the phytochrome, like PIF3 (Khanna *et al*., 2004). All these PIFs carry in their N-terminal domain a short motif (13 residues long in PIF3) called APB (for active phyB binding) that mediates the interaction with phyB Pfr (Khanna *et al*., 2004). The APB motif is found exclusively in the PIF3-related subgroup and it appears to be sufficient for phyB Pfr interaction (Khanna *et al*., 2004). PIF1, as well as PIF3, also interacts with the Pfr form of phyA (Zhu *et al*., 2000; Huq *et al*., 2004). PIF3 has stronger affinity for phyB (Zhu *et al*., 2000), whereas PIF1 has similar affinity for phyA and phyB (Huq *et al*., 2004). In PIF3, the interaction with phyA Pfr is mediated through a short motif (18 residues long in PIF3) called APA (for active phyA binding) that is necessary for the interaction (Al-Sady *et al*., 2006).

De-etiolation studies of *pif1*, *pif4*, and *pif5* mutants under Rc showed that they all participate in phyB signalling, consistent with their binding affinity. Surprisingly, *pif4* and *pif5* mutants displayed short hypocotyls under Rc, a phenotype opposite to that of *phyB* and indicative of a negative role in the phyB pathway (Huq and Quail, 2002; Fujimori *et al*., 2004). Likewise, *pif3* mutants were also shown to be hypersensitive to Rc (Kim *et al*., 2003; Monte *et al*., 2004). This led to the conclusion that PIF3 acts negatively in the phyB pathway under Rc (Kim *et al*., 2003; Monte *et al*., 2004), in contradiction to the initial studies using antisense plants (Ni *et al*., 1998). For PIF1, although the *pif1* mutant did not show any apparent phenotype in Rc, PIF1-overexpressor seedlings displayed a long hypocotyl in Rc suggestive of a negative role in phyB signalling (Huq *et al*., 2004; Oh *et al*., 2004). De-etiolation analyses under FRc of mutant seedlings deficient in the phyA-binding PIF1 and PIF3 factors suggest that they act as negative regulators in the phyA pathway. The *pif1* mutant displayed a short-hypocotyl phenotype in FRc that is opposite to that of *phyA* (Oh *et al*., 2004), and although PIF3 does not appear to mediate hypocotyl elongation in FRc (Kim *et al*., 2003; Monte *et al*., 2004), *pif3* and *pif3-ox* seedlings had larger and smaller cotyledons, respectively, under these conditions (Kim *et al*., 2003). All the above results showed that there is good correlation between the phytochrome binding properties of the PIFs and their role in phytochrome-regulated de-etiolation under long-term Rc or FRc irradiation. Furthermore, they led to the suggestion that the PIFs are negative regulators of phytochrome signalling.

The negative regulatory model

A pivotal study by Bauer *et al*. (2004) showed that the PIF3 protein is stable in the dark and it is rapidly degraded upon exposure to light. This light-induced degradation is promoted by phyA, phyB, and phyD (Bauer *et al*., 2004; Al-Sady *et al*., 2006) and is mediated by the ubiquitin–26S proteasome pathway (Park *et al*., 2004; Al-Sady *et al*., 2006). Similarly, it has been shown that PIF1 is also stable in the dark and rapidly degraded upon light treatment through the ubiquitin–26S proteasome machinery (Shen *et al*., 2005). Together, these results suggest that light-induced degradation of the PIFs might be a general mechanism to regulate their activity. Phytochrome-induced degradation of the PIFs would thus promote photomorphogenesis by removing repressors of the pathway. This negative regulatory model could be considered analogous to the stimulus-induced degradation of repressors in the auxin and gibberellin pathways (Huq, 2006). Protein degradation is a common regulatory system that equips the cells with a fast response mechanism to inactivate proteins rapidly (Moon *et al*., 2004). In particular, it has been shown that the degradation of transcription factors is important in the regulation of light signalling (Hoecker, 2005; Yang *et al*., 2005). Regulated proteolysis in light signalling also takes place in the dark to remove positive regulators such as HY5, LAF1, and HFR1, and keep photomorphogenesis repressed (Osterlund *et al*., 2000; Seo *et al*., 2003; Duek *et al*., 2004; Jang *et al*., 2005; Yang *et al*., 2005). Interestingly, these factors are stabilized upon transfer to light, in contrast to the PIFs

(Osterlund *et al*., 2000; Seo *et al*., 2003; Duek *et al*., 2004), providing a balance of negative- and positive-acting regulators to optimize photomorphogenesis (Huq, 2006).

Recently, it has been shown that phyA and phyB induce rapid phosphorylation of PIF3 (Al-Sady *et al*., 2006). This occurs redundantly by direct interaction prior to the ubiquitination and degradation of PIF3. The requirement for direct binding of phyA and phyB to PIF3 was shown in transgenic plants expressing a PIF3 variant doubly mutated in the APA and APB motifs in which phosphorylation and degradation were not detectable (Al-Sady *et al*., 2006). These results confirmed the direct involvement of the phytochromes in the removal of PIF3 upon light exposure and provided further evidence supporting a negative regulatory model for PIF action. The phytochrome-induced phosphorylation of proteins such as PIF3 might be the primary biochemical modification following phytochrome–PIF interaction. It has been proposed that this phosphorylation following phytochrome–PIF interaction could represent a tagging system for the proteasome pathway to inactivate a previously active PIF3 (Al-Sady *et al*., 2006). Alternatively, phytochrome–PIF3 interaction, phosphorylation, and/or ubiquitination could be necessary to activate PIF3 transiently prior to degradation (Al-Sady *et al*., 2006), an attractive regulatory strategy common in other systems (Lipford and Deshaies, 2003).

However, although for most responses the PIFs act as negative regulators, for some responses they have a positive role in phy signalling. For example, PIF3 has been shown to be a positive regulator of chlorophyll and anthocyanin accumulation (Kim *et al*., 2003; Monte *et al*., 2004). These results suggest that the PIFs might act in different branches of phytochrome signalling that might be regulated through distinct mechanisms.

The long second look: diversity of function early following light exposure

Old perceptions of phytochrome signalling in photomorphogenesis harboured two essential concepts: first, that the photomorphogenic phenotypes observed in different phytochrome mutants following long-term irradiation represent the sum of the actions of the individual phytochrome photoreceptor over time; and, secondly, that the extent of the phenotype observed is directly linked to the differences in gene expression dependent on that photoreceptor. A recent series of papers examining gene expression profiles in dark-grown seedlings exposed to different durations of R or FR light in different *phy* backgrounds have shed doubts on these central beliefs and also greatly broadened understanding of the roles of different phytochromes, phyA and phyB, in particular, in establishing the photomorphogenic programme. First, while both R and FR light exposure of dark-grown seedlings affects expression of over ~10% of the *Arabidopsis* genome (Tepperman *et al*., 2001, 2004, 2006), the phytochromes responsible for particular expression pattern changes, and the temporal nature of the gene expression profile were surprising: both R and FR light affect different kinds of genes early (within 1–3 h) and late (3–24 h) following light exposure. Signalling and transcription factor genes tend to be induced/repressed early, while genes in energy metabolism and photosynthesis were affected later. More interestingly, the effect of phyA and phyB on the gene expression profile did not in fact correlate with their previously postulated roles: given their visible phenotypes, particularly in hypocotyl and cotyledon growth inhibition, phyA and phyB had been postulated to be the only or major photoreceptor for FR or R light, respectively. The gene expression studies showed, however, that phyA dominates the gene expression programme early under both FR and R, and that phyB has a relatively minor role early in R (Tepperman *et al*., 2006). PhyB controls a somewhat larger gene set later during photomorphogenesis, but that still only amounts to 5% of the genes induced or repressed >2-fold under long-term Rc (Tepperman *et al*., 2004). This is consistent with the protein lability profile of phyA in R (Clough and Vierstra, 1997). The dominant role of phyA in control of early response genes also became apparent in a gene profiling study on PIF3 in dark-grown seedlings exposed for 1 h to Rc, similar to the above studies (Monte *et al*., 2004). Interestingly, PIF3-dependent genes at 1 h Rc are co-regulated by phyA, but not phyB, despite *pif3* mutants being hypersensitive in hypocotyl growth in Rc (but not FRc), a pathway dominated by phyB. Importantly, rather than affecting a broad range of the genes induced/repressed by phytochromes in R or FR, as previously predicted (Martínez-García *et al*., 2000), PIF3 affects a very specific and small cluster of genes at 1 h Rc (chloroplast function-related genes).

In summary, when analysing phytochrome signalling from a gene expression perspective, one is led to the conclusion that first, most of the gene expression changes following initial exposure to FR or R light are under phyA control, and, second, that phytochrome-interacting transcription factors such as PIF3 affect specialized subsets of these early response genes that control a particular early photomorphogenic phenotype. This contrasts with the older views about PIF3 that placed it as a general regulator of photomorphogenesis in the phyB pathway (Martínez-García *et al*., 2000). These studies also emphasize the possibility that phytochrome signalling, early following initial irradiation (meaning in the initial hours following light exposure of the etiolated seedling) and in the long term (meaning growth in Rc or FRc for 3–5 d), may be implemented in different ways: early gene expression changes as executed mainly by phyA, and

carried out by PIFs, may in fact not correlate with the ultimate outcome as measured by changes in gross morphology after 4–5 d in Rc; and conversely, phyB-dependent effects on hypocotyl and cotyledon morphology may not be the outcome of the observed early changes in the gene expression profile.

In fact, the notion that the classical long-term morphological phenotype may not represent the early action of the phytochromes has prompted a search for morphological markers closer to the initial light signal. One direct way to assess changes early is to examine de-etiolation phenotypes at earlier stages, a path previously hampered by technical difficulty. Some early studies have addressed early morphological markers such as the apical hook opening, cotyledon unfolding, and chlorophyll accumulation following initial light exposure, and the dependence of these responses on the phytochromes (Liscum and Hangarter, 1993; Reed *et al*., 1994). Recent advances in live imaging techniques have allowed real-time measurements of the hypocotyl growth rate as well as the growth direction of etiolated seedlings following light exposure (Parks and Spalding, 1999). Such studies have revealed that phyA and phyB, as consistent with the gene expression studies, impact hypocotyl growth in different ways: phyA acts initially to achieve transient growth inhibition, and phyB takes over later for the duration of the irradiation period. Studies focusing on early morphological responses such as hook opening and cotyledon separation have shown that both phyA and phyB are involved in these responses and that they are most active in different temporal windows following initial light exposure (E Monte and P Quail, unpublished results).

Collectively, the above studies suggest that key aspects of primary phytochrome signalling are difficult to uncover by analysis of the end-point phenotype after a 4–5 d light treatment; and cannot capture the dynamic interplay between the different receptors. The same can be said for *pif* mutants. While initially most of the PIF genes, such as PIF3, PIF4, PIF5/PIL6, and PIF1/PIL5, were analysed for their effect on long-term growth phenotypes, and concluded to be negative regulators of phytochrome-mediated morphological responses, recent studies discussed below have focused on how PIFs affect the initial transition of the dark-grown seedling to light. While much remains to be analysed, early indications are that PIFs in fact affect a large diversity of early photomorphogenic responses, and appear to have occupied niches of specialization. An above-mentioned gene profiling study on PIF3 [examing gene expression changes after 1 h Rc treatment of etiolated seedlings (Monte *et al*., 2004)] revealed that most PIF3-dependent genes fall into one broad category, nuclear-encoded genes for chloroplast components. This finding is consistent with the observation that *pif3* mutants are defective or delayed in the greening process of etiolated seedlings when first exposed to light.

PIF3 plays a critical positive role in the expression of these genes in Rc (Monte *et al*., 2004), as well as for the expression of anthocyanin pathway genes in FRc (Kim *et al*., 2003; Shin *et al*., 2007). Recent studies further indicate that PIF5/PIL6 may affect early phytochrome responses mediated by ethylene (R Khanna, Y Shen, and P Quail, unpublished results), specifically affecting opening of the apical hook, and that other PIFs including PIF3 and PIF4 may differentially affect early morphological responses such as apical hook opening and cotyledon separation (P Leivar, E Monte, and P Quail, unpublished results). A more highly specialized role is filled by PIF1, which does not appear to play a role during light signalling *per se* but even before initial light exposure as a regulator of both germination (Oh *et al*., 2004) and protochlorophyllide synthesis in the dark (Huq *et al*., 2004). PIF1 acts to decrease active gibberellic acid levels in the seed by both down-regulating gibberellic acid biosynthesis enzymes and up-regulating gibberellic acid-catabolizing enzymes (Oh *et al*., 2006). Further, PIF1 negatively regulates protochlorophyllide synthesis in the etiolated seedling to prevent accumulation of free protochlorophyllide which can result in oxidative damage and bleaching, as evident in *pif1* mutants exposed to light following prolonged growth in darkness (Huq *et al*., 2004). PIF1 therefore keeps both the seed and etiolated seedling poised for light signals, removal of PIF1 by phyA and phyB allows gibberellic acid accumulation and germination, and, later, PIF1 removal from the etiolated seedling relieves the block on chlorophyll synthesis (Shen *et al*., 2005). It therefore appears that first, several of the complexities and dynamics of phytochrome signalling can be better captured by focusing on early morphological phenotypes rather than growth phenotypes under prolonged continuous irradiation and, second, PIFs have a specific role in assisting the phytochrome photosensory system in efficiently and rapidly implementing the photomorphogenic programme following initial light exposure.

Positive and negative signalling roles of PIFs: dichotomy between early and long-term signalling

While PIFs have a variety of different roles early, carried out in conjunction with phyA, which dominantly implements the early light-induced gene expression profile, their long-term growth phenotypes and strong interaction with phyB (Khanna *et al*., 2004) has placed them as negative regulators of phyB-controlled growth responses (see above) (Duek and Fankhauser, 2005).

Collectively, this indicates that PIFs mediate two distinct types of phenotypic responses. A problem in trying to understand how PIFs signal early and late is the

phytochrome-induced degradation to lower steady-state levels of several of them: PIF1 (Shen *et al*., 2005; Oh *et al*., 2006), PIF3 (Monte *et al*., 2004; Park *et al*., 2004; Al-Sady *et al*., 2006), and PIF4 (B Al-Sady and P Quail, unpublished results). This degradation happens in a time frame where PIFs are also most active in gene regulation (Monte *et al*., 2004). It is not clear what role this degradation may play towards the signalling activity of the PIFs, given that they can act, under different circumstances, as positive regulators (PIF3 in dark to light transition) or negative regulators of growth responses under long-term Rc. It is possible that the PIFs use similar mechanisms in early and long-term signalling, and that the mechanism involved in degradation may either activate some of their functions or adjust their protein level to the specific response. However, recent evidence (B Al-Sady and P Quail; E Monte, P Leivar, and P Quail; R Khanna and P Quail, unpublished results) raises the question of whether the processes of early and long-term signalling through the phytochromes, as mediated by the PIFs, could be fundamentally different in nature. Whereas the dark-grown seedling has adapted to implement the photomorphogenic programme rapidly and precisely, the fully de-etiolated seedling needs to optimize its long-term growth responses to the prevalent light quality and quantity rather than to initiate new gene expression programmes. The lack of correlation between these two phases of photomorphogenesis has further become evident from a functional profiling study (Khanna *et al*., 2006). The effect on growth morphology under long-term irradiation of reverse-genetic disruption of 32 genes, previously shown to be strongly induced or repressed by light in the first hours, revealed that these genes mostly do not affect the long-term phenotype. Further, a time-course analysis of the *pif3* hypocotyl phenotype indicates that it appears very late, only after 2–3 d of Rc (Monte *et al*., 2004), far removed from the *pif3* effect on greening following initial light exposure.

What are the mechanisms of PIF action early and in the long-term phase of de-detiolation? The mechanism of action of PIFs early does seem to involve their action as transcription factors. The lines of evidence supporting this conclusion are (i) that PIF3 is found at target promoters *in vivo* (in this case at anthocyanin biosynthesis pathway genes) (Shin *et al*., 2007); and (ii) that the DNA binding capacity of PIF3 is required for the light induction of PIF3-dependent genes (B Al-Sady and P Quail, unpublished results). These studies, as well as studies on *phyA* and *pif3* early mutant phenotypes (Monte *et al*., 2004; Tepperman *et al*., 2006), and their respective gene expression profiles, have suggested a general framework model where PIFs implement subsets of primarily phyA-dependent gene expression programmes to achieve certain early cellular and morphological light responses. However, little is understood about the mechanism of PIF-mediated phytochrome signalling, and phyB signalling in particular, under long-term irradiation. Importantly, this function of the PIFs is apparently facilitated by the lower steady-state level established by the phytochromes during long-term irradiation. While it is possible that the long-term phenotype is a residual manifestation of early PIF action, prior to its degradation, it appears more likely that PIFs act more immediately on phytochrome signalling while at this low level, especially since the *pif* mutants have different early phenotypes, and almost identical phenotypes under long-term Rc. What then could be a mechanism by which PIFs act late, during long-term irradiation? One clue comes from the well-established observation that the hypocotyl and cotyledon growth responses are highly dependent on the absolute levels of the active phyB photoreceptor (Koornneef *et al*., 1980; Wester *et al*., 1994; Wagner *et al*., 1996). This raised the possibility that PIFs might act to inhibit active phyB in long-term irradiation. Indications are that this is indeed the case and that inhibition may be occurring directly as a result of the interaction of the PIFs and the phyB photoreceptor. This appears to be true at least for PIF4, which requires its ability to interact specifically with phyB to implement its negative regulatory role on hypocotyl growth (Khanna *et al*., 2004). In this context, degradation of the PIF proteins could also be critical in the regulation of the long-term phenotype. PIF3 is reduced to ~15% of its dark levels during Rc (Monte *et al*., 2004), and the same appears true for PIF1/PIL5 (Shen *et al*., 2005; Oh *et al*., 2006). This lower level may be optimal for the regulation of phyB signalling output, possibly via the regulation of the absolute active Pfr phyB pool. However, the mechanism by which PIFs could act on Pfr phyB to reduce its activity remains to be defined. One possibility is that PIFs simply titrate Pfr phyB into an inactive pool in an interaction-dependent and reversible manner, or, alternatively, that PIFs act to inactivate a fraction of the phyB molecules irreversibly, by modification or degradation.

Prospects

Our understanding of phytochrome signalling has changed considerably over the last few years, away from the old monolithic idea of one diagnostic phenotype representing one mechanism, to more complex models. The enticing molecular properties of the PIF proteins initially seemed to support, erroneously, previous phytochrome signalling models, but have since served as excellent tools to dissect the apparent intricacies of phytochrome signalling. First, close study of the role of PIFs has uncovered the complex nature of early phytochrome signalling, with different PIF factors controlling separate facets of photomorphogenesis, such as control of germination, protochlorophyllide synthesis, chloroplast biogenesis, and co-ordination of

rapid morphological responses (such as hook and cotyledon unfolding). Second, scrutiny of early and long-term irradiation phenotypes has indicated that the mechanisms of PIF action may be different in these two time frames. PIF3 is a good example. It acts positively as a regulator of chloroplast-related genes, but acts negatively on phyB-controlled hypocotyl inhibition, with the *pif3* mutant effect on the latter only appearing after 2–3 d of long-term irradiation. This early versus long-term mechanistic dichotomy has yet to be fully explained, but there are already some exciting hints from ongoing research. Most of the PIFs studied negatively impact phyB long-term signalling, in an unknown manner. It is known that growth responses are highly sensitive to the absolute amounts of available phyB–Pfr. Cytologically, this may correlate with the finding that phyB accumulates in large nuclear speckles, specifically during long-term irradiation (Nagy *et al*., 2001; Kircher *et al*., 2002; Chen *et al*., 2003). While the significance of the late speckles has been doubted for long-term growth control (Matsushita *et al*., 2003), it has been hypothesized that the speckles may serve as storage vehicles for available phyB. It is not clear whether PIFs may participate in controlling phyB storage in late speckles. If PIFs do not participate in such a mechanism, what could be an alternative model? It is becoming more evident that PIFs in fact regulate phyB protein levels. Evidence is pointing in this direction for at least four PIFs, PIF3, PIF4, PIF5/PIL6, and the previously unpublished PIF7 protein (Monte *et al*., 2004; B Al-Sady and P Quail; P Leivar, E Monte, and P Quail; R Khanna, Y Shen, and P Quail, unpublished results). The mechanism appears to be at the post-transcriptional level, and requires direct interaction with the phyB photoreceptor, and, for the case of PIF3 where it has been examined, does not require its ability to target DNA. Therefore, rather than participating directly in the phytochrome signalling pathway, PIFs appear to be homeostatic regulators of the phytochrome output under long-term irradiation by controlling photoreceptor abundance. What about mechanisms of signalling through PIFs early after initial light treatment? Mounting evidence, as discussed above, suggests that PIFs (especially PIF1 and PIF3) do act as transcription factors controlling the light response of differing sets of genes at this stage of the de-etiolation process. Recently, PIF3 has been shown to bind to anthocyanin biosynthetic gene promoters *in vivo* in FR light and also in the dark (Shin *et al*., 2007), suggesting that PIF3 can bind to the promoters of early light-regulated genes in a phytochrome-independent fashion, presumably allowing for the rapid light induction upon the initial illumination.

These studies have helped sharpen the notion of a mechanistic dichotomy in phytochrome signalling early, following initial light exposure, and later during long-term irradiation. This dichotomy may be implemented via the PIFs, acting in both phases by radically different mechanisms: acting alternatively as transcription factors of sets of genes critical to certain aspects of photomorphogenesis early, and as regulators of the phyB photoreceptor itself, to fine-tune phytochrome output during long-term irradiation. Future studies will have to address by which mechanism PIFs regulate phyB levels, and how they function early with phytochromes to act on photoresponsive genes.

References

Al-Sady B, Ni WM, Kircher S, Schafer E, Quail PH. 2006. Photoactivated phytochrome induces rapid PIF3 phosphorylation prior to proteasome-mediated degradation. *Molecular Cell* **23,** 439–446.

Bailey PC, Martin C, Toledo-Ortiz G, Quail PH, Huq E, Heim MA, Jakoby M, Werber M, Weisshaar B. 2003. Update on the basic helix–loop–helix transcription factor gene family in *Arabidopsis thaliana*. *The Plant Cell* **15,** 2497–2502.

Balasubramanian S, Sureshkumar S, Agrawal M, Michael TP, Wessinger C, Maloof JN, Clark R, Warthmann N, Chory J, Weigel D. 2006. The PHYTOCHROME C photoreceptor gene mediates natural variation in flowering and growth responses of *Arabidopsis thaliana*. *Nature Genetics* **38,** 711–715.

Bauer D, Viczian A, Kircher S, *et al.* 2004. Constitutive photomorphogenesis 1 and multiple photoreceptors control degradation of phytochrome interacting factor 3, a transcription factor required for light signaling in Arabidopsis. *The Plant Cell* **16,** 1433–1445.

Chen M, Schwabb R, Chory J. 2003. Characterization of the requirements for localization of phytochrome B to nuclear bodies. *Proceedings of the National Academy of Sciences, USA* **100,** 14493–14498.

Clough RC, Vierstra RD. 1997. Phytochrome degradation. *Plant, Cell and Environment* **20,** 713–721.

Duek PD, Elmer MV, van Oosten VR, Fankhauser C. 2004. The degradation of HFR1, a putative bHLH class transcription factor involved in light signaling, is regulated by phosphorylation and requires COP1. *Current Biology* **14,** 2296–2301.

Duek PD, Fankhauser C. 2005. bHLH class transcription factors take centre stage in phytochrome signalling. *Trends in Plant Science* **10,** 51–54.

Fairchild CD, Schumaker MA, Quail PH. 2000. HFR1 encodes an atypical bHLH protein that acts in phytochrome A signal transduction. *Genes and Development* **14,** 2377–2391.

Fankhauser C, Casal JJ. 2004. Phenotypic characterization of a photomorphogenic mutant. *The Plant Journal* **39,** 747–760.

Fankhauser C, Chory J. 2000. *RSF1*, an Arabidopsis locus implicated in phytochrome A signaling. *Plant Physiology* **24,** 39–45.

Franklin KA, Davis SJ, Stoddart WM, Vierstra RD, Whitelam GC. 2003*a*. Mutant analyses define multiple roles for phytochrome C in Arabidopsis photomorphogenesis. *The Plant Cell* **15,** 1981–1989.

Franklin KA, Praekelt U, Stoddart WM, Billingham OE, Halliday J, Whitelam GC. 2003*b*. Phytochromes B, D, and E act redundantly to control multiple physiological responses in Arabidopsis. *Plant Physiology* **131,** 1340–1346.

Franklin KA, Whitelam GC. 2004. Light signals, phytochromes and cross-talk with other environmental cues. *Journal of Experimental Botany* **55,** 271–276.

Fujimori T, Yamashino T, Kato T, Mizuno T. 2004. Circadian-controlled basic/helix–loop–helix factor, PIL6, implicated in light-signal transduction in *Arabidopsis thaliana*. *Plant and Cell Physiology* **45,** 1078–1086.

Halliday KJ, Salter MG, Thingnaes E, Whitelam GC. 2003. Phytochrome control of flowering is temperature sensitive and correlates with expression of the floral integrator FT. *The Plant Journal* **33,** 875–885.

Halliday KJ, Whitelam GC. 2003. Changes in photoperiod or temperature alter the functional relationships between phytochromes and reveal roles for phyD and phyE. *Plant Physiology* **131,** 1913–1920.

Heim MA, Jakoby M, Werber M, Martin C, Weisshaar B, Bailey PC. 2003. The basic helix–loop–helix transcription factor family in plants: a genome-wide study of protein structure and functional diversity. *Molecular Biology and Evolution* **20,** 735–747.

Hiltbrunner A, Viczian A, Bury E, Tscheuschler A, Kircher S, Toth R, Honsberger A, Nagy F, Fankhauser C, Schafer E. 2005. Nuclear accumulation of the phytochrome A photoreceptor requires FHY1. *Current Biology* **15,** 2125–2130.

Hiltbrunner A, Tscheuschler A, Viczian A, Kunkel T, Kircher S, Schafer E. 2006. FHY1 and FHL act together to mediate nuclear accumulation of the phytochrome A photoreceptor. *Plant and Cell Physiology* **47,** 1023–1034.

Hoecker U. 2005. Regulated proteolysis in light signaling. *Current Opinion in Plant Biology* **8,** 469–476.

Hudson ME. 2000. The genetics of phytochrome signalling in Arabidopsis. *Seminars in Cell and Developmental Biology* **11,** 475–483.

Huq E. 2006. Degradation of negative regulators: a common theme in hormone and light signaling networks? *Trends in Plant Science* **11,** 4–7.

Huq E, Al-Sady B, Hudson M, Kim C, Apel K, Quail PH. 2004. PHYTOCHROME-INTERACTING FACTOR 1 is a critical bHLH regulator of chlorophyll biosynthesis. *Science* **305,** 1937–1941.

Huq E, Quail PH. 2002. PIF4, a phytochrome-interacting bHLH factor, functions as a negative regulator of phytochrome B signaling in *Arabidopsis*. *EMBO Journal* **21,** 2441–2450.

Jang IC, Yang JY, Seo HS, Chua NH. 2005. HFR1 is targeted by COP1 E3 ligase for post-translational proteolysis during phytochrome A signaling. *Genes and Development* **19,** 593–602.

Jiao Y, Lau OS, Deng XW. 2007. Light-regulated transcriptional networks in higher plants. *Nature Reviews Genetics* **8,** 217–230.

Khanna R, Huq E, Kikis EA, Al-Sady B, Lanzatella C, Quail PH. 2004. A novel molecular recognition motif necessary for targeting photoactivated phytochrome signalling to specific basic helix–loop–helix transcription factors. *The Plant Cell* **16,** 3033–3044.

Khanna R, Shen Y, Toledo-Ortiz G, Kikis EA, Johannesson H, Hwang Y-S, Quail PH. 2006. Functional profiling reveals that only a small number of phytochrome-regulated early-response genes in *Arabidopsis* are necessary for optimal de-etiolation. *The Plant Cell* **18,** 2157–2171.

Kim J, Yi H, Choi G, Shin B, Song P-S, Choi G. 2003. Functional characterization of phytochrome interacting factor 3 in phytochrome-mediated light signal transduction. *The Plant Cell* **15,** 2399–2407.

Kircher S, Gil P, Kozma-Bognar L, Fejes E, Speth V, Husselstein-Muller T, Bauer D, Adam E, Schafer E, Nagy F. 2002. Nucleocytoplasmic partitioning of the plant photoreceptors phytochrome A, B, C, D, and E is regulated differentially by light and exhibits a diurnal rhythm. *The Plant Cell* **14,** 1541–1555.

Kircher S, Kozma-Bognar L, Kim L, Adam E, Harter K, Schaefer E, Nagy F. 1999. Light quality-dependent nuclear import of the plant photoreceptors phytochrome A and B. *The Plant Cell* **11,** 1445–1456.

Koornneef M, Rolff E, Spruit CJP. 1980. Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. *Zeitschrift für Pflanzenphysiologie* **100,** 147–160.

Lipford JR, Deshaies RJ. 2003. Diverse roles for ubiquitin-dependent proteolysis in transcriptional activation. *Nature Cell Biology* **5,** 845–850.

Liscum E, Hangarter RP. 1993. Light-stimulated apical hook opening in wild-type *Arabidopsis thaliana* seedlings. *Plant Physiology* **101,** 567–572.

Martínez-García JF, Huq E, Quail PH. 2000. Direct targeting of light signals to a promoter element-bound transcription factor. *Science* **288,** 859–863.

Matsushita T, Mochizuki N, Nagatani A. 2003. Dimers of the N-terminal domain of phytochrome B are functional in the nucleus. *Nature* **424,** 571–574.

Monte E, Alonso JM, Ecker JR, Zhang Y, Li X, Young J, Austin-Phillips S, Quail PH. 2003. Isolation and characterization of *phyC* mutants in Arabidopsis reveals complex crosstalk between phytochrome signaling pathways. *The Plant Cell* **15,** 1962–1980.

Monte E, Tepperman JM, Al-Sady B, Kaczorowski KA, Alonso JM, Ecker JR, Li X, Zhang Y, Quail PH. 2004. The phytochrome-interacting transcription factor, PIF3, acts early, selectively, and positively in light-induced chloroplast development. *Proceedings of the National Academy of Sciences, USA* **101,** 16091–16098.

Moon J, Parry G, Estelle M. 2004. The ubiquitin–proteosome pathway and plant development. *The Plant Cell* **16,** 3181–3195.

Nagatani A. 2004. Light-regulated nuclear localization of phytochromes. *Current Opinion in Plant Biology* **7,** 708–711.

Nagy F, Kircher S, Schaefer E. 2001. Intracellular trafficking of photoreceptors during light-induced signal transduction in plants. *Journal of Cell Science* **114,** 475–480.

Ni M, Tepperman JM, Quail PH. 1998. PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix–loop–helix protein. *Cell* **95,** 657–667.

Ni M, Tepperman JM, Quail PH. 1999. Binding of phytochrome B to its nuclear signaling partner PIF3 is reversibly induced by light. *Nature* **400,** 781–784.

Oh E, Kim J, Park E, Kim J-I, Kang C, Choi G. 2004. PIL5, a phytochrome-interacting basic helix–loop–helix protein, is a key negative regulator of seed germination in *Arabidopsis thaliana*. *The Plant Cell* **16,** 3045–3058.

Oh E, Yamaguchi S, Kamiya Y, Bae G, Chung WI, Choi G. 2006. Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. *The Plant Journal* **47,** 124–139.

Osterlund MT, Hardtke CS, Wei N, Deng XW. 2000. Targeted destabilization of HY5 during light-regulated development of *Arabidopsis*. *Nature* **405,** 462–466.

Park E, Kim J, Lee Y, Shin J, Oh E, Chung W-I, Liu J-R, Ghoi G. 2004. Degradation of phytochrome interacting factor 3 in phytochrome-mediated light signaling. *Plant and Cell Physiology* **45,** 968–975.

Parks BM, Spalding EP. 1999. Sequential and coordinated action of phytochromes A and B during Arabidopsis stem growth revealed by kinetic analysis. *Proceedings of the National Academy of Sciences, USA* **96,** 14142–14146.

Penfield S, Josse EM, Kannangara R, Gilday AD, Halliday KJ, Graham IA. 2005. Cold and light control seed germination

through the bHLH transcription factor SPATULA. *Current Biology* **15,** 1998–2006.

Quail PH. 2000. Phytochrome-interacting factors. *Seminars in Cell and Developmental Biology* **11,** 457–466.

Quail PH. 2002*a*. Phytochrome photosensory signalling networks. *Nature Reviews Molecular and Cell Biology* **3,** 85–93.

Quail PH. 2002*b*. Photosensory perception and signalling in plant cells: new paradigms? *Current Opinion in Cell Biology* **14,** 180–188.

Reed JW, Nagatani A, Elich TD, Fagan M, Chory J. 1994. Phytochrome A and phytochrome B have overlapping but distinct functions in *Arabidopsis* development. *Plant Physiology* **104,** 1139–1149.

Sakamoto K, Nagatani A. 1996. Nuclear localization activity of phytochrome B. *The Plant Journal* **10,** 859–868.

Schäfer E, Nagy F. 2006. *Photomorphogenesis in plants and bacteria*. Dordrecht: Springer.

Seo HS, Yang J-Y, Ishikawa M, Bolle C, Ballesteros ML, Chua N-H. 2003. LAF1 ubiquitination by COP1 controls photomorphogenesis and is stimulated by SPA1. *Nature* **423,** 995–999.

Shen H, Moon J, Huq E. 2005. PIF1 is regulated by light-mediated degradation through the ubiquitin–26S proteasome pathway to optimize photomorphogenesis of seedlings in Arabidopsis. *The Plant Journal* **44,** 1023–1035.

Shimizu-Sato S, Huq E, Tepperman JM, Quail PH. 2002. A light-switchable gene promoter system. *Nature Biotechnology* **20,** 1041–1044.

Shin J, Park E, Choi G. 2007. PIF3 regulates anthocyanin biosynthesis in an HY5-dependent manner with both factors directly binding anthocyanin biosynthetic gene promoters in Arabidopsis. *The Plant Journal* **49,** 981–994.

Soh MS, Kim YM, Han SJ, Song PS. 2000. REP1, a basic helix–loop–helix protein, is required for a branch pathway of phytochrome A signaling in Arabidopsis. *The Plant Cell* **12,** 2061–2074.

Tepperman JM, Hudson ME, Khanna R, Zhu T, Chang H-S, Wang X, Quail PH. 2004. Expression profiling of *phyB* mutant demonstrates substantial contribution of other phytochromes to red-light-regulated gene expression during seedling de-etiolation. *The Plant Journal* **38,** 725–739.

Tepperman JM, Hwang Y-S, Quail PH. 2006. phyA dominates in transduction of red-light signals to rapidly responding genes at the initiation of Arabidopsis seedling de-etiolation. *The Plant Journal* **48,** 728–742.

Tepperman JM, Zhu T, Chang H-S, Wang X, Quail PH. 2001. Multiple transcription-factor genes are early targets of phytochrome A signaling. *Proceedings of the National Academy of Sciences, USA* **98,** 9437–9442.

Toledo-Ortiz G, Huq E, Quail PH. 2003. The Arabidopsis basic/helix–loop–helix transcription factor family. *The Plant Cell* **15,** 1749–1770.

Tu S-L, Lagarias CJ. 2005. The phytochromes. In: Briggs WR, Spudich JL, eds. *Handbook of photosensory receptors*. Weinheim: Wiley-VCH, 121–150.

Wagner D, Koloszvari M, Quail PH. 1996. Two small spatially distinct regions of phytochrome B are required for efficient signaling rates. *The Plant Cell* **8,** 859–871.

Wang H, Deng XW. 2002. Phytochrome signaling mechanism. In: Somerville CR, Meyerowitz EM, eds. *The* Arabidopsis *book*. American Society of Plant Biologists, Rockville, MD: doi: 10.1199/tab.0074.1, http://www.aspb.org/publications/arabidopsis/.

Wester L, Somers DE, Clack T, Sharrock RA. 1994. Transgenic complementation of the *hy3* phytochrome B mutation and response to *PHYB* gene copy number in *Arabidopsis*. *The Plant Journal* **5,** 261–272.

Yamaguchi R, Nakamura M, Mochizuki N, Kay SA, Nagatani A. 1999. Light-dependent translocation of a phytochrome B–GFP fusion protein to the nucleus in transgenic Arabidopsis. *Journal of Cell Biology* **145,** 437–445.

Yamashino T, Matsushika A, Fujimori T, Sato S, Kato T, Tabata S, Mizuno T. 2003. A link between circadian-controlled bHLH factors and the APRR1/TOC1 quintet in *Arabidopsis thaliana*. *Plant and Cell Physiology* **44,** 619–629.

Yang J, Lin R, Sullivan J, Hoecker U, Liu B, Xu L, Deng XW, Wang H. 2005. Light regulates COP1-mediated degradation of HFR1, a transcription factor essential for light signaling in Arabidopsis. *The Plant Cell* **17,** 804–821.

Zhu Y, Tepperman JM, Fairchild CD, Quail PH. 2000. Phytochrome B binds with greater apparent affinity than phytochrome A to the basic helix–loop–helix factor PIF3 in a reaction requiring the PAS domain of PIF3. *Proceedings of the National Academy of Sciences, USA* **97,** 13419–13424.