

# ACTIN FILAMENTS IN SENSORY HAIRS OF INNER EAR RECEPTOR CELLS

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## ABSTRACT

Receptor cells in the ear are excited through the bending of sensory hairs which project in a bundle from their surface. The individual stereocilia of a bundle contain filaments about 5 nm in diameter. The identity of these filaments has been investigated in the crista ampullaris of the frog and guinea pig by a technique of decoration with subfragment-1 of myosin (S-1). After demembration with Triton X-100 and incubation with S-1, "arrowhead" formation was observed along the filaments of the stereocilia and their rootlets and also along filaments in the cuticular plate inside the receptor cell. The distance between attached S-1 was 35 nm and arrowheads pointed in towards the cell soma. It is concluded that the filaments of stereocilia are composed of actin.

**KEY WORDS** actin · inner ear · hair cell  
sensory hairs · crista ampullaris · sensory  
transduction

Auditory and vestibular receptor cells are equipped with sensory hairs, the bending of which causes excitation of the sensory cells (2). A signal is generated in response to this stimulus and is in turn coded into an impulse message in the auditory nerve. The bending of the hairs is brought about by displacement of auxiliary sensory structures to which the tips of the sensory hairs are attached. The physical displacement of the sensory hairs is thus the first step in the cellular excitation process, and it is of obvious importance to obtain information about the mechanical properties of the sensory hairs and about the arrangement and nature of the responsible structures.

The sensory hairs are tubular projections of the cell membrane which arise from the cell surface, 50–100 in number. One of these, the kinocilium (29), resembles an ordinary flagellum and is present in the vestibular system and the hearing organs of lower vertebrates; all the others are called ster-

ecilia and are the only ones present in the mammalian hearing organ. The present paper deals with the stereocilia. Inside each stereocilium is a core of fine filaments which run down its length and collect to a narrow bundle at the slender base of the cilium, continuing as a rootlet into a cuticular plate which occupies the cell apex. The filament core has recently been found to exhibit considerable stiffness, as determined by micromanipulation of sensory hairs where the membrane has been removed by Triton X-100 (10).

There are certain structural similarities between stereocilia and microvilli of the intestine as described by Mooseker and Tilney (20). In particular, we were interested in testing the possibility that the core of the stereocilium is composed of actin filaments as is the case in gut microvilli. For this purpose, we used the technique of decoration of actin with the S-1 fragment of myosin (14).

## MATERIALS AND METHODS

### *Preparation*

The crista ampullaris of frogs of the species *Rana*

*temporaria* and *Rana pipiens* was used. Some experiments were done on the guinea pig crista. The ampulla of the posterior semicircular canal was exposed by a posterior-ventral approach. The ampulla was freed from the canal by cutting with fine scissors, making sure that both openings to the ampulla remained open.

### *Incubation with Myosin Subfragment-1*

Myosin subfragment-1 (S-1) was prepared from a suspension of rabbit skeletal myosin at pH 7.2 with a low concentration of soluble papain essentially according to Margossian and Lowey (17). The isolated S-1 was fractionated with  $(\text{NH}_4)_2\text{SO}_4$  and the protein obtained between 49% and 58% of  $(\text{NH}_4)_2\text{SO}_4$  saturation was retained and used in this work. Before use, the protein was first dialyzed against a solution containing 60 mM KCl, 5 mM  $\text{MgCl}_2$ , 1 mM dithiothreitol (DTT), and 50 mM Tris at pH 7.3, and the dialyzed protein was adjusted to 10 mg/ml. The protein concentration was determined spectrophotometrically by means of  $E_{280}^{1\%} = 8.0 \text{ cm}^{-1}$ . The sensory hairs were demembrated by exposure for 10 min to a 1% solution of Triton X-100 in frog Ringer's solution, followed by a wash in Ringer's. The ampullae were then incubated in a solution containing S-1 for 10 min at room temperature and were then fixed after a wash in Ringer's solution. Control specimens followed the same routine, except that the S-1 was omitted from the incubating medium.

### *Electron Microscopy*

The following fixatives were used: (a) 2% glutaraldehyde, 10 mM  $\text{MgCl}_2$  in 0.1 M phosphate buffer at pH 6.0 (20); (b) 2% paraformaldehyde, 2.5% glutaraldehyde, 10 mM  $\text{MgCl}_2$  buffered with Na-cacodylate at pH 6.6-6.8 (15); (c) 4% tannic acid, 2.5% glutaraldehyde in phosphate buffer at pH 6.4 saturated with digitonin (18). Postfixation was done with 2% osmium tetroxide buffered to pH 6.0-6.8 (11) with phosphate or Na-cacodylate buffers. 1% tannic acid was sometimes used as a mordant after fixation 1 or 2 (23), or else the tissue was block-impregnated with uranyl acetate. Sections of Epon-embedded tissue were cut with a diamond knife, stained with uranyl acetate (28) and lead citrate (21), and examined with a Zeiss EM 9 electron microscope. Magnification was calibrated with a carbon grating.

## RESULTS

The stereocilia of the frog crista ampullaris range in length from 5-60  $\mu\text{m}$ . The longest of them are situated close to the kinocilium, the shorter ones away from it. In Figs. 1 and 4, filaments are seen inside stereocilia in longitudinal and transverse sections. Their diameter is about 5 nm. The filaments appear to extend throughout the length of the cilium, at the tip of which the filaments end in close contact with the membrane. Towards the

narrow base of the sensory hair the number of filaments becomes reduced. Filaments of the rootlet are continuous with those of the core. There are bridges which connect the filaments to one another sideways, and there are also connections between peripheral filaments of the core and the membrane.

After treatment with Triton X-100, the membranes of the cells in the sensory epithelium dissolve. In spite of this, the protein matrix of the cellular constituents remains in place throughout the various changes of media. This is probably because the epithelium is enclosed in the ampulla, which forms a cavity protecting against turbulence which would otherwise decompose the epithelium. In the organ of Corti and the macula sacculi, which are not protected in this way, the sensory epithelium is washed away during processing. In the crista, the sensory hairs retain their relationships to one another and to the cuticular plate after demembration. Splaying of the filaments of the core may occur as seen in Fig. 2. This makes the cross-bridges visible and gives access to the comparatively large S-1 molecules.

In organs which have been incubated with the S-1 fragment of myosin, the filaments have a serrated appearance (Fig. 3). This is true also for the rootlet (Fig. 5) and for filaments in the cuticula which run parallel to the cell surface. The points of origin and attachment of these cuticular filaments have not yet been determined. Other filaments, in the supporting cells, do not become decorated. The serrated appearance is due to the attachment to the filaments of short rods (S-1) which insert at an angle of about  $45^\circ$  (Fig. 6). The rods are all inclined in the same direction and resemble arrowheads. The interval between these rods was measured in one animal and found to have a mean of 35 nm. Where the direction of the arrowheads could be determined, they pointed from the tip of the stereocilia down towards the cuticular plate.

## DISCUSSION

Although filaments have previously been observed in lateral line and inner ear stereocilia (1, 3, 4, 5, 6, 7, 9, 24), they have not received much attention. Much more interest has been devoted to the kinocilium and the structure of its internal axoneme. However, in the mammalian cochlea the kinocilium is lacking (16), and the stereocilia are the sole structures responsible for transduction of mechanical energy.

Investigation of the physical properties of the

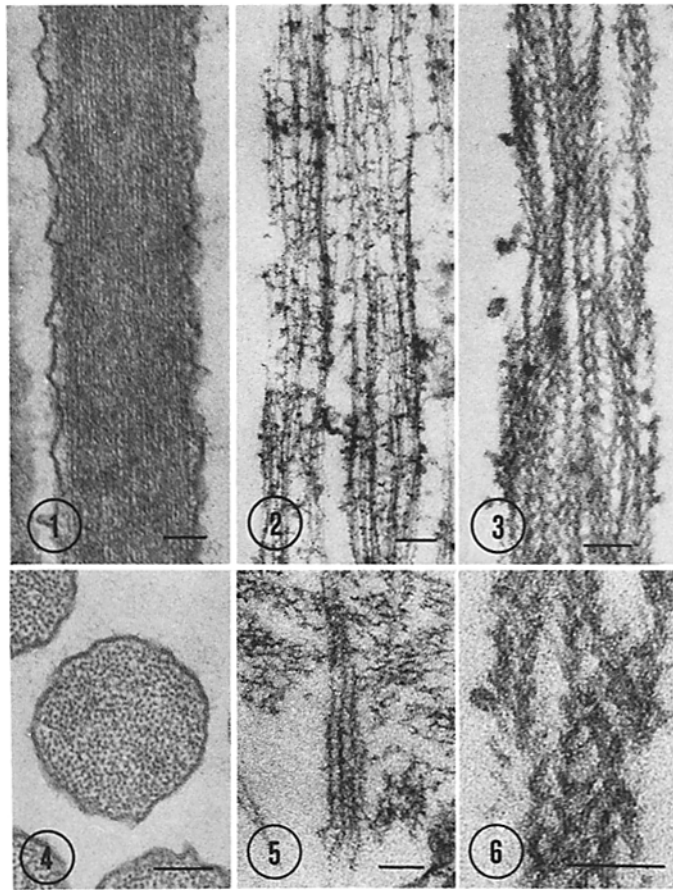


FIGURE 1 Longitudinal section through a stereocilium in the frog crista ampullaris. Bar,  $0.1 \mu\text{m}$ .  $\times 53,000$ .

FIGURE 2 After treatment with Triton X-100 the membrane dissolves and the filaments may separate as seen in this figure. Control for Fig. 3. Bar,  $0.1 \mu\text{m}$ .  $\times 54,000$ .

FIGURE 3 After incubation with myosin S-1, the filaments have a serrated appearance. Tannic acid in fixative. Bar,  $0.1 \mu\text{m}$ .  $\times 62,000$ .

FIGURE 4 Cross section through a normal stereocilium showing internal filaments. Bar,  $0.1 \mu\text{m}$ .  $\times 77,000$ .

FIGURE 5 Rootlet of demembrated stereocilium in S-1-treated guinea pig crista ampullaris. Bar,  $0.1 \mu\text{m}$ .  $\times 59,000$ .

FIGURE 6 Arrowhead orientation can be distinguished in these S-1-decorated filaments. The tip of the cilium is up; the cuticular plate is down. Tannic acid in fixative. Bar,  $0.1 \mu\text{m}$ .  $\times 132,000$ .

sensory hairs (8, 10) has shown that demembrated stereocilia retain their appearance and mechanical characteristics to a remarkable degree and that this can be attributed to their bulk of filaments. The diameter of the filaments in the crista is about 5 nm which compares with 3-4 nm in the lateral line organ (7) and 4 nm in the cochlea of reptiles (4). The diameter of F-actin is

about 5 nm (25). During muscle contraction, the head of the myosin molecule attaches to the actin filament at specific sites separated by about 37 nm (14). We find that the filaments of stereocilia in the frog and guinea pig also bind the myosin head. This binding is selective in that other filaments in neighboring supporting cells do not become decorated. The angle of insertion of the S-1 molecules

is about 45°, as it is in the rigor state in muscle, and the interval is 35 nm, which is in the same range as that in microvilli of the intestine where the period is 33 nm (20). On the whole, the stereocilia of the ear resemble, in some respects, intestinal microvilli which have been shown to contain actin (26). We conclude that the filaments of inner ear stereocilia are likely to be composed of actin.

The filamentous cores of the stereocilia in the frog crista possess considerable stiffness and can by themselves, in the absence of the membrane, convey motion to the cuticular plate (10). This is in agreement with the observation that actin filaments elsewhere are relatively rigid (27). However, the mechanical constants of a structure such as the stereocilium can be different from those of the individual elements depending on their interconnections and packing. It is of interest to note that F-actin can exist in a paracrystalline state (12) and that a change from one state to the other can occur as a result of physiological processes (25). This is of importance from the viewpoint of transduction in the ear, because Harris (13) has calculated that a change from stiff to loose coupling of the hair cells to the tectorial membrane via the sensory hairs can mean a difference in sensitivity of as much as 55 dB.

Microvilli in the brush border are motile, and the actin filaments of the rootlets are apparently pulled in by myosin molecules in the terminal web (19, 22). The polarity of the stereocilium filaments, as determined by arrowhead orientation, is the same as in microvilli. With the demonstration of actin in inner ear sensory hairs, the possibility that these are also capable of some form of movement becomes a reality. This has a bearing on the possible mechanisms which underlie sensory excitation in the ear (8).

We are indebted to Britta Flock for excellent technical assistance.

This work was supported by grants from the Swedish Medical Research Council (04X-02461), Karolinska Institutet, Söderbergs Stiftelse, King Gustaf V Memorial Fund, Bergvalls Stiftelse, and the U.S. National Institute of Health grant no. AM-14589.

Received for publication 11 April 1977.

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