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Stereocilia mediate transduction in vertebrate hair cells

(auditory system/cilium/vestibular system)

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ABSTRACT **The vertebrate hair cell is a sensory receptor that responds to mechanical stimulation of its hair bundle, which usually consists of numerous large microvilli (stereocilia) and a single true cilium (the kinocilium). We have examined the roles of these two components of the hair bundle by recording intracellularly from bullfrog saccular hair cells. Detachment of the kinocilium from the hair bundle and deflection of this cilium produces no receptor potentials. Mechanical stimulation of stereocilia, however, elicits responses of normal amplitude and sensitivity. Scanning electron microscopy confirms the assessments of ciliary position made during physiological recording. Stereocilia mediate the transduction process of the vertebrate hair cell, while the kinocilium may serve primarily as a linkage conveying mechanical displacements to the stereocilia.**

Mechanical stimuli reach hair cells, the sensory receptors of the vertebrate inner ear and lateral line system, through hair bundles at their apices. These bundles generally consist of numerous, closely packed microvilli, termed stereocilia, and a single true cilium, the kinocilium. The association of ciliary derivatives with sensory transduction in various other sensory systems (1, 2), including the hair cells of invertebrates (3), has led to the suggestion that the kinocilium is the essential component of the transduction apparatus in vertebrate hair cells (4), and to uncertainty as to how transduction occurs in the mammalian cochlea, whose hair cells lack kinocilia (5). By microdissection of hair bundles in living vestibular hair cells from the bullfrog, we have found that transduction does not require a normal relationship of the kinocilium to the stereocilia; indeed, stereocilia by themselves suffice to mediate a response.

MATERIALS AND METHODS

Sacculi were dissected from the vestibular apparatus of the inner ear of the bullfrog (*Rana catesbeiana*) and maintained *in vitro* for electrophysiological recording as described (6). The otolithic membrane, a sheet of proteinaceous material to which the distal tips of the hair bundles normally adhere, was dissected away after a 30-min digestion in collagenase (3 mg/ml, 22°C, type I, Sigma). This proteolytic procedure loosens the attachments of hair bundles to the otolithic membrane and reduces trauma to the cells when the membrane is removed.

The preparation was placed in a 0.5-ml experimental chamber and superfused with a saline solution containing 113 mM Na^+, 2 mM K^+, 4 mM Ca^{2+}, 123 mM Cl^-, and 3 mM D-glucose, buffered to pH 7.3 with 1 mM Hepes. Individual hair cells were then impaled with fine glass microelectrodes bent to allow vertical penetrations during visual observation through a ×40 water-immersion objective lens and Nomarski differential interference optics (7).

Each impaled cell was mechanically stimulated with a glass probe, about 2.5 μm in diameter at its end, which engaged the distal tip of the hair bundle. In some experiments, the stimulus probe terminated as a hollow tube that engulfed the end of the hair bundle (6). In other cases a blunt stimulus probe, rendered "sticky" by either of two procedures, adhered to the hair bundle. In one procedure, probes were covalently derivatized with charged amino groups by refluxing for 8 hr at 111°C in 10% γ-aminopropyltriethoxysilane (Pierce) in toluene. Such probes presumably bond to negative surface charges on the hair cell membrane. Alternatively, stimulus probes were made adherent by treatment with 1 mg/ml solutions of lectins (concanavalin A, grade IV, or castor bean lectin, type II; Sigma), which evidently bind to sugars on the cell surface. Probes of either type adhere well to kinociliary bulbs, but not to stereocilia or kinociliary shafts.

Microdissection and stimulation were performed with glass probes mounted on micromanipulators constructed from piezoelectric bimorph benders (Vernitron Piezoelectric Division, Bedford, OH). Each manipulator consisted of two or three bimorphs mounted so as to generate displacements along perpendicular axes; linkages between bimorphs were constructed of balsa wood, and hinges, of silicone cement. A joystick controlled the micromanipulator used for microdissection; signal generators delivered stimulus signals to the stimulating manipulator, whose output was calibrated under microscopic observation.

After physiological recordings, preparations were fixed for 30–100 min at 4°C in 40 mM OsO_4 buffered with 80 mM sodium cacodylate to pH 7.3 and containing 10 mM $CaCl_2$. Specimens were dehydrated in ethanol, critical-point dried from liquid CO_2, sputter-coated with gold, and photographed in an Autoscan scanning electron microscope (ETEC Corp., Hayward, CA). The collagenase treatment employed in loosening the otolithic membrane proved valuable in histology inasmuch as it greatly reduced the amount of fibrillar material adherent to hair bundles and to the apical surfaces of hair cells.

RESULTS

Hair cells maintained *in vitro* under the experimental conditions give resting potentials of 55–70 mV when stably penetrated. Mechanical stimulation of their hair bundles with a stimulus probe (Fig. 1*c*) evokes receptor potentials that are ordinarily about 10 mV in amplitude (Fig. 2, trace a) but occasionally reach as much as 24 mV. Stimuli directed toward the kinocilium cause depolarization, while those directed towards the stereocilia produce a hyperpolarization (6); orthogonal stimuli yield little or no receptor potential. The operating range over which deflections of a hair bundle's tip elicit a changing response is roughly 0.5 μm; stimuli exceeding this range saturate the transducer, producing maximally depolarized or hyperpolarized responses.

Hair bundles were separated into their component kinocilia and stereocilia in either of two ways. In a small fraction (roughly 1%) of collagenase-treated cells, the kinocilium lost its close association with the remainder of the hair bundle and could be

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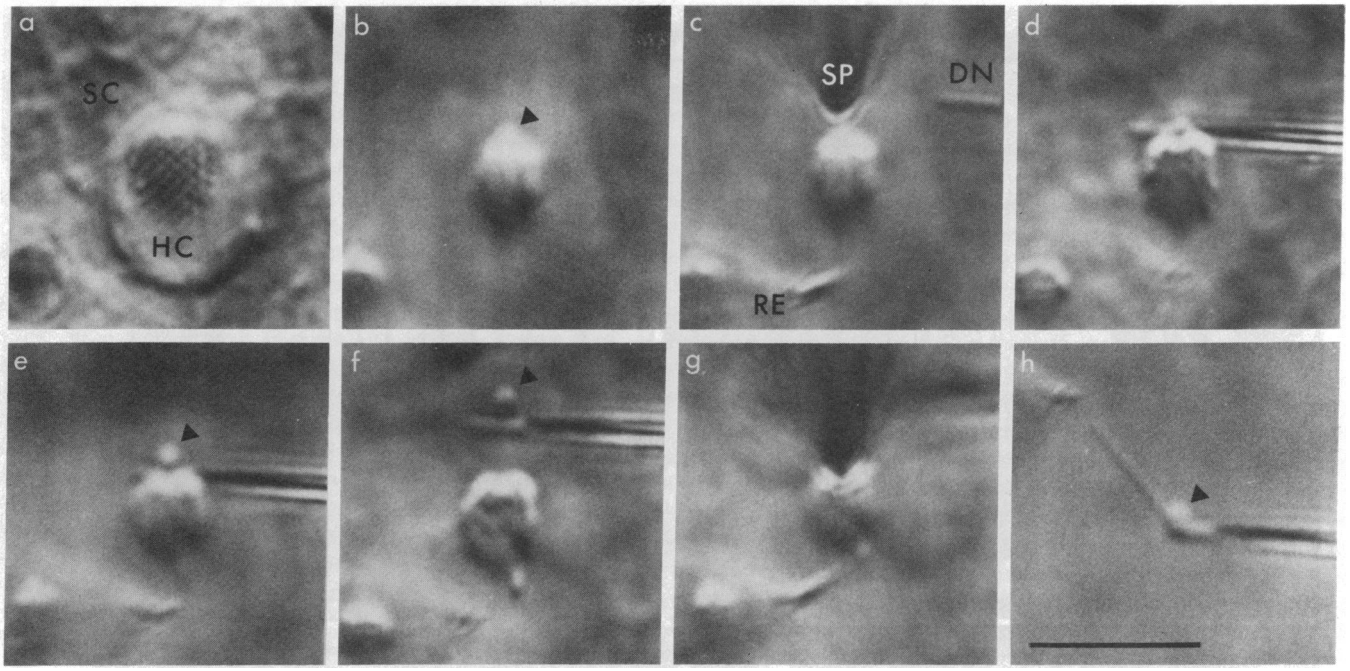

FIG. 1. Nomarski light micrographs of a saccular hair cell during electrophysiological recording and dissection of the hair bundle. (×2200; scale bar = 10 μm.) (*a*) A large hair cell (HC) with trigonally arrayed stereocilia is surrounded by supporting cells (SC); a small hair cell lies at the lower left. (*b*) The same cell at a focal level 7 μm above the foregoing; the kinociliary bulb (▼) protrudes from one side of the hair bundle. (*c*) The normal recording configuration, with a recording electrode (RE) penetrating one edge of the cell's apex and a lectin-coated stimulus probe (SP) attached to the kinociliary bulb. The horizontally oriented dissecting needle (DN) is also shown. (*d*) The dissecting needle is passed between the shaft of the kinocilium and the cluster of stereocilia; the focal level is midway between the hair bundle's base and tip. (*e*) As the kinocilium is displaced from the hair bundle by the dissecting probe, its bulb (▼) becomes more obvious. (*f*) The kinocilium (bulb at ▼) has been displaced from the remainder of the hair bundle and is held by the dissecting needle. (*g*) The remaining cluster of stereocilia is stimulated by pushing the longest stereocilia from the side opposite the kinocilium. (*h*) The kinocilium, which in this case broke free of the cell during dissection, is shown attached to the dissecting needle. Note that the cilium, including its bulb (▼) is over 8 μm in length, and thus fractured at or near its base.

manipulated independently of the stereocilia. Alternatively, it was possible, by employing a glass microelectrode with a broken tip as a dissecting needle, to pull an individual kinocilium away from the accompanying stereocilia (Fig. 1). This was most reliably accomplished by insinuating the dissecting needle horizontally between the shaft of the kinocilium and the stereocilia, about halfway up the hair bundle (Fig. 1*d*). Gentle traction on the kinocilium usually separated its distal end from the stereociliary cluster (Fig. 1 *e* and *f*). The free kinocilium could then be manipulated with the stimulus probe or held horizontally, flat against the epithelial surface, by the dissecting needle. The loosened kinocilium often remained rather stiff and firmly attached at its base; released by the probes, it would, over a period of a few seconds, gradually resume its erect position at one edge of the hair bundle.

No significant intracellular receptor potential (<1 mV) was observed when a loosened kinocilium's tip was deflected by a stimulus probe, in a plane parallel to the epithelium, through distances (3 μm) much larger than the operating range of intact cells. Because their terminal bulbs adhered tightly to "sticky" stimulating probes, kinocilia could also be stretched by vertical movements of the stimulating probes; again, no response occurred. Loose kinocilia could be held flat against the epithelium without producing any response. Extensive micromanipulation, strong tension, and large stimuli occasionally detached kinocilia from their hair cells altogether (Fig. 1*h*); a few cells in the *in vitro* preparations lacked a kinocilium even prior to such manipulation.

When the stimulus probe was applied to a residual hair bundle consisting wholly of stereocilia (Fig. 1*g*), a definite receptor potential was observed. Both static and dynamic stimuli were transduced, yielding peak-to-peak responses as large as 13 mV (Fig. 2, trace b). As with normal cells, deflections toward the site of the kinocilium produced depolarizations, while deflections in the opposite direction elicited hyperpolarizations; orthogonal stimuli were ineffective. The total operating range over which stimulation of dissected hair bundles yielded a response was about 0.5 μm, as was found for normal cells. Whether the loose kinocilium was left undisturbed, held flat

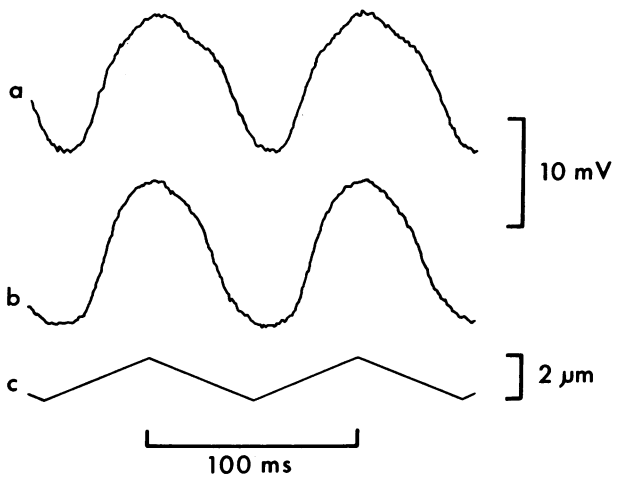

FIG. 2. Receptor potentials obtained from two saccular hair cells, one with a normal hair bundle (a), the other with its kinocilium held flat against the epithelial surface while the stereocilia were stimulated (b). Sixteen responses obtained with 200-MΩ intracellular microelectrodes were digitally averaged to produce each of the traces shown. The stimulus probe deflected the tip of each hair bundle at 10 Hz along the cell's axis of bilateral symmetry; the time course and amplitude of the stimulus is shown (c).

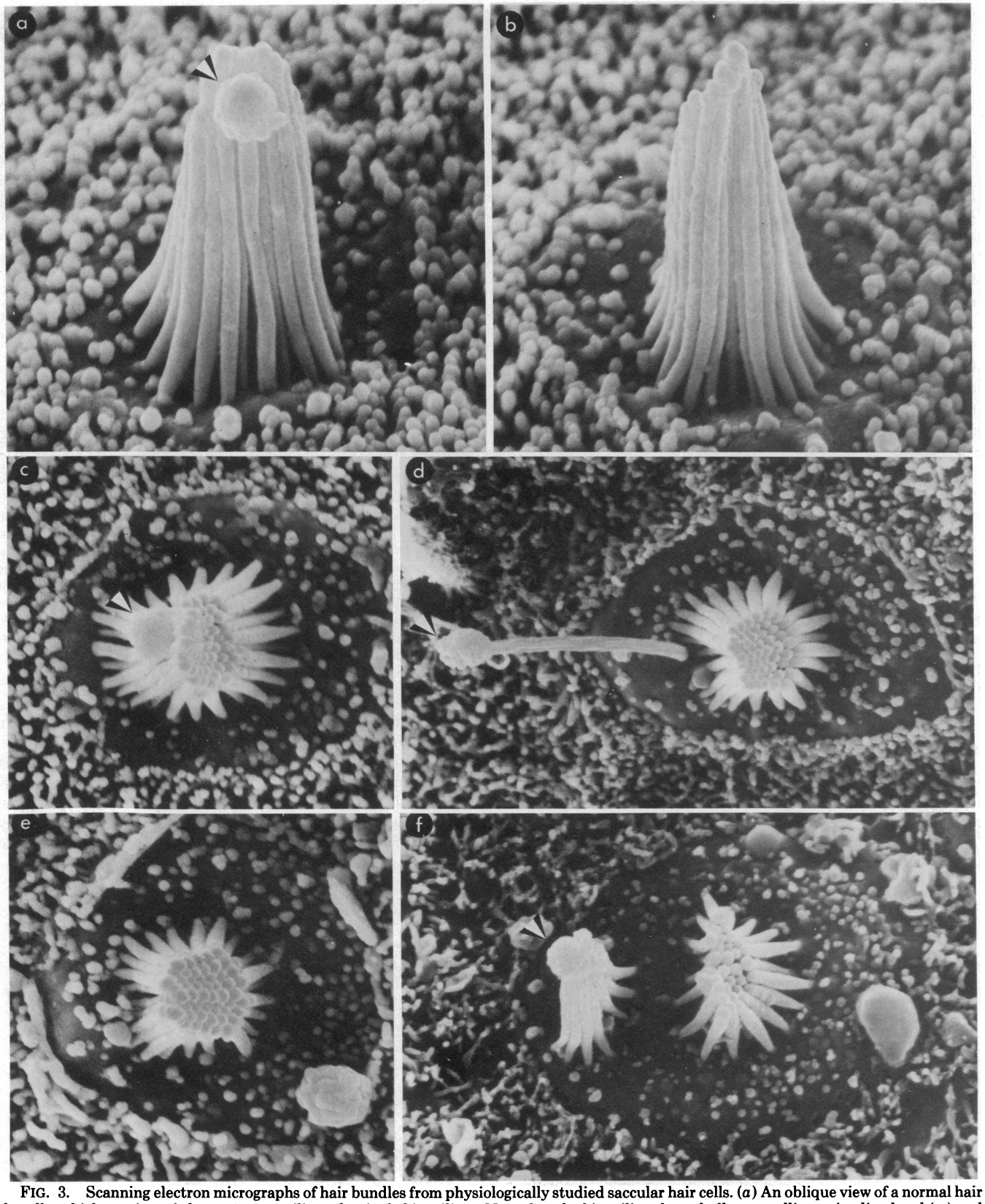

FIG. 3. Scanning electron micrographs of hair bundles from physiologically studied saccular hair cells. (*a*) An oblique view of a normal hair bundle, which consists of about 50 stereocilia and a single kinocilium. Note that the kinocilium has a bulbous swelling at its distal end (▼) and that, unlike the stereocilia, it does not taper at its base. (×12,000.) (*b*) A hair cell whose kinocilium was removed during the experiment; little or no stub remains at the site of separation of the cilium. (×12,000.) (*c*) A normal hair bundle, seen from directly above. The kinocilium is eccentrically located in the bundle; it is distinguished from the stereocilia by its bulbous tip (▼) and its larger basal diameter. (×10,000.) (*d*) A cell whose kinocilium (bulb at ▼) was dissected free and held flat against the epithelial surface during electrophysiological recording. The cell gave normal responses to stimuli. (×10,000.) (*e*) Hair bundle of a cell lacking a kinocilium; this cell also responded normally. (×10,000.) (*f*) Apex of a cell with a bifid hair bundle. One component of the bundle lacks a kinocilium; the other portion has a normal kinocilium with a terminal bulb (▼). Mechanical stimulation of either component elicited responses. (×10,000.)

against the epithelial surface, or actually detached, the response upon stimulation of the stereocilia alone was indistinguishable from that obtained on stimulating intact hair bundles. Similar findings were made, on over 100 hair cells, whether micromanipulation or enzymatic digestion was used to separate the kinocilia from normal hair bundles, and whether hollow-tube or "sticky" stimulus probes were used to stimulate the bundles.

To test whether the condition of the kinocilium was accurately assessed during physiological experiments, cells were examined in the scanning electron microscope. The position of each cell studied was marked at the end of recording by using the stimulus probe, oscillating through a distance of several micrometers at 100 Hz, to shear off the hair bundles of adjacent cells; the preparation was then fixed and prepared for microscopy. Of a total of 38 hair cells treated in this manner, 34 were unequivocally identified in the scanning electron microscope. Of 11 responsive cells whose kinocilia were considered wholly detached during physiology, nine were found to lack kinocilia upon electron microscopic examination (Fig. 3 *b* and *e*); the remaining cells had kinocilia of abnormally small diameter. In several instances the site of the kinocilium was marked by a 0.3-μm-long stump, which suggests that the cilium fractured just above its ciliary necklace. Seven of the nine cells judged at recording to have their kinocilia loosened from the stereocilia were confirmed to be in that state during electron microscopy (Fig. 3*d*); in the other two instances it was likely that the kinocilia became reattached to the stereocilia during fixation. Each of 11 control cells with hair bundles that appeared normal under the light microscope was found to have an intact hair bundle in the scanning electron microscope (Fig. 3 *a* and *c*). It is thus evident that observation with the light microscope permits an accurate estimate of the status of a hair bundle during physiological studies.

The notion that stereocilia are alone sufficient to mediate transduction is also supported by results obtained from four cells with their hair bundles fortuitously divided into two parts. In each instance, stimulation of the short portion of the hair bundle distant from the apparent kinocilium, independently of the longer portion containing the kinocilium, elicited a response. Electron microscopic observations in two instances confirmed that the short bundle components contained only stereocilia (Fig. 3*f*); a third cell was found to have two kinocilia with similar orientations, one in each half-bundle.

Some of the stereocilia in a bundle can also be selectively stimulated after microdissection. After the kinocilium is removed from a hair cell, it is possible, by inserting the dissecting needle horizontally through the residual hair bundle, to divide the cluster of stereocilia. While some stereocilia are held aside with the dissecting needle, others may then be stimulated with the stimulus probe. Under these conditions it was found that clusters of stereocilia can give responses independently of one another. When the hair bundle was split in half, for example, each half was found to be responsive while the other was held stationary. The amplitude of the response to stimulation of half a bundle was roughly half that derived from stimulation of the intact bundle; smaller fractions of the bundle gave smaller responses still. The smallest cluster of stereocilia yet to give a response consisted of a hair bundle's ten shortest stereocilia; stimulation evoked a response 2 mV in amplitude.

DISCUSSION

These observations indicate that an intact kinocilium is not required for the transduction process of the vertebrate hair cell. It remains a possibility that the basal body and/or associated structures of the kinocilium are essential for transduction (8), and that, regardless of the fate of the remainder of the cilium, these structures are effectively coupled to stimuli by motions of the stereocilia, cuticular plate, or surface membrane. The observation that some cochlear hair cells lack basal bodies (5) somewhat diminishes this possibility. The kinocilium might conceivably mediate responses in some particular frequency or amplitude range. Although most cells in the present experiments were stimulated at 10 Hz, several hair cells without kinocilia also responded normally to 100-Hz stimuli. Stereocilia thus suffice for transduction over a broad range of stimulus amplitudes and orientations, and through at least a modest range of frequencies.

It is uncertain what functions kinocilia serve in those vertebrate hair cells that possess them, including the hair cells of the bullfrog's sacculus. Although the kinocilium may be necessary for ontogenetic reasons—e.g., initially to polarize the hair bundle—its role in transduction may be limited to transmitting movements from a cupula or otolithic membrane to the sensitive stereocilia. The various forms that kinocilia assume (9) would then represent adaptations optimized for transmitting particular forms of stimulation to a relatively standardized transducer associated with the stereocilia. The loss of the kinocilium in the mature mammalian cochlea (5), and its regression in the basilar papillae of some reptiles (10) and birds (11), might serve to eliminate an elastic linkage in the interest of improving responsiveness to high-frequency stimuli.

There appears to be a fundamental difference between the hair cells of invertebrates, which usually have only kinocilia (1), and those of vertebrates, at least in the location and perhaps in the operation of the mechanosensitive elements. On the other hand, vertebrate hair cells—in the vestibule as well as in the cochlea—seem to employ a similar mechanism: transduction is mediated by stereocilia, through flexion at their bases, deformation along their lengths, or lateral interactions between one another.

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