Development of macrociliary cells in *Beroë*

II. Formation of macrocilia

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Summary

Two patterns of macrociliary growth occur in *Beroë*. Early differentiation described previously (Tamm & Tamm, 1988) leads to the first pattern of ciliogenesis. A tuft of 10–20 single cilia initially grows out from basal bodies that have migrated to the cell surface and are axially aligned. Ciliary membranes then begin to fuse along their length, except at the base, resulting in thicker groups of cilia on each cell. Progressive fusion of ciliary membranes, together with addition and elongation of new axonemes, finally results in mature macrocilia, 5 μm thick and 40 μm long, enclosed by a single membrane distally. The second pattern of ciliogenesis begins with the simultaneous appearance of several hundred ciliary buds on the apical surface. The short cilia possess individual membranes with bulbous tips, and are not axially aligned. Subsequent elongation is accompanied by progressive fusion of neighbouring ciliary membranes, except at the base, leading to flat-topped 'stumps' surrounded by a single membrane distally. Further elongation then proceeds asymmetrically within each stump. Axonemes on the aboral side of the macrocilium stop elongating, while those towards the oral side increase progressively in height, resulting in a slanted profile. Basal feet and central-pair microtubules are now uniformly aligned. Unequal elongation of axonemes on the oral and aboral sides of the macrocilium continues until the macrocilium resembles a lobster's claw, with a long slender shaft projecting from a broad base. Finally, the polarity of unequal growth reverses: the shorter axonemes on the aboral side elongate and almost catch up with the longer ones on the opposite side, resulting in a mature macrocilium of uniform diameter. The unusual membrane architecture of the macrocilium is thus a consequence of selective fusion of the distal regions of originally separate ciliary membranes. The polarized, asymmetrical growth of axonemes on the two sides of the macrocilium illustrates a remarkable control of microtubule elongation at the subcellular level.

Key words: *Beroë*, macrociliary growth.

Introduction

In the preceding paper (Tamm & Tamm, 1988) we described one pathway (pattern I) of early differentiation of macrociliary cells in *Beroë*; in particular, the formation of a massive bundle of actin filaments and its relationship to basal body migration.

Here we describe how the macrocilium grows. Although we found two different patterns of ciliogenesis, we will spend most of this paper describing pattern II, because it illustrates several remarkable features of membrane dynamics and axonemal elongation during ciliary growth. Preliminary reports of this work have appeared previously (Tamm & Tamm, 1986, 1987).

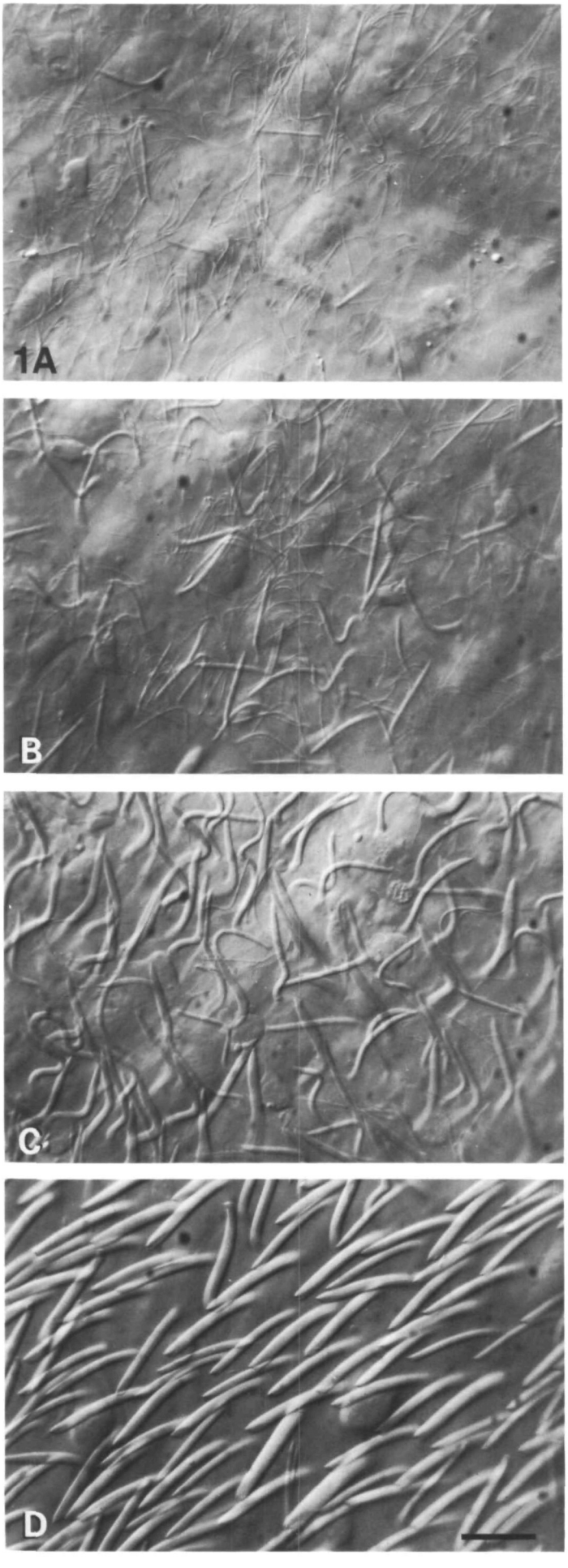
Materials and methods

See preceding paper in this series (Tamm & Tamm, 1988).

Results

Normal structure

Mature macrocilia are ≈40 μm long and 4–5 μm in diameter. A single macrocilium consists of a hexagonal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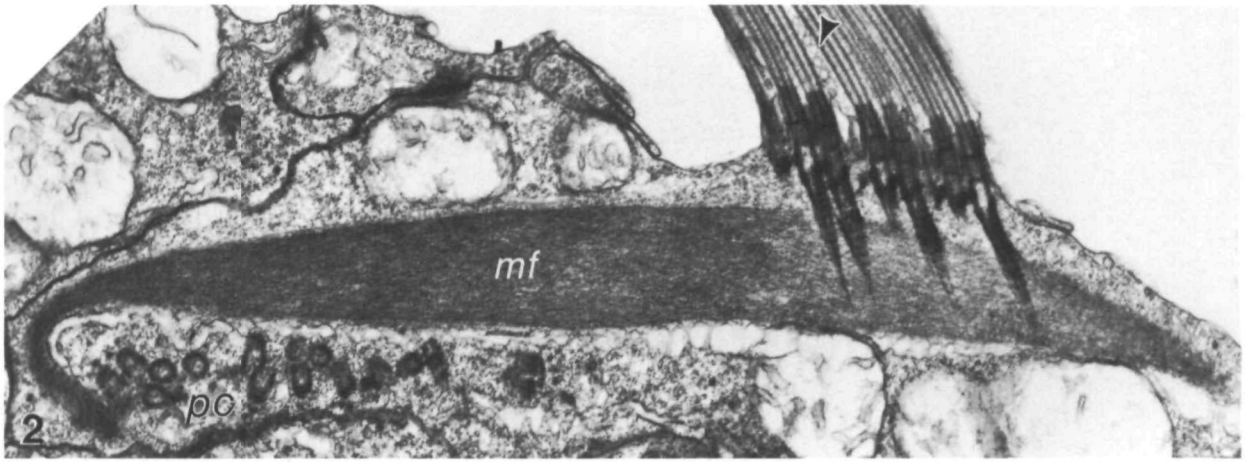


Fig. 2. Longitudinal section through a pattern I macrociliary cell with fusing ciliary groups (arrowhead), on regenerating lips of *B. cucumis*. Developing procentrioles (*pc*) lie in a group beneath the narrow end of the growing bundle of microfilaments (*mf*). ×16 900.

array of several hundred 9+2 axonemes surrounded by a common membrane, except at the base where each axoneme is enclosed by its own membrane (Tamm & Tamm, 1984, 1985).

The axonemes in a macrocilium are connected into ≈20 parallel rows, which run at right angles to the plane of beat and the oral–aboral axis. Axonemes within a row are approximately the same length. However, axonemal rows are shorter on the aboral side of the macrocilium, resulting in a notched appearance of the tip at rest (Tamm & Tamm, 1985). In addition, the A and central-pair microtubules of axonemes on the oral side extend distally to form a dense pointed cap (Tamm & Tamm, 1985).

The basal bodies are axially aligned with their basal feet projecting aborally in the direction of the effective stroke. The striated rootlets of the basal bodies extend downwards into the actin bundle, with the flat sides of the rootlets lying parallel to the paths of the microfilaments and the aboral–oral axis.

Ciliogenesis

In the preceding paper we described the early stages of macrociliary cell differentiation leading up to the first pattern of macrocilia formation. This pattern is described briefly, but the second pattern of ciliogenesis is the main subject of this paper.

Fig. 1. Stages in pattern I development of macrocilia on regenerating lips of *B. cucumis*. A. Tufts of long single cilia, and several early stages of ciliary membranes fused into thin groups, occur near the aboral edge of the lip. B. Progressively later stages of membrane fusion into thicker ciliary groups are found further orally on each cell. The number of axonemes gradually increases as well. C. Further orally, ciliary membranes of individual cells have completely fused, resulting in slender macrocilia of varying diameter. D. Almost mature macrocilia lie at rest near the oral edge of the lip. Macrocilia are thicker due to the addition of new axonemes. Bar, 20 μm.

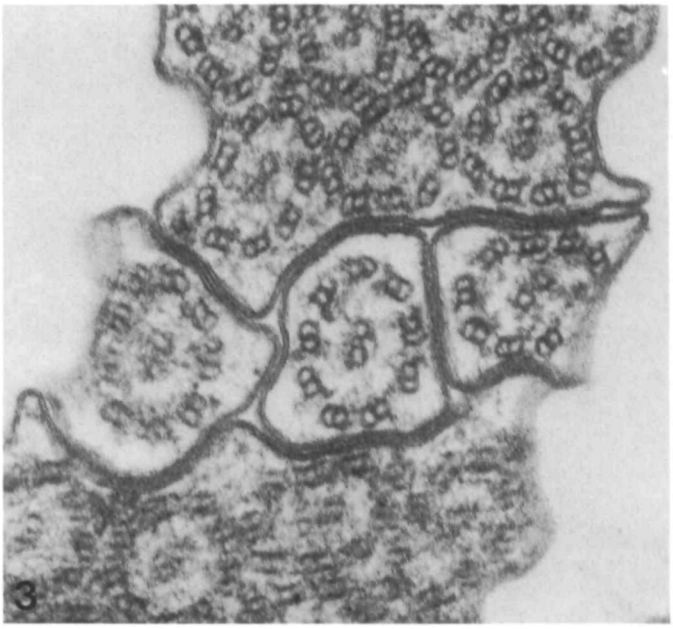
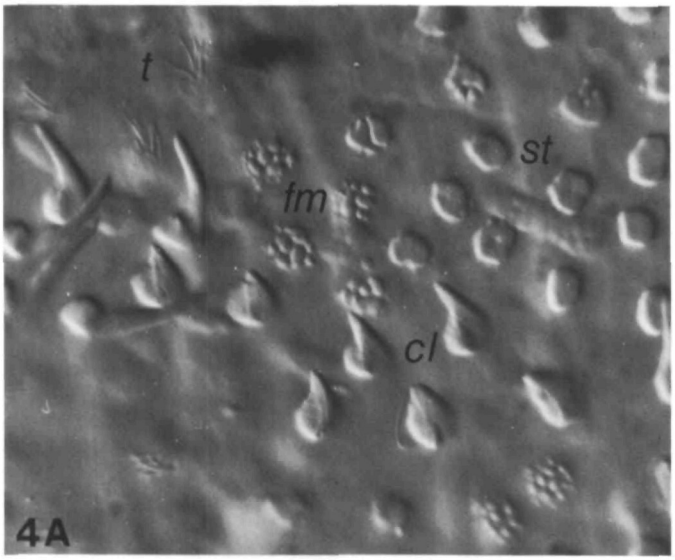
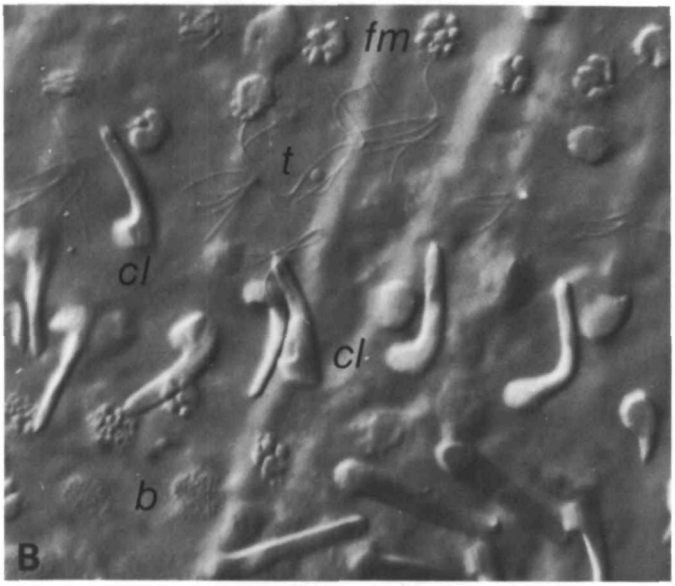


Fig. 3. Transverse section through ciliary groups undergoing membrane fusion in a pattern I macrocilium. The membranes of three unfused cilia are closely apposed to already fused membranes of ciliary groups. Note the uniform gap of ≈50 Å separating ciliary membranes prior to fusion. The central-pair microtubules are regularly aligned ×112 600.

Pattern I ciliogenesis

This is the only type of macrociliary formation found in *B. cucumis*, during both normal growth and lip regeneration. This pattern also occurs in *B. ovata* and





B. sp. (hurricane Gloria), but less commonly than pattern II (Figs 4, 5).

Ciliogenesis is initiated by the growth of a small number of single cilia from basal bodies that have tilted upwards out of the actin bundle to contact the plasma membrane. Because the tilt plane of the basal bodies is parallel to the flat sides of the rootlets and basal feet, the basal bodies are already aligned in their proper axial orientation when they reach the surface (see figs 5 and 7 in preceding paper). In contrast, basal bodies are randomly oriented during the initial stages of ciliogenesis in pattern II (see below).

The cilia of each cell elongate as a tuft of 10–20 individual cilia, which often beat together if not mechanically disrupted by compression on a microscope slide (Fig. 1A).

The membranes of cilia within a tuft then begin to fuse along their lengths, resulting in a smaller number of thicker ciliary groups on each cell (Figs 1B, 2). The membranes around the bases of the cilia remain unfused, however (Fig. 2). Progressive membrane fusion between adjacent groups of cilia is accompanied by continued elongation and addition of new axonemes. Prior to fusion, the adjoining ciliary membranes are often closely opposed and separated by a uniform gap of ≈50 Å (Fig. 3). Macrocilia with completely fused distal membranes are 25–30 μm long and noticeably more slender than mature organelles (Fig. 1C). The beat direction is toward the aboral pole, as in fully grown macrocilia. As new axonemes continue to be added, macrocilia become progressively wider, eventually reaching a maximum diameter of 4–5 μm (Fig. 1D).

By light microscopy we do not see continued outgrowth and fusion of single cilia during this subsequent increase in macrociliary diameter. The new ciliary axonemes must therefore be added within the existing common membrane of the long slender organelle.

During this process, a large group of developing basal bodies is found under the narrow end of the actin bundle, at some distance from the base of the enlarging macrocilium (Fig. 2). The pattern of procentriole development is similar to that described in the preceding paper. Basal body production, which ceased during the initial phase of centriole migration, therefore resumes during later stages of ciliogenesis. Migration of

Fig. 4. Development of macrocilia on regenerating lips of *B. ovata*. Pattern I stages occur less commonly, and are represented by tufts of single cilia (*t*) in A and B. Pattern II stages are more typical and include multiple ciliary buds (*b*), fusion of ciliary membranes into small groups (*fm*), ciliary stumps with completely fused distal membranes (*st*), claws of varying lengths with longer axonemes on the oral sides (*cl*), and mature macrocilia of uniform diameter with tridentate tips (C). *a–o*, aboral–oral axis in A–C. ×810. Bar, 10 μm.

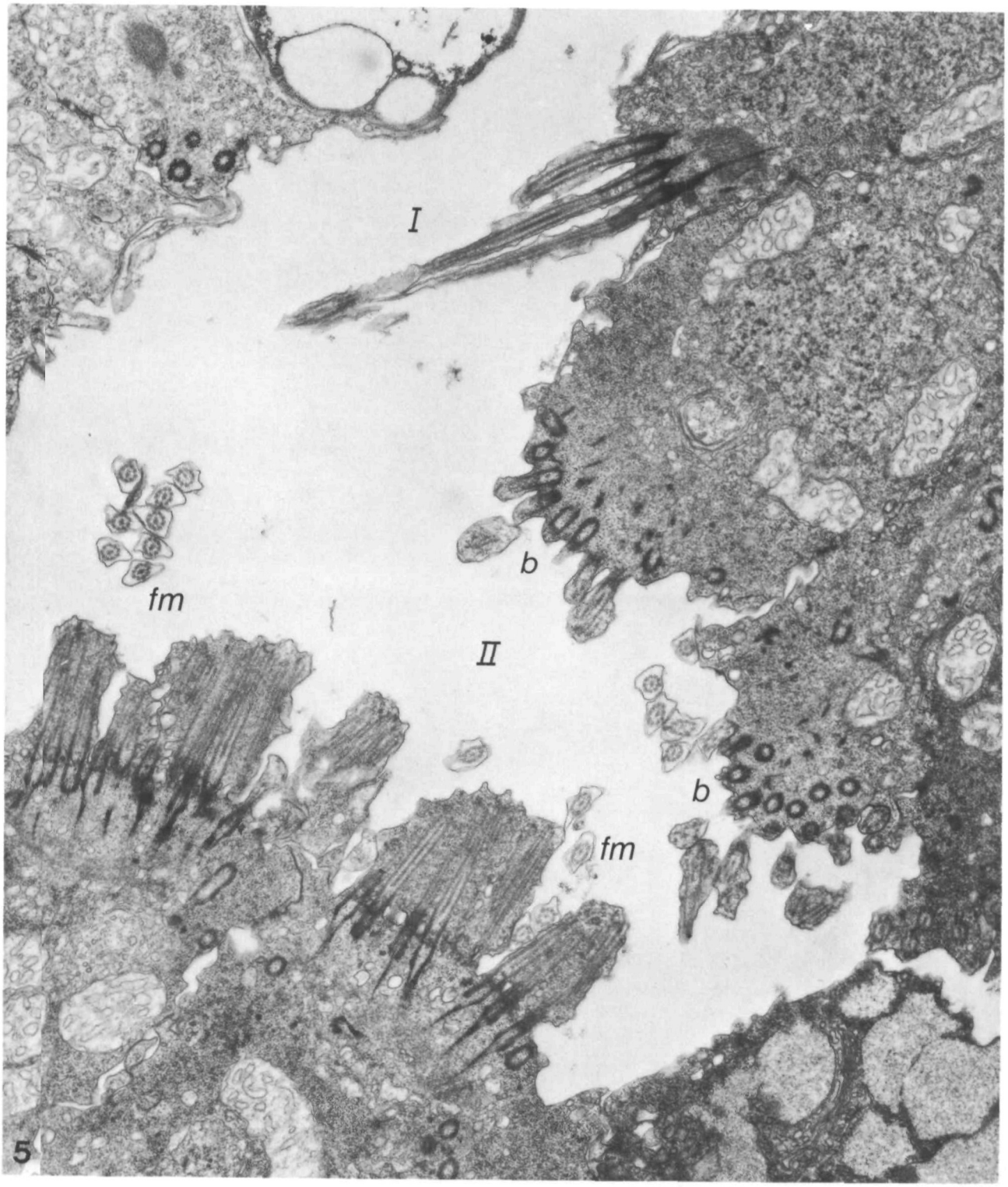


Fig. 5. Survey section showing two patterns of macrociliary development (*I* and *II*) in larvae of *B. sp.* In pattern *I*, a tuft of single cilia is shown on the cell at upper right. Pattern *II* stages include multiple ciliary buds (*b*) arising from the convex apical surfaces of neighbouring cells, and elongating cilia undergoing membrane fusion on neighbouring cells at lower left (*fm*) ×17 400.

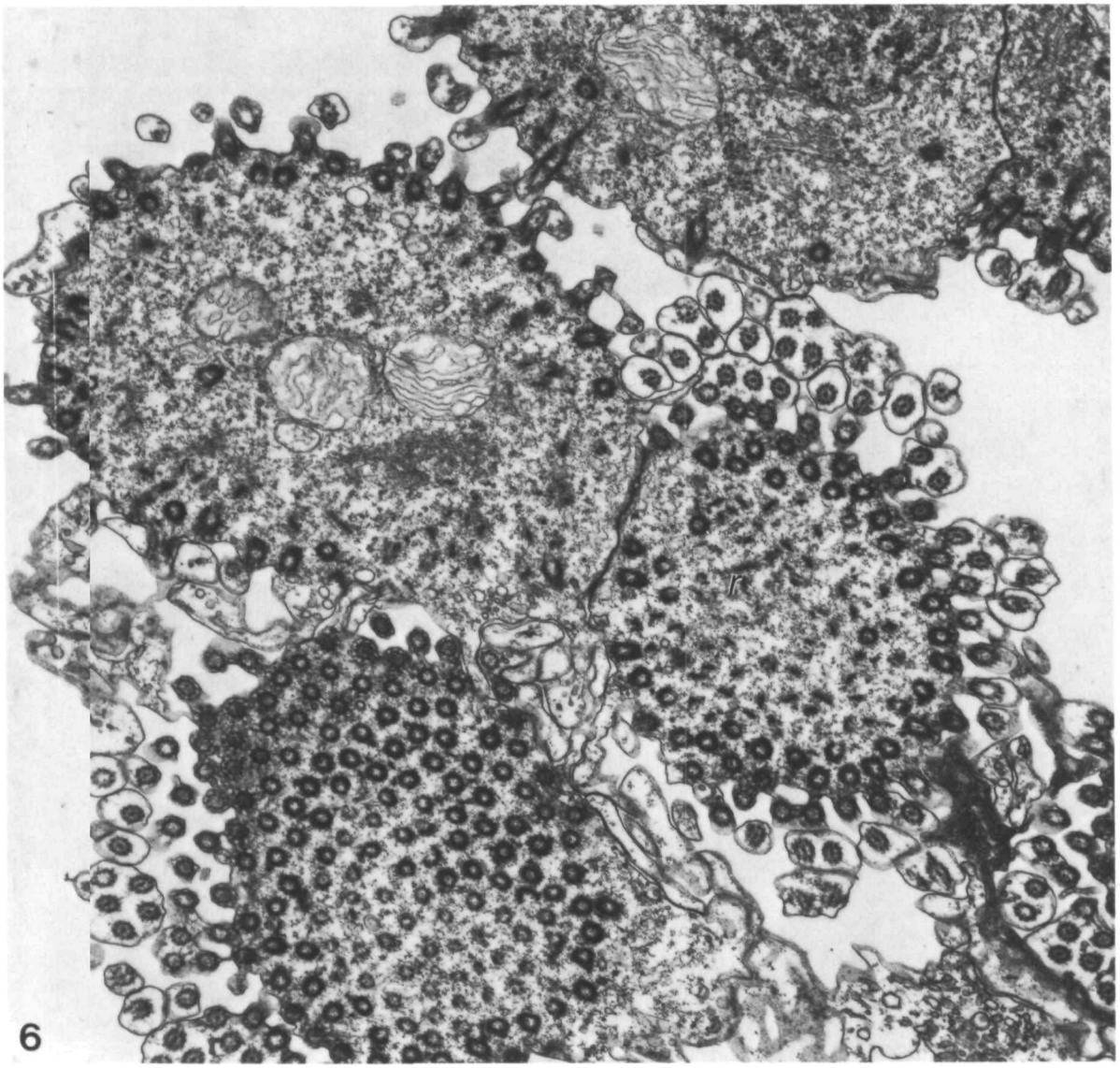


Fig. 6. Pattern II ciliogenesis: buds. Transverse section through the convex apical surfaces of cells with numerous ciliary buds (see Fig. 4, *b*). The short cilia have individual membranes with bulbous tips. Some ciliary membranes have already begun to fuse into small groups of axonemes. Note random axial orientation of basal body rootlets (*r*), here cut transversely. ×14 300.

these additional basal bodies to the base of the developing macrocilium apparently occurs discontinuously, since stages of basal body transport are rarely seen.

Pattern II ciliogenesis

A second pattern of macrociliary growth is found exclusively in *B. ovata* and *B. sp.*, during both larval development and regeneration of lips by adults (Fig. 4). For convenience, we have divided the developmental sequence into the following stages.

Multiple buds. The first sign of ciliogenesis is the simultaneous appearance of several hundred short ciliary buds, 0·5–1·0 μm long, on the apical surface of the cell (Figs 5, 6). The cell surface is markedly convex, and mitotic spindle remnants are often present. The number of ciliary buds is similar to the total number of axonemes found in a mature macrocilium. We have not yet identified earlier stages in the formation or migration of the basal bodies preceding the appearance of the cilia.

Ciliary buds possess individual membranes with bulbous tips (Figs 5, 6). The ciliary buds of a given cell are arranged in a circular field, 8–8·5 μm in diameter, resembling a round cluster of grapes by DIC microscopy (Fig. 4B).

Transverse thin sections through the apical cytoplasm show that the basal bodies are not hexagonally packed into parallel rows as in adult macrocilia, but are more widely and irregularly spaced. The basal feet and

striated rootlets are not uniformly oriented, but point in various directions, indicating a random axial orientation of the basal bodies (Fig. 6).

Ciliary membrane fusion. Subsequent elongation of ciliary buds is accompanied by gradual fusion of the distal regions of adjacent ciliary membranes. Progressive fusion of distal ciliary membranes leads to larger groups of axonemes enclosed by common membranes (Figs 5–7). The central-pair microtubules are still not uniformly aligned at this stage (Fig. 7). Fusion remnants are evident as rows of membranous vesicles between the axonemes (Figs 2, 5).

Fused groups of short cilia appear by light microscopy as blebs or pads of irregular shape on the surface of macrociliary cells (Fig. 4A,B). The overall diameter of the group of blebs on an individual cell is 7·5–8·0 μm, slightly smaller than that of the field of pre-fusion buds. Ciliary membrane fusion often occurs earlier on the oral side of the cell (Fig. 4). No motility of the fused ciliary groups is evident at this stage.

The membranes surrounding the bases of individual axonemes remain unfused, resulting in a membranous reticulum that is continuous with the external sea water. These membrane collars surround the basal 0·25–0·5 μm of each axoneme, from the distal end of the basal body to a level above the transition zone and beginning of the central pair (Fig. 8B).

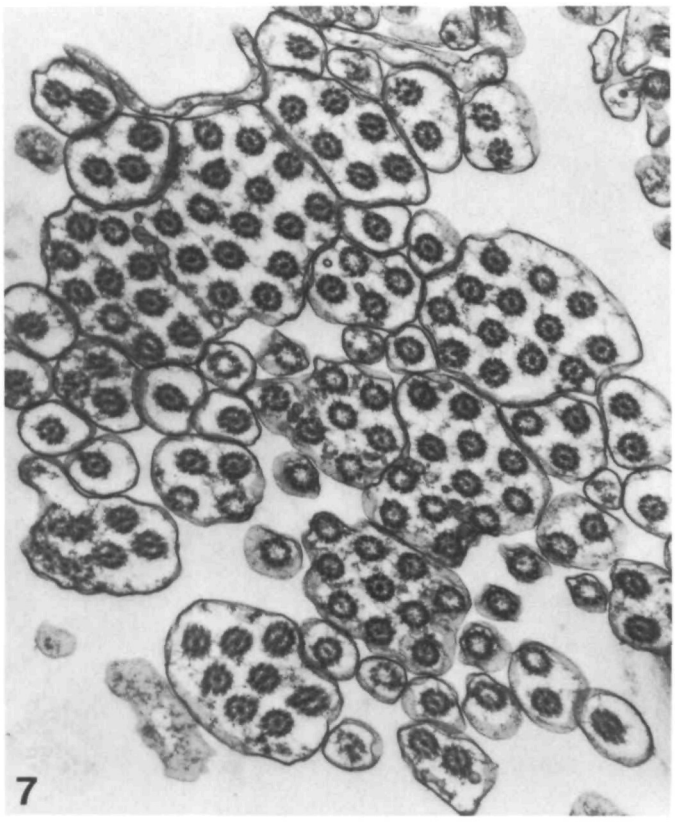


Fig. 7. Pattern II ciliogenesis: membrane fusion. The distal membranes of elongating cilia fuse progressively into larger groups of axonemes enclosed by common membranes (see Fig. 4, stage *fm*). Note that the central-pair microtubules are not yet uniformly oriented. ×20 500.

Stumps. Fusion of the distal regions of all the ciliary membranes of a cell is completed by the time the cilia are 3–5 μm long (Fig. 8). Short macrocilia with fused common membranes are roughly circular or oval in cross-section, with a smaller overall diameter of 6·5–7 μm (Fig. 4A,B). Since all the axonemes are the same length, macrocilia at this stage appear flat-topped or stump-like (Fig. 8).

Motility of some stumps has been observed. As viewed from above, a wave of bending in the oral direction begins at the aboral side of the stump and slowly travels to the oral edge. This 'recovery stroke' is followed by a bending wave in the opposite direction, the 'effective stroke'.

The central-pair microtubules extend 0·4–0·6 μm beyond the distal tips of the peripheral doublets and terminate in an electron-dense cap under the macrociliary membrane (Fig. 8B,C).

Transverse sections reveal that the basal bodies and proximal ciliary regions are often ordered into parallel rows. However, neither the axis of the central-pair microtubules, the basal feet, nor the plane of the striated rootlets shows uniform orientation at this stage (Fig. 9A). Distally, the axonemes often remain irregularly spaced without alignment into rows.

The macrociliary cells are at this stage roughly cuboidal, and have not begun to elongate.

Slanted stumps. Further elongation of axonemes then proceeds asymmetrically along the aboral–oral axis within each macrocilium. Axonemes on the aboral side of the stump stop elongating, while those towards the oral side increase progressively in height, reaching the greatest length (6–7 μm) at the extreme oral edge of the macrocilium. As a result, the tops of the macrocilia slant downwards in the aboral direction (Fig. 10). Membranous vesicles of various sizes are commonly found at the distal end of the slanted stumps (Fig. 10B).

The basal bodies are now axially aligned, with their basal feet pointing in the aboral direction (effective stroke direction) and the flat plane of the striated rootlets oriented parallel to the oral–aboral axis (Figs 9B, 10B).

Claws

Asymmetrical elongation of axonemes within each macrocilium becomes more pronounced. Axonemes in the oral half of the shaft continue to grow, while those in the aboral half remain arrested. Macrociliary profiles resemble a lobster's claw without the opposable part. A slender shaft, up to 25 μm long, projects from the oral side of a broad base, 3–5 μm high and 6–6·5 μm in diameter (Fig. 4A,B, 11, 12A). A cap of electron-dense

material, characteristic of mature organelles, is now evident at the tip of the claw (Figs 11, 12A).

Later stages

The macrocilium attains its mature form by reversing the polarity of unequal growth on the two sides of the organelle. The shorter axonemes in the aboral half elongate selectively, so that the macrocilium becomes uniform in width along its length. The diameter of the full-grown macrocilium is 4–5 μm, significantly smaller than the diameter of the claw stage, and considerably reduced from the width of the original field of ciliary buds (Fig. 12B). However, axonemes on the aboral side of the macrocilium never catch up with those on the opposite side, but remain several μm shorter. As a result, the tip of the mature macrocilium at rest has a notched appearance (Fig. 12B) (Tamm & Tamm, 1985).

The complete sequence of macrociliary development by pattern II is summarized in Fig. 13.

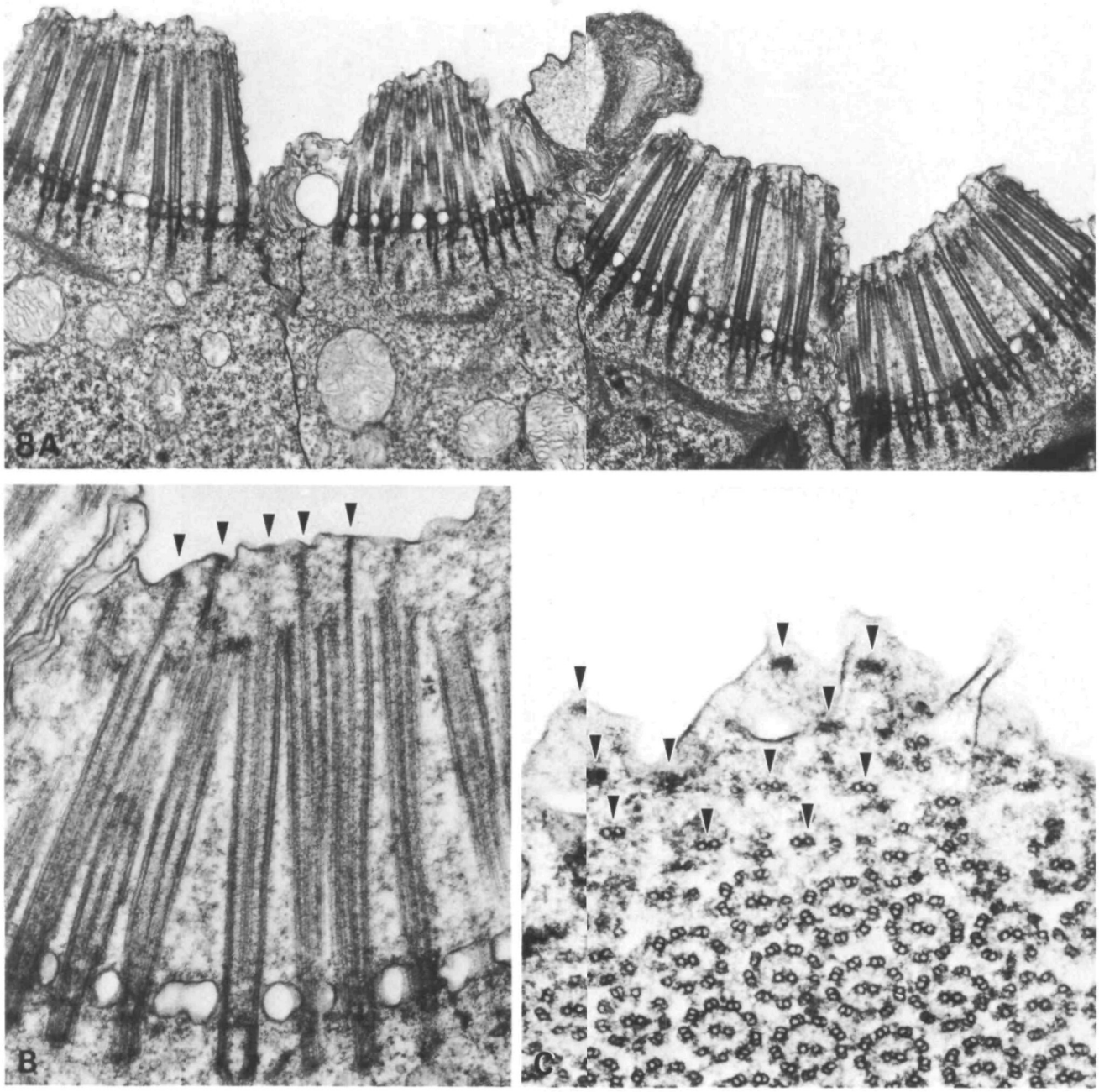


Fig. 8. Pattern II ciliogenesis: stumps. A. Ciliary membranes of each cell have fused into a single common membrane, except at the base. All axonemes are the same length. Longitudinal (B) and transverse (C) sections through stumps show that the central-pair microtubules extend distally 0·4–0·6 μm beyond the peripheral doublets, and terminate at an electron-dense cap under the membrane (arrowheads in B,C). A, ×12 200; B, ×35 200; C, 63 300.

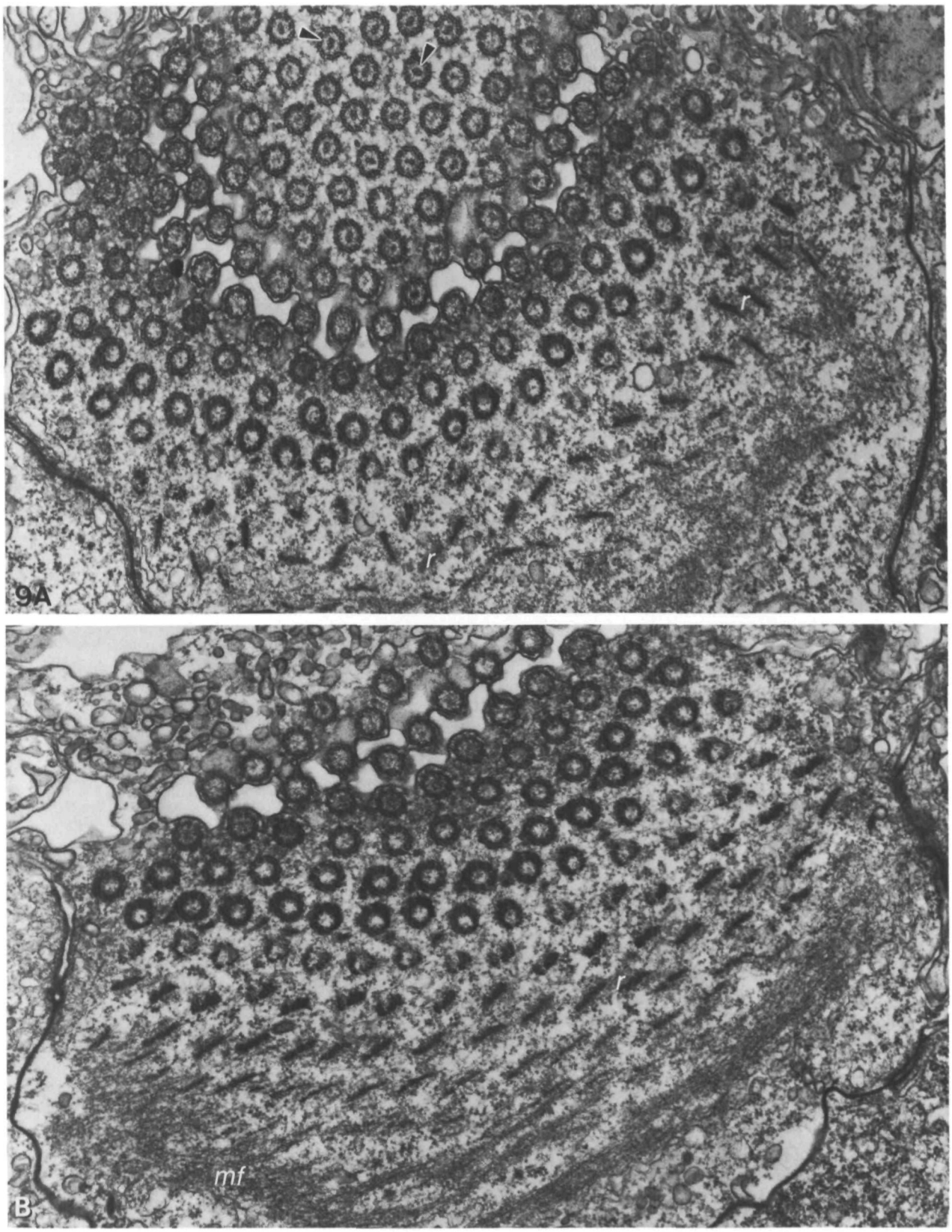


Fig. 9. Pattern II ciliogenesis: orientation of basal bodies in oblique transverse sections near the base of individual cells. A. Non-uniform axial orientation of central-pair microtubules (arrowheads, upper), basal feet and rootlets (*r*). ×31 600. B. Uniform alignment of basal feet and rootlets parallel to the microfilament bundle (*mf*) and plane of beat. ×32 400. A reticulum of unfused ciliary membranes is evident between the macrociliary shaft and basal bodies in A and B.

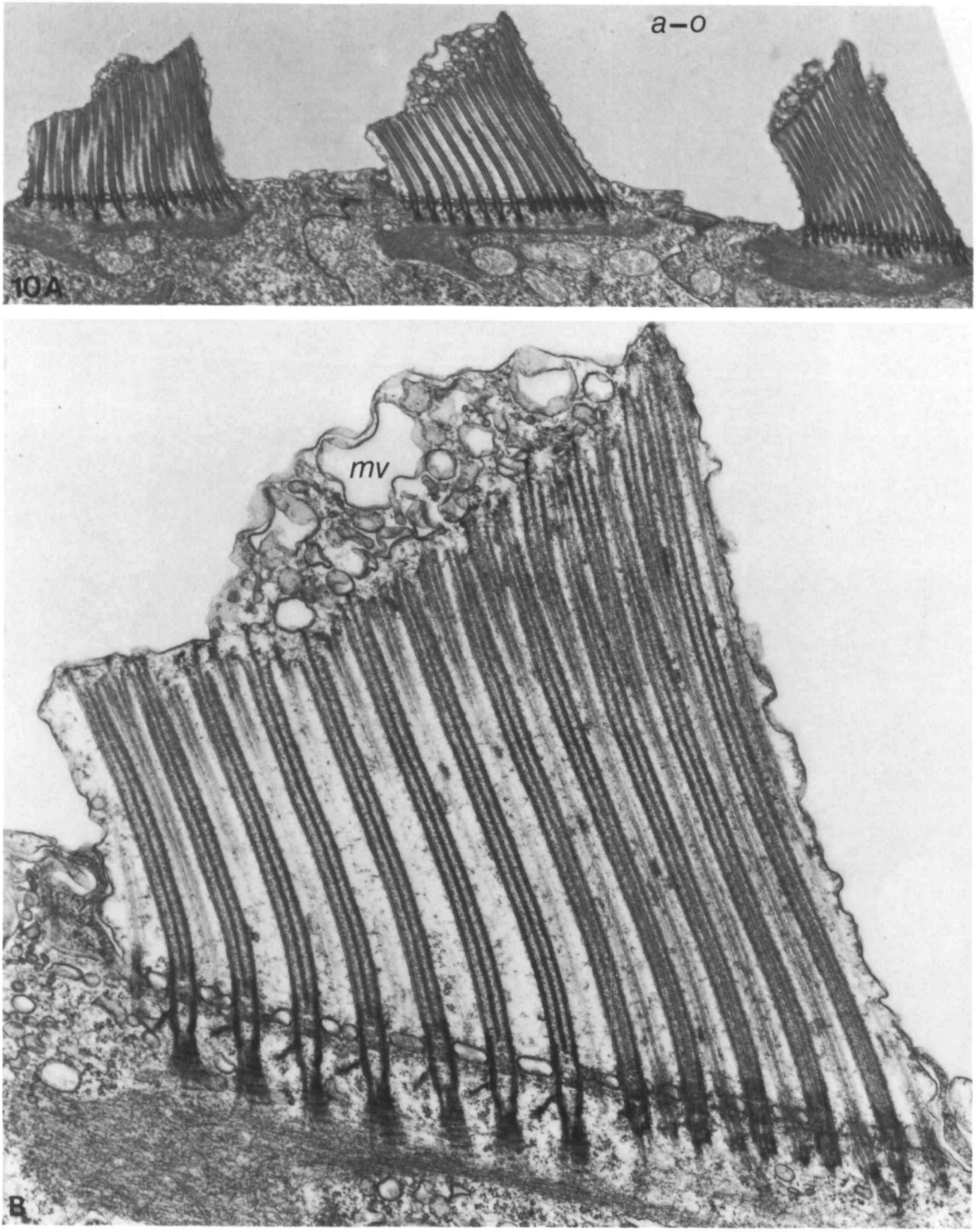


Fig. 10. Pattern II ciliogenesis: slanted stumps. Axonemal rows elongate asymmetrically within each stump, increasing progressively in height from the aboral to the oral sides (*a–o*, aboral–oral axis). B. Enlargement of the central macrocilium in A. Numerous membranous vesicles (*mv*) lie between the distal ends of the axonemes and the overlying membrane. Note that the basal feet point uniformly to the left (aborally) in the direction of the power stroke. A, ×6400; B, ×27 500.

Discussion

The two patterns of macrociliary formation differ in several important respects. In pattern I, basal bodies migrate to the apical surface in close association with assembling actin filaments, continuously, and are properly oriented at the time cilia first appear. Small tufts of single cilia initially grow out, and only later fuse into longer, slender macrocilia of uniform width. The number of axonemes gradually increases as the macrocilium becomes progressively thicker.

In pattern II, ciliary growth begins synchronously from a full complement of basal bodies present at the apical surface. It is not known whether the basal bodies migrate to the surface in a similar fashion to that for pattern I. The basal bodies and short cilia initially have no definite axial orientation, but later become uniformly aligned. Fusion of the distal regions of ciliary membranes is completed at an early stage. Axonemal elongation within a macrocilium occurs uniformly at first, but later proceeds asymmetrically along the oral–aboral axis.

Since both patterns of ciliogenesis occur in larvae (Fig. 5) as well as in regenerating lips of *B. ovata*, the patterns do not reflect differences between normal development *versus* regeneration. At present we do not understand the reason for two pathways of differentiation.

Orientation of basal bodies and cilia

In ciliated cells it is well-established that the uniform axial orientation of basal bodies and cilia corresponds to the direction of beat. The basal feet of the basal bodies point in the direction of the normal effective stroke, whereas the central-pair microtubules of the cilia are aligned in a plane perpendicular to the direction of beat (Afzelius, 1961; Fawcett & Porter, 1954; Gibbons, 1961; Hard & Rieder, 1983; Holley, 1984; Holley &

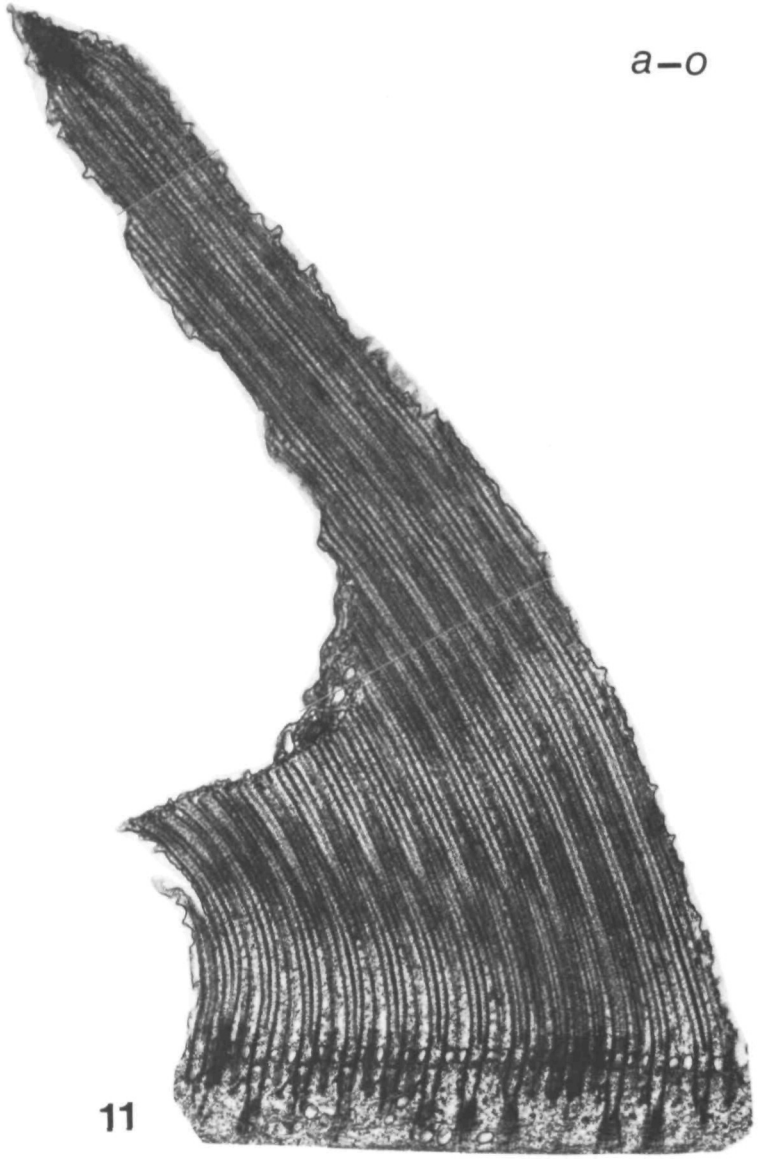


Fig. 11. Pattern II ciliogenesis: early claw. Rows of axonemes on the oral half of the macrocilium elongate further, whereas those on the aboral side remain arrested. Note pointed cap of electron-dense material at the tip of the claw. *a–o*, aboral–oral axis. ×16 500.

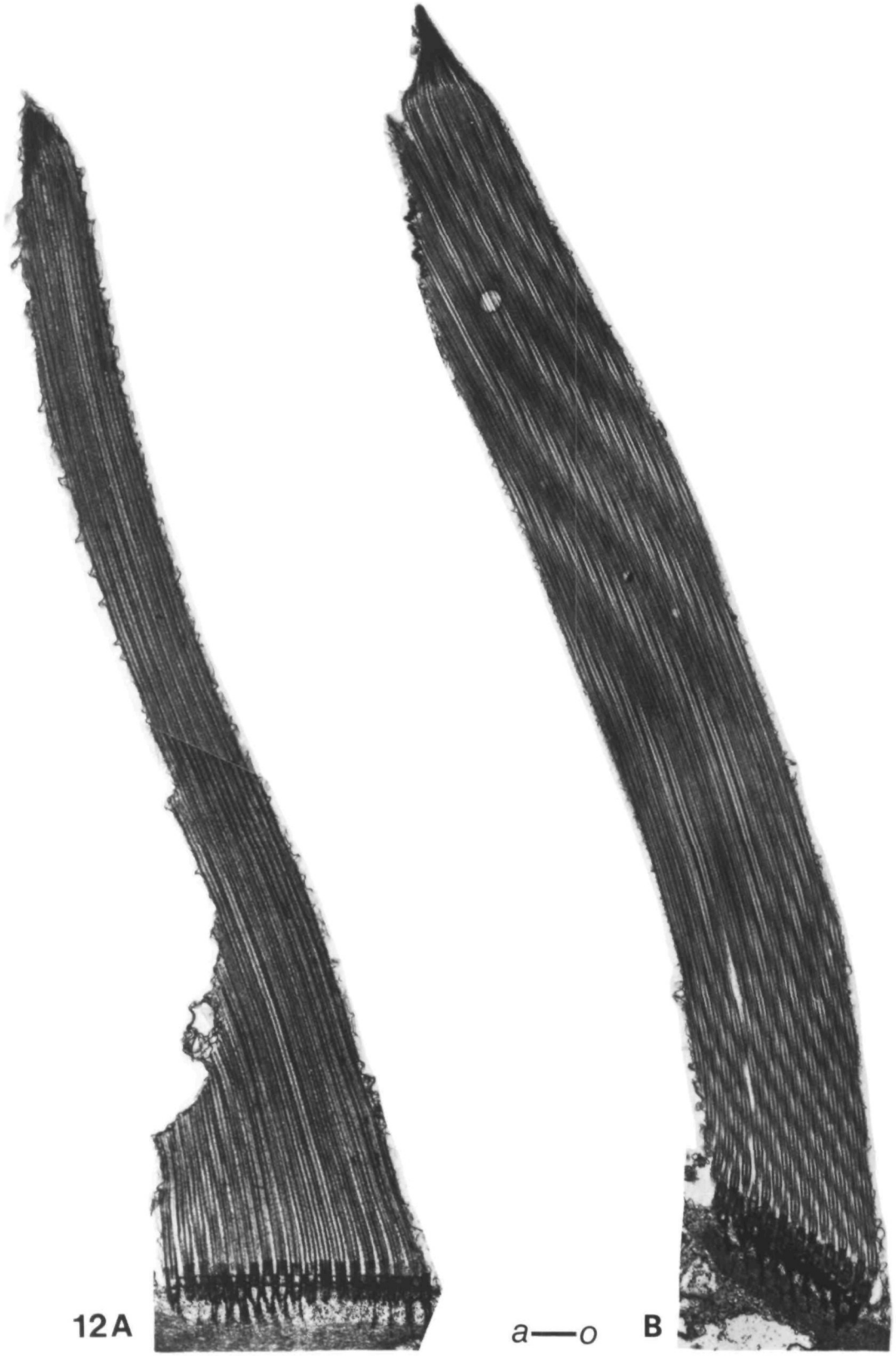


Fig. 12. Pattern II ciliogenesis. A. Later claw resulting from the continued selective elongation of axonemal rows on the oral half of the macrocilium. Note electron-dense cap at the tip of the long slender shaft. ×10 900. B. Mature macrocilium. Axonemes on the aboral half have now elongated, almost catching up with those on the oral side. The shaft is uniform in diameter, capped on the oral side of the tip, and notched on the aboral side. ×7600.

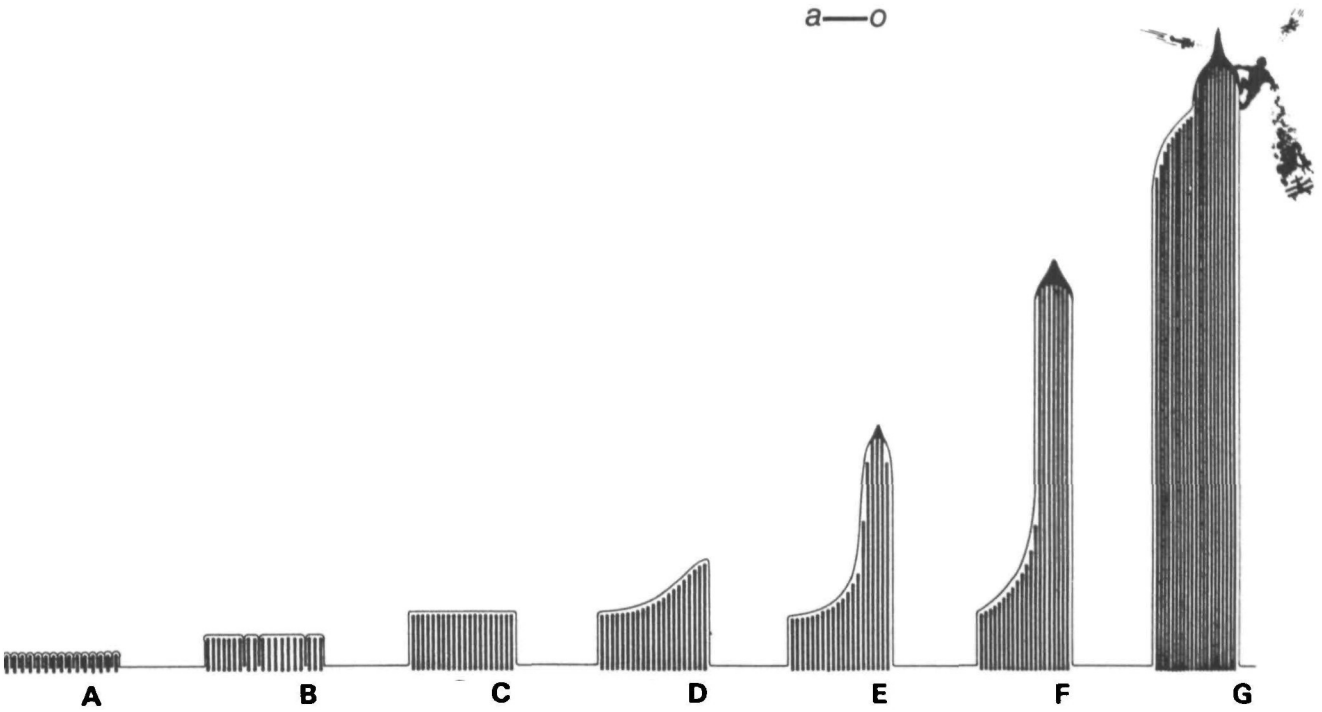


Fig. 13. Summary of development of macrocilia by pattern II. Successive stages are depicted in median longitudinal sections in an aboral–oral sequence (*a–o*, aboral–oral axis). Vertical lines represent rows of axonemes in profile view. A. Ciliary buds; B, membrane fusion; C, stumps; D, slanted stumps; E, early claws; F, later claws; G, mature macrocilium. Note that the spacing between axonemal rows decreases during development, resulting in a progressive reduction in diameter of the macrocilium from A to G.

Afzelius, 1986; Tamm & Horridge, 1970; Tamm & Tamm, 1981).

In macrocilia the axonemes are uniformly ordered in a similar fashion (Tamm & Tamm, 1985).

The mechanism(s) responsible for the spatial ordering of basal bodies and cilia is unknown. In several ciliated epithelia, development of rootlets, basal feet and cilia precedes their alignment, and they remain randomly disposed for a period of time (Boisvieux-Ulrich *et al.* 1985; Frisch & Farbman, 1968; Sorokin, 1968). In quail oviduct, alignment of cilia is closely related to the initiation of beating, as well as to the differentiation of the cortical cytoskeleton, particularly of microtubules and microfilaments associated with the basal feet (Boisvieux-Ulrich *et al.* 1985).

These correlations suggest that spatial orientation of basal bodies and cilia may be achieved by the act of beating *via* hydromechanical forces (Afzelius, 1981), or by cytoskeletal elements associated with the basal bodies (Lemullois *et al.* 1987).

The first possibility seems unlikely *a priori*, since entrainment of randomly beating cilia should not always lead to orientation in the proper direction. More convincingly, the first pattern of macrociliary development clearly shows that ciliary beating does not play a role in basal body/ciliary alignment. We found that the basal body–rootlet units already have the proper orientation when they reach the cell surface, i.e. before cilia grow out and start beating. The basal bodies tilt upwards out of the actin bundle with their basal feet pointing aborally, and the flat sides of their rootlets lying parallel to the path of the microfilaments.

Therefore, ciliary orientation in this case is not caused by beating, but instead is related to the orientation of the underlying actin filaments. Pattern I thus provides the most direct structural evidence to date for the role of cytoskeletal elements in orientation of basal bodies and cilia.

The second pattern of macrociliary development is similar to previous studies, in that alignment of basal bodies and cilia is achieved secondarily after the development of rootlets, basal feet and cilia. Although ordering of basal bodies and cilia appears to coincide with the onset of beating of the stumps, the significance of this correlation is not known.

A novel finding in pattern II ciliogenesis is the progressive reduction in diameter of the macrocilium during development. Starting with an initial width of 8–8·5 μm as a field of short ciliary buds, the diameter of the developing macrocilium progressively decreases to a final diameter of 4–5 μm as a mature organelle. This is due to decrease in the distance between neighbouring basal bodies at the base of the macrocilium. Although

reduction in diameter during early stages (i.e. multiple buds to slanted stumps) is correlated with ordering and alignment of basal bodies into rows, the later reduction in diameter reflects a decrease in spacing between hexagonally packed basal bodies. The mechanism responsible for this aspect of basal body orientation is not known.

Ciliary membrane fusion

Macrocilia are unusual in possessing a common membrane surrounding multiple axonemes. However, at the base each axoneme has its own membranous collar. The present study shows how this remarkable architecture of the macrociliary membrane is achieved.

In both patterns of ciliogenesis, elongating axonemes initially have individual ciliary membranes, similar to ciliary growth in other systems. Fusion of the distal membranes of adjacent cilia begins earlier in pattern II, when the numerous cilia are still quite short. Nevertheless, it is clear that in both patterns the membranous collars around the bases of the axonemes are not formed secondarily, but represent unfused remnants of originally separate ciliary membranes.

This restructuring of ciliary membranes raises several interesting questions. First, why do the distal regions of ciliary membranes fuse? Membrane fusion cannot be due simply to close proximity of adjacent cilia, since axonemes of other compound ciliary organelles are just as closely spaced, yet retain individual membranes. In particular, cilia of ctenophore comb plates are hexagonally packed exactly like axonemes in macrocilia, but each cilium in a comb plate is enclosed by a separate membrane (Afzelius, 1961). Even more convincingly, compound cilia in the oesophagus of *Beroë* are structurally identical to macrocilia, except that the ciliary membranes are not fused (Tamm & Tamm, unpublished data).

Membrane fusion in developing macrocilia therefore appears to be due to unique biochemical properties of these membranes. If so, one may then ask why the basal region of the macrociliary membranes does not fuse. Again, this may reflect biochemical differences between the proximal and distal regions of the macrociliary membrane.

Finally, is there a functional advantage for membrane fusion in macrocilia? And do separate unfused ciliary membranes at the base of the organelle serve some function?

In this regard, macrocilia are used as teeth to grasp or tear through prey during feeding (Swanberg, 1974; Tamm, 1982). During the power stroke into the stomach, macrocilia develop large bending moments, which are transmitted to the prey. The single membrane around all the axonemes may keep the macrocilium intact and prevent it from fraying into individual cilia under mechanical stresses associated with prey ingestion, thereby conserving the full power output of the organelle. A common, fused membrane therefore seems well-designed to fulfil the mechanical requirements for macrociliary function.

The unfused membrane collars enclosing axonemes at the base of the macrocilium are continuous with the surrounding sea water. This membranous reticulum provides direct ionic communication between the sea water and the individual axonemes. Macrocilia can be activated to beat at high frequency by local application of calcium to the base of the organelle, but not to more distal regions (Tamm, 1987). Separate ciliary membranes at the base of the macrocilium may permit rapid access of calcium to all the axonemes, thereby ensuring a synchronous and complete activation response.

Asymmetrical growth of axonemes

In the second pattern of ciliogenesis, all axonemes in a macrocilium initially grow together and at the same rate until they are 3–5 μm long (stump stage). Elongation within each macrocilium then becomes unequal along the aboral–oral axis: rows of axonemes on the oral side of the organelle continue growing, whereas those in the aboral direction elongate progressively less until complete arrest of elongation occurs at the aboral edge of the macrocilium. Later in ciliogenesis, the shorter axonemal rows on the aboral side elongate once again, and almost catch up in length with those on the oral side.

Thus, elongation of axonemes within a macrocilium is initially uniform, but switches to an oral–aboral gradient of growth, which reverses in polarity during the final phase of elongation.

Differences in the lengths of axonemal rows on opposite sides of a growing macrocilium could result from unequal *rates* of elongation, or different *durations* of elongation at a constant rate. Our static pictures at different stages do not allow us to distinguish between these possibilities. We plan to follow the kinetics of elongation in living macrocilia to resolve this question.

Regardless of how it occurs, this pattern of macrociliary growth shows the remarkable degree of control of microtubule elongation that is possible within a single organelle.

It should be noted that length-dependent differences in elongation rates of flagella on the same cell, as well as simultaneous elongation and shortening of adjacent flagella on a single cell occur commonly in protists (Rosenbaum *et al.* 1969; Tamm, 1967). Another example of complex regulation of organelle length (and width) is found during differentiation of the bird cochlea. Rows of actin-containing stereocilia (microvilli) elongate to different heights to produce the staircase-like pattern of the stereociliary bundle on each hair cell (Tilney *et al.* 1986).

Finally, why should growth of macrocilia by pattern II be so asymmetrical? Is there some functional advantage in having a claw-like shape during development? Answers to these questions may help us understand the mechanisms controlling macrociliary growth.

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