# Microtubule Polarities Indicate that Nucleation and Capture of Microtubules Occurs at Cell Surfaces in *Drosophila*

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*Abstract.* Hook decoration with pig brain tubulin was used to assess the polarity of microtubules which mainly have 15 protofilaments in the transcellular bundles of late pupal *Drosophila* wing epidermal cells. The microtubules make end-on contact with cell surfaces. Most microtubules in each bundle exhibited a uniform polarity. They were oriented with their minus ends associated with their hemidesmosomal anchorage points at the apical cuticle-secreting surfaces of the cells. Plus ends were directed towards, and were sometimes connected to, basal attachment desmosomes at the opposite ends of the cells.

The orientation of microtubules at cell apices, with minus ends directed towards the cell surface, is opposite to the polarity anticipated for microtubules which have elongated centrifugally from centrosomes. It is consistent, however, with evidence that microtubule assembly is nucleated by plasma membrane-associated sites at the apical surfaces of the cells (Mogensen, M. M., and J. B. Tucker. 1987. *J. Cell Sci.* 88:95–107) after these cells have lost their centriole-containing, centrosomal, microtubule-organizing centers (Tucker, J. B., M. J. Milner, D. A. Currie, J. W. Muir, D. A. Forrest, and M.-J. Spencer. 1986. *Eur. J. Cell Biol.* 41:279–289). Our findings indicate that the plus ends of many of these apically nucleated microtubules are captured by the basal desmosomes. Hence, the situation may be analogous to the polar-nucleation/chromosomal-capture scheme for kinetochore microtubule assembly in mitotic and meiotic spindles. The cell surface–associated nucleation–elongation–capture mechanism proposed here may also apply during assembly of transcellular microtubule arrays in certain other animal tissue cell types.

DURING the final stages of *Drosophila* wing morphogenesis, a large transcellular microtubule bundle (≤1,500 microtubules/bundle) assembles in each trichome-bearing wing epidermal cell (the major wing cell type; there are ~30,000 such cells per wing). Assembly takes place after the cells have apparently lost their centriole-containing centrosomes (38). Such centrosomes are generally considered to be the main microtubule-organizing centers in metazoan tissue cells (see references 2, 4, 23, and 37) and they evidently nucleate microtubule assembly during early stages of *Drosophila* embryogenesis (14, 19, 31, 40).

Ultrastructural analyses of early stages in assembly of the wing cell microtubule bundles indicate that most microtubules elongate from the apical end of each cell, where their assembly is apparently nucleated by numerous plasma membrane-associated plaques (26). Assessments of microtubule polarity based on application of the tubulin hook-decoration technique to several cell types have shown that most microtubules are oriented with their minus ends associated with a microtubule-organizing center, so that their plus (fast-growing) ends are distal to centers of this type, such as centrosomes, and project outwards towards the cell periphery (11, 12, 25). The investigation of microtubule polarity reported here was undertaken to test a prediction based on our previous studies; namely, that microtubules in the transcellular bundles of wing cells are oriented with their minus ends in contact with the apical surfaces of the cells and therefore exhibit the opposite polarity to that anticipated if the microtubules elongate from centrosomes to contact the cell periphery.

Hook decoration has also been undertaken to explore the question of whether transcellular bundles represent single populations of apically nucleated microtubules of uniform polarity that are captured by the large attachment desmosomes to which they are anchored (26, 38) at cell bases. Do the desmosomes act rather like giant kinetochores and capture (see references 12 and 17) the plus ends of microtubules? Alternatively, are bundles composed of two interdigitating sets of antiparallel microtubules that elongate from surface nucleating sites at opposite ends of a cell in a similar fashion to the coordinated bipolar nucleations used during spindle assembly (see reference 12)? These issues are of general importance for a number of reasons.

It is becoming increasingly apparent that naturally occurring microtubule nucleation can take place at sites which are not associated with centrally positioned, centriole-containing centrosomes in some metazoan cell types (for example, references 3, 6, 16, 20, 21, 29, 33, and 34). In most but not

all of these instances, the cells in question lack, or are losing, centriole-containing centrosomes. So far as certain myocytes and myotubal syncitia are concerned, microtubule nucleation seems to take place at sites associated with the outer surfaces of nuclear envelopes (21, 34), while in mouse oocytes microtubules are apparently nucleated by sites associated with acentriolar clumps of material which have an appearance similar to that of the pericentriolar material of centrosomes (33). In these three cases, the sites include a component which is serologically related to one in centrosomal pericentriolar material. Studies of transcellular microtubule bundles in *Drosophila* (26, 38) raise the possibility that certain metazoan tissue cells can also nucleate microtubule assembly at sites associated with the plasma membrane and capture microtubule ends at other cell surface sites. This possibility has wide-ranging implications for the potential involvement of surface contact interactions between cell neighbors and with the extracellular matrix, and involvement of surface receptor-mediated responses to signals originating from more distant sources, during control of microtubule assembly (36).

Most of the microtubules in the transcellular bundles have 15 protofilaments (38). Although microtubules with more or less than the usual 13 protofilaments have been detected in certain cell types in four animal phyla including mammals (see reference 9), in no case have the sites for nucleation of such microtubules been definitely identified. Furthermore, it has yet to be ascertained whether microtubules with more or less than 13 protofilaments can be hook decorated with exogenous tubulin. Admittedly, there is no obvious reason to suppose that this cannot be accomplished. However, it is well worth finding out, bearing in mind the potential value of this procedure for assessing polarity and obtaining evidence concerning the direction of elongation and likely location of nucleating sites, for what are probably rather highly specialized types of microtubules.

## Materials and Methods

### Preparation of Microtubule Protein

Pig brain microtubule protein was prepared by two cycles of depolymerization and polymerization and was stored before use at −70°C as described elsewhere (32). Aliquots of microtubule protein were thawed immediately before use and adjusted to give a final concentration of 1–2 mg/ml of protein in a buffer (0.5 M Pipes, pH 6.9) containing 1 mM EDTA and 1 mM $MgCl_2$.

### Preparation of Wing Blades

Developing wings of *Drosophila melanogaster* (Oregon S) were dissected from pupae (that had been maintained at 25°C) after immersion of pupae in a *Drosophila* tissue-culture medium and at a point 87 h after the start of pupariation, as described previously (38). At this stage, two or three small cuts were made in each wing with tungsten needles. Each cut was 100–150-μm long, and passed right through the thickness of a wing blade from a point on its margin towards its midregion. These cuts were effected to facilitate penetration by solutions because wings had started to secrete the highly impermeable adult cuticle.

### Hook Decoration

Hook decoration was carried out essentially using the procedure described by Euteneur and McIntosh (10). Two modifications were used.

Procedure 1 was as follows. Freshly isolated wings were rinsed for 1 minute at 37°C in a buffer (0.5 M Pipes, pH 6.9) containing 1 mM EDTA, 1 mM $MgCl_2$, and 1 mM GTP. After this, wings were extracted for 20 min at 37°C in a buffer (0.5 M Pipes, pH 6.9) containing 1% Triton X-165, 0.5% sodium deoxycholate, 0.02% SDS, 1 mM EDTA, 1 mM $MgCl_2$, 3.5% DMSO, and 1 mM GTP. This was followed by two 1-min rinses as described above. Wings were then incubated for 5 min at 4°C, followed by 1 h at 37°C in a decoration buffer (0.5 M Pipes, pH 6.9) containing 1 mM EDTA, 1 mM $MgCl_2$, 3.5% DMSO, 1 mM GTP, and microtubule protein (1 or 2 mg/ml). Subsequently, wings were fixed for 30 min in 2% glutaraldehyde in a buffer (0.1 M Pipes, pH 6.9) containing 1 mM EDTA, 1 mM $MgCl_2$, 1 mM GTP, and 1% tannic acid. After two 1-min rinses in buffer (0.1 M Pipes, pH 6.9) containing 1 mM EDTA and 1 mM $MgCl_2$, they were fixed in 1% osmium tetroxide dissolved in this buffer, dehydrated in ethanol, and embedded in Araldite resin.

Procedure 2 was as described above except that instead of being incubated in a decoration buffer after incubation in a separate extraction buffer, wings were incubated for 30 min at 37°C in an extraction/decoration buffer (0.5 M Pipes, pH 6.9) containing 1 mM EDTA, 1 mM $MgCl_2$, 1% Triton X-165, 0.5% sodium deoxycholate, 0.02% SDS, 2.5% DMSO, 1 mM GTP, and microtubule protein (2 mg/ml). Procedure 1 provided higher yields of hook-decorated microtubules than procedure 2 (see below).

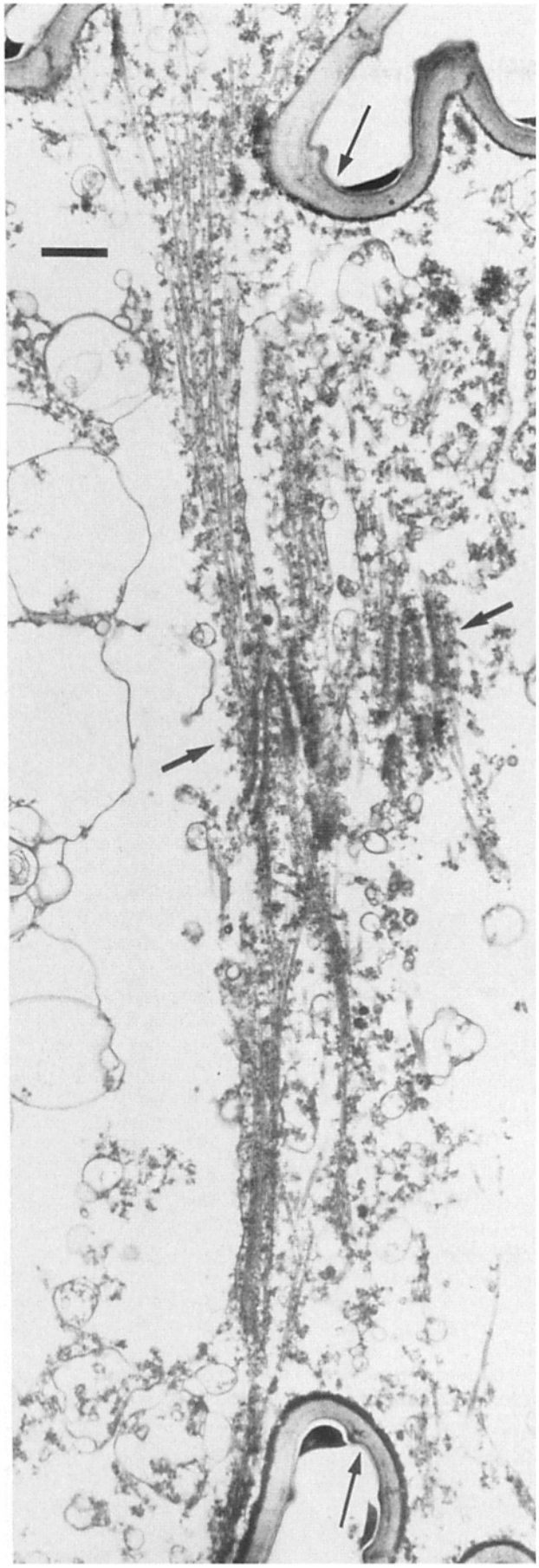
## Results

### Microtubule Arrangement in Extracted Wings

Microtubule arrangement and cell-surface association in *Drosophila* wing epidermal cells containing mature transcellular microtubule bundles fixed 87 h after the start of pupariation has been described elsewhere (26). It is outlined here so that the extent to which the extraction/decoration procedure perturbs normal microtubule arrangement can be appreciated. This is essential for interpretation of the microtubule polarities found in these preparations.

During the final stages of wing morphogenesis each wing blade consists mainly of two layers of trichome-bearing epidermal cells (a dorsal layer and a ventral one). A transcellular microtubule bundle spans the longitudinal axis of each cell. The apical ends of the apically branched bundles make end-on contact with the cuticle-secreting surfaces of cells where the microtubules are attached to hemidesmosome-like structures. The basal portion of a bundle projects from the cell body into a slender basal cell extension. The ends of microtubules at the base of a bundle are connected to an attachment desmosome complex. Each desmosome complex unites a pair of basal extensions, one from a dorsal epidermal cell and the other from a ventral epidermal cell. Hence pairs of transcellular microtubule bundles are united via attachment desmosomes to form transalar microtubule arrays that run right across a developing wing blade.

Thin (50-nm) sections of ten developing wing blades that had been subjected to the hook-decoration procedures and fixed 87 h after the start of pupariation were examined. The plasma membranes of epidermal cells had been extensively fragmented. Nevertheless, most of the transcellular microtubule bundles remained with their longitudinal axes oriented perpendicular to the cuticular surfaces of wing blades, and retained their transalar arrangement (Fig. 1). The basal ends of bundles were still interconnected by remnants of extracted, basal-attachment, desmosome complexes (Fig. 1, *short arrows*). The apical ends of microtubules were often associated with clumps of dense material (Fig. 2, *arrows*). Such clumps presumably represent remnants of the apical hemidesmosomal anchor points which frequently remained close to their original locations. Some were still associated with the inner surface of the imaginal cuticle by fine dense strands (Fig. 2).



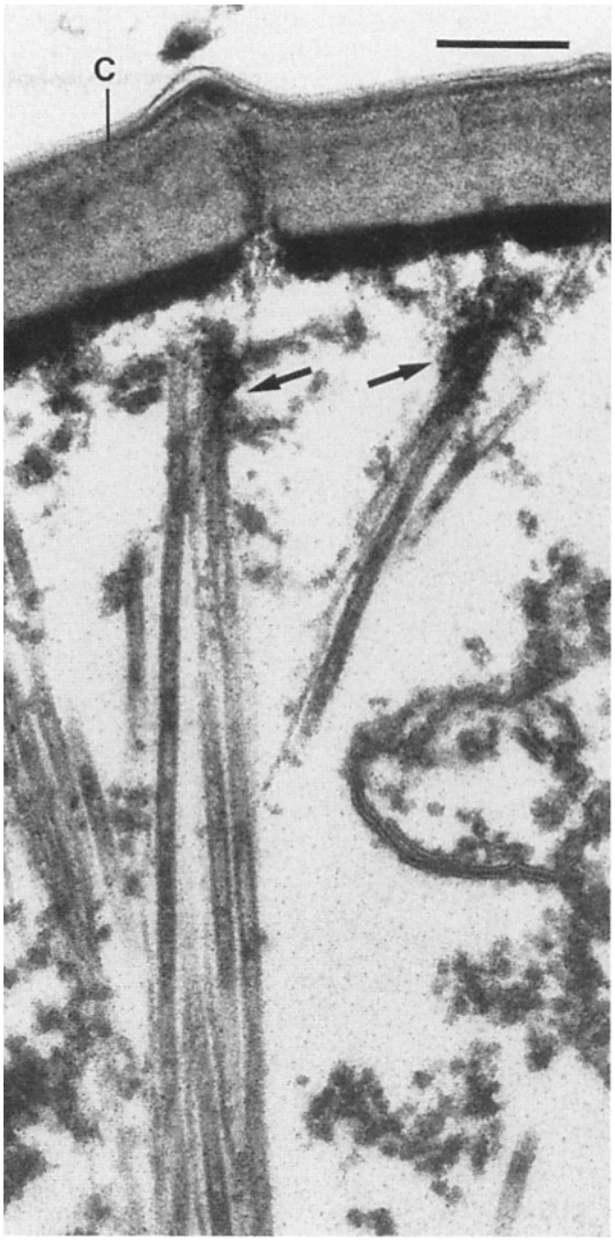



*Figure 2.* Longitudinal section through part of the apical end of a transcellular microtubule bundle in a wing prepared using hook-decoration procedure 2. Some of the dense remnants (*arrows*) of the hemidesmosomal anchorages for the apical ends of microtubules are still attached to the cuticle (*C*). Bar, 0.2 $\mu$m.

*Figure 1.* Part of a wing blade prepared using hook-decoration procedure 1 and cut at right angles to the plane of the blade. A pair of longitudinally sectioned transcellular microtubule bundles are connected by a partially extracted basal attachment desmosome complex (*short arrows*) and have retained their transalar arrangement. The apical ends of the bundles are positioned close to the cuticular layers (*long arrows*) on opposite sides of the wing blade. Many of the vesicles, such as those towards the left of the micrograph, were probably generated by fragmentation and vesiculation of plasma membranes. Bar, 0.5 $\mu$m.

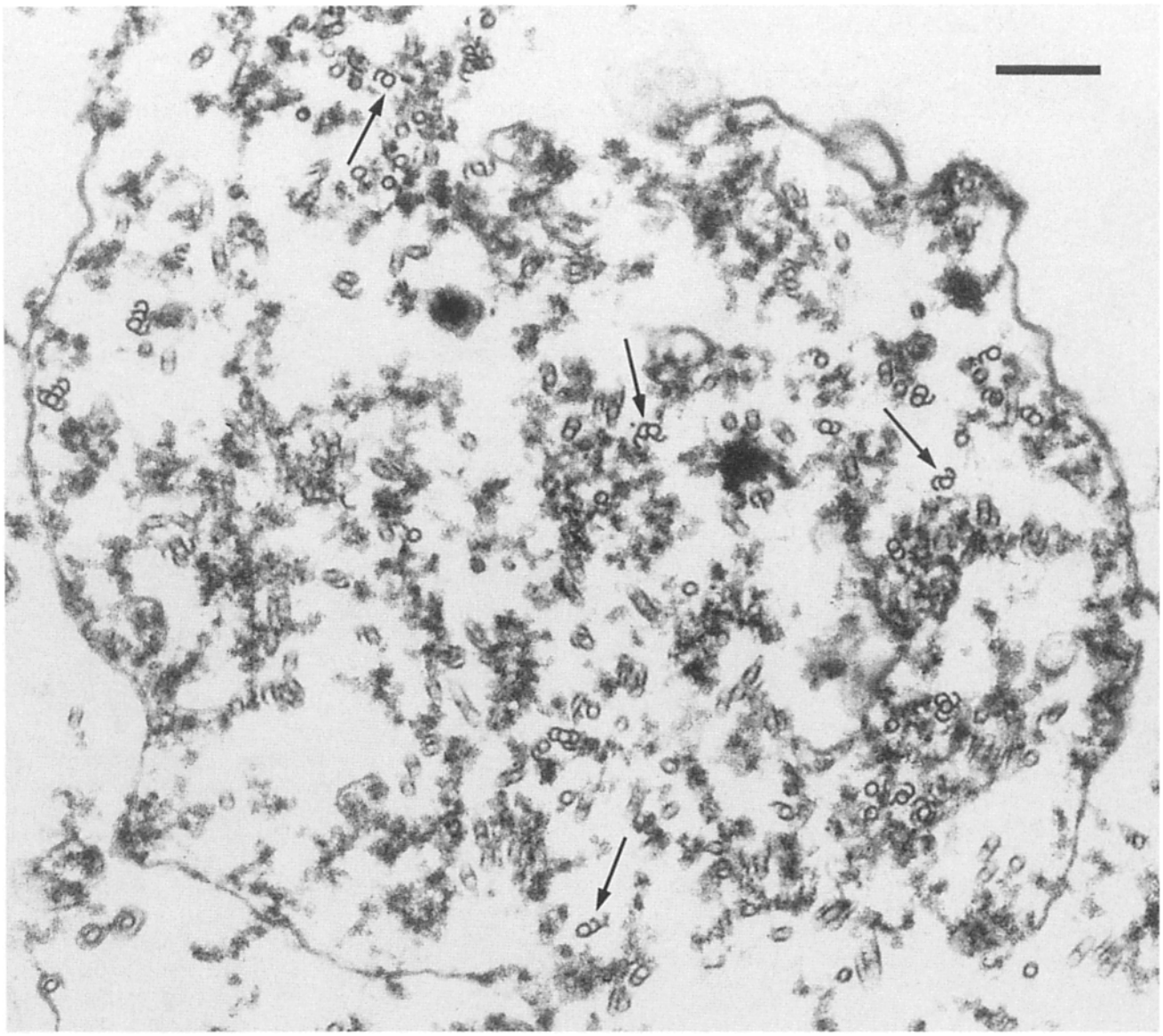


*Figure 3.* Cross section of a basal cell extension and its microtubule bundle prepared for hook decoration using procedure 1 and oriented so that the microtubules are viewed looking apicobasally along their longitudinal axes. Hooks on the decorated microtubules all curve in an anticlockwise direction (when curvature is followed outwards from a microtubule profile, *arrows*). In this instance, much of the plasma membrane has remained more or less in place around the cell extension and microtubule bundle. Bar, 0.2 μm.

Many of the bundle microtubules retained an apicobasal alignment with each other (Figs. 1 and 2). However, microtubule portions with a variety of other orientations were found within extracted bundles, and adjacent to them, at a much higher frequency than in unextracted cells (Fig. 3). Furthermore, intermicrotubular spacings were substantially greater than normal. The number of microtubule profiles per bundle cross section was not obviously reduced below the normal level in many of the basal portions of bundles that had been subjected to the decoration procedures; each such bundle portion could be distinguished because most of its microtubules remained grouped together, and it was sometimes surrounded to some extent by the plasma membrane of the basal extension (Fig. 3). Quantitative assessments of the degree to which microtubules were displaced, and perhaps depolymerized, during hook decoration were not possible for more apical levels. This was because fragmentation of plasma membranes at the level of cell bodies, and the apically splayed configuration of bundles, prevented discrimination of discrete groupings of microtubules belonging to individual bundles.

### *Microtubule Polarities and Patterns of Hook Decoration*

Serial thin-section sequences of portions of three different wings were cut parallel to the planes of wing blades and from one wing surface to the other. Hence, sectioning progressed through both dorsal and ventral wing epidermal layers and tracked through transalar pairs of transcellular microtubule bundles to provide sequences of bundle cross sections.

*Table I. Hook Curvature for Apicobasally Viewed Microtubules in Transcellular Bundles*

| Bundle* | Microtubules per bundle with anticlockwise hooks/hooks per microtubule‡ | | | | | | Microtubules per bundle with clockwise hooks | Microtubules per bundle with ambiguous hook decoration§ | Microtubules decorated per bundle‖ |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Total | | | |
| | | | | | | | | | % |
| A | 26 | 4 | 1 | 0 | 0 | 31 | 0 | 5 | 31 |
| B | 45 | 7 | 4 | 0 | 0 | 56 | 0 | 11 | 38 |
| C | 43 | 9 | 8 | 0 | 0 | 60 | 2 | 32 | 58 |
| D | 44 | 10 | 1 | 0 | 0 | 55 | 4 | 50 | 64 |
| E | 15 | 13 | 6 | 1 | 0 | 35 | 1 | 10 | 71 |
| F | 23 | 14 | 11 | 0 | 1 | 49 | 4 | 18 | 74 |
| 1 | 19 | 11 | 1 | 0 | 0 | 31 | 2 | 12 | 43 |
| 2 | 27 | 13 | 11 | 0 | 0 | 51 | 6 | 29 | 49 |
| 3 | 29 | 10 | 1 | 0 | 0 | 40 | 4 | 21 | 50 |
| 4 | 36 | 8 | 2 | 0 | 0 | 46 | 0 | 17 | 53 |
| 5 | 34 | 9 | 4 | 0 | 0 | 47 | 2 | 9 | 56 |
| 6 | 15 | 4 | 0 | 0 | 0 | 19 | 1 | 25 | 57 |
| Totals | 356 | 112 | 50 | 1 | 1 | 520 | 26 | 239 | Mean = 54 |

* Table shows the numbers of microtubules (classified according to three main categories of hook decoration) in cross sections of twelve different bundles viewed apicobasally and cross sectioned at the level of the cell body (bundles *A–F*) or more basally in cell extensions (bundles *1–6*).
‡ Details of the numbers of anticlockwise hooks per microtubule are shown for each bundle. All microtubules bearing clockwise hooks only possessed one hook each.
§ Further details of the ambiguous patterns of decoration are given in the text.
‖ The right hand column shows the percentage of cross-sectional microtubule profiles in each bundle that had been decorated.

The proportion of microtubule profiles bearing hooks in each bundle cross section varied from 0–74% (procedure 1) and 0–21% (procedure 2). The yield of decorated microtubules did not obviously differ in wings which had been incubated with microtubule protein at concentrations of either 1 or 2 mg/ml when procedure 1 was used. It was not uncommon to find a complete lack of decorated profiles in bundles which were only separated by distances of <15 $\mu$m from bundles that included numerous hook-decorated profiles. Hence, these differences in the extent of decoration were not entirely due to variations in tubulin penetration through a wing blade from the access sites provided by the cuts made through the cuticle.

The vast majority of the hooks on microtubules curved anticlockwise when microtubules were viewed apicobasally (Fig. 3). Hence, hook decoration indicated that virtually all of the decorated microtubules in the bundles had the same polarity. They were oriented with their minus ends attached to their apical hemidesmosomal anchor points, and with their plus ends directed towards the basal desmosomes.

Sections of two regions of a wing which included bundles with the highest proportions of hook-decorated microtubules, compared with regions in the other wings studied, were examined to monitor the different categories of hook decoration (24) in more detail. 12 cross sections of bundles were used. They consisted of six bundles cut in cross section at levels where they occupied cell extensions, and six portions of bundles that included high concentrations of good cross-sectional microtubule profiles at levels in cell bodies where the bundles have a splayed configuration. These sections included 785 hook-decorated microtubule profiles in bundles that were viewed apicobasally. There were 520 microtubules bearing anticlockwise hooks and 26 microtubules bearing clockwise hooks (see Table I). Thus 95% of the unambiguously decorated microtubules in this sample bore anticlockwise hooks. The other decorated microtubules included three with hooks of opposite curvature and 236 with other ambiguous patterns of decoration such as closed hooks, and hooks on closed hooks. There was no distinct difference in the percentage of unambiguously decorated microtubules bearing anticlockwise hooks at the level of cell bodies (96%) compared with that at the level of basal cell extensions (94%).

Evidence for uniformity of polarity was found in sections cut along the entire lengths of bundles until section sequences started to include portions of the basal desmosomes. As sections progressed into a desmosome complex the proportion of microtubules bearing clockwise hooks increased relative to those bearing anticlockwise hooks in section sequences that were continuing to advance apicobasally out through the base of a cell extension. Sections cut near the middle of a desmosome complex included microtubules with opposite polarities but microtubules with the same polarity tended to be grouped together (Fig. 4). Microtubules with clockwise hooks became predominant as a section sequence continued through a desmosome complex and further into the basal extension of the epidermal cell on the other side of the wing blade. Such predominance became complete, at more apical levels in bundles, as soon as portions of basal desmosomes were no longer included in a section sequence. This spatial sequence for the switch in microtubule polarities is consistent with the zig-zag profiles of basal desmosome complexes where the bases of cell extensions interdigitate (Fig. 1). As a consequence, the bottoms of a pair of microtubule bundles effectively overlap each other although they are contained in different cells. It is also consistent with the notion that most microtubules in individual bundles have the same polarity, and the corollary, that a transalar array consists of two antiparallel, transcellular, microtubule bundles. No evidence was obtained for basally located microtubule populations of opposite polarity to that found at more apical levels in bundles with plus ends directed

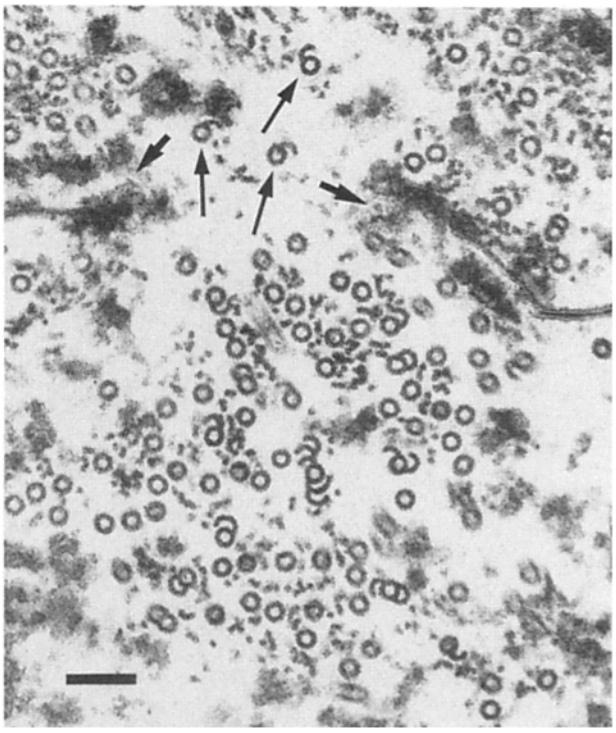


*Figure 4.* Cross section of bundle microtubules at a level where remnants of a basal attachment desmosome complex (*short arrows*) are situated and interdigitation between the bottoms of the basal extensions of two cells located on opposite sides of a wing blade occurs. Decorated microtubules near the center and bottom of the micrograph possess anticlockwise hooks but those which seem to have been located in the cell extension on the opposite side of the desmosome complex exhibit clockwise hooks (*long arrows*). Bar, 0.1 $\mu$m.

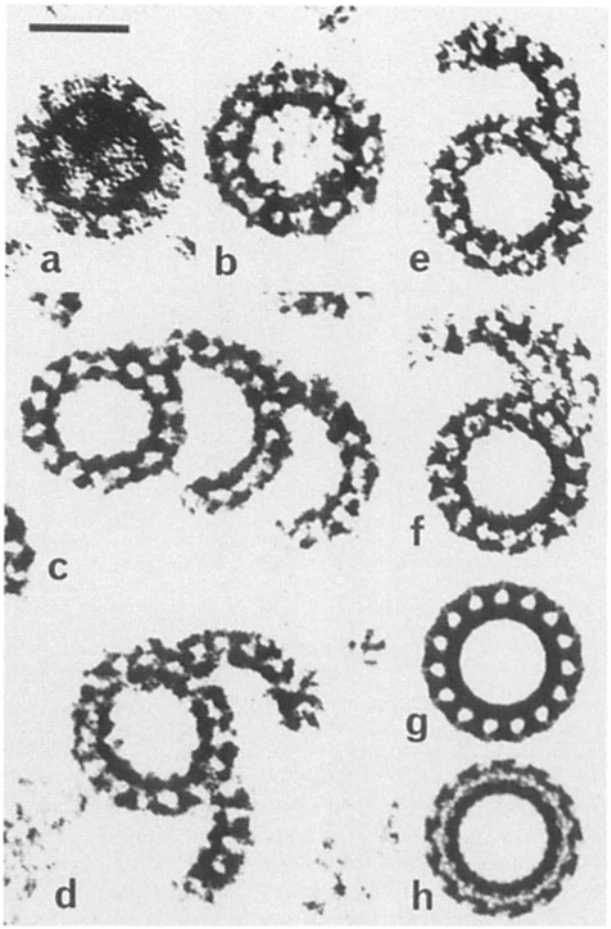



*Figure 5.* (*a* and *b*) Microtubules with 16 protofilaments. (*c*) Hook-decorated microtubule with 14 protofilaments, (*d* and *e*) Hook-decorated microtubules which appear to be composed of 15 protofilaments. (*f–h*) Rotational photographic reinforcements of the microtubule image shown in *e*. (*f*) One rotation of 360/13° reveals 15-fold radial symmetry. (*g*) 15 rotations of 360/15°. (*h*) 13 rotations of 360/13°. Bar, 20 nm.

away (apically) from basal desmosomes. Such microtubules, even if relatively short, should have been detectable as an increase in the incidence of microtubules with opposite polarities in section sequences progressed down basal cell extensions at some point before sections included portions of basal desmosomes.

### *Hooks on Microtubules with More than 13 Protofilaments*

Inclusion of tannic acid in the glutaraldehyde fixative used after detergent extraction and hook decoration rendered protofilaments apparent around the cross-sectional profiles of some microtubules when procedure 2 (but not 1) was used. A few hook-decorated microtubules for which protofilament number could be assessed were found. They all possessed >13 protofilaments and the protofilamentous substructure of hooks was discernable (Fig. 5, *c, d,* and *e*). Two of the decorated microtubules had 14 protofilaments (Fig. 5 *c*). In addition, for five other decorated microtubules it was not quite possible to clearly distinguish individual protofilaments around their entire cross-sectional profiles. However, it was evident from the dimensions of the portions of the profiles where clarity was lacking, and the numbers of protofilaments which could be unequivocably discriminated, that these microtubules possessed ⩾15 protofilaments (Fig. 5, *d* and *e*). Rotational photographic reinforcement analysis (22) confirmed this evaluation. 15 rotations of cross-sectional profiles through 24° (360/15°) about their centers gave strong reinforcements (Fig. 5 *g*). 13 rotations through 360/13° (Fig. 5 *h*), 14 rotations through 360/14°, and 16 rotations through 360/16° did not result in substantial reinforcement. Importantly, one rotation was sufficient to provide reinforcement for protofilament number assessment in some instances. Furthermore, even if the angle of rotation was any one selected to highlight *n*-fold symmetry in the range 13–16 for a full set of rotations (360/$n \times n$), 15-fold reinforcements were always obtained for the single rotation (Fig. 5 *f*). Most of the undecorated microtubules had 15 protofilaments, a few had 13, and two with 16 protofilaments (Fig. 5, *a* and *b*) were found.

## *Discussion*

### *A Nucleation–Capture Model for Assembly of the Transcellular Microtubule Bundles*

*Drosophila* wing cells provide the first reported example of

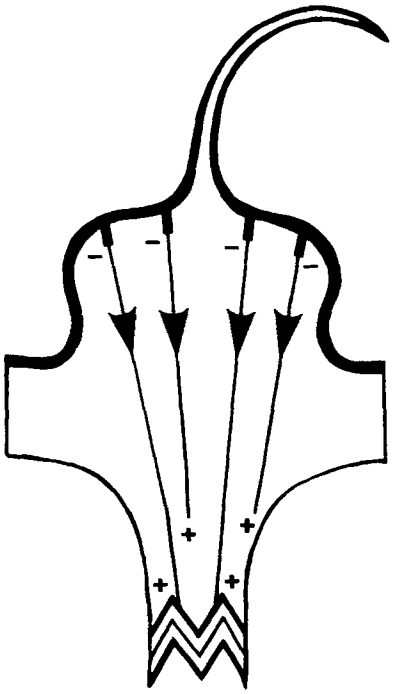


*Figure 6.* Schematic diagram of a wing epidermal cell summarizing the operation of a cell surface-associated nucleation-capture model during the assembly of its transcellular microtubule bundle. The trichome-bearing apical surface of the cell, where cuticle is secreted, is oriented towards the top of the diagram. The basal cell extension and desmosome attachment complex are towards the bottom of the diagram. Microtubule minus ends are attached to apically located hemidesmosomal sites where microtubule assembly is nucleated. The direction of microtubule elongation based on evidence obtained from an investigation of early stages in bundle assembly (26), is indicated by the arrowheads. The plus ends of microtubules are located towards the base of the cell and some are captured by the basal desmosomes.

a situation in which microtubules have their minus ends associated with the cell surface. This finding is compatible with an earlier study (26) which indicated that assembly of these microtubules is nucleated by dense plasma membrane-associated plaques at the apical surfaces of the cells. Microtubule polarity at the apical ends of *Drosophila* wing cells is opposite to that which would be anticipated if the microtubules had grown out from centrosomes to accomplish tip contact with the apical surfaces of the cells and significantly these cells lack centrosomes (38). Certainly, centrosomal promotion of an asterlike array, such as that well documented for initial stages in the outgrowth of interphase and spindle microtubule arrays in certain metazoan cell types (see references 2, 4, 19, and 23), is not the most obvious and architecturally appropriate method to begin construction of a transcellular microtubule bundle. A cell surface-associated nucleation-elongation-capture procedure would be more straightforward.

We propose a model (Fig. 6) in which microtubules elongate down the longitudinally tapered cells (to some extent perhaps guided by the sides of cells) and into the basal cell extensions. Here it is likely that the basal desmosomes provide another example of an organelle which, like certain kinetochores (see references 12 and 17), can capture the plus ends of microtubules. We suggest that capture of the plus ends of bundle microtubules by these basal desmosomes facilitates and initiates events leading to anchorage of the bottoms of the transcellular bundles to the basal desmosome complexes. Some microtubules apparently span the entire lengths of the cells (rather than transcellularity being achieved by an overlapping arrangement of microtubules, such as the interdigitation of two sets of microtubules which elongate from opposite ends of each cell). However, not all of the microtubule plus ends are captured at cell bases since the transcellular bundles include about four times as many microtubules in sections cut across cell bodies than they do in cross sections of the basal cell extensions (26). Hence, many microtubules in a bundle either cease elongation at some stage, or elongate very slowly thereafter, and do not reach the level of the basal desmosomes.

Close associations between the densely plugged "proximal" ends of 15 protofilament microtubules and the axolemma in certain neurons of the nematode *Caenorhabditis* (7) may provide another instance of the involvement of cell surface-associated nucleating sites. The cell surface-associated nucleation-capture procedure suggested above may also operate during control of assembly and positioning of other transcellular microtubule bundles; namely, those that have been described for epidermal muscle attachment cells in a range of arthropods (see reference 28), ommatidial cone cells in certain insects (27, 39), and supporting cells in the mammalian organ of Corti (see reference 30). Furthermore, it may transpire that some other animal tissue cell types which contain longitudinally oriented microtubule arrays, such as neurons (see reference 1), and lens cells of the vertebrate eye (5), also use cell surface-associated microtubule nucleation and/or plus end capture to assist in the positioning and alignment of polarized microtubule arrays.

### *Noncentrosomal Nucleating Sites and Infidelity of Protofilament Number*

Most of the bundle microtubules (~85%) are composed of 15 protofilaments, many of the remainder have 13 protofilaments (38) and in this study a few microtubules with 14 and 16 protofilaments were also found. Thus, protofilament number is not determined with very great precision in these cells. Furthermore, the number which predominates is not thirteen, which is the number specified with considerable fidelity for most microtubules in most cell types that have been examined (35) and for microtubules reassembled from partly purified microtubule proteins isolated from *Drosophila* eggs and embryos (15). The lack of centrosomal microtubule-organizing centers in wing cells is significant because there is evidence that protofilament number is specified by these microtubule-organizing centers (see reference 13). It has been argued above that loss of centrosomes is related to special requirements for the construction of transcellular microtubule bundles. This raises the question of whether the lack of 13 protofilament fidelity arose as an inevitable consequence of centrosomal loss. For example, it is mainly, perhaps only, 13 protofilament microtubules which assemble in certain plant tissue cells (see 18), the heliozoan *Echinosphaerium* (35), and the early mitotic micronuclei of the ciliate *Nyctotherus* (8) which all lack centriole-containing cen-

trosomes. Hence, a lack of centrosomal nucleation does not necessarily lead to unrestrained infidelity. Thus, the exploitation of microtubules with more than 13 protofilaments in wing cells may be a separate issue from loss of centrosomes. The 15 protofilament microtubules may have properties different from those of 13 protofilament microtubules (7), which are of particular value in so far as the function and/or formation of transcellular bundles are concerned.

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