# Microfilament-Supported Macrovilli in the Hindgut of the Polychaete *Dinophilus gyrociliatus*

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Large macrovilli of 7.7 μm maximal length and a diameter of 0.4 μm with several hundred cytochalasin B-sensitive 6 nm microfilaments are found as bordering lateral structures in the ciliary groove of the hindgut of *Dinophilus gyrociliatus*. The microfilament bundle, accompanied by microtubules, extends with its tapering rootlets to the base of the cells. Another cell type in the middle of the groove bears the cilia and equally broad, but shorter macrovilli with a cytochalasin-sensitive microfilament core.

## Introduction

The small marine polychaete *Dinophilus gyrociliatus* has been mainly studied because of its extreme sexual dimorphism which is preceded by an egg dimorphism [1, 2]. Small eggs give rise to neotenic dwarf males of about 50 μm size and large ones to females which reach a size of about 1.3 mm when sexually mature. The females have protonephridia [3] and resemble an elongated trochophora. Their single developed ovary, usually at the right side, is located in the posterior third of the body next to the irregularly folded hindgut with its hitherto undescribed ciliated groove. The epithelial cells lining the groove laterally bear stiff extensions with densely packed microfilaments which are the subject of this communication. Because of their extraordinary size which by far exceeds that of all known microvilli of intestinal epithelial cells, the term macrovilli is proposed. Although intestinal ciliated grooves are known mainly from sedentary polychaetes, but also sipunculids and echiurids [4], there is no report about macrovilli-like structures as described in this paper.

## Material and Methods

The organisms were cultured at 18 °C in previously filtered and boiled sea water and fed with unicellular green algae of the genus *Dunaliella*. Both living *Dunaliella* and heat-killed suspensions were used.

Prior to fixation, some cultures were treated with cytochalasin B (10, 20 or 40 μg/ml) for 1 h or with 35 μg/ml for 20 h. The drug was dissolved in 1% aqueous dimethyl sulphoxide (DMSO). The highest concentration of DMSO in cultures treated with cytochalasin B was 0.4%. Controls were treated with 0.4% DMSO only.

The following fixations were employed.

a: 1 h 3% glutaraldehyde, followed by 50 min 1% $OsO_4$, both fixatives applied in PIPES buffer (pH 7.3) containing 5 mM $CaCl_2$.

b: 1 h 3% glutaraldehyde, 5% DMSO, 0.15% tannic acid in 0.1 M cacodylate buffer pH 7.4, followed by 30 min 0.5% $OsO_4$ and 0.8% $K_3Fe(CN)_6$ in the same buffer. En bloc staining was carried out for 1 h in 2% aqueous uranyl acetate [5, 6].

c: 1 h in a mixture of 3% glutaraldehyde and 2% $OsO_4$ in 0.094 M PIPES buffer (pH 7.4), containing 5 mM $CaCl_2$, followed by 30 min 1% tanic acid in 0.05 M cacodylate buffer, pH 7.25, and two rinses in 1% $Na_2SO_4$ in the same buffer [7].

d: 50 min in a mixture of 3% glutaraldehyde and 1% $OsO_4$ in 0.15 M cacodylate buffer (pH 7.4), followed by 1 h 0.2% tannic acid in 0.05 M cacodylate buffer. After two rinses in the same buffer (10 min each) and in distilled water, en bloc staining for 15 min with 1% uranyl acetate dissolved in 25% ethanol.

The specimen were embedded in Epon. Ultrathin sections were stained with aqueous uranyl acetate and lead citrate and viewed at 80 kV with a Philips EM 410 electron microscope.

## Results and Discussion

Fig. 1 shows a light micrograph of the ciliated groove (cg) from a semithin section of a specimen embedded for electron microscopy. The dark borders of the groove are due to the peculiar macrovilli to be described below. The groove originates dorsally at the entrance to the hindgut and extends posteriorly to its ventral side in a right-handed helix in

*Abbreviations:* a, anterior; bl, basal lamina; c, cilia; cg, ciliary groove; coe, coelom; d, dorsal, ed, epidermis; g, Golgi apparatus; l, gut lumen; m, mitochondrion; mav, long macrovilli; mc, middle cell; mf, rootlet bundle of macrovilli; mt, microtubule; mvc, macrovilli cell; r, ciliary rootlet; rer, rough endoplasmic reticulum; sj, septate junction; smv, short macrovilli.

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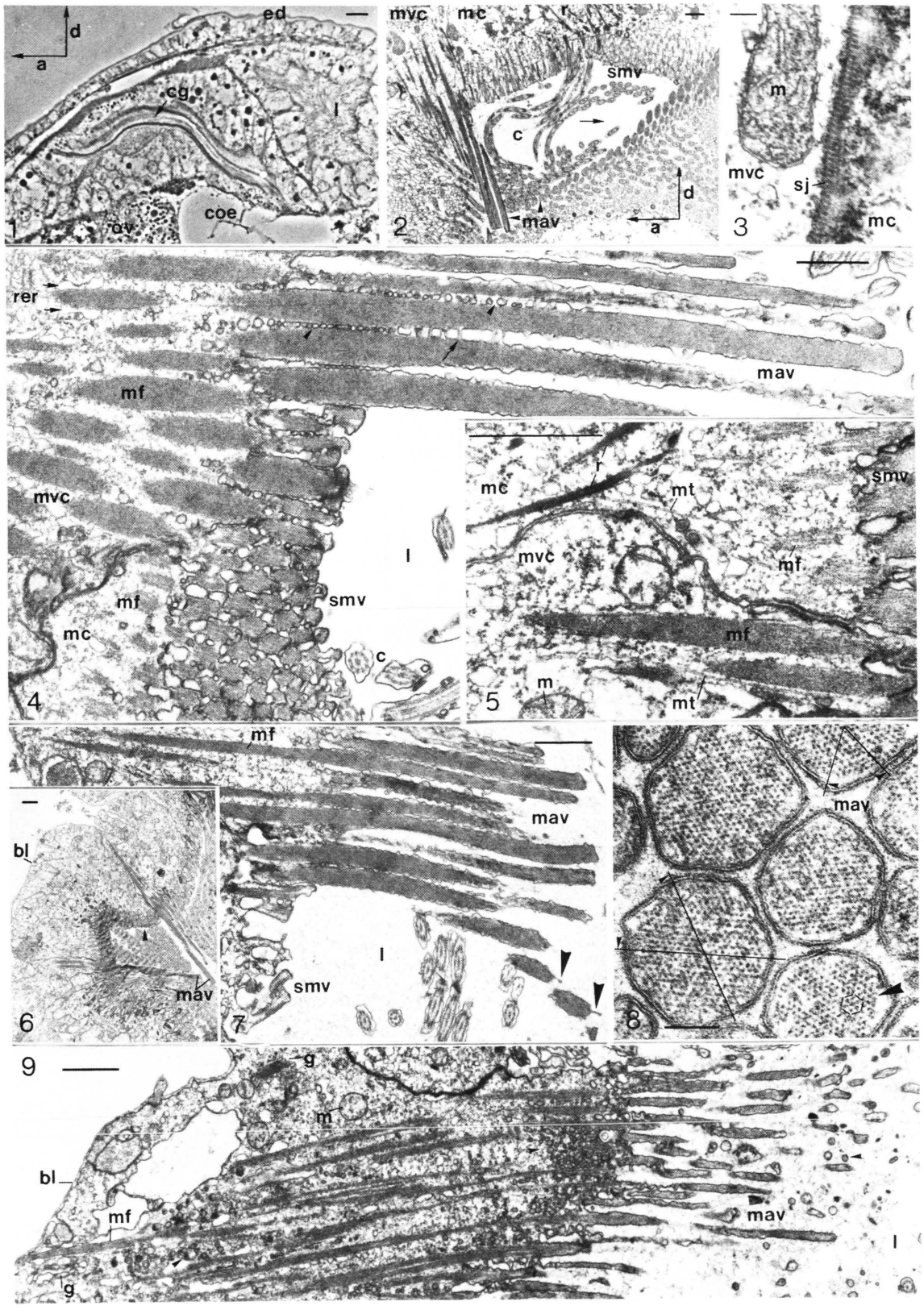
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which the antiplectic metachronal beat of long cilia creates a caudally directed current. Fig. 2 shows in a low power electron micrograph the structures bordering the ciliary groove. The cilia, connected to long rootlets (r) extend into the lumen of the obliquely cut groove from the dorsal side. They arise from special cells, the middle cells (mc) which also bear short macrovilli (smv). The middle cells are flanked by two rows of slender cells, each about 24 μm long (mvc) from which long macrovilli (mav) arise. The long macrovilli of both cells approach each other at an angle of 45° thus delimiting a space within which the cilia of the middle cell are beating. The membranes of adjacent cells are connected by septate desmosomes (Fig. 3). While the short macrovilli of the middle cell are stubby, the macrovilli of the lateral cells attain the unusual average length of about 7 μm which is more than the height of the cell body from which they arise (about 5 μm). Their microfilament cores extend deep into the cytoplasm and taper off close to the basal cell membrane (Fig. 7). Cross sections through the ciliated groove show that the length and width of the macrovilli decreases from the inside toward the outside, leading to a staircaselike profile as in some sensory stereocilia [8, 9] from which, however, they differ in other respects (see below). Adjacent macrovilli are frequently interconnected by short membrane-bound bridges as shown in Fig. 4. In the vicinity of the cell apex, short bridges gradually change to rows of vesicles. At the present, it can not be decided whether this is an indication of a physiological process or an artefact of fixation. Incubation with cationized ferritin did not give any indication of pinocytosis at this site. In contrast to the long macrovilli of the lateral cells, the stubby macrovilli of the middle cell are only about 0.9 μm long.

Details of the microfilament core of both types of macrovilli can be studied in exact cross sections (Fig. 8). The microfilaments are 6–7 nm thick and their average spacing was determined as 11.6 nm from counts along the rows. The rows cross at a measured average angle of 61° indicating a hexagonal packing. The number of microfilaments in a macrovillus depends solely on its diameter since the density of packing is constant. It can amount to 1000 in macrovilli of maximally 0.4 μm diameter. Exactly

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Fig. 1. Sagittal semithin section in the region of the hindgut. Note the position of the ciliated groove (cg) which begins at the level of the ovary (ov). Phase contrast, bar: 10 μm.

Fig. 2. Oblique section of the ciliary groove. The ciliary rootlets (r) are oriented away from the direction of the effective ciliary beat (arrow). Fixation a, bar: 1 μm.

Fig. 3. Septate junction between middle cell and macrovilli cell. Fixation b, bar: 0.1 μm.

Fig. 4. Oblique section showing long macrovilli (mav) with their microfilament cores (mf) extending into the cytoplasm and short macrovilli (smv) of the middle cell. Note vesiculation at the bases of the long macrovilli (arrows heads) which change gradually into anastomoses (arrow), and rough endoplasmic reticulum surrounding the basal part of the macrovillar rootlets (double arrows). Fixation c, bar: 1 μm.

Fig. 5–7, 9. Cytochalasin B treatment and controls (DMSO), fixation d.

Fig. 5. Control. Note microtubules in middle cell with short macrovilli and a cell with long macrovilli, in this case at a constant spacing of 12 nm along the rootlet bundle. Bar: 1 μm.

Fig. 6. Control. DMSO has no influence upon the length of macrovilli (6.4 μm measured). Note also the regularly arranged short macrovilli (arrow heads) of the middle cell. Bar: 1 μm.

Fig. 7. 40 μg/ml cytochalasin B, 1 h. Long macrovilli are shortened (to about 4.8 μm), their diameter is unaffected. Large arrow heads: typical structures which appear at the tips of the long macrovilli after drug treatment. Note that the microfilament core of the short macrovilli has almost totally disappeared.

Fig. 9. 35 μg/ml cytochalasin B, 20 h. Both length and thickness (~150 nm) of the long macrovilli is reduced. Arrow heads point to vesicles within the cell and in the lumen at the tips of macrovilli. Bar: 1 μm.

Fig. 8. Cross section through long macrovilli showing the hexagonal packing of the microfilaments (large arrow head). The angle of about 60° between the filament rows is indicated. Fixation a, bar: 0.1 μm.

longitudinal sections do not show the microfilaments clearly due to section thickness and the density of packing.

In microvilli of the intestinal brush border of vertebrates the central microfilament bundle consists of actin. These microfilaments are attached with their plus ends at the tips of the microvilli which have a width of about 0.1 μm and a length of 1 μm. Their minus ends reach into the terminal web of the cells [5]. The width of the microfilaments described here suggests that they are also composed of actin and their spacing is almost the same as that of the actin microfilaments in sensory stereocilia [8]. Actin is also indicated by the effect of cytochalasin B on the macrovilli. After 1 h in 40 μg/ml cytochalasin B the maximal length of the macrovilli is reduced from 6.5 μm in DMSO-treated controls to 4.7 μm while their thickness does not decrease below the average 0.3 μm observed in controls. The regularly arranged small macrovilli of the middle cell (Fig. 6) become highly irregular after 1 h drug treatment (Fig. 7, smv). Their microfilaments have disappeared except for some peripheral ones and the rootlets of the core bundles have vanished. While the effect upon the long macrovilli and their rootlet bundle appears to be less drastic (Fig. 7), their overall length has decreased by the same amount (1.8 μm) as that of the short macrovilli which have become almost totally disassembled in one hour.

After prolonged treatment with cytochalasin B (35 μg/ml, 20 h) the length of the large macrovilli is further reduced. While their width was unaffected by a short-term treatment, their maximal diameter has now decreased to about half of the DMSO controls, *i.e.* to about 150 nm. However, the filament core bundles still reach to the base of the cell (Fig. 9). It should be pointed out that there are no signs of gross disorder in the macrovilli due to cytochalasin action. The core bundle of microfilaments merely becomes shorter (but at first not thinner). Assuming the presence of actin filaments of the same polarity as in brush border microvilli, this would be in accord with cytochalasin binding at the fast growing ends of the microfilaments [10] and gradual shortening from the minus end in the course of treadmilling [11]. Since the fast growing end in brush borders is at the tips of the microvilli, further study of the unusually long macrovilli described in this paper may provide clues regarding the mechanism of G-actin transport to the distal polymerization site.

The rootlets of the short macrovilli extend into a sparse terminal web. Besides microfilaments, it contains microtubules. In the long macrovilli whose rootlets reach far into the basal region of the cell, the bundles are accompanied by microtubules which seem to be cross-bridged to the bundles (Fig. 5).

The only known structures of dimensions comparable to the macrovilli described here are some sensory stereocilia [8, 9]. They differ from the macrovilli of *Dinophilus gyrociliatus* in their diverging (instead of bundled) rootlets and their pencil-like pointed base which provides a weak point for bending. In addition, the arrangement of the actin filaments within the stereocilium may be different. In the alligator lizard they are arranged in a festooned pattern while bird stereocilia show the hexagonal pattern which is typical for the actin bundles in brush borders, *Mytilus* sperm and the spike-like microvilli of sea urchin oocytes [8]. The mean lateral spacing of the microfilaments in macrovilli (11.6 nm), on the other hand, is practically the same as determined by optical diffraction in stereocilia of the alligator lizard (11.5 nm, [8]).

Because of the lack of a pre-determined bending point and the strictly parallel arrangement of the microfilaments in their rootlets, the macrovilli are not thought to serve a sensory function. Instead, they may stabilize the ciliated groove against the pressure of the surrounding tissues, especially the large ovary which can swell to more than half of the body volume prior to ovulation. Thus, their major function may be to keep the channel open for the ciliary sweep. The function of the ciliated groove is unknown. Since food particles have never been found in it while typical resorptive cells with normal microvilli are abundant in the adjoining hindgut, the groove has probably no function in digestion. The current created by the continuous beating of its cilia may serve some other function.

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*Note added in proof:* In the meantime, HMM decoration has provided further evidence that the microfilaments are composed of actin. The polarity is the same as in intestinal microvilli, the arrow heads pointing towards the cytoplasm.

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