Three microtubule-organizing centres collaborate in a mouse cochlear epithelial cell during supracellularly coordinated control of microtubule positioning

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SUMMARY

Large cell surface-associated microtubule bundles that include about 3,000 microtubules assemble in certain epithelial cells called inner pillar cells in the mouse organ of Corti. Microtubule-organizing centres (MTOCs) at both ends and near the middle of each cell act in concert during control of microtubule positioning. In addition, the three cell surface-associated microtubule-organizing centres are involved in coordinating the connection of bundle microtubules to cytoskeletal components in neighbouring cells and to a basement membrane.

The precisely defined locations of the three MTOCs specify the cell surface regions where microtubule ends will finally be anchored. The MTOCs are modified as anchorage proceeds. Substantial fibrous meshworks assemble at the surface sites occupied by the MTOCs and link microtubule ends to cell junctions. This procedure also connects the microtubule bundle to cytoskeletal arrays in neighbouring cells at two of the MTOC sites, and to the basilar membrane (a substantial basement membrane) in the case of the third site. A fourth meshwork that is not positioned at a major MTOC site is involved in connecting one side of the microtubule bundle to the cytoskeletons of two other cell neighbours. The term surfoskelosome is suggested for such concentrations of specialized cytoskeletal materials and junctions at cell surface anchorages for cytoskeletal arrays.

The large microtubule bundle in each cell is composed of two closely aligned microtubule arrays. Bundle assembly begins with nucleation of microtubules by a centrosomal MTOC that is attached to the apical cell surface. These microtubules elongate downwards and the plus ends of many of them are apparently captured by a basal MTOC that is attached to the plasma membrane at the bottom of the cell. In the lower portion of the cell, the microtubule bundle also includes a basal array of microtubules but these elongate in the opposite direction. This investigation provides evidence that they extend upwards from the basal MTOC to be captured by a medial MTOC which is attached to the plasma membrane and situated near the mid-level of the cell. However, there are substantial indications that the basal array’s microtubules are also nucleated by the apically situated centrosomal MTOC, but escape from it, and are translocated downwards for capture of their plus ends by the basal MTOC. If this is the case, then these microtubules continue to elongate after translocation and extend back up to the medial MTOC, which captures their minus ends.

Key words: microtubule-organizing centre, centrosome, cell junction, cochlea, mouse

INTRODUCTION

The relative positioning of cytoskeletal components in neighbouring cells is often effected with considerable precision during tissue morphogenesis (see Tucker, 1981; Bard, 1990; Ingber et al., 1994). Such supracellular coordination plays an important role during control of tissue shaping (Tucker and Meats, 1976; Tucker et al., 1986; Priess and Hirsch, 1986; Fristrom et al., 1993) and/or is an essential functional require-
neighbouring cells. The spatial pattern of intercellular linkage is specified with great precision. This investigation was conducted to learn more about the progress and control of microtubule positioning when such specifications need to be effected. Furthermore, relatively little is known about microtubule assembly for tissue cells that are differentiating in situ and experiencing the particular microenvironmental cues provided by their usual contacts with cell neighbours and extracellular matrices. Do previously unsuspected procedures operate that have not been detected in studies of cultured tissue cells? This study reveals that control of microtubule positioning in terminally differentiating epithelial cells can sometimes involve a more sophisticated combination of mechanisms than has been generally appreciated.

Inner pillar cells are one of the organ’s several epithelial cell types that are referred to as supporting cells. These cells transmit vibrations from the basilar membrane (a highly specialized basement membrane) to the sensory hair cells during hearing (see Pickles, 1988; Hudspeth, 1989). Hair cells do not make direct contact with the basilar membrane (see Lim, 1986). Their cytoskeletons are linked to it via the large transcellular microtubule bundles and associated cell junctions in inner pillar cells and certain other types of supporting cells (Iurato, 1967; Engström and Ades, 1973; Kimura, 1975; Gulley and Reese, 1976; Slepecky and Chamberlain, 1983; Henderson et al., 1994). How is the spatially precise and supracelluarly coordinated deployment of microtubules and cell junctions achieved?

So far as inner pillar cells are concerned, supracellular coordination is facilitated because each cell has three major microtubule-organizing centres (MTOCs), which are located in particular cell surface regions (Henderson et al., 1994). This account deals with the modification of the cell surface-associated MTOCs as the three ends of each branched microtubule bundle, at the cell’s top, middle and bottom, are connected to the cytoskeletons in neighbouring hair cells, in other supporting cells, and to the basilar membrane, respectively. The involvement of the MTOCs during control of microtubule positioning has also been investigated.

One of the MTOCs is apically situated and corresponds to the main MTOC in animal tissue cells generally. It is a modified centrosomal region, which includes two centrioles and nucleates the assembly of about 3,000 microtubules when bundle construction is initiated (Tucker et al., 1992). However, the mature bundle includes a population of about 2,000 microtubules that is remotely located (more than 10 µm distant at its closest point) with respect to the centrosomal MTOC (Henderson et al., 1994). Have these microtubules escaped from the centrosome and been captured by the two other cell surface-associated MTOCs to which their ends are attached? Alternatively, does at least one of the non-centrosomal MTOCs provide the cell with a second major microtubule-nucleating site? These questions have been addressed in an examination of the microtubule assembly sequence in regions of the cell that are remotely located with respect to the centrosome.

**MATERIALS AND METHODS**

Inner pillar cells have been examined at 8, 9, 21 and 60 days after the birth of Swiss CD1 mice (the period 0-24 hours after birth = day 0, etc.). Such cells in adult Wistar rats and Hartley guinea pigs have also been studied. Each organ of Corti was prepared for electron microscopy using previously described procedures (Tucker et al., 1992; Henderson et al., 1994) after fixation in situ in a cochlea that had been dissected away from surrounding tissues. 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) and in addition, there is a graded baso-apical advance in the progress of morphogenesis during mammalian cochlear development (Lim and Rueda, 1992; Walsh and Romand, 1992).

**RESULTS**

**MTOCs and surfoskelosomes**

In the new-born mouse and for a period of about 7 days thereafter, an inner pillar cell has a simple columnar shape (Tucker et al., 1992). A large straight microtubule bundle has started to

![Fig. 1. Schematic diagram showing the shape of the mature inner pillar cell and the arrangement of the two arrays in its microtubule bundle. The outer side of the cell faces towards the right of the diagram. The flat basal surface of the cell (towards the bottom of the diagram) indicates the plane of the upper surface of the basilar membrane (not shown) on which it is situated. Black lines inside the cell show the orientation of microtubules and regions where high concentrations of microtubules occur (in much larger numbers than represented by the lines). The transcellular microtubule array is depicted by the continuous black lines and the basal array by the broken lines. Black rectangles show the position of the two centrosomal centrioles near the tip of the cell’s phalangeal process. Phalangeal (p), medial (m) and basal (b) surfoskelosomes assemble at sites where MTOCs are situated and anchor microtubule ends to the cell surface. The apical surfoskelosome (a) is attached to one side of the microtubule bundle. The number of microtubules/bundle cross-section has been assessed at levels 1-4 (see Fig. 10). Bar, 10 µm.**
elongate from an apical centrosomal nucleating-site by day 1; on day 6 it includes about 3,000 microtubules near its top. On 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 60° as the cell takes up its final flying buttress-like shape (Tucker et al., 1993). A cell

Fig. 2. Longitudinal section through the upper portions of cells in part of the organ of Corti in a mature rat showing the way in which inner pillar cells are associated with neighbouring cells. The section passes through dense junctional material between two adjacent pillar cells at the point indicated by the large arrow. A phalangeal surfoskelosome (p) is situated where an inner pillar cell contacts an outer hair cell (o). An apical surfoskelosome (a) is located where contact is made with an inner hair cell (i). A medial surfoskelosome (m) is positioned where contact is made with part of a very large surfoskelosome (op) in a neighbouring outer pillar cell. Microtubules at the top of the basal array (small arrows) splay away from those of the transcellular array at levels where they approach and penetrate the medial surfoskelosome. An intercellular space called the tunnel of Corti (t) is situated between much of the lower portions of inner and outer pillar cells. Bar, 2 µm.
Fig. 3. A cross-section through the curved portion of a mature inner pillar cell (day 21), which passes through its apical surfoskelosome (a). Cell junctions connect this cell to: the cuticular plates of two inner hair cells (i), surfoskelosomes in two outer pillar cells (op), and two neighbouring inner pillar cells (ip). Bar, 0.5 \( \mu \text{m} \).

Fig. 4. Longitudinal sections through the tips of phalangeal processes that each include part of an adjacent outer hair cell (o). The apical surfaces of the processes are oriented towards the tops of the micrographs. (A) Day 9. The minus ends of many microtubules are positioned closely against the cell surface at the tip of the process. One of the centrosomal centrioles is included near the bottom of the micrograph. Bar, 0.5 \( \mu \text{m} \). (B) Day 21. The phalangeal surfoskelosome (p) has assembled and connects microtubule ends to the compact fibrous cuticular plate of an outer hair cell (o) via cell junctions at the tip of the process. Bar, 0.2 \( \mu \text{m} \).
Microtubule assembly and anchorage

...extension called the phalangeal process is generated during this manoeuvre (Fig. 1). It projects in an outward direction (towards outer hair cells) (Fig. 2) in terms of the established convention for describing cell arrangement in the organ of Corti (see Lim, 1986). The two centrosomal centrioles are situated near the tip of the phalangeal process (Fig. 1).

Cell and cytoskeletal morphogenesis have been completed by day 21. Accounts of mature cell organization that follow are mainly based on examinations of 21-day-old cells. Such cells exhibit no marked structural differences compared with those of 2-month-old mice, and adult rats and guinea pigs, which have also been studied. With the exception of Fig. 2, all illustrations and results are based on studies of mouse cells.

Two substantial cavities are present on the outer side of a mature cell (Fig. 1). The upper concavity accommodates the rounded upper portion of another supporting cell type (outer pillar cell). The lower concavity flanks an intercellular space called the tunnel of Corti, which separates much of the lower portions of adjacent inner and outer pillar cells (Fig. 2).

In addition to the apical centrosome there are two other MTOCs. They are located near the cell’s mid-level (the medial MTOC) and base (the basal MTOC) at sites where microtubule ends are close to the cell surface. However, meshworks of dense material become concentrated at all three of these organizing sites (Fig. 1) and connect microtubule ends to cell...
junctions. Each of these substantial complexes of dense meshwork material and associated cell junctional components will be referred to as a surfoskelosome (SSS).

The mature microtubule bundle consists of two distinct microtubule arrays (Fig. 1). A transcellular array spans the curved longitudinal axis of a cell from a phalangeal SSS to a basal SSS. The phalangeal SSS occupies much of the region where the centrosomal MTOC is situated. Unlike the other SSSs, the basal SSS is a well documented structural feature of the cell, which is known as the basal cone (see Angelborg and Engström, 1972). A basal microtubule array is confined to the lower portion of a cell; it runs between a medial SSS and the basal SSS.

In a mature cell the microtubule bundle is connected by SSSs to neighbouring cells (Fig. 2). There is a single row of inner pillar cells in the organ of Corti. The inner sides of the apical portions of inner pillar cells are situated against a row of inner hair cells and their outer sides are positioned against a row of outer pillar cells. Cells in adjacent pillar and hair cell rows are off-set like rows of bricks in a wall. Hence, each inner pillar cell contacts two cells in each cell row to either side of it (Fig. 3). The medial SSS connects to two outer pillar cells. This SSS also connects to the medial SSSs of its two inner pillar cell neighbours because it runs right across a cell (in a

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**Fig. 7.** Microtubule number and arrangement at level 3 (Fig. 6) on day 9. The positions of cross-sectional profiles of microtubules are shown by the black dots (for clarity these have been drawn disproportionately large). The cross-sectional profile of the cell (black line) is oriented with its inner side at the top of the diagram. The cell sides, which are juxtaposed against adjacent inner pillar cells in the same pillar cell row are at the sides of the diagram. The diagram was prepared from an electron micrograph of a cross-section of a cell cut at a level about halfway along the length of its basal array (level 3 in Fig. 6); this array’s microtubules are near the middle of the diagram and in general are less closely grouped together than most microtubules in the transcellular array (towards the top of the diagram). Bar, 1 μm.

**Fig. 8.** Longitudinal section through part of the base of a mature cell (day 21) and the upper surface of the basilar membrane (b). The section grazes through the edge of the basal surfoskelosome where its fibrous material is associated with the ends of microtubules that are also located close to a layer of dense material (small arrows) that coats the cytoplasmic surface of the basal plasma membrane. The transcellular array runs alongside the inner side of the cell (towards the left side of the micrograph); most of its microtubules (large arrow) are more closely grouped together than those of the basal array (in the middle and to the right of the micrograph). Bar, 0.5 μm.
Microtubule assembly and anchorage

direction parallel to the inner pillar cell row). The apical SSS is not positioned at the end of a microtubule array (Fig. 1) and hence, does not occupy a major MTOC site. It connects to two inner hair cells. The phalangeal process extends over the tops of neighbouring outer pillar cells and the phalangeal SSS connects to two outer hair cells (Fig. 2).

An inner pillar cell’s SSSs are mainly connected to those of neighbouring cells by a type of junction that apparently only occurs in the mammalian organ of Corti. Dense material on the cytoplasmic surfaces of a junction’s plasma membranes has a beaded appearance (Fig. 3). Such junctions are evident in micrographs of pillar cells published by others (see Introduction).

Microtubule anchorage in the apical portion of a cell

Most of the main features of the assembly sequence for the centrosomally nucleated apical portion of the microtubule bundle have been described previously (Tucker et al., 1992, 1993; Henderson et al., 1994).

There is considerable variation in the distance separating the minus ends of centrosomally nucleated microtubules from the tip of the phalangeal process on day 9. In many cells they are separated by a gap of up to 0.7 μm (Henderson et al., 1994), but in others the ends are closely applied to junctions that link the tip of the process to outer hair cells (Fig. 4A). Most minus ends are not so closely applied to the cell surface after assembly of the phalangeal SSS (Fig. 4B). Hence, having approached from their initial locations about 1 μm away from the cell surface region in question (Henderson et al., 1994), minus ends must sometimes be separated by the process to permit interpolation of a compact slab-like concentration of surfoskeletal material.

The apical SSS is located between junctions with inner hair cells and the upper side of part of the curved portion of the bundle’s transcellular microtubule array (Figs 1, 2, 3, 5). A few microtubules run into the SSS (Fig. 5). Whether such microtubules terminate within the SSS, or pass right through it, has not been ascertained.

Microtubule assembly in the lower portion of a cell

The portion of a cell that is located below the level where the top of the medial SSS is eventually situated (Fig. 1) will be
referred to as its lower portion. By day 9, assembly of both of a bundle’s main microtubule arrays is progressing in the lower portion of a cell (Fig. 6). Most of the microtubules in the transcellular array are situated towards the inner side of the cell and are more closely packed together than those in the basal array, which flanks its outer side (Fig. 7). There are 1,152 microtubules on average in the transcellular array at level 1 which is 4 μm above level 2 at which the top of the basal array is finally situated (Fig. 6). There are similar numbers of microtubules in the array at level 2 (1,035 on average) and 12 μm lower down at level 3 (1,171 on average); (Fig. 6). However, the number of microtubules progressively decreases below level 3 until at the most basal levels in a cell (those where the nucleus is situated, such as level 4 in Fig. 6) the closely packed groupings typical of the transcellular array at higher levels are absent. By day 21, the basal ends of many of the arrays’ microtubules either contact the basal plasma membrane or are situated within a few nanometres of it (Fig. 8). Hence, the transcellular array is still elongating towards the cell base on day 9, which is more than a week after nucleation of its microtubules was initiated by the apically located centrosomal MTOC on day 1 (Tucker et al., 1992).

The basal microtubule array is apparently elongating in the opposite direction to the transcellular array on day 9. The basal array includes progressively greater numbers of microtubules as the base of the cell is approached, in contrast to the decrease in microtubule number for the transcellular array (Fig. 9). At the level just below that at which the top of the basal array is finally located (Fig. 6, level 2) an average of 108 relatively widely spaced microtubules flank the outer side of the transcellular array. The basal array includes 917 and 1,429 microtubules on average at levels 21 μm and 6 μm above the cell base, respectively (Fig. 9). Hence, on day 9, the number of microtubules/bundle cross-section is greatest near level 3, where both of the elongating arrays include substantial

**Fig. 11.** Cross-section of a mature microtubule bundle cut about halfway down a mature cell (day 21) at level 3 as marked in Fig. 1. The inner side of the cell and the transcellular microtubule array are oriented towards the top of the micrograph. Most of the basal array’s microtubules are situated in the somewhat less closely associated groupings (arrows) in the lower portion of the micrograph. Bar, 0.5 μm.

**Fig. 12.** Longitudinal section through part of the base of a cell and the transient microtubule array that is oriented parallel to the direction in which the cell base is increasing in breadth on day 9 (and as depicted in Fig. 6). Part of the nucleus (n) and basilar membrane (b) are also shown. Bar, 0.5 μm.
numbers of microtubules in a region where they overlap and seem to be growing past each other (Figs 6, 7, 9).

Cross-sectional analyses of cells on day 8 reveal a similar spatial microtubule distribution to that reported above. Both arrays include fewer microtubules than on day 9. Microtubule number decreases apicobasally in the transcellular array, and basoapically in the basal array, as on day 9.

The account of microtubule elongation, and deployment on day 9 as summarized in Figs 6 and 9, which has been presented above makes an important assumption. It assumes that the apicobasal increase in microtubule number in the basal array on day 9 is not due to microtubules splaying out from the more compact groupings of microtubules in the transcellular array. The assumption has been made because such splaying cannot account for the increasingly greater number of microtubules in the basal array between levels 2 and 3. For example, there are 917 microtubules on average in the basal array at level 3 but there is no decrease in the number of microtubules in the transcellular array at this level compared with the more apical levels 1 and 2 (Fig. 9). Splaying of the transcellular arrays’ microtubules below level 3 on day 9 seems unlikely because the basal portion of the array is largely composed of compactly grouped microtubules on day 21 (Fig. 8).

There is another aspect of the cross-sectional analysis of microtubule deployment on day 9 that is not completely straightforward. Assessment of which of the two arrays a particular microtubule belongs to is not an unequivocal procedure in regions where the two arrays are juxtaposed. There is no clear-cut boundary in terms of microtubule packing and spacing (Fig. 7). Hence, although the values presented above for the total number of microtubules/bundle are accurate, those for microtubule number/array are less so, but they provide a useful indication of the marked changes in number along the lengths of the arrays (Fig. 9). For example, and importantly, the basal array definitely includes relatively few microtubules at level 2 close to where its top is finally situated (about 100

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**Fig. 13.** Longitudinal section through part of an inner pillar cell (day 9) and its microtubules cut near the level marked 2 in Fig. 6. Microtubules in the transcellular array run along the left side of the micrograph. Tufts of fibrous material (arrows) are present at the site of the medial MTOC and are associated with the upper ends of some of the microtubules of the basal microtubule array (which have started to populate this level by day 9). The tufts represent an early stage in the assembly of the medial surfoskelosome that is involved in linking these microtubules to the surface of an adjacent outer pillar cell (op). The convoluted plasma membrane profiles towards the right side of the micrograph are situated at levels where the top of a large intercellular space (the tunnel of Corti, see Fig. 2) is opening up between the two types of pillar cells. Bar, 0.5 µm.
where it extends above the top of the basal array. It included tubules in the mature transcellular array were detected at levels (Figs 1, 2). No substantial variations in the number of micro -
scellular array, where they approach the medial and basal SSSs of the basal array splay apart slightly, and away from the tran -
a square packing pattern. Microtubules at the top and bottom of a highly regular nature; in most regions it approximates to
the inner side of the cell (Fig. 1). Microtubule packing is not located in the less highly fasiculated part of the bundle towards
bundle; most of the transcellular array’s microtubules are included in groupings that contribute to the outer side of the
Figs 7, 11). Most of the basal array’s microtubules are mainly distinguished from each other as they can on day 9 (compare
microtubular members of the two arrays cannot be so readily distinguished from each other as they can on day 9 (compare
Figs 7, 11). Most of the basal array’s microtubules are mainly included in groupings that contribute to the outer side of the
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cellular array, where they approach the medial and basal SSSs (Figs 1, 2). No substantial variations in the number of micro -
tubules in the mature transcellular array were detected at levels where it extends above the top of the basal array. It included

Microtubule anchorage in the lower portion of a cell
Early stages in the assembly of the medial SSS can be detected on day 9. Pointed tufts of dense material project from cell
junctions at the site where this SSS is subsequently located. Some of the tufts contact the tops of microtubules in the basal
array, which are starting to populate this region (Fig. 13).

The mature medial SSS (Fig. 1) is positioned against cell junctions that connect it to large SSSs in the two neighbouring
outer pillar cells (Fig. 2). Most of the microtubules at the top of the basal array penetrate into the dense material of the
medial SSS (Figs 2, 14). The ends of most microtubules do not make direct contact with the cell junctions. Connection is
affected indirectly by the meshwork of dense material in which the ends of the microtubules are embedded.

Tufts and irregularly shaped clumps of dense material project from the plasma membrane at the base of a cell on day
9 in the region where the bottom of the basal SSS is assembling. This region includes the locality that is being populated
by microtubules at the bottom of the assembling basal microtubule array (Fig. 15). Tufts of dense material that are very
similar to those described above for the medial and basal SSSs also project from cell junctions at the site where the apical SSS
is assembling on day 9.

The mature basal SSS has a compact cone-shaped central core of dense material. Very few microtubules penetrate this
cone (Fig. 16). Most microtubules are splayed around the sides of the cone (Fig. 1) but are connected to it by an intermicro -
tubular meshwork of dense material that is more finely divided than that in the cone (Fig. 16). The transcellular array’s micro -
tubules are mainly situated around the inner side of the central cone and those of the basal array around its outer side (Fig. 8).
The basal ends of many microtubules are positioned within about 100 nm of the plasma membrane where they contact a
layer of dense material that coats the cytoplasmic surface of the membrane. This portion of the basal cell surface presumably
functions in a fashion analogous to a large hemidesmosome or focal contact and helps to anchor the base of the cell
and its microtubule bundle to the basilar membrane (Fig. 8).

The fabric of the basal SSS’s central cone-shaped meshwork of dense material is not uniform. There are several irregularly
shaped regions of greater density where its fibrous meshwork either has a different composition from elsewhere and/or where
its components are clumped together in a more compact fashion (Fig. 16). The appearance of these regions resembles
that of similar regions in the cell’s other SSSs and in the large apically situated SSS of each outer pillar cell (compare Figs 2,
4B, 5, 16). The sizes and densities of these especially compact

![Fig. 14. Cross-section near the top of a mature basal array (day 21) where many of its microtubules are embedded in the fibrous meshwork of the medial surfoskeleton. Bar, 0.2 µm.](image)
clumps of surfoskelosomal material are usually greater in more mature (60-day-old) mice than in 21-day-old pups.

**DISCUSSION**

**Functional roles of the three MTOCs**

Three MTOCs collaborate during control of microtubule bundle assembly in inner pillar cells. To which functional category (nucleating site or capturing site) does each of these MTOCs belong?

The apically situated centrosomal MTOC nucleates microtubules that contribute to the transcellular array and elongate towards the basal MTOC (Tucker et al., 1992; Henderson et al., 1994). This investigation has provided evidence that the apical ends of many of the basal array’s microtubules are captured by the medial MTOC. Does the basal MTOC have a dual function? Does it capture the ends of the transcellular array’s microtubules that have elongated down from the centrosome and also nucleate microtubules for the basal array, which elongates upwards? There is an alternative possibility. The basal MTOC may only act as a capturing site because all the bundle’s microtubules are nucleated by the centrosomal MTOC. Basal array microtubules might escape from the centrosome, migrate down to the basal MTOC for plus end capture, but continue elongating after this until their upper minus ends are captured by the medial MTOC (Fig. 17). Such elongation could continue to be effected by plus end addition of tubulin after these ends have been captured, since microtubule polymerization apparently proceeds at plus ends that have been captured by kinetochores (Mitchison, 1989).

Much evidence supports a translocation/elongation hypothesis to account for the generation of microtubule arrays in neuronal axons that are remotely located with respect to the centrosomal nucleating sites (see Joshi and Baas, 1993; Yu et al., 1993). Evidence that supports a very similar assembly scheme for inner pillar cells (Fig. 17) is outlined below.

**Escape and capture**

About 2,000 microtubules are required for assembly of the basal microtubule array. About 2,000 microtubules are subtracted from the apically situated centrosomal end of a bundle (Henderson et al., 1994). Exact temporal correlation of these two events remains to be demonstrated but they both progress at about the same time during the lengthy period (12 days or so) of bundle construction. For example, the basal microtubule array has started to assemble by day 8 and it includes about 900 microtubules on day 9. By day 10, about 1,200 microtubules have been subtracted from the centrosomal end of the bundle (Henderson et al., 1994).

The basal microtubule array is attached to the cell surface at both ends. If its microtubules have escaped from the centrosome, then it provides an example of the capture of the minus ends of microtubules (at the medial MTOC) as well as that of

![Fig. 15. Longitudinal section through part of the base of a differentiating cell (day 9), the basal microtubule array, and the upper surface of the basilar membrane (b). Tufts of fibrous material (arrows) that project from the basal plasma membrane are associated with the basal ends of some of the microtubules. These tufts represent an early stage in the assembly of the basal surfoskelosome. Bar, 0.5 µm.](image-url)
the capture of plus ends (at the basal MTOC). Plus end capture has been described for other cell types (see Mogensen et al., 1989) but minus end capture has not.

The conclusions presented above are based on data obtained by a procedure that provides only snapshots of microtubule arrangement at certain stages during an assembly sequence that progresses for several days. However, the assembling microtubules are likely to be dynamically unstable and their lengths may fluctuate markedly within periods of a few minutes (see Kirschner and Schulze, 1986; Holy and Liebler, 1994). No information has been obtained about any exploratory probing and shrinkage that may be performed as microtubules search for the three main target sites at the cell surface. To what extent might the assembly sequence depicted in Fig. 17 need to be reinterpreted if such information were available? How valid is the suggestion that two capturing sites are present? If the microtubules are in such a marked state of flux that snapshots of the assembly sequence are not meaningful, then snapshots obtained at a particular assembly stage, for the same region of different cells, should reveal substantial variations in microtubule number and arrangement. Such variation is not evident. Nor should the steady temporal increases in the numbers of microtubules populating most cell regions have been found. Furthermore, the relatively slow build-up described for microtubules at the two sites where capture is suggested to be occurring, compared with the rapid establishment of a high microtubule concentration near the centrosomal nucleating region (Tucker et al., 1992; Henderson et al., 1994), is compatible with a sequence in which progressively greater numbers of dynamically unstable microtubules are captured and stabilized.

The escape-and-capture hypothesis (Fig. 17) can be tested using tubulin hook decoration to reveal microtubule polarities.

Fig. 16. Cross-section of a mature basal surfoskelosome (day 21). Very few microtubules are embedded in the central core of compact fibrous material in contrast to the situation in the medial surfoskelosome (see Fig. 14). Surrounding this core is somewhat more finely divided fibrous material, which interconnects microtubules and joins them to the core. Bar, 0.2 µm.
Nucleation and capture by cell surface-associated MTOCs evidently plays a key role in the supracellular coordination of microtubule positioning in the organ of Corti. The big challenge now is to learn how positional information is interpreted to define the spatial organization of the organizing sites.

**Surfoskelosomal organization**

The ultrastructural appearance of the fabric of all four SSSs is similar although their overall shapes differ. They all connect the microtubule bundle to the cell surface. Hence, it is sensible and useful to use a common collective term for these components, which seem to be related so far as function and composition are concerned. The term *surfoskelosome* is suggested for each such cell *surface*-associated *cytoskeletal* body (Gk *soma*) that includes components associated with one or more cell junctions.

It is not the authors’ intention that the well established term basal cone (see Angelborg and Engström, 1972) should be superseded by basal SSS, except in cases where it is particularly useful to draw attention to its relationship to the other SSSs. The term cone is inappropriate for the phalangeal, apical and medial SSSs which are shaped like slabs, triangular prisms and wedges, respectively.

The surfoskelosomes contain β- and γ- non-muscle actin isoforms (Slepecky and Savage, 1994) and α-actinin (Drenkhahn et al., 1985). There are indications that they are associated with intermediate filaments (Oesterle et al., 1990; Kuipers et al., 1991). To some extent, pillar cell SSSs ultrastructurally resemble the SSSs at the apical surfaces of hair cells to which they are connected by cell junctions. The hair cell SSSs are called cuticular plates and include actin, α-actinin, fodrin, profilin, myosin and tropomyosin (see Drenkhahn et al., 1985; Slepecky and Ulfendahl, 1992; Ylikowski et al., 1992).

The supracellular deployment and intercellular continuity of apically situated dense stiffening material in the mammalian organ of Corti has long been appreciated by investigator of its structure and function. The apical portions of supporting and hair cells collectively contribute to a dense sturdy layer called the reticular lamina (see Gulley and Reese, 1976). It is the high concentration of SSSs and associated microtubule bundles that imparts the dense laminar appearance documented in light microscopical accounts of the organ’s histology.

The inner pillar cell SSSs of other mammals are evident in electron micrographs published by others (Engström and Ades, 1973; Kimura, 1975, 1984; Gulley and Reese, 1976; Slepecky and Chamberlain, 1983). Such micrographs show that the SSSs have positions, shapes and ultrastructural textures that closely resemble those described here for the mouse.

**Surfoskelosomal assembly**

So far as the apical, medial and basal SSSs are concerned, assembly involves the formation of several fibrous tufts for each SSS, which build out from the plasma membrane and its junctions at the site where a mature SSS will be attached to the cell surface. The single fibrous mass of a mature SSS presumably results because more fibrous material accumulates around the tufts until they fuse together. Such assembly progresses while microtubules are assembling near the cell surface regions in question. It is possible that the fibrous material is transported.
to such regions along these microtubules. However, most microtubules do not penetrate the compact central regions of SSSs, which effectively act as ‘microtubule-avoiding centres’. Those microtubules that are centrally embedded probably occupied such regions before much fibrous material had accumulated. For example, the compact central core of the basal SSS occupies a region between the bottoms of the transcellular and basal microtubule arrays. This SSS starts to assemble before most of the transcellular array’s microtubules have reached the cell base.

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