Morphological Basis for a Mechanical Linkage in Otolithic Receptor Transduction in the Frog

Abstract. *Observations made with a scanning electron microscope confirm the binding of the stereocilia to a matchhead-like bulbous terminal at the apex of the kinocilium in frog saccular receptor cells. Since the kinocilium is shown to rest on a portion of the receptor cell that lacks the rigid cuticular base of the stereocilia, movement of the ciliary ensemble results in a "plunging-like" effect of the kinocilium which produces a distension of the membrane at its base. This membrane distension is envisaged as bringing about the ionic conductance changes necessary for the production of a generator potential and, thus, for the transduction of movement into vestibular nerve activity.*

The directional sensitivity of the vestibular system (*1*) has been correlated with the polarity of ciliary tufts on the receptor cells (*2*). This polarization has been shown to be related to the eccentric position of the kinocilium on the cell's luminal surface (*3*) (Fig. 1). Thus, in the vestibular and lateral line systems the kinocilium is located at the periphery of the stereociliary panache (*4–6*) (Fig. 2a). Furthermore, in unidirectional motion detectors such as the semicircular canals, the kinocilia of the receptor cells on a crista are all aligned in the same direction (*2*). After canal rotation so that endolymph pressure is directed toward the kinociliary side of the tufts, electrical activity is increased in the vestibular nerve. A force in the opposite direction (toward the stereociliary side of the tufts) causes a decrease in spontaneous activity of the vestibular nerve (*2*). Likewise, receptor de- and hyperpolarization have been correlated to the location of the kinocilium (*3*). Although there has been considerable speculation on the means by which the receptor cells of the lateral line, the vestibular, and the auditory systems are activated (*3*, *7*), the actual mechanism of the transduction process remains obscure.

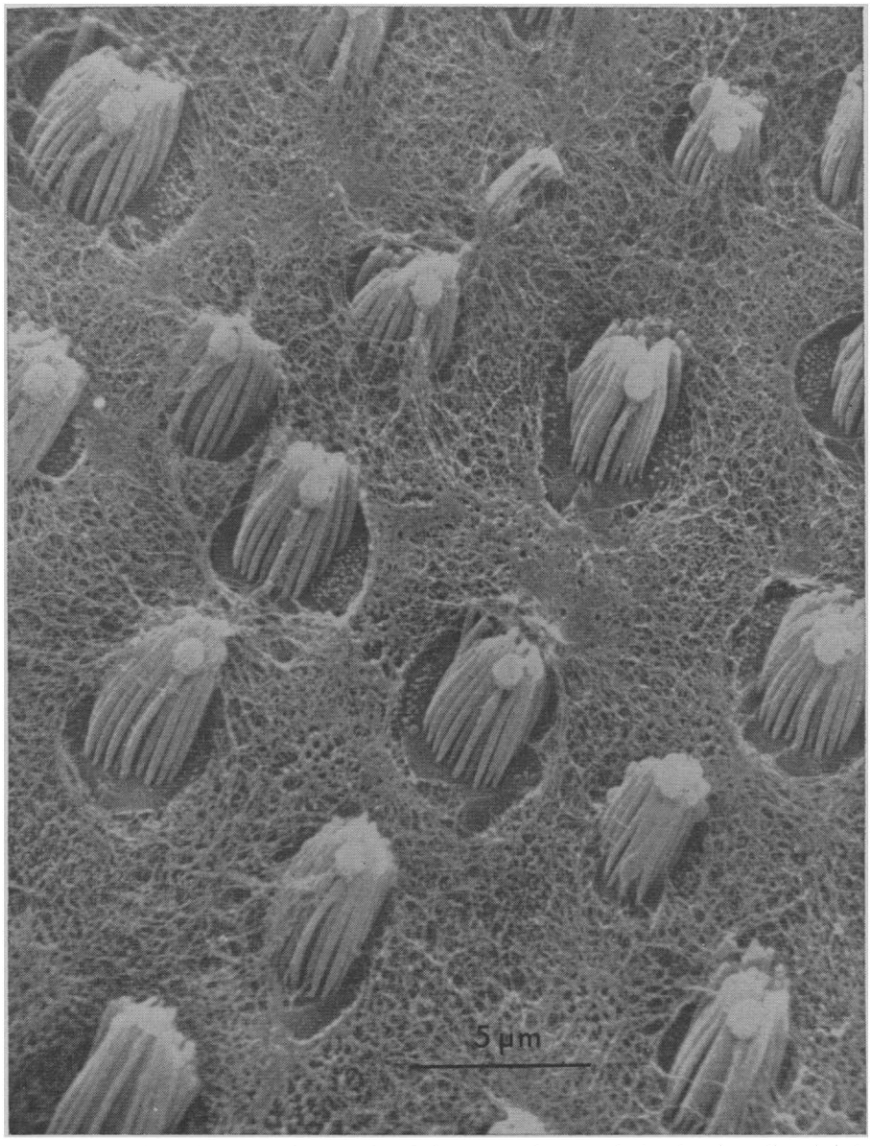

Fig. 1. Scanning electron micrograph of frog macula saccularis showing directional orientation of ciliary tufts and a matlike base from the otolithic membrane. The unidirectional orientated kinocilium terminates in a large bulb, making it distinct from the numerous stereocilia.

A simple model for mechanical transduction, based on the ultrastructure of frog vestibular hair cells, has been proposed previously by one of us (*8*). The present report describes, with the aid of scanning and transmission electron microscopy, some of the mechanical properties of the vestibular receptor cilia in the otolithic organ.

The vestibular membranous labyrinth from a frog (*Rana catesbeiana*) was fixed in situ with osmium tetroxide while the animal was positioned either upright or on its back. The labyrinth was carefully dissected and the maculae of the utriculus and the sacculus were separated from the labyrinth. The otolithic membranes of the maculae were gently detached from the epithelial surface with fine forceps, and the receptor epithelia were dehydrated in alcohol, dried, and mounted. These specimens were coated with gold and viewed in a Cambridge scanning electron microscope (SEM). Similarly prepared, embedded, and sectioned material was viewed with an Hitachi 7S transmission electron microscope (TEM).

Scanning and transmission electron microscopy of the ciliary tufts on the maculae of the sacculus and utriculus demonstrate distinct structural ensembles, which indicates that each tuft can function as a directional sensitive apparatus. The basic features of this complex consist of (i) an apical attachment of the kinocilium to adjacent stereocilia (*8*) (Fig. 2d); (ii) the position of the stereocilia on a relatively rigid cuticular base (*6*, *8*, *9*); and (iii) the loca-

tion of the kinocilium over a notch in the cuticle so that, in contrast with the stereocilia, the kinociliary base is in direct contact with the cell cytoplasm (*6, 8, 9*) (Fig. 3, d–f).

It has been suggested that this special anatomical ensemble serves (through the axial motion of the kinocilium and a distension-sensitive membrane area at the kinociliary base) as a mechanical coupling system for the transformation of shearing motion of the otolithic membrane into a generator potential (*8*). This directional action of the ciliary tuft can be observed in the same preparation along a dividing line (*6, 10*) that separates the cells on approximately one half of the saccular macula from oppositely oriented cells on the remaining half (Fig. 2c). By comparing the two tufts in Fig. 2c, the sliding action of the stereocilia in relation to each other is evidenced by the differential displacement at the apical ends of the stereociliary rows (*11*). The action of the kinocilium, however, is different; the displacement occurs at the kinociliary base (Fig. 2d and Fig. 3, d–f). This is due to an attachment between the apical ends of the kinocilium and adjacent stereocilia (Fig. 2d) (*8*). In the sacculus the attachment occurs at the terminal bulb (Fig. 1) while in the utriculus, where the kinocilia are three to four times as long as the stereocilia, the attachment occurs at the level of the ste-

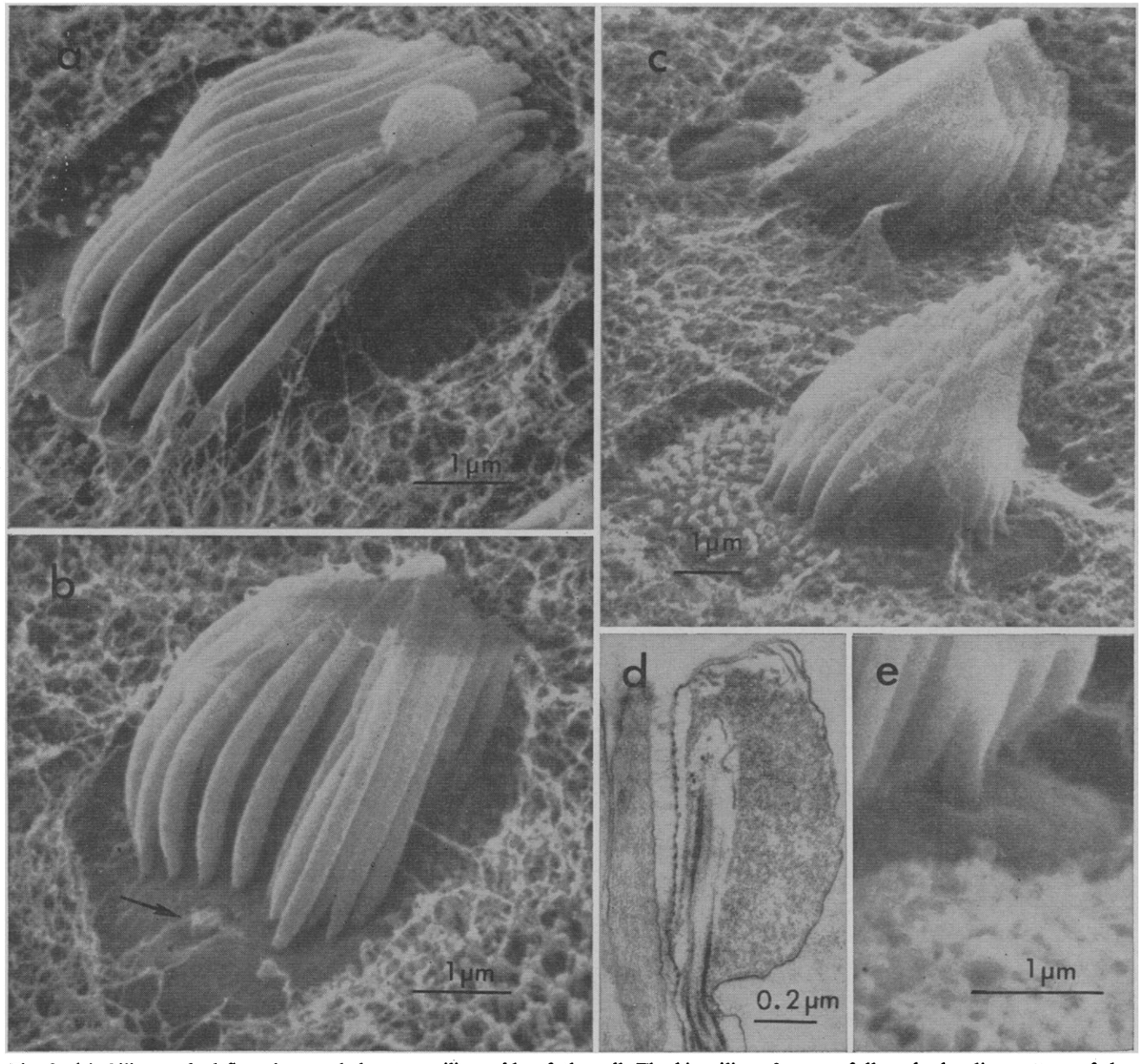

Fig. 2. (a) Ciliary tuft deflected toward the stereociliary side of the cell. The kinocilium does not follow the bending pattern of the stereocilia and its base is raised. (b) The kinocilium is broken away at the cell surface leaving a stump (arrow) and allowing the visualization of the pliable portions of the membrane at the base of the kinocilium. (c) Scanning electron micrograph from frog sacculus showing two opposing ciliary tufts near a dividing line. The longer stereocilia are at opposite poles of these two tufts. Bending of the cilia is due to inversion of the head during fixation. In the top receptor cell the bending is away from the kinocilium, while at the bottom it bends toward the kinociliary side. (d) Transmission electron micrograph of the kinociliary bulb showing the attachment between the kinocilium and an adjacent stereocilium. (e) Scanning electron micrograph of frog saccular receptor cell which has been fixed by inversion of the head so that hairs are bent toward the direction of the kinocilium. The base of the kinocilium is depressed into the cells in a region that corresponds to the cuticular notch.

reociliary apices. In each case, examination by means of TEM shows distinct filaments between the kinocilium and up to five adjacent stereocilia (*8*).

The presence of such an attachment causes the base of the kinocilium to dimple the surface of the receptor into the cuticular notch (Figs. 2e and 3d) when the ciliary tufts are bent toward the kinocilium (Fig. 2c, top, and Fig. 3a). The same force acting on the ciliary tuft that is oriented in the opposite direction (Fig. 2c, bottom) causes the shaft to raise the base of the kinocilium (Fig. 2, a and b, and Fig. 3e).

The membrane surrounding the base of the kinocilium is particularly interesting (Fig. 2b) since it lies over the cuticular notch and, therefore, is far more easily deformed than the membrane over the cuticular base (Fig. 3, d–f). At rest the kinocilium produces a small dimple in the plasma membrane (Fig. 3f) which may act as a bias, resulting in the spontaneous activity of afferent fibers which was first observed by Löwenstein and Sand (*1*). Plunging action of the kinociliary base generates a distension of the membrane which results in a larger dimple over the cuticular notch (Figs. 2e and 3d). On the other hand, a withdrawing motion of the kinocilium reduces the distension of the membrane and the surface becomes convex (Fig. 2, a and b, and Fig. 3, b and e). It is postulated that this distension of the receptor membrane produces changes in ionic conductance which would lead to depolarization of the hair cell and, by means of an increase in the release of a synaptic transmitter, would increase the response in the vestibular nerve. Conversely, a reduction of the amount of dimpling would decrease the depolarization and reduce the activity (*1*) in the vestibular nerve.

Deformation-sensitive membranes have been demonstrated in other mechanoreceptors (*12*). In the pacinian corpuscle, which is an elliptical cylinder (*13*), force applied along the main cross-sectional axis generates receptor hyperpolarization. The opposite effect, a depolarization, is observed if the force is applied 90 deg from this point (that is, along the smaller cross-sectional axis) (*14*). According to Ilyinsky (*14*), these directional effects are subserved by membrane conductance changes produced by distension. Conductance is assumed to increase when the receptor is flattened from its elliptical shape. Deformation, which tends to make the receptor more cylindrical, hyperpolarizes the receptor by reducing the distension and thus the ionic conductance (*14*). In the auditory system, Davis (*7*) depicts transduction as being produced by ionic conductance changes across the membrane of the hair cell between the endolymph and the cytoplasm. Support for an ionic mechanism in the vestibular system has been given by Matsuura *et al.* (*15*). The morphological features and action of the ciliary tuft indicates a mechanical apparatus which could have a directional effect on a stretch-sensitive membrane.

The homologous mechanism in the mammalian auditory system, where the kinocilium is lacking but the basal kinociliary remnant is present and similarly placed in the cuticular notch (*4*, *16*), could be envisaged as deformation of this diaphragm-like, pressure-sensitive spot.

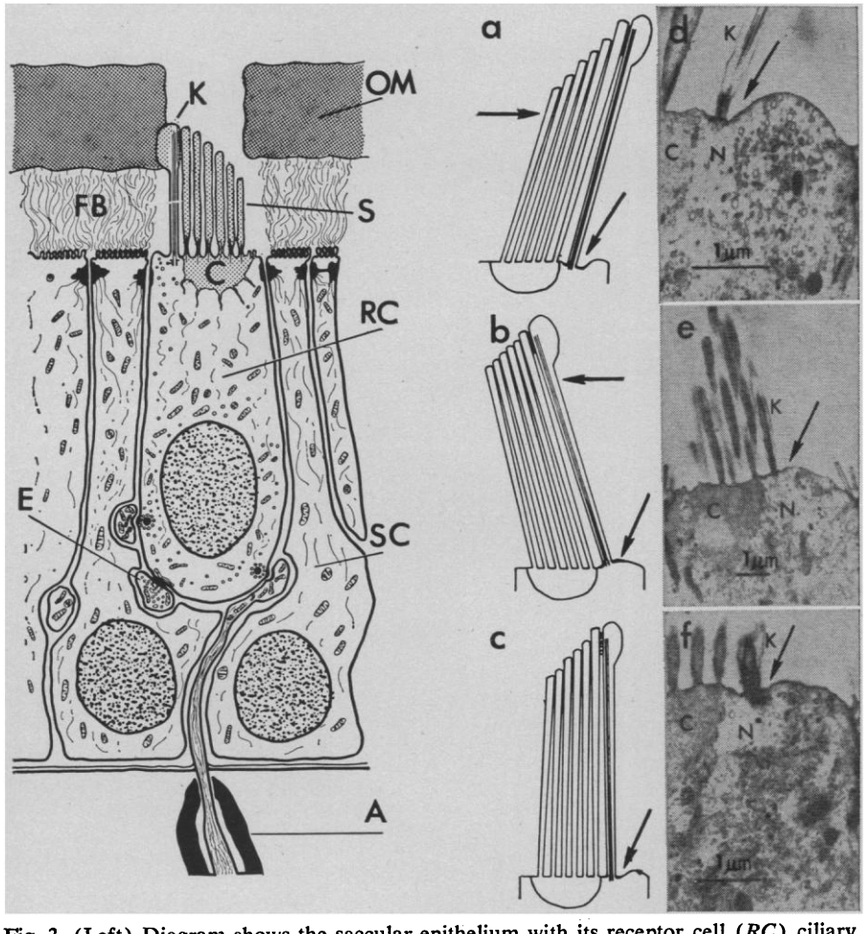

Fig. 3. (Left) Diagram shows the saccular epithelium with its receptor cell (*RC*) ciliary apparatus [kinocilium (*K*), stereocilia (*S*), cuticle (*C*)] and otolithic membrane (*OM*), and the filamentous base (*FB*) which supports the otoliths. (*E*) Efferent endings; (*A*) afferent ending. (Right) Diagrams and electron micrographs to show the effect of bending the cilia toward and away from the kinocilium. The relatively firm cuticular base (d, e, and f) and the attachment of the kinocilium to adjacent stereocilia (a, b, and c) causes the pliable receptor cell membrane in the region of the cuticular notch (*N*) to be thrust up or down with respect to the movements (a and d, and b and c). In the vertical position a slight dip is usually noted in both scanning and transmission electron microscopy (c and f). *K*, kinocilium; *C*, cuticle.

D. E. Hillman
Division of Neurobiology, Department of Physiology and Biophysics, University of Iowa, Iowa City 52240
E. R. Lewis
Department of Electrical Engineering and Computer Sciences, University of California, Berkeley 94720

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17. We thank Dr. R. Llinás for his constructive criticism and encouragement and Dr. T. E. Everhart for use of the scanning electron microscope facility. This research was supported in part by a special grant from the AMA/ERF Institute for Biomedical Research, NSF grant GK-3845, and PHS grant GM-17523.

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Molecular Structure of Starch-Type Polysaccharides from Hericium ramosum and Hericium coralloides

Abstract. *Starch isolated from the fungi* Hericium ramosum *and* Hericium *coralloides differs from that of higher plants in that it consists only of short-chain amylose molecules (32 to 45 glucose units long).*

Despite the use of amyloidity as a taxonomic character in fungi since 1869 (*1*) the chemical structure of the "starch" has not been investigated. Among the Ascomycetes three major families of Pyrenomycetes are characterized by amyloid apical rings in the wall of the asci (*2*) and nine families of Discomycetes feature amyloidity in the ascus wall (*3*). Among the Basidiomycetes, amyloidity occurs in the spore walls of 35 genera of Agaricales (although in a relatively small number of species) and of at least 20 genera of the Aphyllophorales. Fungal starch differs from typical plant starch in three ways. (i) It is not produced in plastids; (ii) it is not found in granular form; and (iii) it is apparently a cell wall component (*4*). Consequently it seemed essential to determine the molecular structure of this anomalous starch. Specimens of *Hericium* spp. offer unique material for such analysis because the amyloid material is stored in the hyphal walls of the fruit bodies as well as in the spores.

Portions of dried fruiting bodies of *Hericium ramosum* and *H. coralloides* were ground up and extracted in hot water for 5 minutes. The cooled, filtered extract was made to 80 percent ethanol, and the resulting precipitate was freeze-dried. The freeze-dried material was soluble in cold water and gave a purple color with iodine. Both salivary amylase and β-amylase (*5*) completely degraded the fungal polysaccharide, as evidenced by the progressive loss of iodine staining properties. Chromatography (*6*) of the β-amylase digest showed that the polysaccharide had been completely degraded to maltose. Consequently the polysaccharide extracted from *H. ramosum* and *H. coralloides* is a linear starch-type molecule containing α-1,4 glucosidic linkages only. The peak value (*7*) of the iodine spectrum was 540 nm indicating an average chain length for the amylose of about 32 glucose units in the *H. ramosum* extract. The *H. coralloides* amylose showed a peak value of 555 nm indicating an average chain length of 38 glucose units. This result contrasts with typical plant amyloses, which have chain lengths from 600 to 4000 glucose units, depending upon the source (*8*).

Not all of the freeze-dried polysaccharide of *H. ramosum* was soluble in cold water; some could only be dissolved in hot water or 1*N* NaOH. Analysis of the iodine spectra of the amylose soluble in hot water indicated an average chain length of about 45 glucose units. Thus the amylose of *H. ramosum* can be readily separated into two fractions, each having average chain lengths of 32 and 45 glucose units, respectively.

The initial treatment of the fungal tissue with hot water may have led to preferential leaching out of amylose molecules as it does from starch granules. However, overnight extraction of the tissue with 1*N* NaOH (which would completely solubilize starch granules) yielded a carbohydrate fraction identical with that from extraction with hot water. Consequently the starch of *H. ramosum* and *H. coralloides* consists of amylose only.

Since they produce only amylose, these organisms may be useful in resolving a fundamental problem of starch biosynthesis—namely, whether starch is produced by debranching of glycogen (*9*) or by initial synthesis of amylose, some of which may later be converted to branched amylopectin molecules (*10*). These organisms could also be used to study regulation of enzyme activity in vivo in that the amylose molecules produced are very short even though both starch phosphorylase (E.C. 2.4.1.1) and adenosine diphosphoglucose–glucosyltransferase (E.C. 2.4.1.b) can extensively elongate a linear molecule in vitro (*11*).

D. A. McCracken
J. L. Dodd
Department of Biological Sciences, Illinois State University, Normal 61761

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