Identification of Actin Filaments in the Rhabdomeral Microvilli of *Drosophila* Photoreceptors

Kentaro Arikawa,[*‡] Jennifer L. Hicks,[*] and David S. Williams[*]

[*] School of Optometry and Institute for Molecular and Cellular Biology, Indiana University, Bloomington, Indiana 47405; and
[‡] Department of Biology, Yokohama City University, 22-2 Seto, Kanazawa-ku, Yokohama 236, Japan

Abstract. The phototransductive microvilli of arthropod photoreceptors each contain an axial cytoskeleton. The present study shows that actin filaments are a component of this cytoskeleton in *Drosophila*. Firstly, actin was detected in the rhabdomeral microvilli and in the subrhabdomeral cytoplasm by immunogold labeling with antiactin. Secondly, the rhabdomeres were labeled with phalloidin, indicating the presence of filamentous actin. Finally, the actin filaments were decorated with myosin subfragment-1. The characteristic arrowhead complex formed by subfragment-1 decoration points towards the base of the microvilli, so that the fast growing end of each filament is at the distal end of the microvillus, where it is embedded in a detergent-resistant cap. Each microvillus contains more than one actin filament. Decorated filaments extend the entire length of each microvillus and project into the subrhabdomeral cytoplasm. This organization is comparable to that of the actin filaments in intestinal brush border microvilli. Similar observations were made with the photoreceptor microvilli of the crayfish, *Procambarus*. Our results provide an indication as to how any myosin that is associated with the rhabdomeres might function.

THE rhabdomere is the site of phototransduction in arthropod and molluscan photoreceptors. It is made up of closely packed parallel microvilli. In squid (20, 21), flies (5, 6), and crayfish (8) each microvillus has been shown to contain an axial filament that appears to be linked to the plasma membrane by lateral "cross-bridges." At least in flies, regulation of this cytoskeleton appears to be closely related to regulation of photoreceptor membrane turnover (6).

The composition of the axial filament has not been determined. Blest et al. (5) found that in a blowfly it was labile and calcium sensitive, and suggested it could be either an actin filament or a neurofilament. There is biochemical and histochemical evidence for the presence of actin in the rhabdomeral microvilli. Saibil (20) analyzed the Triton X-100-insoluble fraction of isolated microvilli from the squid photoreceptors by SDS-PAGE and found a 42-kD protein that was identified as actin by peptide mapping after papain digestion. De Couet et al. (8) showed that rhabdom of the crayfish, *Cherax destructor*, contained large amounts of a 42-kD protein which comigrated with insect flight muscle actin in two-dimensional PAGE, inhibited pancreatic DNase 1, and bound to vertebrate myosin. They also employed light microscopic immunocytochemistry using antiscallop actin to show that the crayfish rhabdoms had actin-like immunoreactivity.

Although the presence of actin in the microvilli has been demonstrated, there is no direct evidence that the axial filament is made up of actin. This possibility is particularly relevant in view of the localization of at least one of the myosin

I-like, *ninaC* proteins in *Drosophila* rhabdomeres (14; J. L. Hicks, C. Montell, and D. S. Williams, manuscript in preparation) and ideas about how this apparent motor might function. An additional point is whether the axial filament that has been observed represents a single filament or the coalescence of several.

In the present study we have shown that each microvillus contains at least two actin filaments. We have labeled the rhabdomeres of *Drosophila* with rhodamine-phalloidin and antiactin, and have decorated the filaments themselves with myosin subfragment 1 (S1).[1] We also present some comparative information using *Procambarus clarkii* photoreceptors and, in discussion, compare the cytoskeleton of arthropod photoreceptor microvilli with that of the extensively studied intestinal brush border.

Materials and Methods

Animals

Electron Microscopy with Minimal Extraction

1. *Abbreviation used in this paper:* S1, subfragment 1.

M sodium cacodylate buffer (CB), pH 7.4, for 10 min, fixed with 2% glutaraldehyde, 2% paraformaldehyde in CB plus 50 mM lysine for 30 min on ice, and then fixed with the same fixative without lysine overnight at 4°C. After washing with CB, they were postfixed with 2% OsO_4 in CB for 2 h, dehydrated with acetone, infiltrated, and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope.

Phalloidin Labeling

Light-adapted *Drosophila* eyes were fixed for 1 h at room temperature with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB, pH 7.4). The tissue was infiltrated with 2.3 M sucrose in PB for 1 h and rapidly frozen with liquid nitrogen. Semi-thin (700 nm) sections were cut with a cryo-ultramicrotome and collected on glass slides. The sections were first washed with 0.1 M PBS (PB plus 0.5 M NaCl) and then permeabilized with cytoskeletal buffer (10 mM Hepes, 200 mM sucrose, 3 mM $MgCl_2$, 50 mM NaCl, 0.5% Triton X-100, 0.02% NaN_3, pH 7.4) for 10 min. Next, they were washed in 0.1 M PBS and blocked with 4% BSA in 0.1 M PB for 45 min. The sections were then labeled with rhodamine-conjugated phalloidin or FITC-conjugated phallacidin for 1 h in the dark. After rinsing, the slides were mounted and examined with a fluorescence microscope.

Immunocytochemistry

For EM immunocytochemistry, light-adapted compound eyes were fixed with 2% glutaraldehyde, 2% paraformaldehyde in PB for 1 h at 4°C. After fixation, tissues were dehydrated in a methanol series, and infiltrated and embedded in L. R. White. We used a monoclonal mouse IgM antibody against chicken gizzard actin (Amersham Corp., Arlington Heights, IL) as ascites fluid at 10-25 μg/ml for the immunocytochemistry.

Ultrathin sections were collected on Nickel grids covered with formvar. All steps for the immunogold labeling were done by floating the grid with section-side down on 15-50 μl of the appropriate solution. Sections were first etched with saturated sodium metaperiodate for 1 h, blocked with 4% BSA in PBS for 30 min, and then incubated overnight with the primary anti-body at 4°C. The sections were reacted with buffered biotinylated goat anti-mouse IgG and IgM (Jackson) for 30 min and then labeled with buffered streptavidin-gold (5 nm, Janssen Life Sciences Products, Piscataway, NJ) for 30 min at room temperature. Control sections were incubated with 4% BSA or normal mouse IgG (100 μg/ml), instead of the antiactin. Sections were examined with a Siemens 101 or JEM 1200EX electron microscope.

Immunoblot Analysis

Light-adapted compound eyes were collected and solubilized in Laemmli sample buffer with 2 mM EGTA and 5% 2-mercaptoethanol. Proteins were separated in a 10% SDS-polyacrylamide slab gel, and they were electrophoretically transferred to Immobilon (Millipore Continental Water Systems, Bedford, MA) in buffer containing 25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3. Western blots were first blocked with 4% BSA for 4 h and labeled with the antiactin (1-2.5 μg/ml as ascites fluid) overnight at 4°C. The primary antibody was detected with alkaline phosphatase conjugated to goat anti-mouse IgG and IgM. Control blots were incubated with normal mouse IgG (10 μg/ml), instead of the antiactin.

Decoration of Actin Filaments with Myosin Subfragment-1

This procedure followed that of Arikawa and Williams (3). Briefly, isolated light-adapted compound eyes (with dioptric apparatus removed, in the case of *Procambarus*) were first incubated with 1.0% Triton X-100 in buffer A (150 mM KCl, 2 mM DTT, 20 mM Tris-HCl, pH 7.4) at room temperature for ∼40 min with gentle agitation. After a wash with the same buffer A for 30-40 min, the eyes were incubated with S1 (10-15 mg/ml) in buffer A for 2 h at room temperature. They were then prefixed overnight at 4°C with 2% glutaraldehyde, 2% paraformaldehyde, plus 1% tannic acid in 0.1 M sodium cacodylate buffer (pH 7.4) and postfixed with 2% OsO_4 in the same buffer for 2 h. After fixation, the tissues were dehydrated by a graded ethanol series, then infiltrated and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate.
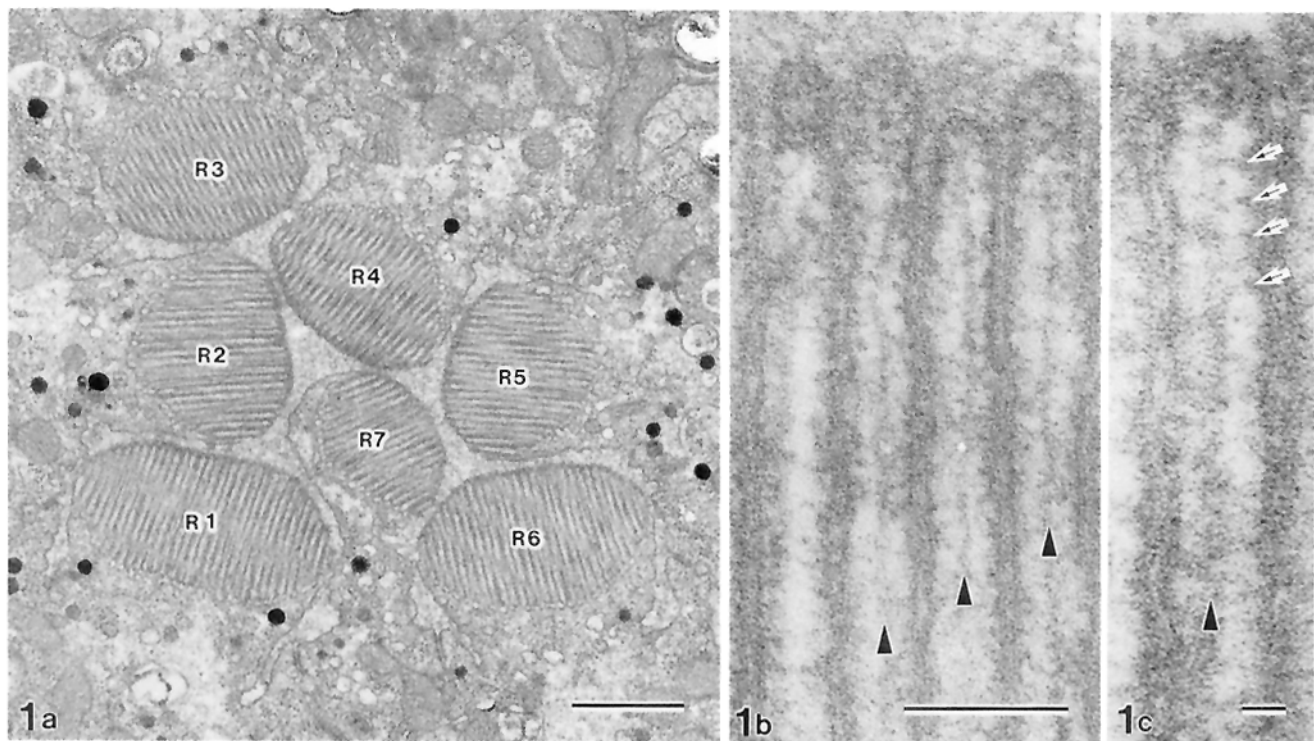
Figure 1. (a) Transverse section of *Drosophila ommatidium*, showing the rhabdomeres of seven photoreceptors (*R1-7*). (b) Higher magnification of rhabdomeral microvilli. Each microvillus contains an axial structure (*arrowheads*). (c) Higher magnification, still. Lateral connections (*arrows*) are apparent between the axial structure (*arrowhead*) and the plasma membrane. The tissues were prepared with minimal extraction. Bars: (a) 1.0 μm; (b) 0.1 μm; (c) 0.02 μm.
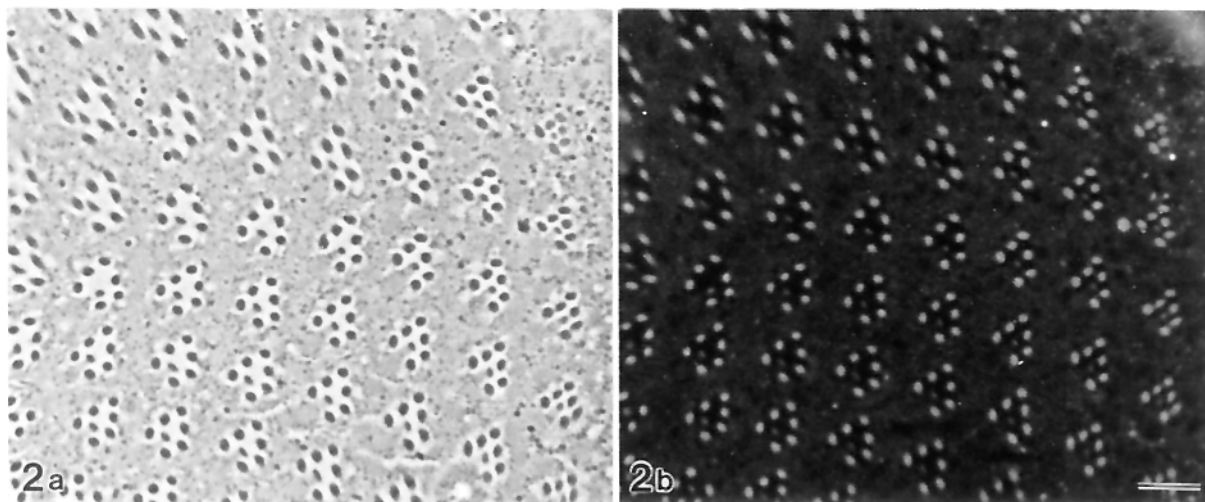
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Figure 2. Transverse cryosection (0.7 μm thick) of a *Drosophila* retina labeled with rhodamine-phalloidin. (*a*) Phase-contrast image; (*b*) fluorescence micrograph. Rhabdomeres appear dark in *a* and are labeled in *b*. Bar, 10 μm.

Results

A *Drosophila* ommatidium contains eight photoreceptor cells (R1-8) each bearing a rhabdomere. R1-6 provide the six peripheral rhabdomeres, and R7 and R8 form a tiered rhabdomere in the center of the ommatidium. Thus, only seven rhabdomeres are observed in any given transverse section of an ommatidium (Fig. 1 *a*). Each rhabdomeral microvillus contains an axial structure (Fig. 1 *b*). In some cases lateral connections between the axial structure and the plasma membrane are evident (Fig. 1 *c*).

Phalloidin Labeling

We used rhodamine-phalloidin and FITC-phallacidin to localize F-actin in the *Drosophila* compound eye by fluorescence microscopy. Both labeled the rhabdomeres of the retina (Fig. 2).

Electron Microscopic Immunogold Labeling of Actin in the Photoreceptors

Monospecificity of the antiactin was confirmed by blot analysis. The antiactin detected only one band with apparent mo-
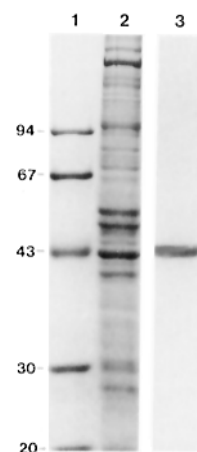
lecular weight of ~42 kD on a Western blot of *Drosophila* head homogenate (Fig. 3).

All observations, from here on, were made on R1-6. On ultrathin sections labeling was found throughout the rhabdomere and in regions of the subrhabdomeral cytoplasm (Fig. 4). No labeling was detected on control sections that were incubated with 4% BSA or normal mouse IgG (100 μg/ml) instead of antiactin.

Myosin Subfragment 1 Decoration

Filaments that extend from the distal ends of the microvilli into the subrhabdomeral cytoplasm were decorated by S1. The periodicity of the complex was 28–33 nm, and the decoration indicated a uniform polarity. At the distal end of each microvillus, the barbed end of each filament appeared to be attached to the plasma membrane via a detergent-resistant cap (Fig. 5). At the other end, the pointed end of each filament projected beyond the bases of the microvilli into the subrhabdomeral cytoplasm (Fig. 6). More than one filament was evident in some microvilli (e.g., Fig. 5).

A similar arrangement of actin filaments was found in the rhabdomeres of *Procambarus*. Each microvillus contains at least two filaments with their barbed ends at the distal end of the microvillus, and their pointed ends extending into the subrhabdomeral cytoplasm (Fig. 7).

Discussion

Presence of F-Actin in the Photoreceptors

Immunogold labeling of *Drosophila* photoreceptors with antiactin indicates that the rhabdomeres and subrhabdomeral cytoplasm contain actin. Phalloidin labeling shows that the actin in the rhabdomere is at least in part filamentous (F-actin). Finally, S1 decoration demonstrates that actin filaments contribute to the cytoskeletal core of each rhabdomeral microvillus. The distribution of immunogold labeling with antiactin corresponds well with that of S1-decorated filaments. Both were observed in the rhabdomeres and in the cytoplasm subjacent to the bases of the rhabdomeral microvilli.
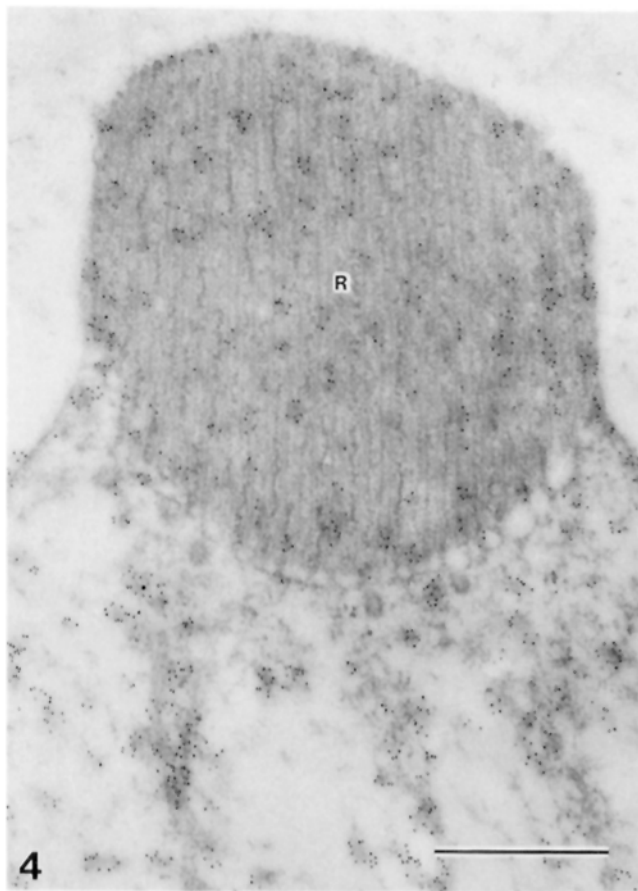

Figure 3. Western blot of proteins from *Drosophila* head. Electrophoresis was done under reducing conditions in a 10% polyacrylamide gel. Lanes *1-2*, part of the gel stained with Coomassie blue. Lane *1*, molecular mass standards; lane *2*, *Drosophila* head homogenate. Lane *3*, transblot of gel on to Immobilon, and incubated with antiactin, followed by goat anti–mouse IgG conjugated to alkaline phosphatase.

al. (5) noted that the axial filaments they preserved in the microvilli of a blowfly photoreceptor were inserted into electron-dense caps. The brush border actin filaments are also embedded in a "dense plaque of unknown composition" (15) at the microvillar tip. Thus, whatever the cap is made of, this structure seems to be a common characteristic of the cytoskeleton in both photoreceptor and brush border microvilli.

Number of Actin Filaments in a Single Microvillus

A brush border microvillus has a diameter of ~0.1 μm and contains ~20 actin filaments (18, 19). With a similar density of actin filaments, a *Drosophila* rhabdomeral microvillus (diameter, 0.05 μm) would contain five filaments. However, previous reports on the cytoskeleton of arthropod photoreceptor microvilli have shown only a single axial structure (5, 6, 8, 20, 21; see also Fig. 1 b).

The filaments we have decorated in the present study are likely to be single actin filaments. Firstly, they appear similar to single filaments decorated by S1 or heavy meromyosin in vitro (10). Secondly, a tight bundle of actin filaments usually has to be frayed out for decoration to occur at all (11). In some cases, microvilli might have fused as a result of detergent exposure. Nevertheless, observations of at least two decorated filaments associated with just one detergent-resis-
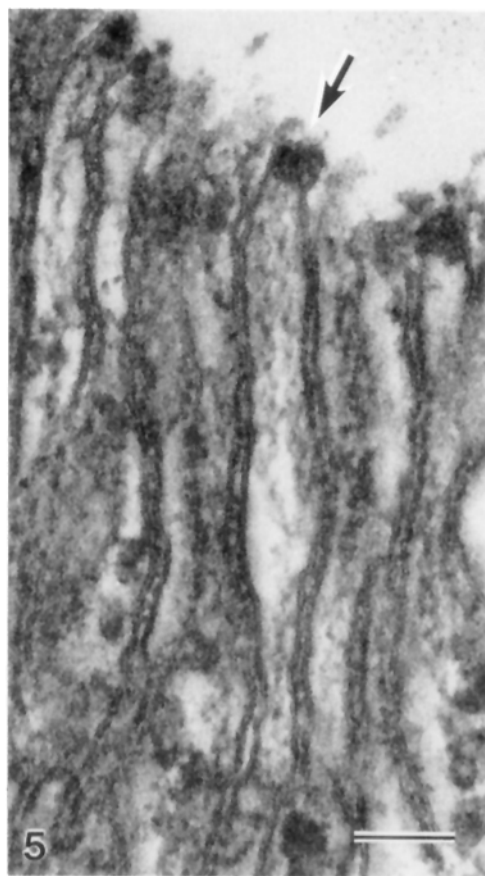

Figure 4. Indirect immunogold labeling of a cross section of a *Drosophila* photoreceptor. *R*, rhabdomere. Bar, 0.5 μm.

Organization of the Actin Filaments

S1 decoration has revealed that the actin filaments in the microvilli of the arthropod photoreceptors have similarities with those in the brush border microvilli. Both the periodicity of the arrowhead complex and the indicated polarity (4, 17) are the same. Fig. 8 illustrates diagrammatically the observed arrangement of actin filaments in the *Drosophila* rhabdomere and compares it with that in the microvilli of the intestinal brush border.

The actin filaments in the brush border microvilli extend beyond the base of the microvilli and are cross-linked in a "terminal web" (9, 15). It is not known if there is a structure that is equivalent to the terminal web in fly photoreceptors. Adjacent to the bases of the photoreceptor microvilli there are cisternae of smooth ER (12). In our preparations, Triton X-100 treatment disrupted these subrhabdomeral cisternae so that their relation with the actin filaments could not be determined. The decorated actin filaments projected well into the photoreceptor cytoplasm, beyond the region of the subrhabdomeral cisternae, and into areas where multivesicular bodies (e.g., Fig. 6) and pigment granules were found. This subrhabdomeral arrangement is similar to that of the "robust filaments" Blest et al. (7) described for *Drosophila* and a blowfly.

The barbed ends of the actin filaments appeared to be embedded in a cap at the distal end of each microvillus. This cap was resistant to solubilization by Triton X-100. Blest et

Figure 5. Part of a *Drosophila* rhabdomere, treated for S1 decoration, showing a microvillus with two decorated filaments (*arrow*). The filaments attach to a single distal cap with their barbed ends. Bar, 0.1 μm.
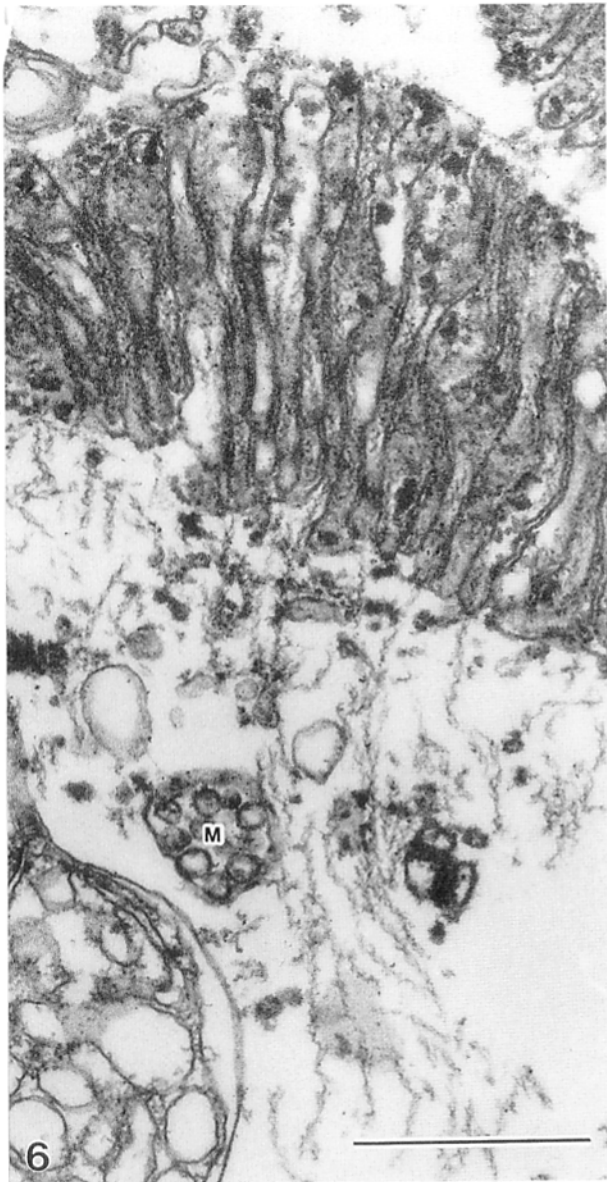
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Figure 6. S1 decorated filaments in the rhabdomeral microvilli and subrhabdomeral cytoplasm of a *Drosophila* photoreceptor. The filaments extend from the microvilli into the cytoplasm, maintaining the same polarity. *M*, multivesicular body. Bar, 0.5 μm.

tant cap (e.g., Fig. 5) indicate that each microvillus contains more than one actin filament.

The present results suggest that the single axial filaments observed previously in rhabdomeral microvilli (5, 6, 8, 20, 21) probably represent a coalescence of at least two actin filaments. Nevertheless, our results do not rule out the possibility that each microvillus contains a nonactin filament in addition to the actin filaments; such a filament may not have been preserved in our procedure for S1 decoration.

Implications for Interaction with Myosin I-like Proteins

The actin filaments of brush border microvilli appear to be linked to the plasma membrane by lateral structures or "cross-

bridges" comprised of a 100-kD protein–calmodulin complex (see reference 15 for review). Similar linkage structures are evident in the microvilli of photoreceptors (5, 6, 8, 20, 21, Fig. 1 c). The 110-kD-calmodulin complex shares many properties with myosins, including the ability to move beads along actin cables; it has been called a myosin I (16). In addition to supporting the translocation of membranous vesicles along actin cables (1), the myosin Is of *Acanthamoeba* have been shown to bind to the plasma membrane (2, 13). Based on their amino acid sequences, the *ninaC* proteins of *Drosophila* appear to be a form of myosin I (14). At least one of the *ninaC* proteins is present in the rhabdomeres (14; J. L. Hicks, C. Montell, and D. S. Williams, manuscript in preparation), so that the actin filaments of rhabdomeral microvilli might also be linked laterally to the membrane by a myosin I.

Figure 7. Part of a crayfish photoreceptor treated with S1. The polarity of the actin filaments is the same as that in *Drosophila* (barbed end, distal). The filaments also project into the subrhabdomeral cytoplasm. *P*, pigment granule. Bar, 0.5 μm.
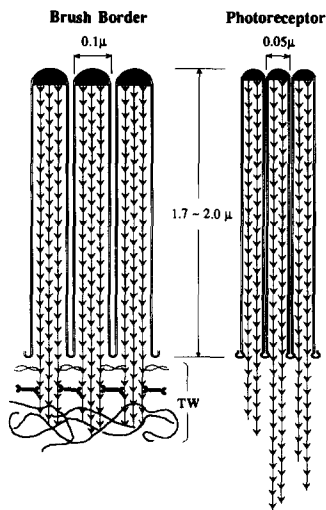
Brush Border **Photoreceptor**

Figure 8. Diagrammatical comparison of the microvilli in the intestinal brush border and in a *Drosophila* rhabdomere. The microvilli of the *Drosophila* rhabdomere are narrower, but their actin filaments are organized similarly; the filaments are illustrated as they are decorated. It is not known if the rhabdomere has a structure that is equivalent to the terminal web (*TW*) of the brush border.

Our results indicate that any myosin interacting with the actin filaments, either within a microvillus or in the subrhabdomeral area, should move towards the distal end of the microvillus (i.e., towards the barbed ends of the filaments). A myosin that is linked to the plasma membrane would thus move the membrane in this direction. Interestingly, at least in primitive dipterans, photoreceptor membrane is shed from the distal ends of the microvilli (22).

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