Development of macrociliary cells in *Beroë*

I. Actin bundles and centriole migration

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Summary

Differentiation of macrociliary cells on regenerating lips of the ctenophore *Beroë* was studied by transmission electron microscopy. In this study of early development, we found that basal bodies for macrocilia arise by an acentriolar pathway near the nucleus and Golgi apparatus, in close association with plaques of dense fibrogranular bodies. Procentrioles are often aligned side-by-side in double layers with the cartwheel ends facing outward toward the surrounding plaques of dense granules. Newly formed basal bodies then disband from groups and develop a long striated rootlet at one end. At the same time, an array of microfilaments arises in the basal cytoplasm. The microfilaments are arranged in parallel strands oriented toward the cell surface. The basal body–rootlet units are transported to the apical surface in close association with the assembling actin filament bundle. Microfilaments run parallel to and alongside the striated rootlets, to which they often appear attached. Basal body–rootlet units migrate at the heads of trails of microfilaments, as if they are pushed upwards by elongation of their attached actin filaments. Near the apical surface the actin bundle curves and runs below the cell membrane. Newly arrived basal body–rootlets tilt upwards out of the microfilament bundle to contact the cell membrane and initiate ciliogenesis. The basal bodies tilt parallel to the flat sides of the rootlets, and away from the direction in which the basal feet point. The actin bundle continues to enlarge during ciliogenesis. These results suggest that basal body migration may be driven by the directed assembly of attached actin filaments.

Key words: *Beroë*, actin filaments, centriole migration, macrocilia.

Introduction

Giant structures often lend themselves to the analysis of general biological problems. Likewise, study of the formation of such structures often provides insights into developmental processes of general significance. A good example is macrocilia, which are giant compound ciliary organelles found on the lips of the ctenophore *Beroë* (Horridge, 1965). We previously used macrocilia to gain new insights into the mechanosensitivity of ciliary motility, and the mechanism regulating microtubule sliding during the ciliary beat cycle (Tamm, 1983; Tamm & Tamm, 1984). We also exploited the giant capping structure at the tip of macrocilia to visualize sliding displacement of microtubules during bending of living cilia (Tamm & Tamm, 1985).

Besides possessing this unique type of ciliary organelle, macrociliary cells contain one of the largest bundles of actin filaments found in non-muscle cells (Tamm & Tamm, 1987*a*). This massive actin bundle extends 25–30 μm from the base of the macrocilium to the opposite end of the cell, where it terminates at a junction with underlying muscle cells. The actin bundle is thought to serve as an intracellular tendon to couple the activities of macrocilia and muscles during ingestion of prey (Tamm & Tamm, 1987*a*).

In this paper we describe a pathway of early development of macrociliary cells. By relating the formation and growth of the actin filament bundle to the migration of newly formed basal bodies to the cell surface, we provide evidence for a novel assembly-dependent role of actin in centriole transport.

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In the accompanying paper, we describe unusual features of formation and growth of macrocilia. Preliminary reports of this work have appeared previously (Tamm & Tamm, 1986, 1987*b*).

Materials and methods

Organisms

Several species of *Beroë* were used: *Beroë*, species unknown, which appeared in Woods Hole, MA in October and November 1985, after hurricane Gloria (hereafter called *B. sp.*); *B. ovata* collected in the Gulf Stream by Dr L. Madin in August 1985 and 1986; and *B. cucumis* collected locally and also shipped to MBL from Friday Harbor, WA.

Lip regeneration

To induce large-scale differentiation of macrociliary cells, the lips of *Beroë*, including the entire field of macrociliary cells, were cut off with a pair of dissecting scissors (Fig. 1). The operated animals were maintained in bowls of sea water during regeneration of lips.

Correlated light and electron microscopic observations of regenerating lips were usually made 6–7 days after the operation.

Living observations

For light microscopy, a small piece of lip was excised and mounted in sea water on a microscope slide. Tissue was viewed through Zeiss DIC optics (16× or 40× objectives). Images were recorded by a Dage MTI 67 M video camera on a GYYR videocassette recorder (model 2051), or by an Olympus OM-2N 35 mm camera using Kodak 2415 Tech Pan film and an Olympus T 32 flash tube.

Rhodamine–phalloidin staining

Lip tissue was fixed in 3·7% formaldehyde, 0·14 M-NaCl, 0·2 M-sodium cacodylate (pH 7·8) for 15 min at room temperature, washed in 0·3 M-NaCl, 0·2 M-sodium cacodylate (pH 7·8) for 15 min, and incubated in 0·33 μM-rhodamine–phalloidin (Molecular Probes, Junction City, OR), 0·3 M-NaCl, 0·2 M-sodium cacodylate (pH 7·8) for 30 min. Slide preparations were mounted in 50% glycerol, 0·15 M-NaCl, 0·1 M-sodium cacodylate (pH 7·8) with 5% *n*-propyl gallate (Sigma Chemical Co., St Louis, MO) to retard bleaching.

Slides were examined with a Zeiss IM-35 microscope using epifluorescence optics (TRITC filter set) and 63×/1·4 NA Planapochromat or 25×/0·8 NA Plan neofluar phase-contrast objectives. Photographs were taken on Kodak Tri-X 35 mm film pushed to ASA 1600 with Perfection XR-1 developer (Perfection Photographic Products, Inc., Los Angeles, CA).

Electron microscopy

Parallel samples of lips were fixed for electron microscopy. The primary fixative consisted of 2·5% glutaraldehyde, 1% paraformaldehyde, 1% osmium tetroxide, 0·075 M-NaCl, 0·01 M-$CaCl_2$, 0·2 M-sodium cacodylate (pH 7·8) for 1 h at 0°C. Lip pieces were washed in 0·3 M-NaCl, 0·2 M-sodium cacodylate (pH 7·8) for 30 min at 0°C, postfixed in 1% osmium tetroxide, 0·37 M-NaCl, 0·1 M-sodium cacodylate (pH 7·8) for 20 min at 0°C, washed in distilled water for 15 min at 0°C, and stored overnight in 1% aqueous uranyl acetate at 4°C. Tissue was dehydrated rapidly in an acetone series and flat-embedded in Araldite. Thin sections of oriented blocks were picked up on Formvar-coated grids, stained with uranyl and lead salts, and examined in a Zeiss 10 CA electron microscope at 80 kV.

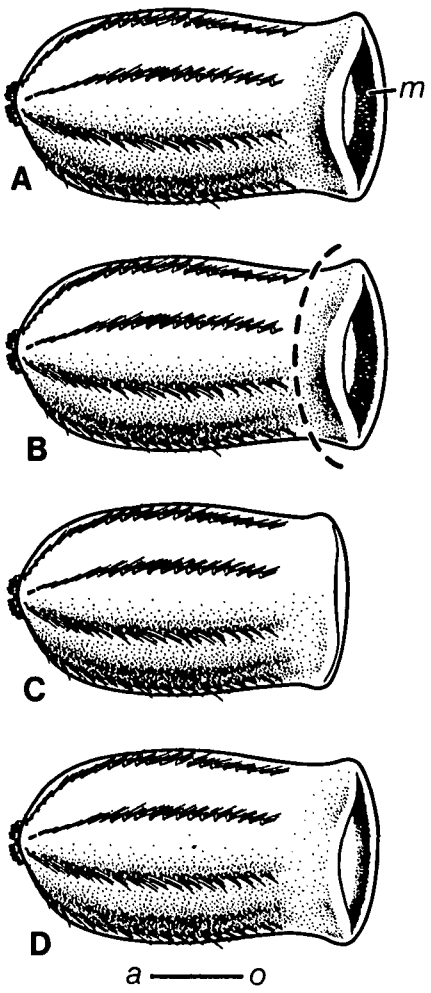

Fig. 1. Regeneration of lips by *Beroë*. A. Side view of intact animal with aboral sensory organ on left and open mouth at right (*a–o*, aboral–oral axis). Around the inside of the lips is the band of macrocilia (*m*). Four of the eight meridional rows of ciliary comb plates are shown. B. Excision of lips to induce regeneration. C. Wound healing soon after the operation. D. At 6–7 days after the operation. The lips and band of macrocilia have partially regenerated, starting at the lip edge (right, latest stages of development) and progressing inward toward the pharynx (left, earliest stages).

Results

Normal structure

Macrocilia are finger-shaped compound ciliary organelles, ≈40 μm long and 4–5 μm in diameter, found in a dense band around the inside of the lips of beroid

ctenophores (Horridge, 1965; Tamm, 1983). A single macrocilium consists of a hexagonal 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).

Each macrocilium arises from the broad end of an elongated epithelial cell. A massive, wedge-shaped bundle of parallel actin microfilaments extends 25–30 μm from the base of the macrocilium to the opposite, tapered end of the cell, terminating at a junction with subepithelial smooth muscle cells (Tamm & Tamm, 1987*a*). The basal bodies of the macrocilium possess striated rootlet fibres that extend downward into the broad end of the microfilament bundle. The actin filaments have a uniform polarity, such that myosin S_1-decorated arrowheads point away from the tapered, membrane-associated end of the bundle (Tamm & Tamm, 1987*a*). The microfilament bundles apparently are not contractile, but serve as 'intracellular tendons' to couple macrocilia mechanically to underlying muscle fibres during ingestion of prey (Tamm & Tamm, 1987*a*).

Regeneration of macrociliary cells

Franc (1970) used lip regeneration of *B. ovata* to describe, at the histological level, cell and tissue interactions during lip differentiation. Like Franc, we found that excision of the lips induces regeneration of macrociliary cells, and that differentiation proceeds in a definite spatiotemporal sequence (Fig. 1). However, we observed that differentiation begins at the lip edge and progresses aborally during both lip regeneration and normal development, contrary to Franc's report that differentiation proceeds in the opposite direction.

For our study we used partially regenerated stages in which fully differentiated macrociliary cells were present at the lip edge, and successively earlier stages of development occurred inwards towards the pharynx (Fig. 1D). This gave us a continuous series of developmental stages for constructing the sequence of events during regeneration.

We found two different pathways of macrociliary cell development, termed patterns I and II. The differences between the two patterns are most noticeable during ciliogenesis (Tamm & Tamm, 1988).

In *B. cucumis*, only the first pattern is found, during normal growth as well as lip regeneration. In the other species both patterns occur, although the second pattern is more common.

We have not determined the complete sequence of development for either pathway. Instead, we present those aspects of each pattern that provide insights into developmental problems of general interest.

In this paper, on early development, we use the first pattern to show how assembly of oriented actin filaments may be responsible for the directed migration of basal bodies to the cell surface.

In the accompanying paper (Tamm & Tamm, 1988), on formation and growth of macrocilia, we emphasize the second pattern of development because it reveals unusual aspects of microtubule elongation and ciliary membrane fusion.

Centriole (basal body) formation

Macrociliary cells in early stages of pattern I differentiation are roughly cuboidal in shape, and are filled with numerous ribosomes and rough endoplasmic reticulum. The plasma membrane is often thrown into folds in certain areas.

Procentrioles, destined to become the basal bodies of the macrocilium, arise in a group near the nucleus and Golgi apparatus (Figs 2, 3). No pre-existing diplosomal centrioles have been detected in the vicinity of the procentrioles.

Electron-dense granules, 60–80 nm in diameter, are closely associated with the procentrioles (Figs 2, 3). The dense granules consist of radially arranged fibrillar and particulate material, and are often surrounded by ribosomes (Fig. 2B).

The dense granules are arranged in plaque-like arrays surrounding the procentrioles (Fig. 2A). The procentrioles initially are aligned side-by-side in one or two layers. Within a layer the proximal or cartwheel ends of the procentrioles, where evident, face outward toward the adjacent dense granules (Fig. 3). In double layers of procentrioles, the cartwheel ends in each layer are opposed and face towards the surrounding dense-granule plaques. The cartwheel ends of the procentrioles are often connected to the dense granules by thin wispy material (Fig. 3). A similar, ladder-like configuration of developing basal bodies was reported previously in protozoan flagellates from an Australian termite (Tamm & Tamm, 1980).

Triplet microtubules of procentrioles are assembled in a stepwise pattern around a hub-and-spokes cartwheel structure: the A or innermost tubules appear first, then the B tubules, and finally the C tubules, as in other developing centrioles (Fig. 2B,C) (Anderson & Brenner, 1971; Dippell, 1968; Fulton, 1971; Kalnins & Porter, 1969; Pitelka, 1974; Tamm & Tamm, 1980; Wolfe, 1972). A thin ring of dense material encircles the lumen of the cartwheel in early stages, but disappears later after microtubule assembly is completed. A cartwheel ring was also found in the lumen of developing basal bodies in Australian termite flagellates (Tamm & Tamm, 1980).

The tilt angle of the triplet microtubule blades at the proximal end of the cylinder is greater in developing centrioles than in mature organelles (Fig. 2B,C). The

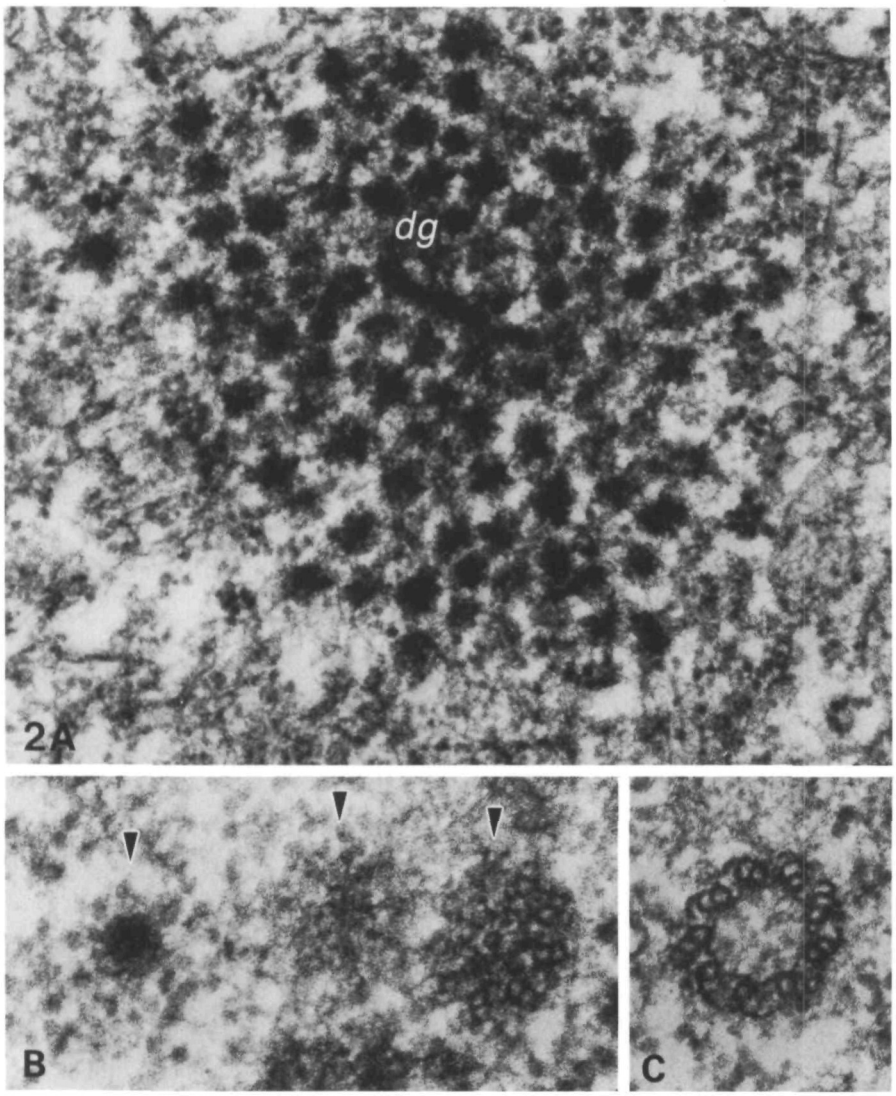

Fig. 2. Procentriole formation. A. Face view of a plaque-like array of dense granules (*dg*) with numerous ribosomes. ×72 700. B. Three successive stages in procentriole development. From left to right (arrowheads): dense granule with surrounding ribosomes (left); appearance of cartwheel and assembling microtubules (centre); hub-and-spokes cartwheel with A and B microtubules (right). Note steep pitch of assembling microtubular blades. ×102 100. C. Distal end of a procentriole with assembling triplet microtubules. Note the decreased angle of the triplet blades. ×130 300.

triplet angle decreases toward the distal end, as reported in other systems (Dippell, 1968; Pitelka, 1974; Stubblefield & Brinkley, 1967; Tamm & Tamm, 1980).

Basal bodies elongate to their mature length of ≈0·2 μm while still arranged in groups. At this stage, striated rootlets and basal feet have not yet developed.

A similar pattern of basal body formation occurs during normal growth of *Beroë*. Groups of procentrioles are located under the narrow basal end of the existing actin bundle, at some distance from the base of the developing macrocilium.

Actin bundle formation and centriole migration

The appearance of actin filaments, the development of striated rootlets at the proximal ends of the basal bodies, and the onset of basal body migration towards the apical cell surface are closely coupled temporally and spatially.

An array of parallel microfilaments arises in the basal region of the cell, between the Golgi apparatus and lateral plasma membrane (Fig. 4). The microfilaments are loosely arranged in parallel strands that are oriented toward the cell surface. Numerous cisternae of rough endoplasmic reticulum surround the developing actin bundle. In some micrographs the microfilaments appear to be associated with dense, folded regions of the cell membrane, but we have not been able to identify with certainty the site(s) of origin of the microfilaments.

The microfilaments are morphologically identical to the actin-containing filaments in the massive filament bundle of mature cells (Tamm & Tamm, 1987*a*). As will be shown below, the filaments develop into this bundle. The microfilaments in differentiating cells are therefore presumed to contain actin. We have not yet determined the polarity of the filaments in these early stages.

The newly formed basal bodies disband from groups and are found close to the developing microfilament array. The cartwheel structure has disappeared from the lumen, and dense granules are no longer evident. A

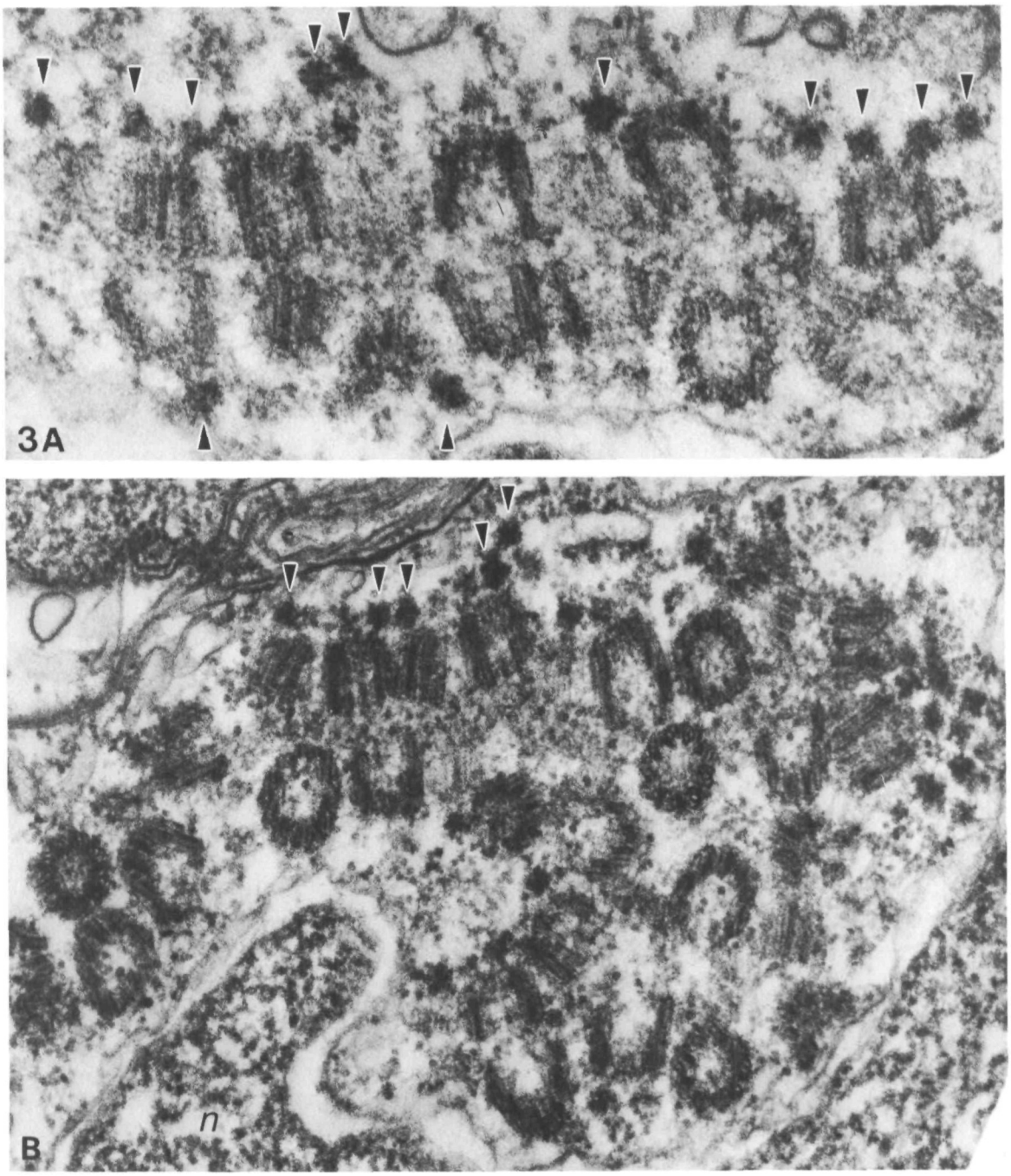

Fig. 3. Procentriole groups. A. Side-by-side alignment of procentrioles in two layers, with cartwheel ends (where evident) facing outward toward the plaques of dense granules (arrowheads). ×82 000. B. Group of procentrioles near the nucleus (*n*), showing side-by-side arrangement and adjacent dense granules (arrowheads). ×65 200. In A and B note wispy connections between dense granules and adjoining cartwheel ends of procentrioles.

long, striated rootlet develops at one end of each basal body (Figs 5–7). The rootlet consists of laterally aligned fine fibrils with periodic transverse densities. The rootlet fibrils arise from dense material, which coats the proximal ends of the centriolar triplet microtubules. The fibrils converge to form a broad flat rootlet that projects directly backward from the basal body cylinder. The opposite (distal) end of the basal body becomes plugged by a cap of fuzzy electron-dense material.

Basal body–rootlet units are transported to the apical cell surface in close association with the assembling actin bundle (Figs 5–7). The microfilaments run parallel to and alongside the striated rootlets, to which they often appear attached (Fig. 6). The microfilaments extend behind the rootlets as 'tails', and anastomose

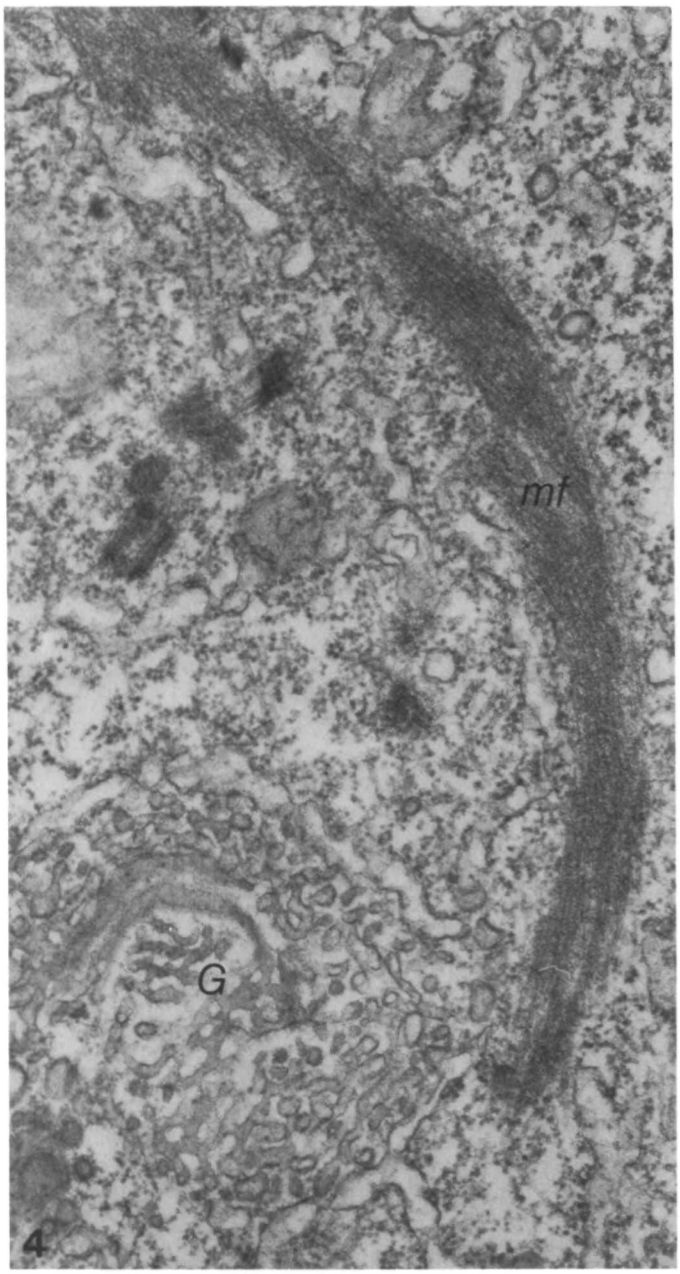

Fig. 4. Early stage of development of the actin bundle. The parallel microfilaments (*mf*) run from near the Golgi (*G*) toward the apical cell surface (top). Note numerous ribosomes and elements of rough endoplasmic reticulum. ×39300.

with other strands to form a loosely woven, three-dimensional filament lattice pointing toward the cell surface. The basal body–rootlet units migrate toward the apical surface at the heads of trails of microfilaments (Fig. 6A). At any one time, migrating basal bodies are found at different positions in the cell. However, they are preferentially aligned parallel to the paths of the microfilaments.

The number and length of the microfilaments increase as more and more basal bodies are transported toward the cell surface. In the apical cytoplasm the microfilament bundle curves and runs almost parallel to the surface, forming a dense mat under the cell membrane (Fig. 7). Most of the newly arrived basal body–rootlet units are embedded in this bundle, with their long axes aligned parallel to the surrounding filaments and overlying cell membrane.

The basal body–rootlet units next tilt obliquely upwards out of the microfilament bundle toward the cell surface (Fig. 7). A basal foot is evident on one side of the wall of each emerging basal body (Fig. 7). The basal foot is a fibrillar, banded cone, which projects from the basal body wall in the same plane as the flat side of the striated rootlet. Cortical microtubules are attached to the basal foot and run parallel to the surface membrane (Fig. 7D). The basal bodies tilt upwards in a plane parallel to the flat sides of the rootlets. The basal feet project uniformly downwards from the lower sides of the centriolar cylinders (Fig. 7). The tilt plane is parallel to the oral–aboral axis, and the basal feet point in the aboral direction. After the distal end of a basal body contacts the overlying plasma membrane, ciliogenesis is initiated. The long axes of the basal bodies are now oriented roughly perpendicular to the apical surface, and the striated rootlets extend downwards into the actin bundle at right angles to the paths of the microfilaments.

During later stages of ciliogenesis, actin bundles increase in size, macrociliary cells elongate, and the number and length of new cilia increase.

Rhodamine–phalloidin staining of fixed material shows a progressive increase in length and width of the actin bundles during pattern I ciliogenesis (Fig. 8). Cells with small tufts of single cilia contain small, spindle-shaped actin bundles, 10 μm long and 2–3 μm maximum width (Fig. 8A,B). In cells at later stages, the cilia are longer and fuse into slender groups. The actin bundles in these cells are longer (12–15 μm) and thicker (3 μm) (Fig. 8C,D). Finally, cells with almost full-grown macrocilia possess greatly elongated actin bundles, 25 μm long and 4–5 μm maximum width (Fig. 8E,F).

The entire sequence of events during early differentiation of macrociliary cells by pattern I is summarized diagrammatically in Fig. 9.

Discussion

Basal body formation

In cells that are replicating their diplosomal centrioles, or doubling their number of basal bodies, new centrioles/basal bodies typically arise at right angles to the pre-existing ones by the so-called centriolar pathway (Allen, 1969; Gall, 1961; Dippell, 1968; Robbins *et al.* 1968).

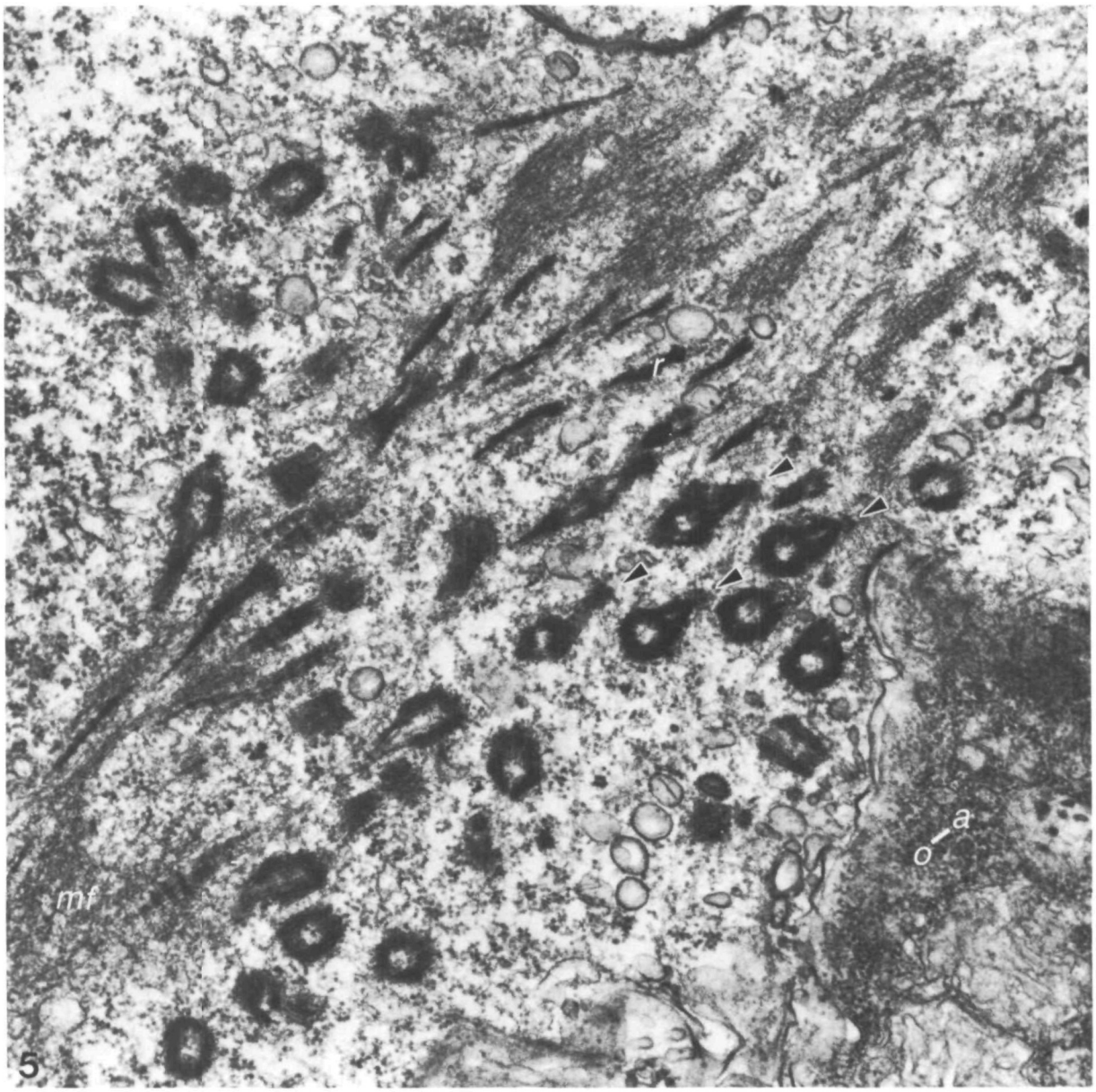

Fig. 5. Oblique survey section near the apical surface showing basal bodies with rootlets migrating from lower left to upper right. At lower left, basal body–rootlet units (here sectioned longitudinally) approach the cell surface with their long axes oriented parallel to the surrounding microfilaments (*mf*). At upper right, basal bodies have tilted 90° towards the reader to reach the cell surface and are now cut transversely. The basal bodies are uniformly oriented with basal feet pointing aborally (arrowheads) and flat rootlets (*r*, shown in cross-section) aligned parallel to the microfilament bundle. ×38 700.

When a large number of centrioles is formed, procentrioles invariably arise in close association with aggregates of dense fibrogranular material that lack centriolar structure (acentriolar pathway) (Anderson & Brenner, 1971; Dirksen & Crocker, 1966; Dirksen, 1971; Kalnins & Porter, 1969; Hepler, 1976; Sorokin, 1968; Steinman, 1968; Mizukami & Gall, 1966; Tamm & Tamm, 1980; Chang *et al.* 1979; Youson, 1982). These intermediate structures have been variously called dense bodies, deuterosomes, condensation forms or blepharoplasts.

The formation of numerous basal bodies for a macrocilium also follows an acentriolar pathway. The dense granules in differentiating macrociliary cells are not associated with diplosomal centrioles, but are found near the Golgi apparatus, as reported in other systems (Anderson & Brenner, 1971; Chang *et al.* 1979; Youson, 1982).

The origin of the various fibrogranular structures is unknown. Their proximity to pre-existing diplosomal centrioles in some systems has suggested that mature centrioles may be involved in generating this material

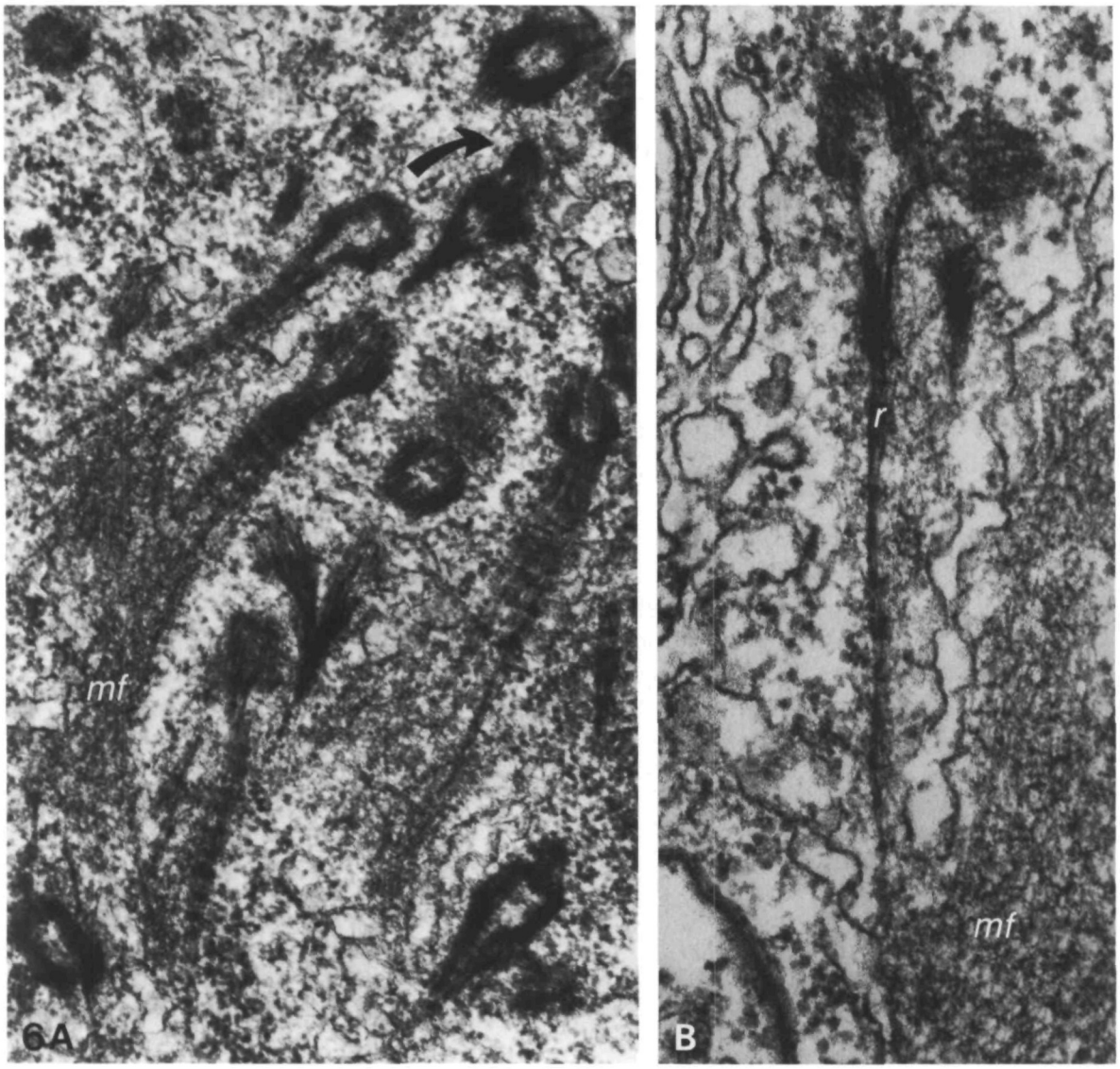

Fig. 6. Migrating basal body–rootlets. A. Basal bodies with rootlets moving upward to the apical surface (arrow) in close association with strands of microfilaments (*mf*). Microfilaments appear attached to the sides of the striated rootlets, and extend behind as anastomosing trails oriented parallel to the direction of migration. ×55 300. B. Longitudinal section of a migrating basal body, perpendicular to the flat side of its rootlet (*r*). Microfilaments run alongside the rootlet and merge behind with a larger strand of microfilaments (*mf*) on right. Note cisternae of rough endoplasmic reticulum. ×66 500.

(Sorokin, 1968; Dirksen, 1971; Kalnins & Porter, 1968).

It should be noted, however, that the blepharoplast, an intermediate structure in the development of flagellated sperm of lower plants, arises in the absence of pre-existing centrioles (Hepler, 1976). Indeed, *de novo* formation of basal bodies is well-documented in many organisms (Cavalier-Smith, 1974; Fulton, 1971; Fulton & Dingle, 1971; Grimes, 1973; Perkins, 1970; Tamm & Tamm, 1980).

In the present study, the close association of ribosomes with the dense granules suggests that the fibrogranular material may be synthesized by the surrounding ribosomes.

The ubiquitous occurrence of fibrogranular material nearby forming procentrioles suggests that these intermediate structures provide material for the production of procentrioles (Anderson & Brenner, 1971; Dirksen & Crocker, 1966; Steinman, 1968; Dirksen, 1971; Chang *et al.* 1979; Youson, 1982). In macrociliary cells, the wispy connections between the dense granules and the procentrioles suggest that the dense granules in our system also may contribute precursor material for the assembly of basal bodies. In this regard, tubulin has recently been localized in the blepharoplast of the fern *Platyzoma* by monoclonal antibody staining (Doonan *et al.* 1986).

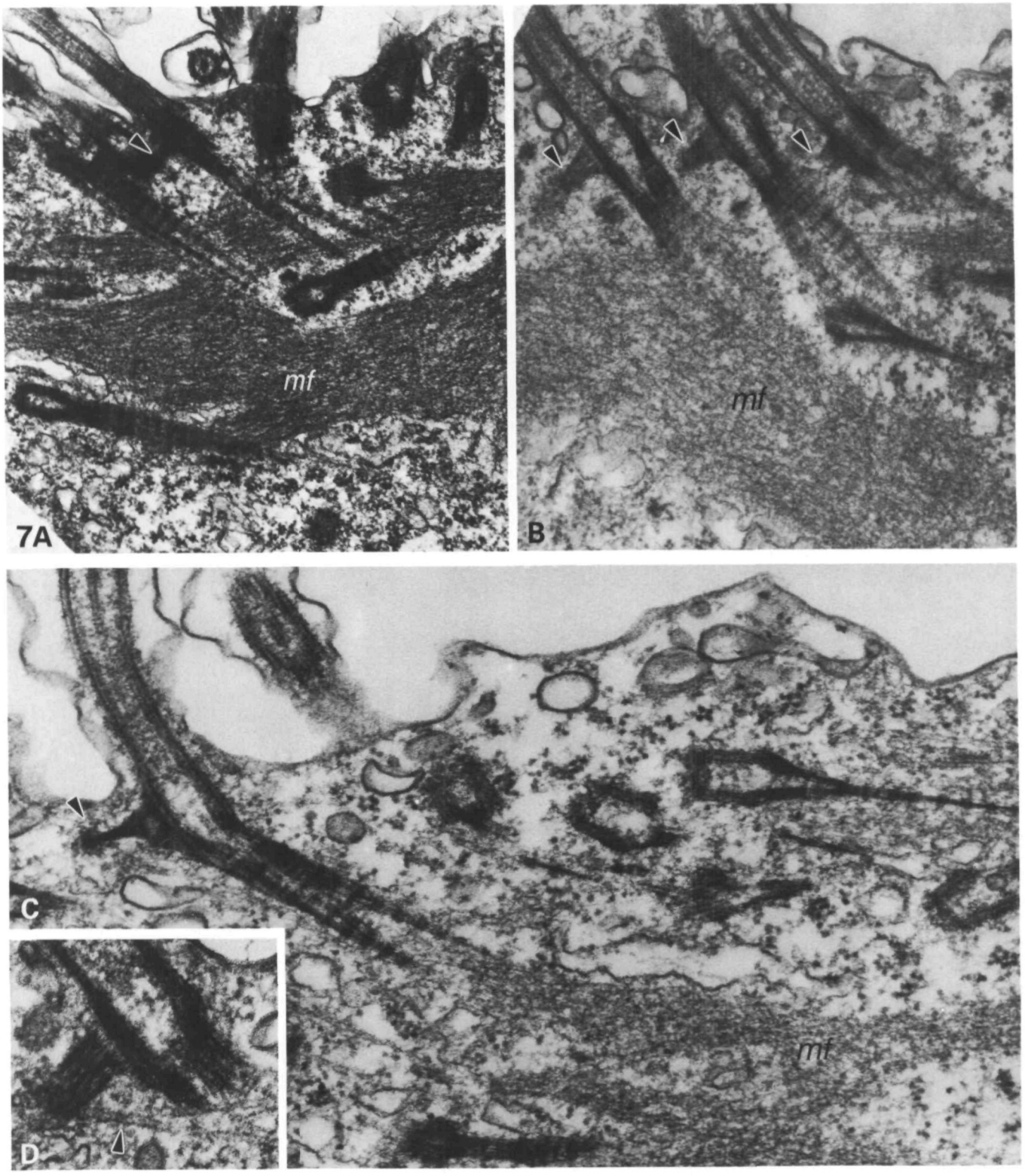

Fig. 7. Longitudinal sections through the apical cytoplasm at the initiation of ciliogenesis. Basal body–rootlets arrive below the surface from the right (oral direction), embedded in the microfilament bundle (*mf*) with their long axes aligned parallel to the filaments. Basal bodies tilt upwards (clockwise direction here) out of the microfilament bundle to reach the surface and initiate ciliary growth. The tilt plane of the basal bodies is uniformly parallel to the flat sides of the rootlets and to the basal feet, which project from the left sides of the basal body wall (arrowheads). D. Cortical microtubules (arrowhead) attach to the basal foot. A, ×34 600; B, ×48 300; C, ×63 900; D, ×78 500.

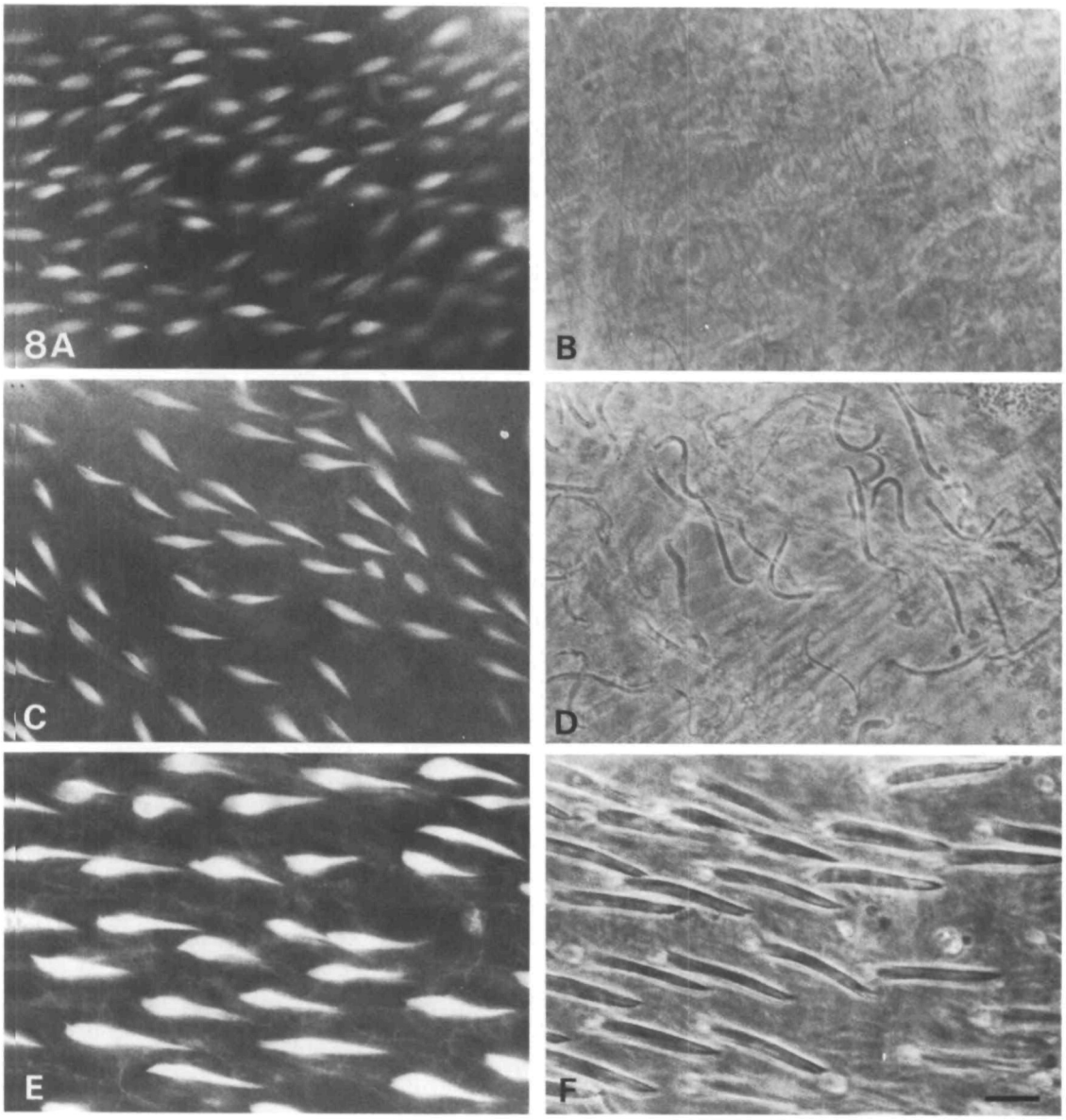

Fig. 8. Corresponding fluorescence (A,C,E) and phase-contrast (B,D,F) face views of rhodamine–phalloidin-stained lip epithelium from regenerating *B. cucumis*. Note progressive increase in size of the actin bundle during growth of macrocilia. A,B. Early stage near aboral edge of regenerating lip. Cells with tufts of single cilia contain small, spindle-shaped actin bundles. C,D. Later stage nearer lip edge. Actin bundles have increased in length and width as the cilia have elongated and fused into slender groups. E,F. Almost completely regenerated macrociliary cells at the oral edge of the lips. Cells with almost full-grown macrocilia contain thick, elongated actin bundles. ×765. Bar, 10 μm.

In summary, the basal bodies of macrocilia arise by an acentriolar pathway, apparently without any contribution by mature centrioles. Dense fibrogranular bodies surrounded by ribosomes are closely associated with the forming basal bodies, and may provide precursor material for the assembly of the procentrioles.

Actin bundle formation

Actin bundles of microvilli, stereocilia and sperm acrosomal processes are nucleated from membrane sites, and the filaments elongate by addition of actin monomers to their membrane-associated (preferred) ends (Tilney *et al.* 1981; Pollard & Mooseker, 1981). Myosin S_1 decoration demonstrates that the filaments have uniform polarity, with the arrowheads pointing away from the membrane-associated end of the bundle (Begg *et al.* 1978; DeRosier & Tilney, 1982; Tilney, 1975).

The relevant questions for our study were: how and where does the massive actin filament bundle of macrociliary cells arise? How are the orientation and polarity of the filament bundle established?

Clearly, much work remains to be done to answer these questions. Some of our images suggest that initial

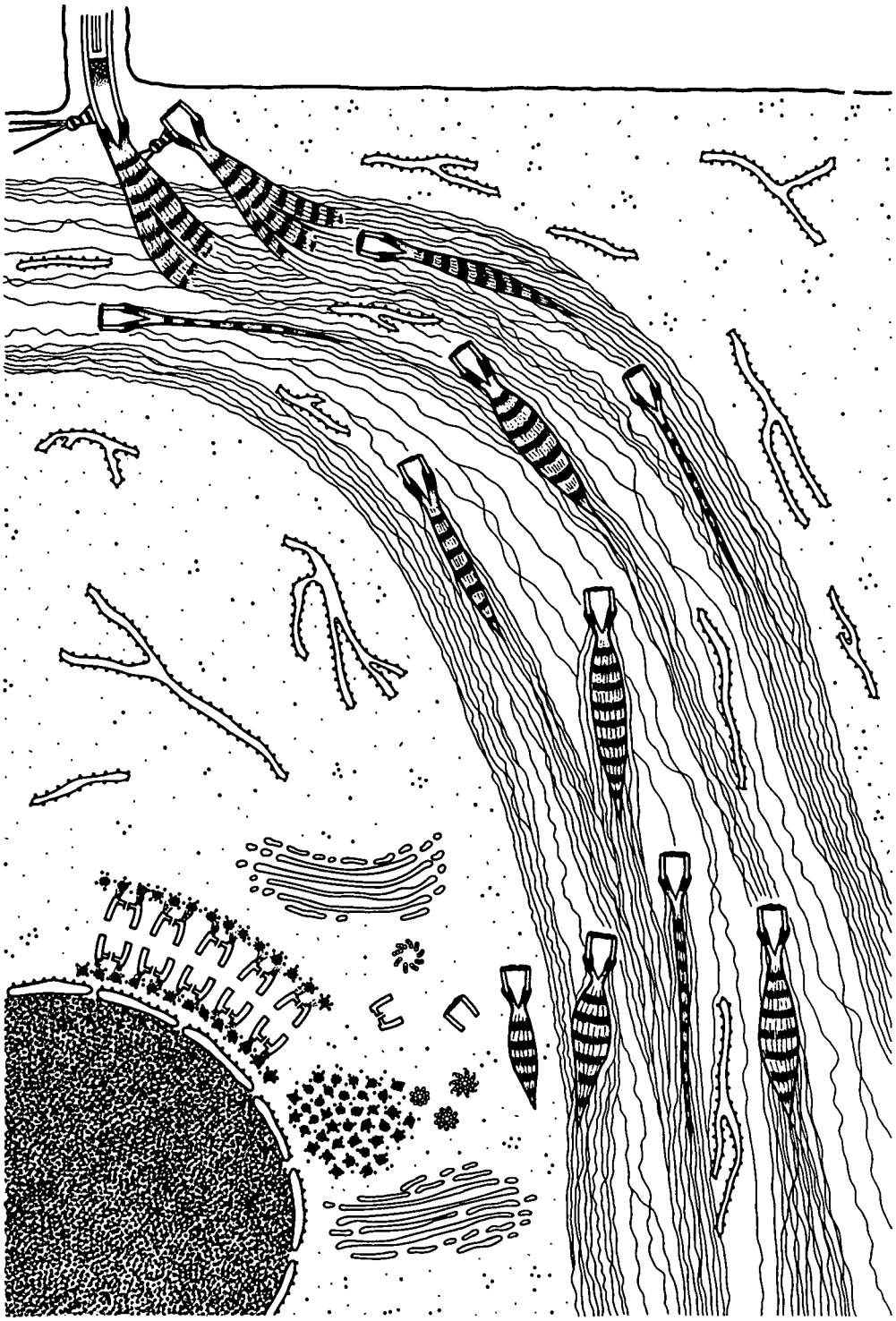

Fig. 9. Sequence of events during early development of macrociliary cells by pattern I. At lower left, procentrioles arise in organized groups near the nucleus and Golgi apparatus. The procentrioles lie side-by-side in layers with their cartwheel ends facing out towards adjacent plaques of dense granules. A face view of a dense-granule plaque with assembling procentrioles is shown immediately to the right. Proceeding counterclockwise in the diagram, a long flat striated rootlet develops from the proximal end of each newly formed basal body. The basal bodies then migrate toward the apical cell surface in close association with the developing bundle of parallel actin microfilaments. Many of the surrounding microfilaments are attached to the sides of the basal body–rootlets and trail behind, merging with other strands of filaments. Near the surface, the actin bundle curves and runs under the plasma membrane. Basal body–rootlets tilt upwards out of the bundle in a plane parallel to the flat rootlets and newly formed basal feet, which project from the aboral side of the basal body wall and serve as foci for cortical microtubules (upper left). Ciliary growth begins when the distal ends of the basal bodies contact the plasma membrane.

formation of the actin bundle is associated with electron-dense areas of the plasma membrane, in agreement with other reports. However, because the actin bundle develops as a loose three-dimensional network of parallel strands, assembly is probably initiated at multiple sites within the cell, perhaps even from the basal body–rootlet units themselves (see below).

We have not yet determined the polarity of the microfilaments in the developing bundle. In the mature actin bundle, S_1-decorated arrowheads point away from the tapered, membrane-associated end (Tamm & Tamm, 1987*b*). Knowledge of filament polarity in differentiating cells would help elucidate possible assembly sites, as well as the role of filaments in directing basal body migration.

Centriole migration

Ciliogenesis by an acentriolar pathway typically involves migration of newly formed centrioles from various intermediate structures to the apical surface.

The mechanism(s) of centriole migration is poorly understood. The use of cytoskeletal-disrupting drugs suggests that actin is somehow involved in this motility. For example, the positioning and mobility of centrosomes during activation of neutrophils are inhibited by cytochalasin D, an actin-depolymerizing agent (Euteneuer & Schliwa, 1985). Likewise, cytochalasin D inhibits centriole migration during ciliogenesis in the quail oviduct (Boisvieux-Ulrich *et al.* 1984). In addition, myosin has recently been localized to migrating centrioles in the quail oviduct by immunocytochemistry (Lemullois *et al.* 1987).

Disassembly of microtubules with nocodazole or colchicine prevents centrosome splitting in neutrophils (Euteneuer & Schliwa, 1985), but does not inhibit centriole migration in quail oviduct (Sandoz *et al.* 1987).

On the basis of these results, Sandoz and co-workers have proposed that an actomyosin system may be involved in centriole migration during ciliogenesis (Lemullois *et al.* 1987). It should be pointed out, however, that structural evidence for participation of actin microfilaments in centriole motility has not been obtained previously.

Our work provides the first clear morphological demonstration that actin filaments are closely associated with migrating basal bodies. The uniform orientation of basal body–rootlets and surrounding microfilaments parallel to the direction of migration leaves little doubt that actin filaments play an important role in basal body transport.

How could this occur? The formation and growth of the actin bundle is correlated temporally and spatially with the migration of the basal bodies to the cell surface. In addition, the direction of basal body migration coincides with the orientation of the growing microfilaments. Moreover, we do not see tracks of microfilaments *ahead* of the migrating basal bodies, but only trailing *behind* them. Finally, the basal body–rootlets with their tails of microfilaments appear to migrate as a unit.

We therefore conclude that basal body–rootlets are pushed toward the apical cell surface by elongation of their attached microfilaments during growth of the actin bundle. Basal body migration in our system would thus be due to the oriented assembly of attached actin filaments.

An assembly-dependent role of actin in organelle movements has a precedent. Explosive polymerization of actin provides the force for extension of the acrosomal process of *Thyone* sperm (Tilney, 1975). In addition, the controlled disassembly and assembly of microtubules is involved in chromosome movement (Inoué, 1981).

How is the basal body–actin filament interaction initiated in developing macrociliary cells? That is, how do newly formed basal bodies make contact with and 'capture' the polymerizing actin filaments? Do the basal body–rootlet units serve as nucleation sites of actin filament assembly as well? Like the organization of mitotic spindle microtubules onto kinetochores of chromosomes, these questions are important for understanding the functional interactions between cytoskeletal elements and organelles during cell development and motility.

The last step in the migration of basal bodies is their emergence from the actin bundle to contact the plasma membrane for initiation of ciliary outgrowth. Unlike the preceding migration towards the surface that occurred parallel to the microfilaments, movement to the surface itself involves tilting of the basal body–rootlet out of the path of the filaments, resulting in a mutually perpendicular orientation of the basal body–rootlets to the actin filaments.

It does not seem likely that this final phase of basal body movement could also be driven by elongation of actin filaments. Instead, the appearance of basal feet and associated cortical microtubules on tilting basal bodies suggests that these structures may play a role in the final movement of basal bodies to the cell surface.

In conclusion, the early differentiation of macrociliary cells shows that, rather than development depending on motility, motility (in this case, migration of basal bodies) may depend on a developmental process (actin filament assembly).

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