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Cell-wall structure and supramolecular organization of the plasma membrane of marine red algae visualized by freeze-fracture

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SUMMARY

The cell-wall structure and the supramolecular organization of the plasma membrane in 29 species of red algae were studied both in replicas of rapidly frozen cells and in ultrathin sections.

Most of the marine red algae investigated have a random distribution of the microfibrils of the cell walls; in a few cases there is a tendency to parallel alignment. *Laurencia obtusa* is an exception in which apart from a random distribution, microfibrils are arranged parallelly in a certain wall layer. The microfibrils have a cylindric or ribbon-like morphology. In a number of species, microfibrils consist of two, three or four linear subcomponents (sub-fibrils). In certain species two or three microfibrils can be bundled.

In *Erythrocladia subintegra*, *Radicilingua reptans* and *Laurencia obtusa* the plasma membrane exhibits randomly distributed linear microfibril–terminal complexes. All results favour the suggestion that the linear terminal complexes in the plasma membrane of the cells of the above mentioned species are involved in the biosynthesis, assembly and orientation of microfibrils. In the plasma membrane a number of other intramembranous particles are aggregated in various complexes (tetrads, complexes of six subunits, crystalline complexes, particle strings). Intramembranous particle complexes composed of four subunits 'membrane tetrads' have been observed in the plasma membrane and in the membranes of mucilage sacs of all red algae investigated. The 'membrane tetrads' are thought to be membrane-bound multi-enzyme complexes participating in the synthesis of the matrix polysaccharides.

Observations of ultrathin sections suggest that the Golgi system and the inflated Golgi-derived vesicles with fibrillar contents contribute to the formation of the wall.

Our results support the view that the biosynthesis of cell-wall skeletal and matrix polysaccharides in red algae are spatially separated.

Key-words: cell-wall structure, freeze-fracture, plasma membrane, supramolecular organization.

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This paper is dedicated to Professor Dr M.M.A. Sassen on the occasion of his retirement.

Abbreviations: CW cell wall; EF exoplasmic fracture face; MS mucilage sac; PF protoplasmic fracture face; PM plasma membrane; VM vacuole membrane.

INTRODUCTION

The cell wall of red algae consists of a microfibrillar (crystalline) phase embedded in a more amorphous phase (the matrix) (Kloareg & Quatrano 1988; Tsekos & Reiss 1991a,b, 1992a). The microfibrillar phase is composed mostly of cellulose, mannan or xylan (Gretz & Vollmer 1989), while the amorphous mucilaginous matrix material of the wall is usually a sulphated galactan polymer (Percival & Foyle 1979; Craigie 1990; Gretz *et al.* 1991a), such as agar, carrageenan, funoran, furcellaran or porphyran (Craigie 1990).

When the cell wall is formed, the polysaccharides must traverse the plasma membrane. In red algae the matrix polysaccharides are synthesized within dictyosomes (Ramus 1972; Evans *et al.* 1974; Tsekos 1981, 1985; Gretz *et al.* 1991a) and mucilage sacs, which make up a category of vacuoles in red algae (Pueschel 1979; Tsekos 1981). They are then transported to the cell exterior by exocytosis of either Golgi-vesicles or the whole mucilage sacs (Tsekos 1981, 1985; Tsekos & Reiss 1988; Gretz *et al.* 1991a). During the elongation of the galactan chain, or immediately after its formation before its release from the Golgi vesicles, the polymer is enzymatically sulphated (Millard & Evans 1982). Linkage of wall proteins to the matrix polysaccharides also seems to occur within the Golgi apparatus (Tsekos 1981, 1985, 1991). In red algae, particle aggregates 'membrane tetrads' in the biomembranes seem to be involved in the synthesis of the amorphous mucilaginous matrix material of the wall (Tsekos *et al.* 1985; Tsekos & Reiss 1988; Tsekos 1991). The formation of the microfibrillar phase, however, is different since it is generally a plasma membrane-associated process (Delmer 1987).

In the red alga *Erythrocladia subintegra*, highly ordered plasma membrane protein complexes associated with the ends of microfibrils or microfibril imprints ('terminal' enzyme complexes, TCs) seem to be involved in the biosynthesis, assembly and orientation of the cellulose microfibrils (Tsekos & Reiss 1991a,b, 1992a).

Kloareg & Quatrano (1988 and the literature cited therein) support the idea that the walls of marine red algae are characterized as: 'microfibrils contained within lamellae parallel to the cell surface. Within each lamella, microfibrils do not exhibit any preferential orientation.' Myers & Preston (1959) showed that the fifteen red algae investigated all have a similar wall structure consisting of numerous lamellae each of which is made up of random microfibrils embedded in an amorphous matrix.

To evaluate the cell-wall structure and supramolecular organization of the plasma membrane, 29 species of Rhodophyta (belonging to various orders and families) were studied employing the freeze-fracture technique.

MATERIALS AND METHODS

Thalli of the following species of red algae were collected from various biotopes of the Aegean Sea:

Subclass Bangiophycidae
Order Compsopogonales,

Family Erythropeltidaceae: 1. *Erythrocladia subintegra* Rosenv.

Order Bangiales,
Family Bangiaceae: 2. *Porphyra leucosticta* Thuret. (conchocelis phase)

Subclass Florideophycidae
Order Bonnemaissoniales,

Family Bonnemaisoniaceae: 3. *Falkenbergia rufolanosa* (Harv.) Schmitz

Order Cryptonemiales,

Family Kallymeniaceae: 4. *Kallymenia* sp.

Order Gigartinales,

Family Gracilariaceae: 5. *Gracilaria verrucosa* (Hudson) Papenf.

Family Gigartinaceae: 6. *Gigartina teedii* (Roth.) Lamour.

Order Ceramiales,

Family Ceramiaceae: 7. *Antithamnion* sp. 8. *Callithamnion corymbosum* (Smith) Lyngb. 9. *Callithamnion caudatum* J. Ag. 10. *Pterothamnion crispum* (Ducluzeau) Naegeli 11. *Spermothamnion johannis* G. Feldmann-Mazoyer 12. *Spyridia filamentosa* (Wulf.) Harv. 13. *Ceramium rubrum* (Huds.) J. Ag. 14. *Ceramium diaphanum* var. *strictum* (Kutz.) Feldmann-Mazoyer

Family Dasyaceae: 15. *Dasya rigidula* (Kutz.) Ardissone

Family Delesseriaceae: 16. *Hypoglossum woodwardii* Kutz. 17. *Radicilingua reptans* (Zan.) Papenf.

Family Rhodomelaceae: 18. *Polysiphonia deusta* (Roth) J. Ag. 19. *Polysiphonia variegata* (C.Ag.) Zan. 20. *Polysiphonia nigrescens* (Dillw.) Grev. 21. *Polysiphonia furcellata* (C.Ag.) Harv. 22. *Herposiphonia tenella* f. *secunda* (C.Ag.) Hollenb. 23. *Laurencia obtusa* (Huds.) Lamour. 24. *Laurencia pinnatifida* (Gm.) Lamour. 25. *Alsidium corallinum* C.Ag. 26. *Chondria tenuissima* (Good. et Woodw.) 27. *Halopitys incurvus* (Huds.) Batt. 28. *Rytiphlaea tinctoria* (Glem.) C.Ag. 29. *Acanthophora delilei* Lamour.

The thalli were shipped immediately to the Institute of Cytology, University of Heidelberg, where they were maintained in a seawater aquarium. The thalli were cut into small pieces, and were immediately frozen in nitrogen slush. The specimens were fractured with the double-replica device in a Balzers BAF 400-T apparatus (Reiss *et al.* 1984).

Material for ultrathin sectioning was prepared according to Tsekos (1981). Ultrathin sections were stained with uranyl acetate and lead citrate. The replicas and ultrathin sections were examined either on a Phillips EM 400 or on a Zeiss EM 9 S-2 electron microscope.

RESULTS

Cell walls

The cell walls of the marine red algae studied, consisted of a succession of wall layers surrounding the plasma membrane of each cell, embedded in a less organized material which fills the spaces between the cells (Figs 1a,b,c). Material similarly stained to that of the cell wall appears to be present both in the Golgi cisternae and vesicles and in the mucilage sacs (Figs 1a,b,c). Fractures through epidermal cells of the cortical layer show the thick cell walls to be lamellated (Fig. 1d, arrows).

Microfibrils within layers lie in all directions (Figs 2a,d, 3a,b,d,e,g, 4d,e, 5a,c,f, 6d,e). In some of the species studied a tendency for microfibrils to be parallel is also evident (Figs 4b,f, Table 1).

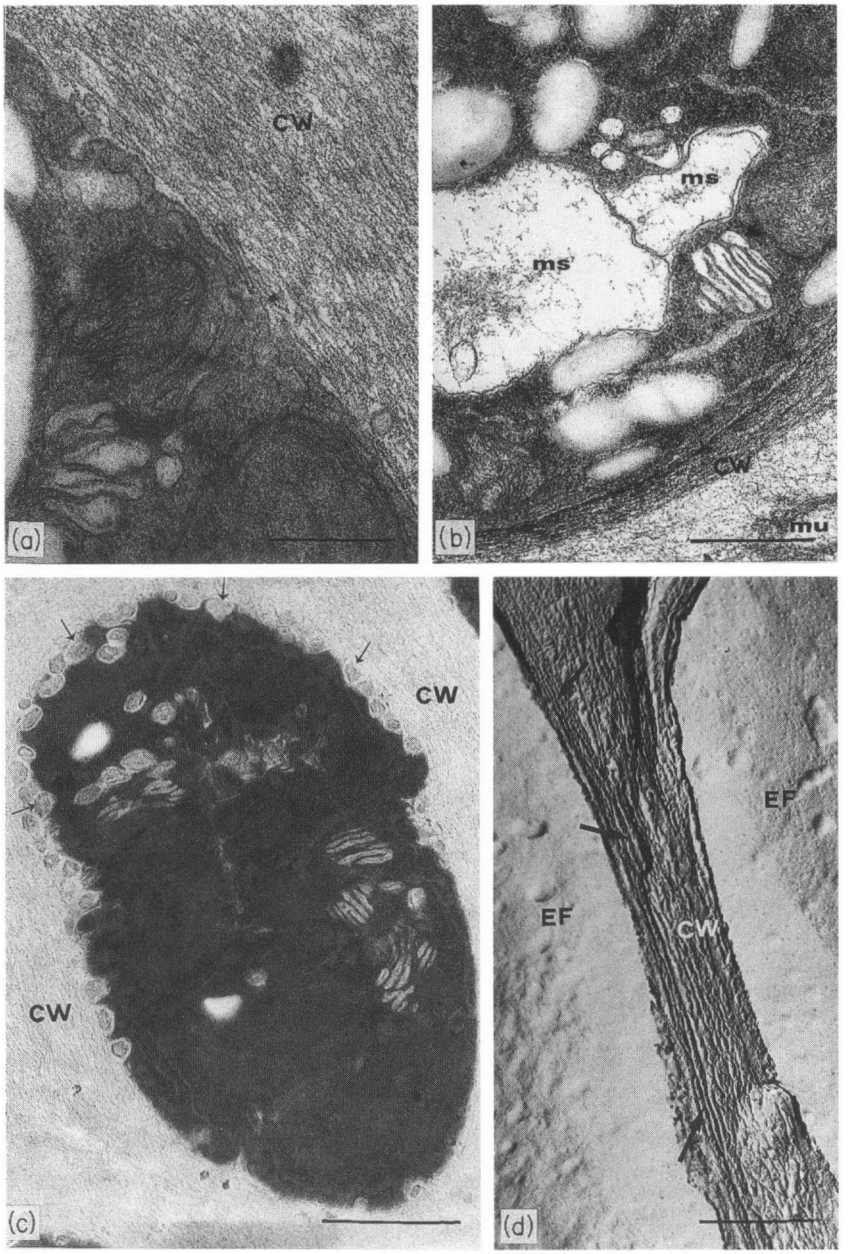

Fig. 1. (a) *Gigartina teedii.* Part of a nearly mature carpospore with hypertrophied dictyosome producing considerably inflated cisternae with fibrous material, which is similar to that of the cell wall (CW). The inner fibrous matrix surrounding the cell becomes oriented parallel to the plasma membrane surface. Bar: 0·4 μm. (b) *Gigartina teedii.* Part of an immature carpospore with mucilage sacs (ms) and hypertrophied dictyosome. Note that the dense fibrillar structure of the immediate cell wall becomes oriented parallel to the plasmalemma surface and is replaced by a fine granular material outer (mu). Bar: 0·6 μm. (c) *Gigartina teedii.* Epidermal cell of the cortical layer. Golgi vesicles have discharged their contents into the apical periplasmic space (arrows). Bar: 1 μm. (d) *Gigartina teedii.* Fracture through freeze-etched epidermal cells of the cortical layer showing the EF faces of the plasma membrane. Note the lamellated cell walls (arrows). Bar: 1 μm.

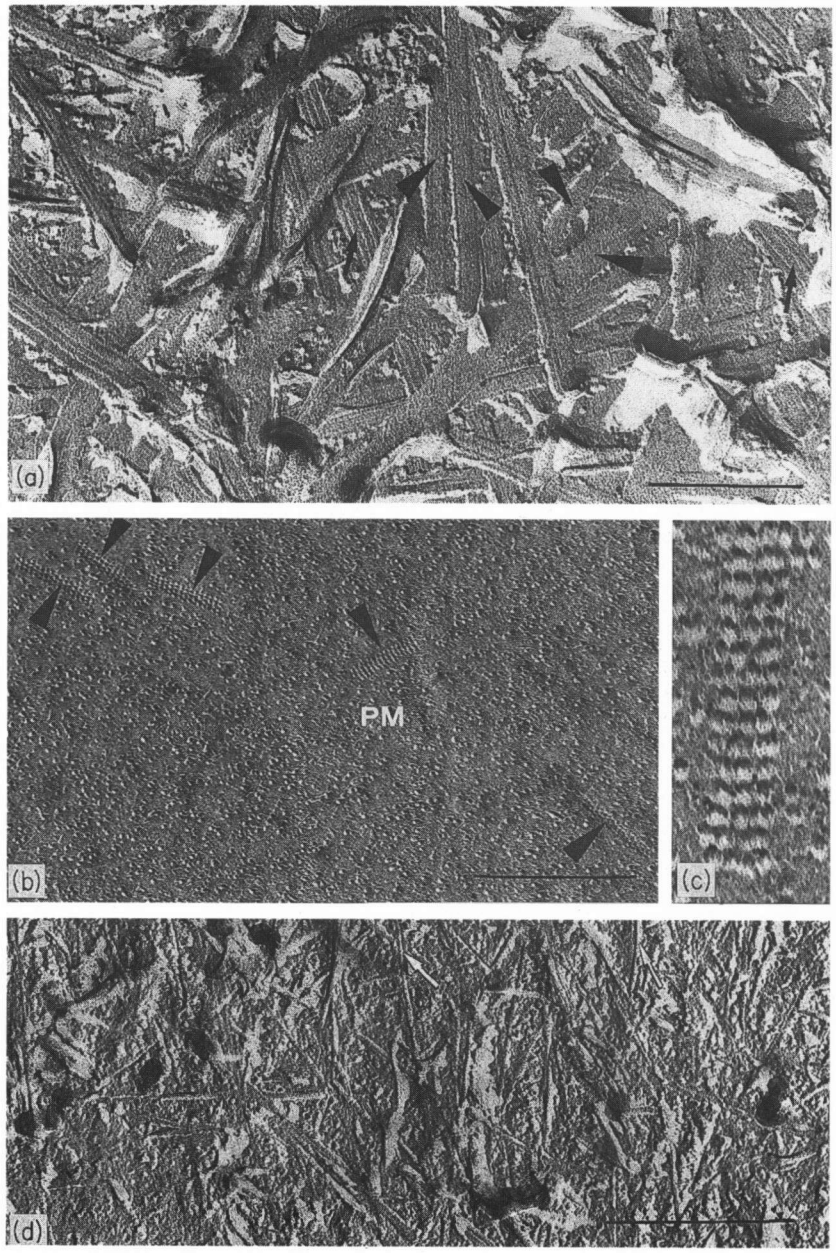

Fig. 2. (a) *Erythrocladia subintegra.* Fractured plane through the cell wall showing random microfibrils. Some fibrils clearly reveal four linear subcomponents (arrows). Note also the bundle of two microfibrils (arrowheads). Bar: 0·3 μm. (b) *Erythrocladia subintegra.* Fracture through a freeze-etched cell at the stage of cell-wall synthesis, showing the PF face of the plasma membrane (PM). Several linear TCs (arrowheads) are shown at the ends of randomly oriented microfibril imprints. Bar: 0·4 μm. (c) *Erythrocladia subintegra.* A TC at higher magnification. × 280 000. (d) *Porphyra leucosticta* (conchocelis phase). Fractured plane through the cell wall showing random microfibrils. Some fibrils clearly reveal two or three linear subcomponents (arrows). Bar: 0·3 μm.

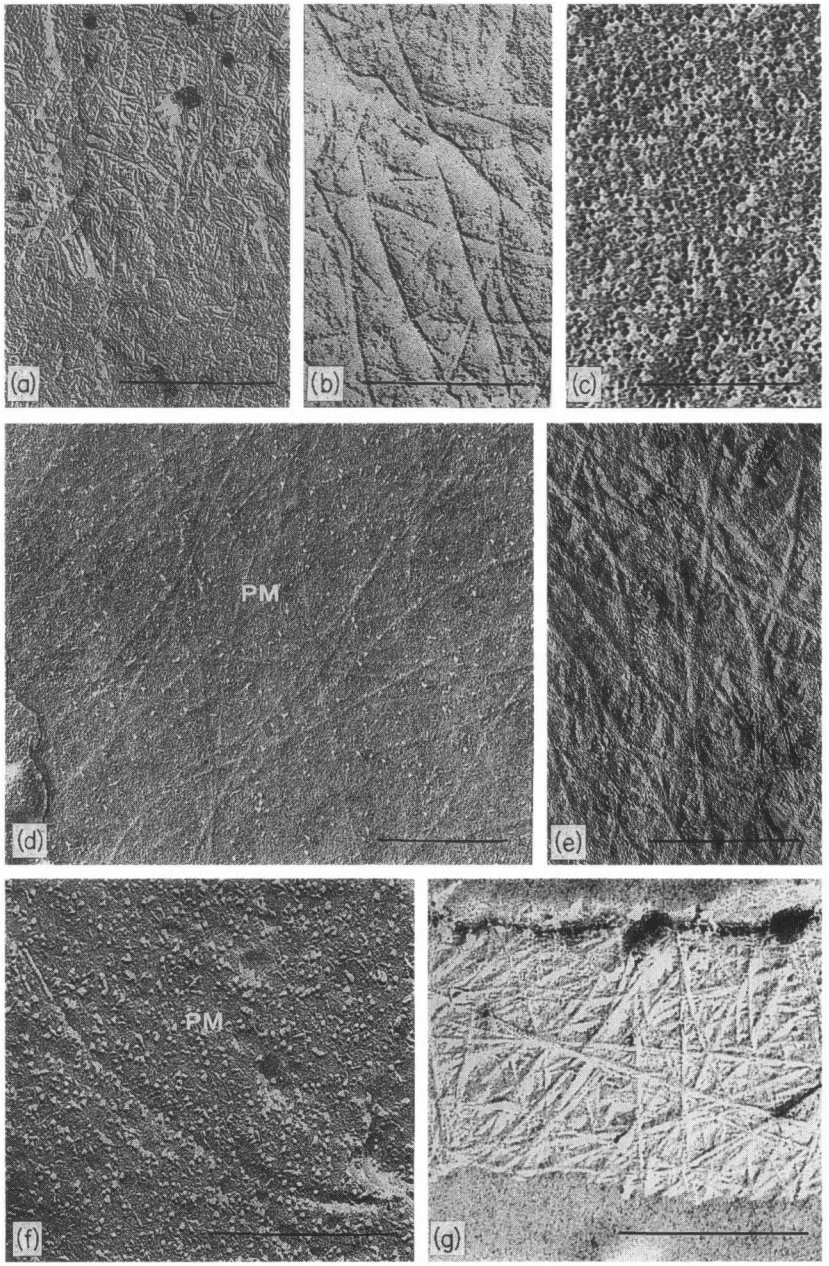

Fig. 3. (a) *Falkenbergia rufolanosa.* Fracture plane through the cell wall of a pericentral cell showing random microfibrils. Bar: 0·3 μm. (b) *Kallymenia* sp. Fracture plane through the cell wall of a cortical cell showing random microfibrils. Bar: 0·2 μm. (c) *Falkenbergia rufolanosa.* Fracture through a freeze-etched pericentral cell showing the PF face of the plasma membrane (PM). Note the presence of crystalline particle complexes. Bar: 0·2 μm. (d) *Gracilaria verrucosa.* Fracture through a freeze-etched cell of a 9-day-old carpospore-seedling showing the EF face of the plasma membrane (PM). Note the numerous randomly distributed imprints of microfibrils. Bar: 0·4 μm. (e) *Callithamnion caudatum.* Fracture through a freeze-etched cell showing the EF face of the plasma membrane. Note the numerous randomly distributed imprints of microfibrils. Also note that the plasma membrane has been removed in certain positions where microfibrils are distinguished (arrows). Bar: 0·4 μm. (f) *Spermothamnion johannis.* Fracture through a freeze-etched cell showing the PF face of the plasma membrane (PM). Note that a particle string (arrow) is associated with the end of a microfibril imprint. Bar: 0·3 μm. (g) *Spyridia filamentosa.* Fractured plane through the cell wall of a cortical cell showing random microfibrils. Bar: 0·3 μm.

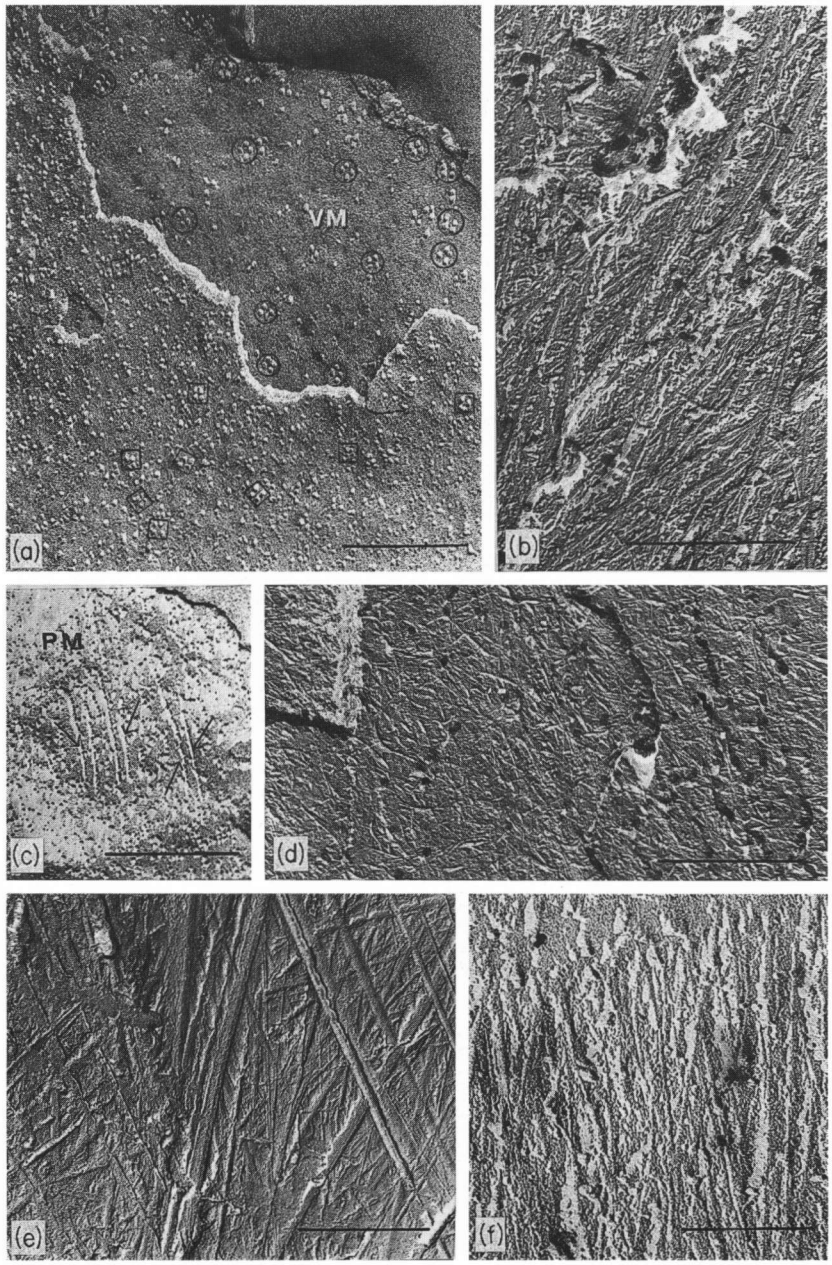

Fig. 4. (a) *Spermothamnion johannis.* EF of vacuole membrane (VM) with 'tetrads' (circles) and PF of plasma membrane (PM), which apart from single particles also contains 'membrane-tetrads' (rectangles). Bar: 0·2 μm. (b) *Spermothamnion johannis.* Fractured plane through the cell wall with a tendency for microfibrils to be parallel. Note the bundle of two microfibrils (arrows). Bar: 0·4 μm. (c) *Ceramium rubrum.* Fracture through a freeze-etched cell showing the PF face of the plasma membrane (PM). Note the presence of particle strings (arrows). Bar: 0·3 μm. (d) *Ceramium rubrum.* Fractured plane through the cell wall showing four lamellae. Within each lamella microfibrils do not exhibit any preferential orientation. Bar: 0·6 μm. (e) *Dasya rigidula.* Fractured plane through the cell wall showing random microfibrils. Bar: 0·6 μm. (f) *Radicilingua reptans.* Fractured plane through the cell wall with parallel microfibrils. Some fibrils clearly reveal two linear subcomponents (arrows). Bar: 0·2 μm.

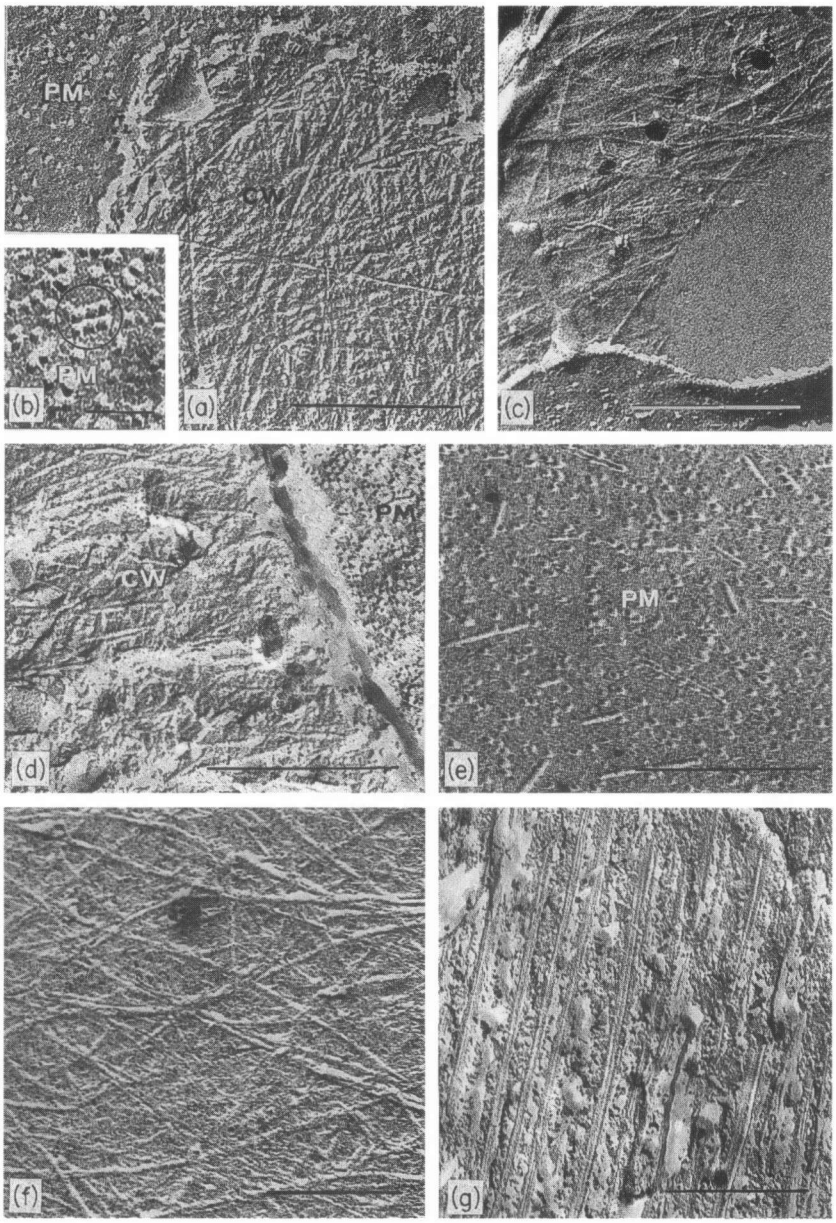

Fig. 5. (a) *Hypoglossum woodwardii*. Fracture through a freeze-etched cell showing the cell wall (CW) and the EF face of the plasma membrane (PM). Note the randomly distributed microfibrils. Bar: 0·3 μm. (b) *Radicilingua reptans*. Fracture through a freeze-etched cell showing the PF face of the plasma membrane (PM). Note the occurrence of a complex of six subunits (circles). Bar: 0·05 μm. (c) *Polysiphonia deusta*. Fractured plane through the cell wall of a pericentral cell showing random microfibrils. Bar: 0·3 μm. (d) *Herposiphonia tenella* f. *secunda*. Fracture through a freeze-etched pericentral cell showing the cell wall (CW) and the PF face of the plasma membrane (PM). Note the tendency of microfibrils to be parallel. Bar: 0·3 μm. (e) *Laurencia pinnatifida*. Fracture through a freeze-etched cortical cell showing the PF face of the plasma membrane (PM). Note the presence of particle strings. Bar: 0·3 μm. (f) *Laurencia obtusa*. Fracture plane through an outer layer of the cell wall in a cortical cell showing random microfibrils. Bar: 0·2 μm. (g) *Laurencia obtusa*. Fracture plane through an inner layer of the cell wall in a cortical cell showing parallelly arranged microfibrils. Note that the microfibrils clearly reveal two or three linear subcomponents. Bar: 0·3 μm.

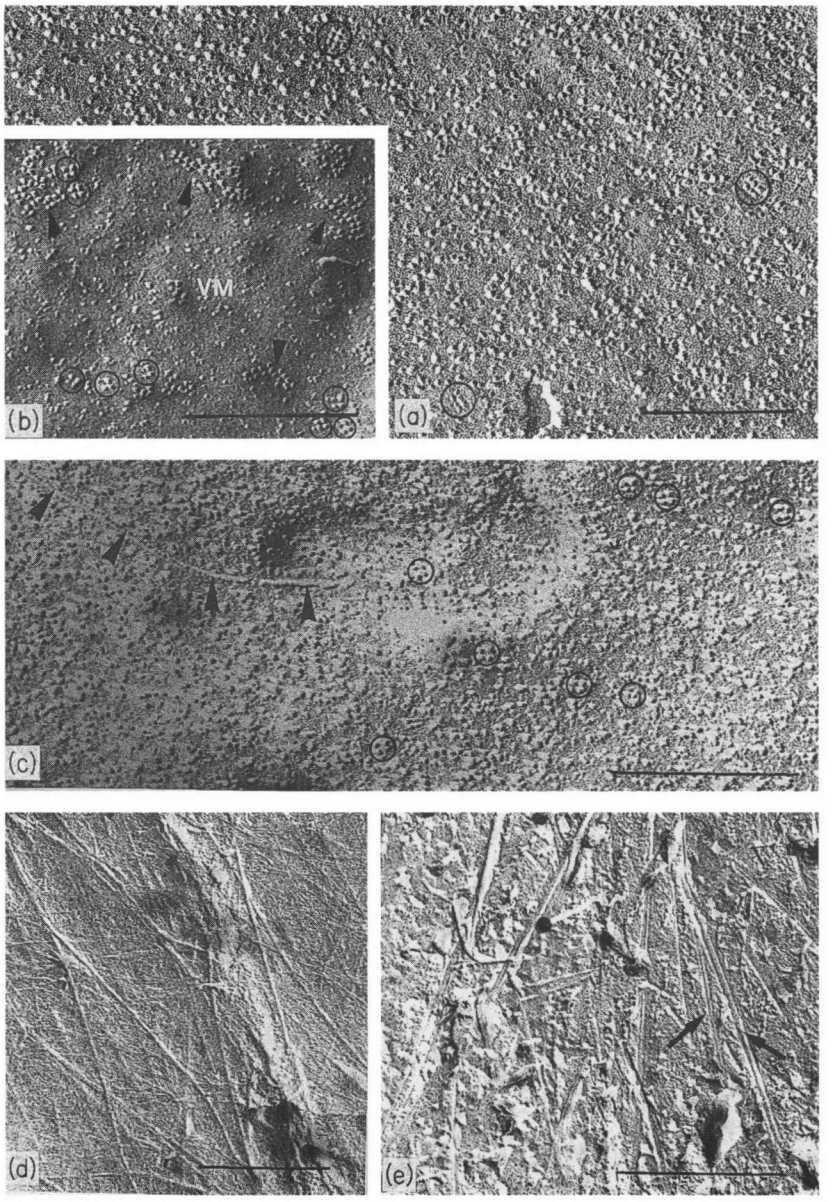

Fig. 6. (a) *Laurencia obtusa.* Fracture through a freeze-etched cortical cell showing the PF face of the plasma membrane (PM). Note the occurrence of three complexes of six subunits (circles), of which two seem to be associated with the ends of microfibril imprints. Bar: 0·2 μm. (b) *Alsidium corallinum.* Many single tetrads (circles) and tetrad clusters (arrowheads) on the EF face of the vacuole membrane (VM) of a cortical cell. Bar: 0·2 μm. (c) *Alsidium corallinum.* Fracture through a freeze-etched cortical cell showing the PF face of the plasma membrane (PM). Note the presence of particle strings (arrowheads) and particle tetrads (circles). Bar: 0·3 μm. (d) *Alsidium corallinum.* Fracture plane through the cell wall of a cortical cell showing random microfibrils. Bar: 0·3 μm. (e) *Rytiphlaea tinctoria.* Fracture plane through the cell wall of a cortical cell showing random microfibrils. Some clearly reveal two linear subcomponents (arrows). Bar: 0·4 μm.

Table 1. Structural characteristics of cell walls of marine red algae

Species	MF-shape	MF-orientation	MF substructure no. of subfibrils	MF bundle*
1. *Erythrocladia subintegra*	ribbon‡	random	four	+
2. *Porphyra leucosticta* (conchocelis phase)	ribbon	random	two or three	+
3. *Falkenbergia rufolanosa*	cylindric	random	—	—
4. *Kallymenia* sp.	cylindric	random	—	—
5. *Gracilaria verrucosa*	cylindric	random	—	—
6. *Gigartina teedii*	cylindric	random	—	—
7. *Antithamnion* sp.	ribbon	random	two	—
8. *Callithamnion corymbosum*	ribbon	random	two	—
9. *Callithamnion caudatum*	ribbon	random	two	—
10. *Pterothamnion crispum*	ribbon	random	two	—
11. *Spermothamnion johannis*	ribbon	tendency to be parallel	two	+
12. *Spyridia filamentosa*	cylindric	random	—	—
13. *Ceramium rubrum*	cylindric	random	—	—
14. *Ceramium diaphanum* var. *strictum*	cylindric	random	—	—
15. *Dasya rigidula*	ribbon	random	two or three	+
16. *Hypoglossum woodwardii*	cylindric	random	—	—
17. *Radicilingua reptans*	cylindric	parallel	two	—
18. *Polysiphonia deusta*	cylindric	random	—	—
19. *Polysiphonia variegata*	cylindric	tendency to be parallel	—	—
20. *Polysiphonia nigrescens*	cylindric	random	—	—
21. *Polysiphonia furcellata*	cylindric	random	—	—
22. *Herposiphonia tenella* f. *secunda*	cylindric	tendency to be parallel	—	—
23. *Laurencia obtusa*	cylindric	outer layers (= random)	—	—
		inner layer (= parallel)	two, three or four	—
24. *Laurencia pinnatifida*	cylindric	random	—	—
25. *Alsidium corallinum*	cylindric	random	—	—
26. *Chondria tenuissima*	cylindric	random	—	—
27. *Halopithys incurvus*	cylindric	random	two	+
28. *Rytiphlaea tinctoria*	cylindric	random	two	+
29. *Acanthophora delilei*	cylindric	random	two	+

*+: Microfibrils (MFs) can be bundled; —: No bundling possible.
‡Microfibrils show a flat-ribbon like morphology.

In many species microfibrils seem to have a cylindric shape (Figs 3a,b,d,g, 4d,f, 5a,c,d,f, 6d), while in others they show a flat-ribbon like morphology (Figs 2a,d, 3e, 4b,e, Table 1). In a number of algae, microfibrils consist of two, three or four small sub-fibrils (Figs 2a,d, 3e, 4b,e,f, 5g, 6e; see also Tsekos and Reiss 1992a). Bundles of two or three microfibrils can be seen (Figs 2a,d, 3e, 4b, 5g, 6e).

In *Laurencia obtusa*, apart from the randomly distributed cylindric microfibrils (Fig. 5f), wall layers also exist with parallelly aligned microfibrils, which are constituted of two

or three sub-fibrils (Fig. 5g); the latter are found in the immediate vicinity of the plasma membrane. All results are given in Table 1.

Plasma membrane

Randomly distributed linear terminal complexes (TCs) (Figs 2b,c), associated with the ends of randomly-oriented microfibrils or microfibril imprints (cf. Tsekos & Reiss 1992a), were encountered on both fracture faces (PF and EF) of the plasma membrane of *Erythrocladia* (Fig. 2b).

On the PF face some intramembranous particles are aggregated to form crystalline structures, particle strings (Figs 3c,f, 4c, 5e, 6c), or complexes with four or six particles (respectively tetrads or complexes of six particles organized as two rows of three particles; Figs 4a, 5b, 6a,c). The complexes of six particles seem to be constantly present in the species *Radicilingua reptans* (Fig. 5b) and *Laurencia obtusa* (Fig. 6a), which belong to different families; these complexes were associated with the ends of microfibril imprints (Fig. 6a).

Vacuole membrane

Tetrads, similar to those in the plasma membrane have been observed in the vacuole membranes of all species investigated (Figs 4a, 6b, cf. Tsekos & Reiss 1992b) as well as in membranes of the Golgi cisternae, Golgi vesicles and cytoplasmic vesicles of *Porphyridium* (Tsekos & Reiss 1988).

DISCUSSION

The cell wall of red algae consists of two components: an inner framework of microfibrils and an outer more amorphous component consisting of mucilage or slime (Craigie 1990).

Evidence exists for the participation of the Golgi apparatus (Ramus 1972; Evans *et al.* 1974; Tsekos 1981, 1985, 1991; Millard & Evans 1982; Tsekos *et al.* 1985; Tsekos & Reiss 1988, 1991b), but also of the mucilage sacs (vacuoles) (Pueschel 1979; Tsekos 1981, 1985, 1991, 1992b; Delivopoulos & Kugrens 1984) in the synthesis and secretion of wall matrix materials of the red algae. The present data support the above-mentioned view. The simultaneous presence of the intramembranous tetrads both in mucilage sacs and plasma membrane added to the results acquired so far (Tsekos *et al.* 1985; Tsekos & Reiss 1988, 1991b; Tsekos 1991) reinforces the view that 'membrane tetrads' are involved in matrix polysaccharide synthesis.

Biosynthesis of the microfibrillar phase differs from that of the amorphous phase (the matrix) as it generally is an exclusively plasma membrane-associated process (Delmer 1987). The unique, randomly distributed linear terminal complexes (TCs) in the plasma membrane of the marine red algae *Erythrocladia subintegra* (cf. Tsekos & Reiss 1991a,b 1992a) and *Porphyra leucosticta* (conchocelis phase) (unpublished data) have been observed in association with ends of microfibrils or microfibril imprints. These linear terminal complexes (TCs) probably represent the morphological equivalents of the plasma membrane-bound multi-enzyme complexes involved in the biosynthesis, assembly and orientation of the microfibrils in the red algae *Erythrocladia subintegra* and *Porphyra leucosticta* (conchocelis phase) (cf. for other algae, Brown & Montezinos 1976; Giddings *et al.* 1980; Herth 1983; Brown 1985; Hotchkiss 1989; Itoh 1990; Quader 1991).

In the red algae *Radicilingua reptans* (family Delesseriaceae) and *Laurencia obtusa* (family Rhodomelaceae) particle complexes consisting of six subunits (two rows of three

closely arranged particles) are probably associated with the ends of microfibril imprints. This could indicate that they are linear microfibril-terminal synthesizing complexes (TCs).

In the plasma membrane of the red algae *Ceramium rubrum, Spermothamnion johannis, Laurencia pinnadifita* and *Alsidium corallinum* particle strings occur similar to those in the embryos of the brown alga *Pelvetia* (Peng & Jaffe 1976). However, no evidence exists that these particle strings are involved in the synthesis of microfibrils. It cannot be completely excluded that these particle strings may be artefacts.

Structure and morphology of the marine red algal microfibrils allow one to distinguish them as two types: the cylindric type and the flat-ribbon like type.

The typical bundling of microfibrils in the cell walls of a good number of the red algae studied may be explained by their wall composition. The Rhodophyta have cellulose, mannan or highly crystalline β-1,3-xylans as skeletal polysaccharides (Kloareg & Quatrano 1988 and the literature therein; Gretz & Vollmer 1989; Gretz *et al.* 1991b). Cellulose and xylan microfibrils strongly adhere to each other and are encrusted with mucilages which cannot be easily removed (Kloareg & Quatrano 1988: 266 f; Tsekos & Reiss 1992a).

Our experiments show that the cell walls of marine red algae are lamellated with microfibrils generally randomly distributed in each lamella and that the lamellae are parallel to the cell surface. This conclusion is in accordance with previous results (Myers & Preston 1959; Kloareg & Quatrano 1988) on the architecture of the wall skeletal phase of marine red algae. It should be kept in mind, however, that in four of the 29 species investigated, namely *Spermothamnion johannis, Radicilingua reptans, Polysiphonia variegata* and *Herposiphonia tenella* f. *secunda*, there exists a tendency for microfibrils to be parallel. In *Laurencia obtusa*, microfibrils are randomly distributed in the outer layers of the cell wall, whereas in the inner layer they are parallel.

In some red algae, microfibrils consist of two (*Porphyra leucosticta, Callithamnion caudatum, Radicilingua reptans, Rytiphlaea tinctoria, Laurencia obtusa*), three (*Porphyra leucosticta, Laurencia obtusa*) or four subfibrils (*Erythrocladia subintegra*). The cellulose-synthesizing linear terminal complexes (TCs) of *Erythrocladia subintegra* and *Porphyra leucosticta* (conchocelis phase) consist of four and three rows of linearly-arranged particles respectively. It is probable that the number of particle rows coincides with the number of subfibrils within a microfibril (cf. Tsekos & Reiss 1992a). Therefore, it is suggested that each particle row in the TC forms individual sub-fibrils in the microfibril.

Taking into account our results and the relevant references (Ramus 1972; Evans *et al.* 1974; Tsekos 1981, 1985, 1991; Millard & Evans 1982; Tsekos *et al.* 1985; Tsekos & Reiss 1988, 1991a,b, 1992a,b; Gretz *et al.* 1991a) we conclude that the biosynthesis of skeletal and matrix polysaccharides by red algae is separated spatially and eventually also temporally.

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