Biosynthesis of chlorophylls from protoporphyrin IX

Robert D. Willows

Department of Biological Sciences, Macquarie University, North Ryde 2109, Australia

Received (in Cambridge, UK) 23rd December 2002
First published as an Advance Article on the web 3rd March 2003

Covering: 1989 to 2002. Previous review: *Nat. Prod. Rep.*, 1989, **6**, 171

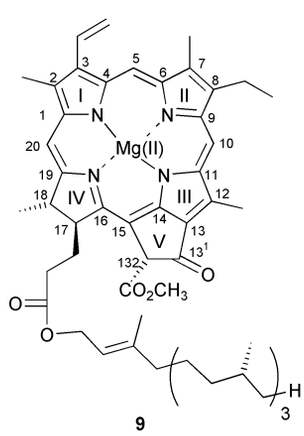
A review of the biosynthesis of chlorophylls and bacteriochlorophylls from protoporphyrin IX with 235 references. The literature on the enzymes magnesium chelatase, *S*-adenosyl-L-methionine:magnesium protoporphyrin IX *O*-methyltransferase, magnesium-protoporphyrin IX monomethyl ester oxidative cyclase, protochlorophyllide oxidoreductase, chlorophyll synthase, bacteriochlorophyll synthase, protochlorophyllide 8-vinyl reductase and chlorophyll *a* oxidase from 1989 is discussed.

Robert Willows was born in 1965 in Sydney, Australia. He received his BSc in Biochemistry and Microbiology at the University of New South Wales in 1987. In 1991 he completed his PhD under the guidance of Professor Barry Milborrow on the biosynthesis of the plant hormone abscisic acid. From 1992–1995 he took up a postdoctoral position at the Carlsberg Laboratory in Denmark to work with Dr Gamini Kanangara, Dr Simon Gough and Professor Diter von Wettstein on chlorophyll biosynthesis. His work on chlorophyll biosynthesis continued at Brown University in the laboratory of Professor Samuel Beale from 1996–1998 before he moved to Macquarie University in 1998 where he is currently a senior lecturer in biochemistry. The focus of the research in his laboratory is primarily on the biosynthesis of chlorophylls.

Robert Willows

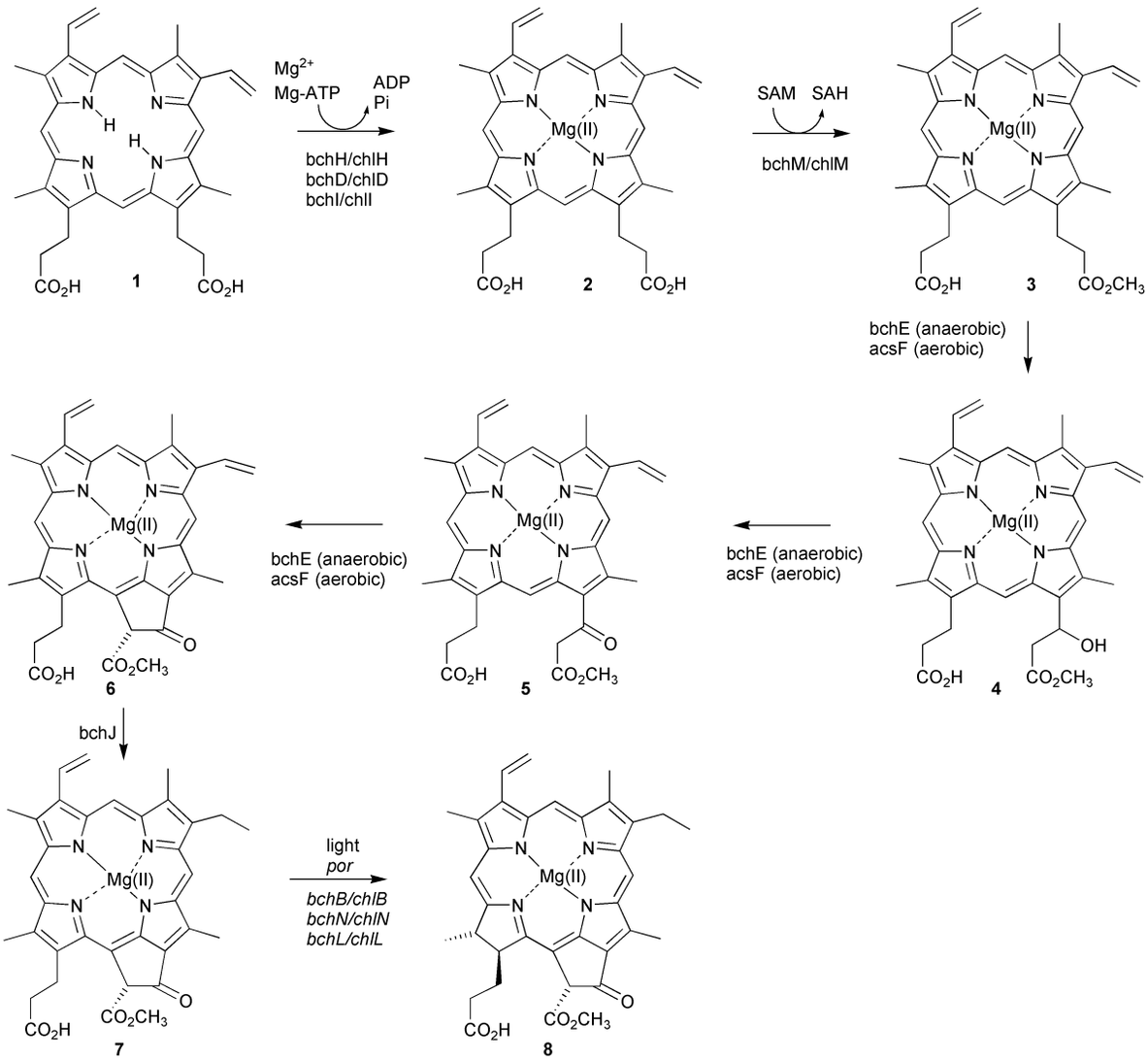
1 Introduction

This review follows the pattern of previous reviews by F. J. Leeper (see Ref. 233–235) but is limited to the biosynthesis of chlorophylls and bacteriochlorophylls from protoporphyrin IX onwards and covers the literature in this field from 1989. Major advances have been made in this period, particularly in regard to the identification of genes encoding the biosynthetic enzymes, characterisation of the enzymes and confirmation of the structures of intermediates in the pathways. The overall scheme from protoporphyrin IX (**1**) to chlorophyllide *a* (**8**) is shown in Scheme 1. This scheme and the identity of the genes involved are generally accepted although additional genes may be required for some steps. The IUPAC numbering scheme is used for the description of intermediates as shown on the structure of chlorophyll *a* (**9**). The details of the enzymology and regulation of each of these steps are covered in sections 2–6. Other steps involved in the synthesis of other chlorophylls and bacteriochlorophylls are covered in sections 7–9.



9

The identification and characterisation of the enzymes responsible for the biosynthesis of both chlorophyll and bacteriochlorophyll have been largely a result of molecular genetic analyses of the photosynthetic gene clusters in *Rhodobacter capsulatus* and *Rhodobacter sphaeroides*. These gene clusters are



Scheme 1

approximately 45 kbp in size and contain most, if not all, of the genes required for assembly of the photosynthetic apparatus in these organisms. This includes the genes encoding the enzymes involved in bacteriochlorophyll biosynthesis from protoporphyrin IX.[1] Directed mutagenesis has identified the genes encoding these bacteriochlorophyll biosynthetic enzymes and heterologous expression in *E. coli* has in many cases confirmed their identity. In addition molecular genetic studies of plants have identified many of the plant genes encoding these enzymes, many of which are orthologous to the bacterial genes which suggests a similar enzymatic mechanism and similar evolutionary origins.

2 Magnesium chelatase

Magnesium chelatase is the first committed step in chlorophyll biosynthesis with the enzymes prior to this step also shared with the heme biosynthetic pathway. Superficially Mg^{2+} insertion into protoporphyrin resembles Fe^{2+} insertion which is catalysed by ferrochelatase. Ferrochelatase is a single subunit enzyme of *ca.* 40 kDa coded for by a single gene which catalyses Fe^{2+} insertion into protoporphyrin without the involvement of any additional cofactors.[2] In contrast, magnesium chelatase has a requirement for ATP and consists of three different protein subunits, known as BchI, BchD and BchH, in organisms that synthesise bacteriochlorophylls and ChlI, ChlD and ChlH in organisms that synthesise chlorophylls, see Walker and Willows 1997.[3] The magnesium chelatase protein subunits from different sources have sequence similarity and are similar in size; the BchI/ChlI homologues being ~40 kDa, the BchD/ChlD homologues being ~65 kDa and the BchH/ChlH homologues being ~140 kDa.[3] The discovery of these genes and the *in vitro* characterisation of magnesium chelatase activity occurred concurrently.

2.1 Magnesium chelatase subunit genes and mutants

Molecular genetic studies of the *R. capsulatus* and *R. sphaeroides* photosynthetic gene clusters have been important for connecting genes with enzymatic functions in the bacteriochlorophyll biosynthetic pathway.[1] Mutations in the *bchI*, *bchD* and *bchH* genes abolish bacteriochlorophyll biosynthesis and cause accumulation of protoporphyrin IX, suggesting a link with magnesium chelatase.[4–6] Mutants in the *bchH* gene lacked both magnesium chelatase and magnesium protoporphyrin IX methyltransferase activity and the two activities were believed to be obligately coupled.[5,7] However, *bchH* was subsequently shown to encode a magnesium chelatase subunit.[8,9] Essentially similar results have also been obtained with the orthologous gene products from *Synechocystis* PCC6803[10] and *Chlorobium vibrioforme*.[11]

Twenty barley mutants at three genetic loci, termed *xantha-f, xantha-g* and *xantha-h*, have been characterised as magnesium chelatase mutants due to their chlorophyll-deficient yellow phenotype and their accumulation of protoporphyrin IX.[12] Based on homology to known BchI, BchH and BchD proteins, the barley *chlI*, *chlH* and *chlD* genes were cloned and sequenced. Analysis of the mRNA transcript levels of these

genes in a number of the *xantha-f, xantha-g* and *xantha-h* mutants indicated that the *chlI* gene is the *xantha-h* locus, the *chlH* is the *xantha-f* locus,[13] and *chlD* is the *xantha-g* locus.[14] Three semidominant alleles of the *xantha-h* locus, originally isolated as pale green *chlorina* mutants, each have single missense mutations.[15]

Arabidopsis thaliana mutants *cs* and *ch-42* have been identified that have defective magnesium chelatase *chlI* genes.[16] These mutants can still make small amounts of chlorophyll using a second *chlI* gene.[17] Two *A. thaliana* mutants having mutations in the *chlH* gene were described and characterised.[18] These two mutants are called *cch* (conditional *chlorina*) and *gun5* (genomes uncoupled).[19] The genomes uncoupled mutants were selected for their ability to express the chlorophyll *a/b* binding protein of photosystem II, *Lhcb1*, under conditions where it is normally not expressed. The *gun5* and *cch* alleles were each found to have single missense mutations. The ChlH protein thus appears to have a dual function in chlorophyll biosynthesis and chloroplast-to-nucleus signal transduction.

Other *chlH* subunit gene mutants have been described in *Antirrhinum majus* (snapdragon) and *Chlamydomonas reinhardtii*. The snapdragon mutant *oli*-605[20] was found to have a transposon insertion in the *ChlH* gene, which encodes a large protein of 1359 amino acids with strong sequence identity to cobaltochelatase CobN subunit and to BchH protein. This was the first indication that the plant and bacterial magnesium chelatase genes were similar.[21] The br_s*-1* mutant in *C. reinhardtii* as well as another chlorophyll-deficient mutant called *chl1* also have mutations in the *chlH* gene.[22] These mutations are insertions that cause a frame-shift resulting in a truncated protein product which does not accumulate.[22]

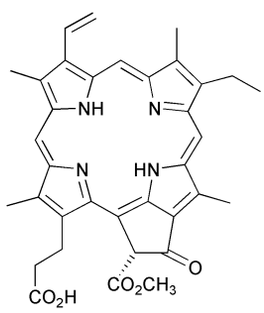
Regulation of magnesium chelatase gene expression has been examined in a number of plant species. In etiolated barley, the *chlI* and *chlH* genes are up-regulated by light.[13] In green barley seedlings grown in normal daylight cycles the *chlH* gene then follows a circadian rhythm with maximal expression in the light phase.[13] The tobacco *chlH* and *chlI* genes follow a similar circadian pattern of expression but the *chlD* gene has an inverse expression pattern with maximal mRNA levels in the dark phase. In *Arabidopsis thaliana*[23] and *Antirhinnum majus*[21] the *chlH* gene has maximal expression in the dark and is down-regulated in the light. In addition, the *chlI* gene from barley and *A. thaliana* is constitutively expressed except during the initial phases of greening.

2.2 Characterisation of magnesium chelatase activity

Magnesium chelatase activity requires ATP, Mg^{2+} and all three magnesium chelatase subunits. The reaction has been dissected into two phases. The first phase involves formation of a complex between the BchI/ChlI and BchD/ChlD proteins and is dependent on both the protein concentration and ATP.[9,24–27] This complex catalyses magnesium insertion into protoporphyrin only when combined with the BchH/ChlH protein, Mg-ATP, protoporphyrin IX and Mg^{2+}. The BchH/ChlH protein behaves as a substrate in the magnesium chelatase reaction and has a K_m in the low micromolar range.[9,28,29]

The characterisation of magnesium chelatase activity prior to 1989 was limited to measurements made *in organello* using developing chloroplasts from cucumber or in spheroplasts of *Rhodobacter sphaeroides* (see Leeper 1989, Ref. 233). Magnesium chelatase activity has since been demonstrated using isolated chloroplasts from tobacco,[30,31] barley[13,32,33] and pea.[25,34] True *in vitro* measurements of activity have also been obtained from lysed chloroplasts using methods developed by Walker and Weinstein,[25] from whole cells and lysates of *Rhodobacter sp.*,[35,36] and with purified protein subunits from a number of different species that were heterologously expressed in *E. coli*.[8,9,11,26,27,37]

The substrate specificity and effect of substrate analogues on magnesium chelatase activity were determined using *in organello* assays. Cucumber magnesium chelatase used protoporphyrin IX as the preferred substrate but deuteroporphyrin IX, mesoporphyrin IX, 3-ethyl, 8-vinyl protoporphyrin IX and 3-vinyl, 8-ethyl protoporphyrin IX were also effective substrates.[38] *N*-Methylprotoporphyrin and *N*-methylmesoporphyrin were both effective inhibitors with 50% inhibition at 3 μM but protochlorophyllide (**7**), chlorophyllide (**8**), heme or magnesium-protoporphyrin (**2**) had very little effect on activity.[38] Pheophorbide (**10**) was a potent inhibitor of barley magnesium chelatase with 50% inhibition at 0.92 μM while chlorophyllide and zinc pheophorbide were only slightly inhibitory, and chlorophyll (**9**) and pheophytin were not inhibitory.[32] In contrast, when magnesium protoporphyrin (**2**) or its monomethyl ester (**3**) is generated *in situ* in pea seedlings, magnesium chelatase activity is strongly inhibited and is inversely dependent on the total concentration of **2** and **3**.[39,40] The possibility exists that the metalated porphyrins may not effectively penetrate the chloroplast in these studies. Inhibition by pheophorbide may have a physiological function as it forms during leaf senescence when chlorophyll is being degraded, thus it may act to prevent further chlorophyll biosynthesis by inhibition of magnesium chelatase.[32]



10

True *in vitro* magnesium chelatase activity was obtained using broken pea chloroplasts[25] and activity was fractionated into soluble and non-thylakoid membrane fractions, both of which were largely devoid of chlorophyll.[41,42] A minimum protein concentration of 2 mg ml^{-1} was required to measure activity in the *in vitro* assay and the assay had a lag phase before the insertion of magnesium into protoporphyrin began.[41] The lag phase could be eliminated by preincubation with ATP and extended by preincubation in the absence of ATP. The slowly hydrolysable ATP analogue, ATP-γ-S, substituted for ATP in elimination of the lag phase of the reaction but not in the metal ion insertion phase. Complete elimination of the lag phase required a minimum protein concentration of 12 mg ml^{-1}, while the magnesium insertion phase was not dependent on a minimum protein concentration after the preincubation to eliminate the lag phase.[42] These results suggested that the lag phase involved the formation of an ATP-dependent and protein concentration-dependent complex.

In contrast to the results obtained with pea, magnesium chelatase activity from lysed cucumber chloroplasts was found in the membrane fraction and required stabilization with protoporphyrin IX, $MgCl_2$ and ATP. It was also found that addition of the soluble fraction inhibited the membrane-associated activity.[43] Cross-species complementation between subunits of the pea and cucumber magnesium chelatases was possible. A membrane fraction of the cucumber chloroplasts obtained without the stabilizing agents was inhibitory to pea magnesium chelatase, while the soluble component stimulated magnesium chelatase activity when combined with the membrane fraction from pea chloroplasts.[25]

Complete solubilisation of the pea magnesium chelatase

proteins was achieved by lysis of the chloroplasts in magnesium-free buffer and subsequent concentration to a protein concentration of 10–20 mg ml^{-1}.[24] Fractionation of the pea chloroplast magnesium chelatase components into separate ChlI, ChlD, and ChlH protein fractions was achieved by chromatography on Cibachrome-Blue followed by size fractionation using ultrafiltration.[24] Reconstitution of activity required all three components and preincubation of the ChlD and ChlI proteins with ATP overcame the lag phase[24] as also found for the bacterial enzymes.[9,26,28] These results suggested that an ATP- and protein concentration-dependent complex needs to form between the BchI/ChlI and BchD/ChlD subunits before interaction with the BchH/ChlH subunit for metal ion insertion.

The ChlD subunit from pea was heterologously expressed in *E. coli* and crude protein extract from this expression combined with partially purified ChlI and ChlH to yield magnesium chelatase activity. The expression of the ChlD required co-expression of the chaperone, DnaK, to fold correctly and reconstitute activity.[44]

Barley magnesium chelatase was also fractionated into soluble and membrane fractions and both fractions were required for activity. The membrane fraction contained both the ChlD and ChlH proteins. Further fractionation of the membrane fraction, from both barley and *R. sphaeroides*, showed that the BchD/ChlD protein from both species was associated, or at least copurified, with ribosomes.[45]

The first *in vitro* assay of a bacterial magnesium chelatase was with the BchH, BchI, and BchD proteins of *R. sphaeroides* that were heterologously expressed in *E. coli*.[8] Magnesium chelatase activity was only reconstituted when a cell extract from *E. coli* coexpressing both BchI and BchD was mixed with cell extract from *E. coli* expressing the BchH protein in a buffer containing an ATP regenerating system. Magnesium chelatase subunits from *C. vibrioforme*,[11] *Synechocystis* PCC6803[27] and *R. capsulatus*[9] have also been heterologously expressed in *E. coli* and in all cases reconstitution of activity required all three subunits, an ATP regenerating system and protoporphyrin IX. Heterologous expression in *E. coli* of the BchI, BchD, and BchH subunits from the aerobic acidophilic bacterium *Acidophilium rubrum*, which has Zn-containing bacteriochlorophyll as its major light harvesting pigment, was also performed. However, *in vitro* activity could not be reconstituted, although all three subunits were able to complement the corresponding *R. capsulatus* mutants, indicating that they coded for functional magnesium chelatase subunits.[46]

The *R. sphaeroides* BchH and BchI proteins expressed in *E. coli* were the first magnesium chelatase proteins to be purified to homogeneity. The purified BchH protein was red in colour due to bound protoporphyrin. Added protoporphyrin IX was not absolutely required as it was already bound to the purified BchH protein, but additional protoporphyrin IX yielded higher activity and deuteroporphyrin could substitute for protoporphyrin.[26,41] As in all magnesium chelatases studied to date,[28] a lag period in activity time courses was observed, which could be overcome by preincubation of the BchI and BchD (or ChlI and ChlD) proteins with Mg-ATP.[26]

The BchI, BchD and BchH proteins of *R. capsulatus* were heterologously expressed in *E. coli* both with and without an *N*-terminal 6xHis-Tag and all proteins with the exception of the non-His-tagged BchH were purified to apparent homogeneity.[9] The His-tagged and non-tagged BchD proteins were expressed as inclusion bodies and were purified and then solubilised in 6 M urea. Magnesium chelatase activity was reconstituted when all three non-His-tagged BchI, BchD, and BchH proteins were mixed. The urea-solubilised BchD protein was added directly to the assay but it could also be refolded by rapid dilution in buffer containing Mg^{2+}, ATP, DTT at 0 °C. Optimal refolding of the BchD protein also required the presence of the BchI protein. The His-tagged BchD and BchH proteins could substitute for the non-His-tagged proteins, but the His-tagged BchI protein was inactive.[9] This was in contrast to His-tagged BchI/ChlI proteins from *R. sphaeroides*,[47] *C. vibrioforme*[11] and *Synechocystis* PCC6803[28] which were all active in magnesium chelatase assays.

The *R. sphaeroides* and *C. vibrioforme* BchI, BchD and BchH proteins were also heterologously expressed in *E. coli* with an *N*-terminal 6xHis-tag.[11,47] The proteins were purified and could reconstitute Mg chelatase activity. The BchI protein from *C. vibrioforme* had two potential start sites for translation, which when expressed yielded products of 42 kDa and 38 kDa. Both products could be used in magnesium chelatase assays with the BchD and BchH proteins.[11] Both products are produced *in vivo* by *C. vibrioforme*; the 42 kDa protein predominated in the exponential growth phase while the 38 kDa band was the predominant protein in the stationary phase.[11]

Similarly the three *Synechocystis* PCC6803 magnesium chelatase proteins were all heterologously expressed in *E. coli* with[27] and without a 6xHis-tag.[28] The proteins without a His-tag were not purified but the proteins with a 6xHis-tag were purified.[28] The ChlD proteins were both soluble, in contrast to the *R. sphaeroides*[47] and *R. capsulatus*[9] BchD proteins which were insoluble. Also, the ChlH protein lost its bound protoporphyrin IX when purified by anion exchange chromatography,[28] in contrast to the *R. sphaeroides* and *R. capsulatus* BchH/ChlH proteins which retained protoporphyrin IX during purification.[9,26]

The tobacco ChlI protein was expressed in *Saccharomyces cerevisiae* together with ChlD and ChlH fusion proteins and magnesium chelatase activity was measured in a soluble extract. Activity required expression of all three proteins, ATP, protoporphyrin IX and Mg^{2+} but the product of the reaction was magnesium protoporphyrin monomethyl ester.[48] This unusual result was due to an endogenous methyltransferase present in *S. cerevisiae*.[48]

The effect of protein-modifying reagents on magnesium chelatase activity was investigated using intact developing cucumber chloroplasts[38] and lysates from *R. sphaeroides*.[36] Reagents that modify cysteine residues in proteins were potent inhibitors of magnesium chelatase with 50% inhibition achieved using 50 μM *N*-ethylmaleimide (NEM), 100 μM *p*-chloromercuribenzoyl sulfonate or 50 μM *p*-chloromercuribenzoate,[38] and 60% inhibition with 22 μM thiomersal.[36] This suggests that a cysteine residue is involved in catalysis. *N*-Ethylmaleimide was found to specifically inhibit the ChlI and ChlH proteins of *Synechocystis* PCC6803.[49] Four cysteine residues are present in the sequence of the *Synechocystis* PCC6803 ChlI protein and three of these react with NEM. The NEM inhibited both the ATPase and magnesium chelatase activities but it did not prevent ChlI–ChlD complex formation, which means that nucleotide binding is sufficient for this complex formation. The ChlI activity could be completely protected from NEM inactivation if either Mg-ATP, ATP or ADP were present. The ChlH protein could be partially protected from *N*-ethylmaleimide inactivation only if ATP, Mg^{2+} and protoporphyrin IX were all present. There are 11 cysteines in the ChlH of *Synechocystis* PCC6803, which suggests that at least one of these is essential for activity. The most likely candidate is Cys638, *R. capsulatus* numbering, which is conserved in all BchH/ChlH proteins.[49]

In addition to protein modifying agents and tetrapyrrole substrate analogues magnesium chelatase can be inhibited by a variety of other compounds. These inhibitors can be classified according to their probable mode of inhibition as: ATPase inhibitors, metal ion chelators, and other non-classifiable inhibitors. The cucumber[38] and *Synechocystis* PCC6803[50] magnesium chelatases were both similarly inhibited by the non-hydrolysable ATP analogues, β,γ-methylene ATP and

β,γ-imino ATP. The cucumber magnesium chelatase was also inhibited by the metal ion chelators 2,2-dipyridyl and 1,10-phenanthroline.[38] The metal ion chelators inhibit both the ATPase activity of individual subunits as well as magnesium chelatase activity.[51] In addition, Co(III)–ATP–1,10-phenanthroline, a reagent which labels ATPases, inhibited magnesium chelatase activity and bound to all three subunits.[51] Another common ATPase inhibitor, sodium fluoride, had no effect on the ATPase activity associated with the BchD or BchI subunits from *R. sphaeroides* but caused ATP to bind to the BchH subunit.

A number of other inhibitors of magnesium chelatase activity have also been described but their mode of action is unclear. All three hydroxybenzoic acid methyl ester isomers were found to inhibit magnesium chelatase of cress and barley seedlings.[33] Magnesium chelatase from barley and *R. sphaeroides* was inhibited by chloramphenicol and *p*-aminosalicylic acid.[45] The *R. capsulatus* magnesium chelatase was 50% inhibited by 300 mM urea which may act by disrupting the interactions between magnesium chelatase subunits.[9] Light has also been shown to inhibit the magnesium chelatase of barley[32] and *Rhodobacter*[9] and inhibition probably occurs *via* photo-oxidative damage of the BchH/ChlH subunit.[9] The inhibition by light of barley magnesium chelatase was demonstrated with isolated chloroplasts and contrasts to what occurs *in planta* where the activity from isolated chloroplasts of barley from etiolated barley seedlings exposed to 4 h of light is considerably higher than the activity from chloroplasts not exposed to light.[13] This increase in activity *in planta* can be attributed to the increased synthesis of the ChlI and ChlH subunits[13] and is supported by data showing that magnesium protoporphyrin and magnesium protoporphyrin monomethyl ester levels increase dramatically in leaves from barley or tobacco when transferred from dark to light.[52]

2.3 Analysis of individual magnesium chelatase subunits

The magnesium chelatase subunits of *R. sphaeroides*,[47,51] *Synechocystis* PCC6803[50,53] and *C. vibrioforme*[53] have been analyzed for ATPase activity since BchI/ChlI and a number of the BchD/ChlD protein sequences have highly conserved ATPase motifs. The specific activities vary considerably between the studies as well as which subunits have activity. All studies found that the BchH/ChlH and BchI/ChlI subunits had ATPase activity while the ATPase activity of the BchD/ChlD subunit was variable and may be due to the purity of the BchD/ChlD protein used. Variation in specific activity may also reflect the method used to detect ATPase activity. Two studies used radiochemical methods to directly measure ATPase activity[51,53] while the remaining studies used a coupled assay which detected the phosphate released as a result of ATP hydrolysis.[47,50] Some general features can be gleaned from these studies. Firstly, BchI/ChlI seems to have the highest ATPase activity, with lower activity for the BchH/ChlH protein and no activity for BchD/ChlD protein. The ATP hydrolysis is dependent on Mg^{2+}, but the porphyrin substrate or the addition of other subunits does not in general stimulate the ATPase activity of individual subunits. Two studies of ATP hydrolysis by the magnesium chelatase subunits have reported an ATP-to-ADP phosphate exchange activity, where the γ phosphate from an ATP molecule was transferred to an ADP,[51,53] although its relevance in the magnesium chelatase reaction remains unclear.

Although the ATPase activity is well documented its function is not understood. It was hypothesized that the ATPase activity by BchI/ChlI is required for the initial activation step in the magnesium chelatase reaction involving BchI/ChlI, BchD/ChlD, ATP and Mg^{2+}.[51] More recent studies showed that binding of ATP rather than ATP hydrolysis is sufficient to form a BchI/ChlI:BchD/ChlD complex[47,50] and previous studies with pea magnesium chelatase using the non-hydrolysable analogue ATP-γ-S[42] have also suggested that the complex formation does not require ATP hydrolysis.

It seems likely that the ATP hydrolysis by BchI/ChlI subunits is the driving force behind the magnesium insertion reaction which has been shown to increase markedly when all of the subunits and substrates are present. As ATP and magnesium appear to be required for the efficient binding of porphyrin onto the BchH/ChlH subunit[37] it is hypothesized that the function of ATP hydrolysis by BchH/ChlH is for this binding process.

The BchH/ChlH subunit from bacteria and cyanobacteria binds protoporphyrin IX.[9,26,28] When deuteroporphyrin is bound to the BchH/ChlH protein, the absorbance and excitation peaks are red shifted by up to 8 nm and the fluorescence yield is reduced compared to deuteroporphyrin in solution.[37] This is also true of protoporphyrin IX bound to BchH/ChlH.[9] These spectral properties of the porphyrin bound to the BchH/ChlH protein are consistent with the porphyrin being in a distorted nonplanar conformation.[37] The quenching of tryptophan fluorescence in the BchH/ChlH protein when porphyrin is bound enabled the calculation of the K_D for deuteroporphyrin binding to the BchH/ChlH proteins of *Synechocystis* PCC6803 ($K_D = 0.53 \pm 0.12$ μM) and *R. sphaeroides* ($K_D = 1.22 \pm 0.42$ μM).[37] A single porphyrin binding site was evident which is consistent with previous estimates.[9,26] Somewhat surprisingly, magnesium deuteroporphyrin also had similar K_D values but it was not determined if the magnesium deuteroporphyrin binding site was the same as the deuteroporphyrin binding site.[37] If the binding sites are the same this would most likely result in strong product inhibition. Attempts to calculate the K_D for protoporphyrin IX were not successful because of the tendency of protoporphyrin IX to aggregate.[37]

The identity of the subunit that binds the magnesium ion for insertion into protoporphyrin IX is difficult to address because magnesium is also required as magnesium ATP in the reaction. The CobN subunit of cobaltochelatase, which is homologous to the BchH/ChlH protein, binds both metal and corrinoid substrates (hydrogenobyrinic acid a,c-diamide and Co^{2+}) forming a ternary enzyme–Co^{2+}–corrinoid complex.[54] This suggests that as the BchH/ChlH protein binds protoporphyrin IX,[26] it will also bind the magnesium ion which is inserted into protoporphyrin IX in an analogous manner to the binding of both metal and tetrapyrrole substrates to the CobN subunit.

2.4 Structural studies and proposed mechanism for magnesium chelatase

Crystals of BchI from *R. capsulatus* were obtained[55] and the structure of this subunit has been determined by X-ray crystallography (PDB accession code 168P).[56] Both the structure and primary sequence of BchI show that it belongs to the AAA+ or extended ATPases Associated with a variety of cellular Activities class of proteins. This is one of the largest and most diverse classes of proteins known and is present in all organisms from all kingdoms.[57,58] These proteins have numerous roles in cellular activity including: proteolysis, protein folding, membrane trafficking, cytoskeletal regulation, organelle biogenesis, DNA regulation and intracellular motility. The AAA+ proteins are known to form nucleotide-dependent ring structures, which are usually hexameric, and many form double hexameric rings. In the double ring structure the second ring of AAA+ modules often has an inactive ATPase and this ring presumably has a structural role. AAA+ proteins have also been called mechanoenzymes due to observed large conformational changes in AAA+ proteins on ATP hydrolysis and the mechanical nature of the processes in which AAA+ proteins are involved.[58] The closest structural relatives to BchI are the NSF-D2 domain involved in vesicle fusion, the δ′ subunit of the clamp loading complex of the DNA polymerase and the HslU subunit of the ATP dependent protease.[56]

The *N*-terminus of BchD/ChlD, which is homologous to BchI/ChlI, also has an AAA+ module. Three other domains or features are evident in all BchD/ChlD subunits. The *C*-terminal region of BchD/ChlD has a polyproline immediately followed by a 20–40 amino acid negatively charged region then by an integrin-I domain containing a metal ion dependent adhesion site or MIDAS motif.[56] Integrin-I domains are known to interact with RGD and LDV sequences through the carboxyl group of the aspartate in these sequences[59] and conserved LDV and RGE motifs are present in both the BchH/ChlH and BchI/ChlI sequences. This suggests that the integrin-I domain may mediate interactions between all three subunits.

Using electron microscopy it was shown that BchI forms a nucleotide dependent hexamer[56] and gel filtration indicates that this hexamer is not stable.[60] Experiments using mutants of BchI that lack ATPase activity demonstrated that coordinated ATP hydrolysis of a number of subunits in the hexamer is required for magnesium chelatase activity.[60]

Based on these observations the most likely mechanism is one in which nucleotide dependent homohexameric rings of BchI/ChlI and BchD/ChlD form. These two rings would then form a stacked double hexameric ring structure.[56] This double hexamer can then act on the BchH/ChlH subunit which has protoporphyrin IX and magnesium bound. Concerted ATP hydrolysis from the majority of subunits within the BchI/ChlI hexamer is required for magnesium chelatase activity. This hydrolysis would presumably cause a conformational change in BchH/ChlH to effect metal ion insertion.[60]

3 *S*-Adenosylmethionine:magnesium protoporphyrin IX *O*-methyltransferase (EC 2.1.1.11)

The next step in the pathway is the *S*-adenosylmethionine dependent methylation of the carboxyl group of the 13-propionate side-chain on magnesium protoporphyrin IX catalysed by *S*-adenosylmethionine:magnesium protoporphyrin IX *O*-methyltransferase. The *O*-methyltransferase from wheat chloroplasts was shown to have a ping-pong type mechanism based on the exchange of unlabeled *S*-adenosyl-L-methionine with radiolabeled *S*-adenosyl-L-homocysteine.[61] When wheat leaves were fed δ-aminolevulinic acid, magnesium protoporphyrin IX monomethyl ester and protochlorophyllide accumulated and the methyltransferase activity in extracts decreased. This decrease in activity was attributed to product inhibition by magnesium protoporphyrin IX monomethyl ester.[62] Sinefungin, an inhibitor of *S*-adenosylmethionine-dependent methylation reactions, was found to be an effective inhibitor of the barley enzyme when delivered *via* the transpiration stream.[63]

The *bchM* gene encoding the *S*-adenosylmethionine:magnesium protoporphyrin IX *O*-methyltransferase was identified from *Rhodobacter*. The protein product of the heterologously expressed *bchM* gene of *R. capsulatus* and *R. sphaeroides* had the *O*-methyltransferase activity when assayed *in vitro*.[64,65] The *R. capsulatus* methyltransferase is stimulated by the BchH subunit of magnesium chelatase[66], which suggests that magnesium protoporphyrin may be channelled directly to this enzyme from the magnesium chelatase by the BchH subunit. The *R. sphaeroides bchH, bchI*, *bchD*, encoding magnesium chelatase genes, and the *bchM* gene were heterologously expressed together in *E. coli* causing accumulation of magnesium protoporphyrin IX monomethyl ester.[67]

The *O*-methyltransferase gene of *Synechocystis* PCC6803 was cloned by complementation of an *R. capsulatus* strain with a defined mutation in the *bchM* gene.[68] There was only 29% protein sequence identity between the *R. capsulatus* and the *Synechocystis* PCC6803 *S*-adenosylmethionine:magnesium protoporphyrin IX *O*-methyltransferases. When this gene was placed into this *R. capsulatus* mutant under the control of the strong *R. capsulatus puc* promoter the mutant produced nearly wild-type levels of bacteriochlorophyll *a*.[68] The *O*-methyltransferase gene has also been cloned and sequenced from tobacco and *A. thaliana*.[69] The *A. thaliana* enzyme was found to localise to both the thylakoid and envelope membranes within the chloroplast.[69]

4 Magnesium protoporphyrin IX monomethyl ester oxidative cyclase

An oxidative cyclization is required to create the fifth ring of chlorophyll and this reaction is catalysed by magnesium protoporphyrin IX monomethyl ester oxidative cyclase. Prior to 1989, the cucumber oxidative cyclase had been fractionated into two components and the hydroxy (**4**) and keto (**5**) derivatives shown in Scheme 1 were identified intermediates (see Leeper 1989, Ref. 233). Since then activity has been described from a number of other sources, the oxidation mechanism has been investigated using isotopic labelling and a number of protein-encoding genes involved in this reaction have been identified.

Oxidative cyclase activity has been demonstrated with chloroplasts of *C. reinhardtii*,[70] developing chloroplasts from cucumber cotyledons,[71] lysed cucumber and *C. reinhardtii* chloroplasts[72–75] and with cell free extracts from cyanobacteria.[72] The oxidative cyclase from cucumber chloroplasts was resolved into membrane and soluble components. Reconstitution of activity required both fractions, NADPH, magnesium protoporphyrin IX monomethyl ester and a pH optimum of 9.0. The soluble fraction was purified 40-fold by ammonium sulfate fractionation and chromatography on phenyl sepharose. Pretreatment of the pellet fraction with either 8-hydroxyquinoline or desferal mesylate inhibited cyclase activity, indicating that there is a metal ion requirement in this fraction.[75] Dialysis of the reconstituted system eliminated activity and could only be restored by addition of Na^+, K^+ and Mg^{2+} ions.[74] Inhibition of the cucumber cyclase by inhibitors of P450 enzymes was not consistent. The reconstituted system was inhibited by hemoprotein inhibitors such as azide and KCN but very little inhibition was achieved in intact chloroplasts using these same inhibitors. Benzoquinone and quinol were also strong inhibitors of the cyclase.[73] The soluble fraction from cucumber chloroplasts used for cyclase activity degraded magnesium protoporphyrin IX monomethyl ester and other pigments found in thylakoid membranes to colourless compounds. This suggests that some of the variability of cyclase activity may be caused by changes in the balance between cyclase activity and degradation of substrates and products.[76]

The *Synechocystis* cyclase was also separated into soluble and membrane components, both of which were required to reconstitute activity together with O_2 and NADPH. The membrane component was partially purified after solubilising with n-octyl-β-D-glucopyranoside in the presence of glycerol and Mg^{2+}. Inclusion of catalase and isoascorbate increased the yield of product, possibly by protecting against oxidative damage.[72] In contrast to the cyclases from *Synechocystis* and cucumber, *C. reinhardtii* cyclase activity was found associated with the membranes and did not require a soluble component. The *C. reinhardtii* cyclase was not inhibited by the flavoprotein inhibitor quinacrine or by the hemoprotein inhibitors CO, KCN, or NaN_3.[72]

β-Thujaplicin is a tropolone compound that is an effective chelator of Fe^{2+}.[77] When plants are treated with β-thujaplicin and aminolevulinic acid, protochlorophyllide synthesis is inhibited and accumulation of magnesium protoporphyrin monomethyl ester occurs.[78] One common feature of all cyclases studied is that they are all inhibited by chelators of Fe^{2+}, suggesting that nonheme iron is involved in the reaction. As only hydrophobic Fe^{2+} chelators appear to be effective inhibitors, it was suggested that the Fe^{2+} requirement is associated with the membrane fraction.[72]

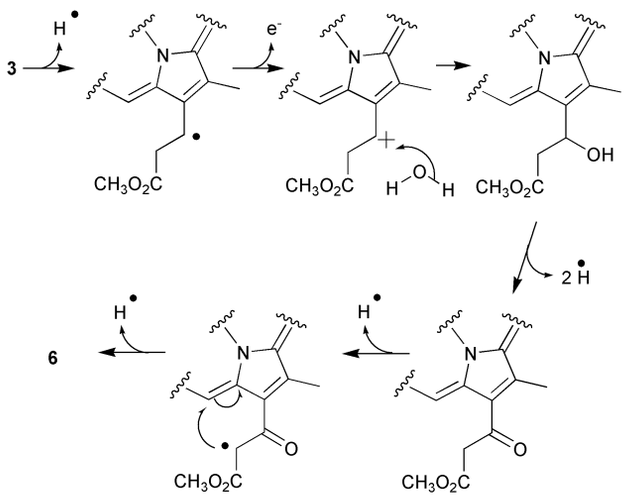
The origin of the oxygen atom in the fifth ring was studied by ^{18}O labelling studies using both $^{18}O_2$ and/or $H_2{}^{18}O$. Cucumber cotyledons incubated in a nitrogen atmosphere containing 20% $^{18}O_2$ in the dark accumulated protochlorophyllide which was isolated, converted to methyl phaeoporphyrin a5 and analyzed by mass spectrometry. The molecular ion of the methyl phaeoporphyrin a5 derived from this treatment was 2 mass units greater than that of the control, establishing that the oxo group of the isocyclic ring is derived from atmospheric oxygen.[79] Similar experiments using $H_2{}^{18}O$ in *R. sphaeroides* indicated that the oxygen atom in the fifth ring of bacteriochlorophyll is derived from water, suggesting a different mechanism for the oxidative cyclase than that in higher plants.[80] *Rhodovulum sulphidophilum*, which, unlike *R. sphaeroides*, is capable of producing large amounts of bacteriochlorophyll under aerobic conditions, incorporates O into the fifth ring using two different mechanisms. When *Rv. sulphidophilum* is grown aerobically ^{18}O from $^{18}O_2$ is incorporated into the fifth ring and when it is grown anaerobically ^{18}O from $H_2{}^{18}O$ is incorporated. This suggests that *Rv. sulphidophilum* has two enzymes for oxidative cyclisation: a dehydrogenase/hydratase which uses H_2O and an oxygenase which uses O_2.[81]

Directed mutagenesis of the *bchE* gene of *R. capsulatus* results in accumulation of magnesium protoporphyrin IX monomethyl ester which suggests that this gene encodes part of the oxidative cyclase.[82] A homologue of this gene has also been identified in *Synechocystis* PCC 6803 (see Suzuki *et al.*[83]). No homologues of the *bchE* gene have been identified in the *A. thaliana* genome, which suggests that a different gene or genes code for this activity in plants. Considering that oxygen-dependent and oxygen-independent mechanisms exist for cyclisation it seems reasonable to assume that the *bchE* gene codes for an oxygen-independent cyclase.

The purple bacterium *Rubrivivax gelatinosus* synthesizes bacteriochlorophyll *a* under both aerobic and anaerobic conditions in a similar manner to *Rv. sulphidophilum*. Disruption of the *acsF* gene of *Rx. gelatinosus* prevents bacteriochlorophyll *a* synthesis and causes accumulation of magnesium protoporphyrin IX monomethyl ester under aerobic conditions but not under conditions of low aeration. The designation *acsF* stands for aerobic cyclization system Fe-containing subunit, as AcsF and its homologues have a conserved putative binuclear-iron-cluster binding motif.[84] The AcsF protein is homologous to previously identified genes in *Chlamydomonas reinhardtii* called Crd1[85] and Cth1[86] and homologues of AcsF were also identified in *A. thaliana* and *Synechocystis*.[84]

Crd1 and Cth1 expression in *C. reinhardtii* is reciprocal and is regulated by copper and/or oxygenation conditions. Crd1 is expressed under low aeration and/or low copper conditions and Cth1 is expressed under oxygenated and copper-sufficient conditions. Mutation of either of these genes and growth under conditions where the alternative protein is not expressed result in a chlorotic phenotype with reduced photosystem I and light harvesting 1 accumulation.[85,86] These results suggest that the Crd1 and Cth1 proteins probably encode two isoforms of the oxidative cyclase. Two mutant loci in barley called *xantha-l*[35] and *viridis-k*[23] also have defective cyclase activity,[87] raising the possibility of two isoforms of the enzyme in barley. However, the barley genes have not been identified.

A mechanism for the oxygen-independent cyclase has been suggested by Gough *et al.* based on sequence analysis of *bchE* and studies with vitamin B_{12} deficient mutants of *R. capsulatus*. The BchE proteins have four conserved cysteines. These are thought to make up a putative Fe–S cluster which would explain the iron requirement for the functional enzyme. In addition a section of the BchE protein sequence aligns to a B_{12}-dependent P-methylase from *Streptomyces hygroscopicus*. Vitamin B_{12} deficient strains of *R. capsulatus* accumulate magnesium protoporphyrin IX monomethyl ester and this accumulation is reversed when vitamin B_{12} is supplied to the cells. A free radical mechanism in which adenosylcobalamin is a hydrogen atom acceptor and the Fe–S cluster is an electron acceptor was proposed as shown in Scheme 2.[88]



Scheme 2

5 Reduction of the 8-vinyl group

The majority of the work on the 8-vinyl reduction step continues to come from the group of Rebeiz. They have shown that the reduction of the 8-vinyl group can probably occur at any stage from protoporphyrin IX to chlorophyllide *a*. This finding is supported by numerous studies in which 8-vinyl and 8-ethyl derivatives of these intermediates have been detected.[89–92] The relative amounts of 8-vinyl and 8-ethyl intermediates and the stage at which reduction occurs are complex and depend on numerous factors such as species, developmental stage, time in the dark or light, the age of the tissue and light intensity.[89] Virtually all photosynthetic organisms require reduction of the 8-vinyl group of chlorophyll or bacteriochlorophyll to an ethyl group. However, certain marine *Prochlorococcus* species accumulate 8-vinyl chlorophylls *a* and *b* in addition to or instead of the 8-ethyl pigments.[93,94]

In the previous review (see Leeper 1989, Ref. 233) it was reported that the 8-vinyl reductase reducing 8-vinyl-protochlorophyllide to 8-ethyl-protochlorophyllide was membrane associated. Since then a method has been described for the separation of 8-vinyl-protochlorophyllide and 8-ethyl-protochlorophyllide using a solid phase polyethylene column. This was used to analyse the biosynthesis of both of these intermediates in wheat and cucumber cotyledons. The activity in wheat was higher than in cucumber and it was suggested that the reaction is reversible.[95] An 8-vinyl reductase activity was detected in plastid membranes from cucumber that converts 8-vinyl-chlorophyllide *a* to chlorophyllide *a* (**8**) but is unable to convert 8-vinyl-protochlorophyllide to 8-ethyl-protochlorophyllide.[90,96] It has been suggested that a soluble component may mediate the substrate specificity of the 8-vinyl reductase allowing other 8-vinyl intermediates to be converted to 8-ethyl forms which would explain the diversity of 8-ethyl intermediates that have been observed.[92]

No genes have yet been identified that are absolutely required for reduction of the 8-vinyl group. However, disruption of the *bchJ* gene of *R. capsulatus* alters the ratio of 8-ethyl- (**7**) to 8-vinyl-protochlorophyllide (**6**) in mutants that accumulate protochlorophyllide.[97]

6 Protochlorophyllide oxidoreductases

Two types of enzymes have been identified that reduce the D pyrrole ring of protochlorophyllide to form chlorophyllide. The most studied of these two enzymes is the light-dependent

NADPH-protochlorophyllide oxidoreductase (EC 1.3.1.33 or EC 1.6.99.1, abbreviated LPOR), which has been the subject of a number of reviews.[98–107]

LPOR is a single subunit enzyme that requires light as a substrate and it appears to be present in all organisms that synthesize chlorophyll but has not been found in bacteriochlorophyll synthesizing organisms. The second type of enzyme, known as the light-independent protochlorophyllide oxidoreductase or DPOR, is a multisubunit enzyme that consists of at least three subunits (reviewed by Armstrong[107]). The multisubunit DPOR has not been found in flowering plants (angiosperms) but appears to be present in most other chlorophyll and bacteriochlorophyll synthesizing organisms and allows these organisms to make chlorophyll in the dark. When angiosperms are germinated in the dark they accumulate small amounts of protochlorophyllide bound together with NADPH and LPOR in a ternary complex and they are unable to synthesize chlorophyll until this bound protochlorophyllide is converted to chlorophyllide when exposed to light. This has led to the widespread belief that angiosperms are unable to synthesize chlorophyll in the dark. However, there are numerous reports that mature green leaves of some angiosperms can synthesize chlorophyll in the dark (reviewed by Adamson *et al.*[105]). This suggests that DPOR may be present in mature leaves of some angiosperms or that another as yet uncharacterised mechanism exists for chlorophyll synthesis in the dark in these plants.

6.1 Light-dependent oxidoreductases (EC 1.6.99.1 or EC 1.3.1.33) (LPOR)

The first LPOR encoding gene, *por*, was isolated from barley (*Hordeum vulgare*).[108] Since then, *por* genes have been isolated and sequenced from many plants and algae including *A. thaliana*,[109] *Triticum aestivum*,[110] *Nicotiana tobacum*,[111] cucumber,[112] *Pisum sativum*,[113,114] *Pinus taeda*,[115] *Pinus mugo*,[116] *C. reinhardtii*[117] as well as from the cyanobacterium *Synechocystis* PCC6803.[118] Multiple isoforms of LPOR have been found in *A. thaliana*,[109,119,120] *P. taeda*,[121] *P. mugo*,[116] barley[122] and tobacco.[111] In barley and *A. thaliana* the isoforms are differentially expressed and the isoform called LPORA appears to have a role only in the de-etiolation process.[109,122] However, this type of differential regulation of isoforms does not appear to be universal as the two tobacco *por* genes are similarly regulated.[111] Some plants such as cucumber only have a single *por* gene, indicating that multiple isoforms are not essential for plant growth and development.[112]

Phytochrome, circadian clocks, cytokinin, abscisic acid and leaf age have all been implicated in control of *por* gene expression. The amount of LPOR protein and LPOR mRNA decreases rapidly in many species when etiolated plants are exposed to light,[123] suggesting phytochrome involvement in this process. Experiments using *A. thaliana* with red and far-red light treatments have confirmed the involvement of phytochrome A in this process and the LPORA mRNA levels are regulated by phytochrome.[124–126] Phytochrome has also been shown to regulate the expression of the *por* gene from a lower plant (*Marchantia paleacea*).[127] In barley the phytochrome- and/or light-dependent regulation of LPORA mRNA levels is dependent on a 3′ untranslated region in the LPORA mRNA.[128] In addition to the reduction in message, a light-dependent degradation of the LPORA bound to chlorophyllide, but not protochlorophyllide, occurs and a light-induced protease has been shown to be responsible.[129] In contrast, cucumber, which only has a single *por* gene, shows an increase in *por* message levels during the de-etiolation process[130] and a decrease in message levels occurs when plants are transferred from dark to light.[131]

The effect of plant age and leaf age on *por* gene expression has been studied in pea,[114] barley,[122,132] wheat[133] and *A. thaliana*.[109] In barley and *A. thaliana* the LPORA mRNA is only expressed in young etiolated tissue while the LPORB mRNA is expressed throughout development. In light-grown seedlings of pea and wheat the youngest leaves contained the highest POR message levels.

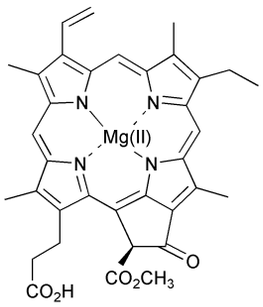
The plant hormones cytokinin and abscisic acid also appear to have a role in regulation of *por* gene expression. The involvement of cytokinin in *por* regulation was inferred from the finding that cytokinins overcame the inhibition of greening caused by treatment with cadmium and mercury,[134,135] although cadmium and mercury also have a direct effect on LPOR enzyme activity.[136,137] It was subsequently found that cytokinins directly activated *por* gene expression in cucumber[138] and *Lupinus luteus*.[139] It was also found that abscisic acid inhibits *por* gene expression in *L. luteus*.[139]

Analysis of LPOR protein sequences has revealed that they belong to the secondary alcohol reductase family of enzymes[140] and are most similar to the short chain alcohol dehydrogenases in this class.[141] Based primarily on secondary structure predictions, the LPOR enzyme is thought to consist of a β-sheet surrounded by α-helixes.[142–144] Sequence-based comparisons and site directed mutagenesis studies have also been important in identifying residues that may be involved in catalysis.[110,141]

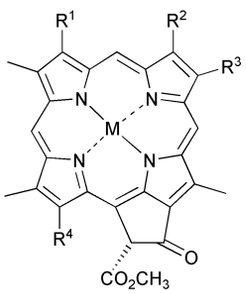
A complementation system using pea LPOR with a DPOR mutant of *R. capsulatus* has been used together with site directed mutagenesis to probe the putative catalytic and structurally important residues contributing to LPOR activity.[141,145] The availability of systems to express LPOR in *E. coli* and purify active enzyme[143,144,146–151] or to purify LPOR to homogeneity from etiolated plants and algae[152–154] has allowed kinetic studies as well as the generation and analysis of site directed mutants.

The substrate specificity of LPOR enzymes from various sources has been investigated. Substrate analogues of protochorophyllide with modifications on rings A and B can be tolerated and substitution of magnesium for zinc can also be tolerated. However, analogues of protochlorophyllide with modifications to rings C and D of protochlorophyllide, specifically at positions 13^2 and 17, are inactive. Thus, the recombinant POR from barley[151] and the purified POR's from *Scenedesmus obliquus*[155] and oat[154] are able to convert both **6** and **7** to the corresponding chlorophyllides. The purified LPOR from oat used analogues modified on rings A and B and the Zn derivatives of these, **18**–**21**, but was unable to use analogues with modifications at the C-13^2 or on C-17, **12**–**16** and **22**–**27**.[154] LPOR from barley was not able to utilise protochlorophyllide *a*′ **11** or tolerate any other changes at the 13^2 position.[156] LPOR from wheat was able to use Zn protochlorophyllide *a* (**18**) and *b* (**19**) but could not reduce Zn protochlorophyll *a* (**23**) or *b* (**24**).[157] The barley LPORA was reported to have a tenfold higher affinity for Zn protochlorophyllide *b* (**19**) than for Zn protochlorophyllide *a* (**18**), and LPORB had a tenfold higher affinity for Zn protochlorophyllide *a* (**18**) than for Zn protochlorophyllide *b* (**19**). A 5 : 1 complex of NADPH–LPORA–Zn protochlorophyllide *b* to NADPH–LPORB–Zn protochlorophyllide *a* was generated *in vitro* and irradiation only yielded Zn-chlorophyllide *a*. This led to the conclusion that LPORA and Zn-protochlorophyllide *b* (**19**) transfer energy to the Zn-protochlorophyllide *a* bound to LPORB and it was suggested that this might occur *in vivo* with protochlorophyllide *b* (**14**).[158] However, protochlorophyllide *b* (**14**) does not occur in barley and hence this finding appears to have no significance *in vivo*.[159,160]

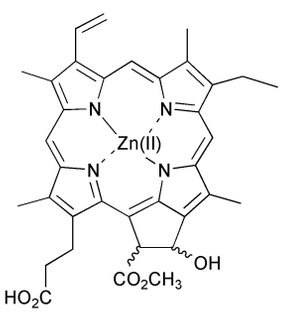
NMR studies using stereospecifically labelled NADPH confirmed the origin of the hydrogen atoms in the reduction reaction catalysed by LPOR. It was found that a hydride is delivered to the C-17 position of protochlorophyllide from the pro-*S* face of NADPH and that the C-18 position is protonated by water or an active site acid.[161] Site directed mutagenesis has indicated that a conserved tyrosine is the most likely proton donor.[141] The photoreduction phase of the reaction has been



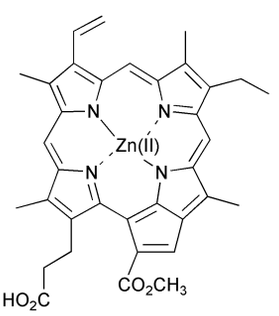
11



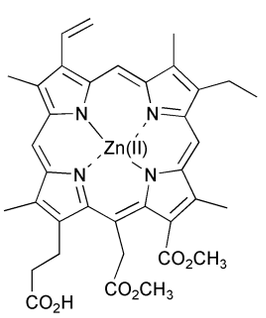
12 M = Mg(II), R^1= $CH{=}CH_2$, R^2 = Me, R^3 = Et, R^4 = $CH_2CH_2CO_2$phytyl
13 M = Mg(II), R^1= $CH{=}CH_2$, R^2 = CHO, R^3 = Et, R^4 = $CH_2CH_2CO_2$phytyl
14 M = Mg(II), R^1= $CH{=}CH_2$, R^2 = CHO, R^3 = Et, R^4 = $CH_2CH_2CO_2H$
15 M = Mg(II), R^1= $CH{=}CH_2$, R^2 = Me, R^3 = Et, R^4 = $CH{=}CHCO_2H$
16 M = Mg(II), R^1= $CH{=}CH_2$, R^2 = Me, R^3 = $CH{=}CH_2$, R^4 = $CH{=}CHCO_2H$
17 M = Mg(II), R^1= $CH{=}CH_2$, R^2 = CO_2Me, R^3 = $CH{=}CH_2$, R^4 = $CH{=}CHCO_2H$
18 M = Zn(II), R^1= $CH{=}CH_2$, R^2 = Me, R^3 = Et, R^4 = $CH_2CH_2CO_2H$
19 M = Zn(II), R^1= $CH{=}CH_2$, R^2 = CHO, R^3 = Et, R^4 = $CH_2CH_2CO_2H$
20 M = Zn(II), R^1= $COCH_3$, R^2 = Me, R^3 = Et, R^4 = $CH_2CH_2CO_2H$
21 M = Zn(II), R^1= CHO, R^2 = Me, R^3 = Et, R^4 = $CH_2CH_2CO_2H$
22 M = Zn(II), R^1= $CH{=}CH_2$, R^2 = Me, R^3 = Et, R^4 = $CH_2CH_2CO_2$Me
23 M = Zn(II), R^1= $CH{=}CH_2$, R^2 = Me, R^3 = Et, R^4 = $CH_2CH_2CO_2$phytyl
24 M = Zn(II), R^1= $CH{=}CH_2$, R^2 = CHO, R^3 = Et, R^4 = $CH_2CH_2CO_2$phytyl



25



26



27

investigated by low temperature spectroscopy by numerous groups. The presence of a free radical has been detected during this phase of the reaction by ESR[162] and a free electron has also been detected by EPR spectroscopy.[149] A nonfluorescent intermediate was recently detected which had an absorbance maximum at 696 nm and this intermediate was suggested to be a radical ion.[147]

6.1.1 Properties and assembly of prolamellae bodies

When plants are placed in the dark, protochlorophyllide, NADPH and LPOR form a ternary complex within the chloroplast which is poised waiting for the final factor, light, to allow photoconversion of the protochlorophyllide to chlorophyllide. This ternary complex is membrane associated and forms crystalline-like structures which are visible by electron microscopy in the etioplasts of angiosperms. These crystalline structures are called prolamellae bodies (PLBs) and their formation is dependent on the ternary LPOR complex arranged in aggregates on membranes. One of the main spectral forms of protochlorophyllide observed *in vivo* is due to these NADPH–LPOR–protochlorophyllide ternary complex aggregates that make up the PLBs.[163–165] Pigment-binding to LPOR is essential for the formation of PLBs as mutants that are unable to make protochlorophyllide do not make PLBs.[12] PLB formation can also be inhibited by treatment of plants with gabaculine, which inhibits protochlorophyllide formation.[166] Lipids are also essential for the formation of the PLBs.[167] Flavins,[168] violaxanthin and zeaxanthin[169] have also been detected in PLBs and may be involved in their formation. PLBs have been detected in yellow in the dark mutants of *C. reinhardtii*, suggesting that most LPORs are capable of forming PLBs even where they are not normally found. The demonstration that both LPORA and LPORB of *A. thaliana* are able to form PLBs supports this.[125,170] On exposure to light, protochlorophyllide (**7**) is converted to chlorophyllide (**8**) and then rapidly to chlorophyll (**9**). The PLBs then rapidly disperse or disaggregate as the photosystems are assembled. Protein phosphorylation appears to be involved in both this disaggregation process and in the formation of the PLBs.[165,171]

The photoconversion catalysed by light with LPOR occurs in a single photochemical step and requires a single photon of light.[172,173] However, multiple spectral forms of protochlorophyllide occur in etiolated tissue and can be classified as photoconvertible and nonphotoconvertible forms based on their ability to be reduced to chlorophyllide by a single flash of light. Two photoconvertible forms have been suggested: the form with fluorescence emission in the range 638–645 nm may involve the formation of a dimer[146] and the form with emission maxima in the range 650–657 nm may be an aggregated form which appears to be dependent on lipid binding.[163,167,174] The nonphotoconvertible forms tend to predominate in etiolated tissue and are spectrally and chemically heterogeneous[173,175] Two of the nonphotoconvertible forms are thought to be unbound protochlorophyllide and an $NADP^+$–LPOR–protochlorophyllide ternary complex.

The targeting and assembly of LPOR to the inner membrane of etioplasts and chloroplasts have been studied by numerous groups with many conflicting reports. The pea preLPOR protein is efficiently imported into pea chloroplasts and assembly of the ternary complex on the membrane can occur with both the mature and preLPOR proteins.[176,177] Reinbothe's group reported that the import of barley LPOR into barley and pea chloroplasts required protochlorophyllide[178,179] and that the transit peptide of preLPORA conferred this substrate dependent transport.[180] This group has suggested that preLPORA is imported by this alternative import pathway in a wide range of plants.[181] These reports are in contrast to studies which do not show protochlorophyllide-dependent import of wheat,[110] *A. thaliana*[182] or pea[176,177,183] preLPORs into their corresponding chloroplasts. In addition barley preLPOR was crosslinked to the TOC75 in barley chloroplasts which is part of the normal import machinery of the chloroplast, suggesting that the same import route is used.[184]

6.2 Light-independent (dark) POR (DPOR)

Light-independent protochlorophyllide reduction has been extensively reviewed by Armstrong.[107] The anoxygenic photo-

synthetic bacteria do not have the LPOR enzyme and use a DPOR. Thus disruption of the *R. capsulatus* genes, *bchL*, *bchN* and *bchB*, results in accumulation of protochlorophyllide in both the light and dark as these genes encode subunits of light-independent protochlorophyllide oxidoreductase (DPOR).[6,185–188] Cyanobacteria, green algae and most non-flowering plants have both an LPOR and a DPOR. Thus mutants in the DPOR from cyanobacteria can still synthesize chlorophyll in the light but not the dark. This has enabled identification of DPOR subunit genes of the cyanobacterium *Plectonema boryanum* which are homologous to *bchL*, *bchN* and *bchB*.[185,186,189] A *bchL* homologue in *Synechocystis* was also identified in this way.[190]

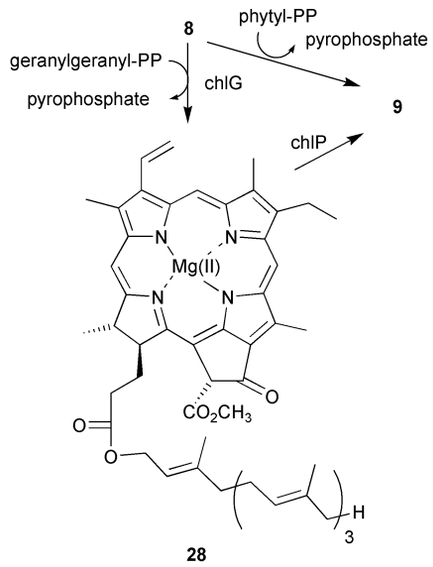
Green algae and most nonflowering land plants are able to make chlorophyll in the dark and have homologues to *bchL*, *bchN* and *bchB* called *chlL*, *chlN* and *chlB*, indicating the presence of a DPOR enzyme. Mutation or deletion of these chloroplast encoded *chlL*, *chlN* and *chlB* genes in the green algae *C. reinhardtii* prevents chlorophyll synthesis in the dark.[191–194] Seven *C. reinhardtii* nuclear mutants have a similar chlorophyll-less phenotype in the dark and in all cases these mutations prevent the translation of the chloroplast-encoded *chlL* gene.[195] The *chlL* gene of *C. reinhardtii* hybridises to DNA from distantly related bacteria and nonflowering land plants but not to DNA from the representative angiosperms, *Zea mays*, *A. thaliana*, *N. tabacum* and *Bougainvillea glabra*.[194] When homologues of the *chlL*, *chlN* and *chlB* genes are present, they are invariably found in the chloroplast genomes.[83,188,196](also see ref. 107)

The predicted BchL/ChlL, BchN/ChlN and BchB/ChlB protein sequences show strong similarity to the NifH, NifD and NifK subunits of nitrogenase, respectively.[103,185,186,188] The BchL/ChlL and NifH share an *N*-terminal MgATP binding motif and four conserved cysteine residues, which make a 4Fe–4S centre in NifH. Conserved cysteines are also found in the BchN/ChlN and BchB/ChlB which are also thought to be involved in formation of Fe–S centres. The BchN/ChlN and BchB/ChlB proteins have been suggested to form an $\alpha_2\beta_2$ dimer by analogy to the NifD and NifK proteins of nitrogenase.[107] The copurification of BchN/ChlN and BchB/ChlB proteins in a 1 : 1 molar ratio supports this suggestion. However, BchN/ChlN and BchB/ChlB are unlikely to form an Fe–Mo centre, analogous to the Fe–Mo centre in the NifD and NifK complex, as the ligands responsible for forming this complex are not conserved.[197]

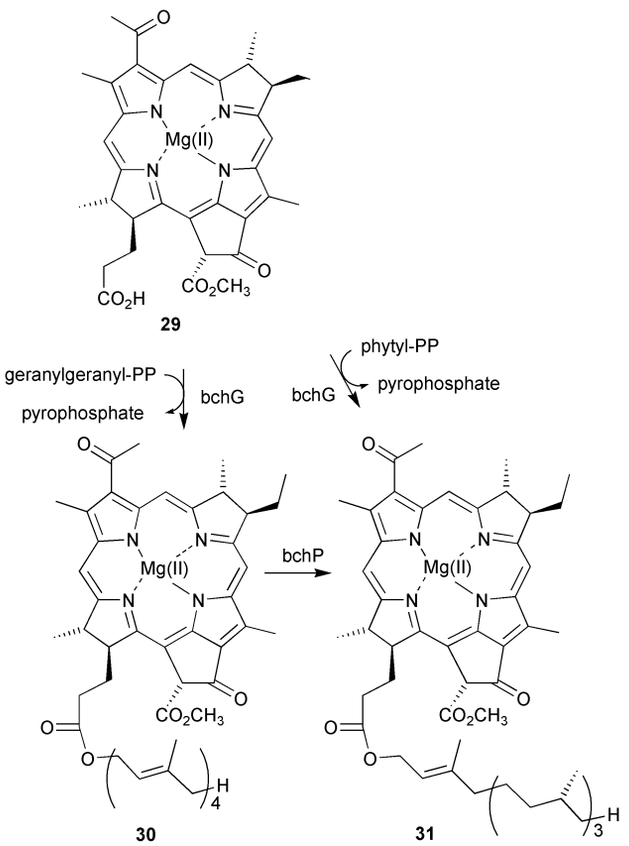
There are only three reports of *in vitro* DPOR activity. DPOR activity was demonstrated using membranes from the cyanobacterium *Anacystis nidulans* and required NADPH.[198] In the second report DPOR from pine chloroplasts was reported and NADPH stimulated activity but was not absolutely required.[116] In the most recent report, the BchL, BchN and BchB proteins from *R. capsulatus* were purified to apparent homogeneity in an anaerobic chamber. The BchN and BchB proteins purified as a complex in an apparent 1 : 1 stoichiometric ratio. Dithionite was required as an electron donor and all three proteins were required to reconstitute DPOR activity. Ferridoxin was suggested to be the normal electron donor *in vivo*.[197] The requirement for NADPH reported previously is thus most likely for the NADPH-dependent reduction of ferridoxin by ferridoxin reductase.

7 Chlorophyll *a* and bacteriochlorophyll *a* synthases

Chlorophyll *a* (**9**) synthesis is completed with the esterification of chlorophyllide *a* (**8**) with phytol. This reaction is catalysed by chlorophyll synthase, although esterification with geranylgeraniol as shown in Scheme 3 can also occur. The corresponding enzyme in photosynthetic bacteria catalyses the esterification of bacteriochlorophyllide *a* (**29**) to bacteriochlorophyll a_{gg} (**30**) or a_p (**31**) as shown in Scheme 4. Mutational analysis



Scheme 3



Scheme 4

of the photosynthetic gene cluster of *R. capsulatus* indicated that the *bchG* gene encoded the bacteriochlorophyll synthase enzyme.[6,199] Bacteriochlorophyll synthase genes, *bchG*, and the homologous chlorophyll synthase genes, *chlG*, have been cloned and the enzymes heterologously expressed in *E. coli*. The plant enzymes are nuclear encoded and have a chloroplast transit sequence for translocation into the chloroplast. Phytyl pyrophosphate and geranylgeranyl pyrophosphate are both substrates for chlorophyll and bacteriochlorophyll synthases from different sources. However, *A. thaliana* chlorophyll synthase preferred geranylgeranyl pyrophosphate as the substrate while *Synechocystis* chlorophyll synthase and *R. capsulatus* bacteriochlorophyll synthase preferred phytyl pyrophosphate as the isoprene donor.[200,201] A similar result was obtained

with the bacteriochlorophyll synthase from *Rhodobacter sphaeroides*.[202]

Chlorophyll synthase can use chlorophyllide *a*, chlorophyllide *b*,[200,201] and zinc-containing chlorophyllides[203] as substrates. However, bacteriochlorophyllide *a*,[200] pheophorbide *a*, and the C-13^2-(*S*) epimer of chlorophyllide *a*, called chlorophyllide *a*′,[204] were not used as substrates. Similarly the bacteriochlorophyll *a* synthase utilized bacteriochlorophyllide *a*, but not chlorophyllide *a*.[200] Two *bchG* genes have been sequenced from *Chloroflexis aurantiacus* and it was suggested that one is involved in bacteriochlorophyll *a* biosynthesis and the second is involved in bacteriochlorophyll *c* biosynthesis in this organism.[205]

Further characterisation of chlorophyll synthase has been with the heterologously expressed enzyme from oat (*Avena sativa*). The oat *chlG* gene encodes a 378 amino acid protein with a presequence of 46 amino acids. Activity depended on the presence of magnesium ions although manganese ions yielded partial activity. Deletion of the first 88 amino acids had no effect on activity and site directed mutagenesis revealed that 2 of 4 Arg residues and one of five cysteine residues were essential. *N*-Phenylmaleimide inhibited activity by binding to one of the nonessential cysteine residues.[206]

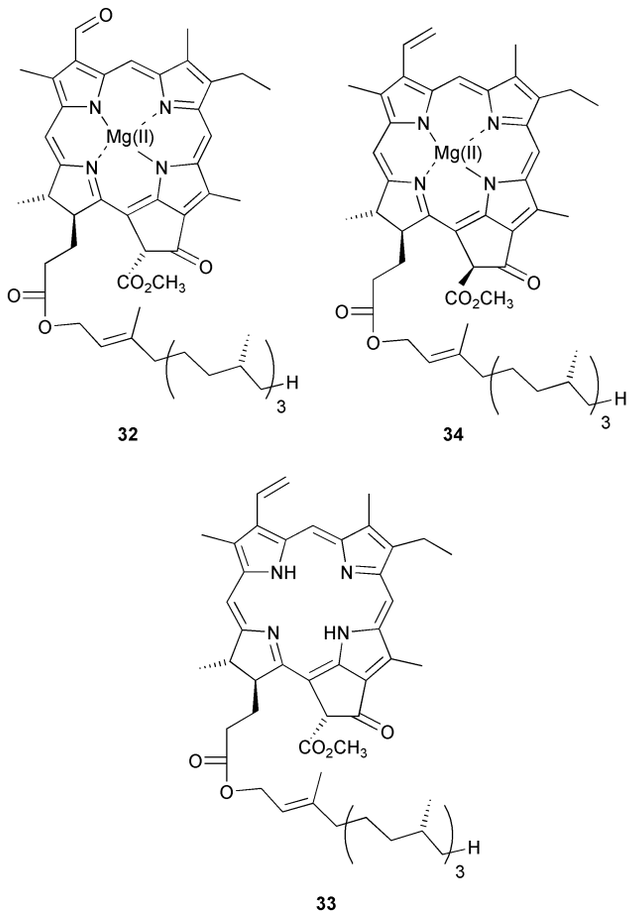
Reduction of the geranylgeranyl chain to the phytyl chain can occur either before or after esterification to chlorophyllide *a* (**8**) or bacteriochlorophyllide *a* (**29**). *BchP* is the gene encoding the enzyme involved in the reduction of geranylgeranyl bacteriochlorophyll *a* (**30**) to bacteriochlorophyll *a* (**31**) in *R. sphaeroides*.[6] Partial functional complementation of a *bchP* mutant of *R. sphaeroides* was used to identify the *chlP* gene from an oxygenic bacterium, *Synechocystis* PCC6803.[207] The *chlP* genes in *N. tabacum* and *A. thaliana* are found in the nuclear genome and encode a 52 kDa precursor protein. Transgenic tobacco plants expressing antisense ChlP RNA have both reduced tocopherol and chlorophyll synthesis, indicating that this enzyme provides phytol and/or phytyl pyrophosphate for both of these pathways.[208] A reductase activity in the chloroplast envelope converts geranylgeranyl pyrophosphate to phytyl pyrophosphate and a second in the thylakoids converts geranylgeraniol esterified to chlorophyllide to chlorophyll *a*. Therefore, a second gene may be present which encodes the reductase which converts geranylgeraniol esterified to chlorophyllide to phytol esterified chlorophyllide *a*.[209]

8 Other chlorophylls

Apart from chlorophyll *a*, other chlorophylls which are functionally important in photosynthesis are synthesized by oxygenic photosynthetic organisms. Chlorophyll *d* (**32**) was reported as the major pigment in an oxygenic photosynthetic prokaryote called *Acaryochloris marina*.[210] Chlorophyll *b* represents the major chlorophyll variant since all plants and green algae contain up to 25% of their total chlorophyll as chlorophyll *b*. The synthesis of chlorophyll *b* has recently been reviewed and is discussed separately below.

Two important minor chlorophyll pigments in higher plants are pheophytin *a* (**33**) and the 13^2-(*S*) epimer of chlorophyll *a* called chlorophyll *a*′ (**34**). Both of these compounds are important components of the photosynthetic reaction centres yet little is known about how they are synthesized.[211,212] Chlorophyllide *a*′ and pheophorbide *a* are not substrates for chlorophyll synthase[204] and protochlorophyllide *a*′ and protopheophorbide *a* are not substrates for LPOR.[156] These results suggest that pheophytin *a* and chlorophyll *a*′ may be synthesized directly from chlorophyll *a*. Interestingly chlorophyll *d*′ has been found in the reaction centres of the chlorophyll *d*-containing prokaryote *Acaryochloris marina*.[213,214]

The chlorophyll *c* series of pigments are found as accessory antenna pigments together with chlorophyll *a* and *b* in chromophyte algae and in some Prasinophyceae. Chlorophyll c_1 (**14**) and c_2 (**15**) were reported in the previous reviews and the structure of a third member named chlorophyll c_3 (**16**) has now been determined.[215] Most chlorophyll *c* pigments have a 17 acrylic acid side-chain, however there have been two reports where this group is esterified with phytol.[216,217]

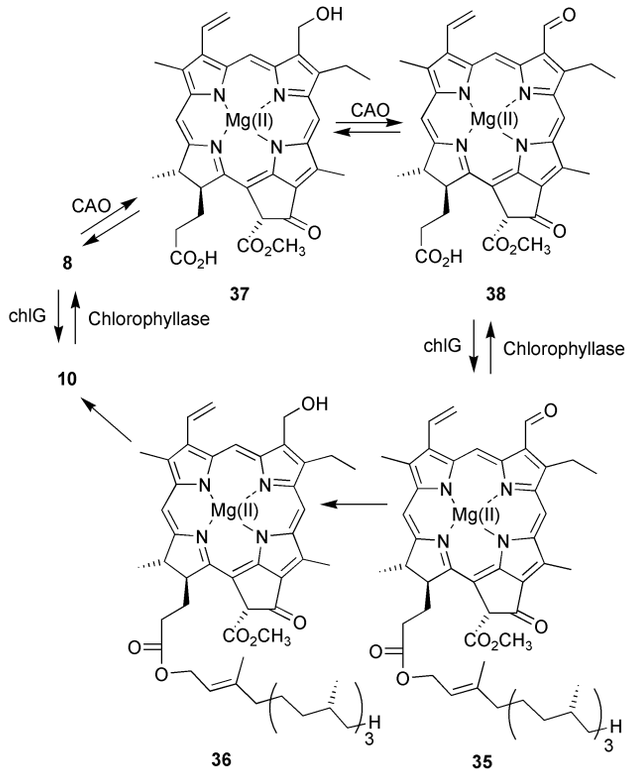


8.1 Interconversion of chlorophyll *a* and chlorophyll *b*

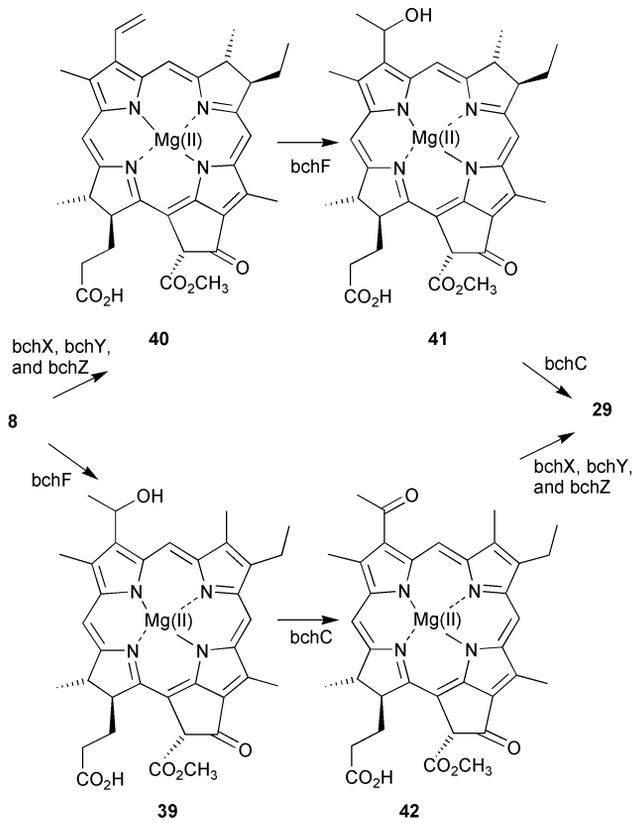
Chlorophyll *b* (**35**) only occurs in oxygenic photosynthetic organisms, suggesting that the formyl oxygen comes from molecular oxygen. This was confirmed by $^{18}O_2$ labelling of the formyl oxygen of chlorophyll *b* in *Chlorella vulgaris*[218] and later in *Zea mays*.[219] Chlorophyllide *a* oxygenase (CAO) is the enzyme which catalyses the conversion of chlorophyllide *a* to chlorophyllide *b* and a reductase activity is found in chloroplasts of senescent leaves which can catalyse the reverse reaction but on chlorophyll *b* itself. Thus interconversion of chlorophylls *a* and *b* is possible by the cycle shown in Scheme 5.[220]

Reductase activity involved in converting chlorophyll *b* to chlorophyll *a* has been found in chloroplast membranes from cucumber and barley.[221–224] The conversion is through a 7^1-hydroxy intermediate[221] (**36**) and this process is thought to be important in chlorophyll *b* degradation.[220]

The *C. reinhardtii CAO* gene was cloned and sequenced by insertional mutagenesis. The *CAO* gene encoded a protein of 463 amino acids in length with a Rieske-type 2Fe–2S cluster and a mononuclear Fe binding site.[225] *CAO* genes have since been identified in *A. thaliana, Oryyza sativa, Marchantia polymorpha, Dunaliella salina, Prochlorothrix hollandica* and *Prochloron didemni*.[226,227] The *A. thaliana* CAO was heterologously expressed in *E. coli* and required oxygen and reduced ferridoxin to convert chlorophyllide *a* (**8**) to chlorophyllide *b* (**38**). Traces of the 7^1-hydroxy intermediate (**37**) were detected and the enzyme could also use Zn-chlorophyllide *a* as a substrate but not pheophorbide or chlorophyll *a*.[228]



Scheme 5



Scheme 6

9 Bacteriochlorophylls

Bacteriochlorophylls are the photosynthetic light harvesting "chlorophylls" found in photosynthetic bacteria. The number of bacteriochlorophylls is enormous and their structures and biosynthesis have been reviewed by Senge and Smith.[229] Bacteriochlorophyll *a* is the major pigment found in purple bacteria and green sulfur bacteria and bacteriopheophytin *a* is found as an accessory pigment in the green sulfur bacteria reaction centre. A variety of alcohols are found esterified to the 17-propanoate of bacteriochlorophylls. Geranylgeraniol and phytol have already been mentioned but esters of farnesol, hexadecanol, octadecanol and 2,10-phytadienol have also been found. Two major structural differences separate the bacteriochlorophylls *a*, *b* and *g* from the bacteriochlorophylls *c*, *d* and *e*. The pyrrole ring II of bacteriochlorophylls *a*, *b* and *g* is reduced to produce a bacteriochlorin ring system. In bacteriochlorophylls *c*, *d*, and *e* this ring remains oxidised as in the chlorophylls. Conversely, bacteriochlorophylls *a*, *b* and *g* have a 13^2-carboxymethyl group like chlorophyll which is absent from bacteriochlorophylls *c*, *d* and *e*.

The reduction of pyrrole ring II to make bacteriochlorophyll *a* in *R. capsulatus* requires the *bchX*, *bchY* and *bchZ* genes.[230] The protein sequences of BchX, BchY and BchZ are similar to the BchL, BchN and BchB subunits of the DPOR reductase, suggesting that this reduction is probably achieved *via* a similar mechanism.[231] The reduction of ring II can occur either before or after conversion of the 3-vinyl group to an acetyl group as shown in Scheme 6. The *bchF* gene encodes an enzyme that adds water across the 3-vinyl group to produce a 3^1-hydroxy 3^2-hydro derivative **39** or **41**.[188] This is then presumably oxidised to an acetyl group to produce bacteriochlorophyllide *a* and it has been suggested that the *bchC* gene encodes the enzyme required for this step.[6] Labeling studies have confirmed that the oxygen in this acetyl group is derived from water, which is consistent with an enzymic hydration.[80]

Experiments with *Chlorobium vibrioforme* have shown that anaesthetic gases such as N_2O, ethylene and acetylene are potent biosynthetic inhibitors of the antennae pigment bacteriochlorophyll *d* but not of the reaction centre pigment bacteriochlorophyll *a*. In addition these inhibitors resulted in accumulation of magnesium protoporphyrin IX monomethyl ester.[232] These results suggest that the biosynthesis of bacteriochlorophyll *d* diverges from that of bacteriochlorophyll *a* at an early stage and this may also be the case for the bacteriochlorophyll *c* and *e* pigments which also lack a carboxymethyl group at C-13^2.

10 References

1 M. Alberti, D. H. Burke and J. E. Hearst, in *Anoxygenic Photosynthetic Bacteria*; R. E. Blankenship, M. T. Madigan and C. E. Bauer, Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1995, 1083–1106.
2 G. C. Ferreira, *Int. J. Biochem. Cell Biol.*, 1999, **31**, 995.
3 C. J. Walker and R. D. Willows, *Biochem. J.*, 1997, **327**, 321.
4 S. A. Coomber, M. Chaudhri, A. Connor, G. Britton and C. N. Hunter, *Mol. Microbiol.*, 1990, **4**, 977.
5 A. Gorchein, L. C. Gibson and C. N. Hunter, *Biochem. Soc. Trans.*, 1993, **21**, 201S.
6 D. W. Bollivar, J. Y. Suzuki, J. T. Beatty, J. M. Dobrowolski and C. E. Bauer, *J. Mol. Biol.*, 1994, **237**, 622.
7 D. W. Bollivar and C. E. Bauer, *Plant Physiol.*, 1992, **98**, 408.
8 L. C. D. Gibson, R. D. Willows, C. G. Kannanagara, D. v. Wettstein and C. N. Hunter, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 1941.
9 R. D. Willows and S. I. Beale, *J. Biol. Chem.*, 1998, **273**, 34206.
10 P. Jensen, B. Stummann, N. Hansen, K. Karlebjerg and K. Henningsen, *Plant Mol. Biol.*, 1996, **30**, 1075.
11 B. L. Petersen, P. E. Jensen, L. C. Gibson, B. M. Stummann, C. N. Hunter and K. W. Henningsen, *J. Bacteriol.*, 1998, **180**, 699.
12 K. W. Henningsen, J. E. Boynton and D. v. Wettstein, *Mutants at xantha and albina loci in relation to chloroplast biogenesis in barley (Hordeum vulgare L.)*; The Royal Danish Academy of Sciences and Letters: Copenhagen, 1993; Vol. 42.
13 P. E. Jensen, R. D. Willows, B. L. Petersen, U. C. Vothknecht, B. M. Stummann, C. G. Kannangara, D. von Wettstein and K. W. Henningsen, *Mol. Gen. Genet.*, 1996, **250**, 383.
14 B. L. Petersen, M. G. Moller, P. E. Jensen and K. W. Henningsen, *Hereditas*, 1999, **131**, 165.
15 A. Hansson, C. G. Kannangara, D. von Wettstein and M. Hansson, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 1744.
16 C. Koncz, R. Mayerhofer, Z. Koncz-Kalman, C. Nawrath, G. P. Redei and J. Schell, *EMBO J.*, 1990, **9**, 1337.

17 H. M. Rissler, E. Collakova, D. DellaPenna, J. Whelan and B. J. Pogson, *Plant Physiol.*, 2002, **128**, 770.
18 N. Mochizuki, J. A. Brusslan, R. Larkin, A. Nagatani and J. Chory, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 2053.
19 R. E. Susek, F. M. Ausubel and J. Chory, *Cell*, 1993, **74**, 787.
20 D. Luo, E. S. Coen, S. Doyle and R. Carpenter, *Plant J.*, 1991, **1**, 59.
21 A. Hudson, R. Carpenter, S. Doyle and E. S. Coen, *EMBO J.*, 1993, **12**, 3711.
22 E. Chekounova, V. Voronetskaja, J. Papenbrock, B. Grimm and C. F. Beck, *Mol. Gen. Genet.*, 2001, **266**, 363.
23 L. C. Gibson, J. L. Marrison, R. M. Leech, P. E. Jensen, D. C. Bassham, M. Gibson and C. N. Hunter, *Plant Physiol.*, 1996, **111**, 61.
24 R. Guo, M. Luo and J. D. Weinstein, *Plant Physiol.*, 1998, **116**, 605.
25 C. J. Walker and J. D. Weinstein, *Proc. Natl. Acad. Sci. U. S. A.*, 1991, **88**, 5789.
26 R. D. Willows, L. C. D. Gibson, C. G. Kanangara, C. N. Hunter and D. von Wettstein, *Eur. J. Biochem.*, 1996, **235**, 438.
27 P. E. Jensen, L. C. D. Gibson, K. W. Henningsen and C. N. Hunter, *J. Biol. Chem.*, 1996, **271**, 16662.
28 P. E. Jensen, L. C. D. Gibson and C. N. Hunter, *Biochem. J.*, 1998, **334**, 335.
29 L. C. D. Gibson, P. E. Jensen and C. N. Hunter, *Biochem. J.*, 1999, **337**, 243.
30 J. Papenbrock, H.-P. Mock, E. Kruse and B. Grimm, *Planta*, 1999, **208**, 264.
31 J. Papenbrock, H. P. Mock, R. Tanaka, E. Kruse and B. Grimm, *Plant Physiol.*, 2000, **122**, 1161.
32 G. Pöpperl, U. Oster, I. Blos and W. Rüdiger, *Z. Naturforsch., C*, 1997, **52**, 144.
33 J. Wubert, U. Oster, I. Blos and W. Rüdiger, *Plant Physiol. Biochem.*, 1997, **35**, 581.
34 C. J. Walker, G.-H. Yu and J. D. Weinstein, *Plant Physiol. Biochem.*, 1997, **35**, 213.
35 A. Gorchein, *Biochem. J.*, 1994, **299**, 869.
36 A. Gorchein, *Biochem. Soc. Trans.*, 1997, **25**, 82S.
37 G. A. Karger, J. D. Reid and C. N. Hunter, *Biochemistry*, 2001, **40**, 9291.
38 C. J. Walker and J. D. Weinstein, *Plant Physiol.*, 1991, **95**, 1189.
39 N. G. Averina, E. B. Yaronskaya, V. V. Rassadina, N. V. Shalygo and G. Walter, *Photosynth.: Light Biosphere, Proc. Int. Photosynth. Congr., 10th*, 1995, **3**, 925.
40 N. G. Averina, E. B. Yaronskaya, V. V. Rassadina and G. Walter, *J. Photochem. Photobiol.*, 1996, **36**, 17.
41 C. J. Walker, L. R. Hupp and J. D. Weinstein, *Plant Physiol. Biochem.*, 1992, **30**, 263.
42 C. J. Walker and J. D. Weinstein, *Biochem. J.*, 1994, **299**, 277.
43 H. J. Lee, M. D. Ball, R. Parham and C. A. Rebeiz, *Plant Physiol.*, 1992, **99**, 1134.
44 M. Luo, J. D. Weinstein and C. J. Walker, *Plant Mol. Biol.*, 1999, **41**, 721.
45 C. G. Kannangara, U. C. Vothknecht, M. Hansson and D. von Wettstein, *Mol. Gen. Genet.*, 1997, **254**, 85.
46 T. Masuda, K. Inoue, M. Masuda, M. Nagayama, A. Tamaki, H. Ohta, H. Shimada and K.-I. Takamiya, *J. Biol. Chem.*, 1999, **274**, 33594.
47 L. C. Gibson, P. E. Jensen and C. N. Hunter, *Biochem. J.*, 1999, **337**, 243.
48 J. Papenbrock, S. Grafe, E. Kruse, F. Hanel and B. Grimm, *Plant J.*, 1997, **12**, 981.
49 P. E. Jensen, J. D. Reid and C. N. Hunter, *Biochem. J.*, 2000, **352**, 435.
50 P. E. Jensen, L. C. Gibson and C. N. Hunter, *Biochem. J.*, 1999, **339**, 127.
51 M. Hansson and C. G. Kannangara, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 13351.
52 G. Pöpperl, U. Oster and W. Ruediger, *J. Plant Physiol.*, 1998, **153**, 40.
53 B. L. Petersen, C. G. Kannangara and K. W. Henningsen, *Photosynth.: Mech. Eff., Proc. Int. Congr. Photosynth., 11th*, 1998, **4**, 3241.
54 L. Debussche, M. Couder, D. Thibaut, B. Cameron, J. Crouzet and F. Blanche, *J. Bacteriol.*, 1992, **174**, 7445.
55 R. D. Willows, M. Hansson, S. I. Beale, M. Laurberg and S. Al-Karadaghi, *Acta Crystallogr., Sect. D*, 1999, **55**, 689.
56 M. N. Fodje, A. Hansson, M. Hansson, J. G. Olsen, S. Gough, R. D. Willows and S. Al-Karadaghi, *J. Mol. Biol.*, 2001, **311**, 111.
57 F. Confalonieri and M. Duguet, *Bioessays*, 1995, **17**, 639.
58 R. D. Vale, *J. Cell Biol.*, 2000, **150**, F13.
59 J. O. Lee, L. A. Bankston, M. A. Arnaout and R. C. Liddington, *Structure*, 1995, **3**, 1333.
60 A. Hansson, R. D. Willows, T. H. Roberts and M. Hansson, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 13944.
61 W. Yee, C. S. J. Eglsaer and W. R. Richards, *Biochem. Biophys. Res. Commun.*, 1989, **162**, 483.
62 E. B. Yaronskaya, N. V. Shalygo, V. V. Rassadina and N. G. Averina, *Photosynthetica*, 1993, **29**, 243.
63 U. C. Vothknecht, R. D. Willows and C. G. Kannangara, *Plant Physiol. Biochem.*, 1995, **33**, 759.
64 D. W. Bollivar, Z. Y. Jiang, C. E. Bauer and S. I. Beale, *J. Bacteriol.*, 1994, **176**, 5290.
65 L. C. Gibson and C. N. Hunter, *FEBS Lett.*, 1994, **352**, 127.
66 S. B. Hinchigeri, B. Hundle and W. R. Richards, *FEBS Lett.*, 1997, **407**, 337.
67 P. E. Jensen, L. C. Gibson, F. Shephard, V. Smith and C. N. Hunter, *FEBS Lett.*, 1999, **455**, 349.
68 C. A. Smith, J. Y. Suzuki and C. E. Bauer, *Plant Mol. Biol.*, 1996, **30**, 1307.
69 M. A. Block, A. K. Tewari, C. Albrieux, E. Marechal and J. Joyard, *Eur. J. Biochem.*, 2002, **269**, 240.
70 D. W. Bollivar and S. I. Beale, *Photosynth. Res.*, 1995, **43**, 113.
71 P. Vijayan, B. J. Whyte and P. A. Castelfranco, *Plant Physiol. Biochem. (Paris)*, 1992, **30**, 271.
72 D. W. Bollivar and S. I. Beale, *Plant Physiol.*, 1996, **112**, 105.
73 B. J. Whyte and P. A. Castelfranco, *Biochem. J.*, 1993, **290**, 355.
74 B. J. Whyte, P. Fijayan and P. A. Castelfranco, *Plant Physiol. Biochem. (Paris)*, 1992, **30**, 279.
75 C. J. Walker, P. A. Castelfranco and B. J. Whyte, *Biochem. J.*, 1991, **276**, 691.
76 B. J. Whyte and P. A. Castelfranco, *Biochem. J.*, 1993, **290**, 361.
77 T. Tanaka, N. Muto, N. Itoh, A. Dota, Y. Nishina, A. Inada and K. Tanaka, *Res. Commun. Mol. Pathol. Pharmacol.*, 1995, **90**, 211.
78 U. Oster, H. Brunner and W. Rudiger, *J. Photochem. Photobiol. B*, 1996, **36**, 255.
79 C. J. Walker, K. E. Mansfield, K. M. Smith and P. A. Castelfranco, *Biochem. J.*, 1989, **257**, 599.
80 R. J. Porra, W. Schafer, I. Katheder and H. Scheer, *FEBS Lett.*, 1995, **371**, 21.
81 R. J. Porra, M. Urzinger, J. Winkler, C. Bubenzer and H. Scheer, *Eur. J. Biochem.*, 1998, **257**, 185.
82 Z. M. Yang and C. E. Bauer, *J. Bacteriol.*, 1990, **172**, 5001.
83 J. Y. Suzuki, D. W. Bollivar and C. E. Bauer, *Annu. Rev. Genet.*, 1997, **31**, 61.
84 V. Pinta, M. Picaud, F. Reiss-Husson and C. Astier, *J. Bacteriol.*, 2002, **184**, 746.
85 J. Moseley, J. Quinn, M. Eriksson and S. Merchant, *EMBO J.*, 2000, **19**, 2139.
86 J. L. Moseley, M. D. Page, N. P. Alder, M. Eriksson, J. Quinn, F. Soto, S. M. Theg, M. Hippler and S. Merchant, *Plant Cell*, 2002, **14**, 673.
87 C. J. Walker, C. G. Kannangara and D. VonWettstein, *Plant Physiol.*, 1997, **114**, 708.
88 S. P. Gough, B. O. Petersen and J. O. Duus, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 6908.
89 C. A. Rebeiz, R. Parham, D. A. Fasoula and I. M. Ioannides, *Ciba Found. Symp.*, 1994, **180**, 177.
90 R. Parham and C. A. Rebeiz, *Anal. Biochem.*, 1995, **231**, 164.
91 J.-S. Kim and C. A. Rebeiz, *J. Photosci.*, 1995, **2**, 103.
92 J. S. Kim, V. Kolossov and C. A. Rebeiz, *Photosynthetica*, 1997, **34**, 569.
93 R. Goericke and D. J. Repeta, *Mar. Ecol.: Prog. Ser.*, 1993, **101**, 307.
94 R. Goericke and D. J. Repeta, *Limnol. Oceanogr.*, 1992, **37**, 425.
95 B. J. Whyte and W. T. Griffiths, *Biochem. J.*, 1993, **291**, 939.
96 R. Parham and C. A. Rebeiz, *Biochemistry*, 1992, **31**, 8460.
97 J. Y. Suzuki and C. E. Bauer, *J. Biol. Chem.*, 1995, **270**, 3732.
98 W. Hendrich and B. Bereza, *Photosynthetica*, 1993, **28**, 1.
99 N. Lebedev and M. P. Timko, *Photosynth. Res.*, 1998, **58**, 5.
100 S. Reinbothe and C. Reinbothe, *Eur. J. Biochem.*, 1996, **237**, 323.
101 B. Schoefs, *Photosynthetica*, 1999, **36**, 481.
102 S. Reinbothe and C. Reinbothe, *Plant Physiol.*, 1996, **111**, 1.
103 Y. Fujita, *Plant Cell Physiol.*, 1996, **37**, 411.
104 B. Schoefs, *Photosynth. Res.*, 2001, **70**, 257.
105 H. Y. Adamson, R. G. Hiller and J. Walmsley, *J. Photochem. Photobiol. B*, 1997, **41**, 201.
106 S. Reinbothe, C. Reinbothe, K. Apel and N. Lebedev, *Cell*, 1996, **86**, 703.
107 G. A. Armstrong, *J. Photochem. Photobiol.*, 1998, **43**, 87.
108 R. Schulz, K. Steinmuller, M. Klaas, C. Forreiter, S. Rasmussen, C. Hiller and K. Apel, *Mol. Gen. Genet.*, 1989, **217**, 355.
109 G. A. Armstrong, S. Runge, G. Frick, U. Sperling and K. Apel, *Plant Physiol.*, 1995, **108**, 1505.

110 G. R. Teakle and W. T. Griffiths, *Biochem. J.*, 1993, **296**, 225.
111 T. Masuda, N. Fusada, T. Shiraishi, H. Kuroda, K. Awai, H. Shimada, H. Ohta and K. Takamiya, *Photosynth. Res.*, 2002, **74**, 165.
112 N. Fusada, T. Masuda, H. Kuroda, T. Shiraishi, H. Shimada, H. Ohta and K. Takamiya, *Photosynth. Res.*, 2000, **64**, 147.
113 A. J. Spano, Z. He, H. Michel, D. F. Hunt and M. P. Timko, *Plant Mol. Biol.*, 1992, **18**, 967.
114 Z. H. He, J. Li, C. Sundqvist and M. P. Timko, *Plant Physiol.*, 1994, **106**, 537.
115 A. J. Spano, Z. He and M. P. Timko, *Mol. Gen. Genet.*, 1992, **236**, 86.
116 C. Forreiter and K. Apel, *Planta*, 1993, **190**, 536.
117 J. Li and M. P. Timko, *Plant Mol. Biol.*, 1996, **30**, 15.
118 J. Y. Suzuki and C. E. Bauer, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 3749.
119 N. Oosawa, T. Masuda, K. Awai, N. Fusada, H. Shimada, H. Ohta and K. Takamiya, *FEBS Lett.*, 2000, **474**, 133.
120 Q. Su, G. Frick, G. Armstrong and K. Apel, *Plant Mol. Biol.*, 2001, **47**, 805.
121 J. S. Skinner and M. P. Timko, *Plant Cell Physiol.*, 1998, **39**, 795.
122 H. Holtorf, S. Reinbothe, C. Reinbothe, B. Bereza and K. Apel, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 3254.
123 C. Forreiter, B. Van Cleve, A. Schmidt and K. Apel, *Planta*, 1991, **183**, 126.
124 S. A. Barnes, N. K. Nishizawa, R. B. Quaggio, G. C. Whitelam and N. H. Chua, *Plant Cell*, 1996, **8**, 601.
125 U. Sperling, F. Franck, B. van Cleve, G. Frick, K. Apel and G. A. Armstrong, *Plant Cell*, 1998, **10**, 283.
126 U. Sperling, B. van Cleve, G. Frick, K. Apel and G. A. Armstrong, *Plant J.*, 1997, **12**, 649.
127 T. Suzuki, S. Takio, I. Yamamoto and T. Satoh, *Plant Cell Physiol.*, 2001, **42**, 576.
128 H. Holtorf and K. Apel, *Plant Mol. Biol.*, 1996, **31**, 387.
129 C. Reinbothe, K. Apel and S. Reinbothe, *Mol. Cell Biol.*, 1995, **15**, 6206.
130 H. Kuroda, T. Masuda, H. Ohta, Y. Shioi and K. Takamiya, *Biochem. Biophys. Res. Commun.*, 1995, **210**, 310.
131 H. Kuroda, T. Masuda, N. Fusada, H. Ohta and K. Takamiya, *Plant Cell Physiol.*, 2000, **41**, 226.
132 P. H. Schunmann and H. J. Ougham, *Plant Mol. Biol.*, 1996, **31**, 529.
133 J. L. Marrison, P. H. D. Schunmann, H. J. Ougham and R. M. Leech, *Plant Physiol.*, 1996, **110**, 1089.
134 R. M. Thomas and V. P. Singh, *Indian J. Plant Physiol.*, 1995, **38**, 313.
135 R. M. Thomas and V. P. Singh, *Photosynthetica*, 1996, **32**, 145.
136 B. Boddi, A. R. Oravecz and E. Lehoczki, *Photosynthetica*, 1995, **31**, 411.
137 K. Lenti, F. Fodor and B. Boddi, *Photosynthetica*, 2002, **40**, 145.
138 H. Kuroda, T. Masuda, N. Fusada, H. Ohta and K. Takamiya, *J. Plant Res.*, 2001, **114**, 1.
139 V. Kusnetsov, R. G. Herrmann, O. N. Kulaeva and R. Oelmuller, *Mol. Gen. Genet.*, 1998, **259**, 21.
140 M. E. Baker, *Biochem. J.*, 1994, **300**, 605.
141 H. M. Wilks and M. P. Timko, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 724.
142 S. J. Birve, E. Selstam and L. B. Johansson, *Biochem. J.*, 1996, **317**, 549.
143 C. Dahlin, H. Aronsson, H. M. Wilks, N. Lebedev, C. Sundqvist and M. P. Timko, *Plant Mol. Biol.*, 1999, **39**, 309.
144 H. E. Townley, R. B. Sessions, A. R. Clarke, T. R. Dafforn and W. T. Griffiths, *Proteins*, 2001, **44**, 329.
145 N. Lebedev and M. P. Timko, *Photosynth. Res.*, 2002, **74**, 153.
146 G. E. Martin, M. P. Timko and H. M. Wilks, *Biochem. J.*, 1997, **325**, 139.
147 D. J. Heyes, G. E. Martin, R. J. Reid, C. N. Hunter and H. M. Wilks, *FEBS Lett.*, 2000, **483**, 47.
148 N. Lebedev, O. Karginova, W. McIvor and M. P. Timko, *Biochemistry*, 2001, **40**, 12562.
149 N. Lebedev and M. P. Timko, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 9954.
150 H. E. Townley, W. T. Griffiths and J. P. Nugent, *FEBS Lett.*, 1998, **422**, 19.
151 R. Knaust, B. Seyfried, L. Schmidt, R. Schulz and H. Senger, *J. Photochem. Photobiol. B.*, 1993, **20**, 161.
152 T. Urbig, R. K. C. Knaust, H. Schiller and H. Senger, *Z. Naturforsch., C*, 1995, **50**, 775.
153 T. Urbig, H. Schiller and H. Senger, *Physiol. Plant*, 1995, **95**, 141.
154 H. Klement, M. Helfrich, U. Oster, S. Schoch and W. Rüdiger, *Eur. J. Biochem.*, 1999, **265**, 862.
155 R. Knaust, T. Urbig and H. Senger, *Physiol. Plant*, 1995, **95**, 134.
156 M. Helfrich, S. Schoch, W. Schafer, M. Ryberg and W. Rüdiger, *J. Am. Chem. Soc.*, 1996, **118**, 2606.
157 S. Schoch, M. Helfrich, B. Wiktorsson, C. Sundqvist, W. Rüdiger and M. Ryberg, *Eur. J. Biochem.*, 1995, **229**, 291.
158 C. Reinbothe, N. Lebedev and S. Reinbothe, *Nature*, 1999, **397**, 80.
159 G. A. Armstrong, K. Apel and W. Rüdiger, *Trends Plant Sci.*, 2000, **5**, 40.
160 V. Scheumann, H. Klement, M. Helfrich, U. Oster, S. Schoch and W. Rüdiger, *FEBS Lett.*, 1999, **445**, 445.
161 T. P. Begley and H. Young, *J. Am. Chem. Soc.*, 1989, **111**, 3095.
162 O. B. Belyaeva, W. T. Griffiths, J. V. Kovalev, K. N. Timofeev and F. F. Litvin, *Biochemistry*, 2001, **66**, 173.
163 B. Wiktorsson, M. Ryberg, S. Gough and C. Sundqvist, *Physiol. Plant*, 1992, **85**, 659.
164 B. Wiktorsson, S. Engdahl, L. B. Zhong, B. Boddi, M. Ryberg and C. Sundqvist, *Photosynthetica*, 1993, **29**, 205.
165 B. Wiktorsson, M. Ryberg and C. Sundqvist, *Plant Physiol. Biochem.*, 1996, **34**, 23.
166 S. Younis, M. Ryberg and C. Sundqvist, *Physiol. Plant*, 1995, **95**, 336.
167 H. Klement, U. Oster and W. Rudiger, *FEBS Lett.*, 2000, **480**, 306.
168 O. B. Belyaeva, C. Sundqvist and F. F. Litvin, *Membrane Cell Biol.*, 2000, **13**, 337.
169 M. A. O. Chahdi, B. Schoefs and F. Franck, *Planta*, 1998, **206**, 673.
170 F. Franck, U. Sperling, G. Frick, B. Pochert, B. van Cleve, K. Apel and G. A. Armstrong, *Plant Physiol.*, 2000, **124**, 1678.
171 S. Kovacheva, M. Ryberg and C. Sundqvist, *Photosynth. Res.*, 2000, **64**, 127.
172 W. T. Griffiths, T. McHugh and R. E. Blankenship, *FEBS Lett.*, 1996, **398**, 235.
173 B. Schoefs, H.-P. Garnir and M. Bertrand, *Photosynth. Res.*, 1994, **41**, 405.
174 B. Boeddi, A. Lindsten, M. Ryberg and C. Sundqvist, *Physiol. Plant*, 1989, **76**, 135.
175 B. Boddi and F. Franck, *J. Photochem. Photobiol. B*, 1997, **41**, 73.
176 H. Aronsson, C. Dahlin, C. Sundqvist and M. P. Timko, *Photosynth.: Mech. Eff., Proc. Int. Congr. Photosynth., 11th*, 1998, **4**, 3257.
177 C. Dahlin, C. Sundqvist and M. P. Timko, *Plant Mol. Biol.*, 1995, **29**, 317.
178 S. Reinbothe, C. Reinbothe, S. Runge and K. Apel, *J. Cell Biol.*, 1995, **129**, 299.
179 S. Reinbothe, S. Runge, C. Reinbothe, B. van Cleve and K. Apel, *Plant Cell*, 1995, **7**, 161.
180 C. Reinbothe, N. Lebedev, K. Apel and S. Reinbothe, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 8890.
181 S. Reinbothe, R. Mache and C. Reinbothe, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 9795.
182 P. Jarvis, L. J. Chen, H. Li, C. A. Peto, C. Fankhauser and J. Chory, *Science*, 1998, **282**, 100.
183 H. Aronsson, C. Sundqvist, M. P. Timko and C. Dahlin, *FEBS Lett.*, 2001, **502**, 11.
184 H. Aronsson, K. Sohrt and J. Soll, *Biol. Chem.*, 2000, **381**, 1263.
185 Y. Fujita, H. Matsumoto, Y. Takahashi and H. Matsubara, *Plant Cell Physiol.*, 1993, **34**, 305.
186 Y. Fujita, Y. Takahashi, M. Chuganji and H. Matsubara, *Plant Cell Physiol.*, 1992, **33**, 81.
187 Z. Yang and C. E. Bauer, *J. Bacteriol.*, 1990, **172**, 5001.
188 D. H. Burke, M. Alberti and J. E. Hearst, *J. Bacteriol.*, 1993, **175**, 2414.
189 Y. Fujita, H. Takagi and T. Hase, *Plant Cell Physiol.*, 1996, **37**, 313.
190 Q. Wu and W. F. J. Vermaas, *Plant Mol. Biol.*, 1995, **29**, 933.
191 C. Roitgrund and L. J. Mets, *Curr. Genet.*, 1990, **17**, 147.
192 J. Li, M. Goldschmidt-Clermont and M. P. Timko, *Plant Cell*, 1993, **5**, 1817.
193 X. Q. Liu, H. Xu and C. Huang, *Plant Mol. Biol.*, 1993, **23**, 297.
194 J. Y. Suzuki and C. E. Bauer, *Plant Cell*, 1992, **4**, 929.
195 A. B. Cahoon and M. P. Timko, *Plant Cell*, 2000, **12**, 559.
196 J. Lidholm and P. Gustafsson, *Plant Mol. Biol.*, 1991, **17**, 787.
197 Y. Fujita and C. E. Bauer, *J. Biol. Chem.*, 2000, **275**, 23583.
198 G. A. Peschek, B. Hinterstoisser, B. Pineau and A. Missbichler, *Biochem. Biophys. Res. Commun.*, 1989, **162**, 71.
199 D. W. Bollivar, S. Wang, J. P. Allen and C. E. Bauer, *Biochemistry*, 1993, **33**, 12763.
200 U. Oster, C. E. Bauer and W. Rüdiger, *J. Biol. Chem.*, 1997, **272**, 9671.
201 U. Oster and W. Rüdiger, *Botanica Acta*, 1997, **110**, 420.
202 H. A. Addlesee, L. Fiedor and C. N. Hunter, *J. Bacteriol.*, 2000, **182**, 3175.
203 M. Helfrich and W. Rüdiger, *Z. Naturforsch., C*, 1992, **47**, 231.

204 M. Helfrich, S. Schoch, U. Lempert, E. Cmiel and W. Rüdiger, *Eur. J. Biochem.*, 1994, **219**, 267.
205 J. C. Lopez, S. Ryan and R. E. Blankenship, *J. Bacteriol.*, 1996, **178**, 3363.
206 H. C. Schmid, U. Oster, J. Kogel, S. Lenz and W. Rüdiger, *Biol. Chem.*, 2001, **382**, 903.
207 H. A. Addlesee, L. C. Gibson, P. E. Jensen and C. N. Hunter, *FEBS Lett.*, 1996, **389**, 126.
208 R. Tanaka, U. Oster, E. Kruse, W. Rüdiger and B. Grimm, *Plant Physiol.*, 1999, **120**, 695.
209 J. Soll, G. Schultz, W. Rüdiger and J. Benz, *Plant Physiol.*, 1983, **71**, 849; reviewed in S. I. Beale, *Photosynth. Res.*, 1999, **60**, 43.
210 H. Miyashita, K. Adachi, N. Kurano, H. Ikemoto, M. Chihara and S. Miyachi, *Plant Cell Physiol.*, 1997, **38**, 274.
211 H. Maeda, T. Watanabe and M. Kobayashi, *J. Photochem. Photobiol. B.*, 1992, **13**, 267.
212 H. Maeda, T. Watanabe, M. Kobayashi and I. Ikegami, *Biochim. Biophys. Acta*, 1992, **1099**, 74.
213 M. Akiyama, H. Miyashita, H. Kise, T. Watanabe, M. Mimuro, S. Miyachi and M. Kobayashi, *Photosynth. Res.*, 2002, **74**, 97.
214 M. Akiyama, H. Miyashita, H. Kise, T. Watanabe, M. Mimuro, S. Miyachi and M. Kobayashi, *Plant Cell Physiol.*, 2002, **43**, S170.
215 C. J. R. Fookes and S. W. Jeffrey, *J. Chem. Soc., Chem. Commun.*, 1989, 1827.
216 M. Zapata and J. L. Garrido, *J. Phycol.*, 1997, **33**, 209.
217 J. R. Nelson and S. G. Wakeham, *J. Phycol.*, 1997, **25**, 761.
218 M. A. Schneegurt and S. I. Beale, *Biochemistry*, 1992, **31**, 11677.
219 R. J. Porra, W. Schafer, E. Cmiel, I. Katheder and H. Scheer, *Eur. J. Biochem.*, 1994, **219**, 671.
220 W. Rüdiger, *Photosynth. Res.*, 2002, **74**, 187.
221 H. Ito, T. Ohtsuka and A. Tanaka, *J. Biol. Chem.*, 1996, **271**, 1475.
222 H. Ito, S. Takaichi, H. Tsuji and A. Tanaka, *J. Biol. Chem.*, 1994, **269**, 22034.
223 H. Ito, Y. Tanaka, H. Tsuji and A. Tanaka, *Arch. Biochem. Biophys.*, 1993, **306**, 148.
224 V. Scheumann, S. Schoch and W. Rüdiger, *Planta*, 1999, **209**, 364.
225 A. Tanaka, H. Ito, R. Tanaka, N. K. Tanaka, K. Yoshida and K. Okada, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 12719.
226 A. Tomitani, K. Okada, H. Miyashita, H. C. Matthijs, T. Ohno and A. Tanaka, *Nature*, 1999, **400**, 159.
227 C. E. Espineda, A. S. Linford, D. Devine and J. A. Brusslan, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 10507.
228 U. Oster, R. Tanaka, A. Tanaka and W. Rüdiger, *Plant J.*, 2000, **21**, 305.
229 M. O. Senge and K. M. Smith, *Adv. Photosynth.*, 1995, **2**, 137.
230 D. H. Burke, M. Alberti and J. E. Hearst, *J. Bacteriol.*, 1993, **175**, 2407.
231 D. H. Burke, J. E. Hearst and A. Sidow, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 7134.
232 J. G. Ormerod, T. Nesbakken and S. I. Beale, *J. Bacteriol.*, 1990, **172**, 1352.
233 F. J. Leeper, *Nat. Prod. Rep.*, 1989, **6**, 171.
234 F. J. Leeper, *Nat. Prod. Rep.*, 1987, **4**, 441.
235 F. J. Leeper, *Nat. Prod. Rep.*, 1985, **2**, 561.