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A Model for the Pattern of Deposition of Microfibrils in the Cell Wall of *Glaucocystis*

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Abstract. Freeze-fracturing of *Glaucocystis nostochinearum* Itzigsohn cells during cell-wall microfibril deposition indicates that unidirectionally polarized microfibril ends are localized in a "zone of synthesis" covering about 30% of the surface area of the plasma membrane. Within this zone there are about 6 microfibril ends/μm^2 cell surface. It is proposed that microfibrils are generated by the passage of their tips over the cell surface and that the pattern of microfibril organization at the poles of the cells, in which microfibrils of alternate layers are interconnected at 3 "rotation centres", results directly from the pattern of this translation of microfibril tips. In a model of the deposition pattern it is proposed that the zone of synthesis may split into 3 sub-zones as the poles are approached, each sub-zone being responsible for the generation of one rotation centre. It is demonstrated that the microfibrillar component of the entire wall could be generated by the steady translation of the microfibril tips (at which synthesis is presumed to occur) over the cell surface at a rate of 0.25–0.5 $\mu m\ min^{-1}$. Microcinematography indicates that the protoplast rotates during cell-wall deposition, and it is proposed that this rotation may play a role in the generation of the microfibril deposition pattern.

Key words: Cell wall – Cellulose – Freeze-etching – *Glaucocystis* – Microfibrils (cellulose) – Morphogenesis – Plasma membrane.

Introduction

It is widely held that the plant cell wall plays a pivotal role in the determination of plant-cell shape (see discussions in Roelofsen, 1959; Preston, 1974). A major variable in plant cell-wall structure is the pattern made by the long cellulosic microfibrils which form the structural framework of the cell wall. Accumulating evidence (Brown and Willison, 1977; Willison and Brown, 1978) supports hypotheses (Roelofsen, 1958; Heath, 1974) that during cell-wall deposition microfibrils elongate by tip growth, and that the growing tip moves in intimate association with the fluid plasma membrane. If this is the case, the microfibril pattern within the cell wall must arise as a result of the paths taken over the cell surface by the growing microfibril tips. Studies of the pattern of microfibril deposition in the formation of morphologically well-defined walls should therefore provide suitable information for testing these hypotheses, as well as valuable insights into one of the many complex processes which determine the form of biological entities.

In recent studies (Brown and Willison, 1977; Willison and Brown, 1978) we have derived a model of the microfibril pattern within the complete wall of the unicellular ellipsoidal alga *Glaucocystis*. *Glaucocystis* cells reproduce by autospore formation. Wall development around autospores is initiated by the formation of a flimsy "primary" wall, and is completed by the deposition of a robust "secondary" wall consisting of a microfibrillar component and a matrix component (Willison and Brown, 1978). Deposition of these components appears to be separated temporally. In the "secondary" wall, microfibrils are arranged about the cell as alternating right-handed and left-handed helices which converge upon the poles (i.e. the ellipsoidal vertices) of the cell (Robinson and Preston, 1971; Willison and Brown, 1978). Careful observation of the cell wall has shown that the microfibrils do not terminate at the poles, but are continuous between wall layers. That is, each microfibril makes a loop at the pole, thereby undergoing a change of the hand of the helix (Willison and Brown, 1978). Microfibrils do not loop about a single point at the pole however, but about three points arranged equilaterally, described as "rotation centres" (Brown and

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Willison, 1977; Willison and Brown, 1978). Twelve wall layers, resulting from these changes in microfibril orientation, may be usually differentiated (Schnepf, 1965; Willison and Brown, 1978).

In the present paper evidence is presented which indicates that the polylamellate wall of *Glaucocystis* is formed by the consistent and orderly translation over the cell surface of a well-defined "zone of synthesis" which contains the microfibril tips.

Material and Methods

The alga, the culture methods, the method of isolating mother-cell walls and the electron microscopical methods were precisely as described in Willison and Brown (1978).

Glaucocystis nostochinearum Itzigsohn, obtained from the culture collection of algae at the University of Göttingen, FRG, was cultured axenically by spraying washed cells onto the surface of modified Kantz medium (Kantz and Bold, 1969) containing 1.5% agar and 1% beef extract (Difco Labs., Detroit, Mich., USA). Cells were scraped gently from the agar surfaces for microscopical examination. Measurements of cell dimensions were made from micrographs obtained using the differential interference contrast ("Nomarski") method on cells mounted in water. Mother-cell walls were obtained from old cultures which had been allowed to settle in distilled water. The mother-cell walls formed a white upper horizon; this was extracted, washed and air-dried as thin layers on electron-microscope grids. Platinum-carbon replicas for observation in the electron microscope were prepared in a conventional manner (Bradley, 1965) using a Balzers (Balzers, Liechtenstein) freeze-etch apparatus. Freeze-etch replicas were prepared from material frozen on specimen supports directly after scraping from the agar surfaces, without chemical pre-fixation or treatment with a cryoprotective agent.

Results

1. Light Microscopy and Cell Dimensions

The general form and dimensions of the mature *Glaucocystis* cell have been described previously (Griffiths, 1919; Geitler, 1924; Echlin, 1967; Robinson and Preston, 1971), but in order to attempt to model the paths taken by microfibril termini over the surfaces of the ellipsoidal cells, it became clear that it was necessary to estimate the cell surface area at the time of microfibril deposition.

Prior to cytokinesis, the cells reach a maximum size (Fig. 1). Cytokinesis divides the protoplast into 4 or 8 uninucleate units (Fig. 2), each of which begins to generate a wall and to increase in volume. The changes in relative dimensions of the long (vertex to vertex) and short (equatorial) axes of the cells during expansion are presented graphically in Figure 3.

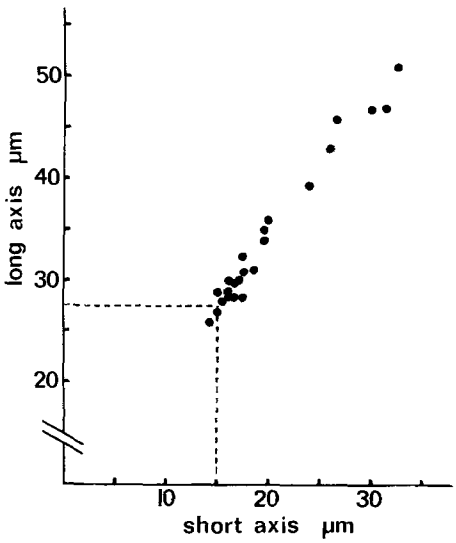


Fig. 3. The relationships between the two principal axes (long, pole to pole; and short, equatorial diameter) of *Glaucocystis* cells. Values were obtained from measurements of cells in an actively growing culture. The dashed lines indicate the cell dimensions used in calculating cell surface area at the time of microfibril deposition

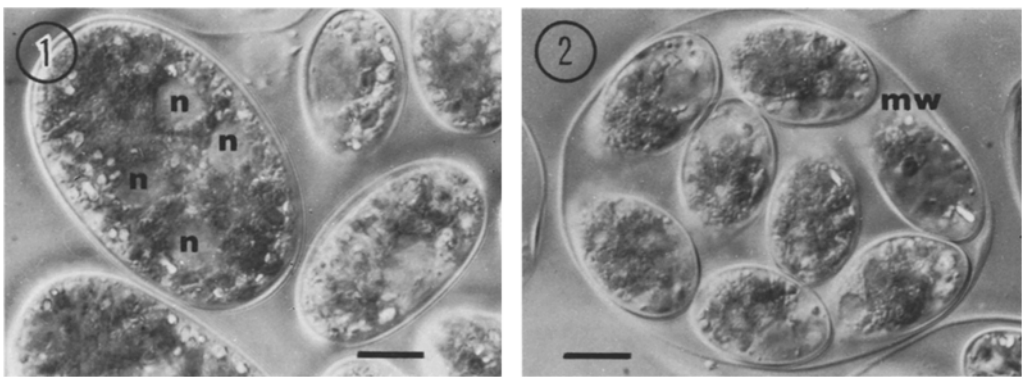


Fig. 1. A mature *Glaucocystis* cell prior to cytokinesis, but containing at least 4 nuclei (n). Uninucleate cells of various sizes are also present. Differential interference contrast. ×880; bar = 10 µm

Fig. 2. Eight daughter cells within a mother-cell wall (mw). Differential interference contrast. ×880; bar = 10 µm

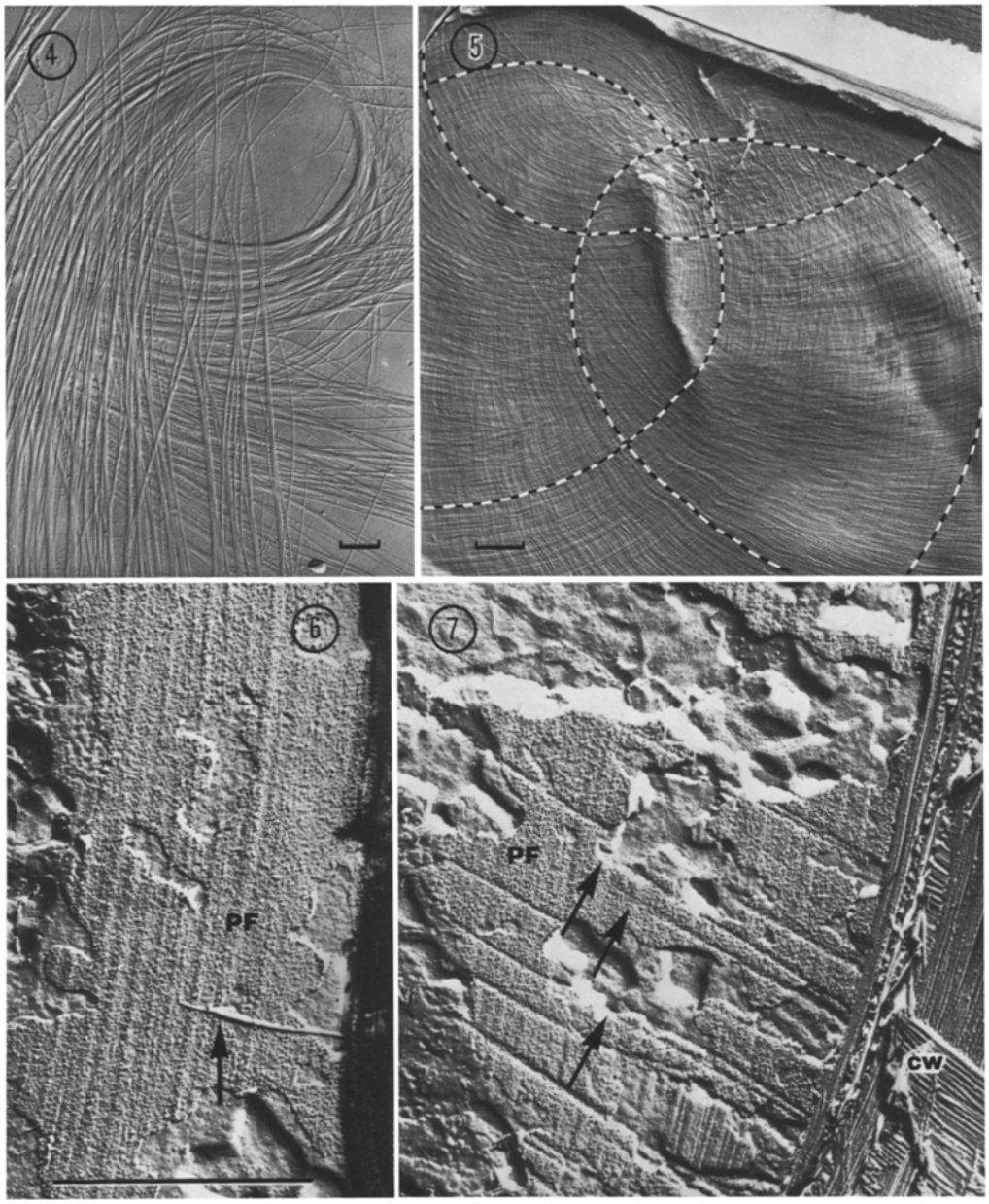


Fig. 4. A fragment of the wall from a preparation of *Glaucocystis* mother-cell walls. The manner in which the microfibrils are looped is very similar to the manner in which microfibrils encircle rotation centres at the poles of mature walls, and the fragment is therefore probably derived from this source. ×5,050; bar = 1 μm

Fig. 5. Surface replica of the polar zone of a mother-cell wall. The manner in which microfibrils encircle 3 "rotation centres" is indicated by the dotted lines. Matrix substances are interspersed between microfibrils. The fold at the top of the micrograph has arisen as a result of the flattening which occurs during drying. ×6,400; bar = 1 μm

Fig. 6. Freeze fracture electron micrograph of the P fracture face of the plasma membrane (PF), at the time of microfibril deposition, in a region of the cell surface not displaying microfibril ends. A microfibril (arrow) has been artefactually displaced from the cell wall. ×35,050; bar = 1 μm

Fig. 7. P fracture face of the plasma membrane (PF) in a region of the cell surface which displays microfibril ends (arrows). Magnification as in Figure 6

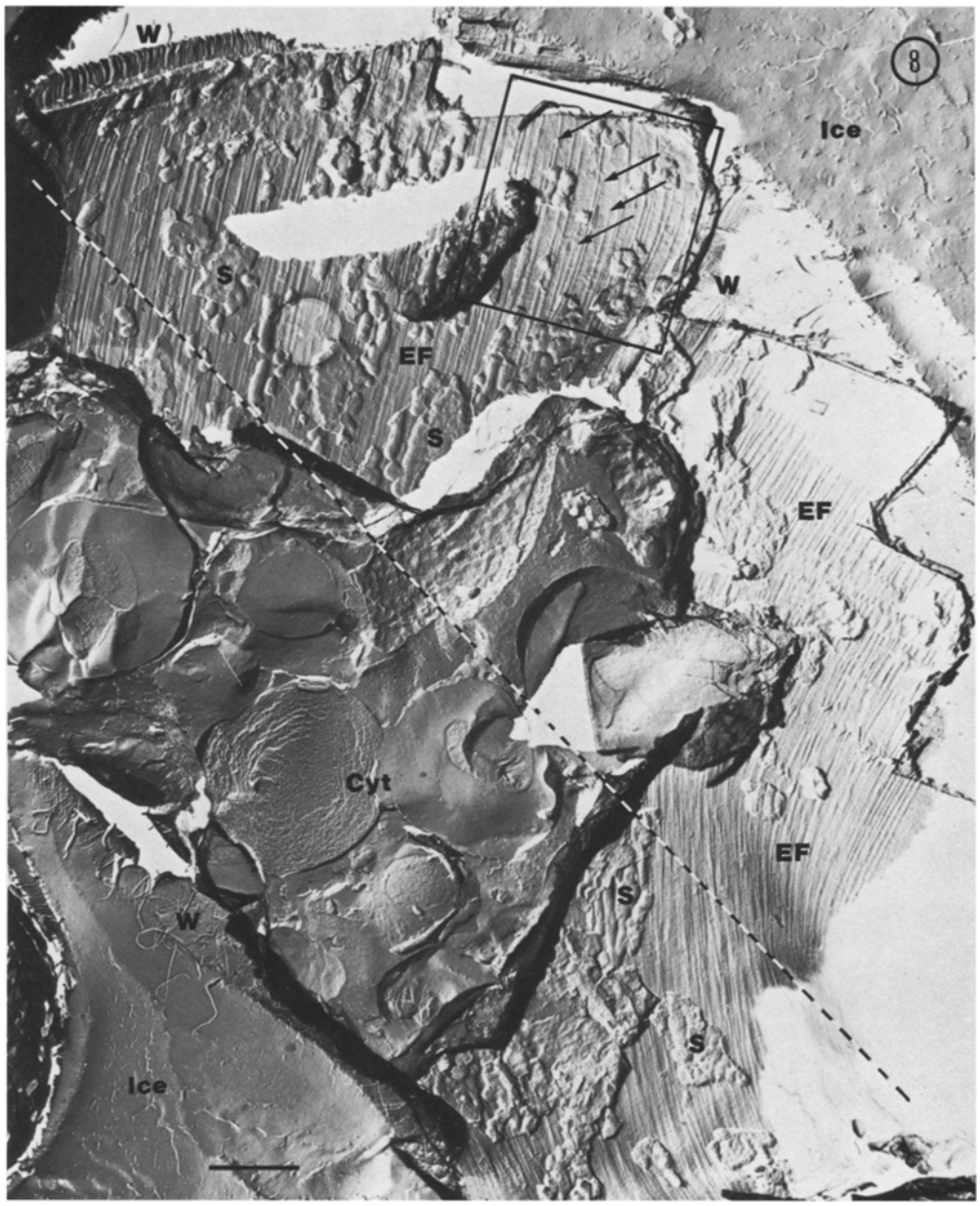


Fig. 8. An extensive region of the E fracture face (EF) of the plasma membrane of *Glaucocystis* in a region displaying microfibril ends (see arrows in boxed region). The pole of the cell lies close to the bottom right hand corner of the micrograph, and the dashed line indicates the long axis of the cell. That the proportion of the plasma membrane bearing impressions of newly formed microfibrils remains approximately constant along lines drawn at right angles to the microfibril long axes, indicates that synthetic zones pass over the cell surface as waves. Cytoplasm (Cyt); frozen suspending medium (Ice); shields (S); wall (W). ×12,025; bar = 1 μm

The cellular expansion is allometric. Since 'secondary' wall deposition begins shortly after cytokinesis, the values shown by the dotted lines were used in calculating the cell surface area at the time of microfibril deposition, and the distance travelled by each microfibril tip during wall formation.

2. Surface Replicas

Several fragments of the microfibrillar wall were found in preparations of mother-cell walls. One of these (Fig. 4) was looped in a fashion similar to the loops formed around polar "rotation centres" (Willison and Brown, 1978). Polar regions of mother-cell walls (Fig. 5) could be clearly differentiated on the basis of their characteristic microfibril disposition (Brown and Willison, 1977; Willison and Brown, 1978). A band of microfibrils passed around each of 3 rotation centres so as to form 3 sets of loops. The 3 polar bands of microfibrils, associated with any one wall layer, overlapped partially (Fig. 5). Nevertheless, interweaving between polar bands of micro-

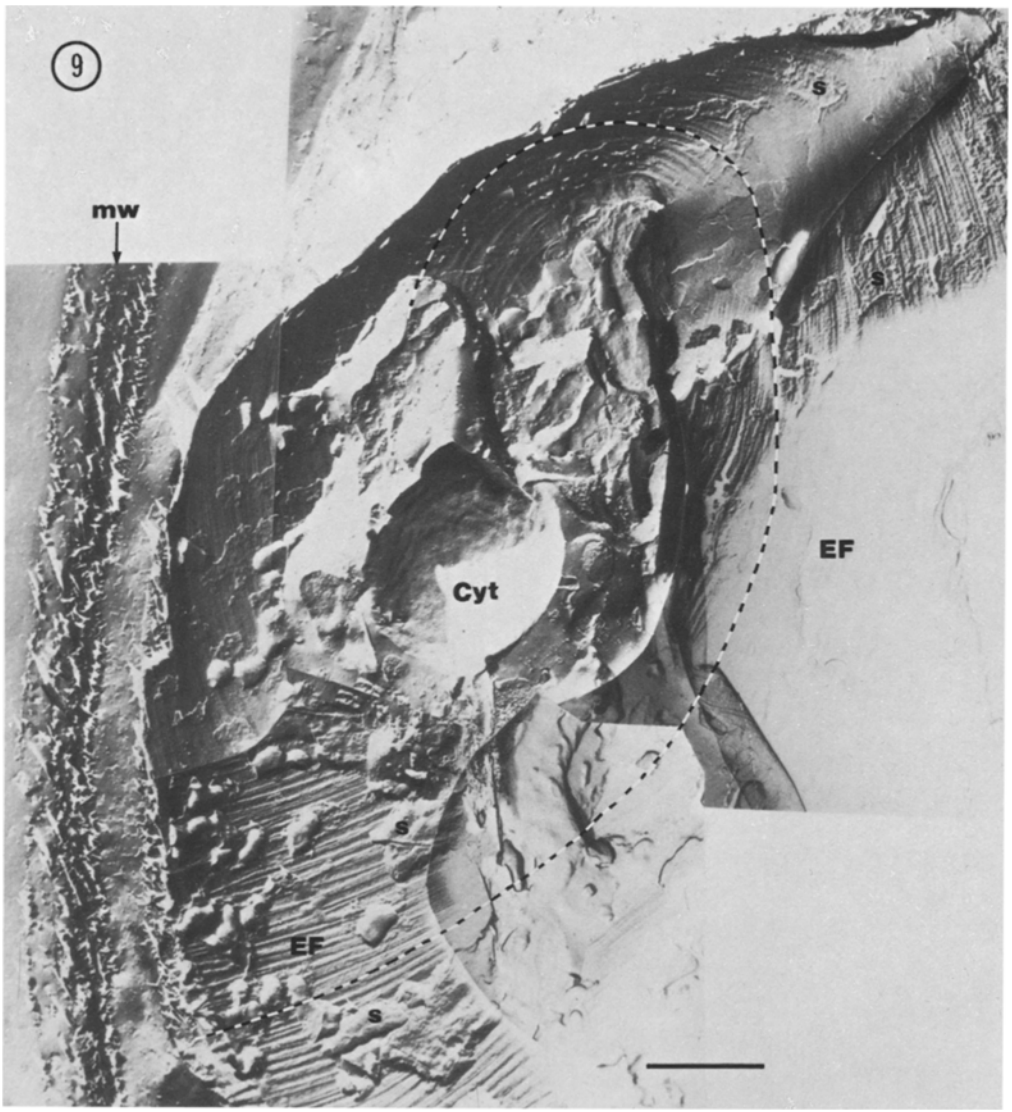


Fig. 9. Composite freeze fracture electron micrograph showing part of the E fracture face (EF) of the plasma membrane at the time of microfibril deposition. The passage of microfibril impressions around a rotation center is indicated by the dashed line. The lower left hand region (EF) contains microfibril ends. Cytoplasm (Cyt); mother-cell wall (mw); shields (s). ×15,500; bar = 1 μm

fibrils appeared not to occur, that is microfibrils of adjacent polar bands were never interspersed between the helically left-handed and helically right-handed arms of the polar loops (Fig. 5).

3. Freeze-etching

The membrane fracture face nomenclature of Branton and colleagues (1975) has been adopted in this work.

In earlier reports (Brown and Willison, 1977; Willison and Brown, 1978), we have shown that impressions of microfibrils, and of microfibril tips, may be observed on plasma membrane E fracture faces during the stage of 'secondary' wall deposition. In order to model the microfibril deposition pattern, we needed to make two qualitative determinations. Firstly, were microfibril ends evenly distributed over the cell surface or were they grouped in some manner; and if they were grouped, what was the spatial relationship between this grouping and the cellular axes? Secondly, was there evidence that microfibril ends encircle rotation centres in the generation of the polar pattern of microfibrils, or might rotation centres arise only after microfibril deposition has been completed? It was also clear that an estimate of the number of microfibril ends per unit cell-surface area would be useful. The above determinations were not easy to make because of the tendency for *Glaucocystis* cells to fracture either within planes within the wall, or to fracture at the extensive "shields" which lie immediately beneath the plasma membrane, rather than

at the plasma membrane itself (Willison and Brown, 1978).

In *Glaucocystis* the developmental stage during which microfibril deposition occurs may be recognized by the absence from the plasma membrane of indentations associated with shields (Willison and Brown, 1978). Initial observations showed clearly that microfibril ends were not evenly distributed over the cell surface during microfibril deposition since contrasting regions demonstrating either the presence, or the absence, of microfibril ends could be found (Figs. 6, 7).

An extensive region of the E fracture face of the plasma membrane representing 5–6% of the entire surface area of the cell and stretched over slightly more than half the cell length, is shown in Figure 8. Microfibril ends are to be found over much of the exposed plasma-membrane fracture face in this case, showing that it represents part of a zone of active microfibril synthesis. From this micrograph, as from other micrographs (Willison and Brown, 1978), it is clear that the microfibril ends are unidirectionally polarized. The micrograph further indicates that microfibril ends probably lie within an extended zone having its long axis lying approximately at right angles to the microfibril axes. Careful observation of the distribution of microfibril ends within such "zones of synthesis" indicates that microfibril ends are roughly evenly distributed within a zone of synthesis. Two parts of zones of synthesis, having all microfibril ends so clearly defined that all could be identified, were found (e.g. the boxed region in Fig. 8). Within these two regions there were about 6 microfibril ends per square micrometre of fractured membrane surface area (5.8 ends/μm^2 in 6.5 μm^2, and 7.2 ends/μm^2 in 2.5 μm^2). The dimensions of the zone of synthesis could not be determined properly, but the micrograph, Figure 8, indicates that it may be of the order of 10 μm wide and at least 16 μm in length.

Freeze-fracturing of the plasma membrane in the polar zone showed clearly that the rotation centre pattern of microfibril deposition may be observed at the time of microfibril deposition (Fig. 9). The ends of some of the microfibrils which encircle this rotation centre are visible near the bottom of this micrograph (Fig. 9).

Discussion

The morphological evidence that microfibril ends are concentrated in a "zone of synthesis" is supported by quantitative data. Measurements made from surface replicas of *Glaucocystis* mother-cell walls indicate

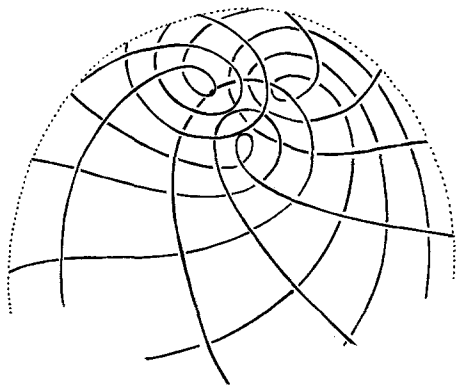
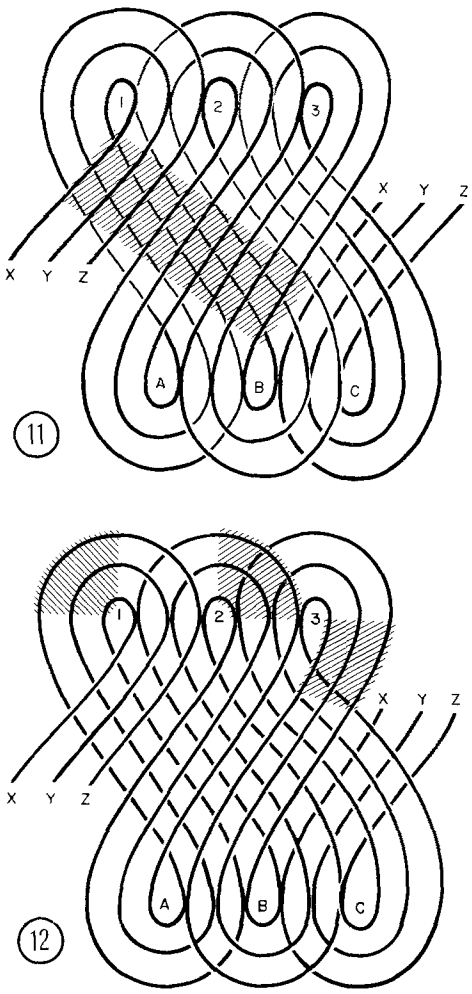


Fig. 10. A diagrammatic representation of the disposition of microfibrils in the wall of *Glaucocystis*, showing part of the ellipsoid with one pole tilted towards the viewer. 3 microfibrils are shown encircling each of 3 rotation centres. This polar pattern is repeated at the other pole. The periphery of the ellipsoid is indicated by a dotted line

that there are roughly 2500 microfibrils in each of the wall layers (Willison and Brown, 1978). If the cell dimensions at the time of microfibril synthesis are taken to be 27.5 μm × 15 μm, then the cell surface area is about 1300 μm^2 ($4\pi R_1 R_2$, where R_1 and R_2 are minimal and maximal radii). If 2500 microfibril ends were evenly distributed over this area, then there would be less than 2 ends/μm^2 of cell surface. That there are about 6 microfibril ends/μm^2 of cell surface in a zone of synthesis indicates that the synthetic zone has a total area of about 400 μm^2 (assuming that the microfibril ends are evenly distributed within the zone of synthesis and that microfibrils grow from one end only). If, as indicated by the freeze fracture results, the zone of synthesis is about 10 μm wide, then it must be about 40 μm in length if uninterrupted. This is slightly less than the 45° helical distance between poles in an unexpanded cell.

The simplest model for microfibril deposition is that microfibril tips, together with the associated "terminal complexes" of membrane intercalated particles (Willison and Brown, 1978), move across the surface of the cell in a pattern which reflects the pattern of microfibrils in the completed wall. The proposed polar organization of microfibrils is shown diagrammatically in Figure 10 for reference. On this basis a model may be constructed in which microfibril tips within the zone of synthesis move in a coordinated fashion, such that the integrity of the zone of synthesis is not disrupted during the synthesis of the wall except in the formation of the poles (Figs. 11, 12). It is proposed that during the deposition of microfibrils in the non-polar regions of the cell surface, an elongated zone of synthesis, containing the microfibril tips, lies at right angles to the microfibril long axes (Fig. 11). As microfibril synthesis progresses and the zone of



Figs. 11 and 12. A model showing the way in which complete wall layers can be formed despite the presence of 3 rotation centres at each pole. The freeze-etching and mother-wall data support this model. One complete wall layer is shown, layed out flat, with the 3 rotation centres of each pole separated. Thus, the 3 microfibrils (X, Y, Z) at the right of the figures, having completed the wall layer, are superimposed on those to the left of the diagram, just as in a world map the left and right hand edges are coincident

Fig. 11. The zone of synthesis (i.e. that region containing the microfibril tips), occupying about one third of the cell-surface area, lies with its long axis normal to the long axes of the microfibrils and is progressing up the page

Fig. 12. Progression results in the zone of synthesis reaching the upper pole. Because of the width of the zone of synthesis, overlapping in the passages of the three sub-zones of synthesis does not occur, and there is no interweaving between microfibrils at the poles of the cells. After transit around the polar rotation centres, the 3 sub-zones of synthesis would recombine in the formation of the next wall layer

synthesis approaches either pole, the zone breaks progressively into 3 sub-zones, each encircling one of the rotation centres (Fig. 12). In this way, interweaving of microfibrils associated with different rotation centres is avoided. Once the polar microfibrils have been completed, the 3 sub-zones would recombine and the zone of synthesis would progress to the other pole. If a pattern of synthesis such as this does operate, then there seems no need for microfibril synthesis to be interrupted until the wall has been completed. That is, once synthesis had been completed, each microfibril would be some 750 μm in length, wrapped elaborately about the cell in 12 interconnected layers. While the results presented in this paper do not exclude models of microfibril deposition other than that described above, this model is compatible with the results and does demonstrate that the *Glaucocystis* wall might be constructed by the orderly progression of microfibril tips over the surface of the cell.

Unfortunately, we have not been able to determine the time period of microfibril synthesis with any degree of accuracy, but it appears to be between 1 and 2d (the life-cycle period is about 4d). Microfibrils 750 μm in length will therefore elongate at the rate of 0.25–0.5 μm min^{-1}. This rate is considerably slower than the measured rate (2 μm min^{-1}) for bacterial cellulose synthesis (Brown et al., 1976), but is comparable with the (possibly non-cellulosic) growth rate (0.4 μm min^{-1}) reported in *Poteriochromonas* by Schnepf et al. (1975).

The problem of the means by which this elegant wall is constructed is not as simple as our model might indicate. The translation of the microfibril tips (and their presumed microfibril-synthesizing terminal complexes) across the plasma membrane need not be the only means by which microfibril orientation is determined. Movement of the protoplast as a whole can occur. In the scale-bearing alga, *Pleurochrysis*, deposition of the scales evenly over the cell surface is obtained by rotation of the protoplast, with its scale-forming Golgi complex, within the wall (Brown, 1975). That microfibrils are deposited without displacement in the flagellar zone of *Glaucocystis* despite the irregular topology of the flagella (Willison and Brown, 1978) stimulated us to observe the cells using time-lapse microcinematography. The protoplasts of smaller cells, presumably those depositing a wall, could be seen rotating unidirectionally about the long axis of the cell. We cannot at present determine whether this rotation is simple cytoplasmic streaming or whether it might be driven by the rudimentary flagella. Protoplast rotation co-ordinated with pole-to-pole translation of the microfibril tips could account for the pattern of wall microfibril deposition. There is a well-defined "equatorial band" of amorphous material lying in the periplasmic space at the equator of *Glaucocystis* cells (Schnepf et al., 1966; Willison and Brown, 1978). The flagella are located in this same region of the cells. The equatorial band might thus be evidence of this protoplast rotation,

its amorphous contents being secreted by the Golgi complexes located at the flagellar bases (Schnepf et al., 1966).

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