Chlorophyll: Structure and Function

Bernhard Grimm, *Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*

Chlorophyll is the dominant pigment on Earth and serves as the light-trapping and energy transferring chromophore in photosynthetic organisms. In recent years research has contributed enormously to a better understanding of the metabolic pathway of chlorophyll synthesis and its biochemical, biophysical and structural properties in close association with the pigment binding proteins of the reaction centre and the antenna complexes of the photosynthetic units.

Secondary article

Article Contents

Introduction

The dominant green pigmentation on Earth originates from the macrocyclic compound, chlorophyll. An average plant leaf has around 70 million cells with 5 billion chloroplasts, each containing about 600 million molecules of chlorophyll (Simpson and Knötzel, 1996). Chlorophyll and bacteriochlorophyll serve as light-trapping and energy-transferring chromophores in antenna pigment protein complexes and thus facilitate all energy-consuming processes in photosynthetic organisms. A central molecule in the photosynthetic reaction centre finally drives the primary charge separation for the conversion of radiant energy to chemical energy.

Chlorophyll/bacteriochlorophyll is one of the end products of tetrapyrrole biosynthesis. Most of the tetrapyrrolic end products possess a flexible macrocycle with extensive conjugated double bonds, which might explain the diverse functions of tetrapyrroles. Haem functions as cofactor in various enzymatic reactions. Open-chain tetrapyrroles can be intermediates of haem degradation, but function also as phycobilins in light-harvesting antenna complexes of Cyanobacteria and red algae, or as phytochromobilin in the signal perceptor phytochrome.

Apart from the photosynthetic eukaryotes, algae and plants, five of 11 phyla of Eubacteria comprise photosynthetic organisms: green nonsulfur bacteria, green sulfur bacteria, purple bacteria, Gram-positive bacteria and Cyanobacteria. Different derivatives of chlorophyll are used in (chemo)autotrophic and photoheterotrophic organisms, and the different pigment combinations in the photosynthetic complexes specify taxonomically related groups of organisms. In spite of the biological diversity among the photosynthetic organisms, the photochemical reactions driven by these various derivatives of chlorophyll/bacteriochlorophyll are essentially the same. The structure of pigment-binding proteins in the photosynthetic complexes is not necessarily conserved among the photosynthetic organisms. This article examines the structural and functional mutualities as well as the diversity of the pigment–protein association in the photosynthetic complexes. First, the structural and functional properties of chlorophyll, the pathway of tetrapyrrole biosynthesis, and some aspects of its metabolic control will be described.

Structure of Chlorophyll

Chlorophylls are lipophilic compounds composed of a chlorin moiety and a long alcoholic carbon chain, which is usually the diterpene phytol. In higher plants, chlorophyll *a* and *b* are the dominant pigments in the photosynthetic units (**Figure 1**). They absorb visible light with maximal absorbency in the blue and red and low absorbency in the green spectral region. Chlorophyll *a* is present in the light-harvesting antenna pigment complexes and in the reaction centre complexes and functions as an accessory pigment as well as the primary electron donor in the reaction centres of photosystems I and II (PS I and PS II

Chlorophyll *b* differs from chlorophyll *a* by a formyl group instead of a C7 methyl substituent and is characterized by slightly different spectroscopic properties. Chlorophyll *b* is present in the light-harvesting antenna complexes, preferentially in the major antenna of PS II, and is absent in the reaction centre.

Bacteriochlorophylls *a* and *b* are synthesized in purple bacteria and green photosynthetic bacteria that do not evolve oxygen (**Figure 1**). Bacteriochlorophyll *b* differs from bacteriochlorophyll *a* by the presence of a C8 ethylidene group. The reaction centre of photosynthetic bacteria contains either the *a* or the *b* type as the primary donor, yielding absorption maxima at around 870 nm and 1000 nm, respectively. Other forms of bacteriochlorophyll

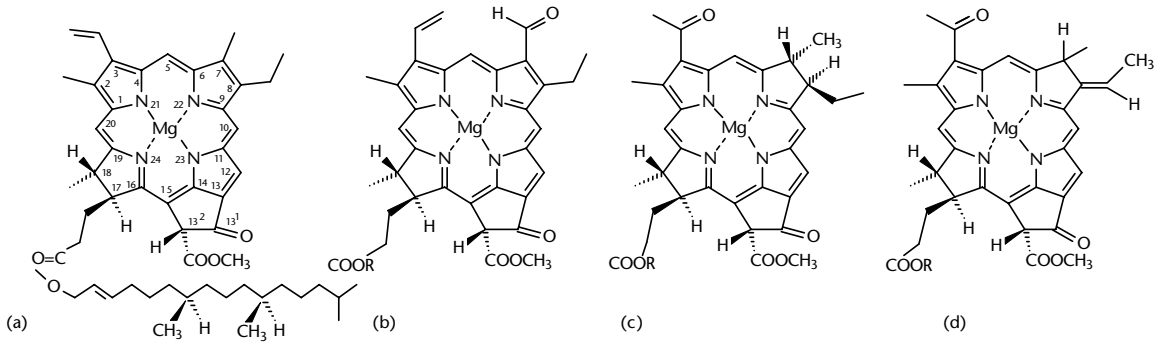

Figure 1 Structure of (a) chlorophyll *a*, (b) chlorophyll *b*, (c) bacteriochlorophyll *a* and (d) bacteriochlorophyll *b*. The major esterifying alcohol (= R) is phytol in chlorophyll *a* and *b*, geranylgeraniol in bacteriochlorophyll *a*, and phytadienol in bacteriochlorophyll *b*.

exist in specific microorganisms. (For a detailed review, see Scheer, 1991.)

Tetrapyrrole Biosynthetic Pathway

Chlorophyll, bacteriochlorophyll and their derivatives arose as variants of the evolutionarily conserved biosynthetic pathway of tetrapyrroles, which consists of at least 18 different enzymatic steps (**Table 1**). The enzymes are encoded by single copy genes or small gene families, providing an individual control dependent on spatial, developmental or environmental factors. The sequential mutational analysis of the 45-kb long gene cluster of *Rhodobacter capsulatus* has given the most comprehensive insight into the genetic organization of the bacteriochlorophyll metabolic pathway and revealed that almost all enzymatic steps for the formation of bacteriochlorophyll are encoded in this cluster (Alberti *et al*., 1995). The identity of cyanobacterial genes homologous to *Rhodobacter* was confirmed by sequencing of the entire genome of *Synechocystis* PCC 6803. Coding sequences involved in tetrapyrrole synthesis were also identified and characterized by genetic dissection of mutants with lesions in chlorophyll synthesis. More genes from other organisms have been identified by means of complementation of *Escherichia coli* and *Rhodobacter* mutants and by sequence homology comparison, resulting in an equivalent set of genes from plants (**Table 1**). Sequencing has revealed that, apart from a few exceptions, the pathways of chlorophyll and bacteriochlorophyll use homologous proteins. All enzymes, as long as their genes have been identified in higher plants, are encoded in the nucleus.

5-Amino laevulinic acid (ALA), the first committed precursor of chlorophyll, is synthesized from glutamate in most bacteria and plants. Glutamate is aminoacylated to a $tRNA^{(glu)}$ by a glutamyl-tRNA synthetase. Glutamyl-tRNA reductase catalyses the NADPH-dependent reduction of glutamyl-$tRNA^{(glu)}$ to yield glutamate 1-semialdehyde. The vitamin B_6-requiring glutamate 1-semialdehyde aminotransferase catalyses the intramolecular transfer of the amino group of the substrate to produce ALA. Two molecules of ALA are condensed to yield one molecule of porphobilinogen. This reaction is catalysed by ALA dehydratase. Then four molecules of porphobilinogen are fused to form hydroxymethylbilane by porphobilinogen deaminase.

The further conversion of hydroxymethylbilane to uroporphyrinogen III involves ring closure and simultaneous isomerization of the acetyl and propionyl groups at the pyrrole ring D and is catalysed by uroporphyrinogen III synthase. The enzyme uroporphyrinogen III decarboxylase catalyses the oxidation of uroporphyrinogen III to coproporphyrinogen III, including the decarboxylation of acetic acid side-chains to methyl groups. Coproporphyrinogen III oxidase converts two propionic acid side-chains into vinyl groups to form protoporphyrinogen IX, which is oxidized to protoporphyrin IX by protoporphyrinogen IX oxidase.

At the subsequent branch point protoporphyrin IX is directed either to the chlorophyll or the haem-synthesizing pathway. For chlorophyll formation, Mg-chelatase inserts Mg^{2+} into protoporphyrin IX by a complex ATP-dependent catalytic reaction. Mg-chelatase is made up of three different subunits. A subsequent esterification of Mg-protoporphyrin IX to Mg-protoporphyrin IX monomethylester is catalysed by the *S*-adenosyl-L-methionine Mg-protoporphyrin IX methyltransferase. This reaction is followed by the formation of a fifth isocyclic ring of the tetrapyrrolic macrocycle mediated by Mg-protoporphyrin IX monomethylester cyclase. The product, divinyl protochlorophyllide, is reduced at the C8 vinyl group to yield monovinyl protochlorophyllide by vinyl reductase.

Table 1 Abbreviations of the gene names for each enzyme in the tetrapyrrolic pathway as far as the coding sequences have been published[a]

Enzyme	Bacteria	Cyanobacteria	Yeast	Plant
Glutamyl-tRNA synthase	*gltX*	*gltX*		*GluRS*
Glutamyl-tRNA reductase	*hemA*	*hemA*		*Hem A*
Glutamate 1-semialdehyde aminotransferase	*hemL*	*hemL*		*gsa*
5-Aminolaevulinate dehydratase	*hemB*	*hemB*	*Hem2*	*Ala D*
Porphobilinogen deaminase	*hemC*	*hemC*	*Hem3*	*Pbg D*
Uroporphyrinogen III-synthase	*hemD*	*hemD*		
Uroporphyrinogen decarboxylase	*hemE*	*hemE*	*HEM12*	*UroD*
Coproporphyrinogen oxidase	*hemN*	*hemN*	*HEM13*	*Cpo*
	hemF	*hemF*		
Protoporphyrinogen oxidase	*hemX*		*HEM14*	*PpxI*/*PpxII*
Ferrochelatase	*hemH*		*HEM15*	*FchI*
				FchII
Mg-Protoporphyrin IX chelatase	*bchD*	*chlD*		*Chl D*
	bchI	*chlI*		*Chl I*
	bchH	*chlH*		*Chl H*
Mg-Protoporphyrin IX methyltransferase	*bchM*	*chlM*		
Mg-Protoporphyrin IX monomethylester cyclase	*bchE*	*chlE*		
Vinylreductase	*bchJ*			
Protochlorophyllide oxidoreductase	*bchB*	*chlB*		*Por A*/*Por B*
				Ch lB
	bchN	*chlN*		*Chl N*
	bchL	*chlL*		*Chl L*
		por		
Geranylgeranyl reductase	*bchP*	*chlP*		*Chl P*
Chlorophyll synthase	*bchG*	*chlG*		*Chl G*
Chlorophyll *a* oxygenase				*CAO*

[a] Sequence data of the microbial database are not considered.

Subsequently, in angiosperms the double bond between C17 and C18 in ring D of the macrocycle is reduced in a light-dependent manner to yield chlorophyllide *a* by protochlorophyllide oxidoreductase (angiosperms have lost the ability to use light-independent protochlorophyllide reductase). All other photosynthetic organisms employ a second mechanism of chlorophyllide formation. The genes encode polypeptides that are involved in the light-independent reduction of protochlorophyllide; these polypeptides show sequence similarity to the three subunits of the nitrogenase. The final steps of chlorophyll biosynthesis link to the isoprenoid pathway, which provides the intermediate geranylgeranyl diphosphate for the synthesis of phytyl diphosphate by geranylgeranyl reductase. Chlorophyllide *a* is esterified with a phytol or a geranylgeraniol chain catalysed by chlorophyll synthase. Chlorophyll *b* is synthesized by oxidation of the methyl group of chlorophyll *a* to a formyl group via a hydroxymethyl intermediate. A chlorophyll *a* oxygenase is involved in chlorophyll *b* formation. The conversion of chlorophyll *a* to *b* and vice versa could be relevant for the regulation of the photosynthetic efficiency by altering the size of the light-harvesting antenna complex.

Protoporphyrin IX can also be directed towards haem synthesis by the iron-chelating enzyme ferrochelatase. Protohaem can be further metabolized to form the linear tetrapyrrole biliverdin and subsequently phytochromobilin, the chromophore of the photoreceptor phytochrome. (For recent reviews on chlorophyll biosynthesis, see von Wettstein *et al*., 1995; Suzuki *et al*., 1997.)

Differences Between the Bacterial, Animal and Plant Metabolic Pathway

In contrast to bacteria, eukaryotic tetrapyrrole biosynthesis is located in distinct compartments. There are some differences between the plant and the animal tetrapyrrolic pathway. Apart from chlorophyll being the privilege of photosynthetic organisms, ALA synthesis and the subcompartmental distribution of the entire pathway differ between both kingdoms. In plants and most bacteria the first committed precursor of the tetrapyrrolic pathway, ALA, is formed from glutamate in three enzymatic steps. Animals, yeast and Rhodobacteriaceae catalyse condensa-

tion of succinyl–CoA and glycine and its subsequent decarboxylation by ALA synthase.

The animal pathway begins in mitochondria with the formation of ALA. The metabolic route continues in the cytoplasm and is completed in the mitochondria by ferrochelatase, which is located at the inner site of the inner membrane. The metabolic flux into mitochondria is directed by the two preceding enzymes of the pathway, coproporphyrinogen oxidase located in the intermembrane space and protoporphyrinogen IX oxidase located on the outer site of the inner membrane of mitochondria.

In plants the synthesis of chlorophyll is located in plastids. Haem synthesis is simultaneously completed in plastids and mitochondria. Genes encoding distinct isoforms of the last two enzymes, protoporphyrinogen oxidase and ferrochelatase, have been identified. Their expression should allow a controlled supply of tetrapyrroles and protein trafficking into different cellular compartments. The plastidal protoporphyrinogen oxidase is encoded as a precursor protein and is found in the thylakoid and envelope membranes. The mitochondrial enzyme has no obvious transit sequence and is assumed to be targeted to the outer site of the mitochondrial inner and outer membranes. Two isoforms of ferrochelatase have been presented; these are apparently transferred as precursors either to chloroplasts and mitochondria, or exclusively to mitochondria and subsequently processed to their final size.

In addition to protoporphyrinogen IX oxidase activity, Mg-chelatase or protochlorophyllide oxidoreductase activity has been found in the envelope membranes. Biosynthesis of phytol also takes place in envelope membranes. Their relevance for the biosynthesis of chlorophyllide is emphasized, as protochlorophyllide is required for the import of POR A, the isoenzyme of protochlorophyllide oxidoreductase that accumulates in etiolated plant tissue (Reinbothe *et al.*, 1996). It remains to be elucidated how the synthesis of the tetrapyrrole intermediates is connected with the sites of pigment protein assembly in the thylakoids.

Regulation of Gene Expression in Tetrapyrrole Biosynthesis

The entire pathway is embedded in a network of a control system that matches gene expression and enzyme activity with the demands of the substrate flux. Control of the tetrapyrrolic pathway depends on exogenous stimuli such as light, temperature, light period and endogenous programmes such as circadian clock, hormones, development and tissue specificity. Light quality and quantity in particular determine rate and turnover of chlorophyll synthesis and its assembly with chlorophyll-binding proteins. Two photoreceptors play the major role in light-dependent control of numerous processes in plants including tetrapyrrole biosynthesis: the photoreversible red/far-red light phytochrome system and the blue light receptor(s).

It is reasonable to assume that the determining regulatory sites of tetrapyrrole metabolism are positioned at the beginning and at the branch points of the pathway. The metabolic flow is limited at the level of ALA synthesis, the first committed product of the pathway. Apart from light induction, ALA synthesis also depends on feedback control triggered by tetrapyrroles (**Figure 2**). In yeast and animals haem exerts its regulatory effects on various levels of expression of ALA synthase by the changes of its level of free haem. Haem represses transcription and translation of ALA synthase and directly inhibits mitochondrial import and activity of the enzyme. Consistent with its role in animals, it is suggested that haem also contributes to the feedback regulation of plant tetrapyrrole biosynthesis. Haem directly inhibits the activity of glutamyl-tRNA reductase. The mode of action of feedback control by haem

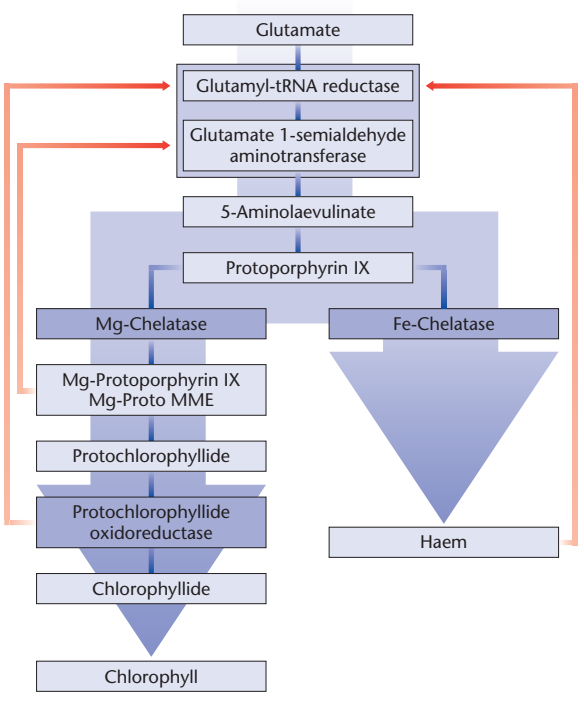

Figure 2 Potential feedback control mechanisms in the metabolic pathway of tetrapyrroles. The synthesis of 5-aminolaevulinate (ALA) from glutamate is the rate-limiting step of tetrapyrrole biosynthesis. Feedback regulation on the enzymes of the ALA pathway, glutamyl-tRNA reductase or glutamate 1-semialdehyde aminotransferase, can start out from three possible sites: at haem formation, at the reduction of protochlorophyllide, and at the level of Mg-protoporphyrin IX and Mg-protoporphyrin IX monomethylester (Mg-Proto MME) formation.

is still elusive. Future experiments are needed to elucidate the control of haem synthesis in plastids and mitochondria and the utility and transfer of haem to other subcompartments.

Protochlorophyllide oxidoreductase functions as a photoreceptor involved in the light-dependent reduction of protochlorophyllide. The repression in protochlorophyllide reduction in the dark parallels the decrease of ALA synthesis, suggesting that the ALA-synthesizing capacity is adjusted with the level of protochlorophyllide to avoid accumulation of photoreactive porphyrins (Reinbothe *et al*., 1996). More recently an additional feedback control mechanism at the level of Mg-porphyrins has been described. Parallel to the decrease of Mg-chelatase activity in transgenic plants caused by reduced synthesis of one of the Mg-chelatase subunits, lower ALA synthesizing capacities were found. This could be explained not only by reduced enzyme activities, but also by reduced transcriptional activity of the gene(s) encoding glutamyl-tRNA reductase and ALA dehydratase.

The regulation of tetrapyrrole biosynthesis is still far from being understood. It will be the objective of future work to compile the *in vivo* and *in vitro* expression and activity data of the pathway and to predict which transcriptional and/or posttranslational modifications are important control steps. Most likely a concert of control mechanisms determines the actual metabolic flow through the pathway.

Chlorophyll Utilization in the Photosystems of Plants

Chlorophyll molecules function either as accessory or reaction centre pigments. The accessory pigments collect the photons in the plant light-harvesting antenna complexes or in the cyanobacterial phycobilisomes. The excitation energy absorbed by these pigments is transported in a series of ultrafast transfers from one chlorophyll molecule to the next. The reaction centre pigment, having a long wavelength for maximum absorption, acts as a kinetic trap for light energy that is funnelled from the light-harvesting complexes and used for photochemical charge separation.

These photoreactions occur in two different photosystems of plants: at P700 and at P680, respectively, in combination with the accessory pigments and electron acceptors and carriers that are organized in PS I and PS II. Excitation of PS II drives electron transfer from P680 to a molecule of pheophytin, which in turn reduces a quinone, Q_A (plastoquinone). The electron flow proceeds to another quinone, Q_B, which is part of a mobile plastoquinone pool, and finally reaches the oxidized chlorophyll in PS I ($P700^+$) through carriers such as several cytochromes, a sulfur protein and plastocyanin. Excitation of PS I results in electron transfer from the P700 chlorophyll dimer to the primary acceptor, which is another molecule of chlorophyll *a*. This further electron transfer generates the strong reductant Chl a^-, which eventually produces high-energy compounds via a set of iron–sulfur proteins and flavoproteins. A molecule of phylloquinone (vitamin K) has been implicated as an intermediary electron carrier between the chlorophyll *a* and the first iron–sulfur centre. The electron transport from PS II to PS I is coupled with proton translocation across the thylakoid membrane, setting up an electrochemical gradient that is used to synthesize ATP. In consequence, one photon is required in each of the two photosystems to result in oxygen evolution at the beginning and the generation of reduction equivalents at the end of the photosynthetic reaction.

Destruction Caused by Excited Chlorophyll

Excessive photon energy, which is funnelled towards the reaction centres of the photosynthetic complexes, can be re-emitted by fluorescence or quenched by nonradiative decay. But high light-exposed chlorophyll and its precursors can also form the long-lived excited triplet state ^{3}Chl, which has the potential to produce free radicals by electron or hydrogen transfer (type I mechanism) or involves the transfer of energy to oxygen giving rise to the formation of toxic singlet oxygen (type II mechanism) (**Figure 3**) (Spikes and Bommer, 1991). These photosensitized processes are harmful when protective components, such as carotenoids or tocopherols, cannot sufficiently detoxify the photochemically generated reactive pigment and oxygen species. The cellular targets of the reactive oxygen species include lipids, proteins and DNA.

Pigment and Protein Composition of Reaction Centres

Comparative studies reveal that the reaction centres in which the two types of photoreactions take place can be categorized into two major groups. These may have evolved from different prokaryotic lineages. The type I reaction centre contains an iron–sulfur centre that functions as electron acceptor. It is found in green sulfur bacteria and PS I. The type II is the quinone-type reaction centre present in purple bacteria, green nonsulfur bacteria and PS II. A pheophytin and a pair of quinones serve as electron acceptors. The determination of the X-ray structure for each type of reaction centre reveals a similarity of their structural arrangement of pigments and cofactors (Allen and Williams, 1998). **Table 2** lists the pigment composition of reaction centres from purple

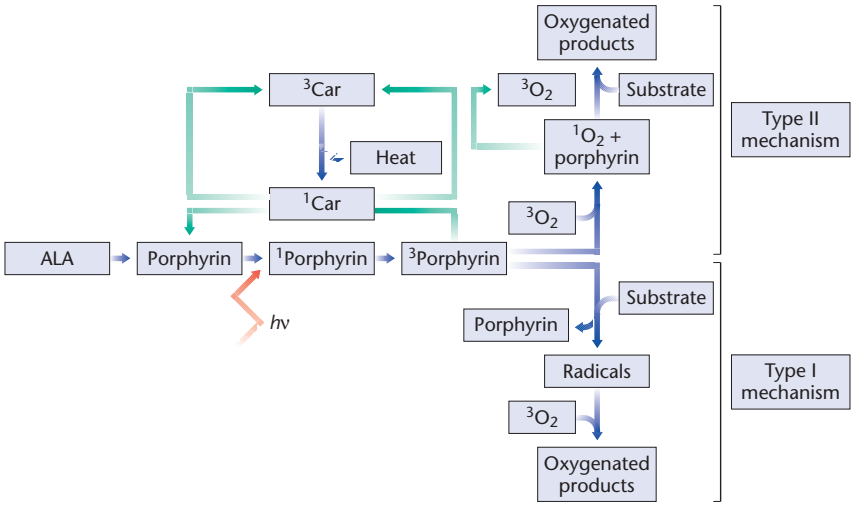

Figure 3 The possible hazardous reactions of excited porphyrins after formation of their long-lived excited triplet state. Free radicals can be generated by electron or hydrogen transfer (type I reaction) or energy is transferred to oxygen giving rise to the formation of toxic singlet oxygen (type II reaction). Additionally, the quench mechanism of excited porphyrins and singlet oxygen by carotenoids (Car) are illustrated (green arrows). Zeaxanthin is assumed to dissipate efficiently excitation energy.

Table 2 Pigments, cofactors and protein composition of the photosynthetic reaction centres of plants and bacteria

	Purple bacteria	Green nonsulfur bacteria	Photosystem II	Photosystem I
Type of reaction centre	Type II	Type II	Type II	Type I
Pigments and cofactors	Four bacterio-chlorophyll *a* or *b*	Three bacteriochlorophyll *a* 3	Four–six chlorophyll *a*	< 100 chlorophyll *a*
	Two bacterio-phaeophytin *a* or *b*	Bacteriophaeophytin	Two phaeophytins	12–15 β-carotenes
	Two quinones	Two quinones	Two quinones	Two phylloquinones
	One nonhaem iron atom	One nonhaem iron atom	One nonhaem iron atom	4Fe–4S cluster
			Internal antenna proteins: 20 chlorophyll *a* 4–5 β-carotene	
Reaction centre proteins	H subunit L subunit M subunit	L subunit M subunit	D1 and D2 CP43 and CP47	Psa A, Psa B[a] Five small proteins

[a]Reaction centre proteins of PSI.

bacteria, green nonsulfur bacteria and plant photosystems I and II.

The reaction centre of photosynthetic purple bacteria and green nonsulfur bacteria contains two major polypeptides with molecular weights of approximately 32 and 34 kDa, which are designated L and M subunits (**Table 2**). They are integral membrane proteins and the pigments are packed in a highly symmetrical arrangement with an axis of two-fold rotational pseudosymmetry. The L and M subunits have homologous amino acid sequences and also show homology to the secondary and tertiary structure of the subunits from other bacterial phyla. The reaction centre of PS II of plants, algae and Cyanobacteria also consists of two main polypeptides of 39 kDa molecular mass, designated D1 and D2, which are structurally and functionally related to their bacterial counterparts. The core antenna protein CP43 and CP47 are associated with the PS II reaction centre and contribute with their pigments to the protein environment of the oxygen-evolving complex.

The plant PS I reaction centre dissociates into two major polypeptides of 83 kDa with homologous amino acid sequences. The two proteins combine reaction centre and internal antenna in one protein complex. In these proteins, chlorophyll and other cofactors are again arranged in a twofold symmetry axis. Five smaller peptides complete the reaction centre core complex. The reaction centre of green sulfur bacteria and Gram-positive bacteria appears to be structurally and functionally similar to PS I with one or more polypeptides of an apparent molecular weight of 65 kDa in addition to several smaller polypeptides.

Light-harvesting Antenna Complexes

Both photosystems in plants are made up from a large family of light-harvesting chlorophyll-binding proteins. The functional antenna sizes are adjusted in response to light conditions, both by dissociation/association with the other constituents of the antenna system and by changing the efficiency of energy transfer via the action of the xanthophyll cycle. These specific functions are presumably attributed to individual members of the protein family.

There are three types of antenna proteins in higher plants (Jansson, 1994).

1. The major light-harvesting complex (LHC), which is organized in a trimeric arrangement of the two members of the protein family, LHCB1 and LHCB2. They form the peripheral antenna complex of PS II, but dissociate from it under certain conditions and can be functionally attached to PS I.
2. The minor light-harvesting complex consists of the LHCB3–6 proteins, which are grouped as monomers around the dimeric PS II core antenna and the reaction centre. Apart from energy transfer between peripheral antenna, and the core antenna, these proteins function in the dissipation of excess energy.
3. The PS I antenna proteins are designated LHCA1–4. Two copies of these are arranged in a single layer around the PS I core under all physiological conditions.

The structure of the trimeric peripheral light-harvesting complex of PS II has been analysed to atomic resolution of 0.34 nm by electron crystallography (Kühlbrandt *et al.*, 1994). The model of the light-harvesting complex provides a general overview for the overall folding of all chlorophyll *a*/*b* binding proteins because they share structural similarities in certain parts of the protein structure. Homologous light-harvesting chlorophyll binding proteins from different plant species show 80–90% similarity among angiosperms. Differences among the different members of this protein family are more substantial (only up to 65% homology).

The light-harvesting antenna proteins have three membrane-spanning helices. The first and the third helices and their amino terminal regions show significant similarities to each other. Twelve chlorophyll molecules (7 and 5 chlorophyll *a* and *b,* respectively) and two carotenoid molecules, which were tentatively assigned as luteins, are associated in the peripheral light-harvesting antenna protein. The chlorophyll molecules are positioned precisely to optimize energy transfer within and between the complexes. Their chlorin rings are arranged roughly perpendicular to the membrane plane in two layers near the surface of the membrane. The chlorophyll *a* molecules are in close contact with photoprotective carotenoids. Horizontal energy transfer between the trimeric light-harvesting complexes is facilitated by chlorophylls that are exposed at the periphery.

Photosynthetic prokaryotes possess no structural counterparts to the plant light-harvesting complexes, but contain functionally analogous proteins. The antenna complexes of purple bacteria are oligomeric proteins forming large rings of overlapping bacteriochlorophyll molecules. The antenna systems of Cyanobacteria and of the chloroplasts of red algae are the water-soluble phycobilisomes that are independently derived structures. They are composed of phycobiliproteins that form rod-like structures and contain 300–800 phycobilin pigments. The proteins constrain the bilins into their extended conformation and their spectral properties are determined by the type of bilin, phycoerythrobilin and phycocyanobilin (between 450 and 630 nm).

Requirements for the Stability of Pigment–Protein Complexes

Expression of numerous nuclear and plastid-encoded proteins ensures chloroplast development and thus the functioning and structural integrity of the photosynthetic apparatus. The stability of the pigment-binding proteins depends on the accumulation of chlorophylls. Experiments performed with mutants deficient in content and synthesis of carotenoids and chlorophylls show the necessity of pigment binding for stabilization of the apoprotein. Plant mutants with chlorophyll deficiency are designated *albina*, *xantha, chlorina*, *viridis* or chl *b*-deficient mutants (Henningsen and Stummann, 1982). Light-harvesting proteins did not accumulate in *Chlamydomonas* and higher plant mutants when pigment synthesis was impaired (Plumley and Schmidt, 1995). Among higher plants many mutants with chlorophyll *b* deficiency have been reported. For example, the barley mutant *chlorina f2* lacks chlorophyll *b* and fails to accumulate the major polypeptide of the light-harvesting antenna complex.

Proteins, chlorophyll and carotenoids are simultaneously required for the stability of the associated

pigment–protein complexes. All constituents are degraded if one partner is missing. It is apparent that the nuclear-encoded apoproteins are first inserted into the thylakoids before binding of pigments occurs. However, synthesis of some plastid-encoded proteins of PS II seems to require chlorophyll that is apparently cotranslationally bound to the proteins.

Reconstitution studies have been carried out with recombinant or isolated light-harvesting chlorophyll binding proteins to predict the requirement of pigments for stable integration into the photosystems. The reconstituted complex harbouring the pigments resembles native light-harvesting complexes. Experiments carried out with mutant and truncated proteins, or with a supply of different ratios of chlorophyll and cofactors, revealed the requirements of protein structure as well as pigments for the reconstitution of stable complexes and for maintenance of the unmodified spectral and photosynthetic properties (Paulsen, 1997).

Expression studies of light-exposed embryonic and etiolated plant tissue revealed the strong light, developmental and circadian control of genes encoding the light-harvesting chlorophyll-binding proteins. The importance of chlorophyll synthesis has already been underlined as a determinant for the assembly of pigment-binding proteins. It is therefore conceivable that light-harvesting antenna protein genes are coordinately regulated at the mRNA level in parallel to chlorophyll formation. The 'crosstalk' for the coordinate expression remains an open question.

Final Remarks

The levels of chlorophyll synthesis are carefully regulated. Stimulation or repression of chlorophyll synthesis as well as chlorophyll degradation are important for regulating tetrapyrrole biosynthesis and functioning of the pigments in the assembly of the photosynthetic apparatus. The genetic and biochemical analysis of function and biosynthesis of chlorophylls has progressed greatly and provides tools for identification and characterization of the regulatory determinants. Different experimental approaches are used to dissect genetically the tetrapyrrole biosynthetic pathway or to screen mutants with deficiency in pigment contents (Grimm, 1998). These experiments lead to the identification of novel genes involved in tetrapyrrole biosynthesis. Studies with transgenic plants expressing these genes in antisense and sense orientation are undertaken to examine the regulatory and regulating competence of each step in the pathway for the metabolite flux in the pathway and the expression and stability of other constituents of the pathway. It is expected that the analysis of transgenic plants with deregulated tetrapyrrole biosynthesis will reveal the regulatory interdependence of the synthesis of chlorophylls and pigment-binding proteins and the assembly of the photosynthetic apparatus or the protective mechanism against photooxidative stress.

References

Alberti M, Burke DH and Hearst JH (1995) Structure and sequence of the photosynthesis gene cluster. In: Blankenship RE, Madigan MT and Bauer CE (eds) *Anoxygenic Photosynthetic Bacteria*, pp. 1083–1106. The Hague: Kluwer Academic.

Allen JP and Williams JC (1998) Photosynthetic reaction centers. *FEBS Letters* **438**: 5–9.

Grimm B (1998) Novel insights into the control of tetrapyrrole metabolism of higher plants. *Current Opinion in Plant Biology* **1**: 245–250.

Henningsen KW and Stummann BM (1982) Use of mutants in the study of chloroplast biogenesis. In: Parthier B and Boulter D (eds) *Encyclopedia of Plant Physiology*, vol. 14B. *Nucleic Acids and Proteins in Plants II*, pp. 597–644. Berlin: Springer-Verlag.

Jansson S (1994) The light-harvesting chlorophyll *a*/*b*-binding proteins. *Biochimica Biophysica Acta* **1184**: 1–19.

Kühlbrandt W, Wang DG and Fujiyoshi Y (1994) Atomic model of plant light-harvesting complex by electron cystallography. *Nature* **367**: 614–621.

Paulsen H (1997) Pigment ligation to proteins of the photosynthetic apparatus in higher plants. *Physiology of Plants* **100**: 760–768.

Plumley FG and Schmidt GW (1995) Light-harvesting chlorophyll *a*/*b* complexes: interdependent pigment synthesis and protein assembly. *Plant Cell* **7**: 689–704.

Reinbothe S, Reinbothe C, Apel K and Lebedev N (1996) Evolution of chlorophyll biosynthesis – the challenge to survive photooxidation. *Cell* **86**: 703–705.

Scheer H (1991) Structure and occurrence of chlorophylls. In: Scheer H (ed.) *Chlorophylls*, pp. 3–30. Boca Raton FL: CRC Press.

Simpson DJ and Knötzel J (1996) Light-harvesting complexes of plants and algae: introduction, survey and nomenclature. In: Ort DR and Yocum CF (eds.) *Oxygenic Photosynthesis*: *The Light Reaction*, pp. 493–506. Dordrecht: Kluwer Academic.

Spikes JD and Bommer JC (1991) Chlorophyll and related pigments as photosensitizer in biology and medicine. In: Scheer H (ed.) *Chlorophylls*, pp. 1181–1204. Boca Raton FL: CRC Press.

Suzuki JY, Bollivar DW and Bauer CE (1997) Genetic analysis of chlorophyll biosynthesis. *Annual Review of Genetics* **31**: 61–89.

von Wettstein D, Gough S and Kannangara CG (1995) Chlorophyll biosynthesis. *Plant Cell* **7**: 1039–1057.

Further Reading

Chadwick DJ and Ackrill K (1994) *The Biosynthesis of the Tetrapyrrole Pigments*. Ciba Foundation symposium 180. Chichester: Wiley.

Dailey HA (1990) *Biosynthesis of Heme and Chlorophylls*. New York: McGraw Hill.

Henningsen KW, Boynton JE and von Wettstein D (1993) Mutants at *xantha* and *albina* loci in relation to chloroplast biogenesis in barley (*Hordeum vulgare L.*). *Biologiske Skrifter* **42**: Munksgaard, Copenhagen. [Whole issue.]

Ort DR and Yocum CF (1996) *Oxygenic Photosynthesis: The Light Reaction*. Dordrecht: Kluwer Academic.

Scheer H (ed.) (1991) *Chlorophylls*. Boca Raton, FL: CRC Press.