Pergamon

PII: S0020-7322(97)00021-4

Int. J. Insect Morphol. & Embryol., Vol. 26, No. 3/4, pp. 191–203, 1997
© 1997 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0020–7322/97 $17.00+0.00

CYTOSKELETAL ELEMENTS IN INSECT SENSILLA

Uwe Wolfrum*

Zoologisches Institut I, Universität Karlsruhe (TH), Kornblumenstrasse 13, D-76128 Karlsruhe, Germany

(*Received* 25 *February* 1997; *accepted* 29 *April* 1997)

Abstract—Insect sensilla have evolved prominent cytoskeletal elements as part of their functional specialization. The cytoskeleton present in sensory cells as well as in auxiliary cells may play an important role in sensilla function. The scolopale, the characteristic cytoskeletal component in the innermost auxiliary cell of mechanosensitive scolopidia and thermo-/hygrosensitive sensilla, is mainly composed of bundles of 10 nm filaments. Cytochemical approaches for light and electron microscopy identified these structures as actin filaments that exhibited a unique filament orientation and uniform filament polarity. None of these approaches has provided evidence for the presence of myosins in the scolopale. In contrast, tropomyosin and the microtubule-associated protein 2 are associated with the actin filament bundles in the scolopale of scolopidia. All data taken together suggest that the actin filaments of scolopale have a stabilizing rather than a contractile function. In scolopidia, in addition to cellular stabilization, filament elasticity would appear to be important during stimulation. Owing to the high number of microtubules, the scolopale in thermo-/hygrosensitive sensilla seems more rigid than in scolopidia and may protect sensory dendrites from mechanical forces. In sensory cells of scolopidia, regularly cross-striated ciliary rootlets are additional prominent cytoskeletal structures. Immunohistochemistry reveals that these rootlets contain the Ca^{2+}-binding protein centrin, which forms contractile filaments in other systems, e.g., unicellular green algae. Accordingly, contractions of ciliary rootlets may also be part of the filament function in insect sensilla. © 1997 Elsevier Science Ltd. All rights reserved.

Index descriptors (in addition to those in the title): scolopidia; thermo-/hygroreceptors; actin; tropomyosin; centrin; microtubules; MAP2; cryofixation; immunohistochemistry; immunogold.

INTRODUCTION

Insects gather information about internal and environmental conditions using specialized sensory organules, the sensilla. The cells composing insect sensilla also exhibit a rich inventory of cytoskeletal elements that are important for sensory function in other sensory systems (e.g., in the vertebrate inner ear: Slepecky and Chamberlain, 1985; and the mammalian photoreceptor cells: Wolfrum, 1995). A prerequisite for an understanding of structure-function relationships is a detailed structural and molecular characterization of cytoskeletal components present in sensilla. Therefore, those studies can be expected to provide further details regarding transduction mechanisms and adaptive processes.

Sensory cells of insect sensilla are enveloped by a small number of auxiliary cells (for reviews see Altner and Prillinger, 1980; Keil and Steinbrecht, 1984; Steinbrecht, 1984; Zacharuk, 1985). Prominent cytoskeletal elements are present in sensory cells as well as in auxiliary cells of insect sensilla (Wolfrum, 1990, 1992). A scolopale structure typifies the innermost auxiliary cell (thecogen or scolopale cell) of a variety of arthropod sensilla (cf. Schmidt, 1969, 1989, 1990; Schmidt and Gnatzy, 1984), and thermo-/hygrosensitive sensilla in insects (Altner and Loftus, 1985; Wolfrum, 1990). According to this prominent structure, the non-cuticular, mechanosensitive scolopidia of insects and various other arthropods were named (Howse, 1968; Schmidt, 1970; Moulins, 1976; McIver, 1985). In addition, in a second auxiliary cell of scolopidia, the so-called attachment cell, numerous microtubules form a prominent cytoskeleton. Finally, in sensory cells of scolopidia, long, cross-striated ciliary rootlets represent a characteristic and prominent cytoskeletal element.

For a more detailed insight into the cellular function of the prominent cytoskeletal components in insect sensilla, their structural and molecular composition has been analysed by light and electron microscopical methods. On the basis of their features, cellular functions are suggested and consequences on sensilla function are discussed.

MATERIALS AND METHODS

Animals

Three species were used: *Periplaneta americana* (Blattodea) was taken immediately after molting; workers of *Schedorhinotermes lamanianus* (Isoptera) were kindly provided by Dr M. Kaib, University of Bayreuth, Germany. *Antheraea pernyi* (Lepidoptera) were obtained from various local breeders.

Cryofixation for electron microscopy

High-pressure freezing. Specimens were frozen in the Balzers high-pressure freezer HPM 010 (Balzers Union, Liechtenstein) (Müller and Moor, 1984; Moor, 1987) at about 2100 bar according to Studer *et al.* (1989) and Wolfrum (1990).

Ambient pressure freezing. The antennae were cryofixed by rapid injection into liquid propane according to Steinbrecht (1980) and Haug and Altner (1984). The frozen specimens were substituted in dry acetone

*Tel: +49 721 608 3913; Fax: +49 721 608 4848; e-mail: dc13@rz.uni-karlsruhe.de

containing 2% OsO_4 at −90°C, −60°C and −30°C (8 h for each step) and for 1 h at 0°C using a freeze-substitution apparatus of the author's own design. The substituted specimens were embedded in Durcupan ACM (Fluka, Buchs, Switzerland).

Freeze-substitution and embedding. For conventional electron microscopy, freeze-substitution in dry acetone containing 2% OsO_4 and embedding procedures in Epon/Araldite were performed as described by Wolfrum (1990).

For immunogold staining procedures, frozen specimens were substituted in dry acetone containing 0.1% glutaraldehyde at −90°C, −63°C and −30°C (8 h for each step). The substituted specimens were infiltrated stepwise with 30%, 70%, and twice with 100% Lowicryl K4M (Chemische Werke Lowi, Waldkraiburg, Germany) at −30°C for 3.5 h for each step. The UV-polymerization was performed for 24 h at −30°C, and 48 h at room temperature. All procedures took place in the freeze-substitution apparatus FSU 010 (Balzers Union) or a copper cooling block, temperature controlled by the flow of liquid nitrogen.

Series of sections were cut with diamond knives on an Ultracut E ultramicrotome (Reichert–Jung, Nußloch, Germany), stained with 2% ethanolic uranyl acetate and Reynolds lead citrate, and examined in a Zeiss EM 10/CR or 912C electron microscope.

Cryofixation for light microscopy

Fixation and sectioning. Unfixed specimens were placed in embedding media, surrounded by boiled liver and cryofixed in melting iso-pentane at −165°C. Cryosections (approximately 10 μm thick) were cut in a cryostat (2800 Frigocut, Reichert–Jung) at −20°C, and placed on coverslips precoated with 0.05% aqueous poly-l-lysine (Sigma, Deisenhofen, Germany).

Cytochemistry

Antibodies. Anti-actin. The monoclonal mouse antibody clone C4 against chicken gizzard actin was a generous gift from Dr J. Lessard, Cincinnati, U.S.A., which has previously been characterized (Lessard, 1988).

Anti-tropomyosin. Mouse monoclonal antibodies to tropomyosin were kindly provided by Dr M. Knipper, University Clinic of Tübingen, Germany. It was prepared against tropomyosin from *Locusta* and was characterized by Krieger *et al.* (1990).

Anti-centrin. A polyclonal rabbit antiserum against centrin from the green algae *Tetraselmis striata* (Salisbury *et al.*, 1984) was a generous gift of Drs M. Melkonian (University of Cologne, Germany) and J. L. Salisbury (Mayo Clinic Foundation, Rochester, MN, U.S.A.).

Anti-MAP2. Rabbit antisera raised against microtubule-associated protein 2 from pig brain (sera no. 32) were kindly provided by Dr G. Wiche (University of Vienna, Austria).

Phalloidins. Rhodamine-coupled phalloidin (a generous gift from Dr H. Faulstich, MPI for Medicine, Heidelberg) and fluorescein isothiocyanate (FITC)-coupled phalloidin (Sigma) were used as specific probes for filamentous actin (Wulf *et al.*, 1979; Faulstich *et al.*, 1988).

Double-fluorescence staining. Cryosections were incubated first with 0.01% Triton-X 100 in phosphate-buffered saline (PBS) (pH 7.2) for 10 min and blocked for 30 min with PBS containing 0.1% ovalbumin/0.5% fish gelatin (blocking solution). After washing in PBS, sections were incubated with primary antibodies diluted in blocking solution for 12 h at 4°C. Unbound antibodies were subsequently washed out with PBS. A mixture of specific secondary antibodies (FITC-coupled or rhodamine-coupled anti-mouse IgG antibody or anti-rabbit IgG antibody; all raised in sheep and obtained from Sigma) and fluorescent phalloidins (rhodamine-coupled: 0.01 mg/ml; FITC-coupled: 0.1 mg/ml) were placed on sections for 1 h at room temperature in the dark. Sections were then mounted in Mowiol 4.88 (Farbwerke Hoechst, Frankfurt, Germany) containing 2% n-propyl-gallate. The mounted frozen sections were examined and photographed with a Zeiss Axiophot microscope.

Immunogold staining. Ultrathin Lowicryl sections of high-pressure frozen and freeze-substituted specimens were incubated first with 0.01% Tween 20 in PBS for 10 min. Free aldehyde groups were blocked with 50 mM NH_4Cl in PBS for 5 min. After being washed in PBS, the sections were preincubated with blocking solution, incubated with primary antibodies in blocking solution for 60 h at 4°C, followed by secondary 1 nm or 10 nm colloidal-gold-coupled antibodies (AroProbe, Amersham, Braunschweig, Germany) in 10 mM phosphate buffer, containing 0.5 M NaCl 0.1% ovalbumin/0.5% fish gelatin and 0.01% Tween 20 (pH 7.4) for 1 h at room temperature. Washed sections were fixed with 2.5% glutaraldehyde in PBS for 10 min. After washing and drying, silver enhancement using the method of Danscher (1981) was performed with some sections. Sections were counterstained with 2% ethanolic uranyl acetate and analysed in a Zeiss EM 10 or 912C.

Controls. For immuno-cytochemical controls, the primary or secondary antibodies were omitted. A secondary antibody against antibodies differing from the primary antibody was used. In no case was a reaction observed. Phalloidin specificity was tested in preincubation studies according to Wolfrum (1991a).

Heavy meromyosin decoration. Pedicelli of cockroach antennae were dissected and infiltrated with Tris–HCl buffer (pH 7.6) for 10 min. After treatment with buffered 1% Triton X-100 (Sigma) for 10 min at room temperature, samples were incubated for 2 h on ice with heavy meromyosin (HMM) from rabbit muscle in Tris–HCl buffer (5 mg/ml) (Sigma); controls were incubated with the Tris–HCl buffer only. Specimens were fixed for 30 min with buffered 2% glutaraldehyde, for 3 h with a mixture of 1% tannic acid and 2% glutaraldehyde, and postfixed in buffered 1% OsO_4 for 45 min at 0°C. They were then stained *en bloc* in 1% uranyl acetate in 50% ethanol, followed by dehydration in graded ethanol and embedding in Durcupan ACM (Fluka).

RESULTS

Scolopidia

Two propioreceptive sense organs, the antennal chordotonal organ and the Johnston's organ are present in the pedicel, the second segment of insect antennae (Toh, 1981). Elementary units of these sense organs are mononematic scolopidia or amphinematic scolopidia, respectively. Scolopidia are non-cuticular mechanosensitive sensilla that are composed of 1, 2 or 3 sensory cells enveloped by 2 auxiliary cells: the attachment cell and the innermost scolopale cell (Fig. 1). In amphinematic scolopidia, the dendritic outer segment of one sensory cell projects to the joint between the pedicel and first flagellar segment. The dendrites of the other sensory cells of amphinematic scolopidia, as well as both sensory cells in mononematic scolopidia, terminate in an extracellular cap structure (Fig. 1). Distally, the cap is enveloped by the attachment cell, which also connects a scolopidium with the cuticle at the segment joint. Prominent cytoskeletal structures are present in all cells of scolopidia. Bundles of longitudinally oriented microtubules are characteristic structural elements of the attachment cell (Fig. 2(A)). These microtubules are associated with electron-dense filaments. The scolopale, an intracellular cytoskeletal tubular cylinder, typifies the innermost auxiliary cell or scolopale cell (Fig. 2(B)). It extends from the cap to the apical part of the dendritic inner segments of the sensory cells. In the sensory cells, long, regularly cross-striated ciliary rootlets extend from the base of the dendritic outer segments through the dendritic inner segments into the perikarya (Figs 1 and 7(A)). Electron microscopy reveals that all components composing a scolopidium are linked by desmosomal structures (Fig. 2(A)). The attachment cell, the scolopale cell and the dendritic inner segments of the sensory cells are linked by desmosomes. Hemidesmosomes are developed between the extracellular cap and the attament cell as well as the scolopale cell. Additional linkages are

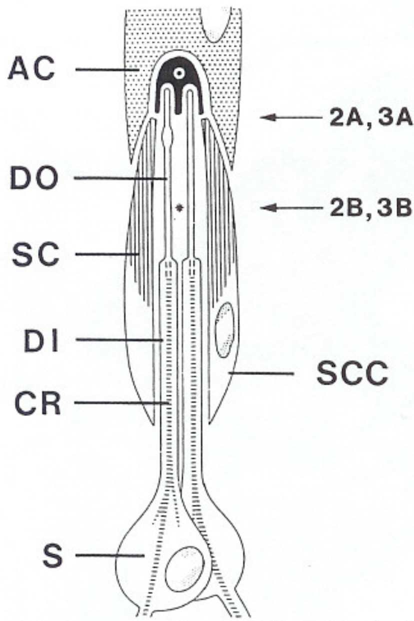


Fig. 1. Diagram of a mononematic scolopidium. The dendritic outer segment (DO) of the sensory cells (S) project into the cap (white circle) enveloped by the the attachment cell (AC). The scolopale (SC) is an elongated structure in the scolopale cell (SCC). Ciliary rootlets (CR) project from basal bodies at the tip of the dendritic inner segments (DI) down to the perikaryon of the sensory cells (S). Asterisk indicates receptor lymph cavity. Membrane junctions are omitted. Arrows show section planes in Figs 2(A,B) and 3(A,B).

present between the cap structure and the tips of the dendritic outer segments (Fig. 2(A)).

Ultrastructural and molecular characterization of the scolopale in scolopidia

The analysis of high-pressure frozen and freeze-substituted specimens reveals that the scolopale is composed of bundles of longitudinally oriented, regularly spaced filaments, approximately 10 nm in diameter (Fig. 2(A,B)). In addition, few longitudinally oriented microtubules, surrounded by an electron-lucent halo, are visible within these filament bundles. Whereas the ratio of 10 nm filaments to microtubules is approximately 200:1 ($n=5$) in the scolopale, in the attachment cell, in which filaments and microtubules are also present, the ratio is 2:1 ($n=5$) (see also Fig. 2(A,B)).

The 10 nm filaments composing the filament bundles in the scolopale and in the attachment cell have been identified as actin filaments by 3 principally distinct cell-biological methods: (i) cytochemistry using fluorescent phalloidins as specific probes for filamentous actin (F-actin) (Faulstich *et al.*, 1988); (ii) immunoelectron microscopy applying monoclonal anti-actin antibodies; (iii) decoration with myosin fragments.

In cryosections, fluorescent phalloidins stain filament bundles in both types of scolopidia. Superposition of phalloidin fluorescence images on Normarski pictures reveals that the labelled F-actin bundles are localized in the scolopale (Figs 4(C), 5(C) and 7(C)).

Immunoelectron microscopy identifies the molecules composing the filaments in the scolopale and in the attachment cell as globular actin (G-actin). The monoclonal antibodies raised against a conserved epitope of G-actin are visualized in ultrathin sections by a secondary anti-mouse IgG coupled to colloidal gold particles. They react with the filaments in the scolopale and in the attachment cell of scolopidia (Fig. 3(A–C)).

In a third set of experiments, Triton X-100-treated scolopidia are incubated with heavy meromyosin (HMM) fragments. HMM binds in a typical way to actin filaments. The filaments in scolopales as well as in the attachment cell are also decorated in this typical "arrowhead"-like manner (Fig. 3(D)). HMM decoration additionally reveals a unidirectional polarity of the actin filaments in the scolopale as well as in the attachment cell. Barbed ends of the actin filaments are directed apically (Fig. 3(D)).

Identification of actin-associated proteins in the scolopale

The association of specific proteins determines the cellular function of actin filaments. In an immunohistochemical approach, specific antibodies against myosin-II, tropomyosin and α-actinin are used for protein identifications. The polyclonal antibodies raised against vertebrate α-actinin and myosin-II cross-react with both insect proteins. However, they do not react in the scolopale of scolopidia. In contrast, monoclonal antibody against tropomyosin co-localizes with actin filaments in the scolopale. Double-staining of cryosections through scolopidia with fluorescent phalloidin and tropomyosin by indirect immunofluorescence reveals co-localization in staining patterns (Fig. 4). Furthermore, conclusive evidence of tropomyosin localization in the scolopale is provided by immunogold labelling (not shown here; Wolfrum, 1991b).

MAP2-localization in scolopidia

Electron microscopy and immunocytochemistry have revealed that, in addition to actin filaments, microtubules are cytoskeletal components in the cells of scolopidia (see also Wolfrum, 1990). Because microtubule-associated proteins function as linkers between microtubules as well as between microtubules and actin filaments, the presence of microtubule-associated protein 2 has been tested here. In cryosections, antibodies against MAP2 react in both types of scolopidia. Double-stainings with anti-MAP2 and fluorescent phalloidin reveal co-localization of phalloidin fluorescence with MAP2 immunofluorescence (Fig. 5(A–C)). Immunogold labelling with anti-MAP2 antibodies demonstrates MAP2 localization at the actin filament bundles of the scolopale (Fig. 5(D)). However, in cryosections through scolopidia, much brighter anti-MAP2 fluorescence is present in the attachment cell enveloping the extracellular cap (Fig. 5(A–C)).

ac

c

do

scc

A

B

sc

*

do

Fig. 2. Transverse sections through mononematic scolopidia of *S. lamanianus*. (A) A section cut at the level of the extracellular cap. Numerous microtubules and filaments stabilize the scolopale cell (scc) as well as the attachment cell (ac), which are linked by desmosomes (small arrows); both cells are also connected with the fenestrated cap structure (c) by hemi-desmosomes (small arrows). Electron-dense links are also present between the cap and the dendritic outer segments (do) (large arrows). (High-pressure freezing preparation). Bar $= 0.2\ \mu m$ ($\times$ 53,500). (B) Section at approximately the median of the longitudinal extension of the scolopale (sc). The scolopale cell encloses receptor lymph cavity (asterisk) with dendritic outer segments (do). The scolopale is composed of filament bundles containing few microtubules. (high-pressure freezing preparation). Bar $= 0.2\ \mu m$ ($\times$ 53,500).

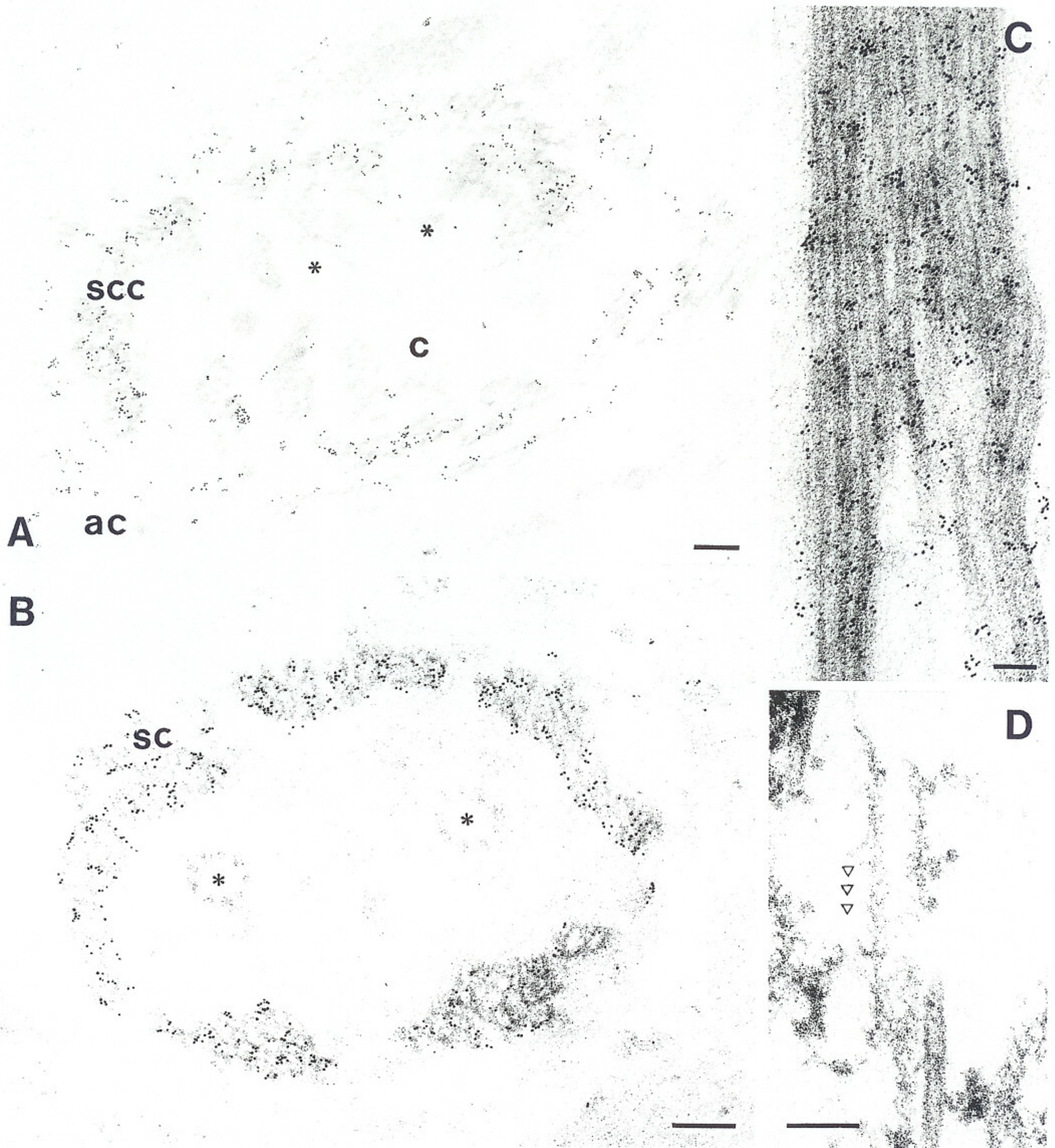


Fig. 3. Ultrastructural localization of actin in insect scolopidia. (A) Transverse section through a mononematic scolopidium of *S. lamanianus* at about the same plane of Fig. 2(A). Monoclonal antibodies against actin react with cross-sectioned filaments in the scolopale cell (scc) and the attachment cell (ac). Dendritic outer segments (asterisks) terminate at this plane enclosed by the extracellular cap (c). The cap is not contrasted after fixation for immunoelectron microscopy. (High-pressure freezing; freeze substitution; low-temperature embedding). Bar $= 0.2\ \mu m$ ($\times$ 39,000). (B) Transverse section through a mononematic scolopidium of *S. lamanianus* at about the same plane of Fig. 2(B). The filament bundles of the scolopale (sc) are immunogold-labelled by anti-actin. Dendritic outer segements (asterisks) are not stained. (High-pressure freezing; freeze substitution; low-temperature embedding). Bar $= 0.2\ \mu m$ ($\times$ 58,500). (C) Longitudinal section through a scolopidium of *S. lamanianus*. Monoclonal anti-actin antibodies react with the electron-dense filaments of the scolopale. (High-pressure freezing; freeze substitution; low-temperature embedding). Bar $= 0.1\ \mu m$ ($\times$ 72,000). (D) Heavy meromyosin decoration of a Triton-extracted scolopale in a scolopidium of *P. americana*. Longitudinal sectioned filaments are decorated in the way characteristic for actin filament. Arrowheads indicate filament polarity and point towards the "pointed end". Bar $= 0.1\ \mu m$ ($\times$ 121,500).

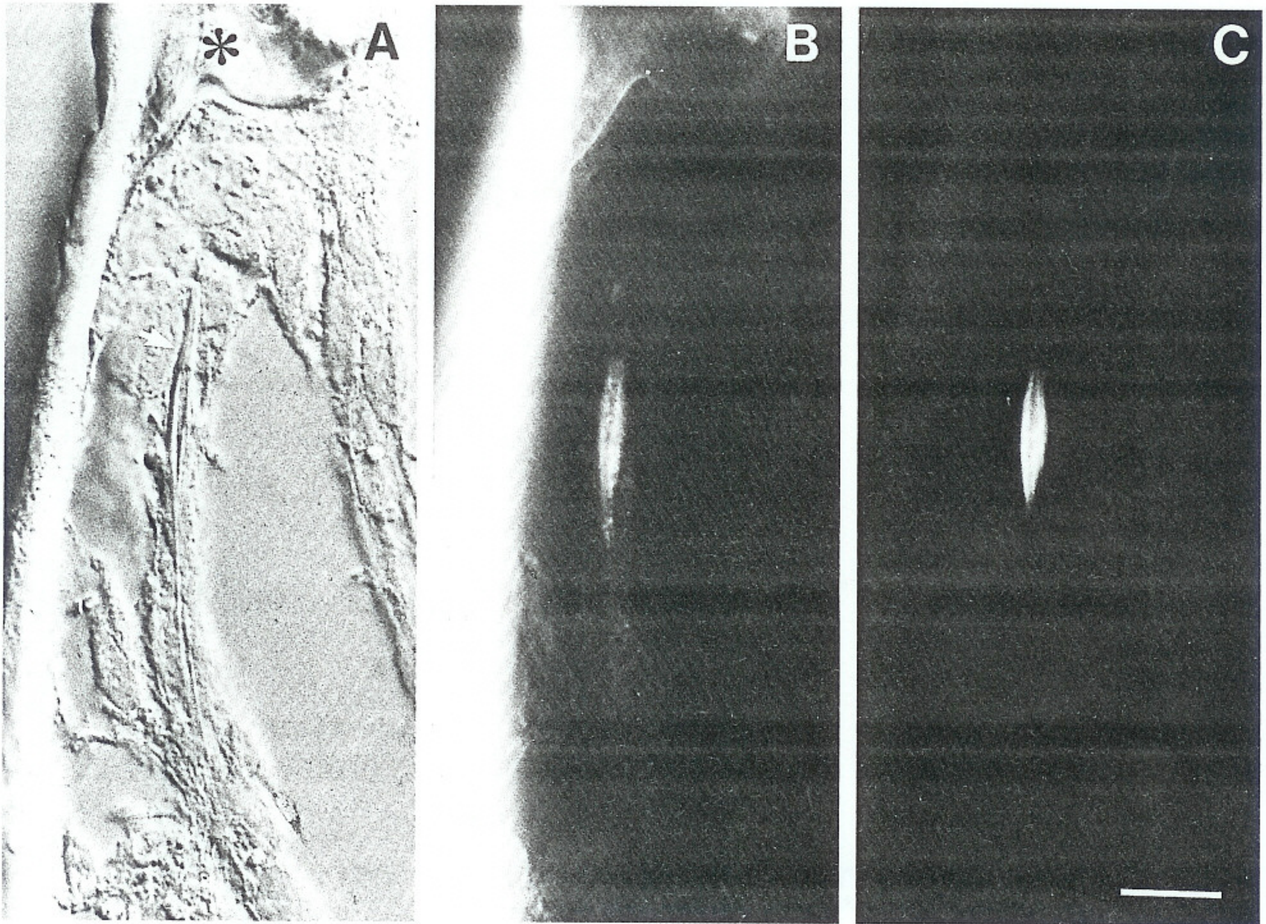


Fig. 4. Double-fluorescence staining of filamentious actin (F-actin) and tropomyosin in a longitudinal section through an amphinematic scolopidium of *P. americana*. (A) Nomarski (differential interference contrast) picture. Asterisk indicates segment joint. (B) Indirect immunofluorescence caused by the presence of anti-tropomyosin (FITC). (Note intense non-specific auto-fluorescence of the cuticle.) (C) Phalloidin-rhodamine fluorescence. Anti-tropomyosin reacts specifically within the scolopale proximal to the cap (arrow). Phalloidin staining is co-localized with anti-tropomyosin immunofluorescence. Bar = 10 μm (× 1250).

Thermo-/hygrosensitive sensilla also contain a prominent scolopale-like structure

Two types of thermo-/hygrosensitive sensilla, no-pore sensilla (np-sensilla; Altner, 1977), are present on the antennae of the moth *A. pernyi*; the styloconic sensilla and a smaller coeloconic np-type. Three sensory cells are present in these np-sensilla. Two of these possess unbranched dendritic outer segments projecting into the hair peg. The third has a lamellated dendritic outer segment and ends at a more proximal level (Fig. 6(A); Haug, 1985; Steinbrecht *et al.*, 1989). In both types of sensilla, the thecogen cell (the innermost enveloping cell) contains a scolopale-like structure (Fig. 6(A)). This scolopale terminates distally at the level of the proximal end of the dendrite sheath and extends proximally for about one quarter of the length of the inner dendritic segments. Within the thecogen cell, the scolopale ribs form a tubular cylinder which surrounds the inner receptor lymph cavity containing the sensory dendrites (Fig. 6(A)). The scolopale is composed of microtubules and 10 nm filaments oriented longitudinally. The ratio of the number of 10 nm filaments to the number of microtubules is smaller in the scolopale of np-sensilla than in the scolopidia. There are about 10 times more actin filaments per microtubule towards the middle of the scolopale in a scolopidium than in the corresponding area of an np-sensillum (200:1 and 20:1, respectively; $n = 6$; Wolfrum, 1990).

Phalloidin cytochemistry and immunogold labelling also identify the 10 nm filaments in the scolopale-like structure of np-sensilla as actin filaments. In frozen sections through both types of np-sensilla of *A. pernyi*, fluorescent phalloidins label a cylinder with a central unstained lumen representing the scolopale (Fig. 6(B–E)). Monoclonal antibodies against actin react with the longitudinally oriented filaments in the scolopale of the thecogen cell of np-sensilla (Fig. 6(F)).

Ciliary rootlets

In 2 sensory cells of both types of scolopidia, extremely long ciliary rootlets originate at the basal bodies in the tips of the dendritic inner segments. The rootlets extend through the perikarya into the axons over a distance of

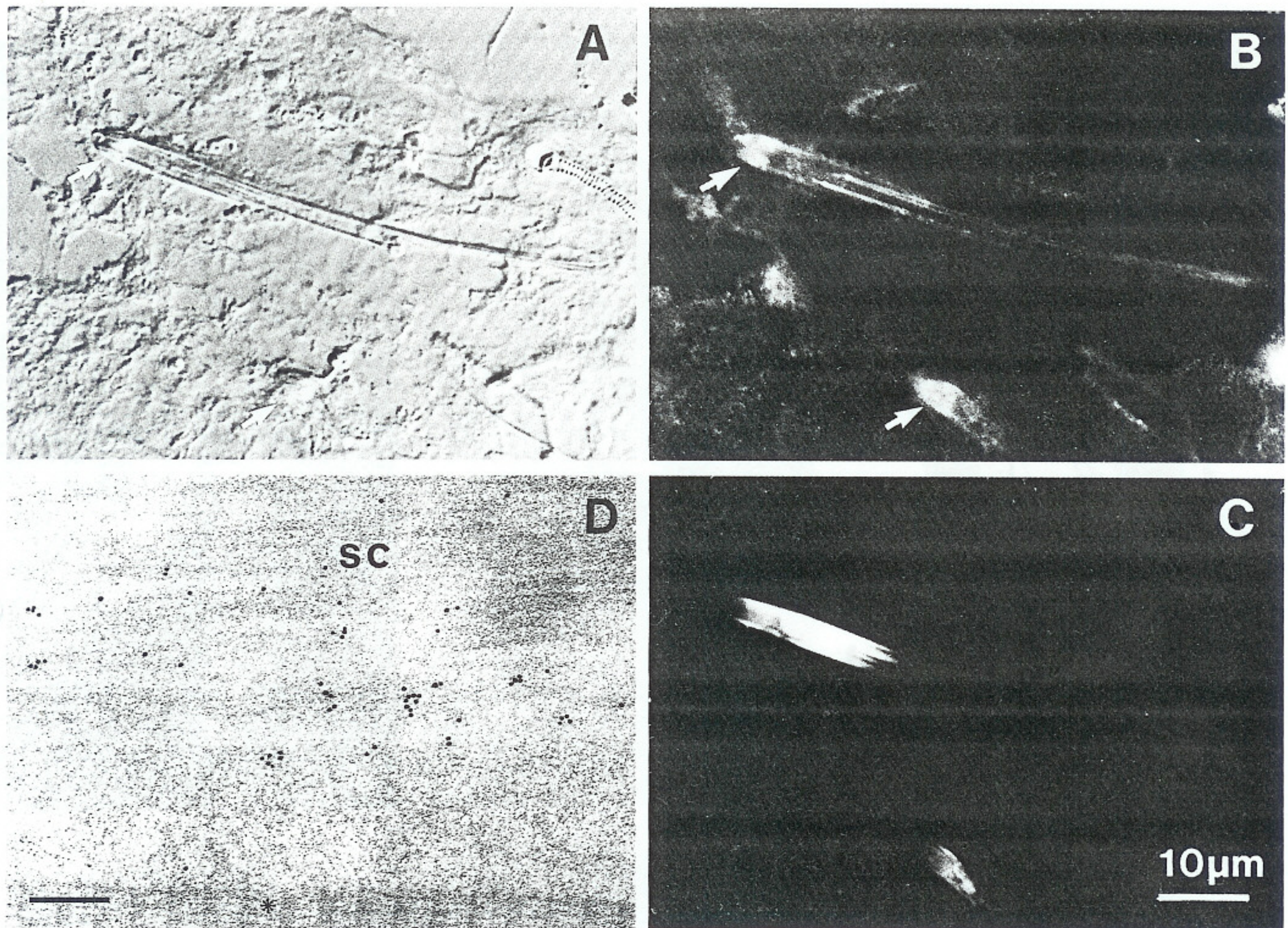


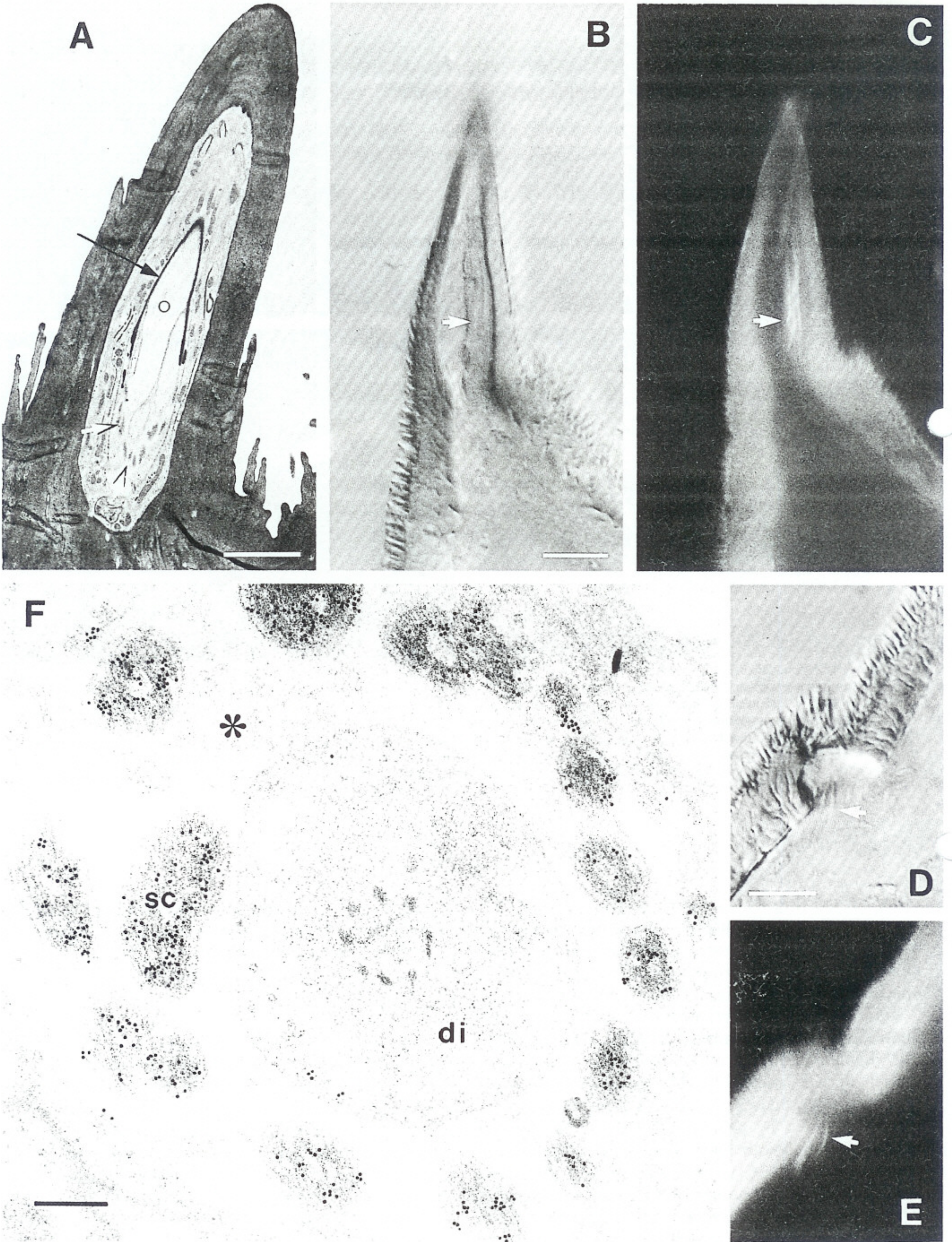
Fig. 5. MAP2 localization in insect scolopidia. (A–C) Double-fluorescence labelling of MAP2 and filamentous actin (F-actin) in a longitudinal cryosection through mononematic scolopidia of *P. americana*. (A) Nomarski picture. (B) Indirect immunofluorescence of anti-MAP2. (C) Phalloidin-rhodamine fluorescence. Bright anti-MAP2 immunofluorescence is visible in the attachment cell, which envelopes the cap structure (arrows). In addition, superposition of (B) and (C) indicates that the anti-MAP2 immunofluorescence co-localizes with the phalloidin staining of actin filament bundles in the scolopale. Bar = 10 μm ($\times$ 1250). (D) Ultrastructural localization of MAP2 in a longitudinal section through the scolopale of a scolopidium of *S. lamanianus*. Antibodies raised against MAP2 react in the filamentous scolopale. The cross-striated ciliary rootlet (small asterisk) localized in the dendritic inner segement lacks staining. (High-pressure freezing; freeze substitution; low-temperature embedding). Bar = 0.15 μm ($\times$ 72,000).

up to 100 μm (Fig. 1). Ciliary rootlets are composed of longitudinally oriented highly ordered fine filaments 2–4 nm in diameter. In cryofixed specimens, their characteristic regular cross-striation shows a periodicity of approximately 68 nm (Fig. 7(A)).

Anti-centrin immunocytochemistry. Indirect immunofluorescence and immunogold labelling reveal that the Ca^{2+}-binding protein centrin is localized at the ciliary rootlets of insect scolopidia. The localization of centrin is ascertained by fluorescence double-labelling using anti-centrin, followed by phalloidin staining (Fig. 7(B,C)). Rhodamine-phalloidin stains actin filament bundles of the scolopale (Fig. 7(C)), whereas the anti-centrin FITC-immunofluorescence occurs within the scolopidia in strands that project proximally resembling the rootlets (Fig. 7(B)). In addition, immunogold labelling on ultrathin sections through high-pressure frozen, freeze-substituted, and low-temperature embedded specimens of scolopidia of *Schedorhintermes lamanianus* shows that the ciliary rootlets are also the most obviously labelled structure by antibodies against centrin (Fig. 7(D)).

DISCUSSION

Cumulative evidence indicates that the 10 nm filaments composing the filament bundles in the scolopale of insect sensilla are filamentous actin (see also Wolfrum, 1990, 1991b). (i) After cryofixation and freeze-substitution, the diameter of the filaments corresponds to X-ray diffraction measurements of single actin filaments (Egelman and Padrón, 1984). (ii) Fluorescent phalloidin-specific probes for F-actin (Faulstich *et al.*, 1988) stain the filament bundles of the scolopale. In addition (iii) in immunogold labelling experiments on ultrathin sections, monoclonal


A
B
C
F
sc
di
D
E

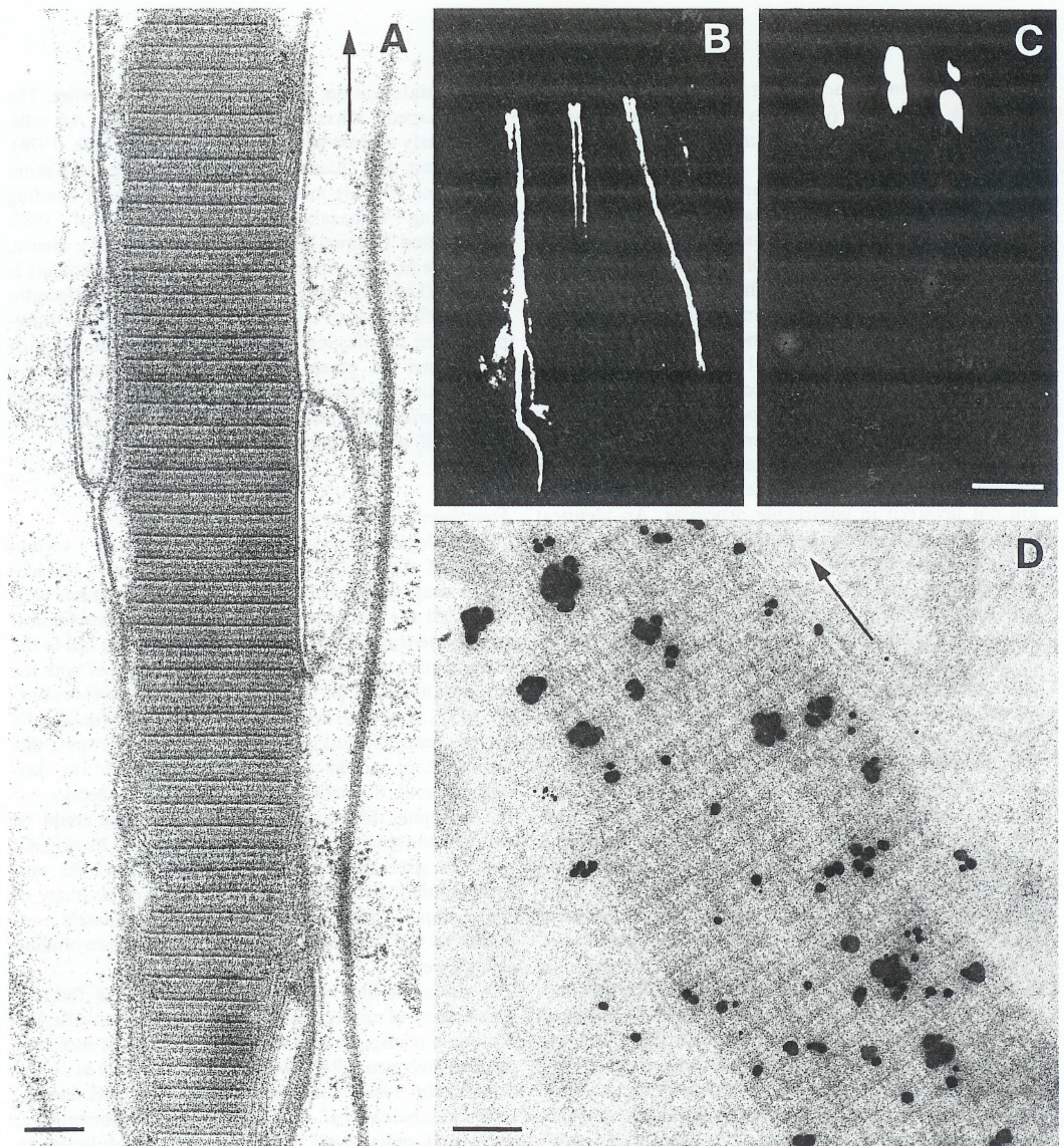


Fig. 7. Ciliary rootlets in insect scolopidia. (A) Ultrastructure of a longitudinally sectioned ciliary rootlet in a scolopidium of *S. lamanianus*. The ciliary rootlet within the dendritic inner segment is composed of fine filaments forming a regular cross-striation. Arrow points apically. (High-pressure freezing preparation). Bar = 0.2 μm (× 58,500). (B, C) Anti-centrin/phalloidin double-staining in longitudinal section through a scolopidium of *P. americana*. (B) Indirect immunofluorescence of anti-centrin (FITC) and (C) phalloidin-rhodamine fluorescence. Rhodamine-phalloidin reacts specifically with the actin filament bundles of the scolopale. Anti-centrin immunofluorescence corresponds to the location of the ciliary rootlets of scolopidia. Bar = 20 μm (× 750). (D) Ultrastructural localization of centrin in a longitudinal section through a ciliary rootlet in a scolopidium of *S. lamanianus*. Labelling of antibodies raised against centrin is most obviously in the cross-striated ciliary rootlet. Arrow points apically. (High-pressure freezing; freeze substitution; low-temperature embedding; silver enhancement of 1 nm colloidal gold-coupled secondary antibody). Bar = 0.2 μm (× 58,500).

Fig. 6. The scolopale-like structure in thermo-/hygrosensitive sensilla of *A. pernyi*. (A) Longitudinal section through the hair of a styloconic np-sensillum of *A. pernyi*. Unbranched dendritic outer segments (black circle) are enclosed by the electron-dense dendrite sheath (black arrow). The scolopale (white arrows) in the thecogen cell encloses the inner receptor lymph cavity containing sensory dendrites. (Ambient-pressure freezing preparation). Bar = 0.5 μm (× 29,000). (B, C) Phalloidin staining of longitudinal section through a styloconic sensillum of *A. pernyi*. (B) Nomarski optics. (C) Phalloidin-rhodamine fluorescence. (Note intense non-specific auto-fluorescence of the cuticle.) Staining is present in the region of the scolopale (arrows). Bar = 10 μm (× 1100). (D, E) Phalloidin staining of longitudinal section through coeloconic sensillum of *A. pernyi*. (D) Nomarski optics. (E) Phalloidin-rhodamine fluorescence. (Note intense non-specific auto-fluorescence of the cuticle.) Staining is also restricted to the scolopale (arrows). Bar = 10 μm (× 1100). (F) Ultrastructural localization of actin in a transverse section through a styloconic np-sensillum of *A. pernyi*. Monoclonal antibodies against actin label the filament bundles in the scolopale structure (sc) present in the thecogen cell. di, Dendritic inner segment. Asterisk indicates the receptor lymph cavity. (Ambient-pressure freezing; freeze substitution; low-temperature embedding). Bar = 0.2 μm (× 72,000).

antibodies against G-actin react with the filaments of the scolopale. Moreover (iv) heavy meromyosin decorates the filaments of the scolopale in the actin-characteristic way.

Cellular actin filaments are part of a contraction mechanism or possess mechanical stabilizing functions (Schliwa, 1986; Bershadsky and Vasiliev, 1988). All present results indicate that, in the scolopale of insect sensilla, actin filaments serve as stabilizing structures. In systems where actin filaments are involved in contractile processes, the function is dependent on the interaction with the motorprotein, myosin. However, all attempts to detect myosin in the scolopale so far have been negative (Wolfrum, 1990, 1992). The scolopale thus most probably lacks myosins. The uniform polarity of the actin filaments with their barbed ends directed apically towards the cap structure, also indicates that the actin filament bundles are not involved in contractile processes. An identical filament orientation has been described in cellular components where actin filaments provide mechanical stabilization, e.g., the microvilli of intestinal brush borders, and stereovilli of vertebrate hair cells (Mooseker and Tilney, 1975; Tilney *et al.*, 1980; Bonfanti *et al.*, 1990). In addition, the present localization of MAP2 in the scolopale indicates linkages between actin filaments and microtubules. In neurons, MAP2 has previously been described as a cross-linker between microtubules and actin filaments stabilizing the heterogeneous cytoskeletal system (Morales and Fifkova, 1989; Chen *et al.*, 1992; Slepecky and Ulfendahl, 1992). Therefore, MAP2 may also provide stabilization to the scolopale of insect sensilla. Moreover, in the scolopale, the co-localization of tropomyosin with actin filaments indicates stabilization of the actin filaments themself. While in skeletal muscle cells, tropomyosin is involved in the regulation of muscle contraction, in non-muscle cells it stabilizes actin filaments. It promotes actin filament assembly, prevents actin filament disassembly, and increases the rigidity of actin filaments (e.g., Fujime and Ishiwata, 1971; Lemanski, 1979; Broschat *et al.*, 1989; Ishiwata *et al.*, 1989). Furthermore, tropomyosin binding is highly regulated by caldesmon and calcium/calmodulin (Yamashiro-Matsumura and Matsumura, 1988). The affinity of tropomyosin to actin filaments increases by complexation with caldesmon, which is promoted by free Ca^{2+}. Therefore, in scolopidia, an increase in the concentration of intracellular free Ca^{2+} may increase the rigidity and decrease the elasticity of the actin filaments within the scolopale.

As mentioned above, actin filament bundles are not the only cytoskeletal element in the scolopale of insect sensilla. The actin filaments of the scolopale are associated with microtubules, which is probably promoted by linkages of MAP2. As in other systems, in scolopidia, microtubules may also contribute to cellular rigidity (Slepecky and Chamberlain, 1983; Weiss *et al.*, 1987; Mogensen and Tucker, 1988). Although both cytoskeletal elements, the actin filaments and microtubules, lead to cellular stabilization, they have different properties. The "tube-shaped" microtubules provide stability to cells, particularly against lateral forces (Bereiter-Hahn, 1978). In contrast, the "filamentous" actin filaments are more elastic and provide more freedom for filament bending or twisting (Yamazaki *et al.*, 1982; Tilney *et al.*, 1983; Pollard and Cooper, 1986; Weiss *et al.*, 1987). Hence, reversible bending of entire bundles of actin filaments is possible (DeRosier and Tilney, 1984). Therefore, the ratio of actin filaments to microtubules as well as their interplay define the elasticity or rigidity of a cell. In comparison, based on present results on their cytoskeleton composition, the attachment cell of scolopidia containing more microtubules should be more rigid than their scolopale cell where more actin filaments are present.

Functional significance of the scolopale

In scolopidia, the stabilizing character and a certain degree of elasticity of actin filament bundles in the scolopale may be important during stimulation. It has been suggested that, in mononematic scolopidia, mechanical stimuli are transmitted by the rigid attachment cell to the extracellular cap, causing lateral displacement of both the cap and the tips of the dendritic outer segments (Moran *et al.*, 1977). However, because all components of scolopidia are mechanically linked by desmosomal structures, this displacement should also affect the scolopale; the elasticity of actin filament bundles should allow bending of the scolopale. During this process, the force could be restored to return the whole system back into its original position after stimulation. The sensitivity of the scolopidia might be governed by the degree of elasticity of the scolopale. This, in turn, should be ultimately controlled by Ca^{2+}-concentrations and tropomyosin binding (see above).

A scolopale-like structure is also present in thermo-hygrosensitive np-sensilla of insects. In these sensilla, the scolopale is also located within the innermost auxiliary cell, the thecogen cell (Yokohari, 1981; Altner *et al.*, 1983; Steinbrecht, 1984; Altner and Loftus, 1985; Steinbrecht, 1989; Steinbrecht *et al.*, 1989). Scolopale components are, as in scolopidia, actin filaments and microtubules. In comparison, the number of actin filaments per microtubule in the scolopale is smaller in np-sensilla than in scolopidia. Therefore, the scolopales in np-sensilla are possibly more rigid than those in scolopidia (see above; Yamazaki *et al.*, 1982; Weiss *et al.*, 1987).

Mechanical forces may also play a role in hygroreception of insect np-sensilla. The current hypothesis for the primary process of hygroreception is that changes in humidity are transformed into mechanical forces. These forces may act on the membranes of the 2 unbranched dendritic outer segments in their distal regions. These membrane areas are tightly apposed to the cuticle of the hair wall (Yokohari and Tateda, 1976; Altner *et al.*, 1978; Yokohari, 1978; Haug, 1985).

By reason of their size, shape and position in the sensil-

lum, both the dendrite sheath and the scolopale may protect the dendritic outer segments from mechanical forces other than those that affect the distal region of their outer segments. In np-sensilla, the inner receptor lymph cavity containing the sensory dendrites is enveloped by the dendrite sheath proximally from the hair base and by the thecogen cell more proximally. Whereas the extracellular dendrite sheath is generally thought to be rigid (Seidl, 1992), the cytoskeletal elements of the scolopale can be assumed to stabilize the thecogen cell.

Ciliary rootlets of insect sensilla are probably contractile

The long ciliary rootlets in sensory cells of scolopidia are composed of filaments forming their regular cross-striation. On the basis of the appearance of their striation, some authors have speculated a collagen nature of the rootlets (e.g., Fawcett, 1961; Schmidt, 1969, 1970, 1974). However, previous studies on insect scolopidia have revealed that ciliary rootlets are probably not composed of intracellular collagen fibres (Wolfrum, 1991c). Phalloidin cytochemistry and immunogold staining with anti-actin antibodies have also demonstrated that the filaments composing the rootlets are not actin filaments.

Anti-centrin immunolabelling on both the light and electron microscopical level reveals that centrin is localized in ciliary rootlets of insect scolopidia. Centrin, also known as caltractin (Huang *et al.*, 1988), is a small phosphoprotein (molecular weight 20 kDa) and a member of the EF-hand superfamily of Ca^{2+}-binding proteins (Salisbury, 1995; Schiebel and Bornens, 1995). Forming fine filaments, it is the main component of the contractile ciliary or flagellar rootlets in green algae and, therefore, is largely responsible for their Ca^{2+}-modulated contractions (Salisbury *et al.*, 1984; Salisbury, 1995). The present immunohistochemical localization of centrin in the ciliary rootlets of the sensory cells of scolopidia indicates that Ca^{2+}-modulated rootlet contractions may also occur in insect sensilla. Such contractions or relaxations are probably involved in sensory transduction as well as in adaptation; during stimulation of scolopidia the distal basal body of the ciliary base is shifted (Moran *et al.*, 1977; Toh and Yokohari, 1985). Rootlet contractions could affect the degree of this displacement or replace the basal body to its original position after stimulation. In addition, if such contractions are transmitted to the dendritic outer segment they probably affect the degree of tension of the ciliary necklace at its base. In this area of the dendritic membrane, the localization of mechanosensitive membrane channels has been suggested (Moran *et al.*, 1977; Stockbridge *et al.*, 1990).

In conclusion, present investigations on prominent cytoskeletal elements in insect sensilla have revealed structural and molecular characteristics of these elements. Recent studies on *Drosophila* mutants in genes encoding for actin filament-binding proteins have indicated that cytoskeletal elements are also functionally important during sensillum development (Tilney *et al.*, 1995; Hopmann *et al.*, 1996). It will be interesting to analyse specific *Drosophila* cytoskeleton mutants, testing the hypotheses on functional roles of the cytoskeletal elements of insect sensilla characterized in the present study.

Acknowledgements—I am grateful to Drs H. Altner, R. Paulsen, S. C. Seidl, and B. Zimmermann for their help and critical discussions. I wish to thank Dr M. Müller, ETH Zürich, for generously providing the high-pressure freezing equipment and Drs M. Knipper, J. Lessard, M. Melkonian, J. L. Salisbury and G. Wiche for generously supplying the antibodies. I also thank Dr J. Bentrop for attentive linguistic corrections, Mrs G. Thies for skilful sectioning, and Dr B. Zimmermann for providing the specimen used in Fig. 6(F) for immunolabelling. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 4: G1) and the FAUN-Stiftung, Nürnberg.

REFERENCES

Altner, H. (1977) Insect sensillum specificity and structure: an approach to a new typology. In *Olfaction and Taste*, eds, J. Le Magnen and P. MacLeod, pp. 295–303. Information Retrieval, London.

Altner, H., Tichy, H. and Altner, I. (1978) Lamellated outer dendritic segments of a sensory cell within a poreless thermo- and hygroreceptive sensillum of the insect *Carausius morosus*. *Cell Tissue Res.* **191**, 287–304.

Altner, H. and Loftus, R. (1985) Ultrastructure and function of insect thermo- and hygroreceptors. *Annu. Rev. Entomol.* **30**, 273–295.

Altner, H. and Prillinger, L. (1980) Ultrastructure of invertebrate chemo-, thermo- and hygroreceptors and its functional significance. *Int. Rev. Cytol.* **67**, 69–139.

Altner, H., Schaller-Selzer, L., Stetter, H. and Wohlrab, I. (1983) Poreless sensilla with inflexible sockets. A comparative study of a fundamental type of insect sensilla probably comprising thermo- and hygroreceptors. *Cell Tissue Res.* **234**, 279–307.

Bereiter-Hahn, J. (1978) A model for microtubular rigidity. *Cytobiologie* **17**, 298–300.

Bershadsky, A. D. and Vasiliev J. M. (1988) *Cytoskeleton*. Plenum, New York.

Bonfanti, P., Cappelletti, A., Colombo, A., Giordana, B., Maci, R. and Camatini, M. (1990) Cytoskeletal characterization of insect brush border. In *Cell Biology International Reports*, Vol. 14 [Suppl], ed. G. Bullock, pp. 206. Harcourt Brace Jovanovich, London.

Broschat, K. O., Weber, A. and Burgess, D. R. (1989) Tropomyosin stabilizes the pointed end of actin filaments by slowing depolymerization. *Biochemistry* **28**, 8501–8506.

Chen, J., Kanai, Y., Cowan, N. J. and Hirokawa, N. (1992) Projection domains of MAP2 and Tau determine spacings between microtubules in dendrites and axons. *Nature* (*Lond.*) **360**, 674–676.

Danscher, G. (1981) Histochemical demonstration of heavy metals: a revised version of the sulphide silver method suitable for both light and electron microscopy. *Histochemistry* **71**, 1–16.

DeRosier, D. J. and Tilney, L. G. (1984) How to build a bend into an actin bundle. *J. Mol. Biol.* **175**, 57–73.

Egelman, E. H. and Padrón, R. (1984) X-ray diffraction evidence that actin is a 100 Å filament. *Nature* (*Lond.*) **307**, 56–58.

Fujime, S. and Ishiwata, S. (1971) Dynamic study of F-actin by quasielastic scattering of laser light. *J. Mol. Biol.* **62**, 251–265.

Faulstich, H., Zobeley, S., Rinnerthaler, G. and Small, J. V. (1988) Fluorescent phallotoxins as probes for filamentous actin. *J. Muscle Res. Cell Motil.* **9**, 370–383.

Fawcett, D. (1961) Cilia and flagella. In *The Cell. Biochemistry, Physiology, Morphology*, 2nd edn, eds, J. Brachet and A. E. Mirsky, pp. 217–298. Academic Press, New York.

Haug, T. (1985) Ultrastructure of the dendritic outer segments of sensory cells in poreless ("no-pore") sensilla of insects. A cryofixation study. *Cell Tissue Res.* **242**, 313–322.

Haug, T. and Altner, H. (1984) A cryofixation study of presumptive hygroreceptors on the antennule of a terrestrial isopod. *Tissue Cell* **16**, 377–391.

Hopmann, R., Cooper, J. A. and Miller, K. G. (1996) Actin organization, bristle morphology, and viability are affected by actin capping protein mutations in *Drosophila*. *J. Cell Biol.* **133**, 1293–1305.

Howse, P. E. (1968) The fine structure and functional organisation of chordotonal organs. *Symp. Zool. Soc. (Lond.)* **23**, 167–198.

Huang, B., Mengerson, A. and Lee, V. D. (1988) Molecular cloning of cDNA for caltractin, a basal body-associated Ca^{2+}-binding protein: homology in its protein sequence with calmodulin and the yeast CDC31 gene product. *J. Cell Biol.* **107**, 133–140.

Ishiwata, R., Yamashiro, S. and Matsumura, F. (1989) Annealing of gelsolin-severed actin fragments by tropomyosin in the presence of Ca^{2+}-potentiation of the annealing process by caldesmon. *J. Biol. Chem.* **264**, 16764–16770.

Keil, T. and Steinbrecht, R. A. (1984) Mechanosensitive and olfactory sensilla. In *Insect Ultrastucture*, Vol. 2, eds R. C. King and H. Akai, pp. 477–516. Plenum, New York.

Krieger, J., Raming, K., Knipper, M., Grau, M., Mertens, S. and Breer, H. (1990) Cloning, sequencing and expression of Locust tropomyosin. *Insect Biochem.* **20**, 173–184.

Lemanski, L. F. (1979) Role of tropomyosin in actin filament formation in embryonic salamander heart cells. *J. Cell Biol.* **82**, 227–238.

Lessard, J. L. (1988) Two monoclonal antibodies to actin: one muscle selective and one generally reactive. *Cell Motil. Cytoskeleton* **10**, 349–362.

McIver, S. B. (1985) Mechanoreception. In *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, Vol. 6. Nervous System: Sensory, eds G. A. Kerkut and L. I. Gilbert, pp. 71–132. Pergamon Press, Oxford.

Moran, D. T., Varela, F. J. and Rowley, J. C. (1977) Evidence for active role of cilia in sensory transduction. *Proc. Natl. Acad. Sci. USA* **74**, 793–797.

Mogensen, M. M. and Tucker, J. B. (1988) Intermicrotubular actin filaments in the transalar cytoskeletal arrays of *Drosophila*. *J. Cell Sci.* **91**, 431–438.

Moor, H. (1987) Theory and practice of high-pressure freezing. In *Cryotechniques in Biological Electron Microscopy*, eds R. A. Steinbrecht and K. Zierold, pp. 175–191. Springer, Berlin.

Mooseker, M. S. and Tilney, L. G. (1975) Organization of actin filament-membrane complex. *J. Cell Biol.* **67**, 725–743.

Morales, M. and Fifkova, E. (1989) Distribution of MAP2 in dendritic spines and its colocalization with actin. An immunogold electron-microscopy study. *Cell Tissue Res.* **256**, 447–456.

Moulins, M. (1976) Ultrastructure of chordotonal organs. In *Structure and Function of Proprioceptors in the Invertebrates*, ed. P. J. Mill, pp. 387–426. Chapman & Hall, London.

Müller, M. and Moor, H. (1984) Cryofixation of thick specimens by high pressure freezing. In *Science of Biological Specimen Preparation*, eds J.-P. Revel, T. Barnard and G. H. Haggis, pp. 131–138. SEM, AMF O'Hare, Chicago.

Pollard, T. D. and Cooper, J. A. (1986) Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. *Annu. Rev. Biochem.* **55**, 987–1035.

Salisbury, J. L. (1995) Centrin, centrosomes, and mitotic spindle poles. *Curr. Opin. Cell Biol.* **7**, 39–45.

Salisbury, J. L., Baron, A., Surek, B. and Melkonian, M. (1984) Striated flagellar roots: isolation and characterization of a calcium-modulated contactile organelle. *J. Cell Biol.* **99**, 962–970.

Schiebel, E. and Bornens, M. (1995) In search of a function for centrins. *Trends Cell Biol.* **5**, 197–201.

Schliwa, M. (1986) *Cytoskeleton*. Springer, New York.

Schmidt, K. (1969) Der Feinbau der stiftführenden Sinnesorgane im Pedicellus der Florfliege *Chrysopa* Leach (Chrysopidae, Planipennia). *Z. Zellforsch.* **99**, 357–388.

Schmidt, K. (1970) Vergleichende morphologische Untersuchungen über den Feinbau der Ciliarstrukturen in den Scolopidien des Johnstonschen Organs holometaboler Insekten. *Verh. Dtsch. Zool. Ges.* **64**, 88–92.

Schmidt, K. (1974) Die Mechanorezeptoren im Pedicellus der Eintagsfliege (Insecta, Ephemeroptera). *Z. Morphol. Tiere* **78**, 193–220.

Schmidt, M. (1989) The hair-peg organs of the shore crab, *Carcinus maenas* (Crustacea, Decapoda): ultrastructure and functional properties of sensilla sensitive to the changes in seawater concentration. *Cell Tissue Res.* **257**, 609–621.

Schmidt, M. (1990) Ultrastructure of a possible new type of crustacean cuticular strain receptor in *Carcinus meanus* (Crustacea, Decapoda). *J. Morphol.* **204**, 335–344.

Schmidt, M. and Gnatzy, W. (1984) Are the funnel-canal organs the "campaniform sensilla" of the shore crab, *Carcinus maenas* (Decapoda, Crustacea)? II. Ultrastructure. *Cell Tissue Res.* **237**, 81–93.

Seidl, S. (1992) Structure and function of the thecogen cell in contact chemosensitive sensilla of *Periplaneta americana* L. (Blattodea: Blattidae). *Int. J. Insect Morphol. Embryol.* **21**, 235–250.

Slepecky, N. and Chamberlain, S. C. (1983) Distribution and polarity of actin in inner ear supporting cells. *Hearing Res.* **10**, 359–370.

Slepecky, N. and Chamberlain, S. C. (1985) Immunoelectron microscopic and immunofluorescent localization of cytoskeletal and muscle-like contractile proteins in inner ear sensory hair cells. *Hearing Res.* **20**, 245–260.

Slepecky, N. B. and Ulfendahl, M. (1992) Actin-binding and microtubule-associated proteins in the Organ of Corti. *Hearing Res.* **57**, 201–215.

Steinbrecht, R. A. (1980) Cryofixation without cryoprotectants. Freeze substitution and freeze etching of an insect olfactory receptor. *Tissue Cell* **12**, 73–100.

Steinbrecht, R. A. (1984) Chemo- hygro- and thermoreceptors. In *Biology of the Integument, Vol. 1: Invertebrates*, eds J. Bereiter-Hahn, A. G. Matoltsky and K. S. Richards, pp. 523–553. Springer, Berlin.

Steinbrecht, R. A. (1989) The fine structure of thermo-/hygrosensitive sensilla in the silkmoth *Bombyx mori*: receptor membrane substructure and sensory cell contacts. *Cell Tissue Res.* **255**, 49–57.

Steinbrecht, R. A., Lee, J.-K., Altner, H. and Zimmermann, B. (1989) Volume and surface of receptor and auxiliary cells in hygro-/thermoreceptive sensilla of moths (*Bombyx mori*, *Antheraea pernyi* and *A. polyphemus*). *Cell Tissue Res.* **255**, 59–67.

Stockbridge, L. L., French, A. S. and Sanders, E. J. (1990) Dissociation and culture of mechanosensory neurons for patch clamp analysis. *Brain Res.* **523**, 161–166.

Studer, D., Michel, M. and Müller, M. (1989) High pressure freezing comes of age. In *The Science of Specimen Preparation*. SEM, eds R. Albrecht and R. Ornberg, pp. 253–269. AMF O'Hare, Chicago.

Tilney, L. G., DeRosier, D. J. and Mulroy, M. J. (1980) The organization of actin filaments in the stereocilia of cochlear hair cells. *J. Cell Biol.* **86**, 244–259.

Tilney, L. G., Egelman, E. H., DeRosier, D. J. and Saunders, J. C. (1983) Actin filaments, stereocilia, and hair cells of the bird cochlea. II. Packing of actin filaments in the stereocilia and in the cuticular plate and what happens to the organization when the stereocilia are bent. *J. Cell Biol.* **96**, 822–834.

Tilney, L. G., Tilney, M. S. and Guild, G. M. (1995) F-actin bundles in Drosophila bristles. 1. Two filament cross-links are involved in bundling. *J. Cell Biol.* **130**, 629–638.

Toh, Y. (1981) Fine structure of sense organs on the antennal pedicel and scape of the male cockroach *Periplaneta americana*. *J. Ultrastruct. Res.* **77**, 119–132.

Toh, Y. and Yokohari, F. (1985) Structure of the antennal chordotonal sensilla of the American cockroach. *J. Ultrastruct. Res.* **90**, 124–134.

Weiss, D. G., Langford, G. M. and Allen, R. D. (1987) Implications of microtubules in cytomechanics: static and motile aspects, In *Cytomechanics*, eds J. Bereiter-Hahn, O. R. Anderson and W.-R. Reif, pp. 100–113. Springer, Berlin.

Wolfrum, U. (1990) Actin filaments: the main components of the scolopale in insect sensilla. *Cell Tissue Res.* **261**, 85–96.

Wolfrum, U. (1991) Distribution of F-actin in the compound eye of *Calliphora erythrocephala* (Diptera, Insecta). *Cell Tissue Res.* **263**, 399–403.

Wolfrum, U. (1991) Tropomyosin is co-localized with the actin filaments of the scolopale in insect sensilla. *Cell Tissue Res.* **265**, 11–17.

Wolfrum, U. (1991) Centrin- and α-actinin-like immunoreactivity in the ciliary rootlets of insect sensilla. *Cell Tissue Res.* **266**, 231–238.

Wolfrum, U. (1992) Cytoskeletal elements in arthropod sensilla and mammalian photoreceptors. *Biol. Cell* **76**, 373–381.

Wolfrum, U. (1995) Centrin in the photoreceptor cells of mammalian retinae. *Cell Motil. Cytoskeleton* **32**, 55–64.

Wulf, E., Deboben, A., Bautz, F. A., Faulstich, H. and Wieland, T. (1979) Fluorescent phallotoxin, a tool for the visualization of cellular actin. *Proc. Natl. Acad. Sci. USA* **76**, 4498–4502.

Yamashiro-Matsumura, S. and Matsumura, F. (1988) Characterization of 83-kilodalton nonmuscle caldesmon from cultured rat cells: stimulation of actin binding of nonmuscle tropomyosin and periodic

localization along microfilaments like tropomyosin. *J. Cell Biol.* **106**, 1973–1983.

Yamazaki, S., Maeda, T. and Miki-Noumura, T. (1982) Flexural rigidity of singlet microtubules estimated from statistical analysis of fluctuating images. In *Biological Functions of Microtubules and Related Structures*, eds H. Sakai, H. Mohri and G. G. Borisy, pp. 41–48. Academic Press, Tokyo.

Yokohari, F. (1978) Hygroreceptor mechanism in the antenna of the cockroach *Periplaneta*. *J. Comp. Physiol.* **124**, 53–60.

Yokohari, F. (1981) The sensillum capitulum, an antennal hygro- and thermoreceptive sensillum of the cockroch *Periplaneta americana* L. *Cell Tissue Res.* **216**, 525–543.

Yokohari, F. and Tateda, H. (1976) Moist and dry hygroreceptors for relative humidity of the cockroach *Periplaneta americana* L. *J. Comp. Physiol.* **106**, 137–152.

Zacharuk, R. Y. (1985) Antennae and sensilla. In *Comprehensive Insect Physiology, Biochemistry, and Pharmacology*, Vol. 6. Nervous System: Sensory, eds G. A. Kerkut and L. I. Gilbert, pp. 1–69. Pergamon, Oxford.