NEW EMBO MEMBER'S REVIEW

Nuclear and cytosolic events of light-induced, phytochrome-regulated signaling in higher plants

Ferenc Nagy[1] and Eberhard Schäfer[2]

Institute of Plant Biology, Biological Research Center, PO Box 521, H-6701 Szeged, Hungary and [2]Universität Freiburg, Institut für Biologie II/Botanik, Schänzlestrasse 1, D-79104 Freiburg, Germany

[1]Corresponding author
e-mail: nagyf@nucleus.szbk.u-szeged.hu

Introduction

Plants as sessile organisms cannot walk away but have to adapt to changes in their natural environments. Light is obviously the most important external factor, being the energy source. To regulate their fitness, the photosynthetic capacity of plants must be adapted to changes in the ambient light environment. Therefore, plants have evolved a large set of photoreceptors to monitor light quality and quantity, ranging from the UV to the infrared part of the spectrum. At least three different photoreceptor classes have been identified and analyzed: (i) UV-B receptors, primarily characterized by action spectroscopy (Wellmann, 1983); (ii) the blue UV-A photoreceptors, cry1 and cry2 and phototropine (Ahmad and Cashmore, 1993; Christie *et al.*, 1998; Lin *et al.*, 1998); and (iii) the red/far-red reversible phytochromes. These photoreceptors control different aspects of plant growth and development, and in this review we focus on the physiological and molecular events regulated by the most characterized plant photoreceptors, the phytochromes. In higher plants, phytochromes are encoded by a small multigene family; five genes (*PHYA–PHYE*, Sharrock and Quail, 1989; Clack *et al.*, 1994) have been identified in *Arabidopsis*. Phytochromes exist as dimers composed of two 125 kDa polypeptides, each carrying a covalently linked tetrapyrrol chromophore in the N-terminal domain and dimerization domains in the C-terminal domain. The photosensory function of the molecule is based on its capacity for reversible interconversion between the red light-absorbing Pr form and the far-red light absorbing Pfr form following sequential absorption of red and far-red light. Photosignal perception by the receptor is followed by conformational changes, and activates, through an as yet poorly understood mechanism, signaling pathways leading to changes in the expression of genes that underlie developmental responses to light (Quail *et al.*, 1995).

In order to finely tune their adaptation to regular dark–light cycles in the ambient light environment brought about by the Earth's rotation, plants, similarly to other eukaryotic and prokaryotic organisms, have evolved an endogenous biological clock. Therefore, the majority of light-regulated physiological responses are modulated with an ~24 h rhythm generated by the circadian clock. To provide information about the local time, circadian clocks are synchronized and entrained by environmental clues, of which the most important is light. Light-driven entrainment of the plant circadian clock has been shown by Somers *et al.* (1998) to be mediated by phytochromes (phyA and phyB) and by cryptochromes (CRY1 and CRY2). Thus photoreceptors have a dual role, i.e. inducing light-responsive gene expression and entraining the circadian clock, whereas the clock modulates phototransduction by the so-called gating phenomenon as shown by Millar *et al.* (1996). Moreover, we have shown that expression of the *PHYB* gene is regulated by the circadian clock and exhibits a robust circadian rhythm (Kozma-Bognar *et al.*, 1999). It follows that photoreceptor-controlled development should be discussed in that context, yet the scope and length of this review are not sufficient for such a complex approach.

It is well documented that light plays a major role throughout the entire life cycle of plants starting from germination, through seedling development and neighbor detection, to the regulation of flowering and senescence (Coupland, 1997; Whitelam and Devlin, 1997). On the one hand, these responses were shown to be light-dependent modulations of the transcription of specific genes and of enzyme activities. On the other hand, for decades until recently, the dominating view has been that plant photoreceptors are localized in the cytosol. Therefore, the central questions of photomorphogenesis (light-dependent development) for nearly two decades have been to address the function of the photoreceptors in the cytosol, the nature of communication between cytosol and nucleus and the mechanism of nuclear events. Our present knowledge of the components and molecular events identified for light-induced signaling is shown in Figure 2.

Signaling in the cytosol

Physiological studies in algae, mosses and ferns showed an action dichroism for chloroplast orientation, polarotropism and phototropism. These observations suggested an ordered localization of photoreceptors within the cytosol and were interpreted as indications that the photoreceptors regulating these responses are localized in or are at least associated with the plasma membrane in lower plants (Kraml, 1994).

Biochemical studies provided some compelling evidence for the cytosolic localization of phytochromes, cryptochromes and phototropine even in higher plants. For example, in the case of phytochromes, immunocytochemical localization assays demonstrated that the majority of phytochromes, both the Pr and Pfr forms, are associated with and localized in the cytosol in light- and/or dark-grown plants (Pratt, 1994). The view that the dominating part of phytochrome-controlled signaling

occurs in the cytosol was strengthened further, although indirectly, by observations derived from analyzing the mode of phytochrome-dependent signaling by using micro-injection techniques in a chromophore biosynthesis-deficient mutant of tomato, *aurea* (Neuhaus *et al.*, 1993; Bowler *et al.*, 1994). These experiments suggest that the phytochrome-controlled signaling cascade includes steps that affect levels of some of the well-known second messengers identified in other eukaryotic cells: namely, light absorption by phytochrome triggers activation of a trimeric GTP-binding protein that is followed by a bifurcated signal transduction pathway. One branch of this pathway modulates the cGMP level that leads to the induction of anthocyanin formation and the expression of chalcone synthase (CHS); the other branch regulates calmodulin/Ca^{2+} levels and promotes chloroplast development. Additional observations derived from these assays indicate cross-talk between these pathways and a cell-autonomous response to light. Pharmacological studies using various inhibitors supported this model of phytochrome signaling. However, identification of the putative target proteins necessary for the regulation of these molecular events is still awaited and nearly nothing is known about the potential contribution of these molecules to blue receptor-controlled signaling.

The assumption of the cytosolic localization of the photoreceptors and their undoubted role in controlling the transcription of specific plant genes invited a search for a molecular mechanism that mediates communication between cytosol and nucleus. In animal systems, the communication between cytosolic or membrane-localized receptors and the nucleus is often achieved by regulating, via different mechanisms, the nucleo/cytoplasmic partitioning of transcription factors controlling the expression of specific genes.

There is fragmented, circumstantial evidence that this type of regulation indeed plays a role in light-regulated transcription of plant genes. It has shown that, among other types of transcription factors, various members of the G-box-binding transcription factor family (GBF) do interact specifically with promoters of light-regulated genes (for a review, see Terzaghi and Cashmore, 1995). It appears that light-dependent partitioning of some of the GBFs between the cytosol and nucleus is regulated by phosphorylation (Harter *et al.*, 1994) and, at least in one case, a blue light-mediated partitioning of GBF2 between the cytosol and nucleus has been described (Terzaghi *et al.*, 1997). It was also demonstrated by immunocytochemical and transient expression studies in suspension culture cells that CPRF2, a member of the common promoter-binding transcription factor family (CPRF), is localized, in the dark, almost exclusively in the cytosol. The cytosolic retention of the protein is abolished by red light treatment (Kircher *et al.*, 1999b). As the nuclear translocation of CPRF2 is red/far-red reversible, the involvement of phytochrome in controlling the cytosolic/nuclear partitioning of this transcription factor is demonstrated. Although an N-terminal domain of the CPRF2 protein was identified as being responsible for the retention, and a rapid phytochrome-mediated phosphorylation was observed (Wellmer *et al.*, 1999), the mechanism controlling retention and release of this transcription factor is still far from being understood.

Light-regulated transcription in the nucleus

Parallel with the 'coming-of-age' of plant molecular biological techniques such as transformation and regeneration of plants, the pursuit to identify the terminal steps, specific *cis-* and *trans*-acting regulatory elements of light-induced signaling, began ~15 years ago and has continued ever since, although with limited success. During these years, it has become gradually accepted that the majority of *cis*-acting elements necessary and sufficient for light-regulated transcription of plant genes are commonly localized in the near proximity of transcription start sites (between −400 and −1). In general, it is likely that in order to achieve high level, regulated transcription, at least two but in some cases an even higher number of *cis*-regulatory elements must be present and act in cooperation. A number of *trans*-acting factors binding to these elements have been cloned and analyzed (for reviews, see Terzaghi and Cashmore, 1995; Fankhauser and Chory, 1997).

Unfortunately, nearly all of these transcription regulatory proteins, like the MYB, GBF and CPRF factors, are encoded by various members of large gene families (containing >100 members in the case of the *myb* family). This fact and the absence of a reliable *in vitro* transcription assay for light-regulated transcription of plant genes make the identification of the factor combination responsible for light regulation of a specific gene very time-consuming, if not impossible, at present.

Mutations affecting phytochrome signaling

The combinations of molecular and genetic approaches have proven to be helpful in addressing some other interesting aspects of photomorphogenesis. Seedling development in higher plants involves a choice between photomorphogenesis, which is the pathway followed under light conditions, and skotomorphogenesis, which is the pathway followed upon germination in darkness. The decision to follow one or the other pathway is reversible and controlled by photoreceptors. The majority of early genetic screens aimed at isolating mutants affecting light-regulated physiological responses fell into two categories. One type of these screens, first performed by Koorneef, was aimed at isolating mutants whose morphology, although grown in the light, resembles that of dark-grown plants. This and other similar screens resulted primarily in the isolation and characterization of genes that either control chromophore biosynthesis or encode the photoreceptors themselves (*hy1*, *cry1*, *cry2* and *phyA–E*). The other screening approach, pioneered by Chory and later by Deng, searched for plants exhibiting light-grown phenotypes, although the plants were grown in darkness. This search led to the isolation of the so-called *COP* (constitutive photomorphogenic) and *DET* (de-etiolated) mutants. Characterization of the *COP/DET* and later the *FUS* mutants revealed that the switch between photomorphogenesis and etiolation is regulated by a complex suppressor system that, in contrast to the photoreceptors, promotes the etiolation pathway by repressing photomorphogenesis in darkness.

More recently, it has been shown that *Arabidopsis* mutants defective in the biosynthesis of brassinosteroids

also exhibit partially de-etiolated phenotypes (Li *et al.*, 1996; Szekeres *et al.*, 1996), suggesting that this plant hormone plays an important role in orchestrating the etiolation response. However, the partial rescue of some severe *cop1* mutants by brassinosteroid treatment indicates that these two pathways are likely to be separate (Szekeres *et al.*, 1996).

All of the 10 identified *COP/DET/FUS* genes operate by directly or indirectly repressing the transcription of light-inducible genes (Misera *et al.*, 1994; Kwok *et al.*, 1995) and thereby repressing the default pathway of photomorphogenesis in darkness. Interestingly, the *COP/DET/FUS* genes seem to be evolutionarily conserved and their homologs have been detected recently in mammalian cells (Seeger *et al.*, 1998; Wei *et al.*, 1998). The COP1 protein, which plays an essential role in this repression, is localized in the nucleus in darkness and disappears after light treatment (von Arnim and Deng, 1994). By studying the intracellular distribution of various COP1–green fluorescent protein (GFP) fusion proteins, it has been shown recently that the COP1 protein contains a bipartite nuclear localization signal (NLS) and a cytoplasmic localization signal (CLS) motif, but the exact mechanism that ensures light-dependent partitioning of the COP1 protein still remains elusive (Stacey *et al.*, 1999).

How do COPs repress, and photoreceptors, most notably phytochromes, induce transcription of specific genes in a light/dark-dependent fashion? For the signal transduction pathway controlled by COP, only two genes encoding COP-interacting proteins have so far been cloned. These proteins are HY5, a basic zipper-type DNA-binding protein of nuclear localization mediating photoregulation by several photoreceptors (Ang *et al.*, 1998), and the putative transcriptional activator CIP7 (Yamamoto *et al.*, 1998). More interestingly, all genes so far identified and shown to affect phytochrome-regulated phototransduction, including *hy5*, also code for proteins that are localized in the nucleus. *SPA1*, a suppressor of a weak phyA mutant, encodes a nuclear protein, possibly a transcription factor (Hoecker *et al.*, 1999). The phytochrome-interacting protein PIF3, which appears to interact with both phyA and phyB, is a nuclear helix–loop–helix (HLH)-type protein and, similarly to phytochromes, it contains a PAS domain known to mediate protein–protein interaction (Ni *et al.*, 1998).

Novel features of phototransduction in the cytosol and nucleus

It was reported that the *Synecocystis* genome has an open reading frame encoding a prokaryotic phytochrome (Yeh *et al.*, 1997). This photoreceptor is homologous to the classical prokaryotic two-component histidine sensor kinase and is capable of transducing signals via phosphorelay (Yeh *et al.*, 1998). During evolution from prokaryotic to multicellular eukaryotic systems, the function of phytochromes has dramatically changed. This statement is supported by a set of articles published in the last 6 months that drastically changed our view of phytochrome-mediated phototransduction.

Phytochrome is an atypical serine/protein kinase
First, it was demonstrated that higher plant phytochromes also function as kinases. It has been shown that purified

oat phyA autophosphorylates and phosphorylates other proteins *in vivo* (Fankhauser *et al.*, 1999). However, in sharp contrast to prokaryotic phytochrome, phyA autophosphorylates on serine rather than on histidine residues. The level of phyA autophosphorylation is slightly higher in Pfr than Pr form, but it is not yet proven whether these forms autophosphorylate at identical residues. Moreover, the only known phytochrome substrate, PKS1 (phytochrome kinase substrate 1) is also phosphorylated on serine/threonine residues rather than aspartate. PKS1 is a novel protein, detected in plants in the phosphorylated form, but red light increases the level of PKS1 phosphorylation. Since overexpression of PKS1 results in long hypocotyls in red light, it is postulated that PKS1 negatively regulates phytochrome signaling. The PKS–GFP fusion protein is constitutively localized in the cytosol; therefore, it is likely that PKS1 interacts with phytochromes in the cytosol but its exact role in phototransduction remains to be seen.

Light controls interaction of phytochrome B with the transcription factor PIF3
Only very recently, Ni *et al.* (1999) reported that the nuclear localized basic HLH protein PIF3, shown earlier to function in phytochrome signaling *in vivo* (Ni *et al.*, 1998), binds to phyB in a light-regulated fashion *in vitro*. These authors found that PIF3 binding occurs only after red light-induced conversion of phyB into its biologically active Pfr form and it is abolished by reconversion of phyB by far-red light into its inactive Pr form. These observations suggest that the photosensory signaling by phyB occurs via light-induced, conformation-specific recognition of a putative transcriptional regulator, thereby providing a potential mechanism for direct photoregulation of gene expression.

Phytochromes are imported in the nucleus in a light-dependent fashion
It was generally believed until recently—based on physiological, biochemical, immunocytochemical and molecular analyses—that phytochromes are cytosolic proteins. It was entirely forgotten that MacKenzie *et al.* (1975) had originally described nuclear localization of oat phyA using immunocytochemical methods until results obtained by Sakamoto and Nagatani (1996) indicated a light-dependent nuclear localization of *Arabidopsis* phyB and phyB–GUS fusion proteins. More recently, the same laboratory used the phyB–GFP fusion protein to complement an *Arabidopsis* phyB-deficient mutant *in vivo* and thereby demonstrated that the phyB–GFP fusion protein is a functional photoreceptor and that nuclear translocation of the fusion protein is indeed inducible by red light (Yamaguchi *et al.*, 1999).

These findings were extended further by results reported by Kircher *et al.* (1999a). These authors studied partitioning of the tobacco phyB–GFP fusion protein in transgenic tobacco plants by overexpressing this fusion protein in a wild-type background or by complementing the phenotype of the phyB-deficient *Nicotiana plumbaginifolia hlg2* mutant (P.Gil, S.Kircher, E.Adam, E.Bury, L.Kozma-Bognar, E.Schäfer and F.Nagy, unpublished). The cytosolic localization of the *Arabidopsis* and tobacco phyB–GFP fusion proteins in dark-grown seedlings or dark-adapted
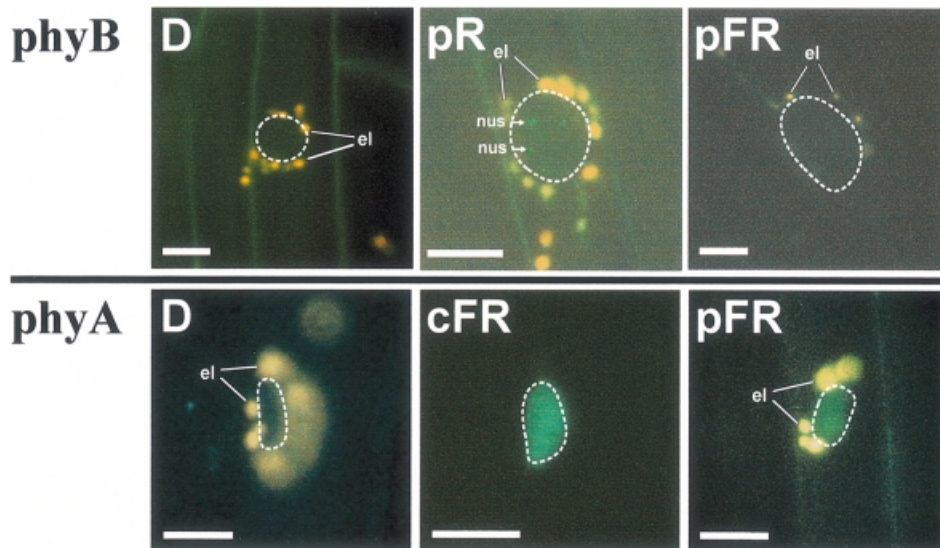
Fig. 1. Nuclear import of different phytochromes is regulated differentially by light quality and quantity. Phytochrome–GFP fusion proteins (phyA–GFP and phyB–GFP) are localized in the cytosol in the dark (D). Nuclear import of phyB–GFP is induced by repeated, short, 5 min pulses of red light (pR). It is completely reversible with a 5 min far-red light (pFR) treatment and not inducible by far-red light alone. In contrast, nuclear translocation of phyA–GFP can be induced either by continuous (cFR) or by a single 5 min pulse of far-red light illumination (pFR). Nuclei are encircled by dashed lines, selected etioplasts (el) are marked and phyB–GFP containing speckles (nus) are also indicated. Filled bars represent 10 μm.

light-grown plants and a red/far-red reversible induction of nuclear localization provided convincing evidence for the light-dependent nuclear transport of the photoreceptor phyB (see Figure 1). This view was supported further by the observation that a chromophore-deficient mutant of tobacco phyB is constitutively localized in the cytosol (Kircher *et al.*, 1999a). These authors also reported import of rice phyA–GFP into the nucleus. In contrast to phyB–GFP, this was induced by far-red light pulses; thus, no red/far-red light reversibility was observed. This means that the nuclear import of rice phyA is controlled by the very low fluence response (VFL), a hallmark of physiological responses regulated by phyA. Furthermore, it was shown (see also Figure 1) that the light-induced nuclear localization of the phyA photoreceptor is preceded by rapid cytosolic spot formation that is reminiscent of the previously described light-induced formation of sequestered areas of phytochrome in oat seedlings (Speth *et al.*, 1987). Using *Arabidopsis* phyA–GFP, nuclear localization in transgenic *Arabidopsis* seedlings could also be induced by continuous far-red light, classifying this reaction as an example of the extensively studied far-red light-related high irradiation response (HIR), shown to be mediated by phyA in dicotyledonous seedlings.

Interestingly, light-driven accumulation of phyB–GFP as well as phyA–GFP fusion proteins was accompanied by spot/speckle formation, although the number and size of these speckles were different for phyA and phyB. In the case of phyB–GFP, up to 25 speckles per nucleus were observed, similar to results reported recently for the COP1–GFP fusion (Stacey *et al.*, 1999).

Relatively fewer data are available about the subcellular localization of the three blue light receptors identified to date namely CRY1, CRY2 and NPH1. It is thought that the phototropin receptor NPH1 is localized in the cytosol, possibly in the plasma membrane (Huala *et al.*, 1997), while it has been reported that the CRY1–GFP fusion

protein is found in the nuclei in darkness (Cashmore *et al.*, 1999). Since all experimental tools are now available, a detailed photobiological characterization of the nuclear import of CRY1 and CRY2 photoreceptors is expected to occur in the near future.

Conclusions

Light can easily penetrate plant tissues; therefore, photoreceptors are found in all cells. In lower plants, the primary role of phytochromes is detection of the direction of light. These reactions involve primarily cytosolic events. The observed action dichroism of phytochrome-mediated responses in ferns, mosses and algae clearly points to a rigid cytosolic, probably plasma membrane-associated, localization of the photoreceptor. In the evolutionary development to gymnosperms and especially angiosperms, a new developmental strategy in darkness, skotomorphogenesis, has evolved. Whereas the sporophytes of algae, mosses and ferns show almost identical development in light or darkness, in angiosperms photomorphogenesis is suppressed in darkness. This is in agreement with the observation that mutations in genes of the *COP–DET–FUS* class lead to the default pathway of photomorphogenesis, i.e. de-etiolation in darkness. This light-mediated transition from skotomorphogenesis to photomorphogenesis needs phytochrome-mediated gene expression. Therefore, a switch from primarily cytosolic toward a more dominating nuclear function has evolved in the course of the evolution of gymnosperms and angiosperms. A hypothetical model illustrating the molecular events involved in phytochrome-regulated gene expression is shown in Figure 2.

Future prospects

Photoreceptors serve to monitor precisely the quantity, quality, direction and duration of light, whereas gene
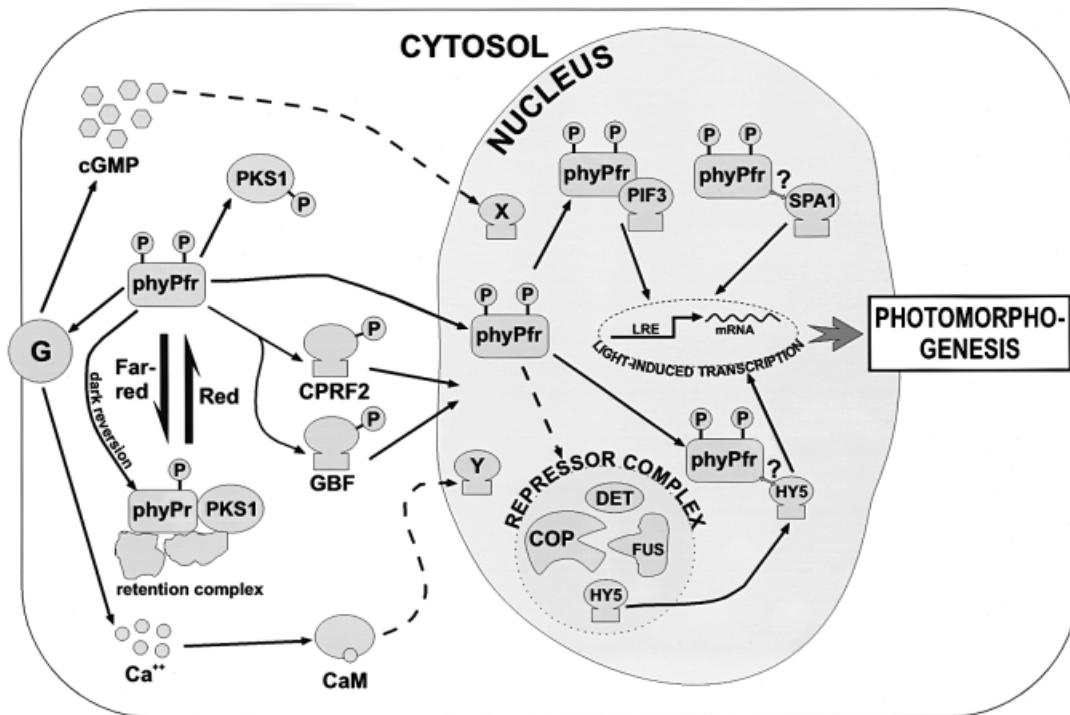
Fig. 2. Expression of genes mediating light-dependent growth, development and adaptation of higher plants is controlled by a complex regulatory network. Light absorption (red light) changes the conformation of phytochromes (phyPr to phyPfr) and triggers translocation of Pfr conformers of phyA–phyE to the nucleus. Light-induced conformation alterations are accompanied by autophosphorylation of the photoreceptor, and phosphorylation of PKS1 and other proteins such as the transcription factor CPRF2. Phosphorylation abolishes cytosolic retention of these proteins as well as that of the photoreceptors themselves (P; phosphorylated residues). Through an as yet unidentified mechanism, light absorption is also followed by activation of cholera toxin-sensitive heterotrimeric GTP-binding proteins (G) and leads to altered levels of cGMP and Ca^{2+}. Modulation of these second messengers then results in activation of transcription factors (X and Y), possible components of transcription complexes that are required for high level, light-dependent expression of various genes. Phytochrome imported into the nucleus in the Pfr form recruits transcription factors such as PIF3 and induces transcription of light-regulated genes after interacting with the light regulatory *cis*-acting elements (LRE) of the target genes. Recruitment of transcription factors (such as HY5) required to form an active transcription complex can also be achieved by disassembling the inhibitory COP–DET complex. Irradiation with far-red light inactivates phytochrome by changing its conformation back to Pr. Inactivation is followed by disassembling the active transcription complexes, and phytochrome is degraded and/or exported out of the nuclei. The levels of activated phytochrome (Pfr) are fine tuned by dark reversion (Pfr to Pr) and by interactions of other signaling pathways. Dashed lines and question marks indicate hypothetical steps.

expression is regulated differentially by the different photoreceptors. Phytochromes are atypical serine/threonine kinases that translocate into the nuclei, interact with transcription regulators in a light-dependent fashion but also pass signals along phototransduction pathways to modulate Ca^{2+} and cGMP levels and regulate partitioning of specific transcription factors. It will be a challenging task to separate the nuclear and cytosolic events and determine the number and functional relationships of various regulatory circuits associated with light-regulated growth and adaptation.

At present, the physiological function of phytochromes in the cytosol is poorly understood. Although detection of light direction in higher plants (phototropism) is mediated by a specialized, plasma membrane-associated blue light receptor (Huala *et al.*, 1997), the sensitivity of this receptor is strongly controlled by phytochromes (Lasceve *et al.*, 1999). In addition, there are data indicating that phyA phosphorylates not only PKS1 but also the blue light receptor CRY1 (Ahmad *et al.*, 1998). These phenomena possibly can be interpreted as an evolutionary remnant of the cytosolic functions of phytochromes in lower plants.

A better characterized cytosolic function of phytochromes and cryptochromes is to regulate light-dependent partitioning of transcription factors such as CPRF2 and

GBF2, respectively. Although the molecular details of these processes are not yet known, it is predictable that signal transduction pathways abolishing cytosolic retention of specific transcription factors following dark to light transition will be found.

The light-dependent nuclear import of phytochromes is so far unique to higher plants. All phytochromes tested so far show light-dependent nuclear localization, although with strikingly different features as far as light quality and quantity and the kinetics of translocation are concerned (Kircher *et al.*, 1999a). As a result, the induction of nuclear localization of different phytochrome species reflects the fluence and wavelength requirements described previously with regard to the differential roles of the individual members of the phytochrome photoreceptor family. Therefore, it is tempting to conclude that molecular events involved in determining the specificity of phytochrome-controlled responses occur mainly in the cytosol rather than in the nucleus.

After nuclear transport of the phytochrome–GFP fusion proteins, spots are formed in the nuclei, similar to those described for COP1 in darkness. Biochemical data show interaction of phyA and phyB with possible transcription factors such as PIF3. It might be interesting, therefore, to speculate that a light-dependent nuclear transport of

phytochromes displaces COP1 and a possible COP9 complex from their site of function; an event followed by phytochrome-regulated recruitment of various factors involved in controlling transcription of specific genes. The challenging question in this context is to determine how specificity is ensured in the course of this process.

At present, we assume that a signal transduction cascade(s) exists, utilizing the kinase activity of phytochromes, to bring about differential light-dependent nuclear import/retention of various phytochromes. This cascade functions as a regulatory circuit (cytosolic) to achieve specificity. Data presently available seem to support a model which predicts that differences established in the cytosol are then amplified specifically within the nucleus by another regulatory circuit that is based on the differences in the capacity of various phytochromes to recruit interacting transcription regulatory proteins. Fine tuning can then be achieved by additional regulatory circuits that also affect partitioning of photoreceptors, e.g. by the circadian system and/or by regulating their turnover rates via cross-talking to signaling pathways controlled by other factors such as hormones, sugar metabolism or stress.

Acknowledgements

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