Chapter 2: Review of Literature
2.1 Light is an important factor for plant growth and development

Light functions as an important signal in plant growth and development (Kendrick and Kronenberg, 1994; Deng and Quail 1999; Quail 2002). To perceive the light signal, plants have developed several photoreceptors that are specific to a particular wavelength of light. 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 subcellular differentiation and gene expression in light as compared to darkness. Arabidopsis seedlings are genetically capable of following two distinct developmental pathways: skotomorphogenesis in the dark and photomorphogenesis in the light (von Arnim and Deng, 1996: Fankhauser and Chory, 1997). Skotomorphogenic development is characterized by etiolation with long hypocotyls, closed cotyledons with apical hooks and inhibition of chlorophyll and anthocyanin biosynthesis. Upon exposure to light, seedlings switch to photomorphogenic development or deetiolation. During this developmental stage, hypocotyls cease rapid elongation, and chlorophyll and anthocyanin biosyntheses are initiated with the formation of true leaves (McNellis and Deng, 1995; von Arnim and Deng, 1996). Light-inducible genes are expressed at high level in light grown seedlings, whereas the dark grown seedlings have very low level or no expression of light-inducible genes.

Photoreceptors allow plants to utilize a broad spectrum of light, ultraviolet (UV <400nm) to far red (>700 nm), to control plant growth and development. Plants contain three major groups of photoreceptors. Phytochromes (phys), which are involved in the absorption of red and far-red light. On the other hand, cryptochromes (crys) and phototropins (phots) are involved in the absorption of blue/UV-A 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). A battery of genes acting immediate downstream to photoreceptors are involved in transducing the light signal from photoreceptors to downstream regulatory components. The downstream regulatory components integrate the signal, regulate gene expression and metabolic activities that
eventually lead to photomorphogenic growth. Some regulatory molecules capable of directly interacting with the photoreceptors have also been reported recently.

2.1.1 Perception of light signals by photoreceptors

A five-member gene family, PHYA to PHYE, is involved in Arabidopsis to perceive, interpret and transduce red and far-red light signals (Sharrock, and Quail, 1989; Clack et al., 1994). Genetic studies have been successful in identification and understanding the functional role of phytochromes. 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). The other phytochromes such as phyC, phyD and phyE have photosensory activity similar to phyB and probably aid in phyB action, as the function of these phytochromes can only be detected in the phyB background.

Each phytochrome is a soluble chromoprotein and is localized in the cytosol as dimers, which is composed of two 120kD polypeptides, each carrying a covalently attached tetrapyrrole chromophore called as phytochromobilin (Spiegelman, et al., 1996; Lagarias and Rapaport, 1980). Phytochromes can interconvert between the red-light-absorbing Pr form and the far-red-light-absorbing Pfr form upon absorption of red and far-red light (McCurdy and Pratt, 1986; Speth et al., 1987; Vierstra, 1994; Ni et al., 1999). The domains of phytochrome proteins have been identified by mutational studies. Domain swapping experiments between PHYA 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). Recent studies have revealed that the N-terminal domain of phytochromes could be divided into four domains: P1 domain is rich in serine residues and important for function; P2 domain is related to PAS domain; P3 domain has the GAF motif that gets covalently attached to bilin chromophore; and the P4 domain is known as the PHY domain. The C-terminal domain is divided into two subdomains: the PRD domain (PAS related domain) containing two PAS repeats and the HKRD (histidine-kinase-related domain).
Cryptochromes are blue and ultraviolet-A light photoreceptors. Genetic studies have identified several mutants that are deficient in the perception of blue light. The hy4 mutants grown under blue light showed elongated hypocotyls with less expanded cotyledons, while retaining the wild type phototropic response to directional blue light. CRY1/HY4 gene has been cloned and the CRY1 photoreceptor has been characterized at molecular level (Ahmad and Cashmore, 1993; Koornneef et al., 1980; Lin et al., 1995). Another member of this gene family, CRY2, has been cloned and studied (Hoffman et al., 1996; Lin et al., 1996). Similar to CRY1, CRY2 is also involved in the photomorphogenic development mediated by blue light. Late flowering mutant fhal has a genetic lesion in CRY2 (Guo et al., 1998) demonstrated that CRY2 is involved in sensing the day length. Both CRY1 and CRY2 are flavoproteins with sequence homology in their N-terminal domains to the NDA photolyases but have no photolyase activity (Ahmad and Cashmore, 1993; Hoffman et al., 1996). Both these proteins have been demonstrated to be constitutively present in the nucleus (Kliener et al., 1999; Cashmore et al., 1999; Guo et al., 1999).

Unlike CRY1 and CRY2, NPH1 (non-phototropic hypocotyl1) and NPL1 (NPH1 like protein) photoreceptors are involved in blue light mediated phototropism (Christie et al., 1998; Briggs and Huala, 1999). NPH1 gene was cloned through the molecular genetic analysis of a phototropism mutant, JK224, originally isolated by Khurana and Poff (1989). 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 (Taylor and Zhulin, 1999; Khurana et al., 1998). These PAS domains are involved in protein–protein interaction and ligand binding. It is interesting to note that very recent studies with a lower vascular plant, Adiantum capillus-veneris, have identified a hybrid photoreceptor superchrome or phytochrome3 (phy3) which has both phytochrome and phototropin properties (Nozue et al., 1998; Briggs and Olney, 2001). The identification and characterization of signaling components of blue light signaling pathway have just started. RPT2 protein is recently isolated, which is NPH3 like protein.
RPT2 encodes a protein with BTB/POZ and coiled–coil protein–protein interaction domains. RPT2 is involved in root phototropism.

The mutant analysis studies have indicated that there is a complex network of interaction between photoreceptors both within and between families. In early seedling development, phyA and phyB show antagonistic response to FR-enriched light. The suppression of internode elongation mediated by phyE is revealed in the absence of phyA and phyB (Reed et al., 1994). phyA and phyB are necessary for full activity of cry1 and from yeast two hybrid interaction studies it has been demonstrated that cry1 interacts with phyA and phyB (Ahmad et al., 1998; Mas et al., 2000). Flowering is regulated by phyB and cry2 in an antagonistic manner: while phyB represses, cry2 stimulates floral induction (Guo et al., 1998). The overlapping functions of phy and cry genes in the regulation of the light regulated gene expression has recently been demonstrated (Chattopadhyay et al., 1998; Yanovasky et al., 2002)

2.1.2 Early phytochrome signaling components

Although the phytochrome family of photoreceptors has been well studied in regulating the gene expression in response to light signals, the early phytochrome signal transduction steps have recently started to be unraveled. Genetic screens have identified various mutants, which function immediate downstream to phytochrome signaling (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. 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, sril, poc1, elf3, srr1 (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 pef1 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 share the light signaling pathways mediated by both phytochromes and cryptochromes (Guo et al., 2001).

Biochemical and pharmacological studies have revealed that G proteins, cGMP and Ca/calmodulin play an important role in phytochrome mediated signaling and gene regulation (Nehause et al., 1993; Sharma et al., 1993; Bowler et al., 1994; Nehause et al., 1997; Sharma et al., 1997; Sopory and Munshi 1998). 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-Garcia et al., 2000). Nuclear localization studies of several of these early phytochrome signaling components in addition to the nuclear localization of phytochromes reveal the emerging concept that early steps in phytochrome signaling could be centered in the nucleus (Quail 2002).

PIF3, and PKS1 are the phytochrome interacting factors, which are identified with the help of yeast two hybrid screen. PIF3 is a 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, 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 phy kinase activity or subcellular localization. NPH3 gene encodes a NPH1 (blue light photoreceptor)-interacting protein (Motchoulski et al., 1999) that belongs to a large protein family and may function as an adaptor or scaffold protein to bring together the enzymatic components of NPH1-activated phosphorelay.

Recent studies have strongly supported the presence of parallel pathways operative 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. subl mutant show short
hypocotyls phenotype not only in blue light but also in far-red light and it does not show any sign of photomorphogenic development in the dark. *SUB1* gene encodes a calcium binding protein and it defines a point of crosstalk between cryptochrome and phyA signal transduction pathways (Guo et al., 2001). Yeast two hybrid studies have shown that CRY1 interacts strongly with COP1 protein (Yang et al., 2001). An interaction is also observed between C-terminal domain of phyB and COP1. These observations suggest a molecular link between CRY1, phyB and COP1 proteins acting in parallel pathways.

**2.1.3 Downstream regulators of photomorphogenic development**

Molecular genetics studies using *Arabodopsis* as a model plant have identified several downstream regulatory components, which have unraveled the downstream molecular mechanism of light regulated plant development. 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.

**2.1.3.1 Negative regulators: COP1, the master regulatory switch, acts as an ubiquitin ligase**

By photomorphogenic mutant screening and purple seed color screening a set of at least ten loci has been identified that are required for full establishment of dark developmental pathway and suppression of photomorphogenic development in darkness (Chory, 1993; Deng, 1994). The ten loci have been designated as *COP1/FUS1, DET1/FUS2, COP8/FUS8, COP9/FUS7, COP10/FUS9, COP11/FUS6, FUS4, FUS5, FUS11,* and *FUS12* and mutations in each result in dark grown seedlings with a pleiotropic de-etiolated or constitutively photomorphogenic phenotype (Wei and Deng, 1999). And the recessive nature of the mutation indicated that their gene products normally function to repress photomorphogenesis in the absence of light, whereas light perceived by the photoreceptors lead to abrogation of their repressive activities (Misera et al., 1994; Kwok et al., 1996, von Arnim and Deng, 1996; Fankhauser and Chory, 1997; Wei and Deng, 1999). The supression 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., 1994a; 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 560kDa consisting of COP9, FUS6 (COP11), 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).

Sequence analysis indicated that COP1 is a novel protein which forms homodimer into and into, 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 substantiated the notion that COP1 acts downstream of multiple photoreceptors (Osterlund and Deng, 1998; Stacey et al., 1999).

In addition to the multiple photoreceptors involved in the signaling cascade(s) that leads to the depletion of COP1 from nucleus, a number of downstream regulators are required to maintain the nuclear localization of COP1 in the darkness. One example of
such regulators is pleiotropic COP/DET/FUS, which maintains the COP1 level in the nucleus. So far, DET1, COP9, and FUS/COP11 of this group have been characterized molecularly and all are nuclear encoded proteins. Except for COP10 and DET1, all other genes seem to be required for the structural stability of COP9 signalosome which exhibit 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.

Mutational studies have recently shown that PIF3 negatively regulates phyB mediated inhibition in hypocotyl elongation (Kim et al., 2003). SPA1 is a phytochromeA (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. The quadruple mutants that are defective in SPA1, SPA2, SPA3 and SPA4 show constitutive photomorphogenic growth in the dark. 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 to the phytochromeA-specific branch of light signaling to COP1.

2.1.3.2 Positive regulators: promotion of photomorphogenesis

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 and UV-A light, indicating that HY5 is required for mediating
developmental responses to phytochromes and blue and UV-A light receptors. \textit{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 \textit{HY5} gene using a T-DNA-tagged mutant has revealed that the gene encodes a 168 aminonacid protein with a bZIP motif, one of the motifs found in transcriptional regulators (Oyama et al., 1997; Ang et al., 1998). \textit{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 bZIP protein, \textit{HYH} has been reported, mutation in which leads to blue light specific partial etiolation (Holm et al., 2002). Mutations in bHLH protein HFR1/REPl/RSF1 lead to an etiolated phenotype only in the far red light (Fairchild et al., 2000; Soh et al., 2000; Spiegelman et al., 2000). Two other bHLH proteins, PIF3 and PIF4, 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). Mutational studies have recently shown that PIF3 negatively regulates phyB mediated inhibition in hypocotyl elongation (Kim et al., 2003). LAF1, a MYB protein, has been shown to be involved in far red light mediated signaling (Ballesteros et al., 2001). Two other MYB proteins, LHY and CCA, are involved in circadian rhythm (Schaffer et al., 1998; Wang and Tobin 1998).

2.1.3.3 Molecular interactions of \textit{COP1} with photomorphogenesis promoting transcription factors: \textit{HY5}, \textit{HYH} and \textit{LAF1}

From yeast two-hybrid assays it has been found that \textit{COP1} which acts as the light inactivable master repressor of photomorphogenic pathway directly interacts with \textit{HY5} which is the only known genetically defined positive regulator of photomorphogenesis (Ang et al., 1998). Recent genetic evidence has suggested that \textit{HY5} also interacts with \textit{DET1}, one of the \textit{COP/DET/FUS} gene products that is not part of \textit{COP9} complex, this indicated a coordinated effort between \textit{COP1} and \textit{DET1} in the repression of positive regulators of photomorphogenesis (Pepper and Chory, 1997).
COP1 is found to be enriched in the nuclei in the dark and which is shown by GUS-COP1 subcellular localization studies, where it probably directly interacts with HY5, which is constitutively present in the nucleus. HY5 consists of at least two distinct functional modules, one is the DNA binding domain (bZIP domain) and other for interacting with COP1. The deletion analysis of COP1 demonstrated that C-terminal WD-40 repeats play a direct role in mediating interaction with HY5. With proper self association, the C-terminal WD-40 repeats are essential for mediating the repression of photomorphogenesis, possibly by directly interacting with and negatively regulating HY5.

A recently emerging picture of how COP1 regulates the activity of its target proteins indicates that COP1 negatively regulates HY5 by targeted degradation mediated by the 26S proteosome and this degradation is enhanced in the darkness, which coincides with nuclear enrichment of COP1 (Hardtke et al., 2000; Osterlund et al., 2000). HY5 exists in two isoforms: phosphorylated and unphosphorelated forms. The Unphosphorylated form of HY5 interacts strongly with COP1 and is the preferred substrate for degradation (Hardtke et al., 2000; Osterlund et al., 2000). It has been reported recently that LAF1, which is a nuclear localized MYB transcription activator functions in transducing the signals from phyA to downstream responses. Furthermore, it has also been demonstrated that the LAF1 ubiquitination by COP1 is further stimulated by SPA1 (Ballesteros et al., 2001; Soe et al., 2003). Recently, a bZIP protein, HYH, has been reported, mutation in which leads to blue light specific partial etiolation (Holm et al., 2002). COP1 interacts with HYH and mediates the degradation of the protein in the dark involving the CSN complex.

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; Yamamoto et al., 2001). CIP7 is the positive regulator of light regulated gene expression and is localized in the nucleus, therefore a possible downstream target of COP1. It is possible that in the dark, nuclear localized COP1 binds to CIP7 and inhibits its transcriptional activation activity (Yamamoto et al., 1998). CIP4 is also a nuclear protein and a potent transcription coactivator (Yamamoto et
al., 2001). It acts as positive regulator of photomorphogenesis, downstream of multiple photoreceptors as well as COP1 in mediating light control of development. CIP4 is light inducible and is regulated by COP1 (Yamamoto et al., 1998). CIP1 is encoded by single gene in *Arabidopsis* and its protein levels are not regulated by light. CIP1 is predominantly alpha-helical and most likely involved in coiled-coil formation. It interacts specifically with the putative coiled-coil region of COP1 *in vitro* (Matsui et al., 1995). CIP1 is demonstrated to be associated with a cytoskeletal structure and has a possible role in mediating control of COP1 nuclear activity by regulating its nucleocytoplasmic partitioning.

### 2.1.4 Light regulated gene expression

Light regulated plant growth and development is triggered in many cases by altering the regulation of transcription of specific genes (Tobin and kehoe, 1994; Terzagi and Cashmore, 1995; Millar and kay, 1996). Some of these 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, 1991; Nehause et al., 1997).

### 2.1.4.1 Light responsive elements and their interacting protein factors

Studies on light control of transcription by combination of deletion and mutagenesis analysis of light–regulated promoters such as *CAB*, *RBCS* and *CHS* have identified regulatory cis-acting sequences called Light Responsive Elements (LREs). These are defined as small DNA sequences that is present upstream of the transcription start site and sufficient to confer light regulated expression onto the minimal promoter. It was found that if upstream regions were attached to some reporter genes like chloramphenicol transferase (CAT) and β glucuronidase (GUS) their expression could
be regulated by light irradiation. Although numerous cis-acting elements have been identified, relatively few have been functionally characterized.

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). It is worth mentioning here that some of these LREs are also found to be present in non light regulated promoters such as CaMV35S. Using footprint or gel retardation assays, several DNA binding proteins have been identified that bind to LREs within light regulated promoters and some of these have been cloned and their role in light signaling have also been studied (Tobin and Kehoe, 1994; Terzaghi and Cashmore, 1995; Feldbrugge et al., 1997; Wang et al., 1997).

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. All contain trihelix (helix-loop-helix-loop-helix) structures predicted to bind DNA. Functional analyses however have not been performed for any of the cloned GT1 factors.

GATA (I box) elements have the core sequence GATAA, 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; Giuliani 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). GATA-box binding factors have been identified in nuclear extracts from both monocots and dicots plants. 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). All cloned plant proteins factors that bind to G-box contain bZIP motif and no function in light regulation has been assigned to any of the G-box binding factor. 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. G-box binding factors bind to DNA as dimers, which can be either homo-and or heterodimer. GBF4 from A.thaliana binds to DNA as heterodimer.

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. 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).

2.1.4.2 Combinatorial interactions of light responsive elements (LREs)

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). Furthermore, it has been demonstrated that paired LRE containing promoters (such as G-GATA-NOS101, GTI-GATA-NOS101 and Z-GATA-NOS101) can respond to phytochrome mediated low-fluence response, whereas the single LRE containing promoter is unable to do so (Puente et al., 1996).

2.2 Cross talk between light signaling pathway and other signaling cascades

Recent studies have demonstrated that various signaling pathways crosstalk. The Arabidopsis DEAD-box RNA helicase mutant los4 is chilling sensitive and is impaired in the cold-regulated expression of CBF genes (Gong et al., 2002). Phytochrome mediated light signaling has recently been demonstrated to be involved in the regulation of TOP2, one of the components of DNA replication and cell cycle machinery (Hettiarachchi et al., 2003). A low temperature promoter determinant, C/DRE, has been demonstrated to be involved in phyB mediated light signaling to cold-induced gene expression (Kim et al., 2002). It has been recently shown that phytochrome signaling interacts with salicylic acid (SA) signal transduction (Genoud et al., 2002). Weatherwax (1996, 1998) earlier demonstrated an interaction of light and ABA in the regulation of plant gene expression in Lemna gibba.

Many plant hormones such as ABA, brassionsteroids, auxins, cytoknins are likely to be involved in the regulation of photmorphogenic responses. ABA is also involved in the repression of expression of light regulated genes such as rbcS and Lhcb (Quatrano et al., 1983; Bartholomew et al., 1991; Chang and Walling, 1991). The transcription of some of the GA biosynthetic genes are regulated by the phytochromes (Kamiya and Martinez-Gracia, 1999). Genetic screens have identified many brassinosteroid mutants which show deetiolated phenotype in the dark (Clouse and Sasse 1998; Li and Chory 1999, 2001).BAS1 which is the gain of function suppressor of phyB missense mutation (phyB-4; Koornneef et al., 1980; Reed et al., 1993) has been identified and its
overexpression resulted in dwarf plants with no shade avoidance syndrome as seen in phyB plants, indicating that \textit{BAS1} acts as a bypass suppressor of phyB acting downstream of phyA and cry1 (Neff et al., 1999).