Supramolecular structure of the thylakoid membrane of *Prochlorothrix hollandica*: A chlorophyll *b*-containing prokaryote

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Summary

Prochlorothrix hollandica **is a newly described photosynthetic prokaryote, which contains chlorophylls *a* and *b*. In this paper we report the results of freeze fracture and freeze etch studies of the organization of the photosynthetic thylakoid membranes of *Prochlorothrix*. These membranes exhibit four distinct fracture faces in freeze fractured preparations, two of which are derived from membrane splitting in stacked regions of the thylakoid membrane, and two of which are derived from non-stacked regions. The existence of these four faces confirms that the thylakoid membranes of *Prochlorothrix*, like those of green plants, display true membrane stacking and have different internal composition in stacked and non-stacked regions, a phenomenon that has been given the name lateral heterogeneity. The general details of these fracture faces are similar to those of green plants, although the intramembrane particles of *Prochlorothrix* are generally smaller than those of green plants by as much as 30%.**

Freeze etched membrane surfaces have also been studied, and the results of these studies confirm freeze fracture observations. The outer surface of the thylakoid membrane displays both small (less than 8·0 nm) and large (greater than 10·0 nm) particles. The inner surface of the thylakoid membrane is covered with tetrameric particles, which are concentrated into stacked membrane regions, a situation that is similar to the inner surfaces of the thylakoid membranes of green plants. These tetramers have never before been reported in a prokaryote. The photosynthetic membranes of *Prochlorothrix* therefore represent a prokaryotic system that is remarkably similar, in structural terms, to the photosynthetic membranes found in chloroplasts of green plants.

Key words: *Prochlorothrix hollandica*, prokaryote, chlorophyll *b*, freeze fracture faces.

Introduction

Prokaryotes have been widely used as model systems for the study of photosynthesis. Their relative simplicity has permitted detailed studies of their photosynthetic membranes and reaction centres, and the information gained from investigations of prokaryotic organisms has been frequently extrapolated to help analyse the photosynthetic systems found in eukaryotic chloroplasts (Barber, 1987). Although prokaryotic systems have served this purpose well, one serious limitation posed by most organisms is the fact that their pigment systems differ from those of green plants: photosynthetic bacteria contain bacteriochlorophylls as their primary photopigments and cyanobacteria contain phycobiliproteins in their light-harvesting systems. The ideal model system for the chloroplast photosynthetic membrane would be a prokaryote utilizing chlorophylls *a* and *b* as its major pigments and possessing a photosynthetic membrane structure similar to that of the chloroplast. Such organisms would also help to clarify the evolutionary relationships between green plants and algae and prokaryotic photosynthetic organisms.

The first organism to be discovered that fulfilled these requirements, *Prochloron didemni*, is an obligant unicellar symbiont of ascidian marine tunicates (Lewin, 1975). It has proved difficult to grow *Prochloron* in the absence of its host (Patterson & Withers, 1982), and *Prochloron*'s usefulness as an experimental organism has therefore been limited. A partial solution to this problem has been provided by the recent discovery of a second prochlorophyte alga: *Prochlorothrix hollandica* (Burger-Wiersma *et al.* 1986). Unlike *Prochloron*, *Prochlorothrix* can be cultured in defined media and grown in quantities suitable for biochemical and physiological studies.

As noted in the original report on *Prochlorothrix*

(Burger-Wiersma *et al.* 1986), electron micrographs of the organism reveal the existence of photosynthetic thylakoid membranes located close to the peripheral cell wall. In this report, we describe the basic structure and orientation of *Prochlorothrix* thylakoid membranes, and discuss the structural similarities that exist between the photosynthetic membranes of *Prochlorothrix*, green plants, and *Prochloron*.

Materials and methods

Subcultures of *Prochlorothrix hollandica* were kindly provided by Drs Louis Sherman and George Bullerjahn of the University of Missouri at Columbia, Missouri. The organism was originally isolated from the waters of the Loosdrecht lakes near Amsterdam (Burger-Wiersma *et al.* 1986). The organism was grown in BG-11 (Allen, 1968) medium with constant illumination ($0{\cdot}35\,W\,cm^{-2}$). Air was gently bubbled through the cultures to promote cell growth, and cultures were harvested in the log phase of growth.

Cells were harvested from growth medium by centrifugation at 1500 ***g***-average for 5 min at 4°C using a Sorvall SS-34 rotor (Dupont Instruments, Wilmington, DE, USA). Pellets were then resuspended in distilled water, containing $0{\cdot}5\,mg\,ml^{-1}$ phenylmethylsulphonyl fluoride, and cells were broken open either by Yeda press disruption (2000 p.s.i.) or by sonic probe homogenization (Branson sonic disruptor at 75 W output for 5 min). Low-speed centrifugation of the crude homogenate (3000 ***g***-average for 5 min, SS-34 rotor) removed intact cells and cell walls. Further centrifugation of the supernatant fluid from the first centrifugation, for 10 min at 20 000 ***g***-average, yielded a pellet enriched in photosynthetic membranes. The membranes were then resuspended in 150 mM-NaCl, 5 mM-$MgCl_2 \cdot 6H_2O$, 5 mM-$CaCl_2$, 50 mM-Tris, pH 7·8 ('isolation buffer').

For freeze-fracture, cells or thylakoids were infiltrated with glycerol to a final concentration of 25 % (v/v) over the course of 1 h at 4°C. Samples for freeze etching were frozen in a buffer containing 2 mM-$MgCl_2$, 10 mM-Tris, pH 7·8 ('dilute buffer') without the addition of a cryoprotectant. Concentrated suspensions of cells or membranes were frozen in liquid Freon-22 (Union Carbide, New York, NY, USA) on copper or gold specimen supports. Freeze fracture was performed at −110°C using a BAF 400 freeze etching unit (Balzers Union, Hudson, NH, USA) and samples were replicated with platinum and carbon from one side. Non-glycerinated samples were fractured and etched for 3 min at −100°C and rotary-shadowed. Electron micrographs were taken with an EM 410 (Philips Electronic Instruments, Mahwah, NJ, USA) at 100 kV.

For immunoblotting, isolated membranes were dissolved in 2 % sodium dodecyl sulphate (SDS), and polyacrylamide gel electrophoresis was carried out as described (Miller *et al.* 1976). Proteins were electrophoretically transferred to Immobilon PVDF transfer membranes (Millipore, Bedford, MA, USA) at 4°C for 2 h at 70 V. Non-specific binding sites in the membrane were blocked by a 30-min incubation in 10 % bovine serum albumin. Antibodies prepared against the beta-subunits of the coupling factor (kindly provided by Dr Nathan Nelson, Roche Institute, Nutley, NJ, USA) were added at a 1:2500 dilution and incubated for 90 min. Antibody binding was detected with the ABC Vectastain kit (Vector Laboratories, Bulingame, CA, USA) for rabbit immunoglobulin G, which employs an avidin–biotin–peroxidase detection protocol.

Results

General cellular morphology

The basic structure of *Prochlorothrix hollandica* is shown in Figs 1, 2. As reported earlier (Burger-Wiersma *et al.* 1986), *Prochlorothrix* is a filamentous species, and a typical filament consists of 5–15 such cells. Within each cell of the filament there is an extensive peripheral network of photosynthetic membranes. These membranes are arranged in paired stacks, often 8–10 membranes high. As noted earlier (Burger-Wiersma *et al.* 1986), these photosynthetic membranes seem to form stacks similar to those of plants and green algae. The freeze fracture image of *Prochlorothrix* is dominated by these internal thylakoid membranes, as shown in Fig. 2.

Thylakoid fracture faces

Thylakoid membranes in many species are appressed at their outer surfaces to form 'stacks', which are known as grana in green plants. One of the first questions that we hoped to answer with our structural studies was whether *Prochlorothrix* would display thylakoid membrane stacking. Figs 3, 4 illustrate the typical appearance of *Prochlorothrix* thylakoid membranes in freeze fracture. By convention, thylakoid membrane fracture faces are described according to a system of nomenclature that refers to exoplasmic (E) and protoplasmic (P) fracture faces (Branton *et al.* 1975), and we have used this system in this and all subsequent micrographs. Fig. 3 illustrates the appearance of stacked and non-stacked regions of the Pf fracture face.

Many studies have shown that the internal organization of thylakoid membranes differs when stacked regions are compared to non-stacked regions (see Staehelin, 1986 for a recent summary). Therefore, a separate terminology exists for thylakoid membranes to point out fracture faces from stacked and non-stacked regions: as shown in Fig. 3, the P fracture face is divided into two regions, with the symbol PF_u used to label the P fracture face from a non-stacked (unstacked) region of the thylakoid membrane, and PF_s from the stacked region. Similar terminology is used to describe E fracture faces: EF_s and EF_u refer to E fracture faces from stacked and non-stacked regions, respectively (Branton *et al.* 1975).

Fig. 3 displays a typical freeze fracture view of *Prochlorothrix* thylakoid membranes. In this micrograph, only P fracture faces are observed, and there are two large stacked regions (PF_s) surrounded by non-stacked membranes (PF_u). The PF_u faces contain a population of small densely packed particles. As the membrane passes into stacked regions the structure of the P fracture face changes, and fewer particles are visible.

Fig. 4 illustrates the EF fracture faces of *Prochlorothrix hollandica*; there is a clear and distinct boundary between the EF_s and the EF_u fracture faces, marking the point at which stacking between two thylakoid membranes begins and ends. The EF_s fracture face is covered with a population of large particles, averaging 11·0 nm in diameter. In contrast, the EF_u fracture face displays fewer particles, and the background of the face is characterized by a series of shallow holes or pits. Small

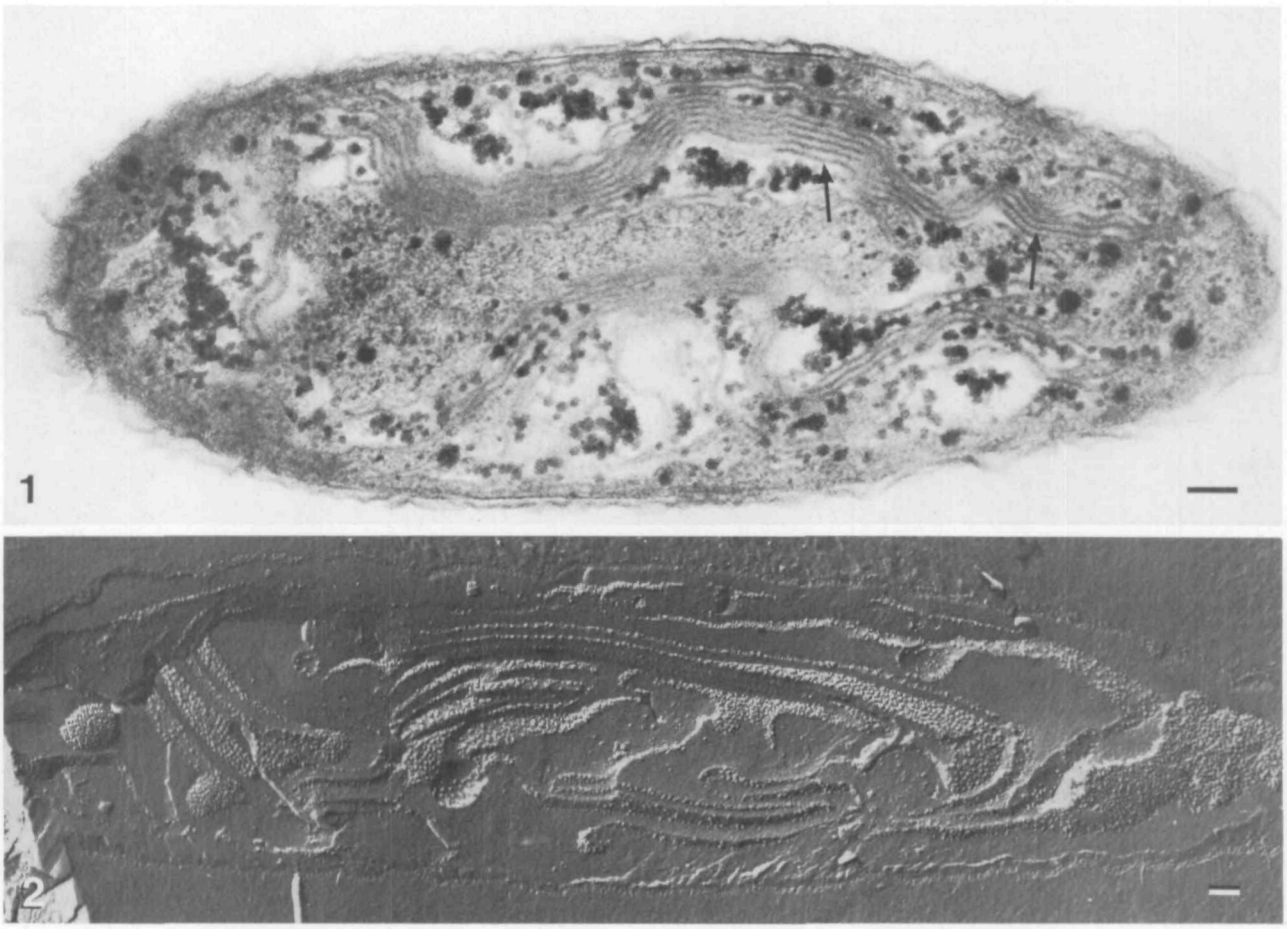


Fig. 1. *Prochlorothrix hollandica* in thin section. The photosynthetic membranes of this organism are seen as dense stacks of paired thylakoids (arrows). Bar, 0·1 μm.

Fig. 2. A single *Prochlorothrix hollandica* cell as viewed by the freeze fracture technique. The major cellular feature of this prokaryote is an extensive system of photosynthetic membranes, arranged as a series of sheets just below the cell membrane and cell wall. Several such thylakoid membranes are visible in this micrograph. Bar, 0·1 μm.

fragments of the P fracture face are still visible on the stacked region in this micrograph (arrows in Fig. 4) confirming that the circular region in the centre of the micrograph is derived from a region of membrane stacking.

The association of membrane fracture faces with thylakoid stacking

The close association of distinct membrane fracture faces with thylakoid stacking in green plants and algae (Goodenough & Staehelin, 1970; Miller, 1980; Staehelin, 1986) is the principal means by which we have identified the four thylakoid fracture faces in *Prochlorothrix hollandica*. To determine if this means of identification was reasonable, we searched our freeze fracture replicas for membranes in which distinct regions of membrane appression and non-appression could be correlated with thylakoid fracture faces. Figs 5 and 6 illustrate two such regions. In each figure, the EF_s and PF_s fracture faces are clearly associated with regions of thylakoid contact. Regions where the thylakoid membranes do not make contact appear in these micrographs and EF_u and PF_u fracture faces.

The coincidence of regions of membrane appression with distinct changes in fracture face morphology was a consistent feature of *Prochlorothrix hollandica* thylakoids, and Figs 5, 6 are typical micrographs in this regard. Such images confirm the validity of thylakoid membrane fracture face identifications noted in Figs 3, 4, and they show a clear correlation of thylakoid differentiation in this prochlorophyte with membrane stacking.

Particle size distributions on fracture faces

We have measured particle diameters and concentrations on the various *Prochlorothrix* fracture faces, and these data are shown in Fig. 7. While there are some similarities between the diameters of fracture-face particles in *Prochlorothrix* and green plants such as spinach (Staehelin, 1986), particles on the E fracture faces are generally smaller than those found on the same fracture face in green plants.

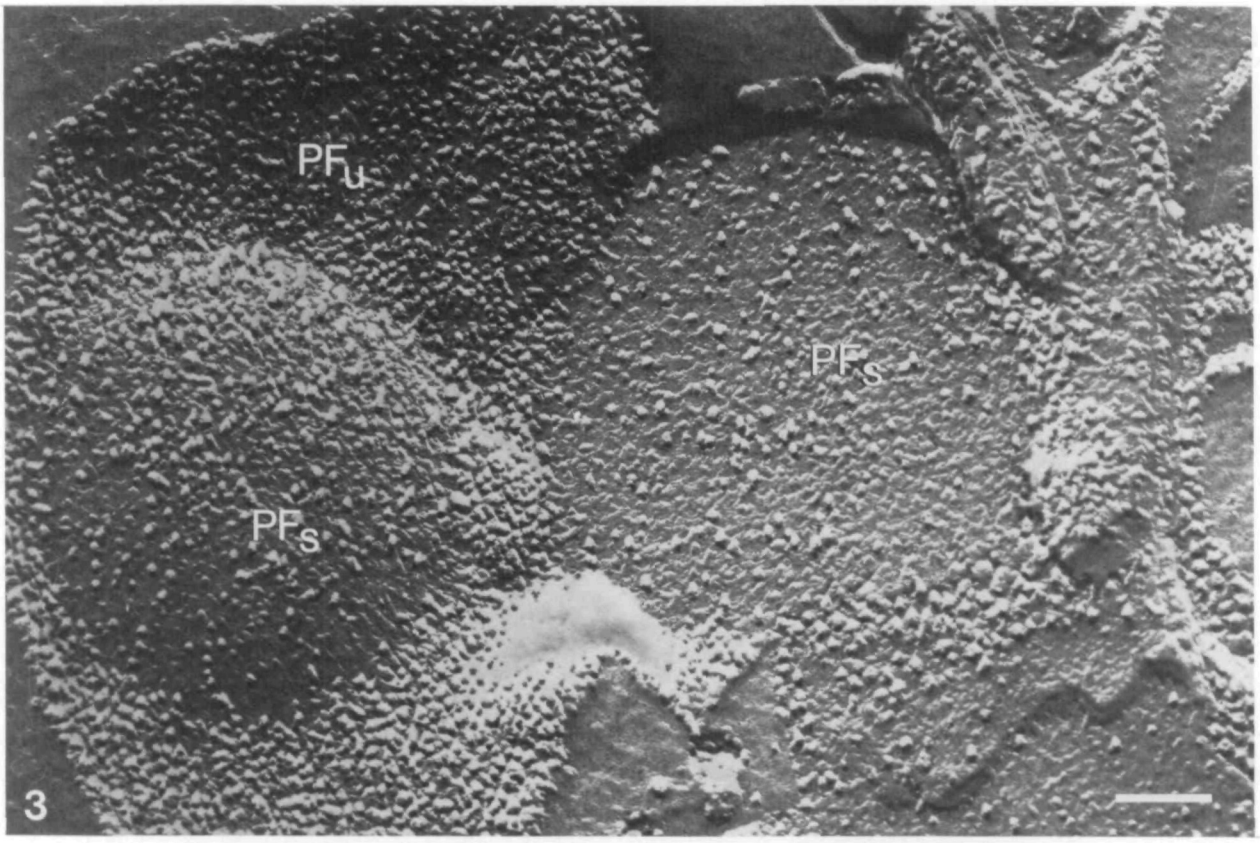


Fig. 3. Isolated photosynthetic membranes (thylakoids) from *Prochlorothrix* display four distinct fracture faces. The EF_s and PF_s fracture faces are derived from membrane splitting in stacked thylakoid regions, while the EF_u and PF_u are derived from membrane splitting in unstacked regions. The nomenclature for fracture face identification is that of Branton *et al.* (1975). The P fracture faces of *Prochlorothrix* display subtle but definite differences when they pass from stacked to non-stacked regions. The two large PF_s regions in this micrograph have fewer particles than the PF_u regions, which surround them, and so they are easy to distinguish. Bar, 0·1 μm.

Etched outer surfaces of the Prochlorothrix *thylakoid membrane*

Isolated thylakoid membranes frozen in a dilute buffer can be fractured and then etched to allow the true outer surface of the membrane to be observed in freeze etch replicas (Miller, 1980). However, it is also necessary to establish that important membrane proteins are not lost by the dilute buffer washes: it is well known that coupling factor can be removed from the surface of higher plant thylakoids by dilute buffers (Miller & Staehelin, 1976). Membranes prepared in isolation buffer as described in Materials and methods were washed several times in a dilute buffer (2 mM-$MgCl_2$, 10 mM-Tris, pH 7·8) in preparation for deep etching. To ensure that the *Prochlorothrix* coupling factor was not removed by this procedure, we carried out immunoblotting using an antibody prepared against the beta subunit of coupling factor. As Fig. 8 illustrates, the coupling factor was removed from the membrane by washing in distilled water (lane 1) but not in dilute buffer (lane 2) or isolation buffer (lane 3). These results show that coupling factor is still present on the membranes used for our deep etch studies, although it is not possible to say from these results whether or not some coupling factor may have been removed during membrane isolation.

We have prepared a series of replicas of isolated *Prochlorothrix* thylakoids using the deep etch procedure. Rotary shadowed and contrast reversed micrographs of *Prochlorothrix* thylakoid membranes prepared by this method are shown in Figs 9, 10. The inner surface of the *Prochlorothrix* thylakoid membrane is shown in Fig. 9. The surface of the membrane is covered with a characteristic particle composed of several subunits. These particles often seem to be composed of four separate subunits, and therefore we will refer to them as 'tetramers'. The inner surface tetramers observed in *Prochlorothrix* are remarkably similar to those found in green plant thylakoids (Miller, 1980; Staehelin, 1986).

The tetramers are not uniformly distributed along the inner surface. Instead, they are largely confined to stacked membrane regions, as shown in Fig. 9. Therefore, we can delineate stacked (ES_s) and non-stacked (ES_u) regions of the thylakoid membrane along the inner surface of the membrane. The high concentration of tetramers found in stacked regions of the inner surface matches the high density of EF_s fracture face particles, which are also concentrated in stacked thylakoid regions, a situation which parallels their distribution in green

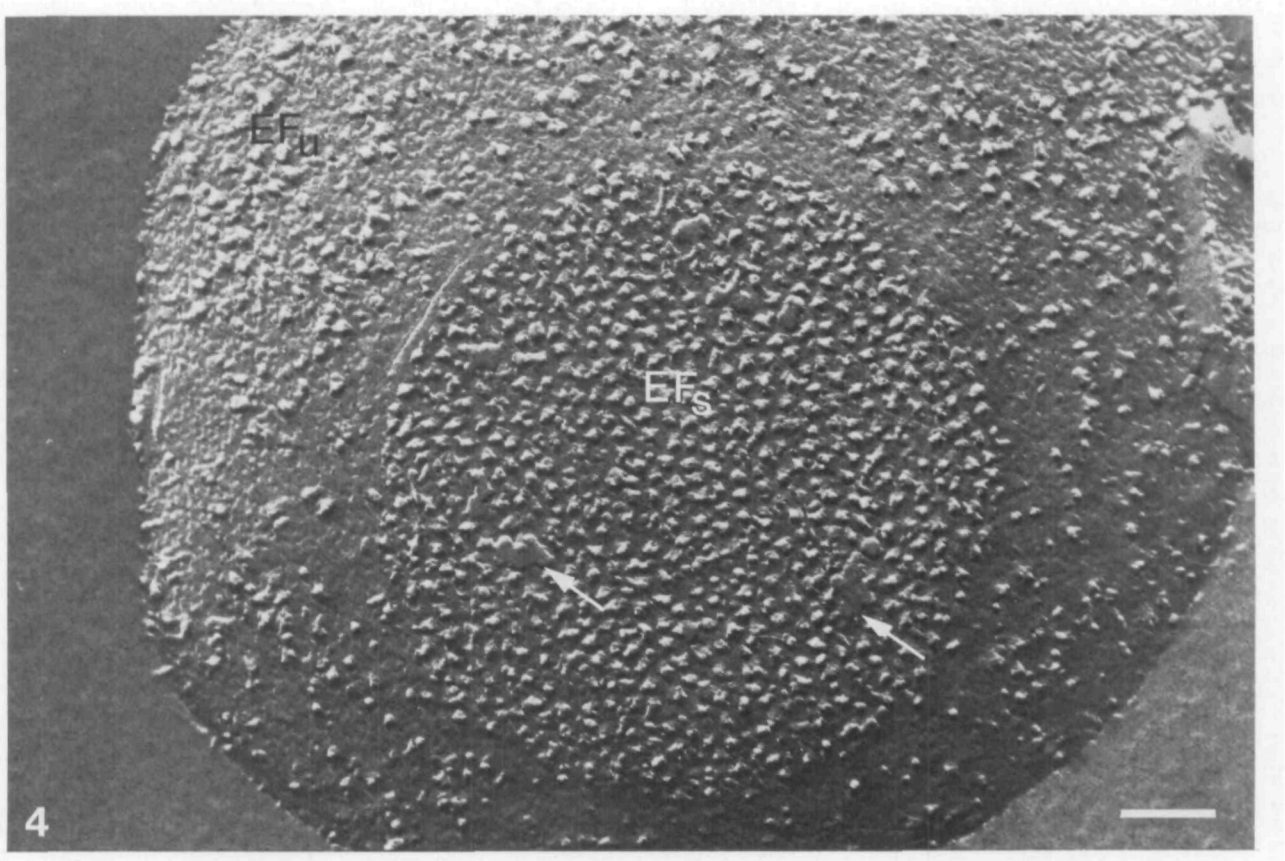


Fig. 4. Lateral heterogeneity of particle distribution on the E fracture face, shown in this micrograph, is particularly clear. EF particles are concentrated into the stacked (EF_s) region near the centre of the micrograph, while the background of the non-stacked (EF_u) face shows a series of regular pockmarks which are not found on other faces. Portions of the PF_s fracture face which are still adhering to the EF_s face are also visible (arrows). Bar, 0·1 μm.

plants (Miller, 1980; Staehelin, 1986).

Fig. 10 shows the outer surface of the *Prochlorothrix* thylakoid. The membrane surface contains a number of large (≈ 12·0 nm) particles (see arrows, Fig. 10), and a larger number of densely packed 8·0-nm particles. In some respects, this image is similar to the spinach thylakoid membrane, which also has large and small particles on its outer surface (Miller, 1980). The outer surfaces of thylakoid membranes in stacked regions are not visible, because these surfaces are closely appressed and not exposed by the etching process.

Discussion

Lateral heterogeneity in the thylakoids of Prochlorothrix

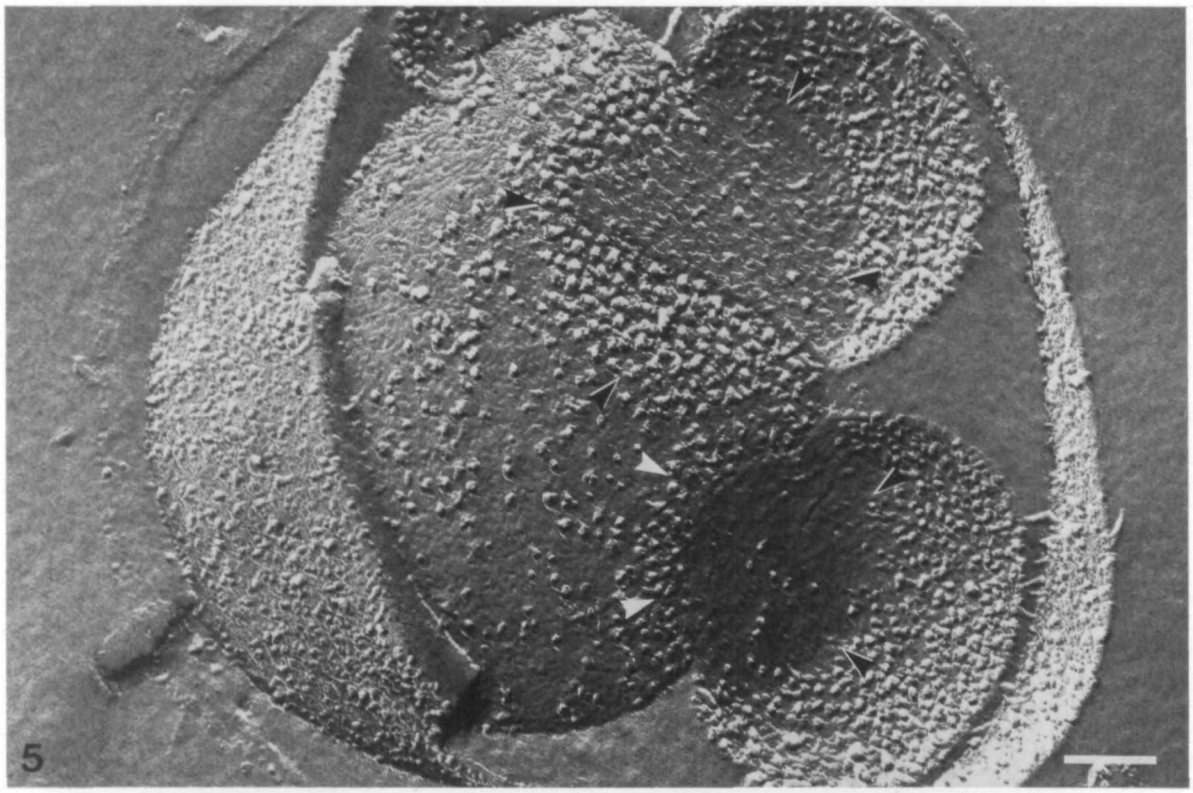
The purpose of these studies was to provide a basic description of the thylakoid organization of *Prochlorothrix*, which would allow the photosynthetic membranes of this organism to be compared to those of green plants and other photosynthetic organisms. Because differences in the lateral distribution of some membrane components can be detected in freeze fracture and freeze etch studies, these investigations were also able to establish the degree of lateral heterogeneity that characterizes *Prochlorothrix* thylakoid membranes.

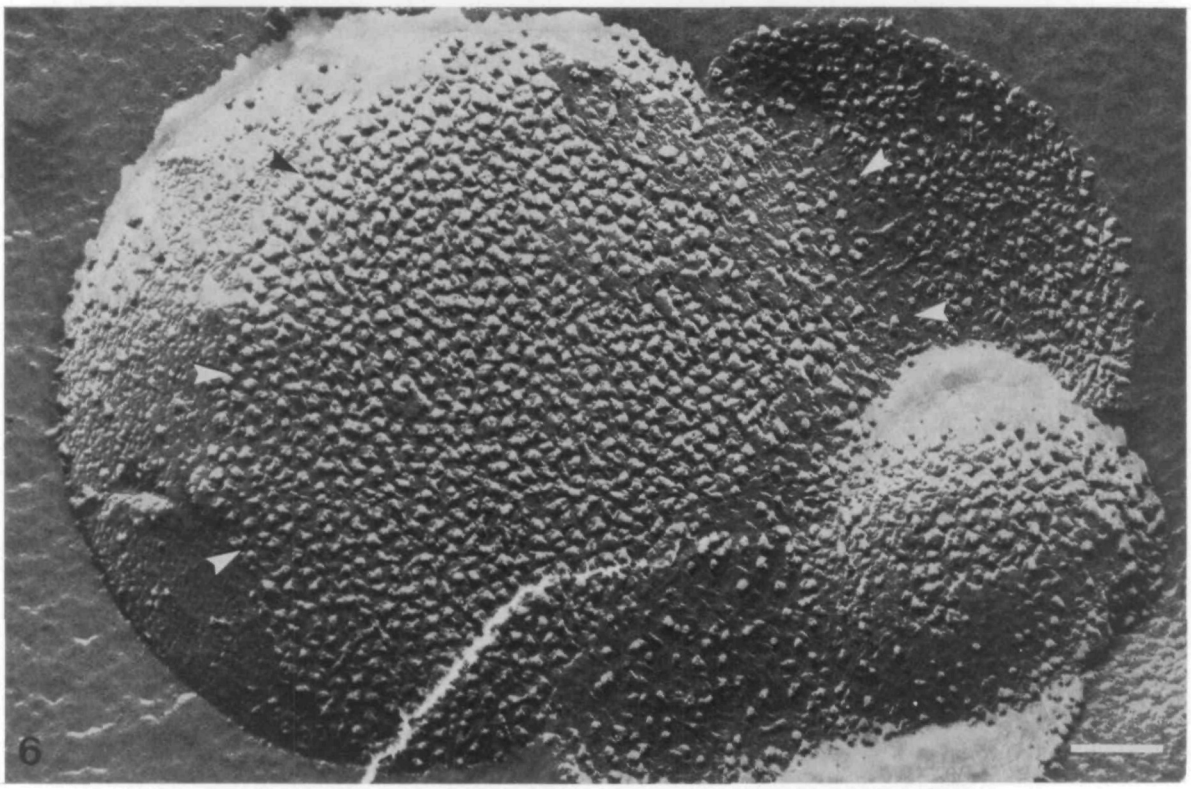
Figs 3, 4 show very clearly that *Prochlorothrix*, like other chlorophyll *b*-containing photosynthetic organisms, displays four distinct thylakoid membrane fracture faces. The association of four thylakoid fracture faces with membrane stacking was first established for *Chlamydomonas* by Goodenough & Staehelin (1970), and has been confirmed in photosynthetic organisms ranging from green plants to cryptophyte algae (Staehelin, 1986). Therefore, we believe that the observation of four fracture faces in *Prochlorothrix* confirms the existence of true thylakoid membrane stacking in this prokaryote.

This conclusion is supported by Figs 5, 6, which show that changes in fracture faces occur precisely at the boundary between stacked and non-stacked membrane regions. While it is possible to argue that one should not infer true membrane stacking in a prokaryotic system merely by analogy to higher plants, Figs 5, 6 show that EF_s and PF_s fracture faces in *Prochlorothrix hollandica* are directly associated with stacked regions of the membrane system. The morphological evidence therefore leads to the conclusion that *Prochlorothrix hollandica* thylakoid membranes display true stacking.

Similarities and differences between Prochlorothrix *and higher plant thylakoids*

The fracture faces of *Prochlorothrix* thylakoid mem-





Figs 5, 6. The association between thylakoid stacking and fracture faces is shown in these freeze-fracture micrographs of isolated *Prochlorothrix hollandica* thylakoid membranes. In each case, the change from unstacked (EF_u, PF_u) fracture faces to stacked (EF_s, PF_s) occurs at the exact point where two adjacent membranes make contact. In these micrographs the edges of stacked membrane regions are indicated by arrowheads. The appression of two membranes occurs at the exact point where each fracture face changes from the unstacked to the stacked form. Images such as these allow an unambiguous connection to be made between membrane appression, true stacking, and each of the four characteristic fracture faces. Bar, 0·1 μm.

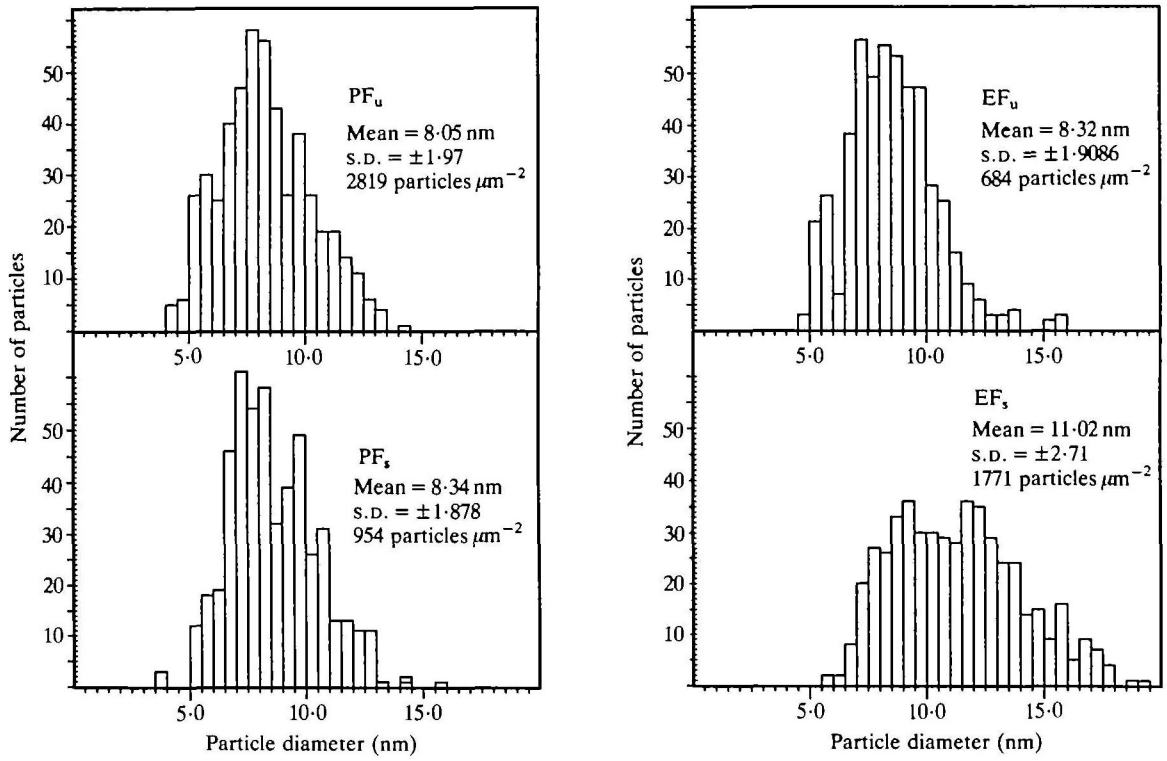


Fig. 7. Particle size measurements on the four *Prochlorothrix* fracture faces, expressed in histogram form. Also included, for each fracture face, is information on the mean and standard deviation of measurements for each fracture face, as well as the distribution density of particles on each fracture face.

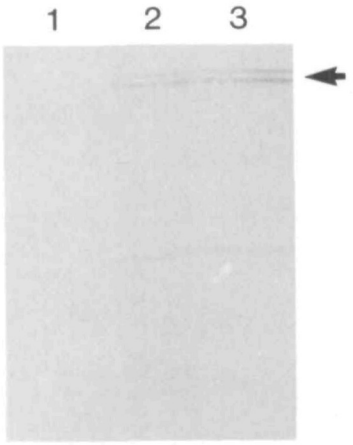


Fig. 8. To ensure that coupling factor proteins were still present on membranes prepared for deep-etching, immunoblots were carried out using an antibody prepared against the beta subunit of the coupling factor. Lane 1 contained membranes washed in distilled water, lane 2 membranes washed in dilute buffer, and lane 3 membranes washed in isolation buffer. A pair of immunoreactive bands (large arrow) are present in lanes 2 and 3, indicating that two polypeptides (possibly the alpha- and beta-subunits of *Prochlorothrix* coupling factor) cross-react with coupling factor antibodies. These immunoblots illustrate that the proteins that cross-react with the coupling factor are not removed from the thylakoid membrane by the washes in dilute buffer required to visualize membrane surfaces in deep-etching.

branes are similar to those described for higher plants, including spinach (*Spinacea oleracea*) (Staehelin, 1986). Similarities between spinach and *Prochlorothrix* E fracture faces are striking: in each case a dense population of large intramembrane particles is found in the stacked membrane regions, and far fewer particles are visible in the non-stacked regions. In contrast, the P fracture faces of spinach and *Prochlorothrix* seem to differ, principally in the stacked regions of the thylakoid system. *Prochlorothrix* PF$_s$ fracture faces contain fewer particles than do their counterparts in spinach, while fracture faces from non-stacked regions seem more similar.

Particle size measurements carried out on *Prochlorothrix* thylakoid fracture faces (Fig. 7) indicate that intramembrane particles on these fracture faces are somewhat smaller than those found in higher plants (Staehelin, 1986). We did not find evidence for distinct size classes of particles on the EF$_s$ fracture face, as reported by Giddings *et al.* (1980) for *Prochloron*, despite the fact that the overall histogram of particle size distributions in our study are quite similar to those reported for *Prochloron*. This may reflect the subjectivity associated with such measurements, and thus we do not see clear evidence in our study or in the earlier work (Giddings *et al.* 1980) for distinct size classes within this fracture face. *Prochlorothrix* and *Prochloron* are the only known prochlorophyte species, and the fact that fracture face structures in these two organisms are quite similar suggests that the sorts of features reported here will be typical for other prochlorophyte species yet to be discovered.

While another group has noted some differences in

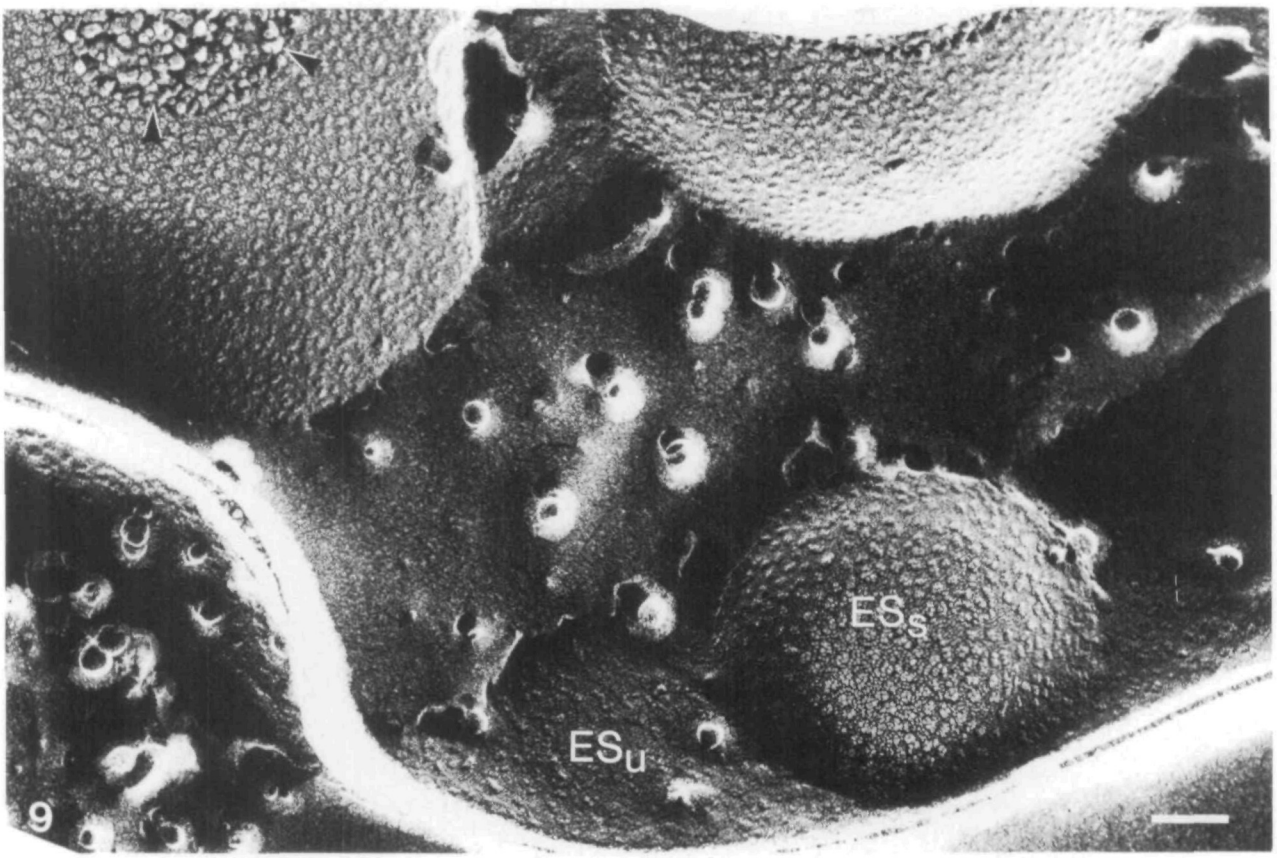


Fig. 9. Freeze etched inner surface of the *Prochlorothrix* thylakoid. Both fractured (PF) and etched surfaces (ES_s and ES_u) are visible. The stacked regions of the inner thylakoid surfaces are covered with tetrameric particles. These particles are largely concentrated into stacked (ES_s) regions, and non-stacked regions (ES_u) can easily be distinguished by virtue of the fact that they contain few such particles. The arrowheads mark a region where the thylakoid membrane has been split open to reveal internal fracture faces. Bar, 0·1 μm.

fracture face structure between the *Prochloron* sp. membranes obtained from *Diplosoma virens* (Giddings *et al.* 1980) and *Prochloron* sp. membranes isolated from *Didemnum molle* (Cox & Dwarte, 1981), some of these differences may result from the length of time required to prepare specimens in the latter study. In the research reported here, the fact that *Prochlorothrix* can be grown directly in the laboratory has made it possible to prepare membrane specimens directly from living cells. Other investigators have not supported Cox & Dwarte's observations of EF_1 and EF_2 fracture faces in *Prochloron* sp., and we find no evidence for such fracture faces in *Prochlorothrix hollandica*. Because our results agree generally with those of Giddings *et al.* (1980), we support Staehelin's (1986) explanation of the results obtained by Cox & Dwarte (1981).

In other systems, techniques of physical and biochemical fractionation have been applied to show that the thylakoid membranes also display differences in the distribution of membrane complexes that parallel the structural differences between stacked and nonstacked thylakoids. These include a preferential localization of photosystem I (Andersson & Anderson, 1980) and the ATP synthetase (Miller, 1980) to non-stacked regions, and localization of photosystem II (Andersson & Anderson, 1980) and the chlorophyll *a*/*b* light-harvesting complex (Andersson & Anderson, 1980; Kyle *et al.* 1983) to stacked regions. Efforts at fractionating the thylakoids of *Prochlorothrix* may yield similar data.

Walsby (1986) has already pointed out some of the implications that studies of *Prochlorothrix* may have for understanding the evolutionary origins of chloroplasts and their relationships to photosynthetic prokaryotes. However, we believe that this analysis (Walsby, 1986) understates the importance of thylakoid stacking in this group by suggesting that membrane stacking may be a 'quasi-mechanical' consequence of the presence of chlorophyll *b* and the absence of phycobilisome structures. Mutants of barley (Miller *et al.* 1976) and clover (Nakatani & Baliga, 1985) have been investigated, which display extensive thylakoid stacking in the absence of chlorophyll *b*, and therefore we believe that membrane stacking reflects a more substantial specialization of membrane components than Walsby (1986) has suggested. This view has been supported by Cox (1986), who has noted that a central issue in understanding the importance of *Prochlorothrix* is determining whether its thylakoid membranes are stacked in the same sense as those of green plants. The evidence presented here, from freeze fracture and freeze etch data, clearly establishes the fact that *Prochlorothrix* thylakoids form true stacked regions.

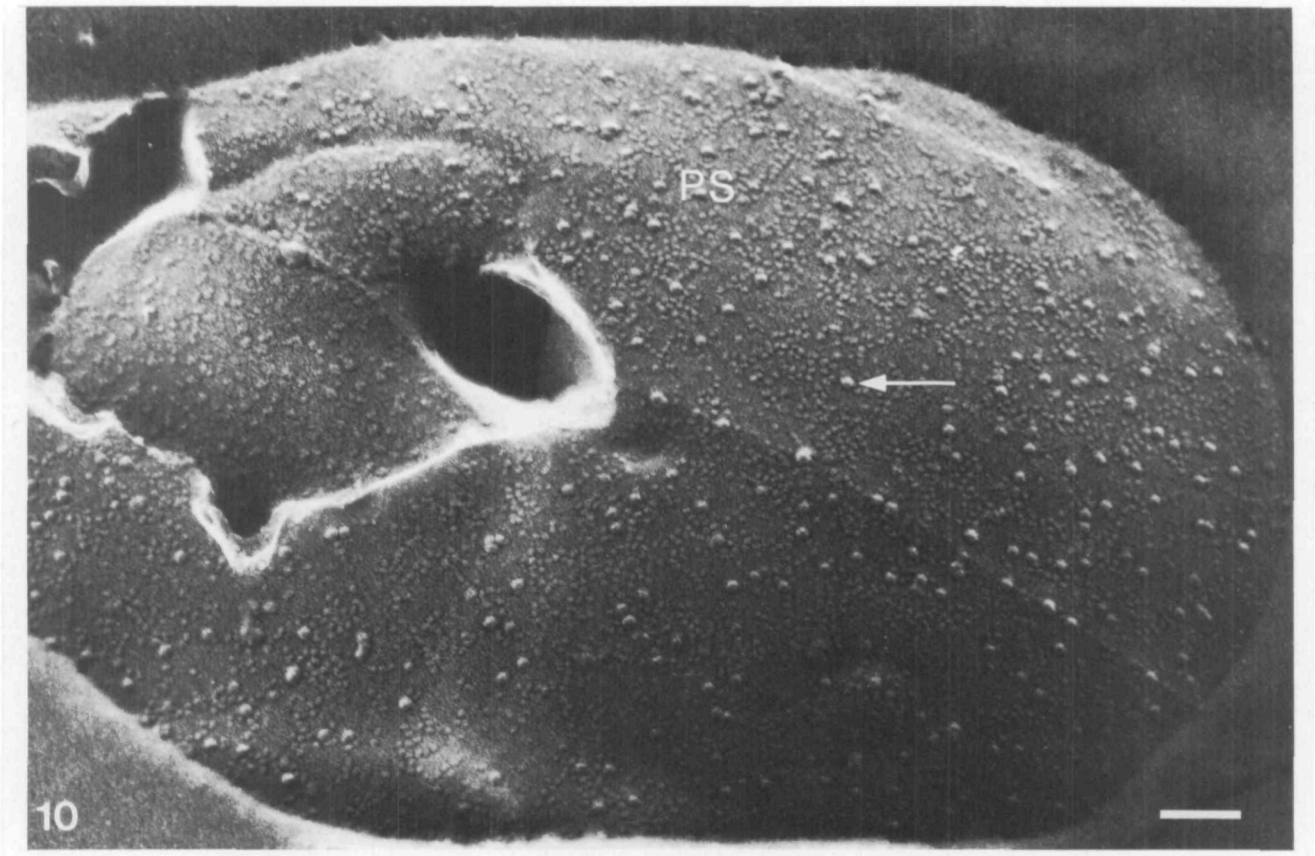


Fig. 10. The freeze etched outer (PS) surface of the *Prochlorothrix* thylakoid. In this procedure, quick-frozen samples of membranes are fractured and then etched to display the true outer surface of the thylakoid membrane. The surface contains both larger (>10·0 nm, one such particle is indicated by an arrow) and smaller (<10·0 nm) particles. The smaller particles are occasionally arranged into regular lattices. Although no biochemical identification of either structure has been made in this organism, by analogy with green plants the larger particles may be ATP synthetase enzymes. Bar, 0·1 μm.

Membrane structure and light-harvesting complexes

A recent study on the polypeptide composition of *Prochlorothrix* thylakoid membranes (Bullerjahn *et al.* 1987) reported the existence of at least five chlorophyll–protein complexes after non-denaturing electrophoresis. One of these complexes (which these authors called CP1) contained a photosystem I reaction centre, and immunological evidence suggested that another (CP4) contained the photosystem II reaction centre. However, immunological studies failed to find any similarities between any of the remaining complexes (CP2,3,5) and the light-harvesting chlorophyll *a*/*b* complex of green plants, sometimes known as LHC-II (Bullerjahn *et al.* 1987). The absence of such a light-harvesting complex may be related to the differences between freeze fracture images of *Prochlorothrix* and green plants. Simpson (1979) has suggested that the LHC-II of green plants forms the majority of particles on the PFs fracture face. It is the PF_s fracture face on *Prochlorothrix* that displays the greatest degree of difference from similar fracture faces in green plants, and the fact that *Prochlorothrix* lacks a LHC-II comparable to that of green plants may explain this structural difference.

Inner surface tetramers

The tetrameric particles found on the inner surfaces of thylakoid membranes in green plants and many eukaryotic algae have now been associated with some of the proteins that make up the oxygen-producing system of the thylakoid (Simpson & Andersson, 1986). When these workers treated inverted membranes with salt washes, which remove specific membrane polypeptides associated with oxygen production, the tetramers lost much of their external mass and visibility on the membrane surface (Simpson & Andersson, 1986).

While we have yet to make any biochemical inferences as to their nature, the great structural similarities between *Prochlorothrix* tetramers and those from green plants (Fig. 9) suggest that these structures are very similar, if not identical, in the two widely separated groups. The fact that *Prochlorothrix* tetramers, like those of green plants and green algae (Staehelin, 1986) are concentrated in stacked membrane regions, lends greater support to this idea. The discovery of an inner surface tetramer in *Prochlorothrix* lends credence to the suggestion that prochlorophytes may be related to the ancestors of eukaryotic chloroplasts.

Prochlorothrix *as a model system for the chloroplast membrane*

The structural similarities between *Prochlorothrix* thyla-

koid membranes and those of green plants are striking. The similarities are especially pronounced when thylakoid inner and outer surfaces are examined, something that was not possible in previous studies of Prochloron because of material size limitations. *Prochlorothrix* membranes display true membrane stacking, lateral heterogeneity, and contain both internal and surface structures, which are very similar to those found in green plants.

Despite these similarities, the high chlorophyll *a*/*b* ratios (Burger-Wiersma *et al.* 1986), and lack of a light-harvesting complex closely related to the LHC-II of higher plants in *Prochlorothrix* indicate that there are genuine differences between the thylakoid membranes found in this prokaryote and those of eukaryotic chloroplasts. Nonetheless, by carefully examining the characteristics that these organisms share with chloroplasts, we may hope to learn more about the evolution and development of photosynthetic organisms.

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References

ALLEN, M. M. (1968). Simple conditions for growth of unicellular blue-green algae on plates. *J Phycol.* **4**, 1–4.

ANDERSON, J. M. & ANDERSSON, B. (1982). The architecture of photosynthetic membranes: lateral and transverse. *Trends Biochem. Sci.* **8**, 288–292.

ANDERSSON, B. & ANDERSON, J. M. (1980). Lateral heterogeneity in the distribution of the chlorophyll-protein complexes of the thylakoid membranes of spinach chloroplasts. *Biochim. biophys. Acta.* **593**, 427–440.

BARBER, J. (1987). Rethinking the structure of the photosystem two reaction centre. *Trends Biochem. Sci.* **12**, 123–124.

BRANTON, D., BULLIVANT, S., GILULA, N. B., KARNOVSKY, M. J., MOOR, H., MUHLETHALER, K., NORTHCOTE, D. H., PACKER, L., SATIR, B., SATIR, P., SPETH, V., STAEHELIN, L. A., STEERE, R. L. & WEINSTEIN, R. S. (1975). Freeze-etching nomenclature. *Science* **190**, 54–56.

BULLERJAHN, G. S., MATTHIJS, H. C. P., MUR, L. C. & SHERMAN, L. A. (1987). Chlorophyll-protein composition of the thylakoid membrane from *Prochlorothrix hollandica*, a prokaryote containing chlorophyll *b*. (in press).

BURGER-WIERSMA, T., VEENHUIS, M., KORTHALS, H. J., VAN DE WIEL, C. C. M. & MUR, L. R. (1986). A new prokaryote containing chlorophylls *a* and *b*. *Nature, Lond.* **320**, 262–264.

COX, G. (1986). The origins of chloroplasts in eukaryotes. *Nature, Lond.* **322**, 412.

COX, G. & DWARTE, D. M. (1981). Freeze etch ultrastructure of a *Prochloron* species – the symbiont of *Didemnum molle*. *New Phytol.* **88**, 427–443.

GIDDINGS, T. H., WITHERS, N. W. & STAEHELIN, L. A. (1980). Changes in the thylakoid structure of stacked and unstacked regions of *Prochloron sp.*, a prokaryote. *Proc. natn. Acad. Sci. U.S.A.* **77**, 352–356.

GOODENOUGH, U. W. & STAEHELIN, L. A. (1970). Structural differentiation of stacked and unstacked chloroplast membranes. Freeze-etch electron microscopy of mutant and wild-type strains of *Chlamydomonas*. *J. Cell Biol.* **48**, 594–619.

KYLE, D. J., STAEHELIN, L. A. & ARNTZEN, C. J. (1983). Lateral mobility of the light-harvesting complex in chloroplast membranes controls excitation energy distribution in higher plants. *Arch. Biochem. Biophys.* **222**, 527–541.

LEWIN, R. A. (1975). A marine *Synechocystis* (Cyanophyta, Chroococales) epizoic on ascidians. *Phycologia* **14**, 153–160.

MILLER, K. R. (1980). Freeze-etching studies of chloroplast membranes. In *Electron Microscopy in Biology*, vol. 1 (ed. J. D. Griffith), pp. 1–30. New York: Wiley-Interscience.

MILLER, K. R., MILLER, G. J. & MCINTYRE, K. R. (1976). The light-harvesting chlorophyll-protein complex of photosystem II: its location in the photosynthetic membrane. *J. Cell Biol.* **71**, 624–638.

MILLER, K. R. & STAEHELIN, L. A. (1976). Analysis of the thylakoid outer surface: Coupling factor is limited to unstacked membrane regions. *J. Cell Biol.* **68**, 30–47.

NAKATANI, H. Y. & BALIGA, V. (1985). A clover mutant lacking the chlorophyll a- and b-containing protein antenna complexes. *Biochem. biophys. Res. Commun* **131**, 182–189.

PATTERSON, G. M. & WITHERS, N. W. (1982). Laboratory cultivation of *Prochloron*, a tryptophan auxotroph. *Science* **217**, 1934–1935.

SIMPSON, D. J. (1979). Freeze-fracture studies on barley plastid membranes. II. Location of the light-harvesting chlorophyll protein. *Carlsberg Res. Commun.* **44**, 305–336.

SIMPSON, D. J. & ANDERSSON, B. (1986). Extrinsic polypeptides of the chloroplast oxygen evolving complex constitute the tetrameris ESs particles of higher plant thylakoids. *Carlsberg Res. Commun* **51**, 467–474.

STAEHELIN, L. A. (1986). Chloroplast structure and supramolecular organization of photosynthetic membranes. In *Photosynthesis III. Photosynthetic membranes and light harvesting systems* (ed. L. A. Staehelin & C. J. Arntzen), pp. 1–83. Munich: Springer-Verlag.

WALSBY, A. E. (1986). Origins of chloroplasts. *Nature, Lond.* **320**, 212.

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