outer pair of membranes surrounding the cryptomonad chloroplast (Fig. 4).

Taylor (*18*) considered the possibility that the chloroplast-mitochondrial complexes of *M. rubrum* may have been able to exist separately from the endosymbiont nucleus because of the presence of a nucleomorph. This appears not to be the case with the blue-green chloroplast of *A. wigrense*, as it lacks a nucleomorph and yet exists as a distinct entity. This could indicate that the cryptomonad nucleomorph may not contain information vital to the chloroplast. Alternatively, in *A. wigrense*, such information may have been transferred, probably to the dinoflagellate nucleus. The absence of both a nucleomorph and a periplastidal compartment in the chloroplast of *A. wigrense* supports the idea that the nucleomorph is necessary for the maintenance of the periplastidal compartment (*7*).

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Evoked Mechanical Responses of Isolated Cochlear Outer Hair Cells

Abstract. *Intracellular current administration evokes rapid, graded, and bidirectional mechanical responses of isolated outer hair cells from the mammalian inner ear. The cells become shorter in response to depolarizing and longer in response to hyperpolarizing currents in the synaptic end of the cell. The cells respond with either an increase or decrease in length to transcellular alternating current stimulation. The direction of the movement with transcellular stimuli appears to be frequency dependent. Iontophoretic application of acetylcholine to the synaptic end of the cell decreases its length. The microarchitecture of the organ of Corti permits length changes of outer hair cells in a manner that could significantly influence the mechanics of the cochlear partition and thereby contribute to the exquisite sensitivity of mammalian hearing.*

Two types of mechanosensory hair cells are found in the mammalian inner ear. Outer hair cells (Fig. 1) differ from inner hair cells in their morphology, afferent and efferent innervation, and biochemistry (*1*). Outer hair cells are required for normal cochlear transduction, but their precise role has been the matter of considerable speculation. Modeling efforts argue that the exquisite tuning and sensitivity of the intact cochlear partition is possible only if a negative damping component (a source of active mechanical energy) is present in the organ of Corti (*2*). Acoustic energy of cochlear origin can be measured in the ear canal, and stimulation of efferent fibers terminating on outer hair cells modulates its intensity (*3*), both of which suggest the presence of an active mechanical process associated with outer hair cells.

We developed procedures to dissociate outer hair cells from the guinea pig organ of Corti by techniques routinely used to isolate solitary vertebrate photoreceptors (*4–6*). Intracellular studies were performed with techniques and equipment identical to those used to study photoreceptors (*5*). Transcellular stimulation was achieved by means of a pair of tungsten electrodes placed in the bathing medium (*4*). Micropipettes filled with 2*M* acetylcholine were used for extracellular iontophoretic applications.

Outer hair cells retained their unique morphological features in vitro (Fig. 1). There was almost a fourfold difference in length between the shortest and longest outer hair cells, a range most likely representing cells originating in the base and apex of the cochlea, respectively (*7*). The passive mechanical properties of outer hair cells differed from those of the other cell types in the organ of Corti. Probing the lateral walls between the nucleus to just below the cuticular plate with micropipettes revealed stiffness and resistance to electrode penetration. The unusual mechanical properties of the lateral membranes may be due to a unique complex of organelles, called the laminated cisterna, located immediately beneath the cytoplasmic membrane in this region of the cell (*8*). Penetration of the cell membrane was achieved most easily in the synaptic region, where the laminated cisterna are not found. Inner hair cell and supporting cell membranes were easily penetrated with the same type of pipette. When a pipette damaged the membrane of a healthy outer hair cell, the cytoplasmic contents were frequently ejected through the resulting hole as if under great pressure. Evidence for a similar cytoplasmic pressure was not observed for inner hair cells or supporting cells.

When current was applied intracellularly (*9*) to the synaptic end of outer hair cells, a conspicuous mechanical response resulted. A 200-msec depolarizing current step resulted in a rapid decrease in cell length and increase in its width. Hyperpolarizing currents evoked the opposite response, an increase in length and a decrease in width. The shape change was not confined to a specific location along the cell but seemed to be distributed over the entire length of the cell between the nucleus and the cuticular plate. The mechanical response was dramatic and easy to detect despite the fact that the absolute differences in cell length were under 1 μm. The magnitude of the response in either direction was graded; at the threshold for visual detection [using ×250 magnification with Hoffman optics (*5*)] the stimulating current was between 100 and 200 pA, with smaller cells requiring lower injection currents. The shape change seemed to be maintained

throughout the current step. A slight response decrease throughout the duration of the current step from a maximum at stimulus onset was associated with larger stimulus currents. Response speed seemed to be similar in both directions, although precise measures of its time course were not made (*10*). For current steps near the visual detection threshold, the cell would return to its prestimulation shape directly after the end of the stimulus. If currents in excess of 500 pA were used, complete recovery was seldom achieved and the cell would change its shape over repeated stimulations, typically becoming shorter and wider.

Motion was evoked in cells maintained in vitro as long as 48 hours after being removed from the cochlea. Many cells with damaged or missing stereociliar bundles still showed a robust mechanical

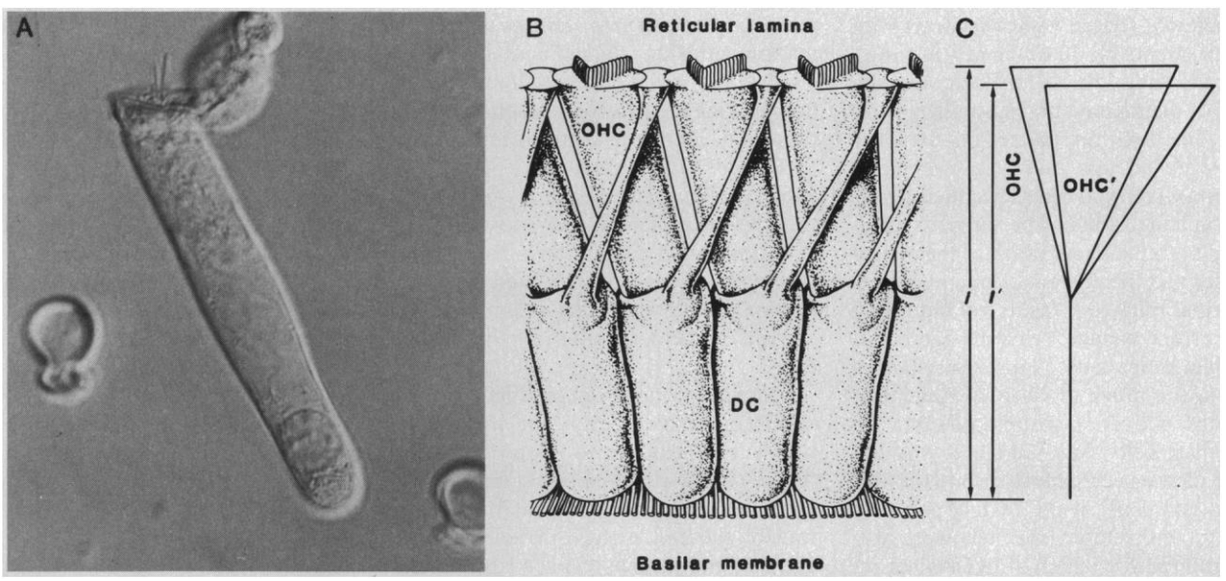

Fig. 1. (A) An outer hair cell isolated from a guinea pig organ of Corti, displaying the elongated cylindrical shape and the eccentrically placed nucleus characteristic of the outer hair cell. Note the collection of organelles beneath the nucleus at the synaptic region of the cell as well as the increase in organelle density towards the cuticular plate at the other end of the cell. Some of the mechanoreceptive stereocilia can be seen emerging from the cuticular plate. The photomicrograph was made using a Zeiss ×63 planapochromat objective and Nomarski interference contrast optics after the cell had been maintained several hours in vitro (diameter of nucleus, 9.1 μm). (B) Microarchitecture of a portion of the cochlear partition, representing a single row of outer hair cells as it would appear if viewed from slightly above the organ of Corti looking toward the central axis of the cochlear spiral from outside the bony capsule. This view reveals how the microarchitectural features of the organ of Corti permit free movement of outer hair cells (*OHC*) along their length. Outer hair cells make contact with afferent and efferent eighth nerve terminals and Deiters' cells (*DC*). Nerve terminals are found on the outer hair cell below the level of its nucleus, and the entire synaptic complex rests in a cup formed by the Deiters' cell. Each Deiters' cell sends a phalangeal process that angles toward the base of the cochlea, inserting between two adjacent outer hair cells. The precise cellular arrangement shown in the drawing is repeated more than 1000 times along the spiraling length of the organ of Corti. There are three parallel rows of the outer hair cells and one row of inner hair cells. The stereociliar end of the outer hair cells and the portion of the Deiters' cell phalangeal process to which they are attached make up the reticular lamina. (C) A decrease in outer hair cell length (*OHC* to *OHC'*) results in a decrease in the separation between the basilar membrane and the reticular lamina (*l* to *l'*).

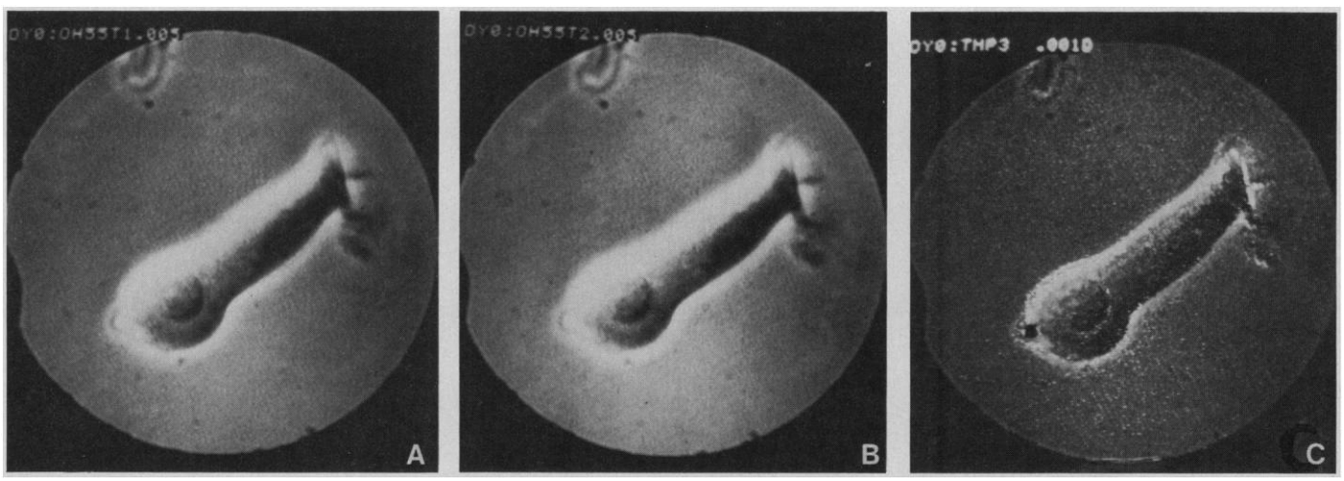

Fig. 2. Increase in length of an isolated outer hair cell in response to transcellular alternating current stimulation at 4 kHz. Digitized video images of an isolated outer hair cell before (A), during (B), and after (C) being stimulated. In (C), the difference between (B) and (A) is superimposed on the poststimulus image, which results in the white line adjacent to the cuticular plate and represents a length increase of less than ½ μm during stimulation (diameter of nucleus, 8.7 μm). The length of the same cell decreased in response to a 400-Hz stimulus. Fine tungsten wire electrodes were located along the major axis of the cell outside the image field. Images were processed by a KUKAM video memory and processor attached to a Wild inverted microscope, with phase contrast optics.

response. We were unable to observe an evoked inner hair cell or supporting cell movement nor were we able to observe stimulus-related stereociliar movements. Evoked motion in these structures, if it exists, may be too small to be observed with optical microscopy.

Motile outer hair cells had resting membrane potentials that were typically between -6 and -12 mV and rarely greater than -20 mV. These values contrast with measurements of -30 to -50 mV for inner hair cells and -40 to -80 mV for supporting cells maintained under similar conditions. The input resistances of outer hair cells were so low that relatively large currents were required to produce significant voltage shifts. The currents challenged both the linear range of the micropipettes and the current clamp amplifiers (*5*) so that reliable measures of outer hair cell current-voltage relations were not possible. Inner hair cell input resistances were in the range of 20 to 40 megohms. The absence of a resting potential in outer hair cells was related to the absence of the mechanical response. Motion would cease if the resting potential went to zero. Outer hair cells were also encountered for which a mechanical response was not observed even though a resting membrane potential was measured. Outer hair cells with membrane potentials greater than -20 mV tended not to move. We were able to record stable resting membrane potentials and observe bidirectional motile responses for as long as 20 minutes in several cells.

Transcellular stimulation with sinusoidal alternating current was also effective in evoking motile responses in outer hair cells (Fig. 2). The position of the stimulating electrodes with respect to the major axis of the cell was important. The optimal electrode placement—that which produced the greatest potential gradients along the length of the cell—was achieved by placing both electrodes on the long axis of the cell, one near the stereociliar end and the other near the synaptic end. We were unable to determine the presence of cycle-by-cycle synchronized movements, but d-c responses of a magnitude comparable to those evoked by intracellular currents were observed. Effective frequencies were >100 Hz and <100 kHz. The optimal frequency for evoking a response varied from cell to cell and for the same cell over time. The alternating current response displayed a frequency-dependent bidirectional feature in several cells. These cells became shorter in response to one stimulus frequency and longer in response to another (Fig. 2).

Iontophoretic application of acetylcholine, the probable neurotransmitter of the efferent fibers that synapse on outer hair cells (*11*), to the synaptic end of the cell caused cells to become shorter. This response was not observed when similar currents were passed from micropipettes that did not contain acetylcholine, nor was it observed if acetylcholine was introduced by iontophoresis on the cell at locations away from the synaptic end. The transcellular electrical and acetylcholine-evoked responses were less conspicuous than those evoked by intracellular stimulation because their time course was longer. Time-lapse video processing was often required for a convincing demonstration of their presence.

Among the more remarkable features of the outer hair cell is the manner in which it is attached to supporting cells (Fig. 1). Cell attachment occurs only at the ends of the cell, so that the lateral wall of the cell is surrounded by large extracellular spaces (spaces of Nuel). The volume of the extracellular space found in most mammalian organs and around inner hair cells is several orders of magnitude smaller. The observation of an active mechanical response in outer hair cells suggests that the functional significance of the spaces of Nuel is to permit the free movement of the receptor cells. A change in length of the hair cells would result in a change in the separation between the reticular lamina and basilar membrane (Fig. 1) that would influence the mechanosensitive stereocilia and modulate the compliance of the cochlear partition. The triangle formed by an outer hair cell, the phalangeal process of the Deiters' cell on which it sits, and that portion of the reticular lamina between the phalangeal process and the hair cell can be considered a mechanical unit within the cochlear partition. Increases in outer hair cell length would make this unit more rigid whereas decreases would make it more compliant.

These observations provide direct evidence of an active mechanical process in the organ of Corti. The cellular mechanism underlying the observed movements is unknown. Conventional molecular mechanisms as well as the possibility of an electrokinetic ionic process for the motility must be entertained. The length changes may be associated with the source of energy that has been proposed to result in the great sensitivity and narrow tuning of the cochlear partition (*2*). Shape changes induced by acetylcholine are compatible with the fact that stimulation of efferent fibers to the cochlea modulates cochlear transduction (*12*) and the mechanics of the cochlear partition (*3*). The possibility of a sensory-motor interaction in the cochlea similar to the gamma efferent modulation of muscle spindle sensitivity offers a new perspective for understanding the central control of the auditory periphery.

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