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Cytoskeletal elements in arthropod sensilla and mammalian photoreceptors *

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Summary – Ciliary receptor cells, typified by cilia or modified cilia, are very common in the animal kingdom. In addition to the cytoskeleton of their ciliary processes these receptors possess other specific prominent cytoskeletal elements. Two representative systems are presented: i) scolopidia, mechanosensitive sensilla of various arthropod species; and ii) photoreceptor cells of the retina of the bovine eye. Two cytoskeletal structures are characteristic for arthropod scolopidia: a scolopale typifies the innermost auxiliary cell, and long ciliary rootlets are extending well into the sensory cells. The latter element is also characteristic for the inner segment of the photoreceptor cells in bovine. The scolopale of scolopidia is mainly composed of actin filaments. In the absence of myosin, the uniform polarity of the actin filaments and their association with tropomyosin all indicate a stabilizing role of the filament bundles within the scolopale. This function and a certain elasticity of actin filament bundles may be important during stimulation of the sensilla. The ciliary rootlets of both systems originate at the basal bodies at the ciliary base of the sensory cells and project proximally. These rootlets are composed of longitudinally oriented, fine filaments forming a characteristic regular cross-striation. An α-actinin immunoreactivity was detected within the ciliary rootlets of scolopidia. In addition, antibodies to centrin react with the rootlets of both types of receptors. Since centrin is largely responsible for the contraction of the flagellar rootlets in green algae, contraction may also occur in the ciliary rootlets of insect sensilla and vertebrate photoreceptors. In both systems, contraction or relaxation of the ciliary rootlets could serve in sensory transduction or adaptation.

mechanoreceptors / photoreceptors / cytoskeleton / mammals / Insecta

Introduction

Animals gather information about environmental conditions using modality-specific sense organs. These organs are composed of one or more receptor cells and additionally associated structures. While these sensory cells show a wide range of modalities, they frequently possess sensory processes which are based on the same basic structure: cilia or modified cilia [45]. In contrast to motile cilia, the modified sensory cilia, in most cases, lack some cytoskeletal elements of a typical cilium, for instance the central pair of microtubules and its associated structures [71]. Such ciliary receptor systems are very common in the animal kingdom. They occur for example in Coelenterates (in some Medusae), plathelminthes, polychaetes, arthropods up to the vertebrates and man (*eg* [11, 16, 28, 36, 41, 49, 61, 68]). In addition to their microtubule-based ciliary structure these receptors contain other prominent cytoskeletal elements [11, 48, 49, 61, 68, 73]. These elements may play an important role in receptor function. Therefore, an investigation of the composition of these structures was undertaken to provide more information concerning the properties and function of these elements within ciliary receptor systems.

The main focus here is on two very different ciliary receptor systems: i) the arthropod sensilla, particulary the non-cuticular, mechanosensitive scolopidia [36, 41]; and ii) the well-known photoreceptor cells of the mammalian retina [78]. Prominent cytoskeletal structures are present in the sensilla as well as in the photoreceptors; in scolopidia the scolopale typifies the innermost auxiliary cell [36, 41, 73]. Ciliary rootlets are characteristic for the receptor cells in both systems [36, 41, 60, 68, 73, 76]. In the present paper the ultrastructure and composition of the scolopale and of the ciliary rootlets in insect scolopidia and in bovine photoreceptor cells are demonstrated by cytochemistry and immunohistochemistry.

Materials and methods

Animals

Two insect species were used: *Periplaneta americana* (Blattodea), taken from colonies of the Institute; workers of *Schedorhinotermes lamanianus* (Isoptera), kindly provided by Dr M Kaib, University of Bayreuth, Germany. Two species of Crustacea were used: *Porcellio scaber* and *Armadillium* sp (Isopoda) taken from colonies of the Institute. Eyes from bovine were kindly provided by the slaughterhouse of the city of Karlsruhe. They were transported to the institute in the dark on ice and were immediately fixed about 30–45 min post-mortem.

Electron microscopy

Chemical fixation

The prepared retinae of the bovine eyes were cut into small pieces and fixed in a mixture of 2.5% glutaraldehyde and 1% parafor-

* This publication is based on a presentation at the 2nd International Symposium on Cell Motility at Nice/Sophia Antipolis, France, in July 1992. Some of the results on the cytoskeleton of arthropod scolopidia, already published, are summarized here, while the studies on the ciliary rootlets in the photoreceptor cells of the mammalian retina are in progress.

maldehyde in 0.1 Na-cacodylate buffer (pH 7.2). The following processes were performed as described in [73].

High-pressure freezing, freeze-substitution and embedding

Insect antennae were frozen in a Balzers high-pressure freezer HPM 010 (Balzers Union, Liechtenstein) [37] at about 2100 bar according to Studer *et al* [64] and Wolfrum [73]. The freeze-substitution procedure in dry acetone containing 0.1% glutaraldehyde, and embedding in Lowicryl K4M were carried out as described in Wolfrum [75].

Ultrathin sections were cut on an Ultracut E ultramicrotome (Reichert-Jung, Nußloch, Germany) with diamond knives. They were stained with ethanolic uranyl acetate and aqueous lead citrate, and examined in a Zeiss EM 10/CR electron microscope (Zeiss, Oberkochen, Germany).

Immunohistochemistry

Antibodies

Primary antibodies. Several primary antibodies were kindly provided for this study: monoclonal antibody (mAb) c4 against actin by Dr JL Lessard (Children's Hospital Res Fondation, Cincinatti, USA) [34]; polyclonal anti-α-actinin serum from rabbit by Dr BM Jockusch (University of Bielefeld, Germany) and another was obtained from Sigma; polyclonal rabbit antisera to centrin and mAb 20H5 by Drs M Melkonian (University of Köln, Germany) and JL Salisbury (Mayo Clinic, Rochester, MI, USA); mAb against tropomyosin by Dr M Knipper (University of Stuttgart-Hohenheim, Germany) [30].

Secondary fluorescent antibodies. The fluorescein-iso-thiocyanate (FITC)- or rhodamine-conjugated anti-rabbit-IgG and anti-mouse-IgG antibodies were obtained from both Sigma and Jackson Immuno Research (USA).

Phalloidins

Rhodamine-coupled phalloidin (a generous gift from Dr H Faulstich, MPI for Medicine, Heidelberg, Germany) and FITC-coupled phalloidin (Sigma) were used as specific probes for filamentous actin [21, 77]. The specificity of the phalloidins was additionally tested according to Wolfrum [74].

Preparation for fluorescence microscopy

Freezing of unfixed specimens in melting isopentane (− 165°C) and cryosectioning on a cryostat (2800 Frigocut, Reichert-Jung) were performed as described in Wolfrum [73]. The sections were placed for continuing procedures on coverslips precoated with 0.05% aqueous poly-L-lysine (Sigma).

Fluorescence staining

Cryosections were incubated first with 0.01% Tween 20 in 0.15 M phosphate-buffered saline (pH 7.2) (PBS) (in the case of anti-centrin-staining the PBS contains 50 mM $CaCl_2$) for 10 min and washed in PBS. After blocking with 0.5% fish gelatin (Sigma) plus 0.1% ovalbumin (Sigma) in PBS for 10 min, 10 μl of a dilution of one of the primary antibodies in PBS was placed on each section for 12 h at 4°C. Unbound antibodies were subsequently washed out three times with PBS. Subsequently, 10 μl of a dilution of the specific secondary antibodies in PBS were placed on each section for 1 h at room temperature in the dark. After being washed in PBS, the sections were then mounted in Mowiol 4.88 (Farbwerke Hoechst, Frankfurt, Germany) containing 2% n-propyl-gallate.

For double-staining experiments, the sections were covered (after incubation with the secondary antibody) with 10 μl of one of the fluorescent phalloidins in PBS (rhodamine-coupled: 0.01 mg/ml, or FITC-coupled: 0.1 mg/ml) for 1 h at room temperature in the dark.

The mounted cryosections were examined and photographed with a Zeiss Axoplan, Axiophot or Axiovert microscope. Kodak T-max 100 films exposed at 400 ASA were used for photographic documentation.

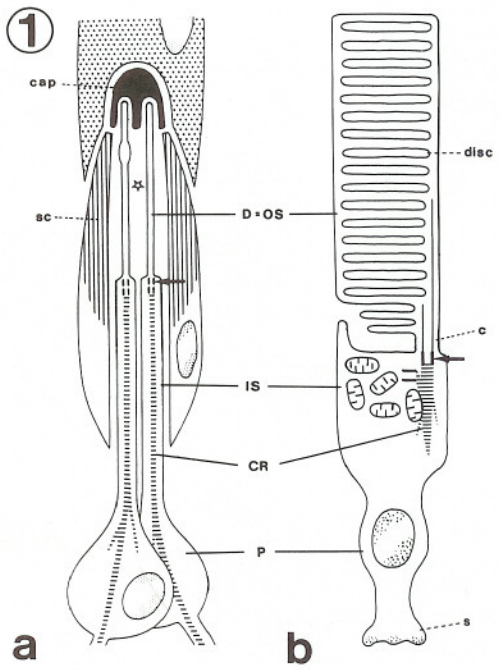


Fig 1. Diagrams of an arthropod scolopidium and a rod photoreceptor cell of the mammalian retina in comparison. **a.** In the scolopidium the dendritic outer segment (D) extends into the extracellular cap structure (cap). Long ciliary rootlets (CR) project from the basal bodies (arrow) of the tip of the dendritic inner segments (IS) into the perikaryon (P) of each sensory cell. The innermost auxiliary cell contains the scolopale (SC), which envelopes the receptor lymph cavity (asterisk). **b.** The photoreceptor cell of mammals is also divided in an inner (IS) and an outer segment (OS). The latter is characterized by membranous discs (disc). At the basal bodies (arrow) of the connecting cilium (C) the ciliary rootlet (CR) arises. S, synapse.

Controls

Muscles of insects were used to demonstrate the specificity of the fluorescent phalloidins and the antibodies used. Anti-actin, anti-tropomyosin and both phalloidins reacted in the light band of the muscles; both anti-α-actinins specifically stained the Z-line. For additional controls of immunofluorescent-staining: i) the primary or secondary antibody was omitted; ii) a secondary antibody against antibodies differing from the primary antibody was used; and iii) non-immunoserum from rabbit was used in the place of the primary antibodies. In no case was a reaction observed.

Immunogold-staining

Immunogold-stainings were performed as described in Wolfrum [75].

Results

A comparison between an insect scolopidium and a mammalian photoreceptor cell is shown schematically in figure 1.

Arthropod sensilla

Scolopidia are present in propioreceptive organs (for example the chordotonal organs) of arthropods [41] as well as in the sense organs of the auditory system of insects

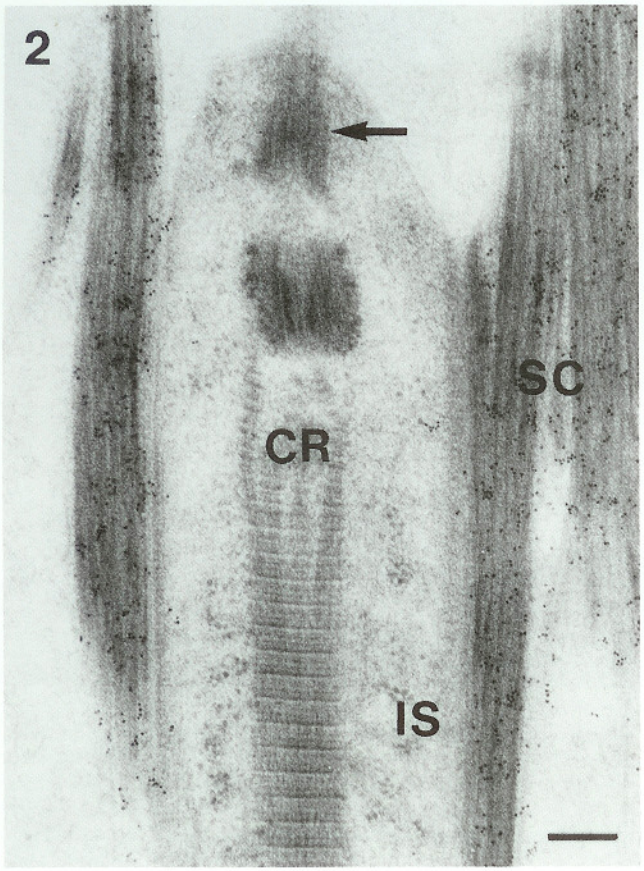


Fig 2. Longitudinal sections through a scolopidium of *Schedorhinotermes lamanianus*. Anti-actin antibodies made visible by 10-nm colloidal-gold particles bound to the filaments of the scolopale (SC). Within the dendritic inner segments (IS) the basal bodies (arrow indicates distal basal body) and the filamentous, regularly cross-striated ciliary rootlet (CR) lack any staining (high-pressure freezing; substitution: acetone/0.1% glutaraldehyde; embedding: Lowicryl K4M). Bar, 0.2 μm (× 45 000).

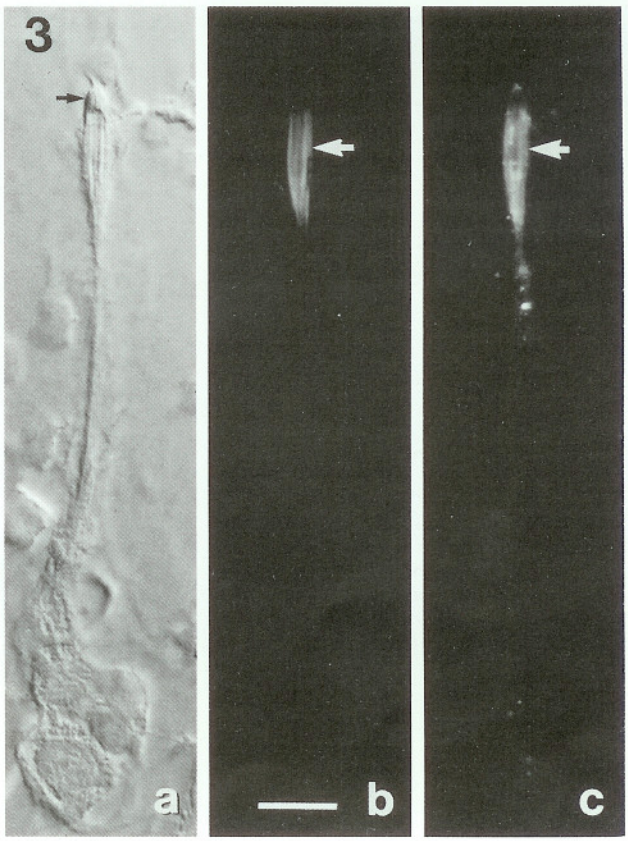


Fig 3a–c. Double-fluorescence staining of a longitudinal section of a scolopidium of *Periplaneta americana*. **a.** Nomarski optics. **b.** Phalloidin-rhodamine fluorescence (arrow). **c.** Indirect immunofluorescence caused by the presence of anti-tropomyosin (FITC) (arrow). Rhodamine-phalloidin reacts specifically within the scolopale proximal to the cap (small arrow in **a**). The indirect immunofluorescence of anti-tropomyosin is co-localized with phalloidin fluorescence. Bar, 10 μm (× 980).

[24]. The scolopidia of the antennal chordotonal organ and the Johnston's organ of various insects were investigated. Additionally, the results found in the insect species were verified in the antennal scolopidia of both Crustacean species used.

Like other sensilla, scolopidia are composed of sensory cells enveloped by some auxiliary cells. Prominent cytoskeletal structures are present in both types of cells: the scolopale typifies the innermost auxiliary cell (scolopale cell) and a long ciliary rootlet is characteristic for each sensory cell (fig 1a).

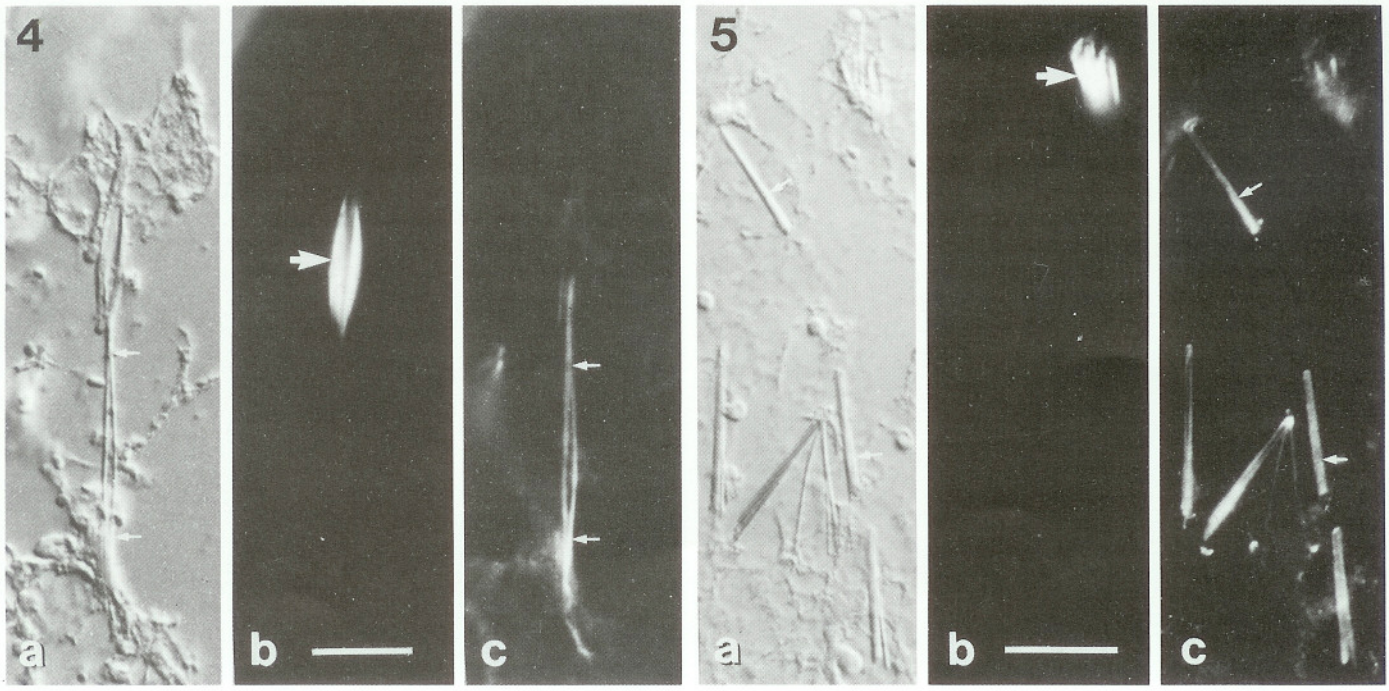
Scolopale

The scolopale structure forms a fenestrated, longitudinally oriented cylinder within the innermost auxiliary cell (figs 1a, 2). Electron microscopical studies of preparations processed by high-pressure freezing and freeze substitution reveal that the scolopale is mainly composed of bundles of longitudinally oriented filaments (filament diameter 7–10 nm) containing a few microtubules [73]. Fluorescent phalloidins, specific dyes for F-actin, stain the filament bundles of the scolopale (figs 3–5). In addition, heavy mero-myosin (HMM) binds to the filaments of the scolopale in an actin-specific way. The resultant 'arrowheads' point proximally [75]. Moreover, monoclonal antibodies raised against actin react within the scolopale (fig 2).

In immunohistochemical tests neither the antibodies to myosin nor to α-actinins stain the scolopale [73, 75]. However, antibodies prepared against tropomyosin react within the scolopale (fig 3).

Ciliary rootlets of scolopidia

The second prominent cytoskeletal element in the scolopidia are the long ciliary rootlets of the sensory cells. These rootlets arise at the basal bodies at the tip of the dendritic innersegment of each sensory cell and project over a distance of up to 100 μm proximally into its cell body (fig 1a). The rootlets are regularly cross-striated with a periodicity of about 68 nm (fig 2). They are composed of rather fine filaments of about 4 nm in diameter. Antibodies produced against actin do not react with these fine filaments (fig 2). Double-staining experiments with fluorescent phalloidins and immunofluorescence of antibodies against α-actinin show an α-actinin-like immunoreactivity within the ciliary rootlets of the scolopidia (fig 4). In addition, antibodies against centrin react with the rootlets within these sensilla (fig 5).



Figs 4, 5. 4a–c. Anti-α-actinin/phalloidin double-labeling in longitudinal section through a scolopidium of *Periplaneta americana*. **a.** Nomarski optics. **b.** Phalloidin FITC-fluorescence. **c.** Indirect rhodamine-immunofluorescence of anti-α-actinin. Phalloidin reacts specifically with the actin filament bundles of the scolopale (arrow). Anti-α-actinin does not co-localize with phalloidin fluorescence; the immunofluorescence corresponds to the location of the ciliary rootlets (small arrows) of the scolopidium. Bar, 10 μm ($\times$ 1250). **5a–c.** Anti-centrin/phalloidin double-staining in longitudinal sections through scolopidia of *Periplaneta americana*. **a.** Nomarski optics. **b.** Phalloidin-rhodamine fluorescence. **c.** Indirect FITC-immunofluorescence of anti-centrin. Rhodamine-phalloidin reacts specifically with the actin filament bundles of the scolopale (arrow). Anti-centrin FITC-immunofluorescence corresponds to the location of the ciliary rootlets (small arrows) of the scolopidia. Bar, 10 μm ($\times$ 1500).

Mammalian photoreceptors

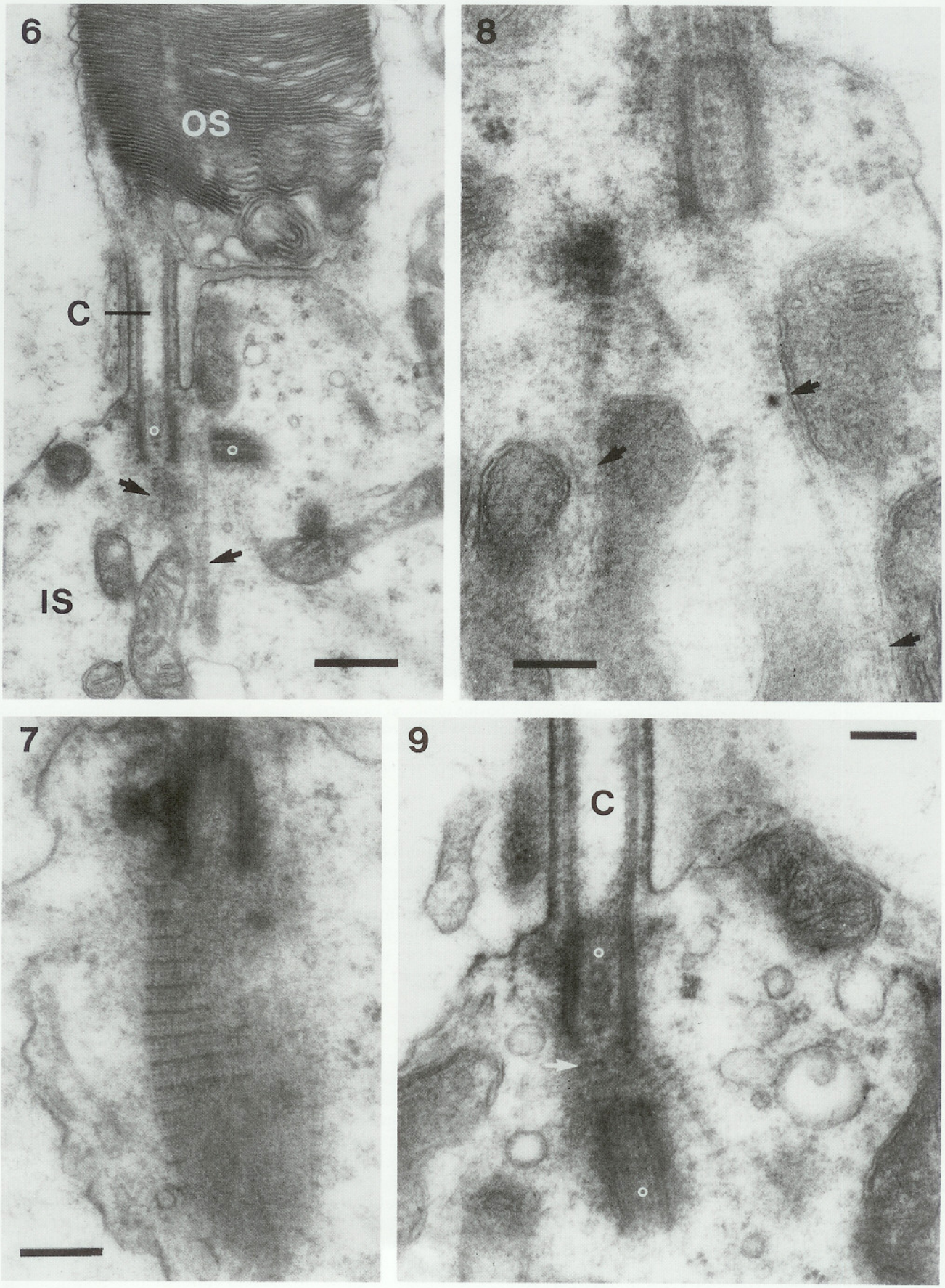
Like the sensory cells of the arthropod sensilla, the photoreceptor cells of the retina of vertebrates and mammals are divided morphologically as well as functionally (fig 1b) in the light sensitive outer segments and the inner segments containing the machinery of biosynthesis. The outer segment is a modified cilium in which the ciliary membrane has been greatly amplified, forming flattened membrane stacks, termed discs (figs 1b, 6). The membranes of these discs are packed with the photopigment rhodopsin. The so-called connecting cilium links the outer segment to the inner segment (figs 1b, 6) [3]. The ciliary arrangment, the 9 $\times$ 2 + 0-structure, is present only over the extension of this connecting cilium. At its base two basal bodies are localized (figs 1b, 6–9). The orientation of these basal bodies relative to each other is variable: after chemical fixation the basal bodies are frequently oriented perpendicularly to each other (figs 1b, 6). In contrast, however, in some of the cases, both are arranged tandem-like in the sensilla (figs 2, 9). Both basal bodies of the photoreceptors are connected by cross-striated material of the ciliary rootlets (fig 9). In the photoreceptor cells these characteristic cross-striated ciliary rootlets originate from both basal bodies (figs 1b, 6–9). Fibers of the ciliary rootlets project predominantly proximally but also laterally into the inner segment (figs 6–9). These strands contact mitochondria localized within the distal part (ellipsoid region) of the inner segment (fig 8). The rootlets of the photoreceptor cells of mammals are composed of fine filaments with about the same diameter as found in the arthropod sensilla. In addition, their regular cross-striation is identical to striation shown in the scolopidia (figs 2, 7). All antibodies used against centrin react specifically within the neuronal bovine retina (fig 10). The indirect immunofluorescence is restricted to the inner segments of the photoreceptor cells. There it occurs within the apical part of cell, just in the region where the connecting cilium, the basal bodies and the ciliary rootlet are localized (fig 10).

Discussion

Scolopale in scolopidia

There is no doubt that the majority of the filaments composing the scolopale are actin filaments. All evidence supports this fact: i) the diameter of the filaments forming the scolopale is identical to the diameter measured for actin filaments [17]; ii) fluorescent phalloidins which are specific dyes for filamentous actin [21] bind in a specific way to the filament bundles of the scolopale of all types of sensilla investigated (figs 3–5) [73]; iii) monoclonal antibodies, raised against actin, react with the filaments of the scolopale as demonstrated by immunoelectron microscopy (fig 2) [75]; iv) moreover, heavy mero-myosin binds to the filaments of the scolopale in the actin-characteristic way [75].

In the cell actin filaments have mechanically stabilizing or contractile functions [55]. All findings on the composition of the scolopale indicate that their actin bundles may



Figs 6–9. Longitudinal section through photoreceptor cells of the bovine retina. **6.** The outer segment (OS) is typified by its membrane discs. It is linked to the inner segment (IS) by the connecting cilium (C). The fibers of ciliary rootlet (arrows) arise at both basal bodies (white circles), which are oriented perpendicularly to each other. Bar, 0.5 μm (× 30 000). **7.** Section through the ciliary rootlet of a photoreceptor cell. The filamentous ciliary rootlet is regulary cross-striated. Bar, 0.2 μm (× 75 000). **8.** Section through the distal part of the inner segment. Contacts between the fibers of ciliary rootlet and mitochondria are visible (arrows). Bar, 0.2 μm (× 75 000). **9.** Section through basal bodies (white circles) arranged in tandem. Both basal bodies are linked by the cross-striated material of the ciliary rootlet (arrow). C = connecting cilium. Bar, 0.2 μm (× 58 500).

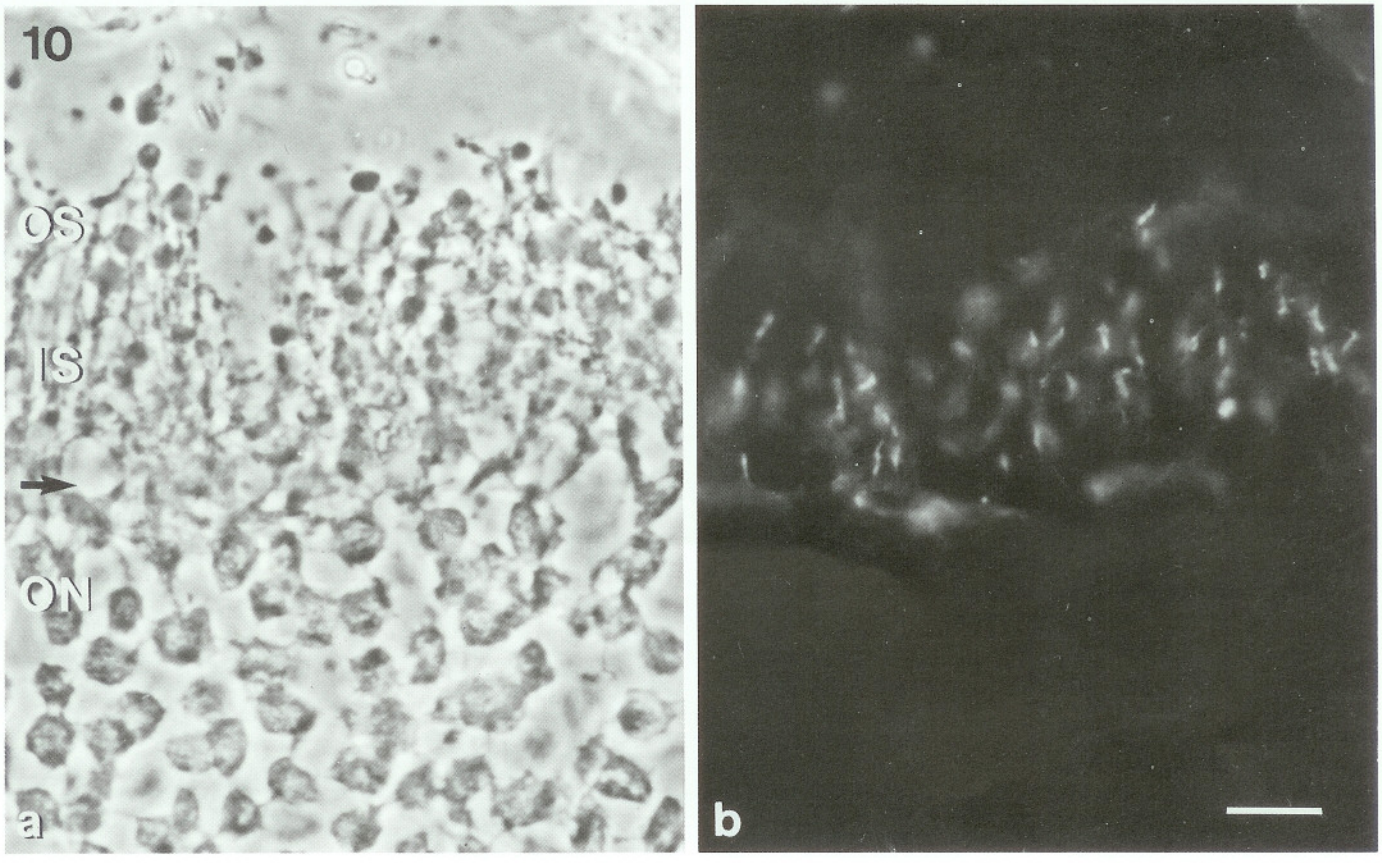


Fig 10. Anti-centrin-staining in longitudinal sections through a bovine retina. **a.** Nomarski optics. **b.** Indirect FITC-immunofluorescence of polyclonal antibodies against centrin. Anti-centrin immunofluorescence corresponds to the location of the connecting cilia, basal bodies and ciliary rootlets within the inner segments (IS) of the photoreceptor cells. The photoreceptor outer segments (OS) and the outer nuclear layer (ON) lack any staining. The *membrana limitans externa* is indicated by an arrow. Bar, 10 μm ($\times$ 1250).

serve as stabilizing elements. First, myosin was not detectable [73]. Second, the orientation of the filaments is uniform and the arrowheads of the pointed-end point proximally [75]. An identical filament orientation is shown in other mechanically stabilized systems of actin filaments. For example, such an orientation is also found in the microvilli of the intestinal brush border in vertebrates [39], in insects [5] and in the unfortunately misnamed stereocilia (a better name would be stereovilli) of the hair cells in the vertebrate inner ear [65], respectively. Moreover, third, tropomyosin is co-localized with the actin filaments of the scolopale (fig 3) [75]. It is known that in non-muscle cells tropomyosin stabilizes actin filaments [7]. Binding of tropomyosin may increase the rigidity of the actin filament bundles [19]. The affinity of tropomyosin binding to the actin filament is increased by caldesmone [79]. The formation of the tropomyosin/caldesmone complex itself is regulated by the calcium/calmodulin system [59, 79]. Therefore, high intracellular calcium concentration may increase the rigidity and decrease the elasticity of the actin filaments within the scolopale.

Their stabilizing character and a certain elasticity of actin filament bundles [14] may be important during stimulation of scolopidia: during stimulation the cap and the tip of the dendritic outer segments are laterally displaced [38]. However, since all components of scolopidia are mechanically linked [67, 73], this displacement should also affect the scolopale: a bend of the bundles of actin filaments should take place according to the situation described for the stereovilli of the hair cells in the vertebrate inner ear [66]. During this process, the force could be restored to return the whole system after stimulation, back into its original position [73]. 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 (see above).

Ciliary rootlets

Ciliary rootlets associated with the base of motile as well as non-motile cilia are composed of fine longitudinally oriented filaments. However, their aspect varies, predominantly in the periodicity of striation [25, 45, 54]. The latter may reflect differences in their composition [54] or may be caused by differences in preparation techniques, including physiologically induced contractions or relaxations of the rootlets [50, 58]. In both sensory systems described here, the regular cross-striation after chemical fixation is nearly identical ([73, 75] and present study). This indicates that the rootlets in the arthropod sensilla and the mammalian photoreceptor cells could be composed of the same basic components.

Composition of the ciliary rootlets in sensory receptors

Based on the appearance of their striation, some authors have speculated a collagen nature of the ciliary rootlets in motile cilia as well as in sensory cilia (*eg* [22, 56]).

However, previous studies revealed that the rootlets are probably not composed of intracellular collagen fibers [62, 73]. In addition, previous investigations [10, 73, 75, 76] and the present observations (figs 2–5) show that ciliary rootlets are not formed from actin filaments. Therefore, it was not unexpected that antibodies against actin-associated proteins, *eg* anti-α-actinin in the mammalian photoreceptor cells would not react within the ciliary rootlets of the inner segment [1, 15]. However, the present immunoreactivity of two different antibodies against α-actinin within the insect scolopidia indicates that α-actinin or a related protein may be present within their rootlets (fig 4). An association of α-actinin with fine non-actin filaments (titin or connectin, nebulin) is known from studies on the composition of the Z-line within skeletal muscle [42, 69, 70]. Therefore, an association with such elastic filaments might also be possible in the striated rootlets of insect sensilla.

Anti-centrin immunostaining reveals that centrin or a protein that is immunologically related to centrin is localized within the ciliary rootlets of both sensory systems investigated here (figs 5, 10). Centrin (or caltractin [26]) is a phosphoprotein with a molecular mass of 20 kDa and a member of the EF-hand superfamily of calcium-binding proteins [2, 34]. Forming fine filaments it is the main component of the contractile ciliary or flagellar rootlets in green algae and, therefore, largely responsible for their Ca^{2+}-modulated contractions [2, 50, 51]. The present immunohistochemical localizations of centrin or an immunologically related protein indicate that Ca^{2+}-modulated contractions of the striated ciliary rootlets may also occur in insect scolopidia and the mammalian photoreceptor cells.

Function of ciliary rootlets in ciliary receptors

Scolopidia

In the scolopidia of arthropods contractions or relaxations may be involved in sensory transduction as well as in adaptation: during stimulation of scolopidia the distal basal body of the ciliary base is shifted [38, 67, 73]. Contractions of the rootlets 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 may affect the degree of tension of the ciliary necklace at its base. In this area of the dendritic membrane the location of the mechanosensitive membrane channels is discussed [38, 63].

Photoreceptor cells

In the mammalian photoreceptor cells the motility of the ciliary rootlet may be involved in adaptive retinomotor movements, in alignments of the photoreceptor cells or in organelle positioning within the inner segment. Motile processes named retinomotor movements are known in the vertebrate retina [8]. During light adaptation, elongation and contraction of the photoreceptor cells and of the pigment epithelium cells occur. For example, in the light the cone cells shorten while the rod cells and pigment cells elongate, thus positioning the cone cells in line for light reception. Based on studies using cytoskeletal inhibitors, it is suggested that cone and rod contraction as well as rod elongation are driven by the actin-myosin system, while, in contrast, the cone elongation seems to be microtubule-dependent [8]. However, retinomotor movements may be limited to lower vertebrates, predominantly to fish, some anurans and birds [8]. In mammals this motility was believed to be absent; however, there is some evidence that less dramatic retinomotor movements are also present in the mammalian retina [32, 44]. The present findings indicate that, in addition to the well known motile systems based on actin-myosin and microtubules, the motile processes based on the ciliary rootlets of the photoreceptors may be additional candidates for adaptive retinomotor movements in mammals. In addition, however, the rootlets may be involved in the alignment of the photoreceptor cells which occur during the photoreceptor orientation within the retina [18, 19]. Moreover, the ciliary rootlets may play an important role in organelle positioning. The filaments forming the ciliary rootlets of ciliated systems are generally associated with organelles, *eg* the endoplasmatic reticulum, mitochondria and the nucleus [4, 12, 42, 52, 53, 57]. Moreover, an active process for positioning the nucleus within the cell based on centrin is described for the green algae *Chlamydomonas* [53, 57]. In mammalian photoreceptor cells ciliary rootlets interact with the membrane of the mitochondria, rough endoplasmatic reticulum, basal bodies and in some cases they encompass the whole nucleus (present study; [35, 60]). They may position these organelles within the inner segment of the photoreceptor cell. Motile processes based on the ciliary rootlets would implicate changes of the organelle location within the photoreceptor cells which may occur under different physiological conditions. The present study reveals additionally that both basal bodies are linked by material which is similar to that of the ciliary rootlets (fig 9). Therefore, the variation in the basal body arrangement, described here (figs 6, 9), could be caused by motile properties of the rootlet material. This is supported by recent experiments on isolated centrosomes, structures which resemble basal bodies: their arrangement is Ca^{2+}-modulated [46] and a centrin-related protein might be involved [40]. Since basal bodies are microtubule organizing centers, any change of their arrangement may affect the whole microtubule system of the cell and all processes where microtubules might be involved, *eg* the very important vesicle transport from the endoplasmatic reticulum and the Golgi apparatus [29] or from the inner segment to the outer segment of the photoreceptor cell [13].

If any of the described motile processes of the ciliary rootlets occurs within the photoreceptor cells, it might be modulated by the intracellular Ca^{2+} concentration within the inner segment of the photoreceptor cell. Ca^{2+} ions play an important role in signal transduction of the vertebrate photoreceptors [27]. Changes of the intracellular Ca^{2+} concentration within the outer segment are involved in recovery after light response [31] and in adaptation [20]. In the retina of teleosts contractions of the cone cells during retinomotor movement are caused by an increase of free Ca^{2+} ions [9, 47]. However, little is known about the effect of changes in Ca^{2+} concentration within the inner segment of the mammalian photoreceptor cells.

The studies on the cytoskeletal elements in the mammalian photoreceptor cells are in progress and future data may provide more information on the role of the rootlets in sensory systems.

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References

1 Arikawa K, Williams DS (1989) Organization of actin filaments and immunocolocalization of α-actinin in connecting cilium of rat photoreceptors. *J Comp Neurol* 288, 640–646

2 Bazinet CW, Baron AT, Salisbury JL (1990) Centrin: A calcium-binding protein associated with centrosomes. *In: Stimulus response coupling: the role of intracellular calcium-binding proteins* (Smith VL, Dedman JR, eds) CRC Press, Boston, 39–56

3 Besharse JC, Horst CJ (1990) The photoreceptor connecting cilium – a model for the transition zone. *In: Ciliary and flagellar membranes* (Bloodgood RA, ed) Plenum, New York, 389–417

4 Boletzky S von (1973) Association of mitochondria with ciliary rootlets in squid embryos. *Cytobiologie* 8, 164–167

5 Bonfanti P, Colombo A, Heintzelman MB, Mooseker MS, Camatini M (1992) The molecular architecture of an insect midgut brush border cytoskeleton. *Eur J Cell Biol* 57, 298–307

6 Borwein B (1981) The retinal receptors: a description. *In: Vertebrate photoreceptor optics* (Enoch JM, Tobey FL, eds) Springer, Berlin, Heidelberg, New York, 11–81

7 Broschat KO, Weber A, Burgess DR (1989) Tropomyosin stabilizes the pointed end of actin filaments by slowing depolymerization. *Biochem J* 28, 8501–8506

8 Burnside B, Nagle B (1984) Retinomotor movements of photoreceptors and retinal pigment epithelium: mechanisms and regulation. *In: Progress in retinal research vol 2* (Osborne N, Charder G, eds) Pergamon Press, Oxford, 67–109

9 Burnside B, Smith B, Nagata M, Porrello K (1982) Reactivation of contraction in detergent-lysed teleost retinal cones. *J Cell Biol* 92, 199–206

10 Chaitin MH, Bok D (1986) Immunoferritin localization of actin in retinal photoreceptors. *Invest Ophthalmol Visual Sci* 27, 1764–1767

11 Cohen AJ (1960) Ultrastructure of the rods of the mouse retina. *Am J Anat* 107, 23–48

12 Cordier AC (1976) Relationship between ciliary rootlets and smooth endoplasmatic reticulum. *Cell Tissue Res* 166, 315–318

13 Deretic D, Papermaster DS (1991) Polarized sorting of rhodopsin on post-Golgi membranes in frog retinal photoreceptor cells. *J Cell Biol* 113, 1281–1293

14 DeRosier DJ, Tilney LG (1984) How to build a bend into an actin bundle. *J Mol Biol* 175, 57–73

15 Drenckhahn D, Wagner H-J (1985) Relation of retinomotor responses and contractile proteins in vertebrate retina. *Eur J Cell Biol* 37, 156–168

16 Eakin RM (1972) Structure of invertebrate photoreceptors. *In: Handbook of sensory physiology VII/1* (Dartnall HJA, ed) Springer, Berlin, Heidelberg, New York, 626–684

17 Egelman EH, Padrón R (1984) X-Ray diffraction evidence that actin is a 100 Å filament. *Nature* 307, 56–58

18 Enoch JM (1975) Vertebrate rod receptors are directionally sensitive. *In: Photoreceptor optics* (Snyder AW, Menzel R, eds) Springer, Berlin, Heidelberg, New York, 17–37

19 Enoch JM (1981) Retinal receptor orientation and photoreceptor optics. *In: Vertebrate photoreceptor optics* (Enoch JM, Tobey FL, eds) Springer, Berlin, Heidelberg, New York, 125–168

20 Fain GL, Matthews HR (1990) Calcium and the mechanism of light adaptation in vertebrate photoreceptors. *Trends Neurosci* 13, 378–384

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

22 Fawcett D (1961) Cilia and flagella. *In: The cell. Biochemistry, physiology, morphology, 2nd edn* (Brachet J, Mirsky AE, eds) Academic Press, New York, 217–298

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

24 Gray EG (1960) The fine structure of insect ear. *Phil Trans R Soc Lond (Biol)* 243, 190–209

25 Harrison F (1989) Primary cilia associated with striated rootlets in granulated and folliculo-stellate cells of the avian adenohypophysis. *Anat Embryol* 180, 543–547

26 Huang B, Watterson DM, Lee VD, Schibler MJ (1988) Purification and characterization of a basal body-associated Ca^{2+}-binding protein. *J Cell Biol* 107, 121–131

27 Kaupp UB, Koch KW (1992) Role of cGMP and Ca^{2+} in vertebrate photoreceptor excitation and adaptation. *Annu Rev Physiol* 54, 153–175

28 Keil T, Steinbrecht RA (1984) Mechanosensitive and olfactory sensilla. *In: Insect ultrastructure, vol 2* (King RC, Akai H, eds) Plenum, New York, 477–516

29 Kreis TE (1990) Role of microtubules in the organisation of the Golgi apparatus. *Cell Motil Cytoskel* 15, 67–70

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

31 Koch KW, Stryer L (1988) Highly cooperative feedback control of retinal rod guanylate cyclase by calcium ions. *Nature* 334, 64–66

32 Kuwabara T (1979) Species differences in the retinal pigment epithelium. *In: Structure and function of the retinal pigment epithelium* (Zinn KM, Marmor MF, eds) Harvard University Press, Cambridge, 58–82

33 Lee VD, Stapleton M, Huang B (1991) Genomic structure of *Chlamydomonas caltractin. J Mol Biol* 221, 175–191

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

35 Matsusaka T (1967) ATPase activity in the ciliary rootlet of human retinal rods. *J Cell Biol* 33, 203–208

36 McIver SB (1985) Mechanoreception. *In: Comprehensive insect physiology, biochemistry, and pharmacology, vol 6. Nervous system: sensory* (Kerkut GA, Gilbert LI, eds) Pergamon, Oxford, 71–132

37 Moor H (1987) Theory and practice of high-pressure freezing. *In: Cryotechniques in biological electron microscopy* (Steinbrecht RA, Zierhold K, eds) Springer, Berlin, Heidelberg, New York, 175–191

38 Moran DT, Varela FJ, Rawley JC (1977) Evidence for active role of cilia in sensory transduction. *Proc Natl Acad Sci USA* 74, 793–797

39 Mooseker MS, Tilney LG (1975) Organization of actin filament-membrane complex. *J Cell Biol* 67, 725–743

40 Moudjou M, Paintrand M, Vigues B, Bornens M (1991) A human centrosomal protein is immunologically related to basal body-associated proteins from lower eucaryotes and is involved in the nucleation of microtubules. *J Cell Biol* 115, 129–140

41 Moulins M (1976) Ultrastructure of chordotonal organs. *In: Structure and function of proprioreceptors in the invertebrates* (Mill PJ, ed) Chapman and Hall, London, 387–426

42 Nave R, Fürst DO, Weber K (1990) Interaction of alpha-actinin and nebulin *in vitro* – support for the existence of a 4th filament system in skeletal muscle. *FEBS Lett* 269, 163–166

43 Olsson R (1962) The relationship between ciliary rootlets and other cell structures. *J Cell Biol* 15, 596–599

44 Pang SF, Yew DT, Tsui HW (1978) Photomechanical changes in the retina and choroid of guinea-pigs, *Cavia porcellus. Neurosci Lett* 10, 221–224

45 Pitelka DR (1974) Basal bodies and root structures. *In: Cilia and flagella* (Sleigh MA, ed) Academic Press, London, New York, 437–469

46 Paintrand M, Moudjou M, Delacroix H, Bornens M (1992) Centrosome organization and centriole architecture – their sensitivity to divalent cations. *J Struct Biol* 108, 107–128

47 Porrello K, Burnside B (1982) A role of calcium in the regulation of reactivited contractin of teleost retinal cone model. *J Cell Biol* 92, 449–454

48 Rodieck RW (1973) *The vertebrate retina – principles of structure and function*. San Francisco, Freeman and Company

49 Rohde K, Cannon LRG, Watson N (1988) Sense receptors with electron-dense supporting structures, and centrioles in nerve fibers of *Gieysztoria* and *Rhinolasius* (Platyhelminthes, Rhabdocoela). *J Submicrosc Cytol Pathol* 20, 153–160

50 Salisbury JL, Floyd GL (1978) Calcium-induced contraction of the rhizoplast of a quadriflagellate green algae. *Science* 202, 975–976

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

52 Salisbury JL, Sanders MA, Harpst L (1987) Flagellar root contraction and nuclear movement during flagellar regeneration in *Chlamydomonas reinhardtii*. *J Cell Biol* 105, 1799–1805

53 Salisbury JL, Baron AT, Sanders MA (1988) The centrin-based cytoskeleton of *Chlamydomonas reinhardtii:* distribution in interphase and mitotic cells. *J Cell Biol* 107, 635–641

54 Sandoz D, Chailley B, Boisvieux-Ulrich E, Lemullois M, Laine MC, Bautista-Harris G (1988) Organization and functions of cytoskeleton in metazoan ciliated cells. *Biol Cell* 63, 183–193

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

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

57 Schulze D, Robenek H, McFadden GI, Melkonian M (1987) Immunolocalization of a Ca^{2+}-modulated contractile protein in the flagellar apparatus of green algae: the nucleus-basal body connector. *Eur J Cell Biol* 45, 51–61

58 Sleigh M (1979) Contractility of roots of flagella and cilia. *Nature* 277, 263–264

59 Sobue K, Muramoto M, Fujita F, Kabiuchi S (1981) Purification of a calmodulin-binding protein from chicken gizzard that interacts with F-actin. *Proc Natl Acad Sci USA* 78, 5652–5655

60 Spira AW, Milman GE (1979) The structure and distribution of the cross-striated fibril and associated membranes in guinea pig photoreceptores. *Am J Anat* 155, 319–338

61 Steinbrecht RA (1984) Chemo- hygro- and thermoreceptors. *In: Biology of the integument, vol 1: Invertebrates* (Bereiter-Hahn J, Matoltsky AG, Richards KS, eds) Springer, Berlin, Heidelberg, New York, 523–553

62 Stephens RE (1975) The basal apparatus. *J Cell Biol* 64, 408–420

63 Stockbridge LL, French AS, Sanders EJ (1990) Dissociation and culture of mechanosensory neurons for patch clamp analysis. *Brain Res* 523, 161–166

64 Studer D, Michel M, Müller M (1989) High pressure freezing comes of age. *In: The science of specimen preparation. SEM* (Albrecht R, Ornberg R, eds) AMF O'Hare, Chicago, 253–269

65 Tilney LG, DeRosier DJ, Mulroy MJ (1980) The organization of actin filaments in the stereocilia of cochlear hair cells. *J Cell Biol* 86, 244–259

66 Tilney LG, Egelman EH, DeRosier DJ, Saunders JC (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

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

68 Uga S, Nakao M, Mimura M, Ikui H (1970) Some new findings on the fine structure of human photoreceptor cells. *J Electron Microsc* 19, 71–84

69 Wang SM, Jeng CJ (1992) Interaction between titin and alpha-actinin. *Biomed Res* 13, 197–202

70 Wang K, Wright J (1988) Architecture of the sarcomere matrix of skeletal muscle: immunoelectron microscopic evidence that suggests a set of parallel inextensible nebulin filaments anchored at the Z line. *J Cell Biol* 107, 2199–2212

71 Witman GB (1990) Introduction to cilia and flagella. *In: Ciliary and flagellar membranes* (Bloodgood RA, ed) Plenum, New York, 1–30

72 Wright BL, Salisbury JL, Jarvik JW (1985) A nucleus-basal body connector in *Chlamydomonas reinhardtii* that may function in basal body localisation and segregation. *J Cell Biol* 101, 1903–1912

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

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

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

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

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

78 Yamada E (1982) Morphology of vertebrate photoreceptors. *In: Biomembranes, PtH, visual pigments and purple membranes. I. Methods in enzymology, vol 81* (Packer L, ed) Academic Press, New York, 3–17

79 Yamashiro-Matsumura S, Matsumura F (1988) Characterization of 83-kilodalton non-muscle caldesmon from cultured rat cells: stimulation of actin binding of non-muscle tropomyosin and periodic localization along microfilaments like tropomyosin. *J Cell Biol* 106, 1973–1983