CHAPTER 2:
LITERATURE REVIEW
2. LITERATURE REVIEW

2.1 CHLOROPHYLL BIOSYNTHESIS:

Light plays an important role in chlorophyll formation. In etiolated plants Pchlide is synthesized. ALA is precursor of chlorophyll and heme biosynthesis. In animals, ALA is synthesized from glycine and succinyl-CoA in one step catalyzed by enzyme ALA synthetase. In plants, ALA is synthesized from glutamate in three steps. tRNA\textsubscript{GLU} is involved in this pathway. This pathway, operating in plants is termed as C5 pathway.

2.1.1 C5 PATHWAY OF ALA BIOSYNTHESIS:

Glutamate tRNA synthetase (GluRS):

This is the first enzyme of C5 pathway. This enzyme is also known as aminoacyl-tRNA synthetase and catalyses ligation of glutamate to tRNA\textsubscript{GLU} (Huang et al., 1984; Kannagara et al., 1984; 1994). Glutamate tRNA ligase has been purified from Chlamydomonas cells (Wang et al., 1984), Chlorella cells (Weinstein et al., 1987), Synechocystis (Rieble and Beale, 1989) and barley leaves (Bruyant and Kannagara, 1977). The molecular weight of the enzyme ranges from 50-60 kDa. In eukaryotic cells two different kinds of glutamate synthesizing enzymes are present; one inside the chloroplast and other outside. Both the enzymes are encoded by nuclear DNA and translated on cytoplasmic ribosomes. One of the enzymes is imported into the chloroplasts where it ligates glutamate to tRNAs containing glutamate (tRNA\textsuperscript{GLU}) as well as glutamine tRNA\textsuperscript{GLN} anticodons (Schon et al, 1988). They have shown that in barley, there are at least two other glutamate-accepting tRNA species. These tRNAs possess differentially modified anticodons for tRNA\textsuperscript{GLN}. These mischarged Glu-tRNA\textsuperscript{Gln} species can be converted in crude chloroplast extracts to Gln-tRNA\textsuperscript{Gln}, which is also accepted by this enzyme. Vothknecht et al (1996) expressed hemA gene from barley in E. coli as a fusion protein having Schistosoma japonicum glutathione S-transferase (GST) at its amino terminus. This protein was found to be active with specific activity of 120pmoles/\mu g/min. This protein used tRNA\textsuperscript{GLU} from barley chloroplast preferentially to E. coli tRNA\textsuperscript{GLU} and its activity was inhibited by hemin.
Fig. A. The three-step reaction of 5-aminolevulinic acid (ALA) synthesis.
tRNA Glu: A cofactor in ALA synthesis:

The first step of the pathway to ALA synthesis is the activation of glutamate by ligation to tRNA$^{\text{Glu}}$. In vitro ALA forming capacity in whole cells of *Chlamydomonas reinhardtii* (Huang et al., 1984), *Chlorella vulgaris* (Weinstein and Beale, 1985) and barley plastids (Kannangara et al., 1984 and Rieble et al., 1989) was inhibited after preincubation of the extracts with RNAaseA. In barley, tRNA$^{\text{Glu}}$ involved in ALA biosynthesis is encoded by chloroplast DNA. The level of tRNA$^{\text{Glu}}$ remains unchanged after light treatment of etiolated seedlings (Schon et al., 1992). The tRNA$^{\text{Glu}}$ was found to be common for both porphyrins and proteins synthesis (Jahn et al., 1992). There are at least two genes encoding tRNA$^{\text{Glu}}$ in *Synechocystis* sp. Strain 6803 (O’Neill et al., 1988). Jahn (1992), have shown the obligatory requirement of aminoacylated tRNA$^{\text{Glu}}$ to act as a substrate for enzyme GluRS. It was also shown that once the GluRS, GluTR and tRNA$^{\text{Glu}}$ forms the complex they become partially protected from ribonuclease digestion (Jahn, 1992).

Glutamyl-tRNA reductase (GluTR):

This enzyme catalyses second step of ALA biosynthesis. It is also known as Glutamyl-tRNA dehydrogenase (Weinstein et al., 1987). It reduces the carboxyl group of glutamyl-tRNA in the presence of NADPH (O’Neill and Soll, 1990a, Chen et al., 1990b) and releases glutamate-l-semialdehyde. Pyridine nucleotides are required for this reaction (Hoober et al., 1988). This step is strongly inhibited by heme (Huang and Wang, 1986; Kannangara et al., 1988). This suggests that it may be a site for feedback regulation of chlorophyll biosynthesis. In greening barley, this enzyme is stimulated by GTP (Kannangara et al., 1988). The enzyme purified from *Chlamydomonas* has molecular mass of 130 kDa and is a monomer (Chen et al., 1990b) and the enzyme from barley is approximately 270 kDa with five identical subunits of 54 kDa (Pontoppidan and Kannangara, 1994). Glutamyl-tRNA reductase from *Synechocystis* has a molecular weight of 350 kDa with subunits of 47.5 kDa (Verkamp et al., 1989; Rieble, 1991) and from *E.coli*, it is a protein with a subunit molecular weight of 46 kDa. Micromolar concentrations of Zn$^{2+}$, Cu$^{2+}$ and Cd$^{2+}$ also inhibited barley glutamyl-tRNA reductase (Pontoppidan and Kannangara, 1994). Bougri and Grimm (1996) have cloned two full-length and one partial clone from barley and by doing northern blot analysis they have shown that BHA1 (a cDNA
clone from barley encoding GluTR) transcripts are present in roots and are elevated after cytokinin treatment whereas BHA 13 (another cDNA clone from barley encoding GluTR) was not present in roots. Also BHA 1 levels show oscillations under circadian rhythm. The level of GluTR increases 10-folds after 4h exposure to light and declined after 16h light. Later on two hemA clones, hemA1 and hemA2 from cucumber were cloned (Tanaka et al. 1996). These clones were homologous to hemA gene sequences. Of these hemA1 accumulated in cotyledons and hypocotyls of greening cucumber seedlings and its accumulation increases upon illumination. The hemA1 mRNA accumulated in response to demand of Chl synthesis in photosynthesising tissues, whereas hemA2 mRNA was expressed in response to the demand of synthesis of porphyrins other than chlorophylls. The occurrence of second functional hemA gene, hemA2 in *Arabidopsis* has also been shown (Kumar et al. 1996). hemA2 is expressed in low levels in roots and flowers of *Arabidopsis* whereas hemA1 is not. On the basis of presence of two independent hemA genes Kumar et al. (1996), have raised a possibility of existence of two C5 pathways. It was postulated that ALA synthesis destined for Chl formation is under the control of phytochrome, whereas ALA targeted for heme biosynthesis is regulated by feedback inhibition (Huang et al., 1990). Light-induced expression of hemA1 indicates the involvement of phytochrome, suggesting the probability of two independent C5 pathways (Kumar et al., 1996). Using transgenic *Arabidopsis* plants expressing antisense HEMA1 mRNA, Kumar and Soll (2000), have shown that HEMA gene expression is essential for the growth of plants, as these transgenic plants exhibited varying degrees of chlorophyll deficiency, decrease in ALA levels and decrease in glutamyl-tRNA levels which was inversely proportional to the expression of antisense HEMA transcript. From genebank the size of hem A1 mRNA was found to be 1.864 kb in barley (Accession no. AF294752).

**Glutamate 1-semialdehyde Aminotransferase (GSA-AT):**

Glutamate 1-semialdehyde (GSA) is immediate precursor of ALA biosynthesis in plants and bacteria. GSA-AT is involved in this conversion (Kannangara and Gough, 1978). This enzyme was purified from barley (Kannangara and Gough, 1978) and was found to be encoded by nuclear DNA. The enzyme has high affinity for GSA. Gabaculine is an inhibitor of this enzyme (Gough et al., 1992). During the conversion of GSA to ALA, amino group from pyridoxamine phosphate is donated to GSA,
leading to formation of an intermediate, 4,5-diaminovalerate. The enzyme then releases amino group from position-4 of this intermediate, releasing δ-ALA and again whole reaction is repeated. In bacteria, the enzyme is a homodimer with 40-60 kDa subunits (Grimm, 1990). The enzyme is produced as a precursor protein of 50 kDa and is then transferred to chloroplast where a 34 amino acid transit peptide is cleaved, forming a mature protein in barley (Kannangara et al., 1994). Pugh et al (1992), purified GSA-AT from pea leaves and found that the enzyme has an absorption spectrum with maxima at 345 and 416 nm. Conversion of the pyridoxaldimine form of enzyme to pyridoxamine form resulted in proportional increase in activity towards glutamate semialdehyde. Enzyme bound diaminovalerate is a central intermediate during the catalytic conversion of GSA to ALA. HemL genes encoding the above enzyme has been cloned from E.coli, Synechococcus (Grimm et al., 1991), Salmonella (Elliot et al., 1990), Hordeum vulgare (Grimm, 1990), Arabidopsis (Ilag et al., 1994), Glycine max (Sangwan and O'Brian, 1993) and Nicotiana tabacum (Hofen et al., 1994). The amino acid sequences of these genes show extensive homology among themselves. 70% homology was found between Synechococcus and barley enzymes (Ilag et al., 1994). In barley enzyme a pyridoxamine 5'-phosphate cofactor binding site was identified (Kannagara et al., 1994), containing an essential lysine, which is at position 272 of the Synechococcus enzyme (Grimm et al., 1992). Light is reported to stimulate transcription of the gene encoding the enzyme in Arabidopsis (Ilag et al., 1994) and Chlamydomonas reinhardtii (Matters and Beale, 1994). The Gsa gene was strongly expressed in leaves of etiolated plantlets independently of light treatment and to a lesser extent, in leaves of mature plants and is expressed in a non-photosynthetic plant organ for nodule heme synthesis in soybean (Sangwan and O'Brian, 1993). The GSA-AT was expressed in soybean leaves and nodules, but not in roots, and that the protein correlated with enzyme activity (Frustaci et al., 1995). A gene gsal, was isolated and appears to be one of the two gsa genes in the soybean genome and was found to be elevated by light. GAGA elements were found to be involved in the transcriptional control of gsal (Frustaci et al., 1995). Zavgorodnyaya et al. (1999) developed some transgenic tobacco plants by introducing a gene encoding a fusion protein of yeast ALA synthase with an amino terminal transit sequence for the small subunit of RuBisCo into the genome of wild type tobacco and a chlorophyll deficient transgenic line expressing GSA-AT antisense RNA. They have found that,
expression of ALA-S in GSA-AT antisense transgenics provided green-pigmented co-transformants similar to wild type in chlorophyll content while transformants derived from wild-type plants did not show any phenotypical change. The transgenic plants formed chlorophyll in the presence of gabaculine, an inhibitor of GSA-AT. Steady-state RNA and protein levels and consequently, the enzyme activity of GSA-AT was reduced in plants expressing ALA-S. Hofgen et al. (1994), found two cDNA clones encoding two isomers of GSA-AT in tobacco. Im and Beale (2000), have shown that a signal transduction pathway involving a heterotrimeric G-protein activation, phospholipase C catalyzed InsP3 formation, InsP3-dependent Ca$^{2+}$ release and activation of a downstream signaling pathway through a Ca$^{2+}$/CaM-dependent protein kinase is operating for light-regulated Gsa expression. Cells of *Chlamydomonas* strain CC-2682, are sensitive to light and do not show phototaxis. In these cells the levels of gsa transcript was low in dark-grown condition but increases significantly after 2h of exposure to dim green (480-585 nm) light (Hermann et al., 1999). They have also shown that, the photoreceptor for light induction in *Chlamydomonas* is a flavoprotein. The transcript size was found to be 1.62 kb in *Hordeum* (Accession no. P18492). The protein size is 469 bp with a transit peptide of 34 bp.

2.1.2 STEPS INVOLVED IN METABOLISM OF ALA TO PROTOIX

Steps involved in metabolism of ALA to protoIX are common to both heme and chlorophyll biosynthesis and in both plants and animals. The enzymes catalyzing these reactions are as follows:

5-Aminolevulinic acid Dehydratase (ALAD):

This enzyme is also known as PBG synthase. This enzyme catalyzes the first common step of tetrapyrrole biosynthesis, and is a metalloenzyme. It catalyses the asymmetric condensation of two molecules of 5-ALA to form a monopyrrole, porphobilinogen. Shemin (1976) first proposed the mechanism of action of this enzyme. The reaction involves formation of a Schiff's base. In *R. sphaeroides* lysine residue was found at the site of formation of Schiff's base (Nandi, 1978). Pea ALAD has a distinct metal binding domain based on aspartate and an active site domain of lysine which is highly conserved (Boese et al., 1991). The aldol condensation between two ALA molecules involves the initial binding of two substrate molecules to two sites and five membered heterocyclic ring of PBG is formed with the help of a
Fig. B.1: The portion of the tetrapyrrole biosynthetic pathway that is common to both chlorophyll and phycobilin branches.
Fig. B.2: The chlorophyll branch.
lysine and a histidine residue (Jordan and Seehra, 1980; Spencer and Jordan, 1994, 1995). In *R. sphaeroides* this enzyme is an octamer (Grune et al., 1977). This enzyme has also been isolated from wheat (Nandi and Waygood, 1967), tobacco (Shetty and Miller, 1969), radish (Shibata and Ochiai, 1977) and Spinach (Liedgens et al., 1980, 1983). Spinach enzyme is found to be a hexamer with molecular weight of 300 kDa (Liedgens et al., 1980) while radish leaves found to have two isozymes of ALAD (Tchounomogne et al., 1992). ALAD enzyme from plant sources found to have an alkaline pH optimum. Enzyme from radish cotyledons showed pH optimum 8.0 (Shibata and Ochiai, 1977). The enzyme from bacteria require K+ for its activity (van Heningen and Shemin, 1971) while the enzyme from yeast require Zn2+ ions for its activity (Wilson et al., 1972) and one Zn2+ is bound to one subunit and all the eight are required for maximum activity of the enzyme. Plant ALAD requires Mg2+ for their activity. The radish cotyledon enzyme require Mg2+ and Mn2+ for its activity while K+ was less effective (Shibata and Ochiai, 1977). Matters and Beale (1995), have shown that, a putative Mg2+ binding domain characteristic to plant ALADs is also present in *Chlamydomonas reinhardtii*. Enzymes from tobacco leaves and radish cotyledons were found to be inhibited by Zn2+ and Fe2+ (Shetty and Miller, 1969; Shibata and Ochiai, 1977). Chauhan and O' Brian (1993) have isolated a gene, hemB, from *Bradyrhizobium japonicum*, which encodes for ALAD. This enzyme was found to be similar to the plant enzyme in metal requirement and this enzyme was found to be required for nodule development. ALAD mRNAs levels were found to be higher in dark grown plants as compared to light grown plants in pea (Li et al., 1991). Matter and Beale (1995), by southern blot analysis, have shown that, the gene for ALAD in *C. reinhardtii*, is a single-copy gene and that there is an increase in both the ALAD mRNA levels and in ALAD enzyme activity during light phase. Kaczor et al. (1994), have shown that in root nodules of soybean ALAD strongly expressed and light is not essential for expression of AIAD in leaves of dark-grown plants as revealed by the mRNA, protein and enzyme activity levels. Using an artificially synthesized PBG synthase in pea (*Pisum sativum* L.) Kervinen et al, (2000) showed that this enzyme is most active at slightly alkaline pH and shows a maximal binding of three Mg(II) per subunit. Disulfide linkages were found to be formed between four cysteines per octamer, each located five amino acids from the C-terminal. In radish seedlings the activity of ALAD decreased in vivo after addition of gabaculine (Kedy et al., 1994). The transcript size is 1.8Kb in Hordeum and protein was 428 amino acid
(Accession no. X92402 and CAA 63139) and in Pisum 1.458 Kb and protein of 398 amino acid (Accession no. M 11235 and AA 33640).

Porphobilinogen Deaminase (PBGD):

The enzyme catalyses the synthesis of uroporphyrinogen by condensing four molecules of Porphobilinogen. Hydroxybilane is the initial product of the reaction. In the absence of uroporphyrinogen cosynthase, the product spontaneously cyclises to uroporphyrinogen I. Uroporphyrinogen III, which is found biologically, and is further, metabolized for chlorophyll biosynthesis is synthesized in presence of enzyme cosynthase. The enzyme has been purified from different bacterial, animal and plant sources. Lim et al. (1994), have isolated a cDNA clone for porphobilinogen deaminase from Arabidopsis and was found to encode a precursor protein of 382 residues, which is then imported to the isolated chloroplasts and processed to a mature protein. They have also shown that the enzyme was encoded by a single gene and is expressed in both leaves and roots. 1.6Kb cDNA was isolated from pea and was found to encode a protein of 40 kDa molecular weight (Spano and Timko, 1991). This protein possesses a 46-amino acid long transit peptide and possesses conserved arginine and cysteine residues. Northern blot analysis have shown that the pea gene encoding PBG deaminase is expressed to varying levels in chlorophyll-containing tissues and is subject to light induction. Smith (1988) has shown that ALAD and PBGD are exclusively plastid enzymes. Spano and Timko (1991), have shown that the enzyme from pea has an acidic isoelectric point and is a single polypeptide and shows different levels of sensitivity to divalent cations and is most sensitive to Fe$^{2+}$. Using western blot analysis and immunoprecipitation studies, Witty et al. (1996), have shown that in Arabidopsis this enzyme is confined to plastids in both leaves and roots. The synthesis of PBGD is regulated by light and cell types (Smith, 1988; Spano and Timko, 1991; Shashidhara and Smith, 1991; He et al., 1994).

Uroporphyrinogen III Cosynthetase:

This enzyme catalyses formation of uroporphyrinogen III from hydroxymethylbilane. The enzyme has been isolated from E. gracilis (Hart and Battersby, 1985) and Wheat germ (Higuchi and Bogorad, 1975). The enzyme was found to be heat labile and Na$^+$ and K$^+$ enhanced the activity. The enzyme PBGD
and cosynthase may be present as a complex (Tsai et al., 1987). HemD gene encodes for the enzyme and has been cloned and isolated from various organisms. The enzyme from *A. nidulans* has an inferred amino acid sequence which shows 43-50% homology to that from *B. subtilis, E. coli* (Jones et al., 1994). The inferred amino acid sequence has conserved arginine residue at codon 146, which has been implicated to be important for cosynthase activity (Hansson et al., 1991).

**Uroporphyrinogen Decarboxylase (4.1.1.37) (UroD):**

This enzyme catalyzes decarboxylation of all the four carboxyl groups of acetic acid moieties of uroporphyrinogen III to yield coproporphyrinogen III. The reaction is substrate concentration dependent and starts at the ring-D acetate group in a clockwise manner (Luo and Lim, 1993). Enzyme has been purified from animal sources and from *Euglena* (Jukant et al., 1989), tobacco leaves (Chen and Miller, 1974), from Yeast (Felix and Brouillet, 1990) and from *R. sphaeroides* (Jones and Jordan, 1993). The molecular weight of the enzymes ranges from 39-57 kDa. All four isomers of uroporphyrinogen are accepted by the enzyme, aromatic porphyrins are not decarboxylated (Castelfranco and Beale, 1981). Porphyrins especially, oxidation products of the substrates, inhibited the enzyme (Smith and Francis, 1981). The enzyme from tobacco showed a pH optimum of 6.5 (Chen and Miller, 1974). The plant enzyme is inhibited by Fe$^{2+}$, Co$^{2+}$, Pb$^{2+}$, Ni$^{2+}$ and also inhibited by Mg$^{2+}$ (Chen and Miller, 1974). Nucleotide sequences encoding UroD have been isolated from *Synechococcus* (Kiel et al., 1992), *E. Coli* (Nishimura et al, 1993), tobacco and barley (Mock et al., 1995). HEM12 gene encodes for UroD in yeast, *Saccharomyces cerevisiae*. Using seven "leaky" mutants from this yeast, Chelstowska et al. (1992), have shown a single active center in UroD and the substitution of glycine leads to a totally inactive enzyme further confirming the existence of a single active center existence in UroD. The amount of UroD mRNA and protein was found to increase during illumination in barley leaves grown under day and night cycles (Mock et al., 1995). Transgenic tobacco plants with reduced activity of either uroporphyrinogen decarboxylase or coproporphyrinogen oxidase accumulate photosensitizing tetrapyrrole intermediates, antioxidative responses and necrotic leaf lesions. These plants also display increased pathogenesis-related protein expression (Mock et al., 1999). Mock et al. (1998,1995), have isolated, sequenced and expressed cDNA sequences encoding uroD from tobacco and barley in *E.coli* and found that a protein
of 43KDa is synthesized and then processed to 39 KDa. Expression of uroD mRNA and protein increased during illumination and maximum uroD-mRNA levels were detected in basal segments of leaf relative to top of the leaf. The mRNA size was found to be around 1.7 Kb in *Hordeum* (Accession no. X82832 and in tobacco the size was found to be 1.5 Kb (Accession no. X82833). In animals reduced activity of UroD causes porphyria cutanea tarda (PCT). PCT is a multifactorial disease where both inherent and environmental factors such as alcohol, estrogens, halogenated aromatic hydrocarbons and viral infection (mainly hepatitis C) are involved in biochemical and clinical expression. In PCT, hepatic iron plays a key role (Mukerji S.K., 2000).

**Coproporphyrinogen oxidase (EC 1.3.3.3) (Coprox):**

This enzyme catalyses the oxidative decarboxylation of propionate side chains on ring A and B to yield protoporphyrinogen IX. In aerobic organisms, oxygen is utilized as the sole electron acceptor for enzymatic activity while in anaerobic organisms NADP⁺ is used (Seehra et al., 1983). The enzyme does not react with coprogen I and II. The coprogen oxidase was first purified from tobacco (Hsu and Miller, 1970). A mutant from *R. sphaeroides*, N1 excretes Coprogen III in growth medium. It is incapable of photosynthetic growth (Coomber et al. 1982). *S. typhimurium* mutants were developed by insertional mutagenesis to block the conversion of 5-ALA to heme. These mutants were defective in two genes, hemN and hemF, which encode alternative forms of coprogen oxidase (Xu et al., 1992). Yeast enzyme was found to be a homodimer of 70 kDa (Camadro et al., 1986). In yeast aerobic coprogen oxidase was stimulated in the presence of divalent ions, whereas anaerobic enzyme activity had an absolute requirement for a metal ion (Poulson and Polgolasse, 1974). The enzyme from tobacco leaves was found to be activated by Fe²⁺, Mn²⁺ and inhibited by EDTA and o-phenanthroline, suggesting that enzyme from plant sources require some metal ions for the activity (Hsu and Miller, 1970). The gene from soybean encodes a polypeptide with a molecular mass of 43 kDa (Madsen et al., 1993). Mock et al (1999), have shown that tobacco plants containing antisense RNA for coprogen oxidase are more resistant to tobacco mosaic virus. Kruse et al. (1995a), produced tobacco plants containing antisense coprogen oxidase mRNA. These plants showed decreased enzyme levels of coprogen oxidase and were characterized by growth retardation and necrosis, showing that these plants were
damaged due to oxidative stress. Kruse et al (1995b), have cloned full-length cDNA from barley and tobacco and found that these encode for precursor proteins of 43.6 and 44.9 kDa respectively and were imported to pea chloroplast and processed to 39 kDa and accumulated in stroma. The plant coprogen oxidase gene was expressed to different extents in all tissues. The level of mRNA did not significantly differ in etioplast and greening barley leaves. The level of RNA reached its maximum in developing cells and decreased drastically when cells were completely differentiated. They have also speculated that enzymes involved in tetrapyrrole biosynthesis are developmentally rather then light-dependently regulated. Madsen et al. (1993), have shown that in soybean a 43 kDa precursor for coprogen oxidase is synthesized and this shows 50% homology to yeast amino acid sequence. The mRNA levels were very high in soybean root nodules followed by its level in leaves. No mRNA for coprogen oxidase was detectable in soybean roots. The enzyme protogen oxidase (EC 1.3.3.4) and coprogen oxidase were found to be localized in both mitochondria and etioplasts and in etioplasts respectively. The size of transcript was found to be 1.45Kb in Hordeum (Genbank).

**Protoporphyrinogen oxidase (EC 1.3.3.4) (Protox):**

The oxidative conversion of Protoporphyrinogen IX (Protogen) to Protoporphyrin IX (Proto IX), is catalyzed by the enzyme protoporphyrinogen oxidase. Protogen is unstable and spontaneously undergoes oxidation in presence of oxygen and its oxidation is enhanced by light. (Jacobs and Jacobs, 1979). Enzyme catalyzed reaction mechanism of Protogen oxidation consists of three consecutive dehydrogenations and a subsequent tautomerization yielding the porphyrin through the stereospecific loss of the fourth meso hydrogen as a proton (Akhtar, 1994). Protox is active only if there are no polar groups on ring A and B. Protox is quite stable towards acids and bases. Some structurally bicyclic herbicides e.g. acifluorofen methyl was shown to inhibit protox activity in chloroplast (Camadro et al., 1991; Matrinage et al., 1992b). It was also shown that there are some extra-organellar oxidase which are resistant to these oxidases which oxidise protogen exported from the plastids into the cytosol (Lee et al., 1993). Protoporphyrinogen oxidase was purified from yeast mitochondrial membranes and found to be a 55 kDa polypeptide with a pI of 8.5 (Camadro et al., 1994). Dailey et al (1994), have expressed hemY gene of the B. subtilis homey cluster in E.coli and got a 53 kDa protein, capable of
oxidizing coprogen III and protogen IX. Enzyme purified from barley etioplasts has a molecular weight of 210 kDa, and gives a band at 36 kDa (Jacobs and Jacobs, 1987). Protox from yeast is synthesized as a higher weight precursor of 58 kDa and is processed to 55 kDa membrane bound protein. Protox was found to be localized in the envelope and thylakoid membranes of chloroplasts (Matringe et al., 1992a). The plastidal protox is preferentially associated with the stromal side of the thylakoid membrane and a small portion of the enzyme is located on the stromal side of the chloroplast inner envelope membrane in spinach (Che et al., 2000). Yamato et al. (1995) have shown that amino acid sequences of protox purified from tobacco cultured cells have shown homology to acid/base catalysis and heme binding regions of plant peroxidases. Narita et al (1996) cloned cDNA for Arabidopsis protox. They have shown that extracts of E.coli cells transformed with Arabidopsis cDNA had high protox activity and encode for a protein of 57.7 kDa. Southern analysis revealed presence of a single gene encoding for Arabidopsis protox. Lermontova et al. (1997), have cloned 2 full length cDNAs from tobacco and shown that first encodes for a protein of 548 amino acids having transit peptide of 50 amino acids and can be translocated to plastids. The second one encodes for a protein of 504 amino acids. Bacterial extracts containing recombinant mitochondrial enzyme exhibit high protox activity where as plastidal enzyme was expressed as inactive peptide. Transcripts of both genes were expressed synchronously during tobacco plant development and diurnal and circadian growth (Lermontova et al., 1997). Transcripts of plastidal protox were very high in Arabidopsis leaves, where as it was low in roots and floral buds (Narita et al., 1996). Spinach protox is a protein with 562 amino acids precursor and is processed at serine-49 i.e., it has a transit peptide of 48 amino acids (Che et al., 2000). Lee et al (2000), have shown that transgenic rice plants expressing a Bacillus subtilis protox gene are resistant to diphenyl ether herbicide oxyfluorofen.

2.1.3 STEPS INVOLVED IN METABOLISM OF PROTOX TO CHLOROPHYLL:

The Mg-chelation step is branch point in chlorophyll and heme biosynthesis. Ferrochelatase catalyses the insertion of Fe-to proto IX leading to synthesis of heme and Mg-chelatase catalyses the insertion of Mg-to proto IX., leading to synthesis of chlorophyll.
Ferrochelatase (EC 4.99.1.1) (fech):

With in the chloroplasts of higher plants, a crucial branch point of the tetrapyrrole synthesis pathway is the chelation of either Fe$^{2+}$ to make heme, or Mg$^{2+}$ for chlorophyll, catalyzed by ferrochelatase or magnesium chelatase, respectively. Ferrochelatase catalyses the insertion of Fe$^{2+}$ into protoporphyrin IX to generate protoheme. A putative mature region of a cucumber ferrochelatase cDNA (hemH) was overexpressed in *Escherichia coli*. The enzyme was found to be 40kDa. The optimum pH was 7.7 and the apparent Km values for deuterium IX and Fe$^{2+}$ were 14.4μM and 4.7μM, respectively. The activity of the ferrochelatase was inhibited by n-methylprotoporphyrin IX (Suzuki et al, 2000). They have also shown that protein from cucumber was present in hypocotyls and roots but not in cotyledons. No cross-reactivity was found with proteins of thylakoid membranes although the activity was mainly associated with the thylakoid membranes. Northern blot analysis of hemH gene from cucumber also showed the presence of expression mainly in hypocotyls and roots and very less in cotyledons (Suzuki et al, 2000). Ferrochelatase has been purified as a monomer of 35-42 kDa from a number of organisms including bacteria, yeast and mammals (Taketani and Tokunga 1981, Dailey and Fleming 1983, Camadro and Labbe, 1988, Miyamoto et al. 1994, Hannson and Hederstedt 1994). In higher plants, ferrochelatase activity has been detected in bean cotyledons, oat seedlings and spinach leaves (Jones 1968, Porra and Lascelles 1968) and barley etiolated seedlings (Little and Jones 1976, Jacobs and Jacobs 1995). In barley etiolated seedlings the ferrochelatase activity was found to be associated with mitochondria, etioplasts and plasma membranes (Jacobs and Jacobs, 1995). In pea chloroplasts, the activity was shown to be associated with thylakoid membranes but not with envelope membranes (Matringe et al, 1994). Chow et al (1998) have shown the presence of two types of ferrochelatases in *Arabidopsis*. One form was shown to be expressed in leaves, stems, roots and flowers and imported into chloroplasts and mitochondria whereas the other one was expressed in leaves, stems and flowers and targeted solely to chloroplasts. Roper and Smith (1997), have shown that ferrochelatase is associated with both thylakoid and envelope membrane suggesting that the control of the branch point can not be by spatial separation of the two chelatases.
Mg-chelatase:

Mg-chelatase catalyzes the insertion of Mg into protoporphyrin and lies at the branch point of heme and (bacterio) chlorophyll synthesis. In all photosynthetic organisms, Mg-chelatase is a three component enzyme and catalyzes the insertion of Mg\textsuperscript{2+} in two steps, with an ATP-dependent activation followed by an ATP-dependent chelation step (Walker et al., 1997; Walker and Weinstein, 1994). In prokaryotes, three genes--BchlD, H and I encode for subunits of this enzyme. In higher plants, homologous cDNAs for the H, D and I subunits have been characterized (Luo et al., 1999). Using immunoassay they have shown that chlD is associated with the membranes in the presence of MgCl\textsubscript{2}. These subunits were found to be of 140, 70 and 42 kDa respectively. Hansson et al. (1999), have found that chlorina mutants in barley possess a single amino acid substitution in 42 kDa subunit. They have also hypothesized that in wild type barley, association of 42 kDa subunit with the 70 kDa subunit allows them to form a specific complex with the 140 kDa subunit and this complex insert Mg\textsuperscript{2+} into proto IX. In soybean, the chlH protein is approx. 153 kDa and its expression was found to be light inducible in soybean suspension cells. Also the transcript levels were under the control of circadian oscillation (Nakayama et al., 1998). Depending upon the concentration of Mg\textsuperscript{2+} in lysis buffer, the enzyme migrated between stroma and the envelope membrane and was localized in the envelope membrane at very higher concentrations of Mg\textsuperscript{2+} (above 5mM), indicating that the activity of Mg-chelatase was regulated by the expression and subchloroplastidic localisation of chlH protein (Nakayama et al., 1998). Kruse et al. (1997), have shown that ChlI and ChlH were strongly expressed in young leaves and less expressed in mature leaves and only traces of both transcripts were found in flowering organs. Using southern blot analysis, they have shown that ChlI is encoded by a single gene and ChlH by several genes. Papenbrock et al. (1997), have isolated and cloned a tobacco cDNA sequence homologous to bchD. The protein was found to be of 758 amino acids (82.9 kDa). The amino terminal half of ChlD cDNA was 46% homologous to that of ChlI, indicating a gene duplication from an ancestral gene. Reconstitution experiments using yeast protein extract expressing the three subunits of tobacco Mg-chelatase showed additional requirement of ATP (Papenbrock et al., 1997). Using Northern blot analysis, Gibson et al. (1996), have shown that the transcripts of ChlH from Arabidopsis undergoes a diurnal variation, rising almost to its maximum level by the end of the dark period, then increasing slightly at the onset.
of light and declining steadily to a minimum by the end of the light period. In contrast the levels of ch-42 (cDNA clone from Arabidopsis encoding ChlI) and ferrochelatase remained constant. Using southern blot analysis, Jensen et al. (1996) and Kannangara et al. (1997), have shown that Xan-f, -h and -g encodes three subunits of the barley. Xan-F protein shows 82% homology to OLI protein of Antirrhinum, 66% to Synechocystis homologue and 34% to Rhodobacter BchH subunit of Mg-chelatase. The Xan-H protein has 85% amino acid sequence identity to the Arabidopsis CH42 protein, 69% identity to the Euglena CCS protein, 70% identity to the Cryptomonas BchA and 49% identity to Rhodobacter BchI subunit of Mg-chelatase. The expression of xan-f and -h gene in wild type barley is light induced in greening seedlings, and in green tissue the genes are under the control of a circadian clock. The ChlI gene was cloned from soybean by Nakayama et al. (1995), and they have shown that this enzyme is localized in stroma and has an ATP-binding motif as found in other BchI homologs. They have also found that ChlI mRNA was reversibly induced by light in cell cultures of soybean. Gibson et al. (1995), were able to reconstitute the activity of Mg-chelatase by combining the products of BchH, BchI and BchD genes from Rhodobacter sphaeroides and expressing them in Escherichia coli. Chlorina mutants in wheat (Triticum) and phenotypically related barley mutant accumulate chlorophyll to varying amounts and the most severely affected possess the least amount of chlorophyll. Using these mutants Falbel and Staehelin (1994), have shown that they have a partial block in chlorophyll biosynthesis and accumulated Proto IX. They have shown that the chloroplasts from these mutants possess a lower activity of the enzyme Mg-chelatase. The ChlH subunit in soybean was 1,383 amino acid long polypeptide (Nakayama et al., 1998) and is 1,382 amino acid long polypeptide in tobacco (Kruse et al., 1997). Grafe et al. (1999), have shown that, in tobacco, only a 110 amino acid long part of ChlH is required for interaction with partner subunits and maintenance of the enzymatic activity. This polypeptide has the capacity to form homodimers and is capable of interacting with Chll and ChlD subunits. Using transformants of tobacco with sense and antisense mRNA for ChlI, Papenbrock et al (2000a), have shown that both elevated and decreased levels of Chl mRNA and ChlI protein led to reduced Mg-chelatase activity and in these plants chlorophyll synthesis was also reduced. Papenbrock et al. (2000b) have also shown that, transgenic tobacco plants expressing antisense RNA for Mg-chelatase ChlH displayed a reduced growth rate and were chlorophyll deficient. In these plants, less
protoporphyrin IX and heme accumulated, and a decrease in 5-aminolevulinate synthesizing capacity was seen. ALAD activity also decreased along with reduction in Mg-chelatase activity. Mochizuki et al (2001), have cloned *GUN5* gene from *Arabidopsis*, encoding chlH subunit of Mg-chelatase and found that mutants in this gene had repressed expression of lhcb whereas those with mutations in ChlII gene had not. This comparison suggests a specific function for ChlH protein in the plastid-signaling pathway. The transcript size of ChlII is 1.1Kb in *Hordeum*, of ChlH is 4.1 Kb in *Hordeum* and of ChlD is 1.7 in *Hordeum* (Genbank).

**S-Adenosyl-L-Methionine : Mg Proto IX Methyltransferase:**

The conversion of Mg-protoporphyrin IX to Mg-protoporphyrin monomethyl ester (MPE) is catalyzed by this enzyme. SAM acts as a methyl group donor. The enzyme was partially purified from *Euglena* (Ebben and Tait, 1969) and wheat (Ellsworth et al., 1974). The gene *bchM* encoding methyltransferase has been identified in *R. capsulatus* and *R. sphaeroides* (Bollivar et al., 1994). The gene of *Glycine max* was 1374bp (Accession no. AW755900). Later on it was shown that the Mg-chelatase and SAM-methyl transferase could be expressed and coupled in *E.coli* extracts (Jensen et al, 1999). ChlM cDNA from tobacco is 1134bp (genebank)

**Mg-Protoporphyrin IX Monomethylester Cyclase (MPE cyclase):**

The enzyme catalyzes the formation of an isocyclic ring from MPE. The reaction is a three-step reaction (Wong et al., 1985). First step involves, hydroxylation of the methyl propionate at the β-carbon atom, followed by oxidation of the hydroxyl group to a carbonyl group and in the last leading to ligation of the α-carbon of the β-ketomethylpropionate to the γ-meso bridge carbon between the C and D pyrrole rings of the tetrapyrrole, thus forming divinyl Pchlide (Chereskin et al., 1983). The carbonyl oxygen of the oxygen atom of DV Pchlide has been found to be derived from O₂ (Walker et al., 1989) while it is derived from H₂O in organisms, which synthesize Chl anaerobically (Porra et al., 1995). The enzyme activity was found in different organisms. In reconstituted system enzyme was found to be inhibited by CN- and N₃-, while these have no effect in intact developing chloroplasts (Whyte and Castelfranco, 1993). Walker et al (1991), have shown that both pellet and soluble fractions are required for the activity of the enzyme from reconstituted system. 6-
methyl acrylate derivatives of Mg-protoporphyrin are inactive as substrate for cyclization. Cyclase activity was also partially lost on reduction of the side chain of Mg-protoporphyrin in the 2-position from a vinyl to an ethyl (Walker et al., 1988). Lipid-soluble metal-complexing agents inhibit the enzyme activity. Zinc protoporphyrin monomethyl ester could act as substrate of the enzyme. However, copper and nickel substituents or demetallated protoIX failed to act as substrate. Mg-protoporphyrin dimethyl ester was inhibitory for enzyme. The activity of the enzyme was unchanged by prior greening of the plants. The activity in isolated etioplasts depends upon intactness of the plastids (Nasrulhaq-Boyce et al., 1987). Fe$^{2+}$ is required for activity of the cyclase from both eukaryotic and prokaryotic sources and Fe$^{2+}$ ion is associated with the membrane component of the enzyme. The enzyme activity in extracts of *Chlamydomonas reinhardtii* and *Synechocystis* sp. PCC 6803 was inhibited by chelators of Fe suggesting that nonheme Fe is involved in the reaction (Bollivar and Beale, 1996). The *Chlamydomonas* cyclase was not inhibited by either CO or Quinacrine (Bollivar and Beale, 1995). This enzyme is encoded by bchE gene in *Synechocystis*. In *Synechocystis* the enzyme is 66kDa and the cDNA is 1499bp (from gene bank accession no. BAA 10457).

**Protochlorophyllide oxidoreductase (POR) (EC 1.6.99.1):**

This enzyme catalyses the only light requiring step of chlorophyll biosynthesis. It converts Pchlide to Chlide, by adding two hydrogen atoms at C7 and C8 on ring D. POR is encoded in the nucleus, translated as a precursor protein in the cytosol and ultimately transported into plastids (Apel, 1981) and the transport is determined by its substrate. Presence of the substrate leads to the transport and the processing to a mature protein. The ChlB gene product is involved in light-independent Pchlide reduction and in *C. reinhardtii* requires three components for Pchlide reduction (Li et al., 1993). H13 mutant of *C. reinhardtii* is unable to synthesize Chl in dark and lacks PSI. A chloroplast gene, ChlN was identified coding for a 545 amino acid protein, involved in reduction of Pchlide to Chlide. This gene was found in liverworts and pine but absent in tobacco and rice (Choquet et al., 1992). The *C. reinhardtii* chloroplast gene chlL is shown to be involved in light-independent conversion of Pchlide to Chlide. The protein has 53% homology to bchlL gene product in *Rhodobacter* (Suzuki and Bauer, 1992). Three isoforms of POR have been identified in angiosperms: PORA, PORB and PORC. PORC was recently identified in
Arabidopsis by Oosawa et al (2000). These three isoforms are differentially regulated by light. The level of PORA mRNA and protein decreases on illumination of etiolated plants (Holtroff and Apel, 1996) while that of PORC increases and was dominantly expressed in both mature and immature tissues (Oosawa et al., 2000). PORB transcript and protein levels remain constant in both dark and on illumination (Armstrong et al., 1995, Holtrof et al., 1995; Holtrof and Apel, 1996). The enzyme POR, was purified from the oat seedlings, and has molecular weight of 37 kDa (Roper et al., 1987). The barley mutants lacking the enzyme POR-A (light regulated) are defective in forming the photosystems and are susceptible to photooxidative damage (Lebedev et al., 1995). Light dependent PORs from barley, oat, wheat, pea and Arabidopsis are 80 to 90% identical at amino acid level as compared to the three polypeptides of light independent POR (DPOR) (Suzuki and Bauer, 1995). The glutamate present with in a conserved tetrapeptide motif was the site of posttranslational cleavage for POR precursor in pea. This motif is also present in other Pchlide reductase precursors (Timko, 1993). In cucumber single POR gene was found to be involved in chlorophyll biosynthesis throughout of the vegetative growth of cucumber, i.e., in cotyledons and fully green leaves and was found to be positively regulated by light (Kuroda et al. 2000). Lebedev and Timko (1999); Griffiths et al (1996), have shown that the PORB-catalyzed Pchlide reduction reaction consists of two steps, first photochemical, involving a single quantum mechanism and leads to the formation of an unstable intermediate and second, nonphotochemical, involving spontaneous conversion of the unstable intermediate into chlorophyllide. Pchlide-b, a form of Pchlide was found to be absent in the etioplasts of barley (Scheumann et al., 1996). Skinner and Timko (1998) have shown that the PORs of loblolly pine are encoded by a large multigene family, composed of two distinct subfamilies encoding porA and porB. The steady-state levels of porA and porB transcripts were found to be same in cotyledons of both dark grown and illuminated seedlings, but the level of transcripts were found to be increased in the stems of dark-grown seedlings upon illumination. The level of POR and the integrity of prolamellar bodies were found to be crucial for cytokinin and abscissic acid controlled greening following transfer of etiolated lupine cotyledons into dark (Kusnetsov et al., 1998). In liverworts the POR gene is expressed in light-dependent manner (Takio et al., 1998). In cyanobacteria, the open reading frame of 2.1 kb was found to be involved in LPOR (light-dependent POR) synthesis. YEP12 mutant of LPOR, which was a por-disrupted mutant, and
wild type cyanobacteria were found to grow normally under low light conditions (10-25 muE m^{-2} s^{-1}), but at high light intensity (85-130muE m^{-2} s^{-1}), YEP12 stop growing and get photobleached. In contrast, a mutant with disrupted ChlL (DPOR less), YEC2, grew irrespective of light intensity, suggesting that the contribution of LPOR in chlorophyll biosynthesis increases with increase in light intensity (Fujita et al., 1998). In Arabidopsis cop1 mutant, both PORA and PORB restore the prolamellar body and photoactive protochlorophyllide-F655 upon etioplast differentiation (Sperling et al., 1998). In ChlB gene of Pine, editing takes place at second position of CCG codon leading to substitution of proline by leucine. Second editing occur at CGG, leading to tryptophan, in place of arginine. In spruce also editing occurs at both sites but in larix only second editing occurs (Karpinska et al., 1997). The secondary structure analysis of POR possesses 33% alpha-helix, 19% beta-sheets, 20% turn and 28% random coil. The Rassmann-fold fingerprint motif was found to be localized in the N-terminal region. A hydrophobic loop-region was supposed to be involved in membrane anchoring (Birve et al., 1996). Schunmann and Ougham (1996) have isolated three cDNA clones on the basis of altered patterns of expression in the leaf extension zone of the development mutant, slender barley. These were 7s, 8s and 24n (slender barley mutants). Of these 24n cDNA clone was found to encoding for POR. Using PORA deficient Arabidopsis mutants, Runge et al. (1996), have shown that PORA is involved in proper greening of the plants and PORB provides a mean to sustain light-dependent chlorophyll biosynthesis in fully greened plants. Reinbothe et al. (1995), have shown that, pPORA-Chlorophyllide complex is more sensitive to the degradation by a light-induced, nucleus-encoded, and energy-dependent protease activity of barley plastids. pPORA devoid of its substrate, protochlorophyllide was less sensitive to proteolysis. Rowe and Griffith (1995) got a fragment amplified from Phormodium laminosum DNA, which shows 73% homology to wheat sequence. Using a wheat reductase antibody, 36, 30 and 60 kDa bands were obtained in Phormidium, which corresponds to a mature reductase protein, a stable proteolytic fragment and soluble dimeric forms of cyanobacterial enzyme. Using in vitro assembly of NADH-potochlorophyllide oxidoreductase in pea chloroplasts, Dahlin et al. (1995), have shown that the POR is a peripheral protein located on the stromal side of the membrane, and that both the precursor and the mature form of the protein can act as substrate for membrane assembly. pPOR with Chlide attached to it is impaired both in the ATP-dependent step of binding to a receptor protein component
of the outer chloroplasts envelope membrane, as well as in the pchlide-dependent step of precursor translocation (Reinbothe et al., 1995). In *Rhodobacter capsulatus*, mutations in genes known to completely block reduction of protochlorophyllide to chlorophyllide (bchN, bchB, bchL) accumulate a pool of monovinyl and divinyl forms of protochlorophyllide but, bchJ-disrupted strains accumulate reduced levels of bacteriochlorophyll concomitant with the accumulation of divinyl Pchlide and thus have an altered ratio of monovinyl to divinyl Pchlide (Suzuki and Bauer, 1995). Wilks and Timko (1995) have shown that Tyr-275 and Lys-279 are critical for enzyme activity. chlB gene encoding one of the subunits of light-independent POR was cloned by Richard et al. (1994) from *Ginkgo biloba* and *Chlamydomonas reinhardtii* and POR from wheat was cloned by Teakle and Griffiths (1993). Lower plants and gymnosperms synthesize chlorophyll and develop photosynthetically competent chloroplasts even when grown in dark. Two PORs were identified in these. One of them is present in prolamellar bodies of etiochloroplasts and its abundance declines upon illumination. Other is found in thylakoid membranes and its level remains same throughout (Forreiter and Apel, 1993). In cotyledons of dark-grown seedlings of pine high levels of POR mRNA accumulates and there was no change in the levels upon illumination whereas, in stem tissue, there was no mRNA accumulation in dark but the level increases on illumination, suggesting that light and developmental age of the tissue affect regulation of light-POR expression in pine (Spano and Timko, 1992). Franck and Strzalke (1992), detected a photoactive Pchlide-protein complex with absorbance and fluorescence maxima at 648 and 653 nm in greening barley leaves without any re-darkening. The Chlide resulting from photoreduction during greening has an absorbance maximum at 684 nm, which shifts towards a shorter wavelength within a few seconds, indicating rapid liberation of the pigment from the enzyme. They have also deduced that chlorophyll accumulation proceeds through continuous regeneration and phototransformation of the photoactive complex. In Arabidopsis, the enzyme activity and concentration of enzyme protein decreases upon illumination of etiolated seedlings, but there was no effect on the mRNA level upon illumination (Joyard et al., 1991). Heyes et al (2000) has shown that the thiol-specific reagent N-ethylmalimide inhibits POR activity. In barley, the size of POR transcript was found to be 1.7kb (Schulz et al., 1989). The transcript level was found to be 1.49 Kb in *Hordeum* and 1.48 Kb in *Triticum* (Accession no. P13653 and Q41578 respectively).
**Chlorophyll A oxygenase (CAO):**

Chlorophyll a and b are two major forms of chlorophyll present in higher plants. Much less was known about chlorophyll-b synthesis until recently. Tanaka et al, (1998), isolated and characterized a gene using mutational studies, which they found to be encoding CAO enzyme, an enzyme involved in conversion of chlorophyll-a to -b. The enzyme was found to be similar to monooxygenases and its coding sequence was found to contain domains for a [2Fe-2S] and for a mononuclear iron binding (Tanaka et al, 1998). During conversion of Chla to Chlb the electron is transferred from Rieske centre to the mononuclear iron with subsequent activation of molecular oxygen for oxygenation of the Chl a methyl group. CAO accept electrons from ferredoxin-NADPH oxidoreductase or from ferredoxin itself. The enzyme was found to be chloroplast-localized. No dehydrogenase was found to be involved in conversion of chl a to chl b (Tanaka et al., 1998). Tomitani et al. (1999) isolated chlorophyllb-synthesizing genes (CAO gene) from two prochlorophytes and from some major chlorophytes and found that the progenitors of oxygenic photosynthetic bacteria, including the ancestors of chloroplasts, had both chlorophyll b and phycobilins. Using leaky and null chlorinal mutants from Arabidopsis Espineda et al (1999), have shown that, the mRNA of CAO was higher in these mutants though these mutants possess reduced levels of chlorophyll b, suggesting that plants that do not have sufficient chlorophyll-b up-regulate CAO gene expression level. The level of mRNA of CAO gene from Arabidopsis was found to be less in plants grown under dim-light conditions (Espineda et al, 1999) major lhcb proteins were also absent in these mutants. Oster et al (2000), have shown that, a recombinant CAO enzyme produced in Escherichia coli catalyses an unusual two-step oxygenase reaction that is the 'missing link' in the chlorophyll cycle of higher plants.

**Geranyl-geranyl reductase:**

Geranyl reductase catalyses a step in isoprenoid biosynthesis. This enzyme catalyses the reduction of geranylgeranyl diphosphate to phytyl diphosphate. This enzyme is encoded by gene ChlP. The transcript size was found to be 1.9Kb and 1.51Kb in Glycine max and tobacco respectively (Genbank). Tanaka et al (1999), have shown that the decrease in CHLP activity affects the chlorophyll and tocopherol contents. Using the transgenic Arabidopsis plants expressing antisense RNA for
geranyl geranyl reductase they have shown that, chlorophyll content decreases in high light-intensity. They have also shown that CHLP provides phytol for both chlorophyll and tocopherol synthesis and is present at a branchpoint of tocopherol and chlorophyll biosynthesis. A bchP gene has been detected by (Mars, 1981; Zsebo and Hearst, 1984) from Rhodobacter capsulatus as a part of photosynthetic gene cluster. The bchP gene encodes for CHLP. Keller et al (1998), have cloned a cDNA encoding a pre-geranyl-geranyl reductase from Arabidopsis thaliana mature protein was 47 kDa. This gene when expressed in E.coli sequentially catalyzed the reduction of geranyl-geranyl-chlorophyll a into phytyl-chlorophyll a as well as the reduction of free geranyl-geranyl diphosphate into phytyl diphosphate, suggesting that this is a multifunctional gene. In Arabidopsis, a higher steady state level of mRNA was observed during deetiolation i.e., in green plants. Its mRNA was high during early stages of chromoplast differentiation.

Chlorophyll Synthetase:

Chlorophylls are esterified with a long chain C-20 alcohol, phytol. The reaction is catalyzed by enzyme chlorophyll synthetase and both Chlidea and Chlideb were equally esterified (Rüdiger et al., 1980). In etioplasts, Geranyl geranyl pyrophosphate(GGPP) is used as a substrate (Rüdiger et al., 1980), while in chloroplasts, the preferential substrate is PhPP (Soll et al., 1983). In the thylakoids, the GGPP is first esterified to Chlide forming ChlGG and reduced stepwise to Chlph but in chloroplast envelope GGPP is reduced by an NADPH-dependent hydrogenase with in the envelope to form PhPP (Soll et al., 1983). Chlorophyll synthetase in chloroplast thylakoid membranes incorporate phytol in presence of ATP and a stromal kinase (Benz and Rüdiger, 1981a). The acceptable tetrapyrrole substrates for etioplasts Chlorophyll synthetase includes Chlides-a and -b, and [3-acetyl]-Chlide a (Benz and Rüdiger, 1981b). The PChlide and Bchlide (bacterial chlorophillide) are not accepted by the plant enzyme indicating that reduction of the 17,18 double bond on the ring D was essential but reduction of 7,8 double bond on ring B was unacceptable (Benz and Rüdiger, 1981b). The chlorophyll synthetase was found to be present in heat-bleached, ribosome-deficient plastids from rye and oat (Hess et al., 1992), indicating its nuclear origin and synthesis by cytoplasmic ribosomes. The enzyme was not affected by the developmental stage of the plastids. In etiolated wheat, the enzyme was found in latent form in prolamellar bodies (Lindsten et al.,
1990). Vezitskii and Sherbakov (1987), have shown that the metallophorbides with a metal having coordination number of 5 (Zn, Mg, Cd) could be esterified. Thus, a fifth axial ligand, presumably a substrate-binding site of the enzyme, is necessary for the formation of an active enzyme-substrate complex (Vezitskii and Sherbakov, 1987). In photosynthetic bacteria Rhodobacter, the essential loci involved in bacteriochlorophylla biosynthesis are clustered in a 46 Kb region of the chromosomes (Bauer et al., 1993). BchG in bacteria (Bollivar et al., 1994b) and G4 (Gaubier et al., 1995) in A.thaliana encodes for the enzyme and shows a homology of 60-75% in amino acid sequences. The final product of the reaction is Chla, which differs from Chlb only by the presence of a methyl group at pyrrole ring II in place of formyl group (Beale and Weinstein, 1990). Schneegurt and Beale (1992); Porra et al (1993,1994), have shown that in C. vulgaris and greening maize respectively, that the 7-formyl oxygen of Chlb is derived from O₂ by an oxygenase mechanism and these oxygenase reactions are irreversible (Hayaishi O., 1987). Despite the irreversibility, Ito et al., 1996 and Ohtsuka et al. (1997), have shown that the 7-formyl group of Chlb can be reduced to a 7-methyl group leading to synthesis of Chla. Schoefs and Bertrand (2000) have shown that the conversion of Chlide to chlorophyll is a four-step process. After reduction of Pchlide to Chlide, three intermediates i.e., Chlide geranylgeraniol, Chlide dihydrogeranylgeraniol, Chlide tetrahydrogeranylgeraniol were detected before the formation of Chlide phytol or chlorophyll (Schoefs and Bertrand, 2000).

2.2 REGULATION OF CHLOROPHYLL BIOSYNTHESIS BY LIGHT:

Light plays an important role in plant photomorphogenesis. It regulates the chlorophyll biosynthesis by regulating the expression of genes encoding chlorophyll biosynthetic enzymes and also by regulating the level of intermediates of chlorophyll biosynthetic pathway.

2.2.1 REGULATION OF C5 PATHWAY ENZYMES:

O’Neil and Soll (1990), have shown that in Synechocystis the tRNA⁶⁰ᵘ and chlorophyll levels are regulated independently. Mayers and Beale (1990,1991) have shown that in Euglena, the tRNA and the enzymes required for chlorophyll biosynthesis are induced by light. The aminotransferase activity was induced
maximally by both red and blue light. They have also shown that, a *Euglena* mutant lacking ALA biosynthetic capacity, possesses all the enzymes of ALA biosynthesis but was deficient in tRNA^{GLU}. In this mutant all the three enzymes were induced by light, but only blue light promoted light induction whereas red light had no effect. 5-ALA also regulates chlorophyll biosynthesis and plant growth via degradation to DOVA. Light elevates the levels of mRNA transcripts of glutamyl-tRNA^{GLU} reductase and GSA transaminase in root, leaf, stem and flower tissue of *Arabidopsis* (Ilag et al, 1994). In greening cucumber cotyledons, light stimulates the activity of glutamyl tRNA^{GLU} reductase (Masuda et al., 1996). The formation of ALA in plastids from cucumber cotyledons is enhanced by treatment with the cytokinin. Cytokinin increased the level of expression of tRNA^{GLU} gene of the chloroplast and of nuclear gene of glutamyl-tRNA^{GLU} reductase (Masuda et al, 1994; Masuda et al, 1995). The levels of expression of tRNA^{GLU} gene was not affected by light in *C. reinhardtii* (Jahn, 1992). Glutamyl tRNA activity was found to be inhibited by (proto)heme (Pontopoddian and Kannagara, 1994). The activity of glutamate-1-semialdehyde was found to increase drastically in light (Kannangara et al., 1978) whereas its mRNA level remained constant in etiolated barley seedlings during illumination (Grimm, 1990). In *Arabidopsis* seedlings, the mRNA levels of glutamyl-tRNA reductase and glutamate-1-semialdehyde aminotransferase were more in plants grown in dark/light cycles than in etiolated seedlings (Ilag et al, 1994). Glutamate-1-semialdehyde aminotransferase activity was strongly induced in light in *Chlamydomonas* grown in dark/light-synchronized cultures (Matters and Beale, 1994). Ilag et al (1994) have shown that the transcript levels of Glutamyl-tRNA reductase and glutamate-1-semialdehyde aminotransferase are found to be elevated in root, stem, leaf and flowers by light. This increase in glutamate-1-semialdehyde aminotransferase mRNA concentration did not coincide with induction of chlorophyll biosynthesis. They have also said that, at the peak of light-induced chlorophyll synthesis during greening, the GSA1 mRNA level was only moderately augmented whereas HEMA mRNA remained at extremely low levels, suggesting that post-transcriptional regulatory mechanisms are likely to be involved in large part to effect maximal ALA synthesis during greening. Klein et al (1977), have shown that a short illumination of etiolated maize leaves with red light causes conversion of Pehlide to Chlide and induces synthesis of δ-ALA. Far-red light has no effect suggesting possible role of
phytochrome and Pchlide. Wang et al (1984) had shown that ALA synthesizing enzymes are sensitive to inhibition by heme but not by protoporphyrin in barley. Im and Beale (2000), have shown that a signal transduction pathway involving a heterotrimeric G-protein activation, phospholipase C catalyzed InsP3 formation, Insitol triphosphate-dependent Ca\(^{2+}\) release and activation of a downstream signaling pathway through a Ca\(^{2+}/CaM\)-dependent protein kinase is operating for light-regulated Gsa expression. Cells of *Chlamydomonas* strain CC-2682, are sensitive to light and does not show phototaxis. In these cells the levels of gsa transcript was low in dark-grown condition but increases significantly after 2 h of exposure to dim green (480-585nm) light (Hermann et al., 1999). They have also shown that, the photoreceptor for light induction in *Chlamydomonas* is a flavoprotein. Matters and Beale, 1995, have shown that the levels of gsa and alad mRNA were found to be induced by blue light and blue light photoreceptor is involved in this process. In barley the gsa mRNA was found to be negatively regulated by light, but in *Arabidopsis* light exhibits a stimulatory effect, in cucumber, light has no effect on gsa expression (Kumar et al., 1996).

### 2.2.2 REGULATION OF ENZYMES INVOLVED IN CONVERSION OF ALA TO PROTOPORPHYRIN IX:

A seven fold increase in ALAD mRNA was found in synchronized cultures of *C.reinhardtii* after 2h light treatment and further six fold increase in ALAD activity after 6h light (Matters and Beale, 1995). Inhibition of chlorophyll biosynthesis by levulinic acid, an inhibitor of ALA dehydratase leads to inhibition of carotenoid biosynthesis (Jilani et al, 1996). mRNA levels of ALA dehydratase, were found to be increased in pea and *Arabidopsis* upon illumination (Witty et al, 1993; Smith et al., 1994). The level of ALA dehydratase transcript remain same and does not increase upon illumination (Boese et al, 1991) and the transcript was present in leaves, stems and to lesser extent in roots. Witty et al (1993), have shown light induction of porphobilinogen deaminase enzyme activity. This increase in light activity was consistent with increase in mRNA levels. Spano and Timko (1991), have found two-fold increase in enzyme levels of PBGD upon illumination in pea. Porphobilinogen deaminase is inhibited by porphyrins produced from ALA but the inhibition is overcome by adding levulinic acid. Mock et al (1995), have shown that, the amount of mRNA and protein of uroporphyrinogen increases upon illumination in barley and
maximum UROD-mRNA was found in basal segments relative to the top of the leaf. Kruse et al (1995), have shown that in barley the level of coproporphyrinogen oxidase does not change upon illumination. This enzyme and the other enzymes of tetrapyrrole biosynthesis are more developmentally regulated rather than regulated by light-dependent signal (Kruse et al., 1995). Madsen et al (1993), have shown that the mRNA of soybean cpo are present maximally in root nodules at a stage when leghemoglobin appears. There are two protoporphyrinogen genes found in the plants. One is mitochondrial form and the other is plastidal. Steady-state RNA analysis indicates a synchronous expression of both genes during tobacco plant development, greening of young seedlings and diurnal and circadian growth (Lermontova et al., 1997).

2.2.3 REGULATION OF FERROCHELATASE EXPRESSION:

Ferrochelatase mRNA was found to be induced by light in etiolated Arabidopsis seedlings (Smith et al, 1994) and this induction was found to be less as compared to the light-induced increase in porphobilinogen deaminase mRNA, suggesting that major part of ProtoIX is channeled through Mg-branch of C5 pathway. Chow et al (1998), have shown existence of two ferrochelatase in Arabidopsis. Of these ferrochelatase I was found to be light-inducible whereas the ferrochelatase II was not. The expression of ferrochelatase from cucumber was found to be light independent. Transcripts of the hemH gene were also not light-responsive (Suzuki et al, 2000).

2.2.4 REGULATION OF ENZYMES INVOLVED IN METABOLISM OF PROTOIX TO PCHLIDE SYNTHESIS:

Mg²⁺-chelatase is first unique enzyme in chlorophyll biosynthesis. The activity of the enzyme increases upon illumination (Beale and Weinstein, 1990). The mRNA level of oli (homolog of ChlH) decreases in light but increases in dark in Antirrhinum grown under light-dark cycle (Hudson et al., 1993). The transcript level of the ChlH was regulated by circadian oscillation. The transcript levels were found to be induced by light for ChlH and the induction was more rapid for ChlH rather then ChlI or cab in soybean suspension cells (Nakayama et al., 1998). When H subunit from Rhodobacter capsulatus is exposed to light and oxygen, the bound proto is converted into photoporphyrin. This porphyrin binds very tightly to the H subunit and renders it inactive, suggesting another way of inhibition of Bchl biosynthesis by high light and
oxygen (Walker and Willows, 1997). In *Arabidopsis*, *Glycine max* and barley the expression of ChlH and ChlI genes is induced by light in developing plants. ChlH subunit seems to fluctuate more than the I subunit under greening conditions or during diurnal conditions. The I subunit is induced by light if tissue is etiolated, but once the chloroplasts are developed, there is very little effect of light on this subunit transcription (Walker and Willows, 1997). Averina et al (1996), have shown that in wheat leaves, with the accumulation of protomonomethyl ester and Pchlide, the activity of SAM:Mg protoporphyrin IX methyltransferase decreases. The Mg-protoporphyrin IX monomethyl ester cyclase was strongly inhibited by CN- and N3- (Whyte and Castelfranco, 1993).

2.2.5 REGULATION OF PCHLIDE-OXIDOREDUCTASE:

Three different POR genes have been cloned from *Arabidopsis*. These genes are differentially regulated by light. The transcript level and enzyme activity of PORA decreases upon illumination (Apel, 1981; Batschauer and Apel, 1984) while that of PORB remains constant (Holtrof et al., 1995). The levels of PORC increases upon illumination (Oosawa et al., 2000). In barley till date only two enzymes are known. Unlike PORA, PORB is not under the control of phytochrome (Holtrof et al., 1995). The level of PORB also depend upon the import of enzyme. This also depends upon the concentration of Pchlide. The level of Pchlide declines upon illumination which in turn affects the translocation of PORA (Reinbothe et al., 1995). POR enzymes also exhibit sensitivity to chloroplast proteases. When incubated with NADPH and Pchlide, they form ternary complex. In this complex, POR enzymes are well protected from proteases. In light the Pchlide of the complex get reduced to Chlide and the complex in turn become susceptible to proteases (Reinbothe et al., 1995). The POR-degrading proteases are not detected in etioplasts but their level increases upon illumination (Reinbothe et al., 1995). The expression pattern of PORA appears to be inversely correlated to lhca/b genes in barley (Batschauer et al., 1986). Johanninger and Howell (1984) have suggested that, the intermediates of chlorophyll biosynthesis, such as protoporphyrin IX, Mg2+-protoporphyrin IX monomethyl ester, Pchlide could be the signals that inhibit cab (lhcb) mRNA accumulation in dark. In light, due to rapid consumption of the pigments during chlorophyll synthesis, the block of lhca/b gene expression could be relieved leading to lhca/b mRNAs to accumulate. Cytokinin-treated cotyledons green faster due to higher level and slower degradation.
of light-sensitive POR, where as ABA has opposite effect. When etiolated lupine cotyledons were exposed to far-red light prior to phytohormone application, the POR level was found to be decreased accompanied by the loss of phytohormone's effect on greening (Kusnetsov et al., 1998). Sperling et al (1997), have shown that, transgenic Arabidopsis overexpressing PORA and PORB, enhance seedling survival in white light and protects the plants from photooxidative damage, in the plants depleted of POR by far-red light. They have also shown that, wild type Arabidopsis thaliana seedlings once grown in non-photooxidative far-red light are yellow and do no green normally even in white light there after. Red light or darkness led to no significant changes to the total level of immunodetected peptides or Pchlide in Phormidium (Rowe and Griffiths, 1995). Barnes et al (1996) have shown that far-red light results in inability of the cotyledons to green in Arabidopsis, upon subsequent white-light illumination. This according to them is due to, inability of the plastids to accumulate prolamellar bodies and due to impairment of the POR gene. The cDNA for geranyl-geranyl reductase was cloned from Arabidopsis thaliana by Keller et al (1998). A higher steady-state geranyl-geranyl reductase mRNA level was observed during deetiolation of Arabidopsis and during the early stage of chromoplast differentiation in pepper.

Thus, it can be said that the chlorophyll biosynthesis is regulated by light and it is the initial enzymes of ALA biosynthesis, which are most affected by light. Light also effect the POR, the enzyme which catalyses the only light requiring step of chlorophyll biosynthesis.

2.3 PROTOCHLOROPHYLLIDE PHOTOREDUCTION AND LOW TEMPERATURE SPECTRA:

Protochlorophyllide is present in three different spectral forms in etioplast membranes (Bovery et al, 1974): (i) non-photoconvertible esterified protochlorophyllide with an absorption peak at 628 nm, P628 (ii) non-photoconvertible protochlorophyllide with an absorption peak at 634 nm, P634 and (iii) photoconvertible protochlorophyllide with an absorption peak near 650 nm, P650. Binding forms the later species from P634 to the photoenzyme POR. In vivo these forms show fluorescence maxima at 632 and 657 nm respectively. P634 internally transfers energy to P650, indicating a closeness between the last two species (Bovery
et al, 1974). Higher content of phototransformable protochlorophyllide is present in prolamellar bodies and higher content of non-phototransformable protochlorophyllide is present in prothylakoids (Ryberg and Sundqvist, 1982). During the early stages of the initial photoconversion of the etiolated leaf at 20°C, a dark stable intermediate photoproduct appears with a fluorescence peak at 674 nm at 77°K. The sequence of fluorescence at photoconversion is protochlorophyllide (E650, F655), I Intermediate (E668, F674), II chlorophyllide (E678, F687), III chlorophyllide (E682, F694) chlorophyll (E672, F680). Thorne (1971) have observed that the A678, F688 transient form of chlorophyllide occur along with another fluorescence transient at 675 nm. Thorne (1971) has also shown by fractional conversion studies that the intermediate F674 gave rise to F687, while its full conversion studies gave chlorophyllide F694.

Böddi et al (1992), have shown the presence of four universal forms of protochlorophyll(ide) from different plant species: at 633 nm, at 645 nm, at 657 nm and one at 670 nm. Their vibrational sublevels were found at 693 nm, 710 nm, 726 nm and 740 nm respectively. Schoefs and Franck (1993), found that in 2 day old leaves of bean a peak at 690 nm was observed after flash illumination. After the photoreduction, the emission maximum is shifted to 675 nm with in 5s in dark. This shift was due to the release of protochlorophyllide from NADPH: protochlorophyllide oxidoreductase. Franck et al (1999), have shown that in etioplast membranes of dark-grown wheat a peak was observed at 681 nm in presence of NADP⁺ and at 690 nm in presence of NADPH after flash illumination and subsequent dark incubation. They have also shown that with in few seconds the peak at 690 nm shifts to 696 nm. This peak at 690 nm is due to Chlde-NADP⁺-POR complex and the shift to 696 nm was due to Chlde-NADPH-POR. The fast regeneration of photoactive Pchlide in intact leaves proves that part of the POR proteins can be reloaded with Pchlide and NADPH in vivo.

The photochemical conversion of protochlorophyllide to chlorophyllide in etiolated leaves involves formation of a series of intermediary compounds (Dujardin et al 1990) and several forms of chlorophyllide. It is possible to accumulate one or more intermediate species between protochlorophyllide and chlorophyllide when illuminating etiolated leaves or extracts at low temperature, e.g. 100K or 77K (Dujardin and Sirnoval, 1977). Dujardin et al (1990), have shown that the fluorescence emission of etiolated leaf segments of barley mutant albozanta2 at 77K occurs in 2 main bands, one at 631 and one at 656 nm. These bands are characteristic
also of wild type etiolated leaves and have been attributed to photoinactive protochlorophyllide absorbing at 628 nm and photoactive protochlorophyllide absorbing at 650 nm.

Shibata shift designates in vivo spectral shift of the chlorophylla absorption maximum from 684 nm to 672 nm following photoconversion of protochlorophyll(ide) to chlorophyll(ide) as performed by a short pulse of saturating light (Shibata, 1957). Mohr (1984) have shown that pretreatment with red light for 36 or 57 h after sowing leads to a strong decrease of the duration of the Shibata-shift.

Böddi et al (1990), have shown that the absorption spectrum and its gaussian components at 625, 637, 650, 668 nm and the fluorescence emission spectrum were same for membrane preparations and for intact leaves. They have proved the findings that 638-640 nm absorbing form of protochlorophyllide was considered to be dimers of protochlorophyllide (Schultz and Saur, 1972) and 650 nm absorbing form to be aggregates with higher number of protochlorophyllide molecules (Simoval, 1972). They have also shown that two forms of chlorophyllide are formed. When the membrane preparations were irradiated with low intensity light flashes a peak at 677 nm with a shoulder at 695 nm appears. After increasing number of flashes the shoulder at 695 nm becomes main peak.

By using fluorescence emission spectroscopy, McEwen et al (1996), have shown that three protochlorophyllide forms were found in hypocotyl of dark grown kidney bean plants. These are (1) Pchl(ide)628-633, (2) Pchl(ide)650-657 and (3) Pchl(ide)636-642 at excitation of 440 nm (1 and 2) and 460 nm (3). In vivo fluorescence spectra and gaussian components of deconvulted spectra showed a decreasing gradient of Pchl(ide)650-657 to Pchl(ide) 628-633 down along the hypocotyl being zero in roots.

By treating the etioplasts or chloroplasts with reagents which changes structural architecture of the pigment-protein complexes, 650 nm peak of the in vivo protochlorophyllide shifts to approximately 635 nm or to approximately 638 nm. The change occurs due to change from dimeric to monomeric form. According to Oliver and Griffiths (1982), photoconversion of protochlorophyllide in etiolated leaves of Avena sativa L., var.pennal or periarth and Phaseolus vulgaris L., var. 'The Prince' results in the sequential appearance of spectrally distinct chlorophyllide complexes (Chlide 678, 684 and 672) in vitro. Excess of NADP⁺ and NADPH stabilize complexes related to Chlide 678 and Chlide 684, respectively, whereas addition of
exogenous Pchlide induces formation of a species related to Chlide 672. They have also shown that Chlide 678 and Chlide 684 represent ternary complexes of the enzyme protochlorophyllide reductase, with Chlide and either NADP⁺ (Chlide 678) or NADPH (Chlide 684). Chlide 672 is seen as 'free' pigment dissociated from the enzyme. The first product of PChlide photoconversion is Chlide absorbing maximally at 678 nm. This is a transient species and is converted in vivo: first to a form absorbing at 684 nm within approximately 2 min (Bonner, 1969 and Griffiths, 1975); second, to a 672 nm absorbing form (Shibata, 1957); and finally to a form absorbing maximally at 675 nm (Broadmann et al, 1971). *In vitro* Pchlide photoconversion in most etiolated leaves, results in initial formation of Chlide 678, which rapidly transforms to Chlide 684, followed by the relatively slower conversion of this to Chlide 672 and in whole leaves the Chlide 678 is rapidly transformed to Chlide 684 whereas in purified membranes this conversion results after addition of excess NADPH (Oliver and Griffiths, 1982). In ALA treated plants, photoconversion produces Chlide 672 directly (Oliver and Griffiths, 1982). In barley and bean same forms of photoactive Pchlides are regenerated by different pathways: 1) photoactive Pchlode regeneration starts immediately after the photoreduction through the formation of a nonphotoactive Pchlide form emitting fluorescence at approximately 651 nm. This form consists of Pchlide-POR-NADP⁺ instead of Pchide-POR-NADPH, which converts to photoactive form with in 10 min phototransformation of Pchlide to Chlide, regeneration of Pchlide begins. The immediate form of Pchlide, which is regenerated, is Pchlide P651, which is nonphotoactive. 2) After dislocation of large aggregates of Chlide-light-dependent POR-NADPH ternary complexes (Schoefs et al, 2000b). There are two phases in the process of Pchl(ide) accumulation during development of bean leaves in darkness: a lag phase (first week) during which photoactive Pchlide(ide) accumulated faster than the non-photoactive Pchlide(ide); and a fast phase (second week), showing parallel accumulation of both forms of Pchlide(ide) (Schoefs et al, 2000a). In the plants grown under 16h photoperiod for 20 days, showed a peak at 653 nm of phototransformable Pchlide instead of 656 nm peak obtained in etiolated seedlings. This P653 form is exclusively associated with prothylakoids or thylakoid membranes and the P656 form is associated with prolamellar bodies (Schoefs et al, 2000a). Le lay et al (2000), have shown that in etiolated barley leaves, a saturating flash of 200ms (0.2sec) induces phototransformation of protochlorophyllide F655 to chlorophyllide, than into
chlorophyll that do not require light. They have also shown that in desiccated leaves the synthesis of Chl is slowed down and Shibata shift appears to be inhibited. In desiccated leaves the peaks goes to 694 nm after 0.2 sec flash illumination but its return to 682 nm wavelength was delayed. Also the regeneration of Pchlide F655 and esterification of Chlide to form Chl is impaired. At high concentrations of glycerol (87%) Chlide blue shift is inhibited whereas the addition of BSA leads to enhancement in blue shift of Chlide (F680 without BSA and F668 with BSA). EDTA and Ca\(^{2+}\) leads to enhancement in disaggregation of oligomeric Chlide-POR complexes (Zhong et al, 1997). Using artificially formed complex containing heterologously expressed photoenzyme POR, protochlorophyllide and NADPH, Belyaeva et al (2001) have shown that the mechanism of Pchlide photoreduction in the complex was practically identical to the \textit{in vivo} mechanism.

Klement et al (2000), have shown that various protochlorophyllide forms and the Shibata shift which disappears upon solubilization of membranes can be restored if the reconstituted complex is treated with plastid lipids and 80% glycerol. Flavins may be the constituents of the active protochlorophyllide-protein complex (Belyaeva et al, 2000).

Dark grown angiosperm seedlings contain achlorophyllous plastid type known as the etioplast, which is transformed into a photosynthetically competent chloroplast during photomorphogenesis. Etioplast contain lattice like prolamellar body, which is composed of inter-connected tubules, and the unstacked prothylakoids. Etioplasts characteristically accumulate the chlorophyll precursor protochlorophyllide (Pchlide), more specifically protochlorophyllide a (Pchlidea). Illumination of etioplasts initiates the dispersal of the prolamellar body and formation of thylakoid membranes containing the pigment protein complexes of the photosynthetic apparatus. Reinbothe et al (1999) have shown that the presence of a novel light harvesting Pchlide a/b binding protein complex and termed it as LHPP. This LHPP was speculated to (i) serve as the central structural determinant of the prolamellar body in etioplasts. (ii) Be essential for the establishment of the photosynthetic apparatus. (iii) Confer photoprotection on greening seedlings by dissipating excess light energy, thereby minimizing Pchlide-induced photo-oxidative damage. But, Armstrong et al (2000) have not supported the idea of existence of LHPP. In etioplasts, large quantities of the strictly light dependent NADPH protochlorophyllide oxidoreductase (POR) are present (Sundqvist and Dalhin, 1997).
When etiolated angiosperm seedlings break through the soil after germination, they are immediately exposed to sunlight, but are unable to perform photosynthesis (Granick, 1950). In the absence of ChlA and ChlB, two other porphyrin species cooperate as the basic light harvesting structure of etiolated plants. Pchlidea and Pchlideb form supramolecular complexes with NADPH and two closely related proteins PORA and PORB in the prolamellar body of etioplasts (Reinbothe et al, 1999). Using Zn substituted protochlorophyllide, they have also shown that PORB is catalytically active in the reconstituted POR pigment complex. Functional LHPP consists of five PORA-Pchlide b-NADPH and one PORB-Pchlide a-NADPH ternary complexes, held together in supramolecular, ring like structures (Reinbothe et al, 1999). When PORA is released from the prolamellar body, it converts PChlide b to Chlide b. This reaction operates only in dark and after de- etiolation expression of PORA is switched off (Armstrong et al, 1995). PORB drives chlide a synthesis not only in etiolated plants at the beginning of illumination, but also in green plants. This PORB derived Chlide a is esterified with phytol to chla, which then initiates chloroplasts development by controlling the intraplastidic synthesis and assembly of photosynthetic complexes in the thylakoid membranes (von Wettstein et al, 1995). The pale green colour of etiolated seedlings is due to the accumulation of Pchlide complexed with a reducing agent (NADPH) and POR in the developing chloroplast. PORA and PORB in LHPP are specific only for photochlorophyllideb and photochlorophyllidea, respectively, as shown by Reinbothe et al (1999). They have also shown that Pchlideb in LHPP initially has a light harvesting function. It then transfers light energy to Pchlidea, for photoreduction to Chlidea by PORB, even under low light intensities. This then leads to assembly of photosynthetic apparatus quickly. Under high light intensity, Pchlide b plays a photoprotective function. Runge et al (1996) have shown that PORA expression can be suppressed in Arabidopsis thaliana if the plant is grown under continuous far-red light. These plants do not make prolamellar bodies and they can not green rapidly. Sperling et al (1997) have shown that the quantity of POR is important for formation of the prolamellar body and greening in Arabidopsis, and the possibility of existence of LHPP containing a PORA-Pchlideb complex occurs in this plant.

PORA has been proposed to play a special role in:

1. Formation of POR ternary complexes containing photoactive Pchlide-F655.
2. Prolamellar body assembly.
(3) Protection against photo-oxidative damage caused by nonphotoactive Pchlide acting as a photosensitizer (He et al, 1994).

2.4 PHOTORECEPTORS:

Light affects the plant growth profoundly. Plants need light for energy and also as a factor in informational signal. Perception, interpretation and transduction of these light signals are accomplished by the use of photoreceptors. Three different classes of photoreceptors operate in plants. These are: phytochromes, which absorbs in red and far-red regions of the light spectrum; cryptochromes, which absorbs light in the blue and UV-A region of spectrum and third are UV-B receptors (about which very less is known).

2.4.1 PHYTOCHROMES:

2.4.1.1 Structure and Localization:

Phytochromes are cytosolically localized dimers and are composed of 125 kDa polypeptides, each carrying a covalently linked tetrapyrrole chromophore in the -NH₂-terminal and dimerization determinants in the COOH-terminal domain. One region near the center of the molecule and one-region near the COOH-terminus may be involved in dimerization of the polypeptide (Viestra, 1993). 124 kDa phytochrome molecule was isolated from Avena by Viestera and Quail (1983) and the proteolysis of 124 kDa phytochrome leads to changes in spectral property of the molecule (Viestera and Quail, 1982). Wang et al (1991) have shown that three phytochromes exist in Avena. 124 kDa phytochrome, is most abundant in etiolated tissue. 123- and 125- kDa phytochromes predominate in green tissue. Phytochromes may function as the light-activated protein kinases, either of the eukaryotic Ser-Thr-Tyr-class or of the prokaryotic two-component His class (Quail et al., 1995). Deletion in the N-terminal domain of phytochrome makes the phytochrome molecule functionally inactive (Cherry et al, 1992). Phytochromes occur in two forms: one Pfr form is morphologically active and the other Pr form is not. Pfr form has absorption maximum in far-red light and Pr form has in red region. Phytochrome genes encode a small family of photoreceptors. Five PHY genes (PHYA to PHYE) exist in Arabidopsis (Sharrock and Quail, 1970). Seven different PHY genes exist in tomato, out of which PHYB1 and PHYB2 were thought to be counter parts of PHYB and
PHYD of *Arabidopsis* (Quail et al., 1995). Single copy of the phy gene in pea and rice were found (Quail et al., 1995). The single-copy gene coding for PhyA in pea produces three distinct transcripts. Expression of phyA shows organ- and tissue-specificity. In etiolated rice seedlings, phyA mRNA was two-fold higher in leaves as compared to shoots, whereas the roots of fully green plants contained higher levels of mRNA than leaves. phyB is constitutively expressed in tissues. phyA mRNA was more in etiolated rice seedlings, whereas the two mRNAs are equally abundant in green tissues because of the light-induced decline of phyA transcript (Smith, 1995).

Two different types of Phytochromes exist in plants: Type I and Type II. Type I phytochromes are light-labile and include the forms in which Pfr form is unstable as compared to Pr form. Type I phytochromes are represented by PhyA. Type II phytochromes are light-stable and here the Pfr form is more stable as compared to Pr form. Type II phytochromes are represented by all the other class of phytochromes except, PHYA (Smith, 1995). Phytochromes are cytosolic proteins with a small fraction associated with membrane (Smith, 1995). PhyA regulates VLFR (Furuya, 2000). PIF3 (phptochrome interacting factor 3) exists in nucleus and interacts with nuclear-translocated PhyB (Halliday et al., 1994). Binding between phytochrome and PIF3 is strictly light dependent. PIF3 might act as a modulator of a response that is transmitted through phytochrome (von Arnim, 1999). PIF3 levels were found to be repressed in high light intensity suggesting that PIF3 levels might not always be rate limiting for phyA signaling (Ni et al., 1998). PIF3 activate or repress the transcription of light-regulated genes through a basic helix-loop-helix (bHLH) domain (von Arnim, 1999). Horwitz et al. (1988) have shown the involvement of phytochrome in greening in *Pisum*. A G1 protein encoded by GIGANTEA gene is involved in phytochrome signal transduction in *Arabidopsis*, this protein is a nucleoplasmic protein and its role is proposed in phyB signaling (Huq et al., 2000). Phytochromes are photoreceptors and they possess a PAS repeat domain and a histidine-kinase related domain. In phytochrome molecules, where there was a mutation in histidine-kinase related domain phenotypes similar to null mutants were obtained whereas in the plants where this domain was truncated phyB molecule with partial activity as obtained, suggesting that this domain is dispensable (Krall, 2000). PIF2 encodes a nuclear localized protein of unknown function. PIF2 antisense plants exhibit reduced sensitivity to red but no phenotype in far red, underlining the involvement of PIF2 in PHYB signaling. NPDK1 phosphorylates PHYA. PhyB is imported in the nucleus upon red light
treatment and phyA upon far-red light treatment where it interacts with PIF3 and other transcription factors leading to activation of target genes (Furuya and Kim, 2000). PHYA signal transduction involves cGMP, Ca2+ and trimeric G-proteins and that phytochromes act as serine and threonine kinases (Bowler and Chua, 1994).

2.4.1.2 Functions:
Phytochrome A and B control hypocotyl elongation in contrasting ways. They also transduce antagonistic signals to the seedling in response to red or far-red light enrichment. Red light absorbed by phyB induces deetiolation, but this response was suppressed by the simultaneous irradiation with the far-red light absorbed by PhyB. Conversely, far-red light absorbed by PhyA induces deetiolation (Smith, 1994). Mutant Arabidopsis seedlings null phyA are strongly retarded in their capacity for deetiolation when grown under vegetational shade (Quail et al., 1995). Phytochrome plays important role in germination of the seeds. Phytochrome mediates regulation of germination by colonization of canopy gaps, by preventing germination in surface seeds in response to low R: FR ratio of the light environment with in the canopy. Phytochromes interact with the blue-light receptor and with the protochlorophyll to regulate stem elongation, leaf development and the synthesis and assembly of the photosynthetic apparatus. Phytochromes also play role in the proximity perception and shade avoidance. Potulaca oleracea, a weed, detects reflected FR from neighboring vegetation and grows in opposite direction (Smith, 1995).

2.4.2 CRYPTOCHROMES:

2.4.2.1 Structure and Localization:
Cryptochromes absorbs in blue light of the spectrum and showed maximal activity in blue (400-500 nm), with weaker responsivity to UV/A (300-400 nm) and green light (500-600 nm) (Ahmad and cashmore, 1993). The responses of cytochromes are very elusive, hence the name Cryptochrome was given. Ahmad and Cashmore (1993) have shown that HY 4 gene of A. thaliana encodes a protein with a characteristic of a blue-light photoreceptor. They have demonstrated that the transcript levels of HY4 RNA remains similar in stems, leaves, flowers, and siliques and after longer exposure in roots. Transcript levels were also similar in the etiolated and green plants suggesting that the gene is not regulated by light. This gene codes for a protein of 75 kDa which has a homology to DNA photolyase at COOH-terminus and with tropomyosin at NH2-terminus (Ahmad and Cashmore, 1993; Lin et al.,
Lin et al (1995) referred this protein as blue light receptor CRY1. Absorption maxima of purified CRY1 from pea resemble that of a flavoprotein. They have shown the association of flavin adenine dinucleotide with blue light receptor CRY1. CRY1 possess a FAD-binding site and upon photoreduction yield FADH (Whitelam, 1995). A gene CPH1 has been cloned from *Chlamydomonas* and was found to encode for a protein, which was another blue-light photoreceptor. This protein was 865 residue long, of which first 500 amino acids showed resemblance to CRY1 and photolyase (Small et al., 1995). CRY1 protein was found to be associated with soluble fraction. Small portion is associated with membrane. CRY1 is expressed ubiquitously in all plant tissues (cotyledon, hypocotyl and roots) and mature plants and the expression does not appear to be light-regulated (Ahmad and Cashmore, 1996). Quiñones and Zeiger (1994) proposed that Xanthophyll and Zeaxanthin play a putative role as blue light photoreceptors, as, the corn coleoptiles devoid of zeaxanthin does not respond to a bluelight pulse. Ahmad et al (1998) have shown that blue light (cryptochrome) photoreceptor from *Arabidopsis*, CRY1, mediate a number of blue light-dependent phenotypes. Two members of the *Arabidopsis* cryptochrome gene family (CRY1 and CRY2) overlap in their function, but their proteins differ in stability. CRY2 is rapidly degraded under light fluences (green, Blue and ultraviolet) that activate the photoreceptor, but CRY1 is not. CRY2 accumulates to high levels under low light intensities. CRY1 functions include inhibition of hypocotyl elongation and blue light-dependent anthocyanin accumulation. CRY2 is involved in the inhibition of hypocotyl growth and anthocyanin accumulation (Ahmad and Cashmore, 1996). Sakai et al (2000) have isolated a RPT2 gene, which encodes for a protein which they suggested as a candidate for blue light reception. This protein has putative phosphorylation sites, a nuclear localization signal, a BTB/POZ domain and a coiled-coil domain. This RPT2 gene was found to be induced by white, blue and green light. This gene plays a role in second-positive phototropic responses in plants Sakai et al (2000).

### 2.4.2.2 Functions:

Using transgenic tobacco plants where *Arabidopsis* CRY1 was overexpressed, Lin et al (1995b) have shown that the transgenic plants showed short-hypocotyl phenotype under blue, UV-A and green light, whereas under red/far-red these plants were phenotypically same to the wild-type plants. The blue light photoreceptors are
required for the inhibition of hypocotyl elongation. Stomatal aperture increases in size in response to a single pulse of blue light (Malhotra et al, 1995). cab genes of pea also respond to a single pulse of blue light (Kaufman, 1993). The rate of stem elongation in dark or red-light-grown seedlings slows in response to blue light. Blue-light-induced suppression of hypocotyl elongation is eliminated in mutants of Arabidopsis thaliana: blu1, blu2, blu3 and hy4 (Koorneef et al., 1980). High-fluence-rate blue light inhibits hypocotyl growth in Arabidopsis. This growth inhibition is preceded by the activation of anion channels. The membrane depolarization results from the activation of anion channels by blue light was only 30% of the wild-type magnitude in HY4, a mutant lacking the HY4 blue light receptor. This inhibition persisted in wild-type seedlings during more than 40 hr of continuous blue light (Parks et al, 1994 and 1998).

2.5 PHOTOMORPHOGENIC MUTANTS AND THE ROLE OF PHYTOCHROME IN PHOTOMORPHOGENESIS:

Photomorphogenic mutants are of two classes:

i) Mutants with lesion within PHY gene or mutations in genes encoding blue light receptor.

ii) Mutants with lesion in downstream components in one or more of the phytochrome transduction pathways.

Most of the mutants were selected from Arabidopsis. The hy mutants were selected for long hypocotyls when grown in white light. hy1, hy2, hy6 chromophore mutants are deficient in all phytochromes. hy3 lack phytochrome B. hy4 is a putative blue light receptor mutant and hy5 is a downstream transduction chain mutant. Some Arabidopsis mutants were also selected on the basis of inhibition of hypocotyl extension, which is FR-mediated. These mutants were named as hy8, fre1, fhy1 (Smith, 1995). aurea mutants from tomato are deficient in phyA apoprotein. long hypocotyl mutant (lh) of cucumber, the elongated internode (ein) mutant of Brassica rapa and the maturity mutant (ma3R) of Sorghum are deficient in B-type phytochrome (Smith, 1995). Ceridan et al (1999) have shown that, phyA acting in VLFR mode (i.e. under light pulses) is antagonistic to phyB signaling whereas phyA acting in the HIR mode (i.e. under continuous far-red light) operates synergistically with phyB signaling, and that both types of interaction require FHY1. eid1, a
A recessive mutant with increased sensitivity to far-red light in Arabidopsis has been isolated. This mutant is hypersensitive in phytochrome A-dependent high-irradiance responses (Buche et al., 2000). The expression of the eid1 phenotype requires the presence of phyA. They have shown that is a negatively acting component in the phyA-dependent HIR-signaling pathway. Triplet mutants of phyAphyB1Phy2 were strongly responsive to the supplementary day time far-red, indicating one of the two remaining phytochromes plays a significant role in tomato photomorphogenesis (Weller et al., 2000). Sullivan and Gray (2000), have isolated a COP1 like transcript and a transcript slightly larger then COP1. These transcripts were similar to lip1 of pea. COP1 forms a subunit of COP9 complex.

Kowk et al (1996) and Pepper (1994) have shown that there is a compliment of at least 10 essential and pleiotropic Arabidopsis genes that are necessary for repression of photomorphogenic development. The mutations in these 10 pleiotropic COP/DET/FUS loci result in lethality after the seedling stage, suggesting that their gene products are essential for normal plant development under light conditions. COP/DET/FUS are required for proper nuclear localization of COP1 protein in dark (von Arnim, 1997). In high intensity continuous white light COP1 levels become undetectable in nucleus (von Arnim and Deng, 1994). The low level of nuclear COP1 is sufficient to carry out its function in normal light development (Deng, 1992) whereas higher levels of COP1 are required for full suppression of the photomorphogenic program in dark-grown seedlings (Misera et al, 1994). Arabidopsis COP9 is a component of a large protein complex that is essential for the light control of a developmental switch and its conformation or size is modulated by light. The complex is acidic, binds heparin and is localized within the nucleus. Biochemical purification of the complex to near homogeneity revealed that it contains 12 different subunit. One of the subunit is COP11, mutations in which result in a phenotype identical to COP9 mutants. COP9 complex may act to regulate the nuclear abundance of COP1, an established repressor of photomorphogenic response, as shown by Chamovitz et al (1996). COP9 complex is larger in the dark than in the light (Wei et al, 1994). Cop1 has direct role in light signaling (von Arnim and Deng, 1994). Since the COP9 complex is larger in dark-grown Arabidopsis seedlings and the nuclear localization of COP1 in the dark is disrupted in Cop9 mutants, it was proposed that COP1 may interact with COP9 complex in the dark to repress photomorphogenesis, while light causes the dissociation of COP1 from the complex and the export of
degradation of nuclear COP1, leaving the smaller "light" COP9. (von Arnim and Deng, 1994) However, the interaction is not direct. Mutations in at least seven of *Arabidopsis* loci (cop8, cop9, fusca, fus6, fus8, fus11 and fus12) result in complete loss of 500 kDa cop9 signalosome (Karniol and Chamovitz 2000). They have also shown that these are composed of eight subunit that is highly conserved in plants and animals. COP9 signalosome was not found in *Saccharomyces* (Wei and Deng, 1999). Six of the subunit contain a novel motif PCI (proteosome-COP9 complex initiation factor 3) domain whereas the other two contain Mpr1-Pad1-amino-terminal (MPN domain) (Wei and Deng, 1999). The COP9 Signalosome is now termed as CSN (Karniol and Chamovitz 2000). CSN7 (initially COP15, FUS5) is a phosphoprotein and effect the electrophoretic mobility of COP9 signalosome. CSN1(COP11, FUS6), interacts with CSN5 and CSN7 (AJH1, AJH2 and COP15, FUS5 respectively). CSN8 (COP9, FUS7) interacts with CSN7. CSN4 (COP8, FUS4) interacts with itself and with other four identified subunit. This suggests a central location of CSN4 in COP signalosome (Karniol and Chamovitz 2000). Osterlund et al (2000), have shown that COP1 and HY5 act antagonistically in regulating the photomorphogenesis in Arabidopsis. They have shown that HY5 transcript as well as protein levels are more in white light grown plants as compared to dark-grown plants and also the HY5 decreases with decrease in light intensity showing that, HY5 abundance is directly correlated to the extent of photomorphogenic development. COP1 interacts directly with HY5 to control photomorphogenesis (Ang et al., 1998). Wei et al, 1994, have shown that light signals perceived by both phytochromes and a blue light receptor converge to repress the action of Arabidopsis COP9 suppresses seedling photomorphogenesis.

Chory (1997) has shown that two groups of key platters in photoregulatory signal transduction pathways have been identified. One group of mutants undergoes photomorphogenic seedling development in darkness and comprises the COP, DET, FUS and SHY2 loci, which act downstream of the phytochromes and cryptochromes. The COP, DET and FUS mutations are recessive, it has been proposed that the corresponding wild-type gene products act to repress photomorphogenic development in the dark. The second group of photomorphogenic mutants shows reduced responses to light stimuli. These include those with defective photoreceptors (such as PHYA, PHYB, PHYD, CRY1, & CRY2) and that in which signaling is perturbed (including RED1, FHY1, FHY3, PEF1 & HY5). COP1 interacts with HY5 and regulate light
dependent development and gene expression in plants. COP1 act as cell autonomous repressor of photomorphogenic development whose repressive activity is overcome by light (McNeillis et al, 1998).

Ang et al (1998) have shown that HY5 consists of at least two distinct functional modules the C-terminus, containing the presumed dimerization and DNA binding activity and N- terminus, required for interaction with COP1. By contrast, the entire structure of COP1 required was shown to be crucial for its interaction with HY5. The phenotypes of mutations in COP1 and HY5 indicate that HY5 acts to repress lateral root initiation and elongation, where as COP1 plays a positive role in lateral root development, possibly by negatively regulating the repressive activity of HY5. Mutsi et al (1995) have shown that CIPI (COPI- interactive protein 1), is encoded by a single gene in Arabidopsis and its mRNA and protein levels are not regulated by light. CIPI is either a part of, or associated with, a cytoskeletal structure in hypocotyl and cotyledon cells, but not in roots. Thus, CIPI mediates light control of COPI nuclear activity by regulating its nucleocytoplasmic partitioning.

Using constitutively expressed PORA & PORB in the Arabidopsis wild type and in the constitutive photomorphogenic COP1-18 (previously DET340) mutant, which is deficient in PLB pchlide-F655, Sperling et al (1998) have shown that etioplast differentiation requires PORA. They found that in both, POR overexpression increased PLB size, ratio of Pchlide F-655.

Genoud et al, (1998) have isolated a new mutant called PSI2 (for phytochrome signaling) in Arabidopsis. This mutant exhibited hypersensitive induction of CAB1, CAB2 and small subunit of Rubisco(RBCS) promoters in the very low fluence range of red light and a hypersensitive response in hypocotyl growth in continuous red light of higher fluences. They have also shown that, PSI2 specifically and negatively regulates both PHYA and PHYB phototransduction pathways. PSI2 gene product negatively regulates phototransduction pathways downstream of both PhyA and PhyB.

2.6 LIGHT SIGNAL TRANSDUCTION IN PLANTS:

Plants sense the light around them. They can sense the light quality i.e., its wavelength, duration, intensity and direction using photoreceptors. The signal perceived in form of light is transduced to downstream elements, which in turn
regulate the expression of different genes involved in chlorophyll biosynthesis and plant development. Phytochrome regulated responses are mediated by calcium and cGMP. The pathway depending on cGMP leads to expression of anthocyanin biosynthetic genes such as chalcone synthase whereas the pathway operating through calcium, and activates genes for apoproteins of light-harvesting chlorophyll-protein complexes I and II, the small subunit of ribulose-1,5-bisphosphate carboxylase, photosystem II and ATP synthase complexes. Third pathway requiring both cGMP and calcium regulates genes of photosystem I and cytochrome b6-f complexes (Wu et al., 1996). Home and Meyer (1997) have shown that Ip3 involving mechanism leads to activation of calcium mediated responses such as the opening of guard cells. Phytochrome A and B in their Pfr form are localized in nucleus where it interacts with PIF3 which in turn regulate expression of genes belonging to COP/DET/FUS group. These genes in turn repress the expression of light-inducible genes (Nagy and Schäfer, 1999). The phytochrome molecule interacts with G-proteins. Bowler et al (1994) have shown that a high concentration of calcium/CaM repress anthocyanin accumulation and a high concentration of cGMP can inhibit chloroplasts development. Calmodulin is reported to be located in both nucleus and cytosol (Bachs et al., 1994). Thus calmodulin can act through nucleus. Calcium dependent protein kinases are known in plants. Two CDPKs, one from soybean (CDPKa) and one from Arabidopsis are known (ATCDPK). ATCDPK is a positive regulator where as ABI1 a type 2C protein phosphatase is a negative regulator of calcium mediated responses (Harmon, 1997). Besides this Ip3 involvement in plant signaling has been established. Phosphatidyl inositol 4,5-bisphosphate (PIP2) is precursor of both Ip3 and diacyl glycerol. PIP2 provide an alternative to guanine nucleotide exchange factors of ARF (ADP ribosylation factor) and rho and other small G-proteins (Chabre et al., 1998). Reed (1998) has shown that, environmental variables induce the sensor kinases. These sensor kinases autophosphorylate on histidine residue. The phosphate group is then transferred from the sensor component to an aspartate residue on the response regulator, which then carries out a downstream control step in plants and yeast. The cyanobacterial phytochrome cph1, converts to Pr form in far-red light or dark and phosphorylates itself at histidine residue. It then transfers the phosphate group to the rcp1 protein, which in turn regulates various responses. In red light, rcp1 is not phosphorylated and hence act in opposite way (Reed, 1998). Phytochromes of higher plants do not autophosphorylate but may act as kinases or phosphatases toward a
downstream regulator of light response (Reed, 1998). Cytokinins and light produces similar effects on etiolated seedlings suggesting a crosstalk between different signaling pathways (Chin-Atkins et al, 1996). CIP7, a gene from *Arabidopsis*, is involved in light activation of a set of light-inducible genes, is also expressed in response to light signal (Yamamoto et al, 1998). Pathways involving MAP kinases have been linked to signal transduction caused by wounding, pathogens and abiotic stresses, as well as, plant hormones abscissic acid, auxin and ethylene. ABA is able to induce MAPK-like activity in barley aleurone protoplasts (Hirt, 1997).

The serine/threonine protein phosphatases catalyse the dephosphorylation of phosphoserine and phosphothreonine in protein substrates. These enzymes are traditionally classified into four subgroups (PP1, PP2A, PP2B and PP2C) based on biochemical and pharmacological properties: okadaic acid and calyculin A inhibit PP1 and PP2A; PP2B is Ca^{2+}/calmodulin dependent; and PP2C requires Mg^{2+} for activity (Cohen, 1989; Luan, 1998). Plant cells respond by a rapid and transient increase in the concentration of cytosolic calcium (Ca^{2+}). These calcium signals modulate cellular processes through calcium-binding proteins, such as calmodulin, which in turn regulate the activity of downstream target proteins. Most proteins that function as intracellular transducers of Ca^{2+} signals contain a common structural motif, the 'EF hand', which is a helix-loop-helix structure that binds a single Ca^{2+} ion. Plants 'forage' for light in plant canopies using a variety of photosensory systems. In *Arabidopsis* and cucumber, perception of reflected far-red requires phytochrome B (Ballaré, 1999).

2.7 PHOTOSYSTEM I, PHOTOSYSTEM II and Cyt b_{6}f COMPLEX:

2.7.1 PHOTOSYSTEM I:
Photosystem I is a light-driven generator of the reducing power in chloroplasts. It catalyses the photooxidation of plastocyanin in thylakoid lumen and the photoreduction of ferredoxin in chloroplasts stroma or cyanobacterial cytoplasm (Chitnis, 1996).

Genes encoding proteins of PSI and their functions are given in Table 1.

2.7.2 PHOTOSYSTEM II:
Photosystem II is a multi-subunit membrane protein complex which functions as a light-driven, water-plastoquinone oxidoreductase converting water to oxygen molecule.

Genes encoding proteins of PSI and their functions are given in table 2.

2.7.3 Cyt b_{6}f COMPLEX:
Genes encoding proteins of Cytb_{6}f complex are given in table 3.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Trivial name</th>
<th>Apparent molecular mass</th>
<th>Location and possible function</th>
<th>Extrinsic/transmembrane</th>
<th>Number of helices</th>
</tr>
</thead>
<tbody>
<tr>
<td>psh A</td>
<td>c</td>
<td>D1</td>
<td>31</td>
<td>RC subunit, binding of redox cofactors</td>
<td>t</td>
<td>5</td>
</tr>
<tr>
<td>psh B</td>
<td>c</td>
<td>CP 47</td>
<td>51</td>
<td>core antenna protein, water splitting?</td>
<td>t</td>
<td>6</td>
</tr>
<tr>
<td>psh C</td>
<td>c</td>
<td>CP 43</td>
<td>46</td>
<td>core antenna protein, water splitting?</td>
<td>t</td>
<td>6</td>
</tr>
<tr>
<td>psh D</td>
<td>c</td>
<td>D2</td>
<td>34</td>
<td>RC subunit, binding of redox cofactors</td>
<td>t</td>
<td>5</td>
</tr>
<tr>
<td>psh E</td>
<td>c  α-subunit of cytochrome b 559</td>
<td>9</td>
<td>water splitting</td>
<td>t</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>psh F</td>
<td>c  β-subunit of cytochrome b 559</td>
<td>4</td>
<td>role in photoinhibition</td>
<td>t</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>(#psb G)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>psh H</td>
<td>c</td>
<td>phosphoprotein</td>
<td>7</td>
<td>RC complex, function unclear</td>
<td>t</td>
<td>1</td>
</tr>
<tr>
<td>psh I</td>
<td>c</td>
<td>4.8 kDa protein</td>
<td>4</td>
<td>subunit of the core complex, function unclear</td>
<td>t</td>
<td>1</td>
</tr>
<tr>
<td>**psb J</td>
<td>c</td>
<td>4 kDa protein</td>
<td>4</td>
<td>subunit of the core complex, function unclear</td>
<td>t</td>
<td>1</td>
</tr>
<tr>
<td>psh K</td>
<td>c</td>
<td>3.8 kDa protein</td>
<td>4</td>
<td>subunit of the core complex, function unclear</td>
<td>t</td>
<td>1</td>
</tr>
<tr>
<td>psh L</td>
<td>c</td>
<td>5 kDa protein</td>
<td>5</td>
<td>subunit of the core complex, function unclear</td>
<td>t</td>
<td>1</td>
</tr>
<tr>
<td>psh M</td>
<td>c</td>
<td>4 kDa protein</td>
<td>4</td>
<td>subunit of the core complex, function unknown</td>
<td>t</td>
<td>1</td>
</tr>
<tr>
<td>psh N</td>
<td>c</td>
<td>5 kDa protein</td>
<td>4.7</td>
<td>subunit of the core complex, function unknown</td>
<td>t</td>
<td>1</td>
</tr>
<tr>
<td>psh O</td>
<td>n</td>
<td>33 kDa protein, OEEC1 manganese stabilizing protein</td>
<td>33</td>
<td>regulatory subunit of the water oxidase, manganese stabilizing</td>
<td>e</td>
<td>—</td>
</tr>
<tr>
<td>*psb P</td>
<td>n</td>
<td>23 kDa protein, OEEC2</td>
<td>23</td>
<td>regulatory subunit of the water oxidase</td>
<td>e</td>
<td>—</td>
</tr>
<tr>
<td>**psb Q</td>
<td>n</td>
<td>18 kDa protein, OEEC3</td>
<td>18</td>
<td>regulatory subunit of the water oxidase</td>
<td>e</td>
<td>—</td>
</tr>
</tbody>
</table>

(Contd)
<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Trivial name</th>
<th>Apparent molecular mass</th>
<th>Location &amp; possible function</th>
<th>Extrinsic/transmembrane</th>
<th>Number of helices</th>
</tr>
</thead>
<tbody>
<tr>
<td>psb R</td>
<td>n</td>
<td>10 kDa protein</td>
<td>10</td>
<td>found in some core complex preparations, function unknown</td>
<td>t</td>
<td>partially membrane integrated</td>
</tr>
<tr>
<td>psb S</td>
<td>n</td>
<td>22 kDa protein, CP22</td>
<td>22</td>
<td>Chl binding; Chl transport? found in some core complex preparations</td>
<td>e</td>
<td>4</td>
</tr>
<tr>
<td>psb T</td>
<td>c</td>
<td>3 kDa protein</td>
<td>3</td>
<td>lumenal polypeptide of unknown function</td>
<td>e</td>
<td>4</td>
</tr>
<tr>
<td>*psb U</td>
<td>n</td>
<td>12 kDa protein</td>
<td>12</td>
<td>supports oxygen evolving activity in PS II</td>
<td>e</td>
<td>4</td>
</tr>
<tr>
<td>*psb V</td>
<td>n</td>
<td>cyt c&lt;sub&gt;550&lt;/sub&gt;</td>
<td>16</td>
<td>hemoprotein of low redox potential on the donor side of PS II, supports oxygen evolving activity</td>
<td>e</td>
<td>4</td>
</tr>
<tr>
<td>psb W</td>
<td>n</td>
<td>6.1 kDa protein</td>
<td>5.9</td>
<td>subunit close to the reaction center complex, unknown function</td>
<td>t</td>
<td>1</td>
</tr>
<tr>
<td>?</td>
<td>n</td>
<td>3.2 kDa protein</td>
<td>3.2</td>
<td>found in PS II membrane fragments</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
Table 2. Photosystem I: Genes, corresponding polypeptides, their possible location, putative topology and function within the complex of cyanobacteria and plants

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene locus</th>
<th>Trivial name</th>
<th>Apparent molecular mass</th>
<th>Function</th>
<th>Extrinsic/transmembrane</th>
<th>Number of helices</th>
</tr>
</thead>
<tbody>
<tr>
<td>psa A</td>
<td>c</td>
<td>(Ia, PS I-A)</td>
<td>83.0 kDa</td>
<td>binding of cofactors: 96+/− 5 Chla; P700 Chla</td>
<td>t</td>
<td>11</td>
</tr>
<tr>
<td>psa B</td>
<td>c</td>
<td>(Ib, PS I-B)</td>
<td>83.0 kDa</td>
<td>dimer: 15-25 β-Car; 2 phylloquinones: 1 [4Fe-4S] Cluster (F₄)</td>
<td>t</td>
<td>11</td>
</tr>
<tr>
<td>psa C</td>
<td>c</td>
<td>(VII, PS I-C)</td>
<td>9.0 kDa</td>
<td>binding of cofactors: 2 [4Fe-4S] (F₄ and F₆)</td>
<td>e</td>
<td>—</td>
</tr>
<tr>
<td>psa D</td>
<td>n</td>
<td>(II, PS I-D)</td>
<td>15.6 kDa</td>
<td>docking site for ferredoxin/flavodoxin; binding/orientation of psa C; no cofactor</td>
<td>e</td>
<td>—</td>
</tr>
<tr>
<td>psa E</td>
<td>n</td>
<td>(IV, PS I-E)</td>
<td>8.0 kDa</td>
<td>important for cyclic electron flow and Fd reduction; no cofactor</td>
<td>e</td>
<td>—</td>
</tr>
<tr>
<td>psa F</td>
<td>n</td>
<td>(III; PS I-F)</td>
<td>15-16 kDa</td>
<td>plastocyanin/cytC₆ docking, binding of Chla?</td>
<td>t</td>
<td>1-2</td>
</tr>
<tr>
<td>#psa G</td>
<td>n</td>
<td>(?, PS I-G)</td>
<td>10-11 kDa</td>
<td>no cofactor; interaction with LHC I?</td>
<td>t</td>
<td>2</td>
</tr>
<tr>
<td>#psa H</td>
<td>n</td>
<td>(VI, PS I-G)</td>
<td>10-11 kDa</td>
<td>no cofactor; interaction with LHC I?</td>
<td>e</td>
<td>—</td>
</tr>
<tr>
<td>psa I</td>
<td>c</td>
<td>(IX; PS I-I)</td>
<td>3.4-4.3 kDa</td>
<td>no cofactor; stabilization of the trimerization domain</td>
<td>t</td>
<td>1</td>
</tr>
<tr>
<td>psa J</td>
<td>c</td>
<td>(VIII; PS I-J)</td>
<td>3.0-4.4 kDa</td>
<td>stabilization of psa F?</td>
<td>t</td>
<td>1</td>
</tr>
<tr>
<td>psa K</td>
<td>n</td>
<td>(PS I-K)</td>
<td>5.0-8.5 kDa</td>
<td>?</td>
<td>t</td>
<td>2</td>
</tr>
<tr>
<td>psa L</td>
<td>n</td>
<td>(V; PS I-L)</td>
<td>14.0-16.6 kDa</td>
<td>no cofactor; important for trimerization</td>
<td>t</td>
<td>2</td>
</tr>
<tr>
<td>*psa M</td>
<td>c(?)</td>
<td>(PS I-M)</td>
<td>2.8-3.4 kDa</td>
<td>no cofactor; electron transport?</td>
<td>t</td>
<td>1</td>
</tr>
<tr>
<td>#psa N</td>
<td>—</td>
<td>—</td>
<td>9 kDa</td>
<td>?</td>
<td>e</td>
<td>—</td>
</tr>
<tr>
<td>#psa O</td>
<td>—</td>
<td>(PS I-O)</td>
<td>9 kDa</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
Table 3: Molecular composition and the function of the cytb₆ complex subunits

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Trivial name</th>
<th>Apparent molecular mass</th>
<th>Location &amp; possible function</th>
<th>Extrinsic/ transmembrane</th>
<th>Number of transmembrane α-helices</th>
</tr>
</thead>
<tbody>
<tr>
<td>pet A</td>
<td>c</td>
<td>cyt f</td>
<td>34 kDa</td>
<td>linear electron transfer, one heme (type c)</td>
<td>partly integrated, heme on the lumen side</td>
<td>1</td>
</tr>
<tr>
<td>pet B</td>
<td>c</td>
<td>cyt b₆</td>
<td>23 kDa</td>
<td>cyclic electron transfer, 2 b-type hemes</td>
<td>transmembrane</td>
<td>4</td>
</tr>
<tr>
<td>pet C</td>
<td>n</td>
<td>Rieske-protein</td>
<td>20 kDa</td>
<td>linear electron transfer, 1 Fe₅S-cluster</td>
<td>partly integrated</td>
<td>1</td>
</tr>
<tr>
<td>pet D</td>
<td>c</td>
<td>subunit IV</td>
<td>17 kDa</td>
<td>quinone binding?</td>
<td>transmembrane</td>
<td>4</td>
</tr>
<tr>
<td>pet F</td>
<td>n</td>
<td>plastocyanin</td>
<td>10 kDa</td>
<td>linear electron transfer, redox active group Cu</td>
<td>extrinsic, lumen, phase</td>
<td>—</td>
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<tr>
<td>pet G</td>
<td>c</td>
<td>subunit V</td>
<td>5 kDa</td>
<td>?</td>
<td>transmembrane</td>
<td>1</td>
</tr>
<tr>
<td>pet L</td>
<td>c</td>
<td>subunit VI</td>
<td>4-5 kDa</td>
<td>?</td>
<td>transmembrane</td>
<td>1</td>
</tr>
<tr>
<td>pet M</td>
<td>n</td>
<td>subunit VII</td>
<td>4 kDa</td>
<td>?</td>
<td>transmembrane</td>
<td>1</td>
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