

Three Different Actin Filament Assemblies Occur in Every Hair Cell: Each Contains a Specific Actin Crosslinking Protein

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Abstract. The apex of hair cells of the chicken auditory organ contains three different kinds of assemblies of actin filaments in close spatial proximity. These are (a) paracrystals of actin filaments with identical polarity in stereocilia, (b) a dense gellike meshwork of actin filaments forming the cuticular plate, and (c) a bundle of parallel actin filaments with mixed polarities that constitute the circumferential filament belt attached to the cytoplasmic aspect of the zonula adhaerens (ZA). Each different supramolecular assembly of actin filaments contains a specific actin filament crosslinking protein which is unique to that particular assembly. Thus fimbrin appears to be responsible for paracrystallin packing of actin filaments in stereocilia;

an isoform of spectrin resides in the cuticular plate where it forms the whisker-like crossbridges, and α actinin is the actin crosslinking protein of the circumferential ZA bundle. Tropomyosin, which stabilizes actin filaments, is present in all the actin filament assemblies except for the stereocilia. Another striking finding was that myosin appears to be absent from the ZA ring and cuticular plate of hair cells although present in the ZA ring of supporting cells. The abundance of myosin in the ZA ring of the surrounding supporting cells means that it may be important in forming a supporting tensile cellular framework in which the hair cells are inserted.

ONE of the most fascinating features of cells is that at the same moment in time a cell can carry out a diverse array of activities, most of which involve the cytoskeleton. For example, a cell can maintain static surface projections or microvilli, nearby it can phagocytose particles, and at another location it can move by amoeboid motion. Thus, an important problem in cell biology is how the cell controls different assemblies of actin filaments. We have chosen the hair cell of the cochlea as a model system that may help us to understand how different actin assemblies are formed and maintained in the same cell at the same time.

Located at the apical end of hair cells in the chick inner ear are three different assemblies of actin filaments. One is a paracrystalline bundle of actin filaments present in each stereocilium. These filaments are parallel, have identical polarities, and are maximally crossbridged together (Tilney et al., 1983). The second, the cuticular plate, is a complex actin gel located immediately beneath the stereocilia. Here the actin filaments are randomly oriented relative to each other, yet connected together by tiny whiskerlike strands of proteinaceous material (DeRosier and Tilney, 1989). The third is a circumferential belt of actin filaments encircling the apicolateral margins of the cell at the zonula adhaerens (ZA)¹. The filaments that make up this belt, although paral-

lel, are of mixed polarities (Hirokawa and Tilney, 1982). Since these three different assemblies of actin filaments all occur in the same cell at the same time and are located a fraction of a micron from each other, the questions that immediately arise are: what actin binding proteins are responsible for producing these strikingly different assemblies; how can they remain distinct from each other; and how does the cell produce, during development, three completely different actin filament assemblies?

The purpose of this paper is to demonstrate that each of the three supramolecular assemblies of actin filaments in hair cells contains separate and different actin crosslinking proteins and thus their organization must in part be due to the preferential localization of these proteins during development. The simplified outcome of our study demonstrates that the hair cell is an excellent model with which to understand how different assemblies of actin filaments can coexist and function differently in the same cell at the same time.

Materials and Methods

Isolation of the Sensory Epithelium and Hair Borders

Chicks 1–14-d of age were decapitated. The entire cochlea was removed and the sensory epithelium (basilar papilla) isolated as described in detail elsewhere (Tilney et al., 1989). Hair border cytoskeletons were prepared by suspending the isolated sensory epithelium in 0.5% Triton X-100, 1 M NaCl, 0.3 mM MgCl₂ in 20 mM imidazole at pH 7.0.

1. *Abbreviations used in this paper:* F-actin, filamentous actin; ZA, zonula adhaerens.

SDS-PAGE, Immunoblotting

Isolated sensory epithelia from which the tectorial membranes had been removed by 30 min incubation at room temperature with dispase (5 mg/ml) (Boehringer Mannheim GmbH, Mannheim, FRG) dissolved in HBSS (Gibco Laboratories, Karlsruhe, FRG) were subjected to SDS-PAGE using 10% minigels. Human erythrocyte membranes (ghosts) served as molecular weight standards. Electrophoretically separated proteins were either stained with Coomassie blue or electroblotted onto nitrocellulose sheets (Schleicher and Schüll Inc., Darmstadt, FRG) and processed for antibody labeling using affinity purified rabbit antibodies. Antibodies against myosin, tropomyosin, fimbrin, villin, and the 110-kD protein were raised against proteins isolated from chicken intestinal brush borders. Antibodies against α actinin and filamin were prepared from chicken gizzard and those against brain spectrin from porcine and bovine brain. Specificity and characterization of antibodies has been published elsewhere (Drenckhahn and Dermietzel, 1988; Broschat and Burgess, 1988). Polyclonal antibodies against α and β spectrin from chicken erythrocytes and chicken brush border (TW 240/260) were kind gifts of Dr. C. Woods, Dr. E. Lazarides, and Dr. J. Glenney (for specificity of these antibodies see Nelson and Lazarides, 1983; Glenney and Glenney, 1983). Actin was detected with a monoclonal IgM antibody specific for actin (Amersham International, Amersham, UK). Antibody-labeled bands were visualized by peroxidase-tagged second antibodies (Sigma Chemical Co. St. Louis, MO) using chloronaphthol as the substrate (Bio-Rad, Richmond, CA).

Immunofluorescence

The isolated sensory epithelium and hair borders were prepared for immunofluorescence as described in detail in a previous study (Tilney et al., 1989). Filamentous actin (F-actin) was visualized with 2 μ g/ml rhodamine-labeled phalloidin (gift from Dr. Faulstich, Max-Planck-Institut, Heidelberg, FRG) dissolved in PBS (25 mM sodium phosphate, 120 mM NaCl, pH 7.4). For staining of tissue sections the isolated cochlear duct was immediately quick frozen by plunging into melting isopentane cooled with liquid nitrogen. The frozen cochleae were freeze-dried, embedded in Epon, sectioned, and processed for immunofluorescence as described elsewhere (Drenckhahn et al., 1982; Drenckhahn and Franz, 1986).

Immunoelectron Microscopy

For the ultrastructural localization of actin and the above listed actin-binding proteins two different approaches were used. In a pre-embedding protocol the sensory epithelium was fixed and permeabilized at 4°C with a solution containing 3.7% formaldehyde (freshly prepared from paraformaldehyde) and 0.1% Triton X-100 in 50 mM phosphate buffer (pH 6.3). After 30 min the sensory epithelium was washed three times in phosphate buffer and blocked with 20 mM Pipes, 0.1% Triton X-100, 1.5 mM MgCl₂ and 1% BSA at pH 6.8 for 30 min. The tissue was then incubated for 2–16 h with antibodies dissolved in PBS at concentrations of 5–20 μ g/ml. After thorough washing with PBS containing 1% BSA (3 \times 30 min) the tissue pieces were incubated with goat anti-rabbit IgG coupled to 5 and 10-nm colloidal gold particles (Janssen Pharmaceutica, Beerse, Belgium) diluted 1:50 with PBS (2–16 h). After three washes with PBS (3 \times 30 min), tissues were fixed by immersion for 30–60 min in 1% OsO₄ and 1% glutaraldehyde in 0.1 M phosphate buffer at pH 6.3 at 40°C, blocked-stained with 0.5% uranyl acetate and embedded in Epon as described elsewhere (Tilney et al., 1989).

Postembedding immunolabeling was performed using ultrathin tissue sections of cochleae fixed at 4°C by immersion with a mixture of 2% formaldehyde and 0.1% glutaraldehyde in PBS (1–4 h), then embedded (at 4°C) in the hydrophilic methacrylate resin, LR-White (London Resin Co., Woking, UK) and processed for immunogold labeling exactly as described in detail in previously published studies (Drenckhahn and Merte, 1987; Drenckhahn and Dermietzel, 1988).

Results

To orient the reader on the three assemblies of actin filaments in the hair cell and their relationship to the encircling supporting cells, we have presented a schematic drawing (see Discussion). In this it can be seen that these assemblies, the stereocilia, cuticular plate, and circumferential ring lie in close association with one another.

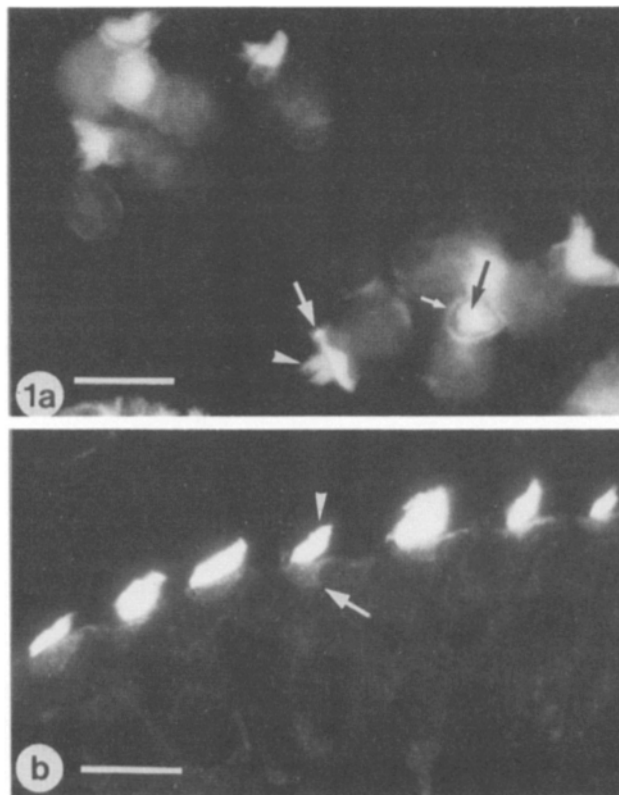


Figure 1. (a) Visualization of F-actin by rhodamine phalloidin in auditory hair cells mechanically separated from the sensory epithelium of the chick cochlea. (b) Tissue sections of the sensory epithelium stained with an actin antibody. Note the bright fluorescence of the three different assemblies of actin filaments, the circumferential ring (small arrow), the cuticular plate (large arrow), and the stereocilia (arrowhead). Bars, 10 μ m.

Stereocilia

Stereocilia were brightly labeled with fluorescent phalloidin (Fig. 1), and showed a strong immunofluorescence specific for actin and fimbrin (Fig. 2). No immunostaining of stereocilia was detected with antibodies to tropomyosin (Fig. 3), α actinin (Fig. 4), brain spectrin (Fig. 5), myosin (Fig. 6), filamin, villin, and the 110-kD protein (not shown). Immunogold labeling of thin sections embedded in LR-White resulted in specific labeling of the stereocilia core with antibodies against actin (extreme density; Figs. 7 and 8) and fimbrin (Fig. 9; moderate density). No obvious differences in the label were measured between short and long stereocilia on the same hair cell or between stereocilia of hair cells located on different parts of the cochlea.

Cuticular Plate

Fluorescence microscopy revealed specific staining of the cuticular plate web with phalloidin (Fig. 1a) and antibodies to actin (Fig. 1b), tropomyosin (Fig. 3), and brain spectrin (Fig. 5). Antibodies to myosin did not bind to the cuticular plate (Fig. 6), and anti- α actinin reacted with small dotlike structures in the periphery of the cuticular plates of outer but not inner hair cells (Fig. 4). Ultrastructural immunolabeling for actin was possible under postembedding conditions (Figs. 7 and 8) whereas antibodies to tropomyosin and brain

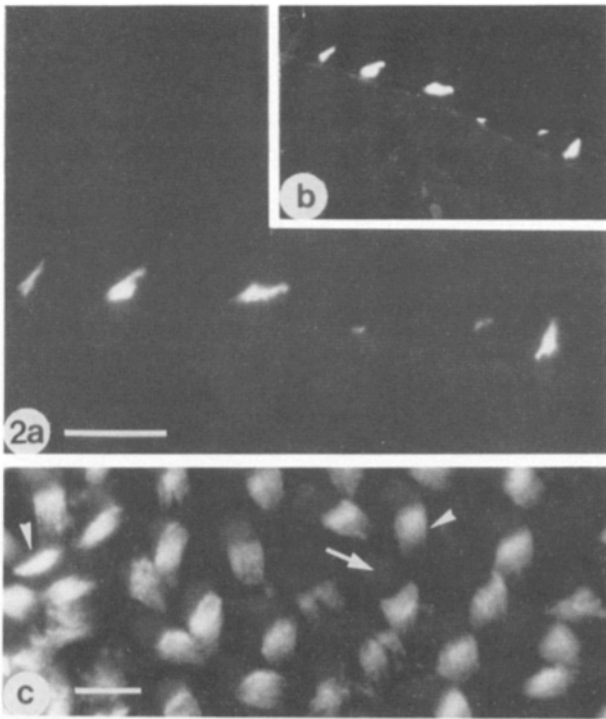


Figure 2. Localization of fimbrin by immunofluorescence in tissue sections (*a* and *b*) and whole mount (*c*) of the auditory organ. Note restriction of fimbrin to the stereocilia (*arrowheads*) and absence from the cuticular plate (*large arrow*). Bars, 10 μm .

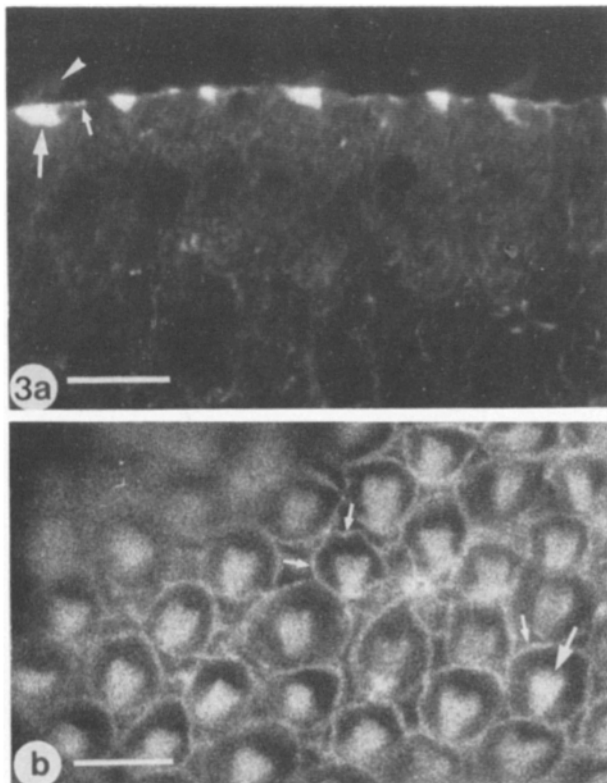


Figure 3. Visualization of tropomyosin in tissue section (*a*) and whole mount (*b*) of the auditory organ. Note the absence from the stereocilia (*arrowhead*), but presence in the cuticular plate (*large arrows*) and the circumferential band (*small arrows*). Bars, 1 μm .

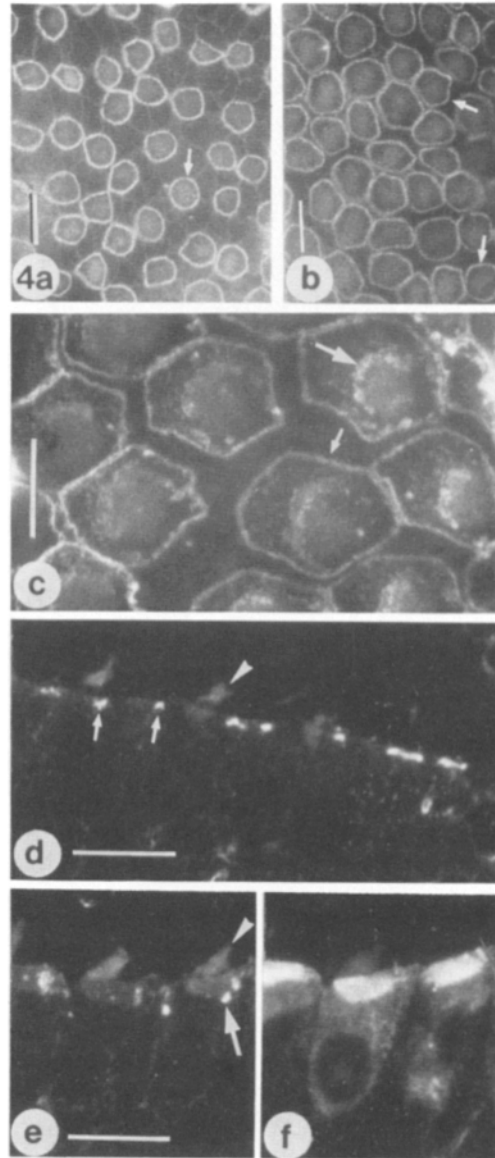


Figure 4. Localization of α actinin (*a-e*) in whole mounts (*a-c*) and sections (*d* and *e*) of the sensory epithelium. Areas with inner (*a* and *d*) and outer (*c* and *e*) hair cells are shown. Note the restriction of α actinin to the circumferential band (*small arrows*). In outer hair cells fluorescent dots are seen in the cuticular plate (*large arrows*). (*e* and *f*) are 0.5 μm serial sections stained with anti- α actinin (*e*) and antitropomyosin (*f*). Antitropomyosin was used to visualize the cuticular plate. The faint α actinin-like immunofluorescence of some of the stereocilia was not seen by immunoelectron microscopy (see text). Bars, 10 μm .

spectrin only bound to mildly fixed detergent-treated cells using a preembedding protocol. Under these conditions most of the stereocilia broke off so that only short pieces of the bases of the stereocilia were seen (Figs. 10 and 11). The immunogold label specific for actin (Figs. 7 and 8), tropomyosin (Fig. 10), and brain spectrin (Fig. 11) was found in association with both rootlets of stereocilia and the cuticular plate web. Both anti-tropomyosin and antiactin showed a higher density of label in association with the rootlets than that of the remaining cuticular plate web, most likely because the concentration of actin in rootlets is higher than in the

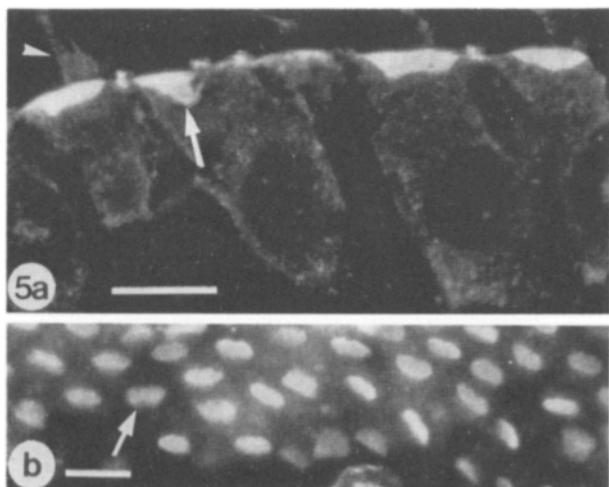


Figure 5. Visualization of brain spectrin on 0.5- μm -thick frozen section (a) and in a whole mount of the sensory epithelium (b). Note restriction of the immunostain to the cuticular plate (large arrows) and absence from stereocilia (arrowhead). Bars, 10 μm .

cuticular plate web. In contrast, the spectrin label appeared to be more dense within the cuticular plate web than within the rootlets. Further binding sites for antibodies against actin in the cuticular plate area were small globular densities ($\sim 20\text{-nm}$ -diam) aligned along the apical plasma membrane (Figs. 7 and 8). These densities displayed a spacing of 60–80 nm, a value which is similar to the spacing of actin filaments within the cuticular plate (DeRosier and Tilney, 1989).

As mentioned before, antibodies against α actinin did not label the cuticular plate and rootlets (Fig. 12 a). However, the antibodies bound to small particles along the basal periphery of outer hair cells. These particles or dots were revealed by immunoelectron microscopy to represent small densities (up to 0.1 μm in size) some of which were seen to be associated with intermediate filaments (Fig. 13). Some of these densities were found within the cuticular plate, but mostly close to its basal margin.

Circumferential Band

By fluorescence microscopy the area of the ZA to which the circumferential band is attached was brightly labeled with phalloidin (Fig. 1) and antibodies against tropomyosin (Fig. 3), α actinin (Fig. 4), and myosin (Fig. 6). At the ultrastructural level actin (Figs. 7 and 8), tropomyosin (not shown), and α actinin (Fig. 13) were shown to label the ZA of both supporting cells and hair cells. In longitudinal (grazing) sections the ZA ring displayed a striated pattern of densities (0.2–0.3- μm -long) interrupted by less dense intersections consisting of parallel-aligned microfilaments. Similar striations have been also observed in the ZA ring of the intestinal epithelium (Drenckhahn and Dermietzel, 1988) and retinal pigment epithelium (Sandig and Kalnins, 1988). In contrast to antibodies against actin, tropomyosin, and α actinin, the myosin antibodies selectively labeled the ZA of only the supporting cells (Fig. 14). No significant immunogold label was seen in association with the ZA of hair cells. Counts of IgG-gold particles associated with the ZA ring of supporting and

hair cells showed that only 5.2% of the particles were found overlaying the ZA ring of hair cells ($n = 1,382$ gold particles).

Immunoblotting

The isolated sensory epithelium contains only hair cells, supporting cells, and some pre- and postsynaptic nerve terminals (Tilney et al., 1989). In immunoblots of the isolated sensory epithelium (Fig. 15) antibodies to myosin labeled a 200-kD band and occasionally a minor band at 80–100 kD (most likely a proteolytic fragment of the heavy chain). Antifimbrin labeled a 68–70-kD band which migrated slightly more slowly than fimbrin from the chicken intestinal

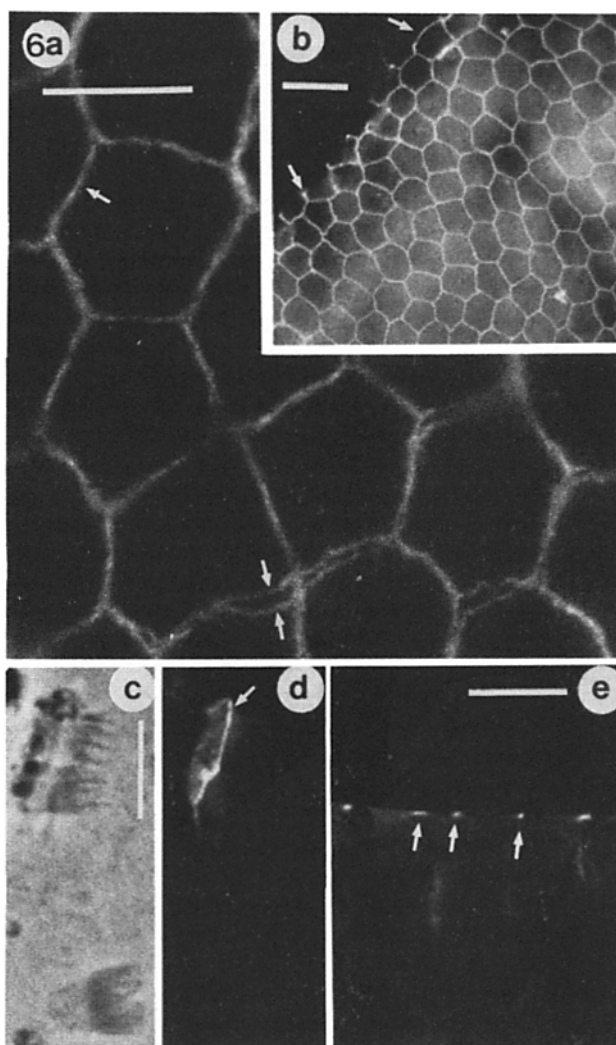
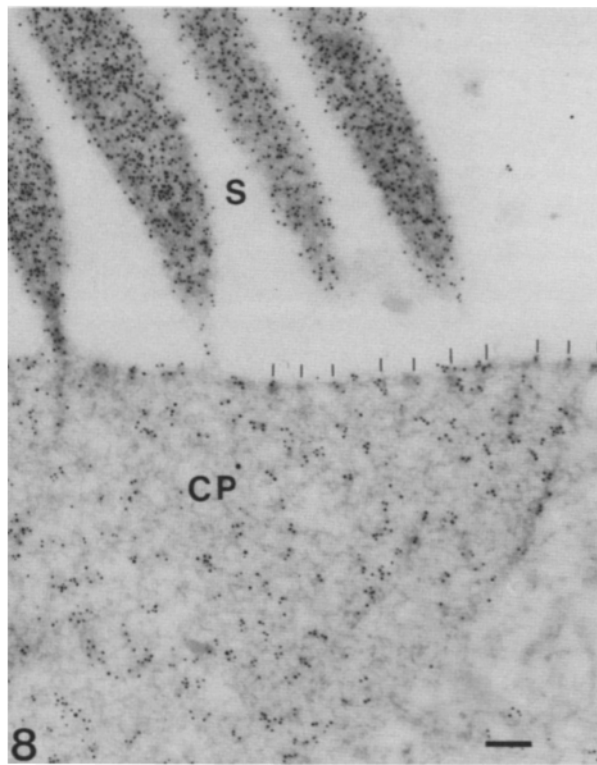
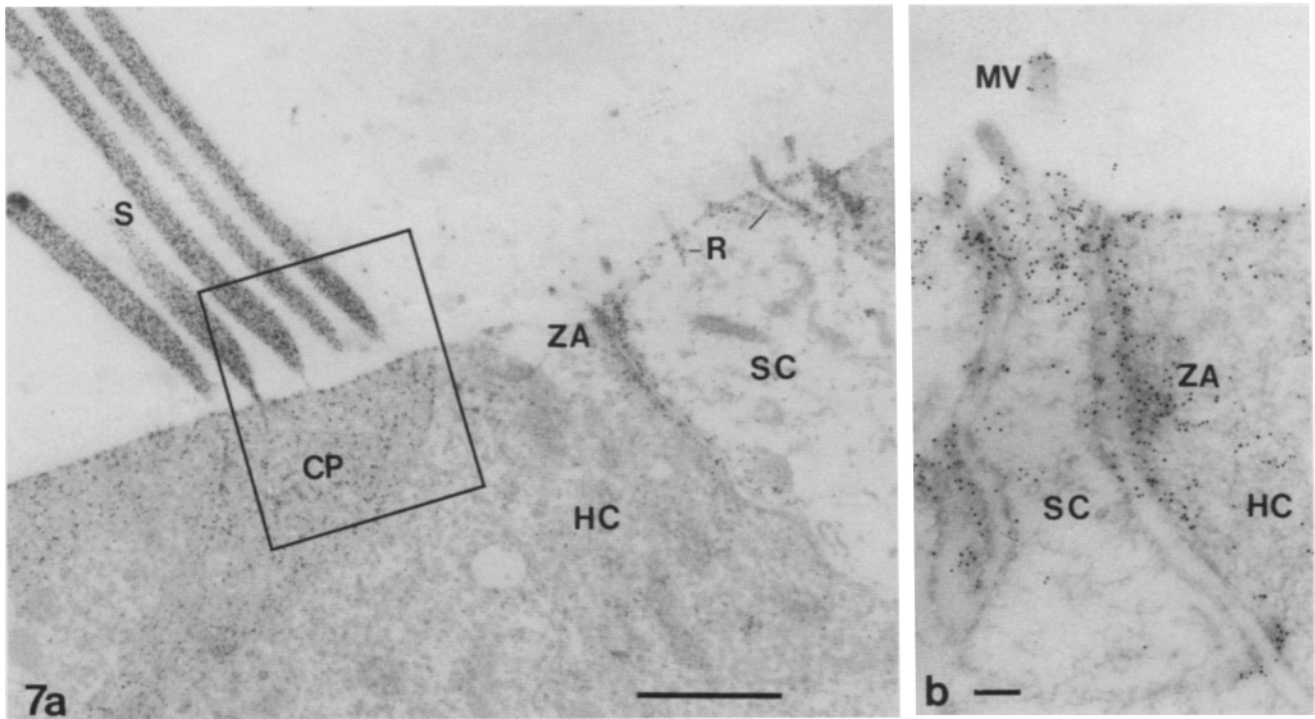


Figure 6. Localization of myosin in whole mounts (a and b), salt extracted hair borders (c and d), and sections of the sensory epithelium (e). The immunostain is confined to the circumferential band (small arrows). In isolated hair borders (d, immunostain; c, phase contrast) the circumferential band (ZA ring) is only stained when remnants of supporting cells remain attached to the cuticular plate. Note absence of fluorescence from the lower ("naked") hair border and presence of the ZA ring in the upper pair of hair borders that still adhere together. Bars, 10 μm .



Figures 7 and 8. Electron micrographs of the apex of hair cells (*HC*) labeled with antiactin and 5-nm-immunogold particles. Note the dense label overlying the stereocilia (*S*), the cuticular plate (*CP*), and the circumferential band that is associated with the ZA (*ZA*). Microvilli (*MV*), rootlets (*R*), and the ZA of supporting cells (*SC*) are also labeled. Boxed area in Fig. 7 is shown in Fig. 8 at higher magnification. Note 60–80-nm-spacing of antiactin-labeled densities (*dashes*) associated with the apical cell surface. Bars: (*7a*) 1 μm ; (*7b*) 0.1 μm ; (*Fig. 8*) 0.1 μm .

brush border (65 kD). Anti- α -actinin labeled a single band at 95–100 kD which precisely comigrated with α actinin purified from chicken gizzard. Antiactin bound to a 42-kD band which is a major polypeptide band seen in Coomassie blue-stained lanes. Antibodies against bovine and porcine brain spectrin (fodrin) and against α spectrin from chicken intestinal brush border (TW 240) and chicken erythrocytes mostly labeled two bands, one comigrating with erythroid α spectrin (240 kD) and the other migrating at 150 kD (Fig. 16). Occasionally the 150-kD band was absent (lane 5 in Fig. 16). The 150-kD band has to be considered as a major proteolytic fragment of nonerythroid spectrin (Carlin et al., 1983). Antibodies to brain spectrin often labeled a further band at 230 kD. This band is probably the β subunit of brain spectrin (fodrin 240/235).

Antibodies against brush border tropomyosin did not react with any polypeptide band of the sensory epithelium indicating that this antibody, which was raised against native tropomyosin does not bind in immunoblots to the SDS-denatured tropomyosin isoform of hair cells. This antibody reacts also only weakly with the tropomyosin band in Western blots of the intestinal epithelium. Antibodies against villin and the 110-kD protein from chicken brush border and filamin from chicken gizzard did not detect any bands in the immunoblots, thus confirming our immunocytochemical observations that these antibodies do not react with any structure of the sensory epithelium (not shown).

Discussion

In the present study we provide evidence that each of the three different assemblies of actin filaments present in the apex of auditory hair cells contains only a single type of actin crosslinking protein that is specific for each kind of assembly (Fig. 17). Thus, the complex apical cytoskeleton of hair cells

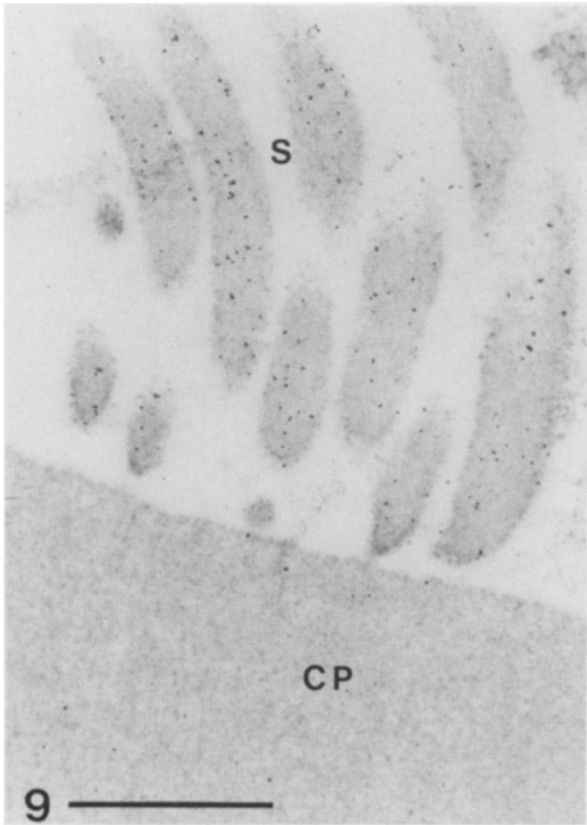


Figure 9. Immunoelectron micrograph showing the association of fimbrin with stereocilia (*S*) but not the cuticular plate (*CP*). Bar, 1 μm .

turns out to represent a very simplified model for studying the basic molecular requirements that are involved in development and maintenance of three major types of actin filament assemblies that are found not only in hair cells, but,

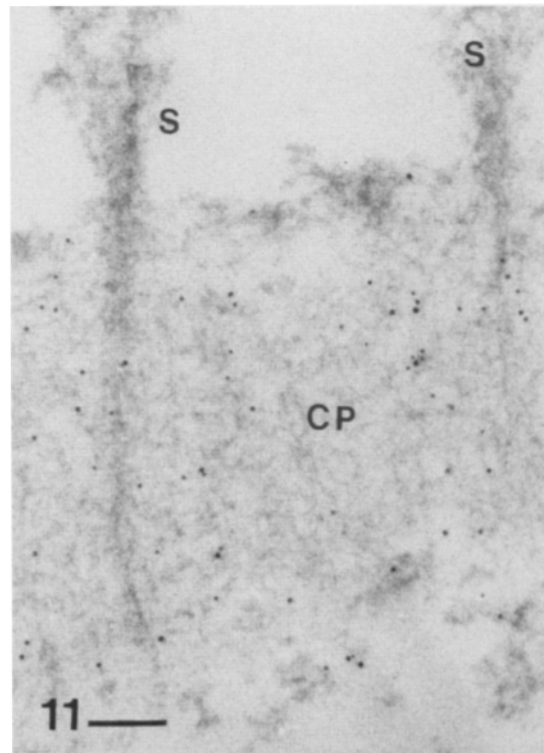


Figure 11. Immunoelectron micrograph (preembedding) showing the random-binding pattern of antibrain spectrin in the cuticular plate (*CP*). (*S*), bases of broken stereocilia. Bar, 0.1 μm .

at least in part, in most cell types of the body (animal and plant kingdom).

Paracrystalline Bundle of Actin Filaments in Stereocilia

Unlike the actin filament core bundles of intestinal micro-

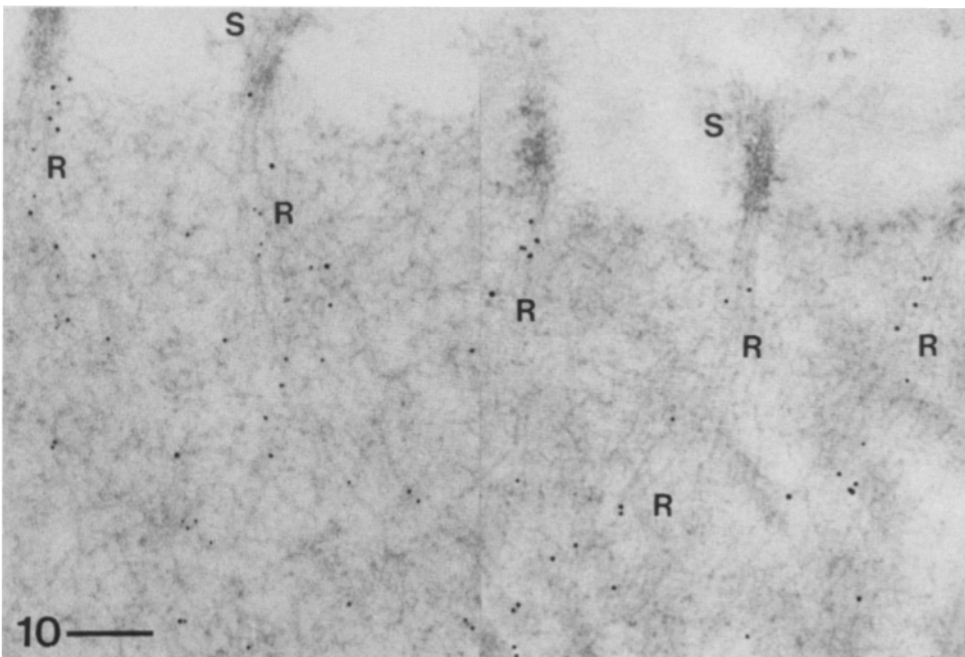


Figure 10. Electron micrograph of the cuticular plate labeled with antitropomyosin using a preembedding protocol. Note the association of immunogold particles (10 nm) with rootlets (*R*) and the cuticular plate. Stereocilia (*S*) are not labeled. Bar, 0.1 μm .

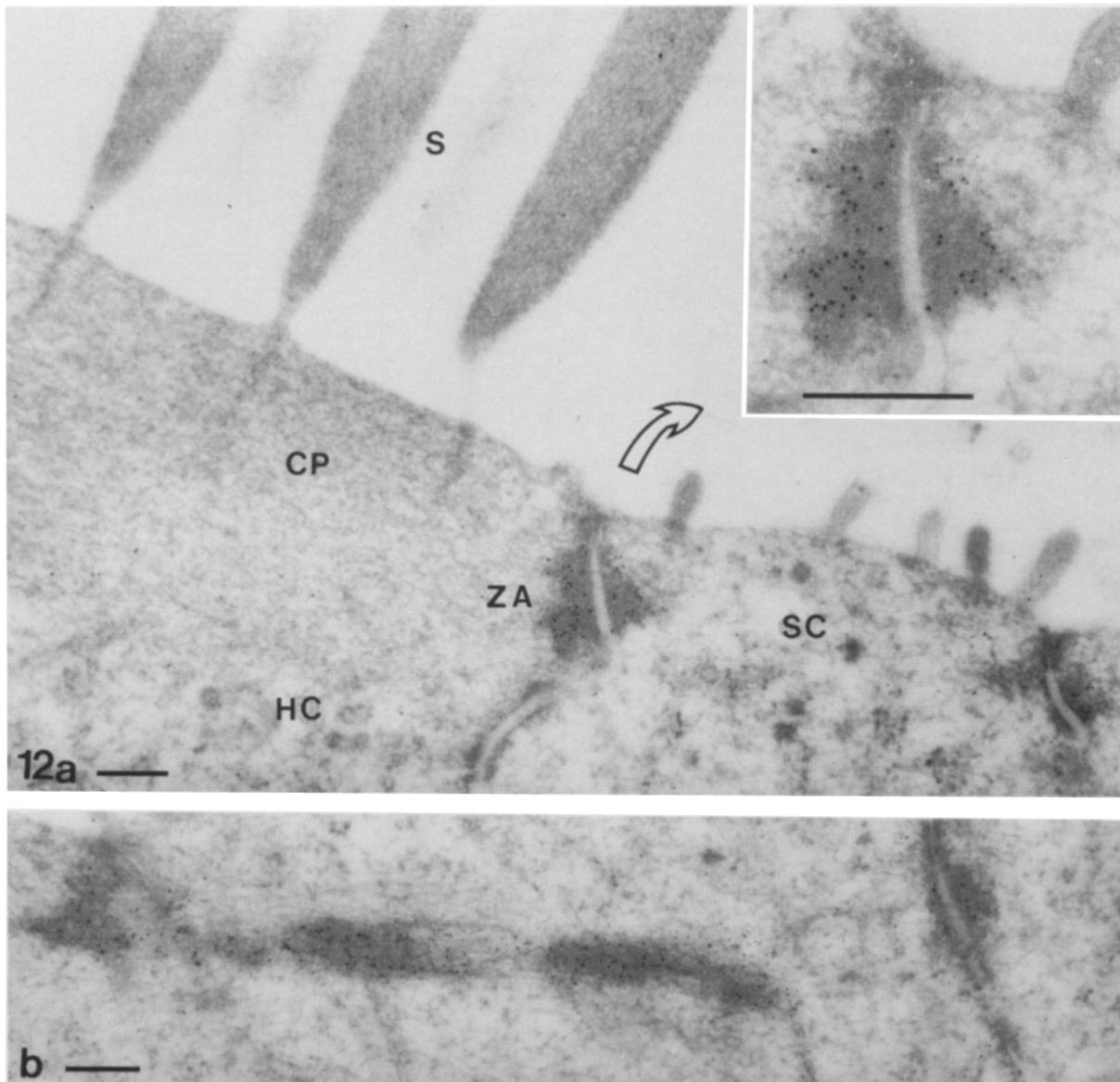


Figure 12. Postembedding immunogold labeling with anti- α actinin. The immunolabel is restricted to the circumferential ZA ring (ZA) of both hair cells (HC) and supporting cells (SC). Note the absence of label overlying the stereocilia (S) and cuticular plate (CP). The inset shows a higher magnification of the ZA. In (b) the ZA ring is cut longitudinally. Note alternating electron-dense (heavily labeled) and electron-lucid (weakly labeled) regions of the ring. Bars, 0.2 μ m.

villi, which contain at least three different actin-crosslinking proteins in high concentration (fimbrin, villin, 110-kD protein) (Mooseker, 1985; Burgess, 1987; Drenckhahn and Dermietzel, 1988; Coluccio and Bretscher, 1989), the actin filament bundle of stereocilia appears to contain only a major single crosslinker, fimbrin (Flock et al., 1982; Drenckhahn et al., 1985; Shephard et al., 1989; Tilney et al., 1989). That fimbrin is essential for paracrystal formation of actin filaments in chicken stereocilia has been indirectly shown by high salt extraction of demembranated sensory epithelia, a procedure that removes fimbrin from stereocilia (Tilney et al., 1989); this treatment resulted in a wavy, disorganized bundle in which the filaments were unevenly spaced, the crossbridges having been lost. Although the loose associa-

tion of actin filaments in fimbrin-depleted and -demembranated stereocilia indicates that there may be further crosslinking proteins in low amounts, the presence of only one major crosslinker in chicken stereocilia is probably the main reason why actin filaments in the stereocilia are more densely and regularly packed than the actin filaments that form the core bundles in intestinal microvilli. Thus, a paracrystalline array of actin filaments appears to require only a single bundling protein, i.e., fimbrin. Once a short array of actin filaments has grown out from nucleation sites thought to be present at the tips of the forming stereocilia, fimbrin may be sufficient by itself to force the actin filaments into a paracrystalline order and may allow the alignment of newly formed actin filaments to the periphery of the paracrystal.

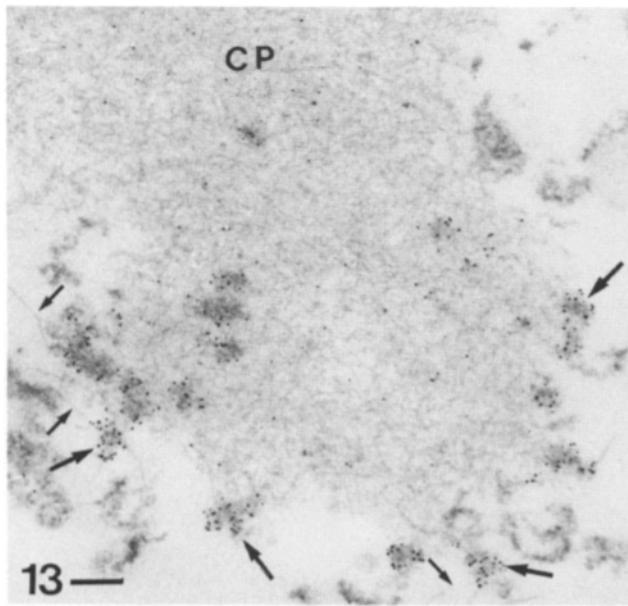


Figure 13. Preembedding immunogold labeling with anti- α actinin. Note the association of gold particles (5 nm) with small dense bodies (large arrows) that are located at the periphery of the cuticular plate (CP). Small arrows indicate intermediate filaments in close proximity to these densities. Bar, 0.1 μ m.

The absence of villin from stereocilia may not only be important for regular paracrystalline packing of actin filaments, but also for the function of stereocilia. Any influx of Ca^{2+} , which is known to occur upon excitatory deflection of the stereocilia (Hudspeth, 1989), could cause fragmentation or at least weakening of actin filaments if villin (which causes fragmentation of F-actin in the presence of $\text{Ca}^{2+} > 10^{-7}$ M) were an additional crosslinker in stereocilia.

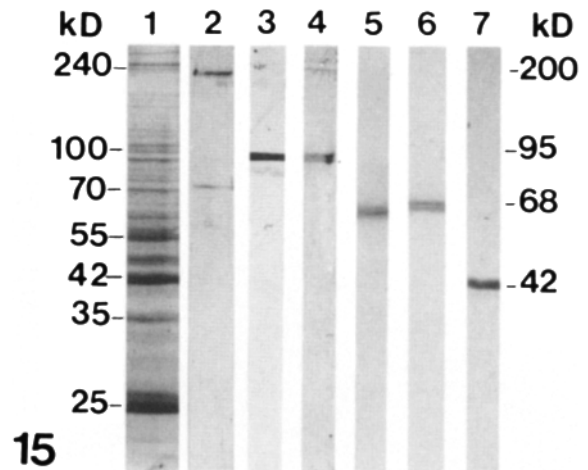


Figure 15. Immunoblot analysis (10% SDS-PAGE) of the chick sensory epithelium (five ear equivalents per lane) incubated with antibodies to myosin (lane 2), α actinin (lane 4), fimbrin (lane 6), and actin (lane 7). Lane 3 was loaded with purified gizzard α actinin and incubated with anti α actinin. Lane 5 was loaded with chicken intestinal brush borders and incubated with antifimbrin. Coomassie blue-stained SDS-PAGE of the sensory epithelium is shown in lane 1.

Cuticular Plate

The most surprising finding was that the actin gel constituting the cuticular plate contains only a single major actin crosslinking protein, an isoform of spectrin. Filamin, gelsolin, α actinin, fimbrin, and myosin were absent from the cuticular plate (this study; Tilney et al., 1989; and unpublished observations). This may explain why the three-dimensional organization of the cuticular plate (DeRosier and Tilney, 1989) differs considerably from that in the cortical cytoplasm of macrophages and neutrophilic leucocytes

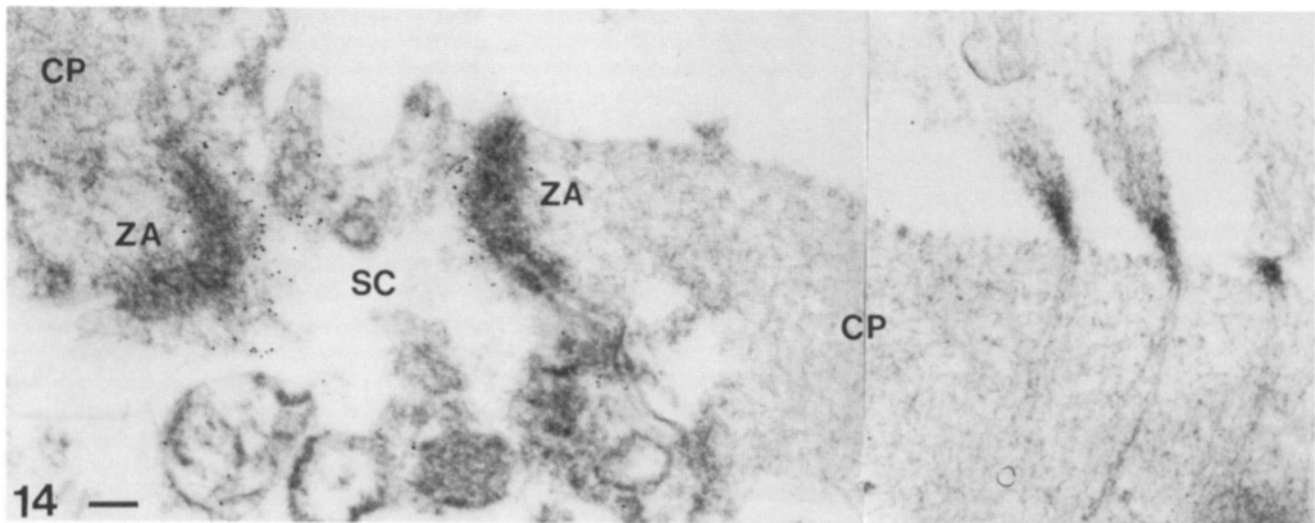


Figure 14. Section of a supporting cell (SC) and neighboring parts of two hair cells labeled with anti-myosin and 5-nm-immunogold particles before embedding. Note restriction of the myosin label to the ZA (ZA) of the supporting cell and the absence of label from cuticular plates (CP) of hair cells. Bar, 0.1 μ m.

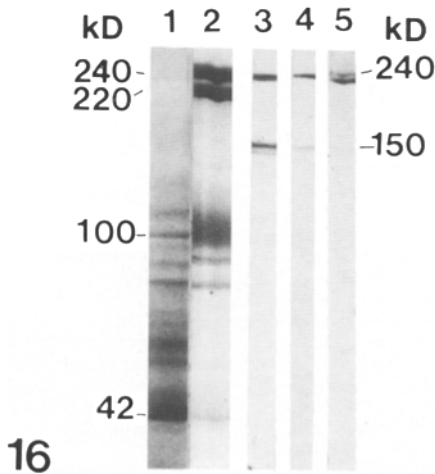


Figure 16. Identification of spectrin in the isolated chick sensory epithelium (7.5% SDS-PAGE) subjected to immunoblotting with antibodies to chicken erythroid α spectrin (lane 3), chicken intestinal brush border α spectrin (TW 240, lane 4) and bovine brain spectrin (fodrin, lane 5). Corresponding nitrocellulose strips of the sensory epithelium (lane 1) and human erythroid membrane (lane 2) stained with Ponceau S are also shown.

which contains at least four different actin filament cross-linkers, filamin, α actinin, myosin, and spectrin (Stendahl et al., 1980; Hartwig and Yin, 1988; Hartwig and Shevlin, 1986; Bennett et al., 1984; Jesaitis et al., 1988). We presume that the biological reason for having these differences between these two actin gels lies in the different functions that they have to fulfill. In macrophages the actin gel is a dynamic cytoskeleton that undergoes myosin-mediated contraction and Ca^{2+} -dependent remodeling. This is probably brought about by gelsolin which causes Ca^{2+} -dependent fragmentation and capping of actin filaments (Hartwig and Yin, 1988), and by nonmuscle α actinin which dissociates from F-actin with an increase of intracellular Ca^{2+} causing a loss of crosslinks between actin filaments (Bennett et al., 1984; Burridge and Feramisco, 1981). In contrast, the cuticular plate is thought to be a static actin gel that serves as a stiff non-flexible plate in which the stereocilia are anchored. Sound-induced deflection of stereocilia causes pivoting of the stereocilia at their tapered bases immediately above the cuticular plate (Flock et al., 1977). Thus, in a figurative sense, the actin gel of the cuticular plate serves as a kind of concrete plate into which flexible poles are inserted. For this function actin filaments need only a single crosslinker. This turned out to be an isoform of spectrin. Indirect evidence in support of this conclusion comes from extraction of cuticular plates with high salt which causes time-dependent disappearance of spectrin immunoreactivity and, concomitantly, disorganization and collapse of the cuticular plate (not shown).

It is very likely that spectrin provides the molecular basis for the 65-nm whiskerlike proteinaceous filaments (DeRosier and Tilney, 1989) that serve as a kind of regular spacer and dictate the three-dimensional organization of the gel. In support of this view recent studies indicate that spectrin tetramers possess properties of a weak, two-stranded spring that exists in a continuum of lengths ranging from 70 to 200 nm (McGough and Josephs, 1990). In erythrocytes in situ the aver-

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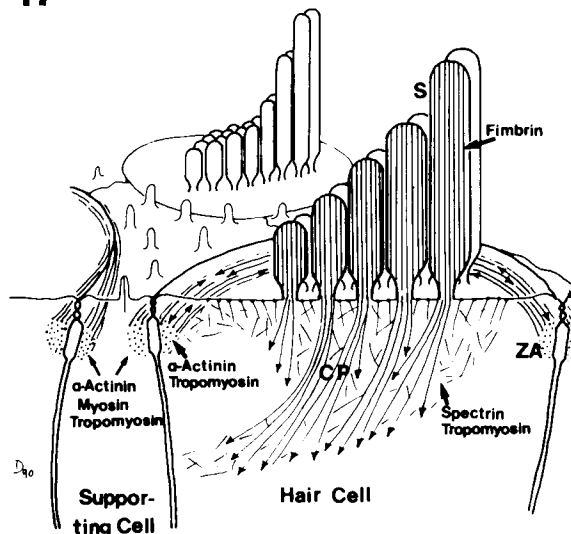


Figure 17. Diagram illustrating the subcellular location of the three different assemblies of actin filaments in auditory hair cells and indicating the major actin-binding proteins that are specific for these different assemblies. (CP), cuticular plate; (S), stereocilia; (ZA), circumferential zonula adhaerens ring.

age end-to-end distance of spectrin tetramers is ~ 70 – 90 nm which is close to the average length of the whiskers in the cuticular plate (65 nm in plastic-embedded cuticular plates that have undergone a 30–50% degree of shrinkage during dehydration). Furthermore, Cohen and Foley (1984) have shown micrographs of actin filaments decorated with spectrin that closely resemble the whisker-studded actin filaments of the cuticular plate both in morphology and dimension. Our immunoblotting studies demonstrated an α isoform of spectrin in the sensory epithelium that is immunologically related to the α isoforms of spectrin from brain, erythrocytes, and intestinal brush border. Antibodies to bovine brain spectrin often detected a further band at 230 kD, which is most probably the β subunit of brain spectrin (fodrin 240/235). Since, however, the sensory epithelium contains several nerve endings it is not clear whether the 230 kD-band belongs to the cuticular plate or to the nervous elements of the basilar papilla. No further attempts have been made so far to identify the variant β isoform (β spectrin) of the cuticular plate. However, our preliminary studies with antibodies to intestinal brush border β spectrin (TW 260) indicate the absence of TW 260 from the sensory epithelium.

The role of tropomyosin that is bound to rootlet filaments and actin filaments of the cuticular plate may be to stabilize these actin filaments and prevent their depolymerization (Weigt et al., 1990; Broschat et al., 1989). In addition, tropomyosin may interfere with binding of fimbrin to the rootlets, thereby inhibiting paracrystal formation of the splying rootlet filaments.

Circumferential Actin Filament Band

Unlike the circumferential band of all cell types studied so far (Owaribe et al., 1981; Hirokawa et al., 1983; Drenckhahn and Dermietzel, 1988; Schnittler et al., 1990), the filament band associated with the ZA of hair cells does not con-

tain myosin. The bright, myosin-like immunofluorescence seen in the area of the junctional complex of the sensory epithelium by light microscopy turned out to result from myosin which was found to be concentrated in the circumferential band of the supporting cells that surround each hair cell. The functional role of myosin in the supporting cells may be to create a tensile cellular framework into which hair cells are inserted and kept in an upright position.

Antibodies against myosin II from chicken intestinal brush border, calf thymus, human platelets, human uterine smooth muscle, and chicken gizzard failed to reveal the presence of myosin II in the circumferential band of hair cells (unpublished data). Antibrush border myosin was the only antibody that bound to the sensory epithelium where 95% of the label was restricted to the circumferential band of supporting cells. This indicates that hair cells have either a unique isoform of myosin II or that myosin is only a minor, negligible component of the circumferential band in hair cells. Deep-etch studies of decorated actin filaments have demonstrated that this band in hair cells is composed of parallel aligned actin filaments with mixed polarities (Hirokawa and Tilney, 1982) thus resembling the structure of the contractile circumferential band of the intestinal epithelium (Hirokawa et al., 1983). In hair cells this particular kind of assembly of actin filaments may therefore depend solely on the presence of α actinin which in muscle can crosslink actin filaments of opposite polarities (Squire, 1981). Linkage of the circumferential band to the plasma membrane probably involves vinculin, which has been demonstrated by immunofluorescence to occur also in the ZA of the chicken sensory epithelium (Drenckhahn et al., 1985).

Chicken Auditory Hair Cells as A Simplified Model for Three Different Assemblies of Actin Filaments

In the present study we have shown that each of the three different types of assemblies of actin filaments contains only a single type of actin crosslinking protein that is specific for each type of assembly. In other cell types and/or species the situation is much more complex. For example, in mammalian outer hair cells and vestibular hair cells the cuticular plate appears to contain three different actin-crosslinking proteins (α actinin, spectrin, and myosin) (Flock et al., 1982, Drenckhahn et al., 1982, 1985; Scarfone et al., 1988; Sans et al., 1989; Ylikoski et al., 1989) and, may also contain fimbrin (Flock et al., 1982; Sobin and Flock, 1983). We know of no other cell type which contains different assemblies of actin filaments in the same cell portion, each regulated by only one specific type of actin crossbridging molecule. Thus the chicken hair cell seems to be a simplified cytoskeletal system which may be an ideal model to study the generation and maintenance of three different specialized assemblies of actin filaments in close spatial proximity. Preliminary observations in our laboratories indicate that part of the explanation for the unique distribution of α actinin, fimbrin, and spectrin is their sequential expression during development.

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