CELL WALL STRUCTURE AND DEPOSITION IN
GLAUCOCYSTIS

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ABSTRACT

Events leading to cell wall formation in the ellipsoidal unicellular alga *Glaucocystis* are described. The wall is deposited in three phases: (a) a thin nonfibrillar layer, (b) cellulosic microfibrils arranged in helically crossed polylamellate fashion, and (c) matrix substances. At poles of cells, microfibrils do not terminate but pass around three equilaterally arranged points, resulting in microfibril continuity between the twelve helically wound wall layers. These findings were demonstrated in walls of both mother cells and freeze-fractured growing cells, and models of the wall structure are presented. Cellular extension results in spreading apart, and in rupture, of microfibrils.

On freeze-fractured plasma membranes, there were 35 nm × 550 nm structures associated with the ends of microfibrils. These are interpreted as representing microfibril-synthesizing centers (terminal complexes) in transit upon the membrane. These terminal complexes are localized in a zone, or zones. The plasma membrane is subtended by flattened sacs, termed shields, which become cross-linked to the plasma membrane after completion of wall deposition. During wall deposition, microtubules lie beneath the shields, and polarized filaments lie between shields and plasma membrane.

The significance of these findings in relation to understanding the process of cellulose deposition is discussed, and comparisons are made with the alga *Oocystis*.

KEY WORDS cell wall • cellulose • freeze-fracture • *Glaucocystis* • morphogenesis • plasma membrane

Cellulose, a major constituent of plant cell walls, is laid down in the form of long strands, known as microfibrils, consisting of \( \beta_1 \rightarrow 4 \) glucan chains arranged in a crystalline form such that the chain long axes lie parallel with the microfibril long axis (6, 11, 25-27). With few exceptions, there are good reasons for considering the plasma membrane as the site of synthesis of the constituent glucan chains, and assembly of the microfibrils from these chains (3, 5, 15, 25, 34, 39, 41, 44). Most authors consider that glucan chain synthesis and microfibril assembly must occur virtually simultaneously under the control of glucan synthetase enzymes located at microfibril tips (3, 4, 6, 17, 26, 27). Heath (17) has proposed that cellulose-synthesizing enzymes might move within the fluid matrix of the cell membrane, using cortical microtubules as guide-tracks, and spinning out the microfibrils as they go. This hypothesis has been lent support by Brown and Montezinos (2),...
who observed regularly arranged accumulations of granules at microfibril termini on freeze-fractured plasma membranes of the unicellular alga *Oocystis*. Further evidence from other sources is required before the proper significance of these observations can be determined. This paper describes a study, comparable with those on *Oocystis* (2, 23), of the alga *Glaucocystis*.

*Glaucocystis nostochinearum* Itzigsohn is a unicellular alga of uncertain affinity. A superficial resemblance to *Oocystis* has resulted in its having been classified in the Chlorococcales (12, 13, 16, 40). However, several recent studies have suggested that this classification is unsatisfactory since the cells have several characteristics which are not usually found amongst the Chlorophyceae (9, 31, 37, 38). As in the case of *Oocystis*, the ellipsoidal cells of *Glaucocystis* expand during the cell cycle, eventually giving rise to autospores by compartmentation of the cytoplasm. Each autospore develops a new wall. Further expansion of the mother-cell wall results, eventually, in its rupture and the release of the autospores and renewal of the cell cycle. The structure of the *Glaucocystis* cell wall has been described by Schnepf (36) and by Robinson and Preston (30), but there is little knowledge of the development of the wall. By contrast, the development of the wall of *Oocystis* has been well documented (2, 23, 32, 33, 35). In this study we show that some of the similarities between *Glaucocystis* and *Oocystis*, both in wall structure and in its development, are superficial and may represent parallel rather than coincident evolution.

The microfibrils of the wall of *Glaucocystis*, like those of *Valonia, Oocystis*, and a relatively small number of other algae (see reference 26), are unusually broad with a near rectangular cross section (2, 26, 35, 36). They are arranged in several distinct layers, and lie parallel with one another in any one layer. The microfibrils of each layer are arranged helically relative to the cell long axis, but the sign of the helix alternates left and right between adjacent layers. As a consequence of the helical arrangement, all microfibrils tend to converge at the vertices of the ellipsoid. The precise disposition of the microfibrils at the vertices has never been determined, however. Preston (26) suggests that the microfibril arrangement in *Glaucocystis* and *Oocystis* is essentially that of *Valonia*. Several studies of the cell walls of *Valonia* (7, 28, 45) demonstrated that the microfibrils of each layer converged to "poles" and that the positions of convergence of alternate layers were coincident. Robinson and White (33) described a cellulose-free "apical nipple" at the vertices of *Oocystis*. No apical nipple is apparent in the polarizing photomicrographs of the poles of *Valonia* prepared by Wilson (45). In *Glaucocystis*, the only evidence comes from intuitive comparison with these other algae. Robinson and Preston (30) stated that they were unable to obtain views of the poles of the cells by freeze-fracturing, and that the poles were obscured by amorphous material in dried and shadowed preparations. They did describe a "convex lens-shaped space" close to the pole, but its relationship to the pole was uncertain. In view of the growing evidence that cellulose microfibrils are synthesized by mobile, plasma membrane-bound, enzyme complexes, it becomes important to determine whether or not there is confluence between the microfibrils of the several wall layers, or whether the microfibrils terminate and restart at these points.

The structure of the plasma membrane of *Glaucocystis* is unusual and controversial. It has been described as "highly convoluted and indentate" (9) and underlain by a series of "lacunae" (38). Robinson and Preston (31) considered, however, that the plasma membrane consists of three layers, the inner two layers being indented to form "shield-shaped structures" (equivalent to the lacunae of Schnepf et al., 38). In this work we clarify the situation.

Some of the results presented in this paper have appeared previously in summary form (3).

MATERIALS AND METHODS

Algal Culture

*Glaucocystis nostochinearum* Itzigsohn used in this study was obtained from the Culture Collection of Algae at Göttingen, West Germany (culture LB 229-1, listed as *G. Geitleri Pringsheim*). This same strain is also listed as *G. nostochinearum* (229/1) in the Culture Collection of Algae and Protozoa (Cambridge, England) and as strain no. 64 in the Culture Collection of Algae at the University of Texas at Austin.

Axenic cultures were obtained by spraying washed cells onto the surface of 1.5% agarized Modified Kantz Medium (18) fortified with 1% Difco Beef Extract (Difco Laboratories, Detroit, Mich.). The same medium was used both for maintenance cultures and for actively growing experimental material. The cells were grown under continuous fluorescent illumination at 25°C. Mother-cell walls were prepared by placing cells from
old cultures in water and allowing them to settle under gravity. The mother-cell walls, which settled as a white upper horizon, were collected, and washed thoroughly in water.

**Replicas**

The replica-making procedure has been described previously (43). In outline: mother-cell walls were pipetted onto Formvar-coated grids and allowed to dry; the material was platinum/carbon shadowed and carbon backed in a Balzers 360M freeze-etch apparatus (Balzers AG, Balzers, Liechtenstein); replicas were removed from the grids by touching to chloroform, followed by immersion in 70% sulphuric acid; after 3-h replica cleaning, they were recollected on Formvar-coated grids.

**Thin Sections**

Actively growing cultures of *G. nostochinearum* were fixed in a standard glutaraldehyde/tannic acid mixture (10, 21). Our modification of the tannic acid procedure consisted of the following: cells were scraped from the surface of the agar medium and fixed for 30 min at 25°C in a 1% vol:vol glutaraldehyde/1-2% wt:vol tannic acid (Polysciences, Inc., Warrington, Pa.) mixture buffered to pH 7.2 and made up to 0.05 M cacodylate. The temperature of the fixative was then lowered to 4°C and the fixation continued for an additional 1.5 h. The cells were washed with 0.1 M cacodylate, pH 7.2, then postfixed at 4°C in 1% osmium tetroxide buffered in 0.05 M cacodylate, pH 7.2, for 1–2 h. They were washed again with 0.1 M cacodylate, then washed with distilled water and embedded in 2.0% Ionagar (no. 25, Wilson Diagnostics, Inc., Glenwood, Ill.). The glutaraldehyde fixation schedule was identical except for the omission of tannic acid. The material was dehydrated in ethanol and acetone and embedded in Spurr’s resin. Sections were cut with a diamond knife on a Reichert Om-U2 ultramicrotome (C. Reichert, Sold by American Optical Corp., Buffalo, N.Y.).

**Freeze-Etching**

The standard procedure of Moor and Mühlethaler (22) was employed, using a Balzers 360M freeze-etch apparatus. Cells were gently scraped from the surface of the agar culture medium and immediately pipetted, without any pretreatment, onto specimen supports for freezing. After fracturing at −105°C with 30-s etching, replicas were prepared and were cleaned with Chlorox bleach (5 h) and 75% sulphuric acid (15 h).

**RESULTS**

**Surface Replicas**

Light microscope observations of ruptured cells from which the cell contents had been expressed (Fig. 1) demonstrated that helically arranged striations in the wall converged to regions at the vertices of the ellipsoidal cells. In order to reveal further detail, a surface replica technique was employed. In several cases, replicas of mother-cell walls in which both ellipsoidal vertices were visible were found (Fig. 2). These allowed examination of the microfibrillar disposition at the external surface of at least half of the cell wall. The microfibrils were clearly arranged in a helically crossed polylamellate fashion, as described in earlier literature (see introductory paragraph). There was never any interweaving between lamellae, either of individual microfibrils or of broad bands of microfibrils. In mother-cell walls, there were always more amorphous matrix materials filling the intermicrofibrillar spaces at the poles than in the equatorial zone (cf. Figs. 3 and 4).

**Thin Sections**

Visualization of ruptured cells in the wall converged to regions at the vertices of the ellipsoidal cells. In order to reveal further detail, a surface replica technique was employed. In several cases, replicas of mother-cell walls in which both ellipsoidal vertices were visible were found (Fig. 2). These allowed examination of the microfibrillar disposition at the external surface of at least half of the cell wall. The microfibrils were clearly arranged in a helically crossed polylamellate fashion, as described in earlier literature (see introductory paragraph). There was never any interweaving between lamellae, either of individual microfibrils or of broad bands of microfibrils. In mother-cell walls, there were always more amorphous matrix materials filling the intermicrofibrillar spaces at the poles than in the equatorial zone (cf. Figs. 3 and 4). Unlike earlier studies (26, 30), it was found that the left-handed helix made by the microfibrils was faster than the right-handed helix (Figs. 1 and 2). Microfibrils had a mean value of 31 μm (maximum 41 μm, minimum 20 μm). Clearly, values could not be obtained in polar zones where matrix substances obscured the microfibrils. If microfibrils are continuous about the poles (see below), this mean value represents half the average length of microfibrils in the fully expanded cell wall. There were 2,000–3,500 microfibrils per wall layer (mean of six values obtained from replicas of three different mother walls gave a mean value of 31 μm (maximum 41 μm, minimum 20 μm). Clearly, values could not be obtained in polar zones where matrix substances obscured the microfibrils. If microfibrils are continuous about the poles (see below), this mean value represents half the average length of microfibrils in the fully expanded cell wall. There were 2,000–3,500 microfibrils per wall layer (mean of six values obtained from the mother wall shown in Fig. 2 was 2,402).

All microfibrils did not, however, converge upon a single point at the poles. Rather, there were always three points arranged roughly equatorially which the microfibrils encircled as broad bands (Fig. 4). In this way, microfibril continuity between wall layers is established. The loops made by microfibrils leave a teardrop-shaped space for which we have proposed the term "rotation center" (3). Distances between rotation centers appeared to be within the range 4.5–6.8 μm (Fig. 4) but were difficult to measure because of the difficulty of establishing a central point. As a result of the three-rotation-center microfibrillar...
Figure 1. A flattened young *Glaucocystis* cell from which the contents have been expressed via the tear (T). Note the helically arranged striations (arrows indicate helical axes) passing to prominent poles (P) situated at the ellipsoidal vertices. Nomarski differential interference contrast. × 1,560. Bar, 10 μm.

Figure 2. Shadowed replica of flattened mother-cell wall showing microfibrils passing to poles (P) and the tear (T) through which the daughter cells were released. Composite electron micrograph. × 1,900. Bar, 10 μm.

disposition at the poles, there were always regions in subpolar zones in which three different microfibril orientations were arranged equiangularly (Fig. 4). This results in an apparent “third orientation” (30, 36), but it is a localized, not a general, phenomenon.

**Thin Sections**

When material was fixed without the addition of tannic acid, spaces appeared between the wall and plasmalemma and are evident in micrographs published previously (9, 37, 38). These spaces were absent when tannic acid was present in the fixative (Figs. 5–12).

The course of the development of the cell wall could be elaborated from thin sections. A thin granular layer is formed initially, both over the two rudimentary flagella and over the remainder of the cell surface (Fig. 6). This layer persists on the outer surface of the wall when the microfibrillar component is produced (Figs. 9, 11, and 12). The microfibrillar component is synthesized in close contact with the plasma membrane (Fig. 7). As reported by Schnepf et al. (38), there are usually about 12 cell wall layers (Fig. 8), each layer being several microfibrils in thickness. The microfibrillar component is apparently laid down uninterruptedly over the flagellar region of the cell surface (Fig. 9). Once all the microfibrils have been laid down, the wall is completed by the addition of matrix substances which mask the microfibrils themselves (Fig. 10). An equatorial annulus of pores in the mature cell wall has been described previously (38). When tannic acid was added to our fixative solutions, we found a densely staining plug lying beneath this annulus (Fig. 11). This plug lies in a groove, filled with amorphous material, made between the plasma membrane and the wall. Both the plug and the amorphous material are not visualized in control material fixed without the addition of tannic acid. The flagella also lie in this groove.

In common with earlier studies (31, 38), we found that the plasma membrane was almost completely subtended by a series of ribosome-free flattened sacs. After the terminology of Robinson and Preston (31), we shall refer to these sacs as “shields.” Shields were absent from the region surrounding the bases of the flagella (Figs. 6 and 9). Until the wall has been completed, the shields are in an extended condition and subdivide a relatively smooth plasma membrane (Figs. 7
FIGURE 3  Microfibrils in the equatorial zone of a *Glaucocystis* mother-cell wall. Note that in the outermost wall layer there are 23 microfibrils at the bottom of the micrograph, but only 19 or 20 at the top. Microfibril ends (arrows) taper to points and are visible in at least two wall layers (vertical and horizontal arrows). $\times 57,700$. Bar, 1 $\mu$m.

FIGURE 4  An inner aspect of a flattened pole of a mother-cell wall. Note that matrix substances are present in greater concentration in the central zone, corresponding with the vertex of the cell. The approximate paths taken by three microfibrils are indicated by dotted lines to show the presence of three rotation centers. Asterisks representing the middles of the rotation centers correspond roughly with the origin of the semicircle made by the innermost microfibril encircling each rotation center. The boxes show regions in which there are three sets of microfibrils making approx. 60° angles with each other. These are a product of the polar organization and do not represent three distinct wall layers. $\times 6,850$. Bar, 5 $\mu$m.
Shields abut each other closely, and commonly overlap partially. Once wall formation, including the interposition of matrix substances, is completed, the plasma membrane and its associated shields undergo a transformation. The plasma membrane becomes deeply grooved, each groove being associated with a shield (Figs. 11-13). It is sometimes possible to see that the shields are bonded to the grooves in the plasma membrane by a series of cross-bridges (Fig. 13). Like Schnepf et al. (38), we found that the shields were partially filled with an amorphous, lightly staining material (Figs. 7, 10, and 13). One or two microtubules per shield lie interior to the shields (Figs. 7 and 12). At cytokinesis, the cleavage furrow appears to be preceded by organization of the shields into layers which will come to lie beneath the advancing plasma membrane (Figs. 14 and 15).

**Freeze-Fracturing**

Unlike Robinson and Preston (30), we were able to obtain fractures of polar zones of the walls of mature *Glaucocystis* autospores still retained within the mother-cell walls (Fig. 16, see also Fig. 24 in reference 3). These showed clearly that the three-rotation-center pattern of microfibril disposition at the ellipsoidal vertices was typical of the wall upon completion, as well as of the fully expanded mother-cell wall. The distances between rotation centers at these freeze-fractured poles appeared to lie in the range 4.5-5.5 μm.

Despite an approximate synchronization of the cultures, it was found that a low proportion of the cells were in stages of active wall deposition. This difficulty was compounded by the finding that there was a distinct preference, during freeze-fracturing, for the locus of fracture to occur at interfaces between layers within the highly ordered cell wall, rather than within the plasma membrane. The observations of plasma membranes reported here have been derived from a study of only seven extensive regions of fractured plasma membranes.

Microfibril ends impressed into plasma membrane fracture faces could be identified (Figs. 17-21). When large areas of fractured plasma membrane were visible, these microfibril ends were never evenly distributed over the entire region. We shall refer to the regions within which microfibril ends were found as "zones of synthesis."

As shown in thin sections, flattened shields lie immediately beneath the plasma membrane at the time of wall formation. Fracture commonly occurs within the plane of the outermost membrane of these sacs, such that the E-fracture face of the plasma membrane itself is obscured (the membrane fracture-face terminology of Branton et al. [1] is adopted in this paper). Extensive bulges, about 550-600 nm in length, could be seen at microfibril termini in a zone of synthesis when shields obscured the plasma membrane itself (Fig. 21). Where the shields had been stripped away, the impression of the microfibril in the E-fracture face of the plasma membrane terminated in a variety of ways (Figs. 17-20). Microfibril ends which were towards the leading edge of the zone of synthesis often showed little substructure (Fig. 17), while those farther back appeared to be led by two or three short rows of granules (Figs. 18 and 19). In all cases, however, the width of the structure was about 35 nm. At the leading edge of a zone of synthesis (Figs. 17 and 21), the plasma membrane was relatively smooth, whereas towards the trailing edge (Fig. 19) its

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**Figure 5** *G. nostochinearum* daughter cells within mother-cell wall (MW). Note that in this glutaraldehyde/tannic acid fixation there has been no withdrawal of daughter protoplasts from their cell walls. Cyanelles (C). × 2,150. Bar, 10 μm.

**Figure 6** Only granular primary wall material (arrows) is present at the earliest stages of cell wall deposition. Flagellum (F), Golgi complex (G). × 24,000. Bar, 1 μm.

**Figure 7** During microfibrillar secondary wall deposition, each forming lamella (large arrows) is found in close contact with the plasma membrane. Microtubules (small arrows) underlie flattened overlapping shields (S). × 66,000. Bar, 500 nm.

**Figure 8** Thin-section of mother-cell wall demonstrating 12 or 13 wall layers. × 86,000. Bar, 500 nm.

**Figure 9** Unmodified forming wall (W) overlying flagella (F). Flagellar base (Fb). × 21,500. Bar, 1 μm.
surface was corrugated. These differences must reflect differences in the composition of the wall against which the plasma membrane is pressed. We believe that the apparent variations in the morphology of the microfibril terminal structures represent slight variations of the precise plane of fracture within these structures, perhaps resulting from these differences in the substratum, rather than representing differences in the microstructure of the body associated with microfibrillar tips. Towards the trailing edge of a zone of synthesis (Fig. 19), it appeared that a wall layer more than one microfibril in thickness was formed as a result of microfibril terminal structures pushing their way beneath the earlier formed, though parallel, microfibrils. P-fracture faces of plasma membranes were densely covered with randomly distributed membrane-intercalated particles. Impressions of microfibril termini in plasma membrane P-fracture faces were relatively deeply inset into the membrane (Fig. 20), indicating that a body having a greater diameter than the microfibril itself had been removed from this place. These microfibril terminal complexes may be equivalent to the terminal complexes described by Brown and Montezinos (2) on fracture faces of *Oocystis* plasma membranes.

As already reported (3), it was sometimes possible to visualize wavy filaments lying between the flattened shields and the plasma membrane in zones of synthesis (Fig. 21). These were oriented parallel with the microfibrils and appeared to precede them.

The indented shields which form after wall development has been completed were well visualized by freeze-fracturing (Figs. 22 and 23). The shields may be spatially arranged in a fairly regular fashion, or in an irregular fashion (Figs. 22 and 23). When shields are fractured open so that P exposures of the membrane immediately apposed to the plasma membrane can be viewed, it is clear that the ridge made by the invagination of the plasma membrane into the shield is grooved and densely speckled with particles (Fig. 22). The E exposure of this same membrane (Fig. 23) reveals a series of fine striations which lie normal to the long axis of the plasma membrane ridge (the "ridge" being a groove in this orientation). As already described (Figs. 14 and 15), the initiation of shield formation begins with cytokinesis. Before wall formation begins, the shields are arranged such that their edges are in close contact with one another. In this way, virtually continuous sheets of membrane are formed (Fig. 24), and persist during wall formation.

**DISCUSSION**

The most important observations reported in this work relate to the mechanisms of microfibril synthesis and the pattern of microfibril deposition *Glaucocystis*. Robinson and Preston (30) have demonstrated the presence of cellulose in these cell walls and state that mother-cell wall hydrolysates yield glucose predominantly. We can therefore expect that the microfibrils that we have observed are composed principally of cellulose. Our results are in accordance with the view that the wall of *Glaucocystis* is produced by the apposition of microfibrillar layers synthesized at the plasma membrane surface.

A zenithal equidistant projection of the pole of *Glaucocystis* is shown in Fig. 25, and a model...
interpreting the observations of the patterns made by microfibrils in mother-cell walls is presented in the diagram Fig. 26. This model is in accordance with the results of Robinson and Preston (30), except that it includes poles having three rotation centers. The model can be most readily understood by taking one of the bands of microfibrils encircling a rotation center and by following it as it passes sequentially between the rotation centers of alternate poles. The rotation centers have been labeled 1, 2, and 3 at the upper pole and A, B, and C at the lower pole. Using (s) and (f) to designate slow and fast helices, respectively, the progression would be thus: 1 (s) A (f) 2 (s) B (f) 3 (s) C (f) 1 (s) A, etc., making one microfibrillar lamella for each passage between poles. In order that there be no interweaving between microfibrillar lamellae, as seems to be the case, then all three bands must be followed coincidentally, enabling the formation of each complete layer before the next is begun. The rotational symmetry relationship between the two poles has not been determined precisely, and that shown in the model (Fig. 26) represents the simplest case. Clearly, many other relationships are possible, as may be seen by halving the model at the equator and rotating one-half about the major axis relative to the other half. Similarly, the helical pitches used in the model are approximations. Nevertheless, the model has the advantage that it is very plastic. It could be adapted readily to conform to virtually any combination of helical pitches or rotational symmetry.

The so-called “third orientation” microfibrils of *Glaucocystis* (30) are an inevitable product of the polar pattern of microfibril deposition and do not represent a true wall layer. They probably do not correspond with the densely interposed third orientation layers of microfibrils which are found erratically, but probably as complete layers, in *Oocystis*¹ and *Valonia* (7, 26).

In freeze-fractured preparations, microfibrils are seen to terminate with a cylinder impressed

¹ D. Montezinos and J. H. M. Willison, unpublished observations.
into the plasma membrane surface (Fig. 21). It is reasonable to propose, on previous evidence (see discussions: 2, 26, 34, 42) that each cylinder might be concerned with the assembly of the microfibril with which it is associated. These cylinders have dimensions very similar to those of the “terminal complexes” of Oocystis (2, 23).

The apparent differences in fine structure of the terminal complexes of Glaucocystis and Oocystis may not indicate real differences in structure. The pattern of fracturing will be influenced by differences in their local environment, particularly by the presence or absence of shields. The linear terminal complexes of Glaucocystis and Oocystis contrast with the near-spherical terminal complexes of higher plants (24, 43), but reflect the differences in cross-sectional dimensions of the microfibrils to which they give rise.

Some form of guide-track under cellular control must direct the progress of the microfibril-synthesizing terminal complexes (3, 17). In Oocystis, microfibril orientation appears to be controlled via cortical microtubules (14, 29) and “granule bands” which can be made visible on P-fracture faces of freeze-fractured plasma membranes (2, 23, 32). No granule bands are present in Glaucocystis, and the cortical microtubules lie beneath the extended shields. There is some evidence that filamentous elements lie between the shields and the plasma membrane. If cortical microtubules are involved in the orientation of Glaucocystis microfibrils, then this influence must be imparted via the shields.

An important consideration in any study of
plant cell walls concerns wall extension. During the cell cycle of *Glaucocystis* the wall extends considerably. Our results indicate that the microfibril pattern at the poles is not altered during cell enlargement, and that the inter-rotation-center distances may not be changed. The retention of matrix substances at the poles no doubt serves to maintain the adhesion of the rotation centers, without which the cell wall would disintegrate. It is clear that cell extension is, in part, the result of the spreading apart of the microfibrils of each wall layer with relatively little change in the helical parameters. However, since the cell enlarges along all axes, the microfibrils must either be discontinuous at the time of synthesis, part during wall extension, or grow intussusceptively. The finding of sufficient microfibril ends to account, approximately, for one break per microfibril between poles demonstrates that intussusceptive growth is unlikely. The tapering ends of microfibril impressions in the E-fracture face of the plasma membrane near the leading edge of a zone of synthesis. At least nine microfibril ends are visible (arrows), each pointing in the same direction. In this fracture, "terminal complexes" are indistinct. × 64,500. Bar, 500 nm.

**Figure 18** A microfibril "terminal complex" in the plasma membrane E-fracture face located in the mid-region of a zone of synthesis. Note the tripartite leading edge and the divided mid-section. The zone lying between the arrowheads shows some structural modification by comparison with the bulk of the microfibril. × 85,000. Bar, 100 nm.

**Figure 19** Plasma membrane E-fracture face at the trailing edge of a zone of synthesis. Note the extent to which the surface is corrugated by comparison with Fig. 18. Microfibril "terminal complexes" may be discerned, e.g., between the arrowheads, apparently pushing beneath the previously formed microfibrils. × 85,000. Bar, 100 nm.

**Figure 20** P-fracture face of plasma membrane showing enlarged indentations made by microfibril ends (arrows). Note the high density of randomly arranged membrane-intercalated particles. × 75,000. Bar, 100 nm.
FigurE 22 Indented shields of a cell after cell wall completion, viewed from the cytoplasmic side. In the upper part of the micrograph the shields are complete (EFi). In the lower part, the shields have been broken open revealing the P-fracture face of the outermost part of the sac (PFo). Note the ribbed granular nature of the shield/plasma membrane junctions (arrows). Magnification as in Fig. 23.

FigurE 23 Outer aspect of indented shields (S) underlying the plasma membrane (P). The discrete nature of the shields is clear. Note the fine striations (arrows) at plasma membrane/shield junctions. × 38,000. Bar, 1 μm.

FigurE 24 Membrane surfaces revealed in a fractured young autospore lacking any wall. Patches of the P-fracture face of the plasma membrane (PF) are present at places where the edges of shields are in contact. One complete shield has been outlined (S). Note that some unconnected grooves are present (arrows), perhaps indicating that subdivision of shields occurs at this time. × 41,500. Bar, 1 μm.
brils in expanded mother walls, described earlier by Schnepf (36), might indicate that the synthetic process is gradually switched on and/or off, but could result equally from microfibril shearing or enzymic dissolution. The relationship between the pattern of microfibril deposition and the pattern of subsequent wall extension requires further detailed observations on walls at various stages during the extension process before any firm conclusions can be drawn.

Structures similar to the shields of *Glaucocystis* are found in the taxonomically disputed organisms *Gloeochaete* (19) and *Cyanophora* (20). The extent to which the structures can be considered homologous is uncertain. It is claimed that *Gloeochaete* and *Cyanophora*, like *Glaucocystis*, retain symbiotic Cyanophytes (loc. citato). The possibility of there being some significance in this correlation between the presence of cyanelles and the presence of shields should not be overlooked. However, that the extracellular structures made by these three organisms are quite distinctive makes any functional role for the shields of *Glaucocystis* problematical. Kies (19) has drawn attention to the similarity between shields and the flattened vesicles which develop at the periphery of Dinophycean cells, and which give rise to the thecal plates of these organisms (see reference 8). The close juxtapositioning of the shields (Fig. 24), and the presence of material within them, might indicate that the shields are either relics or evolutionary progenitors of the Dinophycean structures. Robinson and Preston (31) have suggested that the *Glaucocystis* plasma membrane is a complex consisting of the three membrane layers made by the plasma membrane proper and the shields. That the shields are closed sacs and are absent from the zone close to the flagellar bases makes this proposition unnecessary.

*Oocystis* and *Glaucocystis* are the first organisms in which it has been possible to begin to relate the paths taken by microfibril terminal complexes to the pattern made by the microfibrils to the cell wall. It seems appropriate, therefore, to catalogue the principle similarities and differences relating to cell wall formation between these two organisms (see results of this work and 2, 3, 23, 35, 38). Similarities are: overall cell shape; helically crossed polylamellate nature of the bulk of the wall; coincidence of microfibrillar poles with elliptoidal vertices; microfibril cross-sectional dimensions; dimensions of terminal complexes;
and three-phase deposition of the cell wall (primary wall, microfibrillar secondary wall, and interposition of matrix substances). Differences are: primary wall structure; polar structure; “third orientation” microfibrils; zoning of terminal complexes in Glaucocystis only; unidirectionally polarized (Oocystis), as opposed to bidirectionally polarized (Oocystis), microfibril growth; shields in Glaucocystis only; granule bands in Oocystis only; relative positions of microtubules; and presence of subplasma-membrane filament complexes in Oocystis only. This catalogue indicates that these two organisms have arrived at similar wall constructions via differing evolutionary routes.

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