

REVIEW

Light: an indicator of time and place

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To grow and develop optimally, all organisms need to perceive and process information from both their biotic and abiotic surroundings. A particularly important environmental cue is light, to which organisms respond in many different ways. The responses can be simple, as in phototactic single-celled organisms, or complex, as in higher animals, which use visual inputs to modify their behavior. Many organisms can also detect cycles of light and darkness, which are exploited for seasonal and time-of-day responses.

Because they are both photosynthetic and sessile, plants have to be especially plastic in response to their light environment. In addition to utilizing light as a time-keeping mechanism, plants are unique in that they use light as a source of energy and they analyze light to control such developmental decisions as when to germinate and flower. The diverse responses of plants require sophisticated sensing of intensity, direction, duration, and wavelength of light. The action spectra of light responses have provided assays to identify three photoreceptors absorbing in the ultraviolet, blue/near ultraviolet, and red/far-red (R/FR)-spectral ranges (Kendrick and Kronenberg 1994). The best characterized group of these photoreceptors is the red/far-red (R/FR) absorbing phytochromes. Phytochromes are photochromic proteins composed of a large protein (~125 kD) covalently attached to a linear tetrapyrrole chromophore. Phytochromes are synthesized in a red-light-absorbing form, Pr (λ_{\max} = 660 nm) which, upon exposure to red light, can be phototransformed into a far-red-light-absorbing form, Pfr (λ_{\max} = 730 nm). Upon exposure to far-red light, Pfr is photoconverted to Pr (Kendrick and Kronenberg 1994). Both Pfr and Pr that has been photocycled have been shown to induce developmental responses (Shinomura et al 2000). Thus, phytochrome acts as a light-controlled developmental switch. Phytochromes have been found in all taxa of lower and higher plants examined (Mathews and Sharrock 1997; Mathews and Donoghue 1999), as well as cyanobacteria (Hughes et al. 1997; Lamparter et al. 1997; Yeh and Lagarias 1997).

Phytochromes control development throughout the

plant life cycle beginning with seed germination and seedling deetiolation (the transition from growth in the dark to growth in the light). They also control cotyledon/leaf expansion and stem elongation by regulating cell division and expansion. Phytochromes enable the perception of neighboring plants or shade, and influence the transition to flowering. Because of these profound effects, phytochromes have been studied intensively for >50 years. The impact this family of photoreceptors has had on the history of Botany is extensively covered in the book, *Pigment of the Imagination* (Sage 1992). Many excellent reviews on phytochrome's role in photomorphogenesis have been written recently (Mancinelli 1994; Smith 1995; Fankhauser and Chory 1997; Mustilli and Bowler 1997; Batschauer 1999; Deng and Quail 1999, and references within). In this review we will describe the complexities of phytochrome response pathways and highlight some of the recent accomplishments in elucidating the mechanisms by which phytochromes regulate so many downstream responses. Finally, we will examine the interactions between phytochrome and endogenous developmental programs.

Phytochrome responses

One of the major goals in phytochrome research has been to understand the signal transduction pathways that lead to altered development. Early in the twentieth century it was shown that a pigment, separate from the activity of photosynthesis, was involved in photoperiod detection and floral induction (Garner and Allard 1920), although the nature of this pigment was not discovered until 30 years later. In the 1950's, phytochrome was characterized as the pigment that controls lettuce (c.v. Grand Rapids) seed germination in red and far-red light (Borthwick et al. 1952). This developmental response is a classic example of phytochrome's control of plant development and is still used today in undergraduate lab courses. Red light stimulates seed germination, but this induction can be inhibited by subsequent exposure to far-red light. The seeds can be cycled through sequential red or far-red light treatments; however, the final germination response is determined solely by the last light treatment. This experiment defined the parameters for purification of a dichromic photoreceptor that was later termed phytochrome for "plant color". Reciprocity, the

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dependence on the total number of photons irrespective of the duration of exposure, is exhibited in this response, indicating the involvement of a first-order chemical reaction. Red/far-red reversibility and reciprocity are the hallmarks of the classic phytochrome responses. This class of phytochrome responses is known as the low fluence responses (LFRs) and has been described in many different plant systems (Mancinelli 1994).

In addition to the control of lettuce seed germination, LFRs also include responses that are transient in nature, such as changes in ion flux, leaf movement, and chloroplast rotation (Haupt and Hader 1994; Roux 1994). Other LFRs include changes in gene expression during deetiolation, stem elongation, leaf expansion, and the transition to flowering (Cosgrove 1994; Vince-Prue 1994). It is important to note that although these responses have been identified as being induced by red light and inhibited by far-red light, plants are not generally exposed to simple monochromatic light. In reality, plants sense the R/FR ratio of light in their surroundings and respond accordingly (Fig. 1). Two subsets of LFRs are the end-of-day far-red responses and shade avoidance responses. By detecting the enrichment of far-red light at dusk, the former affects flowering. (Fig. 1B). Shade avoidance is a response to enrichment of far-red light under a leaf-canopy or to reflected light from nearby leaves, and is a mechanism for neighbor detection. In both cases, plants respond by elongating stems and increasing the length-to-width ratio of leaves (Fig. 1C). Because the changes in plant morphology are similar in each case, these responses are often considered together as a single phenomenon. Both responses are controlled by the ratio of R/FR and are thought to be one of the most important functions of phytochrome in natural settings (Smith 1994; Smith and Whitelam 1997).

As more phytochrome-mediated processes were discovered, it became clear that there are at least three physiologically distinct modes of phytochrome action. In addition to the R/FR reversible LFRs, there are two other responses: the very-low-fluence responses (VLFRs) and high-irradiance responses (HIRs). These three response modes can be distinguished by the amount of light required, which varies over eight orders of magnitude. In a typical VLFR, plants respond to between 0.1 and 1 $\mu\text{mole}/\text{m}^2$ of light, whereas the LFRs are typically between 1 and 1000 $\mu\text{mole}/\text{m}^2$ of light. In the HIRs, plants generally respond to >1000 $\mu\text{moles}/\text{m}^2$ of light although it is fluence rate and not total fluence defining these responses (Mancinelli 1994). Reciprocity is not seen for either the VLFRs or the HIRs. Moreover, unlike the LFRs, VLFRs are not reversible by a subsequent pulse of far-red light. Seed germination in response to very low levels of light is a good example of a VLFR (Shinomura et al. 1996), as are some light-induced changes in gene expression (Cerdan et al. 1997, 1999; Hamazato et al. 1997).

In contrast to the LFRs, HIRs are prevalent primarily in far-red light, even though these conditions should create the presumed inactive form of phytochrome. A well-studied far-red HIR is the control of seed germination in

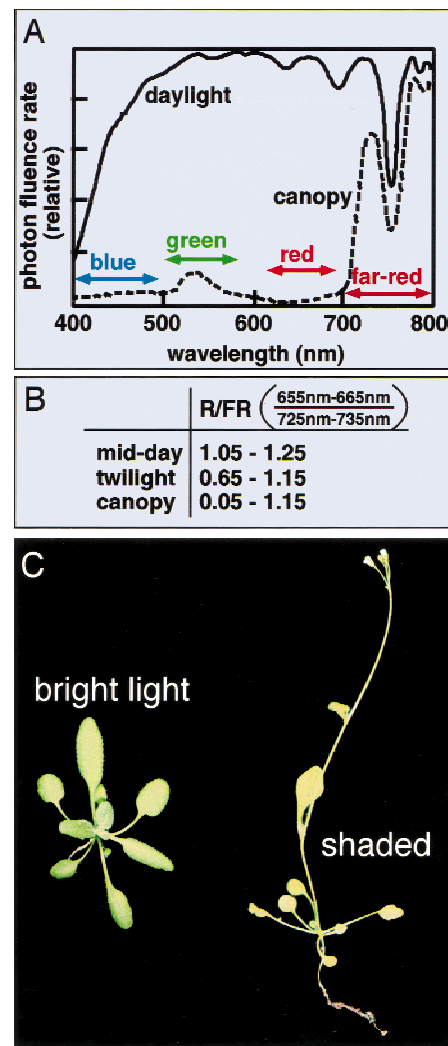


Figure 1. The ratio of R/FR light is a good indicator of time and place. (A) Light spectra of daylight and under a plant canopy. (B) R/FR ratios in different times and places. (C) Phenotype of 3-week-old *Arabidopsis* wild-type (left) and a *phyB* mutant displaying constitutive shade avoidance (right). Both plants were grown under the same light conditions.

certain plant species. For instance, after imbibition, some *Arabidopsis* seeds germinate in the dark without an inductive light pulse. This background germination rate varies with the age of seeds and parental growth conditions. Depending on this background germination rate, continuous far-red light can stimulate or inhibit seed germination (Reed et al. 1994; Shinomura et al. 1994). These observations suggest a biological activity for Pr. Because phytochrome null mutants were used in these experiments, it is also possible that multiple pools of phytochromes are acting antagonistically in wild-type seedlings (Whitelam and Devlin 1997). Recent studies from the Furuya laboratory suggest that the far-red HIR requires a short-lived intermediate generated during Pfr to Pr photoconversion (Shinomura et al. 2000). Most interestingly, this response is FR/R reversible (not R/FR),

which is opposite to what is seen in the LFRs such as far-red inhibition of light-regulated gene expression or lettuce seed germination.

Multiple phytochromes contribute to plant development

The attribution of the variety of light-regulated responses to a single photoreceptor was one of the problems with early phytochrome research. In the 1980's, spectrophotometric studies indicated that there were at least two distinct pools of phytochrome, type I (light labile) and type II (light stable). The light-labile pool degrades fairly rapidly (as fast as a 1-hr half-life, depending on the plant) upon exposure to red or white light. Although there was little spectrophotometrically detectable phytochrome after prolonged exposure to light, R/FR responses still persisted. That this stable phytochrome pool had biological activity was demonstrated in seed germination and end-of-day far-red response experiments where the escape times for far-red reversibility were three to four times longer than the degradation times for light-labile phytochrome (for review, see Mancinelli 1994).

The cloning of multiple phytochrome apoprotein genes has shed some light on the distinct pools and modes of action of phytochromes (Abe et al. 1989; Sharrock and Quail 1989). In *Arabidopsis*, we now know that there are five distinct phytochromes termed phyA–phyE (Sharrock and Quail 1989; Mathews and Sharrock 1997). phyA is a type I phytochrome. phyB–phyE are all type II phytochromes. In dark-grown tissues, phyA is by far the most abundant phytochrome. After exposure to light, the levels of phyA drop up to 100 fold. Degradation of phyA is light dependent and requires selective recognition and ubiquitination of Pfr (Clough et al. 1999). *PHYA* gene expression is also negatively regulated by light (Somers and Quail 1995a). This repression is rapid, occurs at the transcriptional level, and requires phytochrome (Lissemore and Quail 1988). The regulation of phyA protein level by light is therefore the result of a coordinated transcriptional and post-translational regulation. In light-grown plants, phyB becomes the most abundant phytochrome; phyC–phyE are less abundant type II phytochromes (Clack et al. 1994; Hirschfeld et al. 1998). All five phytochromes are expressed throughout the plant with only minor differences in their expression patterns (Somers and Quail 1995b; Goosey et al. 1997).

Although the presence of multiple phytochromes begins to address the light labile and light stable pools, assigning precise roles in development for each of these photoreceptors required genetic analysis of plants lacking one or more of these pigments. Phytochrome apoprotein mutants have allowed an assessment of the function of individual photoreceptors. phyA and phyB play unique, redundant, or antagonistic roles in different responses throughout *Arabidopsis* development (Whitelam and Devlin 1997; Cerdà et al. 1999). phyA is essential for de-etiolation in far-red light (light found under a canopy of plants), whereas phyB is the major red light

photoreceptor during seedling development. phyA also mediates responses to very low fluences of blue, red, and far-red light. Mutants in *phyD* and *phyE* have more subtle phenotypes, only uncovered in a *phyB* mutant background, demonstrating a degree of redundancy between phyB, phyD, and phyE, with phyB playing the most prominent role of the three (Aukerman et al. 1997; Devlin et al. 1998, 1999). These three phytochromes play a major role in regulating shade avoidance. No mutations in *phyC* have been discovered yet, but overexpression studies suggest a role in primary leaf expansion (Halliday et al. 1997; Qin et al. 1997).

In-depth analysis of plants carrying null mutations for different phytochromes has shown that the major phytochrome in *Arabidopsis* is phyB. Mutations in *PHYB* have profound effects on plant development throughout the life cycle (Reed et al. 1993). In general, *phyB* mutant seedlings have long hypocotyls and small cotyledons in continuous red or white light. They also have less anthocyanin, chlorophyll, and fewer chloroplasts than the wild type. As adults, these mutants flower early, have longer petioles and stems, and increased apical dominance. These phenotypes are also observed in mutants with reduced phyB activity from other plant species such as cucumber, pea, tomato, and rape (Whitelam and Devlin 1997). Many of the growth responses regulated by phyB involve cell expansion or elongation. phyB affects nuclear endoreduplication in hypocotyls of *Arabidopsis* (Gendreau et al. 1998), a possible mode of control of cell size (Gendreau et al. 1997). Generally speaking, *phyB* mutants show constitutive shade avoidance and are altered in their end-of-day far-red response (Fig. 1C), indicating that it is primarily phyB mediating this process. However, *phyB* single mutants and *phyA phyB* double mutants still show responses to reductions in R/FR ratios indicating that other phytochromes play significant roles in plant development (Whitelam and Smith 1991; Robson et al. 1993; Halliday et al. 1994; Devlin et al. 1996). This observation was the basis for the genetic screen that identified *phyE* mutants (Devlin et al. 1998).

Genetic analysis has shown that there is a complex web of interactions, not only between the phytochromes, but also between phytochromes and the blue light photoreceptors, cryptochromes. A functional dependency of cryptochrome 1 (*cry1*) on phytochromes has been described based on observations that *phyA phyB* double mutants have a dramatic blue light phenotype (Ahmad and Cashmore 1997). However, phytochromes can absorb blue light (Furuya and Song 1994). Furthermore, phytochrome mutants have blue light-mediated defects in hypocotyl growth inhibition (Whitelam et al. 1993), cotyledon expansion (Neff and Van Volkenburgh 1994), seed germination (Shinomura et al. 1996), *CAB* gene induction (Hamazato et al. 1997), and light-induced shrinking of hypocotyl protoplasts (Wang and Iino 1998). Detailed analysis of plants carrying null mutations in multiple phytochromes and cryptochrome argues against a functional dependency of cryptochromes on phytochromes. Rather, these studies demonstrate a complex web of interactions within and between the two

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classes of photoreceptors including redundancy, antagonism, and effector/modulator relationships (Casal and Boccalandro 1995; Casal and Mazzella 1998; Neff and Chory 1998; Wang and Iino 1998; Hennig et al. 1999; Mockler et al. 1999). However, the mechanisms of these interactions are not clear and are subject to debate (Ahmad 1999).

Effector/modulator relationships describe a situation in which a photoreceptor cannot control a growth response independently yet it can affect that response in the presence of other, controlling, photoreceptors (Mohr 1994). As an example, phototropism is controlled by the blue light-absorbing phototropins, such as NPH1 (Christie et al. 1998), although phytochromes can modulate this response (Parks et al. 1996; Hangarter 1997; Janoudi et al. 1997). Because pretreatments of omnilateral red light can enhance the phototropic response to subsequent exposures of unilateral blue, phytochromes can also act as preprogrammed amplifiers of this phototropin-mediated growth response (Shropshire and Mohr 1970; Woitzik and Mohr 1988). A novel photoreceptor with homology to both phytochrome and NPH1 has recently been isolated from the fern *Adiantum* (Nozue et al. 1998). In this case, the coaction between blue and red light on phototropism of *Adiantum* protonema (Hayami et al. 1986) may be acting through a single photoreceptor.

Molecular properties of phytochrome

One of the major advances made in phytochrome research was the ability to partially purify a species of the pigment, allowing the study of its biochemical properties (Butler et al. 1959, 1964). Because of the pioneering studies of Butler, full-length phyA holoprotein has been purified from multiple plant species. phyA is found as a soluble homodimer with each monomer covalently attached to a linear tetrapyrrole chromophore. Each mono-

mer is composed of two domains separated by a small hinge. The spectral properties of phyA have been well characterized because of the relative abundance of this photoreceptor in dark-grown seedlings (Fig. 2A). The cloning of less abundant phytochromes has allowed a comparison between the different family members. When overexpressed in *Arabidopsis*, phyB displays similar spectral properties to phyA (Wagner et al. 1991). Recombinant phytochromes purified from a variety of sources confirm this view (Gartner et al. 1996; Kunkel et al. 1996; Elich and Chory 1997). However, the use of more sophisticated spectroscopic methods uncovered subtle differences between phyA and phyB, which may account for variation in photoperception in vivo (Remberg et al. 1998).

The chromophore is attached to an invariant cysteine in a well-conserved domain among phytochromes. Light induces an isomerization between rings C and D of the tetrapyrrole, accounting for the interchangeable spectral properties of phytochrome. This Pr-to-Pfr transition is accompanied by rearrangements of the protein backbone (Quail 1997). In plants the chromophore is phytochromobilin (P Φ B), but phycocyanobilin (PCB) will also bind phytochrome resulting in Pr and Pfr spectra that are slightly blue shifted compared with the P Φ B adducts (Lagarias and Rapoport 1980). Heme is the starting point for chromophore biosynthesis. Heme oxygenase (HY1 in *Arabidopsis*) converts heme to biliverdin IX α , which is then converted into 3E-P Φ B by P Φ B synthase (Davis et al. 1999; Muramoto et al. 1999). The final step of chromophore biosynthesis involves an isomerization leading to 3Z-P Φ B. Mutants in a number of plant species are incapable of synthesizing 3E-P Φ B but the gene encoding P Φ B synthase has not been cloned yet (Terry 1997).

The amino-terminal part of phytochromes is necessary and sufficient for chromophore binding and normal spectral properties. The carboxy-terminal half can be re-

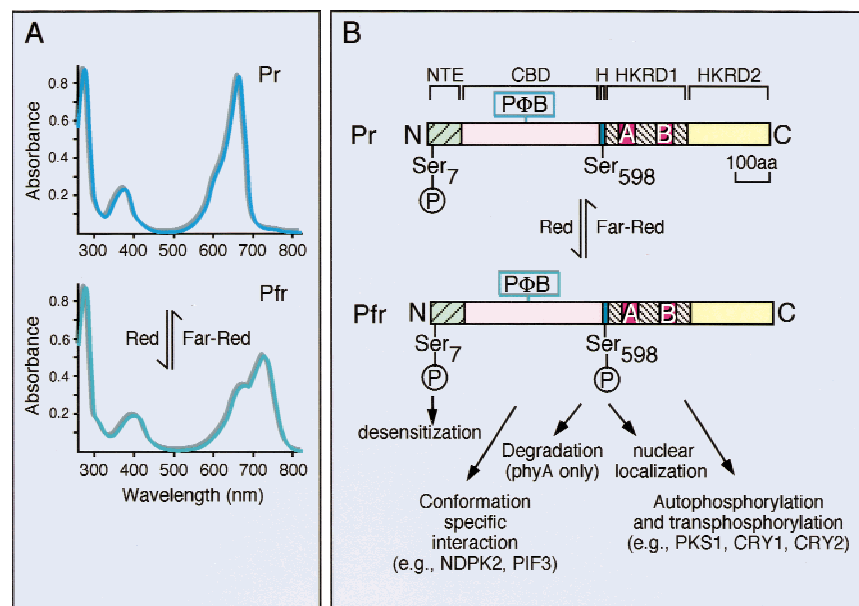


Figure 2. Phytochrome properties are modified by light. (A) Absorption spectra of purified oat phyA in its Pr and Pfr forms. (B) Schematic representation of phytochrome in Pr and Pfr. (NTE) Amino-terminal extension; (CBD) chromophore binding domain; (P Φ B) phytochromobilin; (H) hinge region; (HKRD1) histidine kinase-related domain 1; (HKRD2) histidine kinase domain 2; (A,B) PAS domains. A number of phytochrome properties affected by light are indicated. Whereas phyB translocates into the nucleus as Pfr, phyA becomes nuclear as both Pfr and photocycled Pr.

garded as the output domain. This domain appears to be the result of the duplication of a bacterial histidine kinase-related domain (Schneider-Poetsch 1992; Yeh and Lagarias 1998) (Figure 2B). The first of those domains also contains two repeats with homology to PER-ARN-T-SIM (PAS) domains (Lagarias et al. 1995; Kay 1997), originally found in basic helix-loop-helix (bHLH) containing transcription factors from fly (PER and SIM) and mammals (ARNT and AHR) (Huang et al. 1993). These modules have been found in a wide variety of organisms and play important signaling roles in protein-protein interactions, response to small ligands, and changes in light conditions, oxygen levels, and redox potential (Taylor and Zhulin 1999). The majority of missense mutations in phyA and phyB cluster in the PAS repeats, demonstrating the importance of this domain for phytochrome function (Quail et al. 1995). One of the *phyB* missense mutations, *phyB-101* (Bradley et al. 1996), is in the second PAS repeat. This mutation affects spectral properties of the pigment, causing accelerated dark reversion from Pfr to Pr, and alters the end-of-day far-red response in seedlings (Elich and Chory 1997). Thus, the PAS repeats may be involved in intramolecular interactions within phytochrome itself.

The discovery of phytochromes in cyanobacteria demonstrates that these proteins are not unique to plants (Kehoe and Grossman 1996). *Synechocystis* Cph1 (Cyanobacterial phytochrome 1) has spectral properties very similar to the ones of its higher plant relatives (Hughes et al. 1997). Moreover, Cph1 functions as a light-regulated histidine kinase (Yeh et al. 1997) although the biological function of Cph1 in *Synechocystis* is still unknown. Studies involving prokaryotic phytochromes strongly suggest that plant phytochromes have histidine kinase ancestry. The group of Lagarias has recently shown that two plant phytochromes are also light and chromophore-regulated protein kinases, but unlike their cyanobacterial counterparts they autophosphorylate on serine/threonine rather than histidine/aspartate (Yeh and Lagarias 1998). Phytochromes are not the first eukaryotic serine/threonine kinases with histidine kinase ancestry (Harris et al. 1997), and much remains to be done to characterize this enzymatic activity. Some important questions to be answered include: What is the catalytic domain of phytochrome kinase? Do different phytochromes, which play distinct roles in vivo, phosphorylate different substrates? And, most importantly, what is the biological relevance of this activity?

Interestingly, phyA isolated from plants is a phosphoprotein with at least one serine phosphorylated in a light-dependent fashion, suggesting that phosphorylation of this residue results from autophosphorylation or from phosphorylation by another phytochrome (Lapko et al. 1999). This serine is also a major phosphoacceptor site identified in in vitro phosphorylation studies (Wong et al. 1986). Residues at the very amino-terminus of phyA are also phosphorylated but not in a light-dependent fashion (Lapko et al. 1997, 1999). However, phosphorylation of this portion of the protein has been implicated

in down-regulation of phyA signaling (Stockhaus et al. 1992).

In vitro kinase assays have identified other substrates of phytochrome. Of particular interest are the cryptochrome blue light receptors cry1 and cry2 (Ahmad et al. 1998). Although they are not phosphorylated in a light dependent fashion in vitro, in vivo analysis shows that cry1 phosphorylation is stimulated by red light. These results are particularly interesting in view of the large body of photobiological evidence suggesting an interaction between phytochrome and the blue light receptors (Mohr 1994). We have recently identified the first phytochrome kinase substrate (PKS1) that is phosphorylated in a light-dependent manner in vitro (Fankhauser et al. 1999). Phosphorylation of PKS1 in vivo also appears to be stimulated by red light, suggesting that phytochrome is the kinase. PKS1 was identified as a protein that binds to the carboxyl-terminus of phyA; it also interacts with the carboxyl-terminus of phyB. The phenotypes of plants overexpressing PKS1 are consistent with PKS1 being a negative regulator of phyB signaling. Fusions with the jellyfish green fluorescent protein (GFP) (Chiu et al. 1996) show that PKS1 is a cytoplasmic protein (Fankhauser et al. 1999). It will be important to assess the relevance of cry1, cry2, and PKS1 phosphorylation in phytochrome signaling. Figure 2B presents some of the ways this kinase activity may affect phytochrome signal transduction.

Another property of phytochrome that is affected by light is its subcellular localization. Immunolocalization of phyA performed mainly in dark-grown tissues indicated that phyA is predominantly a cytoplasmic protein (Pratt 1994). However, this view was challenged a few years ago when it was found that a significant portion of the total phyB is present in the nucleus of light-grown plants (Sakamoto and Nagatani 1996). These results have recently been confirmed and extended with both phyA-GFP and phyB-GFP fusion proteins (Kircher et al. 1999; Yamaguchi et al. 1999). Both phyA and phyB are cytoplasmic when plants are kept in the dark but exposure to light triggers the translocation of these photoreceptors to the nucleus (Fig. 3) (Kircher et al. 1999; Yamaguchi et al. 1999). Interestingly, the fusion proteins form speckles in the nucleus (Kircher et al. 1999; Yamaguchi et al. 1999), similar to the structures that factors involved in RNA transcription and processing form in animal cells (Lamond and Earnshaw 1998). COP1, a negative regulator of photomorphogenesis, is also found in nuclear speckles (Ang et al. 1998). This raises the possibility that phytochromes are found in a large complex in the nucleus. However, it should be noted that each of these GFP fusion proteins were expressed from strong constitutive promoters, and it is possible that the speckles in the nucleus are artifacts of overexpression. These speckles are not seen when *phyA-GFP* is driven by the endogenous *phyA* promoter (Fig. 3), even though the fusion protein is nuclear localized and capable of rescuing a *phyA*-null mutant (C. Fankhauser and J. Chory, unpubl.).

The kinetics and light requirements for phyA and phyB nuclear translocation are quite different. Under-

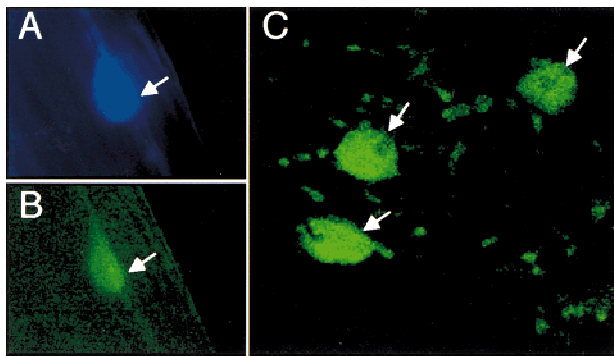


Figure 3. Nuclear localization of phyA–GFP in *Arabidopsis* seedlings exposed briefly to light. Arrows indicate the nuclei. (A) DAPI staining in a hypocotyl cell; (B) GFP fluorescence of the same cell; (C) GFP fluorescence of a confocal section in the hypocotyl. Three nuclei are clearly visible; the smaller structures are plastids that autofluoresce under the wavelengths used to excite GFP.

standing this difference might explain part of the different modes of action of phyA and phyB (Kircher et al. 1999). For phyB there is good evidence that nuclear translocation occurs only in the Pfr conformation. Phytochrome binding to the nonphotoconverting chromophore, phycoerythrobilin, results in brightly orange fluorescent adducts. Under these conditions, phyA remains cytoplasmic—reminiscent of dark-grown seedlings—indicating that photoconversion of the chromophore is required for nuclear translocation of phyA (Murphy and Lagarias 1997). However, phyA migrates into the nucleus even in far-red light (Kircher et al. 1999), suggesting that phyA in its Pr configuration is capable of nuclear accumulation if it has been cycled through Pfr. It is worth pointing out that the light-induced Pr-to-Pfr transformation is very rapid; however, phyB takes several hours to accumulate to significant levels in the nucleus. Thus, the Pfr form of phyB must be present in both the cytoplasmic and nuclear compartments (Kircher et al. 1999; Yamaguchi et al. 1999). Moreover, many phytochrome responses, such as membrane depolarization or changes in hypocotyl growth rates, occur within minutes of irradiation with light (Cosgrove 1994). It is therefore likely that the nucleus is not the only site of action of phytochromes.

Phytochrome signaling components

Four major approaches have been used to identify early signaling components downstream of phytochrome. Physiological approaches have been taken to identify rapid responses associated with phytochrome activity. Pharmacological approaches involved microinjection of possible second messengers into phytochrome mutants of tomato. Molecular approaches identified phytochrome-interacting components using two-hybrid screens in yeast. Finally, genetic approaches focused on the isolation of signaling mutants in *Arabidopsis*.

One of the most rapid physiological actions of phytochrome is its effect on ion fluxes at the plasmamembrane (for review, see Racusen and Galston 1983; Blatt 1987; Kendrick and Bossen 1987). The first demonstration of this effect was the red/far red-reversible electrostatic adhesion of etiolated roots to phosphate-treated glass (Tanada 1967, 1968; Yungmans and Jaffe 1970). In these and other etiolated tissues, phytochrome is directly involved in rapid changes of the membrane potential that occur upon exposure to light (Racusen and Galston 1983). On the other hand, the role of phytochrome in the light-induced electrical responses of green plant cells is less certain. Although these light-induced electrical responses have been shown to depend on the presence of chloroplasts (Tazawa and Shimmen 1980) and full functioning of photosynthesis (Spalding and Goldsmith 1993), there are also reports that demonstrate a direct involvement of phytochrome in the electric responses of green plant cells to light. These include chloroplast movement and ion flux in the green alga *Mougeotia* (Serlin and Roux 1984; Serlin et al. 1996), ion fluxes in the green alga *Nitella* (Weisenseel and Ruppert 1977), and ion fluxes associated with branching in the moss *Physcomitrella* (Ermolayeva et al. 1996, 1997). Experiments with inhibitors and external calcium levels demonstrated that calcium participates in many of these responses as well as in fern spore germination in *Dryopteris* (Wayne and Hepler 1984) and red light-induced swelling of etiolated wheat leaf protoplasts (Tretyn et al. 1990; Shacklock et al. 1992).

The pharmacological approach has identified heterotrimeric G proteins, cGMP, and calcium as being early components in phytochrome signaling (for review, see Mustilli and Bowler 1997). These second messengers induce chlorophyll and anthocyanin biosynthesis in addition to many light-regulated genes such as *FNR* (encoding a ferredoxin NADP⁺ oxidoreductase), *CHS* (encoding chalcone synthase), and *CAB* (encoding chlorophyll a,b-binding proteins) (Neuhaus et al. 1993; Bowler et al. 1994). In addition, these same pathways can repress the *AS1* gene (encoding asparagine synthase) (Neuhaus et al. 1997). Although a role for changes in ion transport across the plasma membrane and second messengers in light mediated signal transduction is apparent, the connection between these responses and specific gene products has not yet been made.

Yeast two-hybrid screens were used to identify PKS1 as a phytochrome-interacting protein. Two other interacting components have been identified in yeast two-hybrid screens. One of these, nucleoside diphosphate kinase 2 (NDPK2) was identified in a screen for proteins interacting with the carboxyl terminus of phyA (Choi et al. 1999). The activity of NDPK2 but not NDPK1 (which does not interact with phytochrome) was shown to increase dramatically when interacting with the Pfr form of phyA. In both the PKS1 and NDPK2 two-hybrid screens, phyA itself was identified as one of the interacting partners with phyA. Given that phytochromes are dimers, this argues that these screens are valid and capable of identifying bona fide phyA interacting partners.

Plants overexpressing PKS1 have longer hypocotyls in red light, suggesting that PKS1 is a negative regulator of hypocotyl elongation downstream of phyB. In contrast, NDPK2 appears to be a positive regulator of phyA and phyB signaling. However, hypocotyl elongation is not affected by this regulatory component. Instead, loss-of-function alleles have a small but significant reduction in cotyledon greening and opening of the hypocotyl/cotyledon hook during deetiolation (Choi et al. 1999).

ndpk2 mutants have altered responses to both red and far-red light, suggesting that this regulator interacts in vivo with both phyA and phyB. *NDPK2-GFP* fusions expressed in tobacco show both cytoplasmic and nuclear localization. Thus, phytochrome may interact with NDPK2 in both the cytoplasm and the nucleus. Red light has been shown to stimulate phosphorylation of NDPK2 in vivo suggesting that this interacting protein may also be a substrate for phytochrome kinase activity (Hamada et al. 1996; Tanaka et al. 1998; Ogura et al. 1999). The PAS domains in phytochrome are clearly important as NDPK2 does not interact with a phyA mutated in one of the two PAS repeats. The mechanism of action for NDPK2 in plants is not known although studies in other model systems implicate this enzyme in many developmental processes (Tanaka et al. 1998; Choi et al. 1999).

A third phytochrome-interacting partner is PIF3, a nuclear-localized, putative bHLH-containing transcription factor isolated by virtue of its binding to both phyA and phyB in the yeast two-hybrid system. Decreasing PIF3 levels results in plants that are defective in both red and far-red light sensing, consistent with the view that this gene acts downstream of both photoreceptors (Ni et al. 1998). Importantly, PIF3 binds phyB in a light-dependent fashion (Ni et al. 1999). As with NDPK2, PIF3 does not interact with phytochromes mutated in the PAS domain. These results again underscore the importance of the PAS domain in phytochrome signaling because mutations in the PAS domain have major effects on both its spectral properties (Elich and Chory 1997) and its ability to interact with downstream signaling partners. It will be important to test if PIF3 is modified in response to light, or if binding to phytochrome directly modulates the activity of this putative transcription factor. For example, the activity of CCA1, a DNA-binding protein acting downstream of phytochromes, can be modulated by phosphorylation (Sugano et al. 1998). However, casein kinase 2, but not phytochrome, appears to be the protein kinase mediating CCA1 phosphorylation.

Genetic and molecular screens have identified a large number of genes acting downstream of light receptors. Because different light qualities trigger the same developmental responses using different photoreceptors, it is very likely that common late-acting signaling intermediates are used. Mutants in such genes are expected to have the same phenotypes irrespective of the light quality. Such loci have been identified and they fall into two classes: mutants that deetiolate even in the absence of light and mutants that are defective for their perception of light at a variety of different wavelengths. The former class is referred to as *det/cop/fus* mutants based on the

different genetic screens from which they were isolated. These are pleiotropic mutations affecting many aspects of plant development and these proteins are generally considered to be late signaling components (for review, see Fankhauser and Chory 1997; Deng and Quail 1999; Osterlund et al. 1999; Wei and Deng 1999).

Early signaling intermediates are expected to have a phenotype only under the specific light conditions activating their photoreceptor (Fig. 4). Such mutants can be further classified into genes that affect phyA signaling (defective in far-red light), phyB signaling (defective in red light) or both phyA and phyB signaling (defective in red and far-red light perception). FHY1, FHY3, FIN2, SPA1, and FAR1 are implicated specifically in phyA signaling. Genetic analysis suggests that, except for SPA1, they all act as positive elements in this pathway (Whitelam et al. 1993; Hoecker et al. 1998; Soh et al. 1998; Hudson et al. 1999). *spa1* was identified as a suppressor of a weak *phyA* mutation, and mutations in this gene increase the "signaling current" in a phyA-dependent manner (Hoecker et al. 1998). The gene codes for a putative protein with a carboxy-terminal domain related to the negative regulator of photomorphogenesis COP1 (Hoecker et al. 1999). *far1* was identified in a screen for suppressors of the exaggerated light response of phyA overexpressing plants. *FAR1* is a member of a small gene family in *Arabidopsis*; homologous sequences can also be identified in other plant species (Hudson et al. 1999). Both SPA1 and FAR1 are localized in the nucleus as determined by transient expression of GUS fusion proteins. It is currently unknown whether this localization is con-

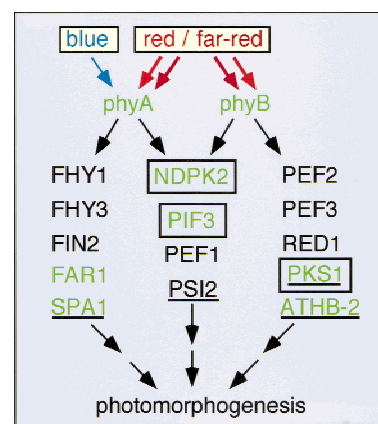


Figure 4. Early intermediates in phytochrome signaling. Cloned genes are indicated in green. Negative regulators are underlined. Proteins that interact with phytochrome are boxed. Not all cloned genes have been tested for physical interaction with phytochrome. This diagram does not imply that all those genes act downstream of the photoreceptors but simply indicates that they affect a specific branch of phy signaling. PKS1 interacts both with phyA and phyB but appears to inhibit phyB signaling. Multiple phytochromes affect ATHB-2 expression, but ATHB-2 acts as a negative regulator of type II phytochromes (phyB). We have indicated only phyA and phyB here; some of those genes could also regulate phyC, phyD, and/or phyE signaling.

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stitutive or regulated by light. A number of earlier studies have suggested that phytochrome signaling involves light-regulated protein phosphorylation (Roux 1994). Now that we know that phytochrome itself is a protein kinase, and that a number of downstream acting factors have been identified, it is important to test if these proteins directly interact with phyA and if they are substrates for phyA's protein kinase activity.

red1, *pef2*, and *pef3* are in a class of mutants specifically affected in red light signaling. They share a number of features with *phyB* mutants, such as early flowering in short days, elongated petioles, and decreased sensitivity to red light (Reed et al. 1993; Ahmad and Cashmore 1996; Wagner et al. 1997). The molecular nature of these genes will certainly provide us with new insights into phyB signaling. The *poc1* mutant shows exaggerated response to red light. This phenotype is caused by a T-DNA insertion, causing the overexpression of the gene encoding PIF3. Although PIF3 interacts with both phyA and phyB, light-grown *poc1* mutants do not show enhanced responses to far-red light (Halliday et al. 1999). PKS1 could also be classified in this group but unlike the above-mentioned genes, PKS1 appears to act as a negative regulator of phyB signaling (Fankhauser et al. 1999). Another mediator of type II phytochrome responses is the homeodomain leucine zipper protein HAT4/ATHB-2 (Schena et al. 1993; Steindler et al. 1999). This transcription factor plays an important role in shade avoidance responses (Steindler et al. 1999). The expression of this gene is regulated by R/FR ratios, and an elegant series of studies indicate that ATHB-2 acts as a negative regulator of gene expression (Carabelli et al. 1996; Steindler et al. 1999). *pef1* and *psi2* mutants are affected in both phyA and phyB signaling. The former is less sensitive to red and far-red light, whereas the latter is hypersensitive to such light treatments (Ahmad and Cashmore 1996; Genoud et al. 1998). As NDPK2 loss-of-function mutants are less responsive to both red and far-red light, they can also be included in this class downstream of both phyA and phyB (Choi et al. 1999).

Models for phytochrome signaling

We are now beginning to develop the tools necessary to model the early mechanisms of phytochrome signal transduction. Light has numerous effects on phytochrome, including changes in both its spectral properties and the overall conformation of the protein (Fig. 2B). In turn, these conformational changes influence the subcellular localization, stability (in the case of phyA), as well as the protein kinase activity of phytochrome. As phytochrome autophosphorylation is light dependent, this post-transcriptional modification could be a direct link between the light conditions and the photoreceptor's subcellular localization, stability, and interaction with signaling partners. The transphosphorylation activity of phytochrome is also regulated by light, allowing light-dependent modification of other signaling components as well (Fig. 2B). One possible model is that phytochromes, in their Pr form, may interact with cytoplas-

mic proteins such as PKS1. Upon exposure to light, phosphorylation of PKS1 by phytochrome may release phytochrome from PKS1, allowing nuclear translocation of phytochrome. Once in the nucleus, phytochrome could interact with nuclear proteins such as PIF3, thus regulating the transcriptional activity of late signal transduction components or genes directly involved in growth responses. In certain cell types, phytochrome may require additional regulatory factors that could be both cytoplasmic and nuclear, such as NDPK2.

A model in which Pr is inactive and Pfr is the active form of phytochrome is compatible with the VLFR and LFR modes of phytochrome action; however this does not explain the phyA-mediated HIR. The photocycling of phyA into the Pr form appears to be responsible for the HIR-, however this is not the same Pr as de novo synthesized phyA that has never seen light (not photocycled) (Shinomura et al. 2000). One could therefore imagine that these two forms of Pr have different post-transcriptional modifications. Because phyA autophosphorylation is light regulated, we propose that additional phosphates could be the difference between Pr and cycled Pr (Yeh and Lagarias 1998; Lapko et al. 1999). This cycled Pr would be active and short lived because one must constantly supply the plant with new photocycled Pr to obtain a sustained response (Shinomura et al. 2000). A model in which both Pfr and cycled Pr is translocated to the nucleus would explain how phyA accumulates in the nucleus after either red or far-red light treatments.

Light-induced, conformation-specific binding of a photoreceptor to a transcription factor is a very attractive model for phytochrome signaling (Smith 1999). Interestingly, earlier studies have suggested that phytochrome signaling involves phosphorylation of nuclear proteins (Roux 1994). Moreover, the activity of a number of transcription factors is regulated by phosphorylation (Komeili and O'Shea 1999). Although this simple model is attractive, attributing this type of model to all of phytochrome signaling is risky. The myriad of phytochrome responses and complexity of interplay between the different species of photoreceptor within the plant point to a more complex, global regulation of phytochrome signal transduction. Perhaps each phytochrome pool controls only a subset of plant developmental responses. Mutations in *PKS1*, *PIF3*, and *NDPK2* each have unique phenotypes affecting different tissues in the seedling. It is certainly possible that these components interact with phytochrome only in certain cell types with *PKS1* and *PIF3* being active in the hypocotyl and *NDPK2* active in cells of the hook region. In addition, there are many phytochrome responses, such as changes in ionic conductance across the plasmamembrane that occur faster than the rate of nuclear translocation or activation of transcription. Thus, it is likely that phytochrome acts in other cellular compartments in addition to the nucleus.

Integrating phytochrome signaling with whole plant development

In addition to light, many plant hormones contribute to

photomorphogenic responses. This is not surprising because the two classes of signals act on similar cells and organs. Cell expansion is one phenomenon that can be affected dramatically by both light and hormones. The quality and quantity of light can both inhibit and induce stem elongation (Chory 1997; Smith and Whitelam 1997). Likewise, phytohormones also affect stem elongation. In most cases ethylene, abscisic acid, and cytokinins inhibit cell elongation. In contrast, brassinosteroids, gibberellins, and auxin can increase cell elongation (Chory et al. 1994; Creelman and Mullet 1997; Kende and Zeevart 1997; McGrath and Ecker 1998). Some photomorphogenic mutants resemble mutants involved in phytohormone biosynthesis or sensing. For example, the gibberellin (GA) signaling mutant *spindly* resembles mutations in *phyB* with long stems, pale leaves and early flowering, a phenotype that can also be mimicked in wild-type plants by the application of GA₃ (Jacobsen and Olszewski 1993). Genetic analysis of GA and phytochrome mutants points to interactions between these two signal transduction systems for certain responses (Chory and Li 1997) although other responses, such as flowering, are likely to be controlled independently by both systems (Blázquez and Weigel 1999). Phytochromes can regulate the transcription of GA biosynthesis genes (Kamiya and García-Martínez 1999). This may be one of the mechanisms of interaction between these two signaling systems and is likely to be involved in *phyB*'s role in generating a graft-transmissible substance that controls tuber formation in potato (Jackson et al. 1996, 1998; Kamiya and García-Martínez 1999).

Many brassinosteroid mutants have been identified in genetic screens for plants which can undergo deetiolation in the absence of a light cue (for review, see Clouse and Sasse 1998; Li and Chory 1999). When these mutants are grown in the dark, their seedlings have short hypocotyls with cotyledons that begin to develop as if growing in the light. As adults these mutants are dwarfs with dark green epinastic leaves, have short stems and petioles, and are slow growing with delayed senescence. Each of these adult phenotypes is essentially the opposite of mutants lacking phytochrome B (Chory and Li 1997; Kamiya and García-Martínez 1999). Although these mutants were identified in photomorphogenic screens, it has been a challenge to describe the molecular mechanism of interaction between photoreceptor and brassinosteroid signaling pathways. Light does not appear to regulate the activity of brassinosteroid biosynthetic genes or the putative receptor. In fact, these genes have similar, ubiquitous expression throughout the plant. In a screen for extragenic, dominant, gain-of-function suppressors of a *phyB* missense mutation (*phyB-4*; Koornneef et al. 1980; Reed et al. 1993), we have identified a cytochrome P450, called *BAS1*, that catalyzes the inactivation/degradation of brassinosteroids (Neff et al. 1999). Overexpression of *BAS1* results in dwarf plants that cannot undergo the shade avoidance syndrome seen in *phyB* mutants. Genetic analysis characterizes this dominant mutation in *BAS1* as a bypass suppressor of *phyB* acting downstream of *phyA* and *cry1*. Although it

is unknown how photoreceptors regulate the activity of *BAS1*, this work demonstrates an interaction between multiple photoreceptors and brassinosteroid catabolism (Neff et al. 1999).

Both physiological and genetic studies indicate a role for auxins in photomorphogenesis. Auxin transport is affected in a light-dependent manner (Jensen et al. 1998). Moreover, *HY5*, mutations in which result in seedlings with long hypocotyls in varying light conditions (Koornneef et al. 1980), encodes a bZIP transcription factor that may be involved in auxin signaling (Oyama et al. 1997). Transgenic plants or mutants with elevated auxin levels also confer seedling phenotypes associated with photomorphogenesis. Although evidence for direct interactions between these signaling pathways is lacking, the possibility for overlap between these two developmental regulation systems is evident (Boerjan et al. 1995; Romano et al. 1995). One possible mechanism is brought forth in studies linking the high-temperature promotion of hypocotyl elongation with elevated levels of free auxins (Gray et al. 1998). An additional link between auxin signaling and photomorphogenesis is found in the cloning of *SHY2*. *shy2* was identified as a suppressor of the long-hypocotyl phenotype found in mutants with reduced levels of all phytochromes (Kim et al. 1998) or a null allele of *phyB* (Reed et al. 1998). This dominant mutation resides in the auxin-induced gene *IAA3* (Tian and Reed 1999). The most recent evidence for interactions between phytochrome and auxin signaling pathways comes from further analysis of the negative regulator *ATHB-2*, a transcription factor whose expression is regulated in response to far-red-enriched light. *ATHB-2*-overexpressing plants confer a shade avoidance phenotype that is in part due to interference with auxin transport or sensing mechanisms (Steindler et al. 1999).

In addition to interactions with phytohormones, phytochromes also effect the activity of the circadian clock (Millar et al. 1995). In studies of the circadian expression pattern of the light-induced *CAB2* gene, *phyA* and *phyB* both affect the acute light response in etiolated seedlings though they have little effect on the expression pattern in green seedlings grown in a 12-hr light/dark cycles (Anderson et al. 1997). When *phyA* or *phyB* mutants are entrained by a circadian light cue then exposed to varying intensities of continuous red light, a role for these photoreceptors is uncovered. *phyA* shortens the clock period in response to low fluence red light whereas *phyB* shortens the period of the clock in response to high fluence red light (Somers et al. 1998). Studies using a *phyB* mutant in *Sorghum bicolor* show that *phyB* is involved in the circadian control of ethylene production. Though *phyB* does not directly affect the clock oscillator, it does modulate this response most likely through a shade avoidance mechanism (Finlayson et al. 1998, 1999). The mechanism by which phytochromes affect circadian rhythms is unknown. However, it is clear that many mutants with aberrant circadian functions also confer seedling phenotypes linking them with photomorphogenesis (Hicks et al. 1996; Schaffer et al. 1998; Wang and

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Tobin 1998; Dowson-Day and Millar 1999; Sugano et al. 1999).

Technology transfer to crops

It is a complex web of molecular and cellular interactions that finally control developmental events such as cell division, expansion, and differentiation. Identification of the components that act at the nodes of interaction between multiple signaling pathways may impact our ability to modify horticultural and agricultural crops (Robson and Smith 1997). For example, overexpression of oat phyA in transgenic tobacco greatly improved the harvest index (Robson et al. 1996) by alleviating some of the shade avoidance response created by close planting. Similarly, when *Arabidopsis* phyB is overexpressed in potato, photosynthetic performance and life span are increased, leading to higher tuber yield (Thiele et al. 1999). Overexpression of oat phyA in hybrid aspen increases dwarfism and reduces shade avoidance (Olsen et al. 1997) possibly allowing for closer crop spacing and increased wood production per area.

Despite these promising gains, the effects of altering photoreceptor expression may not be the best way to manipulate crops. For instance, although the number of tubers is increased in potatoes overexpressing phyB, the tuber size is smaller (Thiele et al. 1999). Hybrid aspen overexpressing phyA have lost the ability to acclimate to cold temperatures due to a lack of end-of-day far-red responses, making them more susceptible to frost (Olsen et al. 1997). However, by cloning and modifying downstream genes that affect a subset of phytochrome-mediated output traits, we should be able to develop a more fine tuned control over altering developmental traits in response to light.

One way to clone and control the activity of photomorphogenesis output genes utilizes high throughput studies of phytochrome-mediated gene expression. A recent paper utilized fluorescence differential display to identify 20 differentially expressed genes between wild type and a *phyA*-null mutant (Kuno et al. 2000). These techniques, coupled with the saturated screening of phytochrome interacting partners and continued identification and cloning of mutations involved in photomorphogenesis, will give us a large genetic tool box. By expanding these approaches to the more primitive phytochrome signaling pathways found in lower plants and bacteria, we may identify components with novel actions in higher plants. With these tools we may be able to manipulate crop production and gain fundamental insight into the complex interactions of phytochrome signaling pathways with other regulatory systems in plants.

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