ELSEVIER

Biochimica et Biophysica Acta 1411 (1999) 21–85

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Review

The biogenesis and assembly of photosynthetic proteins in thylakoid membranes[1]

Francis-Andre Wollman [a,*], Limor Minai [b], Rachel Nechushtai [b]

[a] *UPR/CNRS 1261, Institut de Biologie Physico-chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France*
[b] *Department of Botany, The Hebrew University of Jerusalem, Jerusalem 91904, Israel*

Received 3 November 1998; received in revised form 27 January 1999; accepted 22 February 1999

Keywords: Chloroplast; Thylakoid membrane; Protein biogenesis; Protein assembly; Protein import; Protein processing

Contents

* Corresponding author. Fax: +33 (1) 4046-8331; E-mail: wollman@ibpc.fr

[1] This article is dedicated to the memory of our colleague and friend, Alma Gal. Her presence played a major role in our decision to prepare this review article.

PII: S0005-2728(99)00043-2

1. Introduction

Oxygenic photosynthesis is an energy-transducing process whereby light energy is trapped and converted into biochemical energy. This multi-step process encompasses a series of electron transfer reactions from water molecules to $NADP^+$ (nicotinamide adenine dinucleotide phosphate), coupled to ATP synthesis. Oxygenic photosynthesis occurs within the thylakoid membranes of photosynthetic prokaryotes and eukaryotes and is mainly borne by five types of proteins which are embedded in these membranes: photosystem II cores (CCII); cytochrome b_6f complexes; photosystem I cores (CCI); antenna proteins; and proton-ATP synthases (CF_1–CF_0). These proteins function in a concerted manner to generate ATP and a reducing power in the presence of light.

The major Photosynthetic Proteins, hereafter referred to as PPs, are multiple-subunit protein complexes (see Fig. 1). They span the thylakoid membranes and comprise both peripheral and integral membrane subunits. The functional proteins also encompass a variety of cofactors, pigments, hemes, iron–sulfur clusters and metal ions, that associate with the protein moieties either by covalent or non-covalent binding. The aim of the present review is to present events and steps involved in the biogenesis and assembly of PPs and to raise questions concerning these processes that remain elusive to date.

The study of the biogenesis of these complex multi-molecular structures is entering a new phase for two major reasons. First, the remarkable development of the structural biology of membrane proteins has tremendously improved our knowledge of the actual composition and topological organization of most of the photosynthetic proteins. Second, the field of protein biogenesis should benefit greatly from the implementation of whole genome sequencing projects. This global genetic approach, when combined with new gene transformation strategies and the use of reverse genetics, should complement and extend our knowledge of the numerous biogenesis factors which was originally based only on classical genetic approaches and membrane protein biochemistry.

In recent years, some of the most significant insights into thylakoid protein assembly have come from comparing the phenotype of mutants resulting from similar gene disruptions or similar site-directed mutagenesis in photosynthetic prokaryotes, and photosynthetic eukaryotes. These approaches, together with the availability of in vitro expression systems including the recent development of a promising homologous in vitro translation system [1], paved the way for a refined study of the assembly pathways for photosynthetic proteins.

The present review does not cover the biogenesis of PPs from prokaryotes performing anoxygenic photosynthesis. Some aspects of their gene expression and assembly will occasionally be discussed, together with aspects of mitochondrial protein expression, whenever we feel that they add to our understanding of the PP assembly in organisms performing oxygenic photosynthetic. Tissue specificity and developmental changes of PP assembly in vascular plants, as well as the repair processes and changes in protein expression and assembly under stress con-

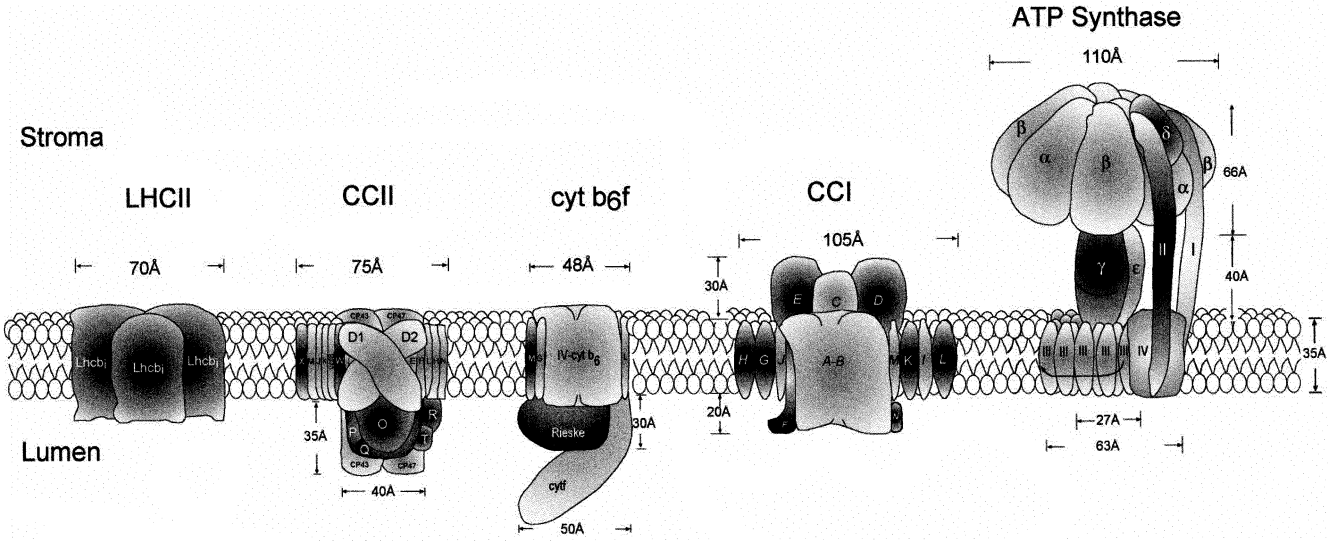


Fig. 1. Photosynthetic complexes of the thylakoid membranes. The compositions of the different photosynthetic complexes and their dimensions are drawn according to structural data available in the literature. CCI is drawn from the 4-Å resolution structure of cyanobacteria [323]. The exact position of PSI-G, PSI-H, and PSI-N that are present only in higher plants, is presently unknown. The sketch of CCII follows the structure of Morris et al. [220]. The only structural data available for some light harvesting complexes comes from the 3.4-Å resolution structure of LHCII [406]. The trimer is drawn based on [621]. Since the nature of the trimer, i.e. homotrimer or heterotrimer, is currently unknown, (i) stands for either LhcbI, II or III. The minor antenna complexes, PSII-S and Lhca antenna are not represented. The structural data available on the bc_1 complex from bovine heart mitochondria [486] served as the basis for drawing the cyt b_6f complex. However, the dimensions of the Rieske protein and cyt f were taken from chloroplast structures [480,489]. The ATP synthase sketch is based on the 2.8-Å resolution structure of the F_1 portion from bovine heart mitochondria and on the model recently suggested by Engelbrecht and Junge [549]. Arrows indicate the direction of movement of the rotor upon ATP synthesis. Black filled subunits are nuclear-encoded; gray filled subunits are chloroplast-encoded.

ditions are beyond the scope of this review. We refer the reader to recent review articles which cover these matters, at least in part [2–5].

In view of the above, we have chosen to address the issue of thylakoid protein assembly under what can be regarded as *steady-state* conditions. These correspond to the conditions that prevail in the expanding mature chloroplast in vascular plants or in the exponential phase of growth for unicellular prokaryotes or eukaryotes. Although each PP may have specific biogenesis features, there should be general rules, or constraints, which govern some aspects of assembly. These rules require a transverse approach that will be presented in Sections 2–4. Subsequently, we will expand upon the more specific biogenesis features of each PP in Sections 5–9.

PP assembly can be regarded as the result of three distinct processes: (1) the biogenesis of the constitutive subunits and their cofactors; (2) their conveyance to the thylakoid membranes; and (3) their assembly in an oligomeric protein. Although the spatial and temporal separation between these three processes may be somewhat virtual, each of these three aspects allows one to raise specific questions.

(1) Regarding the biogenesis of individual subunits, here considered as the substrates in the multiple order reaction leading to protein assembly, a key issue is to identify the critical step(s) that control(s) their actual intracellular concentration. These may vary among organisms since we are considering proteins that are well-conserved between prokaryotic and eukaryotic cells. There is now overwhelming evidence that the limiting steps in protein biogenesis in photosynthetic eukaryotes are post-transcriptional under steady-state conditions. We will show that they are in fact mainly (post-)translational, whereas there is a prominent (but not exclusive) transcriptional contribution, in photosynthetic prokaryotes.

(2) Routing each subunit to its functional location site on the membrane raises two distinct issues. One is common to prokaryotes and eukaryotes, and addresses the energetics and the processing require-

ments for insertion into, and translocation across, the thylakoid membranes. In the case of cyanobacterial or chloroplast-encoded subunits, this may either be a co-translational or a post-translational process. The second is specific to photosynthetic eukaryotes and addresses the post-translational conveyance of the nucleus-encoded and cytosol-translated subunits to the thylakoid membranes inside the chloroplast.

(3) As to the third aspect, we will briefly discuss whether subunit–subunit recognition and interaction in vivo should be considered the result of self-assembly or of an assisted process in which chaperones would be expected to play a critical part.

Sections 5–9 present the current knowledge concerning the assembly of each of the five major PPs. Each of these separate sections starts with a brief description of the constitutive PP subunits and provides some characteristics of their spatial organization in the assembled protein. In Section 7, we focused on the chlorophyll-binding proteins, termed light harvesting complexes (LHCs) from higher plants and green algae. The various biliproteins present in cyanobacteria and red algae are not discussed. The latter are peripheral membrane proteins that display highly ordered structure that has been studied in great detail. The assembly pathways and the regulation of expression of these antenna proteins have received much attention in recent review articles that give a full picture of peripheral antenna systems [6,7].

2. Production of the 'substrate-subunits' for protein assembly

A major issue in the production of PPs is to understand how the subunits and their cofactors are coordinately produced in the stoichiometry required for their assembly in a functional protein. Were this not the case, pools of unassembled components should be present in the photosynthetic cell, for any of the major constituents of these proteins, a situation that has not been reported so far. Chlorophyll pools would be extremely harmful to the cell upon exposure to light, owing to the high reactivity of the triplet state of chlorophylls with molecular oxygen, leading to the deleterious formation of singlet oxygen. Thus, if pools of unassembled PP components were to exist, they would remain insignificant in size. PP assembly is then directly controlled by the level of expression of the constitutive subunits and cofactors. Rate-limiting steps in this process can be sought at the transcriptional, post-transcriptional, (co)-translational, or post-translational levels and they may vary with physiological conditions. For example, changes in the accumulation of chloroplast proteins in differentiating plant tissues, depend on changes in nuclear and chloroplast transcription rates [8]. However, some discrepancy between the amplitudes of changes in transcript and translate levels indicate that other limiting steps soon take over in the control of protein biogenesis [9].

2.1. Some distinctive features of photosynthetic prokaryotes and eukaryotes

Although PPs in oxygenic photosynthetic prokaryotes and eukaryotes are well-conserved both at the functional and biochemical levels, it is immediately apparent that their assembly develops in widely different cellular contexts.

It is well-accepted that chloroplasts from vascular plants and algae originally arose from an endosymbiotic event most likely involving an ancestral prokaryote, phylogenetically related to cyanobacteria [10,11]. Whereas prokaryotes have a single genetic system that is expressed proximal to the site of functional assembly of the encoded proteins, the eukaryotic plant cell has a dual genetic content, in the chloroplast genome (plastom) and in the nucleus. This raises specific questions regarding chloroplast protein assembly. Chloroplast PPs comprise subunits of mixed origins, which are either organellar-encoded or nuclear-encoded. Their assembly thus requires the cooperation of two genetic compartments, the nucleo-cytosol and the chloroplast. While some biogenesis aspects are conserved among prokaryotes and eukaryotes, new strategies have necessarily evolved after endosymbiosis and are progressively uncovered by the current research on the regulation of photosynthetic gene expression.

2.1.1. Physical organization of the photosynthetic genes

In anoxygenic photosynthetic bacteria, the genes encoding the various subunits of each oligomeric

protein involved in photosynthesis are clustered on the bacterial chromosome, and are subject to a common co-transcriptional regulation. These properties identify several operons with properties similar to those found in *Escherichia coli* [12]. The reaction centers and their associated antenna proteins are encoded by the *puf* operon [13], which is part of a superoperon also comprising genes for chlorophyll and carotenoid biosynthesis [14,15]. The ATP synthase is encoded by the *unc* operon and the cytochrome *bc* complex by the *pet* operon.

Operonal organization can be considered as a key mechanism that enables the bacterial cells to rapidly accommodate changes in the oxygen content of their environment [16]. Consistent with this view, anaerobic prokaryotes, like the chlorosome-containing green sulfur bacteria, do not show the same clustering of their photosynthetic genes as do purple bacteria [17]. They exhibit operon splitting as a result of which the genes that encode the subunits of a same oligomeric protein are scattered in various places along the chromosome. For instance, the genes encoding the β- and ε-subunits of the ATP synthase are located apart from the other ATP synthase genes, and they are transcribed independently [18].

Cyanobacteria provide a well-characterized intermediate situation. Those that are capable of chromatic adaptation display an operonal organization for various components of the phycobillisomes with the *cpc*, *apc* and *cpe* operons, the organization of which accounts for the transcriptionally regulated pigment changes in response to environmental changes (reviewed in [19]). Thus, like purple bacteria, cyanobacteria can cope with rapid changes in their environment by achieving the stoichiometric production of subunits for new oligomeric proteins through the activation or repression of their monofunctional operons. In contrast, the other PPs are continually expressed in cyanobacteria because these organisms are obligate aerobic species. It follows that their gene organization displays characteristics similar to those of green bacteria: in most instances, the structural genes for a group of PP subunits are scattered along the cyanobacterial chromosome and their location results from operon splitting. Hence, the cyanobacterial genes for the ATP synthase are split between two operons, and the genes for the various subunits of the cytochrome b_6f complex are split between two operons, namely the *petCA* and *petBD*, plus *petG* and *petM*, which have been mapped at other sites of the *Synechocystis* 6803 chromosome. This gene distribution raises the possibility that a concerted expression of the *pet* genes requires some regulation at the post-transcriptional level. Other genes for major PP subunits, like that for the PSII reaction center subunit D1, are transcribed independently of the genes encoding their assembly partners. D1 polypeptide is particularly susceptible to photodamage [5] and has to be replaced more rapidly than the other PSII subunits. This requirement points to possible regulations of gene expression that is specific to a particular protein subunit.

In eukaryotes, the chloroplast genomes still contain some of the gene clusters found in cyanobacteria, but two major reorganization processes have occurred. Several structural genes for photosynthetic proteins have been transferred out of the chloroplast genome to the nuclear genome. This process has been more extensive in the green lineages than in the non-green ones [20]. In addition, most of the polycistronic transcriptional units in vascular plant chloroplasts are not monofunctional. These gene clusters group information for distinct proteins. For example, the *psaA–psaB–rps14* cluster corresponds to a gene encoding a ribosomal protein subunit associated with two genes encoding the PSI reaction center subunits [21], and the *psbB–psbH–petB–petD* cluster groups structural genes for PSII and cytochome b_6f protein subunits [22].

The chloroplast genomes of unicellular algae, like *Chlamydomonas reinhardtii*, *Chlamydomonas eugametos* or *Euglena gracilis*, show extensive gene rearrangement as compared to that of cyanobacteria but also with respect to that of the chloroplast of vascular plants. These unicellular organisms have lost the majority of the gene clusters present in cyanobacteria. Most of their mRNAs accumulate as monocistronic transcripts, although co-transcription followed by rapid mRNA processing may be more frequent than previously considered (for a review see [23]). For instance, monocistronic transcripts for the cytochrome b_6f genes *petA* and *petD*, which are 3 kbp apart, can be produced from independent promoters, but co-transcription followed by transcript processing also occurs in that gene region [24]. The same situation prevails in the *atpA/psbH* gene region,

which is about 4 kbp long. Here there are monocistronic transcripts for four genes involved in distinct functions, although the whole region can be co-transcribed [25]. If variations in the rates of transcript processing account for the prevalence of monocistronic transcripts in the unicellular eukaryotes, the pre-translational step in the chloroplast of vascular plants and green algae may prove more similar than previously thought.

There is progressive loss of an operon-based co-transcription of the genes for the whole set of subunits of each PP, when going from anoxygenic photosynthetic bacteria to cyanobacteria, vascular plants and green algae. As a major consequence, there is an increasing need for additional post-transcriptional regulations to ensure the stoichiometric accumulation of the subunits of each photosynthetic protein.

2.1.2. *Increasing the half-lives of transcripts, from cyanobacteria to the chloroplasts, sets the scene for extensive post-translational regulation*

A striking feature of chloroplast transcripts is that they are long-lived species. In a systematic analysis conducted in barley, Kim et al. [26] observed that the half-lives of seven different plastid transcripts ranged from 6 to over 40 h. This time range should be compared with that in prokaryotes, typically 1–3 min in *E. coli*, and well-below an hour in cyanobacteria [27].

The determinants for transcript stability in the chloroplast were first sought on their 3′-end, due to hairpin structures that could be predicted in many cases from the nucleotide sequence (reviewed in [23]). Indeed, deletions in this region of the *atpB* chloroplast gene in *C. reinhardtii* caused the destabilization of the corresponding transcript [28]. However, there is now increasing evidence that the major determinants for chloroplast transcript stability are borne by the 5′-untranslated regions (5′-UTR) which contain binding sites for nuclear-encoded proteins acting as protecting agents against exo-/endo-nucleolytic degradation (see Section 2.1.3). These sites may provide a means to modify transcript stability, and thereby transcript concentration, upon translation, which is initiated in the same region.

The physiological consequences of the differential transcript stability between photosynthetic prokaryotes and eukaryotes are illustrated, for instance, by the contrasting strategy these organisms have developed to cope with an increasing demand for the *psbA* chloroplast gene product, D1. As mentioned before, D1 is very susceptible to photodamage in both high-light and low-light conditions (reviewed in [5]). The cyanobacterial strategy to maintain high levels of D1 expression would be based on a transcriptional activation of additional copies of the *psbA* gene [29,30]. In contrast, high-light conditions lead to an increased stability of pre-existing *psbA* transcripts in vascular plant chloroplasts [31]. Thus, the chloroplast can accommodate extensive post-transcriptional regulations, acting at the level of maturation and translation of pools of pre-existing transcripts, both steps bearing consequences on the life-time of transcripts.

2.1.3. *Post-translational degradation of unassembled subunits plays a larger role in the control of protein accumulation in the chloroplast than in cyanobacteria*

Apoproteins which cannot bind their cofactors display shorter half-lives in the chloroplast, most likely because of their improper folding in the membranes, resulting in greater accessibility to a general protein degradation pathway (for a review see [32]). For instance, various chlorophyll-binding apoproteins are short-lived in the absence of chlorophyll or carotenoid biosynthesis [33,34]; apocytochromes and apoplastocyanin are rapidly degraded in the absence of heme and copper binding, respectively [35–37]. This is definitely not specific to photosynthetic eukaryotes. Cyanobacterial strains behave similarly. For instance, *Synechocystis* 6803 loses native apocytochrome cyt *b*-559 when the heme axial ligands are destroyed by site-directed mutagenesis [38]. Similarly, mutation of the putative His ligands of chlorophylls in the PSII core antenna subunit CP47, leads to a destabilization of the whole CCII protein [39].

What is probably more specific to the eukaryotic PP subunits, is their susceptibility to degradation when their assembly partners are absent. The respective stability of unassembled PP subunits in the chloroplast and in cyanobacteria can be compared in mutant strains lacking expression of only one particular PP subunit. With the development of chloroplast gene transformation techniques, it has become possible to compare the effects of a rather large set of gene deletions or interruptions both in cyanobacteria and in the eukaryotic green algae *Chlamydomonas*,

and more recently in tobacco or *Arabidopsis*. The prominent conclusion drawn from such comparative studies is that the steady-state concentration of the unassembled (or partially assembled) subunits is much higher in cyanobacteria than in the chloroplast. In the absence of one assembly partner, the other subunits of a protein complex accumulate in the prokaryotic cell to levels approaching those of the wild-type. However, in *Chlamydomonas*, they do not accumulate beyond 10% of the wild-type level: this holds true for the CCII subunits when expressed in the absence of PSII-K [40,41], PSII-H, PSII-I [42,43] PSII-O [44,45]; or for PSI subunits when expressed in the absence of PSI-C [46,47].

There has been, as yet, no conclusive identification of the chloroplast proteases responsible for the disposal of these unassembled subunits. However, a number of proteolytic activities have been detected in each sub-chloroplast compartment, in the stroma, in the thylakoid membranes and in the lumen [48,49]. Moreover, chloroplast homologs of bacterial proteases have been identified, such as the ATP-dependent Clp protease, which has two components ClpC and ClpP [50], FtsH [51], DegP [52] or various ATP-independent peptidases and amino- or carboxy-terminal processing peptidases (see [32] and references therein).

One could argue that the need for a larger contribution of a post-translational step in the regulation of PP assembly in photosynthetic eukaryotes results from the changes in growth rates, both in cell expansion and cell division rates, whereas the rate of translation remains similar between prokaryotes and eukaryotes. Neosynthesized PP subunits should remain only temporarily unassembled in a prokaryote cell because the membrane system in which they are inserted is permanently expanding. In contrast, the more slowly expanding photosynthetic eukaryotes may have developed more efficient post-translational mechanisms to prevent the erratic accumulation of function-deficient sub-complexes in the thylakoids. However, unicellular eukaryotic algae and cyanobacteria both have doubling times in the range of hours, an observation that weakens the above argument. The efficient proteolytic disposal of unassembled subunits in the chloroplasts is more likely to result from the need to cope with the splitting, between the nucleus and the organelle, of the genes concurring to the formation of a multi-subunit protein. The dual compartmentalization of the genetic information should affect primarily the coordination of the rates at which the subunits are delivered to the thylakoid membranes. Thus, the high proteolytic activity of the chloroplast would reflect one of the alternative regulation steps in protein biogenesis, once transcriptional regulation has lost its prominent role.

2.2. Control mechanisms specifically functioning in photosynthetic eukaryotes

2.2.1. The gene dosage issue

Among the intriguing aspects of chloroplast protein assembly is the tremendous excess in gene copy numbers for chloroplast-encoded subunits as compared with those for nuclear-encoded subunits. The chloroplast polyploidy stands from about 100 up to 100 000 gene copies/cell depending on the organism as opposed to a few copies only of the various nuclear genes [53,54]. To our knowledge, the number of transcriptionally active chromosomes per chloroplast is not known. If all chromosome copies were transcriptionally active in the chloroplast, there would be a dramatic excess of chloroplast gene products relative to nuclear gene products, unless some specific regulation process controls chloroplast gene expression. An elegant experimental approach has been designed to address this question by Hosler and colleagues [54] using *Chlamydomonas* cells treated with Fudr, a thymidine analog that inhibits selectively chloroplast DNA replication [55]. Decreasing the chloroplast gene copy number of *rbcL* and *atpA*, from about 100 to about 10, affects the steady state level of the corresponding chloroplast transcripts, but has no significant effect on the rate of synthesis of the corresponding polypeptides, which are the large subunit of the Rubisco and the α-subunit of the ATP synthase. This observation can be extended to the whole set of polypeptides translated in the chloroplast of *Chlamydomonas*: they retain the same rate of synthesis independent of a drop by an order of magnitude in the number of copies of the chloroplast genome (Drapier and Wollman, unpublished observation). These studies, combined with the analysis of nuclear mutants with altered chloroplast transcript stability [28,56], have conclusively demonstrated that there can be extensive changes in tran-

script levels in the chloroplast with little, if any, effect on the rate of synthesis of the encoded proteins. Thus, chloroplast protein expression in steady-state conditions is not limited by the concentration of transcripts in the chloroplast. One should therefore consider the possibility that a significant fraction of the transcripts is stored in a non-translatable form in the chloroplast stroma.

2.2.2. Cross-talk between genetic compartments

Although PPs in eukaryotes are organelle-located, their biogenesis must still be coordinated by the nucleus during plant cell differentiation. In addition, the mixed genetic origin of the PP subunits argues for some coordination in gene expression in order to get proper protein assembly (see Section 2.1.3). These issues have been discussed in more details in a recent review article [57].

2.2.2.1. The nuclear control. The level of gene expression in the chloroplast is mainly determined by the presence of nuclear factors acting on chloroplast gene expression at the post-transcriptional level (for a review see [58]). It is reasonable to assume that the concentration and life-time of these factors sets an upper limit for chloroplast gene expression. Thus, the subunits encoded by chloroplast genes would be expressed at about the same rate as their assembly partners of nuclear-origin despite the imbalance in gene copy numbers. Understanding how these nuclear factors are expressed and metabolized stands as a prerequisite for a comprehensive view of the coordination of chloroplast and nuclear gene expression.

The most advanced studies in the field of nuclear-control of organelle gene expression have been performed with yeast mitochondria (reviewed in [59]) and *Chlamydomonas* chloroplasts (reviewed in [58]). This nuclear control is exerted mainly at the level of mRNA maturation and translation [60,61]. It is mediated by organellar-targeted proteins that specifically affect the expression of either one gene or of a subset of organellar genes [62]. In most of the cases documented so far, the nuclear factors bind to the 5′-UTR of the chloroplast transcripts, where the *cis*-acting signals that determine translation initiation rates reside. Whether the afore-described nuclear involvement has a regulatory function or whether it has become merely constitutive of chloroplast gene expression, has not yet been addressed experimentally, with the exception of *psbA* gene expression for which a regulatory function of nucleus-encoded factors has been substantiated (see Section 5.2.2.1).

Nuclear gene participation in chloroplast gene expression has also been observed in vascular plants such as *Arabidopsis* [63–65] or maize [66], but the targets have been suggested to be less specific than in *Chlamydomonas*. It would be premature to conclude that the nucleus to chloroplast cross-talk in higher plants obeys a radically different strategy from that operating in *Chlamydomonas*, mainly because the screening procedures used to identify the nuclear mutants are different in the two systems. A number of high chlorophyll fluorescence mutants of *Arabidopsis* have recently been isolated, and shown to be protein-specific ones [67]. They should offer an ideal source of mutagenized strains for the study of nuclear factors involved in chloroplast gene expression in multi-cellular organisms.

2.2.2.2. Chloroplast retrograde signals. It is clear from the above data that the nucleus participates extensively in chloroplast gene expression. However, it is difficult to consider the continuous delivery of nuclear-encoded products to the chloroplast without a feedback signaling which would set a fine-tuning for the concerted expression of nuclear- and chloroplast-encoded gene products.

Indeed, the expression of a number of nuclear genes is light-regulated in vascular plants and green algae. Phytochrome and UV/blue light photoreceptors have been implicated in these light-responses [4]. However, part of the light-sensitivity of nuclear gene expression may be due to chloroplast-based retrograde signals delivered in the course of light-induced photosynthetic electron transfer. Support to this view may be gained from the fact that the functional state of the chloroplast influences the expression of a subset of nuclear genes (see [57] for a review), particularly that of the *Cab* (or *Lhcb*) genes encoding the major antenna proteins [68]. Among the nuclear factors interacting with chloroplast retrograde signal(s) are the products of three nuclear genes (*Gun*) that have been identified by mutations in *A. thaliana* [69].

The chloroplast-based light-regulation of the *Cab*

genes operates both at the transcriptional and translational levels. In *Dunaliella*, transcriptional regulation of the *Cab* genes in the nucleus was found to correlate with changes in the redox state of the intersystem electron carriers in the thylakoid membranes [70]. In tobacco plants, a light signal regulates cytosolic translation of both the *Cab* and *Fed-1* transcripts, the latter encoding the photosystem I acceptor ferredoxin. The signal is DCMU-sensitive and was thus ascribed to photosynthetic electron flow [71].

The two above-mentioned studies point to a possibly independent and direct retrograde signaling from the chloroplast to the translational apparatus in the cytosol acting in parallel with a chloroplast-mediated regulation of transcription in the nucleus. However, three recent reports provide relevant information which should pave the way for a more unified picture of chloroplast retrograde signals to the nucleo-cytosolic compartment: (1) chlorophyll precursors localized in the inner envelope membrane of the chloroplast, namely Mg-protoporphyrin IX, were unambiguously identified as light targets and effectors of the light-induction of nuclear heat shock genes in *Chlamydomonas* (see [72] and references therein); (2) nuclear genes for chloroplast-targeted products, such as the *Cab* genes, are coordinately controlled at the post-transcriptional level by a common nuclear product – their transcripts specifically fail to accumulate in the *Cen* nuclear mutant of *Chlamydomonas* [73]; and (3) transcripts for chloroplast-targeted products, including the *Cab* transcripts, are preferentially localized in the vicinity of the wheat chloroplast envelope [74].

Based on these findings, it is tempting to suggest, as an hypothetical framework, a two stage retrograde chloroplast signal that acts primarily on nuclear transcription but bears consequences on translational activation. Thylakoid-based redox signals, which are intimately associated with the photosynthetic process, may be transduced by protein phosphorylation changes (see [75] for a review) to the chloroplast envelope where they cause changes in the steady state levels of chlorophyll precursors. These transducers are implicated in the regulation of nuclear transcription [72] and may have *Gun*- and *Cen*-related genes among their targets. The corresponding gene products, in turn, participate in translational activation of those transcripts localized next to the chloroplast, which deliver chloroplast-targeted products.

2.2.3. The CES process

Studies with *C. reinhardtii* mutants, in which the genetic lesion primarily prevents the expression of only one subunit of a photosynthetic protein, have often shown as a secondary effect, a specific drop in the synthesis of another subunit of the same protein complex (reviewed in [58,76]). This observation has long been accounted for by the possible early turnover of the unassembled subunit. However, it has

Table 1
The CES process in organelle protein assembly

Organelle	Oligomeric protein	Dominant subunit	CES subunit	Reference
Chlamydomonas chloroplast	Cytochrome b_6f	subunit IV and cytochrome b_6	cytochrome *f*	[78,79]
	CCII	D2	D1	[77,616]
		D1	CP47	[77,617]
	CCI	PSI-B	PSI-A	[385]
	ATP synthase	subunit β	subunit α	[56]
	RUBP	small subunit	large subunit	[618]
Higher plant chloroplast	Cytochrome b_6f	subunit IV	cytochrome *f*	[66]
	CCII	D1	CP47	[82,619]
	RUBP	small subunit	large subunit	[81]
Yeast mitochondria	Cytochrome oxidase	COXII and COXIII	COXI	[84]
	ATP synthase	ATP6 and ATP8	ATP9	[620]

Indicated as CES subunits are those subunits that were identified as showing reduced synthesis in the absence of an assembly partner, indicated here as a dominant subunit.

later been recognized that the poorly labeled subunit was in fact a long-lived species [77,78]. Thus, in several instances, the absence of one PP subunit can lead to a significant drop in the rate of synthesis of one of its assembly partners.

Because of this seemingly hierarchical contribution of polypeptide synthesis to the biogenesis of chloroplast oligomeric proteins, those subunits whose rate of synthesis appears assembly-dependent have been designated as CES subunits (controlled by epistatic synthesis) as opposed to the dominant subunits whose absence causes the attenuated synthesis of their CES partners. Each of the four major PPs contains at least one CES subunit (see Table 1). In one instance (see Section 8), the molecular mechanism for a CES process has been documented in greater detail. It turns out that the CES subunit cytochrome *f* displays an autoregulation of translation [79]. It occurs through a negative feedback mechanism involving a stromal-exposed C-terminal protein motif, which is shielded upon assembly, and the 5′-initiating region of the corresponding mRNA [79]. This mechanism may implicate some additional nuclear factors that have been identified in cytochrome b_6f mutants of *Chlamydomonas* as specifically acting on cytochrome *f* translation [80].

Several observations reported on higher plants support the view that the CES process is ubiquitous in the biogenesis of chloroplast proteins. The rate of translation initiation of the chloroplast-encoded LSu (large subunit of Rubisco), as measured by the proportion of *RbcL* transcripts associated with the polysome fraction, has been found to decrease in tobacco antisense plants that underexpress the nuclear-encoded SSu (small subunit of the Rubisco) [81]. The crp1 nuclear mutant of maize, which lacks synthesis of suIV from the cytochrome b_6f complex because of an impaired processing of its transcript, is also defective in cytochrome *f* synthesis [66]. The vir-115 nuclear mutant in barley, which is primarily altered in D1 expression, also shows reduced synthesis of apoCP47 [82]. Although, other interpretations than a CES process have been suggested for the last two examples, it is remarkable that two subunits, cytochrome *f* and apoCP47, which correspond to CES subunits in *Chlamydomonas*, behave as predicted from a CES process in higher plant chloroplasts. It should also be mentioned that several studies on the biogenesis of mitochondrial proteins in the yeast *Saccharomyces cerevisiae* point to the contribution of a similar CES process. In particular, the COXI subunit of the cytochrome oxidase behaves as a CES subunit: its rate of assembly, but not its half-life, drops when the enzyme does not assemble due to the absence of other COX subunits [83–85].

Fig. 2 summarizes the two major regulation processes, the CES process and the post-translational degradation process, that control the stoichiometric accumulation of subunits from a simple two-component PP protein.

3. Conveyance of the PPs to the thylakoid membranes

3.1. Site of translation of the cyanobacterial and chloroplast-encoded PP subunits

The chloroplast contains its own translation machinery, and the ribosomes therein share extensive similarities with cyanobacterial ribosomes. Therefore, the same question should be addressed in the two cases. Are those polypeptides whose final destination is the thylakoid membranes, first translated elsewhere – for instance on stromal (cytoplasmic, in cyanobacteria) polysomes or next to the inner envelope (plasmic, in cyanobacteria) membrane, and then conveyed to the thylakoid membrane by some specific machinery? Alternatively, are these proteins directly made in situ, that is co-translationally inserted into the thylakoid membranes? These aspects have been discussed, in part, in two recent reviews [86,87].

Early electron microscopy studies of *Chlamydomonas* chloroplasts have shown the presence of ribosome clusters in close vicinity to the thylakoid membranes [88]. This observation is consistent with the subsequent finding that polysomal fractions from chloroplast extracts can be separated in soluble and membrane-bound fractions. Polysomes from the latter fractions can be further distinguished by their susceptibility to membrane binding, high salt-treatment or puromycin treatment [89,90]. Puromycin-sensitive attachment indicates co-translational insertion of the polypeptide into the thylakoid membranes. Identification of those polypeptides that are translated out of membrane-bound polysomes has been performed in two ways: either by probing their

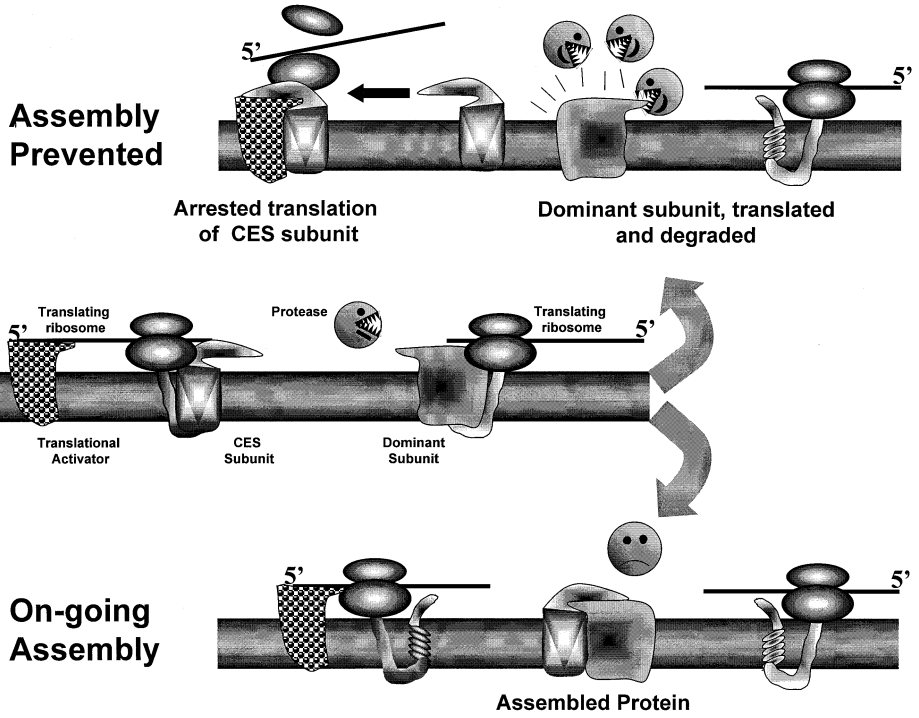


Fig. 2. A simplified view of an assembly mediated production of two transmembrane subunits in the stoichiometry required for the functional accumulation of an oligomeric protein in the thylakoid membranes. The processes depicted in the figure are derived from those which control the biogenesis of cytochrome b_6f complexes in *Chlamydomonas reinhardtii* [78,79]. Central panel: the two subunits are co-translationally inserted in the thylakoid membranes independently. Translation of the CES subunit requires a hypothetical translational activator. Lower panel: the two subunits assemble in a protease-resistant form, either spontaneously or in a chaperonine-mediated process which is not indicated on the figure. Successful assembly allows further co-translational insertion of new subunits. Upper panel: production of each subunit is prevented whenever assembly is impaired. Two distinct regulation mechanisms contribute to the loss of the subunits: (1) a post-translational degradation process according to which the dominant subunit is translated at comparable rates whether assembly is completed or not, but the unassembled subunit exposes protease-susceptible protein motifs which lead to its rapid proteolytic disposal; and (2) an autoregulation of translation according to which the unassembled CES subunit exposes a protein motif which interacts with the translational activator and prevents further synthesis of the CES subunit. Note that the dominant subunit is named after its role, via the assembly process, in translational activation of the CES subunit. Conversely, the CES subunit (controlled by epistatic synthesis) is named after the dependence of its synthesis on the presence of the dominant subunit. In the eukaryotic cell, the dominant subunit could be of nuclear origin and inserted post-translationally in the thylakoid membrane, whereas the CES subunit has to be translated in the subcellular compartment where assembly takes place.

mRNA content or by run-off translation experiments. These two approaches have led to the conclusion that membrane-associated polysomes translate both transmembrane and peripheral membrane polypeptides like the PSII RC subunit D1 [91] or the α/β-subunits of CF_1 [92], respectively. Thus, most, if not all, of the chloroplast-encoded subunits of the PPs are thought to be translated on polysomes bound to the membranes either by electrostatic interactions or by physical attachment of the nascent polypeptide chains.

A novel approach to the identification of the translation site of the chloroplast-encoded PP subunits is emerging with the present efforts to localize those nuclear-encoded factors which interact with the 5′-leader of the chloroplast transcripts. The first evidence that some of these factors were membrane-associated has come from the detergent-dependence of the binding of a 47-kDa protein to the 5′-leader of the *psbD* transcript in *Chlamydomonas* chloroplasts [93]. This observation is reminiscent of the localization to the inner membrane of yeast mitochondria of several translational activators and mRNA-protective proteins (for a review see [94]). In a systematic search for the localization of proteins that bind to the 5′-leader of the *psbC* transcript in *Chlamydomo-*

nas chloroplasts, Zerges and Rochaix [95] have found that five major chloroplast mRNA-binding proteins are indeed associated with membranes. However, these membranes possess characteristics which are different from the bulk of the thylakoid membranes. They are largely devoid of chlorophylls, and they resemble the inner envelope membranes in buoyant density and acyl lipid composition. It should be noted here that the *psbC* transcript used in the experiments of Zerges and Rochaix [95] corresponds to the mRNA for a PSII core antenna subunit. Co-translational insertion of chlorophyll-binding proteins in the inner envelope membrane is a very attractive proposal since the last steps of chlorophyll biosynthesis also occur in the chloroplast envelope. It is then ideally suited as a platform for a concerted biogenesis of the apoproteins and their pigment co-factors. However, the characterization of the particular membrane fraction reported in [95] does not yet rule out a possible heterogeneity among the thylakoid membranes themselves. From this stand point, a subset of the unstacked membrane regions, distinct from the PSI and the ATPsynthase-enriched domains [96], may be specifically devoted to membrane protein biosynthesis.

3.2. Targeting to the organelle and import

The nuclear-encoded PP subunits, whose final destination is the chloroplast, are translated in the cytosol. Hence, some efficient machinery must exist that will target them to the appropriate subcellular/subplastid compartment where they reside. This involves correct targeting of the proteins to the organelle, and transport into or across the organelle membranes. The leader peptide of the cytosolic-translated proteins is believed to be both necessary and sufficient for targeting of the proteins to the organelle, and for their transport across the double membrane envelope of the chloroplast [97,98]. The post-translational import of PP subunits has mainly been studied using in vitro assays, in which radiolabeled precursors are incubated with isolated intact chloroplasts [99,100].

The first step of the import process involves binding to lipids of the outer chloroplast envelope [101–103], and to protease-sensitive receptors on the surface of the chloroplast [104,105]. This step produces the first stable import-intermediate of the precursor, called the early intermediate [106,107]. It requires the presence of low levels of nucleoside triphosphates (10–100 μM) and the presence of outer envelope membrane proteins (OEPs) [106,108,109]. At this stage, the precursors not only bind to the receptors, but also progress into the translocation machinery, being irreversibly bound [106], and frequently partially protected from protease treatment [105,110,111]. Late intermediate proteins span both envelope membranes such that their N-terminal leader peptide is exposed to the stromal processing peptidase while their C-terminus is still accessible to exogenous proteases [107,112]. This is a good indication that the precursors cross the envelope at sites of close contact between inner and outer membranes (contact/joint sites). Complete translocation into the chloroplast is accomplished when the ATP concentration is raised to approximately 1 mM [106,107]. Protein translocation across the chloroplast envelope differs from protein translocation across the mitochondrial envelope in that it does not require a membrane potential [113–115].

In order to translocate across the envelope membranes, the protein should maintain a loosely folded conformation. This is probably achieved by at least three different factors known to date: the leader peptide in the precursor which prevents complete folding of the protein as shown for the ferredoxin precursor [116,117]; an unfoldase activity localized on the surface of the chloroplasts that keeps the preprotein in an unfolded conformation prior to its translocation across the envelope membranes [118–120]; and molecular chaperones, such as members of the Hsp70 family that interact with the precursor proteins, as shown for preLHCII [121].

In recent years, reports coming mainly from the group of J. Soll have elucidated and characterized the components of the protein import machinery of the chloroplast envelope (see [122] for a review and references therein). They have indicated the involvement of at least five OEPs, the fifth being an amino acid-selective channel protein [123], and three inner envelope proteins (IEPs) (see Fig. 3). A fourth IEP has been subsequently suggested to interact with the leader peptide of chloroplast precursor proteins [124]. Precursor proteins are recognized by the outer envelope membrane import complex which include

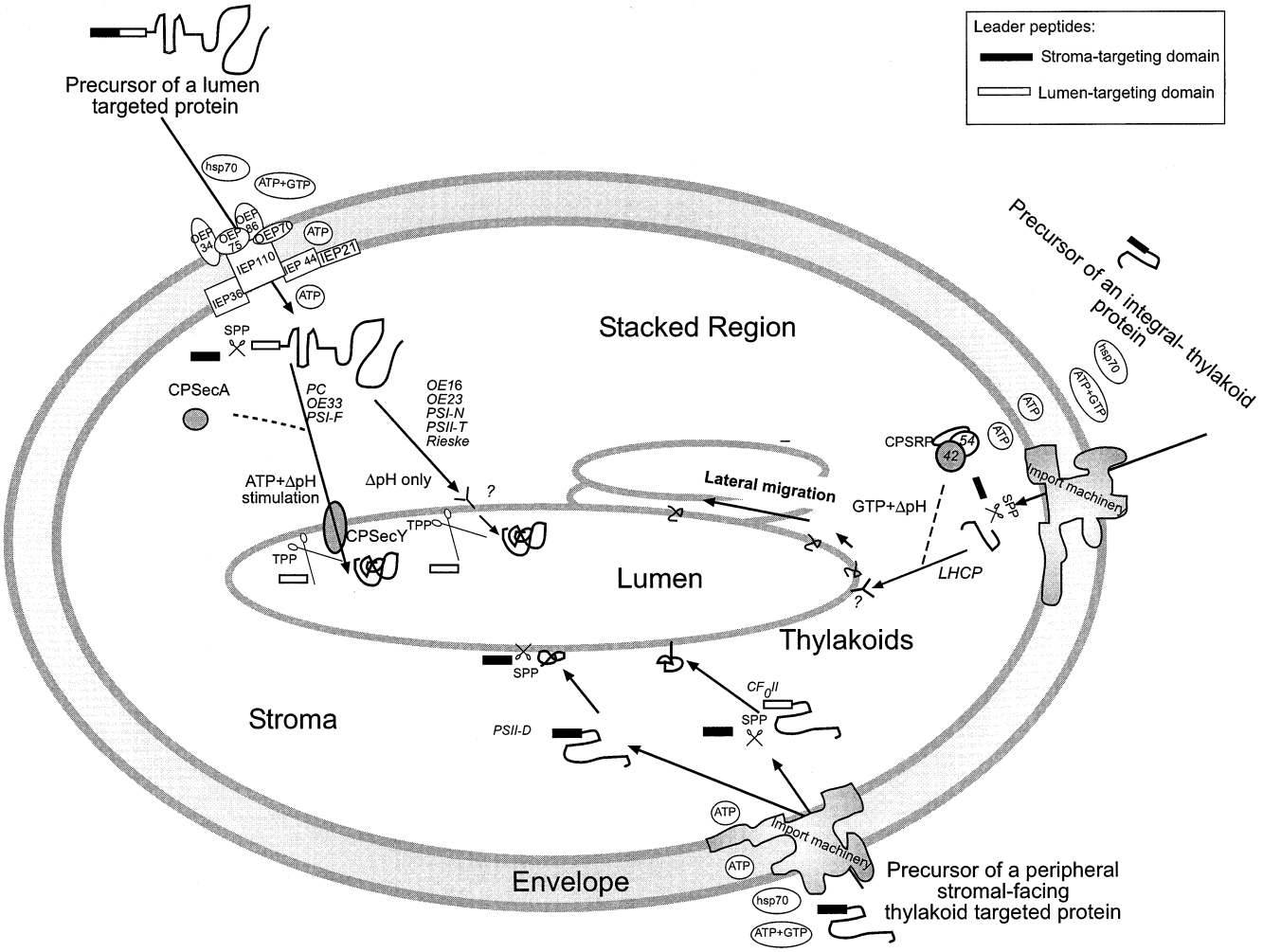


Fig. 3. Import pathways of nuclear encoded photosynthetic proteins into the chloroplast, and their routing to the appropriate suborganellar compartments. The import of nuclear encoded proteins into the chloroplast is mediated by an import apparatus positioned at the chloroplast envelope. The initial step of translocation occurs via binding of the protein to OEP86 that serves as a receptor. OEP75 forms a translocation pore through which the protein translocates with the help of IEP110 the latter probably mediates contact sites between the two envelope membranes (for a detailed presentation see [122,137]). Following import, the stromal leader peptide is cleaved by a stromal processing peptidase (SPP) and the different proteins use several routing mechanisms directing them to their suborganellar compartments. Lumen targeted proteins undergo further processing by a thylakoid processing peptidase (TPP), localized to the thylakoid membrane. OEP, outer envelope protein; IEP, inner envelope protein.

two GTP-binding proteins and a receptor subunit that binds the precursors targeted to the chloroplast. The translocation of the precursors occurs through a pore subunit and it is assisted by a member of the Hsp70 chaperone family situated at the inter-envelope space in association with the surface of the outer envelope membrane. The phosphorylation/dephosphorylation of these outer membrane proteins probably also contribute to the regulation of the precursor import. [122]. A large portion of a 110 kDa IEP is exposed to the intermembrane space between the two envelope membranes [125], suggesting it has a role in the contact sites. The exact function of the other inner envelope proteins involved in the import machinery is not yet known [126].

3.3. Maturation of the precursor: the processing step

During, or shortly after, translocation across the envelope membranes, the leader peptides are re-

moved by SPP, a stromal processing peptidase shown to be a metalo-peptidase [127]. The mechanism of action and the exact nature and characteristics of SPP are not yet understood. The only report claiming to identify SPP is that reported in [128] that has described a purified pea SPP as an oligomer of two proteins of about 140 kDa. The stromal facing PP subunits, as well as some integral membrane proteins, like the LHCII, have been shown to be processed by this SPP.

Lumenal protein maturation has been shown to consist of two-steps. First, the precursors containing a bipartite leader peptide are imported to the stroma where they are processed to intermediate forms by SPP. Then the intermediates are transferred across the thylakoid membranes and processed to the mature size by a thylakoid processing peptidase, TPP localized to the thylakoid membrane. For example, the presequence of oxygen evolving (OE) lumenal proteins consists of two targeting signals in tandem, a stromal targeting domain (STD) and a lumenal targeting domain (LTD). The STD is equivalent to stromal protein targeting presequences that are typically basic, hydrophilic, and rich in serine and threonine. Theoretical analyses have predicted that STDs adopt predominantly random coil conformation [129].

The characteristic features of the lumen targeting domain which serves as thylakoid transfer domain, consist of a charged domain, a hydrophobic core region and short chain residues, usually Ala, at -3 and -1 positions, relative to the TPP cleavage site [130]. These features are very reminiscent of signal peptides of proteins that are exported in prokaryotes by the Sec-dependent mechanism. Pea TPP and *E. coli* signal peptidase have been shown to be virtually identical [131].

In recent years, the question of protein maturation (processing) has become a central issue regarding PP membrane insertion. Different systems have been used in an attempt to determine whether processing the polypeptide is required for membrane integration or whether it is a final step in the assembly process, i.e. does processing enable the protein to obtain the appropriate folded conformation such that it can be integrated into an oligomeric PP in a functional form. This issue, which will also be addressed in the present review article, is especially relevant with respect to PP subunits situated at the stromal surface of the membranes.

3.4. Insertion, assembly and translocation into the thylakoid membranes

Prior to, right after, or concomitantly with the processing step, the polypeptides have to integrate/assemble/translocate across the thylakoid membranes. In recent years, mainly by using in vitro experimental systems, several distinct pathways have been identified for the integration of PP subunits in the thylakoid membranes. The nature of the protein (peripheral/integral) probably determines the pathway by which it will be inserted/integrated into the membrane (see Fig. 3). Several studies have indicated that the thylakoid integration of stromal-facing subunits, like PSI-D [132,133], does not require the presence of NTPs, nor does it need assisting proteins like chaperones/chaperonines or the presence of a *trans*-thylakoid ΔpH. These subunits probably integrate via protein/protein interactions, using specific recognition sites with their assembly partners.

The mode of thylakoid integration of integral membrane proteins, after their import in the chloroplast, remains poorly understood. Whether it is a spontaneous process or a catalyzed process requiring receptor sites at the thylakoid membrane surface is not known. The proteins best characterized in this category are the light-harvesting chlorophyll *a*/*b*-binding proteins (LHCP). The integration of LHCP has been shown to have characteristics of the SRP system used for protein targeting to the ER and the bacterial cytoplasmic membrane [134] (see Section 7). A spontaneous translocation/insertion pathway has been proposed for proteins like CF_0-subunit II (CF_0II) of the ATP synthase. This integral membrane protein which is synthesized with a bipartite presequence similar to those of lumenal proteins [135], seems to possess a stop-transfer signal that prevents its full translocation to the lumen. This spontaneous membrane insertion brings to mind the mechanism reported for bacterial membrane systems [136].

The translocation process across the thylakoids of lumen-facing membrane proteins has been the subject of intensive studies (for recent reviews see [137,138]). In bacteria, the Sec route is employed

for protein export across the cytoplasmic membrane [139,140]. There is now overwhelming evidence that a Sec system also operates in thylakoids. *secA*- and *secY*-homologous genes have been found in the chloroplast genome of several algae and vascular plants [141–143]: a SecA-dependent thylakoid transport mechanism has been identified in pea chloroplasts [143] and in maize [144]; a stromal 110-kDa SecA homolog, designated CPSecA, has been identified and purified [145], thus accounting for the stromal factor required for the membrane integration of PC, PSII-O and PSI-F. Inhibition of the translocation of these proteins by azide, the characteristic inhibitor of *E. coli* SecA, is also indicative of the use of an analogous SecA system by the lumenal targeted proteins [145–147]. Furthermore, a chloroplast homolog of SecY (CPSecY) has been isolated from *Arabidopsis* [148]. Presumably it functions in the thylakoids as in bacteria, by forming part of the membrane translocon. A detailed characterization of the role of SecY in thylakoid transport pathways is still lacking. In addition to soluble stromal factors, translocation through the Sec route requires ATP and is stimulated by a transthylakoid ΔpH [149,150].

Other lumenal proteins, like PSI-N and PSII-P/Q/T, do not require NTPs or stromal factors for their transport across the thylakoid membranes. These proteins are translocated by a mechanism that depends strictly on the thylakoidal ΔpH [146,151–155]. Because these proteins are absent from cyanobacteria, it has been suggested that this novel system for protein translocation to the thylakoid lumen has evolved in photosynthetic eukaryotes [156]. However, three recent findings argue against a phylogenetic interpretation: (1) a ΔpH route is used for exporting folded proteins in bacteria [157]; (2) the Rieske protein, present in cyanobacteria and chloroplasts, is translocated by this route across the thylakoid membranes from vascular plants ([524], Karnauchov and Kloesgen, personal communication); and (3) several bacterial open reading frames have been found to be homologous to the *Hcf106* gene from maize, which encodes a receptor-like thylakoid membrane protein necessary for protein translocation via the ΔpH route [158]. These observations suggest that the ΔpH route is used by passenger proteins in a prefolded state.

4. The biogenesis of PPs: self-assembly or assisted process?

As mentioned above, and detailed for each PP in the next sections, the complex routing of the various PP subunits to the thylakoid membranes is accompanied by a number of co/post-translational modifications which involve numerous possible protein effectors: translational activators, processing enzymes, protein translocators, (un)folding enzymes, cofactor-transferring proteins, etc. Once targeted to the membrane, two major questions arise concerning each subunit. First, the ultimate co/post-translational modifications of a particular subunit could occur in the unassembled state or could be in part catalyzed by some subunit–subunit cooperative interaction. We have not found a single example of the latter requirement among the numerous studies on thylakoid protein biogenesis, whether they have been conducted in vivo or in vitro. Thus, the biogenesis of a PP subunit can be regarded as an assembly-independent process, although the rate of synthesis may be affected by assembly as evidenced by the CES process.

Once the subunits are independently synthesized, they may either assemble spontaneously in the thylakoid membrane or require assisting proteins that would not be part of the assembled PP. In the latter view, a well-ordered series of protein–protein interactions may have to be favored both spatially and temporally in order to get efficient protein assembly. The role of chaperones in such an assisted process has been long described for soluble proteins (for a review see [159]). Although it is the underlying assumption of many biogenesis studies with membrane proteins, there is at present no definite experimental evidence for the participation of unambiguously identified chaperones in the assembly of PPs.

However, there are several polypeptides that can be presently considered contributing to the stable assembly of a PP, without playing some major part in the function of the active protein. This is the case of the numerous small polypeptides having one transmembrane (TM) domain only, the function of which is unknown, but which are found associated with purified PSI, PSII and cytochrome b_6f complexes (see Section 5, Section 6 and Section 8). It is tempting to suggest that they merely contribute to establish a protease-resistant configuration of the

PP in the membrane of the living cell, thereby *assisting* the assembled and active protein. This view has been adopted, in particular, for the chloroplast gene product *psbT* (initially named ycf8) in *Chlamydomonas*, a 3-kDa polypeptide with one TM segment. In its absence, the PSII protein complex still assembles and accumulates in the membranes, but it is destroyed more readily in adverse conditions such as high light or conditions of limited protein synthesis [160]. However, it should be noted that those genuine PP subunits, like PSII-P or the Rieske protein, in the absence of which the partially assembled proteins do accumulate extensively in exponentially growing *Chlamydomonas* cells, also contribute to their stabilization in more adverse conditions or in aging cells (D. Drapier, R. Kuras, C. de Vitry, F.-A. Wollman, unpublished observations). Thus, a lower stability of a PP in *Chlamydomonas* in the absence of a particular subunit should not be taken as a strong evidence that this polypeptide has mainly a chaperone-type function.

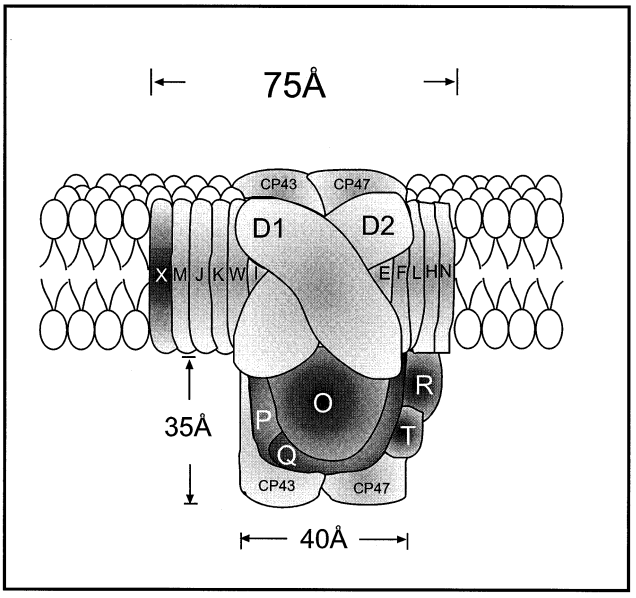
At variance with the above, small subunits of ill-defined function, several new proteins, are candidates for a function in the assembly of PSI [161,162] discussed in Section 6, or of PSII ([163], see Section 5). These are required for the accumulation of the various subunits of the oligomeric protein, although they are not part of the final assembly. Their possible function as a catalyst for cofactor binding to some PSI or PSII subunit, in a very similar way as does the CcsA chloroplast gene product for *c*-heme binding to cytochrome *f* ([164]; see Section 8), has not yet been assessed. It is then premature to consider these proteins as bona fide chaperonines for membrane protein assembly.

Given our present state of knowledge, we would rather consider the assembly process not only as thermodynamically favorable, but also kinetically optimized once the various mature subunits of a PP are membrane-associated within the two-dimensional space of the hydrophobic lipid bilayer. Based on successful reconstitution experiments in vitro with two fragments of bacteriorhodopsin, Popot and Engelman have set a two-stage assembly model for integral membrane proteins [165]. In this model, TM helices are considered as independent folding domains. They first insert and acquire their α-helical structure independently in the lipid bilayer, then undergo helix packing and shuffling which leads them to the fully folded state that corresponds to the active protein. From this stand point, whether TM helices belong to a single subunit or to two distinct subunits of an identical oligomeric protein bears little consequence as to the thermodynamics of the folding pathway. Consistent with this proposal, are various examples of gene fusion or gene splitting for well-conserved TM proteins. The best example is that of the mitochondrial or purple bacterial gene which encodes cytochrome *b* as a single polypeptide with eight TM helices. It has two counterparts in chloroplasts and cyanobacteria, the *petB* and *petD* genes. These genes encode two subunits, cytochrome b_6 and suIV, having four and three TM helices, respectively [166]. They are, respectively, homologous to the N-terminal and C-terminal domains of cytochrome *b*. The well-conserved structural and functional features of cytochrome b_6f and *bc* complexes argues against any major effect of the splitting of a transmembrane protein in two parts, regarding their respective ability to assemble properly. Thus, the major oligomeric PPs, which encompass from 12 TM helices (cytochrome b_6f) to more than 25 TM helices (PSI or PSII), could assemble spontaneously through the packing of their constitutive TM helices, provided that they have already attached their cofactor complement.

5. Photosystem II

Photosystem II mediates electron transfer from water to plastoquinones, as a result of the photoinduced charge separation between a primary chlorophyll donor P680 and a pheophytin acceptor molecule. The light-harvesting capacity of PSII is increased by the association of the core component of PSII (CCII) with other protein complexes bearing a peripheral antenna. In cyanobacteria and red algae, the peripheral antenna is made of phycobiliproteins, the supramolecular organization and assembly of which into phycobilisomes have been described in several recent reviews [7,19,167]. In higher plants and green algae, CCII is associated with chl *a*/*b*-containing light harvesting complexes (for a review see [167]), the biogenesis of which is described in Section 7. A typical PSII complex from higher plants thus

contains more than 20 polypeptide subunits, 200–250 chl molecules per P680, and a chl *a*/chl *b* ratio that equals 2–3. In the past decade, a number of reviews have covered all aspects of the organization and function of the PSII proteins [168–172].



5.1. The assembled protein

5.1.1. Subunit composition and topology in the membranes

CCII is highly conserved among all oxygenic photosynthetic organisms, i.e. cyanobacteria, algae and vascular plants. Table 2 summarizes the current knowledge on its subunit composition. Most of the CCII subunits are in a transmembrane orientation, but a subgroup is associated with the lumenal face of the thylakoid membranes where they contribute to the catalysis of water oxidation.

5.1.1.1. The integral membrane part of CCII. Four major CCII subunits, D1, D2, CP47, and CP43, bind the prosthetic groups and pigments of the complex. These encompass the primary donor P680, a dimer of chlorophyll *a*, the pheophytin electron acceptor, the secondary donor tyrosine Y_z, the alternative donor Y_D, the primary and secondary quinone acceptors Q_A and Q_B, a 40–60 chl *a* core antenna, and 10–12 β-carotenes.

The reaction center II. The isolated reaction center (RCII) [173], which corresponds to the minimal PSII assembly capable of photochemical charge separation, comprises four to six chl *a* molecules, two pheophytin *a*, one plastoquinone, one non-heme iron, one to two β-carotenes and six polypeptide subunits. D1 and D2 are the major constituents of RCII, and bear the two tyrosine donors which are placed at a lumenal loop of D1 and D2, in largely conserved regions of nine amino acid residues. D1 and D2 show a high degree of homology to the L and M subunits of the reaction center of purple bacteria, the structure of which has been elucidated at high resolution [174–176]. The four other RCII subunits are the α (PSII-E) and β (PSII-F) subunits of cytochrome *b*-559 and two low molecular weight subunits, PSII-I and PSII-W, the latter being absent from cyanobacteria [177,178]. D1 and D2 polypeptides have five TM helices each [179], whereas each one of the four other RCII subunits has only one TM helix [178,180,181].

Cytochrome b-559. An intriguing feature of the primary sequence of the two cytochrome *b*-559 subunits, PSII-E and PSII-F, is the presence of only one histidine residue, located in the TM helix near the N-terminus [182]. Thus, the bis-His-coordination of the *b*-type heme [183] points to a requirement for an oligomeric structure of holocytochrome *b*-559. It was first suggested that holocytochrome *b*-559 corresponded to PSII-E/PSII-F heterodimers, based on biochemical and immunocytochemical studies of the transmembrane orientations of the two subunits. They were both found in parallel orientation with stromal-exposed N-termini [184,185]. This conclusion was recently challenged by the creation of a photosystem II competent mutant strain of *Synechocystis*, which expresses a PSII-E/PSII-F fusion protein and forces the two transmembrane helices into an antiparallel orientation [186]. In this construct, the heme-liganding histidines of PSII-E and PSII-F are placed on opposite sides of the mutant membranes. The authors concluded that only PSII-E or PSII-F homodimers could coordinate the *b*-hemes. This proposal supports a stoichiometry of two rather than one *b*-559 heme per RCII, each heme being in a distinct environment on opposite sides of the thylakoid membrane (see [187] for a review of this controversial issue). The rationale for the intimate association of cyt *b*-559 in RCII is far from being understood. It is

Table 2
Photosystem II

Subunit	Topology (no. of TM α-helices)	Approximate molecular mass (kDa)	Gene (C/N)	Function and cofactor binding	Contribution to the accumulating of PSII
PSII-A (D1)	TM (5)	39	*psbA* (C)	RCII, Q_B, Y_Z 4–6 chl *a*, pheo, 1–2 β-carotene	+ CES
PSII-D (D2)	TM (5)	39	*psbD* (C)	RCII, Q_A, Y_D, 4–6 chl *a*, pheo, 1–2 β-carotene	+
PSII-E (cyt *b*-559α)	TM (1)	9	*psbE* (C)	RCII, *b*-heme, photoprotection?	+
PSII-F (cyt *b*-559β)	TM (1)	4	*psbF* (C)	RCII, *b*-heme, photoprotection	+
PSII-I	TM (1)	4	*psbI* (C)	RCII	−
PSII-B (CP47)	TM (6)	56	*psbB* (C)	core antenna, 20 chl *a*, 2-4 β-carotene	+ CES
PSII-C (CP43)	TM (6)	51	*psbC* (C)	core antenna, 20 chl *a*, 5 β-carotene	+/−
PSII-H	TM (1)	8	*psbH* (C)	regulating Q_A to Q_B electron transfer?	− CES
PSII-J	TM (1)	4	*psbJ* (C)	?	−
PSII-K	TM (1)	4	*psbK* (C)	?	+/−
PSII-L	TM (1)	4.5	*psbL* (C)	Role in Q_A binding?	?
PSII-M	TM (1)	4	*psbM* (C)	?	?
PSII-N	TM (1)	4.5	*psbN* (C)	?	−
PSII-T (ycf8 protein)	TM (1)	4	*psbT* (C)	?	?
PSII-O (OE33)	L	26	*PsbO* (N)	Role in Mn cluster stabilization, Ca^{2+} and Cl^- binding?	+/−
PSII-X	TM (1)	4	*PsbX* (N)	?	?
PSII-P[a] (OE23)	L	20	*PsbP* (N)	Ca^{2+} and Cl^- binding?	−
PSII-Q[a] (OE16)	L	16.5	*PsbQ* (N)	Ca^{2+} and Cl^- binding?	−
PSII-R[a]	TM (1?)	10	*PsbR* (N)	?	?
PSII-T′[a,c]	L	3	*PsbT′* (N)	?	?
PSII-W[a]	TM (1)	6	*PsbW* (N)	?	+
PSII-U[b]	L	14	*psbU*	?	?
PSII-V[b] (cyt *c*-550)	L	15	*psbV*	role in water oxidation	−

Topology denotes whether the protein in transmembranal (TM) or lumenal (L). Approximate molecular masses are given according to the amino acid sequence of the mature protein. Note that they may vary markedly upon gel migration and between different organisms. C, chloroplast gene (lower case); N, nuclear gene (upper case). +, Required for the accumulation of other subunits of the complex; +/−, mildly required; −, dispensable; CES, controlled by epistatic synthesis.

[a]Found only in higher plants and in algae.

[b]Found only in *Cyanobacteria*.

[c]A nuclear-encoded subunit found to be associated with the oxygen evolving complex which was also named PSII-T [623]. Here, we call it PSII-T′, in order to avoid confusion with the chloroplast-encoded PSII-T.

suggested to have some protective function by acting as an electron sink in conditions of excess illumination (reviewed in [187,188]).

The core antenna proteins. The two other major integral membrane subunits in CCII are the core antenna proteins CP47 and CP43, each having a bundle of six TM helices, to which about 20 chl *a* molecules bind. CP47/43 have large hydrophilic loops extending in the lumen which participate in the organization of the donor side of PSII, specifically in chloride binding, assembly of the manganese cluster and interaction with the PSII-O subunit [168].

The other transmembrane small subunits of CCII (see Table 2) probably do not bind cofactors, and their function remains elusive. A small transmembrane PSII subunit of about 35 residues, encoded by the *psbT* chloroplast gene, was suggested to play a protective role for PSII in adverse conditions in *Chlamydomonas* [160].

5.1.1.2. The peripheral oxygen evolving enhancer (OEE) subunits. This class of subunits, located on the lumen face of the thylakoid membranes, is significantly different in CCIIs from cyanobacteria and chl *a*/*b*-containing organisms. While the O subunit seems ubiquitous, the P and Q subunits are lacking in cyanobacterial CCII that in turn contains two other specific subunits U and V, the latter being a *c*-type cytochrome [172]. Using combined deletions of the genes encoding the O and V subunits, Shen et al. [189] have shown that the cyanobacterial cytochrome has a function similar to that of the O subunit in O_2 evolution. The three eukaryotic OEE subunits, the O, P and Q subunits, also named after their apparent molecular weights OE33, OE23 and OE16, are present in a 1:1:1 stoichiometry, most likely at one copy per RCII, although two copies have been occasionally suggested (for a discussion see [190]). The O subunit binds directly to intrinsic polypeptides of CCII [191]. It stabilizes the Mn-cluster in an optimized configuration for catalytic activity [168,169]. The P and Q subunits enhance the binding of Ca^{2+} and Cl^- [192–194]. Topological studies have discriminated between regions of subunits O and P that either interact with other proteins or are exposed to the hydrophilic solvent [195–198].

5.1.2. Supramolecular organization

The three-dimensional crystals of the PSII core that have been obtained to date are too small to allow one to determine a structure at high resolution [199,200] (for a review see [201]). However, the supramolecular organization of CCIIs has been approached by a variety of other means. Computational modeling studies have used the bacterial RC structure as a template for positioning the residues of D1 and D2 in a three dimensional model [202–206].

Electron microscopy studies yielded a number of top view projection maps of CCII. The resulting image is that of a central region of low density flanked by two prominent regions of high electron density, which appear to be related by a two-fold rotational symmetry axis. Despite the structural similarities of these EM images, there has been an on going controversy as to the oligomeric state of the CCII. Whereas one group has claimed that the structure is monomeric [207–210], most of the other groups concluded that the two-fold symmetry of the projection maps reflects the dimeric organization of CCII [211–216]. Chromatography analysis and 2D crystallization studies further supported the dimeric nature of CCII [214,215,217].

The dimension of the unit cell in tubular crystals of PSII, containing D1, D2, cytochrome *b*-559, and CP47/43/29/26/24 are those of a [17.0]×[11.4] nm dimer [215]. Similar values of [17.2]×[9.7] nm were reported for PSII-oxygen evolving core complexes of about 450 kDa from vascular plants (spinach) and cyanobacteria (*Synechococcus*) [217]. Analysis of PSII complexes containing peripheral antenna components like LHCII and CP24/26/29, has revealed that these 700-kDa complexes are also dimeric, with dimensions of about [26.8]×[12.3] nm [217]. Comparison of the later PSII particles with the dimeric CCII complex has led to the conclusion that CCII is located in the center of the larger particle. The two LHCII-trimers are located at the periphery of the centrally located CCII dimer [217,218]. Some attempts to obtain 3D information with delipidated thylakoid membranes [207–209,214], added to the monomer to dimer controversy. In these studies, it proved difficult to confirm the precise subunit composition and molecular weight of the unit cell components. Recently, Morris et al. [219] performed a 2D and 3D image analysis of negatively stained crystals formed after reconstitution of an isolated CCII complex into purified thylakoid lipids. The isolated complex comprised D1, D2, CP47, CP43, cyt *b*-559, and associated low molecular weight proteins. In the resulting maps, the PSII complex is composed of two halves related by two-fold rotational symmetry, thus confirming the dimeric nature of the complex. Each monomer appears to contain five domains. The 3D map has shown this CCII dimer to have a rather flat stromal exposed surface and largely protruding bulbs, attributed to hydrophilic loops of CP47 and CP43, in the lumenal space [220].

5.2. Expression and assembly

5.2.1. Gene organization and transcription

In photosynthetic eukaryotes, 14 PSII genes are encoded in the chloroplast genome (see Table 2). The main features of their organization are rather well conserved between cyanobacteria and higher plant chloroplasts with an exception of the two to three extra copies of *psbD* and *psbA* found in cyanobacterial genomes. The differential expression of these genes when varying the illumination conditions has been taken as indicative of their role in the adaptation capability of the cyanobacterium to high light intensities [29,221]. Most of the nuclear-encoded PSII genes are not found in cyanobacteria. In particular, as noted above, there is only one conserved OEE subunit, PSII-O, between the two types of organisms.

Both eukaryotes and prokaryotes show multiple PSII transcripts, some corresponding to single genes transcribed independently, others bearing several PSII genes grouped in a transcriptional unit that may also comprise unrelated genes encoding either tRNAs, ribosomal proteins or subunits of other photosynthetic complexes (reviewed in [222]). Polycistronic transcripts are detected in higher plant chloroplasts which originate from PSII gene clusters also found in cyanobacteria: *psbB*/*psbH*, *psbE*/*psbF*/*psbL*/*psbJ*, *psbD*/*psbC* in which the 3′-end of *psbD* overlaps the 5′-end of *psbC* by about 50 bp. Here again, the chloroplast genome of green algae has distinctive features with respect to the PSII gene organization [223,224]. Studies of PSII mutants from *C. reinhardtii* have identified about 10 nuclear loci which participate specifically in the expression of the major PSII chloroplast genes, *psbA–C*, at the post-transcriptional level (reviewed in [58], see also Section 2.2.2).

5.2.2. Biosynthesis of individual subunits

5.2.2.1. The reaction center subunit D1. The biogenesis of subunit D1 has been extensively studied in the past decade. There are at least two major reasons for this sustained interest. First, D1 binds a class of powerful herbicides, among which is the widely used DCMU or Diuron. Second, D1 is the site of photodamage in photosystem II, leading to a process termed photoinhibition, the recovery from which requires turnover of the D1 polypeptide. Both of these aspects are discussed in [5,203] and will not be developed further here. Several other aspects of D1 biogenesis have been addressed experimentally, namely the translational control of chloroplast D1 by nuclear factors and various co/post-translational modifications which accompany membrane integration of D1, such as its C-terminal processing or its association with pigment cofactors.

Evidence for a nuclear participation in the translation of *psbA*, the gene encoding the D1 subunit, has been described in *Chlamydomonas*. Both a genetic approach, with the identification of the PSII nuclear mutant *TBA-1-F35* [225], and a biochemical approach, show a correlation between translation of the *psbA* mRNA and the binding activity of *trans*-acting proteins to its 5′-untranslated region [226]. A complex circuitry of antagonizing signals has been dissected for the control of D1 translation [227,228]: a possible translational activator, a 47-kDa protein, has been shown to bind to the 5′-UTR of *psbA* mRNA. This binding is antagonized by elevated levels of ADP which allow an ADP kinase to phosphorylate a 60-kDa protein which in turn prevents the binding of the 47-kDa protein. The binding of the 47-kDa protein has been described as being redox controlled, leading to the proposal that the light-induced stimulation of D1 expression would result from the combined increase in reducing power and ATP in the chloroplasts [227,228]. The redox control has been subsequently substantiated by the identification of the 60-kDa protein as a unique chloroplast member of the family of disulfide isomerase proteins, which are usually retained in the endoplasmic reticulum [229]. An additional effect of light, this time on the rate of translation elongation of D1, has been reported based on the increased amounts of polysome-bound translation intermediates of D1 in spinach chloroplasts isolated in their dark phase of growth [230].

D1 inserts co-translationally into the thylakoid membranes from polysomes-bound to the stroma-lamella region [91,231, 232]. Co-translational membrane insertion was observed in the absence of ΔpH and in the presence of azide, provided that ATP is present [233]. The azide insensitivity was taken indicative of a SecA-independent insertion process. D1 inserts in the membranes as a precursor form with a C-terminal extension of about 1.5–2 kDa

[234], the removal of which is required for the assembly of the Mn cluster [235–237].

The enzymes responsible for D1 processing were first characterized as soluble proteins which are active on the lumen side of the thylakoid membranes in *Scenedesmus* and pea [237]. The corresponding *cptA* gene was then cloned and characterized in the cyanobacterium *Synechocystis* [238,239] and subsequently in spinach, barley and *Scenedesmus* [240–242]. In higher plants, CtpA is made as a precursor protein with a bipartite transit sequence, typical of a lumen-targeted protein. Using spinach thylakoids, the CtpA protein from cyanobacteria was successfully imported into its thylakoid location and correctly processed [243]. CtpA translocation depends both on the Sec and ΔpH pathways. The sequence of the mature CtpA protein is well conserved, a fact that probably explains the ability of *psbA* gene from higher plants to successfully replace its *Synechocystis* counterpart [244]. Attempts to further characterize the structural requirement in the site of cleavage in pre-D1 were performed in spinach [245] and *C. reinhardtii* [246]. A *Synechocystis* mutant with inactivated desaturase genes accumulated high amounts of pre-D1 when grown at 18°C [247]. Therefore, membrane fluidity may control, in part, the efficiency of cleavage. Alternatively, the molecular mechanism for D1 processing may involve some specific lipids, a working hypothesis which would be consistent with an older report that palmitoylation of D1 would occur following its insertion into the membrane [231].

At some point of its assembly in RCII, D1 has to bind a pigment complement of chl *a* and β-carotene molecules. A role of β-carotene binding in the control of D1 turnover has been substantiated by the use of inhibitors of phytoene desaturase in *Chlamydomonas* [248]. Mullet and coworkers [33,82,249] had suggested a most stimulating hypothesis according to which pigment-binding to D1 is not only concurrent with its co-translational insertion, but is also required for completion of this process. Two series of experimental evidence supported their proposal. On the one hand, ribosome pausing was detected at some specific stages of translation elongation of D1 [249]. Ribosome pausing increased during chloroplast development, being larger in mature chloroplasts where chlorophyll biosynthesis may become rate-limiting [250]. On the other hand, a barley nuclear mutant, vir-115, showed a developmentally regulated loss of D1 synthesis that results in inactivation of PSII. The analysis of D1 translation in this mutant suggested that vir-115 was deficient in a nuclear-encoded gene product which normally stabilizes D1 translation intermediates and results from ribosome pausing. The latter product was hypothesized to be a chaperonine-like protein that may catalyze chlorophyll binding to D1, thereby permitting its continued synthesis and accumulation [82]. Although translational run-off assays in the absence or presence of chlorophyll did not allow Kim et al. [251] to further substantiate a key role of chlorophyll in the rate of translation elongation of D1, the light stimulation of D1 elongation in mature spinach chloroplast supports this hypothesis [230].

5.2.2.2. Other chlorophyll binding CCII subunits. At variance with the above-described light-stimulation of D1 translation, high-light conditions also stimulate expression of D2 and CP43 at the transcriptional level in higher plant chloroplasts. These two subunits are made up of a *psbD–psbC* co-transcript whose transcription is specifically enhanced by a blue light/UVA responsive promoter [252]. Christopher and Mullet [253] have observed that the blue light promoter allows fully developed chloroplast to keep reasonably high levels of *psbD–psbC* transcripts, while they display an otherwise markedly attenuated chloroplast gene expression [254]. More than 90% of these transcripts are recruited on translating polysomes, suggesting a critical role of the blue light promoter in allowing a replacement of D2 and CP43 in the repair process of photodamaged CCIIs [253]. Participation of nuclear-encoded factors in the expression of the chloroplast genes *psbB*, *psbC* and *psbD* at the post-transcriptional level, has been extensively demonstrated in *Chlamydomonas* [58,255].

Carotenoid accumulation has been shown to play an important role in the formation of PSII subunits [256], and together with chl *a*, in the stabilization and assembly of newly synthesized photosynthetic complexes including CP47 and CP43 [257]. However, the stage at which chlorophyll binds to the apoproteins is still a matter of debate. In one instance, the binding of pigments was reported to occur after mem-

brane insertion of the CP47 apoprotein [258]. The pigment binding was shown to regulate the accumulation of CP47 and CP43 [33,259]. In *Chlamydomonas*, chlorophyll-binding was shown to occur at early stages of apoprotein synthesis, and independent of the assembly of the subunits in a PSII protein complex [77]. By analogy with the proposed biogenesis of pigmented D1, most of the data presently available are consistent with a co-translational binding of the pigment cofactors to CP47/CP43 that develops during membrane insertion of the elongating polypeptide chains.

5.2.2.3. Processing of the small CCII subunits. The nuclear gene contributing the PSII-W subunit of RCII encodes a precursor polypeptide of 14 kDa [177]. Similarly to CF_0II, and despite it being an intrinsic membrane protein, PSII-W carries a bipartite transit peptide of 83 amino acid residues which directs the N-terminus of the 54 amino acids mature protein into the chloroplast lumen [260]. The thylakoid integration of this polypeptide is azide-insensitive and does not require a ΔpH across the membrane. Thompson et al. [261] propose that a central element in the insertion mechanism of PSII-W is a loop structure, the formation of which is driven by hydrophobic interactions.

The insertion of the nuclear-encoded PSII-X subunit into the membranes does not require either nucleoside triphosphates or stromal extracts, both of which are necessary for Sec- and signal recognition particle (SRP)-dependent targeting mechanisms. Furthermore, insertion is unaffected by protease treatments that destroy the known protein translocation apparatus in the thylakoid membrane. Thus, PSII-X is inserted by a Sec/SRP-independent mechanism that probably resembles that used by CF_0II [261,262].

The intronless nuclear *PsbT* gene (hereafter referred to as *Psb*T′ to minimize confusion with the *psbT* chloroplast gene) encodes an 11-kDa protein consisting a transit peptide with stromal and lumenal targeting domains, and a mature 3.0-kDa polypeptide chain of only 28 amino acids. A two-step processing of pre-PSII-T′ has been reported based on in vitro import experiments into isolated pea chloroplasts: a stromal intermediate of approximately 7.5 kDa has been identified together with the lumen-located mature form [263]. The import and membrane insertion of PSII-T′ were found to be insensitive to azide, but sensitive to ionophores. In their presence, PSII-T′ accumulated as a 7.5-kDa intermediate form [146]. Thus, PSII-T′ insertion in the thylakoid membranes is mediated via the ΔpH pathway.

5.2.2.4. The OEE subunits. A regular two-step import process has been documented for the three OEE subunits. First, the larger precursors are imported to the stroma, where they are processed to intermediate forms that are transferred across the thylakoid membranes and processed to the mature size by TPP [137,156,264]. Although all OEE precursors contain a bipartite transit sequence, distinct requirements for protein translocation across the thylakoids have been observed. Their translocation does not depend entirely on interactions between some protein motifs in the transit sequence and the translocation machinery in the thylakoid membrane. It also requires some information from the mature protein sequence. This finding has led to the concept of a functional co-evolution of the transit sequence and its passenger protein [265]. Improper interactions of the transit peptide and the mature part of the protein, may form translocation-incompetent folding, or disable either soluble or membrane bound factors to assist the translocation process [266].

The use of different in vitro and in thylakoido import systems has clearly indicated that the OEE proteins fall into two distinct groups having different requirements for translocation across the thylakoid membranes. PSII-P (OE23) and PSII-Q (OE16) are translocated by a relatively simple mechanism that depends upon a *trans*-thylakoidal ΔpH, but neither the presence of soluble stromal factors nor ATP are required [151–153,155,267,268] (See Fig. 3). In contrast, translocation of PSII-O (OE33) obeys similar requirements as those of PC. It is dependent on the presence of soluble stromal protein(s) and ATP [149,269]. The PSII-O/PC translocation system has been shown to be inhibited by azide, a SecA inhibitor [147], and therefore was concluded to be a Sec-type mechanism. However, Yuan and Cline [150] have also shown that in the presence of ionophores the assembly of PSII-O into isolated thylakoids was dramatically reduced (60–70%), but not abolished. Thus,

they concluded that a transthylakoid ΔpH would stimulate PSII-O translocation in vitro.

Consistent with the use of distinct translocation mechanisms, competition experiments for translocation can be achieved only within each group: PSII-P competes only with PSII-Q, whereas PSII-O competes only with PC. Since cyanobacteria expresses PC and PSII-O but not PSII-P/Q, it has been speculated for long that the latter have co-evoluted with a new translocation machinery alien to the prokaryotic world [156]. The recent report that bacteria are also able to translocate a subclass of proteins by a ΔpH-dependent pathway should lead us to reconsider this view (see Section 3.2).

5.2.3. Stoichiometric production of the PSII subunits

A wealth of information on the process of CCII assembly can be collected from the analysis of PSII mutant strains, obtained either by classical mutagenesis or by gene disruption techniques. Most of these data were obtained with the cyanobacterium *Synechocystis* or the unicellular eukaryote *Chlamydomonas*. Three subclasses of CCII subunits can be distinguished: those whose accumulation is CCII-independent; those whose presence is not required for the accumulation of the major CCII subcomplex; and those which are coordinately accumulating in a CCII subcomplex.

The OEE subunits belong to the first category. In the absence of the transmembrane part of CCII [77] or when it is photodestroyed [270], they still accumulate in the thylakoid lumen of *Chlamydomonas*. The fact that unassembled OEE subunits resist proteolytic degradation in the lumen has been confirmed with higher plant chloroplasts [271,272]. Therefore, the mechanism for the accumulation of the OEE subunits in a stoichiometric ratio required for their assembly in CCIIs remains elusive and highly intriguing. There should be some regulation mechanism, independent of the presence of the major transmembrane CCII subunits, which keeps the presence of OEEs in the lumen at a defined concentration. It may involve those PSII associated proteins, for instance the antenna-like *PsbS* product (see Section 7), or the recently identified HCF169/ycf48 protein [163] which are still present in etiolated spinach seedlings when the chlorophyll-binding subunits are lacking [178]. Whether the regulation occurs at the level of translocation or subsequent to translocation across the thylakoid membranes remains to be determined.

Conversely, the absence of PSII-O in cyanobacteria does not affect the stable accumulation of the rest of CCII in the membrane provided that the cells are grown in low light conditions [45,189,273]. In *Chlamydomonas*, the absence of PSII-O had a stronger destabilizing effect on the other CCII subunits than the absence of PSII-P [44,77,274].

The second category of CCII subunits comprises those whose absence has a moderate effect if any, on the accumulation of the rest of CCII in cyanobacteria, although it has harsher consequences in *Chlamydomonas* (see Section 1). Taking into account these distinctive traits, one can conclude from the various reports in the literature that the small subunits, PSII-H, -I, -J, -K, -L, and -T, are optional since they are not strictly required for phototrophic growth and for the overall assembly and accumulation of the major CCII subunits [40–43,160,275–277]. Whether some of the small subunits show interdependent accumulation is not yet known. A more detailed analysis of their behavior awaits the production of specific antibodies to be used in the various deletion strains presently available.

The third category of CCII subunits was identified by gene disruption studies in *Synechocystis* and *Chlamydomonas*, together with classical mutagenesis in the latter organism. These genetic studies showed that the accumulation of the major transmembrane subunits of the PSII core complex is a concerted process (reviewed respectively in [77,278]). In *Synechocystis* sp. PCC 6803, a drastic loss in all the major CCII subunits was observed in various *psbA* [279], *psbD* [280,281], *psbB* [282], *psbE* and *psbF* mutants [38,283–285]. However, the fate of CP43 is less tightly linked to that of the other major CCII subunits. Its absence had milder effects on the accumulation of the rest of the core complex. A CP43-less cyanobacterium mutant still accumulated 10% of the wild-type amount of CP47/D1/D2 sub-core complexes, capable of electron transfer from TyrZ to Q_A [286]. Conversely, inactivation in *Synechocystis* of *psbA*, the gene encoding D1, still allowed CP43 to accumulate in wild-type amounts, although it prevented accumulation of D2 and CP47 [287].

A somewhat similar picture emerged from studies with *Chlamydomonas* PSII mutants [288]. de Vitry et

al. [77] have demonstrated that part of the concerted accumulation process results from an increased turnover of the unassembled subunits. For instance, CP43 is made at wild-type rates in the absence of either D1, D2 or CP47 in *Chlamydomonas*, but its steady state level does not exceed 10% of what is found in a strain that assembles CCII. The life-time of neosynthesized D2 in vivo, as measured by pulse-chase studies, has been shown to decrease dramatically in the absence of D1 synthesis [77]. However, a major contribution to the concerted accumulation process comes from a CES process. D1, apoCP47 and PSII-H behave as members of the CES subunit family in *Chlamydomonas*: their rate of synthesis is decreased in the absence of an assembly partner. D2 and CP47 for the former and D1 for the latter two ([77], de Vitry unpublished observation, [289,290]). Thus, it can be concluded that there is a concerted synthesis of D2/D1/CP47/PSII-H, by some presently unknown molecular mechanism, which leads to their early stoichiometric assembly and subsequent interaction with CP43, which is synthesized independently [77]. According to the molecular mechanism that is being elucidated for another CES subunit, cytochrome *f* [291], it is tempting to speculate that the CES subunits in CCII, when not assembled, expose some protein motif at the stromal face of the thylakoid membrane which down regulates their translation.

5.2.4. Steps in the assembly of CCII

The above-described studies with PSII mutants from *Chlamydomonas* have suggested that there is a first step whereby a D1/D2/CP47/cyt *b*-559 substructure is formed prior to its association with CP43. These two subcomplexes have been found to migrate to the stacked membrane region independently [77]. This model was largely confirmed by in vivo pulse-chase experiments combined with non-denaturing gel electrophoresis using the wild-type strain of *Chlamydomonas* [292]. It has been shown that D1 associates at very early stages with D2 and CP47 in unstacked membrane regions before integrating in larger protein complexes in stacked membrane regions.

Interestingly, van Wijk and coworkers [293–295], using an independent in vitro approach, based on run-off translation of labeled D1 with isolated thylakoids from spinach and in organello pulse-chase experiments, extended the model for PSII assembly to vascular plants while giving a wealth of more detailed information.

Immediately after completion of translation, neosynthesized D1 has been detected both in assembled RCII and in an unassembled state in the membranes. This dual location indicates that indeed co-translational assembly with D2 may occur in vivo. Unassembled D1 has then been found to incorporate into RCII in unstacked membrane regions before getting in larger CCII complexes in the grana regions. In their more recent study, van Wijk et al. [295] reported a step-by-step association of D1, first with D2, then with cyt *b*-559 and the *psbI* product. CP47 associates with these PSII RC before the final binding of CP43. The requirement for cytosolic and stromal factors in the lateral migration of RCII and CP43 from the stromal to the granal regions of the thylakoids and their incorporation in larger CCII complexes has been suggested based on the inability of isolated thylakoids to perform these steps [294].

As pointed out by the authors [293,294], the time-resolved characterization of the incorporation of D1 in assembly intermediates in the thylakoid membranes has been performed in organello, in experimental conditions where the various biosynthesis cofactors of nuclear origin cannot be delivered properly to the chloroplast. Owing to the dependence of D1 translation on cofactor availability (see synthesis of individual subunits), a shortage in cofactors may explain the slow rate of elongation of D1 which has been estimated to be as slow as 40 residues per minute [293]. Translation in prokaryotes is estimated to occur at rates about 20 times faster [296]. Also, the large proportion of D2, CP43 and CP47 which remained unassembled in the in organello experiments [294] suggests that de novo biogenesis of CCII complexes was poorly operating in these conditions. Thus, the experimental system was best suited for the study of the replacement of D1 in pre-existing CCII and RCII complexes – a physiological situation indeed encountered both in low light and high light conditions (reviewed in [5]). It is remarkable that despite the widely different experimental conditions and organisms used, the in vivo and in vitro approaches yielded consistent observations. These concur to a step-by-step assembly process for CCII in which formation of an RCII/CP47 subcomplex pre-

cedes association with the other core antenna subunit CP43.

C-terminal processing of pre-D1 has been shown to play no critical role in this series of assembly and lateral diffusion steps. According to Adir et al. [292] processing of pre-D1 occurs after the lateral migration and integration into RCII. van Wijk et al. reported that neither processing of pre-D1 is required for its assembly into a PSII (sub)complex, nor is processing prevented in the absence of assembly [294]. *psbA* gene transformation techniques allowed direct expression of a pre-processed D1 in *Synechocystis* [297] and *Chlamydomonas* [298,299]. The transformants grew phototrophically and correctly assembled CCII. Thus, co-translational insertion of a precursor protein followed by its conversion to a mature form is not mandatory in the biogenesis of CCII.

Still, it is likely that for kinetic reasons, processing of D1 occurs at an early stage in the biogenesis of the CCII complex [231,292,295]. Processing of pre-D1 becomes critical at a later stage in PSII assembly. It is required for the photo-activation of CCII that sets up water splitting activity by promoting the assembly of the manganese cluster [237,297]. Indeed, cyanobacterial mutants in which the C-terminal processing of pre-D1 is hampered, are light-sensitive [239,300]. Processing of D1 probably plays a role in the binding of PSII-O to the pre-assembled transmembrane part of CCII. It should be noted that PSII-O and PSII-P have distinct binding sites to the OEE complex, with PSII-Q binding indirectly to CCII via its interaction with PSII-P [77].

The stage at which two other major post-translational changes take place in CCII biogenesis (the dimerization of the protein complex and the phosphorylation of several subunits) remains a matter of debate. The dimerization step should be detected in non-denaturing gels or other chromatographic approaches, as the appearance of larger PSII complexes of similar biochemical composition at the expense of smaller ones. It could correspond to the conversion of a 190–245-kDa small CCII into a 280–360-kDa large CCII described as a late step in PSII assembly [294]. Interestingly, this step was reported to be strictly dependent on the presence of cytosolic factors. This points to a possible involvement of one of the nucleus-encoded CCII subunits, PSII-W, PSII-S or PSII-T, in the dimerization process, similar to that of the PSI-L and PSI-I subunits in the trimerization of CCI (see Section 6). A systematic analysis of the state of oligomerization of those CCIIs which remain in the various PSII mutants deficient in either of the minor PSII subunits should be a suitable way to identify such components. In one such study where the chloroplast *psbH* gene was disrupted in *Chlamydomonas*, it was concluded that PSII-H may participate in the stabilization of CCII dimers [277].

D2, CP43 and PSII-H are the major phosphorylated subunits in PSII in *Chlamydomonas* [301]. In higher plant chloroplasts, D1 is also phosphorylated [302]. Phosphorylation in PSII should occur at a late stage in the assembly of CCII since all the mutants from *Chlamydomonas* which do not accumulate CCIIs because of a defective synthesis in either one of its major subunits, display only the non-phosphorylated forms of the CCII subunits [303,304]. de Vitry et al. [77] have proposed that phosphorylation occurs at a late stage in PSII assembly, namely upon association of CCII with the minor and major chl *a*/*b* peripheral antenna proteins. This proposal is supported by the fact that there is no PSII phosphorylation either in cyanobacterial CCIIs, which do not assemble with such chl *a*/*b* peripheral antenna proteins, or in a *Chlamydomonas* mutant specifically lacking the peripheral antenna complexes [305].

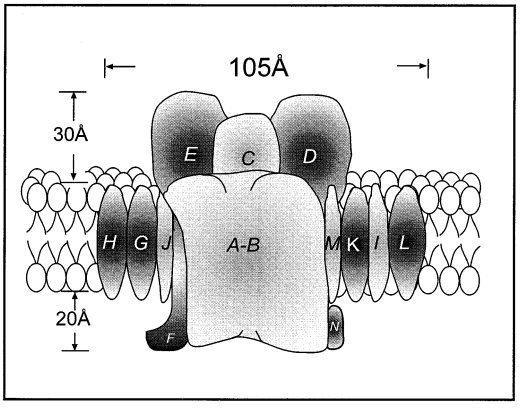
Several studies performed with vascular plants support a role of PSII (de)phosphorylation in the sensitivity of CCIIs to high light conditions. Phosphorylated D1 and D2 would be less prone to light-induced degradation [306] and the phosphorylated dimeric form of CCII would be more stable in high-light conditions [307]. In the two cases, PSII phosphorylation was suggested to play a role in the repair cycle of damaged CCIIs, a proposal substantiated by the observation that dephosphorylation was a prerequisite for degradation of photodamaged D1 [308].

5.2.5. *Are there protein factors specifically involved in CCII assembly?*

Any of the above-described steps in CCII assembly may require some as yet unidentified factors that assist the subunit–subunit recognition process. A candidate for such a function has been recently identified in *Arabidopsis* upon characterization of a high

fluorescence mutant, HCF136, that lacks CCII cores [163]. Interestingly, this mutant is also deficient in the OE subunits, which have been shown in other instances to accumulate in the absence of the transmembrane moiety of CCII (see Section 5.2.3). The protein identified by the mutation is nuclear-encoded and it is translocated to the thylakoid lumen where it resides as a peripheral membrane protein bound to the membrane surface in the stroma lamellae region. It is present in sub-stoichiometric amounts as compared to the bona fide CCII subunits and it accumulates in etiolated seedlings with the OE subunits and cyt *b*-559, whereas the transmembrane subunits of CCII accumulate only upon illumination [163]. Similarly to the Ycf3/4 and BtpA products (see Section 6), HCF136 could act as a chaperonine for PSII assembly. However, it could as well be required for cofactor binding to some PSII subunit, fulfilling a prerequisite for their stable accumulation. For instance, it may participate in the apo-to-holo conversion for cytochrome *b*-559 in a similar way as do the CCB factors for the biogenesis of cytochrome b_6 (see Section 7).

6. Photosystem I



The holo-PSI complex of plants and algae is composed of a light-harvesting complex (LHCI) and a core component (CCI) [309]. The holo complex contains from 11 subunits in cyanobacteria (no LHCI present) up to 17 in higher plant chloroplasts, 100–200 chl molecules per P_{700} and a chl *a*/chl *b* ratio greater than 5. The limited information available on the biogenesis and assembly of the LHCI components that function as a peripheral antenna are described separately in Section 7. Here, we focus on the biogenesis of the core component of PSI, which assembles 60–100 chl *a* molecules that serve as a core antenna, and the primary reactants that perform a plastocyanin-ferredoxin oxido-reductase function.

CCI drives the electron transfer from plastocyanin (PC) which is located in the lumenal space, to ferredoxin (Fd) which is situated in the chloroplast stroma. In some algae and cyanobacteria, the secondary electron donor of CCI is a *c*-type cytochrome. Besides Fd, cyanobacteria may contain a low-potential flavoprotein, flavodoxin, as an electron acceptor [310,311]. The electron transfer within CCI occurs via the primary electron donor P_{700} which is a chl *a* dimer and five electron acceptors: A_0, a chl *a* monomer; A_1, vitamin K1; and A_2–A_4, the three 4Fe–4S clusters, F_X, F_A, F_B. For a recent review on the electron transfer within Photosystem I see [312].

6.1. The assembled protein

6.1.1. Subunit composition and topology in the membranes

Table 3 summarizes the subunit composition of CCI. The subunits are termed PSI-A/N, according to the corresponding *psa A/N* genes. They are well conserved among eukaryotes and prokaryotes performing oxygenic photosynthesis with the exception of three subunits which are not present in cyanobacteria (PSI-G/H/N) and one subunit which is absent in higher plants (PSI-M) (see Table 3). All CCI cofactors, i.e. pigments and electron transfer carriers are situated only within three subunits that define reaction center I (RCI): PSI-A, PSI-B and PSI-C. The function of the various subunits, when known or hypothesized based on reasonable assumptions, is indicated in the table.

CCI comprises both integral and peripheral membrane subunits. Among the former are the two high molecular weight RCI subunits, PSI-A and PSI-B that form the integral membrane core of the complex, each having 11 transmembranal helices [313], and the low molecular weight subunits, PSI-G/I/J/K/L/M each having at least one transmembrane helix [314,315]. The other subunits are peripherally located either on the stromal side of the thylakoids (PSI-C/

Table 3
Photosystem I

Subunits	Topology (no. of TM α-helices)	Approximate molecular mass (kDa)	Gene (C/N)	Function and cofactor binding	Contribution to the accumulation of PSI
PSI-A	TM (11)	84	*psaA* (C)	P700, A_0 (Chl), A_1 (vitamin K_1), F_X [4Fe–4S], heterodimer binds about 100 Chl *a*, 12–15 β-carotene	+ CES
PSI-B	TM (11)	83	*psaB* (C)		+
PSI-C	S	9	*psaC* (C)	2 [4Fe–4S] (F_A, F_B)	+/−
PSI-I	TM (1)	4	*psaI* (C)	trimer formation in *Cyanobacteria*	−
PSI-J	TM (1)	5	*psaJ* (C)	?	−
PSI-D	S	18	*PsaD* (N)	ferredoxin docking	+/−
PSI-E	S	10	*PsaE* (N)	cyclic electron transport	−
PSI-F	TM (1–2?)	17	*PsaF* (N)	plastocyanin docking in eukaryotes	−
PSI-K	TM (2)	9	*PsaK* (N)	?	−
PSI-L	TM (2)	18	*PsaL* (N)	trimer formation in *Cyanobacteria*	−
PSI-G[a]	TM (1–2)	11	*PsaG* (N)	?	?
PSI-H[a]	?	11	*PsaH* (N)	?	?
PSI-N[a]	L	10	*PsaN* (N)	?	?
PSI-M[b]	TM (1)	3	*PsaM*	?	−

Topology denotes whether the protein is transmembranal (TM) or whether it faces the stroma (S) or the lumen (L). Approximate molecular masses are given according the amino acid sequence of the mature protein. Note that they may deviate markedly between different organisms. C, chloroplast gene (lower case); N, nuclear gene (upper case). +, Required for the accumulation of other subunits of the complex; +/−, mildly required; −, dispensable; CES, controlled by epistatic synthesis.
[a]Found only in higher plants and in algae.
[b]Found only in *Cyanobacteria*.

D/E) or on the lumenal side of the membranes (PSI-F/N). Some reports have indicated that PSI-F contains a transmembranal domain as well [313,316].

6.1.2. Supramolecular organization

Two methods, electron microscopy (EM) and X-ray crystallography, have provided valuable information on the supramolecular organization and structure of CCI. While the former has elucidated the global shape and size of different PSI complexes [317–321], the latter has permitted a more detailed molecular knowledge of secondary and tertiary structural elements of the CCI subunits, and of the prosthetic groups [313,322,323].

CCI displays an ellipsoid [324], or disk shape [317] with dimensions of [15–18]×[8–9]×6 nm [317,324–326]. It has a ridge of 2.5–3 nm height projecting from the stromal side of the membrane while the lumenal side is rather flat, with a 3-nm-deep indentation in the center of the complex [318,327]. While the ridge has been attributed to the PSI-C/D/E subunits, the central indentation would correspond to a region in which the two large subunits PSI-A and PSI-B are partly separated [318].

These EM observations have gained support from studies in which averaged top and side view projections of the whole CCI were compared with CCI depleted of PSI-C, -D and -E. The latter showed a reduced height of the complex, by about 2.5–3.3 nm [328]. A recent study has indicated that CCI from higher plants is slightly larger than the cyanobacterial complex, but it shows many similar features, in-

cluding similarities in detecting the stromal facing PSI-C/D/E subunits [321].

More detailed molecular information has been obtained by X-ray crystallography. The most detailed structural data available to date comes from the three-dimensional structure of *Synechococcus elongatus*. CCI determined first at 6-Å and recently at 4-Å resolution [313,322]. The latter has identified 31 transmembrane α-helices and 71 chl *a* molecules whose localization has been determined. PSI-A and PSI-B have been assigned 11 transmembranal helices each. The two subunits relate to each other by a two-fold rotation axis that passes through the Fe–S cluster F_x. In addition to F_x, the location and the spatial arrangement of the other Fe–S clusters have been identified at 15 and 22 Å from F_x. Moreover, the existence of additional chl *a* electron acceptor(s) besides P_{700} and A_0 has been proposed [313]. Although the stromal extension of CCI is presently poorly resolved in the X-ray structure, some detailed structural information is available on PSI-E. Its three-dimensional structure has been determined by two-and three-dimensional NMR in *Synechococcus* sp. PCC 7002 [329,330]. PSI-E folds in a five-strand antiparallel β-sheet with an extensive hydrophobic core.

Whether CCI exists as a trimer and/or a monomer has been a matter of debate. Ford and co-workers [325,326] reported that CCI lies with its shortest axis across the thylakoid membrane. These authors have proposed that the trimeric structure observed in vitro [317] occurs as a result of the detergent environment and the extraction procedure. Hefti and his co-workers, in a detailed study of CCI organization within membranes placed under different ionic strength, concluded that it remained monomeric under all conditions tested [319]. In contrast, several reports have supported the existence of the trimeric form of CCI in thylakoid membranes [331,332]. High resolution electron microscopy and digital image processing studies which made use of a monoclonal antibody isolated from mice immunized with the native trimeric CCI, concluded to the trimeric nature of CCI in situ [333]. The finding, however, that a *Synechocystis* PSI-L mutant failing to form CCI trimers grows autophototrophically supports the view that trimerization is not a prerequisite for efficient photosynthesis in cyanobacteria [334,335].

6.2. Expression and assembly

6.2.1. Gene organization and transcription

In the green lineage of photosynthetic eukaryotes, five PSI subunits are encoded by the chloroplast genes *psaA*, *B*, *C*, *I*, *J*, while the other subunits are encoded by the nuclear genes *PsaD*, *E*, *F*, *G*, *H*, *K*, *L* and *N*. Reith and Munholland [336] have shown that the *psaE*, *psaF* and *PsaL* genes are still present in the plastome of the primitive red algae, *Porphyra purpurea*. Some of the nuclear PSI genes, like the *PsaD* gene are present in more than one copy in higher plants [337–339]. The *PsaE* and *PsaH* genes have also been found in two to three copies in *Nicotiana Sylvestris* [340–342]. Recently, an octamer motif was found to be bound with three phosphoproteins at the 5′-flanking region of *PsaDb*, *PsaEb* and *PsaHa* [343]. This octamer motif is not found in the promoter regions of the plastome genes [344] nor in red-algal plastid-encoded *psaE* [336,345], implying that these CCI genes acquired this octamer motif after they were transferred from the ancestral organelle to the nucleus.

A most noticeable feature of the CCI gene organization is the tandem organization of the *psaA* and *psaB* genes which is conserved in the chloroplast of vascular plants [346] and *Euglena* [347], as well as in cyanobacteria [348–352]. Thus, the two major RCI subunits are co-transcribed in most instances with the noticeable exception of *C. reinhardtii*. In the latter case, the *psaA* and *psaB* genes are transcribed independently. The mature *psaA* transcript is produced after a complex *trans*-splicing process, involving numerous nuclear-encoded factors, which requires the ligation of transcripts from three distinct *psaA* axons that are dispersed along the chloroplast chromosome (for a discussion see [57,58], and references therein). The localization of the *psaC* gene differs between the chloroplast of vascular plants and cyanobacteria. In the former, it is co-transcribed with two adjacent *ndh* genes [353,354], whereas it is transcribed as a monocistronic mRNA in *Synechochoccus* 7002 [355], and in *Synechocystis* 6803 [356].

6.2.2. Biosynthesis of individual subunits

6.2.2.1. The RCI subunits, PSI-A and PSI-B. In *C. reinhardtii*, the translation of the chloroplast *psaB*

transcript is controlled by at least two nuclear-encoded factors that interact with its 5′-UTR [357]. Interestingly, the two PSI mutants which have allowed the identification of these *psaB* targeted factors lacked synthesis of both the PSI-A and PSI-B RCI subunits. Thus, the synthesis of PSI-A depends on the presence of its assembly partner PSI-B, a characteristic typical of the family of CES subunits [358]. At variance with the case of cytochrome *f* [79], the molecular mechanism for this CES process may be at the elongation step of *psaA* rather than at the initiation step, since the expression of a reporter gene fused to the 5′-UTR of *psaA* was preserved whether PSI-A and PSI-B were assembled or not [357]. The insertion of PSI-A and PSI-B into the thylakoids, which forms the hydrophobic core of CCI, is considered as concurrent with their translation on membrane bound ribosomes. However, their membrane insertion becomes protease-resistant only upon their association with chl *a*, as demonstrated in vivo using greening etioplast from barley [251], or by providing intact etioplasts with in vitro synthesized chl *a* or Zn-pheophytin a [359]. The binding of another cofactor, the Fe–S cluster F_x, is required for the protease-resistant assembly of PSI-A and PSI-B since site-directed mutagenesis of F_x-coordinating residues from PSI-B led to the loss of RCI in *C. reinhardtii* [360,361]. Thus, assembly of RCI in a protease-resistant form corresponds to the formation of an *active* RCI that is, an assembly of the PSI-A and PSI-B apoproteins with their functional cofactors.

6.2.2.2. Other transmembrane subunits. PSI-K is a nuclear-encoded integral membrane subunit that is imported into intact chloroplasts in a precursor form where it is converted to a mature form localized exclusively in the thylakoid membrane fraction [362]. Attempts to import a truncated precursor form lacking the seven most N-terminal residues proved unsuccessful [362]. In vitro import studies have indicated that PSI-K, as well as PSI-L, another transmembrane subunit which also contains stroma-targeting pre-sequences, are integrated into the thylakoids in a mechanism which is insensitive to azide and is only slightly affected by uncouplers. It has been proposed that the information for membrane integration lies within the mature part of these proteins [363].

6.2.2.3. The peripheral stromal-facing CCI subunits. The stromal-facing peripheral subunits of CCI, PSI-C/D/E associate with the thylakoid membranes by some specific interaction with other integral or peripheral membrane proteins. Both the precursor and mature forms of PSI-D can integrate into the thylakoid membranes as observed when the radiolabeled protein was expressed in an in vitro translation assay and mixed with intact plastids or isolated thylakoids [132,364]. Assembly of PSI-D into the thylakoids of plants and/or cyanobacteria requires neither the stromal fraction nor ATP [132,133]. Similarly, thylakoid insertion of PSI-H or PSI-E does not require ATP, the proton motive force, or stromal/cyanobacterial cytoplasmic factors ([363,624], Cohen and Nechushtai, unpublished observation). Both PSI-D and PSI-E assemble specifically into holo-PSI. No association of the newly integrated labeled protein subunits with any other membranal complex has been observed [624,625]. Moreover, pre-PSI-D can also assemble into isolated PSI complexes. Upon the addition of the stromal fraction, the precursor, bound to an isolated PSI complex, can be correctly processed by the stromal processing peptidase [365]. While the assembly of pre-PSI-D in the thylakoids is not affected by the presence of salts, the mature form, PSI-D, cannot assemble into the membranes at high salt concentrations [366]. Mature PSI-D becomes resistant to proteolysis only when attached to the thylakoids [364].

This set of observations argues for the involvement of at least two steps in the assembly process of pre-PSI-D in the thylakoid membranes. As a first step, pre-PSI-D binds to RCI, probably in an extended conformation that is responsible for its proteolytic susceptibility. The extended conformation, which may be similar to that of pre-PSI-D in a soluble environment, would be caused by the presence of the leader peptide. Then, pre-PSI-D is processed to its mature form. The latter step is most likely accompanied by a conformational change, thus allowing the formation of electrostatic interactions between PSI-D and some other RCI subunits. These intra-complex interactions probably secure PSI-D on the thylakoid membrane surface and provide protection against proteolytic digestion.

6.2.2.4. The lumen-facing CCI subunits. PSI-N is

a nuclear-encoded subunit, synthesized in a precursor form with a two-domain transit peptide, which first targets the protein into the chloroplast, and then translocates it across the thylakoid membrane into the lumen [367]. The translocation of PSI-N across the thylakoids does not require the presence of stromal factors or nucleoside-tri-phosphates (NTPs). It has been found to depend on the ΔpH across the membrane [154]. Thus, the translocation pathway of PSI-N seems similar to that of the OE16 and OE23 proteins of the oxygen-evolving complex, but differs from that of PC and OE33 [368,369]. Despite the bipartite nature of the PSI-N transit peptide, no intermediate processing form has been detected. Hence, it has been concluded that the full precursor (pre-PSI-N) translocates both across the chloroplast envelope and across the thylakoid membrane, where it is directly processed by the thylakoid processing peptidase to yield the mature PSI-N form [154].

PSI-F is another RCI subunit of nuclear origin. Its final location is on the lumen side of the thylakoid membranes, where it mediates the fast electron transfer from PC to P_{700} [370–372]. The protein has been found to contain a transit peptide that structurally resembles the bipartite targeting signal of lumen proteins [373]. It has been concluded from in vitro import experiments that PSI-F, like OE33 and PC, translocates across the thylakoids by a pathway that involves stromal factors. The fact that thylakoid translocation of PSI-F is impaired in the presence of sodium azide indicates that it is SecA dependent [363,374]. In one report, PSI-F from *Chlamydomonas* could be successfully imported in spinach mitochondria, both in its precursor and mature forms [375]. This unexpected finding suggests that the sorting step for import in either of the two organelles may rely on additional control mechanisms in the cytosol that are independent of the presequence of the imported protein.

6.2.3. Stoichiometric production of the RCI subunits

This issue has mainly been addressed by molecular genetic approaches. These studies concur to the conclusion that the accumulation of the two main RCI subunits, PSI-A and PSI-B, is a concerted process that controls the accumulation of the other CCI subunits. In cyanobacteria and *Chlamydomonas*, inactivation of the genes encoding either PSI-A or PSI-B causes the loss of all transmembrane and peripheral CCI subunits [360,376,377]. In particular, a *psaB* disrupted strain does not accumulate PSI-A homodimers although the PSI-A and PSI-B proteins are highly homologous [377]. The latter observation can be easily explained by PSI-A being a CES subunit whose synthesis requires the presence of PSI-B (see Section 6.2.2.1).

Inactivation of the *psaC* gene gave contrasting observations in *Chlamydomonas* and cyanobacteria. In the former case, the whole set of CCI subunits disappears [46] whereas in the cyanobacterium *Anabena*, the disruption of F_A and F_B that harbor PSI-C subunit has no effect on the functional assembly of PSI-A and PSI-B [47]. This is one of many examples showing that partially assembled protein complexes in the thylakoid membranes are more susceptible to degradation in *Chlamydomonas* chloroplasts than in cyanobacteria.

In cyanobacteria, inactivation of the CCI subunits that do not bind prosthetic groups has limited effects on the accumulation of RCI. In *Synechocystis* 6083, the absence of the CCI subunits like PSI-E, PSI-F and PSI-L, has no effect at all, or has only a minor effect on the function and assembly of the rest of CCI [316,378,379]. Inactivation of the PSI-L subunit prevents trimerization of CCI, but the monomeric protein complex assembles like in the wild-type and remains fully active [334,335]. PSI-I-less cells have revealed that PSI-I plays a major role in the structural organization of PSI-L. Trimeric CCI cannot be isolated from these PSI-I-less cells and the amount of PSI-L in the isolated monomers is reduced by 80%. Besides these two effects, the function and assembly of the other CCI subunits is virtually normal [335,380]. A similar privileged interaction between two subunits was observed for PSI-J and PSI-F. Targeted deletion of *psaJ* from the cyanobacterium *Synechocystis* leads to an 80% reduction in the content in PSI-F [381,382].

The only subunit that contains no prosthetic group, but seems critical for the assembly and/or accumulation of the other CCI subunits is PSI-D. Inactivation of the *Synechocystis psaD* gene resulted in a strain with normal amounts of the PSI-A-PSI-B core subunits, but reduced P_{700} photooxidation and a marked decrease in all low molecular weight RCI subunits [383]. Evidence for a direct interaction be-

tween PSI-D and PSI-B has recently been obtained [384]. In most instances where inactivation of one CCI gene led to a decreased accumulation of some other RCI subunits, the question of their proteolytic disposal versus decreased rate of synthesis was not addressed experimentally. Thus, it remains to be investigated whether the degradation of unassembled subunits is the major contribution to the stoichiometric production of the RCI subunits or whether there are other CES subunits in CCI, besides PSI-A whose synthesis is clearly depressed in the absence of PSI-B in *C. reinhardtii* [385].

6.2.4. Steps in the assembly of CCI

Studies performed during the greening of etiolated seedlings strongly support a step-by-step appearance and accumulation of the CCI subunits. This has been shown by detecting the PSI content in leaves [386,387] and in purified thylakoid membranes [388,389]. While the PSI-A and PSI-B core subunits can be detected in dark-grown seedlings, even in small amounts, the nuclear-encoded peripheral subunits appear sequentially upon exposure to light, with PSI-D being the first to accumulate.

It should be noted, however, that this step-by-step assembly of CCI could very well be confined to developing plastids. Under steady-state conditions, pulse labeling experiments performed in plants, spirodela, green algae *Chlamydomonas*, and cyanobacteria *Synechocystis*, have shown that all CCI subunits are similarly labeled [133,390,391]. Hence, the steady-state assembly of PSI either follows a mechanism different from the assembly in developing leaves, or merely represents the replacement of various CCI subunits, which therefore assemble into pre-existing complexes, and not into a de-novo synthesized CCI [133].

If indeed, a repair mechanism is mainly at work under steady-state conditions, it is of special relevance to understand the intra-complex interactions formed between the different subunits of the assembled complex. These have been studied with CCI mutants of cyanobacteria. The integration of PSI-D and PSI-E increases when it is performed into thylakoids isolated from mutants that lack PSI-E. Similarly, the assembly of PSI-D into thylakoids of PSI-D-deficient mutants is more efficient. In contrast, when the assembly of PSI-E into the PSI-D-deficient mutant is examined, it is found to be significantly reduced [133,392]. When the membranes used for the PSI-E insertion are isolated from a mutant lacking PSI-F and PSI-J, the newly bound PSI-E can be removed by both NaBr and proteolytic treatments [392]. Taken together, these observations suggest that the assembly of PSI-E with the rest of CCI is strengthened by its interactions with PSI-D and PSI-J. The early appearance of PSI-D in greening experiments (see above) led to the suggestion that PSI-D contributes to the binding and/or proteolytic resistance of several other CCI subunits [383,393]. Consistent with this view, PSI-D has also been shown to stabilize the binding of PSI-C to CCI in a reconstitution assay [394]. The type of interaction between the various subunits has not yet been characterized. Mature PSI-D seems to establish electrostatic interactions with other components of CCI [366]. However, there is no clue which residues are involved in those interactions. It is of note, that despite the high homology between PSI-D from cyanobacteria and vascular plants, some specific recognition mechanism may control its membrane integration since the cyanobacterial protein cannot integrate into spinach thylakoids [133,395].

6.2.5. Are there protein factors specifically involved in CCI assembly?

Recently, a new class of proteins has been identified, that could represent specific factors involved in CCI assembly. It encompasses those proteins whose absence prevents accumulation of CCI, although when present they are not found in tight association with CCI.

6.2.5.1. Ycf3 and Ycf4. The function of Ycf3 and Ycf4 was established by reverse genetic approaches. The genes for the Ycf3 and Ycf4 proteins are present in cyanobacteria as well as in the chloroplast of vascular plants and *Chlamydomonas*, with a sequence conservation of about 40% in the case of Ycf4 [396]. Disruption of the *ycf3* gene in Tobacco [397] and in *Chlamydomonas* [161] led to a complete loss in CCI. Disruption of the *ycf4* gene similarly caused a complete loss in CCI in *Chlamydomonas*, but caused only 50% decrease in CCI in *Synechocystis* 6803, as judged from the EPR signal I that remains after *ycf4* inactivation [396]. Interestingly, *Chlamydomonas*

mutants that lack the whole set of CCI subunits because of a disruption of one of the structural *psa* genes, still show wild-type accumulation of Ycf3/Ycf4.

The Ycf3 and Ycf4 proteins were found in the thylakoid membranes of *Chlamydomonas*, [161] with Ycf4 being most likely a transmembrane protein that spans the membrane twice, whereas Ycf3 is probably peripherally located on the stromal face of the membranes. Ycf4 is present in similar amounts at most of the photosynthetic protein subunits, while Ycf3 is present only in sub-stoichiometric amounts. Neither of the two proteins was found to interact tightly with any of the photosynthetic complexes as viewed in fractionation experiments where solubilized thylakoid membrane proteins are separated by sucrose gradient centrifugation [161]. However, a fraction of Ycf4 was found in a large molecular weight protein complex of presently unknown characteristics [161].

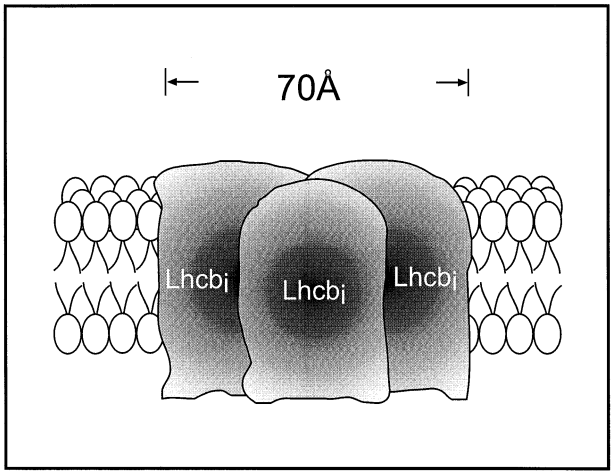
The possibility that Ycf3 and Ycf4 would participate in the regulation of CCI gene expression has been examined. Transcript analysis for the main chloroplast-encoded CCI subunits showed no alteration in the *ycf3*/*ycf4*-disrupted strains [161,397]. Translation of the two major RCI subunits, PSI-A and PSI-B was unaffected by the *ycf3*/*ycf4* disruption in *Chlamydomonas*. Moreover, the presence of the *ycf3*/*ycf4* genes in both photosynthetic prokaryotes and in the chloroplast of photosynthetic eukaryotes, argues against their function in the regulation of CCI gene expression at the post-transcriptional level. Such factors have been consistently found in the nuclear genome of photosynthetic eukaryotes and they have no counterparts in the genome of *Synechocystis* 6803 that has been fully sequenced.

As an alternative hypothesis, Ycf3/Ycf4 were proposed to play a role in the assembly of CCI [161,397]. It is tempting to consider that they could be part of the machinery which catalyzes cofactor binding to the CCI complex, for instance the iron–sulfur cluster assembly, in a very similar way as does the chloroplast ccsA gene product that is involved in *c*-heme attachment to cytochrome *f*. However, the limited loss in PSI activity observed in *Synechocystis* when *ycf4* is disrupted [396] seems not to support this view. Still, one should consider the possibility that a partially assembled cofactor-binding machinery can still operate in a Ycf4 minus context in cyanobacteria but not in *Chlamydomonas* because it shows a better resistance to proteolytic degradation in the former. This proposal is well supported by the many instances where oligomeric proteins lacking one subunit only accumulate in the prokaryote, but not in *Chlamydomonas* chloroplasts (see Section 2.1.3).

6.2.5.2. The BtpA protein. This protein was identified by a random screen for photosynthesis deficient mutants from *Synechocystis* 6803 [162]. BtpA is a 30-kDa protein with two putative transmembrane spanning segments. The BtpA mutant, which harbored a missense mutation in the *btpA* gene, accumulated about 15% of the RCI subunits, PSI-A and PSI-B. Since the transcripts for these subunits were still present in the mutant, and given the fact that the absence of the other CCI subunits does not compromise the accumulation of PSI-A/B in *Synechocystis*, it has been concluded that BtpA acts at a post-transcriptional level on the expression of RCI. A search for possible sequence homologies with either the TAB1 or TAB2 factors identified genetically in *Chlamydomonas* may help understanding whether BtpA acts on the translation of PSI-B. However, given our present understanding that these specific translation factors for chloroplast proteins are of nuclear origin, because they emerged with the eukaryotic nature of *Chlamydomonas* (see Section 2.2.2.1), a role of BtpA in cofactor-assembly or in the assembly/degradation of the major RCI subunits seems a more reasonable assumption.

7. Antenna proteins

This section refers exclusively to the biogenesis of the chl *a*/*b*-containing proteins that form the peripheral antenna in vascular plants and green algae. Three types of peripheral antenna can be distinguished based on their function in light harvesting: the so-called 'minor antenna complexes' CP24, CP26, CP29, associated with CCII, the LHCI antenna associated with CCI, and the LHCII antenna that is mainly associated with PSII, but can also serve as a mobile antenna capable of light-energy redistribution between the two photosystems.



7.1. The assembled proteins

We address the reader to recent reviews of the detailed biochemical and structural characteristics of these proteins [7,167,398–400] which will only be briefly summarized below.

7.1.1. Subunit composition and topology in the thylakoid membranes

The subunits of the three types of peripheral antenna form a family of related polypeptides of molecular mass 22–28 kDa. LHCII is the most abundant chlorophyll–protein complex and it accounts for almost half of the chlorophyll and a third of the protein of the thylakoid membranes. As depicted in Table 4, LHCII preparations from vascular plants, contain three distinct subunits, I:II:III in ratios of 10–20:3:1 depending on plant growth conditions and preparations [401]. In contrast, LHCII from *C. reinhardtii* is made up of three major polypeptides present in equimolecular ratio [402]. LHCI has four distinct polypeptide components present in equimolecular ratio. The minor antenna complexes CP24, CP26, CP29 are made up of one type of subunit only. Despite a characteristic organization in three α-helical transmembrane domains with stromal-exposed N-termini, the various antenna subunits display markedly different chl *a*/chl *b* ratios: CP24 (chl *a*/chl *b* = 1), LHCII (chl *a*/chl *b* is about 1.4)

Table 4
Antenna complexes of eukaryotes

Components	Topology (no. of TM α-helices)	Approximate molecular mass (kDa)	Gene (C/N)	Cofactor binding
LHCI-type I	TM (3)	22	*Lhca1* (N)	LHCI proteins bind Chl *a*, Chl *b* and some carotenoids
LHCI-type II	TM (3)	25	*Lhca2* (N)	
LHCI-type III	TM (3)	26	*Lhca3* (N)	
LHCI-type IV	TM (3)	22	*Lhca4* (N)	
LHCIIb-type I	TM (3)	25	*Lhcb1* (N)	LHCII binds 7 Chl *a*, 5 Chl *b*, 3–4 xanthophylls, 2 luteins and 1 neoxanthin
LHCIIb-type II	TM (3)	25	*Lhcb2* (N)	
LHCIIb-type III	TM (3)	24	*Lhcb3* (N)	
CP29 (LHCIIa)	TM (3)	28	*Lhcb4* (N)	binds 6 chl *a*, 2 chl *b* and xanthophylls
CP26 (LHCIIc)	TM (3)	27	*Lhcb5* (N)	binds 6 chl *a*, 3 chl *b* and xanthophylls
CP24 (LHCIId)	TM (3)	23	*Lhcb6* (N)	binds 6 chl *a*, 4 chl *b* and xanthophylls
PSII-S[a]	TM (4)	22	*PsbS* (N)	stable in the absence of chl
ELIPs	TM (3)	18[b]	(N)	stable in the absence of chl

Topology denotes the number of transmembrane (TM) α-helices of the protein. Approximate molecular masses are given according to the amino acid sequence of the mature protein. Note that they may vary between different organisms. C, chloroplast-encoded gene (lower case); N, nuclear encoded gene (upper case).
[a]Found only in higher plants and algae.
[b]Detected from gel migration.

CP26 (chl *a*/chl *b* = 2), CP29 (chl *a*/chl *b* = 3) and LHCI (chl *a*/chl *b* = 4). In addition, the stoichiometry of chlorophyll per polypeptide chain drops from 12 in LHCII subunits to 10 in LHCI/CP24 and 8/9 in CP29/CP26 [403,404]. Not only LHCII has the highest number of chl molecules per polypeptide, but also the highest xanthophyll content. While each of the minor CPs binds two xanthophylls, LHCII binds at least three and possibly four xanthophylls per polypeptide [405].

The molecular basis for these distinctive features represents one of the intriguing aspects of the biogenesis of this family of proteins. Changes in the distribution of chlorophyll ligand residues among the family of antenna proteins may account for part of the difference in chl *a*/chl *b* ratios. Eight chl binding residues have been identified in LHCII [406] one of which (a histidine residue in helix D) is lacking in CP24. In addition, two glutamine residues are substituted by a glutamic residue in CP24, CP26, CP29, and Lhca 1–4. Besides the nature and distribution of chl binding residues, the protein sequences that provide the environment for porphyrin binding might also be important in selecting chl *a* vs. chl *b*. Accordingly, binding sites have been shown to differ for chl *a* vs. chl *b* selectivity in CP29 [407]. Analysis of some optical properties of a native and reconstituted CP29 revealed that the pigment binding sites that can be occupied by chlorophyll *a* or chlorophyll *b* determine spectral properties of the protein [407]. In CP24 though, at least two of the chlorophyll-binding sites have an absolute requirement for chl *b* [408].

An additional pigment-binding subunit, the S subunit of 22 kDa, has subsequently been identified in CCII. It is predicted to contain four TM helices and to bind only five chlorophyll molecules [409,410]. Topological studies have indicated the protein to be located between CCII and the LHCII complex [409,410].

7.1.2. Supramolecular organization

The three-dimensional structure of LHCII subunits has been elucidated to 3.4 Å resolution by electron crystallography performed on two-dimensional crystals [406]. This structure, which serves as a reference model for the folding of all the other members of the antenna protein family, revealed that two of the three membrane-spanning α-helices are held together by ion pairs formed by charged residues located deep in the membrane. These residues also serve as chlorophyll ligands. A fourth amphipatic α-helix sits next to the lumenal face of the membrane where it contributes to the interaction with a distal chlorophyll molecule. Two xanthophyll molecules and 12 chlorophylls are identified in the structure [406]. However, the resolution obtained so far is insufficient for detection of the small differences between chl *a* and chl *b* or between the three xanthophyll species found in the complex by chemical analysis, thus preventing their attribution to individual sites.

LHCII crystallizes as a trimer, an oligomeric organization which resembles the native state of LHCII in the thylakoid membranes (see Fig. 1). The respective proportions of homotrimers and heterotrimers present in the thylakoids is not known. Interestingly, the LHCI subunits would rather exist as dimers, whereas the minor antenna complexes are found as monomers. These contrasting states of oligomerization may cause changes in the pigment binding properties of several residues of the well-conserved polypeptide sequences.

Attempts to determine the relative stoichiometry between antenna proteins led to several models of the organization of antenna proteins around CCII or CCI (see [398] for a discussion). The monomeric minor antenna proteins are probably in a 1:1 stoichiometry with CCII to which presumably two trimers of LHCII bind to form the supramolecular complex containing two CCII as suggested by combining electron microscopy [217,411] and nearest-neighbor analysis [412]. By a similar combined approach, CCI is proposed to interact with four dimers of LHCI subunits [413,414] (see Fig. 4).

7.2. Expression and assembly

7.2.1. Genes and transcripts

The 10 antenna protein subunits are encoded in the nucleus. The LHCII are encoded by multi-gene (*Lhcb1–3*) families. Depending on species, there are three to 16 copies of the *Lhcb1* gene encoding the most abundant type I subunit, whereas one to four copies of the *Lhcb2* and *Lhcb3* genes encode types II and III subunits (reviewed in [398]). The LHCI subunits (Lhca1–4), and the minor antenna protein com-

plexes (Lhcb4–6) are encoded by only one or two gene copies.

In vascular plants, transcription of the *Lhc* genes is tissue specific, regulated by light and controlled by the developmental stage of the plastid (reviewed in [7]). Light regulation of the *Lhcb* gene expression is mediated in part by phytochrome and UV/blue light photoreceptors [415,416]. However, light may also affect the redox state of intersystem electron carriers of the photosynthetic electron chain, a parameter that has been shown to influence transcription of *Lhcb1* in *Dunaliella* [70,417]. In addition, light stimulates chlorophyll biosynthesis, a pathway that is coupled by some presently unknown mechanism to the rate of transcription of the *Lhcb* genes [418]. There is now increasing evidence that chlorophyll precursors accumulating at the chloroplast envelope membrane build up a chloroplast retrograde signal which leads to an increased *Lhcb* transcription [68,72,419]. Thus, it is very likely that a variety of seemingly distinct parameters that influence the *Lhcb* transcription will turn out to result from a common signaling pathway in which chlorophyll precursors play a key role. This should apply to some of the light effects and to the various studies that have demonstrated that plastid development and the functional state of the chloroplast strongly influence transcription of the *Lhcb* genes [68,420]. As mentioned in Section 2, the recent demonstration that there is a chloroplast-mediated stimulation of translation of *Lhcb* transcripts in tobacco plants [71] may also be an indirect result of upstream changes in the transcription of regulatory genes [73].

7.2.2. Biogenesis of the antenna protein subunits

The fate of the *Lhcb1* products (hereafter referred to as LHCPs) once they are translated in the cytosol is considered typical of the routing of all antenna proteins. In the model depicted on Fig. 3 (see Section 3), pre-LHCP is post-translationally imported into the chloroplast, processed to its mature form and subsequently addressed to the thylakoid membranes where it assembles with its pigment complement. The various steps and factors which contribute to this routing process are detailed below.

7.2.2.1. Import into the chloroplasts. In vascular plants, LHCP is synthesized as a soluble precursor which uses the general post-translational import pathway into the chloroplast (see Fig. 3). In contrast, in *Euglena*, whose chloroplast is surrounded by three, instead of two, envelope membranes, pLHCPII is routed to the chloroplast as a membrane-bound precursor, via the ER/Golgi apparatus [421]. This unusual routing of pLHCP is not surprising in view of the likely evolutionary origin of the most outer envelope membrane from a phagocytic vacuole membrane [422].

Several studies have indicated that the proper import of pLHCP into the chloroplast does not require its authentic transit peptide. Replacement of the LHCP transit peptide with that of the stromal SSU [423,424] or of the lumenal PC [425] did not impair LHCP import and proper insertion into the thylakoid membranes. Cleavage of the transit peptide by a stromal soluble metalloendopeptidase [128] occurs concomitant with, or soon after, pLHCP import into the chloroplast (see Cline and Henry [137] for a discussion). None of the subsequent events, which are described below, depend on the presence or absence of the stromal-targeting N-terminus extension.

7.2.2.2. A journey in the stroma. Following import, LHCP is kept in a soluble form, as demonstrated by its accumulation in the stroma when thylakoid membrane insertion is inhibited [426,427]. It interacts with stromal proteins that are essential for its integration into the thylakoids [428–430]. Chloroplast chaperonine, which belong to the Hsp70 family [431], or to the Hsp60 family [432] were suggested to assist membrane insertion of stromal LHCP. However, Yuan et al. [433] concluded that Hsp70 by itself, although it could preserve an unfolded state of LHCP, would not promote its full integration into the thylakoid membranes. The whole stromal fraction was required to provide full protease resistance to membrane-bound LHCP in the in vitro integration experiments [433]. This conclusion is consistent with earlier studies suggesting that more than one stromal component is involved in maintaining soluble LHCP in a competent form for insertion into the thylakoid membranes [434].

Further insight into the set of stromal proteins which interact with pLHCP was brought about by the novel findings of Hoffmann and coworkers [435,626–628]. A 54-kDa protein, showing homology

with a cytosolic SRP subunit, was found in the chloroplast stroma. Its binding to an LHCP intermediate kept the 'transit complex' in a soluble form. Depletion of the stromal fraction from cpSRP54 (chloroplast SRP) prevented integration of LHCP in the thylakoid membranes. In addition, cpSRP54 exhibits GTPase activity (Li and Hoffman unpublished observation) a property which explains the GTP requirement for membrane integration of LHCP [435].

Two *Arabidopsis* mutants have recently provided more evidence that an SRP-like complex plays a role in LHCP biogenesis. In one study, *Arabidopsis* plants were transformed to express mutated forms of cpSRP54, unable of GTPase activity, in the expectation that they would confer a dominant negative phenotype. The mutant leafs displayed a transient, but marked, reduction in their chlorophyll content. Their biochemical analysis suggested a pleiotropic role of cpSRP54 in chloroplast biogenesis [628]. On the other hand, the CHAOS chlorotic mutant of *Arabidopsis* was shown to be specifically deficient in antenna proteins. Its molecular genetic analysis led to the identification of a new stromal protein, cpSRP42, which interacts with cpSRP54 [629]. Thus, a stromal cpSRP complex, which includes at least two proteins, participates in LHCP biogenesis [630].

7.2.2.3. Integration of LHCP in the thylakoid membranes. Thylakoid treatment with low levels of proteases abolishes membrane integration of LHCP, an observation that suggests a need for a proteinaceous receptor for the integration [137,436]. This view supports the presence of an SRP-homologous translocation mechanism, in which docking of the SRP transit-complex is required for membrane protein integration [134].

Since the N-terminus extension of pLHCP plays no further role in LHCP biogenesis besides its stromal targeting function upon import into the chloroplast, the sequences of mature LHCP which are responsible for its membrane integration have been thoroughly studied. To this end, various truncated forms of LHCP were expressed in vitro using heterologous translation systems, then assayed for chloroplast import and thylakoid membrane integration, or for direct membrane integration. A critical function was first attributed to the C-terminal part of LHCP, including its third transmembrane helix [426,437–440]. However, further integration studies using a variety of chimeric proteins containing single LHCP helices or helix pairs, led to the more stringent conclusion that the entire mature protein, with its consecutively ordered α-helices, is required for proper membrane integration of LHCP, with the exception of a part of its stromal amino terminus and a short lumenal loop [425,441]. However, this conclusion may be questioned in view of the seemingly conflicting observations that two mutated LHCP forms, in which either the first or the second helix were deleted, are found in the thylakoid membranes when assayed in an import system with intact chloroplasts ([437], but see [441] for a different view), whereas they failed to integrate when assayed with purified thylakoid membranes [425]. A possible interpretation for this discrepancy is that only the chloroplast import system mimics the in vivo situation for stromal delivery of the substrate protein, i.e. that pLHCP is taken up by the cpSRP mechanism system for thylakoid insertion. In contrast, the stromal extracts added to the thylakoid insertion systems may not be efficient enough to displace the chaperonine components of the in vitro translation system which are associated with neosynthesized pLHCP. In the latter case, LHCP would escape from its genuine insertion route. Thus, the requirements for thylakoid membrane insertion of LHCP in vivo may be less stringent than it has been concluded from the in vitro experiments.

7.2.2.4. Pigment binding. The apoform of LHCP, when fused with a bacterial signal-peptide, integrates into the inner bacterial envelope membrane [442]. Thus, it may not require pigment binding for its integration into the thylakoid membranes. Consistent with this view, LHCP has been reported to remain in its apoform, in the stroma lamellae [443]. Both in vitro and in vivo studies have conclusively demonstrated the critical role of pigment binding for LHCP integration in a protease resistant form. For instance, LHCP cannot maintain a stable conformation when inserted into barley etioplast membranes in vitro. However, supplementing these etioplast membranes with the chlorophyll analog Zn-pheophytin *a*/*b*, confers protease-resistance to the inserted LHCP. Thus, chlorophyll is the only component lacking in the

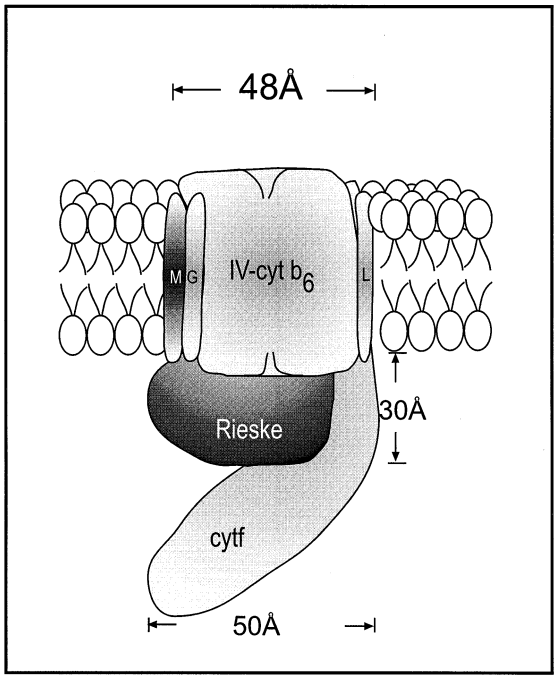
etioplast membranes which is required for a stable LHCP insertion [444]. On the other hand, several carotenoid or chlorophyll-deficient mutants from *Chlamydomonas* [34], barley [445] or *Arabidopsis* [446] insert but fail to accumulate LHCP in their thylakoid membranes. One exception is the cbn-1–113 mutant of *Chlamydomonas* that was reported to accumulate wild-type levels of LHCP despite the absence of chlorophyll *b*. This unique situation has been attributed to a protease-defective activity in this particular mutant [447]. The contribution of chlorophyll binding to the proper folding of LHCP has been elegantly demonstrated by Paulsen et al. [448]. They showed by circular dichroism that upon pigment binding, the rather disordered structure of a solubilized and pigment-free form of LHCP converts into a folded structure with about 60% α-helical organization.

The final site of chlorophyll synthesis as well as the proteins which may store or convey the pigments to their site of assembly with LHCP in the thylakoid membranes remains surprisingly elusive (for a discussion see [449]). It has been suggested that LHCP itself would regulate the final steps of chl *a* and chl *b* synthesis [450]. Among other possible candidates for chlorophyll delivery are those proteins which show sequence homologies with the LHC subunits, such as the ELIPs which are detected at an early stage in thylakoid membrane biogenesis [451] or PSII-S for which there is as yet no definite function [452]. The quasi-exclusive localization of PSII-S in the grana regions [453] implies that LHCP would migrate in its apo-form – and not in its holo-form – from its site of membrane insertion in the stroma lamellae to its site of assembly and oligomerization in the grana region. It is of note that in vitro biogenesis studies led to the conclusion that LHCP was in a 'free' form in the stroma lamellae, while it was found in pigmented LHCIIb complex only in the grana lamellae [443,454,455].

7.2.2.5. The final steps of antenna biogenesis. The picture that results from the above-described experiments is that an association of the antenna complexes with their pigment complement represents an ultimate step in their biogenesis. Indeed, the availability of pigments has been shown to regulate the relative amounts in the three types of antenna complexes that differ in their chl *a*/chl *b* ratios. When limiting amounts of chl *b* are present, a situation achieved experimentally during greening of seedlings grown under intermittent light, the minor antenna complexes accumulate prior to LHCII which require continuous illumination [456]. Moreover, pulse labeling of newly synthesized LHCII has demonstrated that Lhcb subunits accumulate first as monomeric pigmented complexes before their oligomerization in trimers and in even higher-order oligomeric states [456]. Similarly, the Lhca subunits first assemble as pigmented monomers then into oligomers that associate with the pre-existing CCI to form the holo-PSI complex [457]. Trimer formation of Lhcb subunits was also shown to depend on the presence of specific lipid molecules [458,459] whose interaction with the monomers may act as a regulating step in the formation of the grana-based form of LHCII. Last, the distribution of LHCII between the stroma lamellae and the grana regions of the thylakoid membranes is regulated by the extent of reversible phosphorylation of the N-terminus region of the Lhcb subunits (for a review see [460]).

It should be noted that most of the experiments which have contributed to substantiate the above description of LHCP biogenesis have made use of in organello or in thylakoido systems using p/LHCP expressed in vitro in an heterologous translation system. Studies from Hoober and colleagues [461–465] have developed a radically different view, based on in vivo studies using various strains of *Chlamydomonas*. They propose that holo-LHCP assembles at the inner chloroplast envelope. Their conclusion is based on the immunodetection of mature LHCP in vacuolar granules in the cytosol when cells are greened at 38°C. LHCP import in the chloroplast would be arrested when chlorophyll delivery is insufficient and retrograde transport would occur to the cytosol after protein processing. Thus, in physiological conditions, membrane integration would be controlled by pigment binding at the inner envelope level. Subsequent translocation to the thylakoid membranes would occur by envelope invagination, budding and vesicle trafficking and fusion in the stroma, a proposal substantiated by some electron microscopy studies [466,467].

8. The cytochrome b_6f complex



The cytochrome b_6f complex transfers electrons from reduced plastoquinones to a soluble electron carrier located in the lumen of the thylakoids, either the copper-containing plastocyanin or a *c*-type cytochrome. Both types of electron carriers can be found among unicellular photosynthetic prokaryotes and eukaryotes, whereas only plastocyanin is present in vascular plants. The electron transfer reactions through the cytochrome b_6f complex are accompanied by a translocation of protons across the thylakoid membrane, which contributes to the formation of a $\Delta\mu H^+$ used by the chloroplast ATP synthase to generate ATP. The cytochrome b_6f complex is therefore functionally homologous to the cytochrome *bc* complex present in bacteria and mitochondria. However, the subunit composition varies significantly between the various types of cyt *bc* and cytochrome b_6f complexes. For a detailed discussion of the various properties of the cytochrome bc/b_6f complexes, the reader is addressed to recent reviews [468–470].

8.1. The assembled protein

8.1.1. Subunit composition and topology in the membranes

8.1.1.1. Subunit composition. The cyt b_6f complex is made up of seven subunits that are present in a one to one stoichiometry in purified preparations isolated from *C. reinhardtii* [471] (Table 5). There are four major subunits whose contribution to the function of the protein complex is understood: an iron–sulfur protein, the Rieske protein, containing a [2F–2S] cluster (Em ~ +300/370 mV) encoded by the *PetC* gene; a *c*-type cytochrome, cytochrome *f*, encoded by the *petA* gene (Em ~ +330/+370 mV); a *b*-type cytochrome, cytochrome b_6, encoded by the *petB* gene which comprises two *b*-hemes defined according to their relative mid-point potentials, b_h (Em ~ −50/−80 mV) and b_l (Em ~ −160/−170 mV); subunit IV (suIV), encoded by the *petD* gene, which does not carry any prosthetic groups but contributes residues, together with cytochrome b_6 and the Rieske protein, that are required for quinone binding to the protein complex. Comparison of the polypeptide sequences of the various cyt b_6f and cyt *bc* complexes shows that cyt b_6 and suIV are homologous, respectively, to the N-terminus and C-terminus domains of mitochondrial or bacterial cytochrome *b* [166].

Recent biochemical studies of purified cyt b_6f complexes have disclosed three additional subunits of low molecular mass in the 4-kDa region: the *petG* product [472], the *PetM* product [473,474] and the *petL* product, which is present only in higher plants and green algae [475]. Each small subunit displays one single TM helix. Other proteins, of unknown function in the 15–18-kDa range [476,477] or with kinase activity in the 60-kDa region [478,479], have been reported to associate with the cyt b_6f complex in vivo and in partially purified preparations. However, these proteins are either not present, or present in sub-stoichiometric amounts, in highly purified complexes.

8.1.1.2. Topology and lumenal organization of cytochrome f and the Rieske protein. The transmembrane organization of cyt b_6 (four TM helices), suIV (three TM helices) and cyt *f* (one TM helix) is well

established. Most of the cyt *f* residues though, reside in the lumenal space. Martinez and colleagues [480] have obtained a high resolution structure of the soluble part of cytochrome *f*. It shows the presence of a small and a large domain with the heme-binding region being at the edge of the large domain, most likely facing the Rieske protein [481]. On the opposite side of its lumenal domain, cytochrome *f* displays a basic patch that has been suggested to participate in the docking of the electron acceptor plastocyanin through a complementary acidic patch [482]. However, a recent attempt to demonstrate the involvement of these basic residues in electron donation to PC by site-directed mutagenesis has proved unsuccessful [483]. Another striking feature of the fine structure of cytochrome *f* is that the sixth ligand of the *c*-heme is the α-amino-group of the N-terminal tyrosine of the mature polypeptide. This is in contrast to the case of mitochondrial and bacterial cytochrome c_1, in which the ligand is a methionine located more than 150 residues downstream the N-terminal residue (for a detailed discussion see [480]).

The position of the Rieske protein with respect to the thylakoid membranes has been a matter of debate. A hydrophobic stretch of ~25 residues next to the N-terminus of the protein was first considered as a transmembrane anchor for the Rieske protein [484]. This view has been challenged by biochemical studies: the Rieske protein from *Chlamydomonas* behaves as a peripheral membrane protein in as much as it dissociates from membranes upon chaotropic or alkaline treatment. Moreover, it does not aggregate in its isolated form upon removal of detergents [485]. However, the hydrophobic N-terminal stretch of the Rieske protein from mitochondrial bc_1 complexes has recently been shown to adopt a transmembrane orientation in the crystal structure of the protein [486,487]. It is then very likely that the Rieske protein from cytochrome b_6f complexes is a genuine transmembrane protein.

A crystal structure of the lumenal part of the spinach Rieske protein has been recently determined [488,489]. It shows two domains: a small 'cluster-binding' sub-domain, which comprises the [2Fe–2S] cluster and a large sub-domain [489]. The Fe–S cluster on the Rieske protein is coordinated by two cysteines and two histidines, the two histidines being exposed to the solvent, whereas the rest of the cluster is shielded by two loops covalently linked by a disul-

Table 5
Cytochrome b_6f complex

Components	Topology (no. of TM α-helices)	Approximate molecular mass (kDa)	Gene (C/N)	Function and cofactor binding	Contribution to the accumulation of cyt b_6f
Cytochrome *f*	TM (1)	31	*petA* (C)	*c*-heme	+ CES
Cytochrome b_6	TM (4)	24	*petB* (C)	contributing to quinone binding, 2 *b*-hemes	+
Subunit IV	TM (3)	15	*petD* (C)	contributing to quinone binding	+
Pet G	TM (1)	4	*petG* (C)	?	+
Pet L[b]	TM (1)	3	*petL* (C)	?	−
Rieske protein	TM (1?)	19	*PetC* (N)	contributing to quinone binding, 2Fe–2S	−
Pet M	TM (1)	4	*PetM* (N)	?	?
Subunit V[a]	S	18	(N)	?	−
?[a]	?	15	?	?	?
?[a]	M	60	(N)	kinase	?

Topology denotes whether the protein is transmembranal (TM) or stromal (S). Approximate molecular masses are given according to the amino acid sequence of the mature protein. Note that they may deviate markedly between different organisms. C, chloroplast-encoded genes (lower case); N, nuclear-encoded genes (upper case). CES, controlled by epistatic synthesis.

[a] b_6f-associated proteins.

[b] Found only in higher plants and algae.

fide bridge and by a third proline loop [488,490]. In the arrangement of the Rieske protein along the lumenal surface of the membrane, as proposed by Link and Iwata [481], the Fe–S cluster faces the plastoquinol binding site which would be formed by cytochrome b_6, suIV and Rieske motives. According to their model, the bulk of the Rieske protein would be sandwiched between the membrane surface and cytochrome *f* in the lumenal space. Recently, the comparison between two crystal structures of mitochondrial cytochrome bc_1 complex has led to an unexpected finding. A flexible hinge at the lumenal side of its TM helix allows the Rieske protein to orient in two configurations with respect to the membrane: a 'relaxed' configuration, in which it is close enough to cytochrome *f* in the lumen to allow electron transfer between the two, and a 'tight' configuration, in which it is close enough to cytochrome b_6 at the thylakoid membrane surface to allow electron transfer from the Q_o site [487].

8.1.1.3. A mysterious chlorophyll cofactor. Recently, an additional prosthetic group has been identified in the b_6f complex, as a single chlorophyll (Chl) *a* molecule [471,491]. It has been studied in some detail in *Chlamydomonas* where it was reproducibly found in purified cytochrome b_6f preparations, in a 1:1 stoichiometry with cytochrome *f* [492]. An electrochromic response of the chl *a* molecule on a time scale which is similar to that for electron transfer to cyt *f* [493], and the structural precedent for chl binding in the membrane interfacial region of the LHCII pigment protein [406], suggest that it could be located in the region of interaction between the Rieske protein and the surface helices of cyt b_6–suIV (see Cramer et al. [494] for discussion). Solubilization and fractionation studies of cyanobacterial thylakoids in the presence of mild detergents led to the conclusion that the chlorophyll molecule is bound to cytochrome b_6 [495].

8.1.2. Supramolecular organization

Negative staining and freeze-fracturing techniques have shown that the cyt b_6f complex is an elongated particle, 8–9 nm long [491,496,497]. It is asymmetric with respect to the thylakoid membrane plane, as judged from the high-resolution structure of the analogous bc_1 complex from bovine heart mitochondria [486,498,499]. Cytochrome b_6f complexes should present a rather flat surface when viewed from the stromal face of the membrane, but should protrude largely in the thylakoid lumen where most of the Rieske protein and cytochrome *f*, as well as extended loops between TM helices from cytochrome b_6 and suIV, reside (see Fig. 1). As to the prosthetic groups, the structure of bovine heart cytochrome *bc* has shown that the heme irons from b_h and b_l are 21 Å apart. The exact disposition of the *b* hemes may be somewhat different in cyt b_6f complexes because there are 14 residues between the coordinating histidines on helix D of cytochrome b_6, instead of 13 in mitochondrial cytochrome *b* [166]. However, structural homologies are sufficient to predict that heme b_l should sit right at the lumenal surface whereas heme b_h should be more embedded within the stroma-facing leaflet of the thylakoid membrane [498,499].

The packing of the TM helix bundle can be inferred from a comparison of the crystallization data gathered on cytochrome bc_1 complexes [486,487,499], and the projection maps at 8–9 Å resolution of negatively stained or frozen hydrated crystals of cytochrome b_6f complexes from *Chlamydomonas* [500,501]. Cytochrome b_6f complexes crystallize as dimers. Although the possibility that the dimeric cytochrome b_6f complex may adopt a different configuration than the dimeric cytochrome *bc* complex cannot be excluded, the data are consistent with a similar organization of a central bundle of TM helices from cytochrome b_6 responsible for the contacts between the two monomers and more peripherally located helices contributed by cytochrome *f* and the three small subunits.

Biochemical studies of purified cyt b_6f complex have also led to the conclusion that it behaves as a dimer, whether it is isolated from higher plants [491,502], from green algae [497] or from cyanobacteria [495]. It has been suggested that the PetL subunit plays a role in stabilizing the dimeric state of the b_6f complex in *Chlamydomonas* [497]. The Rieske protein was shown to lie next to the interface between the two monomers in the projection map by Mosser et al. [500]. This location is consistent with the observation that the Rieske protein is easily lost upon monomerization of the dimeric form of the cyt b_6f complex from *Chlamydomonas* [497] and spinach

[491]. However, a spinach preparation was reported to preserve the association of Rieske protein with highly active monomeric forms of the cyt b_6f complex [502]. The role of the Rieske protein in cyt b_6f dimerization will be discussed further in Section 8.2.3.1.

It is somewhat paradoxical that neither the functional analysis of cytochrome b_6f complexes, whether they were performed in vivo, in situ or after purification of the protein, nor the current models for cytochrome b_6f activity, point to a mechanism for electron transfer which requires the actual cooperation of two monomers of the cyt b_6f protein. However, the crystal structure of the dimer of cytochrome bc_1, shows that the transmembrane and lumenal domains of the Rieske protein interact with the opposite monomers of the protein complex [499]. On the other hand, Chain and Malkin [502] observed an inactivation of both the dimers and the monomers when reconstituted in phospholiposomes. Interestingly, addition of uncouplers reactivated the dimeric form, but not the monomeric form. One possible interpretation is that a dimer-based proton translocation mechanism is required for the function of the cytochrome b_6f when membrane-embedded. It has indeed been proposed that the cyt b_6f has some proton pumping activity [503,504].

8.2. Expression and assembly of the constitutive subunits

8.2.1. Gene organization and transcription

In cyanobacteria, the four major subunits are encoded in two operons, the *petCA* and the *petBD* [469], which in some instances comprise genes unrelated to the cyt b_6f complex [505]. In addition, *petG* and *PetM* are transcribed independently. In eukaryotes, two subunits are nuclear-encoded (*PetC*/*M*) and five subunits are encoded by the chloroplast genome (*petA*/*B*/*D*/*G*/*L*). The latter are found in a contrasting organization in higher plants and *C. reinhardtii*. In higher plants, *petA* on the one hand and *petB* and *petD* on the other hand, are part of large polycistronic units, which contain other unrelated genes [506,507]. In *Chlamydomonas*, the *petB* gene is located about 10 kbp apart from a 4-kbp region comprising *petA* and *petD* [508]. The *petA*, *petB* and *petD* genes can be transcribed independently and their mRNAs accumulate as monocistronic transcripts [24,78,509]. However, these mRNAs may also result from polycistronic transcription rapidly followed by mRNA processing [24].

Evidence for a nuclear participation in chloroplast *pet* gene expression has come from the characterization of several cyt b_6f mutants which show specific alterations at the levels of mRNA processing, mRNA accumulation or translation of the chloroplast *pet* genes in *C. reinhardtii* [80,510, 511], maize [66] and *Arabidopsis* [63].

8.2.2. Biosynthesis of individual subunits

8.2.2.1. Cytochrome f. Two major maturation steps are involved in the biosynthesis of cytochrome *f*. One is the conversion of a precursor-form to a mature-form, a process which requires translocation of the polypeptide chain across the thylakoid membranes and cleavage of the leader sequence. The other is conversion of the apocytochrome into the holocytochrome, which requires covalent ligation of a *c*-heme to two cysteinyl residues of the polypeptide.

Both processing and heme attachment occur at the lumenal side of the membranes, i.e. following the initiation of translocation [512,513]. The precursor form of pea cytochrome *f* can be processed by the leader peptidase from *E. coli* [514], which is consistent with the expression of pea cytochrome *f* in *E. coli* in a secA-dependent manner [515]. A similar conclusion was reached by Nohara et al. [143], based on in vitro import experiments with pea thylakoids. The conclusion drawn from other studies on cytochrome *f* translocation are somewhat confusing. They all support the view that cytochrome *f* uses the same translocation pathway as some other membrane proteins. The *tha1* nuclear mutant from maize shows altered processing of cytochrome *f*, plastocyanin and OE33, but not of OE16 and OE23 [516], which suggests that cytochrome *f* uses the secA translocation pathway. A plastid mutant from Oenothera shows altered processing of cytochrome *f*, OE23 and OE16, but not of OE33 [517], which suggests that cytochrome *f* uses the ΔpH translocation pathway. Last, mutations in the leader peptide of cytochrome *f* from *C. reinhardtii*, which prevent translocation of the precursor protein and have no

effect on the translocation and maturation of other lumenal proteins, interfere with the levels of D1 and LHCII accumulating in the thylakoid membranes [518]. Since LHCII was reported to use an SRP translocation pathway (see Section 7 on antenna proteins) the possibility exists that cytochrome *f* also interacts with some of the docking sites used in this route.

The translocation machinery has not been characterized. Yet, three nuclear loci were identified in *Chlamydomonas*, the mutant alleles of which restore translocation of a translocation-incompetent variant of precytochrome *f* [518]. This finding opens the way towards the molecular identification of nuclear products, which participate in the translocation across the thylakoid membranes.

The leader peptide of cytochrome *f* plays a key role in the translocation process. Although this cleavable signal sequence comprises 31–44 residues depending on species, its hydrophobic stretch is only 12–18 residues long. The minimal length of the hydrophobic region required for successful translocation, the translocation core, is even shorter. In *Chlamydomonas* it has been found to comprise only seven residues, from T13 to G19, as demonstrated by the contrasting effects of various charged amino acid substitutions in the signal sequence [518]. The exact boundaries of the translocation core appear flexible since chloroplast revertants of translocation-incompetent strains from *Chlamydomonas*, bearing originally a V16D or A15E substitution in the signal peptide of cytochrome *f*, display as an additional mutation, an R10L or R10C substitution [518]. These intragenic suppressions restore a 10–11 residues translocation core, which is N-terminus-shifted by at least three residues as compared to that in the wild-type.

As to the heme attachment process, a genetic analysis has been conducted on several *Chlamydomonas* mutants deficient in the two *c*-type cytochromes found in thylakoid membranes, cytochrome *f* and cytochrome c_6 –an alternative electron carrier to plastocyanin expressed in copper-deficient conditions [519]. At least four nuclear genes (*Ccs1–Ccs4*) and one chloroplast gene (*ccsA*) [164,520] (see Table 7) were shown to participate in *c*-heme attachment. The protein products most likely form a transmembrane protein complex with a *c*-heme attachment activity on the lumen side of the membranes (for a review see [521]).

A soluble form of holocytochrome *f*, generated by a truncation of its C-terminal transmembrane helix, was produced by genetic transformation of *Chlamydomonas* chloroplasts. It accumulated extensively in the thylakoid lumen with no evidence for the production on any significant amount of the apoform [522]. Therefore, the membrane-bound heme-binding activity is still efficient in the strain, despite the fact that its cytochrome *f* substrate in its mature form is soluble in the lumen space. These observations suggest that heme binds to the apocytochrome when the latter is still membrane-associated, that is in its precursor form. Thus, kinetically, heme ligation may well precede protein processing, a proposal consistent with the observation that heme binding to the precursor form can actually be detected in a cytochrome *f* processing mutant of *Chlamydomonas* [36]. Conversely, altered *c*-heme attachment, whether it is achieved by gabaculine treatment in maize [514] and *Chlamydomonas* [523], or by genetic lesions [36,523] does not interfere with apocytochrome *f* processing.

These conclusions do not support a mechanistic intrication of the two processes (protein processing and heme binding) initially suggested upon the identification of the sixth ligand of the heme-iron as the free N-terminus amino-group released upon cleavage of the signal sequence [480]. However, they do not exclude the possibility that the two processes are kinetically coupled during regular cytochrome *f* biogenesis.

8.2.2.2. The Rieske protein. Import experiments with pea chloroplasts suggest that the Rieske protein first associates with Cpn60, then with Cpn70 in the stroma [524]. This is opposite to the order of interaction with mitochondrial chaperonines upon protein import [525]. Whether these transient complexes are intermediates before translocation across the thylakoids or whether they correspond to the rescue and storage of Rieske proteins that have failed to translocate properly remains to be investigated. Non-assembled Rieske proteins have been shown to be degraded in vitro by endogenous proteases which are similar to the bacterial FtsH proteases [526].

It has been suggested that the hydrophobic stretch

next to the N-terminus of mature Rieske proteins functions as an uncleaved lumenal targeting sequence, based on the comparative analysis of the hydropathy index of signal peptides and regular TM helices [527]. Translocation of the Rieske protein across the thylakoid membranes to the lumen compartment, where most of the protein resides, was shown to use the ΔpH pathway ([524], Karnauchov and Kloesgen, personal communication): Rieske translocation proved to be nigericin-sensitive and it competed with that of OE23, a well characterized user of the ΔpH route (see Sections 3.4 and 5.2.2.4). It is noteworthy that the trimethylamine *N*-oxide reductase, folded with its molybdocofactor, was shown to be exported to the periplasm of *E. coli* in a Sec-independent way, via a ΔpH route [157]. Therefore it is tempting to speculate that the Rieske protein translocates to the lumen in a pre-folded state. It implies that the apo-Rieske protein would associate with the 2Fe–2S center in the stroma prior to its translocation, i.e. in the same compartment as for the many other apoproteins, for instance ferredoxins or the three RCI subunits (see Section 6), which associate with iron–sulfur clusters. The formation of the 2Fe–2S center and the molecular mechanism for apo- to holo- Rieske protein conversion in vivo remain fully unknown. Some insight into these processes may be gained with the use of in vitro reconstitution assays that are now available: a native Rieske protein was reconstituted by incubating an apo-Rieske protein from Nostoc *sp PCC7906*, overexpressed in *E. coli*, with iron and sulfide under reducing conditions [528].

8.2.2.3. Cytochrome b_6. The biosynthesis of cytochrome b_6 has been mainly addressed with respect to the process of heme binding. Heme associates spontaneously with peptides that mimic the binding sites of bacterial apocytochrome *b* in vitro [529]. The resulting maquettes displayed spectral properties analogous to those of a bona fide *b*-type cytochrome.

Consistent with their non-covalent binding, the *b*-hemes dissociate from bacterial or mitochondrial cytochrome *b* upon SDS-PAGE [530,531]. In contrast, cytochrome b_6 still behaves as a hemoprotein upon SDS-PAGE [476]. It even resists much stronger denaturation treatments like acetone-acid extraction [37]. At least four nuclear gene products designated CCB1–CCB4 (see Table 7) were shown to be specifically involved in heme attachment to cytochrome b_6 in *Chlamydomonas* [37]. These observations indicate that heme binding to cytochrome b_6 is catalyzed in vivo by a specific enzymatic machinery which probably promotes highly stable, possibly covalent, interactions of at least one of the two *b*-hemes with apocytochrome *b*. It is of note that a similar conclusion that one *b*-heme may be covalently attached to the apoprotein has been reached recently with a homolog of cytochrome b_6 in *B. subtilis* [532].

8.2.3. Assembly and accumulation of the protein complex

8.2.3.1. Reconstitution experiments. Because of the highly hydrophobic nature of most of the cyt b_6f complex subunits, there have been no attempts to reconstitute the oligomeric protein from its individual subunits. However, a successful reconstitution of the isolated Rieske protein from Rieske-depleted cyt b_6f complex has been reported using spinach preparations [533]. The Rieske protein was easily detached from the complex in the presence of Triton X-100 and removal of the excess detergent proved sufficient to reconstitute a functional cyt b_6f complex. Interestingly, detergent treatments that detached the Rieske protein also converted the cyt b_6f dimers into monomers [497]. However, the presence of the Rieske protein is not a prerequisite for dimerization, since, in some instances, dimeric forms of the cyt b_6f complex could be isolated in the absence of the Rieske protein [497,534], in particular using mutant strains of *C. reinhardtii* lacking the Rieske protein (de Vitry, personal communication).

8.2.3.2. Cytochrome b_6f mutants as a tool for assembly studies in vivo. A number of mutants impaired in photosynthetic electron transfer at the level of the cyt b_6f complex have been obtained from the green alga *C. reinhardtii* [78,476,510,519] and also from Lemna [535], maize [66,516,536], and *Arabidopsis* [63]. They conclusively demonstrate that the various cyt b_6f subunits show a concerted accumulation in vivo. In most instances, the whole complement of cyt b_6f subunits was deficient in the cyt b_6f-defective strain, independent of the primary mutational defect. This was observed whether the mutations primarily

affected *c*-heme binding [519,520], *b*-heme binding [37], transcript processing [66,511], transcript stability [510], or protein translocation [537].

Deletion of either the *petA*, *petB*, *petD* [78] or *petG* [538] genes from the chloroplast genome of *C. reinhardtii* led to a decrease of more than 90% in the content of the other cyt b_6f subunits. Further evidence for a concerted accumulation of the cytochrome b_6f subunits has come from site-directed mutagenesis studies with *Chlamydomonas*, which decreased the efficiency of translation initiation of *petA* or *petD* mRNAs [539–542]. The decreased accumulation of the target subunit correlated with a general decrease in the accumulation of other cytochrome b_6f subunits.

In contrast to the above observations, a significant accumulation of the chloroplast-encoded subunits was observed in *C. reinhardtii* in the absence of either the chloroplast gene product PetL [475], or of the nuclear-encoded Rieske subunit [476]. However, it should be noted that the cyt b_6f complexes lacking these subunits accumulated to 30–60% of the wild-type levels, only in exponentially growing cells. The protein complexes were totally lost in aging cells, which therefore displayed the same pleiotropic deficiencies as in the other cyt b_6f mutant strains described above ([475], de Vitry and Wollman, unpublished). This is indicative of a lower stability of the partially assembled complexes, which are more susceptible to degradation in non-dividing cells. The molecular mechanism for this semi-specific degradation process is poorly understood. The possibility that it shares some characteristics of the degradation process specifically acting on native cytochrome b_6f complexes in wild-type cells of *Chlamydomonas*, when deprived in nitrogen sources [543], remains to be investigated. In the latter case, the loss of cytochrome b_6f complexes required the continuing activity of mitochondrial electron transport, which could indicate that intracellular ATP levels may control the degradation process. This proposal is well supported by the recent observation that the ATP-dependent Clp protease is involved in the degradation of cytochrome b_6f complexes. *Chlamydomonas* strains that underexpress ClpP display a delayed and limited degradation of cytochrome b_6f complexes, when deprived of nitrogen sources, and they accumulate wild-type amounts of the cytochrome b_6f moiety of the complex in a mutated context which prevents production of the Rieske protein (Majeran, Wollman and Vallon, unpublished observations).

The variable content of cytochrome b_6f complexes in *petL* or Rieske protein of *C. reinhardtii* mutants may provide a clue for understanding the seemingly conflicting report of a *Lemna* mutant which lacked only the *PetC* transcript for the Rieske protein, but was, nevertheless, deficient in all cyt b_6f subunits [535]. These mutant cells may be in a metabolic situation similar to that of aging cells in *C. reinhardtii* which induces the loss of their cytochrome b_6f complement. Consistent with the view that association of Rieske protein with the rest of the complex may be a late step in the assembly process, is an in vitro study of the import of Rieske protein in isolated tobacco chloroplasts [544]: the neosynthesized product associated with pre-existing cyt b_6f complexes in the thylakoid membranes.

8.2.3.3. Stoichiometric production of cytochrome b_6f subunits. The underlying mechanism for the concerted accumulation of the cytochrome b_6f subunits has been studied in great detail in *Chlamydomonas*. Two major regulation processes have been shown to contribute to their stoichiometric accumulation [78]: (1) a proteolytic disposal of most of the unassembled subunits. This applies, in particular, to the Rieske protein as well as to cytochrome b_6 and suIV, the half-lives of which are considerably shortened in the absence of assembly; and (2) a CES process (see Section 1) in the case of cytochrome *f*, which corresponds to a drop in the rate of cytochrome *f* synthesis in *Chlamydomonas* mutant strains showing impaired assembly. It is not yet known whether cytochrome *f* is also a CES subunit in higher plants. However, the report that a maize mutant defective in *petD* mRNA processing also fails to translate cytochrome *f*, a subunit whose transcript is distinct from the *petD* transcript in maize [66], supports the existence of a similar mechanism in higher plants.

Further analysis of a number of site-directed mutants of the *petA* gene has shown that the CES process operates through an autoregulation of cytochrome *f* translation [291,358]: a protein motif located at the C-terminal domain of cytochrome *f*, down-regulates translation of the *petA* gene, unless it is shielded by assembly in a cytochrome b_6f complex.

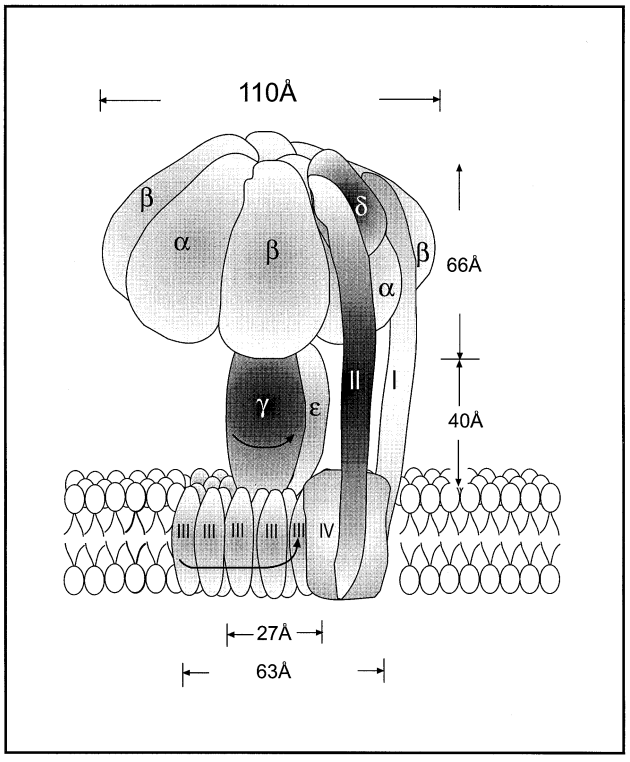
This down-regulation operates through an interaction, either direct or indirect, with the 5′-leader of the *petA* transcript. Thus, in the absence of an assembly partner, cytochrome *f* down-regulates *petA* translation [78] due to exposure of its regulatory motif. Cytochrome *f* lacking its C-terminal anchor [36], or rapidly turning over because of the absence of heme binding [522], does not accumulate the regulatory motif, thereby allowing translation of the *petA* message at maximal rates [291]. As predicted by this regulation mechanism, a strain expressing a chimeric gene, where the *petA* coding region was flanked with an upstream 5′-leader from an unrelated gene, the *atpA* gene, lost the ability to coordinate the accumulation of the cytochrome b_6f subunits. When expressed in a mutant strain blocked in the expression of the assembly partner suIV, the chimeric gene produced cytochrome *f* in wild-type levels despite the absence of all other subunits of the cytochrome b_6f complex [291]. Conversely, a reporter gene flanked by the 5′-UTR of the *petA* gene exhibited the same regulation of expression as *petA*: it was underexpressed in the absence of an assembly partner of cytochrome *f*, but it was overexpressed in the absence of *c*-heme binding to cytochrome *f* [291].

Whether the C-terminus regulatory motif of cytochrome *f*, which is exposed on the stromal side of the membranes, interacts directly with the 5′-*petA* UTR is not known. The regulation may involve a ternary effector that participates in translational activation of the *petA* transcript. This possibility may gain support from the characterization of nuclear mutants specifically altered in cytochrome *f* translation, which have been identified in *Chlamydomonas* [80]. These may help identifying factors involved in the CES process.

The above-described regulation mechanism does not rule out other contributions to the control of protein assembly. For instance, the translation of *petD* mRNA is controlled both positively and negatively by various *cis*-acting elements located far upstream the *petD* initiation codon in *Chlamydomonas* [540]. These may also participate in the fine-tuning of suIV synthesis in circumstances that remain to be defined, and therefore contribute to the regulation of cytochrome b_6f assembly.

8.2.3.4. Conserved features in the assembly processes for cytochrome bc and b_6f complexes. It is worth noting that the most salient features of the subunit assembly are conserved from bacterial cytochrome *bc* complexes to chloroplast b_6f complexes. Studies of *R. capsulatus* mutants defective in either one of the major genes from the *fbc* operon have shown the same properties as the deletion strains from *C. reinhardtii* [545]. In the two organisms, the *b*-type cytochrome is rapidly degraded if not assembled with the *c*-type cytochrome, whereas the latter is a long-lived species in the photosynthetic membranes in the absence of the *b*-type cytochrome. The fact that *c*-type cytochrome accumulates in wild-type levels in the cyt *b*-less bacterial mutant when it reaches only 10% of wild-type level in the cyt *b*-less *Chlamydomonas* mutant, results probably from the absence of a CES process in purple bacteria. Last, the cytochrome moiety of the complex, comprising the *c*- and *b*-type cytochromes, is fairly stable in the membrane in the absence of the Rieske protein, whereas the Fe–S protein is degraded when not associated with the membrane-bound cytochromes [545,546]. Thus, it can be suggested that the biogenesis of cytochrome *bc* and cytochrome b_6f complexes is driven by the biosynthesis of the more stable subunit, the *c*-type cytochrome. This subunit serves as a membrane anchor which associates first with the *b*-type cytochrome, formed by an early interaction between cytochrome b_6 and suIV in the case of cytochrome b_6f complexes, then with the Rieske protein. This mechanism of a step by step pathway of assembly, is in opposite order to the position in which the genes are organized within the bacterial operon: the product of the first gene to be translated will require the next translation product to be fully protected against degradation, etc. Thus, a premature arrest in translation will not lead to the accumulation of unassembled subunits in photosynthetic bacteria. The splitting up of the operonal organization in the chloroplast may have required an additional step of regulation – the CES process operating via translational autoregulation of the *c*-type cytochrome – which aims at preserving its early role in the assembly process, while avoiding an erratic accumulation of this protease-resistant subunit.

9. The chloroplast ATP synthase



The chloroplast ATP synthase belongs to the family of F_1-type ATPases, which are also present in bacteria and mitochondria [547]. It generates ATP from ADP and inorganic phosphate using energy derived from a *trans*-thylakoidal electrochemical proton gradient, $\Delta\mu H^+$.

9.1. The assembled protein

9.1.1. Subunit composition and topology in the membranes

The ATP synthase has long been described as the association of two distinct sectors, a membrane-embedded proton channel, CF_0, and a catalytic sector, CF_1, located on the stromal surface of the thylakoid membranes. The latter part contains the nucleotide binding sites and displays latent ATPase activity. The whole enzyme comprises nine subunits: five CF_1 subunits, $\alpha:\beta:\gamma:\delta:\varepsilon$ in the stoichiometry 3:3:1:1:1, all extrinsic to the membrane and facing the stroma, and four transmembrane F_0 subunits, suI–IV in an assumed stoichiometry of 1:1:9–12:1. Hydropathy analysis suggests that suIV, III, and II/I have four, two, and one TM helices respectively. Owing to the extensive phylogenetic conservation of the bacterial, mitochondrial and chloroplast enzymes, as observed both in the primary sequences of their subunits and in their functional characteristics, it is reasonable to infer the supramolecular organization of the CF_1–CF_0 assembly from data obtained with the enzyme from other sources.

9.1.2. Supramolecular organization

Our view of the supramolecular organization of the ATP synthase has long been restricted to the low resolution EM analysis of the enzyme in situ. It showed the presence of a stalk linking the globular CF_1 to the membrane-embedded CF_0, which is rod-shaped upon detergent solubilization [548]. However, recent advances in structural studies, which combined NMR and X-ray structure determination with cross-linking data, have led to a profound revision of our understanding of the structural arrangement of the enzyme subunits (for a review see [549]). The active enzyme is currently presented as an assembly between a stator portion and a rotor portion, both of which contain peripheral and transmembrane subunits. The stator is composed of the CF_0 subunits I, II and IV and the CF_1 subunits α, β and δ. The rotor comprises CF_1 subunits γ and ε and the 9–12 copies of CF_0 subunit III (see Fig. 1).

The present arrangement of the enzyme stems from the study of a mitochondrial α3β3 subcomplex, which is sufficient to support ATP hydrolysis [550]. Its structure has been determined at 2.8 Å resolution in the presence of suitable nucleotide substrates [551]. Recently, the crystal structure of a nucleotide-free bacterial α3β3 subcomplex has also been determined [552]. The two structures are very similar, despite some difference in asymmetry most likely due to the absence of nucleotide-binding in the crystallized bacterial enzyme. They consist of an hexameric ring of alternating α- and β-subunits that surrounds an α-helical domain containing both the N- and C-terminal regions of the γ-subunit. The top of the αβ-assembly, distal to the membrane, consists of a β-barrel comprised of the N-terminal portion, covering a central nucleotide-binding domain, followed by the C-terminal α-helical domain. Three groups of αβ-heterodimers are thus formed, which can adopt three

distinct nucleotide binding conformations, corresponding to empty sites, ADP/Pi binding sites and ATP tight binding sites. These structural features support a rotatory mechanism [553] where the central γ-subunit rotates within the αβ-hexamer, driving the enzyme through three successive configurations that are required for ATP synthesis/hydrolysis. In recent years, this model has gained increasing support, although it has been challenged, based on the preserved ATPase activity observed in the absence of the γ-subunit or on the preserved binding of γ to CF_1 upon its cleavage into three fragments [554].

Part of the γ-subunit protrudes below the C-terminal domain of the α- and β-subunits. Together with the ε-subunit, the protruding portion of the γ-subunit interacts with CF_0 [555] and may provide the CF_1 contribution to the stalk observed by electron microscopy at low resolution [548,556]. The CF_0 contribution to the stalk is most likely provided by the stromal extended C-terminus domains of the two homologous subunits I and II [557]. Indeed, their *E. coli* counterpart, subunit b, present in two copies in BF_0 [558] interacts with BF_1 through its highly charged C-terminal domain [559]. Subunits I and II are then anchored in the membrane by a single N-terminal α-helix. Subunit δ has also been considered as a member of the stalk portion of the enzyme. However, recent cross-linking data suggest that it is located at the interface between subunits I and II and the N-terminal β-barrel domain of the αβ assembly, in a region distal to the thylakoid membranes [560]. Recent work with site-directed mutants of *E. coli* by Capaldi and coworkers [561,562] has confirmed that δ is a member of the stator portion of the enzyme while ε is part of the rotor as indicated in Fig. 1.

A contribution of suIII to the binding of CF_1 should also be considered. Indeed, specific and stable association between CF_1 and suIII in the total absence of suI/II/IV has been convincingly demonstrated both by in vitro co-immunoprecipitation of detergent-solubilized suIII with CF_1, and by the ability of CF_1 to bind to suIII-containing liposomes [563,564]. There is no doubt that the unraveling of the interactions between the CF_1 and CF_0 sectors awaits better insight on the organization of the stalk region.

The structural features of the transmembrane part of the CF_0 sector remain elusive. Even the exact stoichiometry of the major CF_0 subunit suIII in the active enzyme remains controversial [563] and references therein). Based on the resolved structure of the bacterial homolog of suIII in a monomeric form [565], it has been proposed that subunits III arrange in the shape of a ring. This observation is supported by electron [566] and atomic force spectroscopy [567], which suggest that the proteolipid ring has a diameter of about 7 nm. The proteolipid ring would rotate relative to CF_0 subunit IV that is proposed to play a central part in the functional conductance to protons of the assembled enzyme [568]. Nothing is known on the arrangement of its transmembrane domain, which thus conceals the structural features lacking to understand the proton pumping mechanism of the ATP synthase.

9.2. Expression and assembly of the constitutive subunits

9.2.1. Gene organization and transcription

In most cyanobacteria, the ATP synthase subunits are made up of two operons, a small one encoding *atpB* (β) and *atpE* (ε) and a large one in which the seven other genes are clustered [569,570]. In most photosynthetic eukaryotes, the split organization into a large and a small *atp* cluster is largely conserved [571] even though three genes, *AtpC*, *D*, *G*, encoding subunits δ, γ and suII, respectively, have been transferred to the nuclear genome. It should be noted, however, that *AtpG* and *AtpD* are still part of the large *atp* cluster in the plastid genome of red algae [572]. The clustered genes are co-transcribed and, depending on species, are, either or not, processed to transcripts of smaller size [572–574]. The chloroplast *atp* genes from *Chlamydomonas* display unique organization since they are dispersed throughout the organelle genome, and are therefore transcribed independently [575].

9.2.2. Biosynthesis of individual subunits

Several subunits of the chloroplast ATP synthase undergo post-translational processing. First, nuclear-encoded subunits are processed to their mature form upon translocation from their site of translation in the cytosol to their site of functional assembly in the thylakoid membranes. Intriguingly, two chloroplast-encoded CF_0 subunits (suI and suIV) also undergo

N-terminal processing, although they are not peripherally located on the lumen side of the thylakoid membranes and retain a transmembrane orientation.

9.2.2.1. Processing of nucleus-encoded subunits. The three nuclear-encoded subunits, γ, δ of CF_1 and suII of CF_0 are translated on cytosolic ribosomes as precursors and are post-translationally imported into the chloroplast where they are converted to their mature form. The size of the N-terminal presequences of the γ- and δ-subunits varies between species from about 40 to 60 residues [576,577]. The presequence of suII is longer, e.g. 75 residues in spinach [558], and displays a bipartite organization; the stromal-targeting sequence being followed by a hydrophobic stretch and a consensus site for the thylakoid processing peptidase. Hence, the precursor form of suII contains a presequence that was presented as typical of lumenal-targeted proteins. However, membrane insertion of CF_0II is azide insensitive and does not require the assistance of ΔpH, NTPs, or stromal factors [578]. Moreover, it is not inhibited following treatment of isolated thylakoids with a protease, indicating that there is no involvement of proteinaceous receptors [436].

The N-terminal processing of CF_0II is somewhat unexpected since it is a transmembrane protein with only one putative transmembrane anchor and a long hydrophylic C-terminal domain extending in the stroma. The processing step of cytochrome *f* and PSII-W, whose mature N-terminus faces the lumen (see Sections 5 and 8), argues for the need of a cleavable N-terminal hydrophobic segment, in order to position the N-terminus of the mature protein on the opposite side of insertion into the membrane. It should be noted, however, that some other short bitopic subunits, like PetG or PetM from the cytochrome b_6f complex [473], do not undergo N-terminal processing although their N-termini are positioned at the lumenal face of the thylakoids.

9.2.2.2. Processing of chloroplast-encoded subunits. Since the nuclear-encoded CF_0 suII and the chloroplast-encoded CF_0 suI are both homologous to F_0 subunit b from *E. coli* (see Section 9.1.2), one would expect them also to share a common processing mechanism for positioning their N-termini in the lumen. Indeed, a cleavable N-terminal extension has been identified for suI [579]. Thus, mature suI displays only a very short amino terminal sequence of about 7–8 residues in the lumen, similar to that found for suII. However, the cleavable suI presequences contain 15–17 residues which are difficult to fit with canonic lumenal targeting sequence. They contain shorter hydrophobic stretches than usual and a proline is often found in the −3 position, with respect to the cleavage site. Moreover, in pea, the presequence is only seven amino acid residues long [580]. Therefore the exact role of N-terminal processing in the biogenesis of suI requires further investigation. The same question prevails in the case of suIV. This chloroplast-encoded subunit contains four putative transmembrane segments [581]. The actual N-terminus of the mature protein was shown to start 18 amino acid residues downstream the initiating methionine [582]. This putative cleavable sequence bears no resemblance to lumenal transit sequences. In most cases, cleavage occurs after a serine, and the released peptide is unambiguously hydrophylic. Since *C. reinhardtii* can be used routinely to perform site-directed mutagenesis of chloroplast-encoded proteins, modification of suI and suIV presequences is now feasible and should provide new insights on the function of subunit processing in the biogenesis of the enzyme.

9.2.3. Assembly and accumulation of the protein complex

The biogenesis of the bacterial and mitochondrial enzymes have been reported to occur through independent and stable assembly of soluble F_1 followed by attachment to the transmembrane F_0 subunits [583,584]. No such evidence has been reported for the biogenesis of the chloroplast enzyme. In *C. reinhardtii*, the whole set of chloroplast ATP synthase subunits shows a concerted accumulation in vivo, with much reduced amounts of CF_1 detected in mutants blocked in the synthesis of a CF_0 subunit [585,586]. These contrasting assembly features may reflect the difference in coordination of gene expression due to widely different organization of the structural genes for the ATP synthase. The genes for F_0 and F_1 are either organized in a single operon, the *unc* operon in *E. coli*, or split into two functionally homogeneous groups, with an organelle-encoded F_0 sector and a nuclear-encoded F_1 sector in the case of

the mitochondrial enzyme. In contrast, the chloroplast CF_0 and CF_1 sectors are both of dual genetic origin with subunits encoded either in the nucleus or in the chloroplast.

9.2.3.1. Stoichiometric production of the enzyme subunits. A particularly challenging aspect of biogenesis studies of the ATP synthase is to understand the mechanisms that control the stoichiometric production of the assembled subunits in markedly uneven ratios, with three times more α- and β-subunits than γ, δ or ε in CF_1, and about 10 times more subunit III than subunits I, II and IV in CF_0 (Table 6). The study of various *Chlamydomonas* mutants defective in the synthesis of a particular CF_1/CF_0 subunit, but lacking all of the ATP synthase proteins [585,586], suggests that post-translation degradation of the unassembled subunits is a major tool in keeping the steady-state concentrations of the various subunits to the stoichiometry required for functional assembly. Our present knowledge on other possible contributions remains very elusive. In *E. coli*, it has been suggested that the β/ε ratio results from a translational control exerted through a secondary structure in between their two genes in the *uncDC* mRNA [587]. Genuine translational coupling between β and ε has been demonstrated in the case of maize chloroplast [588]. Its possible contribution to their differential expression has been advocated, but the absence of translational coupling between β and ε in all other chloroplasts examined so far does not support the idea that such a mechanism plays a central role in establishing the subunit stoichiometry of the chloroplast enzyme. It has been suggested that the frequency of ribosome pausing could explain why suIII is made in more copies than suI, but the same explanation would not convincingly hold for subunit α versus suI [574]. The stoichiometry of the subunits has also been suggested to result directly from the ratio of their transcript concentration level in spinach [589]. At present, this proposal is difficult to reconcile with the general observation that chloroplast protein synthesis is not limited by transcript availability (see Section 1).

9.2.3.2. Rates of subunit synthesis control the rate

Table 6
ATP synthase

Components	Topology (no. of TM α-helices)	Approximate molecular mass (kDa)	Gene (C/N)	Stoichiometry	Function	Contribution to the accumulation of ATP synthase
$CF_1\alpha$	S	55	*atpA* (C)	3	stator, nucleotide binding (regulatory)	+ CES
$CF_1\beta$	S	54	*atpB* (C)	3	stator, nucleotide binding (catalytic)	+
$CF_1\varepsilon$	S	15	*atpE* (C)	1	rotor, regulatory	+
CF_0I	TM (1)	21	*atpF* (C)	1	stator	+
CF_0III	TM (2)	8	*atpH* (C)	9–12	rotor; generates torque, proton translocation	+
CF_0IV	TM (4)	25	*atpI* (C)	1	stator, proton translocation	+/−
$CF_1\gamma$	S	35	*AtpC* (N)	1	rotor, energy transducing	+
$CF_1\delta$	S	21	*AtpD* (N)	1	stator, mechanic energy storage?	?
CF_0II	TM (1)	16	*AtpG* (N)	1	stator	+

Topology denotes whether the protein is transmembranal (TM) or stromal (S). Approximate molecular masses are given according to the amino acid sequence of the mature protein. Note that they may deviate markedly between different organisms. C, chloroplast gene (lower case); N, nuclear gene (upper case). +, Required for the accumulation of other subunits of the complex; +/−, mildly required; −, dispensable; CES, controlled by epistatic synthesis.

of assembly. The steady-state concentration of the enzyme is kinetically controlled by the rate of synthesis of its constitutive subunits. In a *Chlamydomonas* mutant showing a considerable drop in its content of monocistronic *atpA* transcripts, a 3-fold decrease in the rate of synthesis of the α-subunit results in a lower accumulation of the assembled enzyme [56]. Short pulse-labeling studies of chloroplast-encoded polypeptides performed in vivo with a number of *C. reinhardtii* mutants defective in the chloroplast ATP synthase have shown that the rates of synthesis of the α- and β-subunits are intimately coordinated [585]. The rate of synthesis of α decreases in mutants lacking β which confers to α the behavior of a CES protein (see Section 1). An opposite effect is observed with strains defective in α-synthesis in which the rate of synthesis of β now increases [56]. A mutant from *C. reinhardtii* bearing two neighboring missense mutations in the coding region of the *atpA* gene (α-subunit) displays increased rates of synthesis of both the α- and β-subunits [590]. These various observations strongly suggest that early interaction(s) between the α- and β-subunits occur during their biogenesis, even though the molecular mechanism of these interactions remains obscure. One possibility, derived from the CES mechanism operating for cytochrome *f* (see Section 8), is that a protein motif accessible from the unassembled α-subunit but largely shielded upon assembly in CF_1, exerts a negative feedback control on the synthesis of both the α- and β-subunits. The target step for this feed-back signal should be translation initiation, since the 5′-UTR of the *atpA* transcript was shown to control the down-regulation of subunit α-synthesis when subunit β is not present: fusion of the coding region of *petA* with the 5′-UTR of *atpA* down-regulates cytochrome *f* synthesis in a mutant of *Chlamydomonas* lacking the β-subunit (Rimbault, Drapier, Girard-Bascou and Wollman, unpublished data).

9.2.3.3. The assembly process as assayed in reconstitution experiments. A number of authors have taken advantage of the conserved features of the F_1-ATP synthase family in order to attempt the production of hybrid enzymes. In several instances, heterologous reconstitution between prokaryote and eukaryote subcomplexes has been obtained in vitro, either by associating F_1 and F_0 sectors from different sources [591], or by exchanging only one subunit with its homolog from another source [592]. However, most of the efforts have been devoted to the expression of a particular subunit of the chloroplast enzyme together with the rest of the bacterial complex in a suitable deletion mutant from *E. coli* [593–596]. These studies were designed to assess the feasibility of a heterologous system to draw structure/function correlations with the chloroplast ATP synthase subunits rather than to understand the biogenesis process itself. They have provided indirect indications as to the specificity of the interactions that develop between the different subunits of the same complex. Genetic complementation between chloroplast and bacterial ATP synthase genes has been observed with CF_1 genes encoding α-, δ-, and ε-subunits [593], but not for the γ-subunit [593], or for any of the CF_0 subunits [594,597]. Nevertheless, a chimeric CF_0-suIII/BF_0-suc has complemented the deletion of the resident bacterial gene when it contained only the first seven N-terminal amino acid residues of the chloroplast sequence. Complementation was lost in constructs expressing the first 13 or 33 N-terminal residues of suIII [594].

E. coli strains have been complemented by the chloroplast δ- or ε-subunits that show only about 25% conserved residues. The complemented strains displayed higher growth rates than those that have been complemented by the chloroplast α-subunit, which shows 54% conservation [593]. In the case of the β-subunit, which shows 66% conservation between *E. coli* and chloroplasts, complementation experiments have led to conflicting reports. In one instance, no complementation was observed with a genuine chloroplast β-subunit [593]. Complementation could be achieved only with a chimeric β-subunit, in which the 96 N-terminal residues from the chloroplast sequence were replaced by the corresponding *E. coli* sequence [595]. This may indicate that the β-barrel domain of the β-subunit plays a critical role in the folding and assembly of F_1, most likely governing the formation of αβ-heterodimers. However, Chen et al. [596] have demonstrated that the intact chloroplast β-subunit could complement a deletion strain from *E. coli* provided that it was expressed at moderate rates. The underlying assumption for this conditional complementation is that a competition between the formation of

inclusion bodies and F_1F_0 biogenesis takes place. However, since all chloroplast subunits produced inclusion bodies in *E. coli*, independent of their ability to complement a deletion strain [593], the deleterious effect of the overexpression of β-subunits on F_1F_0 biogenesis in *E. coli* points to a specific role of this subunit, possibly a nucleation process in the assembly of the enzyme. The general conclusion that stems from the above-described reconstitution experiments is that the efficiency of functional complementation does not match the extent of sequence conservation between bacterial and chloroplast subunits. It reflects both a requirement of some nearest neighbor protein sequences for complementation and the uneven contribution of the various subunits to the functional catalysis of the assembled enzyme.

Following the in vitro disassembling of CF_1 into a mixture of heterodimers and α3β3 hexamers [598], attempts were made to reconstitute ATPase activity by mixing these subcomplexes with purified γ-subunits [599]. A fully functional catalytic core, α3β3γ was restored and displayed both the ε binding properties and the high affinity binding sites for nucleotides, two properties lost by the unassembled subunit preparations. In contrast, it proved difficult to achieve in vitro reconstitution of a catalytic core from the individual α-, β- and γ-chloroplast subunits expressed independently in *E. coli* and then urea-denatured and renatured separately. Approximately 5% of the ATPase activity was recovered using cyanobacterial subunits expressed independently in *E. coli* [600] and no activity was regained with tobacco chloroplast subunits expressed in a similar way [601]. In the latter case, reconstitution was achieved only in the presence of a mixture of chloroplast molecular chaperones, which could not be replaced by the bacterial ones. Moreover, α and β had to be refolded together in order to reconstitute with a γ-subunit folded independently. On the basis of these in vitro studies, Chen and Jagendorf [601] suggested that CF_1 assembly proceeds through the concerted folding of αβ-heterodimers which undergo hexamerization when assembling with a prefolded γ-subunit. The fact CF_1 assembly requires pre-formation of αβ-heterodimers is supported by the early interaction observed between α and β during their synthesis in vivo in *C. reinhardtii* [56]. It should be noted that α has been presented as a possible chaperonine for β in mitochondria [602] and in chloroplasts [603]. These suggestions are consistent with the observation that discrete changes in its amino acid sequence have led to intrachloroplastic inclusion bodies comprising both the α- and β-subunits [590]. This situation resembles the one occurring in *E. coli* when overexpression of a protein exceeds the capability of a chaperonine-assisted folding pathway and leads to inclusion body formation.

9.2.4. The assembly pathway

The resulting pathway for protein assembly that emerges from the experiments described above can be presented as follows: αβ-heterodimers formed at an early stage of synthesis undergo trimerization upon interacting with the γ-subunit, which results in αβ-hetero-hexamers. This core complex, α3β3γ, shows the basic catalytic properties of CF_1 – mainly the asymmetrical features between the three αβ-heterodimers brought about by their differential interaction with the central γ-subunit [551]. As a next step, the core complex converts into CF_1 upon completion of its interactions with the regulatory subunits ε and δ. CF_1 is then fully competent for assembly with CF_0 subunits I and II, the formation of a stalk which is required for the functional interaction with the proton channel, mainly formed of oligomers of subunit III and of subunit IV. The latter has the more peripheral location in the assembled protein, consistent with its lower contribution to the in vivo concerted stabilization process of ATP synthase subunits in *C. reinhardtii* [585].

10. Concluding remarks

10.1. Supercomplex formation

We have described separately the five major oligomeric PPs that contribute to the membrane-embedded photosynthetic apparatus. However, these proteins do not necessarily act as individual entities in the thylakoid membranes. There is strong evidence, from early biochemical and ultrastructural work (see [604–606]) that the antenna complexes are tightly associated with the cores of each photosystem. Thus supercomplex formation is critical for the light-harvesting function of photosynthesis. The

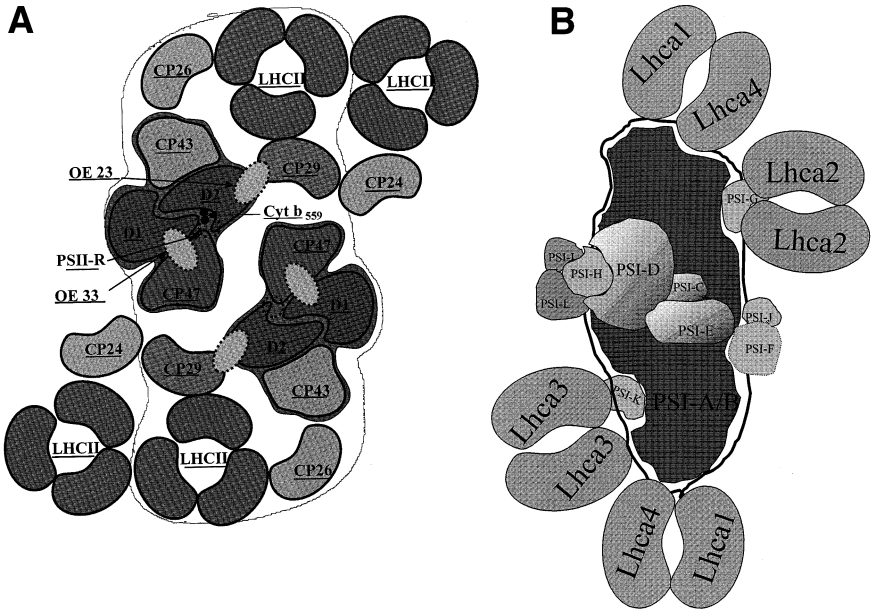


Fig. 4. Photosystems as antenna-core supercomplexes. (A) A model for the organization of Photosystem II: a CCII/CP24/CP26/CP29/LHCII supercomplex. The model is based on electron microscopy [214,217] and nearest-neighbor analysis [412] of supramolecular complexes isolated from grana membranes. The protein complex is shown on the membrane plane from the lumenal side. Subunits embedded in the lipid membrane are depicted with solid contours while water soluble subunits protruding into the lumen are depicted with broken lines. The black line in the background defines the contour of the most stable PSII complex that can be extracted from membranes [217,622] (reproduced by courtesy of R. Bassi, University of Verona). (B) A model for the organization of Photosystem I: a CCI/LHCI supercomplex. The model is based on electron microscopy of cyanobacterial PSI [322,331] and nearest-neighbor analysis using a higher plant PSI preparation [414]. The protein supercomplex is viewed from the stromal face of the thylakoid membranes. Subunits embedded in the lipid membrane are depicted with solid contours while water soluble subunits protruding into the lumen are depicted with broken lines. Stroma-facing subunits are space filled. The positions of LHCI heterodimers are tentative only. Reproduced from Jansson et al. [414]. See [414] for a detailed discussion.

investigation of the actual surpramolecular organization of these supercomplexes is a very difficult task. Several working models have been presented in the literature which are based primarily on the co-purification of the purported protein constituents after mild detergent solubilization of the thylakoid membranes. The polypeptide stoichiometry was assessed in the supercomplexes thus identified, and a nearest-neighbor analysis was conducted using cross-linking experiments. Fig. 4A,B illustrates two such models for PSI [414] and PSII [400]. They should be considered as a framework for a future reassessment of the supramolecular organization of the photosystems upon further biochemical and spectral characterization of the light-harvesting process.

Whether the electron transfer process is also, in part, sustained by supercomplex formation remains an open question (for a review see [607]). There is strong functional and structural evidence that some photosynthetic bacteria display supercomplex formation between reaction centers and cytochrome bc_1 proteins [608,609]. In the case of oxygenic photosynthesis, ultrastructural studies of mutants of *C. reinhardtii* lacking the cytochrome b_6f complex, which were based on the analysis of the particle content of freeze-fractured thylakoid membranes, provided support to the view that cytochrome b_6f complexes could associate with both CCII and CCI [610]. Although some biochemical data also support the existence of supercomplex formation between cytochrome b_6f and the photosystem cores [611], the present body of evidence remains too circumstantial to allow one to draw a definite conclusion as to their existence in the thylakoid membranes.

10.2. Future issues

The comparative examination of studies on the

biogenesis of the various photosynthetic proteins that we have presented in this review article delineates several emerging areas of research that should rapidly contribute to the resolution of issues which are currently poorly understood.

The major features of the translational step in the expression of the chloroplast-encoded PP subunits will have to be revisited. The extent to which an autoregulation of translation contributes to the stoichioimetric production of these subunits, as is the case of cytochrome *f* [79], will deserve a more systematic study. On the other hand, the simple picture that stromal membranes are the site of co-translational insertion of chloroplast-encoded membrane-bound PP subunits, may have to be revisited in light of the finding that inner envelope-like membranes contain several of the regulatory proteins that bind to the 5′-untranslated regions of chloroplast transcripts [95]. This discovery results from the finding that thylakoid membrane fractions prepared from *C. reinhardtii* can be further separated in genuine thylakoid membranes and chlorophyll-less membranes very similar to the inner envelope membranes. The site of translation of cyanobacterial PPs may have to be revisited as well since recent biochemical characterization of cyanobacterial membranes by the two-phase partition system set up by Albertsson and his coworkers [612] has disclosed the presence of a significant proportion of plasma membranes among what has been considered for long as a pure thylakoid membrane fraction [613].

A number of house-keeping chloroplast enzymes (chaperonines of the HSP60 and HSP70 families, proteases analogous to their bacterial counterparts, docking proteins for translating ribosomes) have been already identified, but their participation in the biogenesis and recycling of the PP subunits remains to be characterized. Then come the 'pioneer proteins', most of which are nuclear-encoded and act specifically at the post-transcriptional step in the expression of various chloroplast genes. Their physiological role in protein assembly may have been overlooked since they have been primarily studied for their role in gene expression.

Another aspect, is a growing number of auxiliary enzymes in photosynthesis that are identified as partners in the biogenesis of PPs (see Table 7). They are involved in post-translational modifications of well-

Table 7
Proteins involved in the assembly of the photosynthetic complexes

Photosynthetic complex/subunits	Assembly-assisting protein	Approximate molecular mass (kDa)	Gene (C/N)	Species of identification
PSII	HCF136	37	*Hcf136* (N)	*Arabidopsis*
PSI	Ycf3	19[b]	*ycf3* (C)	*Cyanobacteria*; *Chlamydomonas*; vascular plants
	Ycf4	22[b]	*ycf4* (C)	*Chlamydomonas*
	BtpA	30	*btpA* (C)	*Cyanobacteria*
Cyt b_6	CCB1[a]	unknown	*Ccb1* (N)	*Chlamydomonas*
	CCB2[a]		*Ccb2* (N)	
	CCB3[a]		*Ccb3* (N)	
	CCB4[a]		*Ccb4* (N)	
Cyt c_6/Cyt *f*	CCSA[a]	35	*ccsA* (C)	*Chlamydomonas*; *Synechocystis*
	CCS1[a]	64.9	*Ccs1* (N)	*Chlamydomonas*; *Synechocystis*; *Porphyra purpurea*
	CCS2[a]	unknown	*Ccs2* (N)	*Chlamydomonas*
	CCS3[a]		*Ccs3* (N)	
	CCS4[a]		*Ccs4* (N)	

C, Chloroplast-encoded gene (lower case); N, nuclear encoded genes (upper case).
[a]Cofactor attachment proteins.
[b]Detected from gel migration of the protein [161].

characterized PP subunits, and they act as processing enzymes, kinases and phosphatases or as catalysts for heme-attachment and iron–sulfur cluster formation. The molecular knowledge that would help understanding both their structure and their enzymatic properties remains scarce, but one can expect rapid progress in this field as illustrated by the molecular identification of membrane-bound subunits of the protein complex involved in *c*-heme attachment [614], or of a lumen-located immunophilin-like protein, which probably plays a role in the dephosphorylation of thylakoid membrane proteins [615]. Another issue that lacks characterization is the thylakoid translocon(s) which are experimentally identified through the distinct requirements for protein translocation across, or integration into, the thylakoid membranes. Thus, the field of thylakoid protein biogenesis and assembly remains largely unexplored to date, which is in sharp contrast with our growing knowledge of the structural organization of the assembled proteins.

Acknowledgements

We gratefully acknowledge R. Bassi (University of Verona) and the members of the Paris laboratory, UPR1261-CNRS, for stimulating discussions and critical reading of various sections of the manuscript. Thanks to Deena Oren for her help in looking at 3D structure and to Mezi Berman for her advice on English syntax. We also wish to thank the many colleagues and friends in the field of thylakoid membrane protein biogenesis who communicated some data, before publication. This collaborative work was supported by a French–Israeli Scientific and Technological Cooperation Joint Project 'Arc en Ciel' No. 46.

References

[1] T. Hirose, M. Sugiura, EMBO J. 15 (1996) 1687–1695.
[2] W.F. Thompson, M.J. White, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42 (1991) 423–466.
[3] J.E. Mullet, Plant Physiol. 103 (1993) 309–313.
[4] C. Bowler, N.H. Chua, Plant Cell 6 (1994) 1529–1541.
[5] N. Keren, I. Ohad, in: J.D. Rochaix, M. Goldschmidt-Clermont, S. Merchant (Eds.), The molecular biology of chloroplasts and mitochondria in chlamydomonas, Kluwer, Dordrecht, 1998, 569–596.
[6] A.N. Glazer, Annu. Rev. Biophys. Chem. 14 (1985) 47–77.
[7] A.R. Grossman, D. Bhaya, K.E. Apt, D.M. Kehoe, Annu. Rev. Genet. 29 (1995) 231–288.
[8] J.E. Mullet, Annu. Rev. Plant Physiol. 39 (1988) 475–502.
[9] W. Gruissem, J.C. Tonkyn, Crit. Rev. Plant 12 (1993) 19–55.
[10] A.M. Schwartz, M.O. Dayhoff, Science 199 (1978) 395–403.
[11] M.W. Gray, Trends Genet. 5 (1989) 294–299.
[12] F.C. Neidhardt, in: F.C. Neidhardt, R. Curtiss, J.L. Ingraham, E.C.C. Lin, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, H.E. Umbarger, Escherichia coli and Salmonella Cellular and Molecular Biology, Vol. 1, ASM press, Washington, DC, 1996, pp. 1225–1550.
[13] D.C. Youvan, E.J. Bylina, M. Alberti, H. Begusch, J.E. Hearst, Cell 37 (1984) 949–957.
[14] D.A. Young, C.E. Bauer, C.E. Williams, B.L. Marrs, Mol. Gen. Genet. 218 (1989) 1–12.
[15] C.L. Wellington, T. Beatty, J. Bacteriol. 173 (1991) 1432–1443.
[16] C.E. Bauer, J.J. Buggy, Z.M. Yang, B.L. Marrs, Mol. Gen. Genet. 228 (1991) 433–444.
[17] K. Naterstad, A.B. Kolsto, R. Sirevag, J. Bacteriol. 177 (1995) 5480–5484.
[18] D.L. Xie, H. Hill, G. Hauska, M. Maeda, M. Futai, N. Nelson, Biochim. Biophys. Acta 1172 (1993) 267–273.
[19] W.A. Sidler, in: D.A. Bryant (Ed.), Phycobilisome and Phycobiliprotein Structures, Kluwer, Dordrecht, 1994, pp. 139–216.
[20] K. Valentin, R.A. Cattolico, K. Zetsche, in: R. Lewin, Phylogenetic Origin of Plastids, Chapman and Hall, New York, 1992, pp. 195–222.
[21] B.Y. Meng, M. Tanaka, T. Wakasugi, M. Ohme, K. Shinozaki, M. Sugiura, Curr. Genet. 14 (1988) 395.
[22] C.D. Rock, A. Barkan, W.C. Taylor, Curr. Genet. 12 (1987) 69.
[23] D.B. Stern, R.G. Drager, in: J.D. Rochaix, M. Goldschmidt-Clermont, S. Merchant (Ed.), Kluwer, Dordrecht, 1998, 165–183.
[24] N.R. Sturm, R. Kuras, S. Buschlen, W. Sakamoto, K.L. Kindle, D.B. Stern, F.-A. Wollman, Mol. Cell Biol. 14 (1994) 6171–6179.
[25] D. Drapier, H. Suzuki, H. Levy, B. Rimbault, K.L. Kindle, D.B. Stern, F.-A. Wollman, Plant Physiol. 117 (1998) 629–641.
[26] M. Kim, D.A. Christopher, J.E. Mullet, Plant Mol. Biol. 22 (1993) 447–463.
[27] R.D. Kulkarni, S. Golden, Mol. Microbiol. 24 (1997) 1131–1142.
[28] D.B. Stern, E.R. Radwanski, K.L. Kindle, Plant Cell 3 (1991) 285–297.
[29] R.D. Kulkarni, S.S. Golden, J. Bacteriol. 176 (1994) 959–965.
[30] R. Li, N.S. Dickerson, U.W. Mueller, S.S. Golden, J. Bacteriol. 177 (1995) 508–516.

[31] B.J. Baumgartner, J.C. Rapp, J.E. Mullet, Plant Physiol. 101 (1993) 781–791.
[32] Z. Adam, Plant Mol. Biol. 32 (1996) 773–783.
[33] J.E. Mullet, P.G. Klein, R.R. Klein, Proc. Natl. Acad. Sci. USA 87 (1990) 4038–4042.
[34] D.L. Herrin, J.F. Battey, K. Greer, G.W. Schmidt, J. Biol. Chem. 267 (1992) 8260–8269.
[35] S. Merchant, L. Bogorad, J. Biol. Chem. 261 (1986) 15850–15853.
[36] R. Kuras, S. Buschlen, F.-A. Wollman, J. Biol. Chem. 270 (1995) 27797–27803.
[37] R. Kuras, C. de Vitry, Y. Choquet, J. Girard-Bascou, D. Culler, S. Buschlen, S. Merchant, F.-A. Wollman, J. Biol. Chem. 272 (1997) 32427–32435.
[38] H.B. Pakrasi, P. De-Ciechi, J. Whitmarsh, EMBO J. 10 (1991) 1619–1627.
[39] G. Shen, J.J. Eaton-Rye, W.F. Vermaas, Biochemistry 32 (1993) 5109–5115.
[40] M. Ikeuchi, B. Eggers, G.Z. Shen, A. Webber, J.J. Yu, A. Hirano, Y. Inoue, W. Vermaas, J. Biol. Chem. 266 (1991) 11111–11115.
[41] Y. Takahashi, H. Matsumoto, M. Goldschmidt-Clermont, J.D. Rochaix, Plant Mol. Biol. 24 (1994) 779–788.
[42] M. Ikeuchi, V.K. Shukla, H.B. Parkasi, Y. Inoue, Mol. Gen. Genet. 249 (1995) 622–628.
[43] P. Kunstner, A. Guardiola, Y. Takahashi, J.D. Rochaix, J. Biol. Chem. 270 (1995) 9651–9654.
[44] S.P. Mayfield, P. Bennoun, J.D. Rochaix, EMBO J. 6 (1987) 313–318.
[45] J.B. Philbrick, B.A. Diner, B.A. Zilinskas, J. Biol. Chem. 266 (1991) 13370–13376.
[46] Y. Takahashi, M. Goldshmidt-Clermont, S.Y. Soen, L.G. Franzen, J.D. Rochaix, EMBO J. 10 (1991) 2033–2040.
[47] R.M. Mannan, J. Whitmarsh, P. Nyman, H.B. Pakrasi, Proc. Natl. Acad. Sci. USA 88 (1991) 10168–10172.
[48] I. Adamska, M. Lindahl, M. Roobol-Boza, B. Andersson, Eur. J. Biochem. 236 (1996) 591–599.
[49] A. Sokolenko, L. Altschmied, R.G. Herrmann, Plant Physiol. 115 (1997) 827–832.
[50] J. Shanklin, N.D. DeWitt, J.M. Flanagan, Plant Cell 7 (1995) 1713–1722.
[51] M. Lindhal, S. Tabak, L. Cseke, E. Pichersky, B. Andersson, Z. Adam, J. Biol. Chem. 271 (1996) 29329–29334.
[52] H. Itzhaki, L. Naveh, M. Lindahl, M. Cook, Z. Adam, J. Biol. Chem. 273 (1998) 7094–7098.
[53] A.J. Bendich, Bio-essays 6 (1987) 279–282.
[54] J.P. Hosler, E.A. Wurtz, E.H. Harris, N.W. Gillham, J.E. Boyton, Plant Physiol. 91 (1989) 648–655.
[55] E.A. Wurtz, J.E. Boyntonand, N.W. Gillham, Proc. Natl. Acad. Sci. USA 74 (1977) 4552–4556.
[56] D. Drapier, J. Girard-Bascou, F.-A. Wollman, Plant Cell 4 (1992) 283–295.
[57] M. Goldschmidt-Clermont, Int. Rev. Cyt. 177 (1998) 115–180.
[58] J.D. Rochaix, Plant Mol. Biol. 32 (1996) 327–341.
[59] T.D. Fox, Experientia 52 (1996) 1130–1135.
[60] D.B. Stern, D.C. Higgs, J. Yang, Trends Plant Sci. 2 (1997) 308–315.
[61] N.W. Gillham, J.E. Boynton, C.R. Hauser, Annu. Rev. Genet. 28 (1994) 71–93.
[62] C.R. Hauser, N.W. Gillham, J.E. Boynton, J. Biol. Chem. 271 (1996) 1486–1497.
[63] R.D. Dinkins, H. Bandaranayake, B.R. Green, A.J. Griffiths, Curr. Genet. 25 (1994) 282–288.
[64] J. Meurer, A. Berger, P. Westhoff, Plant Cell 8 (1996) 1193–1207.
[65] J. Meurer, C. Grevelding, P. Westhoff, B. Reiss, Mol. Gen. Genet. 258 (1998) 342–351.
[66] A. Barkan, M. Walker, M. Nolasco, D. Johnson, EMBO J. 13 (1994) 3170–3181.
[67] J. Meurer, K. Meierhoff, T. Westhoff, Planta 198 (1996) 385–396.
[68] A. Batschauer, E. Mosinger, K. Kreuz, I. Dorr, K. Apel, Eur. J. Biochem. 154 (1986) 625–634.
[69] R.E. Susez, F. Ausubel, J. Chory, Cell 74 (1993) 787–799.
[70] J.M. Escoubas, M. Lomas, J. LaRoche, P.G. Falkowski, Proc. Natl. Acd. Sci. USA 92 (1995) 10237–10241.
[71] M.E. Petracek, L.F. Dickey, S.C. Huber, W.F. Thompson, Plant Cell 9 (1997) 2291–2300.
[72] J. Kropat, U. Oster, W. Rudiger, C.F. Beck, Proc. Natl. Acad. Sci. USA 94 (1997) 14168–14172.
[73] D. Hahn, P. Bennoun, U. Kueck, Mol. Gen. Genet. 252 (1996) 362–370.
[74] J.L. Marrison, P.H. Schünmann, H.J. Ougham, R.M. Leech, Plant Physiol. 110 (1996) 1089–1096.
[75] J.F. Allen, K. Alexciev, G. Hakansson, Curr. Biol. 5 (1995) 869–872.
[76] J.D. Rochaix, Annu. Rev. Cell Biol. 8 (1992) 1–28.
[77] C. de Vitry, J. Olive, D. Drapier, M. Recouvreur, F.-A. Wollman, J. Cell Biol. 109 (1989) 991–1006.
[78] R. Kuras, F.-A. Wollman, EMBO J. 13 (1994) 1019–1027.
[79] Y. Choquet, D.B. Stern, K. Wostrikoff, R. Kuras, J. Girard-Bascou, F.-A. Wollman, Proc. Natl. Acad. Sci. USA 95 (1998) 4380–4385.
[80] J. Girard-Bascou, Y. Choquet, N. Gumple, D. Culler, S. Purton, S. Merchant, F. Laquerrière, F.-A. Wollman, in: P. Mathis (Ed.), Vol. III, Kluwer, Dordrecht, 1995, pp. 683–686.
[81] S. Rodermel, J. Haley, C.Z. Jiang, C.H. Tsai, L. Bogorad, Proc. Natl. Acad. Sci. USA 93 (1996) 3881–3885.
[82] J. Kim, P. Gamble-Klein, J.E. Mullet, Plant Mol. Biol. 25 (1994) 459–467.
[83] C.G. Poutre, T.D. Fox, Genetics 115 (1987) 637–647.
[84] K.M. Calder, J.E. McEwen, Mol. Microbiol. 5 (1991) 1769–1777.
[85] T. Nakai, T. Yasuhara, Y. Fujiki, A. Ohashi, Mol. Cell. Biol. 15 (1995) 4441–4452.
[86] A.T. Jagendorf, A. Michaels, Plant Sci. 71 (1990) 137–145.
[87] E.H. Harris, J.E. Boynton, N.W. Gillham, Microbiol. Rev. 58 (1994) 700–754.
[88] N.H. Chua, G. Blobel, P. Siekevitz, G.E. Palade, Proc. Natl. Acad. Sci. USA 70 (1973) 1554–1558.

[89] T. Yamamoto, G.A. Burke, A.T. Jagendorf, Plant Physiol. 67 (1981) 940–949.
[90] A. Friemann, W. Hachtel, Planta 175 (1988) 50–59.
[91] D. Herrin, A. Michaels, FEBS Lett. 184 (1985) 90–95.
[92] D. Herrin, A. Michaels, Arch. Biophys. Biochem. 237 (1985) 224–236.
[93] J. Nickelsen, M.R. van Dillewijn, J.D. Rochaix, EMBO J. 13 (1994) 3182–3191.
[94] T.D. Fox, in: J.W.B. Hershey, M.B. Matthews, N. Sonenberg (Ed.), Genetics of Mitochondrial Translation, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1996, pp. 733–758.
[95] W. Zerges, J.D. Rochaix, J. Biol. Chem. 140 (1998) 101–110.
[96] O. Vallon, F.-A. Wollman, J. Olive, Photobiochem. Photobiophys. 12 (1986) 203–220.
[97] G. Van den Broeck, M.P. Timko, A.P. Kausch, A.R. Cashmore, M. Van Montagu, L. Herrera Estrella, Nature 313 (1985) 358–363.
[98] K. Keegstra, L.J. Olsen, S.M. Theg, Annu. Rev. Plant Physiol. Plant Mol. Biol. 40 (1989) 471–501.
[99] P.E. Highfield, R.J. Ellis, Biochim. Biophys. Acta 447 (1976) 20–27.
[100] N.H. Chua, G.W. Schmidt, Basic Life Sci. 11 (1978) 325–347.
[101] R. van't Hof, R. Demel, K. Keegstra, B. Kruijff, FEBS Lett. 291 (1991) 350–354.
[102] R. van't Hof, W. van Klompenburg, M. Pilon, A. Kozubek, G. de Korte-Kool, R. Demel, P.J. Weisbeek, B. de Kruijff, J. Biol. Chem. 268 (1993) 4037–4042.
[103] B. de Kruijff, FEBS Lett. 346 (1994) 78–87.
[104] K. Cline, M. Werner-Washburne, T.H. Lubben, K. Keegstra, J. Biol. Chem. 260 (1985) 3691–3693.
[105] A.L. Friedman, K. Keegstra, Plant Physiol. 89 (1989) 993–999.
[106] S.M. Theg, S.V. Scott, Trends Cell Biol. 3 (1993) 186–190.
[107] D.J. Schnell, G. Blobel, J. Biol. Chem. 120 (1993) 103–115.
[108] L.J. Olsen, S.M. Theg, B.R. Selman, K. Keegstra, J. Biol. Chem. 264 (1989) 6724–6729.
[109] L.J. Olsen, K. Keegstra, J. Biol. Chem. 267 (1992) 433–439.
[110] K. Waegemann, J. Soll, Plant J. 1 (1991) 149–158.
[111] K. Waegemann, J. Soll, in: D.J. Morre, K.E. Howell, J.J.M. Gergerson (Ed.), Isolation and Characterization of a Functionally Active Protein Translocation Apparatus from Chloroplast Envelopes, Spring-Verlag, Berlin, 1993, pp. 101–104.
[112] H. Alefsen, K. Waegemann, J. Soll, J. Plant Physiol. 144 (1994) 339–345.
[113] U.I. Flugge, G. Hinz, Eur. J. Biochem. 160 (1986) 563–570.
[114] C. Schindler, R. Hracky, J. Soll, Z. Naturforsch. 42c (1987) 103–108.
[115] F.U. Hartl, N. Pfanner, D.W. Nicholson, W. Neupert, Biochim. Biophys. Acta 988 (1989) 1–45.
[116] M. Pilon, A.D. de Boer, S.L. Knols, M.H. Koppelman, R.M. van der Graaf, B. de Kruijff, P.J. Weisbeek, J. Biol. Chem. 265 (1990) 3358–3361.
[117] M. Pilon, B. de Kruijff, P.J. Weisbeek, J. Biol. Chem. 267 (1992) 2548–2556.
[118] A. Guera, T. America, M. van Waas, P. Weisbeek, Plant Mol. Biol. 23 (1993) 309–324.
[119] T. Endo, M. Kawakami, A. Goto, T. America, P. Weisbeek, M. Nakai, Eur. J. Biochem. 225 (1994) 403–409.
[120] D. Walker, A.M. Chaddock, J.A. Chaddock, L.M. Roberts, J.M. Lord, C. Robinson, J. Biol. Chem. 271 (1996) 4082–4085.
[121] K. Waegemann, H. Paulsen, J. Soll, FEBS Lett. 261 (1990) 89–92.
[122] J. Lübeck, L. Heins, J. Soll, Physiol. Plant. 100 (1997) 53–64.
[123] K. Pohlmeyer, J. Soll, T. Steinkamp, S. Hinnah, R. Wagner, Proc. Natl. Acad. Sci. USA 94 (1997) 9504–9509.
[124] Y. Ma, A. Kouranov, S.E. LaSala, D.J. Schnell, J. Cell Biol. 134 (1996) 315–327.
[125] J. Lübeck, J. Soll, M. Akita, E. Nielsen, K. Keegstra, EMBO J. 15 (1996) 4230–4238.
[126] J. Soll, Bot. Acta 108 (1995) 277–282.
[127] C. Robinson, R.J. Ellis, Eur. J. Biochem. 142 (1984) 337–342.
[128] P.S. VanderVere, T.M. Bennett, J.E. Oblong, G.K. Lamppa, Proc. Natl. Acad. Sci. USA 92 (1995) 7177–7181.
[129] G. von Heijne, K. Nishikawa, FEBS Lett. 278 (1991) 1–3.
[130] G. von Heijne, J. Steppuhn, R.G. Herrmann, Eur. J. Biochem. 180 (1989) 535–545.
[131] C. Halpin, P.D. Elderfield, H.E. James, R. Zimmermann, B. Dunbar, C. Robinson, EMBO J. 8 (1989) 3917–3921.
[132] Y. Cohen, J. Steppuhn, R.G. Herrmann, S. Yalovsky, R. Nechushtai, EMBO J. 11 (1992) 79–86.
[133] P.R. Chitnis, N. Nelson, Plant Physiol. 99 (1992) 239–246.
[134] P. Walter, A.E. Johnson, Annu. Rev. Cell Biol. 10 (1994) 87–119.
[135] R.G. Herrmann, J. Steppuhn, G.S. Herrmann, N. Nelson, FEBS Lett. 326 (1993) 192–198.
[136] D.M. Engelman, Science 274 (1996) 1850–1851.
[137] K. Cline, R. Henry, Annu. Rev. Cell Dev. Biol. 12 (1996) 1–26.
[138] G. Schatz, B. Dobberstein, Science 271 (1996) 1519–1526.
[139] A.P. Pugsley, Microbiol. Rev. 57 (1993) 50–108.
[140] W.T. Wickner, Science 90 (1994) 619–629.
[141] C. Robinson, R.B. Klosgen, Plant Mol. Biol. 26 (1994) 15–24.
[142] J. Berghofer, I. Karnauchov, R.G. Herrmann, R.B. Klosgen, J. Biol. Chem. 270 (1995) 18341–18346.
[143] T. Nohara, M. Nakai, A. Goto, T. Endo, FEBS Lett. 364 (1995) 305–308.
[144] R. Voelker, J. Mendel-Hartvig, A. Barkan, Genetics 145 (1997) 467–478.
[145] J. Yuan, R. Henry, M. McCaffery, K. Cline, Science 266 (1994) 796–798.
[146] R. Henry, A. Kapazoglou, M. McCaffery, K. Line, J. Biol. Chem. 269 (1994) 10189–10192.
[147] T.G. Knott, C. Robinson, J. Biol. Chem. 269 (1994) 7843–7846.

[148] V. Laidler, A.M. Chaddock, T.G. Knott, D. Walker, C. Robinson, J. Biol. Chem. 270 (1995) 17664–17667.
[149] A. Hulford, L. Hazell, R.M. Mould, C. Robinson, J. Biol. Chem. 269 (1994) 3251–3256.
[150] J. Yuan, K. Cline, J. Biol. Chem. 269 (1994) 18463–18467.
[151] R.M. Mould, J.B. Shackleton, C. Robinson, J. Biol. Chem. 266 (1991) 17286–17289.
[152] R.B. Klösgen, I.W. Brock, R.G. Herrmann, C. Robinson, Plant Mol. Biol. 18 (1992) 1031–1034.
[153] K. Cline, W.F. Ettinger, S.M. Theg, J. Biol. Chem. 267 (1992) 2688–2696.
[154] V.S. Neilsen, A. Mant, J. Knoetzel, B.L. Moller, C. Robinson, J. Biol. Chem. 269 (1994) 3762–3766.
[155] I.W. Brock, J.D. Mills, D. Robinson, C. Robinson, J. Biol. Chem. 270 (1995) 1657–1662.
[156] C. Robinson, in: D.R. Ort, C.F. Yocum (Ed.), Targeting of Proteins Into and Across the Thylakoid Membrane, Vol. 4, Kluwer, Dordrecht, 1996, pp. 103–112.
[157] C.L. Santini, B. Ize, A. Chanal, M. Muller, G. Giordano, L.F. Wu, EMBO J. 17 (1998) 101–102.
[158] A.M. Settles, A. Yonetani, A. Baron, D.R. Bush, K. Cline, R. Martienssen, Science 278 (1997) 1467–1470.
[159] J.P. Hendrick, F.U. Hartl, Annu. Rev. Biochem. 62 (1993) 349–384.
[160] C. Monod, Y. Takahashi, M. Goldschmidt-Clermont, J.D. Rochaix, EMBO J. 13 (1994) 2747–2754.
[161] E. Boudreau, Y. Takahashi, C. Lemieux, M. Turmel, J.D. Rochaix, EMBO J. 16 (1997) 6095–6104.
[162] V.V. Bartsevich, H.B. Pakrasi, J. Biol. Chem. 272 (1997) 6382–6387.
[163] J. Meurer, H. Plücken, K.V. Kowallik, P. Westhoff, EMBO J. 17 (1998) 5286–5297.
[164] Z. Xie, S. Merchant, J. Biol. Chem. 271 (1996) 4632–4639.
[165] J.L. Popot, D.M. Engelman, Biochemistry 29 (1990) 4031–4037.
[166] W.R. Widger, W.A. Cramer, R.G. Herrmann, A. Trebst, Proc. Natl. Acad. Sci. USA 81 (1984) 674–678.
[167] B.R. Green, D.G. Durnford, Annu. Rev. Plant Physiol. Mol. Biol. 47 (1996) 685–714.
[168] R.J. Debus, Biochim. Biophys. Acta 1102 (1992) 269–352.
[169] B. Andersson, S. Styring, in: C.P. Lee (Ed.), Photosystem II – Molecular Organization, Function and Acclimation, Vol. 16, Academic Press, San Diego, 1992, pp. 1–81.
[170] J.M. Ariksen, J.D. Rochaix, in: J. Barber (Ed.), The Photosystems: Structure, Function and Molecular Biology, Elsevier Science, Amsterdam, pp. 101–177.
[171] A. Siedler, Biochim. Biophys. Acta 1187 (1994) 73–79.
[172] J. Barber, J. Nield, E.P. Morris, D. Zheleva, B. Hankamer, Physiol. Plant. 100 (1997) 817–827.
[173] O. Nanba, K. Satoh, Proc. Natl. Acad. Sci. USA 84 (1987) 109–112.
[174] J. Deisenhofer, O. Epp, K. Miki, R. Huber, I.I. Michel, Nature 318 (1985) 618–623.
[175] J.P. Allen, G. Feher, T.O. Yeates, H. Komiya, D.C. Rees, Proc. Natl. Acad. Sci. USA 84 (1987) 6162–6166.
[176] H. Michel, J. Deisenhofer, Biochemistry 27 (1988) 1–7.
[177] K.D. Irrgang, L.X. Shi, C. Funk, W.P. Schroder, J. Biol. Chem. 270 (1995) 17588–17593.
[178] Z.J. Lorkovic, W.P. Schroder, H.B. Pakrasi, K.D. Irrgang, R.G. Herrmenn, R. Oelmuller, Proc. Natl. Acad. Sci. USA 92 (1995) 8930–8934.
[179] K. Satoh, in: D.R. Ort, C.F. Yocum (Ed.), Introduction to the Photosystem II Reaction Center-Isolation and Biochemical and Biophysical Characterization, Vol. 4, Kluwer, Dordrecht, 1996, pp. 193–211.
[180] A.N. Webber, L.C. Packman, D.J. Chapman, J. Barber, J.C. Gray, FEBS Lett. 242 (1989) 259–262.
[181] M. Ikeuchi, Y. Inoue, FEBS Lett. 241 (1988) 99–104.
[182] W.R. Widger, W.A. Cramer, M. Hermodson, R.G. Herrmann, FEBS Lett. 191 (1985) 186–190.
[183] G.T. Babcock, W.R. Widger, W.A. Cramer, W.A. Oertling, J.G. Metz, Biochemistry 24 (1985) 3638–3645.
[184] O. Vallon, G.S. Tae, W.A. Cramer, D. Simpson, G. Hoyer-Hansen, L. Bogorad, Biochim. Biophys. Acta 975 (1989) 132–141.
[185] G.S. Tae, W.A. Cramer, Biochemistry 33 (1994) 10060–10068.
[186] V.P. McNamara, F.S. Sutterwala, H.B. Pakrasi, J. Whitmarsh, Proc. Natl. Acad. Sci. USA 94 (1997) 14173–14178.
[187] J. Whitmarsh, H.B Pakrasi, in: D.R. Ort, C.F. Yocum (Ed.), Oxygenic Photosynthesis: The Light Reactions, Vol. 4, Kluwer, Dordrecht, 1996, pp. 249–264.
[188] J. Barber, J. Plant Physiol. 22 (1995) 201–208.
[189] J.R. Shen, R.L. Burnap, Y. Inoue, Biochemistry 34 (1995) 12661–12668.
[190] A. Siedler, Biochim. Biophys. Acta 1277 (1996) 35–60.
[191] G.S. Tae, W.A. Cramer, Biochemistry 33 (1994) 10060–10068.
[192] D.F. Ghanotakis, J.N. Topper, G.T. Babcock, C.F. Yocm, FEBS Lett. 170 (1984) 169–173.
[193] M. Miyao, N. Murata, FEBS Lett. 180 (1985) 303–308.
[194] E.J. Boekema, J. Nield, B. Hankamer, J. Bareber, Eur. J. Biochem. 252 (1998) 268–276.
[195] J.J. Eaton-Rye, N. Murata, Biochim. Biophys. Acta 977 (1989) 219–226.
[196] M. Volker, T.A. Ono, Y. Inoue, G. Renger, Biochim. Biophys. Acta 806 (1985) 25–34.
[197] L.K. Frankel, T.M. Bricker, Biochemistry 34 (1995) 7492–7497.
[198] T. Kuwabara, K. Suzuki, Plant Cell Physiol. 3665 (1995) 495–504.
[199] N. Adir, M.Y. Okamura, G. Feher, in: M. Murata (Ed.), Crystallization of the PSII-reaction Centre, Vol. II, Kluwer, Dordrecht, 1992, pp. 195–198.
[200] C. Fotinou, M. Kokkinidis, G. Fritzsch, W. Haase, H. Michel, D.F. Ghanotakis, Photosynth. Res. 37 (1993) 41–48.
[201] B. Hankamer, J. Barber, E.J. Boekema, Annu. Rev. Plant Physiol. Plant Mol. Biol. 48 (1997) 641–671.
[202] H. Michel, K.A. Weyer, H. Gruenberg, I. Dunger, D. Oesterhelt, F. Lottspeich, EMBO J. 5 (1986) 1149–1158.
[203] A. Trebst, Z. Naturforsch. 41C (1986) 240–245.

[204] B. Svensson, I. Vass, E. Cedergren, S. Styring, EMBO J. 9 (1990) 2051–2059.
[205] S.V. Ruffle, D. Donelly, T.L. Blundell, J.H.A. Nugent, Photosynth. Res. 34 (1992) 287–300.
[206] V. Sobolev, M. Edelman, Proteins 21 (1995) 214–225.
[207] A. Holzenburg, M.C. Bewly, F.H. Wilson, W.V. Nicholson, W.V. Ford, Nature 363 (1993) 470–472.
[208] A. Holzenburg, F.H. Shephered, R.C. Ford, Micron 25 (1994) 447–451.
[209] R.C. Ford, M.F. Rosenberg, F.H. Shephered, P. McPhie, A. Holzenburg, Micron 26 (1995) 133–140.
[210] G. Tsiotis, T. Walz, A. Spyridaky, A. Lustig, E. Engel, D. Ghanotakis, J. Mol. Biol. 259 (1996) 241–248.
[211] R. Bassi, A. Ghiretti-Magaldi, G. Tognon, G.M. Giacometti, K.R. Miller, Eur. J. Cell Biol. 50 (1989) 84–93.
[212] K.R. Miller, J.S. Jacob, in: G.W. Bailey (Ed.), Surface Structure of the Photosystem II Complex, San Francisco Press, San Francisco, 1991, p. 197.
[213] M.K. Lyon, K.M. Marr, P.S. Furcinitti, J. Struct. Biol. 110 (1993) 133–140.
[214] C. Santini, V. Tidu, G. Tognon, A. Ghiretti-Magaldi, R. Bassi, Eur. J. Biochem. 221 (1994) 307–315.
[215] K.M. Marr, D.N. Mastronarde, M.K. Lyon, J. Cell Biol. 132 (1996) 823–833.
[216] K.M. Marr, R.L. McFeeters, M.K. Lyon, J. Struct. Biol. 117 (1996) 86–98.
[217] E.J. Boekema, B. Hankamer, D. Bald, J. Kruip, J. Nield, A.F. Boonstra, J. Barber, M. Rögner, Proc. Natl. Acad. Sci. USA 92 (1995) 175–179.
[218] B. Hankamer, J. Nield, D. Zheleva, E. Boekema, S. Jansson, J. Barber, Eur. J. Biochem. 243 (1998) 422–429.
[219] E.P. Morris, B. Hankamer, D. Zheleva, G. Friso, J. barber, Structure 5 (1997) 837–849.
[220] E.P. Morris, B. Hankamer, D. Zheleva, G. Friso, J. Barber, Structure 5 (1997) 837–849.
[221] S.A. Bustos, S.S. Golden, Mol. Gen. Genet. 232 (1992) 221–230.
[222] J.C. Tonkyn, W. Gruissem, Mol. Gen. Genet. 241 (1993) 141–152.
[223] E. Boudreau, C. Otis, M. Turmel, Plant Mol. Biol. 24 (1994) 585–602.
[224] L. Hong, J.K. Stevenson, W.B. Roth, R.B. Hallick, Mol. Gen. Genet. 247 (1995) 180–188.
[225] J. Girard-Bascou, Y. Pierre, D. Drapier, Curr. Genet. 22 (1992) 47–52.
[226] C.B. Yohn, A. Cohen, A. Danon, S.P. Mayfield, Mol. Cell. Biol. 16 (1996) 3560–3566.
[227] A. Danon, S.P. Mayfield, Science 266 (1994) 1717–1719.
[228] A. Danon, S.P. Mayfield, EMBO J. 13 (1994) 2227–2235.
[229] J. Kim, S.P. Mayfield, Science 278 (1997) 1954–1957.
[230] K.J. van Wijk, L. Eichacker, FEBS Lett. 388 (1996) 89–93.
[231] A.K. Mattoo, M. Edelman, Proc. Natl. Acad. Sci. USA 84 (1987) 1497–1501.
[232] A. Boschetti, E. Breidenbach, R. Blätter, Plant Sci. 68 (1990) 131–149.
[233] K.J. van Wijk, T.G. Knott, C. Robinson, FEBS Lett. 368 (1995) 263–266.
[234] J.G. Metz, H.B. Pakrasi, M. Seibert, C.J. Arntzen, FEBS Lett. 205 (1986) 269–274.
[235] J.B. Marder, P. Goloubinoff, M. Edelman, J. Biol. Chem. 259 (1984) 3900–3908.
[236] B.A. Diner, D.F. Ries, B.N. Cohen, J.G. Metz, J. Biol. Chem. 263 (1988) 8972–8980.
[237] J.R. Bowyer, J.C.L. Packer, B.A. McCormack, J.P. Whitelegge, C. Robinson, M.A. Taylor, J. Biol. Chem. 267 (1992) 5424–5433.
[238] S.V. Shestakov, P.R. Anbudurai, G.E. Stanbekova, A. Gadzhiev, L.K. Lind, H.B. Parkasi, J. Biol. Chem. 269 (1994) 19354–19359.
[239] P.R. Anbudurai, T.S. Mor, I. Ohad, S.V. Shestakov, H.B. Parkasi, Proc. Natl. Acad. Sci. USA 91 (1994) 8082–8086.
[240] N. Inagaki, Y. Yamamoto, H. Mori, H. Satoh, Plant Mol. Biol. 30 (1996) 39–50.
[241] R. Oelmuller, R.G. Herrmann, H.B. Pakrasi, J. Biol. Chem. 271 (1996) 21848–21852.
[242] J.T. Trost, D.A. Chisholm, D.B. Jordan, B.A. Diner, J. Biol. Chem. 272 (1997) 20348–20356.
[243] I. Karnauchov, R.G. Herrmann, H.B. Pakrasi, R.B. Klosgen, Eur. J. Biochem. 249 (1997) 497–504.
[244] P.J. Nixon, M. Rogner, B.A. Diner, Plant Cell 3 (1991) 383–396.
[245] F. Taguchi, Y. Yamamoto, K. Satoh, J. Biol. Chem. 270 (1995) 10711–10716.
[246] Y. Takahashi, K. Utsumi, Y. Yamamoto, A. Hatano, K. Satoh, Plant Cell Physiol. 37 (1996) 161–168.
[247] E. Kanervo, Y. Tasaka, N. Murata, N. Aro, Plant Physiol. 114 (1997) 841–849.
[248] A. Trebst, B. Depka, FEBS Lett. 400 (1997) 359–362.
[249] J. Kim, P.J. Klein, J.E. Mullet, J. Biol. Chem. 266 (1991) 14931–14938.
[250] J. Kim, P.G. Klein, J.E. Mullet, J. Biol. Chem. 269 (1994) 17918–17923.
[251] J. Kim, L.A. Eichacker, W. Rudiger, J.E. Mullet, Plant Physiol. 104 (1994) 907–916.
[252] D.A. Christopher, M. Kim, J.E. Mullet, Plant Cell. 4 (1992) 785–798.
[253] D.A. Christopher, J.E. Mullet, Plant Physiol. 104 (1994) 1119–11129.
[254] P.E. Gamble, T.B. Sexton, J.E. Mullet, EMBO J. 7 (1988) 1288–1297.
[255] W. Zerges, J. Girard-Bascou, J.D. Rochaix, Mol. Cell. Biol. 17 (1997) 3440–3448.
[256] K. Humbeck, S. Roemer, H. Senger, Planta 179 (1989) 242–250.
[257] P. Brandt, C. Wilhelm, Planta 180 (1990) 293–296.
[258] E. Minami, K. Shinohara, T. Kuwabara, A. Wanatabe, Arch. Biochem. Biophys. 244 (1986) 517–527.
[259] L.A. Eichacker, J. Soll, P. Lauterbach, W. Rudiger, R.R. Klein, J.E. Mullet, J. Biol. Chem. 265 (1990) 13566–13571.

[260] Z.J. Lorkvoic, W.P. Schroder, H.B. Pakrasi, K.D. Irrgang, R.G. Herrmann, R. Oelmuller, Proc. Natl. Acad. Sci. USA 92 (1995) 8930–8934.
[261] S.J. Thompson, S.J. Kim, C. Robinson, J. Biol. Chem. 273 (1998) 18979–18983.
[262] S.J. Kim, C. Robinson, A. Mant, FEBS Lett. 424 (1998) 105–108.
[263] A. Kapazoglou, F. Sagliocco, L. Dure, J. Biol. Chem. 270 (1995) 12197–12202.
[264] A. Seidler, Biochim. Biophys. Acta 1277 (1996) 35–60.
[265] S. Clausmeyer, R.B. Klösgen, R.G. Herrmann, J. Biol. Chem. 268 (1993) 13869–13876.
[266] A. Mant, I. Schmidt, R.G. Herrmann, C. Robinson, R.B. Klosgen, J. Biol. Chem. 270 (1995) 23275–23281.
[267] R.M. Mould, C. Robinson, J. Biol. Chem. 266 (1991) 12189–12193.
[268] A.M. Creighton, A. Hulford, A. Mant, D. Robinson, C. Robinson, J. Biol. Chem. 270 (1995) 1663–1669.
[269] C. Robinson, D. Cai, A. Hulford, L. Hazell, D. Michl, I.W. Brock, R.G. Herrmann, R.B. Klosgen, EMBO J. 13 (1994) 279–285.
[270] Y. Eisenberg-Domovich, R. Oelmuller, R.G. Herrmann, I. Ohad, J. Biol. Chem. 270 (1995) 30181–30186.
[271] W.F. Ettinger, S.M. Theg, J. Cell. Biol. 115 (1991) 321–328.
[272] A. Hashimoto, Y. Yamamoto, S.M. Theg, FEBS Lett. 391 (1996) 29–34.
[273] J. Komenda, J. Barber, Biochemistry 34 (1995) 9625–9631.
[274] S.P. Mayfield, M. Rahire, G. Frank, H. Zuber, J.D. Rochaix, Proc. Natl. Acad. Sci. USA 84 (1987) 749–753.
[275] S.R. Mayers, J.M. Dubbs, I. Vass, E. Hideg, L. Nagy, J. Barber, Biochemistry 32 (1993) 1454–1465.
[276] P.R. Anbudurai, H.B. Pakrasi, Z. Naturforsch. C. 48 (1993) 267–274.
[277] E.J. Summer, V.H. Schmid, B.U. Bruns, G.W. Schmidt, Plant Physiol. 113 (1997) 1359–1368.
[278] V. Vermas, Annu. Rev. Plant Physiol. 44 (1993) 457–481.
[279] F. Nilsson, D.J. Simpson, C. Jansson, B. Andersson, Arch. Biochim. Biophys. 295 (1992) 340–347.
[280] W.F.J. Vermaas, J.G.K. Williams, C.J. Arntzen, Z. Naturforsh. 42C (1987) 762–768.
[281] J. Yu, W.F. Vermaas, J. Biol. Chem. 268 (1993) 7407–7413.
[282] J.J. Eaton-Rye, W.F. Vermaas, Plant Mol. Biol. 17 (1991) 1165–1177.
[283] H.B. Pakrasi, K.J. Nyhus, H. Granok, Z. Naturforsch. C. 45 (1990) 423–429.
[284] J. Yu, W.F.J. Vermaas, Plant Cell 2 (1990) 315–322.
[285] H. Hiramatsu, A. Mizobuchi, H. Mori, Y. Yamamoto, Plant Cell Physiol. 32 (1991) 881–890.
[286] M. Rogner, D.A. Chisholm, B.A. Diner, Biochemistry 30 (1991) 5387–5395.
[287] W.F.J. Vermaas, M. Ikeuchi, Y. Inoue, Photosynth. Res. 17 (1988) 97–113.
[288] K.H. Jensen, D.L. Herrin, F.G. Plumley, G.W. Schmidt, J. Cell. Biol. 103 (1986) 1315–1325.
[289] J.M. Erickson, M. Rahire, J.D. Rochaix, EMBO J. 3 (1984) 2753–2762.
[290] K.H. Jensen, D.L. Herrin, F.G. Plumley, G.W. Schmidt, J. Cell Biol. 103 (1986) 701–706.
[291] Y. Choquet, D.B. Stern, K. Wostrikoff, R. Kuras, J. Girard-Bascou, F.-A. Wollman, Proc. Natl. Acad. Sci. USA 95 (1998) 4380–4385.
[292] N. Adir, S. Shochat, I. Ohad, J. Biol. Chem. 265 (1990) 12563–12568.
[293] K.J. van Wijk, S. Bingsmark, E.M. Aro, B. Andersson, J. Biol. Chem. 270 (1995) 25685–25695.
[294] K.J. van Wijk, B. Andersson, E.M. Aro, J. Biol. Chem. 271 (1996) 9627–9636.
[295] K.J. van Wijk, M. Roobol-Boza, R. Kettunen, B. Andersson, E.M. Aro, Biochemistry 36 (1997) 6178–6186.
[296] H. Bremer, P.P. Dennis, in: F.C. Neidhardts (Ed.), Modulation of Chemical Composition and other Parameters of the Cell by Growth Rates, ASM press, Washington, DC, 1996, 1553–1569.
[297] P.J. Nixon, J.T. Trost, B.A. Diner, Biochemistry 31 (1992) 10859–10871.
[298] S. Schrader, U. Johanningmeier, Plant Mol. Biol. 19 (1992) 251–256.
[299] A. Lers, P.B. Heifetz, J.E. Boynton, N.W. Gillham, C.B. Osmond, J. Biol. Chem. 267 (1992) 7494–7497.
[300] H.S. Gong, I. Ohad, Biochim. Biophys. Acta 1228 (1995) 181–188.
[301] F.-A. Wollman, P. Delepelaire, J. Cell Biol. 98 (1984) 1–7.
[302] T.D. Elich, M. Edelman, A.K. Mattoo, J. Biol. Chem. 267 (1992) 3523–3529.
[303] P. Delepelaire, EMBO J. 3 (1984) 701–706.
[304] F.-A. Wollman, C. Lemaire, Biochim. Biophys. Acta 933 (1988) 85–94.
[305] C. de Vitry, F.-A. Wollman, Biochim. Biophys. Acta 809 (1988) 444–449.
[306] A. Koivuniemi, E.M. Aro, B. Andersson, Biochemistry 34 (1995) 16022–16029.
[307] O. Kruse, D. Zheleva, J. Barber, FEBS Lett. 408 (1997) 276–280.
[308] E. Rintamaki, R. Kettunen, E.M. Aro, J. Biol. Chem. 271 (1996) 14870–14875.
[309] J.P. Thornber, Encycl. Plant Physiol. 19 (1986) 98–142.
[310] L.J. Rogers, in: P. Fay, C. Van Baalen (Ed.), Ferredoxins, Flavodoxins and Related Proteins: Structure, Function and Evolution, Elsevier, Amsterdam, 1987, 35–67.
[311] S.G. Mayhew, G. Tollin, in: F. Muller (Ed.), General Properties of Flavodoxins, Vol. III, CRC Press, Boca Raton, FL, 1992, 389–426.
[312] K. Brettel, Biochim. Biophys. Acta 1318 (1997) 322–373.
[313] N. Krauss, W.D. Schubert, O. Klukas, P. Fromme, H.T. Witt, W. Saenger, Nat. Struct. Biol. 3 (1996) 965–973.
[314] P. Fromme, Curr. Opin. Struct. Biol. 6 (1996) 473–484.
[315] P.R. Chitnis, Plant Physiol. 111 (1996) 661–669.
[316] P.R. Chitnis, D. Purvis, N. Nelson, J. Biol. Chem. 266 (1991) 20146–20151.
[317] E.J. Boekema, J.P. Dekker, M.G. Van Heel, M. Ronger, W. Saenger, I. Witt, H.T. Witt, FEBS Lett. 217 (1987) 283–286.

[318] B. Bottcher, P. Graber, E. Boekema, Biochim. Biophys. Acta 1100 (1992) 125–136.
[319] A. Hefti, R.C. Ford, M. Miller, R.P. Cox, A. Engel, FEBS Lett. 296 (1992) 29–32.
[320] R.C. Ford, M.A. Cochrane, Biochem. Soc. Trans. 21 (1993) 19–21.
[321] A. Kitmitto, A. Holzenburg, R.C. Ford, J. Biol. Chem. 272 (1997) 19497–19501.
[322] N. Krauss, W.D. Schubert, O. Klukas, P. Fromme, H.T. Witt, W. Saenger, Nat. Struct. Biol. 3 (1996) 965–973.
[323] W.-D. Schubert, O. Klukas, N. Krauss, W. Saenger, P. Fromme, H.T. Witt, J. Mol. Biol. 272 (1997) 741–769.
[324] R.C. Williams, A.N. Glazer, D.J. Lundell, Proc. Natl. Acad. Sci. USA 80 (1983) 5923–5926.
[325] R.C. Ford, A. Holzenburg, EMBO J. 7 (1988) 2287–2293.
[326] R.C. Ford, R.A. Pauptit, A. Holzenberg, FEBS Lett. 238 (1988) 385–389.
[327] R.C. Ford, A. Hefti, A. Engel, EMBO J. 9 (1990) 3067–3075.
[328] J. Kruip, E.J. Boekema, D. Bald, A.F. Boonstra, M. Rogner, J. Biol. Chem. 268 (1993) 23353–23360.
[329] C.J. Falzone, Y.H. Kao, J. Zhao, K.L. MacLaughlin, D.A. Bryant, J.T. Lecomte, Biochemistry 33 (1994) 6043–6051.
[330] C.J. Falzone, Y.H. Kao, J. Zhao, D.A. Bryant, J.T. Lecomte, Biochemistry 33 (1994) 6052–6062.
[331] J. Kruip, D. Bald, E.J. Boekema, M. Rogner, Photosynth. Res. 40 (1994) 279–286.
[332] M. Westermann, A. Emst, S. Brass, P. Boger, W. Wehrmeyer, Arch. Microbiol. 162 (1994) 222–232.
[333] G. Tsiotis, W. Haase, A. Engel, H. Michel, Eur. J. Biochem. 231 (1995) 823–830.
[334] V.P. Chitnis, P.R. Chitnis, FEBS Lett. 366 (1993) 330–334.
[335] W.M. Schluchter, G. Shen, J. Zhao, D.A. Bryant, Photochem. Photobiol. 64 (1996) 53–66.
[336] M. Reith, J. Munholland, Plant Cell. 5 (1993) 465–475.
[337] Y. Yamamoto, H. Tsuji, J. Obokata, Plant Mol. Biol. 22 (1993) 985–994.
[338] J. Obokata, K. Mikami, N. Hayashida, M. Nakamura, M. Sugiura, Plant Physiol. 102 (1993) 1259–1267.
[339] R.G. Herrmann, R. Oelmuller, J. Bichler, A. Schneider-Bauer, J. Steppuhn, N. Wedel, A.K. Tyagi, P. Westhoff, in: R.G. Herrmann, B. Larkins (Ed.), The Thylakoid Membrane of Higher Plants: Genes, their Expression and Interaction, Vol. 2, Plenum Press, New York, 1991, pp. 411–427.
[340] J. Obokata, K. Mikami, Y. Yamamoto, N. Hayashida, Plant Cell Physiol. 35 (1994) 203–209.
[341] M. Nakamura, J. Obokata, Plant Cell Physiol. 35 (1994) 297–302.
[342] Y.Y. Yamamoto, M. Nakamura, Y. Kondo, H. Tsuji, J. Obokata, Plant Cell Physiol. 36 (1995) 727–732.
[343] N. Nakamura, J. Obokata, Plant Cell Physiol. (1995) 1393-1397.
[344] M. Sugiura, Plant Mol. Biol. 19 (1992) 149–168.
[345] M. Reith, Plant Mol. Biol. 18 (1992) 773–775.
[346] T. Berends, P.E. Gamble, J.E. Mullet, NAR 15 (1987) 5217–5240.
[347] J.K. Stevenson, R.B. Hallick, Plant J. 5 (1994) 247–260.
[348] S.R. Rodermel, L. Bogorad, J. Cell Biol. 100 (1985) 463–476.
[349] S.C.G. Chen, M.C. Cheng, M.C. Chen, L.Y. Hwang, Plant Sci. 68 (1990) 213–221.
[350] B.Y. Meng, M. Tanaka, T. Wakasugi, M. Ohme, K. Shinozaki, M. Sugiura, Curr. Genet. 14 (1988) 395–400.
[351] A. Cantrell, D.A. Bryant, Plant Mol. Biol. 9 (1987) 453–468.
[352] L.B. Smart, L. McIntosh, Plant Mol. Biol. 17 (1991) 959–971.
[353] R. Shantz, L. Bogorad, Plant Mol. Biol. 11 (1988) 239–247.
[354] N. Hayashida, T. Matsubayshi, K. Shinozaki, M. Sugiura, K. Inoue, T. Hiyama, Curr. Genet. 12 (1987) 247–250.
[355] E. Rhiel, V.L. Stirewalt, G.E. Gasparich, D.A. Bryant, Gene 112 (1991) 123–128.
[356] K. Stienmuller, Plant Mol. Biol. 20 (1992) 997–1001.
[357] O. Stampachia, J. Girard-Bascou, J.-L. Zanasco, W. Zerges, P. Bennoun, J.-D. Rochaix, Plant Cell 9 (1997) 773–782.
[358] F.-A. Wollman, R. Kuras, Y. Choquet, in: P. Mathis (Ed.), Epistatic Effects in Thylakoid Protein Synthesis: the Example of Cytochrome *f*, Kluwer, Dordrecht, 1995, pp. 737–742.
[359] L.A. Eichacker, M. Helfrich, W. Rudiger, B. Muller, J. Biol. Chem. 271 (1996) 32174–32179.
[360] A.N. Webber, P.B. Gibbs, J.B. Ward, S.E. Bingham, J. Biol. Chem. 268 (1993) 12990–12995.
[361] S.M. Rodday, A.N. Webber, S.E. Bingham, J. Biggins, Biochemistry 340 (1995) 6328–6334.
[362] S. Kjaerulff, B. Andersen, V.S. Nielsen, B.L. Moller, J.S. Okkels, J. Biol. Chem. 268 (1993) 18912–18916.
[363] A. Mant, V.S. Nielsen, T.G. Knott, B.L. Moller, C. Robinson, J. Biol. Chem. 269 (1994) 27303–27309.
[364] L. Minai, Y. Cohen, P.R. Chitnis, R. Nechushtai, Proc. Natl. Acad. Sci. USA 93 (1996) 6338–6342.
[365] Y. Cohen, R. Nechushtai, FEBS Lett. 302 (1992) 15–17.
[366] L. Minai, R. Nechushtai, in: P. Mathis (Ed.), Electrostatic Interactions are Involved in the Assembly of PSaD into Photosystem I, Vol. III, Kluwer, Dordrecht, 1995, pp. 807–810.
[367] J. Knoetzel, D.J. Simpson, Plant Mol. Biol. 22 (1993) 337–345.
[368] C. Robinson, R.B. Klosgen, R.G. Herrmann, J.B. Shackleton, FEBS Lett. 325 (1993) 67–69.
[369] N. Hulford, L. Hazell, R.M. Mould, C. Robinson, J. Biol. Chem. 269 (1994) 3251–3256.
[370] W. Haehnel, T. Jansen, K. Gause, R.B. Klosgen, B. Stahl, D. Michel, B. Huvermann, M. Karas, R.G. Herrmann, EMBO J. 13 (1994) 1028–1038.
[371] F. Drepper, M. Hippler, W. Nitschke, W. Haehnel, Biochemistry 35 (1996) 1282–1295.
[372] M. Hippler, J. Reichert, M. Sutter, E. Zak, L. Altschmied, U. Schroer, R.G. Herrmann, W. Haehnel, EMBO J. 15 (1996) 6374–6384.

[373] M.P. Scott, V.S. Nielsen, J. Knoetzel, R. Andersen, B.L. Moller, Plant Mol. Biol. 26 (1994) 1223–1229.
[374] I. Karnauchov, D. Cai, I. Schmidt, R.G. Herrmann, R.B. Klosgen, J. Biol. Chem. 269 (1994) 32871–32878.
[375] M. Hugosson, G. Nurani, E. Glaser, L.G. Franzen, Plant Mol. Biol. 28 (1995) 525–535.
[376] L.B. Smart, S.L. Anderson, L. McIntosh, EMBO J. 10 (1991) 3289–3296.
[377] L.B. Smart, L. McIntosh, Plant Mol. Biology 21 (1993) 177–180.
[378] P.R. Chitnis, P.A. Reilly, M.C. Miedel, N. Nelson, J. Biol. Chem. 264 (1989) 18374–18380.
[379] V.P. Chitnis, Q. Xu, L. Yu, J.H. Golbeck, H. Nakamoto, D.-L. Kie, P.R. Chitnis, J. Biol. Chem. 268 (1993) 11678–11684.
[380] Q. Xu, D. Hoppe, V.P. Chitnis, W.R. Odom, J.A. Guikema, P.R. Chitnis, J. Biol. Chem. 270 (1995) 16243–16250.
[381] Q. Xu, W.R. Odom, J.A. Guikema, V.P. Chitnis, P.R. Chitnis, Plant Mol. Biol. 26 (1994) 291–302.
[382] Q. Xu, L. Yu, V.P. Chitnis, P.R. Chitnis, J. Biol. Chem. 269 (1994) 3205–3211.
[383] P.R. Chitnis, P.A. Reilly, N. Nelson, J. Biol. Chem. 264 (1989) 18381–18385.
[384] Q. Xu, P.R. Chitnis, Plant Physiol. 108 (1995) 1067–1075.
[385] J. Girard-Bascou, Y. Choquet, M. Schneider, M. Delosme, M. Dron, Curr. Genet. 12 (1987) 489–495.
[386] R. Nechushtai, N. Nelson, Plant. Mol. Biol. 4 (1985) 377–384.
[387] R.G. Hermann, P. Westhoff, J. Alt, J. Tittgen, N. Nelson, in: L. van Vloten-Doting, G.S.P. Groot, T.C. Hall (Ed.), Thylakoid Membrane Proteins and their Genes, Plenum, Amsterdam, 1985, pp. 233–256.
[388] O. Lotan, Y. Cohen, S. Yalovsky, D. Michaeli, R. Nechushtai, in: H. Argyroudi-Akoyunoglou, (Ed.), Characterization of the Sequential Light-Regulated Assembly of Photosystem I Core Complex, Plenum, New York, 1991, pp. 269–276.
[389] O. Lotan, Y. Cohen, D. Michaeli, R. Nechushtai, J. Biol. Chem. 268 (1993) 16185–16189.
[390] R. Nechushtai, N. Nelson, J. Bioenerg. Biomembr. 13 (1981) 295–306.
[391] R. Nechushtai, N. Nelson, A. Mattoo, M. Edelman, FEBS Lett. 125 (1981) 115–119.
[392] Y. Cohen, V.P. Chitnis, R. Nechushtai, P.R. Chitnis, Plant. Mol. Biol. 23 (1993) 895–900.
[393] R. Nechushtai, N. Nelson, J. Biol. Chem. 256 (1981) 11624–11628.
[394] N. Li, J. Zhao, P.V. Warren, J.T. Warden, D.A. Bryant, J.H. Golbeck, Biochemistry 30 (1991) 7863–7872.
[395] Y. Cohen, N. Nelson, P.R. Chitnis, R. Nechushtai, Photosynth. Res. 44 (1995) 157–164.
[396] A. Wilde, H. Härtel, T. Hübschmann, P. Hoffman, S.V. Shestakov, T. Börner, Plant Cell 7 (1995) 649–658.
[397] S. Ruf, H. Kössel, R. Bock, J. Cell Biol. 139 (1997) 95–102.
[398] S. Jansson, Biochim. Biophys. Acta 1184 (1994) 1–19.
[399] H.Y. Yamamoto, R. Bassi, in: D.R. Ort, C.F. Yocum (Ed.), Carotenoids: Localization and Function, Kluwer, Dordrecht, 1996, pp. 539–563.
[400] R. Bassi, D. Sandona, R. Croce, Physiol. Plant. 100 (1997) 769–779.
[401] O. Machold, J. Plant Physiol. 38 (1991) 678–684.
[402] C. de Vitry, F.-A. Wollman, C.R. Delepelaire, Acad. Sci. Paris 297 (1983) 277–280.
[403] P. Dainese, R. Bassi, J. Biol. Chem. 266 (1991) 8136–8142.
[404] R. Bassi, B. Pineau, P. Dainese, J. Marquardt, Eur. J. Biochem. 212 (1993) 297–303.
[405] D. Sandonà, R. Croce, A. Pagano, M. Crimi, R. Bassi, Biochim. Biophys. Acta 1365 (1998) 207–214.
[406] W. Kuhlbrandt, D.N. Wang, Y. Fujiyoshi, Nature 367 (1994) 614–621.
[407] E. Giuffra, G. Zucchelli, D. Sandonà, R. Croce, D. Cugini, F.M. Garlaschi, R. Bassi, R. Jennings, Biochemistry 36 (1997) 12984–12993.
[408] A. Pagano, G. Cinque, R. Bassi, J. Biol. Chem., in press.
[409] S. Kim, E. Pichersky, C.F. Yocum, Biochim. Biophys. Acta 1188 (1994) 339–348.
[410] C. Funk, W.P. Schroder, A. Napiwotzki, S.E. Tjus, G. Renger, B. Andersson, Biochemistry 34 (1995) 11133–11141.
[411] E.J. Boekema, H. van Roon, J.P. Dekker, FEBS Lett. 424 (1998) 95–99.
[412] R. Harrer, R. Bassi, M.-G. Testi, C. Shaefer, Eur. J. Biochem. 255 (1998) 196–205.
[413] E.J. Boekema, R.M. Wynn, R. Malkin, Biochim. Biophys. Acta 1017 (1990) 49–56.
[414] S. Jansson, B. Andersen, H.V. Scheller, Plant Physiol. 112 (1996) 409–420.
[415] E.M. Tobin, J. Silverthorne, Annu. Rev. Plant Physiol. 36 (1985) 569–593.
[416] K.A. Marrs, L.S. Kaufman, Proc. Natl. Acad. Sci. USA 86 (1989) 4492–4495.
[417] D.P. Maxwell, D.E. Laudenbach, N.P. Huner, Plant Physiol. 109 (1995) 787–795.
[418] L. Meehnan, K. Harkins, J. Chory, S. Rodermel, Plant Physiol. 112 (1996) 953–963.
[419] U. Johanningmeier, Eur. J. Biochem. 177 (1988) 417–424.
[420] W.C. Taylor, Annu. Rev. Plant Phys. Plant Mol. Biol. 49 (1989) 211–233.
[421] C. Sulli, S.D. Schwartzbach, J. Biol. Chem. 270 (1995) 13084–13090.
[422] S.P. Gibbs, Ann. New York Acad. Sci 361 (1981) 193–207.
[423] G.K. Lamppa, J. Biol. Chem. 263 (1988) 14996–14999.
[424] J.M. Hand, L.J. Szabo, A.C. Vasconcelos, A.R. Cashmore, EMBO J. 8 (1989) 3195–3206.
[425] A.H. Auchincloss, A. Alexander, B.D. Kohorn, J. Biol. Chem. 267 (1992) 10439–10446.
[426] K. Cline, D.R. Fulsom, P.V. Viitanen, J. Biol. Chem. 264 (1989) 14225–14232.
[427] J.E. Reed, K. Cline, L.C. Stephens, K.O. Bacot, P.V. Viitanen, Eur. J. Biochem. 194 (1990) 33–42.
[428] K. Cline, J. Biol. Chem. 261 (1986) 14804–14810.

[429] P.R. Chitnis, R. Nechushtai, J.P. Thornber, Plant Mol. Biol. 10 (1987) 3–12.
[430] D.R. Fulsom, K. Cline, Plant Physiol. 88 (1988) 1146–1153.
[431] S. Yalovsky, H. Paulsen, D. Michaeli, P.R. Chitnis, R. Nechushtai, Proc. Natl. Acad. Sci. USA 89 (1992) 5616–5619.
[432] T.H. Lubben, G.K. Donaldson, P.V. Viitanen, A.A. Gatenby, Plant Cell 1 (1989) 1223–1230.
[433] J. Yuan, R. Henry, K. Cline, Proc. Natl. Acad. Sci. USA 90 (1993) 8552–8556.
[434] L.A. Payan, K. Cline, J. Cell Biol. 112 (1991) 603–614.
[435] N. Hoffman, A.E. Franklin, Plant Physiol. 105 (1994) 295–304.
[436] D. Robinson, I. Karnauchov, R.G. Herrmann, R.B. Klosgen, C. Robinson, Plant J. 10 (1996) 149–155.
[437] B.D. Kohorn, E. Harel, P.R. Chitnis, J.P. Thornber, E.M. Tobin, J. Cell Biol. 102 (1986) 972–981.
[438] B.D. Kohorn, E.M. Tobin, Plant Cell 1 (1989) 159–166.
[439] S.E. Clark, J.E. Oblong, G.K. Lamppa, Plant Cell 2 (1990) 173–184.
[440] A. Gauly, A. Batschauer, A. von Arnim, H. Kossel, Plant Mol. Biol. 19 (1992) 277–287.
[441] L. Huang, Z. Adam, N.E. Hoffman, Plant Physiol. 99 (1992) 247–255.
[442] B.D. Kohorn, A.H. Auchincloss, J. Biol. Chem. 266 (1991) 12048–12052.
[443] S. Yalovsky, G. Schuster, R. Nechushtai, Plant Mol. Biol. 14 (1990) 753–764.
[444] A. Kuttkat, I. Edhofer, L.A. Eichacker, H. Paulsen, J. Biol. Chemistry. 272 (1997) 20451–20455.
[445] G. Bellemare, S.G. Bartlett, N.H. Chua, J. Biol. Chem. 257 (1982) 7762–7767.
[446] D.L. Murrey, B.D. Kohorn, Plant Mol. Biol. 16 (1991) 71–80.
[447] J.K. Hoober, M.J. Hugeus, Plant Physiol. 99 (1992) 932–937.
[448] H. Paulsen, B. Finkenzeller, N. Kuhlein, Eur. J. Biochem. 215 (1993) 809–816.
[449] H. Paulsen, Physiol. Plant. 100 (1997) 760–768.
[450] F.G. Plumley, G.W. Schmidt, Plant Cell 7 (1995) 689–704.
[451] I. Adamska, K. Kloppstech, I. Ohad, J. Biol. Chem. 268 (1993) 5438–5444.
[452] C. Funk, I. Adamska, B.R. Green, B. Andersson, G. Renger, J. Biol. Chem. 270 (1995) 30141–30147.
[453] C. Funk, W.P. Schroder, A. Napiwotzki, S.E. Tjus, G. Renger, B. Andersson, Biochemistry 34 (1995) 11133–11141.
[454] B.D. Kohorn, D. Yakir, J. Biol. Chem. 265 (1990) 2118–2123.
[455] S. Yalovsky, E. Ne'eman, G. Schuster, H. Paulsen, E. Harel, R. Nechushtai, J. Biol. Chem. 267 (1992) 20689–20693.
[456] B.W. Dreyfuss, J.P. Thornber, Plant Physiol. 106 (1994) 829–839.
[457] B.W. Dreyfuss, J.P. Thornber, Plant Physiol. 106 (1994) 841–848.
[458] A. Tremoliers, P. Dainese, R. Bassi, Eur. J. Biochem. 221 (1994) 721–730.
[459] S. Hobe, S. Prytulla, W. Kuhlbrandt, H. Paulsen, EMBO J. 13 (1994) 3423–3429.
[460] J.F. Allen, Biochim. Biophys. Acta 1098 (1992) 275–335.
[461] J.K. Hoober, R.A. White, D.B. Marks, J.L. Gabrie, Photosynth. Res. 39 (1994) 15–31.
[462] R.A. White, J.K. Hoober, Plant Physiol. 106 (1994) 583–590.
[463] Y. Komine, H. Park, G.R. Wolfe, J.K. Hoober, J. Photochem. Photobiol. B. Biol. 36 (1996) 301–306.
[464] R.A. White, G.R. Wolf, Y. Komine, J.K. Hoober, Photosynth. Res. 47 (1996) 267–280.
[465] H. Park, J.K. Hoober, Physiol. Plant. 101 (1997) 135–142.
[466] J.K. Hoober, C.O. Boyd, L.G. Paavola, Plant Physiol. 96 (1991) 1321–1328.
[467] D.J. Morre, G. Sellden, C. Sundqvist, A.S. Sandelius, Plant Physiol. 97 (1991) 1558–1564.
[468] B.L. Trumpower, R.B. Gennis, Annu. Rev. Biochem. 63 (1994) 675–716.
[469] T. Kallas, in: D.A. Bryant (Ed.), The Cytochrome b_6f Complex, Kluwer, Dordrecht, 1994, pp. 259–317.
[470] W.A. Cramer, G.M. Soriano, M. Ponomerav, D. Huang, H. Zhang, S.E. Martinez, J.L. Smith, Annu. Rev. Plant Phisiol. Plant Mol. Biol. 47 (1996) 477–508.
[471] Y. Pierre, C. Breyton, D. Kramer, J.-L. Popot, J. Biol. Chem. 49 (1995) 29342–29349.
[472] J. Haley, L. Bogorad, Proc. Natl. Acad. Sci. USA 86 (1989) 1534–1538.
[473] C. de Vitry, C. Breyton, Y. Pierre, J.-L. Popot, J. Biol. Chem. 271 (1996) 10667–10671.
[474] S.L. Ketchner, R. Malkin, Biochim. Biophys. Acta 1273 (1996) 195–197.
[475] Y. Takahashi, M. Rahire, C. Breyton, J.-L. Popot, P. Joliot, J.D. Rochaix, EMBO J. 15 (1996) 3498–3506.
[476] C. Lemaire, J. Girard-Bascou, F.-A. Wollman, P. Bennoun, Biochim. Biophys. Acta 851 (1986) 229–238.
[477] S.G. Yu, E. Romanowska, Z.T. Xue, P.A. Albertsson, Biochim. Biophys. Acta 1185 (1994) 239–242.
[478] A. Gal, G. Hauska, R. Herrmann, I. Ohad, J. Biol. Chem. 265 (1990) 19742–19749.
[479] A. Gal, R.G. Herrmann, F. Lottspeich, I. Ohad, FEBS. Lett. 298 (1992) 33–35.
[480] S.E. Martinez, D. Huang, A. Szczepaniak, W.A. Cramer, J.L. Smith, Structure 2 (1994) 95–105.
[481] T.A. Link, S. Iwata, Biochim. Biophys. Acta 1275 (1996) 54–60.
[482] D.C.J. Pearson, E.L. Gross, E.S. David, Biophys. J. 71 (1996) 64–76.
[483] G.M. Soriano, M.V. Ponomerav, G.S. Tae, W.A. Cramer, Biochemistry 35 (1996) 14590–14598.
[484] A. Szczepaniak, D. Huang, T.W. Keenan, W.A. Cramer, EMBO J. 10 (1991) 2757–2764.
[485] C. Breyton, C. de Vitry, J.-L. Popot, J. Biol. Chem. 269 (1994) 7597–7602.

[486] D. Xia, C.A. Yu, H. Kim, J.Z. Xia, A. Kachurin, L. Zhang, L. Yu, J. Deisenhofer, Science 277 (1997) 60–66.
[487] Z. Zhang, L. Huang, V.M. Shulmeister, Y.-I. Chi, K.K. Kim, L.-W. Hung, A.R. Crofts, E.A. Berry, S.-H. Kim, Nature 392 (1998) 677–684.
[488] H. Zhang, C.J. Carrell, D. Huang, V. Sled, T. Ohnishi, J.L. Smith, W.A. Cramer, J. Biol. Chem. 271 (1996) 31360–31366.
[489] C.J. Carrell, H. Zhang, W.A. Cramer, J.L. Smith, Structure 5 (1997) 1613–1625.
[490] S. Iwata, M. Sayoovits, T.A. Link, H. Michel, Structure 4 (1996) 567–579.
[491] D. Huang, R.M. Everly, R.H. Cheng, J.B. Heymann, H. Schagger, V. Sled, T. Ohnishi, T.S. Baker, W.A. Cramer, Biochemistry 33 (1994) 4401–4409.
[492] Y. Pierre, C. Breyton, Y. Lemoine, B. Robert, C. Vernotte, J.-L. Popot, Biol. Chem. 272 (1997) 21901.
[493] P. Joliot, A. Joliot, in: P. Mathis (Ed.), A shift in the chlorophyll spectrum associated with electron transfer within the cyt *b/f* complex, Kluwer, Dordrecht, 1995, pp. 615–618.
[494] W.A. Cramer, G.M. Soriano, H. Zhang, M.V. Ponamarev, J.L. Smith, Physiol. Plant. 100 (1997) 852–862.
[495] C. Poggese, P. Polverino de Laureto, G.M. Giacometti, F. Rigoni, R. Berbato, FEBS Lett. 414 (1997) 585–589.
[496] E. Morschel, L.A. Staehelin, J. Cell. Biol. 97 (1983) 301–310.
[497] C. Breyton, C. Tribet, J. Olive, J.P. Dubacq, J.-L. Popot, J. Biol. Chem. 272 (1997) 21892–21900.
[498] C.A. Yu, J.Z. Xia, A.M. Kachurin, L. Yu, D. Xia, H. Kim, J. Deisenhofer, Biochim. Biophys. Acta 1275 (1996) 47–53.
[499] S. Iwata, J.W. Lee, K. Okada, J.K. Lee, M. Iwata, B. Rasmussen, T.A. Link, S. Ramaswamy, B.K. Jap, Science 281 (1998) 64–71.
[500] G. Mosser, C. Breyton, A. Olofsson, J.-L. Popot, J. Biol. Chem. 272 (1997) 20263–20268.
[501] P. Bron, J.J. Lacapere, C. Breyton, G. Mosser, J. Mol. Biol. 287 (1999) 117–126.
[502] R.K. Chain, R. Malkin, Photosynth. Res. 46 (1995) 419–426.
[503] P. Joliot, A. Joliot, Biochim. Biophys. Acta 849 (1986) 211–226.
[504] P. Joliot, A. Joliot, Biochemistry 29 (1998) 10404–10410.
[505] S.R. Mayes, J. Barber, Plant. Mol. Biol. 17 (1991) 289–293.
[506] Y. Nagano, R. Matsuno, Y. Sasaki, Curr. Genet. 20 (1991) 431–436.
[507] A.G. Smith, R.M. Wilson, T.M. Kaethner, D.L. Willey, J.C. Gray, Curr. Genet. 19 (1991) 403–410.
[508] S. Buschlen, Y. Choquet, R. Kuras, F.-A. Wollman, FEBS Lett. 284 (1991) 257–262.
[509] W. Sakamoto, K.L. Kindle, D.B. Stern, Natl. Acad. Sci. USA 90 (1993) 497–501.
[510] N.J. Gumple, L. Ralley, J. Girard-Bascou, F.-A. Wollman, J.H. Nugent, S. Purton, Plant Mol. Biol. 29 (1995) 921–932.
[511] R.G. Drager, J. Girard-Bascou, Y. Choquet, K.L. Kindle, D.B. Stern, Plant J. 13 (1998) 85–96.
[512] J. Gray, Photosynth. Res. 34 (1992) 359–374.
[513] G. Howe, S. Merchant, J. Biol. Chem. 269 (1994) 5824–5832.
[514] C.M. Anderson, J. Gray, FEBS Lett. 280 (1991) 383–386.
[515] S.J. Rothstein, A.A. Gatenby, D.L. Willey, J.C. Gray, Proc. Natl. Acad. Sci. USA 82 (1985) 7955–7959.
[516] R. Voelker, A. Barkan, Mol. Gen. Genet. 249 (1995) 507–514.
[517] E.M. Johnson, L.S. Schnabelrauch, B.B. Sears, Mol. Gen. Genet. 225 (1991) 106–112.
[518] T.A. Smith, B.D. Kohorn, J. Cell. Biol. 126 (1994) 365–374.
[519] G.M. Howe, S. Merchant, EMBO J. 11 (1992) 2789–2801.
[520] Z. Xie, D. Culler, B. Dreyfuss, R. Kuras, F.-A. Wollman, J. Girard-Bascou, S. Merchant, Genetics 148 (1998) 681–692.
[521] S. Merchant, in: J.D. Rochaix, M. Goldschmidt-Clermont, S. Merchant (Ed.), Kluwer, Dordrecht, 1998, 597–611.
[522] R. Kuras, F.-A. Wollman, P. Joliot, Biochemistry 34 (1995) 7468–7475.
[523] G. Howe, L. Mets, S. Merchant, Mol. Gen. Genet. 244 (1995) 151–159.
[524] F. Madueno, J.A. Napier, J.C. Gray, Plant Cell 5 (1993) 1865–1876.
[525] U.C. Manning-Krieg, P.E. Scherer, G. Schatz, EMBO J. 10 (1991) 3273–3280.
[526] O. Ostersetzer, Z. Adam, Plant Cell. 9 (1997) 957–965.
[527] C. de Vitry, J. Biol. Chem. 269 (1994) 7603–7609.
[528] B. Holton, X. Wu, A.I. Tsapin, D.M. Kramer, R. Malkin, T. Kallas, Biochemistry 35 (1996) 15485–15493.
[529] D.E. Robertson, R.S. Farid, C.C. Moser, Nature 368 (1994) 425–432.
[530] P.O. Ljungdhal, J.D. Pennover, D.E. Robertson, B.L. Trumpower, Biochim. Biophys. Acta 891 (1987) 227–241.
[531] E.A. Berry, L.S. Huang, V.J. DeRose, J. Biol. Chem. 266 (1991) 9064–9077.
[532] J. Yu, N.E. Le Brun, J. Biol. Chem. 278 (1998) 8860–8866.
[533] Z. Adam, R. Malkin, FEBS Lett. 225 (1987) 67–71.
[534] G. Finazzi, S. Buschlen, C. de Vitry, F. Rappaport, P. Joliot, F.-A. Wollman, Biochemistry 39 (1997) 2867–2874.
[535] B.D. Bruce, R. Malkin, Plant Cell. 3 (1991) 203–212.
[536] J.G. Metz, D. Miles, A.W. Rutherford, Plant Physiol. 73 (1983) 452–459.
[537] T.A. Smith, B.D. Kohorn, J. Cell. Biol. 126 (1994) 365–374.
[538] D.A. Berthold, C.L. Schmidt, R. Malkin, J. Biol. Chem. 270, (49) (1995) 29293–29298.
[539] X. Chen, K. Kindle, D.B. Stern, EMBO J. 12 (1993) 3627–3635.
[540] W. Sakamoto, X. Chen, K.L. Kindle, D.B. Stern, Plant J. 6 (1994) 503–512.
[541] W. Sakamoto, N.R. Sturn, K.L. Kindle, D.B. Stern, Mol. Cell Biol. 14 (1994) 6180–6186.
[542] X. Chen, K.L. Kindle, D.B. Stern, Plant Cell 7 (1995) 1295–1305.
[543] L. Bulte, F.-A. Wollman, Eur. J. Biochem. 204 (1992) 327–336.

[544] F. Madueno, J.A. Napier, F.J. Cejudo, J.C. Gray, Plant Mol. Biol. 20 (1992) 289–299.
[545] E. Davidson, T. Ohnishi, M. Tokito, F. Daldal, Biochemistry 31 (1992) 3351–3358.
[546] E. Davidson, T. Ohnishi, E. Atta-Asafo-Adjei, F. Daldal, Biochemistry 31 (1992) 3342–3351.
[547] N. Nelson, Biochim. Biophys. Acta 1100 (1992) 109–124.
[548] J.E. Mullet, U. Pick, C.J. Arntzen, Biochim. Biophys. Acta 642 (1981) 149–157.
[549] S. Engelbrecht, W. Junge, FEBS Lett. 414 (1997) 485–491.
[550] N. Hu, D.A. Mills, B. Huchzermeyer, M.L. Richter, J. Biol. Chem. 268 (1993) 8536–8540.
[551] J.P. Abrahams, A.G. Leslie, R. Lutter, J.E. Walker, Nature 370 (1994) 621–628.
[552] Y. Shirakihara, A.G. Leslie, J.P. Abrahams, J.E. Walker, T. Ueda, Y. Sekimoto, M. Kambara, K. Saika, Y. Kagawa, M. Yoshida, Structure 5 (1997) 825–836.
[553] P.D. Boyer, Biochim. Biophys. Acta 1140 (1993) 215–250.
[554] J.G. Digel, K.E. Hightower, K.E.R.E. McCarty, J. Bioenerg. Biomembr. 28 (1996) 439–442.
[555] S. Engelbrecht, W. Junge, Biochim. Biophys. Acta 1140 (1992) 157–162.
[556] E.P. Gogol, U. Lücken, R.A. Capaldi, FEBS Lett. 219 (1987) 274–278.
[557] J.E. Walker, I.R. Collison, FEBS Lett. 346 (1994) 39–43.
[558] R.G. Herrmann, J. Steppuhn, G. Herrmann, N. Nelson, FEBS Lett. 326 (1993) 192–198.
[559] S.D. Dunn, J. Biol. Chem. 267 (1992) 7630–7636.
[560] H. Lill, F. Hensel, W. Junge, S. Engelbrecht, J. Biol. Chem. 271 (1996) 32737–32742.
[561] R. Aggeler, I. Ogilvie, R.A. Capaldi, J. Biol. Chem. 272 (1997) 19621–19624.
[562] I. Ogilvie, R. Aggeler, R.A. Capaldi, J. Biol. Chem. 272 (1997) 16652–16656.
[563] C.M. Wetzel, R.E. McCarty, Plant Physiol. 102 (1993) 241–249.
[564] C.M. Wetzel, R.E. McCarty, Plant Physiol. 102 (1993) 251–259.
[565] G. Groth, J.E. Walker, FEBS Lett. 410 (1997) 117–123.
[566] R. Birkenhäger, M. Hoppert, G. Deckers-Hebestreit, F. Mayer, K. Altendorf, Eur. J. Biochem. 230 (1995) 58–67.
[567] S. Singh, P. Turina, C.J. Bustamante, D.J. Keller, R. Capaldi, FEBS Lett. 397 (1996) 30–34.
[568] W. Junge, H. Lill, S. Engelbrecht, Trends Biochem. Sci. 22 (1997) 420–423.
[569] A.L. Cozens, J.E. Walker, J. Mol. Biol. 194 (1987) 359–383.
[570] H. Lill, N. Nelson, Plant. Mol. Biol. 17 (1991) 641–652.
[571] J. Henning, R.G. Herrmann, Mol. Gen. Genet. 203 (1986) 117–128.
[572] M. Kostrzewa, K. Zetsche, J. Mol. Biol. 227 (1992) 961–970.
[573] D.F. McCarn, R.A. Whitaker, J. Alam, J.M. Vrba, S.E. Curtis, J. Bacteriol. 170 (1988) 3448–3458.
[574] N.E. Stollar, J.-K. Kim, M.J. Hollingsworth, Plant Physiol. 105 (1994) 1167–1177.
[575] J.P. Woessner, N.W. Gillham, J.E. Boynton, Gene 44 (1986) 17–28.
[576] J.A. Napier, K.H. Larsson, F. Madueno, J.C. Gray, Plant. Mol. Biol. 20 (1992) 549–554.
[577] K.H. Larsson, J.A. Napier, J.C. Gray, Plant. Mol. Biol. 19 (1992) 343–349.
[578] D. Michl, C. Robinson, J.B. Shackleton, R.G. Herrmann, R.B. Klosgen, EMBO J. 13 (1994) 1310–1317.
[579] C.R. Bird, B. Koller, A.D. Auffret, A.K. Huttly, C.J. Howe, T.A. Dyer, J.C. Gray, EMBO J. 4 (1985) 1381–1388.
[580] A.K. Huttly, A.L. Plant, A.L. Phillips, A.D. Auffret, J.C. Gray, Gene 90 (1990) 227–233.
[581] A.S. Höglund, A.L. Plant, J.C. Gray, Curr. Genet. 18 (1990) 471–476.
[582] P. Fromme, P. Gräber, J. Salnikow, FEBS Lett. 218 (1987) 27–30.
[583] D.J. Klionsky, R.D. Simoni, J. Biol. Chem. 260 (1985) 11200–11206.
[584] R.G. Hadikusumo, S. Meltzer, W.M. Choo, M.J.B. Jean-François, A.W. Linnane, S. Marzuki, Biochim. Biophys. Acta 933 (1988) 212–222.
[585] C. Lemaire, F.-A. Wollman, J. Biol. Chem. 264 (1989) 10235–10242.
[586] D. Robertson, J.E. Boynton, N.W. Gilham, Mol. Gen. Genet. 221 (1990) 155–163.
[587] H.G. Dallmann, S.D. Dunn, J. Bacteriol. 176 (1994) 1242–1250.
[588] A.A. Gatenby, S.J. Rothstein, M. Nomura, Proc. Natl. Acad. Sci. USA 86 (1989) 4066–4070.
[589] T. Hotchkiss, M.J. Hollingsworth, Plant. Mol. Biol. 33 (1997) 635–640.
[590] S. Ketchner, D. Drapier, J. Olive, S. Gaudriault, J. Girard-Bascou, F.-A. Wollman, J. Biol. Chem. 270 (1995) 15299–15306.
[591] J.M. Galmiche, S. Pezennec, R. Zhao, G. Girault, E. Baeuerlein, FEBS Lett. 338 (1994) 152–156.
[592] D. Steinemann, H. Lill, W. Junge, S. Engelbrecht, Biochim. Biophys. Acta 1187 (1994) 354–359.
[593] H. Lill, A. Burkovski, K. Altendorf, W. Junge, S. Engelbrecht, Biochim. Biophys. Acta 1114 (1993) 278–284.
[594] A. Burkovski, H. Lill, S. Engelbrecht, Biochim. Biophys. Acta 1186 (1994) 243–246.
[595] A. Burkovski, G. Deckers-Hebestreit, K. Altendorf, Eur. J. Biochem. 225 (1994) 1221–1228.
[596] Z. Chen, A. Spies, R. Hein, X. Zhou, B.C. Thomas, M. Richter, P. Gegenheimer, J. Biol. Chem. 270 (1995) 17124–17132.
[597] G. Schmidt, A.J.W. Rodgers, S.M. Howitt, A.L. Munn, G.S. Hudson, T.A. Holten, P.R. Whitfeld, W. Bottomley, F. Gibson, F.G.B. Cox, Biochim. Biophys. Acta 1183 (1994) 563.
[598] S. Avital, Z. Gromet-Elhanan, J. Biol. Chem. 266 (1991) 7067–7072.
[599] F. Gao, B. Lipscomb, I. Wu, M.L. Richter, J. Biol. Chem. 270 (1995) 9763–9769.

[600] D. Steinemann, S. Engelbrecht, H. Lill, FEBS Lett. 362 (1995) 171–174.
[601] G.G. Chen, A.T. Jagendorf, Proc. Natl. Acad. Sci. USA 91 (1994) 11497–11501.
[602] A.M. Luis, A. Alconada, J.M. Cuezva, J. Biol. Chem. 265 (1990) 7713–7716.
[603] A. Avni, S. Avital, Z. Gromet-Elhanan, J. Biol. Chem. 266 (1991) 7317–7320.
[604] P.A. Armond, L.A. Staehelin, C.J. Arntzen, J. Cell Biol. 73 (1977) 400–418.
[605] J. Olive, F.-A. Wollman, P. Bennoun, M. Recouvreur, Biol. Cell 48 (1983) 81–84.
[606] P.A. Millner, G. Gogel, J. Barber, J. Photosynth. Res. 13 (1987) 185–198.
[607] P. Joliot, A. Vermeglio, A. Joliot, Biochim. Biophys. Acta 1141 (1993) 151–174.
[608] P. Joliot, A. Vermeglio, A. Joliot, Biochim. Biophys. Acta 975 (1989) 336–345.
[609] C. Jungas, J.L. Ranck, J.L. Rigaud, A. Vermeglio, EMBO J. 18 (1999) 534–542.
[610] J. Olive, O. Vallon, F.-A. Wollman, M. Recouvreur, P. Bennoun, Biochim. Biophys. Acta 851 (1986) 239–248.
[611] F.-A. Wollman, L. Bulte, in: D.O. Hall, G. Grassi (Ed.), Photosynthetic Process for Energy and Chemicals, Elsevier, Amsterdam, 1989, pp. 198–207.
[612] L. Wollenbereg, H. Stephansson, S.G. Yu, P. Albertsson, Biochim. Biophys. Acta 1184 (1994) 93–102.
[613] B. Norling, E. Zak, B. Andersson, H.B. Pakrasi, FEBS Lett. 436 (1998) 189–192.
[614] K. Inoue, B.W. Dreyfuss, K.L. Kindle, D.B. Stern, S. Merchant, O.A. Sodeinde, J. Biol. Chem. 272 (1997) 31747–31754.
[615] H. Fulgosi, A.V. Vener, L. Altschmied, R.G. Herrmann, B. Andersson, EMBO J. 17 (1998) 1577–1587.
[616] J.M. Erickson, M. Rahire, P. Malnoe, J. Girard-Bascou, Y. Pierre, P. Bennoun, J.-D. Rochaix, EMBO J. 8 (1986) 1745–1754.
[617] P. Bennoun, M. Spierer-Herz, J. Erickson, J. Girard-Bascou, Y. Pierre, M. Delosme, J.D. Rochaix, Plant Mol. Biol. 6 (1986) 151–160.
[618] I. Khrebtukova, R.J. Spreitzer, Proc. Natl. Acad. Sci. USA 93 (1996) 13689–13693.
[619] P.E. Gamble, J.E. Mullet, J. Biol. Chem. 264 (1989) 7236–7243.
[620] B.G. Ooi, H.B. Lukins, A.W. Linnana, P. Nagley, Nucleic Acids Res. 15 (1987) 1965–1977.
[621] W. Kuhlbrandt, Curr. Opin. Struct. Biol. 4 (1994) 519–528.
[622] E. Boekema, H. van Roon, F. Calkoen, R. Bassi, J. Dekker, Biochem. 38 (1999) 2233–2239.
[623] A. Kapazoglou, F. Sagliocco, L. Dure, J. Biol. Chem. 270 (1995) 12197–12202.
[624] P.R. Chitnis, N. Nelson, Plant Physiol. 99 (1992) 239–246.
[625] Y. Cohen, J. Steppuhn, R.G. Herrmann, S. Yalovsky, R. Nechushtai, EMBO J. 11 (1992) 79–86.
[626] A.E. Franklin, N.E. Hoffman, J. Biol. Chem. 268 (1993) 22175–22180.
[627] X. Li, R. Henry, J. Yuan, K. Cline, N.E. Hoffman, Proc. Natl. Acad. Sci. USA 92 (1995) 3789–3793.
[628] M.L. Pilgrim, K.J. van Wijk, D.H. Parry, D.A. Sy, N.E. Hoffman, Plant J. 13 (1998) 177–186.
[629] V.I. Klimyuk, F. Persello-Cartieaux, M. Havaux, P. Contard-David, D. Schuenemann, K. Meiherhoff, P. Gouet, J.D. Jones, N.E. Hoffman, L. Nussaume, Plant Cell 11 (1999) 87–100.
[630] D. Schuenemann, S. Gupta, F. Persello-Cartieaux, V.I. Klimyuk, J.D.G. Jones, L. Nussaume, N.E. Hoffman, Proc. Natl. Acad. Sci. USA 17 (1998) 10312–10316.