

CILIARY MEMBRANE DIFFERENTIATIONS IN *TETRAHYMENA PYRIFORMIS*

Tetrahymena Has Four Types of Cilia

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ABSTRACT

We have examined thin sections and replicas of freeze-fractured cilia of *Tetrahymena pyriformis*. The ciliary necklace located at the base of all freeze-fractured oral and somatic cilia has been studied in thin sections. Since electron-dense linkers have been found to connect both microtubule doublets and triplets to the ciliary membrane at the level of the necklace, the linkers and the associated necklace seem to be related to the transition region between the doublets and triplets of a cilium. Plaque structures, consisting of small rectangular patches of particles located distal to the ciliary necklace, are found in strain GL, but are absent in other strains examined in this study. In freeze-cleaved material, additional structural differentiations are observed in the distal region of the ciliary membranes of somatic and oral cilia. Somatic cilia contain many randomly distributed particles within their membrane. Oral cilia can be divided into three categories on the basis of the morphology of their freeze-fractured membranes: (a) undifferentiated cilia with very few randomly distributed particles; (b) cilia with particles arranged in parallel longitudinal rows spaced at intervals of 810–1080 Å that are located on one side of the cilium; and (c) cilia with patches of particles arranged in short rows oriented obliquely to the main axis of the cilium. The latter particles, found on one side of the cilium, seem to serve as attachment sites for bristles 375–750 Å long and 100 Å wide which extend into the surrounding medium. The particles with bristles are located at the tips of cilia in the outermost membranelle and may be used to detect food particles and/or to modify currents in the oral region so that food particles are propelled more efficiently into the buccal cavity. Examination of thin-sectioned material indicates that the particles in oral cilia which form the longitudinal rows could be linked to microtubule doublets. Linkage between microtubule doublets and adjacent membrane areas on one side of the cilium could modify the form of ciliary beat by restricting the sliding of the microtubules. It is suggested that membrane-microtubule interactions may form the basis for the various forms of ciliary beat observed in different organisms.

INTRODUCTION

The ultrastructure of the cilia (Allen, 1968; Williams and Luft, 1968; Allen, 1969) and the cortex (Pitelka, 1961 and 1963; Allen, 1967) of *Tetrahymena pyriformis*, a unicellular protozoan, has been well characterized in many studies involving the examination of thin sections. More recent investigations employing the freeze-fracture technique have revealed the presence of membrane specializations within both cortical and ciliary plasma membranes. Satir et al. (1972 a), and Satir et al. (1973), have described small rosettes of particles marking the area of fusion of the membrane of the mucocyst with the plasma membrane of the cortex. In the basal region of the ciliary membrane two rows of closely spaced particles, termed the ciliary necklace, have been observed by Satir and Gilula (1970), Gilula and Satir (1972), Satir et al. (1972 b), Wunderlich and Speth (1972), and Speth and Wunderlich (1972). The strain GL exhibits, furthermore, small groups of particles, termed plaques, immediately distal to the ciliary necklace on somatic and oral cilia (Wunderlich and Speth, 1972).

The cilia on *Tetrahymena* can be divided into two groups: (a) somatic cilia which function in motility, and (b) oral cilia which function in the propagation of food particles towards the cytostome (Nilsson and Williams, 1966). In this study we report additional ciliary membrane specializations, in *T. pyriformis*, in the membrane covering the distal regions of the ciliary shaft. These specializations are found on subgroups of oral cilia and are lacking on somatic cilia. We suggest that the specialized membrane structures could provide individual oral cilia with additional properties and/or affect their beating patterns. Thus, it is conceivable that some differentiated cilia may be responsible for the efficient movement of water containing food particles into and through the buccal cavity, while others could possibly act as food sensors. A preliminary report of this work has been presented elsewhere (Sattler and Staehelin, 1972).

MATERIALS AND METHODS

Three strains of *T. pyriformis*-GL, an amiconucleate, HSM, and an unknown strain containing both a macronucleus and a micronucleus (possibly derived from strain HSM [Nanney, personal communication]), were grown in 2% proteose-peptone supplemented with 0.1% liver extract.

For thin sections, the cells were washed with starvation medium (Cameron and Jeter, 1970), fixed in cacodylate-buffered 2% glutaraldehyde (0.5 h), and postfixed in cacodylate-buffered 1% osmium tetroxide (0.5 h). After fixation, the cells were dehydrated in a graded series of ethanol and then embedded in Araldite 6005. Thin sections were cut on a Porter-Blum MT 1 ultramicrotome (Ivan Sorvall, Inc., Newtown, Conn.) and stained with uranyl acetate and lead citrate.

For freeze-fracturing, the cells were washed with starvation medium and fixed in cacodylate-buffered 4% glutaraldehyde (0.75 h) and then slowly infiltrated over a 2-day period to approximately 30% glycerol. The cells were frozen on copper grids in Freon 12, stored in liquid nitrogen, and fractured at -104°C in a Balzers freeze-etch apparatus (Balzers High Vacuum Corp., Santa Ana, Calif.) according to the method of Moor and Mühlethaler (1963). Thin sections and replicas were examined with a Philips EM 200 or JEM 100 B at 60 kV.

RESULTS

Ciliary Necklace Membrane Differentiation

At the base of all freeze-fractured somatic and oral cilia of the three strains of *T. pyriformis* examined, there are two rows of closely packed particles known as the ciliary necklace (Satir and Gilula, 1970; Satir et al., 1972 b). While the majority of necklace particles remain attached to the A face of the somatic (Fig. 1) and oral (Fig. 3) cilia during the cleaving process, many of the particles can still be seen adhering to the complementary B faces of both somatic (Fig. 2) and oral cilia (Fig. 3, lowermost cilium). The ciliary membrane is constricted in the region of the necklace. This constriction is most obvious in the uppermost oral cilium in Fig. 3.

The transition region between the basal body and ciliary shaft, which contains the ciliary necklace, is shown in thin sections of the unknown strain (Fig. 4 A, 4 D, and 4 E). In a longitudinal section the central tubules of the ciliary shaft end immediately distal to the axosome (Fig. 4 A, large arrow). The ciliary membrane surrounding the cilium is a continuation of the plasma membrane, the outermost membrane of the three membranes forming the pellicle. Three thin bands of densely staining material oriented at right angles to the ciliary shaft can be distinguished in the transition region. One band extends from the axosome to the plasma membrane (Fig. 4 A, upper small arrow) and the other more basal bands extend from the

two dense lines of the terminal plate (Fig. 4 A, lower small arrows) to adjacent alveolar sacs. We have also examined cross sections through the transition region of cilia (Fig. 4 B–4 F) in an attempt to determine more precisely the location of the ciliary necklace in thin sections. Fig. 4 B–4 F corresponds to a section through areas labeled B through F on the longitudinally sectioned cilium in Fig. 4 A. In Fig. 4 B, triplets surround the electron-dense core. Distal to region B (Fig. 4 C) the triplets are distinct and attached on their luminal side to a thin, ringlike structure, the electron-dense core of Fig. 4 B has nearly disappeared, and a membrane surrounds the triplets. The basal body in Fig. 4 D has a lacy, nine-pointed star in the lumen and thus is, according to Allen (1969), a cross section through the distal end of the terminal plate region. At this level (Fig. 4 D) the triplet structure can still be clearly discerned. At the same level there are linkers connecting the microtubules to membrane encircling the basal body. In Fig. 4 E where we have doublets and no longer triplets we can also clearly recognize linkers. Fig. 4 F is a section through the axosome in which the linkers appear less dense and are not found associated with each doublet. The electron-dense material in the lumen forming the axosome is surrounded by two rings, the outer of which is in contact with the nine doublets. The ciliary membrane of the cilium in Fig. 4 F is slightly wavy and thus we believe that this section is from a region distal and not proximal to the ciliary necklace.

Fig. 5 shows that the linkers seen in thin sections (Fig. 4 E) may also be recognized in freeze-fractured preparations. In cross sections through the membranelles of freeze-fractured cells (Fig. 5), we have found linkers apparently composed of globular subunits that extend from the outer microtubules to the ciliary membrane. Fig. 5 also reveals a nonagonal outline of the membrane with the globular linkers leading from the microtubules to the corners of the nonagon. The result is a membrane that is evenly spaced around the circle of microtubule doublets. The ciliary membrane in Figs. 4 D, 4 E, and 5 is tightly apposed to the cytoplasm and thus lacks the waviness found in more distal portions of the ciliary membrane (Fig. 4 F).

Plasma Membrane Differentiation

Both somatic and oral cilia of the GL strain exhibit plaques of particles on the A face distal to

the ciliary necklace (Fig. 6). Each plaque is three particles wide and from three to nine particles long. To date we have only observed plaques on cilia in the GL strain; the other two strains (HSM and an unknown strain) lack plaques. Thus we are in agreement with Wunderlich and Speth (1972) and Speth and Wunderlich (1972) who have also reported plaques in strain GL. In thin sections of GL cells we have not been able to identify structures which correspond to the plaques seen in freeze-fractured cells.

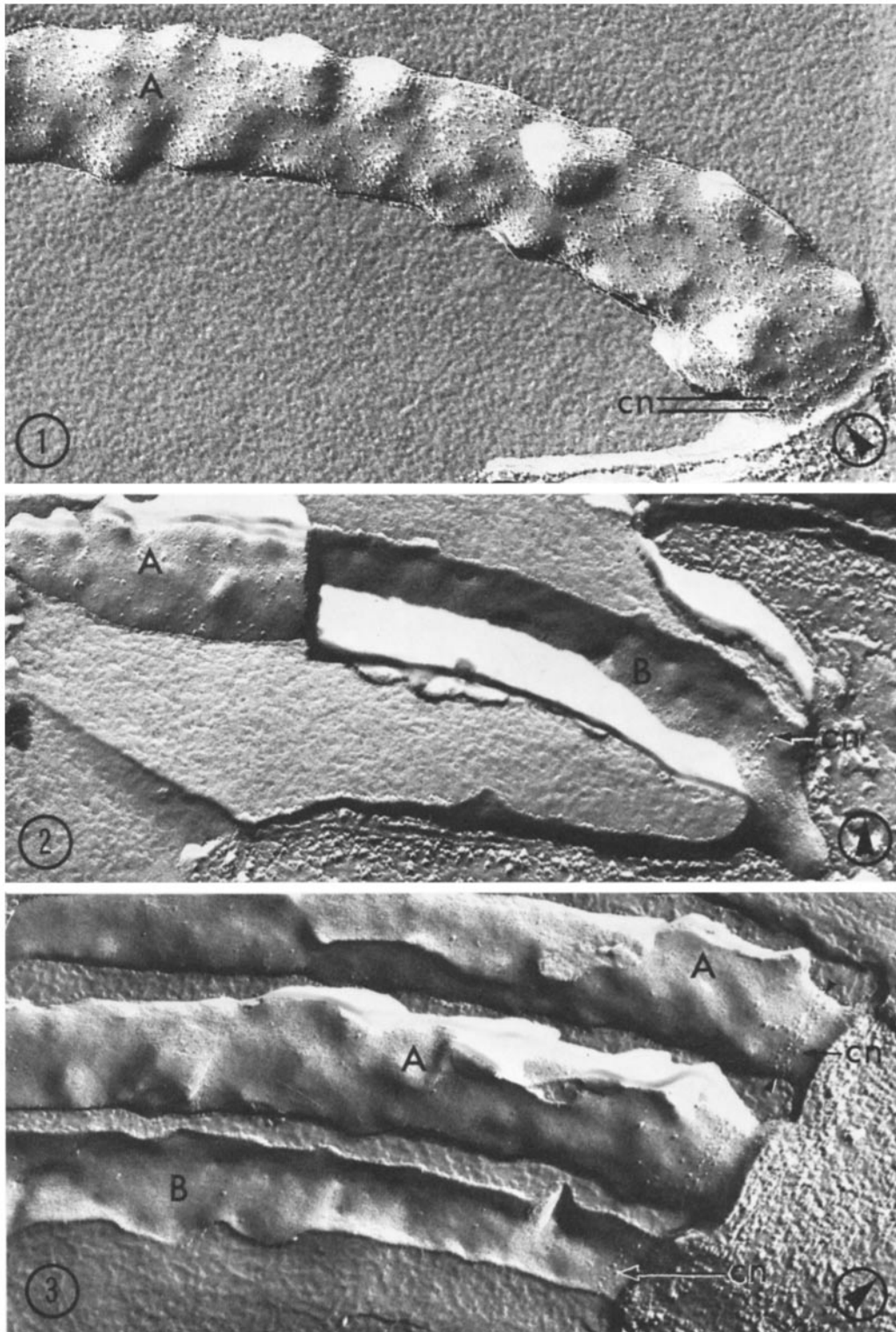
Somatic Ciliary Shaft

The particles on the plasma membrane of somatic cilia distal to the necklace and the plaques, when the latter are present, are randomly distributed (Figs. 1 and 2). During freeze-fracturing, approximately 80% of the particles remain attached to the A face (Fig. 1).

Membrane Differentiations of Oral Cilia

The oral region in *Tetrahymena* is the most highly differentiated area of the cell. Fig. 7 shows how the cilia are arranged in groups called membranelles. The outermost membranelle contains approximately 54 cilia, the second contains approximately 39 cilia, and the third contains approximately 18 cilia (Nilsson and Williams, 1966). On the opposite side of the buccal cavity there are oral ribs which extend from the undulating membrane to the opening of the cytostome which is located at the base of the buccal cavity. Food vacuoles pinch off from the posterior portion of the cytostome.

The cilia located in the membranelles can be divided into three groups on the basis of the appearance of the ciliary membrane in freeze-fractured cells. The first group consists of undifferentiated oral cilia as shown in Fig. 3. The undifferentiated oral cilia can be distinguished from somatic cilia by the near absence of particles associated with their A and B faces (compare Fig. 3 with Figs. 1 and 2). The other two groups of oral cilia have particles arranged in distinct geometrical patterns. For example in Figs. 8–14, there are parallel longitudinal rows of particles which usually begin about 0.5- μm distal to the ciliary necklace (Fig. 12) and extend possibly over the length of the cilium. In other cases patches of particles are arranged in short rows oriented obliquely to the main axis of the cilium (Figs. 17–19). The latter particles are associated with bristles which extend into the surrounding medium.



Oral Cilia with Longitudinal Rows of Particles

Particles in parallel longitudinal rows are found on cilia located in the outer rows of a given membranelle rather than on cilia located in the middle row of the membranelle (Figs. 9 and 10). Cilia with longitudinal rows of particles have been observed in both the second (Fig. 10) and the third membranelles (Figs. 8 and 9). There is no evidence for longitudinal rows of particles on cilia located in the first membranelle. The arrangement of the particles in the rows appears variable. On A faces the particles (approximately 94 Å in diameter) are most frequently arranged in the form of simple but sometimes interrupted rows, with a spacing of approximately 250 Å (Figs. 8 and 9). In other instances the particles are more closely packed in the form of bands containing as many as three rows of particles (Fig. 11). Fig. 12, finally, shows many of the particles organized into short triplet rows that make an angle of about 50° with the main row. B faces usually exhibit fewer of the longitudinal row particles than A faces. The particles and holes on the B faces can be either more or less evenly spaced (Fig. 13) or the particles can be organized into short, closely packed groups (Fig. 14). It has not yet been determined if these interruptions in the particle rows are real or if they simply reflect regions of differential adherence of the particles to the A and B fracture faces. Future studies employing the double replica method (Wehrli et al., 1970) for freeze-fracturing may resolve this question. The longitudinal rows of particles have been observed on oral cilia of all three strains of *T. pyriformis*.

We have detected a maximum of three longitu-

dinal rows of particles on the fracture face of a single cilium (Figs. 8 and 9). Often the third row of particles appears near the edge of the cilium and is difficult to detect. In order to determine how many rows of particles are present in a cilium and if the rows occur on all sides of a cilium, we have examined individual cilia which have been cleaved in such a way as to reveal both A and B faces. In Fig. 13 it can be seen that longitudinal rows of particles do not occur around the entire ciliary membrane. As many as three rows of particles have been seen, but because of the width of the fracture face, the possibility that there may be four to six longitudinal rows of particles could not be eliminated in this study.

In thin sections it is possible to visualize connections between the ciliary membrane and the middle of some of the outer microtubule doublets (Fig. 15). The microtubule-membrane linkers (arrows) are found in cilia adjacent to the oral ribs near the opening to the cytostome and thus the cilia shown in Fig. 15 are probably part of the third membranelle. That the linkers seen in Fig. 15 are indicative of real connections between some microtubule doublets and the ciliary membrane is suggested by the decreased waviness of the membrane where the linkers attach to the membrane. Similarly, a reduction in waviness of freeze-fractured ciliary membranes is associated with the areas containing the longitudinal rows of particles (Figs. 8 and 9). Occasionally, linkers resembling those seen in thin sections (Fig. 15) can also be visualized in cross-fractured cilia (Fig. 16) where they appear to connect outer microtubule doublets with the adjacent ciliary membrane. The distance between adjacent longitudinal rows of particles varies from 810 to 1080 Å. Since the spacing

FIGURE 1 Freeze-cleaved somatic cilium of *T. pyriformis*, unknown strain, exhibiting a ciliary necklace (*cn*) around the basal region. The remaining particles on the exposed split inner face of the cytoplasmic membrane leaflet (A) are randomly distributed. Waviness occurs in the membrane distal to the necklace. Circled triangle in lower right corner of this and following micrographs of replicas shows the angle of shadowing. $\times 82,000$.

FIGURE 2 Somatic cilium of the unknown strain showing that only a few of the ciliary necklace particles (*cn*) remain attached to the outer external membrane leaflet (B). Distal to the necklace, there are fewer randomly distributed particles on the B face than on the A face. $\times 64,000$.

FIGURE 3 Oral cilia from a membranelle of the unknown strain exhibit ciliary necklaces (*cn*). Most of the necklace particles adhere to the A face. The ciliary membrane constricts at the level of the necklace as shown in the uppermost cilium (arrowheads). The membrane distal to the necklace contains few particles on either the A or B face. $\times 53,500$.

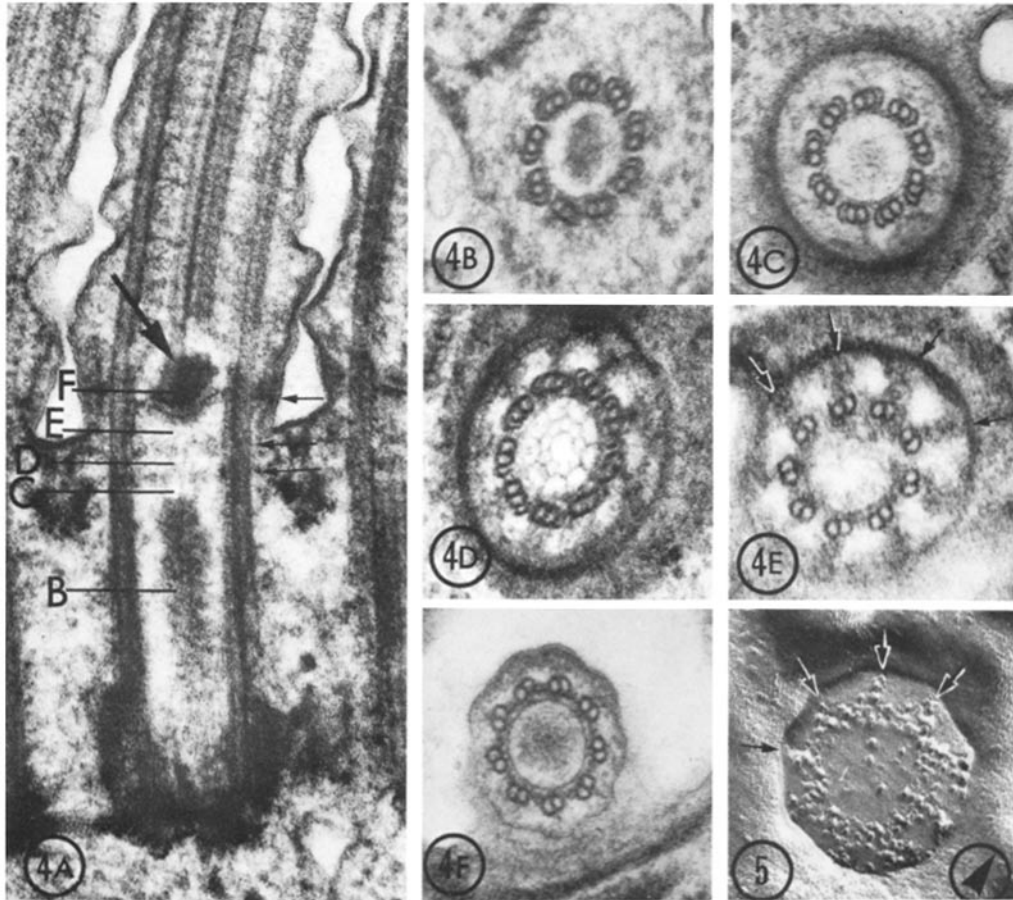


FIGURE 4 A Longitudinal thin section of a cilium with labels B–F corresponding to cross sections of cilia labeled Fig. 4B–4F. An electron-dense structure, the axosome (large arrow), is located proximal to the end of the central microtubules of the ciliary shaft. In the transition region between ciliary shaft and basal body, there are three electron-dense bands. The band directly below the axosome extends to the plasma membrane (uppermost small arrow), while the two dense bands forming the terminal plate (lower two small arrows) extend to the adjacent alveolar membranes. $\times 94,500$.

FIGURE 4 B Cross section of a basal body exhibiting nine triplets surrounding an electron-dense core. $\times 84,000$.

FIGURE 4 C Cross section of a basal body at the level of the zone between the electron-dense core and the terminal plate. The triplets are connected by a thin filament on the luminal side, the electron-dense core is absent, and alveolar membranes encircle the basal body. $\times 86,000$.

FIGURE 4 D Cross section through the terminal plate region of the transition area. The lacy nine-pointed star fills the lumen and connects to the triplet microtubules. Fuzzy linkers connect the triplets to the adjacent membrane. $\times 86,000$.

FIGURE 4 E Cross section through the proximal region of the ciliary shaft. Each doublet is connected to the adjacent ciliary membrane by a fuzzy linker (arrows) which may connect to the particles forming the ciliary necklace structure in freeze-fractured cells. Part of the double ring structure characteristic of the axosome region (4 F) is starting to appear. $\times 87,000$.

FIGURE 4 F Cross section through the axosome region of the ciliary shaft. The ciliary membrane is wavy, the doublets are joined on their luminal side by a double ring structure, and the electron-dense axosome fills the lumen. $\times 86,000$.

FIGURE 5 Cross section of a freeze-fractured oral cilium at the level of the ciliary necklace. The linkers (arrows), composed of globular subunits, extend from the microtubules to the membrane and probably correspond to the fuzzy linkers shown in Fig. 4 E. $\times 81,900$.

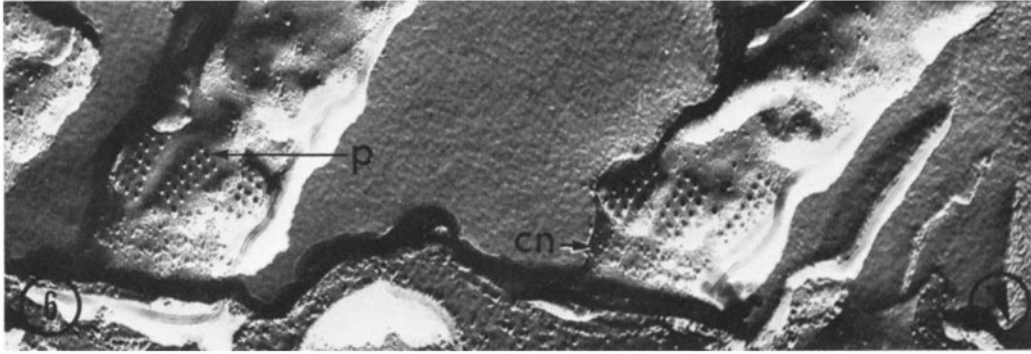


FIGURE 6 Somatic cilium of strain GL. Patches of particles called plaques (*p*), three particles wide to nine particles long, are located on the A face of the ciliary membrane immediately distal to the ciliary neck (*cn*). $\times 67,200$.

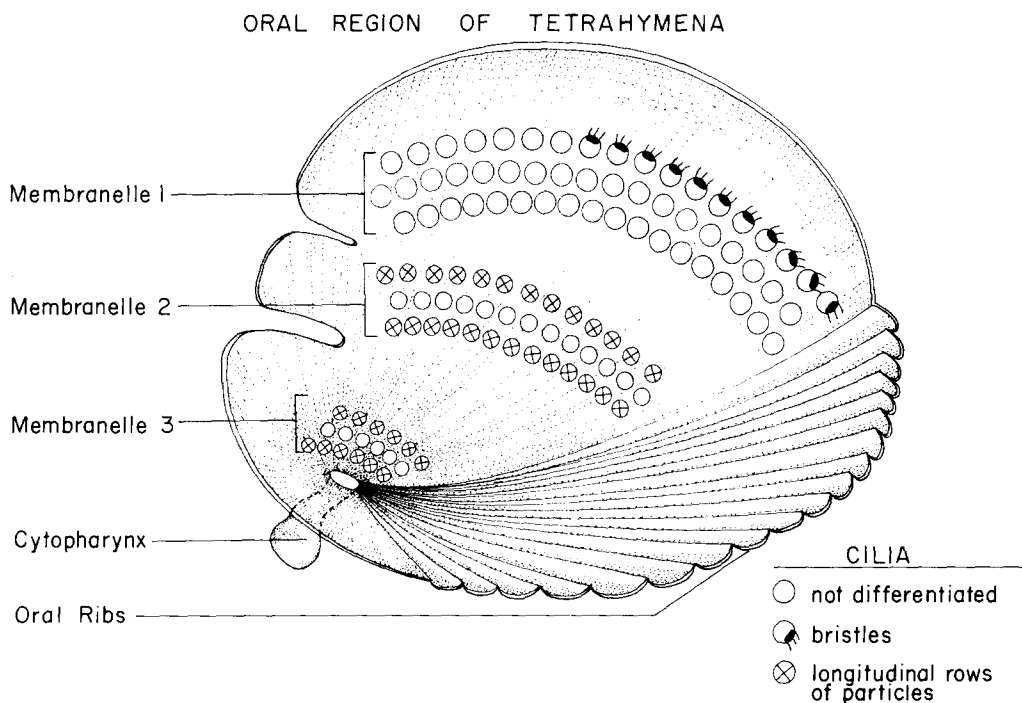


FIGURE 7 A simplified diagram of the oral region of *Tetrahymena* showing the location of ciliary membrane differentiations. Cilia are organized into three membranelles (each contains three rows of cilia, but the length of the rows varies). Some cilia in the outermost row of the first membranelle have bristles protruding from the ciliary membrane. The orientation of the bristles changes with respect to the oral ribs as one moves from cilia located close to oral ribs back along the membranelle. Longitudinal rows of particles are found on the outer rows of cilia in the second and third membranelles.

between the longitudinal rows of particles is close to the calculated spacing between adjacent microtubule doublets, the particles forming the longitudinal rows of particles may represent attachment points for the microtubule-membrane linkers seen in sectioned material.

Oral Cilia with Bristles

The second type of plasma membrane differentiation in oral cilia consists of patches of obliquely oriented rows of particles (Fig. 17) which remain associated with the external membrane leaflet (B



FIGURE 8 High magnification of the cilium from the outer row of cilia of the third membranelle (Fig. 9). The oral cilium exhibits three parallel longitudinal rows of particles (*p*) on the cleaved face of the cytoplasmic membrane leaflet (*A*). The spacing between the rows of particles (810–1080 Å) suggests that the particles could be associated with elements of the underlying microtubule doublets. The ciliary membrane between adjacent rows of particles is tightly apposed to the cytoplasm. $\times 79,000$.

FIGURE 9 Three cilia forming the inner (third) membranelle. Longitudinal rows of particles are located on the cilium adjacent to the oral ribs (*or*) and on the cilium on the opposite side of the membranelle (arrow), but are absent on the cilium in the middle row of the membranelle. $\times 37,700$.

face) during the cleaving process. Fig. 18 shows the complementary cytoplasmic membrane leaflet (*A* face) with rows of holes and only a few particles. The arrays of particles are located on only one side of the tip portion of cilia located in the outer row of the first (outermost) membranelle (Fig. 19). They have been measured over distances as large as $2 \mu\text{m}$ along the main axis of a cilium. The parallel rows make an angle of approximately 48° with the main axis and show an average spacing of 350 Å. Occasionally there is some irregularity in the spacing between rows as is seen in Fig. 18. Fig. 17 indicates that the particles serve as points of attachment for short bristles. These bristles, which

seem to be composed of globular subunits, have a diameter of approximately 100 Å and a length of 375–750 Å. They protrude at approximately right angles from the membrane surface into the surrounding medium. The obliquely oriented rows of particles with bristles are found in all three strains of *T. pyriformis*. The circularity of the cilium is distorted in the region which contains the bristles (Fig. 19). The distortion is due to the presence of material which is associated with the bristles and interposed between the microtubule doublets and the ciliary membrane (see also Fig. 21). In addition, there is a changing orientation of bristles on adjacent cilia with respect to the oral ribs (Fig. 19).

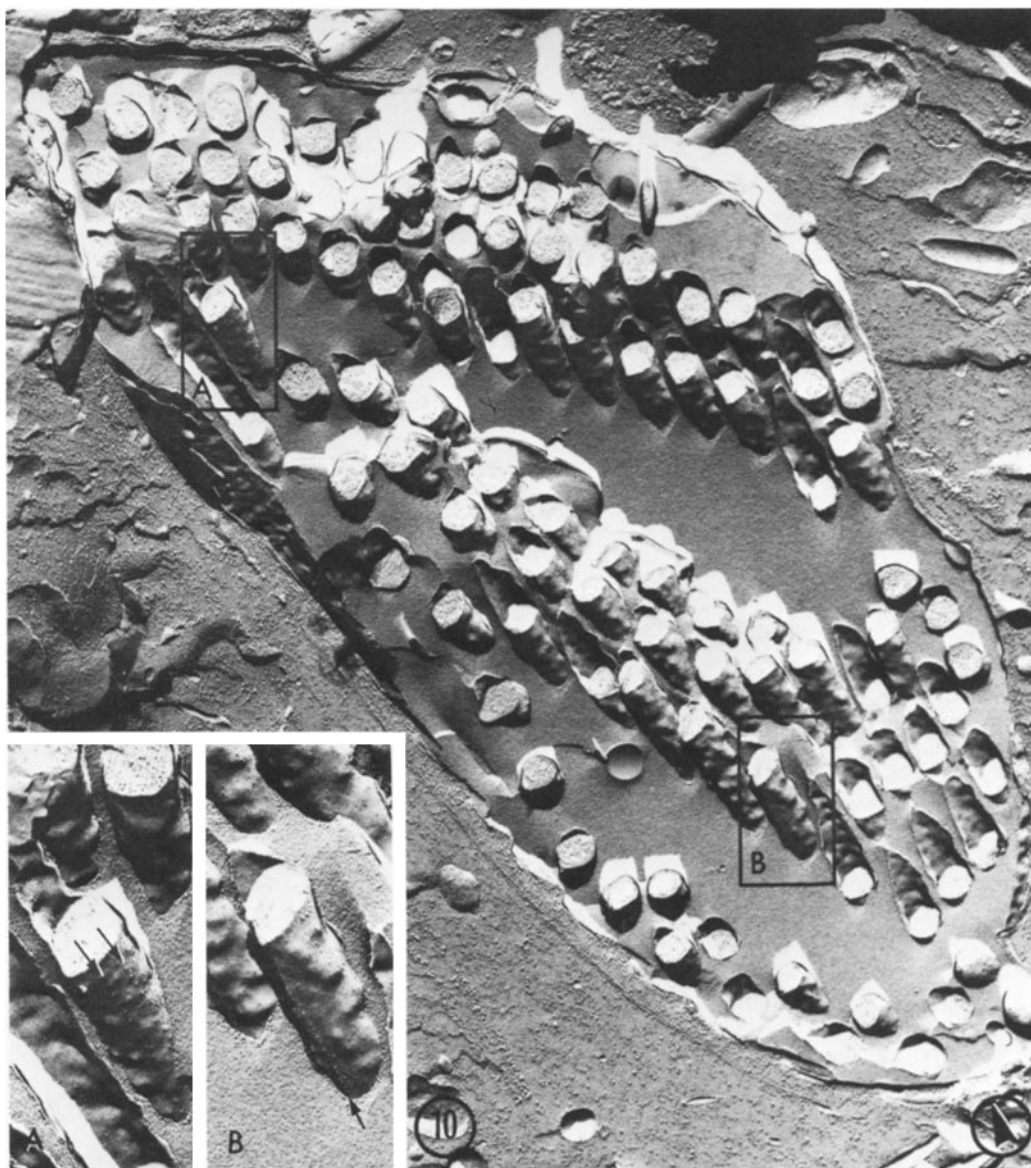


FIGURE 10 Replica of the oral region containing fractured cilia of the first and second membranelles. $\times 16,900$. In Inset A the cilium (located in the outer row of the cilia of the second membranelle) possesses at least two longitudinal rows of particles (arrows). Particles arranged in longitudinal rows (arrow) are also found on cilia in the outer row of cilia on the opposite side of the second membranelle (Inset B). Inset A and B, $\times 34,500$.

In thin sections through the first membranelle we have also observed bristles protruding from the ciliary membrane (Fig. 20). Such bristles are limited to the distal portion of 7-11 cilia in the outermost row of the first membranelle (Fig. 7).

These differentiated cilia frequently extend across the buccal cavity and can be observed in close proximity to the oral ribs which are located on the opposite side of the buccal cavity. The electron-dense material is located in the tip region of the

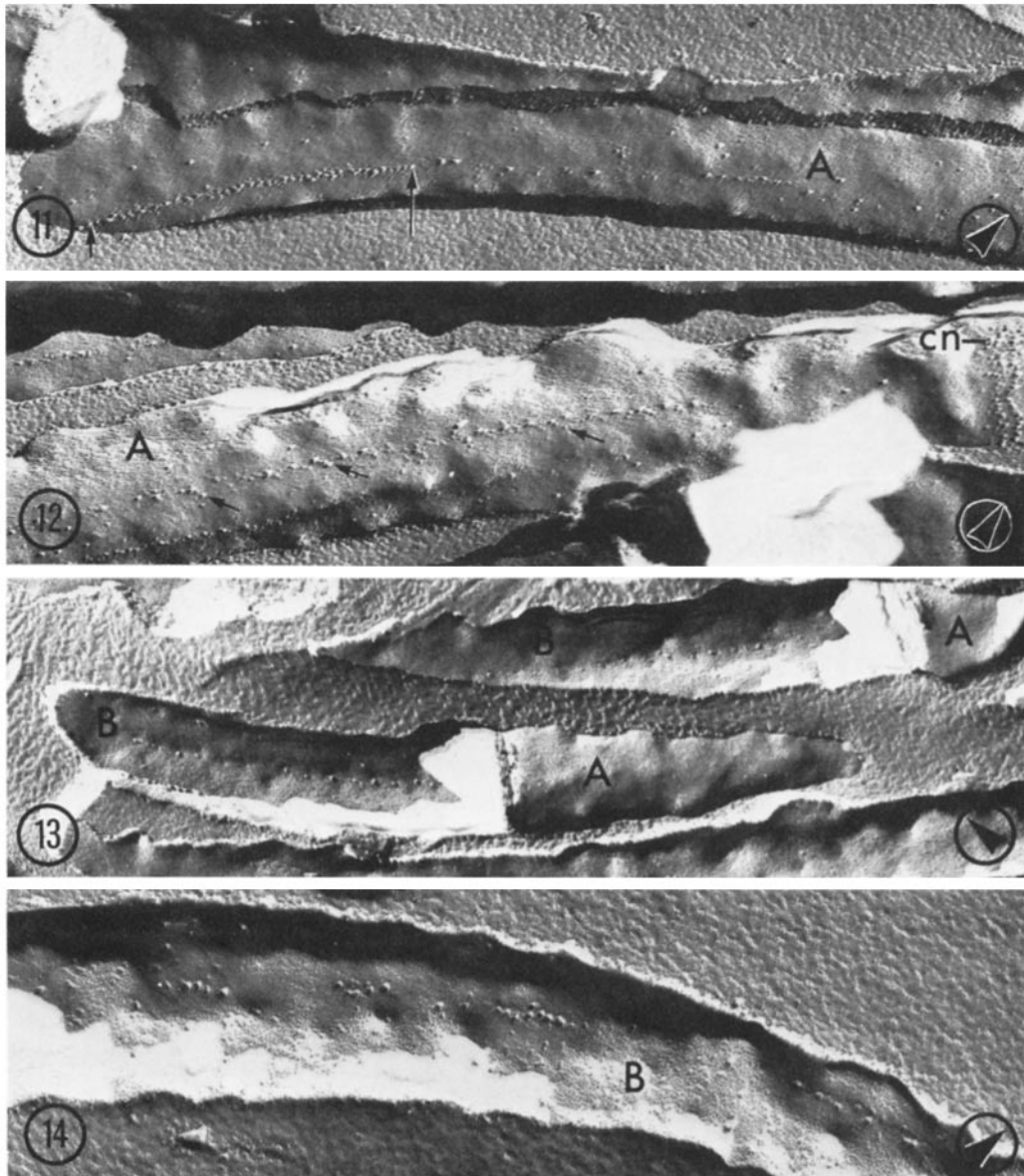


FIGURE 11 Oral cilium exhibiting a longitudinal row of particles. In some areas of the A face (between arrows) the particles are very closely packed and arranged in bands containing up to three particles. $\times 62,300$.

FIGURE 12 Oral cilium exhibiting longitudinal rows of particles on the A face which begin approximately $0.5\text{-}\mu\text{m}$ distal to the ciliary necklace (*cn*). Some of the particles forming the longitudinal rows are organized into triplet rows (arrows) that make an angle of approximately 50° with the main row of particles. $\times 62,700$.

FIGURE 13 Two oral cilia fractured so both the A and B faces in each cilium are exposed. The A faces contain only a few randomly distributed particles while the B faces exhibit a longitudinal row containing evenly spaced particles and holes. $\times 53,100$.

FIGURE 14 Oral cilium exhibiting small clumps of closely packed particles which adhere to the B face and are oriented in a longitudinal row. $\times 75,600$.

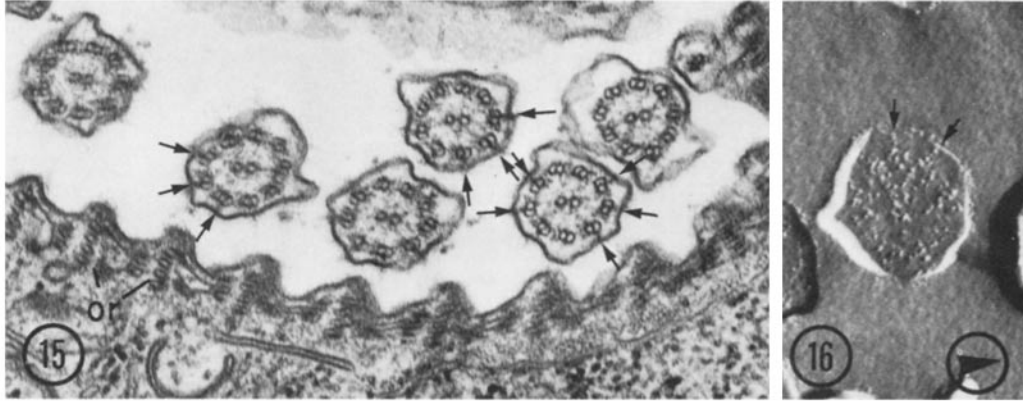


FIGURE 15 Thin section of oral cilia adjacent to oral ribs (*or*) exhibiting linkers (arrows) connecting some of the microtubule doublets to the adjacent ciliary membrane. Waviness in the ciliary membrane occurs in the area of the membrane lacking linkers. $\times 57,800$.

FIGURE 16 Cross fractured replica of an oral cilium. Linkers composed of globular subunits (arrows) extend from some of the microtubule doublets to the adjacent ciliary membrane. The membrane appears to lack waviness in the area associated with the linkers. $\times 58,500$.

cilia since an adjacent membranellar cilium (Fig. 21, arrow) exhibits several single microtubules on one side of the doublet ring which is characteristic of the tip region of a bending cilium. Electron-dense material which is seen immediately under the ciliary membrane in longitudinal sections (Fig. 20) is shown to be associated with three doublet structures in cross sections of cilia (Fig. 21). The middle doublet of the three appears to have a connection with the electron-dense material. Further analysis of Fig. 21 reveals that the electron-dense material is associated with different doublets in adjacent cilia. This can be clearly demonstrated by using the method of Afzelius (1959) to classify the microtubule doublets of a cilium. A line is drawn between the two central microtubules so that it passes through the center of one of the nine outer doublets and is perpendicular to a line through the two central microtubules. The outer doublet through which the line passes is numbered 1 and the rest of the doublets are numbered in the direction defined by the dynein arms from the no. 1 doublet passing around the cilium in the direction in which the arms on subfibril A point. In Fig. 21 the arms on subfibril A are directed clockwise, which is the pattern presented to a viewer looking from the base of a cilium towards its tip (Gibbons and Grimstone, 1960; Gibbons, 1961). In the cilium located closest to the oral ribs (compare Fig. 21 with Fig. 7), the electron-dense material is connected with doublet no. 5, the next two cilia

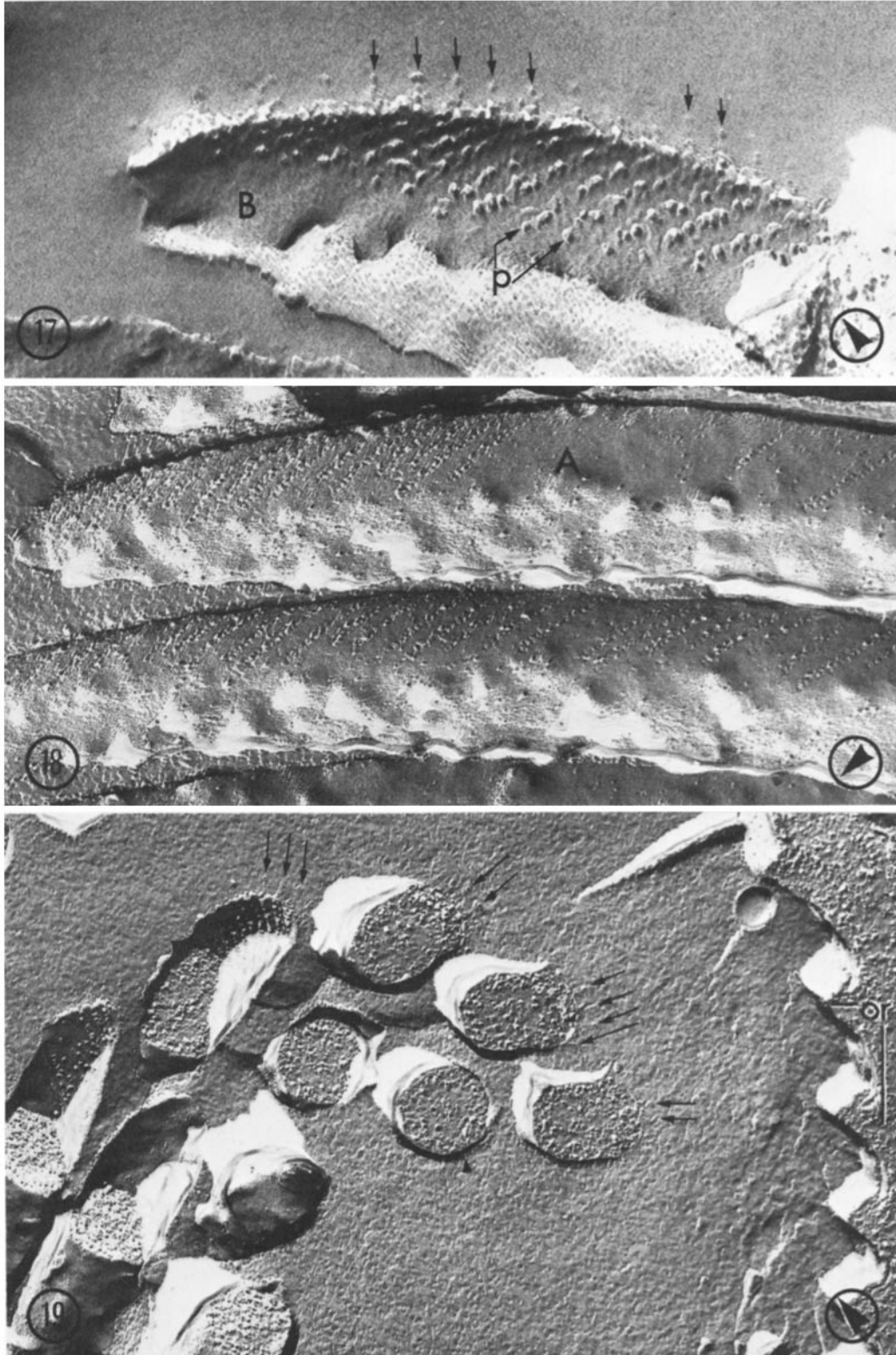
have a connection with doublet no. 4, the fourth cilium with doublet no. 3, and the fifth, sixth, and seventh cilia each with doublet no. 2. There are approximately 18 cilia in the outer row of the first membranelle. We have found bristles and electron-dense material associated with 7–11 of these cilia. Occasionally the ciliary membrane is distended in fixation and in all instances the connection between the electron-dense material and the middle doublet breaks and the electron-dense material remains closely associated with the membrane and bristles.

Rare Ciliary Membrane Differentiations

Occasionally on the B face of some oral cilia we have found three longitudinal rows of particles spaced approximately 300 Å apart (Fig. 22). Since cilia possessing this type of differentiation are rarely seen, we have been unable to determine which cilia in the oral region possess such closely spaced rows of particles.

DISCUSSION

Ciliary necklaces have been observed at the base of many types of flagella and cilia (Satir and Gilula, 1970; Flower, 1971; Gilula and Satir, 1972; Bergstrom and Henley, 1973; Bergstrom et al., 1973). The necklace structure has been examined in an unknown strain of *T. pyriformis* by Satir et al. (1972 *b*) and in strain GL by Speth and Wunder-



lich (1972) and Wunderlich and Speth (1972). In the three strains of *T. pyriformis* which we have investigated, we have confirmed the finding of Satir et al. (1972 b) that the necklace consists of two strands of particles, most of which remain attached to the inner cytoplasmic membrane leaflet (A face) during freeze-fracturing. In contrast to the clearly scalloped ciliary necklace of the cilia of *Elliptio* (Gilula and Satir, 1972), the necklace in *Tetrahymena* appears straight or slightly wavy. A survey of the still limited literature on this subject suggests that scalloped ciliary necklaces may be characteristic of mollusks (Gilula and Satir, 1972, and Bergstrom et al., 1973) and that necklaces with a different morphology may occur in other organisms (Gilula and Satir, 1972; Satir et al., 1972 b; Bergstrom and Henley, 1973; Bergstrom et al., 1973). Gilula and Satir (1972) have also shown that the particles forming the scalloped ciliary necklace of the mussel *Elliptio* are the attachment points of champagne glass-shaped structural linkers that connect the underlying microtubule doublets with the adjacent ciliary membrane. In the present study on *T. pyriformis* we have found linkers between the cytoplasmic doublets and ciliary membrane (Fig. 4 E) as well as between the cytoplasmic triplets and the ciliary membrane (Fig. 4 D), thus suggesting that the linkers may be associated with the transition zone where triplets become doublets. Could the linkers associated with the doublets connect to particles in the upper row of the ciliary necklace and the linkers associated with the triplets connect to particles in the lower row of the two-stranded ciliary necklace? Upon close examination it can further be seen that the linkers of *Tetrahymena* (Fig. 4 D and 4 E) differ from those described by Gilula and Satir (1972) for *Elliptio* in that they appear wider and more diffuse and do not exhibit the typical champagne glass form. Perhaps the champagne glass linkers, like

the scalloped necklaces, are characteristic only of mollusks. Freeze-cleave views of the linkers of *Tetrahymena* (Fig. 5) indicate that they may consist of globular subunits.

The plaque structures, first described by Wunderlich and Speth (1972) and Speth and Wunderlich (1972), located immediately above the ciliary necklace (Fig. 6) are not found in all strains of *T. pyriformis*. The function of the plaques located on the A face of the ciliary membrane is unknown. Since plaque structures are absent on a number of strains, it seems improbable that they are involved in a major way in control of ciliary beat, as has been proposed by Gilula and Satir (1972) for the function of the ciliary necklace-champagne glass linker structure.

It is conceivable that the low number of randomly distributed particles on the oral ciliary membranes as compared to the somatic ciliary membranes could be related to different stages in ciliary development. Several observations suggest, however, that these differences are not developmental differences. Williams and Frankel (1973), studying resorption and redifferentiation of oral structures in *T. pyriformis*, and Buhse et al. (1973), studying oral region formation in dividing *Tetrahymena*, found that the three rows of cilia in each membranelle grow at different rates. That is, during formation of the oral region, the innermost row of cilia located closest to the opening of the cytostome has the longest cilia, the middle row of the membranelle has cilia of intermediate length, and the outermost row of cilia has hardly begun ciliation. If the number of randomly distributed particles is related to the stage of development of a cilium, then we would expect the A face of the cilium in the middle of the membranelle (Fig. 3) to have more particles than the A face of the outermost cilium. There is no difference in the number of particles on the A faces of the two cilia.

FIGURE 17 Tip region of oral cilium illustrating a patch of particles (*p*) arranged in short parallel rows on the split inner face of the external membrane leaflet (B). The rows of particles make an angle of about 48° with the main ciliary axis. The particles function as membrane attachment sites for short bristles (arrows) which protrude into the surrounding medium. × 132,200.

FIGURE 18 A face of tip region of oral ciliary membrane. The two adjacent cilia exhibit patches of short parallel rows of holes oriented obliquely to the main axis of the cilium. × 78,250.

FIGURE 19 Tip region of oral cilia from the outermost row of cilia of the first membranelle. The patches of particles with attached bristles are present only on one side of a cilium. Notice the changing orientation of the bristles (arrows) on each cilium with respect to the oral ribs (*or*). The circularity of the cilium is distorted in the region containing the bristles (compare to cilium lacking bristles, [arrowhead]). × 56,000.

Other replicas of oral membranelles also confirm the low number of randomly distributed particles on oral ciliary membranes.

The bristle differentiation on the oral cilia can be seen both in freeze-cleaved (Figs. 17 and 19) and

in thin-sectioned cilia (Figs. 20 and 21). Williams and Luft (1968) have also reported the bristles with accompanying electron-dense material in thin sections of an amiconucleate strain of *T. pyriformis*. Our study indicates that the bristles have a globu-

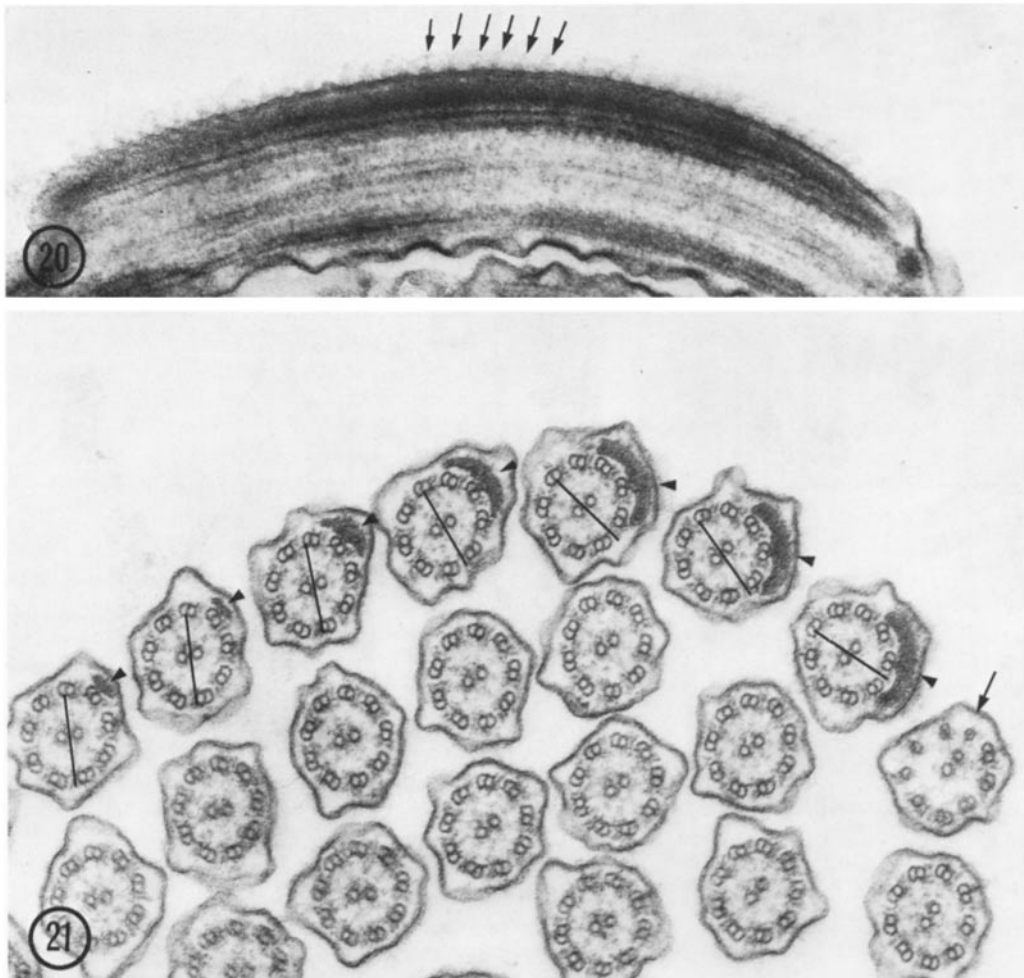


FIGURE 20 Longitudinal thin section of an oral cilium possessing bristles (arrows). Electron-dense material associated with the bristles is found between the ciliary membrane and the microtubule doublets. $\times 83,600$.

FIGURE 21 Cross sections of oral cilia of the first membranelle. The cilia have been sectioned close to the tip since single microtubules are present in the outer microtubule ring of an adjacent cilium (arrow). The electron-dense material has the form of a crescent and is associated with three microtubule doublets. The middle doublet of the three is linked to the electron-dense material. Using the method of Afzelius for numbering the microtubules (line through central tubules which intersects a doublet in the center), we find that the electron-dense material is associated with different doublets in different cilia (arrowheads). Sometimes it is difficult to determine exactly how to draw the line through the central tubules. Nevertheless, it is clear that the orientation of the bristles in thin sections and in replicas (Fig. 19) changes. $\times 72,600$.

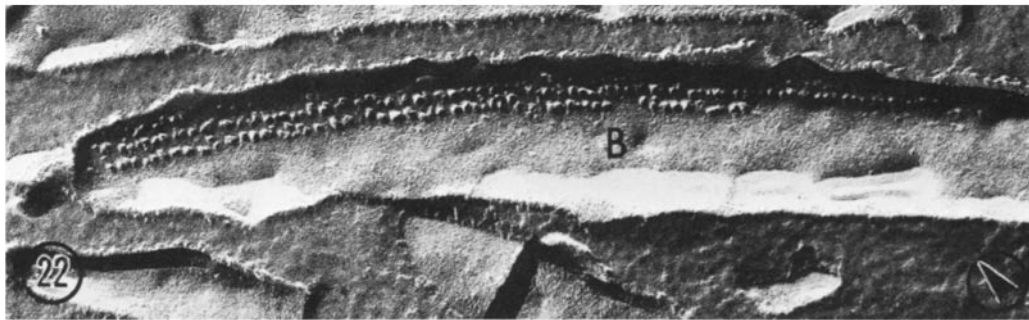


FIGURE 22 Three rows of closely spaced longitudinal rows of particles occasionally found on an oral cilium. The rows are approximately 300 Å apart and are present on the B face. $\times 78,800$.

lar substructure and that the bristles are attached to the membrane through rows of particles, oriented obliquely to the main axis of the cilium, that remain associated with the B face. The distinct array of particles with bristles (Figs. 17–19) suggests possible cross-linking and interaction between the closely associated particles. Cross-linking of the particles could increase the stability of the structure by spreading the strain placed on individual particles over the entire array. Our study indicates that the orientation of the patches of particles and their associated bristles changes on adjacent cilia (compare orientation of bristles on cilia located close to the oral rib side of the buccal cavity with orientation of bristles on cilia located further back in the membranelle, Figs. 7, 19, and 21). The position and orientation of the bristles suggests that they could serve two possible functions that are not mutually exclusive: (a) bristles may be able to sense the presence of food particles and trigger an appropriate response of other oral cilia; and (b) bristles, due to their location on one side of cilia located in the outer row of the first membranelle, may be able to modify currents produced by the cilia at the top of the buccal cavity. It is conceivable, therefore, that the bristles may direct the water current and food particles on to the oral ribs which appear to channel the food particles into the cytostome.

Measurements of the spacing between adjacent longitudinal rows of particles found on some of the oral cilia suggest that the longitudinal rows of particles have a definite geometrical relationship to the peripheral microtubule doublets located in the underlying cytoplasm. When the circumference of freeze-fractured cilia is determined and divided by 9, because of the ninefold symmetry related to

the nine microtubule doublets, a distance of between 800 and 1140 Å is obtained which is close to the measured values of 810 and 1080 Å for the spacing between longitudinal rows of particles.

Satir (1968) has proposed that the sliding of peripheral microtubule doublets along one another accounts for the bending and thus for the movement of cilia. If the particles in the longitudinal rows represent membrane attachment sites of cross-bridging elements linking some of the microtubule doublets to adjacent membrane areas, the sliding of the microtubule doublets in that region may be modified. Restricted sliding of some of the doublets could result in a modified ciliary beat. In thin sections (Fig. 15) and in freeze-cleaved replicas (Fig. 16) we have observed possible connections between the ciliary membrane and the middle of the outer doublets. Allen (1968), using images of thin-sectioned oral cilia of *Tetrahymena pyriformis*, enhanced by rotational superposition, has also observed connections between microtubule doublets and the ciliary membrane. Indirect evidence for these connections comes from the appearance of the ciliary membrane in the area that contains the longitudinal rows of particles. In freeze-cleave replicas (Fig. 8) the membrane associated with the longitudinal rows of particles is tightly apposed to cytoplasm whereas the membrane areas lacking such rows usually appear wavy (Figs. 1 and 3). In thin-sectioned cilia the waviness of the ciliary membrane is also reduced where linkers to microtubules can be recognized (Fig. 15).

The number of longitudinal rows of particles on a single cilium is difficult to determine. If the linkers between the microtubule doublets and ciliary membrane seen in thin sections (Fig. 15) are

related to the longitudinal rows of particles we might expect to find up to six of these rows on one cilium. Fig. 8 demonstrates, however, that for geometrical reasons it is only possible to visualize three rows on one fracture face at a time. This limitation can be partly, but not completely overcome by examining suitably cleaved cilia such as shown in Fig. 13 which exhibit both A and B faces. If, as shown in model I in Fig. 23, it is assumed that the particles forming the longitudinal rows of particles are symmetrically distributed around the membrane (one particle row associated with each doublet), then a replica corresponding to model I would have rows of particles on the A face and longitudinal rows of holes and a few particles on the B face which have pulled out of the A face. If, on the other hand, the longitudinal rows of particles were present only on one side of the cilium as depicted in model II, then the longitudinal row elements would be seen either on the A face or on the B face, but not on both faces. In our replicas we

have never encountered a split cilium possessing longitudinal row structures on both faces. Thus we can conclude that the longitudinal rows of particles are restricted to one side of a cilium and that at the most five or six rows could be present. This arrangement is consistent with the proposed idea that the beating of a cilium could be modified by linkers between the doublets on one side of the cilium and the adjacent membrane.

Studies designed to determine the pattern of ciliary metachronism in ciliates are difficult to perform and to interpret due to the complex interaction of factors such as temperature (Machemer, 1972 *b*) and the viscosity of the medium (Kuźnicki et al., 1970; Preston et al., 1971; Machemer, 1972 *a*). The most reliable data on ciliary beat of a ciliate come from studies using high-speed cinephotomicrography (Machemer, 1972 *a*) in conjunction with studies of micrographs of "instantaneously" fixed *Paramecium* (Párducz, 1967; Tamm, 1972). These latter techniques may

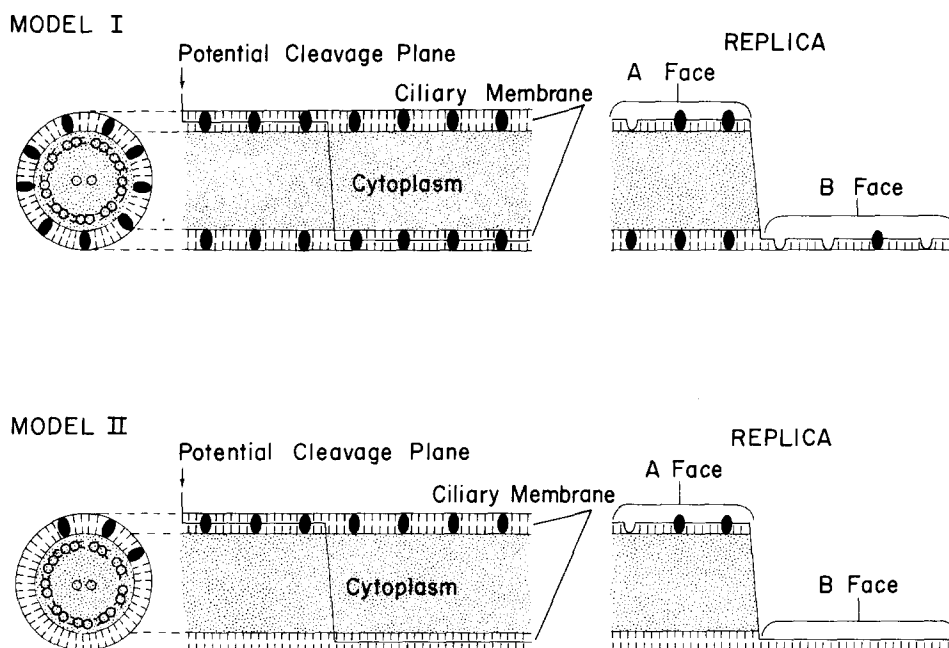


FIGURE 23 A diagram containing two models which illustrate the type of replicas that would be obtained from symmetrical and asymmetrical distribution of the particles forming the longitudinal rows on some oral cilia. In the cross sections of cilia, each row of particles is shown to be associated with a microtubule doublet. Assuming that the longitudinal rows of particles are symmetrically distributed (model I), then the splitting of a cilium to reveal both the A and B faces should reveal particles and holes in longitudinal rows on both the A and B faces. If, on the other hand, longitudinal rows of particles are only associated with one side of a cilium (model II), then a split cilium should exhibit longitudinal rows of particles and holes only on one of the two faces.

enable us to determine accurately the beat of the somatic cilia of *Tetrahymena*, but they cannot be used to determine the beat of approximately 100 closely spaced oral cilia. Thus, it appears that we will have to test our hypothesis that structural elements located within the ciliary membrane affect the form of ciliary beat in an organism other than *T. pyriformis*.

Two avenues seem to be available to test the proposed relationship between membrane specializations and ciliary beating patterns. Gibbons and Gibbons (1972) have shown that after removal of the flagellar membrane of sea urchin sperm with Triton X-100, the sperm can be reactivated and movement resembles that of live sperm. Since the function of the sperm tail is probably solely for locomotion, we believe further studies on cilia which have well-characterized membrane specializations and specific sensory or food-gathering functions should be performed. If the membrane does affect the form of beat as we propose in this paper, removal of the ciliary membrane and activation of the axonemal components should result in a beat pattern which is different from that found in the specialized intact cilium. The second approach is suggested by the fact that ciliary membrane differentiations resembling those described in this paper have also been observed on other organisms. We have briefly examined *Ochromonas danica* and found at least one longitudinal row of particles extending along the shafts of the flagella. Walne, Aldrich, Bartlett, and Pendland (1972, communicated at the American Institute of Biological Sciences meeting) using the freeze-etch technique, have described particles arranged in little necklaces at regular intervals along the length of the flagellum of *Euglena*. Immediately distal to the basal plate region in *Elliptio* and *Mytilus*, Gilula and Satir (1972) have reported that the membrane is fluted and contains longitudinal rows of particles which correspond to the spacing of the microtubular doublets. Although these rows of particles extend only 0.2–0.45 μm above the basal plate, they could influence the pattern of ciliary beat. Bergstrom and Henley (1973) have also observed longitudinal arrays of particles along the flagellar membranes of the sperm of the earthworm, *Lumbricus terrestris*. Thus, by examining organisms in which modified ciliary or flagellar beating patterns have been described (Sleigh, 1968), it may be possible to determine if membrane specializations within the ciliary or

flagellar membrane can be directly related to the form of the beat observed.

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