CHAPTER 2:

Review of Literature
2.1 *Arabidopsis* bZIP transcription factors and light responsive elements

Approximately 5.9% of the total number of predicted genes in the *Arabidopsis* thaliana genome is transcriptional regulators (Riechmann et al., 2000). The dedication of over 5% of the genome to transcription factors indicates the importance of transcriptional control to cell survival. Transcription factors are responsible for controlling cell division, cell identity, metabolic processes and environmental responses (Singh, 1998). There are many different types of transcription factors in *Arabidopsis* responsible for altering gene expression. The basic leucine zipper (bZIP) transcription factors are the eighth largest transcription factor group in the *Arabidopsis* genome, with 81 predicted proteins (Riechmann et al., 2000). In plants, bZIPS are associated with controlling gene expression under drought and high salinity conditions (Hong et al., 1995; Menkens et al., 1995), in response to light (Schindler and Cashmore, 1990) and ABA (Kim et al., 1997; Finkelstein and Lynch 2000; Uno et al., 2000). The name bZIP is given to this group because the members contain a region of basic residues followed by a leucine zipper motif. Most bZIP proteins contain three regions that have been studied: the basic region, the leucine zipper, and a third region of variable composition that is important for additional protein: protein interactions. The basic region is responsible for DNA sequence recognition, binding, and nuclear localization. Several research groups working with plants, animals and yeast have shown the basic region can function as a nuclear localization signal (Terzaghi et al., 1997). *Arabidopsis* bZIP transcription factors are clustered according to sequence similarities of their basic region into ten groups Group A to I. GBF1, along with GBF2 and GBF3 belong to Group G (Jacoby et al., 2002).

The leucine zipper portion of bZIP proteins helps to stabilize binding to DNA by forming dimers (Vinson et al., 1993). In addition to stabilization, the leucine zipper is important for the creation of both hetero- and homo- dimerization (Schindler et al., 1992; Vinson et al., 1993). The formation of hetero-dimers has the potential to recognize additional binding sites and increase the range of DNA binding specificity (Foster et al., 1994). Some bZIP proteins, such as the Fos protein from mammals and the *Arabidopsis* AtGBF4 protein, are capable of forming only hetero-dimers. The leucine zipper of AtGBF4 contains predominately glutamate residues at the e and g positions, which hinders the formation of homo-dimers (Menkens and Cashmore, 1994). Hetero-dimerization also allows the production of new protein configurations. The protein STF1
from soybean can dimerize with GBF proteins. The dimerization brings together the acidic region from STF1 and the proline rich region of the GBF proteins into one binding element (Cheong et al., 1998). Hetero-dimerization is also a form of protein regulation by preventing either a favorable binding association or DNA binding (Menkens and Cashmore, 1994). For example, two bZIP proteins from rice, osZIP2a and osZIP2b, are unable to bind to the G-box sequence, but can hetero-dimerize with EmBP-1 and prevent binding of EmBP-1 to the G-box (Nantel and Quatrano, 1996).

The most common protein modification of bZIP proteins is phosphorylation by protein kinases. Many bZIP proteins can be phosphorylated in several locations, especially the basic region, which can contain one to three serines. In the wheat HBP-1a protein, whose basic region is similar to GBF proteins, all three of the serine residues in the basic region can be phosphorylated in the presence of Ca\textsuperscript{++} (Meshi et al., 1998). Phosphorylation of AtGBF1 by Arabidopsis or broccoli casein kinase II (CKII) enhances DNA binding to the G-box (Klimczak et al., 1992; Klimczak et al., 1995). For the Arabidopsis bZIP protein HY5, phosphorylation reduces the affinity for its target sequence, but also influences its ability to interact with COP1 protein. The association of COP1 and HY5 is an example of a protein: protein interaction, other than bZIP dimerization, that can influence the function of bZIP proteins. The cellular localization patterns of AtGBF1, AtGBF2 and AtGBF4 fused to GUS show that around 50-62% of AtGBF1: GUS and in the dark approximately 50% of AtGBF2: GUS is present in the cytoplasm. Under these conditions, AtGBF4 is different from the other family members in that it shows almost exclusive nuclear localization (Terzaghi et al., 1997).

A 77 base pair (bp) fragment containing the Abscisic Acid Response Element (ABRE) from the drought regulated gene rd29A has been used in a yeast-one hybrid screen to look for interacting proteins. In addition to several other bZIP proteins AtGBF3 and AtGBF1 have also been pulled out of the library as interacting proteins (Uno et al., 2000). However, neither AtGBF1 nor AtGBF3 is able to activate transcription from the 77 bp fragment in yeast cells unlike the other bZIP proteins isolated in this study which activate transcription in the transient assay (Uno et al., 2000).

A combination of deletion and mutagenesis analysis of light regulated promoters such as \textit{CAB}, \textit{RBCS} and \textit{CHS} have identified regulatory cis-acting sequences called Light Responsive Elements (LREs). These are defined as small DNA sequences that are
present upstream of the transcription start site and sufficient to confer light regulated expression onto the minimal promoter. At least four light responsive elements (LREs) G, GATA, GT1 and Z-box are commonly found in different minimal light responsive promoters and have been demonstrated to be important for the light controlled activity (Tobin and Kehoe, 1994; Terzagi and Cashmore, 1995; Millar and Kay, 1996).

GT1 has the core sequence GGTTAA, an is found in the promoters of a number of genes such as RBCS3A, PHYA, CAB, RCA, PETA, and CHS15 (Green et al., 1989; Gilmartin et al., 1992; Sarokin and Chua, 1992; Dehesh et al., 1990; Orozco and Ogren, 1993). GATA (I-box) elements have the core sequence GATAA, and found in light regulated promoters of RBCS genes, which 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). G-box element has the core sequence CACGTG, found in the promoters of many genes such as CAB, RBCS, CHS, (Block et al., 1990; Weishaar et al., 1991; Arias et al., 1993; Orozco and Ogren, 1993; Foster et al., 1994, Menkens et al., 1995). A Z-DNA-forming sequence, 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. 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.

2.2 Importance of light in plant growth and development

Plants are sessile organisms that are forced to adapt to a given environment. Therefore, compared with animals, plants have achieved an enormous developmental plasticity that makes possible very flexible responses to diverse exogenic factors. Among these exogenic factors, light is probably one of the dominating signals that controls gene expression and development throughout all phases of the plant life cycle (Kendrick and Kronenberg, 1994; Neff et al., 2000). The perception of light leads to key developmental transitions throughout the life cycle of the plant, from germination of the seed,
photomorphogenesis or deetiolation of the seedling, phototropism, gravitropism, chloroplast movement, shade avoidance, circadian rhythm to flower induction. (Ma et al., 2001). Perception, interpretation and transduction of light signals are accomplished by a complex molecular network, which ultimately leads higher plants such as Arabidopsis thaliana to develop with contrasting morphologies, cellular and sub-cellular differentiation and gene expression in light as compared to darkness. Depending on the presence or absence of light germinating Arabidopsis seedling is genetically capable of following either of the two distinct pathways, skotomorphogenesis in dark or photomorphogenesis in light. Skotomorphogenesis (etiolation) is characterized by long hypocotyl, presence of apical hook, unopened cotyledons, no pigment accumulation. Where as photomorphogenesis (deetiolation) is characterized by inhibition of hypocotyl elongation, open and expanded cotyledons, accumulation of chlorophyll, anthocyanin and entails a dramatic transcriptional reprogramming (von Arnim and Deng, 1996; Fankhauser and Chory, 1997; Ma et al., 2001).

In higher plants light signals are perceived through at least four distinct families of photoreceptors, which include cryptochromes, phytochromes, phototropins and yet unidentified UV-B photoreceptor(s). These photoreceptors allow plants to perceive and utilize a broad spectrum of light, from ultraviolet (<400 nm) to far-red light (>700 nm) for plant growth and development. Cryptochrome and phototropin (cry) photoreceptors are involved in the perception of blue and UV-A light. On the other hand, phytochrome photoreceptors are involved in the perception of red and far-red region of light (Ahmad and Cashmore, 1996; Furuya, 1993; Quail et al., 1994; Fankhauser and Chory, 1997; Deng and Quail, 1999; Quail, 2002; Schepens et al., 2004). A large group of genes acting downstream to these photoreceptors are involved in transducing the signal from photoreceptors to downstream regulatory components. Downstream genes again modulate the transcriptional regulatory network. This transcriptional regulatory network have a key role in mediating light signaling through the coordinated activation and repression of specific downstream genes leading to photomorphogenesis of light grown seedlings (Jiao et al., 2007).
2.3 Photoreceptor activity

Red and Far-red light signals are perceived by a family of photoreceptors comprising five members, phyA to phyE (Sharrock and Quail, 1989; Clack et al., 1994). Based on the stability, these phytochromes are classified into two groups, Type I phytochromes are light labile and accumulate in etiolated seedlings and are degraded rapidly when seedlings are exposed to light. Where as Type II phytochromes are light stable and accumulate in light grown seedlings. In Arabidopsis phyA is the only Type I photoreceptor and is the only known photoreceptor for the perception of far-red light. And phyB to phyE are Type II photoreceptors (Furuya, 1993; Quail, 1997; Sharrock and Clack, 2002). Phytochrome is a soluble dimeric protein, each monomer consisting of 127 kDa protein covalently attached to a linear tetrapyrrole chromophore (the phytochromobilin) at the N-terminal part (Furuya, 1993). Phytochromes exist in either of the two forms, red light absorbing Pr form (\(\lambda_{\text{max}}\), 666nm) or far-red light absorbing Pfr form (\(\lambda_{\text{max}}\), 730nm). Pfr form is the physiologically active form of phytochrome (Quail, 1991). The modes of phytochrome function have been classified into four groups: VLFR, LFR, red HIR, and far-red HIR. Very low fluence responses (VLFR) are saturated at very low levels of active phytochrome (Pfr) either after light pulses (Mandoli and Briggs, 1981) or under continuous irradiation leading to responsiveness to near infrared light (Schafer et al., 1982). In Arabidopsis, all the five phytochromes accumulate in the cytoplasm in the dark and translocate into the nucleus in a light dependent manner (Sakamoto and Nagatani, 1996; Yamaguchi et al., 1999; Kircher et al., 2002). The domains of phytochrome proteins have been identified by mutational studies. Domain swapping experiments between PHY A and PHYB have revealed that the N-terminal domain of phyA and phyB is involved in the photo-sensory activity. The transmission of light signal to downstream signaling components is mediated by the C-terminal domain of phyA and phyB (Quail et al., 1995).

Cryptochromes are Blue and UV-A light photoreceptors. 'Cryptochrome' was originally coined as a generic term to describe plant blue-light receptors which were known to exist but had not been identified for nearly a century. Now the term is used to designate photolyase sequence homologs with no DNA repair function but with known or presumed blue-light receptor function (Sancar, 2000). In plants, cryptochromes regulate a variety of growth processes in response to blue-light, and are a significant
topic of research in plant biology. Cryptochrome was first discovered as a possible photoreceptor in *Arabidopsis thaliana* when it was cloned as the gene complementing the *hy4* photomorphogenic mutant which has impaired blue-light dependent inhibition of hypocotyl elongation (Ahmad and Cashmore, 1993). With 30% sequence identity to microbial photolyase, a blue-light activated enzyme, it appeared to be a good candidate for a blue-light photoreceptor and was named cry1. A second gene, cry2, has since been cloned in *A. thaliana* (Hoffman et al., 1996; Lin et al., 1998) as well as cryptochromes from other plants including *Sinapis alba* (mustard). Like photolyase, recombinant plant cryptochromes contain FAD (Malhotra et al., 1995; Lin et al., 1995) and folate (Malhotra et al., 1995), but these proteins had no demonstrable photolyase activity (Malhotra et al., 1995; Lin et al., 1995).

Genetic analysis of cry1 and cry2 *Arabidopsis* mutants implicated both genes in blue-light inhibition of hypocotyl elongation (Ahmad and Cashmore, 1993; Lin et al., 1998; Guo et al., 1998), flowering time regulation (Guo et al., 1998), and, following the discovery of the role of CRY in the mammalian circadian clock, cry1 was also implicated in circadian clock control (Somers et al., 1998). Transgenic plants overexpressing plant cryptochromes exhibited enhanced sensitivity to blue-light in terms of hypocotyl elongation (Lin et al., 1996, 1998). Perhaps the most intriguing result of genetic studies of cryptochromes in plants comes from the creation of transgenic *Arabidopsis* plants that overexpress the C-terminal domain of cry1 and cry2. The C-terminus of cryptochrome is postulated to be the mediator of signal transduction; accordingly, overexpression of the C-terminus alone was sufficient to cause a constitutive light response in plants (Yang et al., 2000). These results have led to the following model: cryptochrome in the ground state represses signaling by the C-terminus; upon blue-light activation, the C-terminus is released from its repressed state to initiate signal transduction through protein-protein interactions. Two responses to light have been observed in plant cryptochromes. First, cry2, but not cry1, is rapidly degraded by blue-light through an unknown mechanism (Lin et al., 1998). Second, both cry1 and cry2 are phosphorylated in the C-terminal domain in response to either blue- or red-light (Ahmad et al., 1998; Mas et al., 2000; Shalitin et al., 2002). Although the significance of this phosphorylation event is unknown, the existence of this interaction indicates that cross-talk between photoreceptive systems can be mediated directly between
photoreceptors, and this physical relationship between two different photosensory proteins, cryptochrome and phytochrome, as well as the existence of yet another class of plant blue-light photoreceptors, phototropins (Elich and Chory, 1997; Briggs and Huala, 1999), has complicated the study of the role of cryptochromes in plants. The overlapping functions of phy and cry genes in the regulation of the light regulated gene expression have been demonstrated (Chattopadhyay et al., 1998b; Yanovsky et al., 2002). Full-length cry1, cry2 interact with COP1 in yeast two-hybrid assays and coimmunoprecipitation assays (Wang et al., 2001; Yang et al., 2001). Arabidopsis cry2 directly interacts with phyB (Mas et al., 2000). The cry2-phyB interaction was shown by both yeast two hybrid assays and coimmunoprecipitation tests. In addition, cry1 has also been reported to interact, via its C-terminal domain, with phyA in a yeast two-hybrid assay (Ahmad et al., 1998).

Phototropins are another class of blue light receptors involved in phototropism. NPH1 gene was cloned through the molecular genetic analysis of a phototropism mutant, JK224, originally isolated by Khurana and Poff (1989). Phot1 (originally NPH1 for non phototropic hypocotyl) was identified based on the inability of phot1 mutant hypocotyls to bend towards unilateral blue light (Liscum and Briggs, 1995). NPH1 is a 120kDa plasma membrane associated flavoprotein with C-terminal Ser/Thr protein kinase domain that catalyzes blue-light activated auto-phosphorylation of the photoreceptor. NPH1 or phototropin, contains two PAS domains designated as LOV domains because they are found in proteins regulating responses to light, oxygen, or voltage (Khurana et al., 1998; Taylor and Zhulin, 1999). Phot2, a second Phototropin, is present in Arabidopsis (Briggs et al., 2001). This pair of photoreceptors is extremely important for a number of light responses that ultimately allow optimal photosynthesis, including phototropism, chloroplast movements, and stomatal opening (Briggs and Christie, 2002; Wada et al., 2003).

2.4 Cryptochrome signaling components

Early and recent works have identified a number of blue light signaling components, like HY5, HYH, SUB1, AtPP7, HFR1, HRB1 and ZBF1/MYC2 (Koornneef et al., 1980; Ang and Deng, 1994; Pepper and Chory, 1997; Fairchild et al., 2000; Guo et al., 2001; Holm et al., 2002; Moller et al., 2003; Kang et al., 2005; Yadav et al., 2005). Among these
regulatory proteins, HYH, AtPP7 and ZBF1/MYC2 are exclusively involved in blue light mediated photomorphogenic growth (Holm et al., 2002; Moller et al., 2003; Yadav et al., 2005).

HY5 has been the first 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 in HY5 locus cause defects in 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 phytochrome and cryptochrome and UV-A light receptors. HY5 is responsible for the regulation of fundamental developmental processes of the plant cell such as 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 is constitutively nuclear localized and is involved in light regulation of transcriptional activity of the promoters containing the G-box light responsive element. The characteristic phytochrome-mediated red light- and far-red light reversible low fluence induction of the G-box containing promoters was diminished specifically in hy5 plants (Chattopadhyay et al., 1998a). A number of reports suggest the involvement of HY5 in hormonal signaling (Cluis et al., 2004; Vandenbussche et al., 2007).

Another blue light signaling factor, HYH was isolated from Arabidopsis genome based on the sequence similarity to HY5. HYH is a G-box-binding member of the bZIP family of transcription factors. The cDNA encodes a 149 amino acid protein with a predicted molecular mass of 16.9 kDa, containing a basic leucine zipper (bZIP) transcription factor domain in the C-terminal half. The bZIP protein, denoted HYH for HY5 homolog, is the closest homolog of HY5 in the Arabidopsis genome. HYH is a nuclear localized protein and overexpression can partially compensate for the loss of HY5 in white light suggesting functional overlap between the two proteins. Genetic analysis indicates that HYH is predominantly involved in blue-light regulation of development and gene expression, and that the function of HYH in part overlaps with
that of HY5. The accumulation of HYH protein, not the mRNA, is dependent on the presence of HY5. And further HYH and HY5 can, respectively, act as heterodimers and homodimers, thus mediating light-regulated expression of overlapping as well as distinct target genes (Holm et al., 2002). Short-under-blue-light 1 (SUB1), a calcium binding protein, acts as a negative regulator in blue and far-red light signaling. This is a cytosolic protein suggesting some cryptochrome mediated events in cytoplasm (Guo et al., 2001). HFR1 (long Hypocotyl in FR light 1), a basic helix loop helix (bHLH) protein, acts in both far-red and blue light signaling as a positive regulator (Fairchild et al., 2000). The AtPP7 protein phosphatase acts as a positive regulator in blue light signaling (Moller et al., 2003). HRBl (HYPERSENSITIVE TO RED AND BLUE 1), a ZZ-type zinc 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). Recently, ZBF1/MYC2, a bHLH protein which binds to G-box and Z-box light responsive elements has been reported. ZBF1/MYC2 acts as a negative regulator of blue light mediated photomorphogenic growth and blue light and far-red light mediated gene expression. ZBF1/MYC2 acts as a point of crosstalk between light, ABA, JA and ethylene signaling pathways (Abe et al., 2003; Anderson et al., 2004; Boter et al., 2004; Lorenzo et al., 2004; Yadav et al., 2005).

2.5 COP1, a central switch of light control of Arabidopsis seedling development

Upon light absorption multiple photoreceptors activate multiple, distinct, yet partially overlapping, signal transduction cascades resulting in specific activation of light inducible genes. Many components of the light signaling cascades have been identified over the years using genetic and molecular approaches (Lin, 2002; Quail, 2002; Wang and Deng, 2003, Liscum et al., 2003). Among them, COP1 was the one of the first cloned and is one of the most extensively studied (Deng et al., 1991; Deng et al., 1992). It is established that COP1 functions as essential negative regulator of light mediated plant development, evidenced by cop1 mutant seedlings undergo photomorphogenic development even in the absence of light, and cop1 null alleles never survive to adult (Deng et al., 1991; Deng and Quail, 1992).
COP1 belongs to the COP/DET/FUS loci identified through genetic screens in *Arabidopsis* that searched for mutant seedlings that displayed constitutive photomorphogenic phenotypes (Serino and Deng, 2003). Except for COP1, the rest of the *COP/DET/FUS* loci encode polypeptides that have been shown to form two large protein complexes: the COP9 signalosome (CSN) and the CDD complex (Schwechheimer, 2004). The eight-subunit COP9 signalosome, conserved in both plants and animals, associates with multiple cullin containing E3 ubiquitin ligase complexes and regulates their E3 activities by removing the essential ubiquitin-like RUB/Nedd8 modification from the cullins (Schwechheimer, 2004). The CDD (COP10–DDB1–DET1) complex, so far only reported in plants, is able to enhance E2 ubiquitin-conjugating activity in vitro (Yanagawa et al., 2004). An earlier study revealed that mutations at other *COP/DET/FUS* loci abolish the nuclear accumulation of the COP1 protein in the dark (Von Arnim et al., 1997). Later studies showed that the stability and integrity of the CDD complex but not of the COP1 complex are affected by mutations at CSN subunits, whereas COP10 mutations lead to a size shift of the COP1 complex (Suzuki et al., 2002; Saijo et al., 2003).

The COP1 protein comprises three recognizable domains: a RING-finger motif, a coiled-coil domain and seven WD40 repeats, all of which have been implicated in mediating the interaction of COP1 with other proteins and/or its self dimerization (Holm and Deng, 1999; Hoecker and Quail, 2001; Holm et al., 2001; Wang et al., 2001; Yamamoto et al., 2001; Yang et al., 2001; Holm et al., 2002; Suzuki et al., 2002; Bianchi et al., 2003). Introduction of an N-terminal fragment of *Arabidopsis* COP1 containing the RING-finger and coiled coil domains into a *cop* null allele rescued its lethal phenotype, indicating that COP1 N-terminal region alone is able to sustain a basal function during development of *Arabidopsis* (Stacey et al., 2000). But WD40 domain alone failed to rescue the *cop* mutant phenotype (Stacey et al., 2000). COP1 contains a nuclear import and export signals, and its subcellular localization is regulated by light (Osterlund et al., 2000; Bianchi et al., 2003).

COP1 represses photomorphogenesis by promoting the degradation of positive light signaling regulators, such as HY5, HYH, LAF1, HFR1, phyA and cry2 (Ang et al., 1998; Shalitin et al., 2002; Holm et al., 2002; Seo et al., 2003; Seo et al., 2004; Jang et al., 2005; Yang et al., 2005a) and stabilizing the negative regulators of light signaling
such as PIF3 in dark (Bauer et al., 2004). HY5 a positive regulator of light signaling accumulates to a much higher level in light grown seedlings and upon light-to-dark transition is degraded through proteasome mediated proteolysis (Osterlund et al., 2000). Protein ubiquitination in general requires a specific E3 ubiquitin ligase, which can be a single protein or a multicomponent protein complex. COP1 was then suspected to be the E3 ligase of HY5, as COP1, a RING-finger protein and negative regulator of HY5, had been shown to directly interact and colocalize with HY5 to subnuclear speckles in living plant cells (Ang et al., 1998). This hypothesis was further strengthened by the observation that HY5 degradation during light-to-dark transitions is impaired in cop1 mutant seedlings, transgenic seedling expressing HY5 with point mutations at the HY5 COP1-interacting motif or in COP1 mutants with point mutations in the COP1 WD40 domain abolishing HY5 interaction (Osterlund et al., 2000; Holm et al., 2001). Moreover HY5 becomes stable in white light when the COP1 protein is excluded from the nucleus. (Osterlund et al., 2000). COP1 was also shown to possess intrinsic E3 activity and to ubiquitinate HY5 in vitro (Saijo et al., 2003). Interestingly, another bZIP transcription factor, HYH, which heterodimerizes with HY5 and appears to function primarily in blue light signaling, also binds to the COP1 WD40 domain and is degraded in a COP1 dependent manner under darkness (Holm et al., 2002). LAF1, a myb transcription factor and positive regulator of phyA mediated far-red light signaling, and HFR1, a bHLH transcription factor that is involved in both far-red and blue light signaling, have also been identified as physiological substrates for COP1 (Seo et al., 2003; Yang et al., 2005a). Thus COP1 functions as a master switch in the dark to shut down photomorphogenesis by destroying transcription factors that activate specific light responses.

One of the most important findings came from the report that COP1 also plays an active role in fine tuning light activation (Seo et al., 2004). phyA and cry2, two photoreceptors specific for far-red and blue light, respectively, become rapidly activated upon illumination and are important for the initiation of light signaling. However, both these photoreceptors are extremely unstable under light and are quickly degraded by proteasome when exposed to continuous light (Chen et al., 2004). COP1 was found to directly interact with these light labile photoreceptors and target them for ubiquitylation and degradation, and thus preventing the over-activation of the light signaling pathways.
(Shalitin et al., 2002; Seo et al., 2004). COP1 might also regulate light signal transduction through mechanisms other than protein degradation. For example, COP1 was demonstrated to be required for the nuclear accumulation of the bHLH transcription factor PIF3 in darkness but not for its rapid degradation under red and far-red light (Bauer et al., 2004).

2.6 COP1 and SPA1 interaction plays a critical role in regulation of HY5, LAF1 and HFR1 activity

*Arabidopsis* SPA1 (suppressor of phytochrome A-105), a negative regulator of phyA signaling, is a nuclear localized protein (Hoecker et al., 1999). SPA1 is a member of a small family of four structurally related proteins. SPA proteins contain an N-terminal kinase like domain, followed by a coiled-coil domain and seven WD40 repeats at the carboxyl-terminus. Interestingly, the WD40 domains of the SPA proteins are highly homologous to the COP1 WD40 domain, and the SPA proteins physically interact with COP1 through their mutual coiled-coil domains (Laubinger and Hoecker, 2003; Laubinger et al., 2004). While the quadruple spa mutants exhibit strong cop phenotypes almost indistinguishable from cop1 mutants, single, double and triple spa mutants show no or restricted phenotypes only under certain light conditions and developmental stages, suggesting that the SPA proteins modulate COP1 activity in a partially overlapping fashion (Laubinger and Hoecker, 2003; Laubinger et al., 2004).

How exactly SPA proteins regulate COP1 activity is still a question in debate. The highly conserved WD40 domains, presence of SPA1 in COP1 subnuclear speckles (Seo et al., 2003), together with evidence that the SPA1 WD40 domain also binds to HY5 (Saijo et al., 2003), suggest that the SPA proteins might be required for the nuclear accumulation of COP1. Alternately, the SPA proteins might be essential for the stability of COP1 complexes, since COP1 and SPA1 co-fractionate in large complexes in *Arabidopsis* seedlings (Saijo et al., 2003). SPA1 may function in concert with COP1 to target transcription factors such as HY5, LAF1 and HFR1 for degradation (Saijo et al., 2003; Seo et al., 2003; Yang et al., 2005b). LAF1, a myb transcription activator participates in the transmission of phyA signals to downstream responses (Ballesteros et al., 2001). LAF1 is localized in nuclear bodies along with COP1 and SPA1. LAF1 is ubiquitinated by COP1 and this ubiquitination is dependent on RING motif but not
WD40 repeat domain of COP1. Interestingly, SPA1 coiled-coil domain stimulates LAF1 ubiquitination by COP1 when latter is present at low concentrations (Seo et al., 2003). HY5, a bZIP transcription factor acts as a positive regulator of photomorphogenesis in red light, far-red light and blue light signaling pathways (Oyama et al., 1997). The abundance of HY5 protein correlates with the extent of photomorphogenesis and is primarily regulated at the level of protein degradation via the proteasome pathway (Osterlund et al., 2000). COP1 is known to directly interact with HY5 and subject it for proteasome mediated degradation (Osterlund et al., 2000). Saijo et al., (2003) by the genetic analyses of double mutants reported the synergistic enhancement of the cop1 and spa1 mutations. HY5 was found to accumulate to high levels in spa1-3 mutants in FR conditions and synergistic effect of the cop1-6 and spa1-3 mutations was found on HY5 abundance. Further SPA1 interacts with HY5 through WD40 domain and with COP1 through coiled coil domain. SPA1 acts as a component of the COP1 complex and stimulate the degradation of HY5 (Saijo et al., 2003). HFR1, a photomorphogenesis promoting bHLH protein, is required for a number of exaggerated photoresponses of the spa1 mutants including hypocotyl elongation and light responsive gene expression under specific light conditions (Fairchild et al., 2000; Duek and Fankhauser, 2003; Yang et al., 2005a). HFR1 undergoes proteasome mediated degradation through COP1 (Jang et al., 2005; Yang et al., 2005a). SPA1 physically interacts with HFR1 through coiled-coil domain and degradation of HFR1 is defective in spa1 mutants under all light conditions except dark. Thus SPA1 represses light signaling by modulating COP1-dependent degradation of HFR1 at a post translational level (Yang et al., 2005b).

2.7 Cross-talk between light and hormone signaling pathways

Plant hormones have profound effects on plant growth and development, and in many cases the signals from plant hormones and those derived from light interact either positively or negatively. For example, exogenous application of either auxins or gibberellins can stimulate hypocotyl elongation in light-grown seedlings (Jensen et al., 1998; Saibo et al., 2003). Light inhibits the positive effects on hypocotyl elongation growth of auxin, brassinosteroids and gibberellins. Depending on the species, it regulates biosynthesis and/or signaling of gibberellins (Garcia-Martinez and Gil, 2001). Brassinosteroid biosynthesis genes are generally down regulated by light (Ma et al.,
2001) and photomorphogenesis is modulated by inactivators of brassinosteroids phyB activation- tagged suppressor 1 (BAS1) and suppressor of phyB-4 7 (SOB7) (Turk et al., 2003, 2005). Auxins are influenced by light in their transport and/or distribution, at the level of signal transduction, and in their homeostasis (Morelli and Ruberti, 2000; Tian et al., 2002; Hoecker et al., 2004; Vandenbussche et al., 2005). By contrast, cytokinins mimic the effects of light in dark grown plants (Chory et al., 1994; Deikman and Hammer, 1995). Etiolated seedlings treated with exogenous cytokinins have the short hypocotyls and expanded cotyledons of light-grown plants, and even show true leaf formation. Moreover in tissue culture, exogenous cytokinins enhance greening and shoot formation in calli. In this respect, exogenous cytokinins produce a similar phenotype in seedlings as mutations in COP1 and other subunits of the either COP1 or COP9 signalosomes. Recently phytochrome B and a cytokinin related two component signaling pathway have been suggested to converge (Sweere et al., 2001; Heyl and Schmulling, 2003; Salome et al., 2006), through the demonstration of direct interaction of phytochrome B (phyB) with the Arabidopsis response regulator 4 protein (ARR4) implicated in cytokinin signaling (Sweere et al., 2001). It was suggested that phyB, ARR4 and the circadian oscillator may function as signaling intermediates to integrate light and cytokinin pathways (Zheng et al., 2006). ARR4 has been shown to directly interact with the phosphotransfer protein Arabidopsis histidine phosphotransfer protein 1 (AHP1) (Urao et al., 2000), which is translocated to the nucleus in the presence of cytokinin (Hwang and Sheen, 2001). The connection between Cytokinin and cryptochrome signaling pathways came recently with the work of Vandenbussche et al., (2007), where they demonstrated that cytokinin and cryptochrome act additively and independently on hypocotyl growth inhibition. Cytokinin regulation of anthocyanin accumulation occurs via a mechanism that requires light and act downstream of the photoreceptors. HY5 is involved in the transcriptional regulation of anthocyanin biosynthetic enzymes by both blue light and cytokinins, and significant response to cytokinins in blue light requires prior signal by cry1. Levels of HY5 protein are directly regulated by both light and cytokinins. HY5 is proposed to be a common intermediate in both the light and cytokinin signaling pathways (Vandenbussche et al., 2007). Interaction of light and ABA in the regulation of light inducible gene expression in Lemma gibba was demonstrated by Weatherwax et al., (1996). A report by Yadav et al., (2005),
demonstrated that ZBF1/MYC2, a bHLH transcription factor acts as a negative regulator of photomorphogenesis in cryptochrome signaling pathway and is a point of cross talk between light, ABA, JA and ethylene signaling pathways (Boter et al; 2004; Lorenzo et al., 2004; Yadav et al., 2005). Recently mutation in CULLIN 1 has been show to have altered response to hormone and light signals in Arabidopsis. cul1 mutants show reduced sensitivity to hormones such as auxin and ethylene and to red and blue light conditions (Moon et al., 2007).