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

I. The Cell Covering (Theca)

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In this, the first of three consecutive papers, the complex cell covering of *Ceratium tripos* is examined at different stages of development. During cytokinesis, the cleavage furrow is surrounded by a system of membranes which, together with the thecal plates, will comprise the future cell covering. The outermost membrane completely surrounds the cell and lies over a single layer of large flattened vesicles. An additional membrane, the thecal membrane, lies within the vesicles just below the region of eventual plate formation. The thecal membrane gradually becomes discontinuous in mature cells. Sutures are observed during the initial differentiation of the cell covering. Near the completion of cytokinesis, the portion of cytoplasm still joining the daughter cells is enclosed by only one membrane of the cell covering, the plasma membrane.

INTRODUCTION

This is the first in a series of papers on the fine structural features of morphology and development in *Ceratium tripos.* Special attention is given here to the complex association of membranes and plates which comprise the cell covering. The taxonomy of armored dinoflagellates has been determined largely by thecal morphology: the number, orientation, and ornamentation of the armored plates. During recent years, several workers have used the transmission and scanning electron microscope in an attempt to substantiate or correct previously questionable taxonomic criteria (*1, 2, 3, 7,* and *8*). Extensive observations by Dodge and Crawford (*3*) on numerous members of the Dinophyceae revealed a uniformity in the construction of the theca throughout the class, and reaffirmed its usefulness as a taxonomic criterion. All flagellated members of the Dinophyceae are enclosed by a continuous outer membrane which lies over a single layer of flattened vesicles. Dodge and Crawford (*3*)

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therefore suggest that separation at the generic level could be determined largely by variations in the morphology of the vesicles and the extent to which plate material is deposited within them. Several investigators have raised questions about Dodge and Crawford's (*3*) interpretation of the basic construction of the cell covering. The most notable difference of opinion concerns the exact location of the cytoplasmic or plasma membrane (PM) (*7, 8,* and *12*). As early as 1901, Kofoid (*9*) described a membrane beneath the thecal plates as the probable location of the plasma membrane. Most recent investigators have tended to agree with this interpretation (*7, 8, 10,* and *12*), although Dodge and Crawford (*3*) designate the outer continuous membrane as the PM. The thecal plates are therefore produced above or below the PM depending on one's interpretation. In addition, Kalley and Bisalputra (*8*) have questioned the assumption that the plates are always enclosed within membranebound vesicles. Their examination of *Peridinium trochoideum* often revealed plates which were not completely separated by the typical suture membranes (*8*).

In almost all cases investigators have worked only with mature cells. In the present investigation, the membranes of the cell covering were studied from their initial differentiation during cytokinesis and cell shape development through the formation of the thick thecal plates. The results revealed a pattern of membranes similar to those described by Dodge and Crawford (*3*). However, an additional membrane, the thecal membrane, was observed within each vesicle during early stages of development.

By following the development of the cell and of the cell covering in particular, it was also possible to substantiate the claim of Dodge and Crawford (*3*) that the outermost membrane is the plasma membrane. During one stage in development, a portion of the cell is enclosed within only one membrane of the cell covering, the plasma membrane.

MATERIALS AND METHODS

Ceratium tripos (O.F.M.) Nitzsch was isolated from 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 (*6*) minus silica in a regime of 16 hr light and 8 hr darkness. Cultures are presently maintained by Dr. Robert R. L. Guillard, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543. Cells begin 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 state of development. Approximately one-third of the cells divided at any one time.

The procedure for preparation of *C. tripos* for electron microscopy was the same as described for

thecal plates are synthesized shortly thereafter. The basic structure of this covering is shown in Fig. 1–6 and illustrated in Diagrams 1 and 2. It consists of four unit membranes which overlie a dense region of cytoplasm lined with microtubules. The outermost unit membrane in this system, the plasma membrane (PM), is continuous around the cell and delimits it from the surrounding environment. The layer immediately beneath the PM, the "outer plate membrane" (OPM), is continuous along predetermined lines with the "inner plate membrane" (IPM), to form a number of large flattened vesicles. Mature thecae occasionally reveal discontinuities between the IPM and OPM which has led to their designation as separate membranes (arrow, Fig. 6). The size, shape, and number of vesicles is characteristic of a species, and their differentiation occurs early in development. Each vesicle encloses both a thecal plate and an additional membrane, the "thecal membrane" (TM). The point of contact between two adjoining vesicles is termed a suture (arrows, Figs. 1, 2, and 5), and consists of two unit membranes, one from each vesicle. A vesicle may therefore be visualized as resulting from the union of the OPM and the IPM at a suture. The TM, which is discontinuous at the sutures, lies just above the IPM and below the region of thecal plate formation. A continuous layer of microtubules lies beneath the covering system during cytokinesis and development of the characteristic cell shape (MT, Figs. 1 and 2). This layer becomes dispersed into small groups of one to four in maturing thecae (MT, Figs. 4
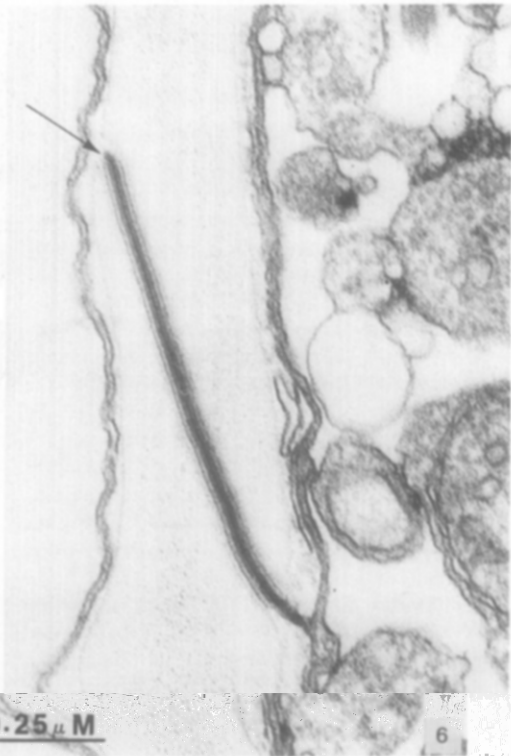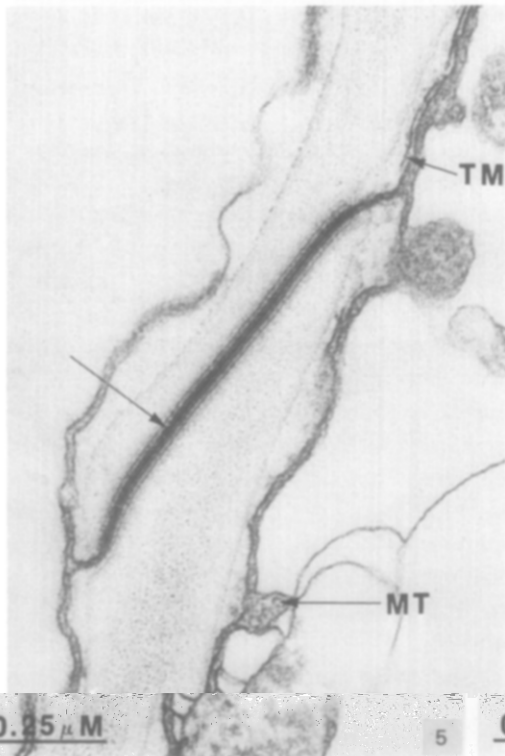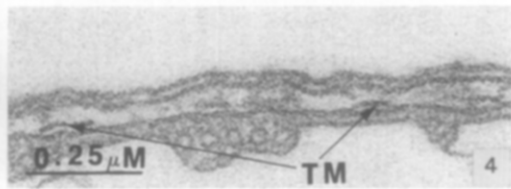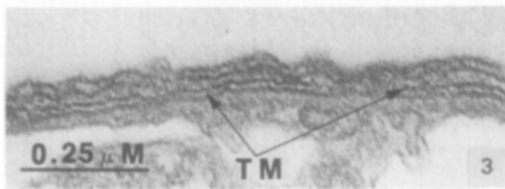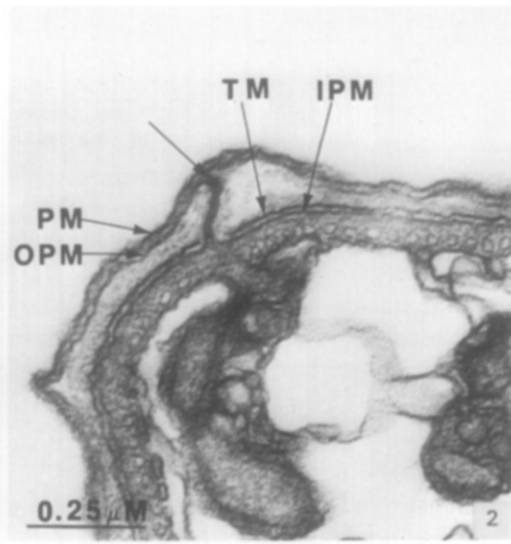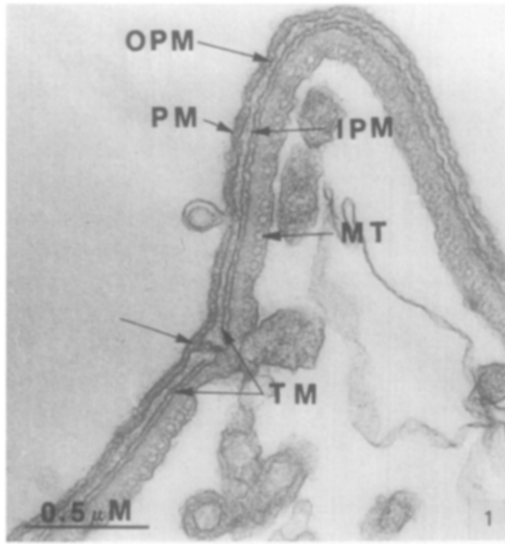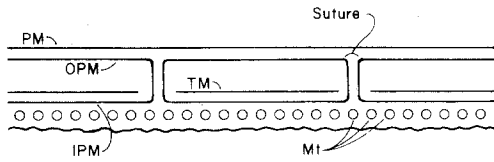
DIAGRAM 1. The orientation of the membranes of the cell covering before thecal plate synthesis. The plasma membrane (PM) is continuous around the entire cell. The outer plate membrane (OPM) fuses with the inner plate membrane (IPM) to form large flattened vesicles which contain an additional membrane, the thecal membrane (TM). A dense layer of cytoplasm containing microtubules (MT) lies under the cell covering.
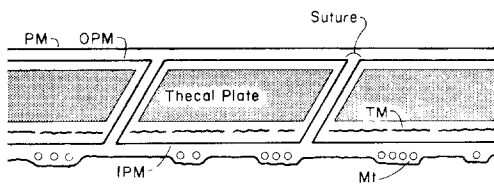

DIAGRAM 2. The structure of the cell covering following plate formation. Thecal plates are formed within the vesicles and above the thecal membrane (TM) which becomes discontinuous in mature walls. The microtubule layer becomes dispersed into small packets of 1–4.

ciated membranes of the covering are absent from a portion of the cell surface near the completion of cytokinesis when two diffuse regions differentiate along the leading edge of the cleavage furrow (Fig. 7). As seen in Fig. 8 (black arrow), there are no additional membranes under the PM in these regions. Though vesicles are present (white arrows, Fig. 8), they are separated from one another and do not form typical sutures. Frequently *Ceratium* daughter cells remain attached so that the actual separation following cytokinesis occurs concurrently with, or subsequent to, the completion of cell shape development

(Figs. 10 and 11). When this situation exists, the final location of the combined diffuse regions mentioned above becomes the area of attachment between the differentiating daughter cells (Fig. 9). At this point the only membrane continuous between the two daughter cells is the PM (black arrow, Fig. 9). No other membrane separates the cytoplasm from the environment. Vesicles (white arrows, Fig. 9) do not form sutures in this region.

DISCUSSION

The thecal structure observed in *C. tripos* is basically similar to that described by Dodge and Crawford (3) in their survey of the Dinophyceae. The outermost membrane is considered the plasma membrane and lies over a single layer of large vesicles in which the thecal plates are synthesized. The presence of an additional membrane within the vesicles, the TM, has not been reported before, although perusal of other published micrographs indicates that it is frequently present. The fact that only mature thecae are customarily studied may explain why the TM has not been previously described. Although it is prominent during early development in *Ceratium tripos*, the TM tends to become less evident in aged cells. In one of the few reports available on developing thecae, Dodge and Crawford (4) described a cross section through a horn of *Ceratium hirundinella* in which initial plate formation was evident and the TM had become discontinuous within the vesicles. Though the TM appeared similar to other membranes of the covering system, it was described as "a discontinuous layer of dark-staining mate-

FIG. 3. The cell covering in the cleavage furrow. Note the unit membrane structures of the TM and other membranes. × 83 025.

FIG. 4. The cell covering following cell shape development with the microtubules dispersed into small groups. The TM is discontinuous but still exhibits unit membrane structure. × 86 895.

FIG. 5. Mature cell covering showing thick thecal plates and a suture (arrow). The thecal membrane (TM) is discontinuous and only a few microtubules (MT) are found beneath the covering. × 86 085.

FIG. 6. Mature wall where the suture (arrow) has become discontinuous and the thick thecal plates are no longer entirely enclosed within vesicle membranes. × 84 735.
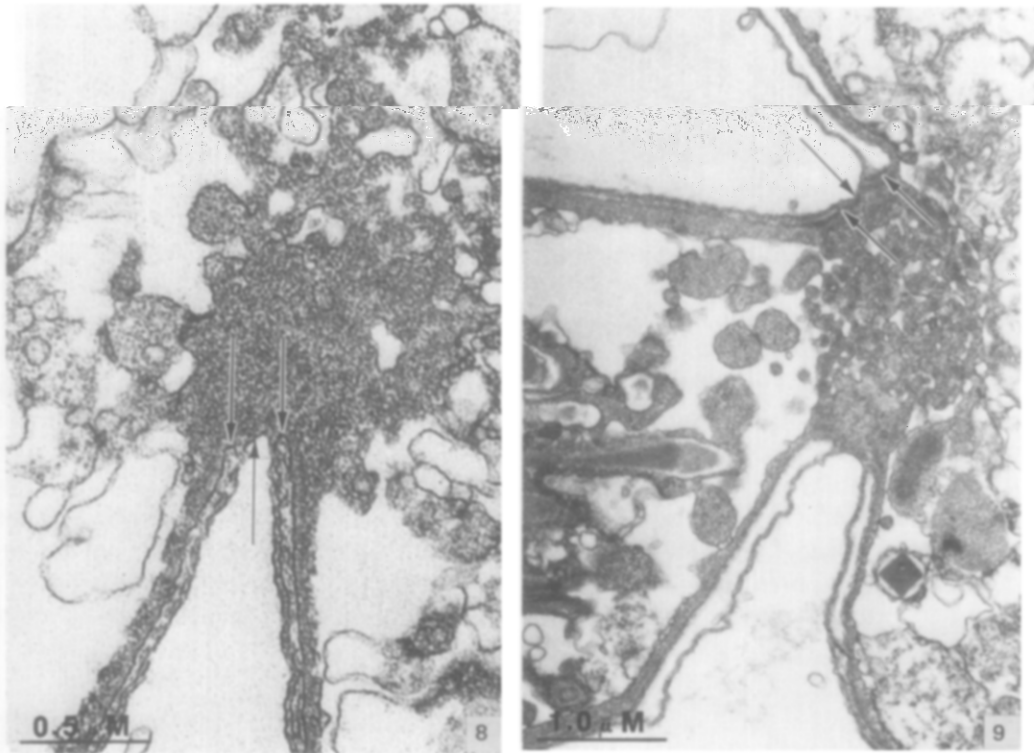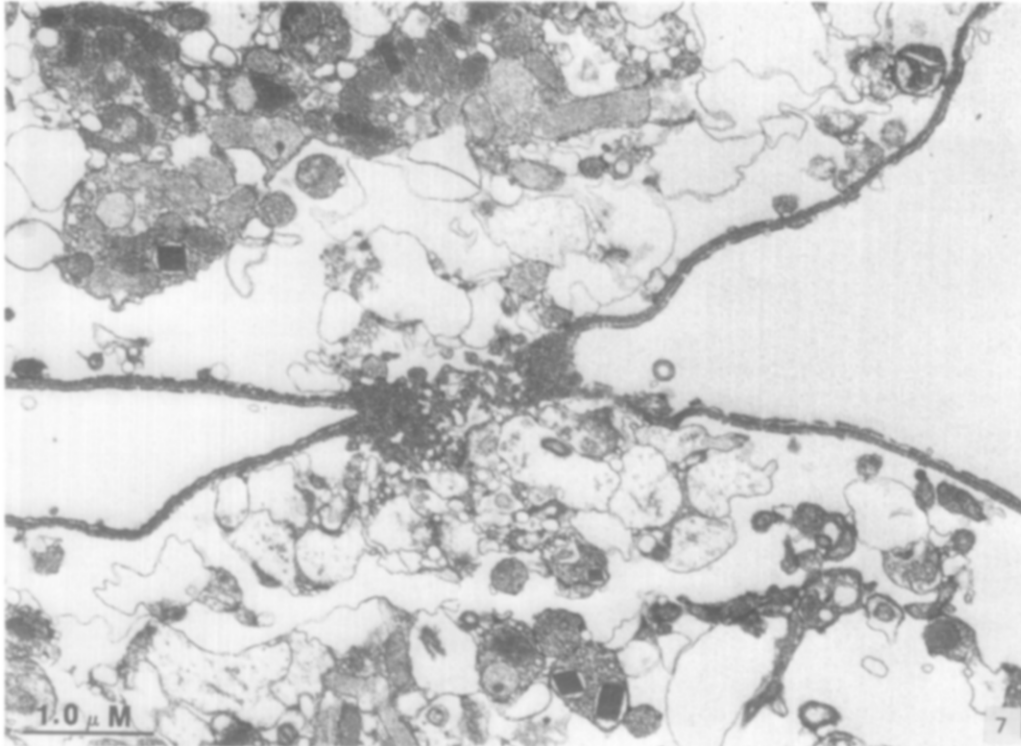
Fig. 7. Two diffuse regions which originate near the completion of cytokinesis along the leading edge of the cleavage furrow. × 14 602.

Fig. 8. Only the plasma membrane (black arrow) is continuous around the diffuse region while the vesicles (white arrows) do not form typical sutures. × 56 295.

Fig. 9. Area of attachment between cells which do not separate following cytokinesis. Only the plasma membrane (black arrow) is continuous between daughter cells while the vesicles (white arrows) are separate from each other and do not form typical sutures. × 23 364.
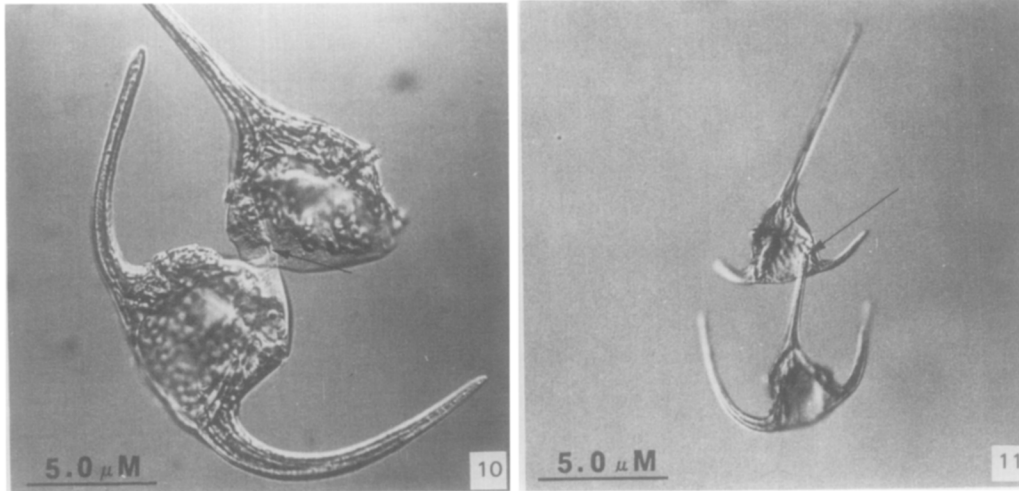
FIG. 10. Area of attachment (arrow) between two daughter cells which have recently divided but have remained attached. × 3 474.

FIG. 11. Two daughter cells almost completely differentiated which have remained attached (arrow). × 1 372.

rial" which Dodge and Crawford (4) suggest is possible "plate precursor material." If a recently differentiated covering had been observed it is likely that this structure would have been identified as an additional membrane. In Dodge and Crawford's (3) survey of thecal structure, at least three other organisms appeared to have a membrane within the thecal vesicles. This is most noticeable in *Amphidinium herdmanii* (plate 1D, 3) where the authors point out that material is present, but make no reference to what appears to be a unit membrane lying at the base of the vesicles. Other micrographs which give indications of this type of structure include unknown species "Plymouth D" (Plate 2A, in 3), *Woloszynskia coronata* (Plate 3E, A, in 3), and possibly *Glenodinium foliaceum* (Plate 3F, in 3) though in this case the membrane within the vesicles does not appear discontinuous at the sutures. Though the TM is likely present in these and other dinoflagellates, only studies which follow the cell covering throughout development can reveal this with certainty.

Loeblich (12) has described another layer of the cell covering termed the pellicular layer. It is reportedly present in typical armored species and consists of one continuous unit lying beneath the vesicles. This structure was not observed in *C. tripos*.

There are two different types of cytokinesis in the armored dinoflagellates (12). The first results in each daughter cell retaining approximately one-half of the parent theca while regenerating the missing moiety. In the second method the parent cell either divides up within its wall, or sheds the wall (ecdysis) before division. In this case, the daughter cells must regenerate an entirely new theca. *Ceratium tripos* is representative of the former method. A subsequent paper in this series will illustrate how, during cytokinesis, complete continuity is maintained between the membranes of the new daughter cell wall and those of the retained parent wall. The PM in *Ceratium* is therefore always continuous around the cell, even during division. Assuming that this membrane has a similar location in all dinoflagellates, cells discarding the parent wall at division would have to regenerate those membranes

located above the region of separation, presumably before the loss of the parent structure. The continuous membranes observed below the vesicles of some dinoflagellate cells (3, 10) might therefore be present in anticipation of division. In *Gyrodinium cohnii* (10) the parent cell encysts before dividing into two or more daughter cells. At the completion of division each new cell is surrounded by four unit membranes. It should be noted that during and subsequent to cytokinesis the two outer membranes of the cyst wall remain intact. It therefore appears that dinoflagellates which divide in such a way will have developed their new covering system before the parent theca is discarded. It is almost impossible to draw a comparison with *Ceratium*, because no definitive work has been done on dinoflagellates which divide in this manner.

The results of this investigation substantiate the claims of Dodge and Crawford (3) that the outermost membrane of the theca is the plasma membrane. No other membrane in the cell covering of *C. tripos* is continuous around the entire cell. This is most noticeable near the end of cytokinesis when a portion of the dividing cell is enclosed only by the PM. It is therefore evident that the armored plates are produced beneath the PM as seen in the cuticular structure of the ciliate *Coleps* (5) and the pellicle of many euglenoid flagellates (11).

Some investigators (8, 10) have proposed that sutures are established by an inward invagination of the OPM between partially synthesized plates, and that the plates are therefore not always completely enclosed within vesicles. In *C. tripos* sutures are present during the initial differentiation of the cell covering, and before the formation of thecal plates. The invaginating membranes reported by the other authors probably represent suture membranes which have become discontinuous in aged cells, a phenomenon often observed in aged cells of *C. tripos* (Fig. 6). Therefore I designated the upper and lower membranes of the vesicles as two separate units (OPM and IPM), though the interpretation of the basic thecal structure proposed by Dodge and Crawford appears correct throughout most of development.

Subsequent communications will demonstrate additional features of thecal structure, including the disposition of covering membranes during plate synthesis.

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