Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 4973–4977, July 1989
Cell Biology

"Bundle blot" purification and initial protein characterization of hair cell stereocilia

(mechanotransduction/auditory system/vestibular system/cytoskeleton/immunocytochemistry)

GORDON M. G. SHEPHERD, BARBARA A. BARRES, AND DAVID P. COREY

Neuroscience Group, Howard Hughes Medical Institute, and Department of Neurology, Wellman 414, Massachusetts General Hospital, Fruit Street, Boston, MA 02114; and Program in Neuroscience, Harvard Medical School, Boston, MA 02115

Communicated by Thomas S. Reese, April 4, 1989

ABSTRACT **Stereocilia were isolated from bullfrog (*Rana catesbeiana*) saccular hair cells by nitrocellulose adhesion. The high purity and high yield of the preparation were demonstrated by microscopy. SDS/PAGE of stereociliary proteins resolved 12–15 major bands. Actin, previously identified as a component of the stereociliary core, was identified in purified stereocilia as a band comigrating with authentic actin and by phalloidin labeling of intact isolated stereocilia. Fimbrin was identified in immunoblots of purified stereocilia. The most abundant other proteins migrated at 11, 14, 16–19, 27, and 36 kDa. Demembranated stereociliary cores consisted primarily of protein bands corresponding to actin and fimbrin and several proteins ranging from 43 to 63 kDa. Because the adaptation mechanism in hair cells is calcium-sensitive and seems localized to stereocilia, we sought evidence for calcium-binding proteins in stereocilia. Calmodulin and calbindin antibodies labeled stereocilia in intact cells. A protein band in purified stereocilia exhibited a Ca^{2+}-dependent shift in electrophoretic mobility identical to that of authentic calmodulin, and the 27-kDa band may represent calbindin. These biochemical data demonstrate that stereocilia consist of a relatively small set of proteins. Most of these, including those involved in transduction and adaptation, are as yet uncharacterized. The availability of purified stereocilia should prove useful in further studies of structure–function relationships in these mechanically sensitive organelles.**

Hair cells, the receptor cells of the auditory and vestibular systems, transduce displacements of their apical hair bundle into electrical signals. The stereocilia constituting the bundle, which share structural properties with intestinal microvilli and growth-cone filopodia, are the mechanically sensitive organelles: displacement is thought to alter tension on filamentous links between their tips, which directly opens ion channels (1–3). The transduction current adapts to maintained stimuli, through a tension-altering mechanism (4, 5) thought to be situated in the tips of stereocilia (6).

Although the physiology of transduction and adaptation is understood in some detail, the proteins involved in these processes are largely unknown. Because of the difficulty in purifying sufficient amounts of stereocilia, protein identification has been pursued through immunocytochemistry. Thus the structural proteins actin (7, 8) and fimbrin (8) have been detected. Calbindin-28 immunoreactivity has been detected in rat and cat stereocilia (10, 11). Some physiological evidence points to the presence of calmodulin (CaM) in stereocilia: the adaptation process in bullfrog vestibular cells is sensitive to Ca^{2+} (4) and is blocked by CaM antagonists (12). Yet CaM antagonists are not particularly specific and could inhibit other calcium-binding proteins.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

To study the biochemistry of stereocilia we developed a purification technique that exploits the adhesion of the apical ends of stereocilia to nitrocellulose paper and the mechanical fragility of the narrow basal ends. A related strategy has been used to adsorb small numbers of piscine stereocilia onto coverslips for ultrastructural studies (13). The nitrocellulose adhesion method, which we term "bundle blot" purification, gave a sufficiently high yield of pure stereocilia for biochemical analysis. Some results have appeared in preliminary form (14).

METHODS

Blotting Stereocilia onto Nitrocellulose. Bullfrogs (*Rana catesbeiana*) were purchased from Ming's Market (Boston, MA) and other suppliers and kept at 20°C. Frogs were pithed and decapitated, and the sacculi were rapidly removed and transferred to a cold (4°C) physiological buffer containing 50 μM Ca^{2+} and protease inhibitors (0.15 μM aprotinin, 20 μM leupeptin, 0.15 μM pepstatin, and 0.1 mM phenylmethylsulfonyl fluoride); this cold buffer was used throughout the dissection. The saccular maculae were trimmed of surrounding epithelium and otolithic membranes were carefully removed with forceps. The apical surfaces were briefly washed with a gentle stream of dissection buffer. To harvest stereocilia, the apical surfaces of the maculae were blotted onto 1-mm^2 pieces of nitrocellulose paper (Schleicher & Schuell; 0.45-μm pores) and removed. Initially a piezoelectric bimorph configured as a force transducer was used to determine the optimal blotting force; thereafter this force of 5–20 mN (equivalent to 0.5–2 g) could, with practice, be delivered manually. The nitrocellulose papers with the adherent bundles were immediately (<60 s) processed for microscopy or biochemistry. Macular epithelia (hair-cell and supporting-cell somata, but not nerve) and otolithic membranes were collected onto similar small squares of nitrocellulose paper.

Characterization of Purity and Yield. Phalloidin, a toadstool alkaloid that binds F-actin, was used to monitor blotting efficiency. Samples were incubated in a solution containing formaldehyde (3.7%), lysolecithin (0.01%), and rhodamine-phalloidin (50 units/ml; Molecular Probes) for 30 min at room temperature and examined with fluorescence microscopy. For scanning electron microscopy, samples were fixed in 2.5% glutaraldehyde and postfixed with 1% osmium tetroxide, dehydrated in ethanol, critical-point-dried, and coated with gold/palladium. An AMR-1000 scanning electron microscope was operated at 20–30 kV.

Demembranation. A membrane extraction method adapted from refs. 15 and 16 was used to isolate stereociliary cores. After stereocilia were blotted onto nitrocellulose paper, the paper was transferred directly into extraction buffer (1% Triton X-100/100 mM NaCl/1 mM $MgCl_2$/0.1 mM EGTA/10 mM Tris, pH 7.6). The detergent-insoluble cores, still attached to the paper, were first washed in "core buffer" (75 mM

Abbreviation: CaM, calmodulin.

KCl/0.1 mM $MgCl_2$/1 mM EGTA/10 mM imidazole, pH 7.3) and then eluted off the paper into the same solution by rapid (1–2 s), mild sonication (Branson ultrasonic cleaner). The cores were pelleted by centrifugation for 10 min at 10,000 × *g* and resuspended in several microliters of core buffer. For electron microscopy, cores were applied to Formvar- and carbon-coated grids and negatively stained with 1% uranyl acetate. A JEOL 1000CXII microscope was operated at 60 kV.

Electrophoresis. Samples were incubated in a Laemmli buffer containing 4% SDS and 10% 2-mercaptoethanol at 100°C for 15 min. SDS/PAGE was performed according to ref. 17 and adapted to gradient minigels (0.75 mm thick, 10–25% acrylamide) (18). For each sample the entire volume of sample buffer (15–20 μl) was loaded. Standards were low and high molecular weight markers (Bio-Rad), actin (Sigma), and bovine brain CaM (Sigma). Protein bands were visualized by silver staining (19). To test for a calcium-dependent shift in the apparent molecular mass of CaM, samples of stereocilia and CaM (100 ng) were incubated in sample buffer containing either Ca^{2+} (1 mM) or EGTA (5 mM) (20). In general, 15, 2, and 3 animals were used for each gel lane of stereocilia, maculae, and otolithic membranes, respectively.

Immunoblotting. Proteins from maculae and purified stereocilia were resolved in gels as above and transferred to nitrocellulose paper (21). Rabbit anti-chick–fimbrin antiserum was used at 1:5; an Immunetics Miniblotter enabled small-volume lanes. Labeling was detected by incubation with gold-conjugated goat anti-rabbit antibody and silver enhancement (Janssen Pharmaceutica). Protein standards were simultaneously transferred to nitrocellulose paper and detected with India ink (22).

Immunocytochemistry. Single cells were dissociated from freshly dissected maculae (23). In brief, maculae were incubated for 30 min in papain (0.5 mg/ml; Calbiochem) and separated with an eyelash in low-Ca^{2+} Ringer solution onto concanavalin A-coated glass coverslips. Affinity-purified anti-CaM antibody raised in sheep against bovine brain CaM, was used at 1:100. Rabbit anti-sheep antibody (Jackson ImmunoResearch) was used at 1:100 to label the CaM antibody. Binding was visualized with a biotinylated secondary antibody and the avidin-biotin-peroxidase technique (Vector Laboratories). Additional sensitivity was achieved by using nickel-diaminobenzidine development. Dissociated frog intestinal epithelial cells were used as positive controls; preabsorption of CaM antibody with CaM provided a negative control. Rabbit anti-chick calbindin antiserum (24) was used at 1:200, and subsequent steps were the same as for CaM. For calbindin, supporting cells provided a negative control.

RESULTS

Purity and Yield of Stereocilia. When hair cell sensory epithelia were gently pressed against nitrocellulose paper, stereocilia adhered to it. Adhesion was sufficiently strong that stereocilia broke at their bases and remained attached to the paper when the macula was lifted from it. The purity and yield of this mechanical isolation procedure were assessed with fluorescence optics and scanning electron microscopy.

Fig. 1 shows scanning electron micrographs of the macula and nitrocellulose paper before and after blotting. The basic form of the hair bundles of intact cells (Fig. 1*A*) was roughly preserved after blotting (Fig. 1*C*). In Fig. 1*B* only hair bundles are missing from the blotted macular surface; the stereocilia appear to have broken at their bases leaving a trigonal array of stubs. The bulbs of the single microtubular kinocilia of each bundle were not evident on the nitrocellulose blot; the bulbs and perhaps shafts were pulled off with the otolithic membrane

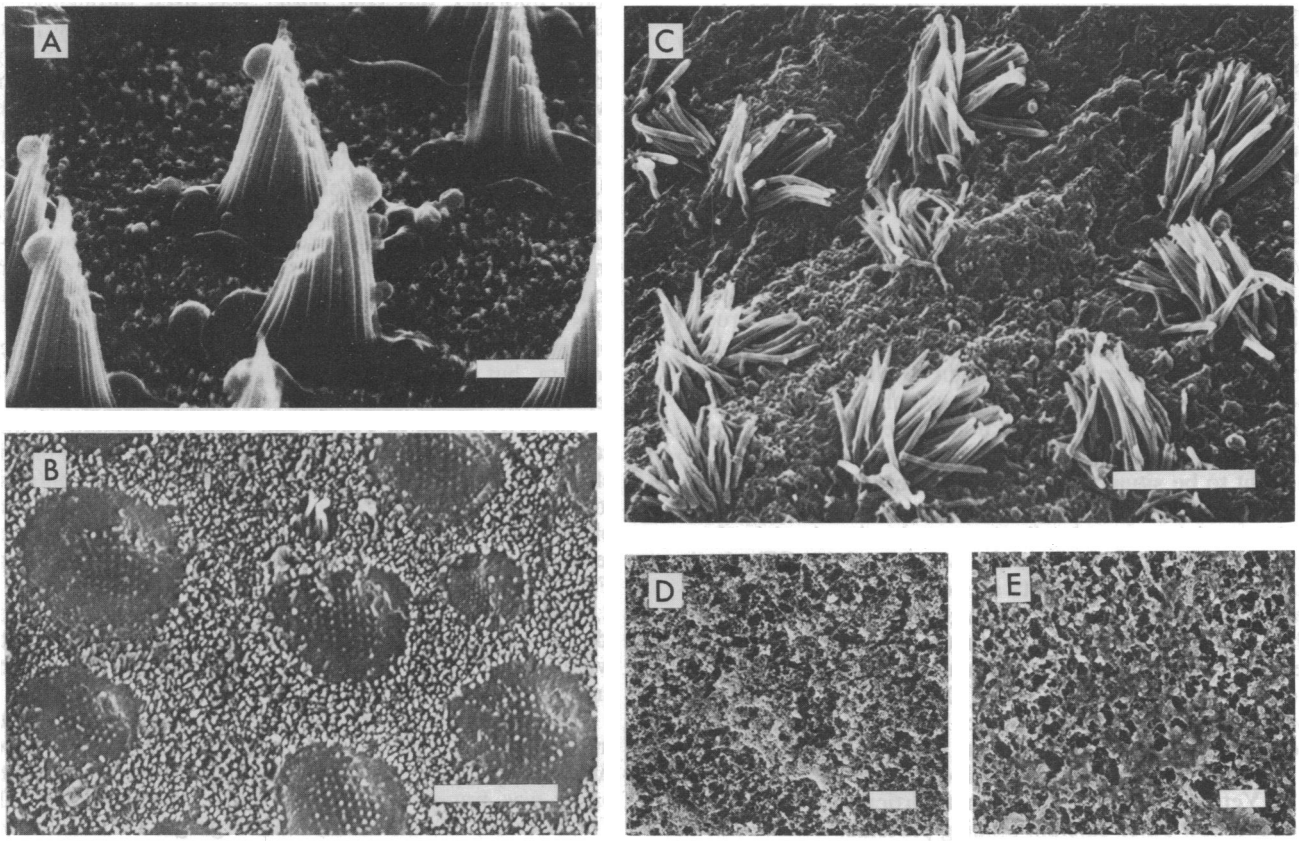

FIG. 1. Scanning electron microscopy of hair cell stereocilia during the bundle-blot isolation technique. (*A*) Sensory epithelium after removal of the otolithic membrane but before blotting. (*B*) Apical surface of epithelium after blotting. The stereocilia broke at their bases, leaving a trigonal arrangement of stubs. (*C*) Surface of nitrocellulose after blotting, showing adherent stereociliary bundles. (*D*) Nitrocellulose paper after elution of stereociliary proteins. (*E*) Unblotted nitrocellulose. (*A*, bar = 2.5 μm; *B* and *C*, bars = 5 μm; *D* and *E*, bars = 10 μm.)

(data not shown; note that kinocilia were intentionally preserved in Fig. 1*A* by enzymatically separating them from the otolithic membrane using subtilopeptidase). After elution of proteins (Fig. 1*D*) the nitrocellulose appeared the same as plain, unprocessed nitrocellulose (Fig. 1*E*).

For an assay of yield, and a further indication of purity, we labeled the stereocilia and the actin-based structures that are just below the apical surface with rhodamine-phalloidin. Labeling of the intact hair cell epithelium (Fig. 2*A*) demonstrated the morphology of three distinct actin-containing structures: stereociliary bundles, cuticular plates, and the circumferential actin belts associated with zonulae adherentes (26). Nitrocellulose blotting of the sensory epithelium caused the transfer of only the stereociliary bundles (Fig. 2*B*); cuticular plates and belts remained with the blotted macula (Fig. 2*C*). Low-power views (Fig. 2 *D* and *E*) showed that the overall shape of the macula was preserved after blotting and that virtually all bundles were transferred. Contamination by components of the hair cell soma, judged by cuticular plates and actin belts, was limited; at most, 5–10 belts contaminated every 3000 bundles. Staining of the same preparation with the nonspecific protein stain amido black gave virtually identical results (data not shown).

Electrophoresis and Immunoblotting. Stereociliary proteins were eluted off the nitrocellulose blots by heating in denaturing sample buffer and resolved by SDS/PAGE. Fig. 3 (see also Fig. 4*B*) shows silver-stained proteins from isolated stereocilia, macular epithelium, and otolithic membranes. Authentic actin comigrated with a major band in stereocilia and macula at 42 kDa. Relatively abundant stereociliary proteins were actin and proteins at 11, 14, 16–19, 22, 23, 27, 36, 51, 60, 63, and 68 kDa. Fainter bands, not all clearly evident in Fig. 3 but consistently present, were seen at 32, 85, 100, 140, and 220 kDa (see Fig. 4*B*, lane 3). In some gels stereocilia and cores show a band at >250 kDa which barely enters the gel (Fig. 4*B*, lanes 3 and 4). Many bands were present only, or in much greater abundance, in macular epithelium; this indicates that a subset of total macular protein was isolated in the purification procedure. Otolithic membrane proteins, seen as broad bands at 33, 40, 59, and 190 kDa, differed almost entirely from those of epithelium and stereocilia.

When sample proteins were resolved in gradient gels and transferred to nitrocellulose sheets, an antibody to chick fimbrin labeled a single band at 68 kDa in both macular epithelium and purified stereocilia. This confirms the immunocytochemical identification of fimbrin (8). Note that the gel in Fig. 3 contains an artifact, common to silver gels (27), at 66 kDa (below fimbrin).

Stereociliary Core Proteins. As previously reported (13), electron microscopy of negatively stained cores showed bare shafts with particles at both ends (Fig. 4*A*). Electrophoresis of the cores resolved approximately seven proteins (Fig. 4 *B* and *C*), a distinct subset of total stereociliary proteins. The most prominent bands corresponded to actin (42 kDa) and fimbrin (67 kDa). All other bands were of intermediate molecular mass, migrating at 52, 63, 60, 55, and 43 kDa (in order of decreasing abundance). No 66-kDa artifact was present in these gels.

SDS/PAGE of Stereociliary CaM. The bands at 16–19 kDa suggested the presence of CaM in stereocilia. Immunoblots of CaM and other proteins in the troponin superfamily are difficult, as these small acidic proteins adhere poorly to nitrocellulose (28). Instead, CaM was identified by its characteristic Ca^{2+}-dependent shift in electrophoretic mobility (20). Stereocilia proteins and authentic bovine CaM were electrophoretically resolved in the presence of high Ca^{2+} (1

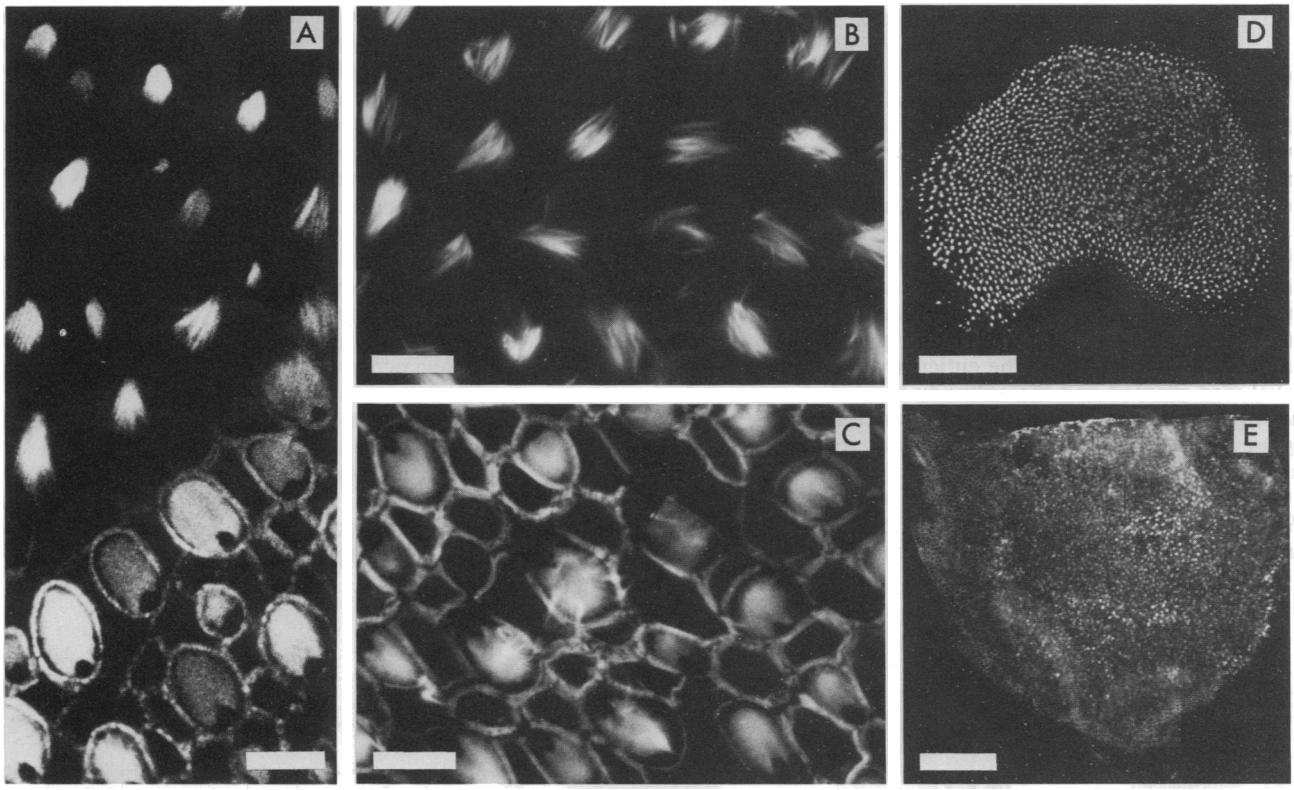

FIG. 2. Fluorescently labeled actin in hair cells before and after nitrocellulose blotting. (*A*) Optical section through intact sensory epithelium, nearly parallel to the apical surface, showing the distribution of actin in stereocilia (upper region of micrograph), cuticular plates, and the actin belts of the zonulae adherentes (lower region). (*B* and *C*) After blotting, stereociliary bundles remained with the nitrocellulose (*B*), but cuticular plates and belts did not transfer (*C*). A few bundles in this field also did not transfer. (*D* and *E*) A low-power view of the nitrocellulose paper shows almost all the bundles transferred to the paper (*D*), whereas all plates and belts remained with the epithelium (*E*). (*A–C*, bars = 10 μm; *D* and *E*, bars = 200 μm.)

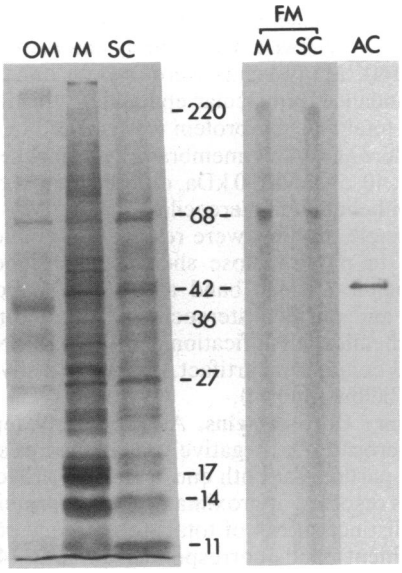

FIG. 3. Gel electrophoresis patterns of stereocilia (SC), blotted maculae (M), and otolithic membranes (OM). Samples were resolved in a gradient acrylamide minigel in the presence of SDS and stained with silver. Authentic actin (AC) comigrated with a band at 42 kDa in purified stereocilia and maculae. An antibody to chick fimbrin (FM) detected a single band at 68 kDa in immunoblots of maculae (M) and stereocilia (SC). Numbers indicate molecular mass in kDa.

mM) or low Ca^{2+} (<1 μM) (Fig. 5). Authentic CaM exhibited a characteristic, pronounced shift in apparent molecular mass in low and high Ca^{2+}, migrating at 20 and 15 kDa, respectively. A protein in purified stereocilia displayed identical behavior. At lane margins both this protein and authentic CaM tended to run at 17 kDa, the molecular mass of CaM in running buffer with no added Ca^{2+}. Additionally, silver-staining of both authentic CaM and the corresponding band in purified stereocilia gave grey bands; almost all other stereocilia proteins stained reddish-brown. Other bands between 15 and 20 kDa did not display Ca^{2+}-dependent migration and are likely to represent different proteins rather than the titration of Ca^{2+}-binding sites on CaM.

Immunocytochemistry. We also used immunocytochemical labeling to look for calcium-binding proteins. Fig. 5 shows anti-CaM labeling of dissociated hair cells. By using nickel-diaminobenzidine development, label was observed throughout the somata and stereocilia, with lighter staining in the base of the stereocilia and the cuticular plate region. When nickel was omitted and the antibody incubation was shortened, the labeling of the stereocilia was confined to the tip region. No labeling of the hair cells was evident with the antibody was preabsorbed with CaM.

Calbindin is a major constituent of whole avian cochlea (29). In frog hair cells, antibodies to calbindin labeled throughout the stereocilia as well as the somata but did not

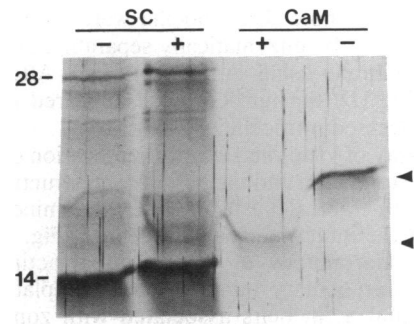

FIG. 5. Relevant portion of gradient acrylamide gel showing migration of stereocilia proteins (SC) and bovine CaM in high and low Ca^{2+}. The sample buffer contained 1 mM Ca^{2+} (shown as +) or 5 mM EGTA (−). A stereocilia protein band displayed the same calcium dependence of migration as authentic CaM; both migrated at 20 kDa in low Ca^{2+} and 15 kDa in high Ca^{2+} (arrowheads). Numbers indicate molecular mass in kDa.

stain supporting cells (Fig. 6*D*). A similar pattern has been observed in mammalian vestibular hair cells (10, 11).

DISCUSSION

Stereociliary Purification. Bundle-blotting provided an efficient method for isolating pure stereocilia from hair cells in quantities sufficient for biochemical analysis. Over 90% of bundles were transferred. Based on phalloidin staining of cuticular plates, contaminants constituted <1%. The electrophoretic map of isolated stereocilia further indicated that the preparation contained a subset of macular proteins. No proteins were found exclusively in stereocilia, perhaps due to protein synthesis in the cell soma. Possible contaminants of the preparation included small blebs of apical membrane, seen as small spheres on the blots, and apical cell components from the surrounding epithelium, seen as phalloidin-stained belts. However, these were infrequent and constituted a small percentage of the material on the blots. Kinociliary shafts could possibly have been minor contaminants; their major protein, tubulin, may be the faint band at 55 kDa. No otolithic membrane components were apparent in the purified stereocilia preparation.

Stereocilia were put into sample buffer within a minute of purification. As such they were probably biochemically intact—that is, core, membrane, and cytosolic proteins were probably all recovered. Although some leakage of cytosolic proteins may have occurred through the open basal ends, it is also possible that the openings rapidly resealed.

Structural and Calcium-Binding Proteins. Our biochemical data verify the presence of two structural proteins in stereocilia. In agreement with ultrastructural and immunocytochemical evidence—e.g., refs. 7 and 8—and with phalloidin labeling, actin was a major component of purified stereocilia. Similarly, immunoblotting confirmed the presence of fimbrin

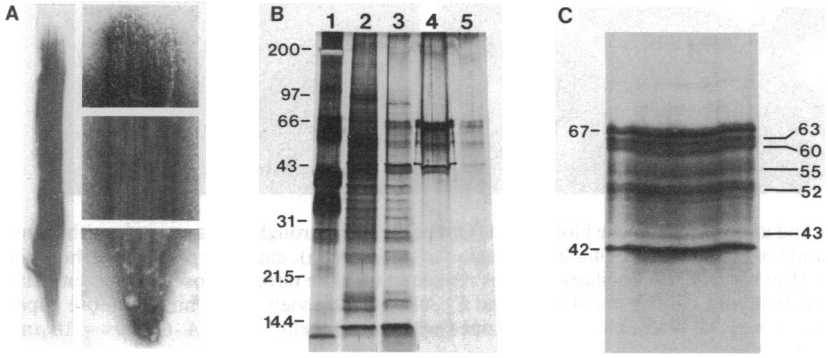

FIG. 4. Stereociliary cores. (*A*) Electron micrographs of negatively stained cores, with details of tip, shaft, and base. (*B*) SDS/PAGE of core proteins. Lane 1, otolithic membrane; lane 2, macula; lane 3, stereocilia; lane 4, cores; lane 5, nitrocellulose after sonication-elution of cores. (*C*) Enlarged view of stereociliary core proteins (from a similar gel). Numbers indicate molecular mass in kDa.

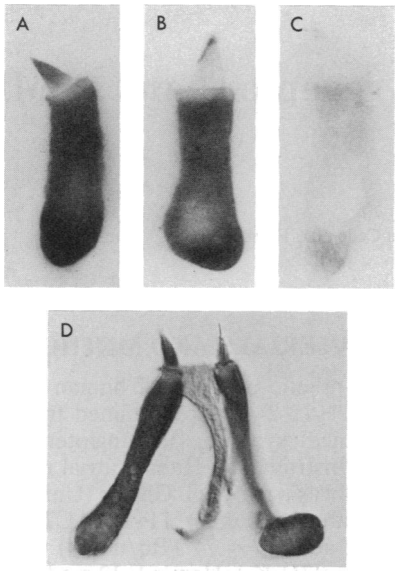

FIG. 6. Immunocytochemical labeling of calcium-binding proteins in dissociated hair cells. (*A* and *B*) Antibodies to CaM labeled stereocilia just at their tips (*B*) or, when nickel-diaminobenzidine development and longer antibody incubations were used throughout their length (*A*). (*C*) Preabsorption of the antiserum with CaM eliminated almost all of the specific labeling. As a positive control, frog intestinal cells showed strong specific labeling of their microvilli (not shown). (*D*) Antibodies to calbindin labeled both somata and stereocilia but not supporting cells.

(8). These two proteins appear to be the major constituents of the core.

Our data also indicate the presence in stereocilia of calcium-binding proteins. CaM was demonstrated both by immunocytochemistry and by a characteristic Ca^{2+}-dependent shift in electrophoretic mobility. High-sensitivity immunocytochemistry with anti-CaM antibody produced label throughout the stereocilia; with shorter antibody incubation, label was localized to the tips. This pattern suggests that specific binding sites for CaM are present in the tips of stereocilia; further experiments will be required to prove this. We also detected calbindin immunoreactivity in frog stereocilia and hair cells. A protein very similar or identical to avian calbindin-28 has been detected immunocytochemically in stereocilia of mammalian cochlear and vestibular hair cells (10, 11), and Oberholtzer *et al.* (29) reported a high abundance of calbindin in whole chick cochlea. Though it is likely that the band we observed at 27 kDa is calbindin, it appears less abundant in these vestibular cells.

Stereocilia and Microvilli. In purified olfactory cilia, candidates for functionally important proteins were initially identified by comparison of their electrophoretic map with that of respiratory cilia (30). Intestinal microvilli provide a similar comparison for purified hair cell stereocilia. Because the nitrocellulose blotting technique proved ineffective for microvilli—probably due to the lack of a tapered base—we referred to previous accounts of (primarily chick) microvillus biochemistry (15, 16). Although actin and fimbrin are major structural proteins in both structures, purified stereocilia and stereociliary cores did not contain bands at the molecular masses of the two other major microvilli core proteins, villin (95 kDa) and the 110-kDa protein. Villin is immunocytochemically absent from stereocilia (8, 31). The 110-kDa protein is the core-membrane crosslinker in microvilli, binds CaM, and has myosin-like properties (reviewed in ref. 9). Its absence in gels of stereocilia proteins suggests that the analogous crosslinker may be a different protein. Indeed, as previously noted (13), demembranated actin cores of stereocilia appear bare, whereas microvillus cores are studded with the 110-kDa protein. The observation that negatively stained cores have particles only at their ends is also consistent with the gel pattern: actin and fimbrin predominate, with several other proteins present in lower quantities.

Although no higher molecular mass proteins appear to be uniquely abundant in stereocilia, several smaller proteins are enriched. These include the proteins at 11 and especially 14 kDa, the triplet at 16–19 kDa, and the 27- and 36-kDa proteins. As none of these are components of the cores, they are presumably either cytosolic or membrane-associated.

We chose the frog as our experimental animal to correlate biochemical and electrophysiological data. However, the bundle blot method has also been effective in preliminary experiments on chick stereocilia, for which more antibodies are available, and it may prove generally applicable to other species and tissues. The method is also suitable for generating antibodies to stereociliary proteins, as nitrocellulose is an effective adjuvant for immunization (25).

We thank H. J. Federoff and P. Matsudaira for comments and advice and J. A. Assad for help with microscopy and uncountable dissections. We appreciate discussions with L. G. Tilney, M. S. Tilney, and D. A. Cotanche, who shared with us their unpublished results on chick stereociliary proteins. We greatly appreciate the gifts of antisera: anti-calbindin from S. Christakos, anti-CaM from J. R. Dedman, and anti-fimbrin from P. Matsudaira. This work was supported by grants from the National Institutes of Health (NS22059 to D.P.C.) and from the Office of Naval Research. D.P.C. is an Associate Investigator of the Howard Hughes Medical Institute.

1. Hudspeth, A. J. (1982) *J. Neurosci.* **2,** 1–10.
2. Corey, D. P. & Hudspeth, A. J. (1983) *J. Neurosci.* **3,** 962–976.
3. Pickles, J. O., Comis, S. D. & Osborne, M. P. (1984) *Hearing Res.* **15,** 103–112.
4. Eatock, R. A., Corey, D. P. & Hudspeth, A. J. (1987) *J. Neurosci.* **7,** 2821–2836.
5. Howard, J. & Hudspeth, A. J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3064–3068.
6. Assad, J. A., Hacohen, N. & Corey, D. P. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2918–2922.
7. Flock, A. & Cheung, H. C. (1977) *J. Cell Biol.* **75,** 339–343.
8. Flock, A., Bretscher, A. & Weber, K. (1982) *Hearing Res.* **6,** 75–89.
9. Mooseker, M. S. (1985) *Annu. Rev. Cell Biol.* **1,** 209–241.
10. Rabie, A., Thomasset, M. & Legrand, Ch. (1983) *Cell Tissue Res.* **232,** 691–696.
11. Sans, A., Brehier, A., Moniot, B. & Thomasset, M. (1987) *Brain Res.* **435,** 293–304.
12. Corey, D. P., Smith, W. J., Barres, B. A. & Koroshetz, W. J. (1987) *Soc. Neurosci. Abstr.* **13,** 538.
13. Neugebauer, D.-C. & Thurm, U. (1984) *J. Neurocytol.* **13,** 797–808.
14. Shepherd, G. M. G., Barres, B. A. & Corey, D. P. (1988) *Soc. Neurosci. Abstr.* **14,** 799.
15. Bretscher, A. & Weber, K. (1978) *Exp. Cell Res.* **116,** 397–407.
16. Matsudaira, P. T. & Burgess, D. R. (1979) *J. Cell Biol.* **83,** 667–673.
17. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
18. Matsudaira, P. T. & Burgess, D. R. (1978) *Anal. Biochem.* **87,** 386–396.
19. Merril, C. R., Goldman, D., Sedman, S. A. & Ebert, M. H. (1981) *Science* **211,** 1437–1438.
20. Glenney, J. R. & Glenney, P. (1985) *J. Cell Biol.* **100,** 754–763.
21. Towbin, H., Staehelin, S. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
22. Hancock, K. & Tsang, V. (1983) *Anal. Biochem.* **133,** 157–162.
23. Lewis, R. S. & Hudspeth, A. J. (1983) *Nature (London)* **304,** 538–541.
24. Pansini, A. R. & Christakos, S. (1984) *J. Biol. Chem.* **259,** 9735–9741.
25. Knudsen, K. A. (1985) *Anal. Biochem.* **147,** 285–288.
26. Hirokawa, N. (1986) *Hearing Res.* **22,** 41–54.
27. Ochs, D. (1983) *Anal. Biochem.* **135,** 470–474.
28. Van Eldik, L. J. & Wolchok, S. R. (1984) *Biochem. Biophys. Res. Commun.* **124,** 752–759.
29. Oberholtzer, J. C., Buettger, C., Summers, M. C. & Matchinsky, F. M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3387–3390.
30. Chen, Z. & Lancet, D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1859–1863.
31. Drenckhahn, D., Kellner, J., Mannherz, H. G., Groschel-Stewart, U., Kendrick-Jones, J. & Scholey, J. (1982) *Nature (London)* **300,** 531–532.