CMLS, Cell. Mol. Life Sci. 55 (1999) 153–165
1420-682X/99/020153-14 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Light perception in higher plants

A. Batschauer

FB Biologie/Botanik, Philipps-Universität Marburg (Germany), Fax + 49 6421 281545,
e-mail: batschau@mailer.uni-marburg.de

Abstract. Photosynthetic plants depend on sunlight as their energy source. Thus, they need to detect the intensity, quality and direction of this critical environmental factor and to respond properly by optimizing their growth and development. Perception of light is accomplished by several photoreceptors including phytochromes, blue/ultraviolet (UV)-A and UV-B light photoreceptors. In recent years, genetic, molecular genetic and cell biological approaches have significantly increased our knowledge about the structure and function of the photoreceptors, and allowed the identification of several light signal transduction components. Furthermore, this research led to fruitful interaction between different disciplines, such as molecular biology and ecology. It is safe to assume that we can expect more milestones in this research field in the upcoming years.

Key words. Phytochrome; blue light receptor; signal transduction; *Arabidopsis* mutants.

Introduction

Plants are able to detect the quality, quantity and direction of light and to use it as an external signal to optimize their growth and development. The majority of developmental processes throughout the entire life cycle of plants are influenced by light: seed germination, seedling development, sensing neighboring plants, phototropism (the bending response in relation to the direction of the light) and induction of flowering. Light is perceived by several photoreceptors, which detect different facets of the solar spectrum. These photoreceptors include the phytochromes (Phy), which are responsible for the detection of far-red (FR) and red (R) light. The blue/UV-A photoreceptors sense the blue and UV-A part of the spectrum and some of them have recently been molecularly cloned [1–5]. Essentially nothing is known yet about the molecular nature of the UV-B photoreceptors, which, for example, regulate the formation of UV-shielding pigments [6–8].

Our knowledge about plant photoreceptors, their function and signal transduction was significantly extended by recent progress in the identification of photoreceptor and signal transduction mutants. Most of these studies were done with the model plant *Arabidopsis thaliana*. Thus, here I focus mostly on results obtained with this species. Several excellent reviews on the same topic have been published recently, and I refer the reader to these for additional information or other points of view [6, 9–24].

The phytochromes

Phytochromes are the best-characterized plant photoreceptors. They are encoded by gene families consisting of five members (*PHY A-E*) in the model plant *Arabidopsis* [16, 25–27]. The phytochromes are responsible for the detection of R and FR. Phytochromes are polypeptides of about 125 kDa carrying a chromophore, the phytochromobilin, which is a linear tetrapyrrole covalently bound to a conserved cysteine residue in the N-terminal region (fig. 1). Phytochromes exist in two forms, the red light absorbing P_r ($\lambda_{max} = 660$ nm) and the far-red light absorbing P_{fr} ($\lambda_{max} = 730$ nm). Upon absorption of red light P_r is converted to P_{fr}, and P_{fr} is converted back to P_r upon absorption of far-red light. The photoreversiblity of phytochrome, which persists in vitro, was an ideal feature for its purification and identification as a photoreceptor. Phytochrome is synthesized in the P_r form [28]. Although the two forms of phytochrome have different spectral absorption ranges, these overlap. Due to this overlap, the photoequilibrium of P_{fr}/P_{tot} depends on the wavelength and is about 80%

in red (660 nm), 3% in far-red (730 nm) and about 40% in blue (450 nm) light (fig. 1). Due to the activation of phytochrome by blue light, a photoresponse induced by blue can therefore be caused by phytochrome or specific blue light receptors. P_{fr} is the active form of phytochrome, although some studies with phytochrome mutants also indicate a physiological function for P_r [29–31]. Phytochromes are dimers, and there is so far only evidence that a phytochrome monomer dimerizes with another identical phytochrome monomer, such as PhyA with PhyA or PhyB with PhyB [19]. The localization of phytochrome has been analyzed with different methods. Most studies support the view that phytochrome is a soluble protein localized in the cytosol [16, 19]. However, recent work by Nagatani and coworkers demonstrated that PhyB is localized in the nucleus, and that the translocation between the cytosol and the nucleus is light-regulated [32]. Besides the localization in different compartments and other differences to be discussed below, the stability of the P_{fr} form of PhyA is about 100-fold less than the stability of other phytochromes. Classical photobiological studies and the use of monoclonal antibodies against different phytochromes had already demonstrated that there are two distinct pools of phytochrome, which had been named type I and type II. Type I phytochrome is much more abundant in dark-grown seedlings than type II. After transfer to light, type I phytochrome rapidly decays, resulting in an about 100-fold decrease of type I phytochrome in green plants compared with dark-grown seedlings, whereas the pool of type II phytochrome is relatively unaffected [9, 15, 16, 19]. Degradation of type I phytochrome is mediated by the ubiquitin system [33]. As a result of this degradation, not is only the total amount of phytochrome reduced but the ratio between the different pools is also changed. It has been demonstrated that PhyA is a type I phytochrome, whereas PhyB–E are light-stable in *Arabidopsis* and very likely constitute the pool of type II phytochrome [27, 34]. Due to the dramatic decrease in the amount of PhyA during deetiolation, phytochrome responses in green plants are dominated by PhyB (and other light-stable phytochromes), whereas phytochrome responses in etiolated seedlings are dominated by PhyA.

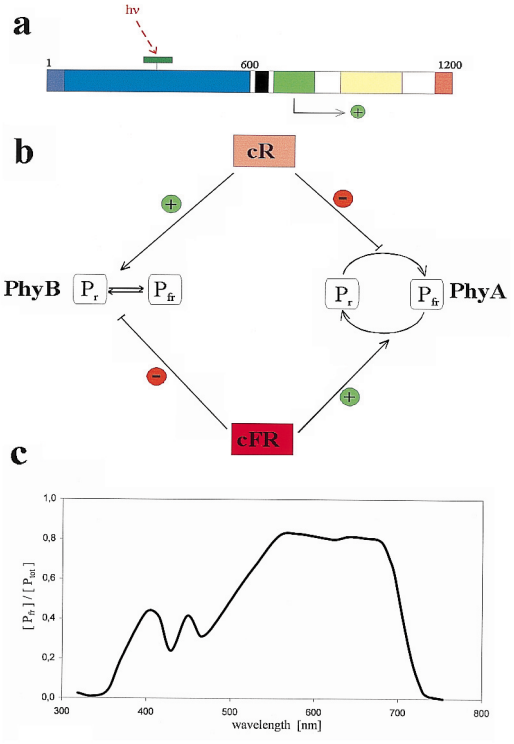

Figure 1. Scheme of the phytochrome domains, the antagonistic effects of PhyA and PhyB in the deetiolation process and the ratio of P_{fr}/P_{tot} under different light regimes. (*a*) The figure highlights the various functional domains of phytochrome. The linear tetrapyrrol chromophore is indicated by a green rectangle. The N-terminal 600-amino acid region (blue) determines the photosensory specificity of phytochrome. The region necessary for signal transduction (green) is separated from the photosensory domain by the protease-sensitive hinge region (black). The C-terminal part contains also the dimerization domain (yellow). Regions affecting the biological activity at the N-terminal and C-terminal end are indicated in violet and red (adapted from [16, 24]). (*b*) Model of the antagonistic effects of PhyA and PhyB in continuous red light (cR) and continuous far-red light (cFR) on the deetiolation process. High-intensity cR promotes the deetiolation (⊕) via PhyB, and high-intensity cFR promotes deetiolation via PhyA (FR-HIR). In contrast, deetiolation is suppressed (*θ*) by high fluence rates of cFR (absorbed by PhyB) or by high fluence rates of cR (absorbed by PhyA). (*c*) Shown is the ratio of the FR-absorbing form of phytochrome (P_{fr}) to the total amount of phytochrome (P_{tot}) as a function of wavelength in photostationary equilibrium (adapted from [101, 104]).

The molecular cloning of phytochromes and the construction or isolation of modified versions allowed the identification of regions and amino acids critical for proper function. For example, recombinant PhyA, PhyB and PhyC were expressed in *Escherichia coli* and yeast cells. Addition of phycocyanobilin (a chromophore very similar in structure to the authentic chromophore) to the purified apoprotein or yeast cells expressing phytochrome, resulted in the formation of photoreversible phytochrome, demonstrating that the chromophore is autocatalytically attached to the target cysteine [35–44]. Subdomains located within the N-terminal 600 amino acids are important for chromophore assembly and spectral integrity [45, 46]. Similarly, subdomains were identified which are necessary for dimerization and located at the center and close to the C terminus of the polypeptide (fig. 1) [47–49].

Phytochrome function

The primary mechanism of phytochrome action is still not known. The discovery of a phytochrome gene in the

moss *Ceratodon purpureus* having a C-terminal extension with similarity to serine-threonine and tyrosine protein kinases [50] led to the hypothesis that the *Ceratodon* phytochrome is a light-regulated kinase [51, 52], and that higher plant phytochromes might also have kinase activity. Nevertheless, kinase activity of the plant phytochromes has not yet been demonstrated. Based on sequence alignments, Schneider-Poetsch and co-workers found similarities to bacterial histidine kinases in the C-terminal region of phytochromes [53, 54]. Recently, a model in which phytochrome has kinase activity has received much support from the discovery that the cyanobacterium *Synechocystis* sp. PCC6803, whose genome has been completely sequenced, contains a gene coding for a protein with about 30% identity to the N-terminal region of plant phytochrome including the chromophore attachment site. The C-terminal part of this *Synechocystis* gene has striking sequence similarity to the two component sensor histidine kinases. It has also been shown that the recombinant *Synechocystis* protein attaches the chromophore (a linear tetrapyrrol) autocatalytically and is photoreversible [55–58]. In addition, the recombinant chromoprotein is able to autophosphorylate and to transfer the phosphate to another protein encoded in the same operon [56]. Thus the *Synechocystis* phytochrome-like protein has all the characteristics of a two-component sensory system. Plant hormone receptors related to two-component systems have been identified before [59–62]. It is therefore attractive to speculate that the plant phytochromes also belong to this class of sensor proteins. So far there is no definite proof for that, and the similarity to histidine kinase could just be a relic of the evolutionary origin of phytochrome.

As already mentioned, the classical criterion for a phytochrome response is reversibility (a response activated by a single R pulse can be suppressed by giving an FR pulse after the R pulse). There are other phytochrome responses, which are induced by such low intensities of (R) light that the P_{fr}/P_{tot} ratio is within the same range as when a saturating FR pulse alone is given. Such a response can therefore not be reversed by FR treatment. The response to such low light intensities is called very low fluence response (VLFR) [15, 19, 21, 63]. VLFR can be induced by R in the range between 10^{-10} and 10^{-7} mol m^{-2}. In addition, there are phytochrome responses which depend on prolonged exposure to relatively high light intensities, the so-called high irradiance response (HIR). Like the VLFR, the HIR is not photoreversible. The 'classical' HIR described by Hartmann [64] operates in continuous FR, but there is also an HIR in R and other light qualities [19, 65]. Since HIRs depend on prolonged light exposure, the reciprocity law is not valid for these response modes. The low fluence response (LFR, 10^{-6}–10^{-3} mol m^{-2} of R) can be reversed by FR treatment. Based on mutant studies (see below) and theoretical calculations it is assumed that the $PhyA_rPhyA_{fr}$ heterodimer can induce a response, whereas PhyB is only active in the $PhyB_{fr}PhyB_{fr}$ homodimeric form [19].

Due to the identification of mutants deficient in defined phytochromes and overexpression of different phytochromes in wild-type and (phytochrome) mutant backgrounds, it was possible to assign specific responses to individual phytochromes. PhyA-deficient *Arabidopsis* do not germinate in high-intensity FR and poorly in very-low intensity light over the whole range from UV-A to FR [66]. The VLFR seen for the expression of the gene encoding the chlorophyll a/b-binding protein (CAB) is missing in the *Arabidopsis phyA* mutant but is present in the *phyB* mutant [67]. Inhibition of the extension growth of the hypocotyl under high-intensity FR is not observed for the *phyA* mutant [25, 26, 68, 69], whereas *phyB* mutants are not affected in these responses. On the other hand, *Arabidopsis phyB* mutants fail to inhibit extension growth of the hypocotyl [26, 68, 70] and to germinate [31, 71] under continuous low to high fluence R. These examples demonstrate that different phytochromes have distinct functions, namely PhyA mediates responses under FR-HIR and VLFR conditions and PhyB under LFR and R-HIR conditions.

Although, PhyA and PhyB are the most prominent phytochromes in *Arabidopsis*, *phyA*/*phyB* double mutants still show phytochrome responses. For example, seed germination in the *phyA*/*phyB* double mutant is still R/FR-reversible, indicating that the involved phytochrome belongs to the LFR type, but shows a fluence rate dependency similar to PhyA [66, 72]. PhyC function has been studied by overexpression. Transgenic tobacco and *Arabidopsis* lines overexpressing PhyC both show an R-dependent increase in leaf expansion [73, 74]. Since neither PhyA nor PhyB overexpression affects leaf expansion, regulation of leaf expansion seems to be a specific function of PhyC. In contrast to the *phyA* and *phyB* mutants which have been identified in mutant screens, PhyD is lacking in a 'normal' ecotype of *Arabidopsis*, the ecotype Wassilewskija [75], indicating that PhyD function is not essential under natural conditions. Nevertheless, the *phyD* mutant shows slight defects in R responses especially when combined with the *phyB* mutation [75]. Little is known so far about the role of PhyE.

In deetiolation and some other processes as well, the different phytochromes can act not only additionally but also antagonistically, depending on the ratio of R to FR (fig. 1). Continuous high-intensity FR induces deeti-

olation via PhyA action (FR-HIR); continuous R induces deetiolation via PhyB action. Conversely, enrichment of the incident light with FR suppresses PhyB, and enrichment of incident light with R suppresses PhyA. Thus, the ratio between R and FR, which alters dramatically under natural conditions (e.g. FR enrichment under the canopy due to absorbance of R by chlorophyll), is the determinant of the relative influence of different phytochromes to mediate a response [15, 16]. Although PhyA plays only a minor role in green *Arabidopsis* plants, it is essential when seeds germinate under the canopy but has almost no effect when seeds germinate in open sunlight [69, 76]. In green plants PhyB (and other light-stable phytochromes) dominates and exhibits among other responses the shade-avoidance response under FR enrichment [15, 77].

As outlined above, different phytochromes have different response modes: PhyA mediates VLFR and FR-HIR; PhyB mediates the LFR. The different response modes of phytochromes may suggest that different phytochromes use different signaling pathways. But domain-swapping experiments performed by Quail and co-workers argue against it. In these experiments, the coding regions for the N-terminal domain (carrying the chromophore binding sites) of *Arabidopsis* PhyA and PhyB were fused to the coding region of the C-terminal domain of PhyB and PhyA, respectively. Overexpression of these constructs in transgenic *Arabidopsis* has demonstrated that the N-terminal domains contain the photosensory specificities, since overexpression of PhyA-PhyB fusion results in the same phenotype as overexpression of PhyA, whereas overexpressors of PhyB-PhyA fusion have the same phenotype as PhyB-overexpressing lines [78]. Since the N-terminal domains of both PhyA and PhyB alone lack biological activity [49, 79, 80], the C-terminal domain is important for signal transduction. As demonstrated by the domain-swapping experiments, the C-terminal domain is exchangeable between the two phytochromes, thus indicating that the primary mechanism of PhyA and PhyB action is the same. In the same line are the observations that overexpression of full-length *Arabidopsis* PhyB as well as either its C- or N-terminal domain interferes with PhyA activity [32, 81]. In addition to the domain-swapping experiments, mutant alleles of *PHYA* and *PHYB* were identified which affect signal transduction. All of the *phyB* and the majority of the *phyA* mutations detected in this screen reside within a 160-amino acid-long region, especially in the stretch between positions 776 and 793 [16, 82]. One can conclude that the 160-residue-long region of both PhyA and PhyB is directly involved in the interaction with a downstream component and, as already concluded from the domain-swapping experiments, that the primary partner could well be the same for both PhyA and PhyB. However, since mutants specific for either PhyA or PhyB signal transduction were identified (see below), it is difficult to understand how a common partner for PhyA and PhyB could channel signals into pathways specific for PhyA or PhyB. Besides the domains necessary for chromophore binding, photoreversibility and signal transduction, further functional domains of phytochrome were identified by overexpression of deletion derivates of phytochrome. Deletion of the N-terminal 52 amino acids of oat PhyA resulted in a loss of FR sensing and signaling [79, 83]. Exchanges of several serine residues in this region to alanine or deletion of the N-terminal serine-rich region results in an enhanced biological activity of the overexpressed phytochrome [49, 84, 85]. It has also been shown that one specific serine residue in this region is phosphorylated [86]. Taken together, mutant studies and analysis of transgenic plants overexpressing different phytochromes have provided detailed knowledge about the function of the different phytochromes and about domains involved in phytochrome function.

Blue light photoreceptors

Blue light affects several processes such as growth of the hypocotyl, stem, cotyledons and the leaves, stomata opening, phototropism, flowering and gene expression [12, 13, 22, 23, 87–89]. Although phytochromes affect most of theses processes and are also activated by blue and UV light, several lines of evidence demonstrated the involvement of specific blue/UV-A photoreceptors. Action spectroscopy indicated that either flavins, pterins or carotenoids could act as chromophores of blue light receptors [90–93]. Very recent studies on the chromophore composition of cloned blue light receptors (see below) support the flavin and pterin models for blue light receptors presented in the past [90, 91]. Up to now, three blue light receptors or likely blue light receptor candidates have been cloned from *Arabidopsis* (fig. 2), and mutants have been identified for each. Koornneef and co-workers isolated several mutants, which in contrast to wild type have a long hypocotyl when grown in the light (*hy* mutants). One of these mutants (*hy4*) is affected in blue light-dependent inhibition of hypocotyl elongation [94]. Besides hypocotyl elongation, *hy4* mutants are also affected in blue light-regulated formation of anthocyanin and expression of the first enzyme in anthocyanin biosynthesis, chalcone synthase (CHS) [1, 94–96]. A transfer DNA (T-DNA)-tagged *Arabidopsis* line was used to isolate the *HY4* gene [1]. The *HY4* gene encodes a protein with about 30% identity within the first 500 amino acids to the enzyme DNA photolyase from *E. coli*. The remaining C-terminal part, which is

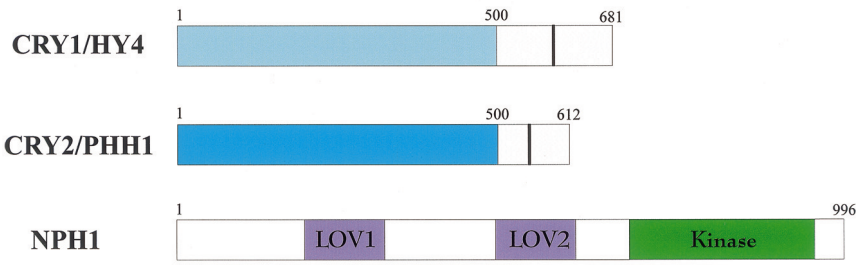

Figure 2. Scheme of the three cloned blue light receptors of *A. thaliana*. CRY1/HY4 and CRY2/PHH1 share significant similarity with class I DNA photolyases in the region between amino acid residues 1–500 (indicated in blue). As far as tested, this region binds the chromophores FAD and MTHF. CRY1/HY4 and CRY2/PHH1 are 54% identical in the N-terminal 500-amino acid region, but are very divergent in the C-terminal extensions except sharing a short serine-rich stretch (indicated in black). In the N-terminal region of NPH1 are two domains of about 100 amino acids (LOV1 and LOV2), sharing 43% identity and thought to be involved in sensing the light signal. The C-terminal part of NPH1 contains 11 sequence motifs typical of serine-threonine kinases (shown in green) (adapted from [101]).

lacking in DNA photolyases but is essential for HY4 function shares some similarity with tropomysin A of rat smooth muscle [1]. The *E. coli* DNA photolyase belongs to the so-called class I DNA photolyases present in many microbial organisms, which repair the cis-syn cyclobutane pyrimidine dimers of UV-B-damaged DNA. Light energy in the blue/UV-A region is used for the catalysis [97]. Screening for further mutant alleles of *hy4* led to the identification of residues essential for HY4 function. These studies demonstrated that amino acid changes in the HY4 protein which affect the growth response of the hypocotyl in blue light also affect the accumulation of anthocyanin [96]. Despite the significant similarity of the HY4 protein, which was named cryptochrome 1 (CRY1) [17], with DNA photolyase, CRY1 has no photolyase activity when expressed in *E. coli* or insect cells [98, 99]. Heterologous expression of CRY1 enabled the identification of the chromophores bound to CRY1. Whereas only flavosemiquinone (FADH) could be identified as a noncovalently bound chromophore of CRY1 in extracts of insect cells [98], *E. coli*-expressed CRY1 contains both $FADH_2$ and the pterin-type chromophore 5,10-methenyltetrahydrofolate (MTHF) noncovalently attached to the apoprotein [99]. The same chromophores are present in DNA photolyase from several species such as *E. coli* and *Saccharomyces cerevisiae*. Photoreduction experiments with CRY1 expressed in insect cells resulted in the semireduced form of FAD ($FADH^{\cdot}$), shifting the absorption spectrum to green light [98]. The presence of a semireduced form of CRY1 in the plant would be consistent with the fact that, in contrast to wild type, the extension growth of the hypocotyl in green light is not inhibited in the *hy4* mutant [98]. Further studies are needed to identify the authentic chromophores bound to the blue light receptors in the plant cell. Besides the lack of photolyase activity of CRY1, all the evidence obtained from studies with the *hy4* mutant and transgenic lines overexpressing CRY1 points to a blue light photoreceptor function of CRY1 [96, 100]. The *hy4* mutant is affected only in some blue/UV-A light responses, indicating that more blue/UV-A photoreceptors exist in higher plants (see also fig. 3). Indeed, in a complementary DNA (cDNA) screen with degenerate oligonucleotides representing conserved regions of class I DNA photolyases, another photolyase-related gene from white mustard (*Sinapis alba* L.) was isolated [2] that shares 54% identity in the deduced amino acid sequence with CRY1 (fig. 2). Expression of this cDNA in photolyase-deficient *E. coli* cells resulted in weak photoreactivation [2], but later studies demonstrated that the *E. coli*-expressed protein has no photolyase activity in vitro, although it binds $FADH_2$ and MTHF as chromophores [99]. cDNA and genomic clones encoding a protein with 89% identity to the white mustard protein were isolated thereafter from *A. thaliana*. The gene was named *AT-PHH1* [3] or *CRY2* [4]. The highest similarity of PHH1/CRY2 with CRY1 is in the region between amino acids 1–500 (fig. 2). Like CRY1, PHH1/CRY2 has an extension at the C terminus which differs in size and sequence from the CRY1 extension, except sharing a short stretch of several serine residues and a few other conserved amino acids [3]. The function of this serine stretch has not been analyzed so far. Antisense expression of part of *PHH1/CRY2* not related to *CRY1* affected blue light-regulated expression of the *CHS* gene (U. Grüne and A. Batschauer, unpublished data), suggesting a blue light receptor function for PHH1/CRY2. Further support for a blue light receptor function for PHH1/CRY2 was provided very recently by the identification of *Arabidopsis* mutants defective in the *PHH1/CRY2* gene

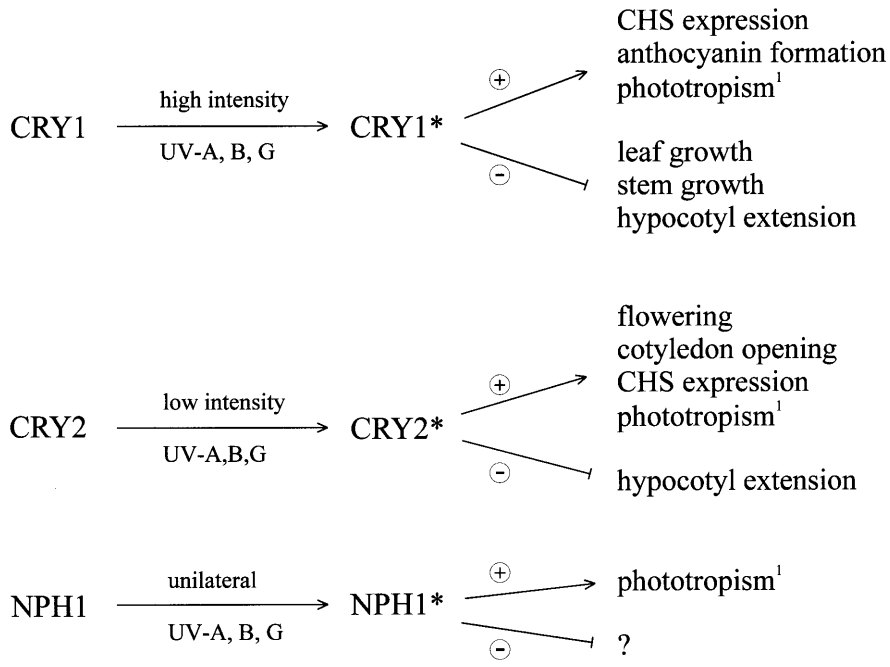

Figure 3. Physiological processes regulated by *A. thaliana* blue light receptors. CRY1 and CRY2 have multiple, partially overlapping functions, whereas NPH1 seems to be specific for phototropism. ([1]) First and second positive curvature in phototropism is lacking in the *nph1* mutant; the *cry1*/*cry2* double mutant is lacking the first positive curvature but is normal in the second one. Mutations in either *CRY1* or *CRY2* alone have no effect on phototropism. Indicated is which process is promoted (⊕) or inhibited (⊖).

[102]. The *Arabidopsis* mutant *fha-1* [103] and additional *Arabidopsis* mutants created by fast-neutron bombardment having mutations or deletions in the *CRY2* gene are late flowering and show an increase in the number of leaves under long-day (LD) but not under short-day (SD) conditions [102]. Transgenic lines overexpressing CRY2 show the opposite effect—slightly earlier flowering than the wild type in SD but not affected in LD [102]. Studies on the LD plant *Sinapis alba*, like *Arabidopsis* a crucifer plant, have shown that the efficiency of blue light in night break experiments is much higher than the efficiency of R [104], suggesting involvement of a blue light receptor in flower induction. Surprisingly, in continuous blue light the *cry2* mutant flowers as early as wild-type plants. Only when blue and red light are given together does the *cry2* mutant show a late flowering phenotype compared with wild type [102]. Since the mutation of *phyB* results in earlier flowering when R is given continuously, PhyB seems to repress flowering. However, it is not clear why the *phyB* mutant flowers as early as wild type, when blue light and red light are given simultaneously. A simple model in which PhyB would just repress and CRY2 promote flowering is not consistent with these data. In addition, the role of phytochromes other than PhyB in the regulation of flowering time have to be analyzed in further detail. For example, the *Arabidopsis phyA* mutant is less able to detect light treatments in the middle of the dark period [31], and overexpression of oat PhyA in tobacco affects flowering time [73]. The molecular mechanism by which CRY2 regulates flowering time is even more complicated, owing to the amount of CRY2 protein is itself light-regulated. In blue light the CRY2 protein is rapidly degraded [105, 106]. Downregulation of PHH1/CRY2 is not affected in *hy4* [105] and nonphototropic *nph1* mutants (U. Grüne and A. Batschauer, unpublished data), indicating that the downregulation is an autoregulatory process. It has also been demonstrated that under low-intensity blue light the amount of CRY2 is virtually unaffected and that CRY1 and CRY2 both affect the extension growth of the hypocotyl but operate under different light intensities [106]. It will be very important to learn in further studies how blue light receptors and phytochromes interact and how downregulation of PHH1/CRY2 in blue light fits with a model in which PHH1/CRY2 is a major player in sensing the photoperiod.

Phototropism (the bending response in relation to the direction of the light) is one of the best-studied blue light responses in plants. Recently, a putative photoreceptor involved in phototropism was identified.

Winslow Briggs and co-workers have characterized in maize coleoptiles and etiolated pea seedlings a plasma membrane-associated protein of about 120 kDa which is rapidly phosphorylated upon blue light treatment in an intensity-dependent fashion [107–110]. In vitro studies indicated that the 120-kDa protein autophosphorylates after blue light treatment [12]. Unilateral irradiation of coleoptiles results in a gradient of protein phosphorylation, demonstrating that the phosphorylated protein is directly involved in the phototropic response [111–113]. The identification of *Arabidopsis* mutants, impaired in phototropism [114, 115], was the starting point for the successful molecular cloning of a putative photoreceptor for phototropism. The *nph1* (nonphototropic hypocotyl 1) mutant does not show first and second positive curvature and lacks the 120-kDa protein mentioned above [115]. This mutant was used to isolate the corresponding gene. The *NPH1* gene encodes a protein of 996 amino acids (112 kDa) and complements the *nph1* mutant phenotype [5]. The C-terminal region of NPH1 has high similarity with the PVPK1 family of serine-threonine protein kinases, and the N-terminal part of NPH1 has two similar regions of about 100 amino acids related in sequence to motifs present in proteins from archaea, eubacteria and eukaryotes—proteins which are known to be regulated by environmental signals, such as light, oxygen and voltage (LOV), that change their redox state. As far as we know, the change in redox state is mediated by a flavin. As outlined above, flavin would also be a likely prosthetic group for a blue light receptor in phototropism. The conserved domain (LOV domain) in the different proteins including NPH1 could act as a flavin-binding site. However, very recent data show that the *cry1*/*cry2* double mutant is deficient in the first positive curvature [116], indicating that the two cryptochromes together could act as photoreceptors for phototropism and that NPH1 could be a component in the signal transduction pathway. Alternatively, NPH1 could still be the photoreceptor for both first and second positive curvature. Phototropism would then depend on three blue light receptors (as well as on PhyA and PhyB), and the activity of NPH1 would just be modulated by the cryptochromes.

In summary, recent work on blue light receptors in higher plants led to the identification of two different classes of proteins, the photolyase-related cryptochromes and the kinase and LOV domain containing NPH1, the likely photoreceptor for phototropism. Although the determination of the chromophore composition of these blue light receptors is not complete, there is good evidence that all the plant blue light receptors and blue light receptor candidates known so far are flavoproteins (O. Kleiner and A. Batschauer, unpublished data; W. R. Briggs, personal communication). Whereas the molecular cloning of the phytochrome photoreceptor family of *Arabidopsis* is complete, it is still not clear whether the same is true for the blue light receptors, or whether further ones (e.g. regulating stomatal opening) await identification.

Light-signaling molecules

As outlined above, most of the plant photoreceptors have been characterized in recent years. Less is known about the components of the light signal transduction pathways, and we can expect a very complicated network consisting of a great number of signaling molecules which are not only affected by light but also by additional factors such as other environmental stimuli or endogenous signals like phytohormones [18]. In principal two different strategies have been used to characterize light signal transduction: the genetic approach and the biochemical/cell biological approach [6, 14, 16, 18, 21, 117–120].

Genetic approach

The genetic approach was, and is currently, very fruitful in identifying genes involved in light signal transduction (fig. 4). Screening for signal transduction mutants was done essentially in two ways: first to identify mutants which show a kind of etiolated phenotype in the light [94], and second to identify mutants which show a light-grown phenotype in darkness [121–124]. Among the *hy* mutants (long hypocotyl of light-grown plants) isolated by Koornneef and co-workers most proved to be affected in photoreceptor genes (*hy3* = *phyB*, *hy8* = *phyA*, *hy4* = *cry1*) [1, 25, 26, 31, 68, 69, 71] or to be impaired in the synthesis of the phytochrome chromophore (*hy1*, *hy2*) [125]. However, the mutant *hy5* is impaired in responsiveness to different wavelengths and thus appears to be a downstream element of both phytochrome and blue light receptor transduction pathways [94]. Indeed, the *HY5* gene was cloned and encodes a bZIP transcription factor [126] which binds to the light-responsive element of the *CHS* gene leading to transcriptional activation [127]. HY5 interacts directly with another protein, COP1 [127–129], previously identified as a repressor of photomorphogenesis (see below). Screening for mutants having a dark-grown phenotype in the light has the advantage that the screens can be done with different light regimes allowing the isolation of mutants affected specifically in the signal transduction pathway of one photoreceptor. The *Arabidopsis* mutants *fhy1* and *fhy3*, for example, differ from wild-type plants under FR-HIR conditions [11, 69, 130, 131] but have normal levels of PhyA. Thus *FHY1* and *FHY3* most likely encode for components specific for the

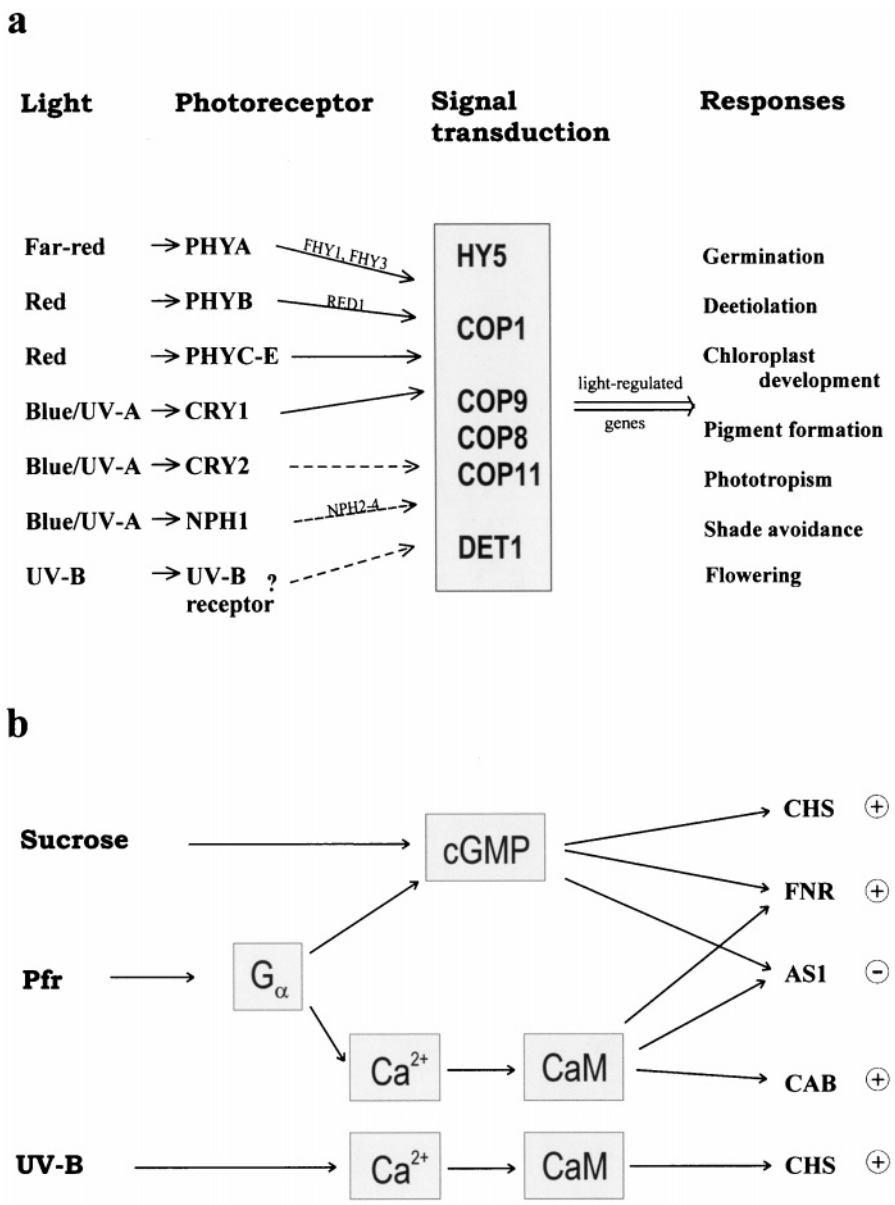

Figure 4. Scheme of components involved in light perception and signal transduction. (*a*) All the genes of the indicated photoreceptors (except for UV-B) and the signaling components (HY5, COP1, COP9, COP11/FUS6, DET1) have been cloned from *A. thaliana*. Genetic studies indicate the involvement of FHY1 and FHY3 in the signal transduction of PhyA, of RED1 in the signal transduction of PhyB and of NPH2-4 in the signal transduction of NPH1. (*b*) The function of G proteins, cGMP and Ca^{2+}/calmodulin as second messengers of the light signal transduction pathway is implicated by inhibitor studies with cell cultures and microinjection experiments with the phytochrome-deficient *aurea* mutant of tomato. The indicated genes regulated by cGMP and/or Ca^{2+}/CaM encode for chalcone synthase (*CHS*), the chlorophyll a/b-binding protein (*CAB*), ferredoxin-$NADP^{+}$ oxidoreductase (*FNR*) and asparagine synthetase (*AS1*). Further signaling molecules not indicated have already been characterized and more can be expected. The schemes, however, cannot explain how the light signals transduced by the different photoreceptors result in specific responses by using common signal transduction components (adapted from [6, 21, 101]).

PhyA signal transduction pathway. Another mutant, *red1*, seems to be affected in PhyB but not PhyA signal transduction [132], whereas *pef1*, a mutant identified in a screen for early-flowering mutants [133], is altered in the signaling pathway of both PhyA and PhyB. All mutants in light signal transduction identified so far are recessive except *shy1* and *shy2* [134, 135]. *Shy1* and *shy2* were isolated in a screen for suppressors of the *hy2*

mutation. Like *cop*/*det*/*fus* mutants (see below), dark-grown *shy* seedlings undergo most of the developmental processes of light-grown wild-type seedlings [134, 135].

Mutant screens performed in darkness led to the identification of *cop* (constitutive photomorphogenesis) and *det* (deetiolated phenotype) mutants with phenotypes similar to that of light-grown seedlings (for review see [14, 18, 21, 118, 129]). The *COP*/*DET* loci are identical to previously identified *FUS* (fusca) loci. The *fus* mutants show enhanced accumulation of anthocyanin, and strong alleles are lethal during seedling development [136]. The recessive nature of the *cop*/*det*/*fus* mutations together with their phenotypes indicate that COP/DET/FUS are repressors of photomorphogenesis and that these repressors are inactivated by the light signals perceived by the photoreceptors. Indeed, genetic interaction studies of photoreceptor mutants with *cop*/*det*/*fus* mutants indicate that *cop*/*det*/*fus* are epistatic to photoreceptor mutations and that COP/DET/FUS are localized downstream of the photoreceptors [123, 137], although this is difficult to conclude from such experiments. Some of the *COP*/*DET*/*FUS* genes have been cloned. *COP1*/*FUS1* encodes a protein with three well-characterized domains: a WD-40 domain typical for the β-subunit of heterotrimeric G proteins, a ring-finger zinc binding domain and a coiled-coil domain, which could act in protein-protein interactions [138–140]. Except for the zinc binding domain, COP1 shares significant homology with d-$TAF_{II}80$ from *Drosophila*, a component of the RNA polymerase II complex, suggesting that COP1 acts in the dark as a repressor of transcription by interacting with the RNA polymerase complex in a nonproductive manner. It has been demonstrated for a fusion of COP1 with the reporter β-glucuronidase (COP1-GUS) that COP1 is enriched in the nucleus in darkness and excluded from the nucleus in light [141]. The translocation of COP1 is a relatively slow process, requiring several hours at least in the cells tested thus far [141]. Most deetiolation processes are much faster. The translocation of COP1 could therefore act in maintaining the light program rather than as a molecular switch of photomorphogenesis. The nuclear localization of COP1 in the dark is impaired in mutants defective in other *COP* genes, *COP8*, *COP9*, *COP11* [142]. *COP9*/*FUS7* and *COP11*/*FUS6* have been cloned [124, 143]. COP9 is exclusively localized in a large protein complex consisting of several proteins including COP11 [124, 142, 144]. The COP9 complex is nuclear-localized, but in contrast to COP1, light has no effect on its localization [144, 145]. It has been hypothesized that the COP9 complex is directly involved in the nuclear localization of COP1, although COP1 is not part of the COP9 complex [129]. Another repressor of photomorphogenesis which has been cloned is DET1 [146]. DET1 has no obvious homology with any other protein in the databases, but is like COP1 localized in the nucleus. In contrast to COP1, the nuclear localization of DET1 is not light-regulated [146]. Despite the localization of DET1 in the nucleus, the protein does not bind DNA [18]. It is therefore likely that the action of DET1 and COP1 requires protein-protein interaction, probably with components of the basal transcription machinery. This interaction could result in the repression of genes that are required for photomorphogenesis.

Biochemical and cell biological approach

Besides the genetic approach, a variety of biochemical approaches have been used to identify signaling molecules downstream of the photoreceptors. Signal transduction in pulvinar movement and guard cell swelling, processes which are regulated by blue light, is beyond the scope of this review, and I refer the reader to recent reviews on this topic [23, 147]. Phytochrome-mediated phosphorylation of a number of mostly unidentified proteins has been demonstrated [148–151]. Phosphorylation and translocation between the cytosol and the nucleus of the bZIP transcription factor CPRF2 from parsley [152–154] and import of the bZIP factor GBF2 from *Arabidopsis* into the nucleus [155] is also light-regulated. In the case of CPRF2 it has been demonstrated that the light response is mediated by phytochrome. Since these processes are faster than the expulsion of COP1 from the nucleus, phosphorylation and translocation of CPRF2 and GBF2 into the nucleus could therefore be a molecular switch in the deetiolation process, as is the release of the bZIP factor HY5 from the COP1/HY5 complex [127]. Studies with cholera and pertussis toxin suggest that heterotrimeric guanosine-triphosphate (GTP)-binding proteins are part of the phytochrome signaling pathway [156]. The aurea mutant of tomato, a mutant affected in the biosynthesis of phytochromobilin [157], was used in microinjection experiments to define components of the phytochrome signal transduction chain (fig. 4). Microinjected purified oat phytochrome into single cells of the hypocotyl resulted in chloroplast development, anthocyanin formation and expression of light-regulated genes [158, 159], processes normally not seen in the mutant. Chloroplast development and anthocyanin formation were induced only in the injected cell, indicating that the phytochrome signal transduction pathway is cell-autonomous. In contrast, microbeam irradiation experiments indicate that the signals which induce the transcription of the *CAB* gene are generated in the irradiated cells but are also transmitted to neighboring cells [160]. Activators of heterotrimeric G proteins ($GTP\gamma S$, cholera toxin) had the same effect as microinjected PhyA, whereas inhibitors of heterotrimeric G proteins ($GDP\beta S$, pertussis toxin) inhibited the action

of coinjected PhyA [158, 159]. Yeast-expressed PhyB injected into aurea cells could stimulate chloroplast development but in contrast to injected PhyA could not stimulate anthocyanin formation [42]. This is consistent with the observation that continuous R treatment of *Arabidopsis* seedlings, which stimulates PhyB, is very inefficient for anthocyanin accumulation and *CHS* expression [42, 161]. Microinjection experiments identified further signaling molecules located downstream of the G proteins, namely cyclic guanosine-monophosphate (cGMP) and calcium/calmodulin [158, 162]. cGMP alone could stimulate anthocyanin accumulation and *CHS* expression, whereas calcium/calmodulin alone could stimulate CAB expression and allow partial development of the chloroplast. Full development of the chloroplast and expression of further chloroplast genes required both cGMP and calcium/calmodulin. Light-responsive cis-elements fused to truncated 35S promoters respond also to these signaling molecules [163]. Repression of genes which are downregulated by light (such as asparagine synthetase) is controlled by the same calcium and cGMP pathways [164]. Surprisingly, the signaling pathway of UV-B leading to the activation of *CHS* in parsley protoplasts and *Arabidopsis* cell suspension cultures involves calcium and is therefore different from the cGMP-dependent phytochrome signal transduction that regulates *CHS* expression [8, 165].

Conclusion

In recent years, progress in the characterization of plant photoreceptors and components of light signal transduction chains has been immense. This progress was made possible by the interaction of different disciplines such as genetics, molecular biology, physiology, biochemistry and cell biology. Although most of this research has focused on molecular aspects of photoreceptor function and signal transduction, the results obtained have also had a significant impact on our understanding of how plants adapt to changing light conditions in the natural environment. We have learned that multiple phytochromes exist which have partially antagonistic functions, and it will be interesting to see whether the same is true for blue light receptors. Despite profound knowledge of the components of the light signal transduction chain, we are far from understanding in detail how light signals are transduced. We must expect further complexity, since other external and internal signals such as hormones, carbohydrates and so on (not discussed here) interfere with light signals. A major challenge in the near future is to understand in greater detail how different photoreceptors interact, to elucidate the structures of photoreceptors and to identify their primary interacting partners.

Acknowledgments. I am grateful for stimulating discussions with several colleagues and apologize to those not cited because of space limitations. I want to thank Oxana Panajotow for preparing the figures, Petra Schreiber for editing and Dr. Helen Steele for critical reading of the manuscript. Our research is supported by a grant from the Deutsche Forschungsgemeinschaft (BA 985/5-2).

1 Ahmad M. and Cashmore A. R. (1993) *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature **366:** 162–166
2 Batschauer A. (1993) A plant gene for photolyase: an enzyme catalyzing the repair of UV-light-induced DNA damage. Plant J. **4:** 705–709
3 Hoffman P. D., Batschauer A. and Hays J. B. (1996) *AT-PHH1*, a novel gene from *Arabidopsis thaliana* related to microbial photolyases and plant blue light photoreceptors. Mol. Gen. Genet. **253:** 259–265
4 Lin C., Ahmad M., Chan J. and Cashmore A. R. (1996) *CRY2*: a second member of the Arabidopsis cryptochrome gene family. Plant Physiol. **110:** 1047
5 Huala E., Oeller P. W., Liscum E., Han I.-S., Larsen E. and Briggs W. R. (1997) Arabidopsis NPH1: a protein kinase with a putative redox-sensing domain. Science **278:** 2120–2123
6 Quail P. H. (1994) Photosensory perception and signal transduction in plants. Curr. Opin. Genet. Dev. **4:** 652–661
7 Beggs C. J. and Wellmann E. (1994) Photocontrol of flavonoid biosynthesis. In: Photomorphogenesis in Plants, 2nd ed., pp. 733–751, Kendrick R. E and Kronenberg G. H. M. (eds), Kluwer Academic Publishers, Dordrecht, Boston
8 Christie J. M. and Jenkins G. I. (1996) Distinct UV-B and UV-A/blue light signal transduction pathways induce chalcone synthase gene expression in Arabidopsis cells. Plant Cell **8:** 1555–1567
9 Furuya M. (1993) Phytochromes: their molecular species, gene families and functions. Ann. Rev. Plant Physiol. Plant Mol. Biol. **44:** 617–645
10 Quail P. H. (1994) Phytochrome genes and their expression. *In*: Photomorphogenesis in Plants, 2nd ed., pp. 71–104, Kendrick R. E. and Kronenberg G. H. M. (eds), Kluwer Academic Publishers, Dordrecht
11 Whitelam G. C. and Harberd N. P. (1994) Action and function of phytochrome family members revealed through the study of mutant and transgenic plants. Plant Cell Environ. **17:** 615–625
12 Short T. W. and Briggs W. R. (1994) The transduction of blue light signals in higher plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. **45:** 143–171
13 Jenkins G. I., Christie J. M., Fuglevand G., Long J. C. and Jackson J. A. (1995) Plant responses to UV and blue light: biochemical and genetic approaches. Plant Science **112:** 117–138
14 McNellis T. W. and Deng X.-W. (1995) Light control of seedling morphogenetic pattern. Plant Cell **7:** 1749–1761
15 Smith H. (1995) Physiological and ecological function within the phytochrome family. Ann. Rev. Plant Physiol. Plant Mol. Biol. **46:** 289–315
16 Quail P. H., Boylan M. T., Parks B. M., Short T. W., Xu Y. and Wagner D. (1995) Phytochromes: photosensory perception and signal transduction. Science **268:** 675–680
17 Ahmad M. and Cashmore A. R. (1996) Seeing blue: the discovery of cryptochrome. Plant Mol. Biol. **30:** 851–861
18 Chory J., Chatterjee M., Cook R. K., Elich T., Fankhauser C., Li J. et al. (1996) From seed germination to flowering, light controls plant development via the pigment phytochrome. Proc. Natl. Acad. Sci. USA **93:** 12066–12071
19 Furuya M. and Schäfer E. (1996) Photoperception and signalling of induction reactions by different phytochromes. Trends Plant Science **1:** 301–307
20 Quail P. H. (1997) The phytochromes: a biochemical mechanism of signaling in sight? Bioessays **19:** 571–579

21 Mustilli A. C. and Bowler C. (1997) Tuning in to the signals controlling photoregulated gene expression in plants. EMBO J. **16:** 5801–5806
22 Briggs W. R. and Liscum E. (1997) The role of mutants in the search for the photoreceptor for phototropism in higher plants. Plant Cell Environ. **20:** 768–772
23 Briggs W. R. and Liscum E. (1997) Blue light–activated signal transduction in higher plants. In: Signal transduction in Plants, pp. 107–135, Aducci P (ed.), Birkhäuser, Basel
24 Chory J. (1997) Light modulation of vegetative development. Plant Cell **9:** 1225–1234
25 Dehesh K., Franci C., Parks B. M., Seeley K. A., Short T. W., Tepperman J. M. et al. (1993) Arabidopsis *HY8* locus encodes phytochrome A. Plant Cell **5:** 1081–1088
26 Parks B. M. and Quail P. H. (1993) hy8, a new class of Arabidopsis long hypocotyl mutants deficient in functional phytochrome A. Plant Cell **5:** 39–48
27 Clack T., Mathews S. and Sharrock R. A. (1994) The phytochrome apoprotein family in Arabidopsis is encoded by five genes: the sequences and expression of *PHYD* and *PHYE*. Plant Mol. Biol. **25:** 413–427
28 Lagarias J. C. and Lagarias D. M. (1989) Self-assembly of synthetic phytochrome holoprotein in vitro. Proc. Natl. Acad. Sci. USA **86:** 5778–5780
29 McCormac A. C., Wagner D., Boylan M. T., Quail P. H., Smith H. and Whitelam G. C. (1993) Photoresponses of transgenic Arabidopsis seedlings expressing introduced phytochrome B-encoding cDNAs: evidence that phytochrome A and phytochrome B have distinct photoregulatory functions. Plant J. **4:** 19–27
30 Liscum E. and Hangarter R. P. (1993) Phototropic mutants of *Arabidopsis thaliana* reveal activities of multiple photosensory systems during light-stimulated apical-hook opening. Planta **191:** 214–221
31 Reed J. W., Nagatani A., Elich T. D., Fagan M. and Chory J. (1994) Phytochrome A and phytochrome B have overlapping but distinct functions in Arabidopsis development. Plant Physiol. **104:** 1139–1149
32 Sakamoto K. and Nagatani A. (1996) Nuclear localization activity of phytochrome B. Plant J. **10:** 859–868
33 Vierstra R. D. (1994) Phytochrome degradation. In: Photomorphogenesis in Plants, 2nd ed., pp. 141–162, Kendrick R. E. and Kronenberg G. H. M. (eds), Kluwer Academic Publishers, Dordrecht
34 Somers D. E., Sharrock R. A., Tepperman J. M. and Quail P. H. (1991) The *hy3* long hypocotyl mutant of Arabidopsis is deficient in phytochrome B. Plant Cell **3:** 1263–1274
35 Deforce L., Tomizawa K., Ito N., Farrens D., Song P. S. and Furuya M. (1991) In vitro assembly of apophytochrome and apophytochrome deletion mutants expressed in yeast with phycocyanobilin. Proc. Natl. Acad. Sci. USA **88:** 10392–10396
36 Wahleithner J. A., Li L. M. and Lagarias J. C. (1991) Expression and assembly of spectrally active recombinant holophytochrome. Proc. Natl. Acad. Sci. USA **88:** 10387–10391
37 Kunkel T., Tomizawa K., Kern R., Furuya M., Chua N. H. and Schäfer E. (1993) In vitro formation of a photoreversible adduct of phycocyanobilin and tobacco apophytochrome B. Eur. J. Biochem. **215:** 587–594
38 Li L. and Lagarias J. C. (1994) Phytochrome assembly in living cells of the yeast *Saccharomyces cerevisiae*. Proc. Natl.Acad. Sci. USA **91:** 12535–12539
39 Hill C., Gärtner W., Towner P., Braslavsky S. E. and Schaffner K. (1994) Expression of phytochrome apoprotein from *Avena sativa* in *Escherichia coli* and formation of photoactive chromoproteins by assembly with phycocyanobilin. Eur. J. Biochem. **223:** 69–77
40 Beale S. I. (1994) Biosynthesis of open-chain tetrapyrroles in plants, algae and cyanobacteria. Ciba Found. Symp. **180:** 156–168
41 Wu S. H. and Lagarias J. C. (1996) The methylotrophic yeast *Pichia pastoris* synthesizes a functionally active chromophore precursor of the plant photoreceptor phytochrome. Proc. Natl. Acad. Sci. USA **93:** 8989–8994
42 Kunkel T., Neuhaus G., Batschauer A., Chua N. H. and Schäfer E. (1996) Functional analysis of yeast-derived phytochrome A and B phycocyanobilin adducts. Plant J. **10:** 625–636
43 Gärtner W., Hill C., Worm K., Braslavsky S. E. and Schaffner K. (1996) Influence of expression system on chromophore binding and preservation of spectral properties in recombinant phytochrome. Eur. J. Biochem. **236:** 978–983
44 Murphy J. T. and Lagarias J. C. (1997) Purification and characterization of recombinant affinity peptide-tagged oat phytochrome A. Photochem. Photobiol. **65:** 750–758
45 Jones A. M., Vierstra R. D., Daniels S. M. and Quail P. H. (1985) The role of separate molecular domains in the structure of phytochrome from etiolated *Avena sativa* L. Planta **164:** 501–506
46 Jones A. M. and Edgerton M. D. (1994) The anatomy of phytochrome, a unique photoreceptor in plants. Semin Cell Biol. **5:** 295–302
47 Edgerton M. D. and Jones A. M. (1992) Localization of protein-protein interactions between subunits of phytochrome. Plant Cell **4:** 161–171
48 Edgerton M. D. and Jones A. M. (1993) Subunit interactions in the carboxy-terminal domain of phytochrome. Biochemistry **32:** 8239–8245
49 Cherry J. R., Hondred D., Walker J. M., Keller J. M., Hershey H. P. and Vierstra R. D. (1993) Carboxy-terminal deletion analysis of oat phytochrome A reveals the presence of separate domains required for structure and biological activity. Plant Cell **5:** 565–575
50 Thümmler F., Duffner M., Kreisl P. and Dittrich P. (1992) Molecular cloning of a novel phytochrome gene of the moss *Ceratodon purpureus* which encodes a putative light-regulated kinase. Plant Mol. Biol. **20:** 1003–1017
51 Algarra P., Linder S. and Thümmler F. (1993) Biochemical evidence that phytochrome of the moss *Ceratodon purpureus* is a light-regulated protein kinase. FEBS Lett. **315:** 69–73
52 Thümmler F., Algarra P. and Fobo G. M. (1995) Sequence similarities of phytochrome to protein kinases: implication for the structure, function and evolution of the phytochrome gene family. FEBS Lett. **357:** 149–155
53 Schneider-Poetsch H. A., Braun B., Marx S. and Schaumburg A. (1991) Phytochromes and bacterial sensor proteins are related by structural and functional homologies. Hypothesis on phytochrome-mediated signal-transduction. FEBS Lett. **281:** 245–249
54 Schneider-Poetsch H. A. (1992) Signal transduction by phytochrome: phytochromes have a module related to the transmitter modules of bacterial sensor proteins. Photochem. Photobiol. **56:** 839–846
55 Hughes J., Lamparter T., Mittmann F., Hartmann E., Gärtner W., Wilde A. et al. (1997) A prokaryotic phytochrome. Nature **386:** 663
56 Yeh K. C., Wu S. H., Murphy J. T. and Lagarias J. C. (1997) A cyanobacterial phytochrome two-component light sensory system. Science **277:** 1505–1508
57 Lamparter T., Mittmann F., Gärtner W., Börner T., Hartmann E. and Hughes J. (1997) Characterization of recombinant phytochrome from the cyanobacterium Synechocystis. Proc. Natl. Acad. Sci. USA **94:** 11792–11797
58 Wilde A., Churin Y., Schubert H. and Börner T. (1997) Disruption of a *Synechocystis sp*. PCC 6803 gene with partial similarity to phytochrome genes alters growth under changing light qualities. FEBS Lett. **406:** 89–92
59 Chang C., Kwok S. F., Bleecker A. B. and Meyerowitz E. M. (1993) Arabidopsis ethylene-response gene *ETR1*: similarity of product to two-component regulators. Science **262:** 539–544
60 Hua J., Chang C., Sun Q. and Meyerowitz E. M. (1995) Ethylene insensitivity conferred by Arabidopsis ERS gene. Science **22:** 1712–1714

61 Wilkinson J. Q., Lanahan M. B., Yen H. C., Giovannoni J. J. and Klee H. J. (1995) An ethylene-inducible component of signal transduction encoded by never-ripe. Science **15:** 1807–1809
62 Kakimoto T. (1996) CKI1, a histidine kinase homolog implicated in cytokinin signal transduction. Science **274:** 982–985
63 Kaufman L. S., Thompson W. F. and Briggs W. R. (1984) Different red light requirements for phytochrome-induced accumulation of cab RNA and rbcS RNA. Science **226:** 1447–1449
64 Hartmann K. M. (1966) A general hypothesis to interpret 'high energy phenomena' of photomorphogenesis on the basis of phytochrome. Photochem. Photobiol. **5:** 349–366
65 Goto N., Yamamoto K. T. and Watanabe M. (1993) Action spectra for inhibition of hypcotyl growth of wild-type plants and the *hy2* long hypocotyl mutant of Arabidopsis thaliana L. Photochem. Photobiol. **57:** 867–871
66 Shinomura T., Nagatani A., Hanzawa H., Kubota M., Watanabe M. and Furuya M. (1996) Action spectra for phytochrome A- and B-specific photoinduction of seed germination in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **93:** 8129–8133
67 Hamazato F., Shinomura T., Hanzawa H., Chory J. and Furuya M. (1997) Fluence and wavelength requirements for Arabidopsis *CAB* gene induction by different phytochromes. Plant Physiol. **115:** 1533–1540
68 Nagatani A., Reed J. W. and Chory J. (1993) Isolation and initial characterization of Arabidopsis mutants that are deficient in phytochrome A. Plant Physiol **102:** 269–277
69 Whitelam G. C., Johnson E., Peng J., Carol P., Anderson M. L., Cowl J. S. et al. (1993) Phytochrome A null mutants of Arabidopsis display a wild-type phenotype in white light. Plant Cell **5:** 757–768
70 Shinomura T., Nagatani A., Chory J. and Furuya M. (1994) The induction of seed germination in *Arabidopsis thaliana* is regulated principally by phytochrome B and secondarily by phytochrome A. Plant Physiol. **104:** 363–371
71 Reed J. W., Nagpal P., Poole D. S., Furuya M. and Chory J. (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. Plant Cell **5:** 147–157
72 Poppe C. and Schäfer E. (1997) Seed germination of *Arabidopsis thaliana phyA/phyB* double mutants is under phytochrome control. Plant. Physiol. **114:** 1487–1492
73 Halliday K. J., Thomas B. and Whitelam G. C. (1997) Expression of heterologous phytochromes A, B or C in transgenic tobacco plants alters vegetative development and flowering time. Plant J. **12:** 1079–1090
74 Qin M., Kuhn R., Moran S. and Quail P. H. (1997) Overexpressed phytochrome C has similar photosensory specificity to phytochrome B but a distinctive capacity to enhance primary leaf expansion. Plant J. **12:** 1163–1172
75 Aukerman M. J., Hirschfeld M., Wester L., Weaver M., Clack T., Amasino R. M. et al. (1997) A deletion in the *PHYD* gene of the Arabidopsis Wassilewskija ecotype defines a role for phytochrome D in red/far-red light sensing. Plant Cell **9:** 1317–1326
76 Yanovsky M. J., Casal J. J. and Whitelam G. C. (1995) Phytochrome A, phytochrome B and HY4 are involved in hypocotyl growth responses to natural radiations in Arabidopsis: weak deetiolaion of the *phyA* mutant under dense canopies. Plant Cell Environm. **18:** 788–794
77 Smith H., Xu Y. and Quail P. H. (1997) Antagonistic but complementary actions of phytochromes A and B allow seedling de-etiolation. Plant Physiol. **114:** 637–641
78 Wagner D., Fairchild C. D., Kuhn R. M. and Quail P. H. (1996) Chromophore-bearing NH_2-terminal domains of phytochromes A and B determine their photosensory specificity and differential light lability. Proc. Natl. Acad. Sci. USA **93:** 4011–4015
79 Boylan M., Douglas N. and Quail P. H. (1994) Dominant negative suppression of Arabidopsis photoresponses by mutant phytochrome A sequences identifies spatially discrete regulatory domains in the photoreceptor. Plant Cell **6:** 449–460
80 Emmler K., Stockhaus J., Chua N. H. and Schäfer E. (1995) An amino-terminal deletion of rice phytochrome A results in a dominant negative suppression of tobacco phytochrome A activity in transgenic tobacco seedlings. Planta **197:** 103–110
81 Wagner D., Koloszvari M. and Quail P. H. (1996) Two small spatially distinct regions of phytochrome B are required for efficient signaling rates. Plant Cell **8:** 859–871
82 Xu Y., Parks B. M., Short T. W. and Quail P. H. (1995) Missense mutations define a restricted segment in the C-terminal domain of phytochrome A critical to its regulatory activity. Plant Cell **7:** 1433–1443
83 Cherry J. R., Hondred D., Walker J. M. and Vierstra R. D. (1992) Phytochrome requires the 6-kDa N-terminal domain for full biological activity. Proc. Natl. Acad. Sci. USA **89:** 5039–5043
84 Stockhaus J., Nagatani A., Halfter U., Kay S., Furuya M. and Chua N. H. (1992) Serine-to-alanine substitutions at the amino-terminal region of phytochrome A result in an increase in biological activity. Genes Dev. **6:** 2364–2372
85 Jordan E. T., Cherry J. R., Walker J. M. and Vierstra R. D. (1995) The amino-terminus of phytochrome A contains two distinct functional domains. Plant J. **9:** 243–257
86 Lapko V. N., Jiang X. Y., Smith D. L. and Song P. S. (1997) Posttranslational modification of oat phytochrome A: phosphorylation of a specific serine in a multiple serine cluster. Biochemistry **36:** 10595–10599
87 Liscum E. and Hangarter R. P. (1994) Mutational analysis of blue-light sensing in Arabidopsis. Plant, Cell and Environm. **17:** 639–648
88 Ninnemann H. (1995) Some aspects of blue light research during the last decade. Photochem. Photobiol. **61:** 22–31
89 Liscum E. and Briggs W. R. (1996) Mutations of Arabidopsis in potential transduction and response components of the phototropic signaling pathway. Plant. Physiol. **112:** 291–296
90 Galland P. and Senger H. (1988) The role of pterins in the photoperception and metabolism of plants. Photochem. Photobiol. **48:** 811–820
91 Galland P. and Senger H. (1991) Flavins as possible blue-light photoreceptors. In: Photoreceptor Evolution and Function, pp. 65–124, Holmes M. G. (ed.), Academic Press, London
92 Quinones M. A. and Zeiger A. (1994) A putative role of the xanthopyll, zeaxanthin cycle in the blue-light photoreception of corn coleoptiles. Science **264:** 558–561
93 Zeiger E. (1994) The photobiology of stomatal movements. In: Photomorphogenesis in Plants, 2nd ed., pp. 683–706, Kendrick R. E. and Kronenberg G. H. M. (eds), Kluwer Academic Publishers, Dordrecht
94 Koornneef M., Rolf E. and Spruit C. J. P. (1980) Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L). Heynh. Z. Pflanzenphysiol. **100:** 147–160
95 Jackson J. A. and Jenkins G. I. (1995) Extension-growth responses and expression of flavonoid biosynthesis genes in the Arabidopsis *hy4* mutant. Planta **197:** 233–239
96 Ahmad M., Lin C. and Cashmore A. R. (1995) Mutations throughout an Arabidopsis blue-light photoreceptor impair blue-light responsive anthocaynin accumulation and inhibition of hypocotyl elongation. Plant J. **8:** 653–658
97 Sancar A. (1994) Structure and function of DNA photolyase. Biochem. **33:** 2–9
98 Lin C., Robertson D. E., Ahmad M., Raibekas A. A., Schuman Jornes M., Dutton P. L. et al. (1995) Association of flavin adenine dinucleotide with the Arabidopsis blue light receptor CRY1. Science **269:** 968–970
99 Malhotra K., Kim S.-T., Batschauer A., Dawut L. and Sancar A. (1995) Putative blue-light photoreceptors from *Arabidopsis thaliana* and *Sinapis alba* with high degree of sequence homology to DNA photolyase contain the two photolyase cofactors but lack DNA repair activity. Biochemistry **34:** 6892–6899

100 Lin C., Ahmad M., Gordon D. and Cashmore A. R. (1995) Expression of an Arabidopsis cryptochrome gene in transgenic tobacco results in hypersensitivity to blue, UV-A, and green light. Proc. Natl. Acad. Sci. USA **92:** 8423–8427

101 Batschauer A. (1998) Photoreceptors of higher plants. Planta **206:** 479–492

102 Guo H., Yang H., Mockler T. C. and Lin C. (1998) Regulation of flowering time by Arabidopsis photoreceptors. Science **279:** 1360–1363

103 Koornneef M., Hahart C. J. and van der Veen J. H. (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Mol. Gen. Genet. **229:** 57–66

104 Hanke J., Hartmann K. and Mohr H. (1969) Die Wirkung von 'Störlicht' auf die Blütenbildung von *Sinapis alba* L. Planta **86:** 235–249

105 Ahmad M., Jarillo J. and Cashmore A. R. (1998) Chimeric proteins between *cry1* and *cry2* Arabidopsis blue light photoreceptors indicate overlapping functions and varying protein stability. Plant Cell **10:** 197–207

106 Lin C., Yang H., Guo H., Mockler T., Chen J. and Cashmore A. R. (1998) Enhancement of blue-light sensitivity of Arabidopsis seedlings by a blue light receptor cryptochrome 2. Proc. Natl. Acad. Sci. USA **95:** 2686–2690

107 Gallagher S., Short T. W., Ray P. M., Pratt L. H. and Briggs W. R. (1988) Light-mediated changes in two proteins found associated with plasma membrane fractions from pea stem sections. Proc. Natl. Acad. Sci. USA **85:** 8003–8007

108 Short T. W. and Briggs W. R. (1990) Characterization of a rapid, blue light-mediated change in detectable phosphorylation of a plasma membrane protein from etiolated pea (*Pisum sativum* L.) seedlings. Plant Physiol **92:** 179–185

109 Short T. W., Porst M. and Briggs W. R. (1992) A photoreceptor system regulating in vivo and in vitro phosphorylation of a pea plasma membrane protein. Photochem. Photobiol. **55:** 773–781

110 Palmer J. M., Short T. W., Gallagher S. and Briggs W. R. (1993) Blue light-induced phosphorylation of a plasma membrane-associated protein in *Zea mays* L. Plant Physiol. **102:** 1211–1218

111 Salomon M., Zacherl M., Luff L. and Rüdiger W. (1997) Exposure of oat seedlings to blue light results in amplified phosphorylation of the putative photoreceptor for phototropism and in higher sensitivity of the plants to phototropic stimuli. Plant Physiol. **115:** 493–500

112 Salomon M., Zacherl M. and Rüdiger W. (1997) Phototropism and protein phosphorylation in higher plants: unilateral blue light irradiation generates a directional gradient of protein phosphorylation across the oat coleoptile. Bot. Acta **110:** 214–216

113 Salomon M., Zacherl M. and Rüdiger W. (1997) Asymmetric, blue light-dependent phosphorylation of a 116-kilodalton plasma membrane protein can be correlated with the first- and second-positive phototropic curvature of oat coleoptiles. Plant Physiol. **115:** 485–491

114 Khurana J. P. and Poff K. L. (1989) Mutants of Arabidopsis thaliana with altered phototropism. Planta **178:** 400–406

115 Liscum E. and Briggs W. R. (1995) Mutations in the *nph1* locus of Arabidopsis disrupt the perception of phototropic stimuli. Plant Cell **7:** 473–485

116 Ahmad M., Jarillo J. A., Smirnova O. and Cashmore A. R. (1998) Cryptochrome blue-light photoreceptors of Arabidopsis implicated in phototropism. Nature **392:** 720–723

117 Millar A. J., McGrath R. B. and Chua N. H. (1994) Phytochrome phototransduction pathways. Ann. Rev. Genet. **28:** 325–349

118 Barnes S. A., McGrath R. B. and Chua N.-H. (1997) Light signal transduction. Trends Cell Biol. **7:** 21–26

119 Bowler C. (1997) The transduction of light signals by phytochrome. In: Signal transduction in Plants, pp. 137–152, Aducci P. (ed.), Birkhäuser, Basel

120 Schäfer E., Kunkel T. and Frohnmeyer H. (1997) Signal transduction in the photocontrol of chalcone synthase expression. Plant Cell Environ. **20:** 722–727

121 Chory J., Peto C., Feinbaum R., Pratt L. and Ausubel F. (1989) *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. Cell **58:** 991–999

122 Deng X.-W., Caspar T. and Quail P. H. (1991) *cop1*: a regulatory locus involved in light-controlled development and gene expression in Arabidopsis. Genes Dev. **5:** 1172–1182

123 Wei N. and Deng X.-W. (1992) *COP9*: a new genetic locus involved in light-regulated development and gene expression in Arabidopsis. Plant Cell **4:** 1507–1518

124 Wei N., Chamovitz D. A. and Deng X.-W. (1994) Arabidopsis COP9 is a component of a novel signaling complex mediating light control of development. Cell **78:** 117–124

125 Parks B. M. and Quail P. H. (1991) Phytochrome-deficient *hy1* and *hy2* long hypocotyl mutants of Arabidopsis are defective in phytochrome chromophore biosynthesis. Plant Cell **3:** 1177–1186

126 Oyama T., Shimura Y. and Okada K. (1997) The Arabidopsis *HY5* gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. Genes Dev. **11:** 2983–2995

127 Ang L.-H., Chattopadhyay S., Wei N., Oyama T., Okada K., Batschauer A. et al. (1998) Molecular interaction between COP1 and HY5 defines a regulatory switch for light control of Arabidopsis development. Molecular Cell **1:** 213–222

128 Ang L.-H. and Deng X.-W. (1994) Regulatory hierarchy of photomorphogenic loci: allele-specific and light-dependent interaction between the *HY5* and *COP1* loci. Plant Cell **6:** 613–628

129 Staub J. M. and Deng X.-W. (1996) Light signal transduction in plants. Photochem. Photobiol. **64:** 897–905

130 Barnes S. A., Quaggio R. B., Whitelam G. C. and Chua N. H. (1996) *fhy1* defines a branch point in phytochrome A signal transduction pathways for gene expression. Plant J. **10:** 1155–1161

131 Barnes S. A., Nishizawa N. K., Quaggio R. B., Whitelam G. C. and Chua N.-H. (1996) Far-red light blocks greening of Arabidopsis seedlings via a phytochrome A-mediated change in plastid development. Plant Cell **8:** 601–615

132 Wagner D., Hoecker U. and Quail P. H. (1997) RED1 is necessary for phytochrome B-mediated red light-specific signal transduction in Arabidopsis. Plant Cell **9:** 731–743

133 Ahmad M. and Cashmore A. R. (1996) The *pef* mutants of *Arabidopsis thaliana* define lesions early in the phytochrome signaling pathway. Plant J. **10:** 1103–1110

134 Kim B. C., Soh M. S., Kang B. J., Furuya M. and Nam H. G. (1996) Two dominant photomorphogenic mutations of Arabidopsis thaliana identified as suppressor mutations of *hy2*. Plant J. **9:** 441–456

135 Kim B. C., Soh M. S., Hong S. H., Furuya M. and Nam H. G. (1998) Photomorphogenic development of the Arabidopsis shy2-1D mutation and its interaction with phytochromes in darkness. Plant J. **15:** 61–68

136 Müller A. J. (1963) Embryonentest zum Nachweis rezessiver Letalfaktoren bei *Arabidopsis thaliana*. Biol. Zentralbl. **82:** 133–136

137 Chory J. (1992) A genetic model for light-regulated seedling development. Development **115:** 337–354

138 Deng X.-W., Matsui M., Wei N., Wagner D., Chu A. M., Feldmann K. A. et al. (1992) *COP1*, an Arabidopsis regulatory gene, encodes a protein with both a zinc-binding motif and a G_{β} homologous domain. Cell **71:** 791–801

139 von Arnim A. G. and Deng X.-W. (1993) Ring finger motif of *Arabidopsis thaliana* COP1 defines a new class of zinc-binding domain. J. Biol. Chem. **268:** 19626–19631

140 McNellis T. W., von Arnim A. G. and Deng X.-W. (1994) Overexpression of Arabidopsis COP1 results in partial suppression of light-mediated development: evidence for a light-inactivable repressor of photomorphogenesis. Plant Cell **6:** 1391–1400

141 von Arnim A. G. and Deng X.-W. (1994) Light inactivation of Arabidopsis photomorphogenic repressor COP1 involves

a cell-specific regulation of its nucleo-cytoplasmatic partitioning. Cell **79:** 1035–1045
142 Chamovitz D., Wei N., Osterlund M., Staub J. M., Matsui M. and Deng X.-W. (1996) The COP9 complex, a novel multi-subunit nuclear regulator involved in light control of a plant development switch. Cell **86:** 115–121
143 Castle L.-A. and Meinke D. W. (1994) A *FUSCA* gene of Arabidopsis encodes a novel protein essential for plant development. Plant Cell **6:** 25–41
144 Staub J. M., Wei N. and Deng X.-W. (1996) Evidence for FUS6 as a component of the nuclear-localized COP9 complex in Arabidopsis. Plant Cell **8:** 2047–2056
145 Chamovitz D. A. and Deng X.-W. (1995) The novel components of the Arabidopsis light signaling pathway may define a group of general developmental regulators shared by both animal and plant kingdoms. Cell **82:** 353–354
146 Pepper A., Delaney T., Washburn T., Poole D. and Chory J. (1994) *DET1*, a negative regulator of light-mediated development and gene expression in Arabidopsis, encodes a novel nuclear-localized protein. Cell **78:** 109–116
147 Cote G. G. (1995) Signal transduction in leaf movement. Plant Physiol **109:** 423–426
148 Romero L. C., Sommer D., Gotor C. and Song P. S. (1991) G-proteins in etiolated Avena seedlings. Possible phytochrome regulation. FEBS Lett. **282:** 341–346
149 Fallon K. M., Shacklock P. S. and Trewavas A. J. (1993) Detection in vivo of rapid red light-induced calcium sensitive protein phosphorylation in etiolated wheat (*Triticum aestivum*) leaf protoplasts. Plant Physiol. **101:** 1039–1045
150 Fallon K. M. and Trewavas A. J. (1994) Phosphorylation of a renatured protein from etiolated wheat leaf protoplasts is modulated by blue and red light. Plant Physiol. **105:** 253–258
151 Harter K., Frohnmeyer H., Kirchner S., Kunkel T., Mühlbauer S. and Schäfer E. (1994) Light induces rapid changes of the phosphorylation pattern in the cytosol of evacuolated parsley protoplasts. Proc. Natl. Acad. Sci. USA **91:** 5038–5052
152 Harter K., Kircher S., Frohnmeyer H., Krenz M., Nagy F. and Schäfer E. (1994) Light-regulated modification and nuclear translocation of cytosolic G-box binding factors in parsley. Plant Cell **6:** 545–559
153 Kircher S., Ledger S., Hayashi B., Weisshaar B., Schäfer E. and Frohnmeyer H. (1998) CPRF4a, a novel plant bZIP protein of the CPRF family: comparative analyses of light-dependent expression, post-transcriptional regulation, nuclear import and heterodimerisation. Mol. Gen. Genet. **257:** 595–605
154 Kircher S., Nick P., Rügner A., Wellmer F., Schäfer E. and Harter K. (1998) Nuclear import of the parsley bZIP factor CPRF2 is regulated by phytochrome. J. Cell. Biol., in press
155 Terzaghi W. B., Bertekap R. L. and Cashmore A. R. (1997) Intracellular localization of GBF proteins and blue light-induced import of GBF2 fusion proteins into the nucleus of cultured Arabidopsis and soybean cells. Plant J. **11:** 967–982
156 Romero L. C. and Lam E. (1993) Guanine nucleotide binding protein involvement in early steps of phytochrome-regulated gene expression. Proc. Natl. Acad. Sci. USA **90:** 1465–1469
157 Terry M. J. and Kendrick R. E. (1996) The *aurea* and *yellow-green-2* mutants of tomato are deficient in phytochrome chromophore synthesis. J. Biol. Chem. **271:** 21681–21686
158 Neuhaus G., Bowler C., Kern R. and Chua N. H. (1993) Calcium/calmodulin-dependent and independent phytochrome signal transduction pathways. Cell **73:** 937–952
159 Bowler C., Yamagata H., Neuhaus G. and Chua N. H. (1994) Phytochrome signal transduction pathways are regulated by reciprocal control mechanisms. Genes Dev. **8:** 2188–2202
160 Bischoff F., Millar A. J., Kay S. A. and Furuya M. (1997) Phytochrome-induced intercellular signalling activates *cab*::luciferase gene expression. Plant J. **12:** 839–849
161 Batschauer A., Rocholl M., Kaiser T., Nagatani A., Furuya M. and Schäfer E. (1996) Blue and UV-A light regulated *CHS* expression in Arabidopsis independent of phytochrome A and phytochrome B. Plant J. **9:** 63–69
162 Bowler C., Neuhaus G., Yamagata H. and Chua N. H. (1994) Cyclic GMP and calcium mediate phytochrome phototransduction. Cell **77:** 73–81
163 Wu Y., Hiratsuka K., Neuhaus G. and Chua N.-H. (1996) Calcium and cGMP target distinct phytochrome-responsive elements. Plant J. **10:** 1149–1154
164 Neuhaus G., Bowler C., Hiratsuka K., Yamagata H. and Chua N.-H. (1997) Phytochrome-regulated repression of gene expression requires calcium and cGMP. EMBO J. **10:** 2554–2564
165 Frohnmeyer H., Bowler C. and Schäfer E. (1997) Evidence for some signal transduction elements involved in UV-light-dependent responses in parsley protoplasts. J. Exp. Biol. **48:** 739–750