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A Freeze-Fracture Study of Cryptomonad Thylakoids

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Summary

The thylakoids of two cryptomonads, *Chroomonas* sp. and *Cryptomonas* sp., were examined in thin sections and freeze-fracture preparations. The thylakoids of both species were organized into stacks of typically 2 thylakoids, with regions of adhesion evident between adjacent membranes. The stacked membranes had a higher EF particle density (EFs face) than unstacked membranes (EFu face). However, no differences in size between EFs and EFu particles was observed; both faces had 15 nm particles. Two types of PF faces were observed in stacked membranes, a PFs face and a PFs_1 face. The PFs_1 face was identical to the PFu face. These results suggest that there is partial segregation of photosystem II into stacked membranes and photosystem I into unstacked membranes. However, the extent to which segregation occurred varied between the two species, as indeed did other aspects of thylakoid organization.

Keywords: *Cryptophyceae*; Freeze-fracture; Thylakoids; *Chroomonas*; *Cryptomonas*.

1. Introduction

The *Cryptophyceae* are unicellular algae which form a very distinct taxonomic group. They can be clearly differentiated from other algae on the basis of their periplast, ejectosome and flagellar structure and in possessing a nucleomorph (GREENWOOD 1974). However, it is the uniqueness of their chloroplast which has generated much speculation about the phylogenetic association between the cryptomonads and other algae. The cryptomonad chloroplasts possess both a unique combination of photosynthetic pigments and thylakoid structure. Their main photosynthetic pigments are chlorophyll *a*, chlorophyll *c* [usually present as chlorophyll c_2 only (JEFFREY 1976)] and biliproteins (HAXO and FORK 1959), thus making them the only organisms which contain both chlorophyll *c* and biliproteins. The cryptomonads also uniquely locate their biliproteins in the intrathylakoid space (GANTT *et al.* 1971, DWARTE and VESK 1982 a), with the other biliprotein-containing organisms, the red algae and cyanobacteria (blue-green algae), having them localized in phycobilisomes attached to the stroma side of the thylakoid membranes.

Virtually no information is known about the organisation of the photosynthetic apparatus within the thylakoids of the cryptomonads. Freeze-fracture techniques have been successfully used to study the photosynthetic organisation in higher plants *e.g.*, spinach (STAEHELIN 1976) and peas (ARMOND *et al.* 1977); green algae *Chlamydomonas* (GOODENOUGH and STAEHELIN 1971); euglenoids, *e.g.*, *Euglena gracilis* (MILLER and STAEHELIN 1973); prochlorons (GIDDINGS *et al.* 1980, COX and DWARTE 1981); red algae, *e.g.*, *Porphyridium purpureum* (NEUSHUL 1970) and *Bangia* (BISALPUTRA and BAILEY 1973); cyanobacteria, *e.g.*, *Synechococcus lividis* (GOLECKI 1979); and a dinoflagellate *Gonyaulax polyedra* (SWEENEY 1981). There has, as yet, been no detailed freeze-fracture study of the thylakoids of the cryptomonads, although LICHTLÉ and THOMAS (1976) have published one micrograph of a freeze-fractured thylakoid of a *Cryptomonas* sp.

This study presents results of the thylakoid structure of two cryptomonads, *Chroomonas* sp. and *Cryptomonas* sp., using both thin sectioning and freeze-fracture procedures.

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2. Materials and Methods

The original cultures were obtained from the CSIRO Division of Fisheries and Oceanography, Cronulla, N.S.W., Australia. The species investigated were *Chroomonas* sp. and *Cryptomonas* sp. (CSIRO culture collection numbers CS-48 and CS-85 respectively).

2.1. Thin Sectioning

Cells were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) plus 0.3 M sucrose for 1 hour, then rinsed 3 × in the same buffer. They were then post-fixed for 1 hour in 1% OsO_4 in 0.1 M phosphate buffer (pH 7.2), dehydrated in acetone and embedded in vinyl cyclohexene dioxide/DER 736/nonenyl succinic anhydride resin. Silver interference colored sections were cut using a diamond knife on an LKB Ultramicrotome IV. Sections were stained in uranyl acetate followed by lead citrate.

2.2. Freeze-Fracture

Cells were concentrated by mild centrifugation then immediately frozen (unfixed and unglycerinated) on gold discs in liquid nitrogen cooled Freon 22. They were fractured in a Balzers BAF 300 Freeze-etch machine at −100 °C. Replicas were produced by shadowing at 45° with 2.5 nm of Pt-C followed by vertical evaporation of 15 nm of C. The replicas were cleaned for 2 hours in 25% chromic acid then transferred to 50% chromic acid overnight, rinsed 2 × in distilled water and picked up on non-coated 600 mesh Gilder Grids (G 600 HH).

The terminology of BRANTON *et al.* (1975) is used for fracture face identification. The thylakoid fracture face closest to the chloroplast stroma is designated the PF face, with the thylakoid fracture face closest to the intrathylakoid space the EF face. STAEHELIN's (1976) terminology for labelling fracture faces from stacked and unstacked regions is used. Fracture faces from stacked regions will be designated PFs or EFs, those from unstacked regions PFu or EFu.

2.3. Electron Microscopy

Both freeze-fracture replicas and thin sections were examined in a Philips EM 400 electron microscope at 100 kV. The microscope magnification was calibrated with cross-grating replicas. All micrographs were taken with the specimen height adjusted to the eucentric position and after pressing the normalization button. Freeze-fracture micrographs for particle size and density measurements were taken at × 32,000 and enlarged to × 100,000. Particle size measurements were measured at right angles to the direction of shadowing using a × 8 magnifier equipped with a graduated scale calibrated in 0.1 mm units.

3. Results

Cryptomonad thylakoids are organised into associations of 2 or more thylakoids. These associations will be referred to as stacks, since they display some similarities with stacks found in higher plants. Before continuing, an explanation of terminology is necessary so that our usage of the terms stacked and unstacked membranes, for the cryptomonads, is made clear. A stack will be defined as an association of 2 or more thylakoids in which adhesion is present in at least some areas of all adjacent membranes. A diagram of a typical cryptomonad thylakoid stack, of 2 thylakoids, is shown in Fig. 1. In a thylakoid stack of this type only the adjacent membranes can be considered as stacked (s), with the other 2 outer membranes being considered unstacked (u). The membranes of any free thylakoid

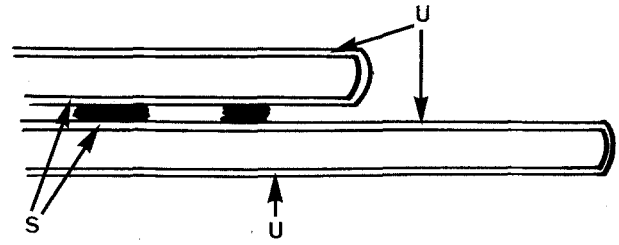

Fig. 1. Diagram of a typical 2-thylakoid stack in the cryptomonads. The adjacent membranes, *i.e.*, stacked membranes (*s*) contain regions of adhesion (electron-dense layer between membranes). The two outer membranes, as well as the membranes of free thylakoids, can be considered as unstacked (*u*)

will also be considered as unstacked. From this then it follows that for stacks consisting of 2 thylakoids 50% of the membranes will be stacked and 50% unstacked. The other point to note about cryptomonad thylakoid stacks is that the adjacent membranes, *i.e.*, stacked membranes, are not necessarily fused throughout their entire lengths (contrasting with the situation in higher plants). Thus cryptomonad thylakoid stacks produce a slightly different image to that found in higher plant chloroplasts that have undergone similar fixation and staining procedures, however by using the same terminology to that of higher plants direct comparisons between thylakoids can be more easily made.

Chroomonas and *Cryptomonas* thylakoids tend to be mainly stacked into pairs (Figs. 2 and 4). However, stacks of three (Figs. 3 and 6) or more thylakoids, which resemble grana (Fig. 5) can be found. Occasionally, the stacked thylakoids separate resulting in areas of free or unstacked thylakoids (Figs. 2 and 6).

A close examination of the stacked thylakoids reveals that although the membranes are free or nonadhering over large areas, regions where the membranes appear fused, *i.e.*, regions of membrane adhesion are evident (Figs. 3 and 6). The extent to which membrane adhesion occurs varies between the two species. In *Chroomonas* membrane adhesion is poorly developed, with regions of membrane adhesion being small, and dispersed randomly through the stacked membranes (Fig. 3). In *Cryptomonas* membrane adhesion is better developed, and hence more easily observed (Fig. 6). *Cryptomonas* shows regions of membrane adhesion of

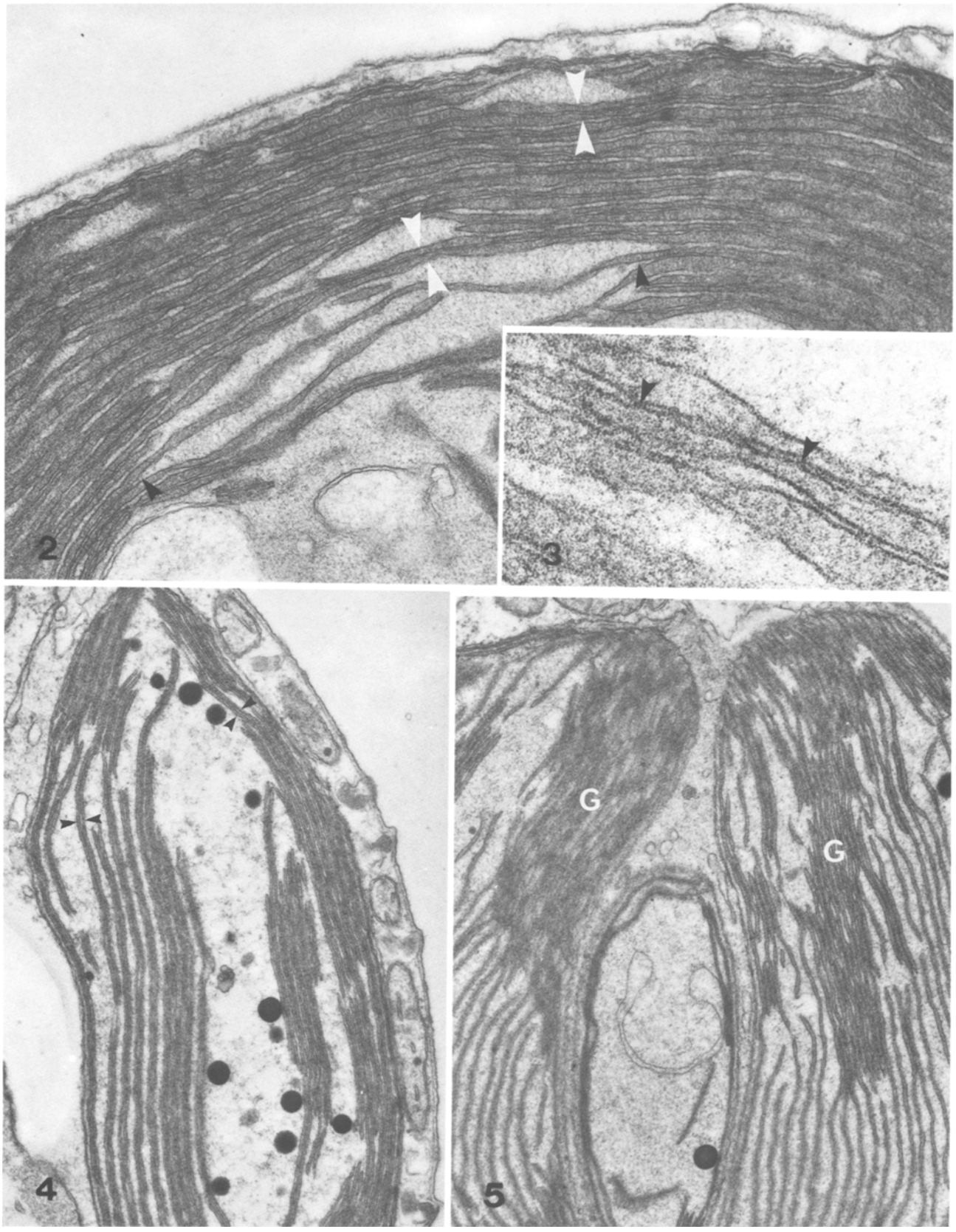

Fig. 2. Section through a chloroplast in *Chroomonas* showing the thylakoids stacked in pairs (white arrows). The black arrows indicate areas where the stacked thylakoids have separated. (× 48,000)

Fig. 3. High magnification of a 3-thylakoid stack in *Chroomonas*. Regions of membrane adhesion, as indicated by the presence of an electron-dense layer between the membranes, can be seen (arrows). (× 180,000)

Fig. 4. Section through a *Cryptomonas* chloroplast with arrows indicating the thylakoids stacked in pairs. (× 26,000)

Fig. 5. *Cryptomonas* thylakoids organized into large stacks which superficially resemble grana (*G*). (× 28,000)

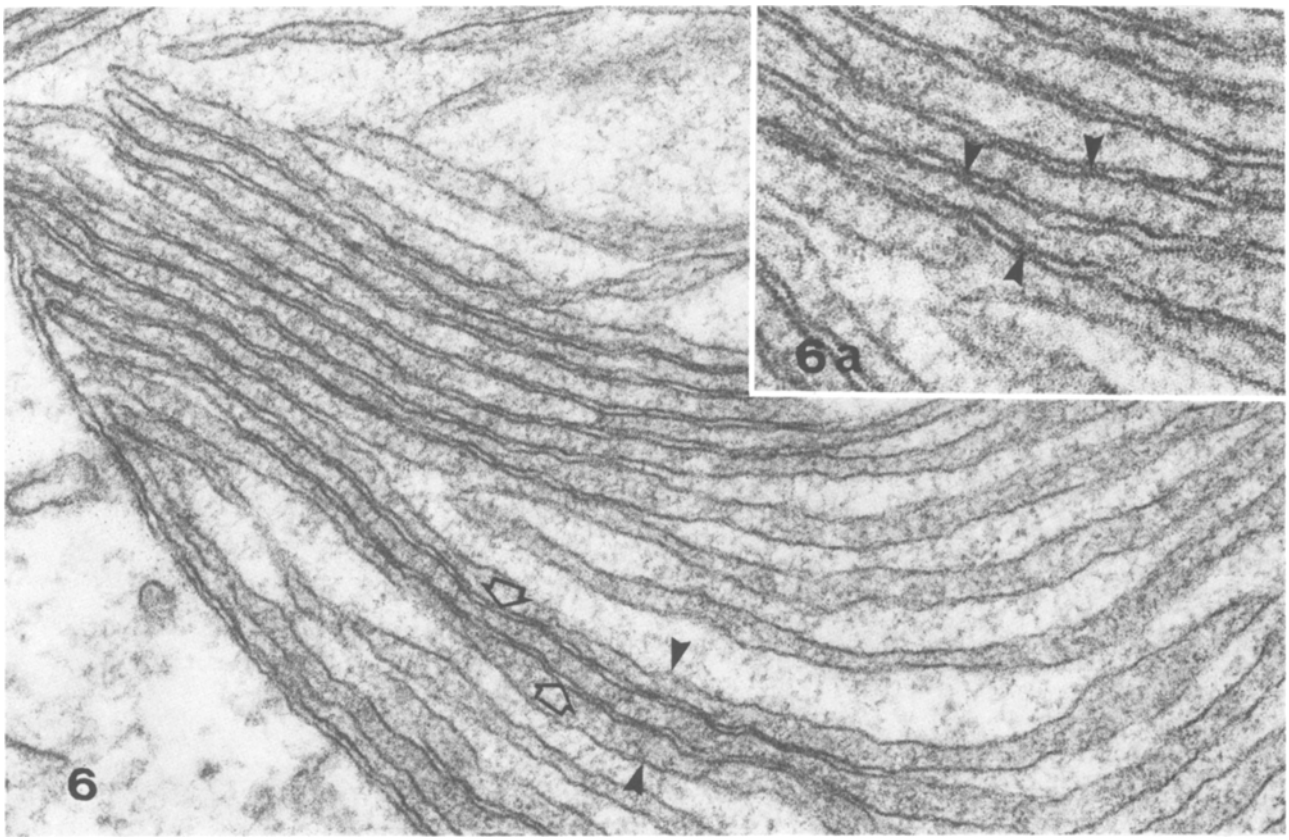

Fig. 6. *Cryptomonas* thylakoids organized into both stacked and unstacked regions. The black arrows indicate a 3-thylakoid stack. The stacked membranes (open arrows) have a greater electron-density than the unstacked membranes (black arrows). (× 108,000)

Fig. 6*a*. Higher magnification of Fig. 6 with the arrows indicating regions of membrane adhesion within a stacked region. (× 176,000)

variable size. Fig. 6 shows appressed regions of approximately 100 nm although larger appressed regions of up to 800 nm have been observed. Where membrane adhesion occurs an electron-dense layer can be observed between the membranes (Figs. 3 and 6). *Cryptomonas*, which has better developed membrane adhesion than *Chroomonas*, is also more likely to have stacks of three or more thylakoids. Stacks of more than two thylakoids are only very rarely observed in *Chroomonas*.

In *Cryptomonas*, the stacked membranes have a greater electron-density than the unstacked membranes (Fig. 6). Where the stacked thylakoids separate and become unstacked this increased electron-density ceases (Fig. 6). Stacked membranes with increased electron-density have not been observed in *Chroomonas*.

In freeze-fracture preparations good ultrastructural preservation of both species was obtained without the necessity for glutaraldehyde fixation and glycerol infiltration. In some cells ice crystal damage was apparent in the cytoplasm, but it was unusual for extensive ice crystal damage to occur in the chloroplasts. In the few instances where such damage was observed these cells were not used in the present study.

Fig. 7 is a diagram of a cryptomonad thylakoid stack,

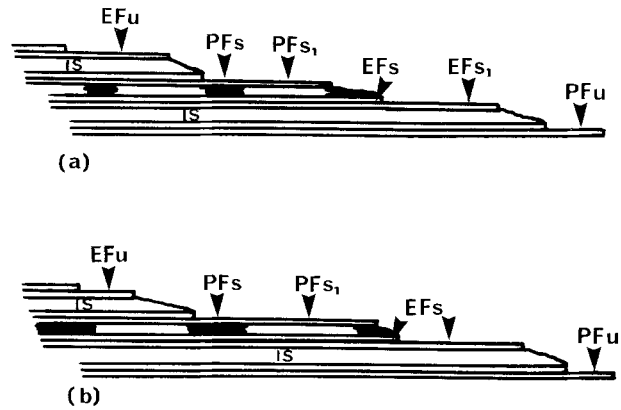

Fig. 7. Diagram of a cryptomonad 2-thylakoid stack showing the location of the different fracture faces (*IS*—intrathylakoid space). (*a*) *Chroomonas*; (*b*) *Cryptomonas*

Table 1. *Particle size and density measurements for EF and PF faces of Cryptomonas and Chroomonas (s – standard deviation)*

	Chroomonas Modal particle size	Particle density ± s	Cryptomonas Modal particle size	Particle density ± s
	(nm)	(μm^{-2})	(nm)	μm^{-2})
PFu	13	2,112 ± 288	14	2,390 ± 410
PFs_1	13	2,042 ± 262	14	2,420 ± 400
PFs	11	2,800 ± 409	11	3,500 ± 420
EFu	15	291 ± 124	15	112 ± 63
EFs_1	15	298 ± 108	not present	not present
EFs	15	825 ± 179	15	1,013 ± 205

consisting of two thylakoids, showing the various fracture faces.

A summary of the particle size and densities of the various fracture faces for *Cryptomonas* and *Chroomonas* is presented in Table 1. As can be seen from this table the *PF* faces have a higher particle density and smaller particle size than the *EF* faces.

Both cryptomonads displayed a non-uniform particle distribution for both *PF* and *EF* faces. In unstacked membranes, *Cryptomonas* and *Chroomonas* produce one type of *PF* fracture face (*PFu*) which had a high density of 14 nm (2,390 μm^{-2}) and 13 nm (2,112 μm^{-2}) particles respectively (Figs. 8, 9, 10, and 11). In stacked membranes, however, two distinct *PF* fracture faces were evident. First, a PFs_1 face which appeared identical to the *PFu* face (Figs. 10 and 12). Second, small, discrete regions of *PFs* were found to have smaller particles (11 nm) occurring at an even higher density (*Cryptomonas* – 3,500 μm^{-2}; *Chroomonas* – 2,800 μm^{-2}) than the *PFu* face (Figs. 10, 11, and 12). The particle size histograms for the *PFu* and *PFs* faces for both cryptomonads are shown in Fig. 16. The PFs_1 face is not shown since it is virtually identical to the *PFu* face. It can be seen that the particle size distributions typically have a single peak. The exception to this is the *PFu* face in *Chroomonas*, which shows a secondary 15 nm peak.

Both cryptomonads produce only one type of *EF* face (*EFu*) in unstacked membranes (Figs. 11, 13, and 14). The *EFu* face in both species has a low density of 15 nm particles. In *Cryptomonas* the *EFu* particle density was 112 μm^{-2} while in *Chroomonas* it was 291 μm^{-2}. *Chroomonas* displayed two fracture faces in stacked regions (Fig. 15), an EFs_1 face which was identical to the *EFu* face and an *EFs* face. The *EFs* face had particles of similar size to the *EFu* face, *i.e.*, 15 nm, but had a greater particle density (825 μm^{-2}). The *EFs* face occurred in discrete regions, in stacked membranes of variable size less than 0.1 μm^{-2} (Figs. 11 and 15). The stacked membranes in *Cryptomonas* only had one type of fracture face (*EFs*), no EFs_1 type faces were ever observed (Fig. 13). The *EFs* face had a density of 1,013 μm^{-2} of 15 nm particles. An examination of the particle size histograms of the *EF* faces shows that they all had only a single peak (Fig. 17). No difference between *EFs* and *EFu* particle sizes were found for either cryptomonad. The *EF* particle size distribution is skewed towards the right of the mode for *Chroomonas* and towards the left for *Cryptomonas*.

4. Discussion

The thylakoids of both *Cryptomonas* and *Chroomonas* are organized into stacks of typically two thylakoids, although larger stacks are not uncommon. The outer, single membranes of the stacks, *i.e.*, unstacked thylakoid membranes, produced different freeze-fracture faces to the stacked thylakoid membranes. This lateral spatial segregation of particles suggest a degree of regional thylakoid specialization which has certain similarities with other thylakoids (more will be said on this later).

The stacked membranes of *Cryptomonas* and *Chroomonas* show regions of membrane adhesion which can be identified by the presence of an electron-dense layer between the membranes. Although areas of membrane adhesion appear to be quite small we feel that they are not artefacts caused by sample preparation or simply local variations in the plane of the membrane within the section. On the contrary, normal chemical fixation

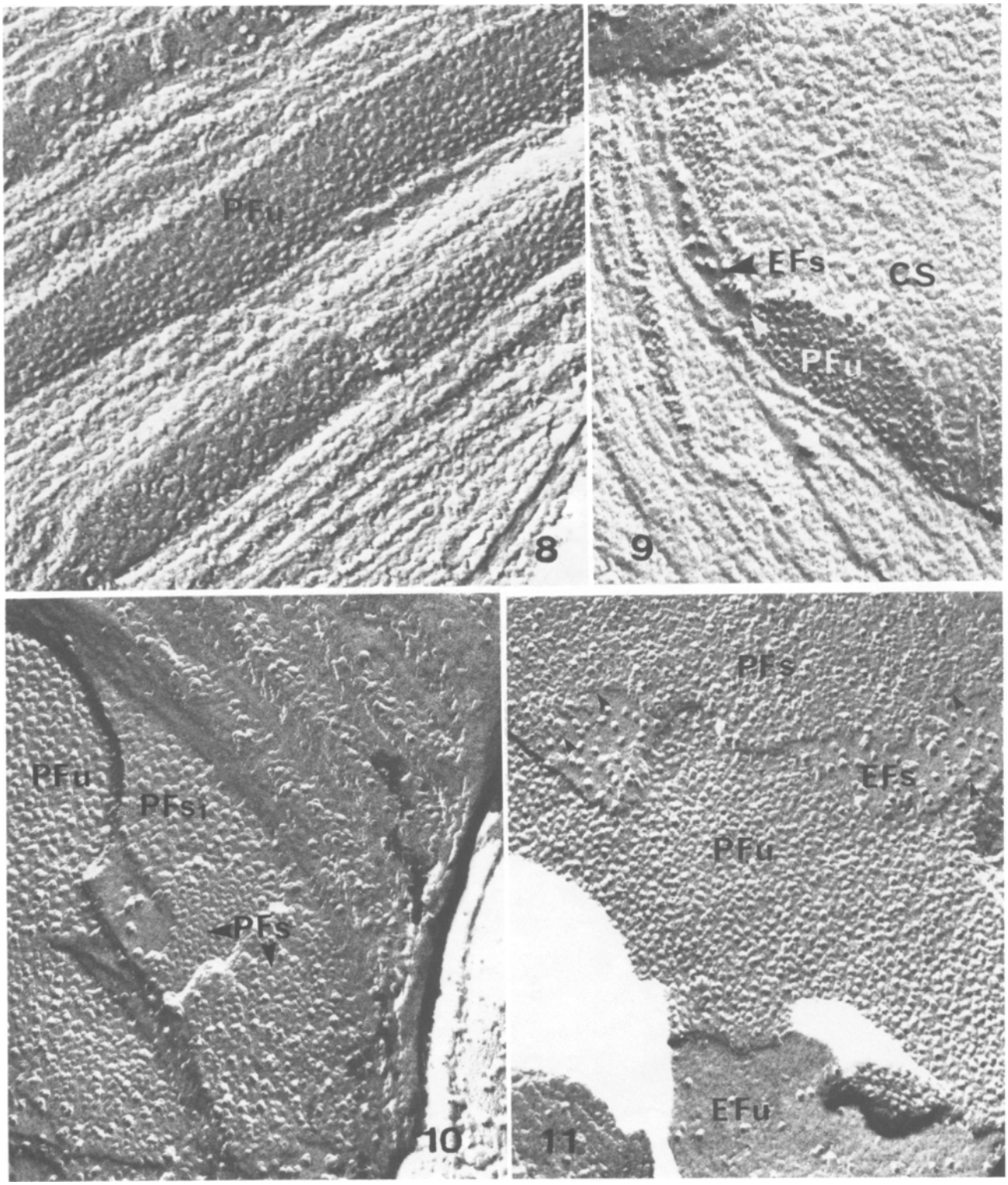

Fig. 8. Fracture through a *Cryptomonas* chloroplast. Most of the thylakoids have cross-fractured but large areas of the *PFu* face have been exposed which have a high density of 14 nm particles (× 100,000)

Fig. 9. The *PFu* face in *Chroomonas* can be recognized since it is the fracture face immediately adjacent to the chloroplast stroma (*CS*). A small area of *EFs* face can also be observed as the membrane immediately in front of the *PFu* face. The white arrows indicates the fracture step between the two faces (× 89,000)

Fig. 10. Fracture through a stacked region in *Chroomonas*. The *PFs* (11 nm particles) and PFs_1 (13 nm particles) faces can be readily differentiated. The PFs_1 and *PFu* faces, however, are identical. (× 80,000)

Fig. 11. A fracture through four membranes in *Chroomonas* revealing the *EFu* (low density of 15 nm particles), *PFu* (13 nm particles), *EFs* high density of 15 nm particles) and *PFs* (11 nm particles) faces. Where the shadowing is unfavourable it is difficult to see the steps between the membrane fracture faces (arrows). A close examination however reveals that the four fracture faces are indeed from four different membranes. (× 89,000)

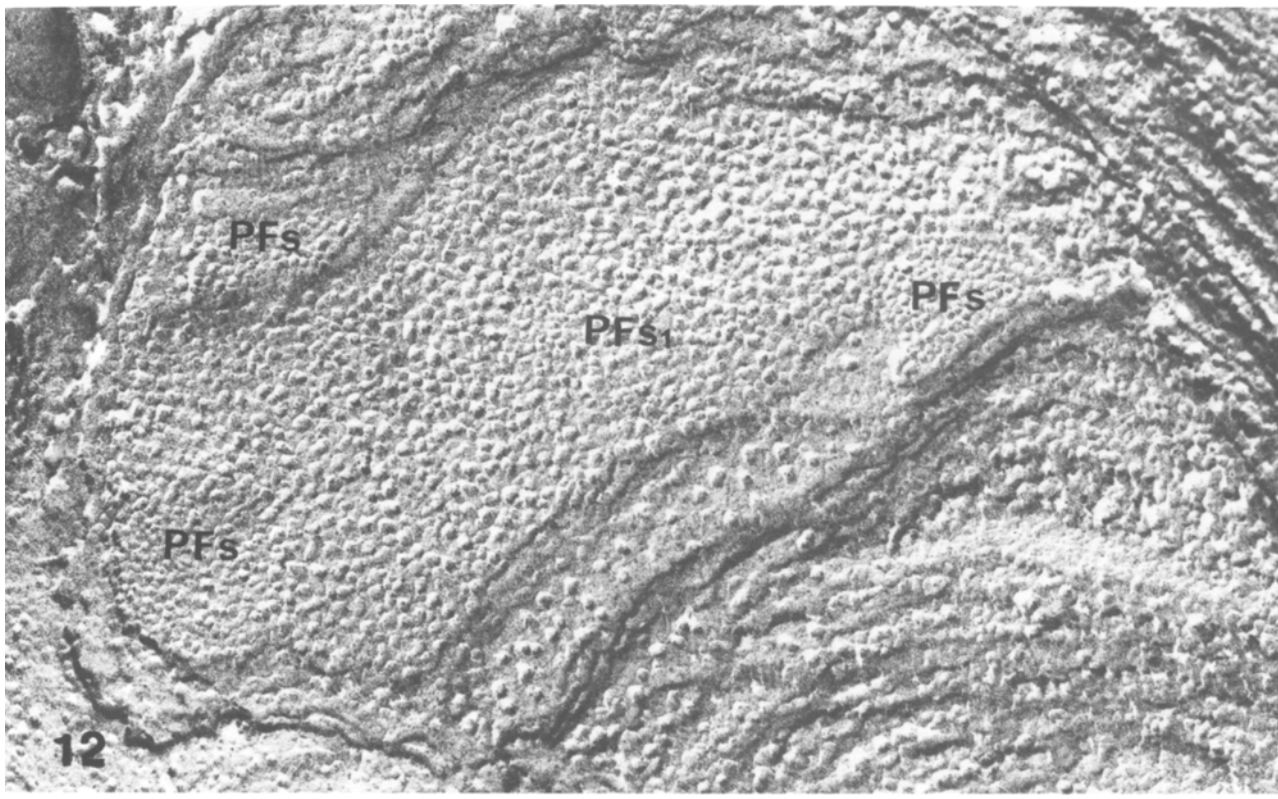

Fig. 12. Fracture through a stacked region in *Cryptomonas*. The micrograph shows a large fracture through the *PF* face showing both the *PFs* (11 nm particles) and PFs_1 (14 nm particles) faces. Smaller fractures of other *PFs* faces can be seen near the top left hand corner of the micrograph. ($\times$ 120,000)

Fig. 13. A region within *Cryptomonas* chloroplast in which the thylakoids are stacked in pairs. The fracture plane has revealed consecutive fractures through the *EF* faces (mainly cross-fracturing through the membranes that would reveal the *PF* faces) producing a sequence of *EFu* (low density), *EFs* (high density), *EFu* (low density), *EFs* (high density). The micrograph also shows an unstacked membrane (labelled *EFu*) which is continuous with two stacked membranes and is therefore similar to stroma thylakoid connections to stacked thylakoids observed in higher plants. ($\times$ 71,000)

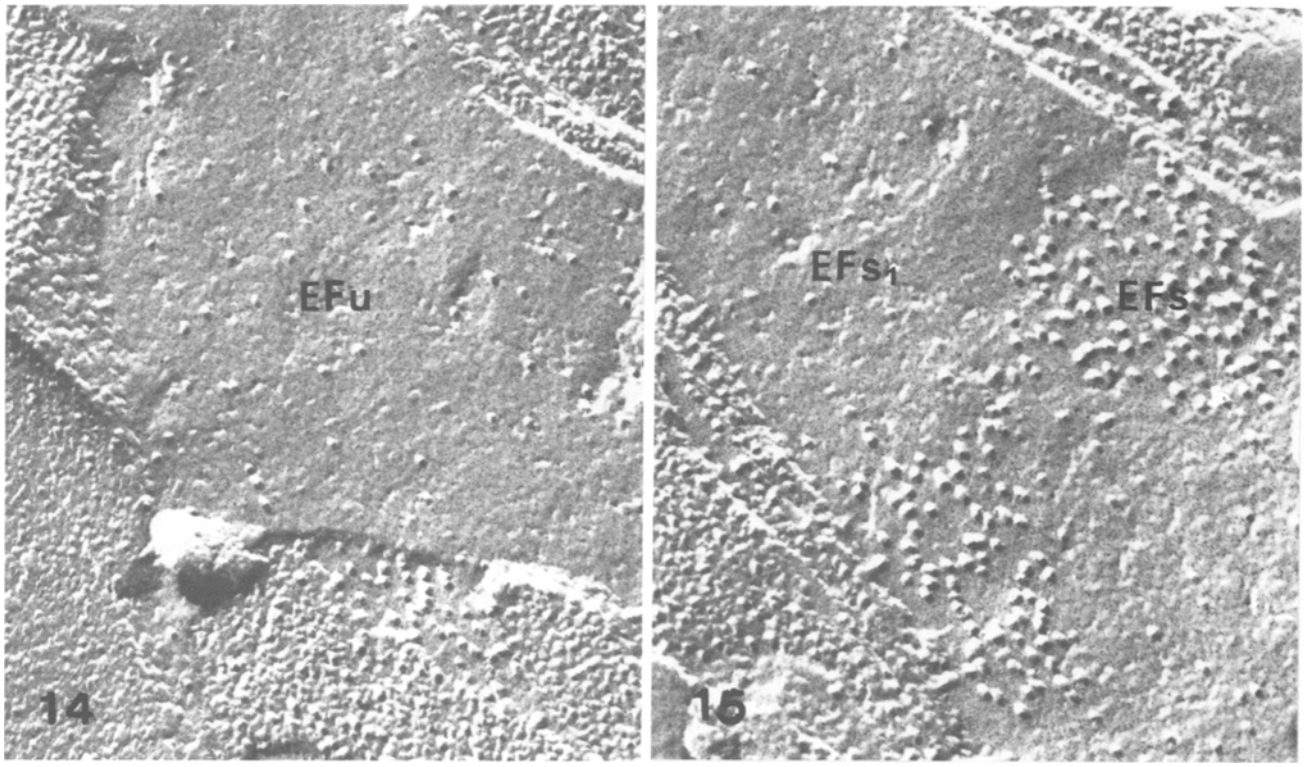

Fig. 14. The *EFu* face in *Chroomonas* showing a low density of 15 nm particles. (× 65,000)

Fig. 15. A fracture through the *EF* face of a stacked membrane in *Chroomonas*. The *EFs* (high density of 15 nm particles) and EFs_1 (low density of 15 nm particles) faces can be easily differentiated (× 95,000)

techniques may tend to obscure the observation of regions of membrane adhesion which can be more readily seen in material prepared by freeze-substitution (VESK, STONE, and DWARTE in preparation). An examination of micrographs in the literature showed that other cryptomonads also exhibited membrane adhesion. The extent to which membrane adhesion occurred varied between species, *e.g.*, *Chroomonas* sp. (a different species to the *Chroomonas* studied in this paper) (VESK and JEFFREY 1977) had only very small regions of membrane adhesion; *Cryptomonas rufescens* (LICHTLÉ 1978) had more extensive areas of adhesion, while in *Cryptomonas* sp. (GLAZER *et al.* 1971) membrane adhesion extended throughout the entire stacked region similar to that found in higher plants. It therefore appears likely that membrane adhesion and hence thylakoid stacks will be commonly and perhaps even universally, found in the cryptomonads.

There is now very good evidence for higher plants, cyanobacteria and red algae, that thylakoid *EF* particles are structurally equivalent to photosystem II complexes (ARMOND *et al.* 1977, GIDDINGS and STAEHELIN 1979, STAEHELIN *et al.* 1978). It therefore appears likely that cryptomonad *EF* particles also represent photosystem II complexes.

The stacked membranes in both *Cryptomonas* and *Chroomonas* had a higher *EF* particle density than the unstacked membranes. Therefore, the majority of the *EF* particles, and hence, photosystem II complexes, were segregated into stacked thylakoid membranes. This increased *EF* particle density in stacked membranes has been found in many organisms, including higher plants, *e.g.*, spinach (STAEHELIN 1976); barley (SIMPSON 1979); green algae, *e.g.*, *Chlamydomonas* (GOODENOUGH and STAEHELIN 1971); prochlorons (GIDDINGS *et al.* 1980, COX and DWARTE 1981) and a dinoflagellate *Gonyaulax polyedra* (SWEENEY 1981). Therefore, it seems quite common throughout the plant kingdom for *EF* particles (photosystem II complexes) to be spatially segregated into stacked thylakoid membranes. The aggregation of photosystem II into stacked membranes will allow increased energy transfer between photosystem II units.

The stacked thylakoid membranes in *Chroomonas* contained many areas with few *EF* particles (the EFs_1 face), which were indistinguishable from the *EFu* face.

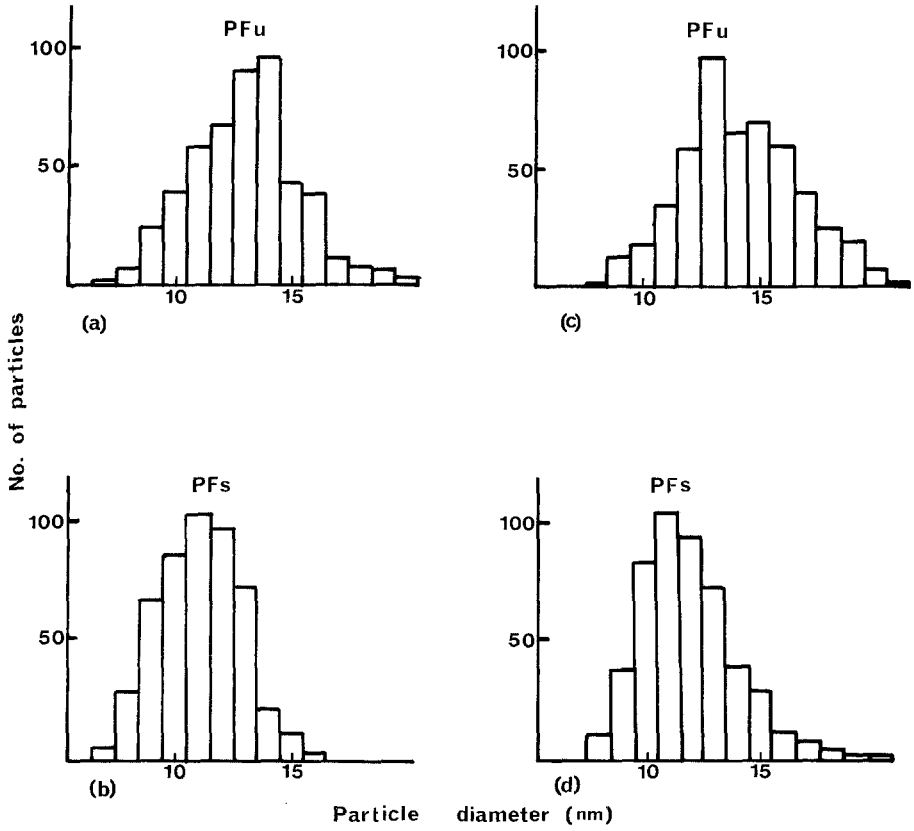

Fig. 16. Histograms of particle diameters on the *PF* faces of *Cryptomonas* (*a*)-(*b*) and *Chroomonas* (*c*)-(*d*). All histograms were based on a sample size of 500 particles

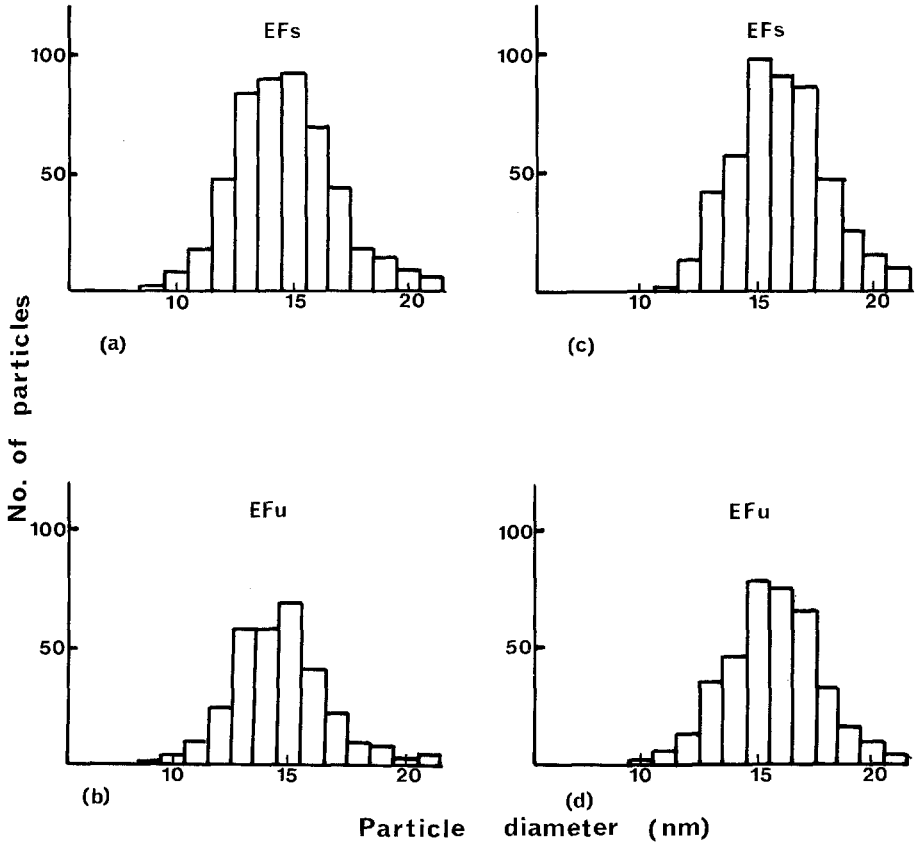

Fig. 17. Histograms of particle diameters on the *EF* faces of *Cryptomonas* (*a*)-(*b*) and *Chroomonas* (*c*)-(*d*). The histograms of the *EFs* faces, *i.e.*, (*a*) and (*c*) were based on a sample size of 500 particles, while those of the *EFu* faces, *i.e.*, (*b*) and (*d*) were based on a sample size of 400 particles

This is an interesting result since most other stacked membranes have a high density of *EF* particles throughout the entire stacked regions, as was obtained with *Cryptomonas*. An exception to this was the *Prochloron* symbiont of *Didemnum molle* where Cox and DWARTE (1981) found an EF_1 face (low density of *EF* particles) in stacked thylakoids. The mosaic distribution of the *EFs* face in stacked regions in *Chroomonas* may reflect the limited thylakoid adhesion observed in these stacked thylakoids. If membrane adhesion were responsible for *EF* particle aggregation then it would be expected that in membranes where adhesion was poorly developed, as in *Chroomonas*, particle aggregation would also be patchy and poorly developed. Conversely, where membrane adhesion was better developed, as in *Cryptomonas*, one would expect better developed *EF* particle aggregation. It is also interesting to note that the ratio of *EFs*:*EFu* particle densities in *Cryptomonas* (where membrane adhesion is well developed) is 9:1 while only 3:1 in *Chroomonas* (where membrane adhesion is poorly developed). It will be interesting to see if this correlation extends to other cryptomonads which show different degrees of membrane adhesion.

No difference in size between *EFu* and *EFs* particles was observed for either *Cryptomonas* or *Chroomonas*. All *EF* faces were characterised by 15 nm particles. In the chlorophyll *b*-containing thylakoids the *EFs* particles (16 nm) are larger than the *EFu* particles (10 nm).

The *EFs* particles in the cryptomonads are smaller than those found in higher plants. The light harvesting complex (LHC) associated with photosystem II appears to play a major role in determining *EFs* particle size. ARMOND *et al.* (1977) correlated changes in *EFs* particle sizes in peas with changes in the amount of chlorophyll *a*/*b* LHC while the barley mutant *chlorina-f2*, which lacks the chlorophyll *a*/*b* protein 2, has smaller *EFs* particles (13 nm) than wild-type barley (SIMPSON 1979). Since the chlorophyll *a*/*b* LHC influences particle size it might be expected that organisms with different LHCs would have different sized *EFs* particles. The cryptomonads do not contain chlorophyll *a*/*b* LHC and therefore it is not surprising that their *EFs* particles have a different size to those of higher plants.

In higher plants photosystem I is confined to unstacked thylakoids (ANDERSSON and ANDERSON 1980). This segregation of photosystem I can be correlated with regional segregation of particles on the *PF* face of thylakoid membranes. Freeze-fracture shows that although 8.5 nm particles occur on the *PF* faces in both stacked and unstacked thylakoids, larger 11 nm particles are confined to unstacked thylakoids, *i.e.*, the *PFu* face (ARMOND *et al.* 1977, SIMPSON 1979). It is thought that photosystem I occurs within these larger 11 nm particles. Regional segregation of particles on the *PF* face also occurs in the cryptomonads, though the particle sizes are different to those of higher plants. In the cryptomonads the *PFu* face also has the largest sized particles but are 13 or 14 nm with the *PFs* particles 11 nm. This result for the cryptomonads raises a few interesting possibilities. It could be argued that the 11 nm *PFs* particles of the cryptomonads are equivalent to the 11 nm *PFu* particles (photosystem I) found in higher plants. If this were true then photosystem I would be segregated into stacked membranes, the opposite to the situation found in higher plants. The thylakoids of other organisms which have been freeze-fractured, *i.e.*, the cyanobacteria and the red algae do not usually contain 11 nm particles and yet presumably some of the particles found on these membranes, probably those on the *PF* face, are structurally equivalent to photosystem I. The *PF* particles in these organisms fall within the range of 5–13 nm. It therefore appears that photosystem I can be represented by different sized particles in thylakoids from different organisms. Therefore, the other possibility exists that the 13–14 nm *PFu* particles in the cryptomonads contain photosystem I. The larger *PFu* particle size in the cryptomonads could be due to the presence of more antenna pigments associated with photosystem I complexes than occurs in higher plants. This second alternative suggests a segregation of photosystem I into unstacked membranes and photosystem II into stacked membranes. In higher plants the separation of photosystem I and photosystem II is thought to represent a photo-adaptive strategy which increases photosynthetic efficiency at low light intensities (ANDERSON 1982), it is likely that the cryptomonads have evolved a similar photo-adaptive strategy to that of the higher plants.

Both *Cryptomonas* and *Chroomonas* fractured to produce two types of *PF* faces in stacked regions, a PFs_1 face (identical to the *PFu* face) and a *PFs* face. If the *PFu* particles represent photosystem I then it would be tempting to speculate that the presence of PFs_1 faces in cryptomonad stacked regions represents incomplete photosystem I exclusion from stacked membranes. However, it is possible that the PFs_1 face is excluded from regions of membrane adhesion. It is not possible to directly prove this because although in freeze-fracture it is easy to identify stacked membranes it is not possible to recognize in which areas of these

membranes adhesion occurs (this can only be seen from thin sections). In the case of *Chroomonas* the area of the stacked membranes occupied by the *PFs* and *EFs* faces were very similar. Also the *PFs* and *EFs* faces were frequently found to be adjacent (see Fig. 11), which suggests that they could be complementary. If we consider the *PFs* and *EFs* occurring in the vicinity of a region of membrane adhesion then a fracture passing through both adjacent membranes in such a region would expose the *PFs* face of one membrane and the *EFs* face of the other. This is indeed what was frequently found. However, the *PFs* and *EFs* faces cannot only be restricted to areas of membrane adhesion, since these fracture faces occupy larger areas than any regions of membrane adhesion observed from thin sections. Nevertheless, it appears likely that there is spatial separation of *EFs* with *PFu* and PFs_1 particles in *Chroomonas*. In *Cryptomonas* the situation is different since the *EFs* face occupied areas much greater than the *PFs* face, therefore the two faces cannot be always complementary. The *EFs* face occurs throughout all stacked membranes, whereas the *PFs* is confined to discrete regions within stacked membranes. Although it is possible that the PFs_1 face is excluded from areas of membrane adhesion, it is apparent that throughout many areas of stacked membranes the *EFs* face must be complementary with the PFs_1 face. It is therefore very unlikely that complete segregation of photosystem I and photosystem II occurs in *Cryptomonas*.

The differences between the two species are intriguing because *Cryptomonas* which has better developed thylakoid adhesion, thylakoid stacking and better developed *EF* particle aggregation into stacked membranes could at the same time have less complete photosystem I and photosystem II segregation than that found in *Chroomonas*. That such differences exist points to a variation in thylakoid architecture within the cryptomonads.

The 14 and 13 nm *PF* particle sizes in the cryptomonads are amongst the largest reported for any thylakoid. The *PF* particles in higher plants, *e.g.*, spinach are typically 8–11 nm (STAEHELIN 1976). Those in the red algae are highly variable ranging from 5 nm in *Porphyridium purpureum* (NEUSHUL 1970) to 13 nm in *Bangia* (BISALPUTRA and BAILEY 1973). The cyanobacteria also show variation in *PF* particle sizes ranging from 5–7 nm in *Skujapelta* (BORDU and LEFORT 1970) to the commonly found size class of 10 nm in *Synechococcus lividus* (GOLECKI 1979). The dinoflagellate *Gonyaulax polyedra* had 10–11 nm *PF* particles (SWEENEY 1981).

The 15 nm *EF* particle sizes in the cryptomonads, however are more intermediate to those reported in the literature. Higher plants can have *EF* particles of up to 16 nm (STAEHELIN 1976). The red algae range from 10 nm in *Porphyridium purpureum* (NEUSHUL 1970) and *Cyanidium caldarium* (WOLLMAN 1979) to 18 nm in *Bangia* (BISALPUTRA and BAILEY 1973). The cyanobacteria *EF* particles vary from 10 nm in *Synechococcus lividus* (GOLECKI 1979) and *Anabaena* (STAEHELIN *et al.* 1978) to 20 nm in *Oscillatoria brevis* (LICHTLÉ and THOMAS 1976). The dinoflagellate *Gonyaulax polyedra* has 10–11 nm *EF* particles (SWEENEY 1981).

The phylogenetic association of the cryptomonads with other algae is not very clear. The presence of biliproteins has often led to them being closely associated with the red algae and cyanbacteria (blue-green algae). However, the cryptomonads, unlike the red algae and cyanobacteria, show thylakoid stacking and subsequently regional differences between stacked and unstacked membranes. Perhaps the most significant of these regional differences is that the majority of the *EF* particles (photosystem II complexes) are confined to stacked membranes. It is becoming increasingly apparent that *EF* particle aggregation into stacked thylakoids is very widespread. The evidence for this in the chlorophyll *b*-containing plants has been well documented, but recent evidence also shows that the chlorophyll *c*-containing plants display a similar *EF* particle aggregation (DWARTE and VESK 1982 b). In this regard the cryptomonad thylakoids show more similarity with the other chlorophyll *c*-containing plants than they do with those of the red algae or cyanobacteria.

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