CHAPTER 2:

Review of Literature
2.1. Light signals perceived by photoreceptors regulate plant growth and development.

Light signals adjust plant growth and development to the prevailing environmental conditions. Photoreceptors allow plants to utilize a broad spectrum of light, ultraviolet (UV<400 nm) to far-red (>700 nm), to control plant growth and development. Three families of photoreceptors that perceive regulatory light signals have been identified: phytochromes (PHY), cryptochromes (CRY) and phototropins (PHOT) and the as yet unidentified ultraviolet-B (UV-B) absorbing receptor molecules. Light induced signal transduction starts with the perception of light by these specialised photoreceptors and culminates in the regulation of the expression of about 2500 genes in Arabidopsis thaliana. Arabidopsis makes use of two CRY (CRY1 and CRY2), two PHOT (PHOT1 and PHOT2), and five PHY (PHYA–PHYE). PHYA is the primary photoreceptor under far-red light, whereas PHYB has a major role under white and red light with the aid of PHYA, PHYC, and PHYD. Both CRY1 and CRY2 are responsible for photomorphogenesis under blue and UVA light (Ahmad and Cashmore, 1996a; Furuya, 1993; Quail et al., 1994; Fankhauser and Chory, 1997; Deng and Quail, 1999; Quail 2002; Schepens et al., 2004).

Genetic screens of Arabidopsis seedlings that show elongated hypocotyls in red light (hy3 mutants) or in far-red light (hy8 mutants) led to the identification of phyB and phyA mutants, respectively (Natagani et al., 1991, 1993; Lopez-Juez et al., 1992; Somers et al., 1995; Reed et al., 1993). All phytochromes exist as dimers that are composed of two 125-kDa polypeptides, each carrying a covalently linked open-chain tetrapyrrol chromophore on the amino-terminus of each subunit. The carboxy-terminal domain of PHY contains two putative dimerisation sites (PAS1 and PAS2), a Quail-box that is responsible for interaction with partner proteins, and a histidine kinase domain. PHY are synthesised in the dark in the physiologically inactive (Pr) form. Upon absorption of red light, Pr undergoes photo-conversion to the active Pfr form; this process is reversible by far red light. All PHY are localised in the cytoplasm in the Pr form but migrate to the nucleus following photo-conversion to the Pfr form. In accordance with the physiological responses mediated by PHYA and PHYB, the rapid migration of PHYA to the nucleus (accumulation accomplished within 15 min) is promoted by very low fluences of red and far red light, whereas the nuclear import of PHYB is activated by red light and inhibited by far red light. The
nuclear import of PHYB is much slower than that of PHYA: PHYB requires several hours to accumulate in the nucleus (Kircher et al., 2002; Yamaguchi et al., 1999). The light-activated nuclear translocation of PHY appears to be linked with their ability to regulate gene expression. CRY are photolyase like flavoproteins that have no DNA repair activity. Their amino-terminal domain, which is related to the microbial photolyses, contains two chromophores: a light-harvesting pterin, methenyltetrahydrofolate, and a catalytic flavin adenine dinucleotide (FAD). The carboxy-terminal part of the CRY bears no significant similarity to other known proteins. Genetic studies have identified several mutants that are deficient in the perception of blue light. The hy4/Cry1 mutants grown under blue light showed elongated hypocotyls with less expanded cotyledons. Arabidopsis, CRY2 is constitutively nuclear localised whereas CRY1 is nuclear in dark but cytoplasmic under light. Nuclear localised cryptochrome closely interacts with the chromatin. Another cryptochrome, CRY3 is very closely related to cryptochrome isolated from cyanobacteria and is significantly different from both CRY1 and CRY2 (Brudler et al., 2003; Klein et al., 2003). CRY3 has dual role targeting signal that mediates its transport to chloroplast and mitochondria, suggesting role in regulating transcription in organelles.

PHOT is the most recently characterized flavin photoreceptors. They contain a serine/threonine kinase domain at their carboxyl terminus and are capable of blue-light induced autophosphorylation. Their amino-terminal region carries two conserved PAS domains, which have been designated LOV1 and LOV2 because of their high homology to domains in light, oxygen and voltage regulated proteins in animals. LOV domains that are flavin mononucleotide (FMN)-binding sites have distinct roles in regulating the function of PHOT in Arabidopsis. Both PHOT1 and PHOT2 are associated with plasma membrane, although following activation by light; a fraction of PHOT1 is released to the cytoplasm (Kinoshita et al., 2001). NPH1/PHOT gene was cloned through the molecular genetic analysis of a phototropism mutant, JK224, originally isolated by Khurana and Poff (1989). RPT2 protein is recently isolated, which is NPH3 like protein (Sakai et al., 2000). RPT2 is involved in root phototropism.

UV-B photoreceptor(s) are still unknown; however, these UV-B responses (280-320 nm) are clearly not triggered by the known photoreceptors. UVB RESISTANCE 8 (UVR8) is a specific signalling component that orchestrates the
expression of a range of genes with vital UV-B protective functions. UVR8 regulates the expression of HY5, when the plant is exposed to UVB. In addition, COP1 a negative regulator of the visible light response is a crucial positive regulator of responses to low level of UVB resulting in UVB tolerance (Brown et al., 2005; Oravecz et al., 2006).

PHYA is known to be autophosphorylated, and itself functions as a kinase that phosphorylates PHYTOCHROME KINASE SUBSTRATE 1 (PKS1 and Aux/IAA (Auxin/Indoleacetic acid) proteins. Recent studies have demonstrated that the overexpression of the carboxy-terminal parts of CRY1 and CRY2 induces a constitutive but still phytochrome induced photomorphogenesis. CRY1 and CRY2 interact with COP1 in the nucleus in the dark. It is postulated, therefore, that blue-light perception by CRY photoreceptors triggers the rapid deactivation/ degradation of COP1 by an unknown mechanism, allowing the accumulation of HY5 in the nucleus, which in turn enhances the transcription of target genes. Arabidopsis cry2 directly interacts with phyB (Mas et al., 2000). The Arabidopsis CRY2 protein undergoes a rapid blue light–induced degradation (Ballesteros et al., 2001; DeBlasio et al., 2003; Harper et al., 2000). cry2 mutant also displays the longer hypocotyls under blue light at low fluence (<10 μmol/m²/sec) but not at high fluence rates (Lin et al., 1998). The inductive effect of blue light on floral initiation in Arabidopsis suggested the involvement of blue light absorbing photoreceptors (Mozley and Thomas, 1995). Under long day condition cry2 mutant flowers significantly later than wild type (Koornneef et al., 1991; Guo et al., 1998) because the transcription level of floral activator CONSTANS (CO) were significantly reduced in cry2 mutant grown in long day photoperiod (Guo et al., 1998). Cry2 and phyA have similar expression profiles and are light labile. It has been shown that phyA colocalises with the E3 ligase COP1 in nuclear speckles. COP1 has an E3 ligase activity that enables the ubiquitination of phyA in vitro.

2.2. Signalling intermediates and molecular mechanisms involved in light-signal transduction

After activation by light, receptors initiate downstream signal propagation that results in transient or sustained physiological responses.
2.2.1. Phytochrome Signaling Involving Calcium/Calmodulin

The *Arabidopsis* genomic mining revealed that there are approximately 232 EF-hands containing proteins encoded in the *Arabidopsis* genome (Day et al., 2002), including seven genes that encode bona fide CaMs and 50 genes that encode CaM-like proteins (CML) composed almost entirely of CaM like EF hands structures (Cormack et al., 2005). In spite of the potential importance in mediating plant calcium signaling, the physiological functions of the *Arabidopsis* CaMs and CMLs remain largely unknown. CaM is ubiquitous among eukaryotes, and essential for life. The EF hands in CaM are organized into two distinct globular domains, each of which contains one pair of EF hand (Zelinski et al., 1998; Reddy et al., 2001; Snedden and Fromm, 2001; Luan et al., 2002). Pairing of EF hands is thought to stabilize the protein and increase its affinity toward Ca\(^{++}\) (Seamon and Kreetsinger, 1983). Each globular domain binds Ca\(^{++}\) and undergoes conformational changes independently, the two domains act in concert to bind target proteins (Nelson and Chazin, 1998). Even small changes in the chemical properties of the Ca\(^{++}\) binding loop (e.g., Glu-12Gln) can drastically reduce the binding affinity to Ca\(^{++}\) (Beckingham et al, 1991; Haiech et al., 1991).

The seven distinct genomic loci represent only four CaM protein isoforms: the first isoform is composed of CAM1 and CAM 4; second group comprised of CAM2, CAM3 and CAM5; third and forth isoforms are represented by CAM6 and CAM7, respectively. CAM7 is the most consensus among all the seven CAMs. CAM1/CAM4 differs from CAM7 by four amino acids, whereas CAM2/CAM3/CAM5 and CAM6 differ from CAM7 by a single amino acid substitution (McCormack et al., 2005). Despite high similarity one documented difference among the CAM’s is that CAM2, also known as TCH1 is the only CaM whose expression is upregulated by touch (Lee et al., 2005; Braam et al., 1990). Studies using inhibitors and agonists indicate that G-proteins and Ca\(^{++}\)/calmodulin act as intermediate in phytochrome-regulated *CAB* gene expression (Lam et al., 1989), and that Pfr regulates the activity of monomeric G-proteins in pea nuclear envelopes. Microinjection studies in cells of tomato PHYA deficient mutants showed that either Pfr or GTP\(^*/S\) could activate the expression of coinjected *CHS-GUS* or *CAB-GUS* fusions, whereas inhibitors of G-proteins prevented expression. Coinjection of Ca\(^{++}\)/calmodulin activated *CAB-GUS* however not *CHS-GUS* expression, whereas cGMP activated *CHS-GUS* expression, and both were needed to assemble functional chloroplasts. These data clearly demonstrate that
Ca\(^{++}\)/CaM plays an important role in the phytochrome signal transduction pathway. Repression of Asparagine synthetase (ASI) in the light is controlled by Ca\(^{++}\)/cGMP dependent pathway that is used to activate other light responses (Neuhaus et al., 1997). Thus these studies proposed three different phytochrome associated transduction pathways, the first is dependent on cGMP, the second is dependent on calcium/calmodulin, and the third is dependent on both (Neuhaus et al., 1993; Bowler et al., 1994a). It was proposed that the Pr to Pfr conversion induced by red light caused an increase in cytosolic levels of calcium that, in turn, had direct effects, or acted through calcium-binding proteins to activate enzymes and alter cellular metabolism. Blue light stimulation also leads to a number of very rapid electrophysiological responses. Most notably, Ca\(^{++}\) concentration rapidly rises in the cytoplasm in a phototropin-dependent manner. Ca\(^{++}\) uptake from the apoplast is mediated by phot1 and phot2. Studies of phytochrome-dependent germination of fern spores showed that Ca\(^{++}\) in the surrounding medium is transiently required for red light induction of germination. Using the CaM inhibitors trifluoperazine (TFP) and chlorpromazine (CPZ), it was possible to inhibit red light-induced spore germination in the presence of calcium. Red light also induced protoplast swelling, whereas subsequent far-red treatment prevented this effect (Shacklock et al., 1992)

**Calcium/CaM Targets in the Nucleus**

CaMs participate in transcriptional regulation either directly, by binding to transcription factors (Szymanski et al., 1996) in the nucleus, or indirectly, by activating kinases or phosphatases that control transcription factor activity (Marechal et al., 1999; Snedden and Fromm, 2001). Several CaM isoforms have been shown to be located in the nuclei of plant cell. The distribution of petunia CaM53 (Rodriguez-Concepcion et al., 1999) and rice OsCaM61 (Dong et al., 2002) between nucleus and plasma membrane is shown to be regulated by influencing their prenylation. Transcription factors are among the targets protein for Ca\(^{++}\)/CaM in plant cell nuclei (Szymaanski et al., 1996; Reddy et al., 2000, Reddy et al., 2002; Bouche et al., 2002). Expression of tobacco NpCaM1 but not NpCaM2, which encodes an identical CaM protein in response to wind was stimulated by a nuclear Ca\(^{++}\) transient, whereas cold-responsive expression was induced primarily by a cytoplasmic Ca\(^{++}\) transient (Van Der Luit et al., 1999). Thus, spatially separated Ca\(^{++}\) signals also can control the function of closely related CaM proteins through the regulation of their genes.
A Ca$^{++}$ binding protein is required for light response has been localized in the cytoplasm (Guo et al., 2001). This protein (called SUB1) also contains a putative domain for DNA binding, suggesting that it may combine the function of Ca$^{++}$ sensing and transcriptional regulation in one protein. SUB1 transcription factor with two EF hands has been implicated in transcriptional responses to blue and Far red light (Guo et al., 2001). The Arabidopsis genome contains two homologues of AtSUB1: AtSUL1 and AtSUL2.

Finally, one should also consider the possible occurrence of transcription factors that directly respond to Ca$^{++}$ signals; such as the mammalian DREAM protein (the human DRE-antagonist modulator) has four EF-hands and specifically interacts with DRE element (Carrion et al., 1999). In this case, Ca$^{++}$ binding to an EF-hand motif of the transcription factor causes its release from its DNA binding site, thus alleviating the suppression of gene expression (Ikura et al., 2002). However, transcription factors of this nature have not yet been characterized in plants. In another example, E histolytica URE3BP protein having EF hands and no other consensus DNA binding motif recognizes URE3 DNA sequence in Ca$^{++}$ dependent manner (Gilchrist et al., 2001). This sequence is important in the regulation of at least two E. histolytica genes, the hgl5 encoding the heavy subunit of the Gal/GalNAc-inhibitable lectin and the fdx gene encoding ferredoxin. Calmodulin is known to interact with many known transcription factors like bHLH, MYB, bZIP and WRKY and modulating there activity. The basic sequence within the bHLH domain is the primary target for calmodulin binding, and sequences modulating the calmodulin interaction reside directly N-terminal to the basic sequence. Both the N-terminal and C-terminal domains of calmodulin can independently bind to and inhibit the DNA binding of bHLH proteins. Miller and Sanders, (1987), using a Ca$^{2+}$ selective intracellular microelectrode, found that the alga Nitellopsis had a basal [Ca$^{2+}$]$_i$ of 400nM in the dark. However, when cultured in light, the [Ca$^{2+}$]$_i$ dropped to 150nM. The interpretation put forth was that the process of photosynthesis, together with ion uptake by chloroplast, caused the reduction of the [Ca$^{2+}$]$_i$. Recent analysis of transcriptome changes revealed 230 calcium-responsive genes, of which 162 were upregulated and 68 were downregulated (Kalpan et al., 2006).
2.2.2 Early phytochrome signaling components

Classical genetic screen and molecular approaches have identified various regulators downstream of photoreceptors (Ahmad and Cashmore, 1996; Genoud et al., 1998; Soh et al., 1998; Hoecker et al., 1999; Hudson et al., 1999; Bolle et al., 2000; Hsieh et al., 2000). These mutants could be divided into three groups depending on their response to individual photoreceptor (Figure 2.1). For example, *fhy1*, *fhy3*, *spa1*, *pat1*, *pat3*, *laf1*, *laf3*, *laf6*, *hfr1*, *far1*, *eid1*, *rep1*, *fin2*, *fin5*, *fin219* act immediate downstream to phyA (Ballesteros et al., 2001; Bolle et al., 2000; Desnos et al., 2001; Fairchild et al., 2000; Hsieh et al., 2000; Hudson et al., 1999; Moller et al., 2001; Soh et al., 1998; Wang and Deng, 1999; Zeidler et al., 2001; Buche et al., 2000; Hoecker et al., 1998; Hare et al., 2003); *pef2*, *pef3*, *red1*, *srll*, *pocl*, *elf3*, *srrl* (Liu et al., 2001; Wagner et al., 1997; Ahmed and Cashmore, 1996; Genoud et al., 1998; Staiger et al., 2003) are specific to phyB; and *pefl* and *psi2* (Ahmed and Cashmore, 1996; Genoud et al., 1998) loci act in signaling pathways shared by both phyA and phyB.

Furthermore, it has also been reported that SUB1, which is one of the calcium binding proteins shares the light signaling pathways mediated by both phytochromes and cryptochromes (Guo et al., 2001). COG1 and OBP3 are involved in red light signalling. COG1 is a negative regulator under both red and far red, whereas OBP3 acts as positive regulator for the inhibition of hypocotyl elongation and negative regulator or cotyledon expansion in both PHYB and CRY1 signalling pathway (Jason et al., 2005). HRB1 (HYPERSENSITIVE TO RED AND BLUE 1), a ZZ-type zinc

Figure 2.1. A simplified overview of transcriptional networks for seedling photomorphogenesis (Nature Review 2007)
finger protein, can act as a negative regulator of red-blue light mediated inhibition of hypocotyl and petiole elongation and positive regulator of red-blue light mediated leaf expansion and gene expression (Kang et al., 2005). Yeast two hybrid screening have identified some phytochrome interacting factors (PIF) such as PIF3, PIF4 and PKS1, which can physically interact with phytochromes and function as regulators of the shared pathway (Ni et al., 1998 and 1999; Halliday et al., 1999; Choi et al., 1999; Fankhauser et al., 1999; Martinez-García et al., 2000). PIF3 is a bHLH transcription factor that has been isolated by its ability to interact with non-photo active C-terminal domains of phytochromes A and B (Ni et al., 1998; Choi et al., 1999; Fankhauser et al., 1999; Martinez-Gracia et al., 2000). PIF4, another phytochrome interacting factor, has a bHLH motif, and is encoded by SRL2 and it binds selectively to the biologically active Pfr form of phyB, but it has little affinity to phyA (Huq and Quail, 2000 and 2002). PKS1 protein has been demonstrated to be interacting with the C-terminal domain of both phyA and phyB by yeast two hybrid assays. This protein is localized in the cytoplasm and interacts with phyA and phyB in the histidine kinase domains, which indicates that PKS1 probably modulate phytochrome kinase activity or subcellular localization. Recent studies have proposed a parallel pathway in the light signaling cascade. Shl (seedling hypersensitive to light), loci was identified by genetic screens in low white light which is a threshold condition in which the normal photoperception pathways are only partially active.

2.2.3. Downstream regulators of photomorphogenic development

Different light colors that selectively activate different photoreceptors activate a highly overlapping set of genes, indicating the presence of shared signalling components. Light signals perceived by photoreceptors and the complex array of light sensing and early signaling processes have been shown in some cases to converge to common downstream regulators that in turn controls cellular developmental decisions.

Negative regulators

Several loci acting as negative regulators of photomorphogenesis have been isolated from genetic screens that sought to identify mutants that display characteristics of light grown seedlings in complete darkness. Mutant analysis has further demonstrated a panel of negative regulators, which keep chloroplast
development suppressed in the cotyledons of dark grown seedlings. Mutations at each of 10 loci known as Constitutively Photomorphogenic (COP1, COP8–COP11), De-etiolated (DET1), and Fusca (FUS4, 5, 11, and 12) result in the absence of etioplasts and in partial chloroplast development incomplete darkness, accompanied by cotyledon expansion, arrest of hypocotyl elongation, and light-specific cell type differentiation. The suppression of photomorphogenic seedling development under defined light conditions by overexpression of COP1 confirmed that at least COP1 can act as a light inactivable repressor of photomorphogenesis, acting downstream to all the photoreceptors and is the master regulator of photomorphogenic development (Deng and Quail, 1999; Holm and Deng, 1999). COP1 is first such loci to be cloned and characterized at the molecular level. COP9, DET1, and FUS6 (COP11) have also been cloned and characterized at the molecular level (Deng et al., 1992; Wei et al., 1994; Castle and Meinke, 1994; Pepper et al., 1994). COP9, DET1 and FUS6 encode novel helical rich proteins that are constitutively localized in the nucleus (Pepper et al., 1994; Wei et al., 1994; Chamovitz et al., 1996; Staub et al., 1996). COP9 has been found to be a part of an eight subunit protein complex of 560 kDa consisting of COP9, FUS6, presumably COP8 and others which is now known as COP9 signalosome (CSN) (Wei et al., 1994; Chamovitz et al., 1996; Wei and Deng, 1999; Serino and Deng, 2003). Mutant analysis reveals that COP1, COP10 and DET1 are not included in CSN complex. Sequence analysis indicated that COP1 is a novel protein which forms homodimer in vitro and in vivo, and it consists of four recognizable structural domains: an N-terminal ring finger zinc binding domain, which is involved in self association, a coiled coil domain (helix) that helps in dimerization, a central core domain, and C-terminal multiple WD-40 repeats characteristic of β subunit of trimeric G protein (Deng et al., 1992; Torii et al., 1998).

The core domain of COP1 has a bipartite nuclear localization signal located in it, which helps in the nuclear import of COP1 and a cytoplasmic localization signal (CLS) mediates the nuclear exclusion of COP1, which overlaps the helix domain (Stacey et al., 1999). GUS-COP1 fusion protein studies indicated that COP1 acts in the nucleus in the dark to suppress photomorphogenesis and light inactivates COP1 which is indicated by reduced COP1 abundance in the nucleus (Von Arnim and Deng, 1996; Osterlund and Deng, 1998; Stacey et al., 1999). It has been found that at least three photoreceptors, phyA, phyB, and cry1, can trigger nuclear depletion of COP1.
under their respective light-responsive regimes by regulating the balance between the competing CLS and NLS activities, which further substantiates the notion that COP1 acts downstream of multiple photoreceptors (Osterlund and Deng, 1998; Stacey et al., 1999).

Another regulator which maintains COP1 level in the nucleus is pleiotropic COP/DET/FUS, which maintains the. Except for COP1, COP10 and DET1, all other genes seem to be required for the structural stability of COP9 signal, some which exhibits similarity to distinct non ATPase subunits of the 19S regulatory particle of the 26S proteosome. This similarity indicated that the regulation of COP1 nuclear abundance may be mediated by a proteosome protein degradation pathway (Wei et al., 1998; Glickman et al., 1998). In the dark COP9 signalosome protects nuclear COP1 from proteosome degradation, whereas light may abrogate this protection, which results in an accelerated degradation of COP1 in the nucleus.

Suppression of photomorphogenesis in darkness also requires the four-member SUPPRESSOR OF PHYTOCHROME A-105 (SPA) gene family. SPA1 is a phytochrome-A (phyA) specific signaling component that acts as a light-dependent repressor of photomorphogenesis. SPA1 belongs to four member gene family SPA1, SPA2, SPA3, SPA4 (Laubinger and Hoecker, 2003; Laubinger et al., 2004). Among these four members SPA1, SPA3 and SPA4 are involved in the repression of photomorphogenesis in the light grown seedlings. Seedlings of the spa1 spa2 spa3 spa4 quadruple mutant exhibit constitutive photomorphogenesis that is very similar to that of cop1 mutants. Yeast two hybrid and in vitro interaction studies showed that SPA1 strongly and selectively binds to COP1 with help of coiled-coil domain of both SPA1 and COP1 (Hoecker and Quail, 2001). SPA1 may function as an important branch of light signaling to COP1.

The repressor of photomorphogenesis PIF3, by contrast, is stabilized by COP1 in darkness, probably by an indirect mechanism. Mutational studies have recently shown that PIF3 negatively regulates phyB mediated inhibition in hypocotyl elongation, cotyledon opening and expansion (Kim et al., 2003). PIF1 negatively regulates several aspects of phyA-phyB mediated photomorphogenesis (Huq et al., 2004; Oh et al., 2004). Two other bHLH proteins, PIF4 and PIF5, have been shown to be involved in phytochrome mediated transcriptional regulation. Furthermore, it has been demonstrated that phyB interacts with the G-box bound PIF3 (Ni et al., 1998). OBP3 negatively regulates the cry1 mediated cotyledon expansion. HRB1 acts as a
negative regulator of red-blue light mediated inhibition of hypocotyl and petiole elongation (Kang et al., 2005; Jason et al., 2005). EID1 (Empfindlicher Im Dunkelroten Licht) is a F-box protein and acts as negative regulator in phyA mediated light signaling (Buche et al., 2000). EID1 forms SCF$^\text{EID}$ ligase which degrades the positive regulators of phyA signal pathway (Dieterle et al., 2001). Recently it has been shown that ATMYC2 a bHLH protein, acts as a negative regulator in blue and far red light mediated signal transduction, whereas GBF1, a bZIP transcription factor, function as a repressor for blue light mediated seedling deetiolation and positive for cotyledon expansion.

Positive regulators: promotion of photomorphogenesis

ELONGATED HYOCOTYL5 (HY5) has been the first such genetically defined positive regulator of photomorphogenesis based on the light insensitivity of hy5 mutants (Koornneef et al., 1980; Ang and Deng, 1994; Pepper and Chory, 1997). This was identified by loss of function mutants exhibiting partial etiolated morphology under light growth conditions. Mutations at HY5 locus cause defects in light inhibition of hypocotyls elongation, light induced chlorophyll accumulation, and extensive root abnormalities in far-red, red, blue, white and UV-A light, indicating that HY5 is required for mediating developmental responses to phytochromes and blue and UV-A light receptors. HY5 is responsible for the regulation of fundamental developmental processes of the plant cell: cell elongation, cell proliferation and chloroplast development (Oyama et al., 1997; Ang et al., 1998).

Molecular cloning of HY5 gene using a T-DNA tagged mutant has revealed that the gene encodes a 168 amino acid protein with a bZIP motif, one of the motifs found in transcriptional regulators (Oyama et al., 1997; Ang et al., 1998). HY5 binds to G-box DNA sequences containing an ACGT core motif, which are present in many cis-acting elements in the promoters of various light inducible genes in plants (Ang et al., 1998; Chattopadhyay et al., 1998a). Recently, a similar nuclear localized bZIP protein, HY5 HOMOLOG (HYH) has been reported, mutation in which leads to blue light specific partial etiolation. HYH protein levels decreases in dark but its mRNA levels increases in responses to red and or blue light compared to darkness but not in response to far red (Holm et al., 2002).

Mutations in bHLH protein HFR1/REP1/RSF1 lead to an etiolated phenotype only in the far red light (Fairchild et al., 2000; Soh et al., 2000; Spiegelman et al.,
It acts as a positive regulator in both far red and blue light signaling (Duek et al., 2003). HFR1 mRNA level expresses in darkness but increases in far red and blue light. Under red light its mRNA level decreases (Duek and Frankhauser, 2003). HFR1 does not bind to phytochromes but it binds PIF3 forming a heterodimer that in turn binds the Pfr form of phytochromes (Fairchild et al., 2000). LONG AFTER FAR-RED LIGHT (LAF1), a MYB protein, has been shown to be involved in far red light mediated signaling (Ballesteros et al., 2001). It is constitutively localizes within the nucleus. FAR1, FHY3 and FHY1 acts as positive regulator in far red light signaling (Hudson et al., 1999; Wang et al., 2002; Zeider et al., 2002, 2004).

Two other MYB proteins, LHY and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), are involved in circadian rhythm (Schaffer et al., 1998; Wang and Tobin 1998). CCA1 is the part of network connecting light and clock signals to the control of gene expression. HRB1 is a positive regulator for red-blue light mediated leaf expansion and gene expression (Kang et al., 2005). OBP3 also acts as a positive regulator of red light mediated inhibition of hypocotyl elongation (Jason et al., 2005). PIF3 also acts as a positive regulator of CHS transcript accumulation (Kim et al., 2003).

2.3. Molecular interactions of COP1 with different signaling components of photomorphogenesis

Regulation of protein degradation is an integral part of the phytochrome signaling mechanism. Post translational regulation in light signaling mainly includes E3 ligase mediated targeted destabilization by 26S proteosome of some positive regulators of the photomorphogenesis. Ubiquitination involves three enzymes namely, Ubiquitin activating enzyme (E1), Ubiquitin conjugating enzyme (E2) and Ubiquitin protein ligase (E3). Out ten COP/DET/FUS loci, six of them are the constituents of COP9 signalosome (CSN) having two more subunits other than COP/DET/FUS loci. This CSN is very similar to the 19S lid complex of 26S proteosome. COP1, DET1 and COP10 are not the functional units of CSN complex because mutation in any of these loci does not degrades CSN complex but mutation in any loci related to CSN components leads to absence of CSN complex (Serino and Deng, 2003). COP1 protein is RING zinc finger protein and acts as an E3 ligase in light signaling pathway.
**Figure 2.2** HY5 is regulated by light in many ways. In addition to transcriptional regulation by light, the nuclear level of COP1 regulates HY5 by targeted ubiquitin-mediated proteolysis. Unphosphorylated HY5 interacts more strongly with COP1, is the preferred substrate for degradation and has higher binding affinity for target promoters.

COP1, which acts as master repressor of photomorphogenic pathway, regulate HY5 (**Figure 2.2**) and other positive factors by targeted ubiquitin mediated proteolysis. COP1 is found to be enriched in the nuclei in the dark where it probably directly interacts with HY5, which is constitutively present in the nucleus. (Sajio et al., 2003). HY5 degradation is regulated by fine tuning of phosphorylation and dephosphorylation by CKII kinase activity (Hardtke et al., 2000). The deletion analysis of COP1 demonstrated that C-terminal WD-40 repeats play a direct role in mediating interaction with HY5. The unphosphorylated form of HY5 interacts strongly with COP1 and is the preferred substrate for degradation and has higher binding affinity for target promoters (Hardtke et al., 2000; Osterlund et al., 2000). Recent genetic evidence has suggested that HY5 also interacts with DET1, one of the COP/DET/FUS gene products that is not part of COP9 complex, this indicated a coordinated effort between COP1 and DET1 in the repression of positive regulators of photomorphogenesis (Pepper and Chory, 1997).

Another a bZIP transcription factor, HYH is involved in blue light specific etiolation at seedling stage. COP1 interacts with HYH and mediates the degradation of the protein in the dark involving the CSN complex (Holm et al., 2002). LAFl is a nuclear localized MYB transcription activator and acts as a positive regulator in far red light signalling pathway. Furthermore, it has also been demonstrated that the LAFl ubiquitination by COP1 is further stimulated by SPA1 (Ballesteros et al., 2001; Soe et al., 2003). HFR1 is a bHLH transcription factor and acts as a positive regulator...
of far red light signaling. It is colocalized in nuclear body and is targeted for ubiquitin-mediated proteolysis by COP1-E3 ligase under darkness (Yang et al., 2005). Recently it has been reported that FHY1, a positive regulator in far-red signalling pathway, is degraded through 26S proteosome with the help of COP/DET/FUS in light dependent manner. Whether it gets ubiquitinated by COP1 E3 ligase activity is not still unknown (Shen et al., 2005). PIF3, a negative regulator in phytochrome signaling pathway, which gets ubiquitin mediated proteolysis by COP1-E3 ligase activity in light dependent manner (Bauer et al., 2004). Recently it has been reported that PIF1 is regulated by light mediated degradation through the ubiquitin-26S proteosome pathway (Shen et al., 2005).

Recent studies suggest that COP1 interacts with multiple transcription factors to regulate gene expression and thus suppress photomorphogenic development. This was supported by identification of transcriptional regulators CIP7, CIP4 and CIP1 as COP1 interactive-protein (Yamamoto et al., 1998, 2001). CIP7 is the positive regulator of light regulated gene expression and is localized in the nucleus, therefore a possible downstream target of COP1. (CIP4 is light inducible nuclear protein and is regulated by COP1 (Yamamoto et al., 1998, 2001). CIP1 protein levels are not regulated by light. It interacts specifically with the putative coiled-coil region of COP1 in vitro (Matsui et al., 1995). It has a possible role in mediating control of COP1 nuclear activity by regulating its nucleocytoplasmic partitioning.

Light mediated signal transduction pathways thus get desensitised by COP1-E3 ligase mediated proteolysis of positive as well as other negative regulators of photomorphogenesis, or directly the photoreceptor itself, in presence or absence of lights.

2.4. Light regulated gene expression

Light-regulated promoters are composed of ubiquitous regulatory elements; the specific combination of elements appears to make a promoter light regulated, and these combinations vary widely. Some of light regulated genes, such as nuclear encoded photosynthesis related genes for chlorophyll a/b binding proteins (CAB) and ribulose 1, 5 bisphosphate carboxylase small subunit (RBCS), spinach ribulose bisphosphate carboxylase/oxygenase (RCA), A subunit of glyceraldehydes 3 phosphate dehydrogenase (GAPA) are induced by light whereas genes such as PHYA,
NADPH-protochlorophyllide reductase, and asparagines synthase are down regulated by light (Silverthorne and Tobin, 1987; Ha and An, 1988; Donald and Cashmore, 1990; Gilmartin et al., 1990; Sun and Tobin, 1990; Quail et al., 1991; Nehause et al., 1997).

2.4.1 Light responsive elements and their interacting protein partners

Light Responsive Elements (LREs) are small DNA sequences that is present upstream of the transcription start site and sufficient to confer light regulated expression onto the minimal promoter.

GT1 has the core sequence GGTTAA. GT1 sites are usually found in tandem, and spacing between two sites is critical. These are found in a number of genes such as RBCS3A, PHYA, CAB, RCA, PETA, and CHS15 (Green et al., 1989; Gilmartin et al., 1992; Sarokin et al., 1992; Dehesh et al., 1990; Orozco and Ogren, 1993). GT1 binding proteins in nuclear extract from numerous species have been reported and three corresponding genes have been cloned: one from tobacco, one from rice and one from Arabidopsis. However functional analyses have not been performed for any of the cloned GT1 factors.

GATA (I box) elements have the core sequence GATAAA, and are found in many light regulated promoters of both monocot and dicot plants (Borello et al., 1993; Buzby et al., 1990; Gidoni et al., 1989; Giuliano et al., 1988; Kehoe et al., 1994; Lam et al., 1989; Schaffner et al., 1991; Luan and Bogorad, 1992). RBCS genes have a single GATA element near G-box whereas CAB has two or three GATA elements arranged in tandem and separated by few base pairs are found near the TATA-box (Batschauer et al., 1994; Borello et al., 1993; Carrasco et al., 1993; Gidoni et al 1989). GATA element is also present in non-light regulated promoters (Lam and Chua, 1990). LRF-1 from L.gibba, ASF-2, 3AF3 from tobacco, GA-1 from N.plumbaginifolia are the factors which have been identified (Buzby et al., 1990; Lam and Chua, 1989; Schindler and Cashmore, 1990; Sarokin and Chua, 1992). The function of these proteins in light regulated transcription is yet to be determined.

G-box element has the core sequence CACGTG, found in the promoters of many genes such as CAB, RBCS, CHS, RCA (Foster et al., 1994, Menkens et al., 1995; Arias et al., 1993; Block et al., 1990; Orozco and Ogren, 1993; Weishaar et al., 1991). G-box binding factors have been identified and cloned from many different plant species (Carrasco et al., 1993; Foster et al., 1994; Gilmartin et al., 1989;
Lubbertstedt et al., 1994; Menkens et al., 1994; Schindler et al., 1992; Schulze-Lefert et al., 1989). O2 from maize is only factor, which has been assigned function suggesting it activates the transcription (Izawa et al., 1993; Ueda et al., 1992). Other factors, which have been identified, are GBF1, GBF2, GBF3, GBF4, CPRF-1 (Weishaar et al., 1991, Schindler et al., 1992; Menken and Cashmore, 1994; Terzaghi and Cashmore, 1995). Only GBF3 and CPRF-1 have been shown to be light regulated.

Z-box element has the core sequence ATACGTGT and is found in light regulated promoter of CAB gene. Deletion analyses of Arabidopsis CAB1 promoter have demonstrated that the Z-box is essential for the light dependent developmental expression of CAB1 gene (Ha and An, 1988). Even though G, GATA and GT1 LREs have been studied in some detail with respect to identification of specific transacting factors and regulation of these LREs by specific light signaling components, corresponding information with the Z-box is not available thus far. Recently two factors AtMYC2/ZBF1 and GBF1/ZBF2 has been shown to interact with Z-box and There are other LREs such as TGACG, H-Box, AT rich sequence, CCAAT Gap-box, ATGAA (A/G)A, AT1&I box are also found in many promoter of light regulated genes (Terzaghi and Cashmore, 1995; Hettiarachchi et al., 2003). Interestingly, the Z-box (ATACGTGT) or G-box (CACGTG) are very similar to the recently identified Ca++ responsive element, (CACGTG[T/C/G]).

Combinatorial role of distinct LREs is an important factor for light regulated promoter activities (Degenhardt and Tobin, 1996; Puente et al., 1996; Feldbrugge et al., 1997). Synthetic promoters with paired LREs such as G-GATA and GT1-GATA are able to respond to a wide spectrum of light mediated by multiple photoreceptors including phyA, phyB and cry1, similar to native light inducible promoters (Chattopadhyay et al., 1998b). On the other hand, light-inducible single LRE (such as G-box, GATA) containing promoters primarily respond to a specific wavelength of light (Chattopadhyay et al., 1998b).

2.4.2. Chromatin remodelling

Chromatin modifications are important for light regulated gene expression. Mutations in two Arabidopsis histone acetyltransferases, HISTONE ACETLYTRANSFERASE OF THE TAFII250 FAMILY (HAF2) and GCN5, repress
photomorphogenesis under a wide range of light conditions (Bertrand et al., 2005; Benhamed et al., 2006), whereas mutation of the histone deacetylase HD1/HDA19 results in the opposite effect. At the molecular level, HAF2 and GCN5 are required for histone H3 and H4 lysine acetylation in several light responsive promoters, with both overlapping and distinct roles. A chromatin remodelling function has been associated with DEETIOLATED (DET1) protein, as loss of function mutants for DET1 develops in darkness in the similar manner as light grown seedlings. DET1 is part of a protein complex (CDD) with DDB1a protein implicated in chromatin modification, possibly through recruitment of histone acetyltransferases and COP10, a ubiquitin conjugating enzyme variant (Schroeder et al., 2002; Yanagawa et al., 2004). The binding between DET1 and chromatin can be specific, occurring through promoter elements and protein such as transcription factors CCA1 and HY5 to induce or repress gene transcription involved in photomorphogenes.