CHAPTER 2

REVIEW OF LITERATURE
Since light provides the energy source for photosynthesis, plants have evolved mechanisms for sensing light parameters such as its presence, the direction, magnitude, quality and photoperiod length (Mancinelli, 1994) to adapt to the light environment. The adaptive responses are seen throughout the life cycle of plant, which include growth changes such as phototropism and developmental changes like de-etiolation and floral induction (Kendrick and Kronenberg, 1994). The physiological study of these responses has defined the basics of how plants detect and respond to changes in light environment but have not led to a detailed understanding of them. In recent years, the studies have been focused to elucidate the mechanisms of detecting light signals by the photoreceptors and transducing the signals into the appropriate intracellular and molecular events (Fairchild and Quail, 1998; Deng and Quail, 1999; Briggs and Huala, 1999; Lin, 2000; Smith, 2000).

LIGHT PERCEPTION - PHOTORECEPTORS AND FUNCTIONS

2.1 DE-ETIOLATION

Among the responses induced by light, the de-etiolation of seedling, is one of the most important phenomena in plant growth and development (Deng, 1994). Seedlings upon germination in darkness show developmentally repressed process known as skotomorphogenesis and have elongated hypocotyls, closed apical hooks and unexpanded cotyledons. There is little or no expression of photosynthetic genes encoded by nuclear and plastid genomes and plastids develop into etioplast that possess no chlorophyll and are not photosynthetically competent. In the presence of light, however, the seedlings undergo a developmental process known as photomorphogenesis, which involves inhibition of hypocotyl growth, opened apical hook, expanded cotyledons, transformation of etioplast to chloroplast, anthocyanin induction and stimulation of a large number different genes. This process has been used
as a model system to study the mechanisms of light signal transduction leading to photomorphogenesis in plants (Deng, 1994).

2.2 PHOTORECEPTORS

The processing of light information by a plant in different photomorphogenic responses involves the function of photoreceptors. These photoreceptors are thought to comprise three classes viz., the red/far-red photoreceptors-phytochromes, the blue/UV-A and UV-B photoreceptors (Kendrick and Kronenberg, 1994), each of which is able to detect light of particular wavelengths. Of these phytochromes have been most thoroughly studied and in recent years blue/UV-A photoreceptors have been characterized (Ahmad and Cashmore, 1993; Haula et al., 1997; Briggs and Huala, 1999; Lin, 2000), however, the nature of UV-B photoreceptors remains elusive although evidences exist for a separate receptor (Young et al., 1992). The environmental light signals perceived by these three classes of photoreceptors apparently contribute to the photomorphogenic development.

2.3 PHOTOCROME RESPONSES

In the red/far-red region of the spectrum, plants sense light with the regulatory photoreceptor phytochromes (Kendrick and Kronenberg, 1994; Smith, 2000). Phytochromes control responses such as seed germination, seedling de-etiolation, gene expression, chloroplast differentiation, floral induction, fruit ripening and senescence (Smith, 1995). The phytochromes are also responsible for shade-avoidance response (Smith, 1995) and end-of-day far-red responses (Furuya, 1993). In addition, phytochromes interact with the gravity sensing mechanism to control gravitropism (Gaiser and Lomax, 1993;
Parks et al., 1996) and enhancement of phototropism (Parks et al., 1996; Janoudi et al., 1997).

2.4 PHYTOCHROME GENE FAMILY

The large diversity of phytochrome-mediated responses is due to the expression of multiple phytochrome species in different plants (Furuya, 1993; Chory, 1994; Pratt et al., 1995; Smith, 1995). Recent genetic and biochemical evidence has shown that a small family of genes in all plant species so far studied encodes the phytochrome apoproteins. The exact number of genes coding for phytochrome is ambiguous. The cDNA sequences derived from Arabidopsis has revealed five phytochrome coding regions and designated them PHY A, B, C, D and E (Shamrock and Quail, 1989; Quail, 1991; Clack et al., 1994). Based upon partial sequences of genomic DNA fragments obtained by the PCR, five tomato PHY genes have been identified (Hauser et al., 1995; Pratt, 1995) and these genes were classified as PHY A, PHYB1, PHYB2, PHYE and PHYF. The five Arabidopsis PHY genes are expressed at both the mRNA (Clack et al., 1994) and protein (Somers et al., 1991) levels, yielding products of the sizes predicted from gene and cDNA sequences. These gene products are present throughout most stages of plant development and in most plant organs (Hauser et al., 1998).

Phylogenetic analysis of the phytochrome genes of higher plants suggests that duplication of an ancestral gene at about the time of origin of the seed plants led to the divergence of two lineages, one giving rise to the phyA and phyC homologs and the other giving rise to phyB, phyD, and phyE homologs. Subsequent duplications are proposed to have occurred near the time of the origin of flowering plants (Mathews and Sharrock, 1997). In Arabidopsis the phyB and phyD proteins share approximately 80% amino acid
sequence identity and are thought to result from a gene duplication in a recent progenitor of the Cruciferae (Mathews and Sharrock, 1997). The phyB and phyD proteins are more closely related to phyE than they are to either the phyA or phyC proteins.

2.5 TYPES OF PHYTOCHROMES

The proteins encoded by the phytochrome genes are differentiated to at least two different types. The type I is highly abundant in dark-grown seedlings and becomes severely depleted once exposed to light whereas type II is stable and is present at relatively similar levels in dark-grown and light-grown seedlings. The type I phytochromes (Furuya 1993) are mainly involved in de-etiolation processes of etiolated plants, while the type II phytochromes (Furuya 1993) in light-grown plants. The PHYA is the abundant light-labile phytochrome in dark grown seedlings and represents species defined by type I phytochrome (Furuya 1993). The light-stable PHYB shows a longer half-life and is the predominant phytochrome species in light-grown tissue, which represents type II along with remaining members of the gene family. Moreover, PHYA and PHYB are immunologically distinct and differ slightly in their molecular mass. In dicotyledons plants, both proteins possess indistinguishable spectral properties (Wagner et al., 1991), whereas PHYB from oat is spectroscopically distinct from PHYA (Tokuhisa and Quail, 1989; Pratt et al., 1991).

2.6 PHYTOCHROME - MOLECULAR PROPERTIES

The molecular properties of phytochrome have been determined most extensively for the abundant protein species purified from dark grown oat seedlings (Virestra and Quail, 1983). The phytochrome is a chromoprotein
existing in vitro, and possibly in vivo as well, as active homodimer (Cherry and Vierstra, 1994) with a monomeric molecular mass of 125 kDa. Each monomer of the phytochrome molecule folds into two major domains separated by a protease-sensitive hinge region: 1. the N-terminal chromophore domain of about 60-70 kDa, which is highly conserved in all phytochrome species and to which the tetrapyrrole chromophore is covalently attached that mediates light perception, and 2. more divergent C-terminal domain of about 30-50 kDa with one or more dimerization domains (Rudiger and Thummler, 1991; Quail, 1991; Furuya, 1993; Furuya and Song, 1994).

The chromophore of phytochrome, phytochromobilin, is synthesized in the plastid from 5-aminolevulinic acid (ALA) via the heme branch of the tetrapyrrole pathway (Terry et al., 1993), and evidence from reconstitution studies using recombinant phytochromes indicate that different phytochromes use the same chromophore. Phytochrome is assembled in its inactive, Pr form, and current evidence indicate that assembly in vivo is autocatalytic and takes place in cytoplasm (Lagarias and Lagarias, 1989; Terry et al., 1993). The absorbance of light by the chromophore, can lead to a Z/E isomerization of the tetrapyrrole (Rudiger et al., 1983; Farrens et al., 1989). This photoreversible process mediates the conformation change of the photoreceptor from the physiological inactive Pr to the active Pfr. Following conversion to the active, Pfr form, phytochrome activates signal transduction pathways that lead to changes in gene expression (Gilamartin et al., 1990; Thompson and White, 1991) which underlie wide range of developmental responses. However, the
On the contrary, recent studies of transgenic overexpressing phytochrome genes in *Arabidopsis* have indicated that the photosensory specificities exhibited by phytochromes are governed by sequences in N-terminal domain of their respective polypeptides (Boylan and Quail, 1989, 1991; Kay et al., 1989; Keller et al., 1989; Cherry et al., 1991; Wagner et al., 1991; McCormac et al., 1993; Quail et al., 1995; Xu et al., 1995; Wagner and Quail, 1995). The C-terminal region is responsible for regulatory capacity of the photoreceptors and contains a proximal PAS homology domain that is delimited by two direct repeats showing sequence similarity to the repeats that define PAS domains (Lagarias et al., 1995; Kay, 1997) which is a hotspot for missense mutations that affect phytochrome function (Quail et al., 1995) and a distal histidine kinase homology domain that shows sequence similarity with transmitter modules of bacterial protein histidine kinases two-component systems (Schneider-Poetsch, 1992). The latter constitute the environmental sensor proteins of bacteria, suggesting that phytochrome acts as a light-regulated protein kinase (Schneider-Poetsch et al., 1991; Thummler et al., 1995a). This proposal has received strong support due to the recent identification of genes homologous to phytochrome in cyanobacteria which also exhibit the canonical motifs of the catalytic domain of histidine kinases within their C-terminal regions (Kaneko et al., 1996; Kehoe and Grossman, 1996).

2.7 PHYTOCHROME REGULATION OF GENE EXPRESSION

Phytochrome affects the expression of several different genes by affecting the abundance of mRNAs. In many photomorphogenic responses the expression of some genes is induced by irradiation, these include the rbc S genes encoding small subunit of ribulose bisphosphate carboxylase/oxygenase
and cab genes encoding chlorophyll a/b binding light-harvesting proteins (Silverthrone and Tobin, 1984), chloroplastic Gln synthetase (Edwards and Coruzzi, 1989) and Fd (Dobres et al., 1987), and others (Tobin and Kehoe, 1994; Terzaghi and Cashmore, 1995). On the other hand some genes, however, become repressed when plants are exposed to light. These include phyA gene encoding phytochrome A (Colbert et al., 1983), Asn synthetase (Tsai and Coruzzi, 1990), and pcr genes encoding protochlorophyllide oxidoreductase (Forreiter et al., 1990).

In oat and rice the reduced PHYA mRNA levels in red or far-red light largely result from a strong and rapid decrease in transcription (Lissemore and Quail, 1988; Kay et al., 1989) which is most likely regulated via the phyA signal transduction pathway (Quail, 1994). Short periods of red light do not detectably reduce PHYA mRNA levels in tomato and Arabidopsis, although continuous white light is effective (Sharrock and Quail, 1989; Somers et al., 1991; Quail, 1994). In contrast, PHYB transcript accumulates in rice, potato and Arabidopsis to similar levels, regardless of light treatment (Sharrock and Quail, 1989; Dehesh et al., 1991; Somers et al., 1991; Heyer and Gatz, 1992; Clack et al., 1994). Consistent with this idea, red light treatment of dark-grown Arabidopsis seedlings has little effect on PHYB polypeptide levels (Somers et al., 1991).

Several promoters of PHYA, rbcS and Lhcb gene families have been examined to characterize the cis elements specifically necessary for mediation of phytochrome regulation (Tobin and Kehoe, 1994; Terzaghi and Cashmore, 1995). For the PHYA gene a cis element involved in repression of activity was found in a 10-bp fragment of promoter (Bruce et al., 1991). A 166-bp fragment
of the pea rbcS-3A promoter was found to be involved in phytochrome regulation (Gilmartin and Chua, 1990). The *Arabidopsis Lhcb1*gene was found to retain phytochrome regulation and circadian responsiveness in a promoter fragment from −111 to -33 (Anderson *et al.*, 1994), and mutations in the region from -74 to -58, which contains multiple GAT A elements, abolished phytochrome responsiveness (Anderson and Kay, 1995). For the *Arabidopsis Lhcb1*gene, sequences downstream of −183 were able to confer phytochrome responsiveness (Sun *et al.*, 1993).

In some instances, it has been shown the **cis-elements** involved in the light responsiveness of the genes are occupied by protein factors. A promoter region of *Lhcb1* gene from -138 to -99 is bound *in vitro* by a protein factor, CA-1 (Sun *et al.*, 1993), and this region is necessary for phytochrome regulation and contains an element involved in maintaining a high level of transcription (Kenigsbuch and Tobin, 1995). In the case of the *cabE* gene, many different factors, including the G-box binding factor (GBF), GA-1, GC-1, AT-1, and GT-1, have been shown to bind to different promoter elements, probably mediating light responsiveness through protein-protein interactions (Schindler *et al.*, 1990, 1992a, 1992c; Dehesh *et al.*, 1992; Gilmartin *et al.*, 1992; Perisic and Lam, 1992).

### 2.8 PHYTOCHROME MUTANTS

Two classes of phytochrome-deficient mutants have been identified viz., mutations affecting specific phytochromes and mutants showing general phytochrome-deficiency (Kendrick and Nagatani, 1991; Reed *et al.*, 1992; Chory, 1993; Koornneef and Kendrick, 1994; Liscum and Hangarter, 1994; Whitelam and Harberd, 1994). The phytochrome A specific mutants are blind
to FR, but respond to R. Mutants deficient in phyA are known in several plant species which have been named as long hypocotyl (hy-8: Parks and Quail 1993), far-red elongated (frel: Nagatani et al, 1993) and far-red long hypocotyl (fhy2: Whitelam et al, 1993) of Arabidopsis, the far red insensitive mutant of tomato (fri; Van Tuinen et al, 1995a), far-red unresponsive mutant of pea (fun1; Weller et al, 1995b). These mutations have been shown to represent in the PHYA gene of independent alleles of Arabidopsis (Dehesh et al., 1993; Whitelam et al, 1993) and due to splicing of mRNA transcript in tomato (Lazarova et al, 1998).

The phytochrome B specific mutants are elongated compared to wild type under red light (R) but not far-red light (FR). In addition, the phyB mutants exhibit reduced cotyledon size, elongated petioles and leaves, stronger apical dominance, and early flowering (Reed et al., 1994). Mutants deficient in phyB are known in the Arabidopsis long hypocotyl (hy-3 = phyB; Koornneef et al., 1980; Nagatani et al, 1993; Somers et al., 1991; Reed et al, 1993), the temporarily red light insensitive mutant of tomato (tli; van Tuinen et al, 1995b), the long hypocotyl mutant of cucumber (lh; Lopez-Juez et al, 1992), the lv mutant of pea (Weller et al, 1995a), the elongated internode of Brassica rapa (ein; Devlin et al, 1992) and the maturity mutant of Sorghum (ma3R; Childs et al, 1992). All these mutants have been shown to be deficient in immunochemically detectable phyB (Devlin et al, 1992; Reed et al, 1993; Weller et al., 1995a; Kerckhoffs et al, 1996). For the phyB mutant of Arabidopsis, the ma3R mutant of Sorghum and the ein mutant of B.rapa it has been directly established that the observed phyB deficiency results from a mutation within the PHYB gene (Reed et al, 1993; Devlin et al, 1997; Childs et al., 1996).
In addition to these specific phytochrome mutants a second class of phytochrome-deficient mutants have an elongated phenotype under both R and FR corresponding to the absence of both phy A and phyB activities (Koomneef et al, 1980; Koomneef et al, 1985; Weller et al, 1996, 1997). The absence of these responses is correlated to a loss of all or most of the spectrophotometrically detectable phytochrome in dark-grown seedlings, indicating that these mutants are lacking the chromophore of phytochrome. The long hypocotyl hyl and hy2 mutants of Arabidopsis (Koomneef et al, 1980; Chory et al, 1989a; Parks and Quail, 1991), aurea (au) and yellow green-2 (yg-2) mutants of tomato (Koomneef et al, 1985; Terry and Kendrick, 1996; Van Tuinen et al, 1996), phytochrome chromophore deficient (pcdl and pcd2) mutants of pea (Weller et al, 1996, 1997) and partly etiolated in white light (pew1 and pew2) mutants of tobacco (Kraepiel et al, 1994) are known to be phytochrome chromophore deficient mutants.

Recent analysis has shown that a specific lesion in chromophore deficient mutants is a consequence of a lesion in chromophore synthesis (Weller et al, 1996, 1997; Terry and Kendrick 1996). The hy-1, and hy-2 of Arabidopsis contain lesions that result in deficiencies in the photochemically functional phytochrome (Koomneef et al, 1980; Chory et al, 1989a; Parks et al, 1989) and affect the synthesis of the phytochrome tetrapyrrole chromophore, since functional phytochrome can be produced by providing these mutants with chromophore precursor biliverdin IXα or phycocyanobilin (Parks and Quail, 1991). HY1 gene has recently been cloned and appears to encode a heme oxygenase (Davis et al, 1999). The pew1 mutant of Nicotiana plumbaginifolia (Kraepiel et al, 1994) can also be rescued by feeding chromophore precursor. The pew2 mutant, however, could not be rescued by
feeding biliverdin, which is also the case of au mutant of tomato. The pcdl and yg-2 mutants are unable to synthesize BV IX from heme (Weller et al., 1996; Terry & Kendrick 1996) and are deficient in phytochromobilin synthase activity (Terry & Kendrick 1996; Weller et al., 1997). The hy6 mutant has also been proposed to be a chromophore-related mutant, defective in either the biosynthesis or attachment of the chromophore to the phytochrome apoprotein (Chory et al., 1989a; Chory 1992).

In all chromophore-deficient mutants, since all phytochromes probably use the same chromophore, it is possible that all phytochromes are deficient in biological function in these mutants and display extreme pleiotropic phenotypes (Koornneef et al., 1980; Chory et al., 1989a). The amount of PHYA which accumulates varies considerably between species ranging from estimates of about 25% of wild type levels for the au (Sharma et al., 1993) and yg-2 (van Tuinen et al., 1996a) mutants to close to 100% in pcdl (Weller et al., 1996) and pewl (Kraepeil et al., 1994). The hy mutants (Chory et al., 1989a; Parks et al., 1989), pcd2 (Weller et al., 1997) and pew 2 (Kraepeil et al., 1994) all have PHYA levels somewhere between these two extremes. In contrast, PHYB levels always appear to be unchanged (Sharma et al., 1993; van Tuinen et al., 1996; Weller et al., 1996). PHYA from the pcdl mutant has been partially purified and assembled in vitro with phycocyanobilin, an analogue of the phytochrome chromophore, to yield a photoreversible holoprotein (Weller et al., 1996).

2.9 PHYTOCHROME FUNCTIONS

The phytochromes integrate a number of parameters in the light environment and thereby regulate a wide range of light-mediated responses
(Smith 1995; Kendrick and Kronenberg, 1994) by functioning in different mode of actions. These are defined by Low Fluence Response (LFR), and Very Low Fluence Response (VLFR) activated by extremely low light intensities and High Irradiance Response (HIR) activated by constant exposure to relatively high photon fluxes. HIRs are further subdivided into red light- and far-red light-mediated HIR. In contrast to LFRs neither HIRs nor VLFRs are photoreversible. In addition to this series of distinguishing characteristics, each response is distinct in that it displays a clear fluence threshold at which the response saturates.

Mutants lacking one or several of phytochromes have provided useful information concerning the role of individual photoreceptors and their interaction in transducing light signals (Barnes et al., 1997). The availability of phytochrome genes from several plant species also facilitated an alternative approach to analyze photoreceptor function through their expression in transgenic plants. The functions of phyA and phyB have been defined through analysis of responses to constitutive overexpression of phytochrome sequences (Boylan and Quail, 1989, 1991; Kay et al., 1989; Keller et al., 1989; Cherry et al. 1991; Wagner et al., 1991; McCormac et al., 1993). Overexpression of monocotyledonous phyA in the dicotyledonous tobacco and tomato has shown that introduced heterologus phyA is biologically active (Boylan and Quail, 1989; Kay et al., 1989; Keller et al., 1989) and act through normal signal transduction pathways (Nagatani et al., 1991).

Based on the physiological and genetic analysis, the phytochromes have been postulated to have both overlapping and distinct functions (Nagatani et al., 1991, 1993; Somers et al., 1991; Dehesh et al., 1993; Parks and Quail,
PhyA is the primary phytochrome mediating various plant responses to continuous FR light (McCormac et al., 1993; Dehesh et al., 1993; Nagatani et al., 1993; Parks and Quail 1993; Whitelam et al., 1993) and have very limited significance under continuous white light, regardless of the fact that PhyA is predominant molecular species in dark-grown tissues (Somer et al., 1991). PhyA is responsible for the FR-HIR inhibition of hypocotyl elongation, cotyledon and apical hook opening (Johnson et al., 1994; Koornneef et al., 1980; Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al, 1993). Phy A also mediates accumulation of anthocyanin in FR light (Kunkel et al., 1996) and the so-called FR-preconditioned blocking of greening (Barnes et al., 1996a; Runge et al, 1996; van Tuinen et al, 1995a).

PhyA regulates the induction responses via the VLF mode of phytochrome action such as seed germination (e.g., Botto et al, 1996; Parks et al, 1996; Mazella et al, 1997; Shinomura et al, 1996) phototropism (Parks et al, 1996; Janoudi et al, 1997) and gravitropic orientation of hypocotyl (Hangarter, 1997; Poppe et al, 1996; Robson and Smith, 1996). The VLFR mediated by phyA do not require continuous light, and are induced by R, FR, or any wavelength between 300 and 800 nm (Botto et al, 1996; Shinomura et al, 1996; Mazella et al, 1997). The VLF mode of phytochrome action are characterized by a lack of R/FR reversibility and it has been suggested that phyA action during induction reactions is not photoreversible (Furuya and Shecafer, 1996). Apart from VLFR and FR-HIR responses, phyA may also play a role in mediating inductive responses to pulses of R. For instance, Parks et
ai, (1996) have shown that, phyA seedlings show no marked enhancement of first positive phototropic curvature.

A major role for phyB in many light-grown seedlings is the perception to R pulses or to continuous R irradiation (e.g Reed et al, 1993, Quail et al, 1995), alteration in R:FR ratio (Smith, 1995) and EODFR. The phyB seems to be the major contributor to the red/far-red reversible low-fluence responses (Furuya and Schafer, 1996; Casal et al., 1998). The phyB also plays a dominant role in the shade-avoidance reaction including the effects of red light and of the R:FR ratio (Smity 1995; Smith and Whitelam 1997; Mc Cormac et al, 1993;Devlin et al.,1992 ;Nagatani et al, 1991,1990; Somers et al., 1991). In light-grown seedlings, the LFR is manifested as responses to end-of-day (EOD) light pulses, such as the promotion of elongation growth or flowering by EOD-FR (e.g. Lopez-Juez et al., 1990; Nagatani et ai, 1991; McCormac et ai, 1993; Parks and Quail 1993; Halliday et ai, 1994; Devlin et ai, 1996). A further role for solanaceous phytochrome B1 has been revealed in potato where it has been demonstrated that plants transformed with an antisense PHYB lose photoperiodic control of tuberization (Jackson et ai, 1996).

Although the responses to continuous R are largely mediated by phy B (Koornneef et al., 1980; McCormac et ai. 1993; Parks and Quail 1993) a residual effect can often to observed that suggests the action of other phytochromes (Casal 1995). An Arabidopsis phyD mutation was identified as a naturally occurring allele, which encoded no functional phyD protein (Aukerman et al., 1997). The monogenic phyD mutant plants had no obvious phenotypic abnormality, whereas plants impaired in both the PHYB and PHYD genes flowered earlier than the phyB monogenic mutation (Aukerman et al.,
1997; Devlin et al., 1999b). This indicates that, like phyB, phyD inhibits flowering. A genetic screen was carried out to look for mutations that exhibited elongated rosette internodes, resulting in the isolation of the phyE mutation (Devlin et al., 1999a). The phyE mutant showed no phenotypic alteration unless it was in the phyB mutant background. This indicated the function of phyE is also similar to that of phyB.

2.10 INTERACTIONS BETWEEN PHYTOCHROMES

PhyA and PhyB are capable of mediating some common responses following brief or prolonged R irradiation and share the control of responses but little is known about their interactions in the mutual signaling regulation. Synergistic interactions between phyA in its HIR mode of action and phyB (Casal, 1995) have been reported for hypocotyl growth and cotyledon unfolding in Arabidopsis. In contrast, phyA in its VLF mode of action may act antagonistically with phyB (Mazzella et al., 1997; Yanovsky et al., 1997). Recent evidences indicate that PhyA acting in VLFR mode is antagonistic to phyB signaling whereas PhyA acting in the HIR mode operates synergistically with phyB signaling in the control of Lhcb1*2 and hypocotyl growth in Arabidopsis. (Cerdan et al., 1999).

The redundant activities of phyA and phyB were revealed when the null mutations, phyA-201 and phyB-8-36, were compared with the double mutant (Reed et al., 1994). Double mutant plants germinated under white, red, or far-red light consistently show longer hypocotyls than do single mutants, suggesting that these two phytochromes partially compensate for each other's activity during hypocotyl elongation. In red light, the double mutant displays enhanced defects in greening and produces a more pronounced apical hook and
smaller cotyledons than either single mutant. The phyA phyB double mutant also displays severely impaired induction of chlorophyll a,b binding protein (CAB) transcription by red light in comparison with CAB accumulation in the single mutants. More recently, Devlin et ai, (1996) have reported another example of redundancy between phyA and phyB. Mature plants of the phyA,phyB double mutant, grown under 10 h light, 14 h dark photoperiods, have reduced stature with only about 30-40% of the biomass of wild type plants. The biomass of individual monogenic phyA or phyB mutants plants is not significantly different from that of wild-type plants (Devlin et ai, 1996).

PhyD and PhyE also perform specific regulatory functions in Arabidopsis that only partially duplicate those of phyB (Aukerman et ai, 1997). Loss of phyD causes alteration of many of the same shade avoidance responses which are affected in the phyB mutant, but comparison of the two null mutants shows that phy B plays a much more prominent role than phyD. Hence, diversification of the PHY gene family, has allowed the evolution of distinct photosensory roles for the photoreceptor subfamilies, with the most divergent genes, exemplified by PHYA and PHYB, having highly divergent functions and the most closely related genes, PHYB and PHYD, having overlapping or even somewhat redundant roles. In addition to redundant actions photoreceptors can also work in antagonistic ways to inhibit responses (Reed et a., 1994; Devlin et al., 1996; Neff and Chory, 1998).

2.11 BLUE/UV-A LIGHT RESPONSES

There are a wide variety of blue-light responses in higher plants. These include the blue-light-induced suppression of hypocotyl elongation, phototropism, anthocyanin induction, expression of specific blue-light
regulated genes, opening of stomata and flowering (Kaufman, 1993). Many such responses show action spectra that are consistent with a flavin chromophore, with peaks of activity in the near-UV (around 350 nm) and blue (450-480 nm) regions of spectrum indicating that these class of responses are mediated by photoreceptors distinct from phytochromes. However, due to the difficulty of obtaining a good in vitro assay system for the pigment, there was only recently little progress made towards identification of these receptors (Briggs and Huala, 1999; Lin, 2000).

2.12 Blue/UV-A Light Mutants

Several mutants have been identified that lack either growth inhibition to blue light. The long hypocotyl (hy-4: Koornneef et al, 1980) and blue light uninhibited (blu:Liscum and Hangarter, 1991) mutants of Arabidopsis show deficient hypocotyl growth responses to blue light. Although the blu mutants were at first identified to be at different loci to hy4, however, subsequent analysis showed that they are alleles of hy4 (Jenkins et al, 1995). The hy-4 mutant selectively lacks the inhibition of hypocotyl elongation when grown under B, however, the mutant is normal with respect to far-red light (FR) and red light (R) hy responses mediated by phyA and phyB (Koornneef et al., 1980). On the other hand, the cryptochrome (cry2) mutant, seedlings have longer hypocotyls than the wild type under relatively low fluence rates of blue light (Lin et al., 1998), which also flowers late in LD (long day) but not in SD (Short day) photoperiods and impaired in photoperiod sensing (Guo et al., 1998). Cry2 is found to be allelic to a previously isolated photoperiod-insensitive flowering time mutant fha (Guo et al, 1998; Koornneef et al, 1991).
The physiology of phototropism is complex and the relationship of phototropic response to the fluence of unilateral light has been studied in different plant species (Lino, 1990). In most species studied, two types of positive responses are observed, separated by indifferent zone in which very small, no or even negative response is seen. 'First positive' phototropism is characterized by relatively weak response induced by short exposures to relatively high fluence rates. In contrast, the magnitude of 'second positive' phototropism induced is large, and is a direct function of exposure time. The phototropism is further complexed by the fact that after red light pre-irradiation an increase in seedling responsiveness takes place, known as enhancement of phototropism (Janoudi et al., 1990, 1991 and 1992). Mutations have been identified that affect first and second positive phototropism in Arabidopsis. Liscum and Briggs (1995) isolated non-phototropic mutants (nph: for non-phototropic hypocotyl) at four loci (NPH1-4) in Arabidopsis. Mutants JK224 and 218 isolated by Khurana and Poff (1989) are now found to be alleles of nph1 and nph3 (Liscum and Briggs, 1995). Two additional mutants, ZR8 and ZR19, that show normal second positive phototropism, but reduced first positive phototropism (Khurana et al., 1989) were also isolated.

2.13 BLUE/UV-A PHOTORECEPTORS

The use of mutants with known phenotypic defects has provided some information about the nature of blue/UV-A photoreceptors. There are now evidences for more than one distinct BL photoreceptor for blue light induced responses (Liscum et al., 1992; Liscum and Briggs, 1995; Ahmad and Cashmore 1996; Short and Briggs 1994; Lazava et al., 1999). For example, studies on blue light mediated hypocotyl growth inhibition and phototropic responses have provided evidence that they are physiologically and genetically
independent (Liscum et al., 1992) and are regulated by different photoreceptors viz., cryptochromes (Ahamad and Cashmore, 1993), and phototropin (Huala et ai, 1997) respectively.

2.14 CRYPTOCHROME RECEPTORS - MOLECULAR PROPERTIES

The HY4 gene, affecting hypocotyl inhibition in BL, has been isolated by T-DNA tagging (Ahmad and Cashmore 1993) which encodes a blue light photoreceptor named cryptochrome I (CRY1; Lin et ai, 1995). This photoreceptor has been shown to be responsible for blue light perception by various lines of evidences. The N-terminal two-thirds of CRY1 shows 30 % sequence homology to microbial class I DNA photolyase, a kind of flavoprotein. However, the CRY1 lacks any detectable photolyase activity, and it also lacks a tryptophan (W227 in the E.coli photolyase sequence; Li and Sancar, 1990) residue found in photolyases and believed to be important for binding to pyrimidine dimers (Ahmad and Cashmore, 1993; Lin et ai, 1995). Disruption of the region adjacent to the conserved chromophore binding motif found in all class I photolyases and in CRY1(Ahmad and Cashmore, 1993) of E.coli photolyase completely inactivates the enzyme. The C-terminal region of CRY1 is functionally important as lesions within the corresponding region of the HY4 gene confer a mutant phenotype (Ahmad and Cashmore, 1993; Ahmad et ai, 1995).

Secondly, analysis of recombinant CRY1 expressed in and purified from insect and bacterial cells shows that the recombinant protein from insect cells binds FAD noncovalently and in its oxidized state, has an absorption spectrum expected for a blue/UV-A light photoreceptor (Lin et ai, 1995a, 1995b). In bacterial cells, the recombinant CRY1 protein binds a pterin,
methenyltetrahydrofolate (MTHF), in addition to FAD (Malhotra et al., 1995). This pterin chromophore is most likely responsible for the majority of the blue-light absorbing properties of CRY1. Finally, overexpression of CRY1 in transgenic tobacco or Arabidopsis plants resulted in plants that were hypersensitive to light; the transgenic plants showed an enhanced hy response and increased production of anthocyanin (Lin et al., 1995, 1996a). These enhanced responses were observed under B, UV-A, green or white light, but not R or FR. Recently, CRY1 gene (TCRY1) has been identified in tomato that shows 78% identity and 88% similarity to Arabiopsis CRY1 (Ninu et al, 1999).

A second gene related to CRY1 was isolated from Arabidopsis, which was named AT-PHH1 (Hoffman et al., 1996), or CRY2 (Lin et al., 1996a). The CRY2 is similar to CRY1 but contains a distinct C-terminal sequence (Lin et al., 1996b). The CRY2 gene encodes a protein of 612 amino acids, which is 54% identical with CRY1 within the N-terminal 500 amino acids. CRY2 does not code for a photolyase (Hoffmann et al., 1996). Another member of cry gene family the Sinapis alba gene SA-PHR-1, lacks a C-terminal extension and, like Arabidopsis CRY1, its protein product is devoid of DNA photoreactivating activity both in vitro and E.coli (Batschauer, 1993; Malhotra et al., 1995). Further, CRY genes have been isolated from organism other than higher plants, including Chlamydomonas (Small et al, 1995).

2.15 PHOTOTROPIN RECEPTOR- MOLECULAR PROPERTIES

The NPH1 gene of Arabidopsis, affecting phototropic responses, encodes blue light photoreceptor known as phototropin that is essential for phototropism and is a plasma membrane-associated phosphoprotein (Lin et at,
The phototropin is an apoprotein of 996 amino acids (112 kDa) and the coding region consists of 20 exons extending for 5.4 kb. The deduced amino acid sequence suggests that the C-terminal region of the gene encodes a serine/threonine protein kinase. The N-terminal region of the gene has two repeated domains, LOV1 and LOV2, that share similarity with diverse proteins of archaea, eubacteria and eukaryotes that detect changes in redox status as affected by light, oxygen, or voltage (hence LOV) (Huala et al., 1997). This LOV domains binds flavin chromophore (Christie et al., 1999).

The putative phototropin photoreceptor has been shown to be blue light photoreceptor for phototropism by the following evidences. In the plant, it becomes phosphorylated on exposure to blue light; this reaction was detected as biochemical step necessary for blue light signal transduction before identifying it as a receptor (Gallagher et al., 1988; Reymond et al., 1992). The spatial and temporal, fluence and dark-recovery kinetics of the phosphorylation response correlate well with phototropic response which led to the conclusion that the phosphorylation event was likely to be a part of the phototropism signal transduction pathway (Short and Briggs, 1990, 1994). In addition, a gradient asymmetric distribution of phosphorylation of the protein within the coleoptile at the irradiated versus the shaded side of oat coleoptiles has been demonstrated (Salomon et al., 1997a, 1997b).

Secondly, the detailed biochemical and genetic analysis of Arabidopsis mutants lacking phototropic responses further supported this correlation. The hy-4, coding for cryptochrome blue light receptor showed normal phototropism indicating that it is not involved in phototropism. On the other hand, Reymond et al., (1992) showed that Arabidopsis strain JK224, which has a threshold
fluence for first positive curvature approximately 100-fold greater than wild-
type, was deficient for the light-induced phosphorylation. Furthermore, the
nph1 mutant allelic to JK224 mutant with the concomitant loss of the 120 kD
protein in all its null alleles and of any blue light-dependent phosphorylation,
gave rise to the hypothesis that phototropism may be regulated by a
photoreceptor that becomes phosphorylated upon irradiation with blue light
(Liscum and Briggs, 1995). Finally, by expressing the NPH1 gene in insect
cells found that the protein produced had exactly the same light sensitivity as
the protein from the plant (Huala et al., 1997). Hence, it was proposed that
NPH1 was the photoreceptor for the phototropic response.

2.16 BLUE/UV-A PHOTORECEPTORS FUNCTIONS

In Arabidopsis, CRY1 has been implicated in developmental responses
like blue light-induced inhibition of hypocotyl elongation, stem growth and
internode elongation, leaf and cotyledon expansion, B-dependent gene
expression, and anthocyanin accumulation (Ahmad and Cashmore 1993;
Ahmad and Cashmore, 1996; Fuglevand et al, 1996; Koornneef et al, 1980; Lin
et al., 1995b; Lin et al., 1995a; Ninu et al, 1999).

Mutant studies and transgenic plants have enabled identification of the
function of CRY2. The late flowering Arabidopsis mutant fha-l (Koornneef et
al, 1991) is defective in the CRY2 gene (Guo et al, 1998), indicating that
CRY2 is a blue light photoreceptor which is involved in measuring the day-
length. Transgenic plants overexpressing CRY2 were hypersensitive to blue
light and developed short hypocotyls under blue light or white light (Lin et al,
1998) and cry2 mutant seedlings had longer hypocotyls than the wild type under
relatively low fluence rates of blue light (Lin et al, 1998), indicating that the
function of *cry2* in hypocotyl inhibition was limited to low intensity light. This result was interpreted as being the consequence of the blue-light-induced degradation of CRY2 protein in high intensities of blue light (Lin *et al.*, 1998). Specifically, overexpression of CRY2 resulted in substantial increase in the sensitivity of cotyledon expansion to blue light (Lin and Cashmore, 1996).

Extensive biochemical, physiological and genetic work done has shown that NPH1 is the only photoreceptor for both first and second positive phototropism (Huala *et al.*, 1997; La 1999). In addition, the CRY1 in combination with CRY2 are implicated in first positive phototropism (Ahmad *et al.*, 1998) but recent analysis showed that phototropin alone is the main photoreceptor and CRY1 and CRY2 only modulate the response downstream of the receptor (Lasceve *et al.*, 1999).

### CO-ACTION OF PHOTORECEPTORS

#### 2.17 CO-ACTION OF PHYTOCHROMES AND BL/UV-A PHOTORECEPTORS

Though phytochromes and blue/UV-A photoreceptors employ distinct signal transduction pathways (Kunkel *et al.*, 1996; Yanovsky *et al.*, 1997), these receptors display an intense interactions (Casal, 2000). Numerous physiological experiments on inhibition of hypocotyl growth and photoregulation of anthocyanin have documented cases of synergy or interdependent co-action between phytochrome and the BL photoreceptor(s) (Mohr, 1994). In independent co-action both photoreceptors can elicit the same response, interdependent co-action can be of several possibilities (Mohr, 1994). Nevertheless, while the mode of co-action and the relative importance of different photoreceptors is still unclear; some species appear to respond only to phytochrome and blue/UV-A photoreceptors (Gaba and Black, 1979).
The study of co-action is inevitably further complicated by the fact that B/UV-A is also absorbed by phytochrome leading to Pfr production and phytochrome cycling. It can absorb blue light both in vitro and in vivo (Butler et al., 1964; Jabben et al., 1982). However, the phytochromes are generally not regarded as blue/UV-A photoreceptors because they operate maximally in red and far-red regions of visible light. Hence, it has been hypothesized that the blue light receptor function is independent of phytochromes (Cosgrove, 1981; Gaba and Black, 1987) or the blue light receptor is completely dependent on the presence of active phytochrome and only modulates the sensitivity of phytochrome signal transduction cascade (Oelmuller and Mohr, 1985). Mohr (1994) proposed a model of co-action between BAJV-A photoreceptors and the phytochrome photoreceptor system. The Pfr is considered as the effector while the B/UV-A effect is considered as amplifying responsiveness toward Pfr. The general observation seems to be that the responsiveness towards Pfr can be strongly increased - even induced - by a pre-irradiation, which is absorbed by cryptochrome. Recently, the stimulation of responsiveness to Pfr was not only detected after B but also after FR pre-irradiation (Beggs et al., 1981; Casal, 1995).

As a consequence crucial question was addressed as to whether blue light responses are mediated by phytochromes and/or blue light photoreceptors. The genetic evidences obtained from phytochrome deficient mutants indicate that phytochrome can also act as blue light receptor in different responses. Under continuous BL, phytochrome deficient mutants show virtually wild-type responses suggesting that phyA and BL photoreceptors act independently in an additive manner and contributes to the blue light induced hypocotyl growth inhibition response (Koornneef et al., 1980; Young et al. 1992; Liscum and
Hangarter, 1994). By using phyA, phyB, and phyAphyB double mutants of *Arabidopsis*, it has been shown that PHYA is the most sensitive blue light receptor for the induction of seed germination (Shinomura *et al.*, 1996) or LHCB gene expression in VLF. Moreover, PHYB and an additional phytochrome of unknown identity contribute to a LF blue light induction of LHCB which shows far-red light reversibility (Hamazato *et al.*, 1997). Similarly, by using double mutants *phyA,phyB* it has been shown that the presence of either phyA or phyB is required for first positive phototropism and time threshold of second positive phototropism (Janoudi *et al.*, 1997; Hangarter, 1997). Furthermore, it has been demonstrated that white and blue light induced accumulation of anthocyanin requires the presence of at least one of the phytochromes: either phyA or phyB (Kunkel *et al.*, 1996; Ahmad and Cashmore, 1997).

Evidence for the blue/UV-A receptor acting through phytochrome is further supported by studies in other plant species. The *au* mutant of tomato deficient in all phytochromes retains some responses to BL and lacks others (Oelmuller and Kendrick 1991; Oelmuller *et al.*, 1989; Casal 1994). Levels for several light regulated nucleus encoded transcripts in wild-type tomato show a blue-light-induced increase beyond the levels produced by red light alone. However, neither red nor blue light leads to accumulation of these transcripts in the *au* mutant, showing that blue light is ineffective in the absence of phytochrome in this system (Oelmuller *et al.*, 1989). In the case of the *lh* mutant of cucumber, deficient in phyB (Lopez-Juez *et al.*, 1992) light-grown plants do not respond to BL (Ballare *et al.*, 1991; Lopez-Juez *et al.*, 1992). In contrast, the overexpression of oat phyA impairs the response mediated by BL receptor(s) (Casal and Sanchez, 1994).
Another example of interacting photosensory systems is evidenced by the ability of phytochrome to enhance blue-light mediated phototropism (Steinitz et al., 1985). It has been found that the red-light preirradiated seedlings usually exhibit an enhanced phototropic curvature in response to subsequent stimulation by unilateral blue light (Chon and Briggs, 1966; Janoudi and Poff, 1991, 1992). Analysis of the various phytochrome deficient mutants indicates that phy A is primary photoreceptor responsible for the enhancement response by low fluences of red light (Parks et al., 1996). Moreover, there are indications for a response-specificity in the action of phy A and phyB (Parks et al., 1996). By studying phy A and phyB mutants and transgenic lines overexpressing these phytochrome species, Janoudi et al., (1997a, b) have indicated that the involvement of phy A is essential in the very-low to low-fluence range for enhancement and either phyA or phyB is required for the high-fluence enhancement by red light. However, the nature of the interaction between phytochromes and phototropic response is not understood.

Different species of phytochromes and cryptochromes are known to interact synergistically or antagonistically following a brief or prolonged B irradiation and share the control of response. Though CRY1 interacts with PHYB, it does not interact synergistically with PHYA (Casal and Boccalandro, 1995). Moreover, measurements of blue-light induced shrinkage of protoplasts indicate that PHYB was mainly responsible for the phytochrome dependence of this CRY1 mediated response and not PHYA (Wang and Linó, 1997). In contrast, Ahmad and Cashmore (1997) observed reduced responses to blue light compared with darkness in phyA,phyB mutant of Arabidopsis and proposed that PHYA or PHYB is necessary for CRY1 activity. Recent investigations indicate an interaction of CRY1 and PHYA in vitro (Ahmad et
Further, the physiological and genetic studies demonstrate that CRY2 suppresses or antagonistic to the function of the PHYB-dependent inhibition of floral initiation (Guo et al., 1998; Mockler et al., 1999).

LIGHT SIGNAL TRANSDUCTION ELEMENTS

2.18 PHOTORECEIVER SIGNAL TRANSDUCTION - BIOCHEMICAL ELEMENTS

Though considerable progress has been made in identifying the photoreceptors that mediate the effects of light and the cis-elements and transcription factors that are involved in the photoregulation of specific genes, however, the signal transduction processes that couple photoreception to transcription remains fragmentary. Several components of phytochrome signal transduction pathways have been identified by biochemical methods (Millar et al., 1994). There is evidence that G protein activation is an early event (Neuhaus et al., 1993; Romero and Lam, 1993), and transient increases in cytosolic calcium ions have been reported (Roux et al., 1986; Chae et al., 1990; Shacklock et al., 1992; Volotovski, 1998).

Using a combination of microinjection of pharmacological agonists and antagonists in au mutant of tomato, the phyA signaling has been shown to involve the activation of one or more heterotrimeric G proteins and the subsequent participation of three different pathways dependent upon calcium and/or cGMP. The calcium-dependent pathway regulates the expression of genes such as CAB and is able to direct partial chloroplast development. The cGMP-dependent pathway regulates expression of CHS and production of anthocyanin pigments. A third calcium/cGMP-dependent pathway is required for FNR gene expression and full chloroplast maturation (Bowler et al., 1994a). These pathways cross-regulate each other by reciprocal control (Bowler et al., 1994a).
1994b). For example, high levels of cGMP can negatively regulate the two calcium-dependent pathways, and high levels of calcium or calcium-activated calmodulin can negatively regulate the cGMP pathway that controls CHS expression.

2.19 Phytochrome Signal Transduction - Genetic Elements

Wagner et al., (1997) recently proposed the hypothesis of specific pathways of signal transduction downstream from phyA and phyB. This possibility is supported by the observations that loci such as fhy1, fhy3 (Whitelam et ai., 1993), vlfl and vlf2 (Yanovsky et ai., 1997), fin 2 (Soh et ai., 1998) and the spal have been genetically identified in the PhyA signaling pathway that affect phyA-mediated responses but not phyB-mediated responses. The mutants denoted fhyl and fhy3, are not linked to the PHYA gene. They resemble phyA-deficient mutants despite possessing normal levels of spectrally active PHYA protein and normal levels of PHYA mRNA (Whitelam et ai., 1993). The fhyl mutant is blocked, however, in only a subset of phyA-mediated responses at the physiological level, as phy-A dependent effects on germination are normal in fhy1 mutants (Johnson et ai., 1994), suggesting that fhy-1 defines a branchpoint in phyA signal transduction pathways. Indeed, this has recently been demonstrated at the level of gene expression; fhy1 is deficient in phyA-regulation of only a subset of genes, such as CHS, whereas the regulation of genes such as CAB and nitrate reductase (NR) is relatively intact (Barnes et ai., 1996). By contrast, the pef2, pef3, (Ahmad and Cashmore, 1996) and redl (Wagner et al., 1997) mutants have reduced de-etiolation only in R, indicating that these loci may be specific to phyB - but not phyA-mediated signal transduction and act as positive regulators in the signaling pathway.
The mutant spal also specifically affect only responses to FR and, hence, phyA signaling (Hoecker et al., 1998). Compared to fliyl and fliy3 mutants which show impairment in phyA-regulated response, the spal mutant shows amplified phyA signaling and is thought to be mutated at a locus encoding a negatively acting signaling component. Very recently, it has been shown that the SPA1 encodes a nuclear-localized 115 kDa protein with WD-repeats as found in COP1 (Hoecker et al., 1999). Because of their resistance to far-red-induced death and their inability to respond to PHYA-mediated gene expression, the pat mutants are also related to PHYA signaling (Cordelia Bolle et al., 1999).

Although phyA and phyB activities occur under different light conditions, the end-point responses (e.g., hypocotyl growth, cotyledon unfolding, flowering etc.,) controlled by phyA and phyB are largely same. The phototransduction pathways of phyA and phyB obviously converge at some point. The relative position of the point of convergence is not known but it has been proposed that phyA and phyB share the same reaction partner (Wagner et al., 1996a, 1996b; Ahmad and Cashmore, 1996). The mutants such as the pefl mutant (Ahmad and Cashmore, 1996) shows reduced R- and FR-mediated responses, whereas the psi 1 (for phytochrome signaling) mutant (Genoud et al., 1998) shows enhanced R-and FR-mediated responses and thought to encode negatively acting component, suggesting disruption in both phyA and phyB signaling.

2.20 PHYTOCHROME SIGNAL TRANSDUCTION - INTERACTING PARTNERS

Several phytochrome interaction partners have been isolated by yeast two-hybrid system. A new type of basic helix-loop-helix type protein (pif3) has
been identified as possible signal-receiving protein and constitutively localized in the nuclei with similarities to b-HLH transcription factors (Ni et al., 1999). Pif 3 contains a single PAS-like domain in its N-terminal region, and specifically interacts with the C-terminal domains of both phyA and phyB. Transgenic plants overexpressing the PIF3 gene in antisense orientation show reduced responsiveness to red and far-red treatments. Furthermore, the interaction of PIF3 with the mutants derivatives of the C-terminal domains of phyA and phyB, was also severely compromised in vitro. These data suggest that PIF3 is indeed required for phyA and phyB signaling and its interaction with phyA and phyB is probably mediated by the PAS-like domains present in these proteins. Recently it has been shown that the photoactive phytochrome B binds PIF3 in vitro only upon light-induced conversion to its active form, and that photoconversion back to its inactive form causes dissociation from PIF3 (Ni et al., 1999) providing a potential mechanism for direct photoregulation of gene expression.

Moreover, recently Martinez Garcia et al., (2000) have shown that phytochrome B binds reversibly to G-box-bound PIF3 specifically upon light-triggered conversion of the photoreceptor to its biologically active conformer, suggesting that phytochromes may directly regulate the transcriptional machinery of specific genes by physically complexing with promoter bound transcriptional regulators. Using a similar approach, Fankhauser et al., (1999) have isolated a protein designated phytochrome kinase substrate 1(PKS1) which binds to both PHYA and PHY C-terminal domains. Choi et al., (1999) have also isolated nucleoside diphosphate kinase 2 as phyA-interactor which is involved in phytochrome signaling. These studies have thus identified potential immediate signaling partners for phytochrome A and B.
2.21 Blue/UV-A Signal Transduction - Biochemical Elements

Warpeha et al., (1991) have identified a blue-light activated heterotrimeric GTP-binding regulatory protein associated with the plasma membrane of pea apical buds. The threshold fluence for blue-light excitation of the G-protein resembles that for the blue-light induced transcription of the Cab gene family in pea. Plasma membranes derived from the apical buds of peas exhibit GTPase activity and GTP-γ-S binding when irradiated with blue light but not when irradiated with red light. The α-subunit was identified as a 40-kD polypeptide by several means, including cross-reactivity with polyclonal antibodies directed against transducin, blue-light-specific binding of a photoaffinity-labeling GTP analog, blue-light specific ADP-ribosylation by chloera toxin, and blue-light-specific inhibition of ADP-ribosylation by pertussis toxin. The receptor driving the G-protein activity is likely to be a flavoprotein (Warphera et al., 1992). Compounds such as phenylacetate and potassium iodide, inhibiting transfer of excitation energy from flavins to nearby proteins, inhibit the ability of blue light to activate the G-protein.

As for the blue light and UV receptors responsible for anthocyanin accumulation in Arabidopsis, the effects of pharmacological agents indicated that an increase in cytoplasmic Ca2+ is somehow involved in, although not sufficient to cause, the light-induced increase in CHS mRNA in suspension-cultured cells (Christie and Jenkins, 1996). Also, the effects of kinase and phosphatase inhibitors indicate a role for phosphorylation in the signal cascade (Christie and Jenkins, 1996).
2.22 BLUE/UV-A SIGNAL TRANSDUCTION - BIOPHYSICAL CHANGES

The blue light dependent inhibition hypocotyl elongation initiates within seconds of blue-light exposure, and results from a change in the cell wall’s ability to relax and expand (Cosgrove, 1988). The most immediate effect of blue-light irradiation is a transient hyperpolarization of the plasma membrane of cucumber hypocotyl cells (Spalding and Cosgrove, 1992). The hyperpolarization, as large as 100 mV, precedes the cessation of stem elongation and is rectified within 2 to 3 min. The inhibitory effects of vanadate and KCN strongly suggest that depolarization is due to plasma membrane H+-ATPase. Repolarization appears to involve calcium channel. Membrane depolarization correlates with the suppression even both temporally and with respect to the threshold of the response. Furthermore, the CRY1 could mediate the activation of anion channels which may lead to plasma membrane depolarization was shown to be involved in blue-light-dependent growth inhibition (Cho and Spalding, 1996; Parks et al., 1998).

2.23 BLUE/UV-A SIGNAL TRANSDUCTION - GENETIC ELEMENTS

Genetic studies have defined the elements acting downstream of phototropin in phototropism by isolating mutants nph-3 and nph-4 (Liscum and Briggs, 1995). Further the studies have shown that nph-4 acts downstream of nph-3 and acts as a conditional modulator of auxin-dependent growth responses in Arabidopsis (Stowe-Evans et al., 1998). Recently, both NPH3 and NPH4 genes were cloned by positional cloning. The NPH3 encodes a protein of unknown function that interacts in vitro with phototropin (Motchoulski and Liscum, 1999). The NPH4 gene product shows high homology to auxin-regulated transcriptional regulators (Harper et al., 2000).
2.24 TRANSLOCATION OF RECEPTORS TO NUCLEUS

The phytochromes and cryptochromes are known to translocate in a light dependent fashion into the nucleus. R-dependent import of PHYB-GUS fusion protein into the nucleus (Sakamoto and Nagatani, 1996). Interestingly, the nuclear import of PHYA is dependent of FR and PHYB upon R treatment. The CRY1 photoreceptor was found to be a soluble protein expressed at similar levels in dark and light grown *Arabidopsis* seedlings (Lin *et al.*, 1996a, 1996b), but was also found to be enriched in the membrane fraction (Ahmad *et al.*, 1998a). Very recently it was shown that a fusion protein consisting of CRY1 and GFP localizes to the nucleus, indicating that CRY1 is a nuclear protein (Cashmore *et al.*, 1999). Arabidopsis CRY2 is also localized to the nucleus (Kleiner *et al.*, 1999; Guo *et al.*, 1999). The CRY2 contains a putative nuclear localization signal (NLS) within its C-terminal region (Kleiner *et al.*, 1999).

2.25 POSITIVE REGULATORS OF LIGHT SIGNAL TRANSDUCTION

Although it appears that the blue-light and phytochrome photosensory systems act in an independent and additive manner, they probably have some common elements in their signal transduction systems. This hypothesis is supported by the hy5 mutant of *Arabidopsis*, which exhibits reduced hypocotyl growth inhibition to red, far-red and blue light, and produces less anthocyanins in the light than do wild type plants (Koornneef *et al.*, 1980). The hy-5 is also unlinked to photoreceptor mutations and possesses normal amounts of spectrally active phytochrome (Koornneef *et al.*, 1980; Chory *et al.*, 1989a; Parks *et al.*, 1989; Nagatani *et al.*, 1991; Somers *et al.*, 1991). Furthermore, the hy5 mutant appears to be at least partially epistatic to the phytochrome-deficient mutants (Koornneef *et al.*, 1980) and hy4 (Chory 1992) suggesting that the HY5 gene product is a common downstream element in the red/far-red-
and blue-light response pathways (Chory 1992, 1993). Recently, the HY5 has been identified as a protein with homology to b-ZIP transcription factors.

2.26 CONSTITUTIVE AND EXAGGERATED PHOTOMORPHOGENIC MUTANTS

Putative constitutive-response mutants have been screened with light-grown morphologies when grown in the dark. Such mutations have been isolated from three plant species: the ‘de-etiolated’ (det), ‘constitutively photomorphogenic’ (cop) and ‘fusca’ (fus) mutants (Chory et al., 1989; 1991; Deng et al., 1991; Misera et al., 1994) of Arabidopsis, Pisum sativum L. (Frances et al., 1992; Kwok et al., 1996), and tobacco (Trass et al., 1995) and all mutations identified to date are recessive. Their dark-grown seedlings have lost some of the characteristics of the dark developmental pathway and exhibit aspects of photomorphogenic development. In addition, two suppressor mutants shy (suppressor for hy2) at two loci (shy 1-2) of long hypocotyl mutant (hy-2) showing photomorophogenic phenotype in dark are identified in Arabidopsis which are dominant. They are partially constitutive and exhibit phenotypic traits in addition to those due to loss of phytochrome activity and involved farther downstream in signal transduction.

Genetic analysis showed that some of det/cop mutants of Arabidopsis are allelic to some fus mutants (Castle and Meinke, 1994; Pepper et al., 1994). The determination of hierarchy of det/cop/fus and the photoreceptor mutants have shown that det/cop/fus mutants are epistatic to the latter and act downstream of both the blue-light and phytochrome response systems (Chory, 1992; Ang and Deng, 1994; Misera et al., 1994). Because all of the mutations at the pleotropic COP/DET/FUS loci are recessive and cause photomorphogenic development in darkness, the proteins encoded by these loci
have been postulated to act as repressors of photomorphogenesis in dark. Light signals absorbed by the various photoreceptors are thought to reverse the repressive activities of the COP/DET/FUS proteins and allow photomorphogenetic development to proceed.

The tomato high pigment (hp-1) mutant shows exaggerated phytochrome responses and is dwarfed and dark green (Peters et al., 1989, 1992). This phenotype is identical to that of transgenic tomato plants overexpressing phytochromes A and B (Boylan and Quail, 1989; Wagner et al., 1991). However, the hp-1 mutant does not accumulate higher levels of phytochrome, nor is it defective in the degradation of phytochrome (Peters et al., 1992). The double mutant analysis of hp-1 with PhyA- and PhyB-deficient tomato mutants has demonstrated that the hp-1 mutation can amplify responses mediated by both phytochromes (Peters et al., 1992; Kerckhoffs et al., 1997b). These results led to the hypothesis that the HP gene product is involved in an amplification step in phytochrome signaling and may act as negative regulator.

2.27 NEGATIVE REGULATORS OF LIGHT SIGNAL TRANSDUCTION

The DET1 gene was isolated by positional cloning, and it encodes a hydrophilic protein (Pepper et al., 1994). Recently, a tomato homolog of the Arabidopsis DET1 has also been found by cloning HP-2 gene (Mustilli et al., 1999). The COPl gene encodes a protein with a novel combination of distinct domains: an N-terminal zinc-binding RING-finger, a putative coiled coil, and a domain at the C-terminal with multiple WD-40 repeats homologous to the b subunits of heterotrimeric G-proteins (Deng et al., 1992; Lovering et al., 1993; Mc Nellis et al., 1994a). The N-terminal portion of COPl, which alone is retained in the mild cop 1-4 alleles, is sufficient to perform a basal set of
functions that prevent seedling lethality, but the C-terminus is required for the repression of light-inducible genes (Mc Nellis et al., 1994a). The FUS6 gene isolated by T-DNA tagging encodes a novel polypeptide with no homology to known metabolic or regulatory proteins and the identification of its homolog in rice suggests that fusca genes may be conserved throughout the angiosperms (Castle and Meinke, 1994). COP9 was cloned from a T-DNA tagged mutant allele, and shown to encode a small hydrophilic protein with a molecular mass of 22.5 kDa (Wei et al. 1994b). However, gel filtration studies indicated that COP9 functions in vivo in a high-molecular weigh protein complex of 560kDa (Wei et al., 1994b).

Based on biochemical analysis of DET1, COP1, COP9 and FUS6 fall into two subgroups. COP9 and FUS6 are representative of one group, because they copurify as subunits of a 500-kD nuclear-localized protein complex, which may also include COP8 (Chamovitz et al., 1996; Staub et al., 1996). The COP9 complex is localized to the nucleus and binds heparin (Chamovitz et al., 1996); however, the biochemical function for this complex is not known. Indirect evidence for the function of the COP9 complex has been provided in animal systems in which proteins highly similar to COP9 complex components have been identified (Chamovitz and Deng, 1995; Spain et al., 1996).

Ecotopic expression of the human FUS6 ortholog, GPS1, inhibits mitogen-activated protein kinase (MAPK) pathways in both yeast and mammalian cells (Spain et al., 1996). The recently reported mammalian COP9 complex contains JAB1, a c-JUN-activating binding protein (Seger et al., 1998; Wei et al., 1998). The human COP9 complex (also termed the signalosome) appears to phosphorylate c and D-Jun in vitro (Seger et al., 1998), suggesting that the COP9 complex may be directly involved in MAPK pathways. Recent
reports have highlighted the similarities among the COP9 complex, eIF3 complex, and the 19S regulatory component of the proteasome (Glickman et al., 1998; Wei et al., 1998). All three complexes are multisubunit and similar in size, and subunits of all three complexes share a similar motif, termed the PCI (for proteasome-COP9 complex initiation; Hofmann and Bucher, 1998) or PINT (for proteasome-Int6-Nip1-Trip15; Aravind and Ponting, 1998) domain.

The second subgroup of COP/DET/FUS proteins includes COPI and DET1. Both COPI and DET1 were identified to be nuclear regulators (Pepper et al., 1994; von Arnim and Deng, 1994). Chimeric proteins of both DET1 and COPI fused to βGUS reporter proteins were shown to be nuclear localized and act in the nucleus to suppress photomorphogenic development (Pepper et al. 1994; von Arnim and Deng, 1994). Moreover, light modulation of COPI activity involves a light-dependent nucleocytoplasmic partitioning of COPI in hypocotyl cells of seedlings (von Arnim and Deng, 1994). The protein localizes to the nucleus in the dark and to the cytoplasm in the light. The relocalization of the COPI-GUS protein to the nucleus in the dark was blocked in several cop\ mutant and in the det\ mutant, implying that the products of these genes are involved in the dark-induced nuclear targeting of COPI. Furthermore, in a cop\ enhancer/suppressor screen, mutants at the hy5 locus were recovered; the COPI and HY5 proteins were subsequently shown to interact in a yeast two-hybrid experiment (Ang et al., 1998).

**COORDINATION OF LIGHT SIGNALLING**

### 2.28 INTERACTION OF LIGHT SIGNALS WITH ENDOGENOUS FACTORS

Physiological and molecular studies have begun to yield information about the interaction between light cues and their modulation by endogenous signals. The mutations in less pleiotropic photomorphogenic developmental phenotypes
in darkness such as $cbb1$ (Kauschmann et al., 1996); $cpd/cbbi$ (Kauschmann et al., 1996; Szekeres et al., 1996); $dwfl$, allelic to $dim/cbb1$ (Kauschmann et al., 1996; Takahashi et al., 1995); $det2$ (Chory et al., 1991); $cop2/ampa/pt-l$, $hsl/cop3$ (Chaudhury et al., 1993; Hou et al., 1993; Lehmannn et ai. 1996); and $cop4$ (Hou et ai., 1993; suppressor of $hy2$ or $shy$ (Kim et ai., 1996) suggest that in addition to photoregulated seedling development, the genes may play a role in other aspects of plant development. Physiological and developmental studies by Castle and Meinke (1994) showed that the $fus6 (copl 1)$ mutation has pleiotropic effects, interfering with normal responses to sugars, hormones, and developmental signals, in addition to light. Indeed, severe mutant alleles of several of these genes (e.g. $copl$ and $detl$) are lethal. Further, the $detl, copl$, and $cop9$ mutations cause inappropriate expression of several genes (Mayer et ai, 1996).

Many of the developmental processes that occur as result of light signals are dependent, at least in part, on the action of phytohormones. The phenotypic defects in three of the less pleotropic genes are rescued by treatment with brassinosteroids, suggesting that this class of plant growth regulators is required for efficient hypocotyl cell elongation during the etiolation response ($det2$; Li et ai. 1996); $cpd/cbb3$ and $dwfl/dim/cbb1$ (Kauschmann et ai, 1996; Szekeres et ai, 1996). The mutants known as $det2$ (Chory et ai, 1991) and diminuto (Takahashi et ai, 1995) are shown to be deficient in the biosynthesis of brassinosteroid plant hormones and show partial de-etiolation in darkness suggesting that brassinosteroid hormones play an important role in coordinating the etiolation response (Li et ai, 1996; Szekeres et ai. 1996). How exactly the brassinosteroid-mediated pathway and the COP/DET/FUS pathway are integrated with each other remains to be shown, but partial rescue
of severe cop1 mutants by brassinosteroid treatment has suggested that the two pathways are separable (Szekers et al, 1996).

In some other cases, light has been shown to alter the levels of IAA (Bandurski et al, 1977; Jones et al, 1991; Behringer and Davies, 1992), GAs (Ross et al, 1992; Foster and Morgan, 1995), ABA (Kraepiel et al, 1994; Toyomasu et al, 1994); Weatherwax et al, 1995), cytokinins (Qumuruddin and Tillberg, 1989; Kraepiel et al, 1995), and ethylene (Kathiresan et al, 1996). Behringer and Davies (1992) proposed that phytochrome regulation of stem elongation is partly the result of changes in IAA levels. Phytochrome-deficient mutants of Arabidopsis require GAs to express the elongated phenotype of these plants (Peng and Harberd, 1997).

On the other hand, carbohydrates have been found to alter responsiveness to light, particularly with respect to specific gene expression (Tsukaya et al, 1991; Cheng et al, 1992; Harter et al, 1993; Dijkwel et al. 1996, 1997). Recent data have shown that metabolizable sugars can overcome the phyA-specific repression of protochlorophyllide oxidoreeductases (Barnes et al, 1996), that Suc represses light-inducible plastocyanin production (Dijkwel et al, 1996), and that Suc can specifically affect seedling growth in FR light (Whitelam et al, 1993; Dijkwel et al, 1997). Furthermore, in Arabidopsis overexpression of phyB inhibits phyA function in the presence of sucrose (Short, 1999). However, it is unclear how carbohydrates and phytochrome signaling mechanisms interact to regulate these functions.