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Surface Architecture of the Plant Cell: Biogenesis of the Cell Wall, With Special Emphasis on the Role of the Plasma Membrane in Cellulose Biosynthesis

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Cell wall structure and biogenesis in the unicellular green alga, Oocystis apiculata, is described. The wall consists of an outer amorphous primary layer and an inner secondary layer of highly organized cellulosic microfibrils. The primary wall is deposited immediately after cytokinesis. Golgi-derived products contribute to this layer. Cortical microtubules underlie the plasma membrane immediately before and during primary wall formation. They function in maintaining the elliptical cell shape. Following primary wall synthesis, Golgi-derived materials accumulate on the cell surface to form the periplasmic layer. This layer functions in the deposition of coating and cross-linking substances which associate with cellulosic microfibrils of the incipient secondary wall. Secondary wall microfibrils are assembled in association with the plasma membrane. Freeze-etch preparations of untreated, living cells reveal linear terminal complexes in association with growing cellulosic microfibrils. These complexes are embedded in the EF fracture face of the plasma membrane. The newly synthesized microfibril lies in a groove of the outer leaflet of the plasma membrane. The groove is decorated on the EF fracture face by perpendicular structures termed "ridges." The ridges interlink with definitive rows of particles associated with the PF fracture face of the inner leaflet of the plasma membrane. These particles are termed "granule bands," and they function in the orientation of the newly synthesized microfibrils. Microfibril development in relation to a coordinated multienzyme complex is discussed. The process of cell wall biogenesis in Oocystis is compared to that in higher plants.

Key words: cellulose biosynthesis, freeze-etching, plasma membrane, cell wall, unicellular alga

INTRODUCTION

The cell walls of higher plants and algae are examples of elaborate and highly organized extracytoplasmic structures. The cell wall is composed of cellulosic microfibrils arranged with varying degrees of organization, and noncellulosic components generally referred to as pectins, hemicelluloses, and cell wall protein (1, 2). In our investigation of Oocystis apiculata, a unicellular alga with large, highly organized microfibrils, we have attempted to formulate a model of cell wall biosynthesis with special emphasis on the role of the plasma membrane in cellulose formation.

METHODS

One- to 2-week-old axenic cultures of Oocystis apiculata (IUC col. #B418) grown on Modified Kantz Medium (3) were fixed with a 1% glutaraldehyde-tannic acid mixture followed by osmication (1%), washing, dehydration through an ethanol series, and embedment in Epon. For carbohydrate localization, sections were exposed to 1.0% periodate followed by silver methenamine (4). For plasma membrane staining, sections were floated on a mixture of 1.0% PTA in 10% chromic acid, according to the procedure of Rambourg (5). To obtain freeze-etch replicas, untreated cells were placed on gold specimen holders, frozen in Freon 22, stored in liquid nitrogen, and processed with a Balzers M360 freeze-etch apparatus. Etching was for 2 min at -100°C. To examine the effects of fixation on the preservation of plasma membrane structures, samples were fixed in a mixture of 1% glutaraldehyde and 1% tannic acid at room temperature for 2.5 hr prior to processing for freeze-etching. For colchicine treatment, cells were grown on nucleopore filters layered on the surface of the solidified growth medium. The filters were floated on liquid growth medium containing 1% colchicine for 2 hr in the dark, then processed for freeze-etching. Liquid medium without colchicine was used as a control. All material was examined with an Hitachi HU11E electron microscope.

RESULTS

The growth cycle of Oocystis becomes semisynchronous if freshly inoculated cultures are placed in total darkness for 2 days and then grown under constant illumination at room temperature. Most of the cells divide during the 4th day following inoculation. Cell division results in 4–16 unwalled autospores enclosed within a mother cell wall (Fig. 1). Each autospore then produces a cell wall during the next 4 days.

Cell Wall Structure

An examination of fixed cells of Oocystis reveals 2 distinct wall layers (Fig. 6). The outermost and first formed layer is the primary wall (Fig. 2). It is fibrillar in structure and uniformly 0.1 micrometers in thickness.

The inner layer is the secondary wall. It is composed of cellulosic microfibrils, the cores of which are not stained in sectioned preparations (Fig. 5). The microfibrillar cores are coated and cross-linked by densely staining components. The microfibril core averages 5.5×7.1 nm in cross-section. These microfibrils are organized into single roles, each layer oriented approximately perpendicular to adjacent layers (Fig. 5).

Early Wall Development

Stages of primary wall biosynthesis have not been observed, indicating that this is a rapid process. Following the synthesis of the primary wall, periplasmic material accumulates between the primary wall and the plasma membrane (Figs. 3–7). Microfibrils then appear adjacent to the plasma membrane. Rows of microfibrils later appear within the periplasm and finally form the secondary wall external to the periplasmic layer (Fig. 4). Although the role of the periplasm is unclear, it could serve as a pool for cell wall precursors or as a site for the addition of material to microfibrils, including possible coating and cross-linking components. Like the coating and cross-linking material, the periplasm stains densely.

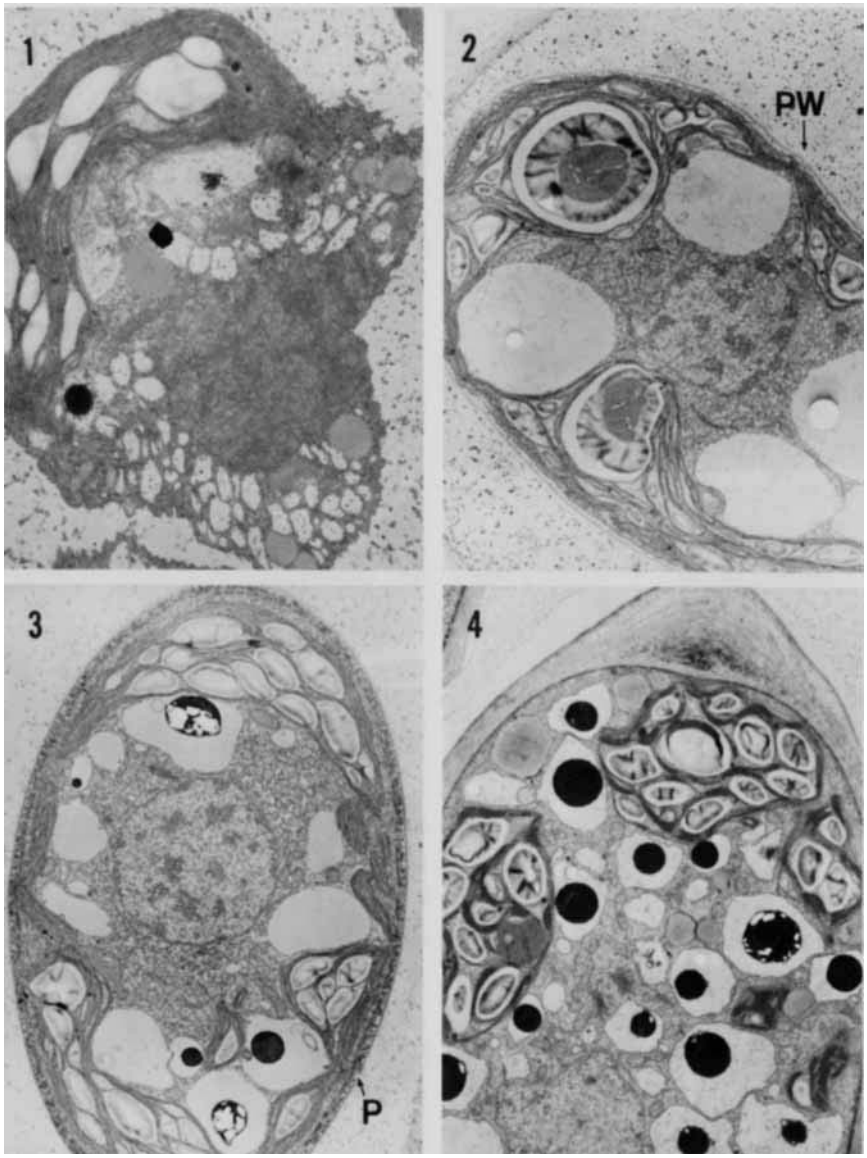
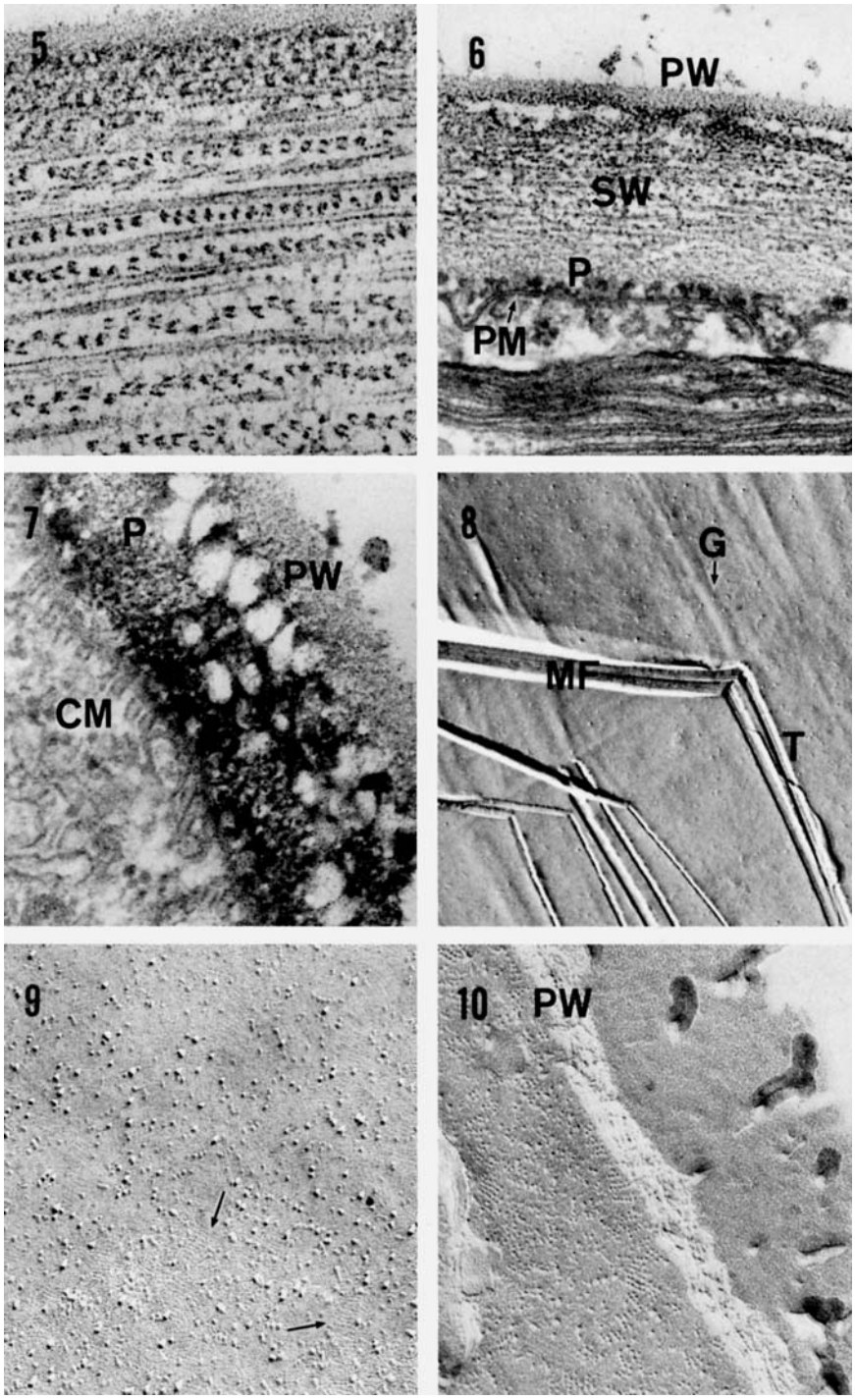


Fig. 1. Young wall-less autospore immediately after cytokinesis. Note loss of ellipsoidal shape due to fixation. × 8,365.

Fig. 2. Young daughter cell at the time of primary wall (PW) production. × 5,295.

Fig. 3. Young daughter cell during secondary wall initiation. Note periplasm (P) between the primary wall and the plasma membrane. × 5,535.

Fig. 4. Mature vegetative cell with a completely synthesized secondary wall. Note the thickening of the cell wall at the pole of the cell. × 5,423.


5
6
PW
SW
P
PM
7
P
PW
CM
8
G
MF
T
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10
PW

Prior to the primary wall synthesis, cortical microtubules form directly subtending the plasma membrane (Fig. 7). These microtubules appear in large numbers during primary wall biosynthesis; however, they disappear during the formation of the secondary wall. It is likely, then, that they act as cytoskeletal structures maintaining the ellipsoidal shape of the cell until the primary wall becomes thick enough to assume this role. This hypothesis is supported by the fact that prior to the accumulation of microtubules in the cell, cell shape is lost following fixation (Fig. 1).

When young unwalled autospores or daughter cells with only primary cell walls are fractured during the freeze-etching process, the EF face of the plasma membrane is commonly exposed (6). This surface of the plasma membrane is covered with randomly but uniformly dispersed particles ranging in size from 9nm to 18nm (Fig. 9). There is no evidence at present linking these particles to primary wall biosynthesis. However, since developing ridges have been observed on the EF fracture face at this stage, it is not unreasonable to assume that early granule band complex formation develops during the later stages of primary wall synthesis (Fig. 9). This is substantiated by the observation of developing granule bands in PF fracture faces (Fig. 10). The granule band complex which functions in orientation of secondary wall microfibrils will be explained below.

Secondary Wall Development

Freeze-etched preparations of cells during secondary wall biosynthesis commonly reveal the EF face of the plasma membrane (Fig. 8). Less often the PF face is exposed (Fig. 17). The innermost microfibrils of the cell wall are closely appressed to the plasma membrane (Fig. 8). Occasionally microfibrils are seen to have torn back through the membrane during the fracturing process leaving a rip in the EF face, and often leaving a piece of microfibril protruding from the surface of the replica (Fig. 8). Such tears in cells actively engaged in secondary wall synthesis are always continuous with grooves seen on the EF face, indicating that microfibrils occupy these grooves on the ES surface of the plasma membrane.

Linear arrays of particles with an average diameter of 8.5nm are found on the EF face of the plasma membrane during secondary wall biosynthesis (Fig. 11). They are often associated with the termini of grooves which contain microfibrils, suggesting their involvement in microfibril assembly and possibly in the synthesis of cellulose. These linear com-

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Fig. 5. Microfibril orientation pattern in the secondary wall. × 87,750.

Fig. 6. Cross-section through a mature cell wall revealing the primary wall (PW), the secondary wall (SW), and the periplasm (P) between the wall and the plasma membrane (PM). × 57,600.

Fig. 7. Cross-section through a young daughter cell at the time of periplasm (P) accumulation. Note numerous parallel cortical microtubules (CM). × 82,350.

Fig. 8. EF fracture face of the plasma membrane during secondary wall formation with tears (T) made by microfibrils (MF) which occupy grooves (G) on the ES surface. × 37,720.

Fig. 9. EF face of the plasma membrane in a young daughter cell prior to primary wall synthesis. Particles of different sizes are scattered on the membrane surface. Note developing ridges (arrows). × 55,200.

Fig. 10. PF face of the plasma membrane during late primary wall (PW) synthesis. Note developing granule band and the edge of the primary wall to the right. × 96,600.

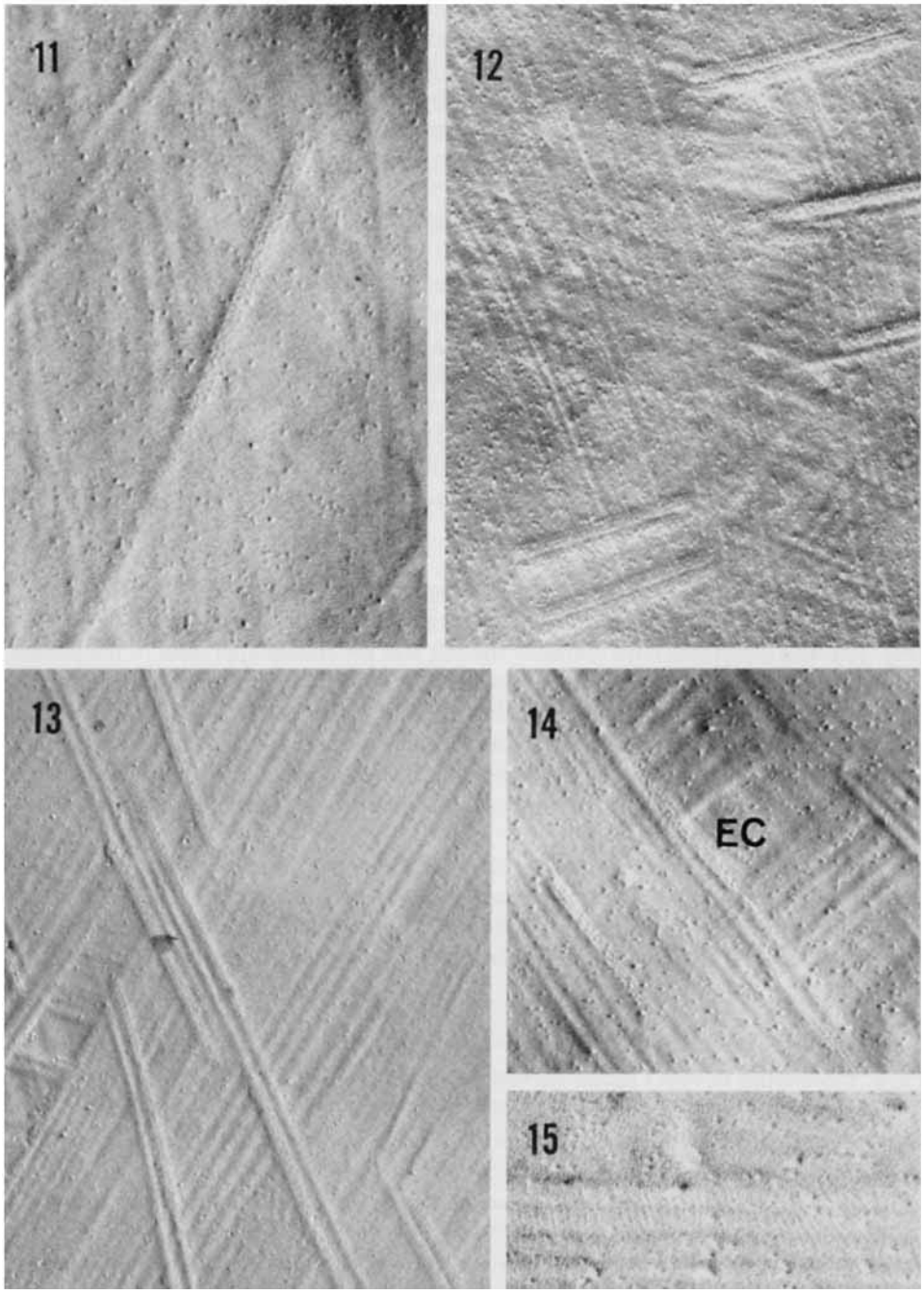

Fig. 11. Terminal complex on the EF face. A groove terminates at one end of the complex. × 76,800.

Fig. 12. Inactive dimer complexes. EF fracture face. Note that all complexes are oriented parallel to one another. × 48,000.

Fig. 13. The initiation of microfibril synthesis. Complexes move in opposite directions with grooves forming behind them. EF fracture face. × 32,400.

plexes have 3 rows of particles. Each row contains 30–40 particles. All the complexes within a given membrane are oriented parallel to each other.

Inactive complexes initially are without grooves, and associated in pairs in a fixed dimer state (Fig. 12). As microfibril assembly is initiated, grooves form at opposite ends of the paired complexes and elongate in opposite directions when the complexes move apart (Fig. 13). The grooves elongate faster than the complexes move, indicating that the assembly process itself (perhaps the crystallization of the microfibrils) may provide motive force for the movement of the complexes. Complexes continue to synthesize microfibrils bidirectionally and in one orientation until the converging microfibrils form wide bands which form the single wall layer (Fig. 14).

Structures interpreted as terminal complexes associated with the ends of microfibrils are seen in sectioned specimens (Fig. 19). Freeze-etched preparations of glutaraldehyde-tannic acid-fixed material indicate that the complexes are present in a modified form on the plasma membrane following fixation (Fig. 20).

Previous studies by Robinson and Preston (7) indicated the existence of granule bands, consisting of lateral aggregates of paired rows, with 8 particles per row (Fig. 16). Our study indicates that the granule bands are associated with the PF face of the plasma membrane, and only during secondary wall biosynthesis do they aggregate to produce extensive rows (Fig. 17). Granule band rows, like grooves, are found to lie parallel to the orientations of microfibrils in the secondary wall. The rows correspond to the last-formed layer of microfibrils, and are continuous, cutting across rows running in other directions (Fig. 17). Granule bands do not lie exactly perpendicular on the rows, but are aligned approximately 70° to the axis of the row (Fig. 17). This angle corresponds to the orientation of ridges which lie across grooves in the EF face of the plasma membrane (Fig. 15). Furthermore, in rare examples, granule band rows terminate with structures which appear to be the depressions left by the terminal complexes on the PF face of the plasma membrane (Fig. 18). It appears, then, that granule band rows of the PF face are associated with grooves of the EF face to form an interlinking structure which traverses the plasma membrane. On the PF face of the plasma membrane in cells which are no longer actively producing cell wall, granule bands occur in patches over the entire surface of the membrane (Fig. 21).

Colchicine Effects

Colchicine is not an inhibitor of cellulose biosynthesis; however, it causes loss of orientation of microfibrils (8). Exposure to colchicine causes no change in the structure of the complexes in Oocystis, but it does cause a loss of orientation in the movement of the complexes, resulting in scattered grooves instead of the normal parallel pattern (Fig. 22). The grooves become irregular with variable ridge length, and the microfibrils are sinusoid within the disrupted grooves (Fig. 24). Colchicine also causes disruption of the organization of granule band rows (Fig. 23). These observations support the contention that granule band-groove complexes are independent structures associated with and maintaining the orientation of newly formed microfibrils.

Fig. 14. Numerous grooves overlying a nearly completed layer of parallel microfibrils. EF fracture face. Note terminal complex (EC). × 54,900.

Fig. 15. Ridges of the EF fracture face positioned across the grooves at an angle complementary to the orientation of granule band rows. × 120,000.

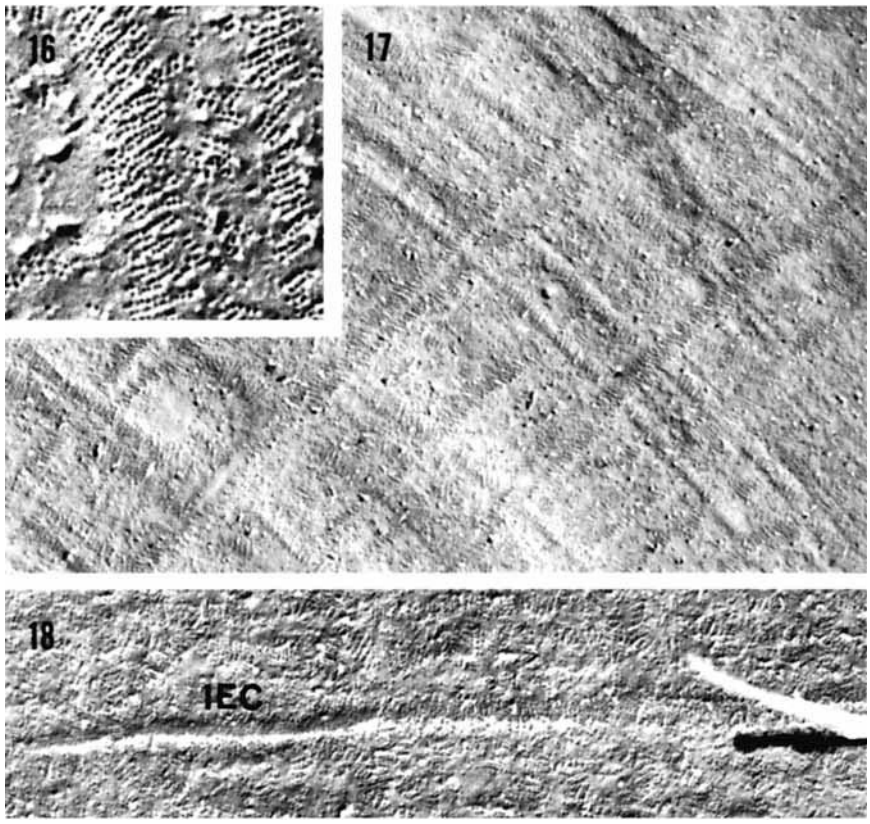

Fig. 16. Granule bands, paired rows 8 particles wide that organize laterally into rows. PF fracture face. × 118,320.

Fig. 17. Granule bands forming extensive rows on the PF face underlying and interlinking with ridges on the EF face. Secondary wall biosynthesis. × 34,500.

Fig. 18. An impression of a terminal complex (IEC) observed at the terminus of a granule band row. × 42,840.

Cytochemical Investigations

Phosphotunstic acid-chromic acid staining is specific for the plasma membrane and membranes differentiating into the plasma membrane in many higher plant systems (9). This specificity appears to hold for Oocystis (Fig. 25). Staining of the secretion faces of the dictyosomes and the membranes of the dictyosome-derived vesicles provides supporting evidence for exocytosis of Golgi-derived products and concurrent incorporation of Golgi-derived membrane into the plasma membrane throughout cell wall biogenesis (Fig. 26).

Carbohydrate localization with the periodic acid-silver methanamine procedure indicates the presence of polysaccharides in dictyosomes, and dictyosome-derived vesicles throughout all stages of cell wall formation (Fig. 28). The primary wall, the secondary wall, and the periplasm also contain large amounts of carbohydrate (Fig. 27). The evidence strongly suggests the role of the Golgi apparatus in the synthesis of noncellulosic

wall polysaccharides, including those of the periplasm. No material corresponding to the fibrillar substructure of the primary wall, nor any material resembling microfibrils, is found in Golgi-derived vesicles.

DISCUSSION

The results of this study demonstrate a complex sequence of events leading to the synthesis and assembly of the wall in a plant cell. First, it should be noted that cell wall biogenesis in the unicellular alga, Oocystis, is comparable to the process in most higher

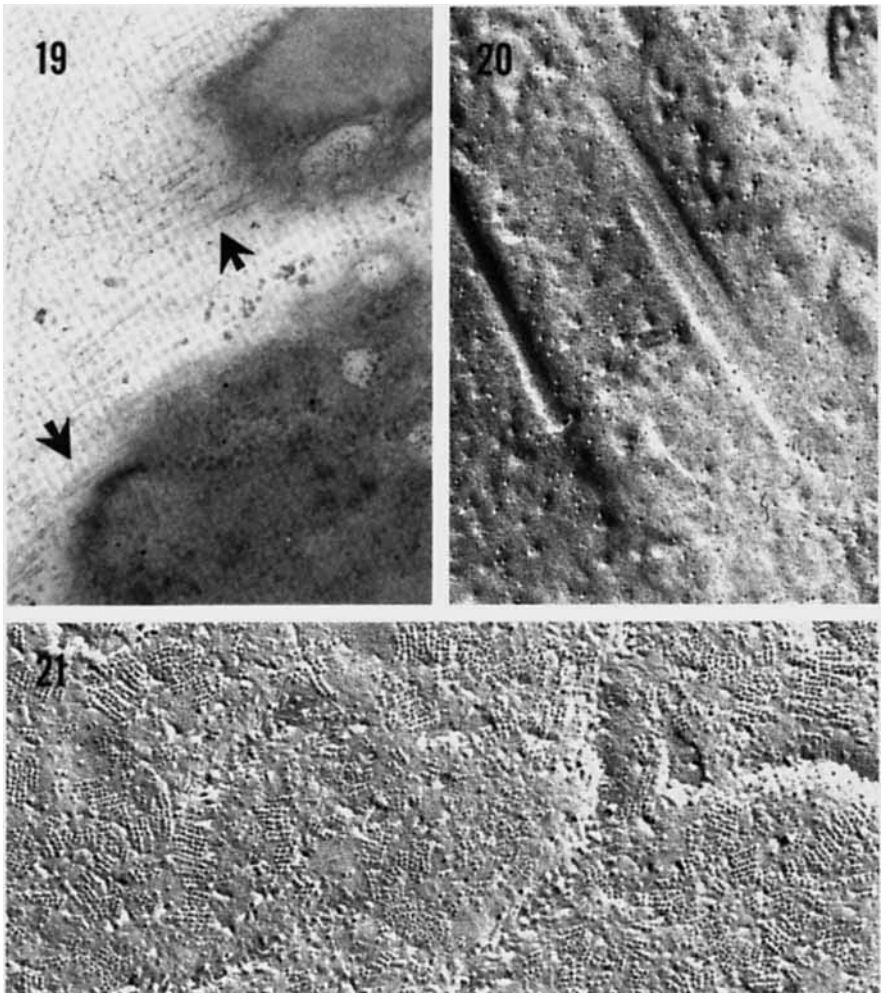

Fig. 19. Near-tangential section of the plasma membrane with associated terminal complexes (arrows). Secondary wall formation. Glutaraldehyde fixation. × 45,750.

Fig. 20. Complexes following fixation with glutaraldehyde-tannic acid. EF fracture. Note the loss of visible substructure. × 54,000.

Fig. 21. PF face of the plasma membrane with scattered granule band patches in a mature cell following secondary wall formation. × 82,800.

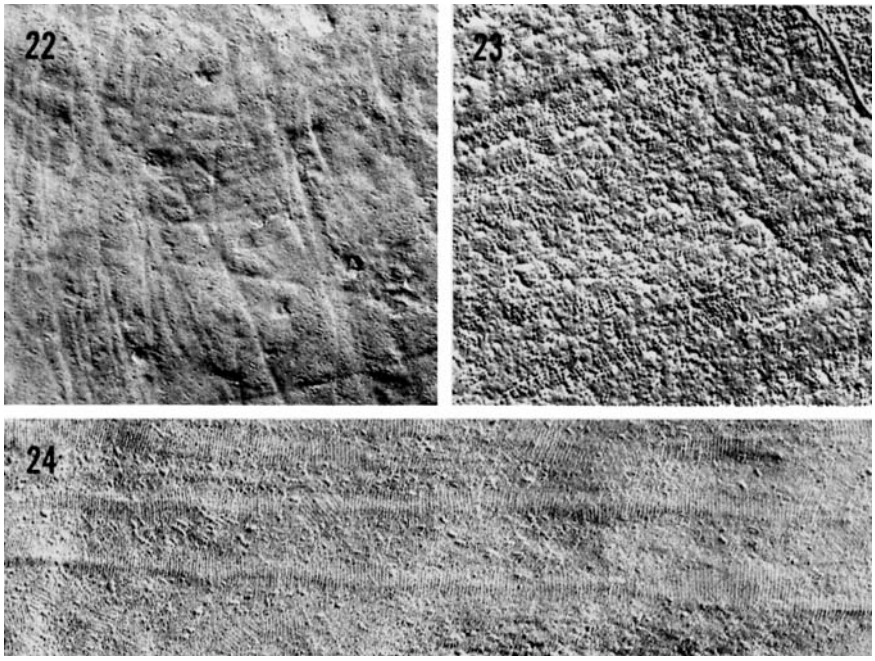

Fig. 22. Disoriented grooves on the EF face following 2 hr colchicine treatment. × 25,500.

Fig. 23. Disorganized granule bands on the PF face following 2 hr colchicine treatment. × 46,920.

Fig. 24. Ridges on the grooves following 2 hr colchicine treatment. Note variable length and angular orientation as well as a slight sinuous curvature of the groove. × 58,140.

plant cells, not only in terms of the similarity of composition of the wall products, but also in relation to the sequential steps of synthesis. Central to the process of cell wall biosynthesis is the membrane system. This includes not only the endomembrane system of endoplasmic reticulum and Golgi membranes (10), but also the plasma membrane.

It is generally accepted that most noncellulosic cell wall components are synthesized intracellularly, packaged, then transported to the surface via exocytosis of Golgi vesicles (9, 10, 11). Our data relating to nonmicrofibrillar cell wall components in Oocystis is in agreement with this concept. We suggest that carbohydrate-containing substances produced by the Golgi apparatus are secreted to form the periplasm. In the periplasm these substances are organized into components which coat and cross-link the cellulosic microfibrils of the secondary cell wall. The biochemical cell wall models of Albersheim and coworkers (1) suggest just such coating and cross-linking properties.

Evidence is rapidly accumulating to implicate the plasma membrane in cellulose biosynthesis. The early autoradiographic experiments of Wooding (12) demonstrated that radioactive glucose fed to cells of the vascular cambium of sycamore seedlings was incorporated into the cell wall before it appeared in any organelles. This implied that the only sites of incorporation could be the plasma membrane or extraplasmic space between the plasma membrane and cell wall. This evidence certainly favors the localization of the active cellulose synthetase near or on the surface of the cell. Bowles and Northcote (13) concluded on the basis of ^{14}C-glucose fed to corn root tips that cellulose was neither

synthesized nor transported by the Golgi apparatus or other cytoplasmic organelles. Evidence favored the plasma membrane as the site of cellulose synthesis. On the basis of cell fractionation experiments, Ray et al. (14) demonstrated that the Golgi fraction contained most of the in vitro β-1,4 glucan glucosyltransferase activity in pea stem homogenates. This reaction yielded an alkali-insoluble product which was digestible by cellulase and consisted of β-1,4 linked glucose residues. Van Der Woude et al. (15) demonstrated similar enzymatic activity, either in the dictyosome fraction, or the plasma membrane fraction, depending on the concentration of the nucleotide sugar used in the in vitro assay. Shore and MacLachlan (16) confirmed the work of Ray et al. (14) but, in addition, found β glucan synthetase activity in the endoplasmic reticulum.

These data suggest that the Golgi apparatus and endoplasmic reticulum may function, at least in higher plants, in the synthesis of β glucan synthetase; however, the site of activation is the plasma membrane.

In one example, Brown and coworkers (4) demonstrated that the Golgi apparatus is fully capable of not only transporting the cellulose synthetase, but also of activating it and synthesizing cellulosic microfibrils within the cisternae. Because of this "incipient" synthesis of microfibrils, special exocytotic mechanisms seem to have evolved for efficiently handling the large Golgi scale product (17).

Intermediate conditions of cellulose synthetase transfer within the Golgi apparatus, but activation upon the plasma membrane, were demonstrated by Kiermayer and Dobberstein (18) in the alga, Micrasterias. Implication of cellulose synthetase transfer from the endoplasmic reticulum to the plasma membrane recently were suggested to occur in the cotton fiber during secondary wall biogenesis (19).

Preston (20) formulated the "ordered granule hypothesis" more than 10 years ago, suggesting on the basis of morphological evidence that cellulose synthesis occurs extracellularly. On the basis of morphological evidence, others have suggested that microfibril synthesis occurs in association with the plasma membrane (21, 22, 23). Ruiz-Herrera et al. (24) have shown that a particulate enzyme complex isolated from the plasma membrane is fully capable of synthesizing chitin microfibrils. Therefore, it is reasonable to assume that such a complex exists for the synthesis of cellulose.

Only through the greater preserving power of rapid freezing without need for cryoprotectants or fixatives, has it been possible to approach the problem of visualizing structures associated with cellulose biosynthesis (25, 26). This approach has been particularly successful with Oocystis by revealing the presence of a particle complex directly associated with elongating microfibrils (27). This compels us to suggest a central role for this complex not only in the synthesis of glucan chains but also in the assembly of the microfibril.

In this regard, we also note that the Meyer-Misch model for the organization of microfibrils of cellulose I (28), and the cross-sectional dimensions of microfibrils in Oocystis (27), indicate the presence of 100 glucan chains per cross-section. Since there are approximately 100 subunits in the complexes, it could be suggested that each subunit is an enzymatic unit for the production of single glucan chains.

In order to achieve microfibril assembly, we believe that each subunit must exist in the form of a coordinated multienzyme complex. Such a complex could function not unlike that found in Neurospora (29), in which 5 different enzymes comprise a complex catalyzing the synthesis of the aromatic complex. In cellulose synthesis, the multienzyme complex might function in catalyzing all major intermediate metabolic steps for glucose phosphorylation and epimerization to nucelotide sugar formation and final transferase

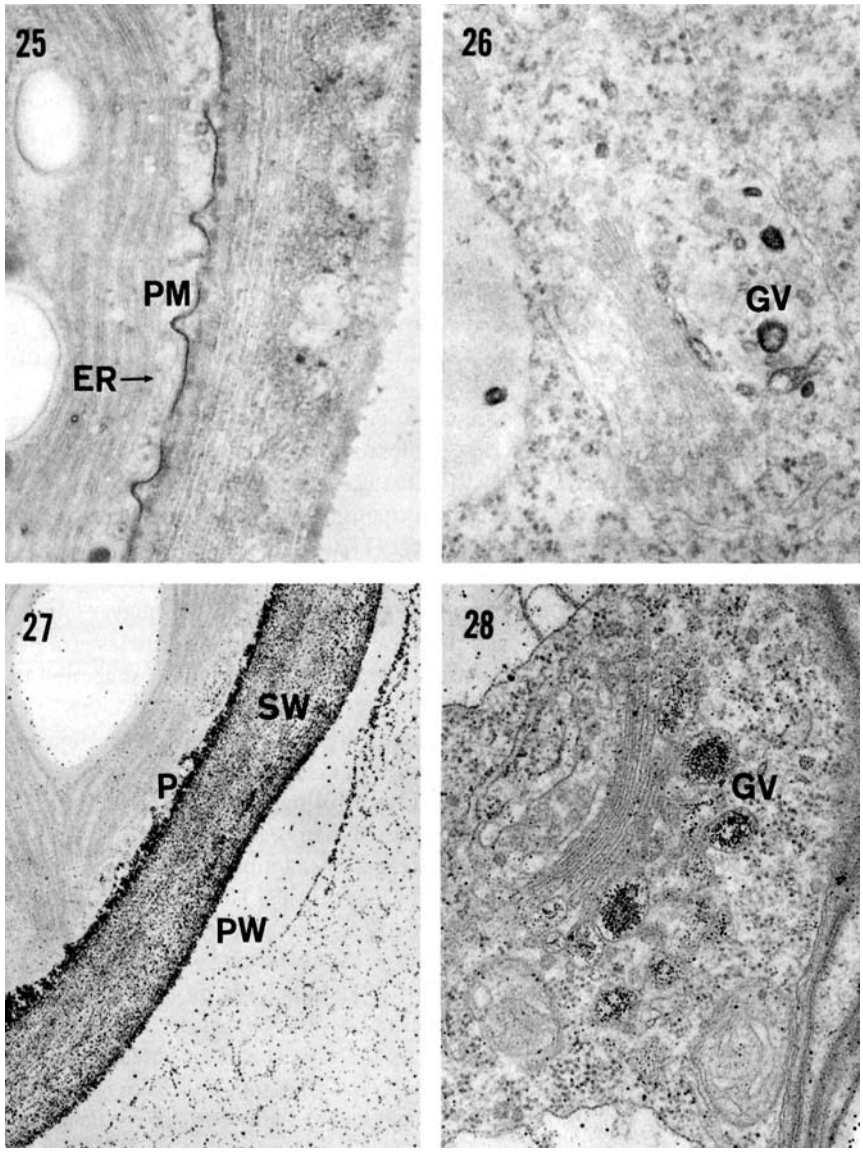

Fig. 25. PTA-chromic acid staining of the plasma membrane (PM) of a cell active in secondary wall formation. Note lack of staining in ER (arrow) and chloroplast membranes. Glutaraldehyde-tannic acid fixation. × 34,425.

Fig. 26. Same cell as in Fig. 25. Note that the secretion face of the Golgi apparatus and its derived vesicles (GV) are stained by PTA-chromic acid. × 4,425.

Fig. 27. Periodic acid-silver methenamine preparation demonstrating reduced silver at the sites of carbohydrates in the primary wall (PW), secondary wall (SW), and periplasm (P). × 19,125.

Fig. 28. Periodic acid-silver methenamine preparation demonstrating carbohydrates packaged into Golgi-derived vesicles (GV) prior to transport and exocytosis. × 33,020.

activity for the synthesis of the β-1,4 cellulosic glucan. As in Neurospora, the complex could have a single, separate site for coordinate activation. Disturbing the sequence of reactions would lead to β-1,3 glucan production. Recent evidence of Delmer (30) indicates that cell-free homogenates of cotton fibers have the capacity to synthesize only β-1,3 glucans, even though these cells normally produce largely β-1,4 cellulosic glucans in vivo.

Traditionally, the classical approach toward in vitro cellulose synthesis has been to use nucleotide sugars to stimulate the in vitro process, in the belief that a greater specificity of incorporation would occur in the terminal steps of synthesis; however, we think it now worthwhile to reexamine the entire problem by using early metabolic precursors and doing so with great care and concern for not disturbing the obviously delicate organization of the complex.

On the basis of the data presented, a model for the secondary wall biosynthesis in Oocystis can be formulated. The wall is composed of 2 types of components, cellulosic microfibrils (7) and densely staining noncellulosic materials which coat and cross-link the microfibrils. The microfibrils are assembled and synthesized by enzyme complexes in association with the plasma membrane. The microfibrils are assembled in a highly specific orientation. Granule band-groove complexes direct and maintain this orientation for each wall layer until it is completed. An acceptance of the fluid mosaic model of Singer and Nicholson (31) is intrinsic in understanding this part of our model, since we are proposing the highly organized movement of large numbers of particles in the plane of the plasma membrane. A review of membrane structure and the evidence supporting the fluid mosaic model recently has been published by Nicholson (32). He describes the relationship of lipid fluidity to the mobility of particles on membranes. Work by Wunderlich et al. (33) indicates the role of colchicine as an inhibitor of membrane fluidity. In Oocystis this, in turn, would account for the loss of orientation of terminal complexes under the influence of colchicine. It would provide an indication that particle complexes indeed move on the surface of the outer leaflet of the plasma membrane.

Although our model for cell wall biogenesis leaves much room for clarification and substantiation, it does provide a framework in which to understand the complex architecture of the plant cell surface. The cell wall is analogous to the stratographic record in geology inasmuch as its morphology is a true time-course reflection of past metabolic events. Elucidation of these events leading to wall synthesis provides a unique opportunity to better comprehend many of the economically important aspects of botanical science such as pathogenicity (34), wood and textile production (35), and renewable hydrocarbon resources (36).

ACKNOWLEDGMENTS

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