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The kinocilium of auditory hair cells and evidence for its morphogenetic role during the regeneration of stereocilia and cuticular plates

H. M. SOBKOWICZ,* S. M. SLAPNICK and B. K. AUGUST

Department of Neurology, University of Wisconsin, Madison, WI, USA

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Summary

Auditory hair cells that survive mechanical injury in culture begin their recovery by reforming the kinocilium. This study is based on cultures of the organ of Corti of newborn mice and two control animals. The axonemal patterns were examined in 165 kinocilia in cross-section. In the immature and regenerating kinocilium, one of the normally peripheral doublets is frequently located inward, forming the modified 8+1 (double) form; the distribution of the remainingmicrotubules is irregular. As the cell matures, the 9+0 form predominates. Overall, 34–61% of auditory kinocilia consist of 9+0 microtubules. The 9+2 (single) form, previously thought to characterize the organelle, occurs only in about 3–14%, whereas the remaining population comprises the modified 8+1 (double) form. Normally, the kinocilium lasts only about 10 postnatal days; however, post-traumatic hair cells reform their kinocilia regardless of age. Concomitant with the regrowth of the kinocilium, the basal body and its cilium take a central location in the cuticular plate, stereocilia regrow, and the cytoplasmic area adjacent to the basal body displays pericentriolar fibrous densities, growth vesicles, and microtubules, all surrounded by actin filaments. Pericentriolar bodies nucleate microtubules. Involvement of microtubules is seen in the alignment of actin filaments and in the formation of the filamentous matrix of the cuticular plate. We propose that reformation of the kinocilium in recovering post-traumatic hair cells indicates the possible role of its basal body in the morphogenesis and differentiation of cuticular plates and stereocilia.

Introduction

The purpose of this paper is twofold. We shall describe for the first time the evolution of the microtubular structure of auditory kinocilia as well as speculate on the developmental role of this organelle in the formation of hair cell cuticular plates.

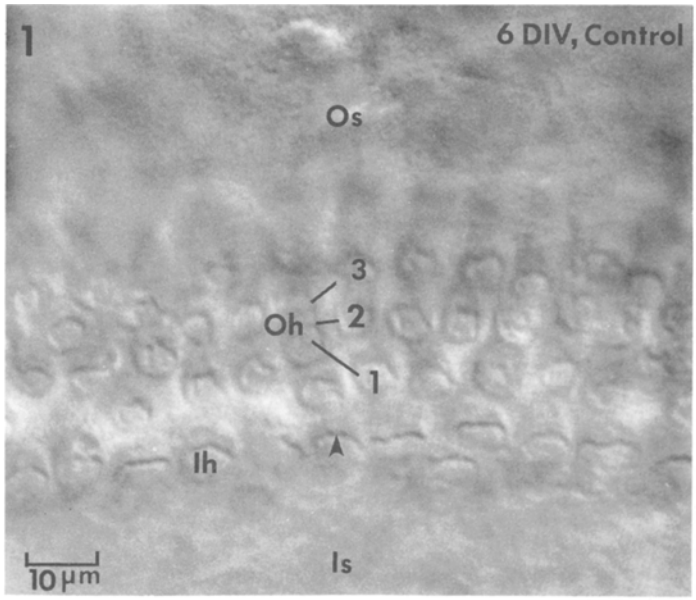
The auditory kinocilium is a primary nonmotile cilium, regardless of the prefix 'kino' which denotes movement (for review, see Wiederhold 1976). The kinocilium of mammalian auditory hair cells lasts only a short time, in the mouse no longer than ten days after birth (Kikuchi & Hilding, 1965; Kimura, 1966). Thus, it has been considered a rudimentary organelle and has received very little attention in the literature.

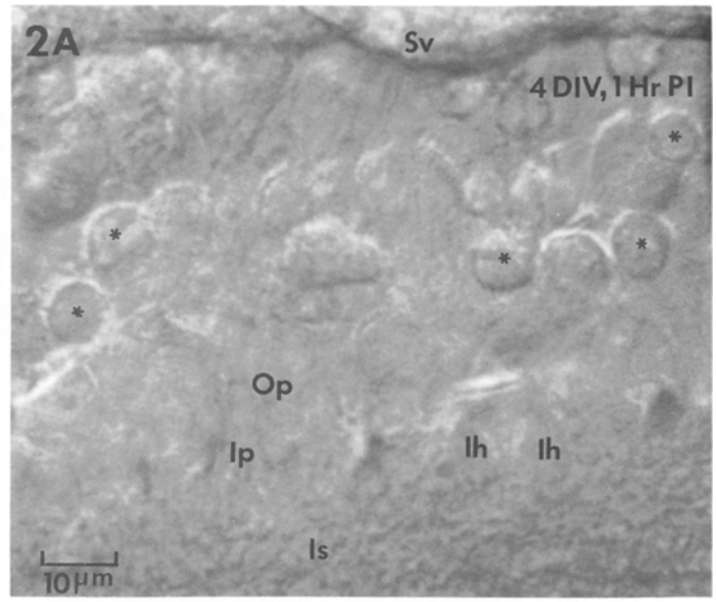
Abbreviations: Ac = actin filament; Cd = cellular debris; Cu = cuticular plate; Dc = Deiters' cell; DIV = days *in vitro;* DPI = days postinjury; HPI and HrPI = hours postinjury; Ih = inner hair cell; Ip = inner pillar cell; Is = inner spiral sulcus cell; m = microtubule; MN = mouse normal; Oh = outer hair cell; Op = outer pillar cell; Os = outer spiral sulcus cell; PN = postnatal (days); Sv = stria vascularis; v = vesicles.

* To whom correspondence should be addressed.

Using tritiated thymidine as the mitotic marker, we reported previously that auditory hair cells survive mechanical injury in culture as nonmitotic cells and show signs of repair of their cuticular plates and regrowth of the stereocilia (Sobkowicz *et al.*, 1992). These mechanically injured hair cells reform kinocilia that conform neither to the 9+2 nor the 9+0 microtubular configurations. Instead, they display a variety of microtubular arrangements characterized by the displacement of one of the microtubular pairs into the centre of the axonemal ring. Cilia, either motile (9+2) or nonmotile (9+0), are structurally the most invariable cellular organelles within the animal and plant kingdoms (Fawcett, 1961). Thus, finding any deviation in their microtubular content or configuration is a considerable rarity.

Questions arose upon our initial discovery: Are the modified axonemal configurations in the newly formed kinocilia unique to postinjury hair cells or are they developmental forms? What is the possible


1
6 DIV, Control
Os
3
Oh
2
1
Ih
10 µm
Is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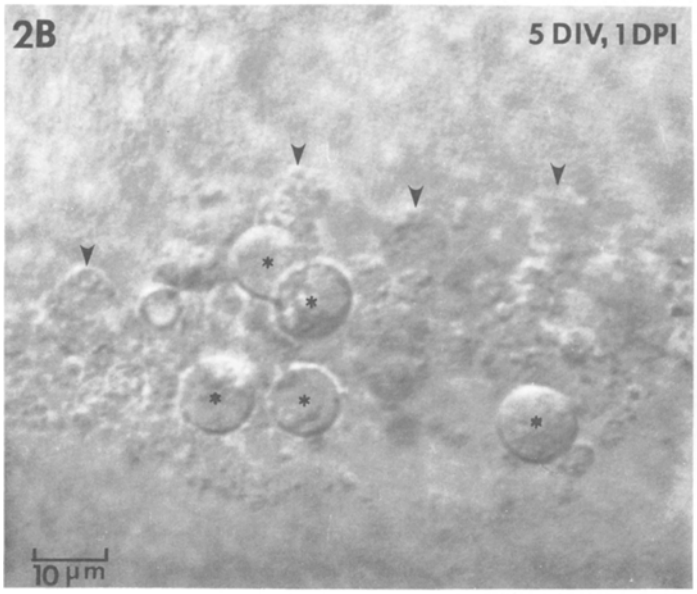
Sv
4 DIV, 1 Hr PI
Op
Ip
Ih
Ih
Is
10 µm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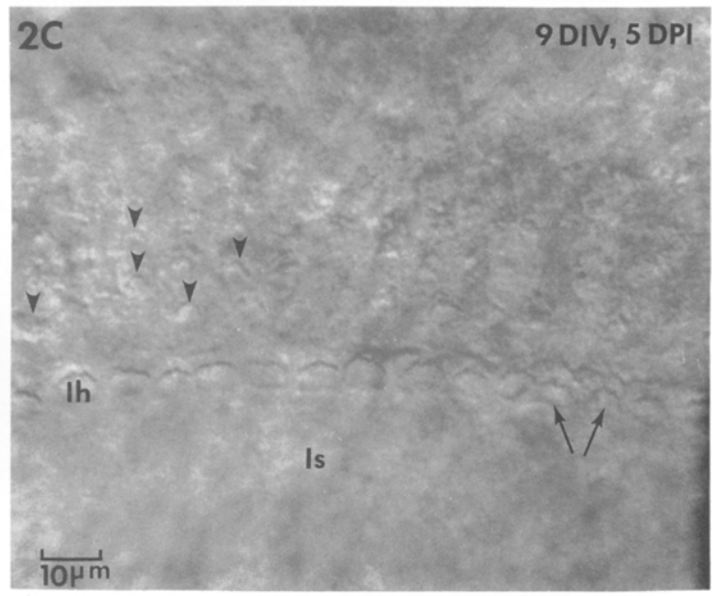
10 µm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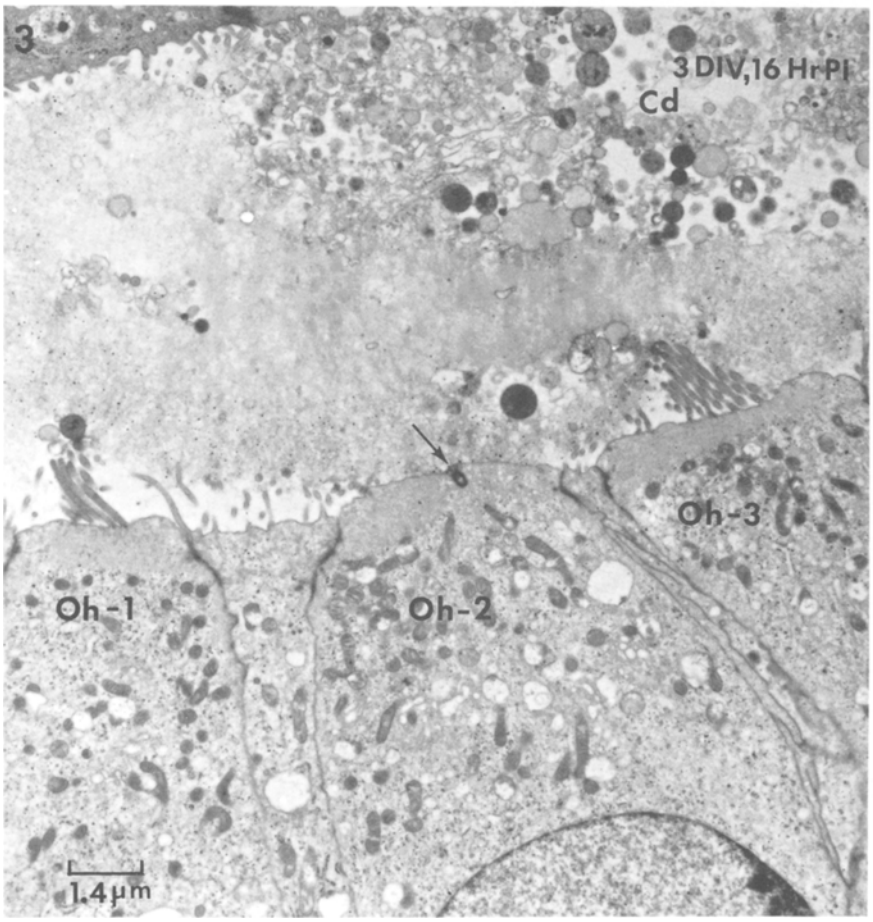
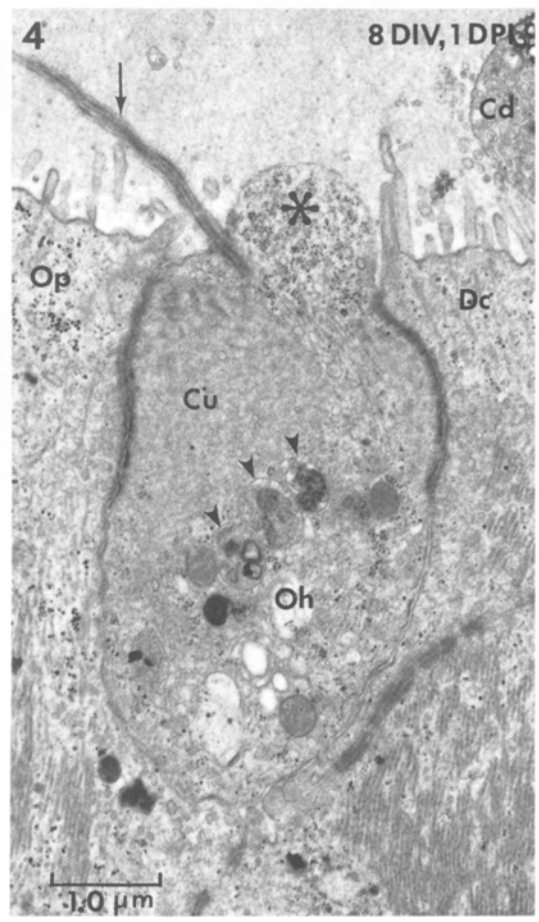
Ih
Is
10 µm


3
3 DIV, 16 Hr PI
Cd
Oh-3
Oh-1
Oh-2
1.4 µm


4
8 DIV, 1 DPI
Cd
Op
Dc
Cu
Oh
1.0 µm

significance of the reformation of this organelle? Does the kinocilium play any role in the process of self-repair in the post-traumatic hair cell?

We will show that the regenerating kinocilium repeats the developmental patterns: the axonemes of both the primary and the regenerating kinocilia display an irregular distribution of microtubular units which mature toward the 9 + 0 and 9 + 2 forms. We shall also demonstrate that the immediate area around the kinociliary basal body displays a concentration of organelles essential for the regrowth of the cuticular plate and stereocilia. Consequently, we suggest that the kinocilium, *i.e.*, its basal body, may play a morphogenetic role not only in the reformation but possibly also in the primary differentiation of the cuticular plate.

Materials and methods

The present work is based on 12 cultures, 1–13 days *in vitro* (DIV), explanted from 11 newborn ICR mice (Harlan Sprague-Dawley). Six of the cultures were injured (as described below). We examined electron microscopically 165 kinocilia of auditory hair cells in single cross-sections; among them, 75 were from injured recovering cells. The controls consisted of kinocilia from 90 uninjured developing hair cells, at 1 and 10 postnatal days (PN), and from cultures at 1, 6 and 10 DIV (see Table 1). All preparations were sectioned serially through the cuticular plates, stereocilia and kinocilia. Cross-sections of basal bodies and centrioles were obtained from the same specimens. All animals were cryoanaesthetized.

Tissue culture

The technique for the preparation and maintenance of cultures of the newborn mouse organ of Corti together with the corresponding segment of spiral ganglion was described by Sobkowicz and colleagues (1975, 1993a). The cultures were explanted from newborn mice, *i.e.*, within 24 h after birth, and were injured between 2 and 12 DIV. The hair cell region was injured with the tip (~5 μm) of a hand-held, pulled-glass pipette under a Zeiss dissecting Stereobody III microscope. Specifically, we scratched and pierced the reticular lamina of the organ, with the intent to ablate the hair cells (Sobkowicz & Slapnick, 1992; Sobkowicz *et al.*, 1992). Despite the severity of injury, most of the hair cells escaped with only damaged cuticular plates and shaved stereocilia. The lateral portions of each explant remained intact and served as controls. The cultures were examined, drawn and photographed immediately after injury and every 24 h thereafter; they were fixed for electron microscopy at 8, 16 and 24 h, and 4 and 5 days, postinjury.

Tritiated-thymidine labelling

To check for mitotic patterns induced by the injury, 1 μm sections from entire cultures or injured areas were mounted on glass slides. Sectioning was done as in the electron microscopy procedure. The mounted sections were dried at 60° C for 12 h, coated with Kodak NTB-2 (1 part emulsion and 3 parts water), and left in the dark for one week at 4° C. They were developed for 2 min in Kodak PK-1 developer and fixed for 2 min in fresh 23% sodium thiosulfate. Finally, the tissue sections were stained with 1% Methylene Blue/Azure II and examined under the light microscope.

Electron microscopy

For electron microscopy, the cultures were fixed in 2.5% glutaraldehyde in 0.1 M Sorenson's phosphate buffer (pH 7.4) for 30 min. They were postfixed in 1% OsO_4 in the same buffer for 30 min, dehydrated in ethanol, and embedded for sectioning in Durcupan epoxy resin. For detailed information in handling the cultures during fixation, see Guillery and colleagues (1970). The control intact organs of Corti were dissected and fixed in a mixture of 1% paraformaldehyde/2.5% glutaraldehyde in 0.1 M Sorenson's phosphate buffer (pH 7.4) for 30–60 min, followed by immersion in 2.5% glutaraldehyde for 30 min. They were then postfixed in 1% OsO_4 for 1 h, dehydrated, and embedded in Durcupan.

The organ of Corti was sectioned in two planes in relation to the hair cell axis; thus, the hair cells were sectioned either longitudinally or transversely. For most of this study, transverse sections were used. It should be noted

Fig. 1. The intact hair cell region in a control culture; Ihs form a continuous row, while Ohs are aligned in three rows. The outlines of hairs (arrowhead) identify each sensory cell. Apex, 6 DIV.

Fig. 2A–C. Basal turn of a live culture at 4–9 DIV, photographed 1 h (A), 1 day (B) and 5 days postinjury (C). (A) Seemingly near total destruction of the hair cell region 1 h after surgery. The line of pillar cells is discontinuous, and only two Ihs can be tentatively identified. Many Ohs are expelled from the organ (asterisks). The injury is so widespread that the status of the remaining cells in the region cannot be assessed. 4 DIV. (B) Hair cell region 1 DPI. The injured hair cell area is covered by granular tissue debris and expelled cells that, judging by their large size, may be either pillar or Deiters' cells (asterisks). Smaller crumbled cells are remnants of Ohs (arrowheads). Ihs are not identifiable in this field. (C) The hair cell region 5 DPI. The Ihs form a continuous row. Most hair-lines are still irregularly aligned, and in places there is more than one cell (arrows). The alignment of the Ohs is not as clearly defined; arrowheads point to their hair-lines.

Fig. 3. The apices of three Ohs 16 HPI. The stereociliary bundle of the cell in the middle is scraped off, the cuticular plate material is pushed aside by the basal body (arrow) that is anchored in the centre to the cell apical membrane. All three hair cells are vacuolated and their tops are covered by cellular debris. Apex, 3 DIV.

Fig. 4. A recovering Oh, 1 DPI. The cuticular plate material is misplaced sideways and downward, while a kinocilium (arrow), together with a bulging cytoplasm (asterisk), occupy a central position. The cell itself is vacuolated and shows many lysosomes (arrowheads). Apex/Mid, 8 DIV.

Table 1. Distribution and types of kinocilia in control and experimental material ($n = 165$).

	9 + 2	9 + 0	*Modified (8+/7+/9 + 3/10+)*	*(Modified & Irregular)*	*Disintegrating*	*Centrioles*
Developmental (1 PN ($n = 15$) + 1 DIV ($n = 17$) = 32 total)	3%	34%	63%	(75%)	0%	0%
Control to the age of injury (6 DIV (29 total))	14%	41%	45%	(15%)	0%	0%
Mature (10 DIV ($n = 20$) + 10 PN ($n = 9$) = 29 total) (67 hair cells did not contain kinocilia)	7%	38%	20%	(33%)	14%	21%
Postinjury cultures (5 DPI, 10 DIV+, 7 DPI, 11 DIV (75 total))	3%	61%	32%	(96%)	0%	4%

Table 1 illustrates the developmental trends in the axonemal forms of the auditory kinocilium as compared with their newly formed counterparts in post-traumatic cells. Note that the group 'Modified & Irregular' refers to the fraction of kinocilia that, in addition to their modified forms, display an irregular distribution of microtubules. Data show that both the 9 + 2 and 9 + 0 forms coexist (in different kinocilia) throughout the life span of the organelle. During development, modified forms decrease from 63% to 20%, and the distribution of microtubules becomes more regular. The presence of centrioles is noted at around 10 days, together with disintegrating kinocilia. Axonemal forms of regenerating kinocilia are comparable to those seen at the developmental age at which injury was inflicted, (except that almost all newly formed, modified kinocilia display an irregular alignment of microtubules). n = number of samples.

that the hair cells in post-traumatic cultures may be misaligned, and thus somewhat oblique sections were often obtained. Also, for this reason no distinction was made between the outer hair cells in different rows. The different angle at which stereocilia of inner and outer hair cells were aligned resulted in the predominance of outer hair cells in this study.

Three micrometre sections were alternated with 1 μm sections through the entire explant. The 1 μm sections were stained with Methylene Blue/Azure II (Richardson's stain); they were used to locate cuticular plates under the light microscope and for autoradiography. The adjacent 3 μm sections (one above and one below) were remounted and thin sectioned serially. This method permits one to keep a 1 μm section as a permanent record. The tissue was photographed using a Hitachi H-600 electron microscope.

Results

INJURY TO HAIR CELLS *IN VITRO*

Initially, the injured hair cell region appears as a distinct strip of disrupted tissue. The repair of the reticular lamina starts almost immediately after insult, and during the next few days the surviving hair cells surface anew. In low-power electron micrographs, the post-traumatic hair cells were identified by their denuded cuticular plates or immature patterns of regrowing stereocilia. In the analysis of cross-sectioned kinocilia, the auditory cilia were differentiated from those of the supporting cells by the presence of stereocilia.

The injuries in live cultures were followed daily, as in Figs 1 and 2. The reticular lamina in a control 6 DIV culture displays the hair outlines in an intact hair cell region (Fig. 1). Figure 2A–C shows approximately corresponding fields in an experimental culture. One hour after injury, a total disruption of the reticular lamina dislocates the cells, which cannot be identified as either hair or supporting cells (Fig. 2A). Twenty-four hours later, the hair cell region is replaced by a strip of debris, with some swollen, expelled cells (Fig. 2B). At 5 days postinjury (DPI), the picture looks strikingly different. The reticular lamina is repaired, the inner hair cells form a continuous row, and hair bundles of the outer hair cells are just reaching the surface of the organ (Fig. 2C).

The variability of the injury is demonstrated in Figs 3 and 4. In a 3 DIV, 16 hours postinjury (HPI) culture, the outer hair cell in the middle is clearly traumatized, while the two remaining hair cells still retain some of their stereocilia (Fig. 3). In an 8 DIV, 1 DPI, heavily traumatized outer hair cell, the cuticular plate material is pushed downward, and the pronounced kinocilium is the sole sign of an impending recovery (Fig. 4).

Degenerative changes in the organ of Corti are usually induced experimentally by damage from sound or from ototoxic drugs; thus, regenerative phenomena perforce involve a sick cell. Unique to our experimental approach is the production of a local injury to an otherwise healthy hair cell. Since the injury always varies, even hair cells in close proximity offer an array of different stages of apical repair.

Beginning immediately after physical trauma, all cultures were continuously exposed to 1.0 μCi ml^{-1} of tritiated thymidine in the feeding solution to check for its incorporation into the nuclei of dividing cells. Control cultures were exposed to tritiated thymidine continuously from the time of explantation to the day of fixation. *None of the hair cells was labelled.*

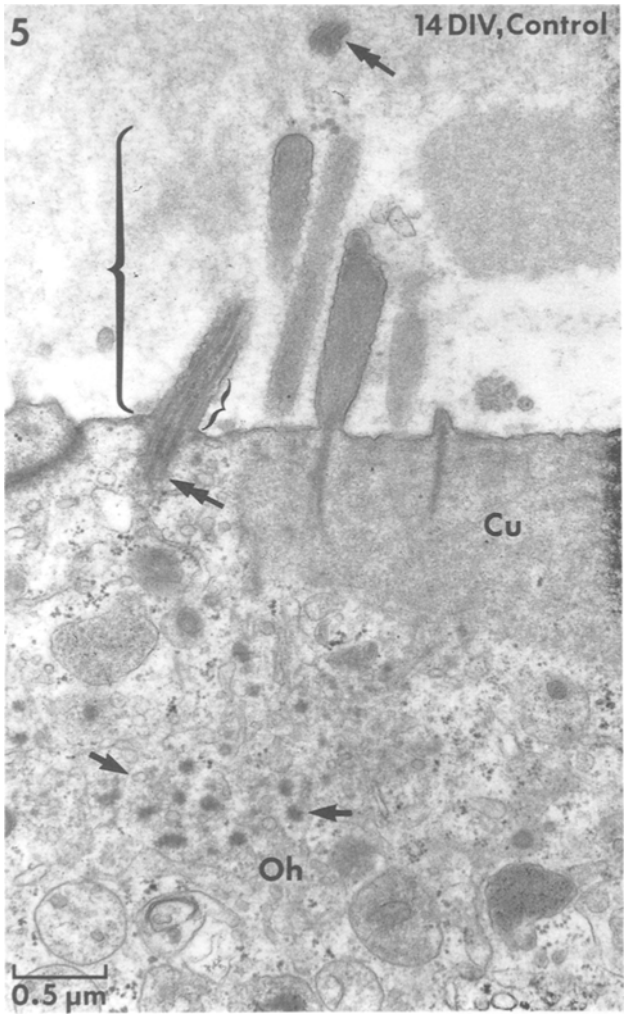


Fig. 5. A longitudinal section through an Oh kinocilium (double arrows) in a control culture at 14 DIV. The kinocilium is inserted laterally. Some distance below it lies a little field of dense pericentriolar bodies (single arrows). The large bracket approximates the level at which the cross-sections of kinocilia were made. The small bracket defines the level 0.3 µm above the cell surface that corresponds to the 9 + 0 axonemes, whereas the levels above correspond to 9 + 2 axonemes (according to Flock (1965)). Apex.

AXONEMAL FORMS OF THE AUDITORY KINOCILIUM

During development, the kinocilium is usually twice as long as the longest stereocilium (Cotanche & Sulik, 1984). Thus, the presence of stereociliary profiles near a cross-sectioned kinocilium helps to approximate the levels at which their shafts were sectioned, *i.e.*, usually proximal to the cuticular plate (Fig. 5).

The results are based on single cross-sections from 165 kinocilia. The classical axonemal forms of kinocilia (9 + 0 and 9 + 2) are shown in Figs 6–8, the centrioles and basal bodies in Figs 9–11, and the modified forms in Figs 12–16.

The regular forms of the auditory kinocilium

We found that, in the mouse, about 30–40% of primary auditory kinocilia consist of nine peripheral double microtubules, the 9 + 0 form (Table 1, Figs 6, 7), whereas the classical 9 + 2 form (Fig. 8) – previously thought to characterize the kinocilium – is rare (about 3–14%). The two forms coexist in different kinocilia within a given population, both in primary and in regenerated kinocilia. All 9 + 2 kinocilia and 94% of the 9 + 0 forms show evenly spaced microtubules.

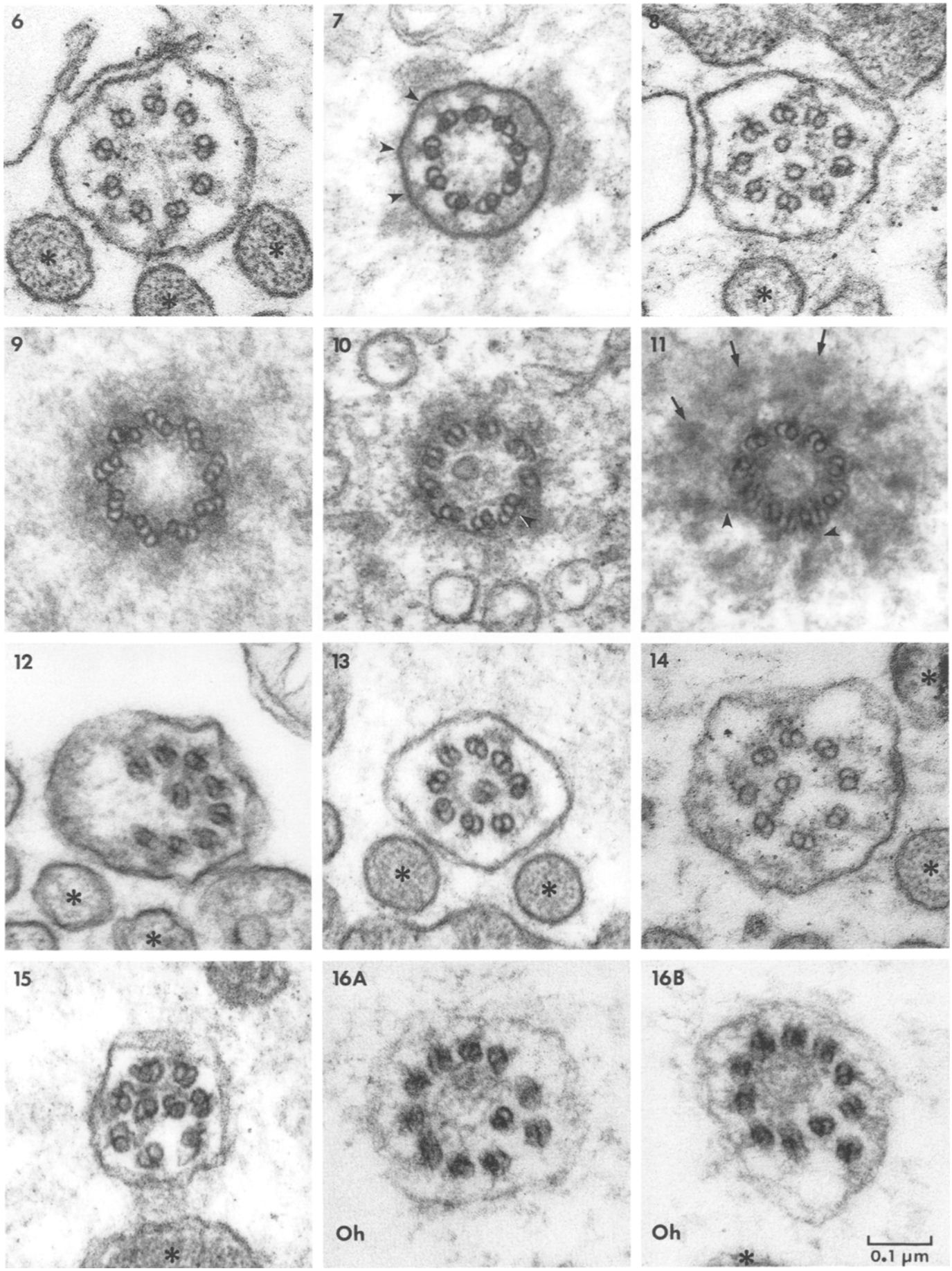
In our material, the presence of central microtubules varied independently of location along the length of the kinocilium. On occasion, the central microtubules could even be found as low as within the basal body itself. Figures 19–24 present examples of different levels of kinocilia that are devoid of central microtubules: from very close to the apical cell membrane (Figs 19, 20) to the more distal shafts (Figs 21–24).

Basal bodies and centrioles

Centrioles differ from basal bodies by their position in the cell. Centrioles are free and may be located at the side of or beneath the fibrous part of the cuticular plate. Their axonemes are characterized by arrangements of triple or a mixture of double and triple microtubules (Figs 9, 10). Basal bodies are attached to the apical plasmalemma and give rise to the cilium (Fig. 5). In fortuitous sections, they show the attachment densities, called alar sheets, formed by trapezoidal structures that arise along their upper half and run obliquely toward the cell membrane (Fig. 11). These densities surround the ring of microtubular triplets, creating the picture of a pinwheel.

The modified forms of the auditory kinocilium

Depending on the *developmental stage*, between 20–60% of auditory kinocilia deviate from the regular 9 + 0 and 9 + 2 forms (Table 1). In the immature and regenerating cuticular plates, an axoneme frequently consists of eight peripheral and one central doublets, the 8 + 1 (double) form (Figs 12–14). It appears that one of the nine peripheral doublets may change its position, leaving a gap (Fig. 12) or causing an irregular distribution among the remaining doublets (Fig. 13). In some cases, when two microtubule doublets are in the centre, the ring may be reduced to seven peripheral pairs, resulting in the 7 + 2 (double) form (Fig. 15). *Thus, the prevalent form of the auditory kinocilium consists of nine double microtubules of varied peripheral and central distribution (9 + 0, 8 + 1 and 7 + 2).* Forms featuring more than ten microtubular pairs are rare, the most striking in our material being a 10 + 1 kinocilium in which the microtubules alter their alignment along the shaft (compare Fig. 16A and B).


6
7
8
9
10
11
12
13
14
15
16A
Oh
16B
Oh
0.1 µm

These illustrations emphasize further the positional instability of the microtubules along the axis of the cilium.

Comparison between primary and regenerated kinocilia

The primary kinocilium exhibits a *developmental trend* toward the 9+0 and 9+2 forms and a regular configuration of microtubular units. The initial number of modified forms in the newborn cochlea – 63% – falls at maturity to 20%, and the percentage of kinocilia with an irregular distribution of microtubules decreases from 75% to 33%. In the 10 PN cochlea, the regular forms of kinocilia and the free centrioles constitute 66%.

The patterns of kinocilia seen in the hair cells of cultures at 6 DIV correspond to an intermediate developmental stage (Table 1): The axonemal configurations resemble those of mature kinocilia; the number of modified forms falls somewhere between the values in the early postnatal organs and in those approaching maturity; neither disintegrating kinocilia nor centrioles are present.

In the intact 10 PN mouse, kinocilia are exceedingly difficult to find; we found only nine kinocilia (all in outer hair cells) in screening 76 sensory cells. Similarly, kinocilia occur rarely in 10 DIV or older cultures (Fig. 5), and show signs of membrane disintegration. Notable exceptions are *injured* sensory cells that regenerate their cuticular plates and stereocilia (Table 1). Regardless of age, a recovering hair cell possesses a kinocilium. *Thus, at the age when a normal sensory cell loses its cilium, an injured cell reforms a new one.*

Compared with primary kinocilia, regenerating kinocilia in postinjury cultures at 10–11 DIV (injured at 4 or 5 DIV) show a higher proportion of regular forms (68% including centrioles) as well as a distinctive group of modified forms (32%), almost all of which are irregular (96%). This combination of mature and immature traits in the axonemal forms might be explained by the fact that the regenerating kinocilia begin their growth in older, more differentiated cells.

MORPHOGENETIC EVENTS IN THE REPAIR OF CUTICULAR PLATES

Spatial relationship between the kinocilium and regrowing stereocilia

Regenerating cuticular plates in injured hair cells can be identified by the distribution of their regrowing stereocilia and the presence of a kinocilium. In young

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Figs 6–16 illustrate axonemal configurations of auditory kinocilia and associated structures in cross-section. Hair cell type is indicated where known. Stereocilia, if present, are marked by asterisks. Scale in Fig. 16B applies to the entire plate.

Fig. 6. A primary kinocilium in a 1 DIV control culture showing the most frequently seen axonemal form: a ring consisting of nine double microtubules (9+0) uniformly distributed. Apex.

Fig. 7. A regenerating kinocilium in an 11 DIV culture at 7 DPI showing a variant of the 9+0 form in which electron-lucent microtubules pair with electron-dense ones. Note the triangular densities (arrowheads) by which the microtubule doublets appear to anchor to the membrane. Base.

Fig. 8. A primary kinocilium in a control culture at 1 DIV showing the classical but infrequent form, 9+2 (single). The axoneme consists of a ring of nine microtubular pairs and two central single microtubules. Apex.

Fig. 9. A classical centriole formed by a ring of triple microtubular units, all shown in the same plane of section, photographed in a Deiters' cell in a 10 DIV culture at 5 DPI. Apex.

Fig. 10. A basal body consisting of a mixture of double and triple (arrowhead) microtubular units in a recovering hair cell, 11 DIV, 7 DPI. A small vesicle is in the centre of the axoneme and large membranous vesicles surround the basal body. Base.

Fig. 11. Basal body of the primary kinocilium in a control 5 DIV culture. The basal body is sectioned through its alar sheets (arrows). All nine alar sheets attached to the outermost microtubules (arrowheads) are in the same plane. The peripheral densities within each sheet indicate the attachment points to the cell membrane (see Figs 1a–c in Anderson, 1972). The axonemal ring is formed by nine triple microtubules, some of which are partially out of the plane of section. Apex.

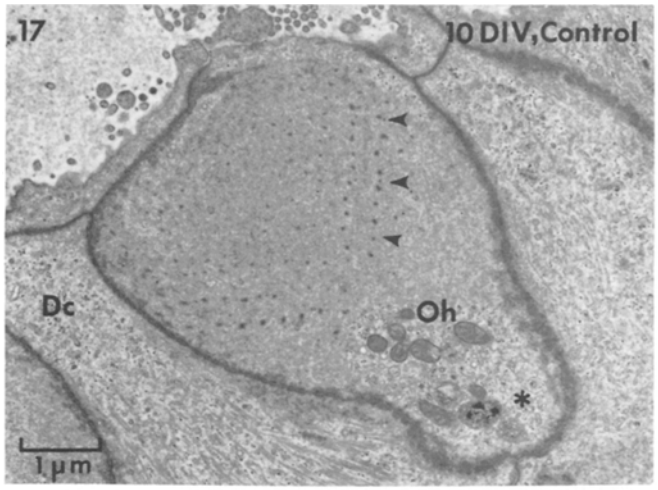
Fig. 12. A primary kinocilium in an intact, 1 PN mouse shows a modified 8+1 (double) form with an irregular distribution of microtubular pairs. The axonemal ring is interrupted by the apparent central shift of one of the nine peripheral doublets. Mid, MN (mouse normal).

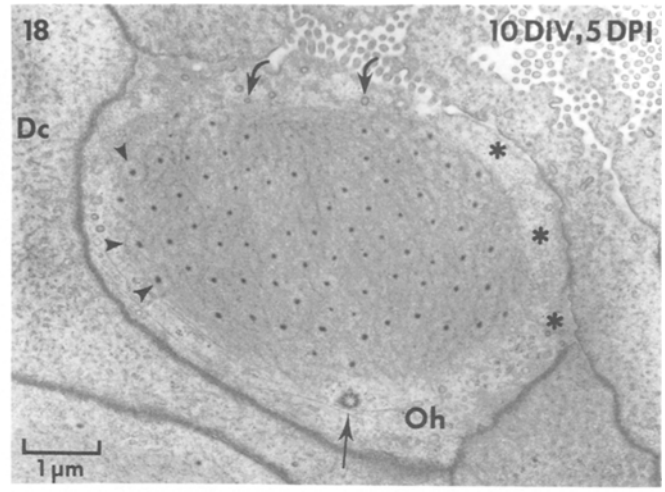
Fig. 13. A primary kinocilium in a control 1 DIV culture shows a modified 8+1 (double) axoneme with an irregular distribution of peripheral microtubular pairs and with the inner microtubular doublet slightly off centre. Apex.

Fig. 14. A primary kinocilium in a control 1 DIV culture shows a modified 8+1 (double) irregular axoneme, characterized by the off-centre placement of the inner doublet. Apex.

Fig. 15. A regenerating kinocilium shows a rare (modified, irregular) 7+2 (double) microtubular arrangement. 11 DIV, 7 DPI, base.

Fig. 16. Two cross-sections showing a modified, irregular axoneme with a 10+1 (double) inward (A) and outward (B) placement of the eleventh microtubule doublet in a regenerating kinocilium. For an overview of this entire stereociliary bundle, see Fig. 21. 10 DIV, 5 DPI, apex.


17
10 DIV, Control
Dc
Oh
1 µm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10 DIV, 5 DPI
Dc
Oh
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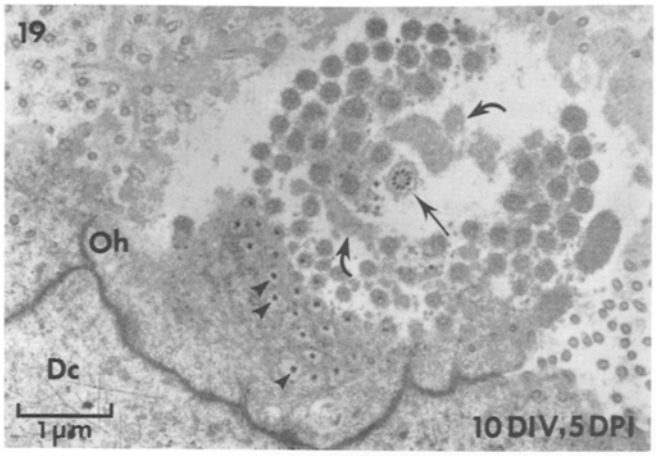
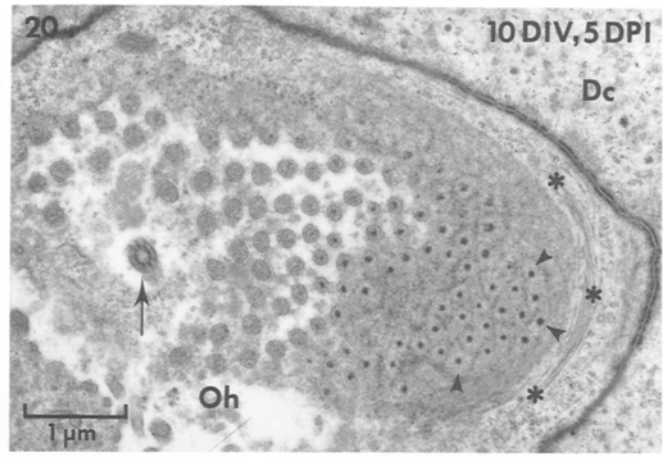

19
Oh
Dc
1 µm
10 DIV, 5 DPI


20
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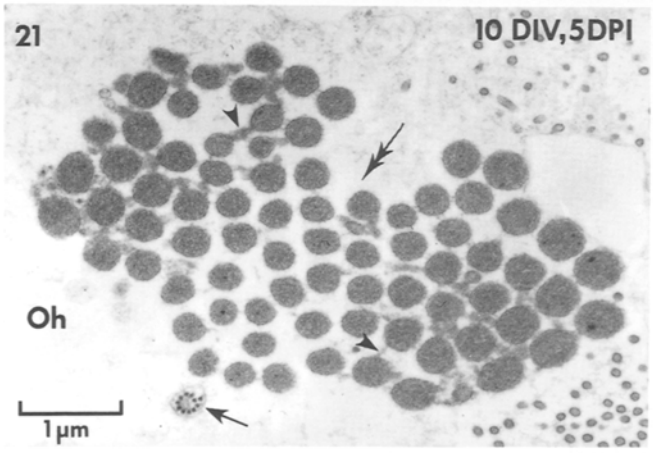
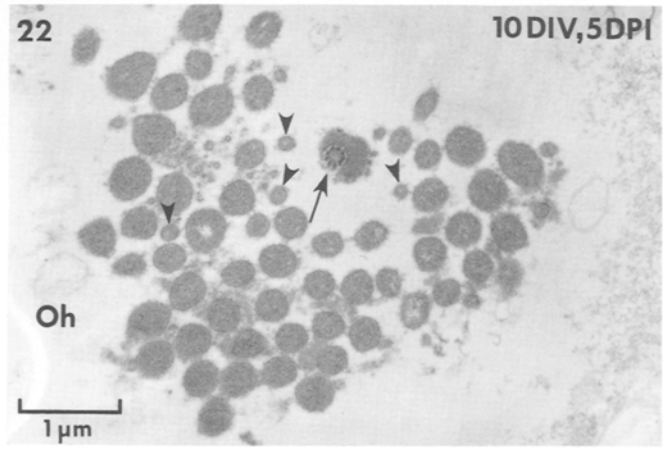
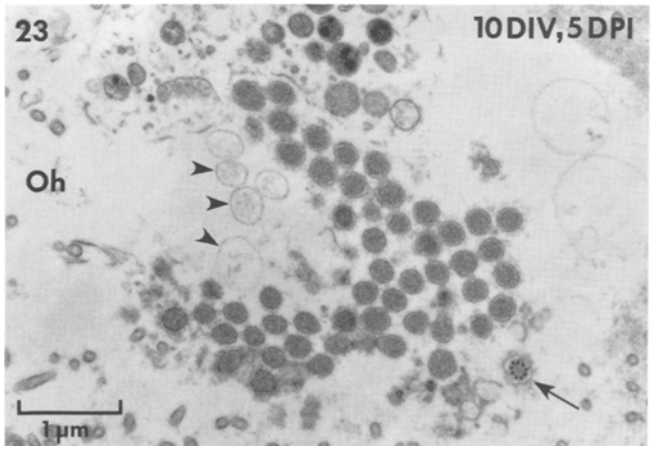
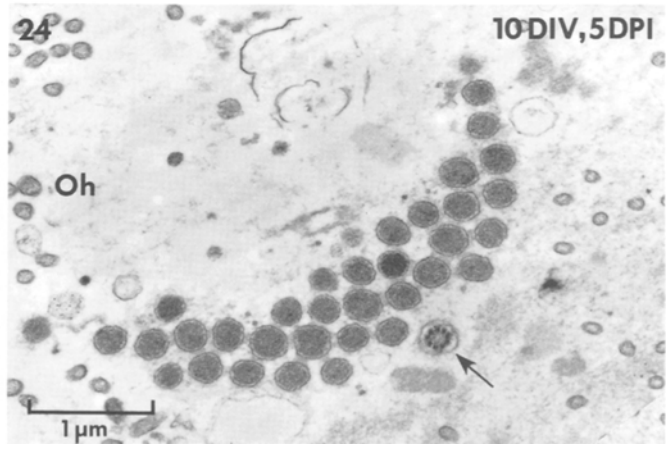
Dc
Oh
1 µm


21
10 DIV, 5 DPI
Oh
1 µm


22
10 DIV, 5 DPI
Oh
1 µm


23
10 DIV, 5 DPI
Oh
1 µm


24
10 DIV, 5 DPI
Oh
1 µm

normal hair cells, the kinocilium occupies a peripheral position, behind the tallest stereocilium. Figure 17 shows the mature stereociliary arrangement (without a kinocilium) in a control intact 10 DIV culture: in cross-section the rows of stereociliary rootlets are aligned in the classical 'V' pattern, while the cytoplasmic area that normally houses the kinocilium is empty.

In contrast, cuticular plates of outer hair cells in a 10 DIV, 5 DPI culture (Figs 18–24) all show the presence of a kinocilium. Its location among the regenerating stereocilia, however, varies from peripheral (Figs 18, 21, 23, 24) to central (Fig. 19); moreover, the kinocilium is not necessarily associated with the largest stereocilium (Fig. 21). As in Tilney and colleagues (1986), the newly-growing stereocilia repeat the initial developmental patterns, sequentially forming roundish, oval and then rectangular patches which further differentiate into the mature 'V' patterns (Figs 18–24). When the kinocilium in regenerating cuticular plates occupies a central position, it splits the filamentous part of the cuticular plate, opening a cytoplasmic channel through which organelles may gain access to the regenerating apex of the hair cell (Figs 25, 26). Among the most characteristic organelles seen here are growth vesicles (Figs 27–30), pericentriolar bodies and microtubules (Figs 31, 32, 34).

The kinocilium and growth vesicles

Our material suggests that the presence of growth vesicles around the basal bodies in auditory hair cells (Figs 26–30) is a constant feature. Figures 27, 29 and 30 show the basal body in association with growth vesicles in a control intact organ of Corti (1 PN) and in culture (7 and 10 DIV), whereas Fig. 28 shows an example of a 13 DIV, 1 DPI hair cell; its basal body too is literally surrounded by growth vesicles.

In the repairing cuticular plates, the vesicles are not confined to the immediate surroundings of the basal body, but are also found adjacent to the plasma membrane and in contact with sprouting stereocilia. Membrane vesicles do not seem to collect around the free centrioles located deep beneath the cuticular plates (Fig. 32).

Pericentriolar bodies

Pericentriolar bodies are electron-dense fibrous organelles belonging to the centriolar/basal body apparatus that provides sites for nucleation of microtubules. In post-traumatic hair cells, pericentriolar bodies figure prominently in the periciliary cytoplasmic region and in the channel that extends into the cell cytoplasm (Fig. 25); they average 60 nm in diameter. The fibrous bodies are repeatedly seen in

Figs 17–24. Cross-sections through the cuticular plates of Ohs in 10 DIV cultures. Figures 18–24 depict regenerating cuticular plates at 5 DPI in an apex.

Fig. 17. A cross-section through the cuticular plate of an Oh in a control 10 DIV culture. The cuticular plate is uniformly filamentous. Arrowheads point to the electron-dense rootlets of the stereocilia forming a characteristic 'V' pattern. The cytoplasmic area (asterisk) of the cuticular plate shows no kinocilium. Base.

Fig. 18. The stereociliary rootlets (arrowheads) form a patch, a characteristic arrangement early in the primary growth of stereocilia. All rootlets are suspended in a fibrillar net throughout the cuticular plate. The basal body, indicating the presence of a kinocilium, is located at the periphery (arrow). Asterisks delineate a strip of cytoplasmic vesicles; curved arrows mark two of the coated vesicles. (Compare with Fig. 16A in Tilney *et al.*, 1986.)

Fig. 19. An oblique cross-section through a roundish patch of stereocilia and their rootlets (arrowheads) embedded in the cuticular plate. Note the central position of the kinocilium (arrow). Curved arrows point to a patch of extracellular matter.

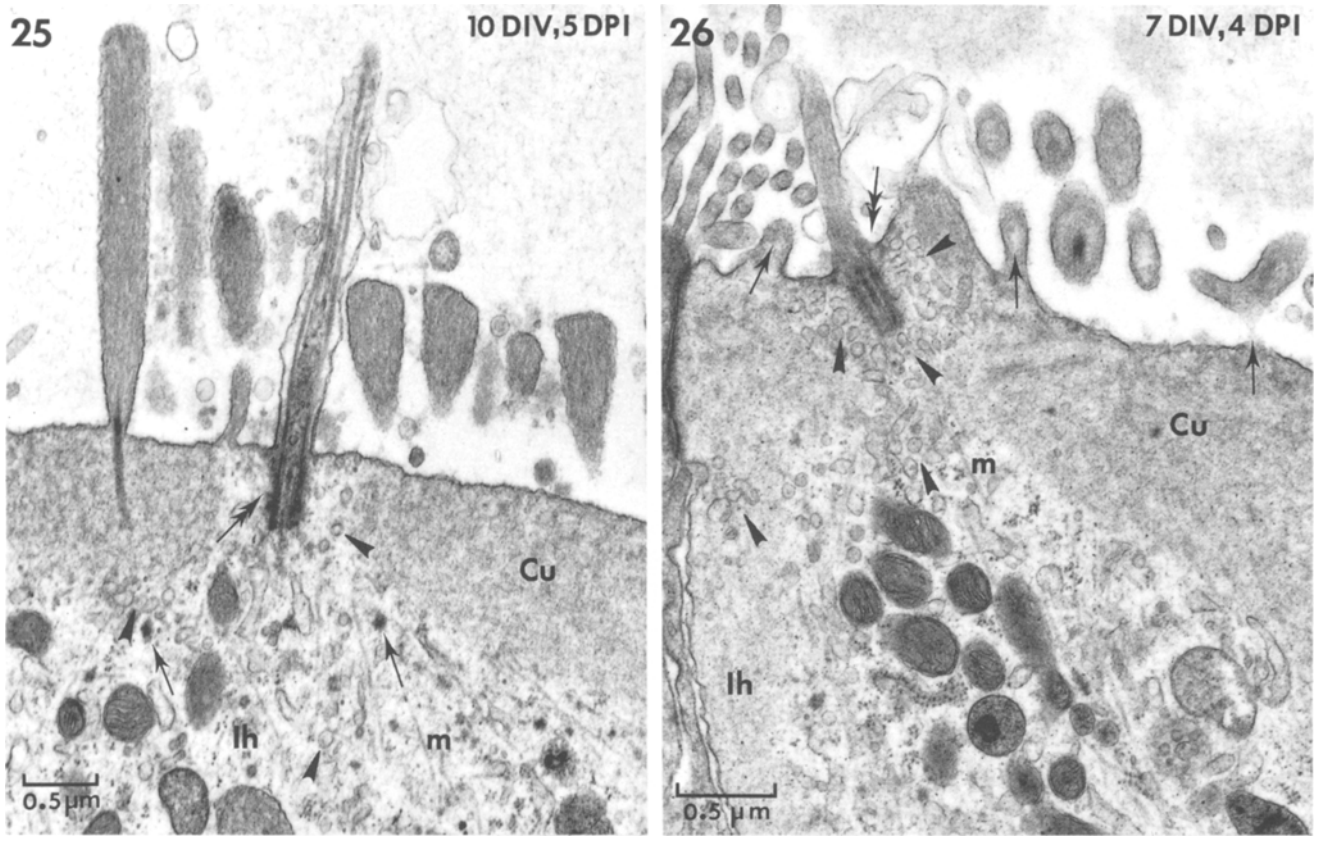
Fig. 20. Off-centre position of a kinocilium (arrow) in a regenerating cuticular plate. Note the oval distribution of the stereocilia and their rootlets (arrowheads) embedded in the distinct fibrillar net. Asterisks delineate the microtubular belt circumscribing the cuticular plate.

Fig. 21. Kinocilium (arrow; also shown in Fig. 16) peripheral to the regrowing stereocilia. Note the predominantly rectangular arrangement of the stereocilia. Most of the stereocilia are connected by distinct links (arrowheads), especially well expressed at the lateral edges of the stereociliary rectangle. There is a suggestion of an incipient regrouping of the stereocilia along the kinociliary axis (double arrow) into the future 'V'-like alignment. Note that the stereocilia closest to the kinocilium are the smallest in diameter.

Fig. 22. In contrast to Fig. 21, in this stereociliary bundle the kinocilium (arrow) is located centrally within the 'V'-forming configuration. Note the variation in diameter of the regrowing stereocilia; some of the smallest ones are marked by arrowheads. Extracellular matter, possibly exuded by the stereocilia, links the growing stereocilia together. (Compare with Fig. 17 in Tilney *et al.*, 1986.)

Fig. 23. In this stereociliary bundle, the kinocilium (arrow) is located at the tip of the forming 'V'. The regrowing stereocilia are of varying diameter and are surrounded by patches and strings of extracellular matter that appear to link them. Ghost-like membrane formations (arrowheads) within the 'V' bundle suggest a resorption of stereocilia. 10 DIV, 5 DPI.

Fig. 24. Near normal arrangement of stereocilia with the kinocilium located at the tip of the 'V' (arrow).



Figs 25–26. Longitudinal sections through the apical region of injured hair cells.

Fig. 25. Paracentral location of the kinocilium in an Ih. The basal body (double arrow) is surrounded by the cytoplasm through which growth vesicles (arrowheads) and microtubules reach the surface membrane of the cuticular plate. Arrows point to pericentriolar bodies. 10 DIV, 5 DPI, apex.

Fig. 26. Cuticular plate in an Ih 4 DPI. The kinocilium is positioned laterally (double arrow) and surrounded by a mound of growth vesicles (arrowheads) that appear to have streamed upward from the cell cytoplasm. Arrows point to budding stereocilia. 7 DIV, 4 DPI, base.

intimate contact with individual microtubules (Figs 31, 32).

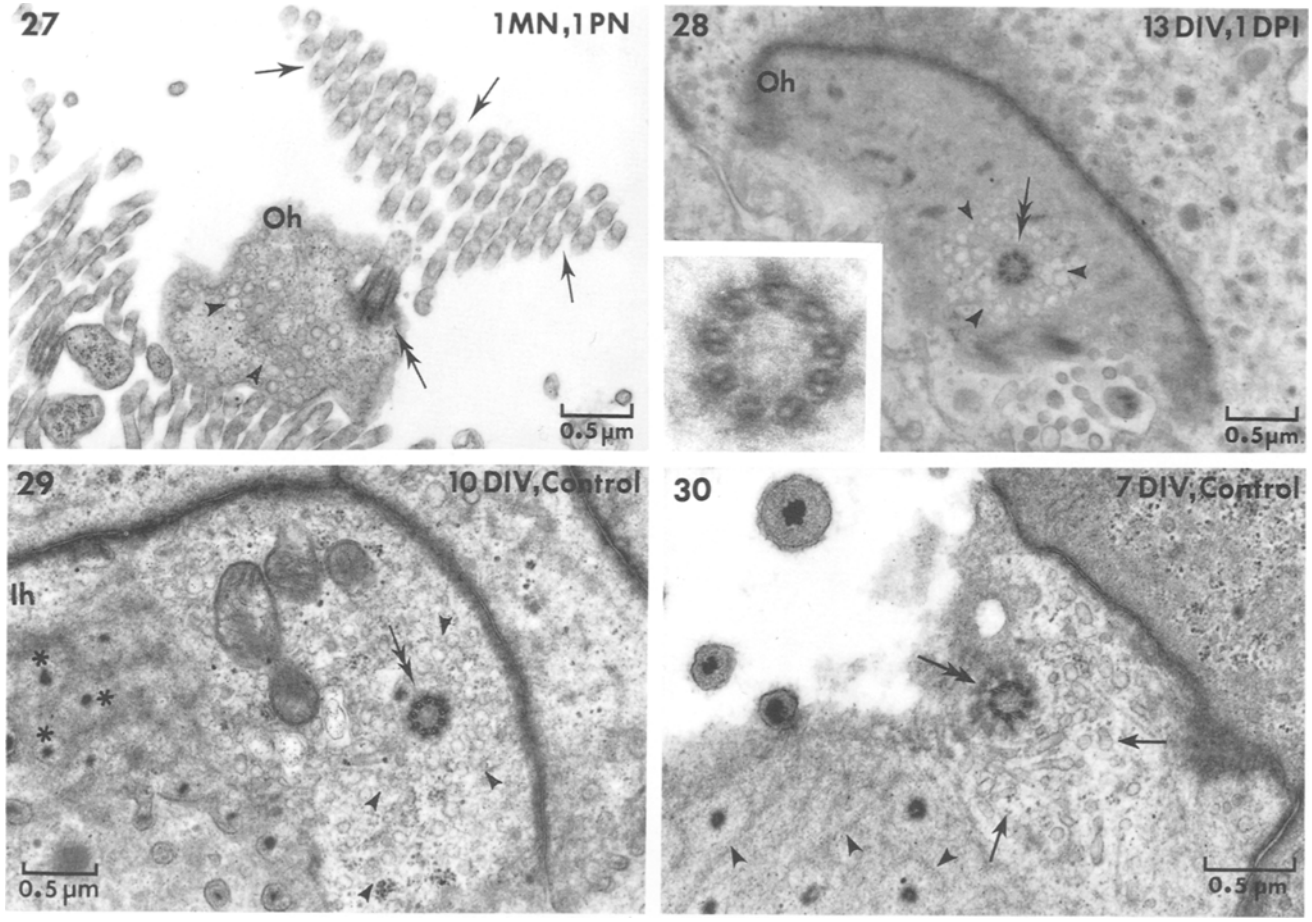
A more striking picture exemplifying the apparent nucleation of microtubules by pericentriolar bodies can be seen in the regenerating top of an inner pillar cell at 10 DIV in Fig. 33. (Pillars are the central supporting cells of the hair cell region; their cytoskeletal microtubules consist of 15 protofilaments (instead of the usual 13) and thus are of larger diameter than those in hair cells (Tucker *et al.*, 1992). In our experience, the pericentriolar bodies in pillar cells are activated by injury and are not a normal component of the quiescent cell. In the injured pillar, pericentriolar bodies appear in fields juxtaposed to the basal body and beneath the subapical part of the cell. They are large (up to 184 nm in diameter), and each appears to give rise to more than one microtubule at a time.

Microtubules

In post-traumatic hair cells repairing their cuticular plates, microtubules become very numerous. They diverge from the basal body to the region of the Golgi apparatus. The microtubules that occupy the cytoplasmic channel are chiefly associated with membrane vesicles.

Microtubules that re-align within or parallel to the plane of the cuticular plate can be divided roughly into two groups: those associated with actin filaments in the reformation of the cuticular plate material itself (Figs 34–36, 38, 39 (dots)), and those surrounding the filamentous part of the cuticular plate in three to five parallel semicircular bands (Figs 20, 38, 40).

The microtubules associated with repairing cuticular plates tend to form loose circular patterns around patches of filamentous matrix as in Fig. 34. Many of these microtubules are seen to join the filamentous matrix, where they gradually blend into the amorphous cuticular plate material (Figs 34, 36, 38, and 39 (dots)). Microtubules are closely associated with actin filaments. Visible linear alignments of microtubules extending into the territory of the cuticular matrix can be seen in Fig. 35. Tracks of microtubules display a periodicity of ~ 145 nm (Figs 34 and 36) that sometimes



Figs 27–30. The association of growth vesicles with basal bodies.

Fig. 27. An oblique cross-section through the base of a kinocilium at the tip of a triangular alignment of stereocilia (arrows). Arrowheads point to the adjacent growth vesicles. Double arrow points to one of the densities that attach the organelle to the plasma membrane. Mid, MN, 1 PN.

Fig. 28. A basal body (double arrow) – as judged by its position beneath the apical plasma membrane – surrounded by growth vesicles (arrowheads) in the cuticular plate of a post-traumatic Oh, 1 DPI. Inset shows its microtubular arrangement. 13 DIV, base.

Fig. 29. A cross-section of a basal body (double arrow) in the cuticular plate of an Ih in a control, 10 DIV culture. An alar sheet attachment density left of the double arrow identifies the level. Note the vesicular content (arrowheads) of the surrounding cytoplasm. At left, electron-dense rootlets (asterisks) indicate the stereociliary patch. Base.

Fig. 30. An oblique cross-section through the cuticular plate and basal body (double arrow) in a hair cell in a 7 DIV control culture. The basal body is identifiable by the pinwheel-like densities (alar sheets) that serve as attachments to the apical plasma membrane. Note the complement of smooth endoplasmic reticulum and vesicles surrounding the organelle (arrows). Note also the delicate fibrillar net surrounding the stereociliary rootlets (arrowheads), somewhat resembling that in Fig. 37. Base.

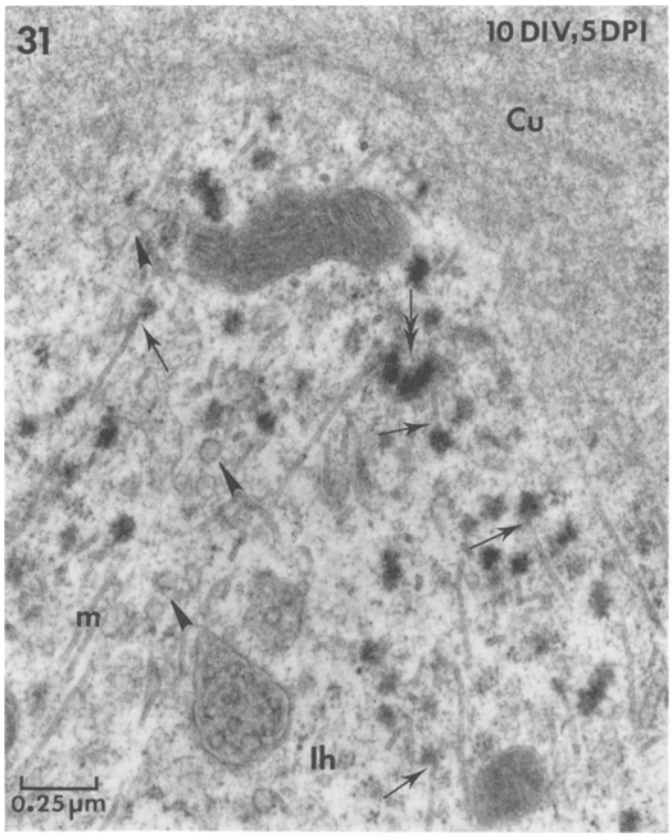
seems to be further bisected by long, fine microfilaments (inset in Fig. 36). The V-like alignment of the fibrils as seen in Fig. 34 suggests that their convergence may lead to a crisscross pattern. Based upon many micrographs, *e.g.* Figs 38 and 39, it is our impression that this periodic fibrillar alignment (initiated by the microtubules) contributes to the latticed structure (Figs 37 and 40) that compartmentalizes the stereociliary rootlets (Fig. 37). This highly organized fibrillar net is a constant feature in the repairing cuticular plates of post-traumatic outer hair cells.

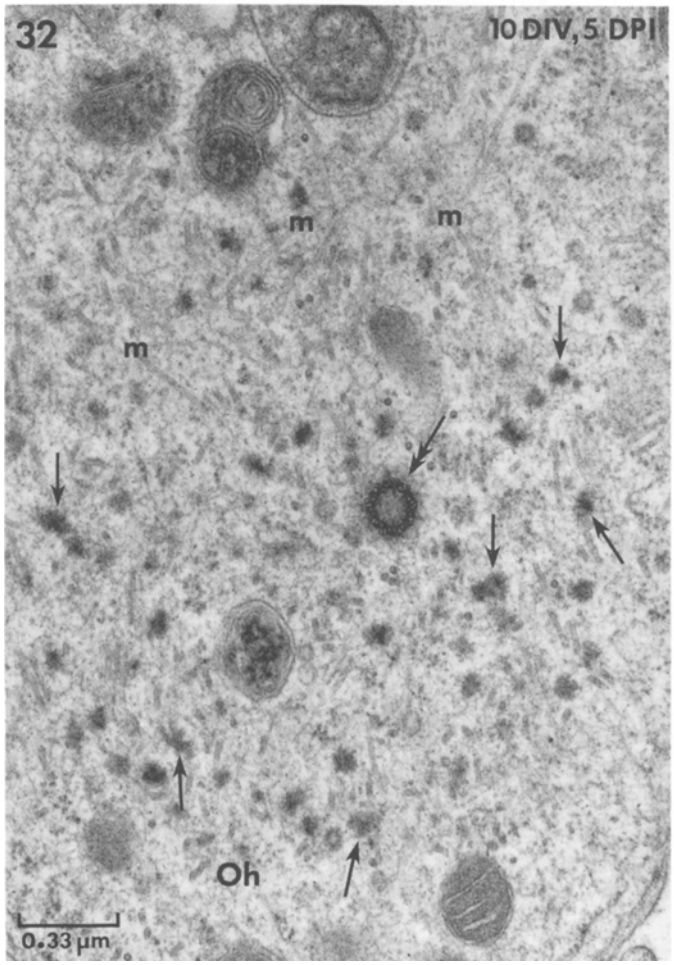
Several sections suggest that the inner part of the net may diverge from a common point in the cell. Figures 38 and 39 show a very regular striation formed by diverging fibrils, mixed with microtubules that originate from the pericentriolar region.

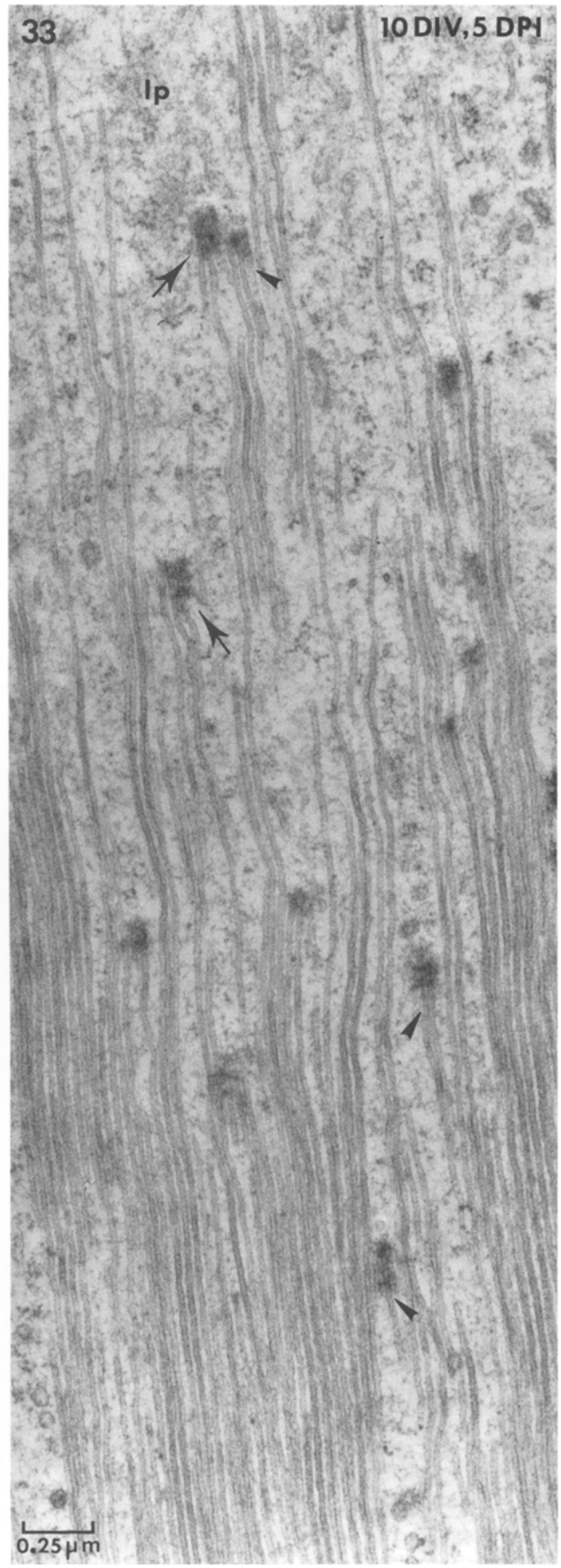
Discussion

RECOVERY OF HAIR CELLS AFTER INJURY

The peripheral hearing organ of mammals is unique among sensory systems in its restricted number of receptors: about 481 per 1 mm of length – 103 inner


31
10 DIV, 5 DPI
Cu
m
lh
0.25 µm


32
10 DIV, 5 DPI
m
m
m
Oh
0.33 µm


33
10 DIV, 5 DPI
lp
0.25 µm

hair cells plus 378 outer hair cells (Burda *et al.*, 1988). The organ of Corti of the ICR laboratory mouse averages 6.41 mm in length and operates using ~3390 sensory cells, *i.e.*, only 800 inner hair cells, the primary receptors (Burda & Branis, 1988).

Until recently, it was believed that the mammalian auditory hair cell is formed once in a lifetime and perishes upon injury (for review see Saunders *et al.*, 1985). We have reported, however, that following mechanical trauma in culture, some hair cells show signs of self-repair and regrowth of their stereocilia (Sobkowicz *et al.*, 1992, 1993b). The first sign of recovery appears to be the reformation of the extinct kinocilium, and thus, as seen in low-power electron micrographs, almost all hair cells (and also the supporting cells) are adorned with cilia.

There is an effort and urgency to induce the formation of new sensory cells in the mammalian acoustic organ, but so far this effort has failed (Staecker *et al.*, 1995). All recovering hair cells shown in our present paper were unlabelled by tritiated thymidine. As we reported, using this mitotic marker (Sobkowicz *et al.*, 1992), injury to the hair cell region induces mitotic division only in the epithelial cells of the inner and outer spiral sulci, while the sensory cells and specialized supporting cells remain unlabelled. These patterns of survival and proliferation are unchanged by the nature of the injury: mechanical versus ototoxic versus laser beam ablation (unpublished observation).

The capacity for self-repair is not limited to the apical parts of sensory cells. Direct injury to the receptor poles of hair cells and the afferent endings induces dendritic sprouting and new synaptogenesis (Sobkowicz & Slapnick, 1992).

A constant feature in post-traumatic hair cells, and the one that prompted our investigations, is a shift of the kinocilium to a central position within the hair cell bundle. This new position is consistent with its initial location in differentiating mammalian hair cells (Anniko, 1983b). Our evidence suggests that the basal body may play a pivotal role in forming, recruiting and aligning the organelles essential to the reformation of the cuticular plate and stereocilia.

Comparison of developmental with regenerative phenomena has to be made cautiously. A 5- or 7-day-old hair cell may employ different, more efficient, molecular mechanisms in reforming its cuticular plate and stereocilia after injury than those used during the primary phases of differentiation. The cellular and structural differentiation of the organ of Corti in isolation progresses to a significant degree but does not correspond exactly to that in the normal animal of the same age (Sobkowicz *et al.*, 1984). The differentiation of hair cell stereociliary bundles in cultures of the postnatal mouse cochlea as compared with that in the intact mouse was described in detail by Furness and collaborators (1989). It should be stressed that the position of the kinocilium within the hair cell bundle in their material was at all times peripheral. The consistently peripheral position of the kinocilium was also observed in the developing postnatal hamster by Kaltenbach and collaborators (1994).

THE MORPHOGENETIC FORMS OF THE AUDITORY KINOCILIUM

In the mouse, the auditory hair cells are formed on foetal days 14–16, of 18–20 days total gestation time (Ruben, 1967). At this age, the kinocilium is already present among the microvilli covering the apical surface of the hair cells (Anniko, 1983a). Thus the kinocilium, which is gone by 10 PN (Kimura, 1966), does not last longer than 16 days.

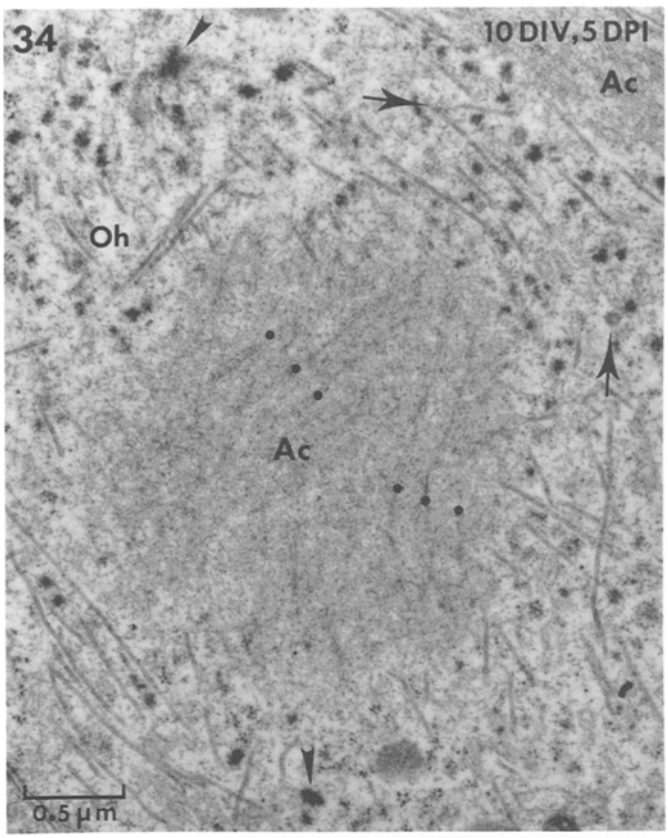
It is generally accepted that the structure of the ciliary axoneme varies along the length of the cilium (Flock, 1965). The basal body is formed by a ring of triple microtubules; the immediate proximal part of the cilium for about 0.5 μm (only 0.3 μm above the cell surface) consists of a ring of nine peripheral microtubule doublets (the 9 + 0 form); at this point, the cilium acquires two single central microtubules, forming the classical 9 + 2 (single) configuration along its main shaft. In both control and experimental material, however, we observed several configurations of axonemal microtubules, from the 8 + 1 (double) to the 9 + 0 and 9 + 2 (single) forms. The frequency of 9 + 2 axonemes seems to be related to the maturation of the organelle (Table I), independent of the level of sectioning. We are not aware, however, of any

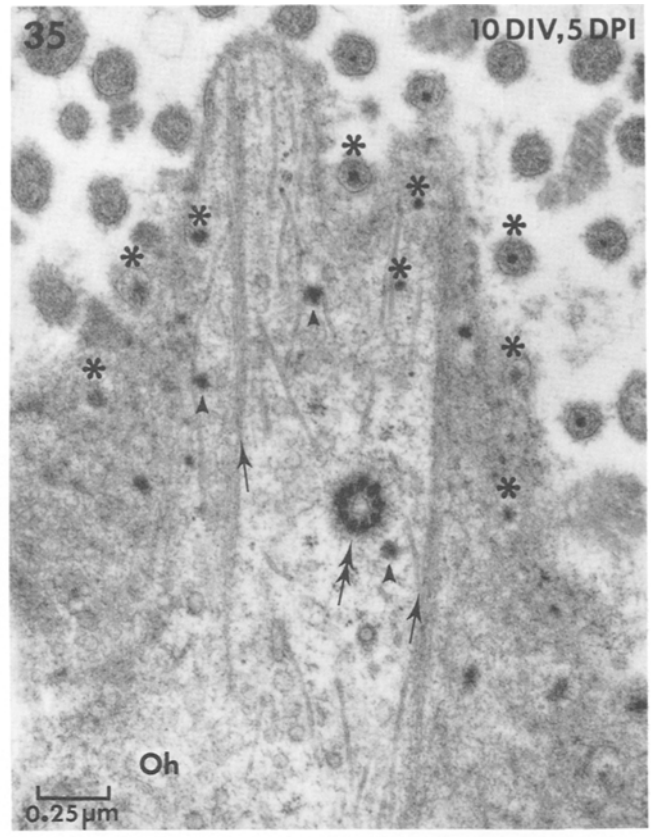
Figs 31–33. Nucleation of microtubules from pericentriolar bodies.

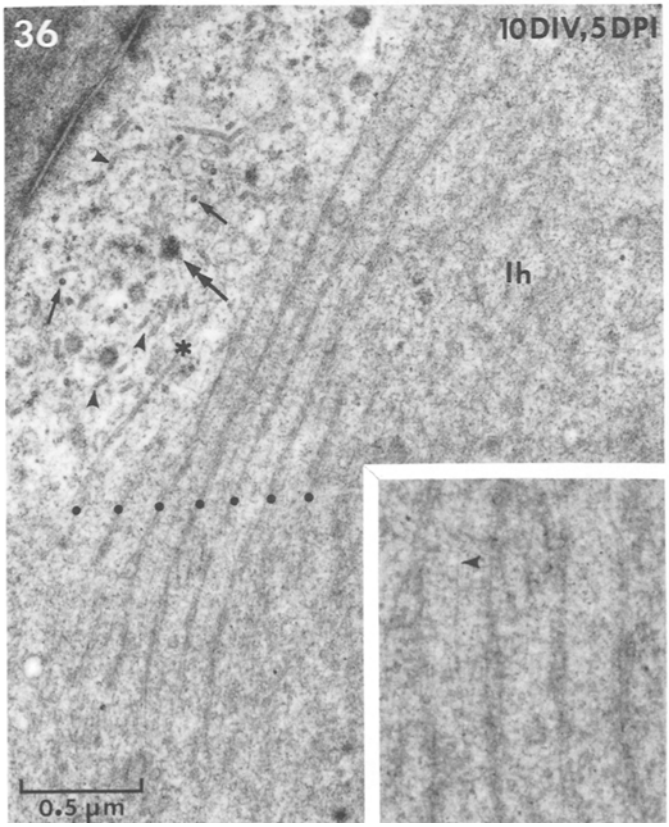
Fig. 31. An oblique cross-section of the area beneath the basal body in an Ih. Double arrow points to the lowest part of the basal body. Dense fibrous pericentriolar bodies extend downward in the cell cytoplasm. Individual microtubules are seen in contact with the pericentriolar bodies (arrows). Plasma membrane vesicles (arrowheads) are discernible among the microtubules.

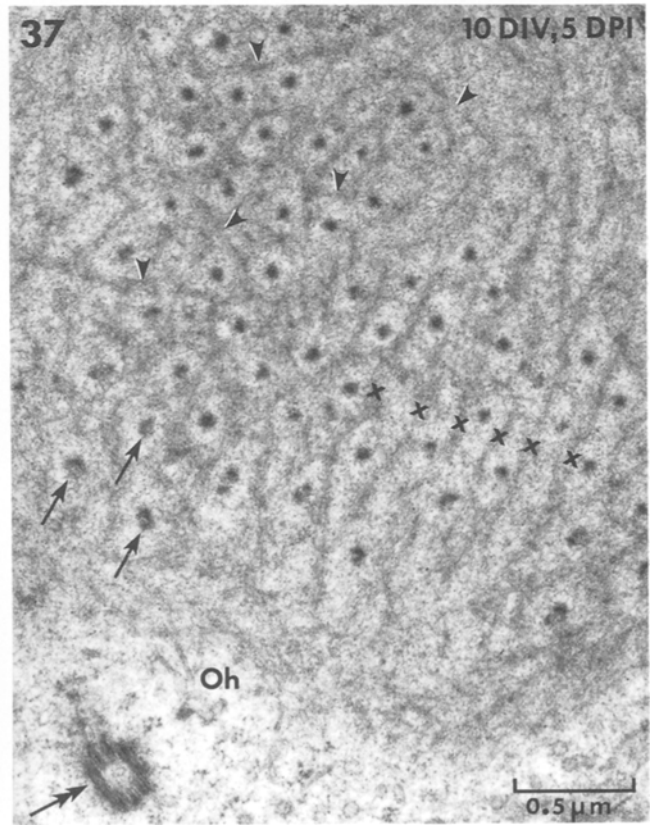
Fig. 32. A cross-section through the centriole and pericentriolar area beneath the cuticular plate in an Oh. The centriole (double arrow) is formed by triplets of microtubules. Multiple pericentriolar fibrous bodies fill the region (arrows). Most of them appear to give rise to short microtubules.

Fig. 33. An example of the nucleation of microtubules from the pericentriolar bodies (arrows) in the head of a recovering inner pillar cell. Microtubules originate as single, double or triple units (arrowheads) from each pericentriolar fibrous body within the subapical part of the cell.


34
10 DIV, 5 DPI
Ac
Oh
Ac
0.5 µm


35
10 DIV, 5 DPI
Oh
0.25 µm


36
10 DIV, 5 DPI
Ih
0.5 µm


37
10 DIV, 5 DPI
Oh
0.5 µm

example in the literature of a population of primary cilia that consists of both the 9 + 0 and 9 + 2 forms.

The kinocilia in post-traumatic hair cells, just as the primary cilia, consist of microtubular doublets which, especially during differentiation, display an 8 + 1 irregular configuration. Usually the 9 + 2 microtubular configuration is confined to motile cilia (Fawcett, 1961), whereas the primary, nonmotile cilia are characterized by the 9 + 0 arrangement (Barnes, 1961). The motile cilia are more constant in form than are the primary cilia. Aberrations of the 9 + 2 form usually concern the number of microtubules, not their distribution (Afzelius, 1963). In contrast, modified 9 + 0 cilia manifest irregularities in the microtubular arrangement, while the total number of microtubule pairs remains constant (Konrádová, 1973; Konrádová *et al.*, 1975).

Among the modified primary cilia, the most frequent is the 8 + 1 form. Notable examples are: cilia in the inner retinal bipolar neurons and in the retinal pigment epithelium of the guinea pig and human (Allen, 1965); cilia in the granular neurons and astroglia of the rat hippocampus (Dahl, 1963); cilia in the stellate neurons of the rat cerebellum (Ruela *et al.*, 1981); cilia in parathyroid glands of the Virginia deer and in the cells of the anterior pituitary of the rabbit (Munger & Roth, 1963); and cilia in the chromaffin cells of the rat adrenal medulla (Coupland, 1965). Most relevant is the 8 + 1 form found in the sensory cells of the basilar papilla in the adult bird (Takasaka & Smith, 1971). The persistence of the 8 + 1 form throughout the life span of the auditory kinocilium may reflect its phylogenetic past. Ontogenetically, the modified forms of 9 + 0 cilia are usually interpreted as expressions of the different developmental stages at which the cilia become arrested (Dahl, 1963). Thus, the imperfectly structured kinocilia in traumatized hair cells may merely reflect the normal developmental sequence.

THE PROPOSED MORPHOGENETIC ROLE OF THE AUDITORY KINOCILIUM

The first observation of the reformation of the kinocilium by injured hair cells was made by Engström and collaborators (1983). They observed thin, regrowing kinocilia in adult rabbit inner hair cells during long-term survival after noise-induced trauma. The recovering cells were forming bundles of actin filaments and giant stereocilia. For this reason, the authors interpreted the newly forming kinocilia as a sign of cell 'regenerative activation.'

A morphogenetic function of the kinocilium in the auditory sensory cells was first proposed by Spoendlin (1964), who found double ectopic cilia adjacent to a fibrous matrix of cuticular plate material in the *infranuclear* portion of the vestibular hair cell in an adult squirrel monkey. In the retina, the cilium plays a pivotal role in the formation of the outer segments of the receptor cells (De Robertis, 1956; De Robertis & Lasansky, 1958; Tokuyasu & Yamada, 1959; Nilsson, 1964; Olney, 1968; Spira, 1975; McArdle *et al.*, 1977).

Generally, a primary cilium is thought to be a connective organelle between the Golgi apparatus and the cell surface or extracellular space (Zeigel, 1962), which regulates protein synthesis (Poole *et al.*, 1985). Its primary role as such might be to activate a synthesis of proteins involved in membrane production.

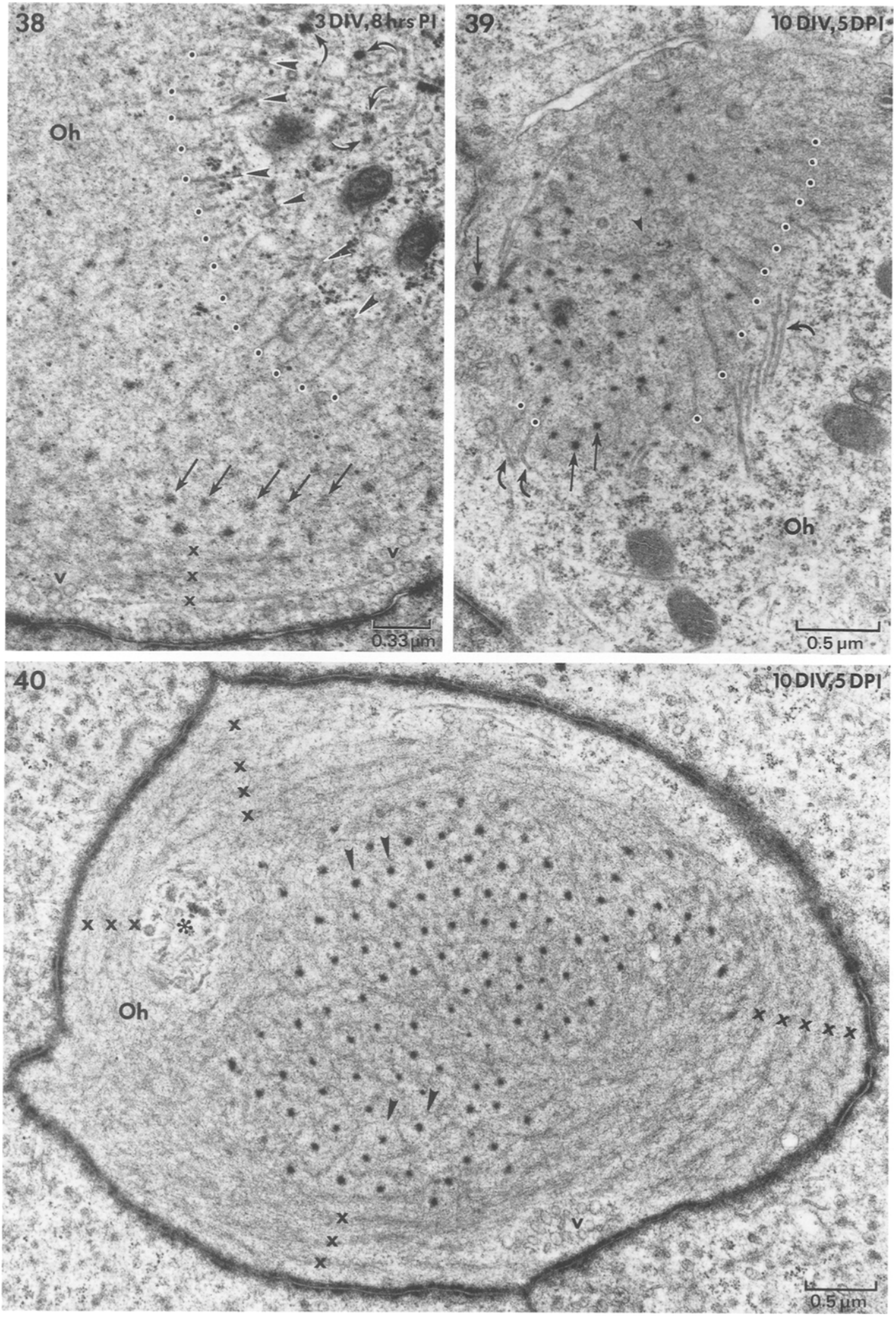
Figs 34–40. Illustrate the alignment of actin filaments in regenerating cuticular plates of outer hair cells in a 10 DIV, 5 DPI apical culture.

Fig. 34. A striking arrangement of microtubules, fibrous pericentriolar bodies (arrowheads), and actin filaments in a cross-section of a regenerating Oh cuticular plate, beneath the basal body. Actin filaments occupy the central and peripheral (upper right) positions in the field. Microtubules and pericentriolar bodies surround the central patch of actin; in places, they exhibit alternate arrangements (between arrows). The dense fibrous pericentriolar bodies vary in size from about 20 to 140 nm. The central patch of actin filaments appears to have incorporated the peripheral microtubules. Note a tendency for the periodic alignment of microtubules and actin filaments (dots) before they blend into the amorphous mass. There is an indication of a crisscrossing alignment of the actin filaments as they converge toward the upper right corner of the central actin patch.

Fig. 35. A cross-section through the area around a basal body (double arrow) in a regenerating Oh cuticular plate. Asterisks mark electron-dense rootlets of stereocilia. Note the close relationship between microtubules and the fibrous pericentriolar bodies (arrowheads). Also note the co-alignment of microtubules and actin filaments (arrows).

Fig. 36. Periodic alignment (at ~145 nm) of microfilaments and microtubular tracks (dots) in an Oh. A microtubule at left (asterisk) appears to be blending with actin filaments (see also Fig. 39). In the upper left part, the pericentriolar body (double arrow) is surrounded by newly formed short segments of microtubules (arrowheads). Single microtubules in cross-section are indicated by a lucent halo (arrows). Inset: higher magnification (approximately two times) of the fading microtubular tracks. Fine lines of longitudinal microfilaments often bisect the distance between the microtubular remnants (arrowhead).

Fig. 37. A cross-section through a regenerating cuticular plate in an Oh at the level of the centriole (double arrow). A crisscross alignment of actin filaments forms an intricate net whose mesh surrounds each individual rootlet (arrows). At right, actin filaments in vertical linear alignment are marked by x (compare with those in Figs 34 and 36); horizontally or diagonally running filaments in the upper field are marked by arrowheads. Compare with the filamentous network seen in the guinea pig in Fig. 4a of Steyger and colleagues (1989).


38
3 DIV, 8 hrs PI
Oh
v
v
0.33 µm
39
10 DIV, 5 DPI
Oh
0.5 µm
40
10 DIV, 5 DPI
Oh
v
0.5 µm

Association of the basal body, pericentriolar bodies and membrane vesicles

Pericentriolar bodies are small, fibrous patches populating the pericentriolar area. They were described as 'dense 70 nm granules beneath the cuticular plates in the inner hair cells' by Furness and collaborators (1990). Both centrioles and pericentriolar bodies provide sites for nucleation of the microtubules (Tilney & Goddard, 1970; Calarco-Gillam *et al.*, 1983) that form a framework for the cuticular plate (Furness *et al.*, 1990).

Growth vesicles are round, clear, membrane vesicles that tend to collect beneath the plasma membrane of growing cells, and are believed to contribute to plasmalemmal expansion (Roth & Shigenaka, 1964; Bunge, 1973; Griffin *et al.*, 1981; Pfenninger & Maylié-Pfenninger, 1981). The presence of membrane vesicles in the immediate surroundings of the basal body is a constant feature during development and reformation of cuticular plates. A similar association was noted previously in post-traumatic hair cells in the mammalian vestibular organ by Engström and collaborators (1962), in the lateral line hair cells by Flock (1965), and in the sensory cells of the basilar papilla by Takasaka and Smith (1971). The role of membrane vesicles may be best observed during ciliogenesis in the brush border (Bonneville & Weinstock, 1970). An accumulation of small membrane vesicles beneath the apical membrane represents the first step in the formation of cilia. These 'surface forming' vesicles are derived from the mature, *trans* face of the Golgi apparatus and may be considered as precursor units of the cell membrane (Bergmann *et al.*, 1981; Kupfer *et al.*, 1982; for review, see Farquhar & Palade, 1981; Lodish *et al.*, 1981).

New plasma membrane units can serve as nucleation sites for actin polymers (Tilney *et al.*, 1981), while the basal body and pericentriolar apparatus, as well as the microtubules, are able to organize actin filaments into compact sheets (Karr & Alberts, 1986; Tamm & Tamm, 1988; Raff & Glover, 1989).

Association of microtubules and actin

Microtubules are integral to the apices of hair cells. The microtubular arrangements in the apical portions of hair cells have been defined ultrastructurally and immunocytochemically by Steyger and collaborators (1989) and by Furness and collaborators (1990). Basically, in adult hair cells of guinea pigs the complex arrays of microtubules enclose the filamentous part of the cuticular plate in an incomplete belt and line the cytoplasmic channel housing the basal body. The microtubules themselves are excluded from the cuticular plate material.

This arrangement is in sharp contrast to the close association of microtubules and actin filaments seen during the reformation of the cuticular plate in our material, where the microtubules that blend into the filamentous matrix seem to align some of the actin filaments into regularly distributed bundles. This highly organized structural pattern could conceivably control the distribution of stereociliary rootlets within the cuticular plate.

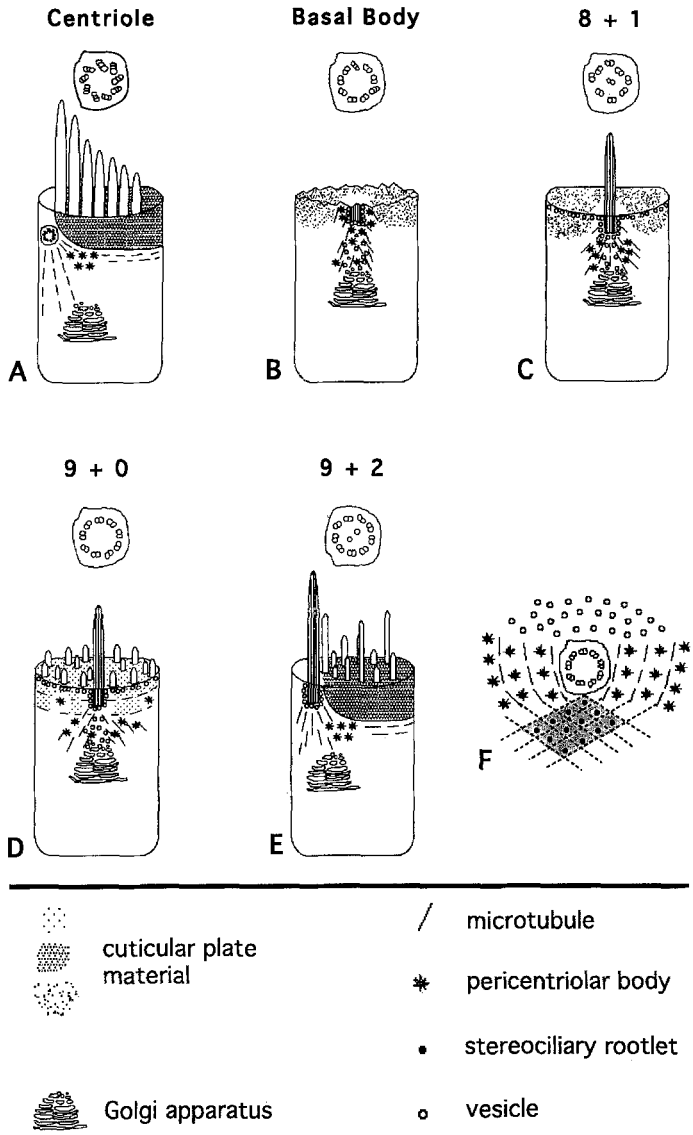
Except for the specific periodicity, the parallel alignment of actin filaments within the matrix of the cuticular plate appears similar to that of the filamentous striae seen in the hair cells of the alligator lizard by DeRosier and Tilney (1989). Until now, this organized fibrillar net has not been observed in the normal developing mouse or in intact cultures, where the cuticular plate was perceived as a uniform filamentous matrix.

Interactions between microtubules and actin filaments were discovered by Griffith and Pollard (1978) and extensively studied *in vitro* (Griffith & Pollard, 1982; Pollard *et al.*, 1984; Selden & Pollard, 1986). It has been shown that the binding of actin filaments to microtubules is activated by microtubule-associated proteins (MAPs) that coat the surface of microtubules (Sattilaro & Dentler, 1982). The addition of MAPs to actin *in vitro* forms a gel that is of a much higher viscosity than the sum of the

Fig. 38. A cross-section through the cuticular plate of an Oh at 3 DIV, 8 HPI. Note the pattern of diverging actin filaments (marked by dots) that emerge from the pericentriolar area (upper right corner); the centriole itself was not in the plane of section (curved arrows point to pericentriolar bodies). The microtubules (arrowheads) seem to participate in guiding the actin filaments (compare with Figs 34–6). At the periphery of the fibrous part of the cuticular plate, the actin filaments form a circumferential band (x); their periodicity is clearly preserved. Note the regular distribution of the forming rootlets (arrows) that appear to correspond in periodicity to that of the actin filaments. 'V's (lower left and right) indicate discrete collections of vesicles along the plasma membrane. Base.

Fig. 39. An oblique section through the fibrous area of the regenerating cuticular plate of an Oh. Note again the diverging pattern of microtubular remnants and actin filaments (dots) that seem to derive from the pericentriolar region (as judged by the conglomeration of membranous vesicles, arrowhead). The regular distribution of fibrous densities suggests that they are forming rootlets (arrows). The curved arrows point to microtubules that appear to join the fibrous network. 10 DIV, 5 DPI, apex.

Fig. 40. An overview in cross-section of the actin filament net within the cuticular plate of an Oh. The net consists of the outer belt that encircles the cuticular plate (x) and the 'inner lattice' whose fibres crisscross around each rootlet (arrowheads). A discrete cluster of vesicles (v) is adjacent to the plasma membrane. The cytoplasmic area, here (asterisk), is free of a centriole. (10 DIV, 5 DPI, apex.)



Figs 41A–F. The proposed sequence of cuticular plate regeneration triggered by injury to a hair cell. (A) In the maturing hair cell, the kinocilium may be extinct. The cytoplasmic channel housing the centriole or the basal body is located laterally, the pericentriolar bodies are subcuticular, and a microtubular belt runs at the periphery of the filamentous cuticular plate. The centriolar axoneme consists of a nine-triplet ring. (B) The injury to the hair cell triggers the centriole to move centrally, to attach to the apical plasmalemma, and to transdifferentiate to the basal body. The cytoplasmic channel is now located in the centre of the cell; membranous vesicles are drawn along the microtubules toward the basal body and the pericentriolar bodies pack around it. The axonemal ring of the basal body often shows a mixture of double and triple microtubules. (C) The basal body sprouts a cilium, and the growth vesicles appose the apical plasmalemma. Pericentriolar bodies nucleate microtubules. The axonemal ring of the new kinocilium is 8+1 and displays an irregular distribution of the peripheral doublets. (D) The cell sprouts stereocilia along the entire apical surface. The area of the subcuticular pericentriolar apparatus enlarges. The filamentous lattice begins to form around the stereociliary rootlets. An axonemal ring is usually of the 9+0 form. (E) The kinocilium gradually returns to a peripheral position, the growth of new stereocilia continues and their alignment approximates the 'V' formation. Their diameter and height are unknown. Some axonemes of the kinocilium may attain a 9+2 form. (F) A simplified model of the proposed fibrillary alignment of actin filaments formed around newly developing stereociliary rootlets. The basal body with its pericentriolar region provides a pool of membranous vesicles and nucleates the microtubules. These in turn interact with actin filaments to form an organizing lattice parallel to the plane of the apical surface.

viscosities of the initial proteins (Griffith & Pollard, 1978). Another property evident in the interaction of MAPs with actin is their ability to align the actin polymers into well-defined bundles (Sattilaro *et al.*, 1981). Finally, MAPs direct the assembly and disassembly of microtubules themselves (Nishida *et al.*, 1982).

In situ, the interaction of microtubules and actin filaments is best documented during the formation of the mitotic spindle (Gawadi, 1974), where there is a

transitory association of both organelles and where microtubules are known to assemble and disassemble depending on the mitotic phase. The integration of microtubules and actin filaments is also described in the apical border of the lateral cells of the freshwater mussel gill (Reed *et al.*, 1984).

The diagrams in Fig. 41 illustrate the proposed sequence of cuticular plate regeneration (see caption). We infer that scattered pericentriolar bodies give rise to an abundance of microtubules, whose depolymerization in turn provides a pool of MAPs available for interaction with actin to provide a high viscosity gel of cuticular plate material. Microtubules also align the actin filaments into bundles, binding them into nondiffusable networks. The apparent integration of microtubules and actin filaments may lead to the formation of a fibrillary net that encompasses rootlets of stereocilia and may play a role in their specific alignment. This interpretation awaits future biochemical and immunohistochemical data.

The integration of microtubules and actin filaments demonstrated here has not been reported previously in hair cells either during primary development or during recovery. The only other example of an association between microtubules and actin filaments may be found in the formation of the subcuticular, Friedmann's laminated bodies (Friedmann *et al.*, 1965) described by Slepecky and collaborators (1981) in inner hair cells. It is noteworthy, however, that a maze of actin filaments perpendicular to the stereociliary rootlets, and showing opposite polarities, was described by Flock and collaborators (1981) in guinea pig hair cells. Within this meshwork, microtubules accompanied by actin filaments ran for considerable distances.

It is surprising that the differentiation of the cuticular plate and stereocilia can be activated anew and at a fairly advanced postnatal age, although we do not know how successful this repair is. If one assumes that the kinocilium plays a key morphogenetic role in this process, then it is easy to comprehend its transitory nature: Shedding of the kinocilium by the tenth postnatal day implies the completion of the differentiation of the cuticular plate and stereocilia. This timing coincides with the onset of hearing in the mouse (Mikaelian & Ruben, 1965; Ehret, 1977; Shnerson & Pujol, 1982).

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