The Fine Structure of *Ceratium tripos*, a Marine Armored Dinoflagellate

III. Thecal Plate Formation

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In this third paper of a series on the fine structure of *Ceratium tripos*, the formation of the thick thecal plates is examined. Cells were sectioned at various times during the formation of the plates. Examination of actively growing areas, especially the tips of differentiating horns, revealed a cell covering devoid of plates. Thecal plate formation began immediately below these regions with the plates becoming gradually thicker towards the main body of the cell. Deposition of plate precursor material occurs by two mechanisms. The first is most active early in development, and is accomplished by the deposition of elongate vesicles at the sutures. Several of these vesicles often originate close together and appear superficially similar to stacks of Golgi cisternae. The second mechanism occurs in areas not immediately active in cell shape development and results from deposition along the entire area at the base of a plate. Vesicles originating within the cytoplasm flatten out and fuse with one another resulting in the formation of membrane enclosed bands of precursor materials. The layer of microtubules beneath the plates disappears prior to the onset of the second process.

INTRODUCTION

Work on the overall structure of fully developed dinoflagellate thecae is relatively extensive (*4, 5, 9, 10, 11, 15*), whereas little, if any, information is available concerning their initial formation and development.

Within the past 5 yr several reviews of plant cell wall metabolism and deposition have appeared (*12, 20, 22, 24*). Mühlethaler (*22*) summarized the literature through 1966 and described the roles of organelles in cell wall synthesis. Following this report, several papers appeared which further defined the roles of cellular organelles, especially the Golgi apparatus, in wall deposition.

Of particular significance is the work on scale and coccolith formation in Chrysophycean, Prasinophycean, and Haptophycean algae. Manton's (*17-19*) descriptions of the Golgi system and its apparent functions have been supplemented by those of Brown (*1-3*), who demonstrated that Golgi cisternae are responsible for the production of the cellulosic wall fragments known as scales. Previous workers had distinguished between the pathway of cellulose synthesis and synthesis of other polysaccharides known to form in cisternae.

The functions of other cellular components in wall formation such as microtubules and the endoplasmic reticulum have been investigated extensively (*3, 13, 24*). However, the accumulated evidence has left many questions unanswered.

The cell wall material of the armored dinoflagellates is referred to as cellulose by both classical and modern authors (*7, 14-16*). Recently, however, Nevo and Sharon (*23*) found the major component of the skeletal cell wall of *Peridinium westii* to differ in physical and chemical properties from cellulose; they established that the glucose units were joined by β-1-4 and β-1-3 linkages but were unable to deter-

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mine whether they occurred in the same polymer. Since little work has been done on the dinoflagellates as a group, further investigations may clarify the presence and extent of cellulose in these organisms.

It is puzzling that of the large number contributions dealing with cell wall formation, none considers the armored dinoflagellates. Thick and intricate thecae are produced within short periods of time by many species. Cell wall formation is most obvious following cytokinesis, when dividing cells either retain half of their parent thecae while reforming the other half or synthesize an entirely new wall after shedding the old (15). Based on descriptions of thecal ultrastructure in armored dinoflagellates, suggestions have been made as to the formation of these plates. Dodge and Crawford (6), working with Ceratium hirundinella, described a "discontinuous layer of dark staining material" which lay beneath the plates. They stated that "in the absence of any known mechanism of plate construction it seems possible that this is plate precursor material." Cox and Arnott (4) described "possible evidence of growth zones" in Ensiculifera loeblichii, though no possible mechanism of formation was discussed. Other literature (4, 5, 15) in the area of thecal ultrastructure is concerned almost entirely with its systematic implications.

In studying wall formation one must be able to obtain material in appropriate sequential stages. Because of their large size, individual cells of Ceratium tripos can be selected according to their stage of thecal plate development and sectioned for electron microscopy.

MATERIALS AND METHODS

Ceratium tripos (O.F.M.) Nitzsch was isolated from water samples taken from the north end of the Cape Cod Canal, Bourne, Massachusetts in June, 1970. Cultures were grown in f/2 enriched seawater medium (8) minus silica in a regime of 16 hr light and 8 hr darkness. Cultures were maintained by Dr. Robert R. L. Guillard, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543. Cells began mitosis 2 hr before the end of the dark period and complete cytokinesis several hours after the beginning of the light period. Development of the vegetative cell shape and cell wall synthesis proceeded for an additional 6 hr or more. Cultures were fixed at various times during this period, and individual cells were selected for sectioning depending on their stage of development.

The procedure for preparation of C. tripos for electron microscopy was the same as described for Polysiphonia (25). Sections were cut on a diamond knife with a Porter-Blum MT-2 ultramicrotome and viewed with a Zeiss 9A electron microscope.

OBSERVATIONS

The best method for observing the formation of the thick thecal plates characteristic of C. tripos was to section apical or posterior horns still in the process of development. The very tips of horns show little, if any, plate formation (Fig. 1). Differentiation, however, begins immediately below the tips with the plates becoming gradually thicker towards the main body of the cell (Figs. 2 and 3). Increase in the thickness of the plates appears to continue for a long time following completion of cell shape development. Active wall thickening is generally observed in cells of all ages, although usually limited to specific areas of the thecae in older cells.

Sections through horns at various stages of differentiation reveal striking differences in the nature of the cytoplasm and patterns of organelles (Figs. 1–3). This would be expected since the zone of maximum extension occurs at the tip. The basal region seems more typical in appearance, though highly active in plate synthesis. The concentration of the microtubules beneath the developing cell covering initially increases towards the base of a developing horn. Areas inactive in development have only scattered microtubules beneath the covering. Figures 1–3 show cross-sections at different levels of one developing apical horn. An initial increase in microtubule concentration (Figs. 1 and 2) and the first signs of plate formation are evident. The dense cytoplasm which contains the mi-
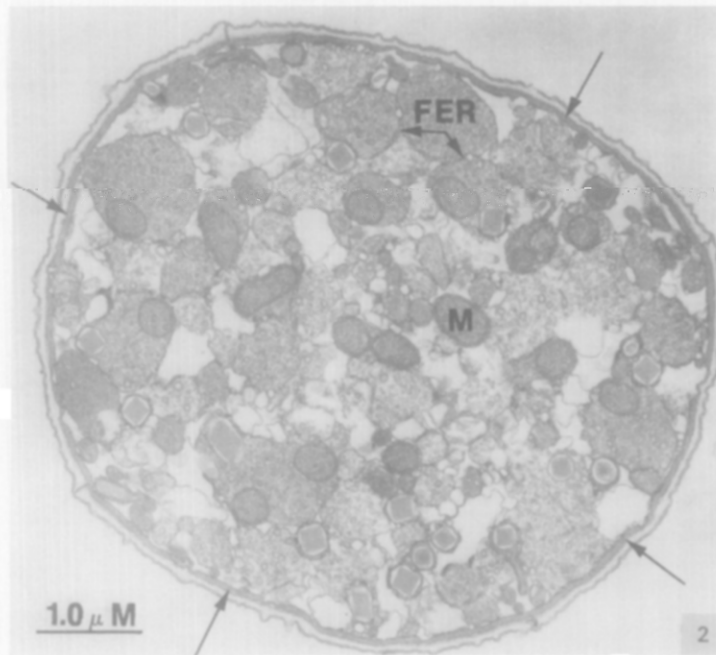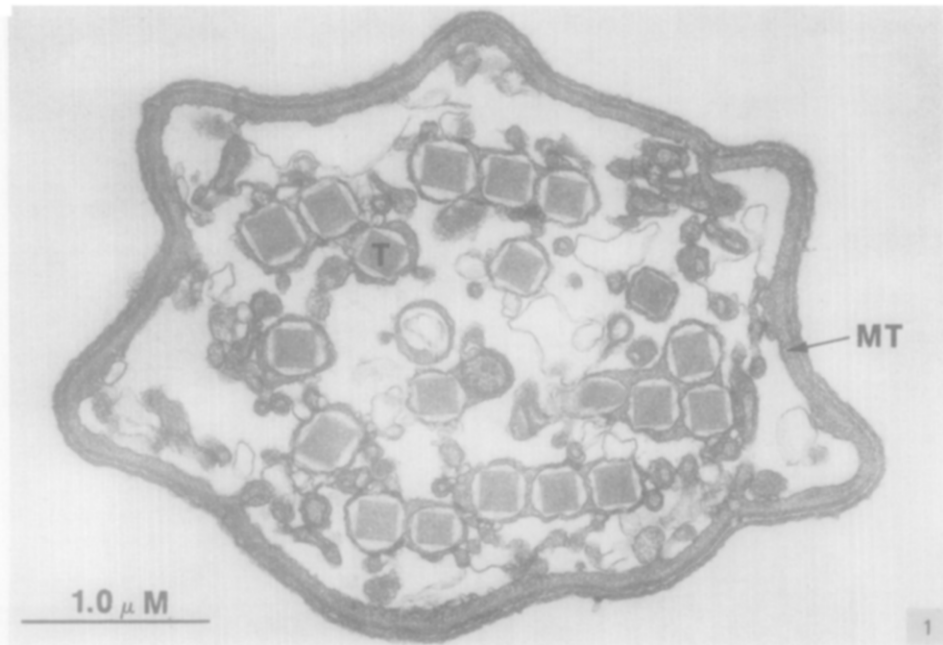
FIG. 1. Cross-section through a developing horn near the apex. The four membranes of the cell covering are underlain by a dense band of cytoplasm lined with microtubules (MT). Plate formation is not evident. Note the high concentration of trichocysts (T). × 25 600.

FIG. 2. Cross-section through a developing horn below the region seen in Fig. 1. Note the high concentration of microtubules beneath the cell covering (arrows) and the initial presence of thecal plates. Mitochondria (M) are oriented longitudinally with their cross-sections visible in this section. Large swollen vesicles and fenestrated ER (FER) divide the cytoplasm. × 14 190.
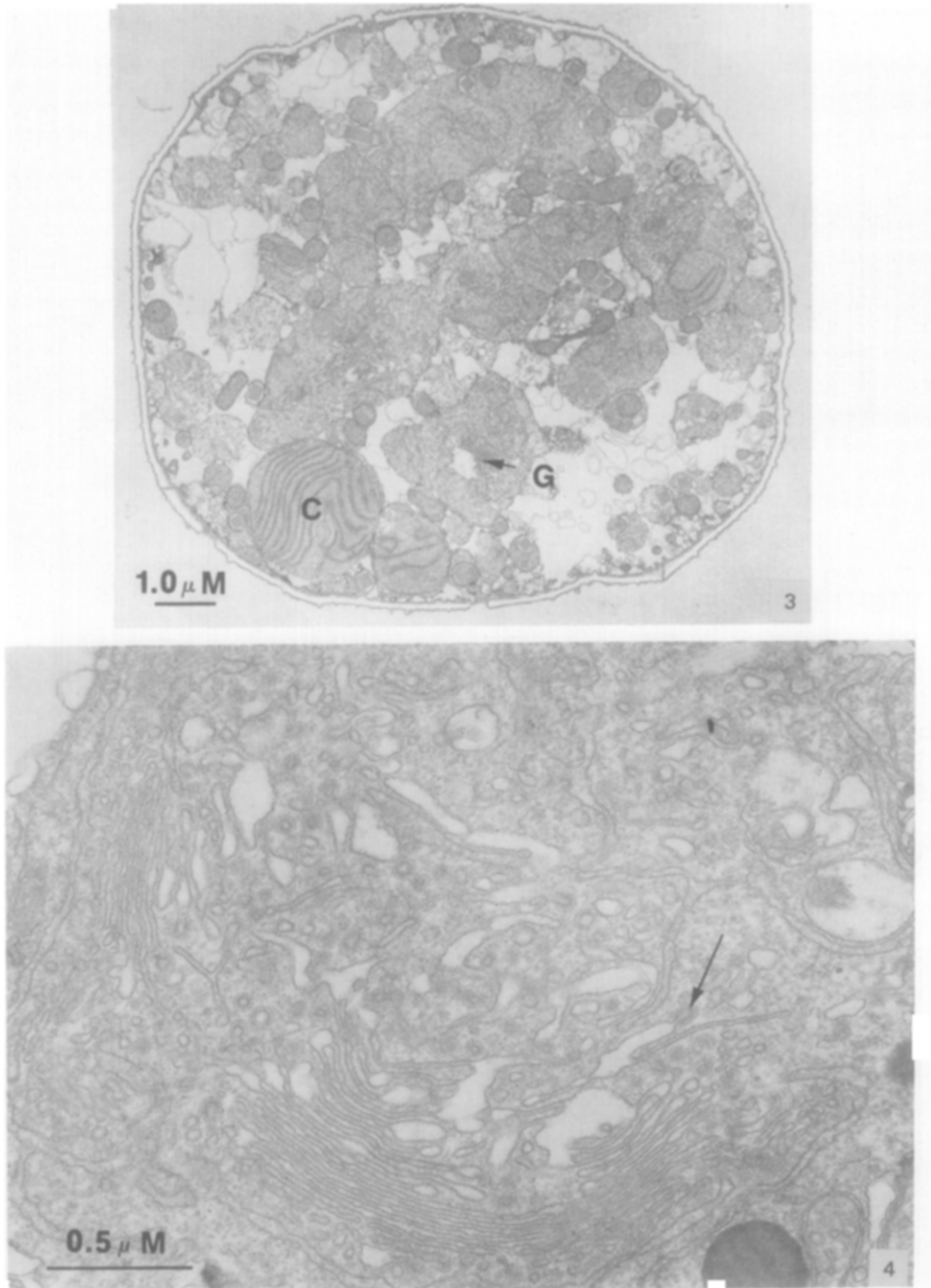
1.0 μM

3

0.5 μM

4

FIG. 3. Cross-section at the base of the developing horn seen in Figs. 1 and 2. Chloroplasts (C) and Golgi (G) are present. Note that the microtubule lined band of cytoplasm is no longer prominent beneath the covering. Thecal plates are thicker. × 8 000.

FIG. 4. Intense Golgi activity at the base of a developing apical horn shows the production of the large swollen vesicles by several cisternae. Small spherical vesicles bud off the larger vesicles (arrow). × 42 600.

crotubules often appears isolated against the cell covering by fenestrated ER and enlarged vesicles (Figs. 1 and 2). These vesicles originate from stacks of Golgi cisternae located at the base of the horn (Figs. 3, 4, and 6), and become increasingly swollen towards the apex. Often several cisternae open into one expanding, membranebound vesicle (Figs. 3, 4, and 6). Small spherical vesicles which appear darkly stained at their periphery arise by a budding process from the sides of the larger Golgi vesicles (arrow, Fig. 4). The presence of Golgi in other portions of the cell is much less extensive than in this area. Above the region of high Golgi activity, layers of fenestrated ER further divide the cytoplasm. These lamellae are often continuous with the expanding vesicles derived from the Golgi (Figs. 2, 4, 7-10). Numerous extremely elongate mitochondria are present, each with the long axis parallel to the direction of horn growth (Figs. 2 and 5).

The large chloroplasts typical of vegetative cells are found only at the base of a developing horn (Fig. 3), and never near the region of actual extension (Figs. 1 and 2). Trichocysts, however, are typically present throughout development. Differentiation of trichocyst pores in the theca appears to occur prior to the synthesis of thecal plates since they are present during early plate formation (P, Fig. 6). Although trichocysts are found in the surrounding cytoplasm during early horn development (T, Fig. 1), it is generally difficult to determine if they are associated with the forming pores.

The structure of the complex cell covering was examined throughout development (26). Basically it consists of an outer plasma membrane (PM) underlain by a series of large flattened vesicles which contain the thecal plates. Early in the development of the theca, an additional membrane, the thecal membrane (TM), lies within the vesicles below the region of plate synthesis. The membranes of the

vesicles are not always continuous and are therefore referred to as the "inner plate membrane" (IMP) and "outer plate membrane" (OPM).

The actual synthesis of the thecal plate material undoubtedly occurs within the flattened vesicles lying beneath the PM and by a process which is similar throughout the development of plate thickness. However, deposition of plate precursor materials within these vesicles apparently is achieved in two different ways. The processes may, or may not, occur simultaneously, depending on the stage of development. The first process appears to involve the fusion of elongate vesicles (EV) at the sutures (Figs. 8-10). This type of deposition is prominent during initial stages of cell shape development while its occurrence diminishes later. The second method appears to result from the fusion of plate precursor materials along the entire area at the base of the plates (Figs. 12-15). This type of deposition is extensive in all regions not active in cell shape development.

It was noted above that a dark band of cytoplasm lined with microtubules lay tightly packed beneath the cell covering during development of the cell shape. Cross-sections of horns at this stage often show the band of microtubules to be discontinuous in the region just below the sutures (arrow, Fig. 7). Although the layer of cytoplasm which contains the microtubules remains continuous, it is observed to be either thinner at this point (arrow, Fig. 7), or broken by the intrusion of an elongate vesicle (EV) (Figs. 9 and 10). Such EVs appear active in the deposition of precursor materials which may be used during the initial synthesis of the thecal plates. Conceivably, constituents could also penetrate inward from the underlying cytoplasm and vesicles. However, fusion of vesicles with the covering is seen only at the sutures.

The fusion of an EV to the cell covering occurs at the point where the IPM enters
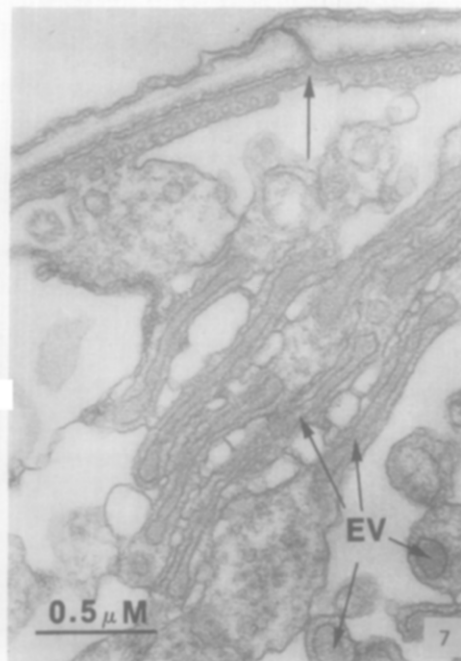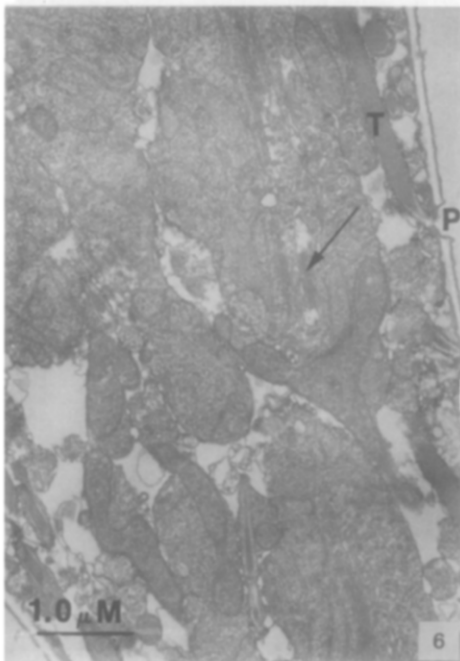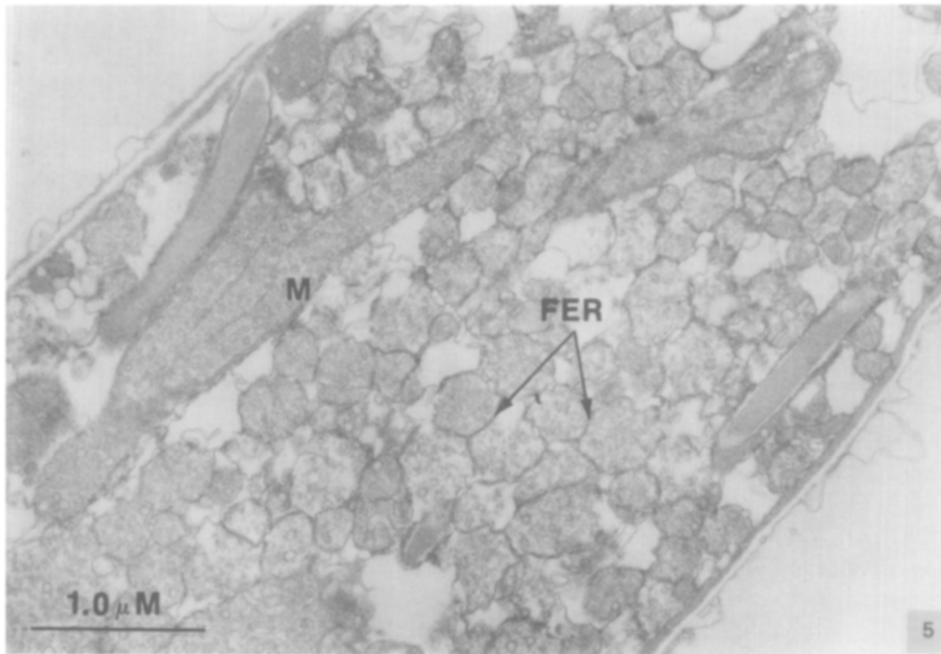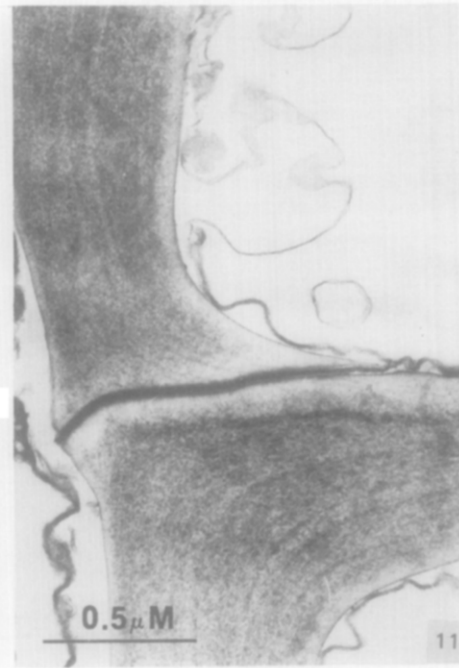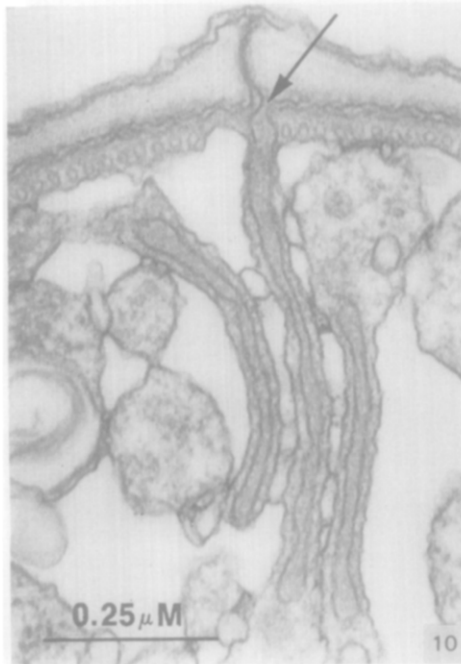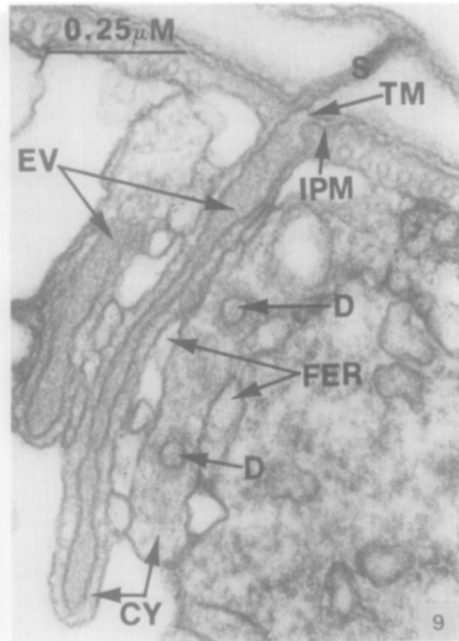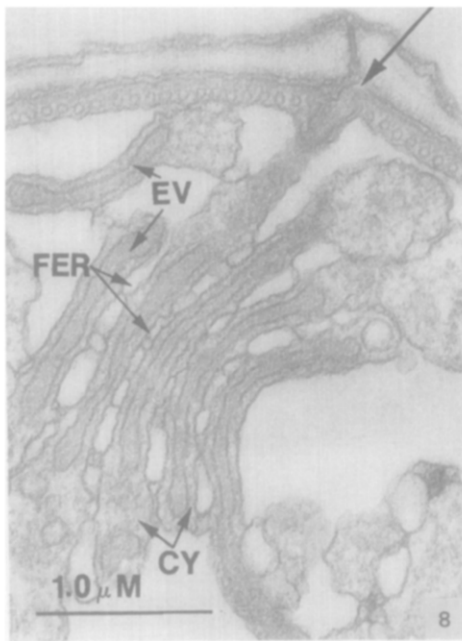
the suture (Fig. 9). One side of the EV wall is thus continuous with one of the unit membranes of the suture, and the other with the IPM of the same vesicle (Figs. 9 and 10). EVs may be seen in various states of penetrating the cell covering and fusing with it. Deposition of EV contents occurs only within the vesicles designated for thecal plate formation.

The EVs apparently originate in the cytoplasm immediately adjacent to sutures (Figs. 7-10). Initially the EVs are relatively small and circular and have darker contents than the surrounding cytoplasm. However, they gradually elongate and increase in size until they occupy most of the patch of cytoplasm in which they originated (Figs. 7-10). Sections taken at various orientations reveal the EVs to be tubular in shape. Single EVs are seen to develop independently of others, though normally several occur side by side in various stages of differentiation. The highly swollen and fenestrated ER which is prominent during this time of horn development always separates adjacent patches of cytoplasm containing EVs (Figs. 8 and 9). Nevertheless the EVs remain closely associated in groups reminiscent of the stacked cisternae of Golgi bodies (Fig. 8).

The TM is generally present at the surface of the IPM, and its morphology is quite variable during the synthesis of the thecal plates. It becomes undulate in appearance and often discontinuous at numerous points within the vesicle (Figs. 7-10).

This method of deposition of precursor materials appears to be employed only where actively developing areas overlie a dense layer of microtubules. In later stages of development fusion at the sutures is still observed but may also have a more specialized function, such as providing the additional amount of material necessary for the production of intercalary growth bands and the high ridges which form around the girdle. Both processes require additional deposition and occur in the region of sutures.

The second method of plate precursor deposition occurs in regions not active in development and appears to be responsible for most of the plate thickness. The EVs evidently are not involved in the accumulation of plate material during this phase of synthesis. Instead of the relatively selective deposition of wall constituents described above, materials are deposited along the entire base of the forming plate. Vesicles originating within the cytoplasm flatten out and fuse with one another (Figs. 12 and 13). The result is a membrane which supports a band of precursor materials deposited by the fusing vesicles. Further fusion below this region results in successive, alternating bands of membrane and precursor material (Figs. 12-15). The innermost band of precursor material becomes incorporated into the thickening plate while the membrane supporting it becomes discontinuous and apparently disintegrates. It is difficult during times of active plate formation to distinguish the IPM and TM of the basic cell covering since they appear to be in a constant state of flux. This type of deposition would explain the layered look of plates which are heavily stained and viewed under low magnification (Fig. 11).

In summary, there appear to be at least two methods of wall formation in *C. tripos*. The first selectively deposits wall constituents at the sutures while the second is active along the entire area at the base of the plates. The former is most prominent early in development, while the latter is not active in areas undergoing cell shape development.

DISCUSSION

The production of thick cellulose walls to the exterior of the plasma membrane is characteristic of most plant cells (24). The mechanisms involved in this type of wall synthesis are assumed to be somewhat

0.25 μ M

12

0.5 μ M

13

0.25 μ M

14

0.25 μ M

15
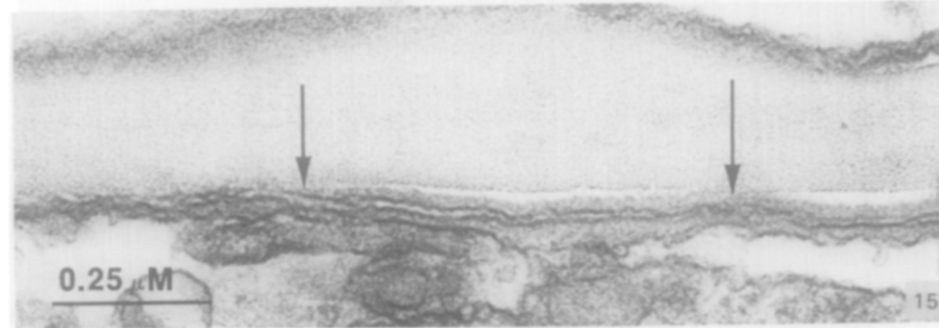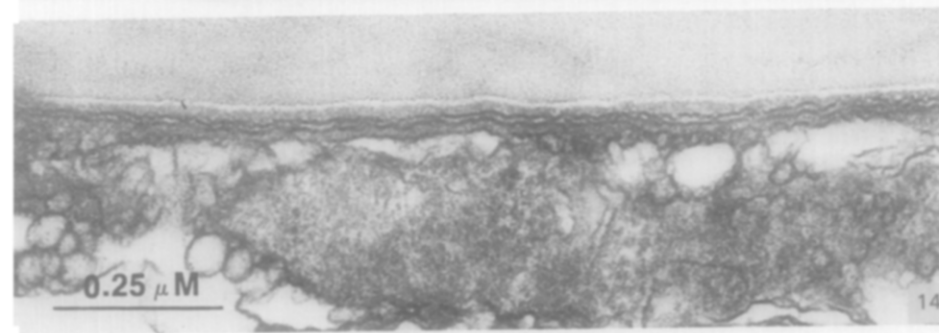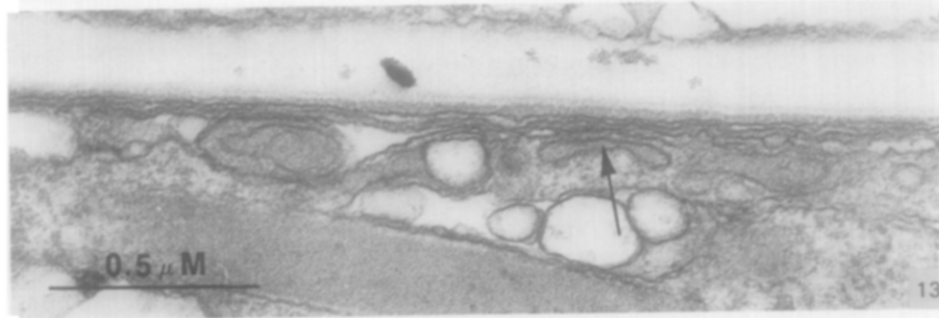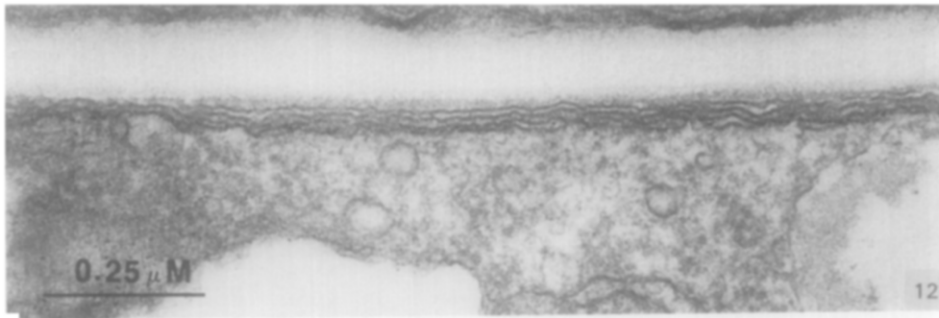
different than those of *Ceratium* where synthesis occurs beneath the PM. The mechanism of cellulose cell wall formation exterior to the PM favored by most investigators (24) involves Golgi-derived vesicles fusing with the PM while depositing precursor materials to the exterior. The vesicle membrane is incorporated into the PM which is believed to serve as the site of synthesis for some or all of the wall constituents (21, 24, 27). The evidence for the role of this membrane in synthesis is hard to evaluate. Although most authors argue that the PM plays a major role in wall synthesis, most agree that its exact function is not known (24, 27). At present the sequence by which walls are synthesized and the origin of their constituents is difficult to ascertain since the pathways involved cannot be marked with sufficient specificity. However, recent work by Manton (17-19) has unequivocally demonstrated that the scales which adorn certain Chrysophycean and Haptophycean algae are derived from Golgi cisternae which were observed in various stages of formation and deposition. Brown (1-3) has shown the scales of the Chrysophyte *Pleurochrysis scherffellii* to be composed of cellulose, indicating that the Golgi may be the site of cellulose synthesis. In these examples, there is a morphological similarity between the scales deposited on the surface of the organism and those found within cisternae in the cytoplasm. Therefore the process of scale formation in these algae is easy to follow ultrastructurally. Similar evidence is not available to describe the sequence of wall formation in organisms where nondescript precursor materials are transported to the surface by several mechanisms (24).

The origin of the vesicles involved in both methods of plate precursor deposition in *Ceratium* is not known. Deposition by EVs at the sutures involves little transport within the cytoplasm since they generally arise adjacent to the site of fusion with the

suture membranes. Several of these vesicles often appear to be arranged in stacks similar to the cisternae of Golgi. This arrangement and the fact that they are involved in the deposition of plate material may indicate at least some similarity to the function of the Golgi apparatus. However, EVs differ from Golgi in several ways. They are not formed and modified in sequence with the mature vesicles occurring at one face nor do their membranes ever come in close contact with each other. The EVs differentiate individually from small vesicles located within the cytoplasm. Mature EVs occupy most of the cytoplasm in which they differentiated and are always separated from adjacent EVs by a layer of fenestrated ER.

The vesicles which fuse to form the alternating bands of membrane and plate constituents are more randomly arranged than the EVs. Fenestrated ER may be present during this period of synthesis, but the vesicles appear to arise randomly in the cytoplasm. Their origin is a mystery. Golgi bodies typical of most plant cells were observed to be active within the cytoplasm during periods of development and plate synthesis, though no direct correlation with plate synthesis could be discerned.

Plate formation by deposition at the sutures does not appear to have much morphological similarity with the second method of deposition, especially since it would be difficult to explain a banded appearance from material which enters from a single point of fusion. However, very little increase in plate thickness is incurred while an area is still active in development of the cell shape. The actual thickness of wall synthesized during this time is minimal compared to the eventual size at maturity. Therefore the thecal plates could easily take on their banded appearance later in development. The EVs are also found in maturing thecae although they are less numerous than in early development. Perhaps they provide additional ma-

terial required for the production of girdle ridges and thecae ornamentation.

The exact composition of the thecal plates in *Ceratium* or any other Dinoflagellate, has not been extensively explored, though all indications appear to confirm the presence of cellulose (14–16, 23). In *C. tripos*, the role of the vesicle membrane which surrounds the region of plate synthesis may be somewhat similar to that of the PM in other plant cells and function as a limiting barrier isolating the region of wall synthesis. Since membranes also have been implicated as being active in wall synthesis, perhaps the TM, which lies at the base of each vesicle during development, acts as a reaction surface for the synthesis of materials deposited at the sutures. The majority of plate constituents are believed to be added by alternating bands of membranes and precursor materials along the entire area at the base of the plates. These successive membranes, which result from the fusion of cytoplasmic vesicles, are at least physically associated with the thickening plates. They seemingly disappear when the band of material they support is incorporated into the plate. The typical membrane associations of the cell covering are altered. The IPM and TM are no longer discernible and microtubules are never found beneath the forming plates. It therefore appears possible that these layers of membranes, like the TM during earlier phases of development, act as a reaction surface for the synthesis of precursor materials to the thickening plate.

As indicated in a previous paper in this series (26), membranes which comprise sutures appear active during periods of plate separation at cytokinesis, suture formation, and production of intercalary growth bands. The dependence of activity dealing with plate phenomena on the presence of membranes is generally indicated.

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