

# ULTRASTRUCTURE OF THE PROXIMAL REGION OF SOMATIC CILIA IN *PARAMECIUM TETRAURELIA*

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## ABSTRACT

The morphology of the transition zone between the terminal plate of the basal body and the 9 + 2 region of the somatic (non-oral) cilium has been examined in *Paramecium tetraurelia*. Freeze-fracture and thin-section techniques disclosed both membrane specializations and various internal structural linkages.

Freeze-fracture material revealed sets of particles interrupting the unit membrane. The more distal of these form plaquelike arrays while the proximal set of particles forms the ciliary "necklace." The plaque regions correspond to anionic sites on the outer membrane surface as revealed by binding of polycationic ferritin. Both the plaque particles and the necklace particles appear to be in contact with outer doublet microtubules *via* a complex of connecting structures.

In the interior of the transition zone an axosomal plate supports an axosome surrounded by a ring of lightly packed material. Only one of the two central tubules of the axoneme reaches and penetrates the axosome. Below the axosomal plate four rings, each approx. 20 nm wide, connect adjacent outer doublets. An intermediate plate lies proximal to these rings, and a terminal plate marks the proximal boundary of this zone. Nine transitional fibers extend from the region of the terminal plate to the plasmalemma.

The observations described above have been used to construct a three-dimensional model of the transition region of "wild-type" *Paramecium* somatic cilia. It is anticipated that this model will be useful in future studies concerning possible function of transition-zone specializations, since *Paramecium* may be examined in both normal and reversed ciliary beating modes, and since mutants incapable of reverse beating are available.

KEY WORDS cilia · *Paramecium* · plaques · polycationic ferritin · transition zone · membrane

Recent studies show that there are specialized structures at the proximal region of the cilium just above the basal body. Electron microscope examination of freeze-fractured material shows rings of

7-8-nm particles, the necklace, in the ciliary membrane at its junction with the plasma membrane in *Mytilus* gill (26). In certain protozoan cilia, many investigators (39, 45, 46, 48, 51) have shown that there are 3 × 5 rectangular arrays of 10-nm particles located above the necklace; there are nine "plaques" of these arrays on the circumference of the ciliary base. During deciliation, the

cilia invariably break in this region between neck-lace and plaques (11, 44).

Electrophysiological experiments on ciliated, deciliated, and reciliated *Paramecium* show that the ion channels (presumably membrane proteins) important in the bioelectric events of ciliary reversal are largely, if not exclusively, located on the ciliary membrane (20, 36). Certain strains of membrane mutants showing electrophysiological defects were found to have deranged plaque structures in the transition region (13). The plaque regions correspond to cation-binding sites on the outside of the ciliary membrane (21). In the ciliary lumen at the plaque region are sites capable of forming calcium deposits when the cells are fixed in the presence of high concentrations of  $\text{Ca}^{2+}$  (23, 39, 55, 56). In *Tetrahymena*, comparison between strains with and without the plaques indicates that the plaques are correlated with an ATPase activity (7).

Electrophysiological (35), biochemical (12), and genetic (30) evidence leaves little doubt that  $\text{Ca}^{2+}$  entering the cell during excitation causes the cilia to beat in a reversed direction in *Paramecium*. However, the nature and the location of the Ca-channels, the nature and the mechanism of the Ca-sensitive "reversal" element, and the microtubular movements related to ciliary reversal are not known. A detailed description of the structure of that region of the cilium from plaques to the terminal plate of the basal body, henceforth designated as the transition zone, may be useful in solving these problems.

The current understanding of the specializations occurring in the ciliary transition zone is based upon observations of several different organisms. Any future attempts to identify the functional significance of the transition zone would be greatly aided by an experimental system in which the ciliary wave form may be modified and identified experimentally and in which mutant cilia, incapable of expressing certain wave forms, existed. Since both of these conditions are possible with the use of *Paramecium tetraurelia*, we have examined the transition region of its somatic (non-oral) cilia with both thin-section and freeze-fracture electron microscopy. Our observations have confirmed many of the findings of previous authors and have revealed some new structural specializations. The morphology described will provide a basis for comparative analysis of forward and reverse beating *Paramecium* cilia as well as a basis for identifying aberrant structures in mutant cilia.

## MATERIALS AND METHODS

Cells of stock 51s of *P. tetraurelia* (previously species 4 of *P. aurelia*; see Sonneborn [50]) were cultured in Cerophyl medium (Cerophyl Laboratories, Kansas City, Kansas) bacterized with *Enterobacter aerogenes* (49). Cells were harvested during late log phase and fixed for 1 h in 2% glutaraldehyde in 0.08 M s-collidine buffer (pH 7.2) at room temperature. After several buffer rinses the material was postfixed in 1%  $\text{OsO}_4$  for 45 min. Cells were subsequently washed and stained *en bloc* with 0.5% aqueous uranyl acetate for 2 h. They were dehydrated in an ethanol series followed by propylene oxide and embedment in Spurr's resin (52). Silver interference sections were taken on a Reichert Om U3 ultramicrotome (C. Reichert, sold by American Optical Corp., Buffalo, N. Y.), stained with lead citrate, and photographed with a Philips 300 electron microscope.

Polycationic ferritin (PCF, Miles Laboratories, Inc., Elkhart, Ind.) was used to test for surface anionic sites. Before exposure to PCF, cells were washed for 10 min in Dryl's solution (19). The cells were then exposed to 0.018 mg/ml PCF in Dryl's solution for 2 min, followed by a wash in Dryl's for 2 min. Next, the cells were fixed for 60 min in 2% glutaraldehyde buffered with 0.05 M sodium phosphate buffer, followed by washing and postfixation in phosphate-buffered  $\text{OsO}_4$ . The remainder of the procedure was identical to that described above. Controls were exposed to Dryl's solution for two more minutes without PCF after the initial 10-min wash.

Cells to be freeze-fractured were fixed in 4% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) for 45 min. Afterward, the fixed cells were resuspended in fresh buffer, and glycerin was gradually added over a period of hours until a final concentration of 30% was reached. Drops of concentrated cells were placed on copper hats, frozen in Freon-22, and stored in liquid nitrogen. Fracturing of the specimens at  $-115^\circ\text{C}$  and  $2 \times 10^{-6}$  torr followed by replication took place in a Balzers' BA-510M apparatus (Balzers AG, Balzers, Liechtenstein). The replicas were cleaned overnight in sodium hypochlorite solution, rinsed with distilled water, and mounted on Formvar-coated copper grids before EM examination.

## RESULTS

### *Plaques and the Necklace*

In freeze-fracture preparations of the basal region of cilia, the most evident structures are the necklace and the ciliary granule plaques, or simply "plaques" (39, 45). These structures are best seen on the P-fracture face of the ciliary membrane (Fig. 1). The necklace consists of two parallel rows of 8-nm particles at the narrowest portion of the ciliary base at the junction of ciliary membrane and plasmalemma. The plaques, on the other hand, are distal to the necklace on a distinctly

swollen portion of the cilium. A typical plaque consists of a  $3 \times 5$  (three columns and five rows) rectangular array of 10-nm particles.

In thin-sectioned material, median longitudinal views at the plaque region show structures which extend into the interior of the cilium (Fig. 2). Each structure consists of electron-dense subunits alternating with transparent areas. The transparent areas of the structures in a column are in register and form two light bands (single arrows, Fig. 2). The band nearer the membrane is seen in all median longitudinal sections: the band farther from the membrane is often obscure. Another possibility is these bands are due to section thickness and each structure is in fact solid. Each of the structures has a thin, fibrous connection with the microtubule (qv reference 39).

In cross sections, as in median longitudinal sections, one can see that the plaque complex consists of discrete units (Fig. 5, brackets). Although the complex is 3 U wide adjacent to the membrane (corresponding to the three particles' width seen in freeze-fracture views of the plaques), there is only a single connection to the junction of the A and B tubules of the peripheral doublet.

The plaque particles pass into the unit membrane of the cilium. Freeze-fracture evidence shows that they protrude beyond the inner half of the bilayer (Fig. 1). In thin sections, we observe that the "railroad track" configuration of the unit membrane at the plaque region is interrupted by outer subunits of the plaque complex (Fig. 2, tandem arrows). These subunits may thus be identical to the particles seen in freeze-fracture.

When cells are treated with low concentrations of polycationic ferritin, a site of deposition is located on that part of the cilium surface associated with the plaque complexes. Not infrequently deposits are observed directly on the plaques (Figs. 3 and 4). Therefore, this area of the ciliary membrane possesses anionic sites on its outer surface. The chemical nature, ion-binding specificity and the possible mutant variation of these anionic sites are now under investigation.

On occasion, one may observe dynein arms and nexin links at the plaque region (Fig. 5). Radial spokes and spurlike arms from one of the central tubules (1) are also present. Such structures do not exist proximal to this region.

As in the plaque region, the peripheral doublets are connected through discrete structures to the membrane at the necklace. Again, the unit mem-

brane appears to be interrupted (Fig. 7, arrows). The gap between a plaque complex and the necklace connections is sometimes bridged by structures, thus giving the appearance of a continuum and making distinction between plaque and necklace impossible (Fig. 6).

### *Central Microtubules and the Axosome*

One of the central microtubules of the axoneme terminates in the region between the lower part of the plaque and the necklace (Figs. 6 and 8). The other member of the pair continues downward and enters the axosome. The axosome (a globule of about 60 nm diameter) rests on the curved axosomal plate (17, 38). Together they give the appearance of a ball-and-socket joint. That one tubule appears to terminate above the axosome is not caused by its turning away from the plane of section. Proof of this comes from thin cross sections of this region in which only one central tubule is present (Fig. 13). The single microtubule that enters the axosome enters it off-center. In higher (more distal) sections of this region, cross sections show that this tubule extends beyond the circle formed by the upper section of the axosome globule (Fig. 7). At the opposite point of this circle, the cross section of the other central tubule is clearly missing. There is no shadow or hole at this point to suggest other elements which may relate to the structure or function of the missing tubule. In a lower section, the one tubule is completely incorporated into the axosome (Fig. 10). Only an electron-transparent circle of the dimension of the microtubule lumen remains which eventually disappears as one views sections farther down (Fig. 11). Throughout the path of entry, the microtubule remains off-center with respect to the axosome. This microtubule does not completely penetrate the axosome to reach the axosomal plate (Fig. 11). On very rare occasions, cross sections give images which might be interpreted as showing two tubules within the axosome. Due to rarity and the section thickness, we could not verify such observations. In no case was the axosome devoid of both tubules.

The position at which the single central tubule enters the axosome bears no fixed relation to the orientation of the kinetodesmal fibers nearby that provide the coordinates of the local topography of *Paramecium*. The point of entry may vary from cilium to cilium even of the same cell. The amount of variance differs among fields of cilia. In some, it is slight; in others, substantial. In fact, there is

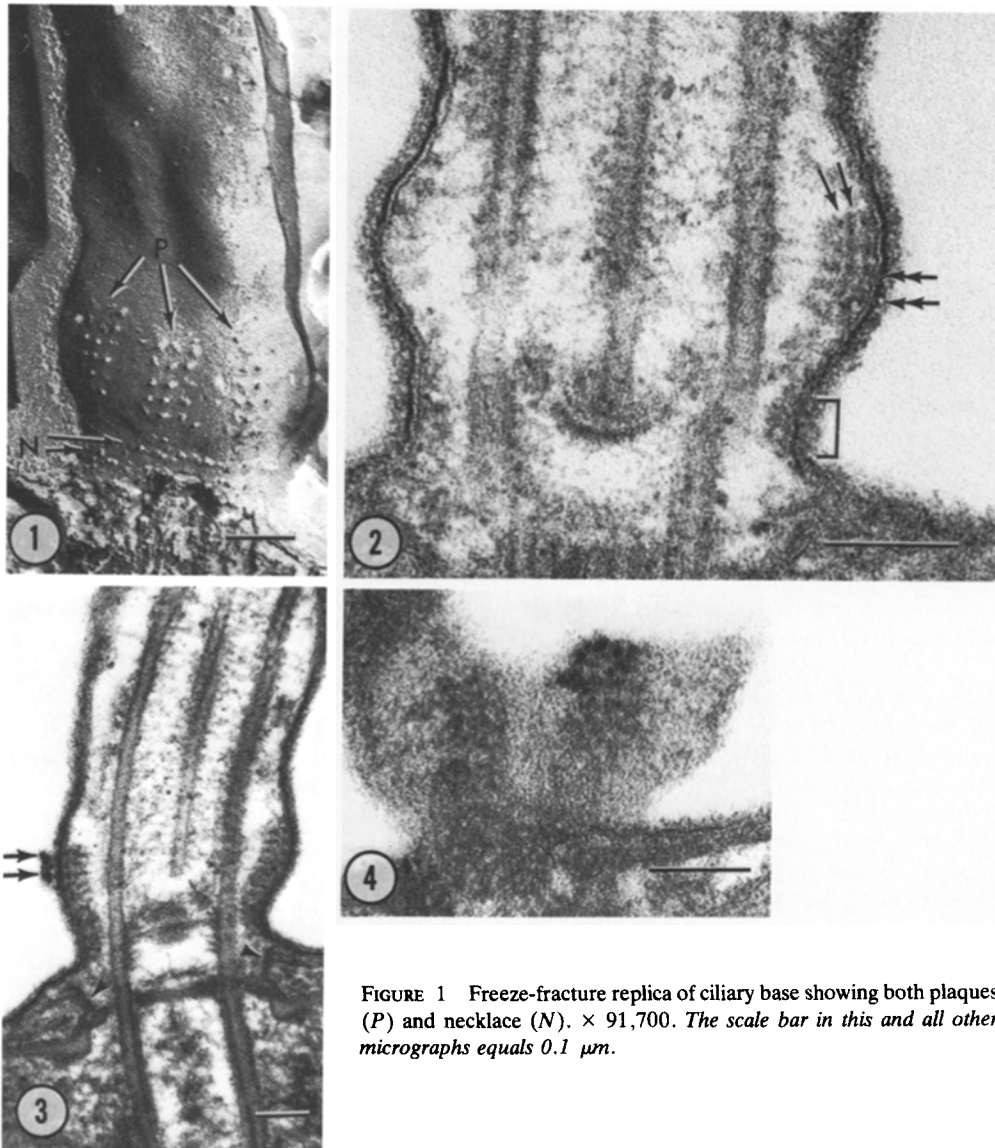


FIGURE 1 Freeze-fracture replica of ciliary base showing both plaques (P) and necklace (N).  $\times 91,700$ . The scale bar in this and all other micrographs equals  $0.1 \mu\text{m}$ .

FIGURE 2 Longitudinal section of ciliary base showing a lateral view of a plaque complex which contains subunits in a banded arrangement, and extends toward the peripheral doublet. The single arrows indicate these bands. The outer units of this complex pass into the unit membrane (tandem arrows). The necklace region is outlined by a bracket.  $\times 180,000$ .

FIGURE 3 Deposition of polycationic ferritin (arrows) on that part of the cilium surface associated with the plaque complex. Staining the sections often obscures the individual ferritin particles. Arrowheads denote transitional fibers.  $\times 76,000$ .

FIGURE 4 Glancing section of ciliary base treated with polycationic ferritin. The plaque region on left is visible, whereas the plaque region on right is partially hidden by ferritin deposits.  $\times 143,000$ .

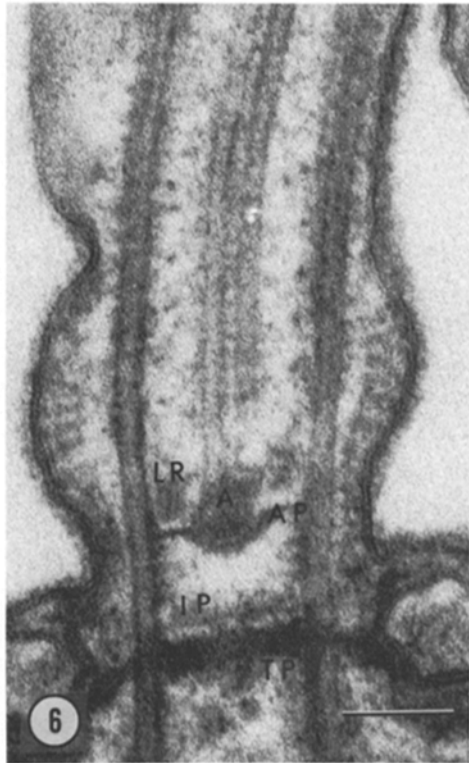
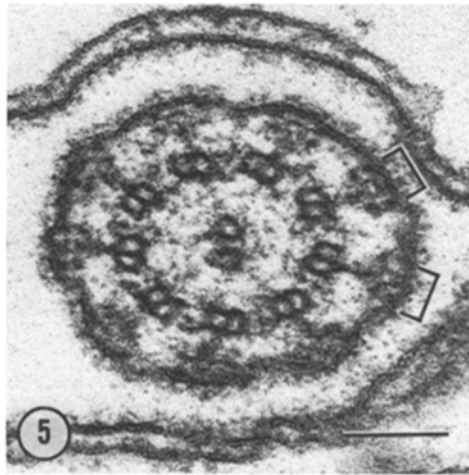


FIGURE 5 Cross section of the cilium in the plaque region. Each plaque complex (2 examples are outlined by brackets) consists of subunits and is connected to the common wall of the peripheral doublets.  $\times 139,000$ .

FIGURE 6 A ciliary base with a continuous set of particle-associated structures from plaque through necklace region (right side of cilium). *TP*, terminal plate; *IP*, intermediate plate; *AP*, axosomal plate; *A*, axosome; and *LR*, loosely packed ring.  $\times 146,000$ .

FIGURE 7 Cross section of the cilium in the necklace region. Unlabeled arrows denote positions where the necklace structures penetrate the unit membrane. Note the presence of but one central microtubule within the axosome (*A*).  $\times 138,000$ .

no evidence showing that the same microtubule of the central pair always enters the axosome (Fig. 9). Such an occurrence as seen in Fig. 9 was rare but not unique. Studies are now underway to determine whether the orientation of the central tubules correlates with the position of the cilium during the beat cycle, or with the direction of the

effective stroke (i.e., forward vs. backward swimming).

#### *Septa, Plates, Rings, and Pinwheel*

A number of structures are associated with the peripheral tubules at the region below the plaques and above the basal body. The upper half of the

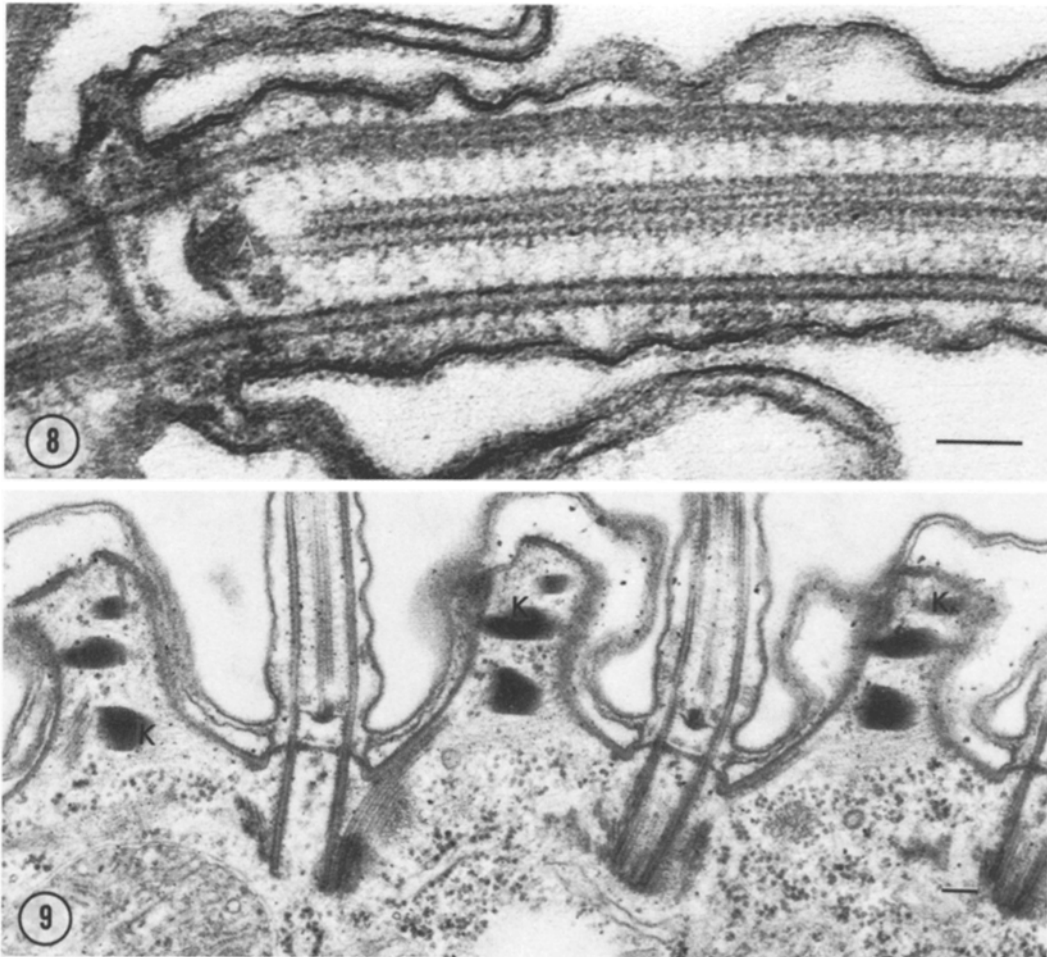


FIGURE 8 Longitudinal section in which only one of the central tubules is observed to enter the axosome (A).  $\times 117,000$ .

FIGURE 9 Two adjacent cilia which have alternate central tubules entering the axosomes. *K*, kinetodesmal fibers.  $\times 44,600$ .

axosome is surrounded by a ring of loosely packed material. In median longitudinal sections (Figs. 6 and 8), it approximates two rectangles 30 nm wide and 45 nm high on either side of the axosome above the axosomal plate. A cross section through the distal portion of this structure shows that it begins as nine discrete masses associated with the inner walls of the nine outer doublets, in particular the A tubules (Fig. 13, left). These masses join to form a continuous ring at a slightly more proximal position (Fig. 13, right). Longitudinal sections indicate that the lower boundary of this ring occurs just above the axosomal plate (Figs. 6 and 8).

About 90 nm below the rim of the axosomal

plate is a dense structure, designated the terminal plate (Fig. 6) (17). This plate marks the boundary between the transition zone and the basal body proper. Below this plate the peripheral tubules are in triplets instead of doublets (Fig. 17). Although it is thought that the C tubules of the basal body terminate at this point (16), they in fact extend to a position just distal to the terminal plate (Fig. 18, arrowhead). Immediately above the terminal plate can be found yet another structure, the intermediate plate (Fig. 6) (17). In some cases, the C tubules seem to extend into the region of the intermediate plate. Note, however, the apparent lack of recognizable C tubules in the

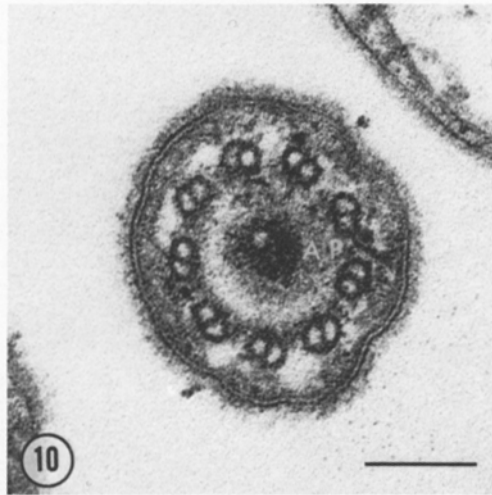


FIGURE 10 Section of a cilium proximal to that of Fig. 7. The tubule is now located entirely within the axosome. AP, rim of axosomal plate.  $\times 150,000$ .

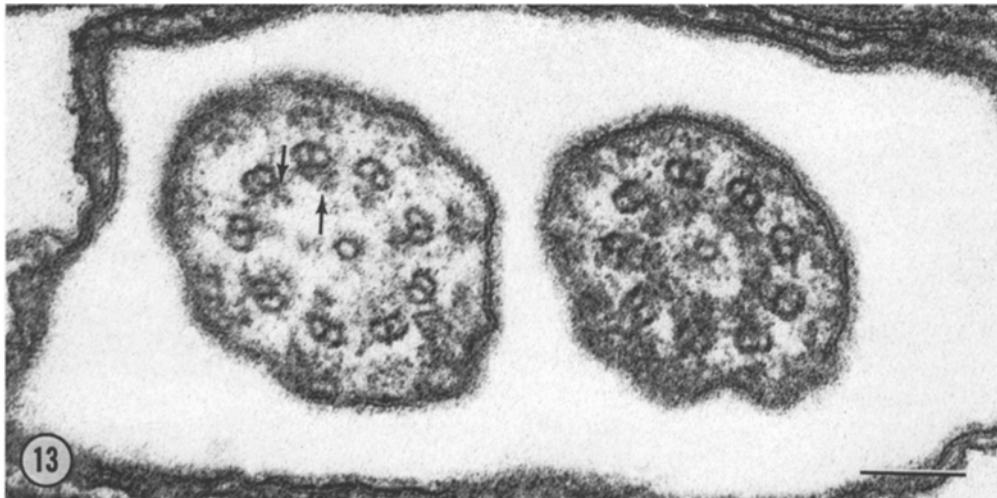
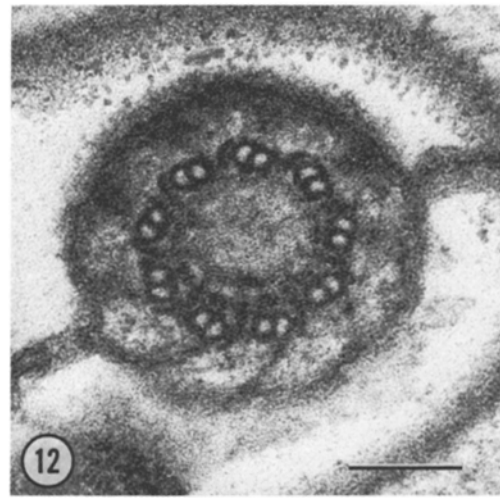
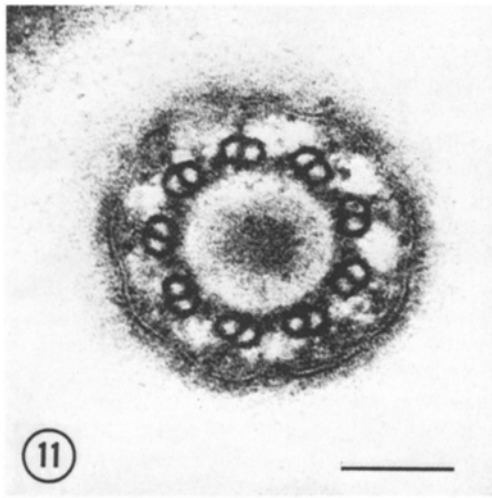


FIGURE 11 A section proximal to that of Fig. 10. The center of the cilium is occupied by that part of the axosomal plate subtending the axosome. There is no evidence of a central microtubule. The inner sides of all peripheral doublets are connected by a dense ring.  $\times 150,000$ .

FIGURE 12 A cross section through the terminal plate region showing the origin of the transitional fibers from the B tubules.  $\times 150,000$ .

FIGURE 13 Two ciliary cross sections slightly above the axosomal region. The section on the right is slightly more proximal than its partner. The loose ring of material begins as nine separate masses confluent to the inner walls of the peripheral doublets, particularly the A tubules (arrows, left cilium). These masses join proximally to become a continuous ring (right cilium).  $\times 137,000$ .

cross section of this region (Fig. 15, left). Figs. 14–17 present a sequence of four serial cross sections of a pair of cilia at the region between the axosome and the basal body. Section 14 is the highest (most distal), and all sections are viewed as if one is inside the cell looking out.

Cross sections containing the terminal plate show an intricate, radially symmetric sculpturing (Figs. 15, right, and 16, left). A central hub with a darkened rim sends processes centrifugally into the gaps between the tubule doublets. The tips of the C tubules are located in this position. Alternatively, the darkened nature of the hub's rim could be due to the superposition of the intermediate plate atop the terminal plate. Concentric with this rim, immediately interior to the peripheral tubules, is yet another darkened ring. This ring, the hub's rim and the processes define nine clear areas facing the peripheral tubules. These clear areas appear to be perforations of the terminal plate. In longitudinal sections, the terminal plate appears thinnest near the center (Figs. 8 and 9). This observation is consistent with the view in cross sections where the center of the hub appears to be less dense than its rim. On occasion, the center of the hub is perforated by and filled with material which may originate from the lumen of the basal body (Figs. 19 and 20).

The intermediate plate, which lies just above the terminal plate, is also nonuniform in thickness (Figs. 8 and 9). It is thin in the center, thickens outwardly and thins again before reaching the tubule doublets. In proper cross sections, one would expect a light-dark-light alternation moving from the center to the periphery of the intermediate plate. This is in fact the case (Fig. 15, left).

We have found yet other structures above the terminal plate. Immediately below the edges of the axosomal plate a dense ring approx. 20 nm wide connects the inner sides of all doublets (Fig. 11). Similar rings can be found in cross sections proximal to this (Fig. 14, right). Such rings not only connect all tubules but also extend into the area immediately interior to the circle of tubules. Serial longitudinal sections show that these structures are discrete rings or a helix of low pitch but not a continuous sleeve (Figs. 18–21). Appropriate sections suggest that there are four rings in a series. (Alternatively, one may interpret the images as the result of a helix of four turns.) These rings are surmounted by the edge of the curved axosomal plate (Figs. 20 and 21) and are distal to the intermediate plate (Fig. 20).

A pinwheel-like structure (the alar sheets or transitional fibers [4, 25]) is observed surrounding the peripheral tubules in cross sections. This structure is seen in thin sections containing the terminal plate, those containing the intermediate plate, and those just above the intermediate plate containing some of the dense rings (Figs. 12 and 14–16). In longitudinal sections, the blades of the pinwheel (or the individual transitional fibers) are dark, upright structures, originating from the portion of the terminal plate peripheral to the microtubules and extending distally, ending immediately beneath the plasmalemma (Fig. 3). Occasionally, there are two linked distal ends instead of one in longitudinal sections (Fig. 18). This may be a reflection of the overlaps of the pinwheel blades as seen in cross sections (Fig. 14, right). Cross sections near the terminal plate show that each blade of the pinwheel radiates from the B tubule of a triplet (Fig. 12). An acute twist (Fig. 22) near the origin of the blades is postulated to rationalize the images from both the cross sections and the longitudinal sections.

Distal to the terminal plate, below the axosome, the doublets have short peglike projections (Figs. 14, right, and 15, left) which may be ridges in longitudinal section. The pegs are attached to the common wall of the A and B tubules and have no association with the pinwheel structure. Fibrous material exists between these pegs.

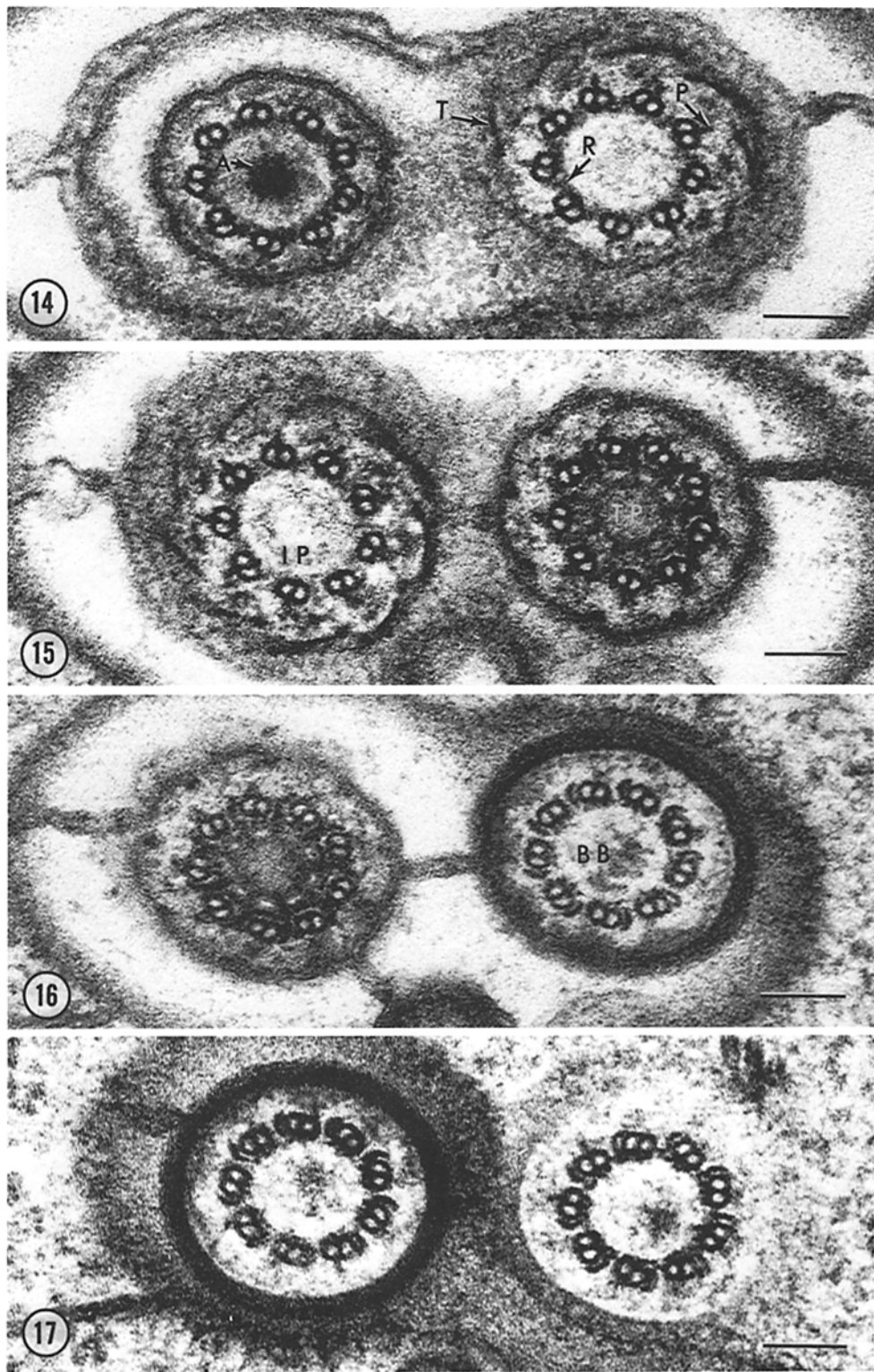
A diagrammatic, three-dimensional reconstruction of all the structures described in this paper is given in Fig. 22 to help in visualizing them.

## DISCUSSION

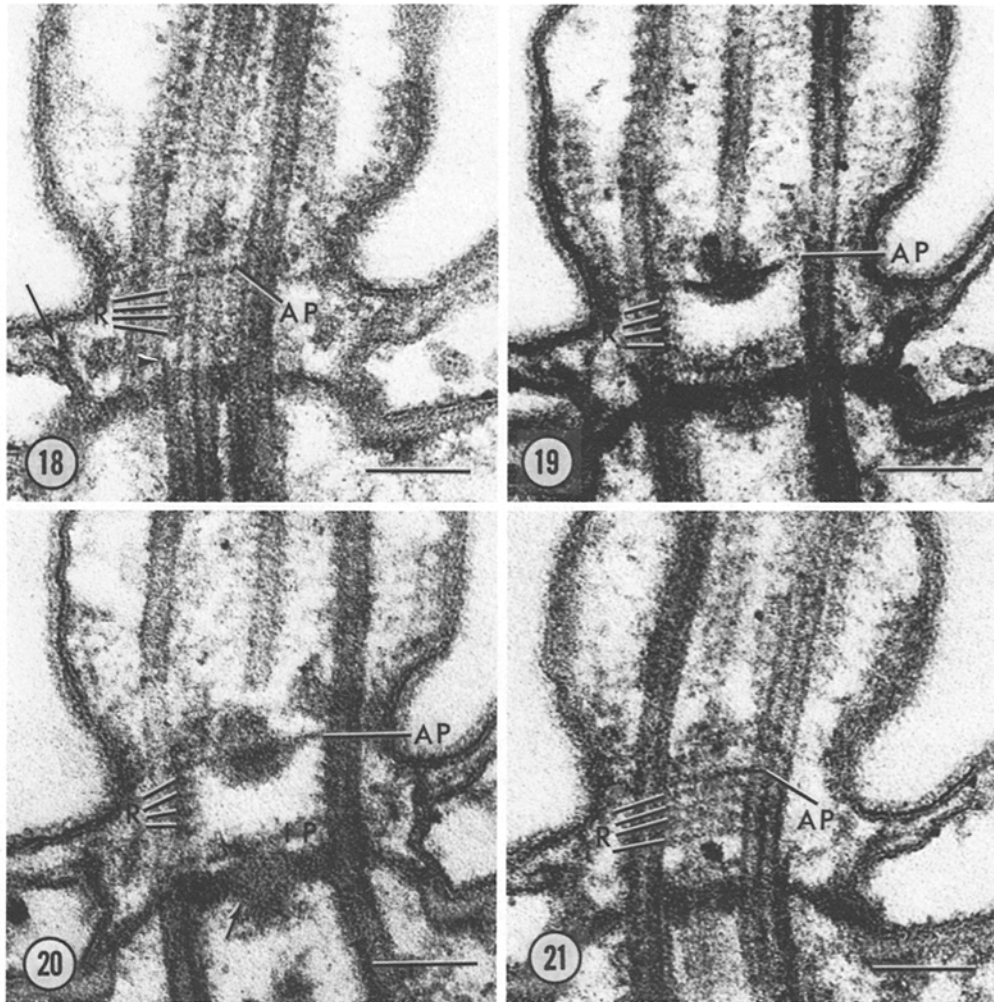
### *Necklace and Plaques*

Since the discovery of ciliary necklaces (24, 43, 46, 61), the list of organisms whose cilia possess these structures has increased appreciably. Necklaces are present in somatic cilia or flagella of protozoa (43, 45, 46, 48, 51, 61), invertebrates (9, 10, 26, 43), vertebrates (26, 43), and algae (58) as well as in sensory cilia (33). Thus, it is safe to say that the necklace is an integral part of the cilium. In most cases in which the necklace has been studied in detailed thin sections, an association between the particles visualized in freeze-fracture specimens and the doublets of the axoneme has been found. Such connections in *Elliptio* cilia have been designated "champagne glasses" by Gilula and Satir (26). These connections also exist in *Paramecium* as noted by Pitelka and Child





FIGURES 14-17 Serial sections through a pair of cilia from axosome to basal body proper. Section 14 is the most distal. All sections are viewed from the inside of the cell. The right cilium is at a slightly lower level than the left member. *A*, axosome; *P*, peg; *R*, dense ring; *T*, transitional fiber; *IP*, intermediate plate; *TP*, terminal plate; and *BB*, basal body proper.  $\times 127,000$ .



FIGURES 18-21 Serial longitudinal sections showing different views of the four dense rings (*R*) surmounted by the axosomal plate (*AP*). The terminal plate seems perforated (arrow, Fig. 20) and filled with material perhaps from the lumen of the basal body. The arrowhead in Fig. 18 denotes the distal termination of a C tubule. The arrow in this same figure identifies the distal tip of a transitional fiber which seems to bifurcate. *IP*, intermediate plate.  $\times 139,000$ .

(38) as early as 1964 as well as other studies including the present one.

The presence of plaques in cilia is more restricted than that of necklaces. Only the protozoan genera *Tetrahymena* (44, 48, 51, 61) and *Paramecium* (39, 40, 45, 46) are known to possess them (however, see Fig. 14 of reference 6). The plaque complexes in *Paramecium*, of which the plaque particles seem to be part, have been noted in thin section by previous workers (17, 39) but never described in detail until the present.

Anderson and Hein (5) using polycationic fer-

ritin (PCF) observed high deposition densities near the base of rabbit oviduct cilia corresponding to the position of the necklace. Deposition was related to ciliary beat, as immotile cilia bound no PCF in this region. We have also shown that cilia in *Paramecium* bind PCF near their bases, but that the area of the plaques rather than the necklace rows is primarily responsible. The PCF binding to the plaque region is strong since it occurs at a lower concentration (0.018 mg/ml) of PCF than the binding to the necklace of oviduct cilia (0.32 mg/ml). Density of surface charge de-

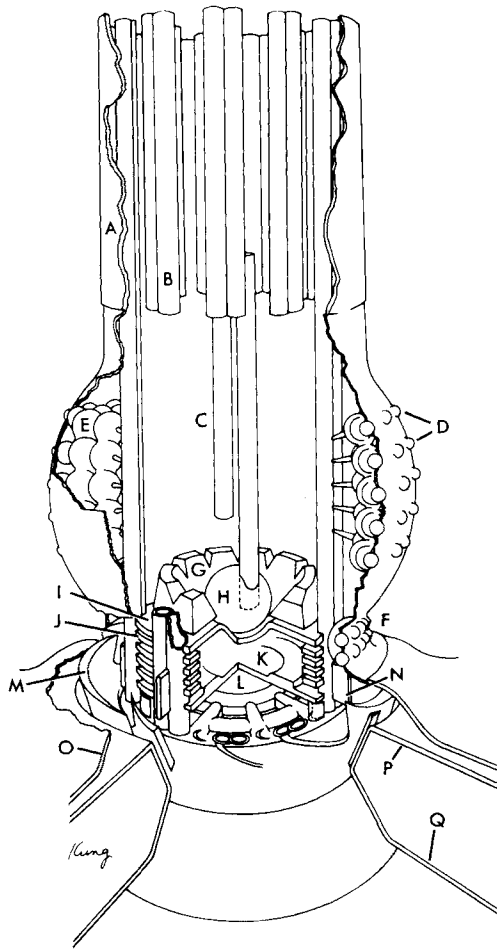


FIGURE 22 A three-dimensional reconstruction of the proximal region of a *Paramecium* cilium. (A) Ciliary membrane. The outer half of the membrane is lifted in parts to show the necklace and plaque particles. (B) Peripheral tubules. Portions of the tubules are deleted to simplify the diagram. (C) Central tubules. Note that only one of the tubules enters the axosome. (D) Plaque particles. (E) Plaque complex. (F) Necklace. (G) Loosely packed ring of material surrounding the axosome. (H) Axosome. (I) Curved axosomal plate. (J) Rings connecting peripheral tubules. Note that they do not enter the tubules. (K) Intermediate plate. (L) Terminal plate. (M) Transitional fibers (pinwheel structures). (N) Projections from the peripheral tubules. (O) Plasma membrane. (P) Outer alveolar membrane. (Q) Inner alveolar membrane.

termines the local environment, e.g., local voltage, ion concentration, and pH, in which membrane protein functions, such as transport and excitation, are carried out (32). The concentration

of negative charges identified here may be related to the function of the plaques.

The plaque complexes may be associated with cation movement between the cilium and the surrounding medium. Such movement could be enhanced by the negative surface charge of this region. Recently, a mutant characterized by abnormal ion flux (29, 47) was also found to have deranged plaques (13). If a relationship between ion flux and plaque complexes exists, one might also expect to find abnormal plaques in other membrane mutants.

### Central Tubules

We provide evidence in this paper that, in *Paramecium*, only one of the two central microtubules of the axoneme typically reaches the axosome and enters it. Conversely, Pitelka and Child (38) contended that both central tubules originate in the axosome. Dippell (17) stated that at least one of the central pair originates from the axosome, and Ehret and McArdle (22) observed only one member of the pair touching it.

Similar confusion attends the central tubule origin in the related genus *Tetrahymena*. Satir et al. (44) noted that both central tubules originated from the axosome. Sattler and Staehelin (48), on the other hand, stated that the central tubules ended immediately distal to the axosome. Their Fig. 4A, however, indicates that one tubule reaches the axosome whereas the other does not. Munn (34) observed the "longer" central tubule to terminate just above the axosome, and Allen (2) concluded that only one member of the pair was continuous with the spheroid. Taken together, these reports seem to indicate that in the majority of cases for both *Paramecium* and *Tetrahymena*, only one member of the central pair touches the axosome: using cross sections, we have observed that it not only touches it, but enters it. Since other workers have shown the distal ends of the central microtubules in *Tetrahymena* to be attached to a cap (14), one central tubule may be longer than the other.

Axosomes containing but one central tubule have been either described or pictured for other ciliates (6, and literature cited therein: 28, 57). Thus, this phenomenon exists in certain ciliates but may not occur in other protozoa (see, for example, reference 42). Various explanations can be given for this phenomenon. Anderson and DuMont (3) suggest a bifurcation of the axosomal central tubule in species of the Buetschliidae and

Paraisotrichidae. We have no evidence for this in *Paramecium*. Certain authors (8, 31, 59) have observed that the two central tubules have different solubilities. Thus, one might suspect that the proximal tip of one member of the pair was digested during preparation for electron microscopy. However, were this the case, one would expect a second hole within the axosome. No such hole is present. Therefore, we are led to conclude that only one tubule actually exists within the axosomes of *Paramecium* cilia. This does not mean that both central tubules do not exist within the axosome during early development of the cilium or at some point during the beat cycle, but at present we have no definite proof of such a condition.

Tamm and Horridge (53) observed that during the three-dimensional beat of the protozoan *Opalina* a line drawn through the central tubules was always normal to the direction of bend. Thus, the position of the central tubules is related to the beat cycle but whether in an active or passive manner is unknown. Cilia of *Paramecium* also have a three-dimensional beat cycle. For the central pair to vary its direction, either it must twist during the beat cycle or the axosome must rotate thus turning it, or both. In the present study, no preferred orientation of the central tubule within the axosome to kinetodesmal fibers was observed and might indicate rotation. In order to clarify this situation, we are at present investigating fields of somatic cilia in cells which have been instantaneously fixed. This fixation preserves the metachronal waves, and with such a system it is possible, with serial thin sections, to observe large numbers of ciliary cross sections in the axosomal region. From this, we should be able to discover any relationship between the position of the central tubule within the axosomal and the beat cycle.

### *Rings and Fibers*

Both the ring of loosely packed material surrounding the upper portion of the axosome and the series of rings beneath the axosomal plate have been pictured previously (e.g., references 16 and 22) but never described. No function can be imagined for the former structure; but the series of rings may act as stabilizing structures, maintaining the proper distance between the microtubule doublets.

The pinwheel-like structures or transitional fibers described in this report appear to be common components of the transition region from basal

body to cilium or flagellum (qv review articles by Wolfe [60] and Goldstein [27]). These structures, first reported in flagellates by Gibbons and Grimstone (25), are attached to the microtubule triplets of the distal part of the basal body and pass obliquely to the cell surface where they make contact with the cell membrane (4, 15, 18, 41, 54). In appearance they may be fibrous or, at least in basal bodies from monkey oviduct, sheet-like (4). The consensus is that these structures anchor the basal body through attachment to the cell membrane (18, 27, 60). Nevertheless, reports exist of unattached basal bodies and centrioles possessing transitional fibers (37, 41).

Many of the structures described here have been studied by previous microscopists. We have provided an inventory of them and a reconstruction of the architecture of the basal region of the cilium. In some cases, we have described for the first time in *Paramecium* the detailed morphology of previously identified parts such as the terminal plate and the plaque complex. Two structures, sometimes seen in published micrographs, have not been described before. They are the loosely packed ring surrounding the axosome and the four rings between the axosomal and the intermediate plate. The complex architecture of this region of the cilium, as summarized in the diagram of Fig. 22, cannot yet be related to the ciliary functions with any confidence. However, its complexity argues for the functional importance of this region.

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