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Phytochrome Signaling Mechanism

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INTRODUCTION

Because of their sessile nature, plants have adapted a high degree of developmental plasticity to optimize their growth and reproduction in response to their ambient environments, such as light, temperature, humidity, and salinity. Plants utilize a wide range of sensory systems to perceive and transduce specific incoming environmental signals. Light is one of the major environmental signals that influences plant growth and development. In addition to being the primary energy source for plants, light also provides them with positional information to modulate many developmental processes, including seed germination, seedling de-etiolation, leaf expansion, stem elongation, phototropism, stomata and chloroplast movement, shade avoidance, circadian rhythms, and flowering time (Deng and Quail, 1999; Wang and Deng, 2003).

The phenotypic changes associated with the seedling photomorphogenic development are among the most dramatic events mediated by light. Dark-grown seedlings undergo skotomorphogenesis (etiolation) and are characterized by long hypocotyls, closed cotyledons and apical hooks, and development of the proplastids into etioplasts. Light-grown seedlings undergo photomorphogenesis (de-etiolation) and are characterized by short hypocotyls, open and expanded cotyledons, and development of the proplastids into green mature chloroplasts (McNellis and Deng, 1995, Figure 1).

Plants can detect almost all facets of light such as direction, duration, quantity, and wavelength by using three major classes of photoreceptors: The cryptochromes and phototropins monitor the blue/ultraviolet-A (B/UV-A) (320-500 nm) region of the spectrum, whereas the phytochromes monitor primarily the red (R) and far-red (FR) wavelengths (600-750 nm) (Kendrick and Kronenberg, 1994; Briggs and Olney, 2001). These photoreceptors perceive, interpret, and transduce light signals, via distinct intracellular signaling pathways, to modulate photoresponsive nuclear gene expression, and ultimately leading to adaptive changes at the cell and whole organism levels.

This chapter aims to highlight some of the most recent progress in elucidating the molecular, cellular and biochemical mechanisms of phytochrome signaling in Arabidopsis. The interested readers are referred to the accompanying reviews on other related subjects, such as photomorphogenesis (reviewed by Jennifer Nemhauser and Joanne Chory), circadian rhythms (reviewed by C Robertson McClung, Patrice A. Salomé, and Todd P. Michael), phototropism (reviewed by Emmanuel Liscum), and chapters of phytohormones (such as brassinosteroids and ethylene).

THE DISCOVERY AND ACTION MODES OF PHYTOCHROMES

Phytochrome, stand for “plant color”, was originally coined to describe the proteinous pigment that controls photoperiod detection and floral induction of certain short-day plants (such as cocklebur and soybean, Garner and Allard, 1920) and the reversible seed germination of lettuce (c.v. Grand rapids) by red (R) and far-red (FR) light (Borthwick et al., 1952). Red light promotes seed germination, whereas subsequent far-red light treatment abolishes red light induction of seed germination. The germination response of lettuce seeds repeatedly treated with R/FR cycles is determined by the last light treatment. Thus R/FR reversibility is a characteristic feature of this response. In addition, the law of reciprocity applies to this response, i.e., the response is dependent on the total amount of pho-

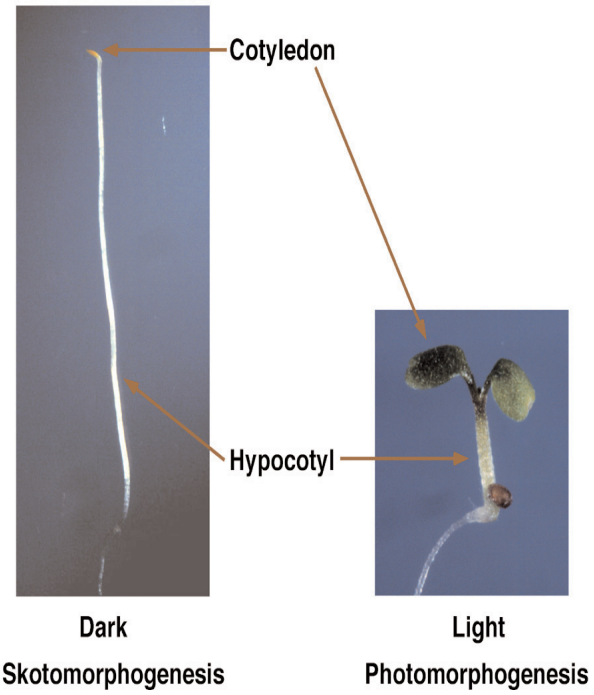

Figure 1. The contrasting phenotypes of dark- vs. light-grown Arabidopsis seedlings.
Dark-grown seedlings undergo a skotomorphogenic development program (etiolation), which is characterized by elongated hypocotyls, closed cotyledons and apical hooks. Light-grown seedlings undergo photomorphogenesis and are characterized by short hypocotyls, open and expanded green cotyledons.

tons received irrespective of the duration of light treatment. This type of responses is now defined as the low fluence responses (LFRs, Mancinelli, 1994). LFRs also induce other transient responses, such as changes in ion flux, leaf movement, chloroplast rotation and changes in gene expression (Haupt and Hader, 1994; Roux, 1994; Vince-Prue, 1994). Besides the R/FR reversible LFRs, there are two other modes of phytochrome action. The very-low-fluence responses (VLFRs) are activated by extremely low light intensities of different wavelengths (far-red, red and blue), such as light-induced expression of the *LHCB* gene and light induction of seed germination, and the high-irradiance responses (HIRs) which depend on prolonged exposure to relatively high light intensities. HIRs are primarily responsible for the control of seedling de-etiolation (e.g. inhibition of hypocotyl elongation and promotion of cotyledon expansion) under all light qualities (Mustilli and Bowler, 1997; Casal et al., 1998; Table 1).

THE PHYTOCHROME GENE FAMILY AND THE CHROMOPHORE

Two Reversible Forms of Phytochromes

The enrichment of phytochrome in dark-grown seedlings and its predicted photoreversibility facilitated its initial purification (Butler et al., 1959, 1964). The purified phytochrome from dark-grown plants is blue in color and absorbs red light (termed Pr, absorbs maximally at 660 nm). On exposure to red light, the Pr form converts to the far-red light absorption form (Pfr) which is olive-green in color and absorbs maximally at 730 nm (Quail, 1997, Figure 2). The Pfr form is considered as the biologically active form. The Pfr form converts back to the Pr form on absorption of far-red light or undergoes slow dark reversion in the absence of light. It should be noted that in addition to their maximal absorptions of red and far-red wavelengths, phytochromes also weakly absorb blue light (Furuya and Song, 1994; Figure 2).

Classification of Phytochromes and The Gene Family

Purified phytochrome from etiolated seedlings exists as a soluble homodimer, with each apoprotein monomer attaching a covalently linked chromophore. The molecular mass of the phytochrome apoprotein is approximately 125 kDa. The chromophore is attached via a thioether linkage to an invariant cysteine in a well-conserved domain among all phytochromes. Previous spectrophotometric studies indicated that there are at least two distinct pools of phytochromes, Type I (light labile) and type II (light stable). The light-labile pool degrades fairly rapidly upon exposure to red or white light, whereas Type II phytochromes are stable in these light conditions. In Arabidopsis, there are five phytochromes (termed phyA-phyE) encoded by five distinct members of the phytochrome gene family (Sharrock and Quail, 1989). phyA is a type I phytochrome, and phyB-phyE belong to type II phytochromes. Sequence analysis suggests that these phytochromes can be clustered into three subfamilies: phyA/phyC, phyB/phyD, and phyE (Figure 3A; Table 2). Counterparts of Arabidopsis *PHY* genes are present in most, if not all, higher plants (Clack et al., 1994; Sharrock and Quail, 1989; Mathews and Sharrock, 1997).

All five Arabidopsis phytochromes are expressed throughout the plant. Although the differences in their expression patterns are minor, their abundance and stability differ dramatically (Somers and Quail 1995; Goosey et

al., 1997). phyA is most abundant in dark-grown seedlings and its level drop up to 100-fold after exposure to light, as a result of combinatorial regulation at both the transcriptional and post-transcriptional levels. Light down-regulates *PHYA* gene transcription (Somers and Quail, 1995; Canton and Quail, 1999) and causes degradation of phyA polypeptide (Clough et al., 1999; Hennig et al., 1999). In light-grown plants, phyB is the most abundant phytochrome, whereas phyC-phyE are the less abundant type II phytochromes (Clark et al., 1994; Hirschfeld et al., 1998).

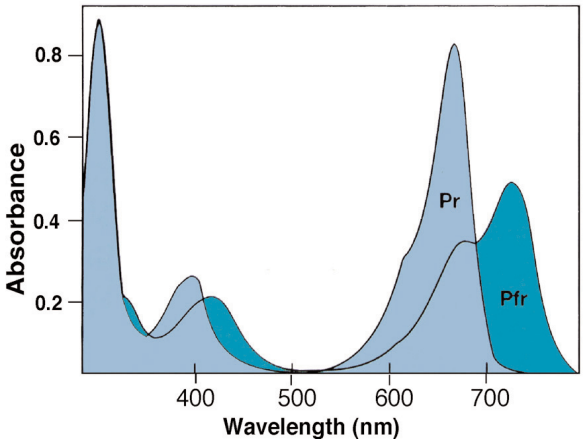

Figure 2. Absorption spectra of phytochromes.
Absorption spectra of the two forms (Pr and Pfr) of phytochromes. The Pr form absorbs maximally at 660 nm, while the Pfr form absorbs maximally at 730 nm.

General Structure of Phytochromes

Studies with biochemically purified phyA holoproteins indicated that the phytochrome molecule consists of two structural domains connected via a flexible hinge region. The N-terminal domain is responsible for chromophore-binding, light absorption and photoreversibility (~ 70 kDa). The C-terminal domain (~ 55 kDa) contains several conserved subdomains/motifs including the regulatory core sequence (Quail box), two dimerization motifs, and the histidine kinase-related domain (HKRD). A pair of Per-Arnt-Sim (PAS) motif overlaps with the Quail box (Figure 3B). PAS domains can be used either as platforms for protein-protein interaction or as response modules to small ligands or changes in light conditions, oxygen levels, and redox potentials (Quail, 1997; Neff et al., 2000). A number of point mutations in the C-terminal domain of both phyA and phyB do not affect photoreversibility but eliminate the biological activity (Quail et al., 1995; Quail, 1997), suggesting that the C-terminal domain is essential for proper downstream signaling. A domain swapping and deletion analysis suggested that the N terminus of phytochrome is essential for its specific photosensory properties and that the C termini of phyA and phyB are the output domains and interchangeable (Wagner et al., 1996). However, this notion was recently confronted by the finding that the N-terminal domain of phyB, when dimerized and localized in the nucleus, confers much higher photosensitivity than the full-length phyB. These results suggest that the N-terminal domain of phyB transduces the light signal to downstream targets, and the C-terminal domain of phyB attenuates the activity of phyB (Matsushita et al., 2003). At present, it is not known whether other phytochrome species share the same structure-function relationship as phyB.

Chromophore Biosynthesis

As mentioned above, functional phytochrome holoproteins require the covalent attachment of a chromophore to each phytochrome apoprotein monomer. Using degradation approaches and proton NMR spectroscopy, the structure of the phytochrome chromophore was determined to

Table 1. Diagnostic Features of Different Phytochrome Action Modes

Action Mode	Fluence Requirements	Photoreversibility	Reciprocity
VLFR	0.1 μmol/m^2 – 1 μmol/m^2	No	Yes
LFR	1-1000 μmol/ m^2	Yes	Yes
HIR	>1000 μmol/ m^2	No	No

VLFR: very low fluence response;
LFR: low fluence response;
HIR: high-irradiance response.

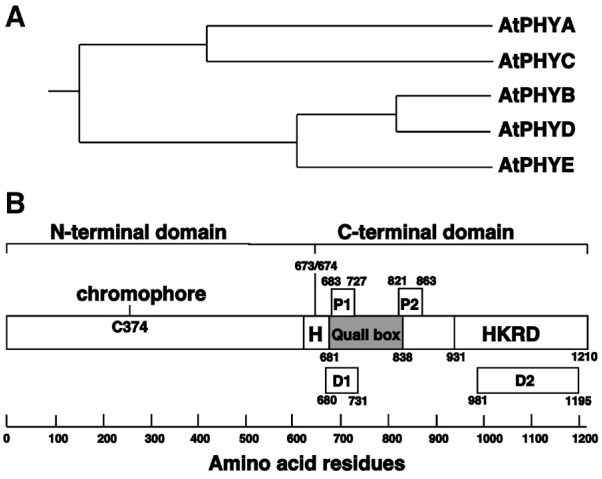

Figure 3. The Arabidopsis phytochrome family and the domain structure map of a generic phytochrome molecule.
(A) The phylogenetic distance tree of the five phytochrome species from Arabidopsis thaliana. PHYB and PHYD share ~80% amino acid sequence identity, and constitute a branch of the gene family. PHYE itself, PHYA and PHYC form two other branches of the family evolution tree (adapted from Clack et al., 1994).
(B) The domain structure map of a generic phytochrome molecule. The coordinates indicate positions of the consensus sequence derived from the alignment of multiple full-length phytochrome polypeptide sequences by Mathews et al. (1995). The N-terminal photosensory domain (CBD, for chromophore binding domain) and the C-terminal regulatory domain are joined by a flexible hinge region (H). The chromophore binding site (C374) is located in the N-terminal photosensory domain. The C-terminal domain contains several conserved motifs, including the regulatory core sequence (Quail box), two dimerization motifs (D1 and D2), two PAS domains (P1 and P2), and the histidine kinase-related domain (HKRD). The positions for the junction site and these individual motifs are indicated (adapted from Quail, 1997).

be a linear tetrapyrrole, phytochromobilin (PΦB), which ligates to a cysteine residue located in the N-terminal half apoprotein of PHYA through its A-ring (Lagarias and Rapoport, 1980).

The synthesis of PΦB is accomplished by a series of enzymatic reactions in the plastid that begins with 5-aminolevulinic acid. The early steps in the PΦB pathway are shared with chlorophyll and heme biosynthesis. The committed step is the oxidative cleavage of heme by a ferredoxin-dependent heme oxygenase (HO) to form biliverdin IX (BV). BV is then converted into 3Z-PΦB by the ferredoxin-dependent bilin reductase PFB synthase. Both 3Z-PΦB and its isomerized form 3E-PΦB can serve as functional precursors of the phytochrome chromophore. PΦB is then exported to the cytoplasm where it binds to the newly synthesized apo-PHYs (Terry et al., 1997, Figure 4 A and B). Absorption of red light triggers a "Z" to 'E" isomerization in the C-15 double bond between the C and D rings of the linear tetrapyrrole, resulting in the far-red light absorbing form Pfr. This Pr-to-Pfr transition is associated with rearrangement of the protein backbone. Pfr can be converted to Pr either by a slow non-photoinduced reaction (dark reversion) or much faster upon absorption of far-red light (Quail, 1997; Fankhauser, 2001; Figure 4C).

Arabidopsis mutants defective in the PΦB-synthetic pathway have been isolated. These mutants (*hy1* and hy2) have dramatically reduced levels of PΦB and consequently functional phytochromes, and thus exhibit severely impaired photomorphogenesis (Parks and Quail, 1991). The Arabidopsis *HY1* locus encodes a HO (designated *At*HO1) responsible for much of PΦB synthesis in Arabidopsis (Davis et al., 1999; Muramoto et al., 1999; Table 2). Three additional HO genes (*AtHO2-4*) exist in the Arabidopsis genome, and these additional HOs may provide alternative pathways for making BV (Davis et al., 2001). The Arabidopsis *HY2* locus, likely a unique gene in the Arabidopsis genome, encodes the phytochromobilin synthase (Kohchi et al., 2001; Table 2).

It is generally assumed that all phys have the same chromophore. The finding that phyB purified from transgenic Arabidopsis shows spectral properties similar to phyA supported this notion (Elich and Chory, 1997). It should be pointed out that besides PΦB, phycocyanobilin (PCB), the chromophore of the light-harvesting pigment phycocyanin, also can bind phytochrome resulting in Pr and Pfr spectra that are slightly blue shifted compared with the PΦB adducts (Lagarias and Rapoport, 1980, Figure 4B). This finding allowed the constitution of photoreversible phytochromes by expressing recombinant phytochrome proteins in yeast and assembling them *in vitro*. Analysis of reconstituted recombinant phyA, phyB, phyC and phyE revealed that they have similar but not identical spectral properties (Kunkel et al., 1996; Remberg et al., 1998; Eichenberg et al., 2000). Further, overexpression of the mammalian billiverdin reductase in Arabidopsis was found to cause the loss of multiple phytochrome activities by degrading phytochromobilin *in vivo* and constituted a new class of chromophore mutants which is phenotypically stronger than the *hy1* or *hy2* mutants, supporting the notion that phytochromobilin could serve as the chromophore for multiple phytochromes (Lagarias et al., 1997).

Table 2. Molecularly Identified Arabidopsis Genes Cited in This Chapter

Gene Name	AGI* Number	Reference
PHYA	AT1g 09570	Sharrock, R.A., and Quail, P.H., 1989
PHYB	AT2g18790	Sharrock, R.A., and Quail, P.H., 1989
PHYC	AT5g35840	Cowl et al., 1994
PHYD	AT4g16250	Clack, T., et al., 1994
PHYE	AT4g18130	Clack, T., et al., 1994
HY1	AT2g26670	Muramoto et al., 1999
HY2	AT3g09150	Kohchi et al, 2001
FHY3	AT3g22170	Wang and Deng, 2002
FAR1	AT4g15090	Hudson et al., 1999
FIN219	AT2g46370	Hsieh et al., 2000
PAT1	AT5g48150	Bolle et al., 2000
HFR1	AT1g02340	Fairchild et al., 2000
LAF1	AT4g25560	Ballesteros, M., 2001
LAF3	AT3g55850	Hare et al., 2003
LAF6	AT4g04770	Møller, S.G., 2001
SPA1	AT2g46350	Hoecker, U., 1999
EID1	AT4g02440	Dieterle, M. et al., 2001
SUB1	AT4g08810	Guo et al., 2001
PIF3	AT1g09530	Ni, M., 1998
PIF4	AT2g43010	Huq and Quail, 2002
PKS1	AT2g02950	Fankhauser et al., 1999
PKS2	AT1g14280	Lariguel et al., 2003
NDPK2	AT5g63310	Choi et al., 1999
GPA1	AT2g26300	Ma et al 1990
AGB1	AT4g34460	Weiss et al., 1994
AGG1	AT3g63420	Mason and Botella, 2001
ELF3	AT2g25930	Liu, X.L., 2001
ELF4	AT2g40080	Doyle et al., 2002; Khanna et al., 2003
CCA1	AT2g46820	Wang, Z.-Y., 1997
LHY	AT1g01060	Schaffer, R., 1998
SRR1	AT5g59560	Staiger et al., 2003
COG1	AT1g29160	Park et al., 2003
PFT1	AT1g25540	Cerdan and Chory, 2003
PRR7	AT5g02810	Kaczorowski and Quail, 2003
SPA2	AT4g11110	Laubinger and Hoecker, 2003
SPA3	AT3g15354	Laubinger and Hoecker, 2003
SPA4	AT1g53090	Laubinger and Hoecker, 2003
TOC1	AT5g61380	Strayer et al., 2000
CO	AT5g15840	Putterill et al., 1995
PIL1	AT2g46970	Salter et al., (2003)
RED1	AT4g31500	Hoecker et al., (2004)
GI	AT1g22770	Fowler, S., 1999
ADO1	AT5g57360	Somers, D.E, 2000
ADO2	AT2g18910	Jarillo, J.A. et al., 2001
ADO3	AT1g68050	Jarillo, J.A. et al., 2001
ARR4	AT1g10470	Imamura, A., 1998
COP1	AT2g32950	Deng et al., 1992
HY5	AT5g11260	Oyama, T., 1997
HYH	AT3g17609	Holm et al., 2002
CSN1	AT3g61140	Castle and Meinke, 1994
CSN2	AT2g26990	Serino et al., 2003
CSN3	AT5g14250	Peng et al., 2001a
CSN4	AT5g4297	Serino et al., 1999
CSN5 (AJH1)	AT1g22920	Kwok et al., 1998
CSN5 (AJH2)	AT1g71230	Kwok et al., 1998
CSN6A	AT5g56280	Peng et el., 2001b
CSN6B	AT4g26430	Peng et al., 2001b
CSN7	AT1g02090	Karniol et al., 1999
CSN8	AT4g14110	Wei et al., 1994
COP10	AT3g13550	Suzuki et al., 2002
DET1	AT4g10180	Pepper et al., 1994

*AGI: Arabidopsis Genome Initiative.

ACTIONS AND INTERACTIONS OF PHYTOCHROME FAMILY MEMBERS

Phytochromes regulate a variety of developmental processes throughout the life cycle of plants. In most instances, the roles of individual phytochromes are studied in the context of specific responses and/or developmental stages. Loss-of-function analyses combined with gain-of-function analyses and higher order mutants of various combinations are revealing the roles of individual phytochrome in regulating different aspects of plant development. It is clear now that different phytochromes play both distinct and overlapping roles within the spectrum of plant photomorphogenesis (Quail, 1998; Table 3).

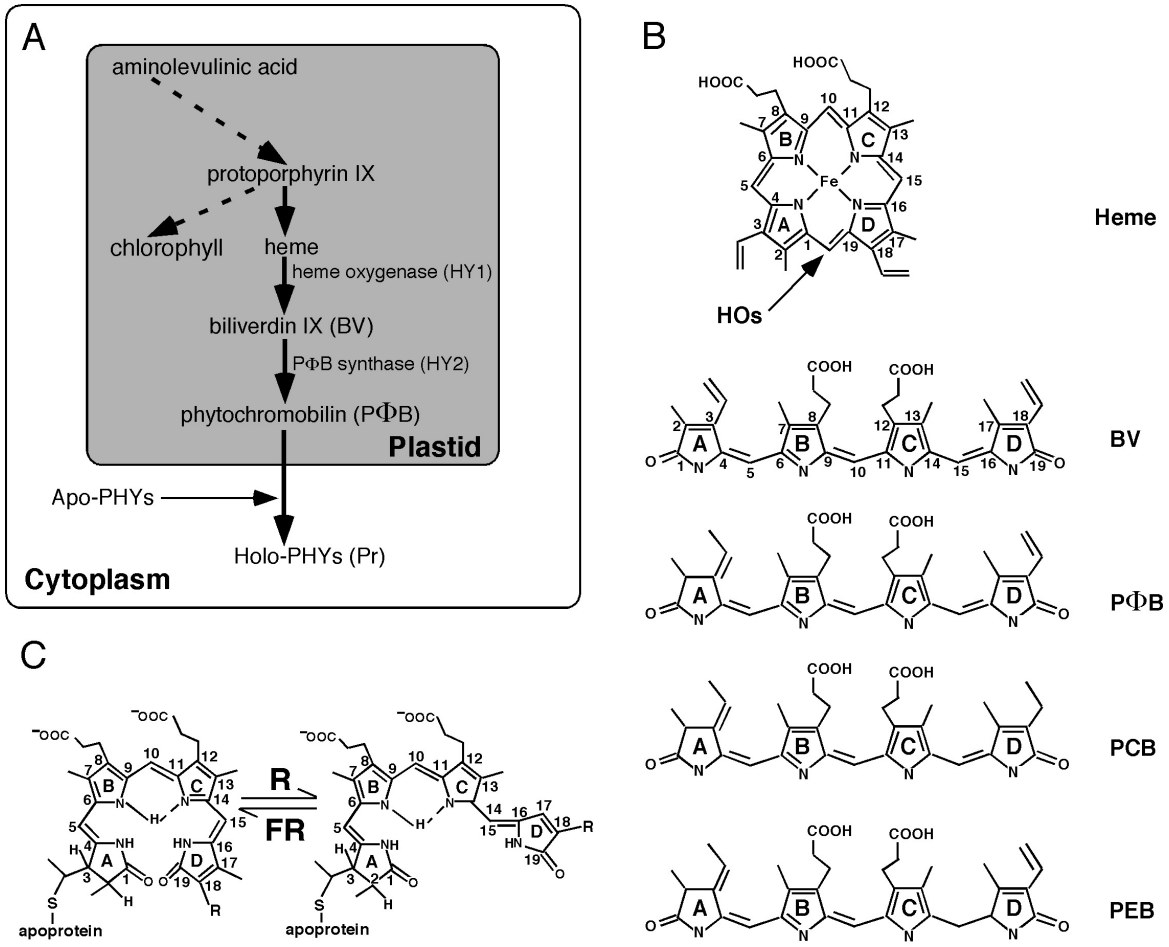

Figure 4. Arabidopsis phytochrome chromophore.
(A) The biosynthesis pathway of Arabidopsis phytochrome chromophore (adapted from Kohchi et al., 2001).
(B) Chemical structures of heme, BV, PΦB, PCB, and PEB. Heme oxygenase converts heme to BV by an oxidative cleavage between rings A and D at the position marked (arrow).
(C) Red light (R) triggers a "Z" to "E" isomerization in the C-15 double bond between the C and D rings of the linear tetrapyrrole, which is accompanied by rearrangement of the apoprotein backbone. This results in the photoconversion of phytochromes from the Pr form to the Pfr form. Far-red (FR) light converts the Pfr form back to the Pr form.

Seed Germination and Seedlings De-etiolation

At least three phytochromes have been documented to be involved in control of Arabidopsis seed germination: phyA, phyB and phyE. phyA is responsible for the photoirreversible VLFR responses triggered by a broad spectrum of irradiations (ultraviolet, visible and far-red light), while phyB controls the R/FR photoreversible effects of low fluence response (Reed et al., 1994; Botto et al., 1996; Shinomura et al., 1996). Recently, it was found that phyE also plays a role in the control of seed germination by FR light. Either phyE is directly involved in the photoperception of FR for this response or the action of phyA in mediating seed germination requires the presence of phyE (Hennig et al., 2002).

Following seed germination, light acts to inhibit hypocotyl elongation and promote cotyledon expansion. Under far-red light, phyA is probably the only active photoreceptor (Nagatani et al., 1993; Whitelam et al., 1993). In white or red light, although phyB plays a major role, the long hypocotyl and reduced cotyledon expansion phenotype of its null mutants is enhanced by mutations in phyA or phyD, suggesting that multiple photoreceptors sense

Table 3. Different Roles of Phytochrome Family Members in Seedling and Early Vegetative Development

Phytochrome Members	Primary Photosensory Activities	Primary Physiological Roles
phyA	VLFRs FR-HIRs	Seed germination under a broad spectra of light conditions (UV, visible, FR); Seeding de-etiolation under FRc; promoting flowering under LD.
phyB	LFRs R-HIRs EOD-FR (R/FR ratio)	Seed germination under Rc; Seedling de-etiolation under Rc; Shade avoidance response (petiole and internode elongation, flowering).
phyC	R-HIRs	Seedling de-etiolation under Rc.
phyD	EOD-FR (R/FR ratio)	Shade avoidance response (petiole and internode elongation, flowering).
phyE	LFRs EOD-FR (R/FR ratio)	Seed germination; Shade avoidance response (petiole and internode elongation, flowering).

VLFRs: very-low-fluence responses; LFRs: low-fluence responses; HIRs: high-irradiance responses; FR: far-red light; R: red light; FRc: continuous far-red light; Rc: continuous red light; LD: long day light condition; EOD-FR: end-of-day far-red light response; R/FR ratio: red/far-red light ratio perception.

white light during de-etiolation (Neff & Van Volkenburgh, 1994; Johnson et al., 1994; Aukerman et al., 1997). The contribution of phyE to seedling de-etiolation seems negligible (Devlin et al., 1998). Interestingly, a recent high-resolution kinetic analysis of the growth of Arabidopsis seedlings revealed that the red light inhibition of hypocotyl elongation is controlled by a sequential and coordinated action of phyA and phyB. phyA contributes to the initial hypocotyl growth inhibition (first 3 hr of irradiation), while phyB functions in the later phase (Parks and Spalding, 1999).

Previous studies with Arabidopsis phyC overexpressors suggested that phyC likely plays a role in primary leaf expansion (Qin et al., 1997). A more accurate assessment of phyC function became possible until recent identification of *phyC* loss-of-function mutants. *phyC* mutant seedlings exhibited a partial loss of sensitivity to red light, with longer hypocotyls and smaller cotyledons than wild type seedlings. Further, hypocotyl of *phyAphyC* double mutant is significantly longer than that of *phyC* single mutant, suggesting that phyA and phyC acts redundantly to inhibit hypocotyl elongation under red light (Franklin et al., 2003a, Monte et al., 2003). Consistent with this, *phyAphyBphyDphyE* quadruple mutant still retains some weak responses to red light, suggesting that phyC has some roles in mediating red light signaling (Franklin et al., 2003b). However, *phyBphyC* double mutants de-etiolated similarly to phyB single mutants, suggesting that phyC function is dependent on phyB (Monte et al., 2003).

Vegetative Development

The incident light signal embedded in ambient light environments usually activates multiple light signaling pathways and the ultimate cellular responses are determined by the combinatorial effects of these signaling pathways. For example, under a leaf canopy, plants sense the low R/FR ratio of light and initiate the shade-avoidance response by increasing the elongation growth of petioles and stems, the length-to-width ratio of leaves, and accelerating flowering (Smith and Whitelam, 1997). The ability of plants to monitor their light environments and change their architecture provides them with a competitive strategy to survive and finish their life cycle in dense stands.

phyB-deficient plants have a constitutive elongated-petiole and early-flowering phenotype, suggesting that phyB plays a major role in the perception of low R/FR signals (Devlin et al., 1996). Although the monogenic *phyD* mutant plants have no obvious abnormal phenotype, plants impaired in both the *PHYB* and the PHYD genes display significantly longer hypocotyls under either R or white light and flower earlier than the *phyB* monogenic mutants suggesting that phyD performs a similar role of phyB (Aukerman et al., 1997; Devlin et al., 1999).

The shade avoidance responses elicited by low R/FR ratios can be effectively phenocopied by end-of-day far-red (EOD-FR) treatments. *phyAphyBphyD* triple mutant still retains the ability to respond to EOD-FR treatments by developing elongated rosette internodes and accelerated flowering responses (Smith and Whitelam, 1997; Whitelam and Devlin, 1997), implicating the actions of phyC and/or phyE in these responses. The isolation of the *phyE* mutant confirmed this hypothesis. The *phyE* mutants show no phenotypic alteration unless it is in the *phyB* mutant background and the *phyBphyE* double mutants flower much earlier than the phyB monogenic mutants (Devlin et al., 1998; Franklin et al., 2003b). These studies show that phyB, phyD and phyE control shade avoidance responses and inhibit flowering in a conditional redundant manner.

Structural and Molecular Basis for the Differential Functions of Phytochromes

The underlying mechanism for the observed functional differences between different phytochromes and their action modes has begun to be unraveled. Previous experiments by deleting regions, random mutagenesis of the full-length clone, or domain swapping between phyA and phyB have provided some useful information regarding the function of various parts of phytochromes. For example, the N-terminal 70 amino acids (the 6-kD domain) of oat phyA is required for correct chromophore/apoprotein interactions and undergoes a substantial conformational change upon photoconversion of Pr to Pfr. In this region, one subdomain (residues 13-62) is necessary for conformational stability and another one (residues 6-12) is involved in attenuating phytochrome responses (Jordan et al., 1996, 1997). An oat phyA deletion mutant lacking amino acids 7 through 69 is inactive in transgenic tobacco (*Nicotiana tabacum,* Cherry et al., 1992), whereas deletion of amino acids 1-52 of oat phyA causes a dominant negative interference in Arabidopsis (Boylan et al., 1994). Clough et al (1999) showed that both the N-terminal and C-terminal halves of phyA are essential for Pfr degradation. The N-terminal region provides recognition signals for ubiquitin conjugation, and an intact C-terminal domain is essential for phyA breakdown.

A recent study demonstrated that continuous FR light treatment could be replaced by intermittent FR light pulses to induce the FR-HIR responses. Analysis of these action spectra suggests that neither phyA in its Pr form synthesized in the dark nor in its photoconverted Pfr form is active in inducing the signal. Instead the short-lived signal was produced during phototransformation from Pfr to Pr (Shinomura et al., 2000). This is in sharp contrast with the case of phyB. Alternative irradiation with R and FR light photoreversibly switches on or off the phyB responses, indicating that Pfr is the active form from which the R-induced signal is derived.

At the molecular level, it has been demonstrated that expression of the nuclear photosynthetic gene *LHCB* in response to red light depends on both phyA and phyB (Reed et al., 1994; Cerdan et al., 1997). However, phyA and phyB respond to light of different wavelengths and fluences (phyA is responsible for VLFRs, and phyB is responsible for LFRs, Hamazato et al., 1997). Further, it has been shown that phyA mediates the activity of the *LHCB* promoter in response to VLFRs and HIRs by targeting distinct regions of the same *LHCB1*2* promoter (Cerdan et al., 2000), suggesting that these different action modes of phytochromes may entail distinct signaling pathways. Consistent with this notion, *VLF1* and *VLF2* are distinct components of the VLFR pathway of phyA response (Yanovsky et al., 1997), whereas *FHY3* primarily acts in the HIR response pathway of phyA (Yanovsky et al., 2000).

PHYTOCHROME SIGNALING AND THE CIRCADIAN CLOCK

Plants use an array of photoreceptors (including phytochromes) to gather information about the light environment for setting the clock to oscillate with a period of about 24 hours. It has been shown that mutations of photoreceptor genes *PHYA* and *PHYB,* cause the circadian rhythm of *CAB2* promoter activity to oscillate at a pace slower (with a longer period length) than that of the wild type under various light conditions (Somers et al., 1998). This study revealed that in the regulation of the Arabidopsis circadian clock, phyA acts in low intensities of red light and blue light, while phyB functions in high-intensity red light. Recently it was shown that phyD and phyE also act as photoreceptors in red light input to the clock, and that phyA and phyB act additively in red light input to the clock (Devlin and Kay, 2000). On the other hand, the circadian clock affects the outputs (such as floral induction) of light signaling mediated by these photoreceptors, a feedback regulatory mechanism referred to as gating (Millar and Kay, 1996; Anderson et al., 1997). For example, the mRNA abundance and transcriptional levels of *PHYA*, *PHYB* and *PHYC* have been shown to be regulated by the clock with robust rhythmicity, although *PHYD* and *PHYE* expression is weakly rhythmic in Arabidopsis (Bognar et al., 1999; McClung, 2001).

Besides the photoreceptors themselves, several Arabidopsis flowering-time genes have been recently isolated and shown to be associated with the function of the circadian clock. Interestingly, these clock-related genes affect both flowering time and hypocotyl elongation (Dowson-Day and Miller, 1999; Somer, 1999), and they include ELF3, ELF4, CO, TOC1, CCA1, LHY, ADO1/ZTL/LKP1, ADO2/LKP2, ADO3/FKF1, and GI (Hicks et al., 1996; Wang and Tobin, 1998; Schaffer et al., 1998; Fowler et al., 1999; Park et al., 1999; Nelson, et al., 2000; Somers et al., 2000; Strayer et al., 2000; Putterill et al., 1995; Doyle et al., 2002; Table 2). CO is a zinc finger type transcription factor (Putterill et al., 1995), whereas CCA1 and LHY are both MYB-type transcription factors (Wang and Tobin, 1998; Schaffer et al., 1998). *ELF3* encodes a nuclear localized putative transcriptional regulator (Liu et al., 2001) and *ELF4* encodes a novel protein with no significant homology to proteins of known function (Doyle et al., 2002; Khanna et al., 2003). *TOC1* encodes a nuclear protein containing an atypical response regulator receiver

domain and two motifs related to transcriptional regulation: a basic motif conserved within the *CO* family of transcription factors and an acidic domain (Strayer et al., 2000). *GI* encodes a novel protein localized to the nucleus (Fowler et al., 1999; Huq et al., 2000a). ADO1/ADO2/ADO3 are products of a three-gene family and they all contain an amino-terminus PAS domain, multiple kelch repeats, and an F-box (Jarillo et al., 2001). It has been proposed that ELF3 and GI are most likely components of the light input pathway to the circadian clock, whereas TOC1, CCA1 and LHY are likely to be components of the central oscillator. Like *phyB* mutants, both the *gi* and the *elf3* mutants display elongated hypocotyls in red light. However, the *gi* mutants are late flowering, which is in contrast with the early flowering phenotype of the *phyB* and *elf3* mutants. This clearly suggests that ELF3 and GI play different roles or use different mechanisms in controlling hypocotyl elongation and flowering responses. Intriguingly, both ELF3 and ADO1/ZTL/LKP1 are capable of directly interacting with phyB (Liu et al., 2001; Jarillo et al., 2001). It has been suggested that ELF3 and ADO1 could modulate phyB signaling through such interactions (Matsushita et al., 2003), however; the mechanism of such regulation is still unknown. It was recently shown that phyB and ADO1/ZTL do not seem to affect each other's protein levels (Somers et al., 2004).

It has been shown that CCA1 binds in a circadian fashion to a short element of the *LHCB1*1* (*CAB2*) promoter, which is sufficient to confer phytochrome responsiveness and circadian transcription (Wang et al., 1997). Interestingly, the expression of *CCA1* and *LHY* itself is under the control of phytochrome signaling (Martinez-Garcia et al., 2000), suggesting a mechanism for phytochrome control of light input into the clock.

PHYTOCHROMES AS LIGHT-REGULATED KINASES

How do phytochromes initiate their signal transduction upon photo-activation? A long-standing, but much disputed hypothesis is that phytochromes act as light-regulated kinases (Wong et al., 1986; Kim et al., 1989). The recent discovery of phytochrome-like photoreceptors in bacteria, collectively called bacteriophytochromes (BphPs), has generated supporting evidence for such a view (Fankhauser, 2000; Vierstra and Davis, 2000). Phytochrome-like sequences were identified in the cyanobacteria *Fremeyella diplosiphon*, *Synechocystis sp. PCC6803* (Hughes et al., 1997; Hughes and Lamparter, 1999; Kehoe and Grossman, 1996; Wu and Lagarias, 2000), the purple photosynthetic bacterium *Rhodospirillum* and non-photosynthetic bacteria such as *Deinococcus radiodurans*, *Pseudomononas putida* and *Pseudomonas aeruginosa* (Jiang et al., 1999; Davis et al., 1999). Some of them, such as Cph1 of *Synechocystis sp. PCC6803*, can bind to the plant phytochrome chromophore (phytochromobilin PΦB or phycocyanobilin PCB) autocatalytically and display red/far-red absorption spectra similar to plant phytochromes. Further, Cph1 was shown to be a light-regulated histidine kinase. Both autophosphorylation of Cph1 and transphosphorylation of Rcp1 (the response regulator for Cph1) are inhibited by red light and stimulated by far-red light (Yeh et al., 1997; Lamparter et al., 1997), suggesting that the phosphorylation/dephosphorylation event of Rcp1 might be physiologically relevant.

Higher Plants Phytochromes

Higher plants phytochromes share limited sequence similarity with BphPs at their C-termini (Schneider-Poetsch, 1992, Figure 5A). However, plant phytochromes have two additional domains: a serine-rich N-terminal extension domain (NTE) and a PAS repeat domain (PRD) located between the CBD and the HKRD (Figure 5). In addition, mutating several critical residues required for bacterial His kinase activity does not affect the activity of plant phytochromes, suggesting that plant phytochromes are not active His kinases (Vierstra and Davis, 2000). Recently, Clark Lagarias's group developed a recombinant system to express and purify plant phytochromes in yeast. Purified oat phyA expressed in *S. cerevisiae* and the green alga *Mesotaenium caldariorum* phytochrome expressed in *P. pastoris* were assembled with PCB *in vitro*. These phytochromes display the expected spectroscopic properties and protein kinase activity in a light and chromophore-regulated manner. In addition, the purified oat phyA can phosphorylate histone H1 and Rcp1, the response regulator substrate of Cph1. However, unlike their cyanobacterial counterparts, they auto-phosphorylate on Ser/Thr rather than His/Asp. These results provided strong evidence for the kinase property of plant phytochromes (Yeh and Lagarias, 1998).

Physiological Roles of Phytochrome Kinase Activity

The claim that higher plant phytochromes function as protein kinases invite many questions such as what is the biological role of the kinase activity? Which form is more active? Pr or Pfr? Answers to these questions have just begun to be unraveled. For example, recombinant oat phyA is a light and chromophore-modulated protein kinase

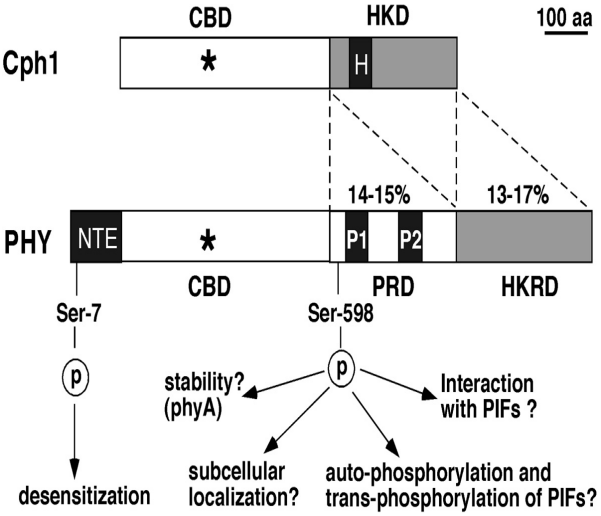

Figure 5. Arabidopsis phytochromes function as light-regulated kinases.
Domain comparison map between the bacterial phytochrome Cph1 and Arabidopsis phytochromes. The conserved cysteine residue for chromophore binding is indicated (*). CBD: chromophore-binding domain; HKD: histidine kinase domain; PRD: PAS related domain; HKRD: histidine kinase related domain. PIFs: phytochrome-interacting factors. (H) highlights the conserved histidine on the HKD domain of Cph1. The percent amino acid identities between the HKD of Cph1 and both PRD and HKRD of Arabidopsis phytochromes are indicated. Also note that Arabidopsis phytochromes have distinct N-terminal extensions (NTE). Autophosphorylation of phytochromes could affect their stability (in case of phyA), their subcellular localization, and their abilities to interact with PIFs. Alternatively, phytochromes could phosphorylate PIFs and modulate their activities. The amino acid numbers correspond to the oat phyA (adapted from Neff et al., 2000).

with Pfr being more active than Pr (Yeh and Lagarias, 1998). A number of phosphorylation sites have been mapped for oat phyA. The Ser-7 is phosphorylated in vivo in both the Pr and Pfr forms, and mutagenesis studies suggest that this residue is implicated in down-regulation of phyA signaling (Stockhaus et al., 1992; Figure 5). The Ser-17 is phosphorylated by protein kinase A *in vitro* only in the Pr form. Mutation of the first 10 Ser of phyA to Ala (all contained within the first 20 aa) or deletion of this region results in a mutant that is hypersensitive to light, suggesting that these Ser residues are involved in desensitization of phyA (Stockhaus et al., 1992; Jordan et al., 1996, 1997).

Another serine residue, Ser-598, is preferentially phosphorylated in the Pfr form *in vivo*, and a S598K mutant loses light-regulated kinase activity (Fankhauser et al., 1999). Further, when an oat *PHYA* cDNA with a Ser-598 to Ala substitution was expressed in the *phyA* mutants, the transgenic plants exhibited hypersensitivity to far-red light, suggesting that the Ser-598 phosphorylation may also serve as a desensitizing mechanism, possibly by disrupting the interactions between phytochromes with their downstream signaling partners (Park et al., 2000, Figure 5). In addition, a recent report showed that phyA is a substrate of a phosphatase, named FyPP (for flower-specific, phytochrome-associated protein phosphatase). FyPP dephosphorylates phyA in a light-dependent manner and modulates phyA control of flowering time (Kim et al., 2002). Taken together, these results suggest that phosphorylation and de-phosphorylation play important roles in regulating phytochrome *in vivo* function.

It should also be noted that there is no evidence for the kinase activity of phyB-phyE and how it may affect light signaling. A recent study showed that although point mutations in the HKRD region of phyB cause strong phenotypes in hypocotyl length and flowering time, indicating that this domain is important for phyB signaling, deletion of this domain resulted in a milder phenotype, suggesting that this domain is dispensable (Krall and Reed, 2000).

PHYTOCHROME-INTERACTING SIGNALING PARTNERS

Phytochrome-interacting Partners (PIFs)

Protein-protein interactions are necessary for many signal transduction cascades. Both general screenings for phytochrome interacting partners and targeted protein-protein interaction studies have identified a number of phytochrome-interacting factors (PIFs). Those include PIF3 (Ni et al., 1998), PKS1 (Fankhauser et al., 1999), NDPK2 (Choi et al; 1999), cryptochromes (both CRY1 and CRY2) and the AUX/IAA proteins (Ahmad et al., 1998; Colón-Carmona et al., 2000; Mas et al., 2000; Figure 6; Table 2). The physiological roles for some of these factors in phytochrome signaling have been substantiated by recent molecular genetic studies. PIF3 is a nuclear localized basic helix-loop-helix (bHLH) protein and was previously thought as a positive regulator of phyB signaling, as transgenic Arabidopsis seedlings with antisense-imposed reduction in PIF3 levels exhibited strongly reduced responsiveness to red light signals (Ni et al., 1998), whereas a T-DNA tagged *pif3* mutant (designated *poc1*) exhibits enhanced responsiveness to red light. This exaggerated response of the *poc1* mutants to red light was originally interpreted as being associated with PIF3 overexpression (Halliday et al., 1999). However, recent re-evaluation of PIF3 function through characteriza-

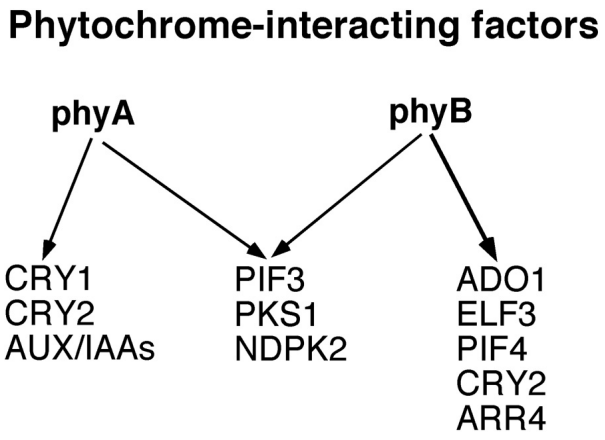

Figure 6. A summary of identified phytochrome-interacting partners (PIFs).
Some PIFs interact with both phyA and phyB, whereas others specifically interact with either phyA only or phyB only. Note that most of these physical interactions were detected with the yeast two-hybrid assay and/or *in vitro* binding assay.

tion of T-DNA insertion loss-of-function mutants as well as overexpression studies with full-length PIF3 showed that PIF3 negatively regulates phyB-mediated hypocotyl elongation and cotyledon opening, but promotes both phyA- and phyB-mediated *CHS* induction (Kim et al., 2003). These studies suggested that phenotypes associated with antisense expression of genes belonging to gene family members (such as PIF3) should be interpreted with extreme cautions, since multiple homologous genes could be simultaneously affected. The exaggerated red light response of the *poc1* mutants might be caused by other complications of T-DNA mutagenesis (such as altered expression pattern) rather than PIF3 overexpression as was claimed originally (Halliday et al., 1999). PKS1 is a basic, soluble, cytoplasmic protein and has been proven to be a substrate for light-regulated phytochrome serine/threonine kinase activity. PKS1 overexpressing plants display less sensitivity to red light, suggesting that it acts as an inhibitor of phyB signaling (Fankhauser et al., 1999). Recent studies with loss-of-function mutants of PKS1 and its closest homolog PKS2 suggested that they are primarily involved in the phyA-mediated VLFR responses. Null mutants of *PKS1* or *PKS2* show enhanced phyA-mediated VLFR responses (with enhanced cotyledon opening, inhibition of hypocotyl elongation, and blocking of greening responses to brief pulses of FR but not to continuous FR or hourly pulses of R), whereas a *pks1pks2* double mutant has no phenotype. In addition, overexpression of either PKS1 or PKS2 causes the same phenotype as the *pks1* or *pks2* single null mutant. These results suggest that PKS1 and PKS2 form a regulatory loop controlling phyA signaling, possibly through their direct physical interaction with each other (Lariguet et al., 2003). NDPK2 (nucleoside diphosphate kinase 2) appears to be a positive regulator of both phyA and phyB signaling. Although hypocotyl elongation is not obviously affected by this locus, its loss of function alleles have a small but significant reduction in cotyledon greening and opening of the hypocotyl/cotyledon hook during de-etiolation (Choi et al; 1999).

Both cryptochromes and the AUX/IAA proteins were identified as *in vitro* substrates of phytochrome kinase activity (Ahmad et al., 1998; Colón-Carmona et al., 2000). Although the phosphorylation of CRY1 is not light-dependent in an *in vitro* experiment, in vivo analysis shows that cry1 phosphorylation is stimulated by red light. Moreover, the identification of the *shy2* mutant as a suppressor of *phyB* and of phytochrome chromophore mutants and the gene encodes *IAA3*, one of the early auxin-inducible genes (Tian and Reed, 1999), suggesting that the interactions between phytochromes and the AUX/IAA proteins and phosphorylation events are likely to be biologically relevant.

A response regulator from Arabidopsis, ARR4, specifically interacts with the extreme N-terminus of phyB both *in vivo* and *in vitro*. Interaction of ARR4 with phyB results in the stabilization of the active Pfr form of the photoreceptor. Accordingly, transgenic Arabidopsis plants overexpressing ARR4 display hypersensitivity to red light with shorter hypocotyls and delayed flowering. Further, transgenic plants overexpressing a mutated form of ARR4 in which the Asp involved in phosphotransfer was substituted by an Asn residue revealed a hyposensitive phenotype regarding all phyB-dependent light responses. These data indicate that phyB is the target of a novel two-component phosphorelay system that modulates red-light-dependent signaling by direct interaction of its response regulator (ARR4) with the photoreceptor (Sweere et al., 2001; Table 2). In addition, *ARR4* expression is rapidly induced by cytokinin (Brandstatter and Kieber, 1998), suggesting a possible link between cytokinin signaling and red light response mediated by phyB.

GENETICALLY IDENTIFIED EARLY INTERMEDIATES OF PHYTOCHROME SIGNALING

Genetic screening for Arabidopsis mutants potentially defective in signaling intermediates either specific to phyA or phyB, or shared by both pathways has identified a number of candidate loci (Hudson, 2000; Figure 7). There are

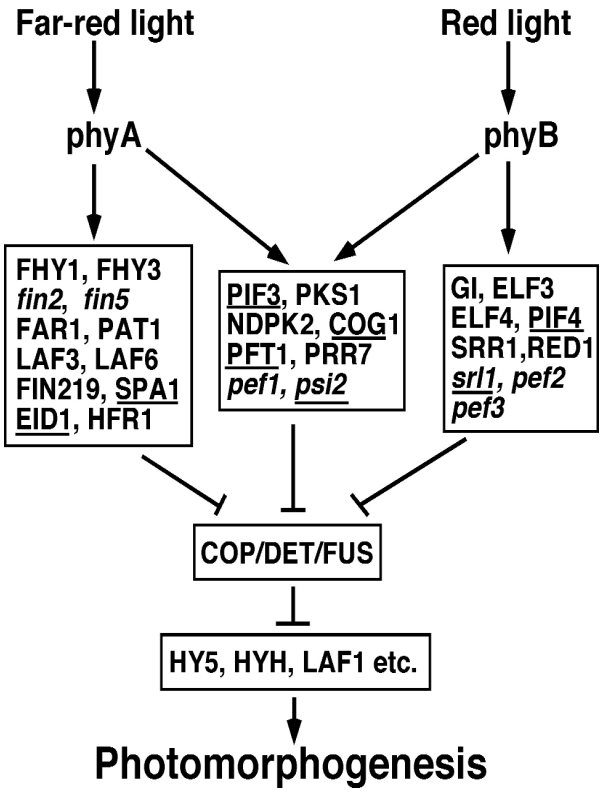

Figure 7. A simplified genetic model for phytochrome-mediated signaling pathways. phyA and phyB signaling entails specific as well as shared components. These loci presumably act upstream of the COP/DET/FUS genes, thus controlling HY5, HYH, LAF1 and the degree of photomorphogenic development. Genes have been characterized at the molecular level are capitalized. The mutant loci have not been cloned are lower case and italicized. Negative regulators of the signaling pathways are underlined. Arrows indicate a positive action, and the bars indicate a repressive effect.

both positively and negatively acting components in each pathway, and many of them have been characterized at the molecular level (for reviews, see Quail, 2002; Wang and Deng, 2003, Table 2).

Phytochrome A-specific Signaling Components

Mutants affected in phyA-specific signaling process were screened under a continuous far-red light (FRc) condition and a number of potential signaling components specific to this pathway have been identified, including FHY1, FHY3 (Whitelam et al., 1993), FIN2 (Soh et al., 1998), FIN5 (Cho et al., 2003); SPA1 (Hoecker et al., 1998), FAR1 (Hudson et al., 1999), FIN219 (Hsieh et al., 2000), PAT1 (Bolle et al., 2000), EID1 (Buche et al., 2000), HFR1/RSF1/REP1 (Fairchild et al., 2000; Fankhauser and Chory, 2000; Soh et al., 2000), LAF1 (Ballesteros et al. 2001) and LAF3 (Hares et al., 2003). The *fhy1, fhy3, fin2, fin5*, *fin219, far1, laf1, laf3, laf6,* and *hfr1/rep1/rsf1* mutants show less sensitivity in continuous far-red light, indicating that their respective genes encode positive regulators of the phyA signaling pathway. On the other hand, mutations in the SPA1 and EID1 genes cause increased sensitivity to the FR light signal, and it is most likely that they act as negative regulators of the signaling cascade.

Among the positive regulators of phyA signaling identified, their loss-of-function mutants (mostly null mutation alleles) only exhibit partial defects with different spectra and strength in phyA signaling, suggesting that phyA signaling involves multiple branches or parallel pathways controlling overlapping yet distinctive sets of far-red light responses (hypocotyl growth, apical hook and cotyledon opening, anthocyanin accumulation, far-red light pre-conditioned blocking of greening, gravitropic response, light-regulated gene expression etc., Barnes et al., 1996; Hudson, 2000; Wang and Deng, 2002). Moreover, the finding that the double mutants *fhy3-1/far1-2, fhy3-1/fhy1-1* and *far1-2/fhy1-1* all display an additive effect, whereas the *fhy3-1/spa1-3* double mutant has an intermediate hypocotyl-length phenotype (Wang and Deng, 2002), also indicates that there is no simple downstream/upstream relationship among these phyA signaling components. Further, complex genetic relationships such as non-allelic non-complementation between *fin2* and *fhy3-1* as well as between *fin219* and *fhy1* have been reported (Soh et al., 1998; Hsieh et al., 2000), suggesting that their gene products may directly interact or engage in extensive cross-talk.

Several phyA signaling intermediates have been characterized at the molecular level (Table 2). LAF6 is a plastid-localized ATP-binding-cassette protein involved in coordinating intercompartmental communication between plastids and the nucleus (MØller et al., 2000). PAT1 is a new member of the GRAS family (Bolle et al., 2000), whereas FIN219 is a GH3-like protein whose expression is rapidly inducible by auxin (Hsieh et al., 2000). Both PAT1 and FIN219 are cytoplasmically localized proteins. *LAF3* encodes two isoforms that differ only at their N termini due to the use of two alternative transcription initiation sites. Both isoforms of LAF3 protein are predicted to contain a transmembrane helical region and have been shown to localize to the perinuclear region, suggesting that LAF3 might regulate nucleo-cytoplasmic trafficking of an intermediate(s) involved in phyA signal transduction (Hare et al., 2003). FHY1, FAR1, FHY3, SPA1, HFR1, LAF1 and EID1 are all nuclear localized factors. Interestingly, *FAR1* and *FHY3* encode two closely related proteins that consti-

tute one branch of a small gene family (Hudson et al., 1999; Wang and Deng, 2002). FHY1 is a novel light regulated protein that accumulates in dark-grown but not far-red grown hypocotyl cells (Desnos et al., 2001). HFR1 is an atypical bHLH transcription factor closely related to PIF3 (Fairchild et al., 2000) and LAF1 is a MYB type transcription activator (Ballesteros et al. 2001). SPA1 possesses a C-terminal WD-40 repeat domain that is most closely related to that of COP1 (Hoecker et al., 1999), whereas EID1 is a novel F-box protein most probably involved in ubiquitin-dependent proteolysis (Dieterle et al., 2001). The biochemical functions of FHY1, PAT1, LAF3, FIN219, FAR1, FHY3 and SPA1 remain largely unknown. Determining the protein-protein interactions among these phyA signaling intermediates and identifying their novel interactive partners should enhance our understanding of the phyA signaling pathway.

Interestingly, a jasmonate response locus, *JAR1*, was mapped to the same locus as *fin219* (Hsieh et al., 2000, Staswick et al., 2002). Unlike *fin219* mutants, several *jar1* alleles are not defective in hypocotyl elongation under far-red light, and unlike the *jar1* alleles, *fin219* shows no increase in resistance to MeJA (Staswick et al., 2002). This discrepancy could be due to the epigenetic nature of *fin219* mutation (Hsieh et al., 2000). Further studies are required to clarify this issue.

Phytochrome B-specific Signaling Components

Putative phyB-specific signaling mutants have also been identified, including *RED1, PEF2, PEF3, GI, ELF3, ELF4,* and *SRR1* (Huq et al., 2000; Liu et al., 2001; Staiger et al., 2003; Doyle et al., 2002; Khanna et al., 2003; Table 2). They share a number of features with *phyB* mutants, such as a long hypocotyl phenotype specifically under red light, early flowering in short days, and elongated petioles, suggesting that these loci positively regulate phyB signaling (Reed et al., 1993; Ahmad and Cashmore, 1996; Wagner et al., 1997). On the other hand, the *srl1* and *srl2* mutants show enhanced responsiveness to red light, suggesting that SRL1 and SRL2 are negatively acting components specific to phyB signaling (Huq et al., 2000b). *SRL2* was shown to encode a PIF3-related protein named PIF4 (Huq and Quail, 2002). *ELF3* encodes a nuclear-localized putative transcriptional regulator that interacts with phyB (Liu et al., 2001). GI is a novel protein that is localized to the nucleus (Fowler et al., 1999; Huq et al., 2000a). *SRR1* codes for a nuclear/cytoplasmic protein that is conserved in most eukaryotes, however; their biochemical activity is not apparent due to the lack of any recognizable domains (Staiger et al., 2003). The *ELF4* gene encodes a novel 111-amino acid protein with no significant homology to proteins of known function (Doyle et al., 2002; Khanna et al., 2003). *red1* is allelic to *sur2* and *atr4* and its wild type gene encodes a cytochrome P450, CYP83B1. The enzyme catalyzes the N-hydroxylation of indole-3-acetaldoxime (IAOx), which is the first committed step in the biosynthesis of indole glucosinolates. IAOx also serves as a precursor for the biosynthesis of IAA. In *red1/sur2/atr4* mutants, synthesis of indole glucosinolates is blocked, leading to hyperaccumulation of auxin and reduced responsiveness to red light. *CYP83B1* transcript levels are induced specifically by continuous red light (Rc) treatment, suggesting that Rc-induction of the *CYP83B1* transcript and auxin homeostasis are necessary for normal seedling de-etiolation under red light (Hoecker et al., 2004). The molecular identities of *SRL1, PEF2* and *PEF3* are currently unknown.

Signaling Components Shared by Both phyA and phyB

Two mutants, *pef1* and *psi2*, affect responses from multiple photoreceptors. The *pef1* mutants show attenuated red and far-red responses, whereas the *psi2* mutant is hypersensitive to red and far-red light, and has necrotic lesions in light-grown plants (Ahmad and Cashmore 1996; Genoud et al., 1998), suggesting that these two loci are shared by both the phyA and phyB signaling pathways. The identities of these genes have not been reported yet.

Recently, COG1, PFT1, PRR7 were reported as signaling components shared by both phyA and phyB (Park et al., 2003; Cerdan and Chory, 2003; Kaczorowski and Quail, 2003; Table 2). *COG1* encodes a Dof transcription factor and its overexpression causes hyposensitive responses to red and far-red light whereas transgenic lines expressing antisense COG1 were hypersensitive to red and far-red light, suggesting that COG1 functions as a negative regulator of both phyA and phyB signaling (Park et al., 2003). *pft1* loss-of-function mutants were hyposensitive to far-red and hypersensitive to red light, and these altered responses require functional phyA and phyB respectively. The opposite effects of *pft1* mutation on phyA and phyB signaling suggest that PFT1 may function at a node where phyA and phyB signaling converge (Cerdan and Chory, 2003). In addition, *pft1* mutation causes late-flowering under long-day conditions (16h light/8h dark) and suppresses the early flowering phenotype of *phyB* in the *pft1phyB* double mutant, suggesting that PFT1 acts downstream of phyB to regulate flowering time. *PFT1* encodes a putative protein of 836 amino acids, with a predicted cWF-A (von Willebrand factor type A) domain in the N-terminus and a glutamine-rich region in the C-terminus. Its ability to activate transcription in yeast suggests that PFT1 could function as a transcriptional co-activator (Cerdan and Chory, 2003). *prr7* loss-of-function mutants exhibit

reduced sensitivity to both red and far-red light, suggesting that PRR7 is a positive regulator of both phyA and phyB signaling. *PRR7* is a member of the Arabidopsis pseudo-response regulator gene family and its gene product is nuclear localized. PRR7 is also involved in the regulated expression of *CCA1* and *LHY*, whose gene products are essential components of the circadian clock. Consistent with this, *prr7* mutants also display a defect in clock regulation of gene expression including *PRR7* itself (Kaczorowski and Quail, 2003).

LIGHT-REGULATED CELLULAR LOCALIZATION OF PHYTOCHROMES

Being synthesized within the cytosol, where phytochrome apoproteins assemble autocatalytically with the plastid-derived chromophore. Early studies using an immunohistological approach and cell fractionation assay suggested that phytochromes are predominantly localized outside the nucleus (Pratt, 1994). Recently, it was shown that upon photoconversion of Pr to Pfr, both phyA and phyB tagged with GUS or green fluorescent protein (GFP) can translocate from cytoplasm into the nucleus where they form intranuclear bodies (NBs) (Sakamoto and Nagatani, 1996; Yamaguchi et al. 1999; Kircher et al., 1999; Nagy et al., 2000). For example, phyB:GFP is localized in the cytosol in etiolated seedlings. phyB:GFP translocates into the nucleus and forms NBs upon treatments with continuous red light treatment, or multiple red light pulses, and this process can be reversed by subsequent far-red treatment, indicating that the nuclear import of phyB is mediated by a low-fluence response (LFR) of phytochrome. On the other hand, nuclear import and NBs formation of phyA:GFP is very rapid (one magnitude of order faster than that of phyB) and can be induced by brief irradiation with red, far-red, or blue light. In addition, nuclear translocation of phyA can also be induced by continuous far-red but not by continuous red light treatment. These observations suggest that the nuclear translocation of phyA is mediated by both the very low fluence response (VLFR) and the far-red high irradiance response (HIR). Further, phyA nuclear translocation is preceded by an even faster cytosolic spot formation of the fusion protein, a phenomenon mimicking SAP (sequestered area of phytochrome) formation, and that the transport seems to be not complete, i.e. a significant portion of phyA:GFP remains cytosolic (Nagy et al., 2000).

A recent report described the steady-state dynamics of phyB localization in response to different light conditions using a 35S-phyB:GFP line. Four different phyB subcellular localization patterns were defined: diffuse nuclear localization, small and numerous NBs only, both small and large NBs, and large NBs only. The amounts of phyB accumulates in NBs is mainly determined by the percentage of active Pfr conformer among the total amount of phyB protein, with large phyB NBs correlate with strong phyB responses (Chen et al., 2003). However, the function of phytochrome NBs is still not clear. They could be sites of sequestration for downregulating phy signaling or active transcription complexes in which phy physically interact with their downstream partners to regulate gene expression.

GFP fusions of full-length phyC-E revealed that the nucleocytoplasmic partitioning of these phytochromes seems not to be regulated by light. They are detected in both the cytosol and the nucleus in etiolated seedlings. However, the formation of NBs for these fusion proteins is light dependent, similar to phyA or phyB:GFP (Nagy et al., 2000). Interestingly, the nuclear import of all phytochrome species is regulated by the circadian clock and displays oscillations under constant conditions, suggesting a new regulatory mechanism modulating phytochrome signaling by the circadian clock (Kircher et al., 2002).

It was until recent that the biological significance of the nuclear translocation events for phytochromes was substantiated by experimental data. Huq et al. (2003) used a glucocorticoid receptor (GR)-based fusion protein system to control the nucleo-cytoplasmic partitioning independent of the light signal. The phyB:GR fusion protein rescued the seedling etiolation phenotype of *phyB* mutant, only in the presence of the steroid dexamethasone (Dex) under continuous red light, suggesting that the Dex-dependent nuclear translocation of phyB:GR is necessary for phyB function (Huq et al., 2003).

At the current stage, very little is known about the molecular machinery and factors modulating the nucleocytoplasmic partitioning and NB formation of phytochromes. It has been speculated that the conformation change to Pfr, their autophosphorylation patterns and their interaction with other proteins may all attribute to the control of their nuclear import (Nagy et al., 2000). In addition, the various phytochrome signaling intermediates described above and other signaling cascades (such as phytohormones) could also affect the intracellular distribution of phytochromes. It is interesting to note that a genetic screen to identify factors required for phyB NB formation resulted in several *phyB* mutants or mutants defective in chromophore biosynthesis, suggesting that functional phytochrome themselves are essential for NB formation (Chen et al., 2003). Further studies aimed to map the possible nuclear localization signal (NLS) and nuclear export signal (NES), and elucidation of the molecular composition of NBs should shed lights on the control of phytochrome localization.

COP/DET/FUS PROTEINS AND REGULATED PROTEOLYSIS IN PHYTOCHROME SIGNALING

Aside from these photoreceptor-specific signaling intermediates, genetic screens have also identified eleven pleiotropic negative regulators of photomorphogenesis (the COP/DET/FUS loci) and one positive regulator (HY5), which function downstream of both phytochromes and cryptochromes (Wei and Deng 1996; Figure 8A and B). *HY5* encodes a bZIP transcription factor whose abundance is correlated with the extent of photomorphogenesis. HY5 activity is primarily regulated at the level of protein stability, which requires the COP/DET/FUS group of constitutive repressors (Oyama et al., 1997; Osterlund et al., 2000). The COP/DET/FUS group of proteins defines four biochemical entities: COP1, COP10, DET1, and the COP9 signalosome [CSN]). Among the COP/DET/FUS proteins, DET1 is distinct and likely involved in controlling chromatin remodeling and gene expression (Pepper et al., 1994; Benvenuto et al., 2002; Schroeder et al., 2002). The other three groups of COP/DET/FUS proteins are all likely involved in regulated proteolysis. COP10 is an E2 ubiquitin conjugating enzyme variant (Suzuki et al., 2002). The COP9 signalosome (CSN) is composed of eight COP/DET/FUS proteins (CSN1-CSN8) and it shares subunit-by-subunit similarity to the lid subcomplex of the 26S proteasome (Kwok et al., 1999; Peng et al., 2001a, 2001b; Serino et al., 1999, 2002; Castle and Meinke, 1994; Karinol et al., 1999; Wei et al., 1994; Schwechheimer and Deng, 2000).

Of these constitutive photomorphogenic repressors, COP1 is capable of directly interacting with HY5 in the nucleus through its WD40 repeat domain and targets HY5 for proteasome-mediated degradation (Ang et al., 1998; Osterlund et al., 2000). Besides a C-terminal WD40 repeat domain, COP1 also contains a RING finger domain in its N-terminal region, followed by a coiled coil in the middle (Deng et al., 1992). RING-finger domains are commonly conserved in a subclass of ubiquitin-protein ligases (E3s, Callis and Vierstra, 2000). It has been recently demonstrated that COP1 could act as an E3 ubiquitin ligase for targeted degradation of HY5, LAF1 (a myb transcription factor) and possibly HYH (a homolog of HY5) (Seo et al., 2003; Osterlund et al., 2000; Holm et al., 2002; Saijo et al., 2003; Figure 9A). Through targeted destabilization of HY5 and other photomorphogenesis-promoting factors, COP1 represses photomorphogenesis. Further, it was recently demonstrated that COP1 could also act as an E3 ligase for the photoreceptor phyA, leading to its elimination, and subsequent termination of phyA signaling (Seo et al., 2004).

Light-induced photomorphogenic development requires the inactivation of these COP/DET/FUS proteins. However, little is known as to how the light-activated photoreceptors (including phytochromes) regulate the activities of those downstream COP/DET/FUS proteins. At least one of the mechanisms is suggested to be the light triggered nuclear depletion of COP1 (von Arnim and Deng, 1994; Osterlund et al., 1999; Osterlund and Deng, 1998; Figure 9B). However, the slow kinetics of COP1 nuclear depletion (could take up to 24 hours, von Arnim and Deng, 1994) suggests an additional, unknown regulatory event (s) is required to account for the early and rapid inactivation of COP1 by light. Blue light signaling has been reported to involve a direct protein interaction of cryptochromes with

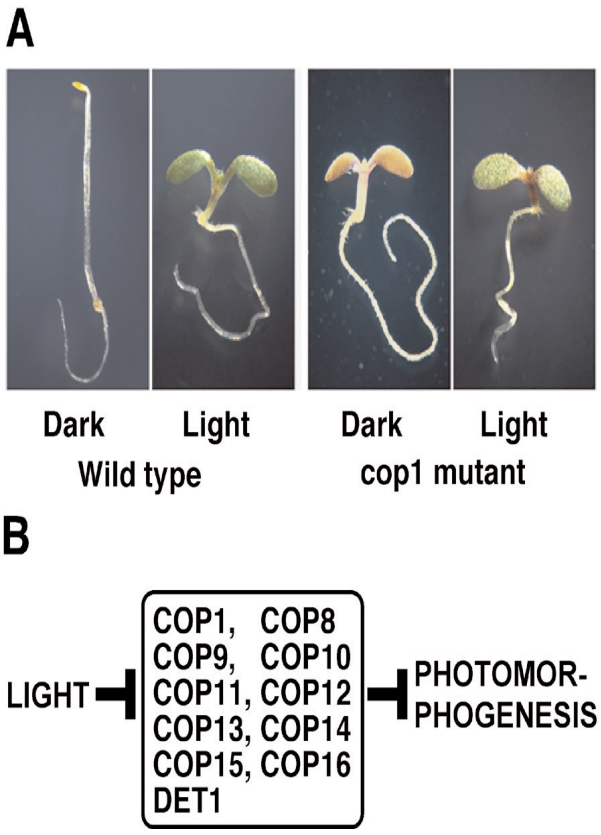

Figure 8. The phenotype of *cop1* (constitutive photomorphogenic) mutants and the proposed roles of the COP/DET/FUS proteins in photomorphogenesis.
(A) Dark-grown *cop1* mutant seedlings phenotypically mimic light-grown wild-type seedlings.
(B) A total of eleven pleiotropic *COP/DET/FUS* loci function as repressors of photomorphogenesis. Light signals perceived by multiple photoreceptors are transduced to inactivate these COP/DET/FUS proteins, and to turn on photomorphogenic development.

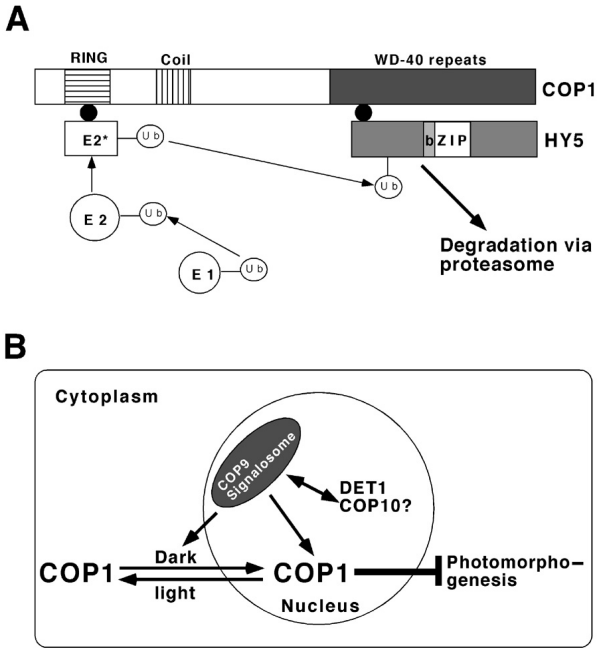

Figure 9. The cellular basis and proposed biochemical mode of COP1 function.
(A) A model depicting COP1 as an E3 ubiquitin ligase. COP1 mediates the ubiquitination of HY5 and its subsequent degradation via the proteasome by recruiting an E2 and HY5 through distinct interacting domains (RING-finger and WD-40 repeat domain, respectively, adapted from Osterlund et al., 2000).
(B) Light-regulated nucleocytoplasmic partitioning of COP1. In darkness, COP1 is enriched in the nucleus to suppress photomorphogenic development. Light signals (perceived by phytochromes and other photoreceptors) trigger the nuclear depletion of COP1, thus abrogating the repressive effect of COP1. Note that the COP9 signalosome may contribute to the control of nuclear localization of COP1 or the stability of COP1 in the nucleus. The roles of DET1 and COP10 in this cellular process have not been determined.

COP1 (Wang et al., 2001; Yang et al., 2001). However, little information is available regarding the molecular link between phytochrome activation and COP1 inactivation.

Recently, three independent studies showed that SPA1, a negative regulator of phytochrome A (phyA) signaling, physically interacts with COP1 *in vitro* and *in vivo,* and that this interaction is reduced by light (Hoecker and Quail, 2001, Saijo et al., 2003; Seo et al., 2003). *SPA1* encodes a 114 kDa nuclear protein with several structural motifs: a protein kinase-like domain at its N terminus, a coiled coil region in the middle, and a C-terminal WD40 domain that is highly related in structure to that of COP1 (Hoecker et al., 1999). It was demonstrated that SPA1 modulates the E3 ubiquitin ligase activity of COP1 on HY5 and LAF1 *in vitro,* leading to their degradation by the 26S proteasome and desensitized light signaling (Saijo et al., 2003; Seo et al., 2003). It is conceivable that SPA1, through direct physical interaction with COP1 and direct interaction with substrate proteins, may help to capture the substrate proteins and bring them to the proximity of COP1. This might be particularly important for light-grown seedlings, as upon light illumination, the abundance of nuclear COP1 is reduced (von Arnim and Deng, 1994) and its efficiency of ubiquitinating substrate proteins in the nucleus will become weakened. Further, the observation that *spa1-3* mutation enhances *cop1* mutant phenotypes in the dark (Saijo et al., 2003) suggests SPA1 has some functional role in darkness as well. SPA1 might synergize with COP1 in the dark to deplete transcription factors, like HY5, HYH and LAF1, more rapidly to prepare plants for upcoming light signals. This model is in good agreement with the results of a physiological study which showed *spa1* mutant was more persistent in both the very-low-fluence response (VLFR) and continuous far-red light induced high-irradiance responses (HIRs) (Baumgardt et al., 2002), possibly due to the delayed turnover of these photomorphogenesis-promoting transcription factors.

The Arabidopsis genome contains three additional *SPA1*-related genes, named *SPA2, SPA3* and *SPA4* (Table 2). Seedlings with mutations in *SPA3* or *SPA4* also exhibit enhanced photomorphogenesis in the continuous far-red, red and blue light, but show no phenotype in the dark, suggesting that SPA3 and SPA4 are also two negative regulators of light signaling. In addition, SPA3 and SPA4 can interact with COP1 in a yeast two-hybrid assay and in vitro interaction assay, suggesting that they may share a conserved mechanism with SPA1 in fine tuning light signaling (Laubinger and Hoecker, 2003).

The finding that EID1, another negative regulator of phyA signaling, encodes an F-box protein and a likely component of a SCF^{EID1} ubiquitin ligase complex (Dieterle et al., 2001) reinforce a possible role of targeted protein degradation in phyA signaling. It is conceivable that phyA signaling may utilize the SCF^{EID1} ligase to mediate regulated proteolysis of various signaling components. Since the level and degradation of phyA are not altered in the *eid1* mutants, FHY3, FAR1, LAF1, PIF3 and HFR1 might be the more likely candidates for the targeted degradation. A defect in the degradation of these positive regulators of phyA signaling could well underlie the observed hypersensitivity of the *spa1* and *eid1* mutants to FR light. It is also possible that SPA1 and the SCF^{EID1} complex may function together with COP1 and the COP9 signalosome to control the timing or the amplitude of the response by timely and precisely regulating the abundance of these signaling molecules.

PHYTOCHROME REGULATION OF NUCLEAR GENE EXPRESSION

The photoregulation of gene expression in higher plants has been extensively studied during the past two decades and a number of photoregulated genes have been identified from several plant species. For example, the transcript level of both the *LHCB* genes for light-harvesting chlorophyll a/b-binding protein of photosystem II and the *RBCS* genes for ribulose-1, 5-biphosphate carboxylase/oxygenase small subunit increases by light illumination, and this light-inducible gene expression is mediated by phytochromes. Consequently, the promoter region of *LHCB* and *RBCS* genes have been isolated and analyzed to identify various cis-regulatory elements responsible for light- or phytochrome regulation, such as the GT-1 boxes of *RBCS*, G-box of tomato *RBCS-3A*, GATA motif, 3AF binding sites and AT-1 binding site. In addition, trans-acting factors which bind to light-responsive cis-regulatory elements (LREs) such as GT-1, GBF, GAF1, 3AF-1 and AT-1 were isolated, and some of which have been shown to be involved in phytochrome-mediated light responsiveness (von Arnim and Deng, 1996; Terzaghi and Cashmore, 1995; Menkens et al., 1995; Kuno et al., 2000; Kuno and Furuya, 2000). Further, it was suggested that the combinatorial interaction of multiple LREs is the key determinant for mediating light control of promoter activity (Puente et al., 1996). However, these traditional studies only revealed limited information on the role of individual phytochromes in the photoregulation of gene expression.

Recently, the newly developed gene chip technology has been applied to study light regulation of gene expression and to define the roles of individual phytochromes (Ma et al., 2001). It was revealed that a large number of genes, possibly over one third of the genome, are coordinately regulated by various light signals. Utilization of the *phyA* and *phyB* null mutants under specific light conditions confirmed that phyA seems to be the primary photoreceptor for mediating far-red light regulation of gene expression, whereas phyB is only one of the phytochromes mediating red light regulation of genome expression (Ma et al., 2001).

The genome profiling study revealed an interesting feature of light-regulated gene expression: many cellular metabolic and regulatory pathways are found to be coordinately regulated by light. Some of them (such as all photosynthetic genes, glycolysis and the TCA cycle etc.) are activated by light, whereas others (such as cell wall-loosening enzymes and water channel protein aquaporins) are repressed by light (Ma et al., 2001). Similar conclusions have also been drawn from studies on circadian clock control of gene expression (Harmer et al., 2000; Schaffer et al., 2001) and genomic analyses of shade avoidance response in Arabidopsis (Devlin et al., 2003).

The microarray technology has also been used to examine the functional relationship of various genetically identified phyA signaling intermediates. A core group of far-red light regulated genes was defined and used as the basis for a comparative analysis of the genome expression profiles of various phyA signaling mutants. The study suggested that FHY1, FHY3 and FAR1 control large numbers of FR-regulated gene expression and likely represent upstream branch components in the phyA signaling network, whereas FIN219, SPA1 and HFR1 control the expression of smaller sets of genes and likely act more downstream in the network (Wang et al., 2003).

AN EMERGING MODEL OF PHYTOCHROME SIGNALING

A Transcription Hierarchy and Roles of a Class of bHLH Proteins

The recent findings that upon photoconversion of Pr to Pfr, phytochromes can translocate from cytoplasm into the nucleus (Kircher et al., 1999; Nagy et al., 2000) and that the Pfr form of phytochromes is capable of physically interacting with a light-responsive element (G box)-bound bHLH protein, PIF3, suggest that phytochromes can function as transcriptional regulators, which would allow plants to change light-regulated gene expression rapidly (Ni et al., 1999; Martínez-Garcia et al., 2000). Further, Dr. Quail's group found that nearly half of the genes responding to the far-red light signal within the first hour are predicted to encode multiple classes of transcriptional regulators (such as the MYB transcription factors CCA1 and LHY, Tepperman et al., 2001). These results suggest a transcriptional cascade may operate in phytochrome control of gene expression. phyA may directly target the light signal to the promoters of a group of early phytochrome-responsive transcriptional regulators (such as PIF3 and HFR1), which in turn regulates the expression of numerous late phytochrome-responsive transcription factors (such as CCA1 and LHY), leading to sequential expression of downstream target genes constituting the phyA-regulated transcriptional profile and the various ultimate cellular responses (Figure 10).

In addition to PIF3, Quail's group found two additional bHLH proteins involved in phytochrome signaling. One of them, PIF4, was isolated as a PIF3-interacting protein and it also interacts specifically with the Pfr form of phyB, but not phyA. PIF4 could form homodimer with itself or heterodimer with PIF3, and all dimeric forms can bind to the

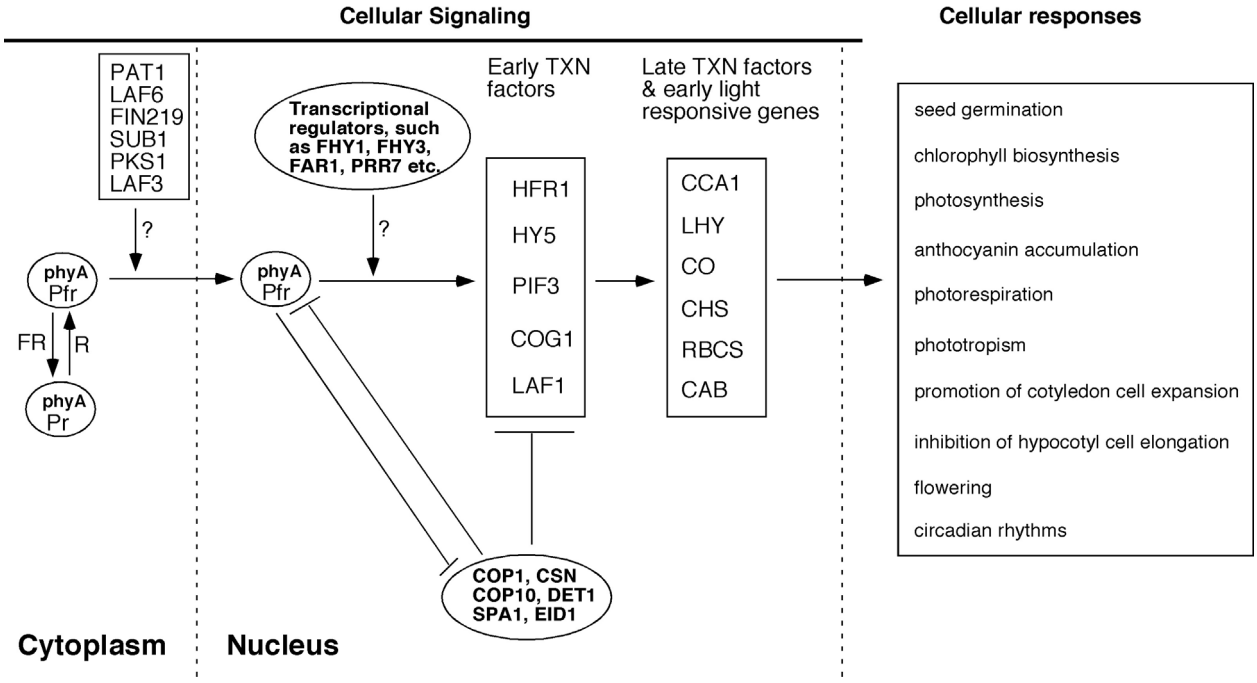

Figure 10. A molecular model depicting phyA control of gene expression and photomorphogenesis.
Upon photoconversion to the Pfr form, phyA translocates into the nucleus, where it regulates the transcription activities of a group of early phytochrome-responsive transcription factors (such as the phyA interactive PIF3 and other genetically defined signaling components HFR1, HY5, LAF1, and COG1 etc.). Many of the encoded early target gene products are transcriptional regulators such as CCA1 and LHY, and they are responsible for orchestrating the expression of somewhat more downstream target genes, thus generating a transcriptional network required for various cellular responses constituting photomorphogenesis. Other nuclear localized phyA signaling intermediates (such as FHY1, FHY3, FAR1 and PRR7) could act as transcriptional co-activators or co-repressors for the various transcription factors. COP1, the COP9 signalosome (CSN), COP10, DET1 and the phyA signaling specific repressors SPA1 and EID1 could interact (directly or indirectly) with these transcription factors and other signaling intermediates and regulate their abundance through a light-regulated proteolysis process. Those cytoplasmically localized phyA signaling molecules, including LAF6, PAT1, FIN219, PKS1, SUB1 and LAF3, could also participate in phyA regulation of gene expression through different mechanisms. Some possibilities include nuclear translocation of phyA and other phyA signaling molecules (such as LAF3 and PKS1), membrane permeability, ion flux and plastid to nucleus signaling (such as LAF6). Activated phyA and subsequent phyA signaling events inactivate COP/DET/FUS gene activities, and abrogate their repressive effect. Conversely, COP/DET/FUS proteins could attenuate phyA signaling through targeted degradation of the phyA photoreceptor itself. R: red light; FR: far-red light; Pr: inactive, Pr conformer of phytochromes; Pfr: active, Pfr conformer of phytochromes; TXN: transcription factor. Arrows indicate a positive action, and the bars indicate a repressive effect. The "?" marks denote undemonstrated mechanisms.

PIF3-binding promoter element. Missense mutants of phyB that are impaired in signaling show reduced binding to PIF4, suggesting a biologically relevant interaction. Overexpressing PIF4 in transgenic Arabidopsis causes a hyposensitive phenotype specific to red light. Conversely, antisense PIF4 plants are hypersensitive to red light. Further, a T-DNA knockout mutant of PIF4, *srl2*, was independently identified from a genetic screen as a hypersensitive mutant under red light (Huq and Quail, 2002; Toledo-Ortiz et al., 2003). Together, these studies suggest that PIF4 acts as a negative regulator of phyB signaling, similar to PIF3. The apparent phenotypes of *pif3* and *pif4* loss-of-function suggest that PIF3 and PIF4 have distinct functions. The construction and analysis of *pif3/pif4* double mutant should reveal more information about their functional relationship in regulating phyB signaling.

On the other hand, HFR1, another bHLH protein, is originally identified as a positive regulator specific to phyA signaling. It has also been demonstrated that HFR1 is capable of forming a homodimer as well as a heterodimer with PIF3 (Fairchild et al., 2000). However, in contrast to PIF3, HFR1 does not bind to either phyA or phyB, although the

HFR1/PIF3 complex can bind preferentially to the Pfr form of both phyA and phyB. Thus, HFR1 may function to modulate phyA signaling via heterodimerization with PIF3. An intriguing question is how HFR1 achieves its specificity in phyA signaling, given that PIF3 binds both phyA and phyB. Further, PIF3 only affects phyB- but not phyA-induced inhibition of hypocotyl elongation, although it affects both phyA- and phyB-induced cotyledon expansion, cotyledon opening, and CHS expression (Kim et al., 2003), suggesting that HFR1 might have a PIF3-indenpendent function (s) in regulating seedling photomorphogenesis, depending on the types of light responses and light conditions. Consistent with this, a recent study showed that HFR1 is a positive regulator of cry1-mediated blue light signaling (Duek and Fankhauser, 2003). Further, HFR1 mRNA is more abundant in far-red and blue light than in continuous red light, suggesting a potential mechanistic basis for the specificity of HFR1 in regulating signaling (Fairchild et al., 2000; Duek and Fankhauser, 2003).

Furthermore, another bHLH protein, named PIL1 (for PIF3-like 1), was recently shown to be required for the rapid shade avoidance responses (such as elongation of internodes and petioles, reduced leaf growth and enhanced apical dominance). *PIL1* transcript level increases rapidly up to 35 folds in response to low R/FR ratio (within an hour), and the increase can be detected as early as 8 minutes of lowR/FR. Interestingly, the change of *PIL1* transcript levels is reversible upon transferring from low R/FR to high R/FR, and is gated by the circadian clock. It has been proposed that in response to low R/FR, plants sense a change in Pfr level of phyB, D and E, and initiate a rapid increase in *PIL1* transcript and protein levels, followed by expression of target genes required for differential elongation growth (Salter et al., 2003). It will be very interesting to define the transcriptional regulators responsible for the rapid increase of *PIL1* levels in response to low R/FR ratio.

The Arabidopsis genome contains a large gene family for bHLH proteins, with up to 162 members (Toledo-Ortiz et al., 2003; Bailey et al., 2003; Arabidopsis Genome Initiative, 2000). Due to the known nature of bHLH proteins to form homodimers and/or heterodimers, other bHLH protein could also be involved in regulating phytochrome signaling. It is imaginable that combinatorial heterodimeric interactions of different members of the bHLH family could potentially generate a vast array of transcriptional activities that are essential for plants to respond to the incidental light signal (Quail, 2000, 2002). It should be noted that how phytochromes regulate the transcriptional activities of these bHLH proteins is currently unknown. By interacting with PIF3, phytochromes could regulate transcription either by functioning as a transcriptional co-activator or co-repressor or by altering the biochemical/allosterical nature of PIF3 (Quail, 2000). With the knowledge that the activities of a number of transcription factors are regulated by phosphorylation (Hardtke et al., 2000) and that phytochrome is a light-regulated kinase, it will be interesting to test whether PIF3 is a substrate of phytochrome kinase activity and how it may modulate the function of PIF3.

Cytoplasmic Signaling Events

Besides nuclear signaling events, phytochrome signaling likely entails cytoplasmic events. First, the nuclear import of some phytochrome species (such as phyB) takes hours (Nagy et al., 2000) and their nuclear actions obviously could not explain some rapid phytochrome responses (occur within minutes of irradiation), such as the change in hypocotyl growth rate (Parks and Spalding, 1999). Second, phytochromes interact with a number of cytoplasmic proteins identified so far (such as PKS1 and NDPK2, Fankhauser et al., 1999; Choi et al., 1999). Third, genetic studies have identified cytoplasmic proteins as signaling intermediates (such as PAT1 and FIN219 for phyA signaling, Bolle et al., 2000; Hsieh et al., 2000).

Previous microinjection and pharmacological studies have suggested the involvement of G-proteins, cGMP and Ca^{2+}/calmodulin in phytochrome signaling (Bowler et al., 1994; Neuhaus et al., 1997). Particularly, heterotrimeric G-proteins have been implicated in several processes during plant growth and development, and they transduce extracellular signals to the cell. In general, heterotrimeric G-proteins consist of three subunits: α, β, and γ. Analysis of the complete genome sequence of Arabidopsis indicated that the Arabidopsis genome contains only a single canonical G_α gene, previously designated *ATGPA1* (Ma et al., 1990), and a single G_β gene, designated *AGB1* (Weiss et al., 1994) and possibly one G_γ subunit (Mason and Botella, 2001; Table 2).

It was reported that transgenic Arabidopsis plants conditionally overexpressing the G_α subunit of the heterotrimeric G-protein under the control of a glucocorticoid-inducible promoter exhibited a light-dependent hypersensitive response as a result of reduced hypocotyl cell elongation (Okamoto et al., 2001). However, a separate study reported that loss-of-function *gpa1* mutants display partial de-etiolation in the dark, with short hypocotyls and open apical hooks typical of light-irradiated seedlings. The short hypocotyl of *gpa1* seedlings is reportedly due to a defect in cell division, not cell elongation (Ullah et al., 2001). To resolve these discrepancies regarding GPA1 function in photomorphogenesis, Jones et al. (2003) re-evaluated the roles of the heterotrimeric G protein employing multiple alleles of both *gpa1* and *gpb1* (in both Col and WS backgrounds) and their double mutants. They concluded that these mutants have wild-type sensitivity to red and far-red

light. In addition, there is no apparent alternation in R or FR sensitivity in transgenic plant overexpressing GPA1 (wild-type or a constitutively active form) or GPB1. The observed shorter hypocotyls and partially open cotyledons of *gpa1* and *gpb1* mutants grown in the dark are mainly caused by a defect in cell division, rather than cell elongation (Jones et al., 2003).

Genetic studies have also provided supporting evidence for the involvement of Ca^{2+} in phytochrome signaling. The *sub1* mutant exhibits hypersensitive responses to both far-red light and blue light. The *SUB1* gene was found to encode a Ca^{2+}-binding protein. Genetic interaction studies suggest that SUB1 is a component of a cryptochrome signaling pathway and is a modulator of the phyA signaling pathway. Further, SUB1 negatively regulates HY5, a bZIP transcription factor and a positive regulator of photomorphogenesis (Guo et al., 2001; Table 2).

Some early studies also suggested that phytochrome could exert its effects by rapidly altering the permeability of the plasma membrane to ions. This might be true for certain light responses,such as the bud induction process of the moss *Physcomitrella patens* and the unrolling of the primary leaf wrapped within the oat coleoptiles (Kendrick and Bossem, 1987; Ermolayeva et al., 1997; Viner et al., 1988). However, there is no report for changes in cytoplasmic ion (such as Ca^{2+}) concentration or ion fluxes in Arabidopsis hypocotyl cells in response to light (Parks and Spalding, 1999; Spalding, 2000). Thus, the role of ion flux and membrane depolarization in phytochrome control of Arabidopsis photomorphogenesis remains elusive.

POTENTIAL APLICATIONS IN AGROBIOTECHNOLOGY

Light is one of the major environmental signals governing many aspects of plant growth and development. Over the past decade or so, we have seen rapid progress in our understanding of the light signaling mechanisms controlling plant development, largely due to the utilization of Arabidopsis thaliana as the model experimental system. As in dicot plants, light controls many aspects of development in monocot plants. For example, seed germination, elongation of coleoptiles and mesocotyls, unrolling of the leaf blade, chloroplast differentiation, pigment synthesis and control of flowering time are all regulated by light in cereals such as oats, maize, barley and rice (Kendrick and Kronenberg 1994; Yano et al., 2001). Light regulation of monocots gene expression, such as that of *LHCB* (Iwasaki et al., 1997), *RBCS* (Kyozuka et al., 1993) and *PHYA* (Kay et al., 1989), has also been reported. The monocots and in particular the grasses, are by far the most agronomically important group of plants. Rice, maize, wheat and sorghum are staple food crops throughout the world and have been under intense human selection pressure for at least 10,000 years. Agronomically important traits such as high yield, early flowering and increased stand densities are all influenced by light (Smith, 1994). Nevertheless, the genetic characterization of light signaling pathways in monocots has just begun. Accumulating evidence suggests that monocots and dicots might share conserved signaling components/mechanisms. For example, mutations in phytochrome genes or function resulted in early flowering in barley, sorghum, rice, and maize (Hanumappa et al., 1999; Childs et al., 1997; Izawa et al., 2000; Basu et al., 2000; Sawers et al., 2002). Homologous proteins for the downstream COP/DET/FUS proteins, a group of general repressors of photomorphogenesis in Arabidopsis, have also been identified in monocots. For example, the rice COP1 is 73% identical to the Arabidopsis COP1 and it can functionally complement an Arabidopsis *cop1* mutant (Tsuge et al., 2001). In addition, attempts have been made to genetically modify some horticultural and agricultural crops and promising results have been achieved (Robson et al., 1996; Robson and Smith, 1997; Halliday et al., 1997; Thiele et al., 1999). For example, overexpressing oat phyA in transgenic tobacco and hybrid aspen alleviates the shade-avoidance response, thus increasing dwarfism and harvest index (Robson et al., 1996; Robson and Smith, 1997; Olsen et al., 1997). Overexpressing Arabidopsis phyB in potato increases potential tuber yield both in greenhouse and under field conditions, particularly at high densities (Jackson et al., 1996; Thiele et al., 1999; Boccalandro et al., 2003). In the future, it should be possible to modify agricultural crop plants with genetically improved phytochrome or their downstream signaling molecules to develop transgenic crop species with more fine tuned light-responsive developmental traits to better fit their ambient light environments.

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