Journal of Cell Science 107, 589-600 (1994)
Printed in Great Britain © The Company of Biologists Limited 1994
JCS2013

Reorganization of the centrosome and associated microtubules during the morphogenesis of a mouse cochlear epithelial cell

Craig G. Henderson, John B. Tucker*, Mark A. Chaplin, John B. Mackie, Steven N. Maidment, Mette M. Mogensen and Calum C. Paton

School of Biological and Medical Sciences, Bute Medical Building, University of St Andrews, St Andrews, Fife KY16 9TS, Scotland

*Author for correspondence

SUMMARY

Reorganization of centrosomal microtubule-organizing centres and the minus ends of microtubules occurs as the centrosomal ends of large microtubule bundles are repositioned and anchored to cell junctions in certain epithelial cells called inner pillar cells in the mouse organ of Corti.

The microtubule bundle that assembles in each cell consists of two distinct microtubule arrays that run closely alongside each other. Both arrays are attached to the cell surface at their upper and lower ends. One of the arrays spans the entire length of a cell but the other is confined to its lower portion.

Initially, about 3,000 microtubules elongate downwards from an apically situated centrosome in each cell. Subsequently, the minus ends of these microtubules, and the centrosome and its two centrioles, migrate for about 12 μm to the tip of a laterally directed projection. Then, a meshwork of dense material accumulates to link microtubule minus ends and the centrosome to cell junctions at the tip of the projection. Pericentriolar satellite bodies, which form after the initial burst of microtubule nucleation, may represent a condensed and inactive concentration of microtubule-nucleating elements.

Suprisingly, as a cell matures, about 2,000 microtubules are eliminated from the centrosomal end of the microtubule bundle. However, about 2,000 microtubules are added to the basal portion of each bundle at levels that are remote with respect to the location of the centrosome. Possibly, these microtubules have escaped from the centrosome. If this is the case, then both the plus *and minus ends* of most of the errant microtubules are captured by sites at the cell surface where the ends are finally anchored. Alternatively, each cell possesses at least one other major microtubule-nucleating site (which does not possess centrioles) in addition to its centrosome.

Key words: centrosome, microtubule, cochlea, mouse

INTRODUCTION

The centrosome is the main microtubule-organizing centre in most animal cell types. The prime function of this central body is to nucleate the assembly of microtubules that radiate from it, during, for example, the construction of mitotic spindles in dividing cells, and the cytoplasmic microtubule complexes of interphase cells. A centrosome usually includes two centrioles. Microtubule nucleation is effected by elements included in pericentriolar material (a less highly ordered complex of components that surrounds the centrioles). These characteristics have mostly been established by studies of oocytes and relatively undifferentiated cells in tissue culture (see Tucker, 1979; McIntosh, 1983; Bornens and Karsenti, 1984; Brinkley, 1985; Mazia, 1987; Vorobjev and Nadezhdina, 1987; Vandré and Borisy, 1989; Kalnins, 1992; Kimble and Kuriyama, 1992; Kalt and Schliwa, 1993).

The notion that microtubules simply radiate out from a central centrosome has to be substantially elaborated upon to account for events that occur during the assembly of certain microtubule arrays. This is especially the case so far as control of microtubule organization during the assembly of some interphase microtubule arrays in terminally differentiating tissue cells is concerned (see Tucker, 1981, 1984, 1992). Studies of differentiating nerve, muscle and epithelial cells are all revealing the operation of previously unanticipated assembly sequences and control mechanisms to effect construction of microtubule frameworks during tissue morphogenesis. For example, microtubule populations can be established that are not directly connected to centrosomes in cytoplasmic regions that are remotely located with respect to centrosomes (see Bré et al. 1987; Baas and Joshi, 1992). In some cases, centrosomal microtubule-organizing centres are either abandoned or are extensively reorganized. Such reorganizations include: relocation so that loss of central positioning close to the nucleus occurs, the formation of new associations with other cell components, and replacement by microtubule-nucleating sites with configurations that do not closely resemble that of typical centriole-containing centrosomes (Tassin et al., 1985; Kronebusch and Singer, 1987; Achler et al., 1989; Bacallao et al., 1989; Mogensen et al., 1989, 1993; Tucker et al., 1992). This report deals with changes in centrosomal and microtubule

organization as the centrosomally nucleated minus ends of microtubules are attached to the cell surface in certain epithelial cells called inner pillar cells in the mouse cochlea.

Each inner pillar cell contains a large microtubule bundle (about 40 µm long with about 3,000 microtubules in mouse) (Tucker et al., 1992, 1993). These bundles are transcellular; they are anchored to the cell surface at both ends (Iurato, 1967; Engström and Ades, 1973; Kimura, 1975; Friedmann and Ballantyne, 1984). Inner pillar cells, and certain other supporting cells in the organ of Corti that contain substantial cell surface-associated microtubule bundles, perform an important function in the mammalian cochlea. They anchor sensory hair cells to the basilar membrane, which vibrates in response to acoustic stimuli (Pickles, 1988; Hudspeth, 1989). The microtubule bundles and other cytoskeletal components in the supporting cells and hair cells (Retzius,1884; Held, 1926; Steyger et al. 1989; Slepecky and Ulfendahl, 1992), are connected by cell junctions to form a structurally contiguous supracellular framework in the epithelium (Iurato, 1967; Engström and Ades, 1973; Kimura, 1975; Gulley and Reese, 1976). This framework must inevitably play a crucial micromechanical role that helps to define cochlear performance and sensitivity. How are the correct connections between microtubules and cell junctions set up?

Microtubules initially elongate from a cell surface-associated centrosome at the apical end of an inner pillar cell (Tucker et al., 1992). Each cell is initially columnar but, appropriately enough for a supporting cell, becomes shaped like a flying buttress as it matures (Tucker et al., 1993). However, this study shows that the minus ends of microtubules in a mature pillar cell are linked to cell junctions (which join the pillar cell to sensory hair cells) at the tip of a 12 µm long lateral projection at the top of the cell. Is the centrosomal microtubule-organizing centre involved during this marked reorganization of the microtubules it has nucleated? This investigation mainly deals with the reorganization of the centrosomal region as the minus ends of microtubules are repositioned and anchored to the cell surface.

MATERIALS AND METHODS

Differentiating inner pillar cells have been examined at 6, 9, 10 and 21 days after birth in mice (Swiss CD1) (the period 0-24 hours after

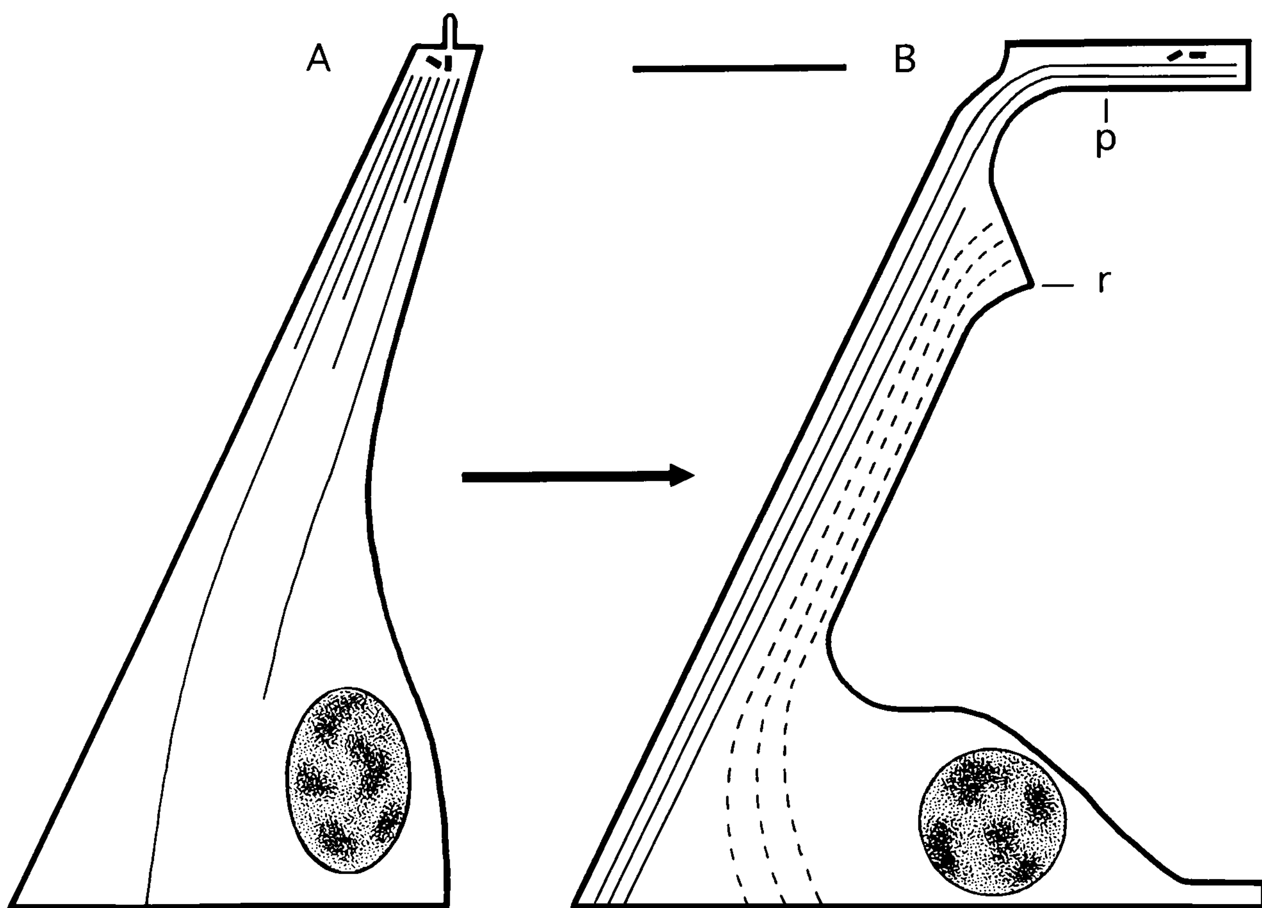

Fig. 1. Schematic diagrams showing the shapes of inner pillar cells and microtubule arrangement. Black lines inside cells show the orientation of microtubules and regions where high concentrations of microtubules occur (in much larger numbers than depicted by the lines). Black rectangles show the positions of the basal body and centrioles. The outer sides of the cells face towards the right of the diagram. The flat basal surfaces of cells (towards the bottom of the diagram) indicate the plane of the upper surface of the basilar membrane (not shown) on which they are situated. (A) Day 6. Cell organization before bending occurs. (B) Day 21. There is a phalangeal process (p) at the apical end of the cell and a ridge (r) projects at the boundary between two concavities in the cell's outer surface. The transcellular microtubule array is depicted by the continuous black lines and the basal array by the broken lines. Bar, 10 µm.

birth = day 0, etc.). Each organ of Corti was prepared for electron microscopy as described previously. Each was fixed after a portion from the basal half of a cochlea had been dissected out from the organ (Tucker et al. 1992), or in situ in a cochlea that had been dissected away from surrounding tissues (Tucker et al., 1993). In the latter case, a small hole (about 1 mm in diameter) was made through the ossifying cochlear capsule at its apex, and a similar one at its base, before immersing the whole cochlea in fixative. The bony capsule and other cochlear tissues were trimmed away from a portion of the organ of Corti in cochleas that had been fixed in this way after they had been embedded in Araldite. The portions of the organ that have been examined were all selected from the basal regions of cochleas because a graded baso-apical decrease in the number of microtubules/mature pillar cell has been reported for rats and guinea pigs (Iurato, 1967; Kikuchi et al., 1991).

In some instances, sodium cacodylate (0.1 M, pH 7.3) was used to buffer the fixative solutions and $CaCl_2$ (2 mM) was also included (instead of the phosphate buffered sucrose solution used in earlier

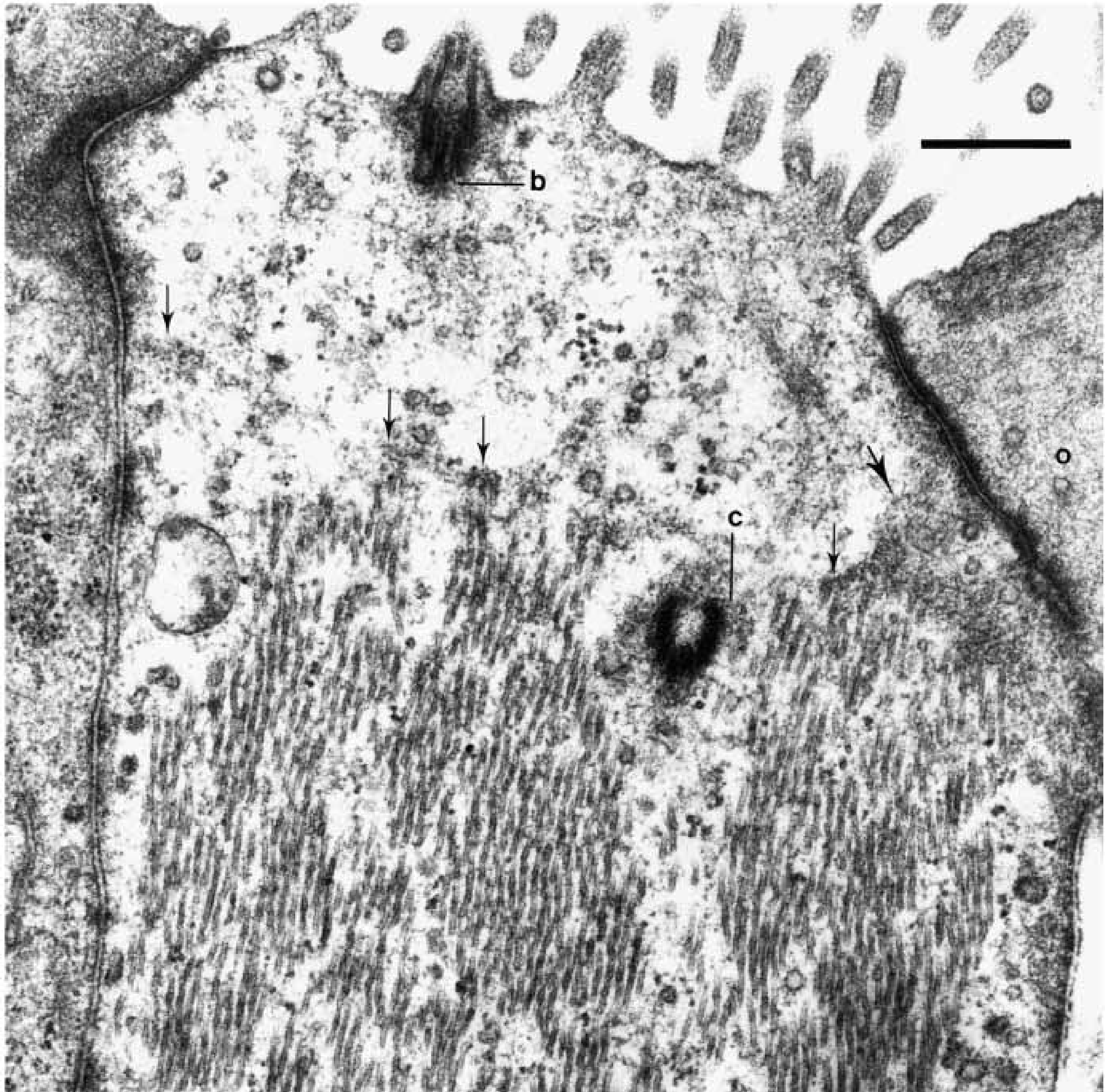

Fig. 2. Median longitudinal section through the cell apex on day 6, which includes its basal body (b) and centriole (c). The minus ends of microtubules are associated with a subapical layer (small arrows), which includes several perforations in regions where there are gaps in the microtubule array. The layer is much thicker in the region (large arrow) where the pillar cell contacts the top of an outer hair cell (o).
Bar, 0.5 μm.

studies). After such fixation and prior to embedding, each cochlea was en bloc stained with uranyl acetate (1% in 100% ethanol) for 18 hours at room temperature. Ultrathin sections were still double stained as usual with ethanolic uranyl acetate and lead citrate. When this procedure was employed, most cell components were stained more densely than was otherwise the case.

RESULTS

Cell morphogenesis and microtubule deployment

In the newborn mouse, and for a period of about 7 days thereafter, an inner pillar cell has a simple columnar shape (Tucker et al., 1992) (Fig. 1A). A large straight microtubule bundle has started to assemble by day 1; on day 6 it includes about 3,000 microtubules. The top of each bundle is situated about 1 µm from the apical surface of a cell. About 1 week after the initiation of bundle assembly (day 8) the longitudinal axis of a cell and its microtubule bundle start to extend (by about 25% with respect to their previous lengths) and bend through about 60º (Tucker et al., 1993). A cell extension called the phalangeal process is generated during this manoeuvre (Fig. 1B). This process projects for about 12 µm in an outward direction (towards outer hair cells) in terms of the established convention used for describing cell arrangement in the organ of Corti (see Friedmann and Ballantyne, 1984).

The basal body of a primary cilium and an adjacent centriole are usually located fairly centrally with respect the apical surface of a cell and above the top of the microtubule bundle prior to cell bending (Fig. 1A). Two centrioles are situated near the end of a phalangeal process and the microtubule bundle after cell and bundle bending (Fig. 1B). In addition, two substantial concavities form on the outer side of a cell (Fig. 1B). The upper concavity accommodates part of the rounded upper portions of another supporting cell type (outer pillar cell). Inner pillar cell phalangeal processes extend over the tops of outer pillar cells. The lower concavity flanks an intercellular space called the tunnel of Corti, which opens up between most of the lower portions of adjacent inner and outer pillar cells. A ridge juts out from the outer surface of an inner pillar cell at the boundary between these two concavities (Fig. 1B).

As an inner pillar cell matures, a substantial change in the architecture of its microtubule bundle is effected. By day 21, the bundle consists of two distinct microtubule populations. One of these spans the curved longitudinal axis of a cell from the tip of its phalangeal process to the cell base. The other bundle is confined to the lower portion of a cell; it runs between the ridge and the cell base (Fig. 1B).

A centrosome initiates assembly of the transcellular microtubule bundle

During the initial stages of bundle assembly (days 1-6), the tops of most bundle microtubules are located more or less in

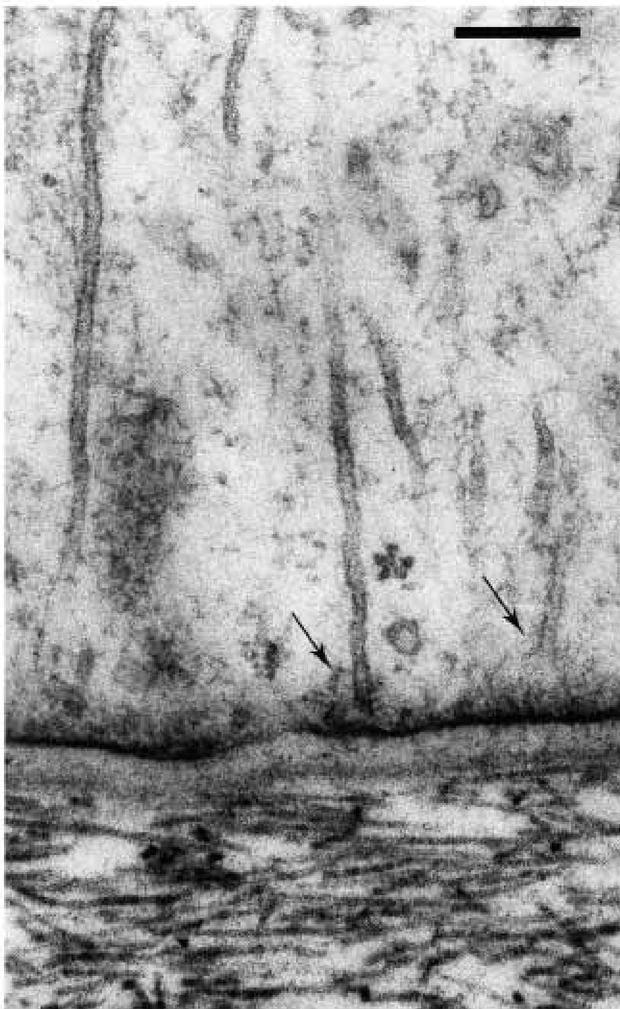

Fig. 3. Longitudinal section through part of the cell base on day 9. Part of the adjacent basilar membrane is at the bottom of the micrograph. Microtubules are less closely grouped alongside each other than is the case at the cell apex (compare with Fig. 2). The ends of some of the microtubules (arrows) are positioned against a layer of dense material that coats the cytoplasmic surface of the basal plasma membrane. Bar, 0.2 µm.

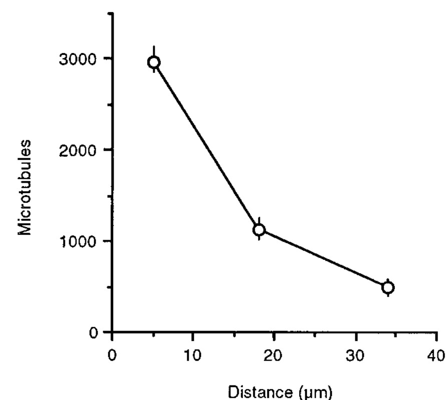

Fig. 4. Number of microtubules/bundle cross-section at different levels on day 6. Each point shows the mean value for microtubule numbers counted in three different cells at each of the different distances from the tops of the bundles. The accompanying vertical line shows the range of each set of counts. Cross-sectional tracking was not sufficiently detailed to determine whether exactly the same cells in the portion of the organ that was used (which included about 25 inner pillar cells) were being assessed at the different levels. Hence, the bundles counted at any one level were often likely to have been different from those counted at another.

register with one another about 1 µm below the apical plasma membrane. The microtubules project downwards from a subapical layer of dense material that is attached to the plasma membrane at the sides of a cell (Fig. 2). A primary cilium projects centrally from the apical surface of a cell and a centriole is positioned near its basal body (usually less than 1 µm away) (Fig. 1A). From days 1-3 both basal body and centriole are usually situated above the subapical layer. By day 6 the centriole is often located at, or just below, the level of the layer and much closer to the top of the microtubule bundle (Fig. 2).

Previous studies led to the conclusion that the basal body/centriole/subapical layer complex is a modified centrosomal configuration in which the layer represents a cell surface-associated deployment of pericentriolar material. This centrosome acts as a microtubule-nucleating site during the inital stages of bundle assembly and the apical tips of the microtubules that contact the layer are the minus ends of the microtubules. Serial cross-sectional analysis on day 3 revealed that each bundle possessed about 2,400 microtubules at its top but was composed of less than 300 microtubules at a level 14 µm below this (Tucker et al., 1992).

At least until day 9, the base of a cell is only sparsley populated with microtubules relative to the more concentrated groupings present at the top of a cell. However, some microtubules make close end-on contact with a layer of dense material that coats the cytoplasmic surface of the plasma membrane at the base of a cell. This contrasts with the much greater separation of the ends of microtubules and the plasma membrane at the top of a cell (compare Figs 2 and 3).

Is the microtubule bundle's transcellularity achieved because a site at the base of a cell nucleates a microtubule population that elongates upwards to meet the centrosomally nucleated population that extends downwards? Bundles include progressively fewer microtubules at successively lower levels along their entire lengths on day 6 (Fig. 4); about 3,000 microtubules near their tops but only about 500 microtubules at a level 5 µm above the bases of the cells. These findings indicate that until

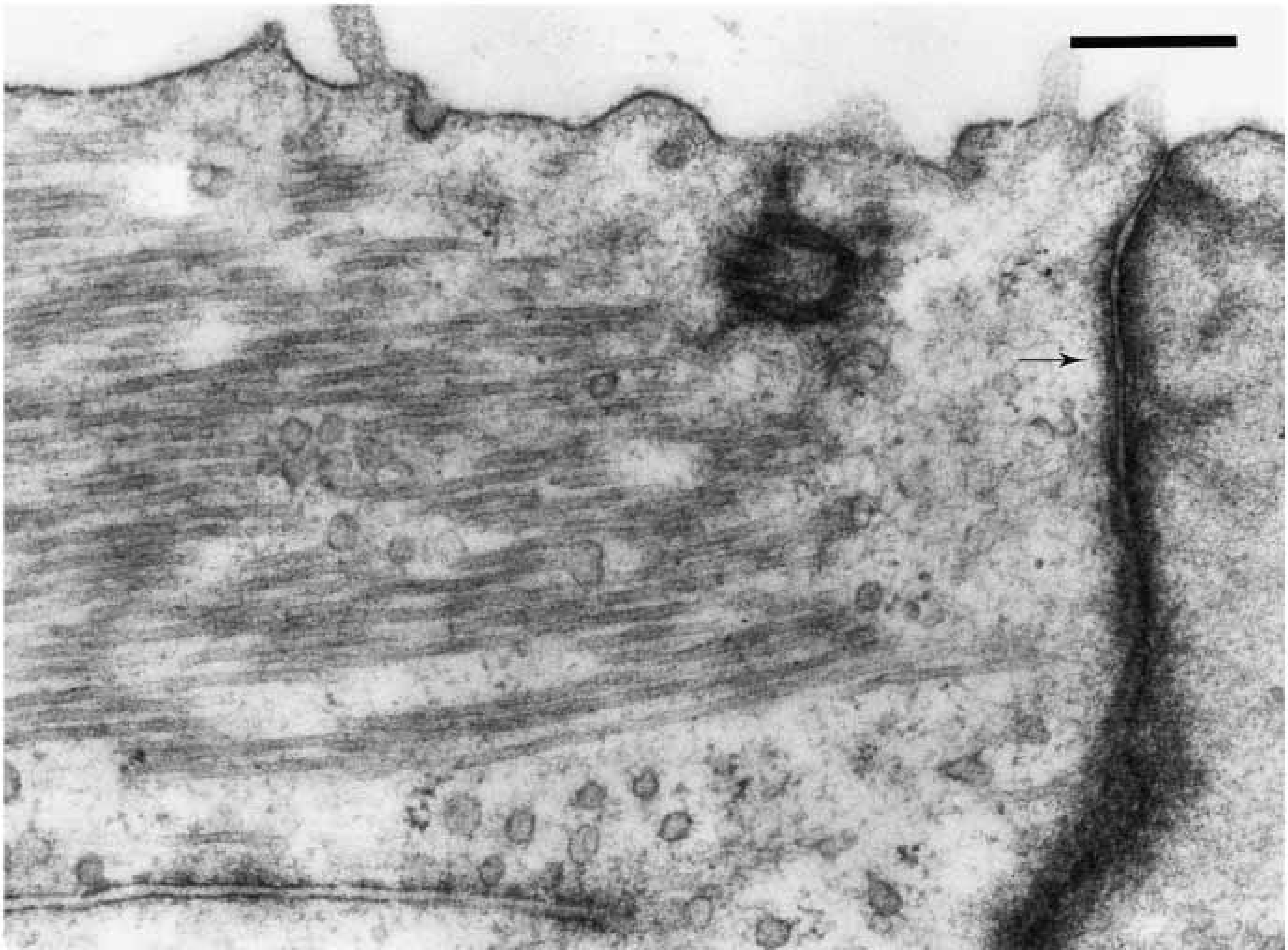

Fig. 5. Longitudinal section through the tip of a phalangeal process on day 9, which includes one of its centrioles. The upper surface of the process is towards the top of the micrograph. The decrease in the breadth of the microtubule bundle that has occurred since day 6 can be appreciated by comparing the number of microtubule profiles with those included in the section shown in Fig. 2, which was cut in the same plane. A junction (arrow) connects the tip of the phalangeal process to a sensory hair cell (towards the right side of the micrograph). Bar, 0.4 µm.

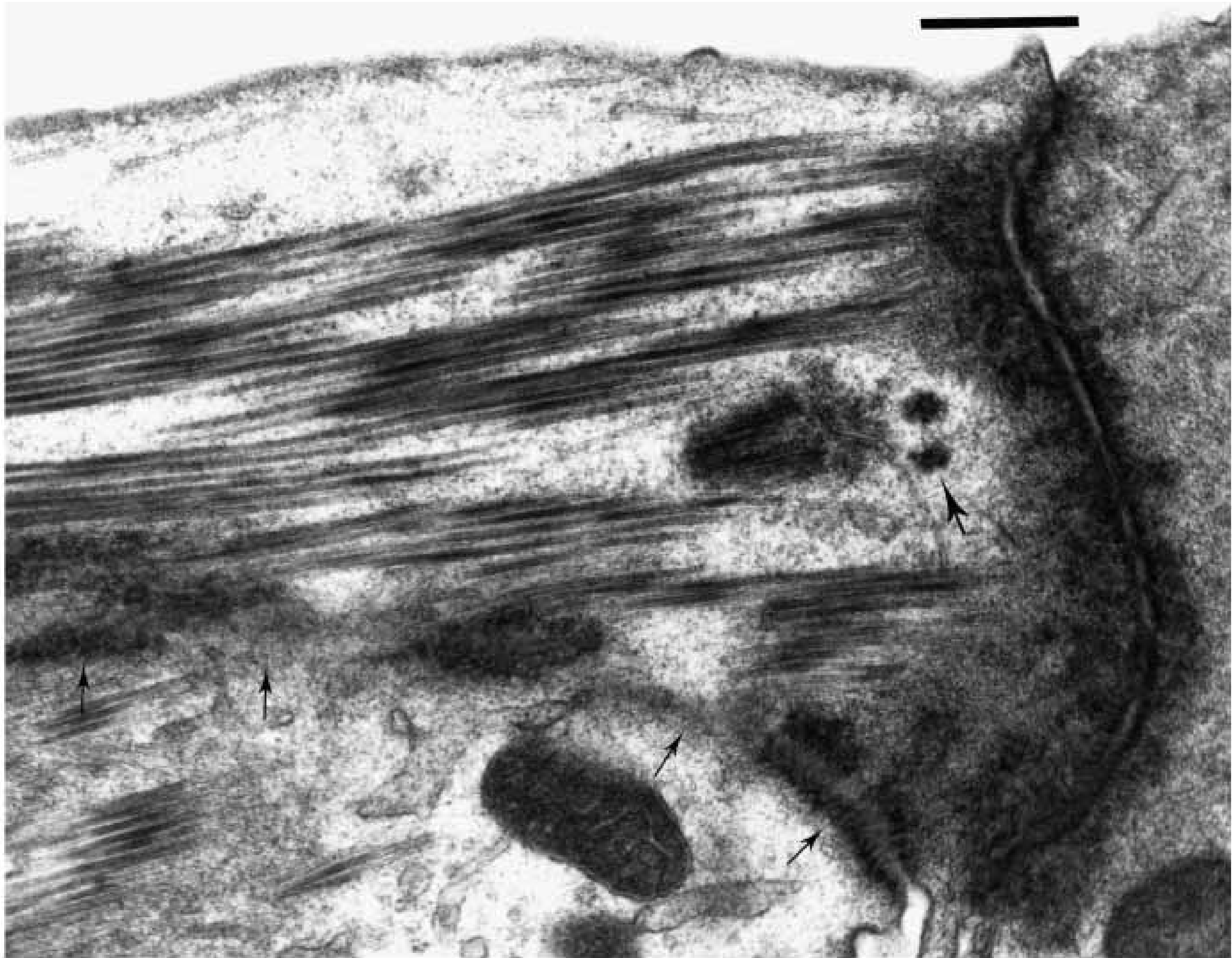

Fig. 6. Longitudinal section through the tip of a phalangeal process on day 21, which includes one of its centrioles and two adjacent satellite bodies (large arrow). A meshwork of dense material is associated with the end of the microtubule bundle and joins it to a junction, which connects the phalangeal process to a sensory hair cell (towards the right side of the micrograph). In this region part of the apical surface of an outer pillar cell (small arrows) is situated beneath the inner pillar cell's phalangeal process. Bar, 0.5 µm.

day 6 the base of the cell is populated by microtubules that have elongated downwards from the apical centrosome.

Repositioning of centrioles and microtubule minus ends

The breadth of the microtubule bundle in a phalangeal process is substantially less than it is in the apical portion of a cell at some earlier stages before the process has formed (compare Figs 2, 5). Most of the decrease in cell and bundle breadth is completed by day 7 before bundle bending and extension of the phalangeal process occurs on day 8 (Tucker et al., 1993).

Two centrioles are situated near the tip of the process and its microtubule bundle (Figs 1B, 5-8). Pericentriolar material connects centrioles to some of the bundle microtubules (Figs 5, 8). Presumably, one of the centrioles is derived from the basal body of the primary cilium, which is present until day 6 (Figs 1A, 2). The ciliary shaft and its axoneme are resorbed shortly thereafter if this is the case, because they have not been detected after day 6.

On day 9, 1 day after extension of a phalangeal process, most microtubule minus ends are situated less than 0.7 µm from the tip of the process (Fig. 5); a few make direct contact with cell junctions, which connect the tip to two adjacent outer hair cells. Attachment to outer hair cells is consolidated after day 9. Most minus ends are usually closer to the cell junctions on day 21 than they are on day 9. In addition, a meshwork of dense material accumulates around the end of the microtubule bundle and links it to the cell junctions by day 21 (compare Figs 5, 6). To some extent the ultrastructure of this meshwork resembles that of a cytoskeletal meshwork called the cuticular plate (see Engström and Ades, 1973), which contacts the opposite sides of the junctions and is concentrated at the apex of each neighbouring hair cell (Fig. 6). Hence, the pillar cells' contribution, to this intercellular linkage of intracellular cytoskeletons, is organized in a region that is also occupied by its centrosomal microtubule-organizing centre. However, most of the material of the meshwork is applied to the sides and ends of bundle microtubules rather than to centriolar microtubules (Fig. 6).

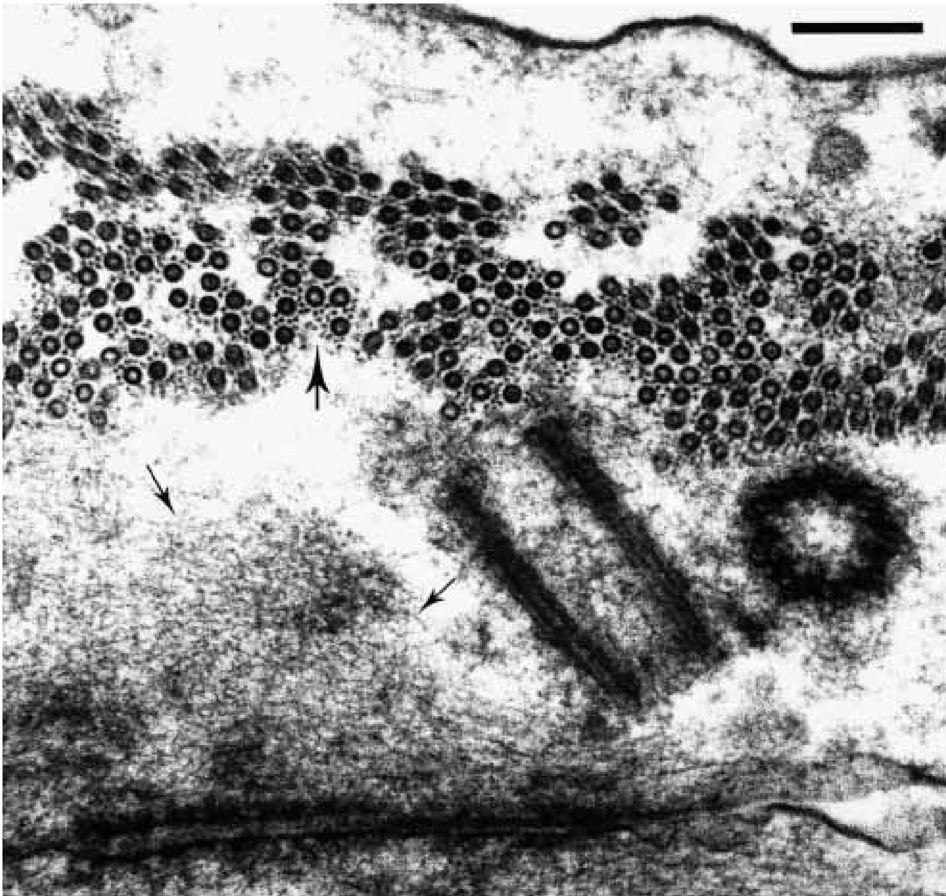

Fig. 7. Cross-section of a phalangeal process near its tip on day 21, which includes both centrioles. The apical surface of the process is towards the top of the micrograph. Cross-sectional profiles of microfilaments that are mostly concentrated between the bundle's microtubules are most clearly evident in the region indicated by the large arrow. Some regions of the dense meshwork have a fibrous appearance (small arrows). Bar, 0.2 μm.

Fibrous material is apparent in some regions of the meshwork. Such material has a much less orderly arrangement than that of filaments, situated between microtubules within a bundle (Fig. 7). The intermicrotubular filaments have started to accumulate between microtubules on day 3 (Tucker et al., 1992), are present along the entire length of a bundle, and are composed of actin (Slepecky and Chamberlain, 1983).

Satellite bodies are situated near centrioles on day 21 (Figs 6, 8) but they have not been detected at any of the earlier stages examined. In addition, there are some microtubules in the cen-

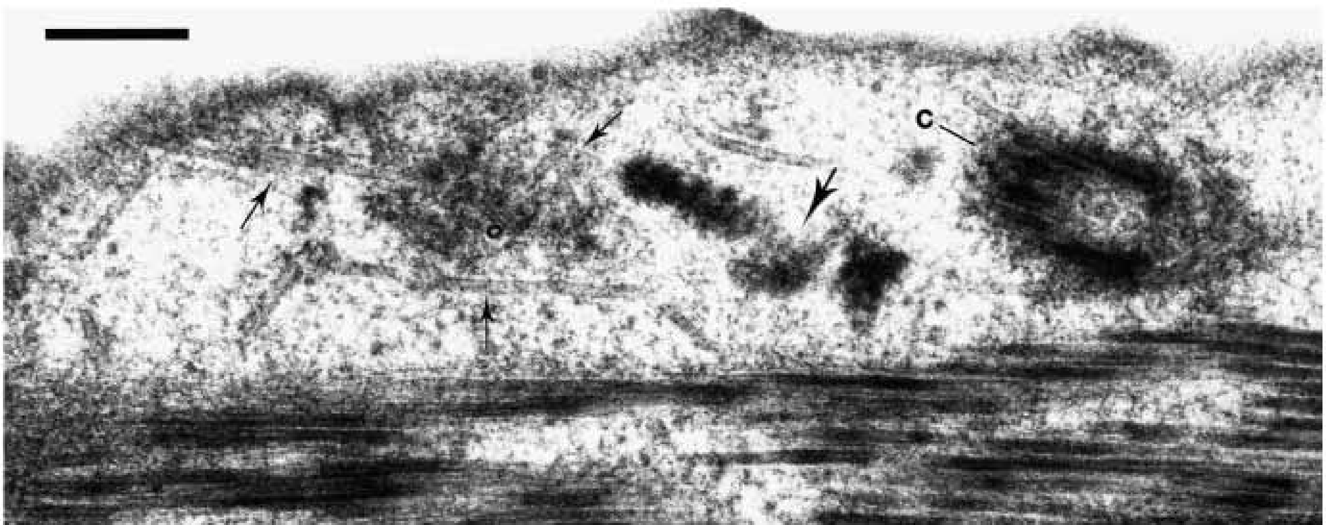

Fig. 8. The centrosomal region of a phalangeal process on day 21 that includes a centriole (c), satellite bodies (large arrow), and regions (small arrows) where there are microtubules that are not oriented alongside microtubules in the main bundle (towards the bottom of the micrograph). Bar, 0.3 μm.

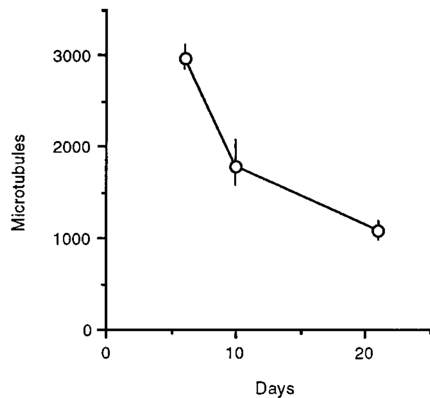

Fig. 9. Number of microtubules/bundle cross-section in the apical portions of bundles as cells mature. Each point shows the mean value for microtubule numbers counted for three different inner pillar cells for days 6, 10 and 21 after birth. The accompanying vertical line shows the range of each set of counts. Cross-sections were situated 5 μm from the centrosomal ends of the bundles.

trosomal region that are not regularly arranged or grouped closely alongside microtubules in the main bundle (Fig. 8).

Apical subtraction and basal addition of microtubules

By day 6, centrosomal nucleation of microtubules has resulted in the assembly of a bundle that includes about 3,000 microtubules at its apical centrosomal end. However, by day 21, when the apical portion of a bundle is situated in a phalangeal process it only includes about 1,000 microtubules (Fig. 9, and compare Fig. 10A with 10B). On day 21, there are about 1,000 microtubules at all other points along the lengths of the apical portions of the bundles down to a level near that of the ridge on the outer side of each cell (Fig. 1B). Hence, about 2,000 microtubules are subtracted from the apical portion of a bundle between days 6 and 21. Much of this subtraction does not occur until after bundle bending and extension of the phalangeal process have occurred on day 8, because the apical portions of bundles include about 1,800 microtubules on day 10 (Fig. 9).

Although the apical portions of microtubule bundles include about 1,000 microtubules on day 21, their basal portions include about 3,000 microtubules (compare Figs 10A and 11) at all levels between the ridge on the outer side of each cell (Fig. 1B) and the cell base. A mean value of 3169 was obtained for microtubule number/bundle for three cross-sections (3383, 2904, 3221) cut at levels 1 μm, 8 μm and 24 μm below the ridge, respectively. Cross-sectional tracking was not sufficiently detailed to determine whether the same cell in the portion of the organ that was used (which included about 25 inner pillar cells) was being assessed at the different levels. Hence, the bundle counted at a particular level was likely to have been different from that counted at another. Compared with the situation on day 6 (Fig. 4), 2,000 or so microtubules have been added to the basal portion of a bundle. The marked and spatially abrupt apicobasal increase in microtubule number at the level of the ridge is due to the presence of the top of a microtubule array that is confined to the lower portion of a cell. The top of this array is closely associated with the side of a cell at the level of the ridge, and its bottom contacts the basal surface of a cell (Fig. 1B). It will be referred to as the *basal array* to distinguish it from the *transcellular array*, which spans the curved longitudinal axis of a cell from the centrosomal region at the tip of the phalangeal process to the cell base (Fig. 1B).

The microtubular pillar can be regarded as being composed of two microtubule arrays rather than one. At the top and bottom of a basal array, its microtubules splay away from those in the neighbouring transcellular array. This occurs near regions where the ends of microtubules in a basal array are associated with the cell surface (Fig. 1B). At the levels in question, the cross-sectional profiles of the two arrays and their microtubular members can readily be distinguished from each other. Microtubules in the basal array are less closely packed together than those in the transcellular array (Fig. 11).

DISCUSSION

Reorganization of the centrosomal region

Each cell possesses a basal body and a centriole until day 6, but subsequently it has two centrioles. Hence, the inner pillar cell provides a rare example of the derivation of a centriole from what was previously a ciliated basal body, in contrast to the more commonly detected reverse procedure (Wheatley, 1982).

The extensive comigration of microtubule minus ends and centrioles during extension of the phalangeal process leaves little doubt that they are firmly attached to each other during the period in question. Connection is effected by pericentriolar material. This is another indication, in addition to that provided by preservation of the structural integrity of spindle poles during anaphase, that this diffuse and apparently insubstantial material has considerable mechanical strength.

Why do centrioles remain associated with the end of a mature microtubule bundle that is repositioned and then anchored to the cell surface? Although the cell is structurally mature on day 21 in terms of having completed assembly of all its main cytoskeletal components (authors' unpublished data), a few microtubules radiate from the centrosomal region that are not closely aligned alongside those of the main bundle. Such microtubules are likely to be ones that have recently been nucleated. The centrosome probably retains a functional role as a microtubule-nucleating site in mature cells. Presumably, microtubule replacement is involved during maintenance of the microtubular pillar throughout the years that lie ahead (several decades for some mammals), since pillar cells are not replaced from a pool of undifferentiated precursor cells. Like nerves and diamonds, pillar cells are forever.

Pericentriolar satellite bodies are present during interphase in animal tissue cells but are usually absent during the stage of the cell cycle (much of M phase) when microtubule nucleation is most pronounced (see Rieder and Borisy, 1982; Wheatley, 1982). The terminally differentiating pillar cells exhibit a sequence of satellite body absence and presence that is not tightly coupled to cell cycle progression. However, it parallels the cell cycle sequence. Centrosomes indulge in intense nucleating activity in non-dividing inner pillar cells during days 1-3 and well defined satellite bodies have not been detected during the period in question (Tucker et al., 1992). The cen-

trosome does include several satellite bodies on day 21 when there is, at most, only a relatively low level of nucleating activity. These findings provide further evidence in support of the suggestion (Rieder and Borisy, 1982) that satellite bodies may represent a condensed and relatively inactive form of the centrosomal components that effect microtubule nucleation.

In mature cells, the centrosomal region is extensively modified and has taken up a major mechanical role in anchoring the end of the microtubule bundle to the cell surface. A dense meshwork has accumulated near the centrioles to link the minus ends of microtubules to cell junctions. The meshwork may include actin and intermediate filaments (certain cytokeratins), since immunocytochemical studies indicate that both are concentrated at the ends of phalangeal

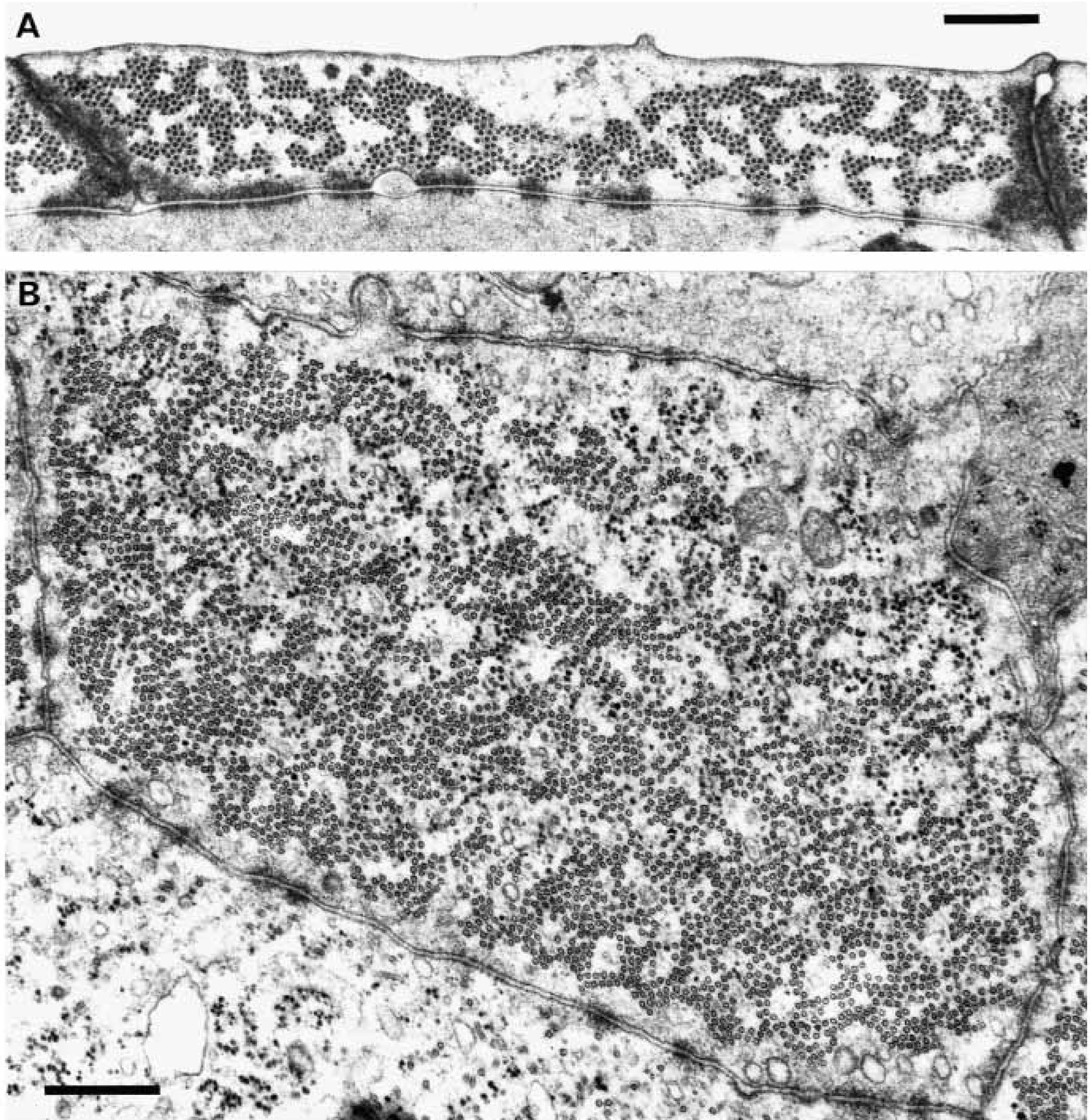

Fig. 10. Cross-sections of microtubule bundles cut 5 μm from their centrosomal ends. (A) Day 21. Cross-section of a phalangeal process oriented with its upper surface towards the top of the micrograph. The bundle includes less microtubules than it does at the same distance from its centrosomal end on day 6 (compare with B below). Bar, 0.5 μm. (B) Day 6. Cross-section of a cell cut 6 μm below its apical surface, and 5 μm below the top of its microtubule bundle, prior to bundle bending and formation of a phalangeal process. Bar, 0.5 μm.

processes (Flock et al., 1982; Kuijpers et al., 1991; Slepecky and Ulfendahl, 1992). Is this meshwork organized by the centrosomal microtubule-organizing centre? Does it represent pericentriolar material that has been augmented and reorganized for mechanical purposes? These possibilities are unlikely because similar meshworks link the ends of microtubules to the cell surface at other levels in the cell at sites remote with respect to the centrosome (authors' unpublished observations).

The procedure for attaching microtubule minus ends to to the plasma membrane in pillar cells is different from that which operates in certain *Drosophila* epithelial cells. In the insect cells, attachment is established at the outset; plaque-like thickenings of the plasma membrane act as microtubule-nucleating sites (Mogensen and Tucker 1987; Mogensen et al., 1993). This contrasts with the sequence reported here for pillar cells where most minus ends are about 1 µm from the plasma membrane to begin with. Subsequently, ends move towards the membrane and attachment is effected by interpolating material to bridge the remaining gaps that separate minus ends from the membrane.

Subtraction of microtubules from the centrosomal end of the bundle

Why are 2000 or so microtubules eliminated from the centrosomal end of each bundle? One can argue that in terms of the mature cell's function there are too many to start with. Why generate such a large surplus? There are three particularly obvious possibilities.

If the microtubules are dynamically unstable (Kirschner and Schulze, 1986) during bundle assembly a large surplus may be required to ensure that about 1,000 microtubules usually encounter their target (the cell base), where they are capped and stabilized. The plus ends of the remainder may fail to achieve this stable transcellular configuration so that such microtubules suffer catastrophic disassembly.

A second possibility is that far more than 1,000 microtubules are required to effect bundle bending and extension of the phalangeal process. Much of the loss of microtubules occurs after bundle bending has occurred. There is evidence that bending is promoted by forces generated within a bundle (Tucker et al., 1993) and big bundles will be more powerful than smaller ones.

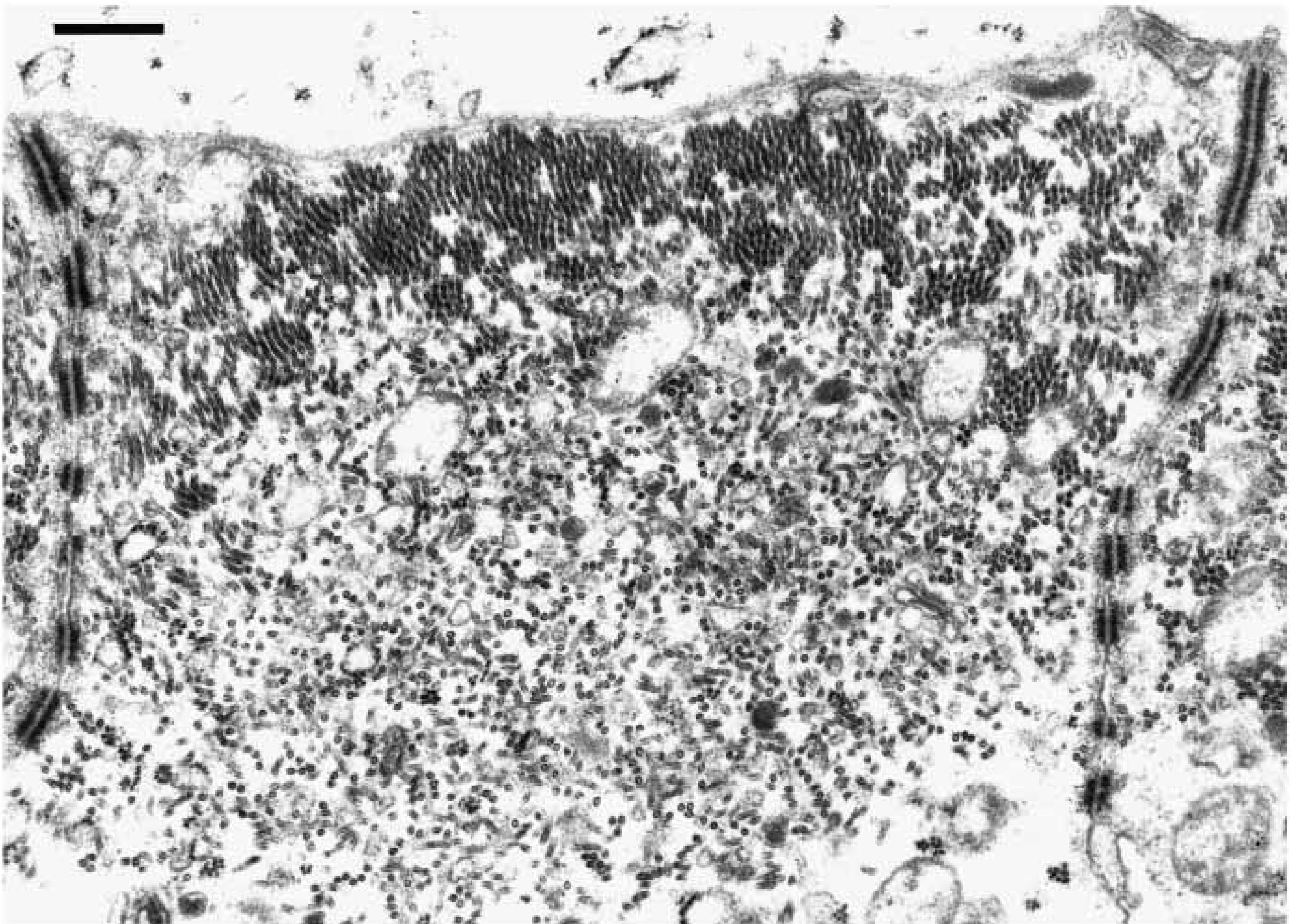

Fig. 11. Cross-section of a microtubule bundle on day 21 cut about 14 µm below the cell's apical surface. The section passes about 3 µm below the top of the basal microtubule array where its microtubules splay apart just below a region where they are associated with the outer side of the cell (see Fig. 1B). Microtubules in the transcellular array, which is positioned towards the top of the micrograph, are more closely packed together than those in the basal array. There is a much greater number of microtubules at this level than at more apically situated levels at this stage (compare with Fig. 10A). Bar, 0.5 µm.

Hence, many microtubules may be functionally redundant after bending has occurred, so that they are eliminated from the apical portion of a bundle as the maturing cell makes adjustments to meet new micromechanical requirements.

Thirdly, there is the possibility that there may not be a real surplus of microtubules near the centrosome during the early stages of bundle assembly. Subtraction of microtubules from the apical portion of a bundle may not involve disassembly. The 'missing' microtubules may have escaped from the centrosome and been captured at another location (see below). This possibility is compatible with studies of the replacement of microtubule arrays in several cell types after microtubule disassembly has been experimentally induced. Such studies have provided substantial evidence that centrosomally nucleated microtubules can be released for translocation to other cytoplasmic venues (Vorobjev and Chentsov, 1983; McBeath and Fujiwara, 1990; Yu et al., 1993).

Addition of microtubules at locations remote from the centrosome

As bundle assembly progresses, about 2,000 microtubules are added to the basal portion of a bundle and about 2,000 are subtracted from its apical portion. Hence, microtubules in the basal array may be nucleated by the centrosome and then translocated to lower levels. If this is occurring, then the minus ends of these microtubules escape from the centrosome but are subsequently captured at the cell surface at the level where they are attached to it at the top of the basal array (Fig. 1B). Evidence for plus end capture is well documented (see Mogensen et al., 1989). The events dealt with here raise the possibility that there are situations where minus end capture needs to be seriously considered.

If minus end escape and capture are not taking place, then each cell must possess at least one other major microtubule-nucleating site (which lacks centrioles) at a location that is remote with respect to its apical centrosome. Are the cell surface regions attached to the top and bottom of the basal microtubule array both capturing sites, or is at least one of them a nucleating site? Answers are being sought by assessments of: the assembly sequence during basal array construction, whether apical loss and basal addition of microtubules is temporally correlated, whether bundle microtubules all have the same polarity, and whether antibodies to centrosomal proteins bind to the two cell surface sites where microtubule ends are situated but where centrioles are lacking. The issues that are being investigated are not parochial ones confined to learning more about how microtubule assembly is controlled in inner pillar cells. For example, they are identical in general terms with those needing elucidation to comprehend control of the assembly of axonal and dendritic microtubules in neurons. These microtubules are also, eventually at least, remotely located with respect to the positioning of the neuronal centrosome (see Baas and Joshi, 1992).

We thank the Medical Research Council (UK) for support (grant nos. G8722419N, G9027373CB) and the Wellcome Trust for award of a Prize Studentship to C.G.H.

REFERENCES

Achler, C., Filmer, D., Merte, C. and Drenckhahn, D. (1989). Role of microtubules in polarized delivery of apical membrane proteins to the brush border of the intestinal epithelium. *J. Cell Biol.* **109**, 179-189.

Bacallao, R., Antony, C., Dotti, C., Karsenti, E., Stelzer, E. H. K. and Simons, K. (1989). The subcellular organization of Madin-Darby Canine Kidney cells during the formation of a polarized epithelium. *J. Cell Biol.* **109**, 2817-2832.

Baas, P. W. and Joshi, H. C. (1992). γ-Tubulin distribution in the neuron: implications for the origins of neuritic microtubules. *J. Cell Biol.* **119**, 171-178.

Bornens, M. and Karsenti, E. (1984). The centrosome. In *Membrane Structure and Function*, vol. 6 (ed. E. E. Bittar), pp. 99-171. John Wiley, New York.

Bré, M.-H., Kreis, T. E. and Karsenti, E. (1987). Control of microtubule nucleation and stability in Madin-Darby canine kidney cells: the occurrence of non-centrosomal, stable detyrosinated microtubules. *J. Cell Biol.* **105**, 1283-1296.

Brinkley, B. R. (1985). Microtubule organizing centers. *Annu. Rev. Cell Biol.* **1**, 145-172.

Engström, H. and Ades, H. W. (1973). The ultrastructure of the organ of Corti. In *The Ultrastructure of Sensory Organs* (ed. I. Friedmann), pp. 83-151. North Holland, Amsterdam.

Flock, Å., Bretscher, A. and Weber, K. (1982). Immunohistochemical localization of several cytoskeletal proteins in inner ear sensory and supporting cells. *Hearing Res.* **6**, 7-89.

Friedmann, I. and Ballantyne, J. (1984). *Ultrastructural Atlas of the Inner Ear.* Butterworths and Co. Ltd, London.

Gulley, R. L. and Reese, T. S. (1976). Intercellular junctions in the reticular lamina of the organ of Corti. *J. Neurocytol.* **5**, 479-507.

Held, H. (1926). Die Cochlea der Säuger und der Vögel, ihre Entwicklung und ihr Bau. In *Handbuch der Normalen und Pathologischen Physiologie, Receptionsorgane I,* vol. 11 (ed. A. Bethe, C. V. Bergmann, G. Emden and A. Ehinger), pp. 467-534. Springer-Verlag, Berlin.

Hudspeth, A. J. (1989). How the ear's works work. *Nature* **341**, 397-404.

Iurato, S. (1967). *Submicroscopic Structure of the Inner Ear.* Pergamon Press, Oxford.

Kalnins, V. I. (1992). *The Centrosome.* Academic Press Inc., San Diego, CA.

Kalt, A. and Schliwa, M. (1993). Molecular components of the centrosome. *Trends Cell Biol.* **3**, 118-128.

Kikuchi, T., Takasaka T., Tonosaki, A., Katori, Y. and Shinkawa, H. (1991). Microtubules of guinea pig cochlear epithelial cells. *Acta Otolaryngol.* **111**, 286-290.

Kimura, R. S. (1975). The ultrastructure of the organ of Corti. *Int. Rev. Cytol.* **42**, 173-222.

Kimble, M. and Kuriyama, R. (1992). Functional components of microtubule-organizing centers. *Int. Rev. Cytol.* **136**, 1-50.

Kirschner, M. and Schulze, E. (1986). Morphogenesis and the control of microtubule dynamics in cells. *J. Cell Sci. Suppl. 5*, 293-310.

Kronebusch, P. J. and Singer, S. J. (1987). The microtubule-organizing complex and the Golgi apparatus are co-localized around the entire nuclear envelope of interphasic cardiac myocytes. *J. Cell Sci.* **88**, 25-34.

Kuijpers, W., Tonnaer, E. L. G. M., Peters, T. A. and Ramaekers, F. C. S. (1991). Expression of intermediate filament proteins in the mature inner ear of the rat and guinea pig. *Hearing Res.* **52**, 133-146.

Mazia, D. (1987). The chromosome cycle and the centrosome cycle in the mitotic cycle. *Int. Rev. Cytol.* **100**, 49-92.

McBeath, E. and Fujiwara, K. (1990). Microtubule detachment from the microtubule-organizing center as a key event in the complete turnover of microtubules in cells. *Eur. J. Cell Biol.* **52**, 1-16.

McIntosh, J. R. (1983). The centrosome as an organizer of the cytoskeleton. Spatial organization of eukaryotic cells. In *Modern Cell Biology*, vol. 2 (ed. J. R. McIntosh,), pp. 115-142. Alan R. Liss, Inc., New York.

Mogensen, M. M. and Tucker, J. B. (1987). Evidence for microtubule nucleation at plasma membrane-associated sites in *Drosophila. J. Cell Sci.* **88**, 95-107.

Mogensen, M. M., Tucker, J. B. and Stebbings, H. (1989). Microtubule polarities indicate that nucleation and capture of microtubules occurs at cell surfaces in *Drosophila*. *J. Cell Biol.* **108**, 1445-1452.

Mogensen, M. M., Tucker, J. B. and Baggalay, T. B. (1993). Multiple plasma membrane-associated MTOC systems in the acentrosomal cone cells of *Drosophila* ommatidia. *Eur J. Cell Biol.* **60**, 67-75.

Pickles, J. O. (1988). *An Introduction to the Physiology of Hearing*, 2nd edn. Academic Press Inc., London.

Retzius, G. (1884). *Das Gehörorgan der Wirbelthiere, Morphologische-Histologische Studien. II. Das Gehörorgan der Reptilien, der Vögel und der Säugethiere*. Samson & Wallin, Stockholm.

Rieder, C. L. and Borisy, G. G. (1982). The centrosome cycle in PtK_2 cells:

asymmetric distribution and structural changes in the pericentriolar material. *Biol. Cell.* **44**, 117-132.

Slepecky, N. B. and Chamberlain, S. C. (1983). Distribution and polarity of actin in inner ear supporting cells. *Hearing Res.* **10**, 359-370.

Slepecky, N. B. and Ulfendahl, M. (1992). Actin-binding and microtubule-associated proteins in the organ of Corti. *Hearing Res.* **57**, 201-215.

Steyger, P. S., Furness, D. N., Hackney, C. M. and Richardson, G. P. (1989). Tubulin and microtubules in cochlear hair cells: comparative immunocytochemistry and ultrastructure. *Hearing Res.* **42**, 1-16.

Tassin, A-M., Maro, B. and Bornens, M. (1985). Fate of microtubule-organizing centers during myogenesis in vitro. *J. Cell Biol.* **100**, 35-46.

Tucker, J. B. (1979). Spatial organization of microtubules. In *Microtubules* (ed. K. Roberts and J. S. Hyams), pp. 315-357. Academic Press Inc., London.

Tucker, J. B. (1981). Cytoskeletal coordination and intercellular signalling during metazoan embryogenesis. *J. Embryol. Exp. Morph.* **65**, 1-25.

Tucker, J. B. (1984). Spatial organization of microtubule-organizing centres and microtubules. *J. Cell Biol.* **99**, 55s-62s.

Tucker, J. B. (1992). The microtubule-organizing centre. *BioEssays* **14**, 861-867.

Tucker, J. B., Paton, C. C., Richardson, G. P., Mogensen, M. M. and Russell, I. J. (1992). A cell surface-associated centrosomal layer of microtubule-organizing material in the inner pillar cell of the mouse cochlea. *J. Cell Sci.* **102**, 215-226.

Tucker, J. B., Paton, C. C., Henderson, C. G. and Mogensen, M. M. (1993). Microtubule rearrangement and bending during assembly of large curved microtubule bundles in mouse cochlear epithelial cells. *Cell Motil. Cytoskeleton.* **25**, 49-58.

Vandré, D. D. and Borisy, G. G. (1989). The centrosome cycle in animal cells. In *Mitosis. Molecules and Mechanisms* (ed. J. S. Hyams and B. R. Brinkley), pp. 39-75. Academic Press Inc., San Diego, CA.

Vorobjev, I. A. and Chentsov, Y. S. (1983). The dynamics of reconstitution of microtubules around the cell center after cooling. *Eur. J. Cell Biol.* **30**, 149-153.

Vorobjev, I. A. and Nadezhdina, E. S. (1987). The centrosome and its role in the organization of microtubules. *Int. Rev. Cytol.* **106**, 227-293.

Yu, W., Centonze, V. E., Ahmad, F. J. and Baas, P. W. (1993). Microtubule nucleation and release from the neuronal centrosome. *J. Cell Biol.* **122**, 349-359.

Wheatley, D. N. (1982). *The Centriole: A Central Enigma in Cell Biology*. Elsevier, Amsterdam.

(Received 18 August 1993 - Accepted, in revised form, 4 November 1993)