The phytochrome family of photoreceptors (there are five phytochromes in Arabidopsis, named phyA to phyE) maximally absorbs red and far-red light and plays important functions throughout the life cycle of plants. Several recent studies have shown that multiple related bHLH (basic helix–loop–helix) class transcription factors play key roles in phytochrome signal transduction. Somewhat surprisingly these transcription factors primarily act as negative regulators of phytochrome signalling. Moreover, in some cases, the phytochromes inhibit those negative regulators.

Phytochrome signalling

To grow and develop optimally, plants must constantly monitor and respond to fluctuations in the ambient light conditions. Several classes of photoreceptors enable them to sense light ranging from UV-B to far-red [1]. The UV-A–blue light-responsive cryptochromes (cry) and phototropins (phot), as well as the phytochromes (phy), are present as small gene families in all higher plants. These different photoreceptors control various facets of the photomorphogenic response, including seed germination, seedling de-etiolation, shade avoidance and photoperiodic induction of flowering [1–3].

Phytochromes exist in two interconvertible conformers: Pr maximally absorbing red light that upon photon capture is converted into Pfr (maximally absorbing far-red light) [2]. Pfr is considered to be the active form for most phytochrome responses. The Pr-to-Pfr phototransformation can act as a switch inducing a given response (e.g. seed germination) but phytochrome-mediated responses are often gradual with the Pr:Pfr ratio defining (e.g. seedling) but phytochrome-mediated responses are often gradual with the Pr:Pfr ratio defining (e.g. seedling) but phytochrome-mediated responses are often gradual with the Pr:Pfr ratio defining the extent of the response (e.g. shade avoidance response) [1,2,4]. Among the phytochromes, the roles of phyA and phyB are the most prominent. In several instances, phyC, phyD and phyE act in conjunction with phyB but phyC, phyD and phyE single mutants do not have any obvious phenotypes [3].

The phytochromes are cytoplasmic in the dark (in their Pr conformation) and light triggers translocation of an important fraction of these light sensors into the nucleus [5]. Nuclear translocation of phyB is essential for signalling, although this does not preclude possible phytochrome functions outside of the nucleus as well [5]. Light also induces rapid phytochrome-dependent changes in gene expression [2]. The exact mechanism involved is unknown but transcription factors from distinct families are required for phytochrome signalling [2]. Members of the bHLH (basic helix–loop–helix) family appear to be particularly important because several of them specifically interact with the light-activated phytochrome (Pfr) [2]. The model derived from early studies was mainly based on analyses of PIF3 (phytochrome-interacting factor 3); from these studies it was inferred that these bHLHs act as positive regulators of the pathway by inducing light-regulated genes [2]. The model was mainly based on the fact that in vitro phytochrome in the Pfr form can bind to DNA-bound PIF3 and that PIF3 binds to G-box motifs that are present on many light-regulated genes. The reduced light response of PIF3 antisense lines supported this idea [2]. However, several recent publications now challenge this working hypothesis.

Several bHLH class transcription factors preferentially interact with the Pfr form of phytochrome

The Arabidopsis genome codes for > 150 putative bHLH class transcription factors [6]. Interestingly all the bHLH proteins involved in light signalling belong to a single evolutionarily related subclass (Figure 1) [6–8]. One outstanding feature of these proteins is that in vitro several of them preferentially interact with phytochrome in the Pfr conformation [9,10]. These bHLH proteins are known as PIF (phytochrome interacting factor) or PIL (phytochrome interacting factor-like) [10,11] (Figure 1). Some PIFs preferentially interact with phyB whereas others interact with equal affinity with both phyA and phyB [12,13]. The results from in vitro studies correlate with the function of these bHLH proteins in vivo (Figure 1). PIF1/PIL5 interacts with both phyA and phyB whereas PIF4 only significantly interacts with phyB [12–14]. These interaction data correlate with the phenotype of pif4 and pif1 mutants (see below) [12–14]. The PIF–phy interaction has not been demonstrated in vivo but a large body of evidence indicates that this interaction is biologically relevant [15]. In particular, the authors of a recent paper have shown that expressing a PIF4 mutant that can no longer interact with phyB does not complement the pif4 phenotype [10].

These bHLH class proteins have closely related bHLH domains and most of them carry a small conserved N-terminal domain [7,10,11]. It was recently demonstrated that this domain, dubbed APB (active
Regulation of the PIFs

The mRNA levels of PIL1, PIL2, PIF4 and PIL6 are under circadian control and several members of the PIF family interact with the circadian oscillator component TOC1
(timing of CAB 1) in vitro [11,19,21]. The functional significance of this interaction remains unknown given that no circadian phenotype has been detected for pif3 and pif6 mutants [19,20]. However, additional studies are required in light of the recent discovery that the shade-avoidance response is under circadian control and requires PIL1 [21].

PIF3 mRNA accumulation is not under light regulation but the protein abundance is [15,20,22]. The protein is stable in the dark and destabilized in the light [15,20,22]. This destabilization is mediated by the 26S proteasome and promoted by light-activated phytochromes. The transient light-induced colocalization of PIF3 and the phytochromes in nuclear bodies suggests that the phytochromes target PIF3 to proteolysis [15,20,22]. The ubiquitin E3 ligase COP1 (constitutively photomorphogenic 1) has been shown to mediate 26S proteasome-dependent degradation of several proteins involved in light signalling [4]. However, COP1 positively regulates PIF3 accumulation in the dark [15].

HFR1 is a distantly related member of the PIF family, positively regulating phyA and cryptochrome signalling [23–25]. HFR1 is light regulated both at the transcriptional and at the protein-stability level [23–26]. However, in contrast to PIF3, HFR1 protein is highly unstable in the dark and stabilized by the light [26]. HFR1 destabilization is COP1-mediated and regulated by phosphorylation. Interestingly, in spite of the lack of evidence for HFR1–phytochrome interaction, the N-terminus of HFR1 that is related to the APB domain is a determinant of HFR1 stability [7,26]. It will be interesting to test if the APB domain of the other PIFs also controls their stability. Because the APB domain is important for the PIF–phytochrome interaction and the phytochromes control PIF3 degradation, the answer is likely to be yes [10,15,20,22].

How do the PIF proteins act in light signalling?
The overall picture that emerges from the study of pif mutants is that the different PIF proteins differentially affect various facets of photomorphogenesis (Figure 1). In most cases they act as negative regulators. The phenotype of dark-grown pif1 mutants is interesting because it indicates that PIF1 might have an activity before activation by the phytochromes [12,13]. The apparent absence of phenotype in some etiolated pif mutants might result from genetic redundancy. The strong inhibition of gravitropism in dark-grown pif1 pif3 double mutants backs up this idea [13]. Until recently it was assumed that the PIF proteins have to be activated by the phytochromes but recent studies indicate that this might not be an absolute requirement [2,12,13]. However, it is difficult to rule out phyB activation of PIFs completely even in etiolated seedlings because seeds contain phyB and have seen light before germination. This argument is unlikely for phyA, which is only synthesized once germination has started.

Upon conversion into Pfr the phytochromes negatively regulate PIF1 and PIF3 [12,13,15,20,22]. The mechanism involved is unknown for PIF1; PIF3 is down-regulated by phytochrome-induced degradation [15,20,22]. The phytochromes might therefore promote photomorphogenesis by inhibiting those negative regulators. However, some data do indicate that phytochromes are required to activate the PIFs, for instance, a PIF4 variant that cannot interact with phyB is inactive in vivo [10]. These seemingly contradictory results might be reconciled if phytochrome-mediated light activation simultaneously marks PIFs for degradation, a situation that can be compared to that of phyA [1,2,4]. The requirement for phytochrome activation might depend on the particular PIF and/or the gene that is being regulated. Phytochrome regulation of the PIFs does not have to work as a binary switch. The different light conditions experienced by the plant lead to a range of Pr:Pfr ratios resulting in a modulation of the PIF activity.

It is currently difficult to make a model that explains all the experimental data. The phenotype of several phy pif double mutants has not been reported and when the data are available the epistatic relationship between pif and phy mutants is complex. For instance phyA mutants are not epistatic to pif1 mutants under conditions where phyA is the only active photoreceptor [13]. By contrast, the phyB phenotype is epistatic to the one of pif4 [14]. These recent publications indicate that the mode of action of PIF proteins is more elaborate than previously anticipated and we expect further progress in the near future.

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Transcriptional networks in plants

Homing into the origin of the AP2 DNA binding domain

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The AP2 DNA binding domain was thought to be plant specific because of its presence in plant, but not animal, transcriptional regulators, particularly members of the AP2/ERF family. Two recent studies have identified the AP2 domain in bacteria, bacteriophage and a ciliate as part of proteins that also encode site-specific endonucleases. The association of AP2 with an enzyme known to catalyze its own movement within populations and between species explains the unusual distribution of AP2 and, as such, adds to a growing list of phenomena where mobile DNA has promoted evolutionary novelty.

Introduction

The cloning of the Arabidopsis APETALA2 (AP2) gene led to the surprising finding that it did not contain a MADS domain like that in previously isolated floral regulators. Instead, the AP2 protein harbored two copies of a 68 amino acid sequence that came to be known as the AP2 domain [1]. Soon after, this domain was recognized in four tobacco proteins where it was shown to be required for light signaling in Arabidopsis. Plant Cell 16, 1433–1445


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