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The Sensory Cilium of Retinal Rods is Analogous to the Transitional Zone of Motile Cilia

Pál Röhlich

Laboratory I of Electron Microscopy and 2nd Department of Anatomy, Histology and Embryology, Semmelweis University of Medicine, Budapest, IX. Tüzoltó u. 58, Hungary

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Summary. The connecting cilium of rat retinal rods was studied by freeze-fracture and thin-sectioning techniques. Transverse strands of intramembranous particles could be observed on fracture face B of the ciliary plasma membrane. The strands were essentially similar to those found at the transitional zone of motile cilia ("ciliary necklace"). The large number of intramembranous particles obscured the pattern on fracture face A of the membrane.

On longitudinal sections of the cilia, beads showing a periodicity similar to the necklace strands were observed. Each bead consisted of two structures apposed to both sides of the plasma membrane. Transverse sections of the cilia revealed radial Y-shaped structures that connected each ciliary doublet with the plasma membrane. Axial tubules, central sheath, radial spokes and dynein arms were missing in the connecting cilium.

Comparing the fine structure of the retinal cilia with that of motile cilia it becomes evident that the connecting cilium is analogous in structure with the transitional zone of motile cilia. The present observations suggest that periodic membrane beads along the plasma membrane on thin sections correspond to strands of necklace particles as observed on freeze-fractured membranes. The arrangement of the particles in transverse strands is probably ensured by the radial connecting structures.

Key words: Sensory cilia — Retinal rods (rat) — Comparison with kinocilia — Freeze fracture — Electron microscopy.

Non-motile cilia, having a microtubular pattern of $9+0$, are regular constituents of many receptor cells ("sensory cilia"). They are usually located at that part of the cell which is exposed to the specific stimulus and this suggests that they play an important role in the primary receptor events. However, very little is known about the mechanism by which excitation is generated in such cilia and, surprisingly, very few data are available on their detailed architecture.

The present study was undertaken to analyse the fine structure of one of these sensory cilia, the connecting cilium of retinal rods. Emphasis was laid on the plasma membrane because the membrane was thought to have special importance in the specific function of the cilium. The freeze-fracturing technique was used to reveal the interior domain of the plasma membrane, and transverse strands of intramembranous particles were found along the length of the connecting cilium. The same observation was made while this paper was in preparation by Matsusaka (1974). The strands were strikingly similar to those found at the 0.1–0.2 μm high transitional zone of motile cilia (Flower, 1971; "ciliary

Send offprint requests to: Dr. P. Röhlich, Cell Pathology Division, Clinical Research Centre, Watford Road, Harrow, HA1 3UJ, Middlesex, England.

necklace", Gilula and Satir, 1972). The similarity in the arrangement of intramembranous particles and the lack of axial microtubules in both the connecting cilium and the transitional zone suggested that both were analogous structures. Therefore, our fine structural study has been extended to thin-sectioned connecting cilia with the aim of finding additional characteristics of the transitional zone of motile cilia: lack of the central sheath, spokes and dynein arms as well as the presence of radial linkers between the doublets and the plasma membrane.

Material and Methods

Freeze-fracturing. Retinae from Wistar rats (10 rats) were separated from the eye wall and fixed in 1.5% glutaraldehyde (in 0.07 M cacodylate buffer, pH 7.2) for 40 min at 4°C. After a wash in 0.15 M cacodylate buffer for 30 min, the retinae were soaked in 25% glycerol (in 0.1 M cacodylate buffer) at 4°C for $1^1/_2$ hour and subsequently frozen in Freon 22 chilled by liquid nitrogen.

The freeze-fracturing device was a modified variety of the Bullivant-Ames type II cold block device (Röhlich, Környei and Balogh, 1974, to be published). The freeze-fracturing equipment, placed into a Zeiss HBA 2A vacuum evaporator (Zeiss Jena, DDR) made internal fracturing under controlled temperature conditions possible. Fracturing and replication was usually made at temperatures between −140 and −160°C. Replicas were cleaned on sodium hypochlorite and chromic acid and mounted on one-hole grids with carbon-strengthened formvar film. Shadows of platinum evaporation are white and the direction of shadowing is indicated on each micrograph by an arrow.

Conventional Electron Microscopy. Retinae were fixed in 2% glutaraldehyde in Millonig's phosphate buffer containing 0.5% tannic acid for 2 hours at 22°C. Tannic acid was used to enhance the contrast of protein constituents at the membrane surface (Futaesaku, Mizuhira and Nakamura, 1972). Thouroughly washed specimens were postfixed in 1% osmic acid (0.1 M cacodylate buffer) and embedded in araldite. Thin sections were made on a Reichert OMU-2 ultramicrotome, stained with aqueous uranyl acetate and lead citrate.

Replicas and thin sections were observed in a JEM 6C electron microscope using 30 and 50 μm apertures.

Results

Rod cells of rats are slender and elongated cells with a long connecting cilium which is easily accessible for observation in the freeze-fractured specimen. The cilium that connects the outer segment of the cell with the inner segment usually originates at the apex of the inner segment in an excentric position, most frequently from the lateral portion of the apex.

The Ciliary Membrane as seen in Freeze-fractured Specimens. In freeze-fractured specimens membranes are cleaved most probably along their hydrophobic interior resulting in two complementary fracture faces. Fracture face A belongs to the membrane half which adheres to the cytoplasm, while fracture face B represents the inner surface of the outer membrane half.

Fracture face A of both inner and outer segments, as well as of the connecting cilium can easily be identified because of the high density of intramembranous particles. While on the outer and inner segments the particles are randomly distributed, the connecting cilium shows occasionally transverse orientation of the particles (Fig. 1). In many cases however, this striation is not evident (Fig. 2).

In certain, probably less adequately fixed specimens, the particles on membrane fracture faces below and above the connecting cilium are not evenly dis-

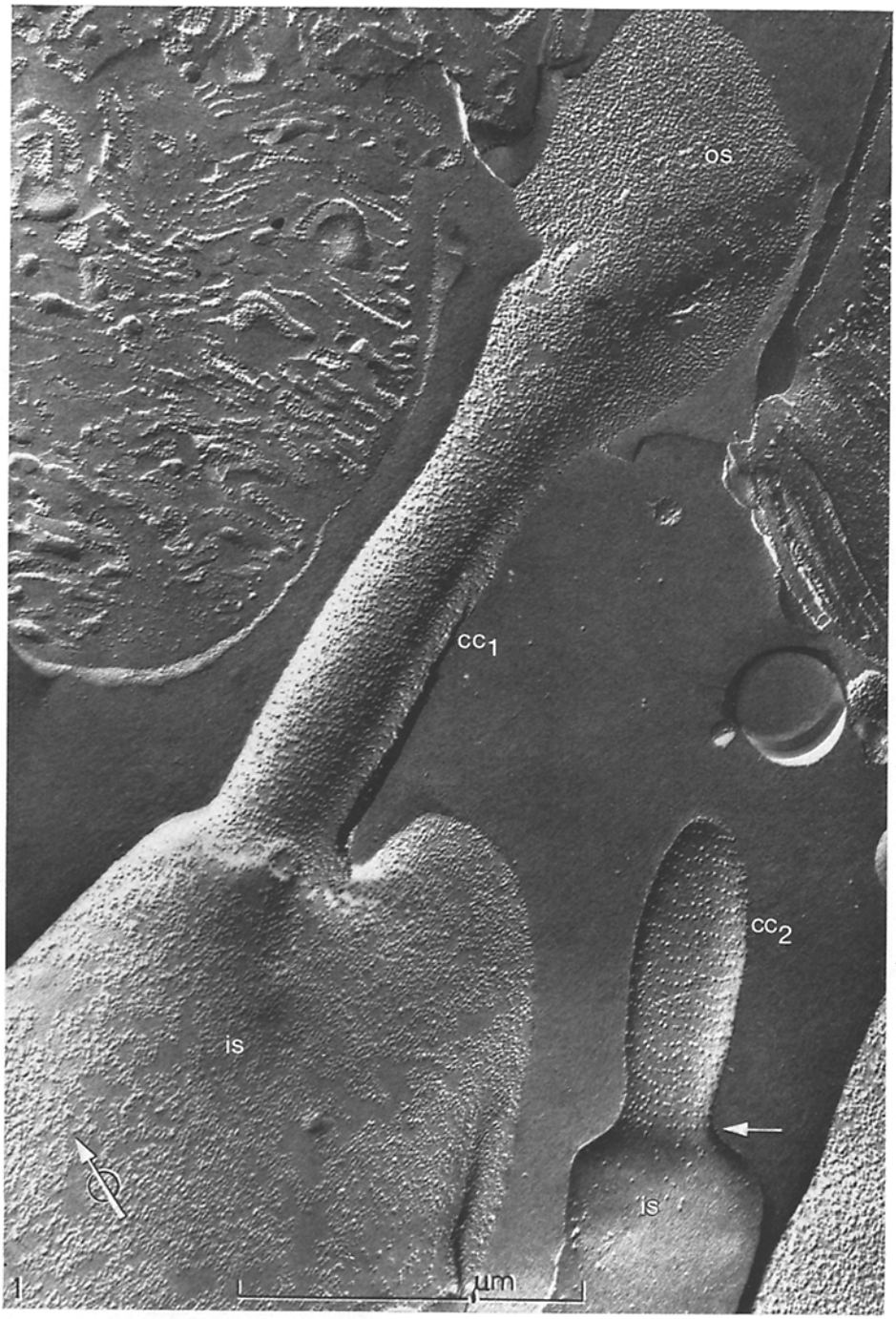


Fig. 1. Two connecting cilia are shown on a freeze-fractured specimen; one of them (cc_1) reveals fracture face A of the ciliary membrane and neighbouring parts of inner (*is*) and of outer segments (*os*). The cilium on the right (cc_2) shows fracture face B of the membrane with adjacent part of the inner segment (*is*). Note the transversely oriented strands of intramembranous particles on this fracture face and their abrupt disappearance at the border between inner segment and cilium (arrow)

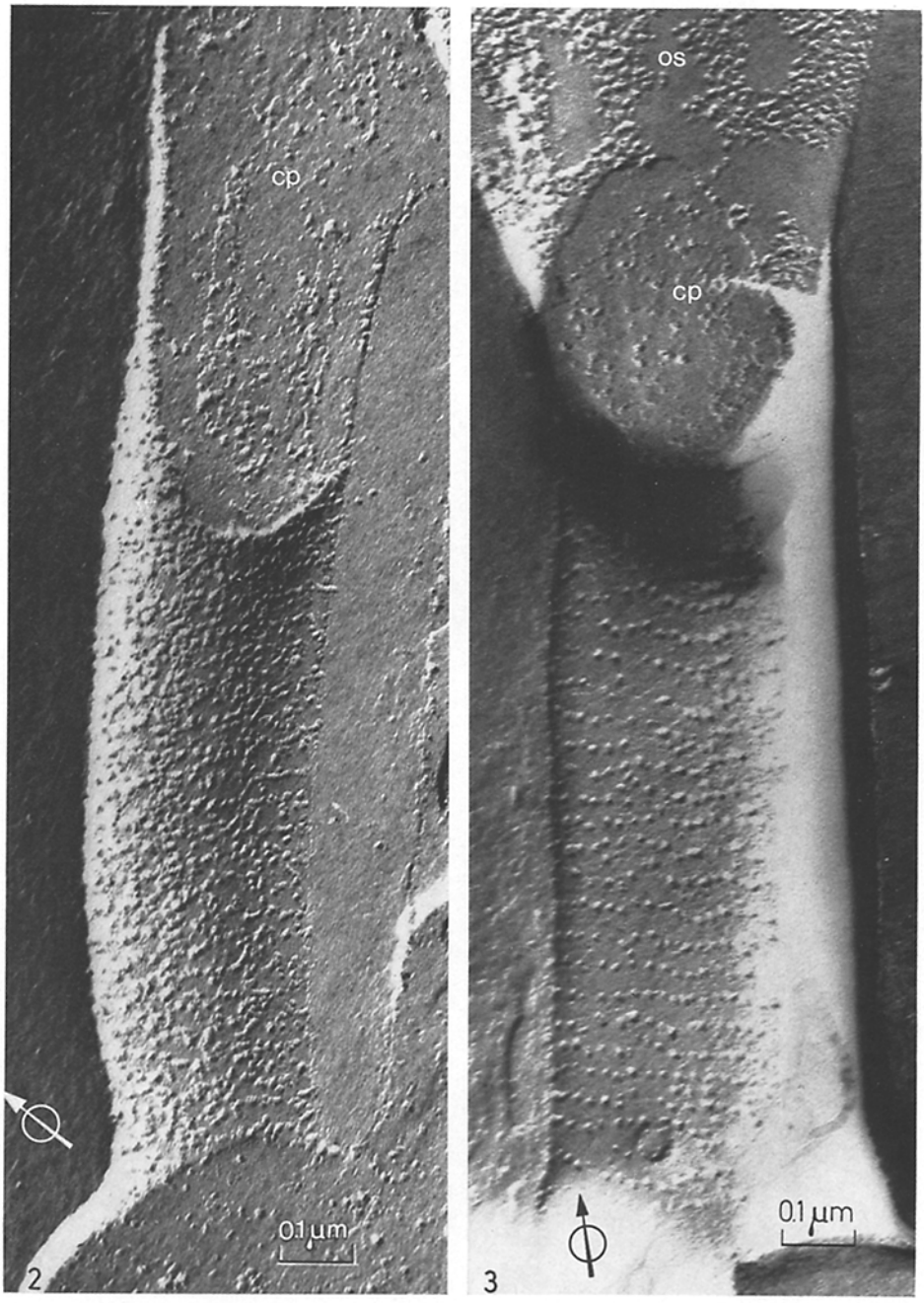


Fig. 2. Fracture face A of the connecting cilium. Many intramembranous particles are presen in a random distribution on this fracture face of the ciliary membrane. On the upper par of the micrograph detail of the cytoplasm (*cp*) with the ciliary tubules can be seen

Fig. 3. Fracture face B of the connecting cilium with transverse strands of intramembranous particles; (*os*) fracture face A of the outer segment, (*cp*) cytoplasm of the cilium

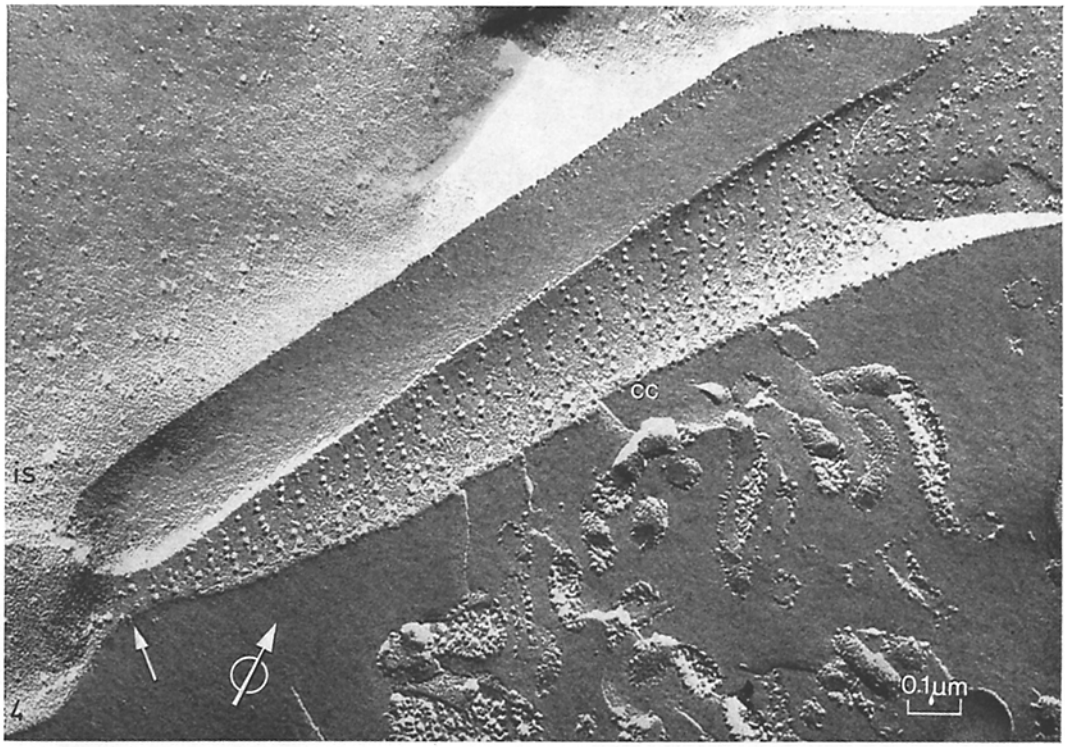
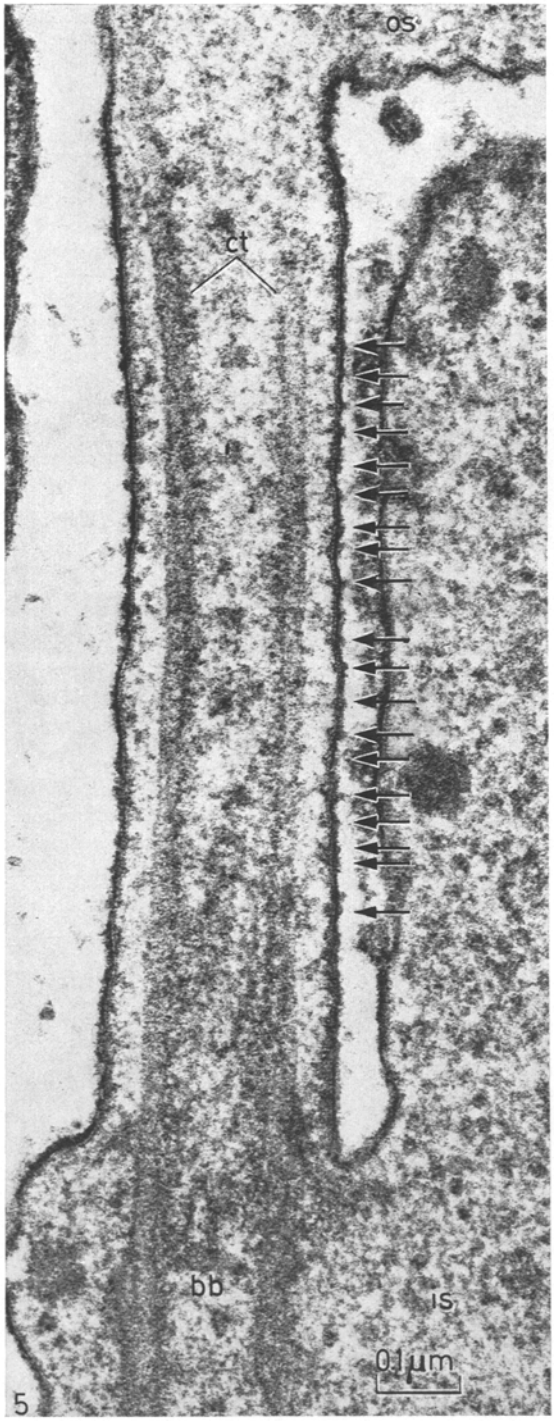


Fig. 4. Fracture face B of a connecting cilium (*cc*). About 35 strands of intramembranous particles are present along this segment which represents almost the whole length of the cilium. Arrow indicates the transition between cilium and inner segment (*is*); note fracture face B of the inner segment plasma membrane with only a few scattered intramembranous particles

tributed but may show smooth areas in between them. This finding was never observed on the connecting cilium itself.

As in many other cell types, *fracture face B* shows only few intramembranous particles (Figs. 1, 4). The only exception is the connecting cilium where transverse strands of intramembranous particles can be seen along the whole length of the ciliary plasma membrane (Figs. 1, 3, 4). The 9–10 nm particles have a centre to centre distance of about 25 nm. The strands are running more-or-less parallel to each other; some of the strands may be incomplete or may fuse with a neighbouring strand. Usually 20 to 30 strands are found per micrometer (30 to 40 along the length of the cilium). At both ends of the cilium where it loses its cylindrical shape and becomes dilated to form neighbouring portions of outer and inner segments, the strands abruptly disappear to be replaced by the characteristic fracture face B poor in intramembranous particles (Figs. 1, 4).

Between the individual strands of particles, especially at those areas which were shadowed at a low angle, there are fine impressions which most probably correspond to the particles at fracture face A. However, such impressions are rather unfrequent in the strands between the particles.



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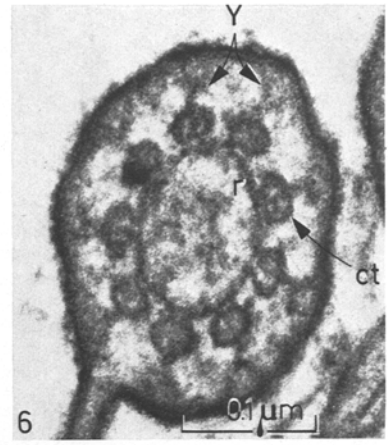

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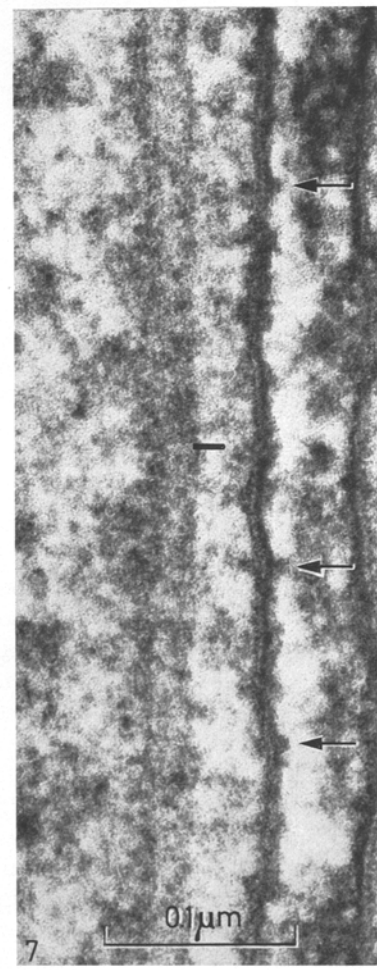

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Inner Structure of the Cilium in Thin Sections. Transverse sections of cilia show radially arranged structures connecting the ciliary doublets with the plasma membrane (Fig. 6). The Y- or V-shaped linker structures are essentially similar to those described for the transitional zone of motile cilia at the level of the ciliary necklace (Gilula and Satir, 1972; Anderson, 1974). The closely packed 9 ciliary doublets are arranged along the outer surface of a ring-like structure that may be analogous to a continuous series of inter-doublet links.

In longitudinal sections of the connecting cilia one can find beads that are more-or-less periodically arranged along the plasma membrane (Fig. 5). The beads consist of dense structures on both sides of the membrane (Fig. 7). The one on the cytoplasmic side is usually elongated with an oblique or perpendicular orientation to the plasma membrane and can be followed for 10 to 30 nm in the cytoplasm. The periodicity of the beads is roughly 25 per micrometer. The beaded appearance of the plasma membrane can be observed only at the connecting cilium and is not present either on the inner or on the outer segments.

Discussion

Both the observation by Matsusaka (1974) and the present study have shown that the plasma membrane of the connecting cilium in rat retinal rods exhibits intramembranous particles arranged in strands transversely oriented to the ciliary axis. About 30 to 40 strands can be found along the length of the cilium. The inner structure of the cilium is characterized by closely packed ciliary doublets that are arranged along a ring-like structure, and by the absence of the central pair of microtubules, as well as of the central sheath, spokes and dynein arms. Instead, it has a series of specialized Y-shaped structures connecting the midpart of each ciliary doublet with the ciliary membrane.

If this structure is compared with that of motile cilia (Fig. 8), it becomes obvious that there is one narrow segment of motile cilia which is strikingly similar in appearance, namely the transitional zone at the base of the cilium. In rat tracheal and mouse oviduct cilia, the membrane of this transitional zone is characterized by about 6 non-scalloped strands of intramembrane particles, the ciliary necklace (Gilula and Satir, 1972). While the central ciliary tubules, central sheath, spokes and arms are absent, the inner structure of this part of the cilium contains radial linkers extending from the midwall of each doublet to the ciliary membrane (Gilula and Satir, 1972; Anderson, 1974). In fact, hardly any dif-

Fig. 5. Longitudinal section of the connecting cilium in ultrathin-sectioned material. Beads (arrows), more-or-less periodically arranged, can be observed along the plasma membrane of the cilium. (*is*) inner segment, (*os*) outer segment, (*ct*) ciliary tubules, (*bb*) basal body

Fig. 6. Transverse section of a connecting cilium. Doublets of ciliary tubules (*ct*) are arranged along a ring-like structure (*r*) and are connected with the plasma membrane by Y-shaped cytoplasmic structures (*Y*)

Fig. 7. Enlarged area from Fig. 5 showing details of beads on the plasma membrane. Beads seem to be composed of two dense structures on both sides of the membrane (arrows)

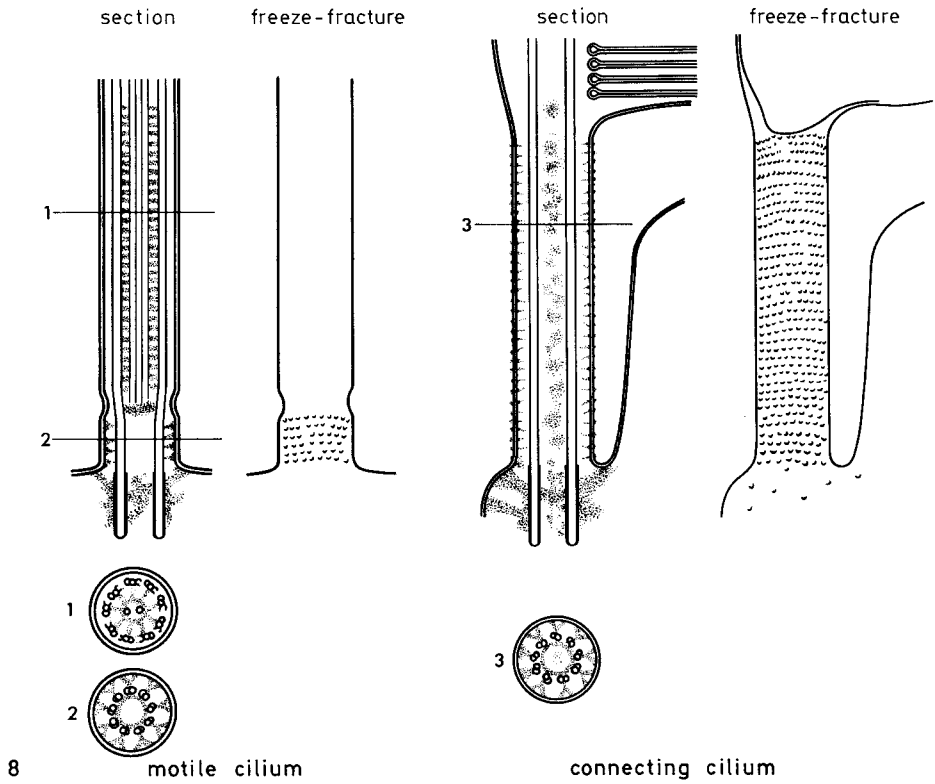

Fig. 8. Schematic drawing summarizing analogies between transitional zone of motile cilium and the connecting cilium of retinal rods. For each cilium, fine structure as seen on both thin sections and freeze-fractured specimens is illustrated

ference can be detected between the basic structure of the sensory cilium and that of the transitional zone of motile cilia. Therefore, purely on morphological evidence, we regard them as analogous structures (Fig. 8). In other words, the sensory cilium is nothing else, but a transitional zone which extends over the whole length of the cilium.

Besides the striking similarities, there are some minor differences between the two structures. One of them, a speciality of the rod cell plasma membrane, is the presence of large numbers of intramembranous particles on fracture face A. Most probably it is due to these particles that necklace strands or depressions corresponding to particles on fracture face B are not evident on fracture face A. Another difference is that in the connecting cilium intramembranous particles belonging to the necklace strands are found in relatively high number on fracture face B, when compared with the transitional zone of motile cilia where they are more numerous on fracture face A (Gilula and Satir, 1972).

The longitudinal section of the connecting cilium shows periodic beads along the plasma membrane. The fact that the beads can be found only at the ciliary plasma membrane and with a similar periodicity to that observed for the necklace

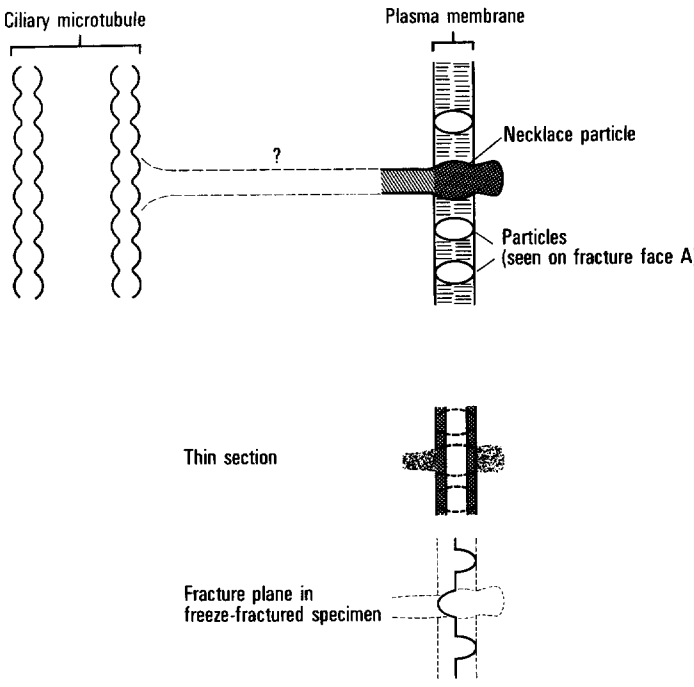

Fig. 9. Hypothetical relation of a necklace particle to the dense bead in the membrane. The two parts of the bead are joined to the necklace particle on both sides. The necklace particle may be held in position in the fluid plasma membrane by a connecting structure supported by the ciliary microtubule

strands on freeze-fractured specimens, suggests a possible relation between the beads and the necklace particles. High resolution reveals that the beads consist of two parts attached to the inner and outer surfaces of the plasma membrane respectively. If one considers the high membrane fluidity in photoreceptor cells (Daemen, 1973) that allows free lateral diffusion of membrane components, the structures on both sides of the membrane can be kept in position only if they are connected with each other by a trans-membrane structure. Such a structure would essentially correspond to the intramembranous particle that is supposed to represent a structure spanning the membrane. We believe therefore that beads on the ciliary plasma membrane are complementary parts of necklace particles (Fig. 9). Observation on ultrathin sections reveals that the outer half of the beads stands out from the external surface of the plasma membrane. If our hypothesis is correct and necklace particles and beads are complementary structures, the necklace particles must have a portion standing out from the membrane surface. This would explain the observation by Flower (1971) that elevations on the etched outer surface of the membrane can be found corresponding to underlying necklace particles.

Considering the high membrane fluidity of photoreceptor cells, a random distribution of intramembranous particles on the plasma membrane fracture faces could be expected. Although membrane particles are in fact randomly

distributed on most parts of the plasma membrane, particles on fracture face B of the connecting cilium form strands oriented perpendicularly to the direction of the cilium. An ordered arrangement can be interpreted by assuming that either the particles exhibit lateral binding sites which enable them to be linearly aggregated, or the orientation of the particles is maintained by an extramembranous structural pattern. The first assumption cannot explain the transverse orientation of the strands. In addition, the particles in the strands are not closely adherent to each other but are rather loosely arranged in each strand. The second hypothesis is supported by the presence of radial connecting structures which extend from the doublets to the inner surface of the plasma membrane. The connection between the Y-shaped radial structures and the necklace particles is not yet clear. However, our observation that inner part of the membrane beads can be followed into the cytoplasm for a certain distance seems to support the idea of such a connection.

A thourough investigation of other sensory cilia is necessary to see whether the ciliary structure described above is characteristc only of the retinal rod cells or is a general feature of all sensory cilia. The connecting cilium is special, however, in that sense that it performs a highly important function by transferring substances (mainly rhodopsin) from the inner segment into the outer segment (Young and Droz, 1968; Young, 1968). Since rhodopsin is a typical intrinsic membrane protein (Heller, 1969) which seems to be represented as intramembrane particles on fracture face A of outer segment membranes (Jan and Revel, 1974; Hong and Hubbell, 1972; Mason, Fager and Abrahamson, 1974; Röhlich, 1974 unpublished) it seems probable that particles on fracture face A of the connecting cilium are identical with rhodopsin molecules in motion towards the outer segment.

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