FEMS Microbiology Reviews 13 (1994) 393–414

Published by Elsevier

FEMSRE 00367

The photosynthetic machinery in Prochlorophytes: Structural properties and ecological significance

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(Received 1 July 1993; accepted 13 December 1993)

Abstract: The Prochlorophytes are a diverse group of photosynthetic prokaryotes which falls within the cyanobacterial lineage, yet lack phycobilisomes as light harvesting structures. Instead, the Prochlorophytes have a light-harvesting apparatus composed of the higher plant pigments chlorophylls *a* and *b*. This review discusses antenna structures, photosynthetic properties and evolutionary relationships among these bacteria, with focus on the role of photosynthesis in their natural habitat. Most of the available information is obtained from studies on *Prochlorothrix*, the model organism of this group in laboratory studies. Our analysis yields a consensus from studies on two Prochlorophytes, *Prochloron* and *Prochlorothrix*, as to how the thylakoid membrane is organized. Lack of laboratory studies on an abundant third Prochlorophyte, *Prochlorococcus*, does not (yet) allow to include this species in the consensus. Overall, we propose that the structure of the light-harvesting complexes from Prochlorophytes is very different from those of chloroplast systems, and is evolutionarily very ancient. The light-harvesting apparatus is considered to maintain a strong structural and functional association with Photosystem I in both *Prochlorothrix* and *Prochloron*. Photosystem II in *Prochlorothrix* differs from other photosynthetic systems in structural and functional properties of both donor and acceptor sides of its reaction center. A demonstrated capacity for Photosystem I-dependent anoxygenic photosynthesis in *Prochlorothrix* may indicate that there is an increased dependence on cyclic photophosphorylation in these organisms. A description of the natural habitats of the Prochlorophytes has been employed as a jumping board for speculation on the role of the photosynthetic apparatus in occupying, proliferating and surviving in their ecological niches. Prochlorophytes seem to thrive in stable environments of low light, sufficient nitrogen supply and possibly the presence of essential organic solutes.

Key words: Prochlorophytes; Chlorophyll *a* / *b* antenna; Light harvesting; Thylakoid organization; Phylogeny; Ecological physiology

Introduction

The combination of the photosynthetic pigments chlorophylls (Chl) *a* and *b* is common to the green algae and the higher plants. These pigments form the core of the light harvesting antenna in these organisms. Nearly all Chl *b* is bound in the light harvesting complex of photosystem II (LHC_{II}). Until 1975 one considered the Chl *a* / *b* antenna as property unique to eukaryotic species, not in the least since the genetic

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SSDI 0168-6445(94)00002-G

information for the apoproteins of the antenna is contained in the nucleus. These so-called *Cab* genes and their protein products show a high degree of homology among the various species [1]. Moreover, eukaryotic antenna structures containing different Chl complements (i.e. from different algal classes) show some degree of structural similarity to the LHC_{II} [2,3].

During the last two decades we have witnessed the discoveries of a few photosynthetic prokaryote species that carry Chls *a* and *b*. Their finding has triggered a vivid discussion in commentary articles regarding their possible role in the evolution of green chloroplasts [4–7]. As if anticipating this discussion, these organisms were given the suggestive name of Prochlorophyta (Prochlorophytes) [8] and were even considered to form a separate taxonomic entity with its own subdivision [9]. Ironically, most research papers did not contribute to this discussion, but instead provided much evidence for phylogenetic linkage between the Prochlorophytes and the cyanobacteria (see below). We will maintain the term 'Prochlorophytes', meaning prokaryotes containing chlorophylls *a* and *b*. This name makes no reference whatsoever to them being prokaryotic chlorophytes and has no intention to infer any phylogenetic relationship among these species distinguishing them from other photosynthetic prokaryotes. This review focuses mainly on the current knowledge of thylakoid structure, organization and functioning in Prochlorophytes. This focus will be used for a critical review of molecular phylogeny and the position of Prochlorophytes among photosynthetic organisms. Special emphasis will be put on the properties of the prokaryotic Chl *a*/*b* antenna in comparison to its eukaryotic counterparts. Its features are discussed in relation to the ecological physiology of Prochlorophytes with the aim of providing explanations on how such features may support competitiveness of Prochlorophytes in their natural environments. Some aspects of Prochlorophyte photosynthetic properties and molecular evolution are of a more speculative nature. For this reason we have separated those aspects from the main text and they are being dealt with in separate sections called intermezzo's.

History of Prochlorophyte discovery

The Prochlorophyte discoveries were reported from widely different environments and they failed to provide any clue with respect to the actual number of Prochlorophyte species, or the diversity of ecosystems they thrive in. Until now three genera with each one species have been described: *Prochloron didemnii*, *Prochlorothrix hollandica* and *Prochlorococcus marinus*. *Prochloron didemnii* is found in a symbiotic relationship with marine tunicates in (sub)tropical coastal waters. Upon its discovery [10] it was at first thought to be a marine cyanobacterium containing two distinct Chl types. Identification of these pigments as chlorophyll *a* and *b* and the apparent absence of cyanobacterial antenna phycobilins [11,12] subsequently formed the basis for the creation of a new phylum, the Prochlorophyta [8]. For a decade, *Prochloron* was the only known photosynthetic prokaryote containing Chl *a* and *b*, and by some it was considered a clear indication for the correctness of the theory of the endosymbiotic origin of plant organelles.

The second Prochlorophyte was discovered much along the line that characterized the finding of *Prochloron*. From a series of enrichment experiments aimed at the isolation of characteristic planktonic photosynthetic organisms in a shallow lake in the Netherlands, a light green subculture lacking phycobilins was obtained. Although never published under that name, it was at first identified as *Oscillatoria limnetica*. After establishing the prokaryotic nature of the organisms, together with the presence of Chls *a* and *b*, it became evident that a second Prochlorophyte was found [13]. It differs from *Prochloron* in that it is filamentous, free-living, and thrives in a freshwater environment; thus it was described as the new species *Prochlorothrix hollandica* in the new family of the Prochlorothricaceae, order of the Prochlorales [5]. With the finding of *Prochlorothrix* came the realization that the Prochlorophytes may form a group of related Chl *a*/*b*-carrying prokaryotes, whose representatives were hitherto overlooked due to lack of criteria for recognition and the apparent difficulties met when trying to bring these organisms into culture.

The discovery of a third Prochlorophyte in part stemmed from work which had described for over a decade the presence of a distinctly different type of Chl in marine waters at various locations in the Atlantic Ocean [14,15]. The occurrence of this Chl species was correlated to the presence of picophytoplankton. It took the on board deployment of a flow cytometer capable of detecting fluorescence emission by individual cells to detect the smallest Prochlorophyte so far [16]. They are free-living photosynthetic coccoid cells, contain divinyl-Chls *a* and *b* and are often the abundant species in picophytoplankton of marine surface waters. After its isolation this Prochlorophyte was named *Prochlorococcus marinus* [17]. The discovery of this organism will most probably effect a shift in the Prochlorophyte research focus away from the evolutionary origin of the chloroplast towards characterization of the photosynthetic apparatus in an ecologically important group of organisms.

Cell structure and morphology of Prochlorophytes

Light and electron microscopy studies of the Prochlorophyte (ultra)structure have clearly established the prokaryotic nature of the three Prochlorophytes. The various Prochlorophytes differ considerably in cell size. *Prochloron* is found as coccoid cells with a diameter of 9–30 μm [18]. The spherical cells of *Prochlorococcus* are 1.2–1.6 μm in length and 0.6–0.8 μm in width [17]. The only filamentous species, *Prochlorothrix*, are cells of 3–10 μm in length and 0.5–1.5 μm in diameter during exponential growth [13]. The filaments or trichomes consist of at least five cells, but may increase to up to 100 cells or more in undisturbed cultures. In all three species, the thylakoid membranes lack the phycobilisomes characteristic for cyanobacteria, and the membranes are found spread in the cytoplasm, often running parallel to one another and to the cell wall. Thin sections show modest membrane stacks seen as parallel arrays of paired membranes [19,20], but it is difficult to distinguish well defined stacked granal and unstacked stromal membranes characteristic of higher plant chloroplasts. The pairing of photosynthetic membranes in Prochlorophytes as opposed to the situation in cyanobacteria has been attributed to the presence of Chl *b* [5], the presence of a LHC type antenna [19] and the absence of phycobilisomes [5].

Prochlorophytes further contain inclusion bodies typical of (cyano)bacteria. Carboxysomes, which contain the enzymes essential for efficient photosynthetic carbon fixation, have been shown to occur in *Prochloron* [21–23], in *Prochlorothrix* [24,25] and in *Prochlorococcus* [16]. Gas vacuoles were found at the poles of the longitudinal cells in the *Prochlorothrix* filaments [25], but their low number does not seem to provide buoyancy capability. The cell wall itself bears great similarity to that of cyanobacteria. Transmission electron micrographs clearly show a cytoplasmic membrane, a periplasmic space, an outer membrane and an outer sheath in all three species [9,13,16,17,23,25]. In summary, Prochlorophytes are morphologically more similar to cyanobacteria than to chloroplasts with limited thylakoid stacking being the only exception.

As cell morphology already suggested, the macromolecular building blocks of the Prochlorophyte cell bear much similarity to those of cyanobacteria. On the outer cell surface of *Prochlorothrix* one finds an abundant S-layer protein, which binds the carotenoid zeaxanthin [26]. This protein has only been observed and characterized for *Prochlorothrix* until now, although a similar carotenoid-protein configuration may exist in *Prochloron* based on the observation that *Prochloron* secretes zeaxanthin into media and buffers used during physiological determinations [27]. An antibody raised against this S-layer protein [26] recognizes proteins of similar molecular mass in *Synechococcus* and *Synechocystis* (Lindell and Post, unpublished results). The cell wall contains a multilayered A1-γ-type peptidoglycan in both *Prochloron* [28,29] and *Prochlorothrix* [30]. Such an outer sheath is very similar to that found in Gram-positive bacteria and cyanobacteria. Prochlorophyte membranes consist of four major lipid components, monogalactosyl-diacylglycerol, digalactosyl-diacylglycerol, sulfoquinovosyl-diacylglycerol and phosphatidylglycerol, but no phosphatidylcholine, which is specific for the green chloroplast [31–34]. Like cyanobacteria,

Prochlorophytes contain monoglucosyl-diacylglycerol, their overall fatty acid compositions are similar [31–34], and so are the buoyant densities of both the thylakoids and the cytoplasmic membrane [27]. Major fatty acids in *Prochloron* and *Prochlorothrix* are 14:0, 14:1ω5, 16:0, 16:1ω7 and two novel fatty acids unique to *Prochlorothrix*: 16:1ω12 (hexadec-4-anoic acid) and a 16:2 isomer [31]. The carboxysomes of Prochlorophytes are membraneless [21] protein structures which contain the enzymes involved in CO_2-assimilation and the CO_2-concentrating mechanism [22–24,37] as we know them from cyanobacteria [38]. The DNA is generally found in the central regions of the cell surrounded by the thylakoid membranes [9,16,23]. Genome sizes of *Prochloron* and *Prochlorothrix* have been estimated at $3.5–4.0 \times 10^9$ basepairs [35,36], while the GC composition of *Prochloron* and *Prochlorothrix* DNA is between 40 and 50 percent [9,36].

Phylogeny and molecular genetics

If one reviews the literature on the Prochlorophytes, it becomes clear that taxonomic classification and the inferring of relationships between organisms is not clear-cut. Some researchers have been eager to show a direct relationship between Prochlorophytes and the chloroplast, whereas others were determined to place them among the cyanobacteria. The underlying problem in such exercises lies both in the danger of overemphasizing one aspect while belittling others and in the import of externally imposed bias into the judgement of a certain property. Study of gene homology is presently considered the most powerful tool for taxonomic and phylogenetic purposes. Partial 5S rRNA [39] and later 16S rRNA sequences [40] established a position for *Prochloron* among the cyanobacteria, distant from other eubacteria and the chloroplast. However, these findings have also been used to correctly support alternative phylogenies positioning *Prochloron* as "comparatively little modified descendant of the common ancestor of blue-green algae and chloroplasts" [41]. The only sequences known for all three Prochlorophytes are partial sequences of the genes encoding 16S rRNA and DNA-dependent RNA polymerase, and these have been employed for phylogenetic study [40,42–44]. This work places the Prochlorophytes as a polyphyletic group in the cyanobacterial radiation, and the results from these studies led to the conclusion that Chl *b* emerged more than once in the evolution of photosynthetic organisms [43].

Of the three bacteria, the most sequence information is available for *Prochlorothrix* and this has been used for phylogenetic comparisons to other phototrophs as well. The cloned genes analyzed mostly encode different components of the photosynthetic apparatus. To date, the following gene sequences are available: *psbA*, encoding the D1 protein of PS_{II} [45], *psbB*, encoding the PS_{II} Chl-protein CP47 [46]; *psbH*, encoding the 10-kDa PS_{II} phosphoprotein [47]; *petBD*, encoding apocytochrome b_6 and subunit 4 of the cyt b_6/f complex [47]; *petE*, encoding plastocyanin (Seeburg and Bullerjahn, unpublished results); and *rbcLS*, encoding the subunits of rubisco, ribulose bisphosphate carboxylase/oxygenase [48,49]. While many of these sequence analysis studies support a close affinity of *Prochlorothrix* to the cyanobacteria, analysis of the *psbA* gene product revealed that both the *Prochlorothrix* and chloroplast homologs lack a seven amino acid domain at the C-terminal end, which is found in all cyanobacterial *psbA* sequences [45] and in the *Prochloron psbA* sequence [50]. This domain is pivotal in the assignment of the *psbA* sequence as more homologous to either the cyanobacteria or the green chloroplast [51]. In combination with other characters, one can ascribe evolutionary significance to the different primary structures of the D1 protein [50]. All other gene sequences and their operon structure reflect that *Prochlorothrix* falls within the cyanobacteria. Additionally, while *rbcLS* are linked in cyanobacteria and *Prochlorothrix*, in eukaryotes *rbcL* is a plastid gene and the RuBisCo small subunit is encoded by a nuclear multigene family [48,49]. Chloroplasts also have a conserved operon transcription unit of *psbBHpetBD*, but in *Prochlorothrix* and cyanobacteria only *petBD* are linked in an operon [47]. Sequence data available on the *atpBE* operon, encoding the β and ϵ subunits of the thylakoid CF1 complex, further support the positioning of

Prochloron within the cyanobacteria [52]. However, these authors recognized a bias against the AT-rich chloroplast plastid when constructing phylogenetic trees of Prochlorophytes, cyanobacteria and the chloroplast [52]. Finally, the *psbO* gene of *Prochloron* has recently be cloned (C. Howe, personal communication). This gene encodes the extrinsic manganese stabilizing protein, MSP, involved in the oxidizing side of PS_{II}.

All these data together allow us to position the Prochlorophytes with confidence outside of the chloroplast lineage, but more information on *Prochlorococcus* and *Prochloron* is necessary to establish their relationship to one another within the cyanobacterial radiation. While it has been stated that the Prochlorophytes are a diverged polyphyletic group, some evidence has accumulated to suggest that the apoproteins carrying the Chl *a*/*b* antenna in *Prochloron* and *Prochlorothrix* are structurally similar [53], and thus that the antennae may share a common origin. A major emphasis of this review is to provide insight into the structure and physiological properties of the Prochlorophyte photosynthetic apparatus and to relate these to photosynthetic properties which support Prochlorophyte proliferation in their natural environment. Particular emphasis will be a detailed discussion of the antenna/reaction center interactions, which so far appear to be unique among photosynthetic systems.

***Intermezzo:* Prochlorophytes, phylogeny and the origin of the plastid**

To date, phylogenetic studies showing Prochlorophytes as being scattered among the cyanobacteria have prompted the theory that Chl *b* arose multiple times during the evolution of phototrophs. Limited sequence data, immunological studies on the Chl *a*/*b* antenna apoproteins and Chl *a*/*b* ratios, among other things, show that the antenna of *Prochlorothrix* is structurally different from LHC_{II} and likely has a separate evolutionary origin. While no data are available on the *Prochlorococcus* antenna so far, the *Prochloron* and *Prochlorothrix* antenna appear to share a common origin. This observation shows that the phylogenetic studies among Prochlorophytes, between Prochlorophytes and cyanobacteria, and between Prochlorophytes and the green chloroplast, do not necessarily lead to forgone conclusions. Two points have to be experimentally addressed in this context.

(i) If the Chl *a*/*b* antenna is found to display a high degree of homology among the Prochlorophytes, than we have to consider the possiblity that Prochlorophytes do after all form a monophyletic group. In that case, the rRNA clock of Prochlorophyte evolution apparently runs at a different pace than its cyanobacterial counterpart.

(ii) The odds are that Prochlorophytes are not related to the progenitor of the green chloroplast, based on studies of gene homology, thylakoid composition and structure, and genome size. However, also here the last word has not been spoken. The endosymbiosis may have caused a loss of the prokaryotic antenna and a concurrent import of the nucleus encoded LHC_{II}-like antenna apoproteins. The genome size of a Prochlorophyte endosymbiont would be rapidly reduced to plastid size due to gene transfer to the host and the loss of redundant genes. Furthermore, a problem remains in explaining the overall high AT content of organellar DNA, while photosynthetic prokaryotes are, by comparison, more GC-rich. It has been argued that the AT bias has arisen following endosymbiosis [52,54].

Photosynthetic apparatus of the Prochlorophytes

The thylakoid complexes involved in light harvesting, charge separation and electron transport have been characterized in some detail in *Prochloron*, but the structure and dynamics of the photosynthetic mechanism are best understood for *Prochlorothrix*. For *Prochlorococcus* little is currently known, but some initial observations suggest that this species has similar properties as found in the other Prochlorophytes. Below we describe the relevant characteristics of the photosynthetic complexes which identify these bacteria as structurally and possibly functionally distinct from the green chloroplast and phycobilisome-

Table 1

Pigment composition of the Prochlorophytes

Organism	major carotenoid	Chl *a* / *b* ratio (cells)	Chl *a* / *b* ratio (antennae)
Prochloron	β-Carotene, zeaxanthin, cryptoxanthin, echinenone, mutatochrome	4–7	2.4
Prochlorothrix	β-Carotene, zeaxanthin	8–9	2.5
Prochlorococcus	α-Carotene, zeaxanthin	< 1–2 [a]	?
Choroplasts	Lutein, violaxanthin, zeaxanthin, neoxanthin, β-carotene	2–3	1.4

Data compiled from references cited in text [7–9,14–15,43–48].

[a] Pigments are the divinyl-chlorophylls *a* and *b*.

containing organisms. The most obvious feature of the Prochlorophytes is the presence of Chl *b* in addition to Chl *a*. While Chl *a* is an essential component mediating both light-harvesting and electron transport functions in all oxygenic phototrophs, Chl *b* is a pigment dedicated solely to light-harvesting. Chl *a* / *b* pigment–protein complexes, dedicated to light-harvesting, will henceforth be referred to as antenna complexes, different from the core Chl *a* complexes that comprise Photosystems I (PS_I) and II (PS_{II}).

Photosynthetic pigments

Table 1 presents the composition of the Chl and the carotenoid pigments in *Prochloron* [11, 12,27,55,56], *Prochlorothrix* ([13]; Post and Sukenik, unpublished results) and *Prochlorococcus* [16,57]. *Prochlorococcus* is unique among this group in the fact that it contains divinyl Chl *a* [16]. Further study of the Chls showed that not only do these cells contain divinyl Chl *a*, but also divinyl Chl *b* [57]. A characteristic feature of the divinyl Chls *a* and *b* is seen from the blue absorption maximum, which is red-shifted 8–10 nm. The Chl *a* / *b* ratios in *Prochloron* [11,56,58–60] and *Prochlorothrix* [13,61] are in general higher than those found in the chloroplast of higher plants [61]. In contrast, *Prochlorococcus* maintains an extremely low Chl *a* / *b* ratio of < 1 in natural populations and in cultured isolates [16]. These high Chl *b* contents raise some important questions with respect to Chl *a* / *b* ratios in the light harvesting antenna(e) and even the possiblity of a light harvesting antenna carrying (virtually) only Chl *b*. No detailed studies of the carotenoid composition of *Prochlorothrix* and *Prochlorococcus* have been reported. The carotenoids apparently do not contribute to light interception in antenna structures and likely serve to protect the cells from photoinhibitory damage. All three Prochlorophytes have a carotenoid composition distinct from chloroplasts of green algae or higher plants [63]. There is no evidence that a xanthophyll cycle as found in green chloroplasts [64] operates in the Prochlorophytes.

The light harvesting antenna

An early studied concluded that the Chl *a* / *b* antenna in *Prochloron* is spectrally and electrophoretically indistiguishable from that of the chloroplast [56]. Subsequent research on *Prochloron* and *Prochlorothrix* indicates that prokaryotic Chl *a* / *b* antennae are probably different in structure from their eukaryotic counterparts. The Chl *a* / *b* antenna in *Prochloron* is bound to a 34-kDa polypeptide [65,66]. For *Prochlorothrix* three polypeptides of 30, 33 and 35 kDa molecular mass could be identified in the Chl *a* / *b* antenna [67,68]. The antenna polypeptides of both *Prochloron* and *Prochlorothrix* are hydrophobic intrinsic thylakoid proteins and their molecular masses suggest that they may be very different from LHC_{II} apoproteins [68]. The antenna proteins appear to form a small, homologous family of structurally related polypeptides as judged from immunological studies; an antibody prepared against the *Prochlorothrix* 30-kDa antenna apoprotein cross-reacted with two additional *Prochlorothrix* proteins of 33 and 35 kDa, and to the major 34-kDa antenna apoprotein from *Prochloron* [53]. The antibody did not cross-react with the chloroplast LHC_{II} apoproteins, and antibodies against LHC_{II} failed to identify these *Prochloron* and *Prochlorothrix* antenna apoproteins

[53,66]. These were the first data to suggest that the Prochlorophyte antenna is structurally dissimilar to that of the green chloroplast. The antibody to the 30-kDa *Prochlorothrix* antenna protein was used to retrieve immunopositive λ_{ZAP} clones encoding fragments of the antenna polypeptides (Bullerjahn, Krugh and Evans, unpublished results). Sequence analysis also indicated that these proteins are structurally dissimilar from the LHC proteins (*Cab* polypeptides) of chloroplast systems; currently it is unclear whether the antenna proteins have evolved independently from the LHC polypeptides encoded by the plant and algal *Cab* multigene family [1].

Pigment analysis of purified antenna complexes yields a relatively high Chl *a*/*b* ratio in comparison to the major green chloroplast antenna, LHC_{II} (see e.g. [69]). The antenna complex of *Prochloron* has been shown to have an *a*/*b* ratio of 2.4 [66], whereas a ratio of 2.5 has been reported for *Prochlorothrix* [68]. Overall, these ratios account for the high *a*/*b* ratio of whole cells as compared to green algae and higher plants (see Table 1). Circular dichroism (CD) studies on the purified pigment–protein complexes also indicate that the organization of Chl *b* molecules is different than in LHC_{II}, as the negative CD band at 470 nm ascribed to trimeric Chl *b* is absent in the *Prochlorothrix* antenna [70].

Unlike the situation in higher plants, there is growing evidence that a very significant fraction of the Prochlorophyte antenna has a structural and functional association with PS_I. A number of papers from five separate laboratories have reported that the bulk of the antenna apoproteins and Chl *b* both co-purify with PS_I and that Chl *b* transfers excitation energy efficiently to PS_I in *Prochloron* and *Prochlorothrix* [36,56,66–68,71–73]. One research group has claimed that the antenna is largely bound to PS_{II} particles after solubilization in Zwittergent 14 [74]. The use of this harsh detergent [75] was previously recognized to strip the Chl *a*/*b* antenna away from PS_I [76]. Furthermore, different publications cite different protein compositions for the Chl *a*/*b* antenna (compare [74] with [76,77]). There is evidence that the 33-kDa antenna apoprotein protein of *Prochlorothrix* is largely associated with PS_{II} [67]. In sum, while the precise partitioning of these antenna proteins in the photosynthetic membrane is currently not fully understood, it is clear that the overall arrangement of the *Prochlorothrix* antenna is quite different from that in green chloroplasts. Characterization of the Chl *a*/*b* antenna as being a PS_I or PS_{II} antenna is less straight forward than in green chloroplasts. The picture emerges that, functionally, energy harvested by the Chl *a*/*b* antenna is delivered to both photosystems, whereas its structural association is not fully resolved. How the antenna organization may contribute to the phototrophic metabolism of the Prochlorophytes will be addressed later in this section. No detailed information is as yet available on the structure and organization of the light harvesting Chl *a*/*b* antenna in *Prochlorococcus*. Initial analysis of fractionated thylakoids showed that not all *Prochlorococcus* strains have abundant proteins in the 30–35-kDa range, which could be candidate antenna proteins (Post and Thomas, unpublished results). Moreover, given the very low Chl *a*/*b* ratio of *Prochlorococcus* [16,17], we can expect either a different organization of the (divinyl) Chl *a* and *b* in its antenna or even the presence of a different antenna type.

Freeze-etch electron microscopy of *Prochlorothrix* and *Prochloron* membranes demonstrates that there is some degree of partitioning between PS_I and PS_{II} centers in the thylakoid membrane [19,20]. This feature, termed lateral heterogeneity, is a widespread feature of chloroplast systems in which the PS_{II} centers are located in the stacked, granal membranes, while PS_I is found in the unstacked, stromal thylakoids. A notable observation from these studies is that the particles of the *Prochloron* and *Prochlorothrix* EF_s (exoplasmic fracture face of stacked thylakoid membrane) fracture face, which have been described as PS_{II} plus antenna particles, are about 30% smaller than those seen in green chloroplasts [19,20]. Overall, EF_u (exoplasmic fracture face of non-stacked thylakoid membrane) EF_s and PF_u (protoplasmic fracture face of non-stacked thylakoid membrane) contain smaller particles than those found in either chloroplasts or cyanobacteria. Hence assigning pigment–protein complexes

to the various freeze fracture particles in Prochlorophytes is uncertain, especially since the ratio of PF_s (protoplasmic fracture face of stacked thylakoid membrane) to EF_s particles is far lower in Prochlorophytes than in green chloroplasts [19,20]. However, we would like to note that EF_u and EF_s particles in *Prochlorothrix* and *Prochloron* are significantly smaller in diameter than their chloroplast counterparts. Whereas EF_s particles have been correlated to the presence of $PS_{II}^{\alpha} + LHC_{II}$ and EF_u to PS_{II}^{β} stripped of its antenna (see e.g. [78,79]), a similar interpretation seems troublesome for Prochlorophytes although it has been attempted [80]. Transmission electron microscopy of *Prochlorothrix* and *Prochloron* membranes revealed that while some degree of membrane appression is evident, the degree of stacking is less widespread than in chloroplast thylakoids [9,13,16,21,53].

***Intermezzo:* prokaryotic versus eukaryotic Chl *a / b* antenna**

A fundamental question which remains concerns the relationship of the Prochlorophyte antenna to other antenna types seen in chloroplasts and cyanobacteria. Current phylogenetic data suggest that Chl *b* evolved more than once, even among the Prochlorophytes. An argument in favor of a monophyletic origin for the Prochlorophyte antenna comes from work demonstrating immunological relationships between the *Prochloron* and *Prochlorothrix* antenna apoproteins [53], and their functional associations with PSI [66–68,71–73].

The discovery of *Prochloron* and *Prochlorothrix* has led to several studies aimed at understanding the structure and dynamics of the photosynthetic apparatus. Such work has provided a great deal of information on the function of the antennae complexes and their relationship to the reaction centers. One conclusion we have drawn from the accumulated data is the apparent lack of a chloroplast LHC_{II}-like complex in these bacteria. In contrast, the major antenna appears to have a strong structural and functional coupling to PS_I, and the characterization of the isolated pigment-proteins suggests profound differences between LHC and the Prochlorophyte antennae. Antenna apoproteins, immunological relationships among antenna proteins, Chl *a / b* ratio, Chl organization within the antenna, energy transfer etc. all distinguish this antenna type from its eukaryotic counterparts. These structural and functional differences are particularly noteworthy as several papers have independently suggested that Chl *b* antennae evolved more than once [42–44]. In light of all these data, we would propose that the term 'LHC', which has been used indiscriminately to denote a Chl *a / b* antenna, be reserved for antennae types seen in chloroplast systems only. To resolve beyond all doubt whether the Prochlorophyte antenna is evolutionarily distinct or divergent from the LHC_{II} antennae will require more detailed sequencing studies examining the primary structure of the *Prochloron*, *Prochlorothrix* and *Prochlorococcus* antenna apoproteins.

Reaction centers

PS_{II} and PS_I particles have been prepared from *Prochloron* and *Prochlorothrix* which exhibit photochemical activity; immunological analysis of these particles and whole thylakoid membranes demonstrate the presence of the homologous reaction center polypeptides found also in cyanobacteria and chloroplasts. For example, PS_I particles from *Prochloron* and *Prochlorothrix* yield a polypeptide composition very similar to PS_I preparations from higher plants or cyanobacteria [67,81]. The composition of PS_{II} particles is somewhat less clear, but the major components of the PS_{II} complex have been identified. These include the Chl *a*-binding polypeptides CP47 and CP43 (47 and 43-kDa molecular mass chlorophyll protein, respectively) [46,67]; the reaction center proteins D1 and D2 ([45,73]; Bullerjahn and Krugh, unpublished results); the 10-kDa psbH protein [47]; and the 33-kDa psbO protein involved in stabilizing the donor (water-splitting) side of PS_{II} [82,83]. The *psbO* gene product or manganese stabilizing protein (MSP) of *Prochlorothrix* was found to be a hydrophobic protein as compared contrasting with the morte hydrophilic MSP found in a green alga and a cyanobacterium [83]. A possible relation to photosynthetic functions is discussed below. As in cyanobacteria *Prochlorothrix* lacked the 23 and 17-kDa proteins of the oxygen evolving complex [83]. Homologs of the small, functionally enigmatic hydrophobic PS_{II}

proteins have been found in *P. hollandica* (Bullerjahn and Krugh, unpublished results); this includes the so-called PsbJ and PsbK proteins whose presence in both cyanobacteria and chloroplasts has been well documented (see e.g. [84]).

Electron carriers

While no active preparations of the cytochrome b_6/f complex from Prochlorophytes have been reported, analysis of whole membranes by difference spectroscopy and tetramethylbenzidine staining have identified both cytochromes f and b_6 in both *Prochloron* and *Prochlorothrix* (Bullerjahn and Sherman, unpublished results). Furthermore, the *petBD* genes encoding apocytochrome b_6 and subunit 4 have been cloned and sequenced [47]. Lastly, examination of soluble fractions from *Prochlorothrix* has shown that the primary electron donor to PS_1 is plastocyanin [36]; this is in contrast to an earlier paper which stated that a cytochrome c_{553} serves this function in *Prochlorothrix* [85]. In summary, virtually all of the components known to be associated with whole chain electron transport in oxygenic photosynthesis have been identified in either *Prochloron* or *Prochlorothrix*, thus it is reasonable to assume that the overall protein composition of such complexes is not substantially different than those reported for cyanobacteria and chloroplast systems.

Modes of photosynthesis

With the two photosystems and all electron carriers known from oxygenic photosynthesis in place, it is hardly surprising that photosynthetic oxygen evolution and carbon fixation do take place in the Prochlorophytes at rates which can support the carbon and energy demands for growth [9,17,18]. The firm positioning of Prochlorophytes within the cyanobacterial radiation has also prompted the search for anoxygenic photosynthesis as an alternative mode of photosynthetic energy conservation in *Prochlorothrix*. Anoxygenic photosynthesis is found among a number of, mainly filamentous, cyanobacteria and it involves a dual role for sulfide. Sulfide acts both as an inhibitor of oxygen evolution at the donor side of PS_{II} [86] and as an electron donor to the plastoquinone pool mediated through an inducible sulfide-quinone oxidoreductase [87]. Green chloroplasts are in general very sensitive to sulfide inhibition and anoxygenic photosynthesis has not been found in these systems. *Prochlorothrix* was shown to have a constitutive but low capacity for anoxygenic photosynthesis at 1–5% of the light saturated oxygenic photosynthesis rates (Post and Arieli, unpublished results). Whereas such a low activity is not sufficient to support significant growth, it may still contribute to the survival of this organism during extended periods of anaerobiosis. This capacity for anoxygenic photosynthesis was accompanied by a continued oxygenic photosynthetic activity up to 5 mM sulfide, indicating a high resistance of the oxygen evolving complex of *Prochlorothrix* against sulfide stress (Post and Arieli, unpublished results). Interesting in this respect is the hydrophobic nature of the MSP in *Prochlorothrix* [82,83], which we suggest may secure the active site of the protein from sulfide intrusion. Whereas (partial) resistance of oxygenic photosynthesis has been observed in a number of species [89], it has never been related to biochemical properties of the MSP.

Photosynthesis–light relationships

The rate of photosynthetic oxygen evolution is a function of quantum flux impinging on the photosynthetic apparatus. Photosynthesis–light relationships have been frequently used to assess competitiveness of phytoplanktonic species under a given set of environmental conditions. The initial slope of the light saturation curve, α, reflects the light utilization efficiency of photosynthesis and is directly related to the quantum efficiency of the photosynthetic process [90]. The maximal photosynthetic rate is a function of the average time needed to transfer electrons from H_2O to NADP through both photosystems [90]. The antenna function and the reaction center function are sometimes fit into photosynthetic units, a functional definition of the number of chlorophylls needed for the production of 1 molecule of O_2 following a single turnover, saturating light

flash [90]. We have summarized the available data on photosynthetic activity of the Prochlorophyte species *Prochloron*, *Prochlorothrix* and *Prochlorococcus* in comparison to *Chlorella* [91–93] and *Oscillatoria* [94,95] as representatives of the green algae and the cyanobacteria respectively (Table 2). Although interpretation of photosynthetic data should be done with care because Chl partitioning among the major chlorophyll–protein complexes may be very different in the representatives of the various groups, we can still make some interesting observations. Firstly, the range in which α, the light utilization efficiency of photosynthesis, of *Chlorella* varied was considerably lower than that for *Prochlorothrix*. Minimum quantum requirements in *Prochlorothrix* were found to be between 10 and 11 [61], comparable to both *Chlorella* [92] and marine phytoplankton species [90]. Hence, differences in α may be explained from different light absorption efficiencies. The absorption cross-section of Chl was between 2 and 4 times smaller in *Chlorella* [92] than in *Prochlorothrix* [61]. This could be due to the absence in the latter of densily stacked grana, in which much of the Chl is shaded by neighbouring pigment molecules. *Prochlorococcus* maintained larger absorption cross-sections of Chl than common marine algae [96]. However, quantum requirements for photosynthetic C-fixation varied between 11–25 mol quanta/mol C in three different strains of *Prochlorococcus* grown over a range of light intensities [96]. P_{max} in *Prochloron* is considerably higher than in any of the other species and it may be indicative of its symbiotic lifestyle, which forces it to optimally utilize high irradiances during daytime. It is further interesting to note that the large photosynthetic units of *Chlorella* (and other planktonic algae) are not matched by the Prochlorophytes. As effective PS_{II} absorption cross-sections were found to be far smaller in *Prochlorothrix* (Post, unpublished results) than those reported for other species, this may be diagnostic for both smaller PS_{II} antenna sizes and a different thylakoid organization in Prochlorophytes as suggested above. This is in agreement with the finding that photosynthesis in *Prochloron* [97] and *Prochlorothrix* [61] reaches saturation at light intensities that are high in comparison to those found for many green algae and cyanobacteria [91–95].

As is true for most if not all algae and cyanobacteria, the light intensity range which allows for maximal cell division rates of the Prochlorophytes species is much lower than that for maximal photosynthesis rates. *P. hollandica* reaches maximal growth rates at < 80 μmol quanta m^{-2} s^{-1} [61]. Current unpublished work by Moore and Chisholm on *Prochlorococcus* has focused on the growth characteristics of the dif-

Table 2

Photosynthetic characteristics of Prochlorophytes

	α [a]	P_{max} [b]	I_s [c]	PSU_{O_2} [d]	τ [e]
Chlorella [91–93]	1.5–2.3	110– 425	> 150	1360–5460	5.5–14.2
Oscillatoria [94,54]	2.4–5.8	313– 680	60–240	920–1660	–
Prochloron [97]	1.9–3.9	815–1062	175–575	1200–3000 [f]	–
Prochlorothrix [61]	2.0–3.5	252– 552	> 250	1512–3672	4.2– 4.4
Prochlorococcus [96]	2.6–8.8	91– 416	40–340	–	–

Photosynthetic characteristics of Prochlorophyte species *Prochloron*, *Prochlorococcus* and *Prochlorothrix* as compared to the common green alga *Chlorella* sp. and the common cyanobacterium *Oscillatoria* sp.

[a] Light utilization efficiency α, μmol O_2 μmol Chl a^{-1} h^{-1} μmol^{-1} quanta m^{-2} s^{-1}.

[b] Light saturated rate of photosynthesis P_{max}, μmol O_2 μmol Chl a^{-1} h^{-1}.

[c] Saturating light intensity I_s, μmol quanta m^{-2} s^{-1}.

[d] Photosynthetic unit size PSU_{O_2}, mol Chl a per mol O_2 produced.

[e] Minimal turnover time τ, ms.

[f] Calculated using the a Chl/P_{700} unit size of 240 [55] and assuming a PSI/PSII ratio of 1.2–2.5 as observed in *Prochlorothrix* [61].

ferent marine isolates. Sargasso Sea strain SS120 yields a maximal growth rate of 0.55 divisions day^{-1}. Its photosynthesis rate becomes light saturated at 40 μmol quanta m^{-2} s^{-1}. While this strain is well adapted for growth in very low light (3 μmol quanta m^{-2} s^{-1}), it seems to be sensitive to photoinhibitory damage to PS_{II} at light intensities as low as 70 μmol quanta m^{-2} s^{-1}. By comparison, the Mediterranean strain MED4 is clearly better adapted to higher light environments; photosynthesis saturates at 60 μmol quanta m^{-2} s^{-1}, while the minimum light intensity supporting growth is approximately 16 μmol quanta m^{-2} s^{-1}. MED4 is also comparatively resistant to photoinhibition (Moore and Chisholm, unpublished results). Future work aimed at understanding antenna structure and function in *Prochlorococcus* should identify the physiological bases for the adaptation to surface vs. deep water light environments.

The Prochlorophytes *Prochloron* and *Prochlorothrix* both showed plasticity in their photosynthetic characteristics as a result of light-shade adaptation [61,97]. Light-shade adaptation was apparent as changes in photosystem ratio [61] and changes in antenna contribution to the pigment bed as observed from changes in Chl *a*/*b* ratio of cells [56,58,61,97]. Field data based on single cell fluorescence detection and HPLC pigment analysis suggest that light-shade adaptation may further occur in the marine Prochlorophyte *Prochlorococcus* [98,99]. These observations were confirmed by culture studies of three strains of *Prochlorococcus* grown at different light intensities [96].

The apparent sensitivity to high light intensities in *Prochlorococcus* is compounded by the observation that *Prochlorothrix* is much more sensitive to photoinhibition than green algae grown under identical conditions [100]. The site of photoinhibition was found to be located on the D1 protein of the PS_{II} reaction center and irreversible damage to this protein was followed by far slower repair than found in green algae [100]. An additional response seen in *Prochlorothrix* during long-term adaptation to supersaturating light is the accumulation of zeaxanthin pigments at the cell surface [26]. This results in the selective screening of wavelengths in the blue region of the spectrum. The zeaxanthin is bound to a protein complex which appears to coat the cell surface, thus this complex has been proposed to represent an S (surface)-layer which assembles under light stress. Cultures shifted to high light exhibit a 2.3-fold increase in the level of this protein within 24 h [26], and Northern blots showed that the mRNA levels for this complex is under transcriptional control by light (Engle and Bullerjahn, unpublished results). Overall, the accumulation of this carotenoid-protein layer may account for much of the 40% decrease in the quantum efficiency for O_2 evolution in high-light adapted cells [61].

Regulation of excitation energy distribution

Light-shade adaptation is responsible for long term regulation of the light harvesting capacity, tuning in to the average photon flux impinging on photosynthetic cells. However, short term changes in light intensity and spectral quality may be cause to imbalances in excitation energy supply to PS_{II} and PS_I and hence in their respective activities. In both chloroplast and cyanobacterial systems, mechanisms operate to regulate energy imbalances between PS_{II} and PS_I. This phenomenon, often termed the state $1 > 2$ transition, acts to maximize linear electron transport should the wavelength of the ambient light favor absorption by one photosystem over the other. In a general sense this is achieved by changes in antenna orientation such that it transfers excitation energy preferentially towards the photosystem with the smallest effective absorption cross-section. For example, in far-red light preferentially absorbed by PS_I, the light harvesting antenna is oriented such that the energy harvested by it is trapped in PS_{II} (state 1). In a state 2 situation, in light preferentially absorbed by PS_{II}, energy is redistributed to PSI. Such transitions are visualized from changes in PS_{II} fluorescence yield; state 1 is the high fluorescence condition, while adaptation to state 2 results in a marked decrease in PS_{II} fluorescence. In green chloroplasts, the major antenna LHC_{II} complex is reversibly phosphorylated in a light-dependent fashion by a thylakoid kinase which is activated by sensing the

redox state of electron carriers between PS_{II} and PS_{I} (for reviews see [101,102]). Upon overexcitation of PS_{II} relative to PSI, the kinase becomes activated, and it phosphorylates the LHC_{II} apoproteins. A phosphorylated subset of the antenna then decouples from PS_{II} and so restores the balance in energy flows towards PS_{I} and PS_{II}. Accompanying these events is the destacking of the thylakoid granal membranes as a consequence of the movement of phosphorylated LHC_{II} away from PS_{II} centers. In the phycobilisome-containing organisms (cyanobacteria and red algae), state transitions do not occur by this mechanism: instead, reorganization of the photosynthetic apparatus occurs such that energy redistribution is enabled by modulating spillover from the PS_{II} Chl *a* core to PS_{I} [103,104].

An early study on *Prochloron* reported that state $1 > 2$ transitions may not occur in this species, based on the presence of a continuously active thylakoid protein kinase [65]. A similar situation was encountered in *Prochlorothrix*, where redox control of a thylakoid protein kinase could be enforced by the addition of strong reducing or oxidizing agents only [76]. Initially, redox controlled kinase activity was related to state $1 > 2$ transitions [105], but this suggestion was discounted in a later publication [76]. Recent observations on *Prochlorothrix* show that the opposite may be true, and the same findings suggest that a similar mechanism may operate in *Prochloron* [61,68,73]. State $1 > 2$ transitions could be clearly shown in the latter species from in vivo measurements of modulated fluorescence [61] and fluorescence induction [72] after red/far red light treatment of the cells. Additional work showed that the 35-kDa Chl *a*/*b* antenna protein forms the major target for the kinase activity located in the thylakoid membrane [69,70]. Under the proper experimental conditions the 35-kDa antenna apoprotein undergoes reversible phosphorylation in a light and/or redox dependent manner both in vivo and in vitro [68]. Since it was shown that the Chl *a*/*b* antenna of *Prochlorothrix* and *Prochloron* are immunologically related [53], it was subsequently suggested that a similar situation may be found in the latter species [68]. White light at moderate to saturating intensity, and PS_{II} light (650 nm) yielded enhanced phosphorylation at the same timescale (approx. 30 min) as the state $1 > 2$ transition [72,73]. Another indication that antenna phosphorylation is related to state $1 > 2$ transitions comes from the finding that phosphatase inhibition yielded a highly phosphorylated antenna, and that cells treated with inhibitor were locked in state 2 in vivo [68]. There is some evidence suggesting that state $1 > 2$ transitions do not just modulate PS_{II} absorption cross-sections, but that in state 2 the Chl *a*/*b* antenna becomes a more efficient PS_{I} antenna. Monitoring PS_{I} function in vivo by measuring non-photochemical fluorescence quenching (indicative of a PS_{I}-driven transmembrane pH gradient), shows that the fluorescence loss following a transition to state 2 is accompanied by an increased diversion of excitation energy towards PS_{I} [73]. All these data suggest an involvement of protein phosphorylation in regulating energy distribution between the photosystems, analogous to what has been observed in chloroplast systems [101,102]. However, so far it is unclear whether protein phosphorylation forms the molecular mechanism of state $1 > 2$ transitions or that it is simply an accompanying process, which in itself has no bearing on the regulation of excitation energy distribution. If the former proves to be true than this would provide strong evidence that kinase activity played a role in regulating photosynthetic activities long before the chloroplast LHC_{II} evolved.

Since state transitions have not been reported for *Prochlorococcus* and *Prochloron*, it remains to be shown that such dynamic short-term responses occur in other Prochlorophytes. However, as described previously, there is a phosphorylation mechanism acting on the *Prochloron* antenna [65] and thus it is possible that state transitions play a role in antenna orientation *in hospite*. On the basis of the information gathered on *Prochlorothrix*, one can now design the experimental conditions for the study of state transitions in *Prochloron* and *Prochlorococcus*.

Thylakoid organization in Prochlorophytes

Studies in recent years have established that the organization of the photosynthetic membrane

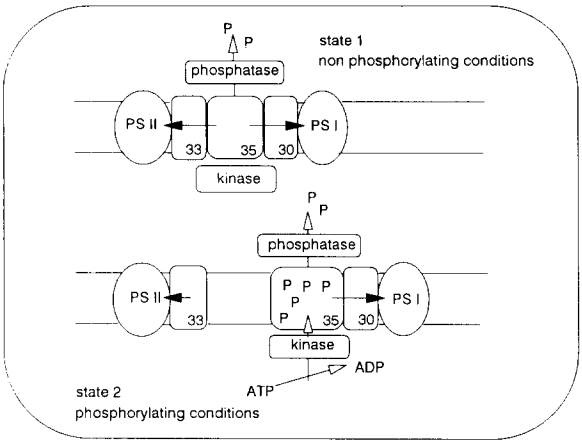

Fig. 1. Model for the molecular mechanism of state 1 > 2 transitions in photosynthetic prokaryotes carrying chlorophyll *a* / *b* antennae. The chlorophyll *a* / *b* antenna consists of a bulk antenna which is located on 30 and 35-kDa apoproteins and associates preferably with PS I. A minor chlorophyll *a* / *b* antenna is carried by a 33-kDa apoprotein and is found to co-purify with PS II. The 35-kDa antenna protein forms the major target protein of light/redox controlled kinase activity. Upon phosphorylation, the bulk antenna excludes PS II centers and enters a more tight association with PS I. Under such conditions, the energy transfer to PS I is enhanced. This process reverses to a state of balanced energy transfer by the bulk antenna following dephosphorylation of the antenna in either darkness or far red illumination.

in both *Prochloron* and *Prochlorothrix* is somewhat different than in cyanobacterial and chloroplast systems. As mentioned briefly above, evidence has accumulated indicating that a large part of the antenna is energy coupled to PS_I at all times. This differs considerably from the situation in green chloroplasts and cyanobacteria, where the bulk antennae were identified as light harvesting antennae of PS_{II}. Many aspects of the Prochlorophyte photosynthetic machinery, particularly with respect to the structure and composition of PS_I and PS_{II} centers, and the reversible phosphorylation of the antenna, are structurally or functionally analogous to other photosynthetic systems. Other observations, mainly that of a Chl *a* / *b* antenna associated with PS_I, have prompted us to construct the model for thylakoid organization in *Prochlorothrix* [73] as presented in Fig. 1. The model predicts that a minor Chl *a* / *b* antenna bound to a 33-kDa protein serves a light harvesting antenna to PS_{II}. However, the bulk of the Chl *a* / *b* antenna is bound to proteins 30 and 35 kDa molecular mass and it is this antenna which is associated with PS_I. Under non-phosphorylating conditions (state 1), both PS_I and PS_{II} share a common light-harvesting antenna composed largely of the 30/35-kDa Chl *a* / *b* antenna in its dephosphorylated state. This condition exists both in the dark and upon treatment with far-red (PS_I) light. Transfer of cells to either high light and red light provokes the phosphorylation of the 35-kDa protein and the decoupling of the bulk antenna from PS_{II} (state 2). This results in the exclusion of PS_{II} centers from the major light-harvesting apparatus in the cell. It is this situation which we argue results in the lateral heterogeneity seen in freeze-etch electron micrographs. As mentioned previously, the EF_S particles seen following freeze-etch are smaller than those in chloroplasts, consistent with our model stating that the PS_{II} centers should lack a large antenna complex. However, the fact that thylakoid membrane stacking occurs in both *Prochloron* and *Prochlorothrix* deserves our future attention. From the limited data available, we would propose that the degree of stacking and lateral heterogeneity in *Prochloron* and *Prochlorothrix* arises from interactions among the PS_{II} centers excluded from the bulk antenna/PSI complexes, thus resulting in increased stacking in a state 2 situation. Ovérall, the lower degree of stacking in Prochlorophytes can be attributed both to the lack of an LHC_{II} complex to stabilize membrane appression, and a high PS_I/PS_{II} ratio in comparison to chloroplasts [61]. Of course, this model is speculative, but it is testable and will help addressing directly the structural and mechanistic requirements necessary to drive thylakoid stacking. E.g. our model predicts that Emerson enhancement does not occur in *Prochlorothrix*, on the basis that action spectra for PS_I and PS_{II} activity are expected to show a strong resemblance. Early photoacoustic measurements on *Prochlorothrix* indeed revealed that far red illumination did not enhance photosynthetic activity and that chemical energy storage was considerable (Post and Canaani, unpublished results).

Although the above model is based on information obtained on *Prochlorothrix*, we propose that it be used as working model for the two marine Prochlorophytes *Prochlorococcus* and *Prochloron*. Properties in the latter two species, like the immunological relationship between the *Prochlorothrix* and *Prochloron* antenna [53] and the dim fluorescence of *Prochlorococcus* [98], suggest that they too have a different thylakoid organization than is found in the green chloroplast. It is therefore of utmost importance to establish whether there is a unifying concept for thylakoid organization in Prochlorophytes, which distinguishes them from other photosynthetic systems. In addition, it should be studied if such a difference bears consequences on the success of Prochlorophytes in occupying their ecological niche(s). The next section addresses the natural habitats of Prochlorophytes and describes properties that may relate to their existence, proliferation and survival strategies.

Intermezzo: Why having a light harvesting antenna be energy coupled to PS_I?

The presence of the Prochlorophytes within the cyanobacterial lineage also suggests that both the *Prochlorothrix / Prochloron* antennae and phycobilisomes are antenna types which could have arisen from a common ancestral phototroph. Such an ancestor would have both a Chl *a / b* PS_I antenna and biliprotein PS_{II} antenna. While no such organism has been described so far, it is consistent with the observation that both these antennae have ancient origins within a prokaryotic lineage. Organisms having only either phycobilisomes or Chl *a / b* antennae arose by losing one of the two antenna types. With a mechanism for excitation energy distribution in place, one can easily understand that a PS_I antenna becomes disposable. However, it is difficult to imagine what selective pressures could result in the loss of the phycobilisome.

The most unusual feature of *Prochloron*, *Prochlorothrix*, and *possibly Prochlorococcus*, is the degree of energy coupling between antenna and PS_I. All other cyanobacteria and chloroplast systems are arranged such that the major antenna complex is tightly coupled to PS_{II}. This arrangement evidently results from a strong dependence on linear electron transport to contribute both to a proton-motive force and an accumulation of reducing equivalents. The antenna organization of *Prochloron* and *Prochlorothrix* appears to yield a situation in which energy transfer to PS_I is optimized. In order for this to make good biological sense, the demands for reducing equivalents must be met by other means. We suggest that *Prochlorothrix* and other Prochlorophytes may have evolved from a bacterium thriving in one of the following environments:

(i) In environment with sources of reduced carbon. Oxidation of reduced substrates is coupled to NAD(P)H accumulation. Consequently, ATP demands could be met by PS_I activity, and pools of reducing power could be maintained by both PS_{II} and oxidative pathways. Another possibility is that reduced carbon is capable of serving as an alternative electron donor. Studying the interactions of *Prochlorothrix* with reduced substrates should be an important priority.

(ii) Considering that *Prochlorothrix* can exhibit low rates of anoxygenic photosynthesis, it may maintain itself in the presence of sulfide. Moreover, the resistance of PS_{II} to sulfide is an indication of the importance of anoxic environments in the evolution of this species.

(iii) *Prochlorothrix* may have evolved in environments with steep gradients in ambient oxygen concentrations. Such environments would enable it to combine properties like photoheterotrophy or anoxygenic photosynthesis with photosynthetic activity involving both photosystems.

(iv) Lastly, the sensitivity to photoinhibition as apparent in *Prochlorococcus* ([96]; Moore and Chisholm, unpublished results) and as has been established for *Prochlorothrix* [100], may have added selective pressure towards the development of an antenna with efficient energy transfer to PS_I. This is further supported by the finding that following a short period of strong PSII activity, the antenna reorganizes itself such that PS_{II} centers are largely excluded from excitation energy supply. No rigorous experiments have been undertaken on any of the Prochlorophytes to test the above hypotheses.

Prochlorophytes in their natural habitat

It is self evident that the photosynthetic apparatus should operate to support cell integrity and growth of the Prochlorophytes in their natural environment. Hence, a closer look at the distribution in nature may give us additional information on the ecological niche of Prochlorophytes and the possible contributions of the photosynthetic apparatus. Ironically, most is known of the ecology of the marine planktonic Prochlorophytes that until recently were not cooperative in laboratory studies.

Prochloron is found as an obligate endosymbiont of marine tunicates, mainly from shallow (< 5 m), warm (20–26°C), (sub)tropical marine waters [19,52,58–60], although it reportedly thrives in deeper waters (> 20 m) as well [60,65, 81,106]. *Prochloron* from deeper waters adapts to the lower light flux by increasing its light harvesting capacity with a comcomitant loss of maximal photosynthetic activity [97]. An early study suggested that *Prochloron* does not function well at osmolarities met in seawater and that it may depend on fast diffusing factors from the animal host into its photosynthetic cell [106]. Doubt was cast on the former observation, when cells were shown to maintain high photosynthesis rates in buffered seawater [97]. Until now, no successful attempts have been made to characterize the nature of the symbiotic relationship or to assess the factors limiting the growth of *Prochloron* populations *in hospite*. A carbon budget of photosynthetically fixed carbon showed that 15–20% of the carbon fixed per day by high light adapted *Prochloron* is translocated to the tunicate host [97]. It is not known whether the symbiosis under low light conditions involves carbon translocation to the host.

Prochlorothrix is found in a number of rather eutrophic, shallow lakes in the center part of the Netherlands. These wind mixed lakes are characterized by a high turbidity, a relatively high background extinction due to water colour and hence light does not penetrate beyond depths of 20–30 cm [107]. Moreover, their water contains considerable amounts of dissolved organic matter [107]. Due to the steep light gradient and possibly activity of heterotrophic bacteria, one finds anoxic conditions at the water-sediment interphase, conditions that may extend into the water column when mixing is interrupted. Anoxic conditions are normally accompanied by the appearance of sulfide in the water phase [108] and hence the observed resistance of PS_{II} to sulfide and the potential for (low) anoxygenic photosynthetic activity [88] may have ecological significance. *Prochlorothrix* populations build up during the summer months when water temperatures reach 15–18°C. [109]. In this respect the population dynamics of *Prochlorothrix* show similarity to those of cyanobacteria for which (late) summer blooms have been ascribed to their favorable light harvesting capability [110,111], photosynthesis/respiration ratio [112,113] and maintenance energy requirements [114] as compared to eukaryotic algae.

Prochlorococcus seems to be ubiquitous and has been reported from temperate and (sub) tropical climate zones, namely the Sargasso Sea, North Atlantic [98,99,115,116] and tropical Atlantic [14,15], the Mediterranean [117], the Red Sea [118] and the Gulf of Aqaba (Post and Lindell, unpublished results), Pacific [119–121] and the Southern California Bight [16] and finally the Banda Sea, Indonesia [122]. On a north-south transect in the eastern North-Atlantic no Prochlorophytes could be found at latitudes above 42°N [123]. Whereas the free-living marine Prochlorophytes are found throughout the photic zone [98,99,116,117], they seem to dominate the deep chlorophyll maximum at densities of 10^4–10^5 cells ml^{-1} [99,116]. The marine Prochlorophytes seem to position themselves under the *Synechococcus* population according to some authors [95,112,113]. This is in itself a surprising observation since part of the *Synechococcus* population possesses phycourobilin enriched phycoerythrin [124], which is optimally suited for light harvesting of those wavelengths that penetrate deepest [125], i.e. to those depths where the Prochlorophytes are found. However, it could well be that the presence of divinyl Chl *b*, with an absorption maximum red shifted in the Soret band by some 8 nm [56], contributes significantly to the light harvesting capacity of Prochlorophytes at

great depth. Interesting in this respect are the low Chl *a*/*b* ratios reported for marine free living Prochlorophytes [16,99], which underscribes a strategy of aiming at optimal light harvesting by these organisms. Culture study showed that marine Prochlorophytes are better absorbers than scatterers in the blue part of the spectrum [126]. This is the only example among phytoplanktonic species so far. There are also indications that their positioning in the water column is related to the depth of the nitracline [98], suggesting that the nitrogen status of the cells is related to their vertical distribution. Differences in cellular pigment contents of the Prochlorophytes were reported to result from different light history [99]. Since Chl contents are known to react strongly to the nutrient status of the cell, the observed lower Chl contents of Prochlorophyte cells from shallow layers may be explained as well from the different nitrogen status of these cells as compared to those thriving in the vicinity of a higher inorganic nitrogen supply. Nitrogen and light were found to be the key environmental factors controlling cell cycling of Prochlorophytes in the western Mediterranean [127]. Prochlorophyte numbers dominate the phytoplankton groups [98,115,116] and they may account for up to 60% of primary productivity in the deepest layers of the marine photic zone [16]. Marine free-living Prochlorophytes form about 40% of the phytoplankton carbon biomass in the Sargasso Sea [115] with an unkown contribution to the bacterial biomass.

Concluding remarks

Taxonomic studies tell us that the Prochlorophytes are cyanobacteria, although the evolutionary origin(s) of the Prochlorophyte light harvesting antenna(e) – the property most distinguishing Prochlorophytes from cyanobaceria – is still unkown. Biochemical/physiological studies indicate that their photosynthetic apparatus is organized in an unique way. The special features of prokaryotic chlorophyll *a*/*b* antenna in *Prochloron* and *Prochlorothrix* may prove useful in understanding general rules for how antenna systems in photosynthetic organisms function and respond to changes in ambient light and nutrient conditions. An in depth study of the photosynthetic features of these prokaryotes is necessary to understand fully both the selective advantage of a chlorophyll *a*/*b* antenna in Prochlorophyte evolution and the ability of Prochlorophytes to colonize very specific niches in marine and fresh water environments.

Much work remains to be done regarding the Prochlorophytes. Our major interest has been to examine the structure-function relationships in the photosynthetic apparatus in an effort to understand how their different antenna systems function, and thus how it contributes to the growth and metabolism of the cell. Two areas need to be addressed in the near future. Firstly, the complete sequence of the *Prochlorothrix* Chl *a*/*b* binding antenna apoproteins should be obtained and analyzed for any domains which might cooperate in Chl binding. This study is essential in establishing the degree of divergence from the antenna apoproteins of green chloroplasts. Secondly, a detailed study on the structure and function of the *Prochlorococcus* antenna is necessary to assess its degree of homology to the *Prochloron* and *Prochlorothrix* antennae. Additional important studies include examining the molecular basis for the anoxygenic, sulfide resistant photosynthesis; perhaps such work can give clues towards understanding the functional significance of a PS_1/antenna complex and the contribution of alternative/additional electron donors in photosynthetic electron transport.

Acknowledgements

Dr. Sallie Chisholm and Lisa Moore provided work prior to publication and helpful discussions. The work compiled from the authors' laboratories was supported by Grants DCB-8903060 and MCB-9204672 from the National Science Foundation (to G.S.B.) and award 88-00341 from the US/Israel Binational Science Foundation (to A.F.P.).

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