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Ca^{2+} Signaling in the Inner Ear

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The inner ear contains delicate sensory receptors that have adapted to detect the minutest mechanical disturbances. Ca^{2+} ions are implicated in all steps of the transduction process, as well as in its regulation by an impressive ensemble of finely tuned feedback control mechanisms. Recent studies have unveiled some of the key players, but things do not sound quite right yet.

Sensory transduction in the inner ear (FIGURE 1) is the job of hair cells that are grouped into three types of sensory epithelia. The organ of Corti is the epithelium of the mammalian cochlea (hearing); the maculae and the cristae are the epithelia of the vestibular system (balance). The epithelia are polarized in that sensory hair cells as well as nonsensory (supporting) cells are divided into apical and basolateral domains. Diffusion of proteins between these subcellular domains is prevented by tight junctions between neighboring cells, which also serve to insulate endolymph, an extracellular fluid low in Na^+ and Ca^{2+} but rich in K^+, from perilymph, the normal extracellular fluid that bathes the basolateral membrane of the cells (FIGURE 1A).

Mechanical stimuli that can be ultimately ascribed to sound, for the cochlea, or acceleration, for the vestibular system, are applied to the hair cell mechanoreceptor organelle, composed of 20–300 actin-filled stiff microvilli, called the stereocilia, that reach into endolymph from the cell's apical surface and are arranged in three to four rows of increasing height. Stimulation of sensory transduction results in an increased flux of K^+ from endolymph through the hair cells, for hair bundle deflection modulates the open probability of specialized cation-selective mechanosensitive transduction (MET) channels found in the stereocilia (FIGURES 1A AND 2A). By altering the ratio of apical to basolateral membrane impedance, MET channel opening (closing) decreases (increases) the potential difference across the basolateral membrane of the hair cell. The ensuing graded receptor potential (i.e., the analog modulation of the hair cell resting potential) follows the movements of the stereocilia. Electrical activity in the hair cell is signaled to the central nervous system by afferent fibers of the acoustic or vestibular nerve that synapse to several active zones in the cell basolateral membrane.

With few exceptions, hair cells of the vestibular system in all animals respond to subacoustic frequencies, rarely in excess of a few tens of Hertz, whereas distinct mechanisms have evolved in the auditory periphery to extract sound frequency information over a far broader range. Thus, in the mammalian cochlea primed by sound, hydromechanical interactions excite specific vibration patterns on the basilar membrane, the elastic structure that supports the organ of Corti (FIGURE 1A). Basilar membrane vibrations are enhanced by a physiologically vulnerable mechanism, known as the cochlear amplifier, permitting frequency discrimination with ~1% accuracy over a range that, in humans, covers seven octaves from 40 Hz to 18 kHz. Frequency limits are species specific, and in other mammals the upper boundary may extend beyond a dazzling 100 kHz. In a subset of these frequencies, what might rightfully be defined as the energetic miracle of sound perception enables the detection of just-audible sounds corresponding to pressure changes as small as ~20 μPa (hearing threshold), i.e., 1 part in 5×10^9 of the static atmospheric pressure. At 1 kHz, these minute pressure changes are reciprocally related to air density fluctuations that alter the mean intermolecular distances by ~8 pm, about 1/10 of an atom's diameter. The sensitivity of the human ear is such that, under ideally quiet conditions in a still atmosphere, a 1-W source emitting sound waves at 3.5 kHz could in principle be heard at a distance of 564 km! Indeed, it has been proposed that yodeling, now a type of folk music, was developed as a method of communication between relatively distant mountain peaks of the Swiss Alps.

Though hair cells are the sensors for hearing and balance, several other cell types play essential roles for maintaining the environment necessary for optimal function of the inner ear. For example, vestibular and cochlear endolymph is secreted, respectively, by dark cells and by marginal cells of the stria vascularis. The stria is additionally responsible for maintaining cochlear endolymph at a potential of +80 to +100 mV (the so-called endochlear potential, or EP) relative to perilymph (FIGURE 1A), thus increasing the driving force for K^+. MET channel activation causes the hair cell to release K^+ ions that are either dispersed into perilymph or taken up by adjacent supporting cells and eventually returned to the stria vascularis. In this complex scenario of vertebrate inner ear, Ca^{2+} ions play numerous and fundamental roles. Here, we summarize the common aspects of transduction that are influenced by Ca^{2+} in vestibular and auditory hair cells, including MET function and neurotransmitter release. In the second part, we concentrate on the auditory periphery, reviewing the control exerted by Ca^{2+} on the cochlear amplifier and ion homeostasis in endolymph.

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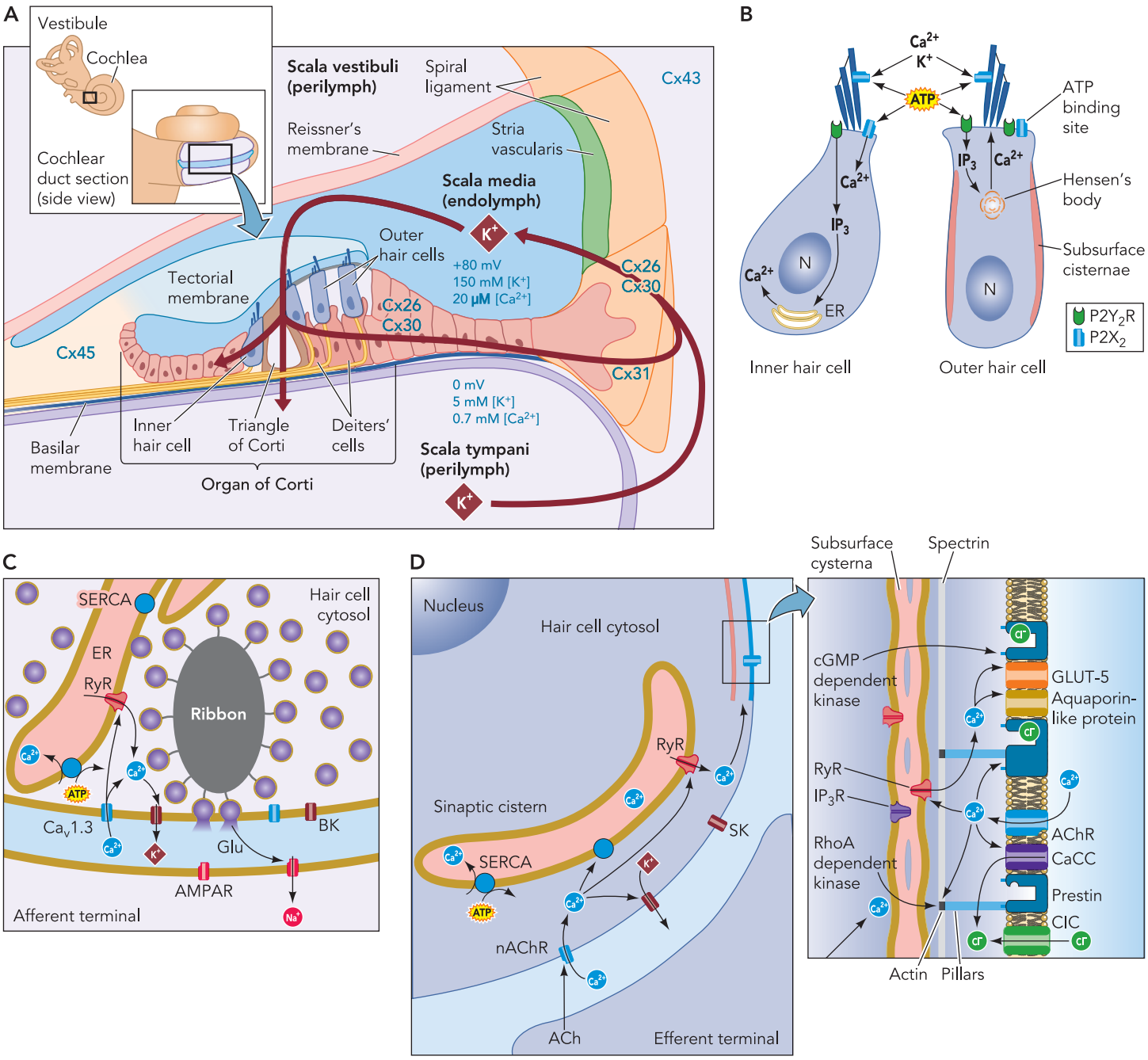

FIGURE 1. **Schematic view of selected Ca^{2+} signaling processes in the inner ear**

A: scheme of the inner ear (*inset*). Endolymphatic (blue) and stria vascularis (SV) compartments are delimited by a tight junction network. Perilymph bathes the rest of the cochlea. Scala media is separated from scala vestibuli by the Reissner's membrane (RM). The organ of Corti rests on the basilar membrane (BM), surmounted by the tectorial membrane (TM). The SV borders the spiral ligament at the lateral wall of the cochlear duct. The organ of Corti hosts sensory inner (IHC) and outer (OHC) hair cells. IHCs mechanotransduction is activated by the fluid viscous drag applied to their stereocilia by the oscillation of the TM. *B*: P2R expression in hair cells. P2YR are associated with release of Ca^{2+} from intracellular stores [endoplasmic reticulum (ER) or Hensen's body], whereas P2XR are localized on the stereocilia of the hair cells, in proximity of the MET channels, as well as in the apical cell surface. *C*: afferent (ribbon) synapse. $[Ca^{2+}]_i$ concentration at the afferent ribbon synapse of the hair cell indirectly controls the activation of glutamate receptors (AMPARs) at the postsynaptic terminal. $[Ca^{2+}]_i$ increase is amplified by CICR from tubular shaped ER extending to within 50 nm of the plasma membrane, close to the ribbon (see Ref. 100). *D*: efferent synapse and CICR. The inhibitory hyperpolarizing potassium current at the OHC efferent synapse is mediated by SK-type Ca^{2+}-activated potassium channels. This mechanism is supplemented and modulated by CICR from a synaptic cistern facing plasma membrane acetylcholine receptors (nAChRs). *Inset*: ACh influences OHC electromotility also by activating Ca^{2+}-dependent signaling pathways mediated by protein kinases that target cytoskeletal and prestin proteins. Function of prestin is influenced by intracellular anion concentrations, in particular Cl^- controlled by Ca^{2+}-dependent (CaCC) and voltage-dependent (e.g., ClC-2) chloride channels. OHC cell volume and turgor is regulated by Ca^{2+}-dependent phosphorylation of an aquaporin-like protein and/or a GLUT-5 sugar carrier, both voltage dependent and located in the lateral plasma membrane.

Ca^{2+} Modulation of Mechanotransduction in Hair Cells

As mentioned in the introduction, hair bundle deflection opens MET channels (FIGURE 2A), one of the few ion channels not yet conclusively identified (6, 26). Stereocilia in hair bundles are organized into rows of graded heights, forming precisely uniform staircase patterns (103). Stimuli that are sensed by hair cells as excitatory deflect a hair bundle toward its tallest stereocilia, thus increasing tension in the tip link, an extracellular filament stretched between the tops of stereocilia in adjacent rows (28, 85). Application of the Ca^{2+} chelator BAPTA to reduce the extracellular calcium concentration ($[Ca^{2+}]_o$) to submicromolar levels cuts the tip links and eliminates transduction (3). Tip-link tension is thought to be conveyed to MET channels through unidentified elastic elements, termed gating springs (78), mechanically in series with the tip links (28, 56). Values of up to two channels per tip link have been estimated for the mammalian cochlea (6), in accord with previous results in nonmammalian vertebrates (36, 76, 133).

Even though K^+ ions carry most of the transduction current, MET channels are highly permeable to Ca^{2+} (43). At the tens of micromolar $[Ca^{2+}]_o$ found in endolymph (12, 27), Ca^{2+} ions carry ~10% of the total MET current (134). But, to complicate matters, it appears that Ca^{2+} ions can both permeate and block the transducer channels (134) by binding with millimolar affinity at a site presumably located halfway through the membrane electric field (40). These and several other observations led to the conclusion that endolymph composition is specialized to allow a compromise between the largest monovalent current and the largest Ca^{2+} influx (43). From a physiological point of view, the key question is the role of Ca^{2+} ions admitted into the stereocilia through MET channels. The most obvious answer is that elevated stereociliary Ca^{2+} levels promote MET channel closure through adaptation, a negative-feedback mechanism that involves a shift of the sensitive range of the MET process (42, 78) (see http://www.rockefeller.edu/labheads/hudspeth/graphicalSimulations.php). Because it is accompanied by the restoration of sensitivity to transient stimuli, adaptation has been postulated to depend on an active motor that readjusts tension in the gating springs (2, 77), whose stiffness may in turn be Ca^{2+} dependent (20). But adaptation is a complex phenomenon in which at least two phases, both Ca^{2+} dependent, can be distinguished on the basis of their different kinetics and mechanical correlates (44, 71, 163). In different species and different epithelia, slow adaptation has a time constant (τ_A) of 10–100 ms (55), whereas fast adaptation in turtle auditory hair cells has millisecond τ_A (at 2.8 mM $[Ca^{2+}]_o$) (135), scaling down to sub-millisecond values in rodents (at 1.5 mM $[Ca^{2+}]_o$) (6, 87, 89). It has been suggested that fast adaptation is mediated by Ca^{2+} entering through the MET channel to shut it by binding at a site on the channel itself or a closely associated intracellular component (20, 29, 136, 163) and that this may tune the channel for small displacements around a resting position that is continually readjusted by slow adaptation (42). However, the calcium-binding site for channel block is thought to be distinct from that mediating fast adaptation (40). The alternative proposal is that of a unified mechanism for both fast and slow adaptation (73, 151) that involves the operation of an adaptation-motor complex formed by myosin-1c (Myo1c) (55), one of the several unconventional myosin isoforms of the stereocilia (43, 65). Myo1c in this scheme interacts in a Ca^{2+}-dependent manner with the rigid cross-linked actin filaments at the core of the stereocilia to adjust the force delivered by the tip link to the MET channel (FIGURE 2). In addition, phosphatidylinositol 4,5-bisphosphate (PIP2) binds directly to the IQ domains of Myo1c, regulating the motor activity, and consequently also transduction and adaptation (70). Ca^{2+} may also influence the activity of MET channels in an indirect way, i.e., via cAMP, because the rise in stereociliary $[Ca^{2+}]$ can activate a Ca^{2+}-calmodulin-activated type I adenyl cyclase of the hair cells (37, 54, 135) followed by activation of protein kinase A and phosphorylation of relevant targets. In summary, adaptation *1*) provides a transient response to slow or static stimuli, emphasizing changes in force; *2*) positions the transduction element in the most sensitive part of its activation curve to maximize responsiveness, both during development and during physiological stimuli; and *3*) prevents saturation of the transducer with larger stimuli (20).

Despite the staggering amount of heroically accumulated data on MET function, several aspects require further investigation and clarification. For instance, in virtually all types of hair cells studied so far, fast and slow adaptation are readily discriminated only for low-amplitude stimuli delivered (in the excitatory direction) under millimolar $[Ca^{2+}]_o$. Indeed, fast adaptation seemingly requires a well-defined range of stereociliary Ca^{2+} levels, but whether this range occurs under physiological conditions in vivo remains an open question. Thus, at 100 μM $[Ca^{2+}]_o$, MET currents of hair cells from mouse utricular macula or bullfrog saccular macula show negligible adaptation (20, 72), consistent with single MET channel recordings of frog auditory hair cells at 50 μM $[Ca^{2+}]_o$ or +80 mV holding potential (133). For larger displacements of the hair bundle, the time course of decline in the MET current slows down considerably, suggesting a prevalence of the slow component of adaptation (44). But MET current kinetics slow down also when the driving force for Ca^{2+} entry is reduced either by depolarizing the hair cell or by reducing $[Ca^{2+}]_o$ from nonphysiological millimolar levels to values between 20 and 100 μM that better approximate those found in endolymph (43). Furthermore, reducing $[Ca^{2+}]_o$ to near endolymphatic values in auditory hair cells of turtles

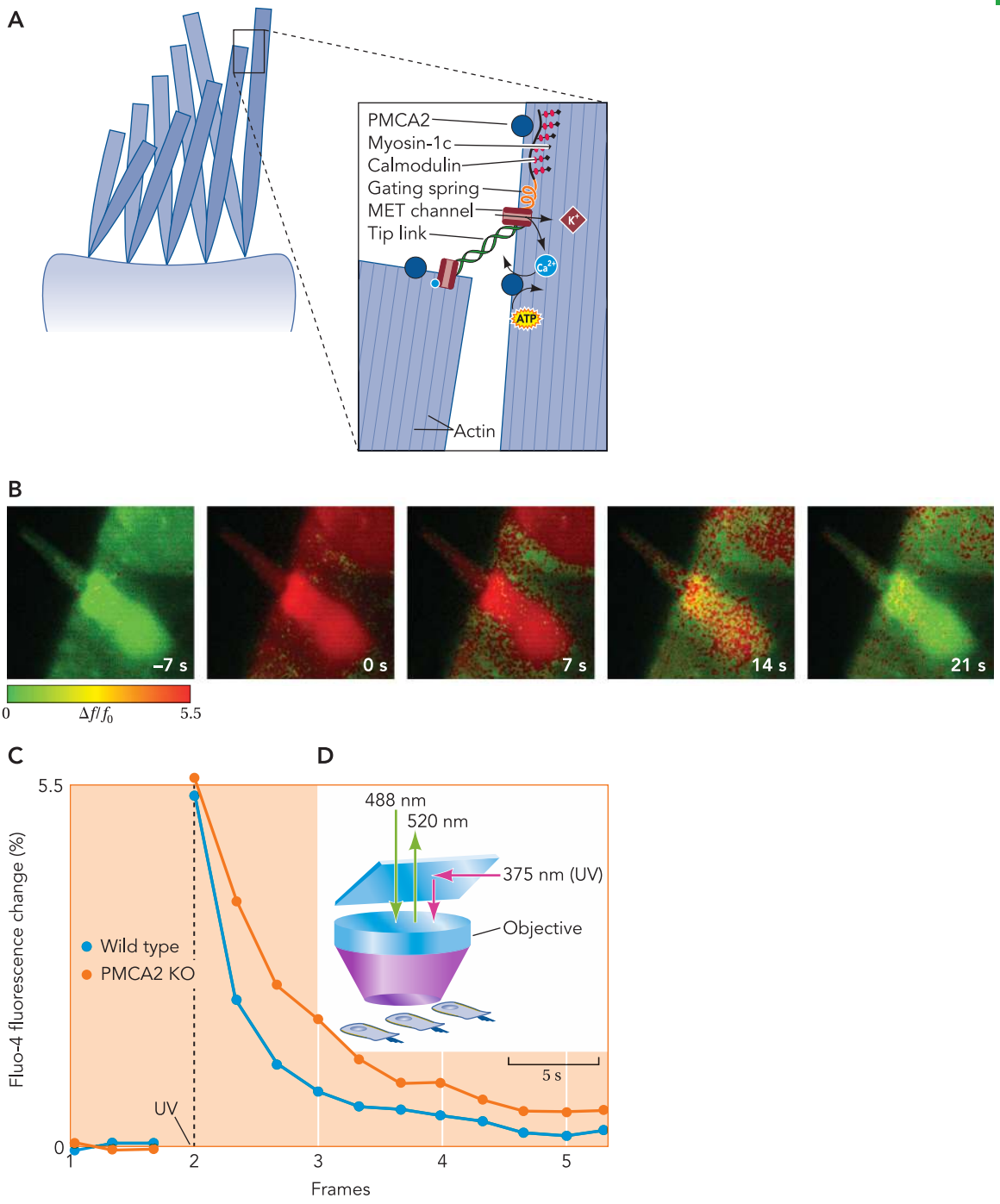

FIGURE 2. **Ca^{2+} dynamics at the apical pole of the hair cell**
A: mechanotransduction and calcium pumps. In this model, Myo1c of the adaptation-motor complex interacts, in a Ca^{2+}-calmodulin-dependent manner, with the rigid cross-linked actin filaments at the core of the stereocilia to adjust the force delivered by the tip link to the MET channel. The elastic component of the motor is represented by an intracellular gating spring connected to the MET channel. Ca^{2+} levels are returned to baseline by the ejection activity of the calcium pump (PMCA2 *w/a*). *B*: time sequence of confocal images before and after UV photolysis of caged Ca^{2+} in hair cells from a mouse utricle culture at *postnatal day 2* (P2). Timing relative to the onset of the 100-ms UV light pulse is shown on each numbered frame. Ca^{2+} concentration changes probed by Fluo-4 emission signal are encoded by the color scale bar beneath frame n.1. *C*: comparing time course of stereociliary fluorescence ratio changes evoked by Ca^{2+} photoliberation in a wild-type control (blue trace) and a PMCA2 KO mouse (red trace, corresponding to the frames in *B*); arrowheads below time axis show time of frame capture. *D*: methods. Fluo-4 fluorescence was excited by the 488-nm line of an argon laser, and fluorescence emission was selected around 520 nm. The UV illumination covered an area of ~5,000 μm^2, comprising a few hair cells, and was generated by a 375-nm laser light beam, reflected off a 400 DCLP dichromatic beam splitter positioned at 45 degrees just above the microscope objective lens. See Ref. 45. Note: the otolith organs (utricle and saccule) respond to translational head movements (linear accelerations) or to the orientation of the head relative to gravity.

and rodents results in doubling the unitary conductance, from 100 to 150 pS to 200 to 300 pS, and slowing of MET channel activation kinetics (6, 133). Interestingly, when turtle explants are exposed to 70 μM $[Ca^{2+}]_o$, the typical (multi)exponential decay of the MET current evoked by step displacements of the hair bundle evolves into damped current oscillations at frequencies comprised between 58 and 230 Hz, in the lower fraction of the animal's auditory range (134, 135). Under some conditions, the damping becomes negligible, and the turtle MET currents oscillate continuously in a limit-cycle mode (43), as found in other lower vertebrates in relation to low-frequency spontaneous hair bundle oscillations (110).

Clearly, fast adaptation ought to be most compelling for the mammalian cochlea, in which auditory information with frequency content in the kilohertz range is transmitted to the brain by the inner hair cells (IHCs) via synapses onto afferent terminals of the auditory nerve (41). It has been known for decades that when the mammalian ear is stimulated by sound with precisely defined frequency (pure tones, up to ~3.5 or 5.0 kHz depending on the species) the firing activity on the auditory nerve is cyclically and precisely concentrated only in one-half of the stimulating sinusoid, a phenomenon known as phase locking (1, 112). But rat IHCs, maintained at room temperature (19–22°C) at a potential of –80 mV, show level-dependent fast adaptation with shortest τ_A of ~0.5 ms (for the smallest stimuli in 1.5 mM $[Ca^{2+}]_o$, both at the 4- and 14-kHz frequency points sampled in this study (6). τ_A should be considerably shorter, of the order of 60–70 μs, when corrected for the environmental conditions in vivo (as done in Ref. 89 for OHCs). Therefore, if the properties of IHC MET channels are indeed invariant along the cochlea (6), during each cycle of, say, a 1-kHz tone, adaptation would be expected to impart a significant and Ca^{2+}-dependent (i.e., level-dependent) distortion to the transduction current and, consequently, receptor potential and afferent firing. Instead, published period histograms of phase-locked firing activity, evoked by pure tones around 1 kHz, track remarkably well the (half-wave rectified) stimulus waveform. In particular, they are symmetric around the peak firing probability over a wide range of input sound levels (1), in accord with the undistorted shape of the phasic (a.c.) receptor potential component (30, 124). In conclusion, it seems difficult to escape the questions of *1*) how fast is fast adaptation under physiological (endolymphatic) $[Ca^{2+}]_o$ in vivo and *2*) what of it is not an artifact due to the highly artificial recording conditions that are commonly employed?

Whatever the answer to these pressing questions, we now have a fairly clear picture of the fate undergone by Ca^{2+} entering through MET channels. It is rapidly bound by endogenous buffers within the stereocilia (63, 137) to be eventually shuttled back to endolymph by the plasma membrane Ca^{2+} ATPases (PMCAs) that are expressed at high density in the stereocilia (~2,000/μm^2) (134, 162, 164, 165). Ablation of the PMCA2 gene causes deafness and balance disorders in mice (93), associated to reduction of the Ca^{2+} extrusion rate (FIGURE 2, B–D). The extrusion task is performed by the *w/a* splicing isoform of PMCA2 (60, 61, 69). The choice of this unusual variant of the otherwise very efficient PMCA2 is surprising, given that this isoform is probably less responsive to the two natural PMCA activators, calmodulin and acidic phospholipids, and should be unable to rapidly enhance its activity in response to a sudden Ca^{2+} increase (14). It is also thought that PMCAs create an ambient of higher extracellular Ca^{2+} in the immediate proximity of the hair bundle (165), possibly with the complicity of the acellular structures overlying the hair cells in the cochlea (FIGURE 1A) (147) and, particularly, in the vestibular system (109) where PMCAs would also contribute to the formation and maintenance of the otoliths in the otolithic membranes (93) (structures in the saccule or utricle comprised of a combination of a gelatinous matrix and calcium carbonate crystals; the inertia of these small particles causes them to stimulate hair cells when the head accelerates). Furthermore, a mutation in the PMCA2 pump in the deaf waddler (*dfw*) mouse (G283S, downstream of the phospholipids binding domain in the loop connecting transmembrane domains 2 and 3) has been found to cause recessively inherited deafness (152). A mutation next to the active site of the pump (V586M) was later found to depress activity of the pump and to increase loss of hearing in heterozygous human patients that also carried a homozygous mutation in cadherin 23 (CDH23) (146), a member of the rapidly growing complement of stereocilia proteins (56). An unrelated T1999S substitution in the CDH23 gene has been recognized as a precondition for the development of a deafness phenotype in a human family when associated to a missense mutation of PMCA2, G293S, close to the *dfw* site (45). As discussed in detail in Ref. 127, an increasing number of other proteins have been found to be associated with hereditary hearing loss, the most common sensory deficit in humans (see the Hereditary Hearing Loss Homepage, http://webh01.ua.ac.be/hhh/), and their analysis is proving essential for the understanding of sensory transduction in the normal ear.

"Clearly, fast adaptation ought to be most compelling for the mammalian cochlea..."

Ca^{2+} Control of Synaptic Function

As mentioned above, MET current flowing into the apex of hair cells (and shaped by adaptation?) produces a

graded receptor potential at the cell basolateral membrane. Therefore, the hair cell can be functionally subdivided into apical and basal compartments, devoted to mechanoelectrical transduction and synaptic dialogue with afferent (and efferent) nerve fibers, respectively (52). At the afferent synapses of all hair cells, glutamate release is triggered by Ca^{2+} influx through L-type voltage-gated calcium channels ($Ca_V1.3$) clustered at such active zones ("hot spots") together with BK-type (Ca^{2+}-sensitive) voltage gated K^+ channels (116). Estimates of the total number of Ca^{2+} channels of these ~300-nm diameter micro- or nano-domains have been obtained in frog saccular hair cells [~90 channels for each of the ~20 active zones (138)] and mouse inner hair cells (IHCs) [~80 for 10–20 active zones (13)]. Domains of presynaptic Ca^{2+} are spatiotemporally restricted by the presence of mobile, proteinaceous Ca^{2+} buffers, calretinin, calbindin, and parvalbumin, which have been found in a variety of cochlear and vestibular hair cells with concentration in the millimolar range (63). However, discrepancies regarding both the amount and kinetic properties of such buffers in different hair cells suggest that their exact role and scope of function need to be analyzed further.

The afferent synapse of both vestibular and auditory hair cells is of the ribbon type (51, 120) (FIGURE 1C)

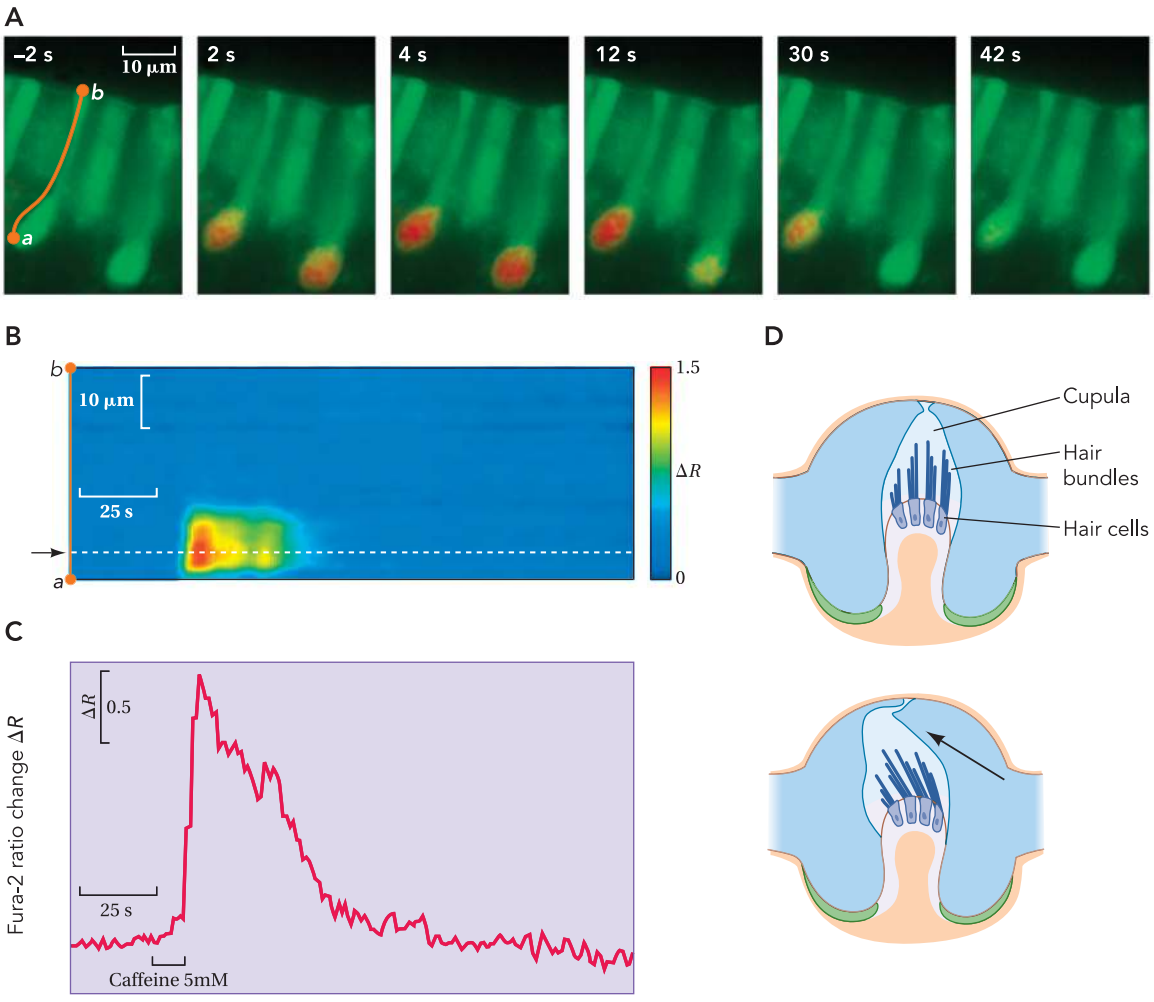

FIGURE 3. **Ca^{2+} stores at the synaptic pole of the hair cells**
A: time course of Ca^{2+} responses evoked by caffeine application (5 mM, 10 s) on hair cells in a slice of frog *Crista Ampullaris* loaded with fura 2-AM. *B*: pseudo-line-scan representation of fluorescence intensity changes ΔR. Abscissas represent time, and ordinates are distance along the line (*a*–*b*) superimposed on the cell in *A*. *C*: response analyzed at the point marked by the black arrow in *B*. See Ref. 99. *D*: schematic representation of the semicircular canal and crista ampullaris. *Top*: within each ampulla, the hair cells and their supporting cells lie embedded in a saddle-shaped neuroepithelial ridge, the crista, which extends across the base of the ampulla. Type I hair cells are concentrated in central regions of the crista, and type II hair cells are more numerous in peripheral areas. Arising from the crista and completely enveloping the stereocilia of the hair cells is a gelatinous structure, the cupula. The latter attaches to the roof and walls of the ampulla, forming a fluid-tight partition that has the same specific density as that of endolymph. *Bottom*: rotational head movements produce angular accelerations that cause the endolymph in the membranous ducts to be displaced so that the cupula is pushed to one side or the other. These cupular movements displace the stereocilia (and kinocilium) of the hair cells in the same direction.

and operates under stringent Ca^{2+} control conditions (8), and even more so in the auditory system where the establishment and maintenance of accurate phase relationship with sound stimuli relay on fast and tonic simultaneous multi-vesicular release (57, 86). Accurate and reliable timing of afferent discharge underlies the phenomenon of phase locking, discussed above, as well as the localization of sound sources in space, which requires discriminating time differences in the arrival of sound down to tens of microseconds (113). At any instant, only about 100 vesicles are tethered to the synaptic "ribbon" (or "dense body") of IHCs, and about 14 of these are docked to the plasma membrane (101, 142), constituting the "readily releasable" pool (115). The presynaptic Ca^{2+} current that closely follows the membrane potential (79) is directly proportional to vesicle release (83, 145). This linear relationship extends across the synapse to the postsynaptic current; therefore, the postsynaptic glutamate AMPA receptors faithfully transmit the information conveyed by the hair cell receptor potential (86). In higher frequency cells, overall higher frequencies of vesicle release are obtained primarily as a result of a greater number of synapses, whereas the lower Ca^{2+} entry per synapse may prevent postsynaptic receptor saturation (154). The Ca^{2+}-dependence of release is non-saturating and independent of hair cell frequency, suggesting that release, not refilling, is the rate-limiting factor (145). This conclusion is supported by the rapid, 1.9 vesicle per millisecond resupply that enables temporally precise and sustained release rates, two orders of magnitude higher than those of conventional synapses (62). How the ribbon replenishes its pool of vesicles, concentrating them at the site of Ca^{2+} influx, remains unclear (52, 120). Although the microtubule motor protein KIF3A was identified on the ribbon (117), evidence accumulates against an active "conveyor belt" for the resupply mechanism (67, 125). Interestingly, vesicles of the ribbon synapse lack synapsins as well as synaptotagmin 1 and 2, which are considered the Ca^{2+} sensors for vesicle fusion in neurons and neuroendocrine cells (140), whereas the core fusion complex contains some components in common with other synapses: SNARE proteins (syntaxin 1, synaptobrevin 1, and SNAP-25); the ribbon structural component RIBEYE; Bassoon and Piccolo, contributing to the anchoring of the ribbon at the active zone (120).

In addition to Ca^{2+} flowing through plasma membrane voltage-gated channels, it has been proposed that the increase of cytoplasmic free calcium concentration ($[Ca^{2+}]_i$) can also depend (or be amplified) by Ca^{2+} released from a presynaptic endoplasmic reticulum network of tubules and cysternae lying only a few tens of nanometers from the hair cell plasma membrane (25, 100, 141, 149) (FIGURE 1C). The mechanism of release depends on a caffeine and ryanodine sensitive Ca^{2+} channel through the mechanism of calcium-induced calcium release, CICR (FIGURE 3). An antagonistic concentration of ryanodine reduced afferent nerve fiber activity both in frog vestibular hair cells (99) and in mammalian IHC (7). In rat cochlear hair cells, CICR may be mediated by type I and II ryanodine receptors (RyR1–2) (59, 114). In IHCs, the highest levels of RyR1 labeling were observed over an area of rough endoplasmic reticulum that sits in the cytoplasmic region beneath the nucleus (59), supporting the conclusion that CICR enhances neurotransmitter release at the afferent region (57, 90). It has also been evidenced that RyRs and BK-type (Ca^{2+}-sensitive) voltage-gated potassium channels, co-localized mainly at the extrasynaptic apical neck of the IHCs, filter the receptor potential, and in turn depress neurotransmission in extreme conditions of enhanced Ca^{2+} accumulation that may occur during sound overstimulation or ischemia (7).

Recently, transient receptor potential ion channels (TRPC) have been characterized as a novel Ca^{2+} entry pathway in cochlear OHCs (132). TRPCs represent a superfamily of nonselective cation channels that have a particular significance in sensory systems (21). The OHC Ca^{2+} entry current is activated by the lowering of cytosolic Ca^{2+} levels and displays biophysical and pharmacological properties consistent with channels incorporating TRPC3 subunits. The TRPC-mediated Ca^{2+} entry channels may also be directly activated by DAG to complement G protein-coupled receptor-mediated release of stored Ca^{2+} (132).

The Cochlear Amplifier and Its Regulation by Ca^{2+}

As early as 1948, there were suggestions that an active mechanism might be necessary to explain the exquisite sensitivity and frequency resolving powers of the cochlea (58). It is now amply documented that a vulnerable active process known as cochlear amplifier (34) enhances up to a thousand-fold the vibrations of the basilar membrane (139) and is thus key to the perception of low-level sounds (107), as well as to other facets of sound processing performed by the cochlea (118, 119) (see The Cochlea Homepage, http://vimm.it/cochlea). This is the job of the outer hair cells (OHCs) (41), in which synaptic function is eclipsed by electromotility, a feature of these receptor/actuators that has no analog in any other cell of the body (15). The source of the electromotile cell length variations in OHCs is prestin (FIGURE 1D, *INSET*), an 81-kDa protein similar to pendrin and other members of the sulfate-anion transporter family but uniquely packed in the OHC lateral plasma membrane at a density of several thousand per square micron (31). Prestin, in contrast to enzymatic-activity-based motors, is a direct voltage-to-force converter that uses cytoplasmic Cl^- and HCO_3^- anions as extrinsic sensors for the receptor potential (122) and can operate at

microsecond rates (up to a frequency of, at least, 70 kHz) (46). The cell membrane fraction containing the prestin motor is a significant voltage-dependent contributor to the cell axial stiffness (66), together with the cytoskeletal cortical lattice and subsurface cisterns (121, 158). The OHC motor function can be modulated by numerous signaling pathways (47), some of which are Ca^{2+} dependent (48, 49), and, due to the strategic location of OHCs in the organ of Corti (FIGURE 1A), any change of OHC mechanical properties, particularly somatic length and axial stiffness, is bound to profoundly affect cochlear mechanics. On a slow time scale, OHC stiffness is controlled by phosphorylation of both cytoskeletal and prestin proteins. The polymerization state of cortical cytoskeleton filaments might be affected by activation of RhoA kinases under the control of intracellular Ca^{2+}, resulting in a stiffness increase (64, 166). Furthermore, OHC electromotility can be modulated by cGMP-dependent protein kinase acting at two prestin phosphorylation sites, as indicated by experiments performed in prestin-transfected cells (35). As mentioned above, prestin's function is influenced by intracellular anion concentrations, in particular by $[Cl^-]$, which may be regulated by Ca^{2+}-dependent and voltage-dependent chloride channels. Finally, water or sugar balance, influencing cell volume and turgor, can be regulated by a Ca^{2+}-dependent phosphorylation of an aquaporin-like protein and/or a GLUT-5 sugar carrier, both voltage dependent and located in the lateral plasma membrane (47) (FIGURE 1D, *INSET*).

A Ca^{2+}-dependent decrease of OHC axial stiffness was observed following the application of acetylcholine (ACh) to the cell basal pole (32). This is related to OHCs being the target of olivocochlear fibers that carry efferent inhibitory feedback from the brain (50) (FIGURE 1D). The efferent synapse uses a Ca^{2+}-permeable excitatory ACh receptor (75) to evoke both fast ionotropic and slow metabotropic responses (48). The fast response is a hyperpolarizing potassium current mediated by small-conductance Ca^{2+}-activated potassium channels (SK2) (123). The slow component, occurring with a delay of ~10 s, mediates an increase in the motile response of isolated unloaded OHCs, attributed to the activation of Ca^{2+}-/calmodulin-dependent phosphorylation of unspecified cytoskeletal proteins (153). OHC efferent synapses rely on the CICR mechanism (39), which is mediated by RyR1 receptors of a "synaptoplasmic" cistern at the postsynaptic site (104) (FIGURE 1D). It could be speculated that ACh triggers some sort of intracellular Ca^{2+} wave propagating in the proximity of the plasma membrane from the base of the cell to the lateral wall, via CICR from subsurface cisternae located beneath the cortical cytoskeleton. However, this phenomenon has not yet been experimentally demonstrated (47). The ensuing decrease in the global axial stiffness of the cell should exert an inhibitory effect on the OHC amplification mechanism in the organ of Corti (32). The biochemical pathway targeting OHC stiffness either after ACh application or following the increase of $[Ca^{2+}]_i$ appears to share a currently uncharacterized common mechanism (49). OHC axial stiffness decreases in response to increases of $[Ca^{2+}]_i$ (49) that also trigger cell elongation with consequent circumferential contraction, known as "Ca^{2+}-dependent slow motility" (24, 130). In summary, current data strongly argue for a crucial role of prestin-dependent amplification of mechanical stimuli in the mammalian cochlea (18, 19, 33, 102, 105, 143). The relevance for amplification of Ca^{2+}-dependent hair bundle movements, identified in rat OHCs as mechanical correlates of fast adaptation (17, 87) similar to those of nonmammalian vertebrates that lack prestin (110, 111), remains highly controversial (82, 88).

Other Ca^{2+} Signaling Pathways that Impact Inner Ear Function and Sound Perception

Extracellular signaling by nucleotides has long been associated with sensory systems, where ATP acts as a co-transmitter and/or neuromodulator (156). In both endolymphatic and perilymphatic compartments, basal level of extracellular ATP is maintained in the low nanomolar range by the action of ectonucleotidases (159). Even minimal departures from this level signal important changes to purinergic receptors (P2R) that play multiple important roles in sensory transduction and neurotransmission, consistent with the widespread expression of ATP gated ion channels (P2XR) and G-protein coupled receptors (P2YR) on inner ear cells, including cochlear hair cells (74). In most cases, Ca^{2+} ions are key intermediates in the ATP-evoked signaling cascades. Thus application of ATP to guinea pig IHC results in a rapid production of NO, presumably via ATP increase in $[Ca^{2+}]_i$. This in turn leads to a cGMP-PKG-dependent inhibition of the ATP-induced Ca^{2+} elevation in these cells (148). In OHCs, focal application of ATP to the hair bundle determines a biphasic increase in $[Ca^{2+}]_i$ (106). Initially, Ca^{2+} enters the cell apex through P2X ionotropic channels. Because it opens a pathway for K^+ ions (FIGURE 1B) in parallel to MET channels (FIGURE 2A), this process has been proposed to mediate a humoral modulation of mechanotransduction (75). But other mechanisms may be activated by the second phase of the ATP-evoked Ca^{2+} response, due to IP_3-induced Ca^{2+} release from internal stores mediated by PLC-coupled P2YR. In guinea pig OHCs, the release site coincides with the system of endoplasmic reticulum membranes and mitochondria known as Hensen's body, located at the base of the hair bundle. Ca^{2+} released in this region, beneath the cuticular plate that supports the hair bundle, effectively bypasses the clearance mechanism of the stereocilia and potentially influences mechanotransduction by mod-

ulation of unconventional myosin isoenzymes and actin-binding proteins involved in maintaining bundle integrity (106). Besides Myo1c, at least two other myosin isoforms, myosin VIIa and XVa, are found in the stereocilia and may alter bundle mechanics by acting on the attachment sites of the later links that hold the bundle together. Tropomyosin in the stereociliary rootlets, at the level of the cuticular plate, may also be involved in this process (38). In summary, myosin, tropomyosin, and actin together regulate the stiffness of bundle and cuticular plate under the control of Ca^{2+} binding proteins whose action is in turn regulated by Ca^{2+} pumps and buffers that control the concentration of intracellular Ca^{2+} at the apical pole of the hair cell, as explained in the relevant section above. Importantly, cochlear models indicate that even minimal disturbance to the mechanotransduction process may significantly alter the motor output of the cochlear amplifier, which operates at the threshold of spontaneous oscillations (107, 118, 119). Indeed, the estimated P2X-mediated conductance of hair cells must be minimal with respect to the one calculated for the Reissner's membrane (FIGURE 1A) because *1*) measurements of cochlear partition resistance (CoPR) in guinea pig endolymphatic compartment show that activation of hair cell P2XR has negligible influence on EP modulation and *2*) the EP value following ATP administration in the endolymph is not significantly altered in kanamicin treated animals lacking OHCs (157). Finally, mechanotransduction may be also affected by reduction of EP brought about by ATP stimulation of marginal cells in the gerbil stria vascularis, which determines a metabotropic Ca^{2+}-dependent inhibition of K^+ channel KCNE1 via $P2Y_4R$ (108).

Gap-Junction Coupling and Ca^{2+} Signaling in the Cochlear Duct

The homeostatic regulation of EP and endolymphatic K^+ levels, key prerequisites for the maintenance of MET function, require K^+ recirculation through the stria vascularis (FIGURE 1A) that houses a dense capillary network, supporting the high metabolic rate of this compartment (131). K^+ recirculation in the inner ear has been recently reviewed in this Journal (68). Briefly, K^+ released from cochlear hair cells following mechanotransduction (84) has been initially proposed to diffuse into scala tympani perilymph before entering the lateral wall of the cochlea (168). Here, it is taken up by fibrocytes in the spiral ligament, a densely vascularized endosteum at the outer limits of the cochlear canals, which is covered by the stria vascularis toward the cochlear duct. The stria vascularis in turn comprises basal, intermediate, and marginal cells. Gap-junction channels interconnect the fibrocytes of the spiral ligament and also medial and intermediate cells of the stria vascularis (22). Therefore, it has been proposed that intercellular transport across the gap junction network of this connective tissue participates in K^+ recirculation. After reaching the stria vascularis, K^+ ions are released by intermediate cells into the strial luminal site and are finally taken up by strial marginal cells to be secreted back into endolymph (81, 160, 161).

The fraction of K^+ being lost to perilymph due to MET channel activation is unknown, but disruption of the K-Cl cotransporters Kcc4 and Kcc3, which causes deafness in mice (10, 11), indicates that active K^+ uptake by the Deiter's cells and other supporting cells of the organ of Corti is essential to cochlear ion homeostasis. These cells belong in distinct gap junction networks formed by nonsensory epithelial cells (22, 80), including interdental cells of the spiral limbus, inner sulcus cells, organ of Corti supporting cells, outer sulcus cells, and cells within the root processes of the spiral ligament (92). Therefore, immediately after being released from the hair cells, a substantial proportion of the K^+ ions is likely to be taken up into these syncytia that may be an integral part of the recycle scheme (91).

In the organ of Corti, most gap-junction channels are assembled from connexin (Cx) protein subunits, primarily connexin 26 (Cx26, *GJB2* gene) and co-localized Cx30 (*GJB6*) (92, 98). Mouse models confirmed that both connexins are essential for cochlear function and cell survival (23, 94, 155), and the complex deafness DFNB1 locus (13q11-q12) has been shown to contain both gap-junction genes. Deafness-causing mutations have been reported in two other connexin genes, Cx31 (*GJB3*) and Cx43 (*GJA1*) (22); however, DFNB1 mutations are responsible for about half of all cases of human deafness in countries surrounding the Mediterranean Sea. In particular, over 50 causative mutations in *GJB2* have been shown to account for the vast majority of prelingual hearing impairment in most populations (see The Connexin-deafness homepage, http://davinci.crg.es/deafness/). DFNB1 is thus almost as frequent as cystic fibrosis (128). Yet deafness associated with mutations in the Cx26 gene may not solely depend on the loss of K^+ coupling and K^+ recirculation. Collectively, secondary messengers and small metabolites are deemed to be the molecular constituents that are directly passed from one cell to another: important molecules include cAMP, cGMP, IP_3, adenosine, ADP, and ATP, to name only a few (97). Indeed, Cx26 channels carrying the deafness-related V84L substitution present normal localization and similar conductance to wild-type protein (16), but significantly impaired permeability to IP_3 (4). Furthermore, direct injection of IP_3 in cochlear supporting cells of neonatal rat organotypic cultures generates a wave of calcium that spreads throughout the tissue and is suppressed when junctional channels (or unapposed Cx hemichannels facing scala media) are blocked by acidification with CO_2 (4). IP_3 permeability defects (167) may interfere with K^+ recycle function, because K^+/Cl^- efflux from supporting cells (10) may be, directly or indirectly, controlled by Ca^{2+}.

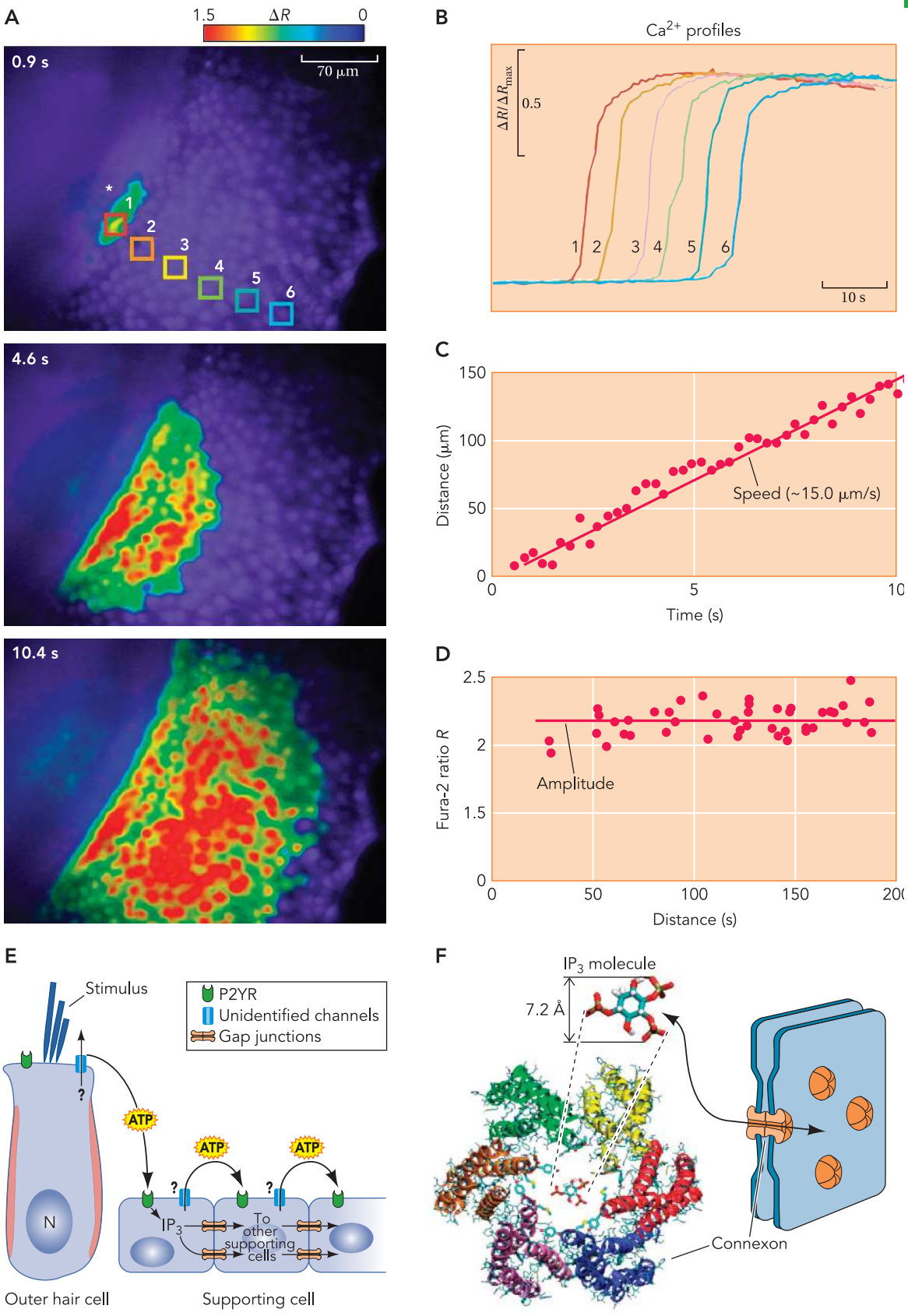

FIGURE 4. **Ca^{2+} waves in the organ Corti**
A: one representative experimental record, showing Ca^{2+} transient responses in fura 2-loaded supporting cells following focal ATP application (50 ms, 4 μM) to OHCs. Shown are pseudocolor images of the Ca^{2+} wave spreading at three different time points. *B*: $[Ca^{2+}]_i$ profiles from the regions of interest (ROIs) superimposed over six selected supporting cells in *A* (*top*). *C*: distance of wave-front points from the point of stimulus application (white asterisk) vs. time; wave speed is estimated by the slope of the least square linear fit (solid line). *D*: the amplitude of mean fura 2 ratio signal vs. distance shows no significant attenuation of the $[Ca^{2+}]_i$ response with distance from the point source, implicating an active mechanism of propagation. *E*: proposed mechanism of Ca^{2+} wave propagation. ATP release following hair cell stimulation induces an auto-sustained spreading of the signal in adjacent supporting cells, determined by further release of ATP into endolymph through an unidentified channel and concurrent IP_3 diffusion through gap-junction channels. *F*: IP_3 diffusion through gap junctions. The differently colored ribbons represent alpha-helices in the transmembrane portion of the six connexin proteins forming the connexon. See Ref. 9.

Pharmacological dissection indicates that ATP, acting through a highly sensitive purinergic-/IP_3-mediated signaling pathway, is the principal paracrine mediator implicated in the propagation of waves through supporting and epithelial cells (129) (FIGURE 4). Interestingly, Ca^{2+} waves generated by brief (50–200 ms) application of low doses of ATP (4–1 μM) from a puff pipette are identical to Ca^{2+} waves evoked by mechanical stimulation of hair cells, mimicking in vitro the effects of sound over-stimulation (53). A fundamental step is ATP release at the endolymphatic surface of the epithelium, which triggers adjacent cell responses (FIGURE 4E). Based on connexin expression models and inhibition by blockers, connexin hemichannels have been proposed as a diffusion pathway for the release of extracellular messengers, including ATP (5). This is the case of retinal pigment epithelium where ATP is released in the extracellular space through Cx43 hemichannels, with pore opening controlled by spontaneous elevations of intracellular Ca^{2+} (126). But transmission of Ca^{2+}-related signals may exploit alternative nonvesicular release mechanisms, along with IP_3 diffusion through gap junctions (4, 144) (FIGURE 4F). Thus anion channels, pannexins, and $P2X_7$ receptors have all been proposed as conduits for ATP to the extracellular milieu in other systems (150). The significance of Ca^{2+} wave propagation through the cochlear tissue, as well as that of oscillations in intracellular Ca^{2+} concentration levels (95, 96), are intimately connected to the role of Ca^{2+} in the control of key cellular processes. The extraordinary sensitivity to ATP of cochlear neonatal culture suggests that spreading of intracellular calcium through the syncitium is an essential component for the perception of sound and is potentially involved in sound-induced gene expression (53).

Conclusions

Ca^{2+} acts as a fundamental signal transduction element in the inner ear, delivering information about sound acceleration and gravity through the small number of hair cell MET channels to the ribbon synapse, thus driving neurotransmission. Ca^{2+} is also a second messenger that induces conformational changes in effector molecules regulating the sensitivity of the cochlear amplifier and inner ear fluid homeostasis. Several aspects regarding Ca^{2+} effects on MET function require further investigation and clarification. For instance, open questions are *1*) how fast is fast adaptation under endolymphatic Ca^{2+} concentrations (i.e., in vivo), and *2*) what is the relevance for amplification of Ca^{2+}-dependent hair bundle movements, mechanical correlates of fast adaptation? Other interesting problems regard Ca^{2+} dynamics near the ribbon synapse and the way in which the dense body replenishes its pool of vesicles, concentrating them at the site of Ca^{2+} influx. Is Ca^{2+} implicated in yet unidentified mechanisms driving ribbon dynamics? The genetic approach is proving fundamental in unraveling the molecular basis of Ca^{2+} function in the control of key cellular processes. It has recently provided interesting advances about cochlear K^+ recycle function and the role of Ca^{2+} waves propagation through the supporting cell network. Calcium wave spreading throughout the cells of the auditory periphery, following mechanical stimulation of hair cells, suggests the possibility of a link between wave generation and sound propagation. Although many of the molecular effectors of wave spreading have been identified, the explanation of the physiological significance of this phenomenon requires additional research efforts. Furthermore, the large number of human genes implicated in deafness and the increasing number of mouse models with a deafness phenotype indicate that we will still learn a great deal more about hearing from the genetic approach, which will undoubtedly pave the way to therapies for auditory dysfunctions in the years to come. ■

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References

1. Anderson DJ, Rose JE, Hind JE, Brugge JF. Temporal position of discharges in single auditory nerve fibers within the cycle of a sine-wave stimulus: frequency and intensity effects. *J Acoust Soc Am* 49, *Suppl* 2: 1131, 1971.
2. Assad JA, Corey DP. An active motor model for adaptation by vertebrate hair cells. *J Neurosci* 12: 3291–3309, 1992.
3. Assad JA, Shepherd GM, Corey DP. Tip-link integrity and mechanical transduction in vertebrate hair cells. *Neuron* 7: 985–994, 1991.
4. Beltramello M, Piazza V, Bukauskas FF, Pozzan T, Mammano F. Impaired permeability to Ins(1,4,5)P3 in a mutant connexin underlies recessive hereditary deafness. *Nat Cell Biol* 7: 63–69, 2005.
5. Bennett MV, Contreras JE, Bukauskas FF, Saez JC. New roles for astrocytes: gap junction hemichannels have something to communicate. *Trends Neurosci* 26: 610–617, 2003.
6. Beurg M, Evans MG, Hackney CM, Fettiplace R. A large-conductance calcium-selective mechanotransducer channel in mammalian cochlear hair cells. *J Neurosci* 26: 10992–11000, 2006.
7. Beurg M, Hafidi A, Skinner LJ, Ruel J, Nouvian R, Henaff M, Puel JL, Aran JM, Dulon D. Ryanodine receptors and BK channels act as a presynaptic depressor of neurotransmission in cochlear inner hair cells. *Eur J Neurosci* 22: 1109–1119, 2005.
8. Beutner D, Voets T, Neher E, Moser T. Calcium dependence of exocytosis and endocytosis at the cochlear inner hair cell afferent synapse. *Neuron* 29: 681–690, 2001.
9. Bicego M, Beltramello M, Melchionda S, Carella M, Piazza V, Zelante L, Bukauskas FF, Arslan E, Cama E, Pantano S, Bruzzone R, D'Andrea P, Mammano F. Pathogenetic role of the deafness-related M34T mutation of Cx26. *Hum Mol Genet* 15: 2569–2587, 2006.
10. Boettger T, Hubner CA, Maier H, Rust MB, Beck FX, Jentsch TJ. Deafness and renal tubular acidosis in mice lacking the K-Cl co-transporter Kcc4. *Nature* 416: 874–878, 2002.

11. Boettger T, Rust MB, Maier H, Seidenbecher T, Schweizer M, Keating DJ, Faulhaber J, Ehmke H, Pfeffer C, Scheel O, Lemcke B, Horst J, Leuwer R, Pape HC, Volkl H, Hubner CA, Jentsch TJ. Loss of K-Cl co-transporter KCC3 causes deafness, neurodegeneration and reduced seizure threshold. *EMBO J* 22: 5422–5434, 2003.

12. Bosher SK, Warren RL. Very low calcium content of cochlear endolymph, an extracellular fluid. *Nature* 273: 377–378, 1978.

13. Brandt A, Khimich D, Moser Few CaV1 T.3. channels regulate the exocytosis of a synaptic vesicle at the hair cell ribbon synapse. *J Neurosci* 25: 11577–11585, 2005.

14. Brini M, Coletto L, Pierobon N, Kraev N, Guerini D, Carafoli E. A comparative functional analysis of plasma membrane Ca^{2+} pump isoforms in intact cells. *J Biol Chem* 278: 24500–24508, 2003.

15. Brownell WE, Spector AA, Raphael RM, Popel AS. Micro- and nanomechanics of the cochlear outer hair cell. *Annu Rev Biomed Eng* 3: 169–194, 2001.

16. Bruzzone R, Veronesi V, Gomes D, Bicego M, Duval N, Marlin S, Petit C, D'Andrea P, White TW. Loss-of-function and residual channel activity of connexin26 mutations associated with non-syndromic deafness. *FEBS Lett* 533: 79–88, 2003.

17. Chan DK, Hudspeth AJ. Ca^{2+} current-driven nonlinear amplification by the mammalian cochlea in vitro. *Nat Neurosci* 8: 149–155, 2005.

18. Cheatham MA, Huynh KH, Gao J, Zuo J, Dallos P. Cochlear function in Prestin knockout mice. *J Physiol* 560: 821–830, 2004.

19. Cheatham MA, Zheng J, Huynh KH, Du GG, Gao J, Zuo J, Navarrete E, Dallos P. Cochlear function in mice with only one copy of the prestin gene. *J Physiol* 569: 229–241, 2005.

20. Cheung EL, Corey DP. Ca^{2+} changes the force sensitivity of the hair-cell transduction channel. *Biophys J* 90: 124–139, 2006.

21. Clapham DE. TRP channels as cellular sensors. *Nature* 426: 517–524, 2003.

22. Cohen-Salmon M. Connexins responsible for hereditary deafness: the tale unfolds. In: *Gap Junctions in Development and Disease*, edited by Winterhager E. Heidelberg, Germany: Springer-Verlag, 2005, p. 111–134.

23. Cohen-Salmon M, Ott T, Michel V, Hardelin JP, Perfettini I, Eybalin M, Wu T, Marcus DC, Wangemann P, Willecke K, Petit C. Targeted ablation of connexin26 in the inner ear epithelial gap junction network causes hearing impairment and cell death. *Curr Biol* 12: 1106–1111, 2002.

24. Coling DE, Bartolami S, Rhee D, Neelands T. Inhibition of calcium-dependent motility of cochlear outer hair cells by the protein kinase inhibitor, ML-9. *Hear Res* 115: 175–183, 1998.

25. Collin T, Marty A, Llano I. Presynaptic calcium stores and synaptic transmission. *Curr Opin Neurobiol* 15: 275–281, 2005.

26. Corey DP. What is the hair cell transduction channel? *J Physiol* 576: 23–28, 2006.

27. Corey DP, Hudspeth AJ. Kinetics of the receptor current in bullfrog saccular hair cells. *J Neurosci* 3: 962–976, 1983.

28. Corey DP, Sotomayor M. Hearing: tightrope act. *Nature* 428: 901–903, 2004.

29. Crawford AC, Evans MG, Fettiplace R. The actions of calcium on the mechano-electrical transducer current of turtle hair cells. *J Physiol* 434: 369–398, 1991.

30. Dallos P. Neurobiology of cochlear inner and outer hair cells: intracellular recordings. *Hear Res* 22: 185–198, 1986.

31. Dallos P, Fakler B. Prestin, a new type of motor protein. *Nat Rev Mol Cell Biol* 3: 104–111, 2002.

32. Dallos P, He DZ, Lin X, Sziklai I, Mehta S, Evans BN. Acetylcholine, outer hair cell electromotility, and the cochlear amplifier. *J Neurosci* 17: 2212–2226, 1997.

33. Dallos P, Zheng J, Cheatham MA. Prestin and the cochlear amplifier. *J Physiol* 576: 37–42, 2006.

34. Davis H. An active process in cochlear mechanics. *Hear Res* 9: 79–90, 1983.

35. Deak L, Zheng J, Orem A, Du GG, Aguinaga S, Matsuda K, Dallos P. Effects of cyclic nucleotides on the function of prestin. *J Physiol* 563: 483–496, 2005.

36. Denk W, Holt JR, Shepherd GM, Corey DP. Calcium imaging of single stereocilia in hair cells: localization of transduction channels at both ends of tip links. *Neuron* 15: 1311–1321, 1995.

37. Drescher MJ, Khan KM, Beisel KW, Karadaghy AA, Hatfield JS, Kim SY, Drescher AJ, Lasak JM, Barretto RL, Shakir AH, Drescher DG. Expression of adenylyl cyclase type I in cochlear inner hair cells. *Brain Res Mol Brain Res* 45: 325–330, 1997.

38. El-Amraoui A, Petit C. Usher I syndrome: unravelling the mechanisms that underlie the cohesion of the growing hair bundle in inner ear sensory cells. *J Cell Sci* 118: 4593–4603, 2005.

39. Evans MG, Lagostena L, Darbon P, Mammano F. Cholinergic control of membrane conductance and intracellular free Ca^{2+} in outer hair cells of the guinea pig cochlea. *Cell Calcium* 28: 195–203, 2000.

40. Farris HE, LeBlanc CL, Goswami J, Ricci AJ. Probing the pore of the auditory hair cell mechanotransducer channel in turtle. *J Physiol* 558: 769–792, 2004.

41. Fettiplace R, Hackney CM. The sensory and motor roles of auditory hair cells. *Nat Rev Neurosci* 7: 19–29, 2006.

42. Fettiplace R, Ricci AJ. Adaptation in auditory hair cells. *Curr Opin Neurobiol* 13: 446–451, 2003.

43. Fettiplace R, Ricci AJ. Mechanoelectrical transduction in auditory hair cells. In: *Vertebrate Hair Cells*, edited by Eatock RA, Fay RR, and Popper AN. New York: Springer Science, 2006, p. 154–203.

44. Fettiplace R, Ricci AJ, Hackney CM. Clues to the cochlear amplifier from the turtle ear. *Trends Neurosci* 24: 169–175, 2001.

45. Ficarella R, Di Leva F, Bortolozzi M, Ortolano S, Donaudy F, Petrillo M, Carella M, Lelli A, Domi T, Fedrizzi L, Lim D, Shull GE, Gasparini P, Brini M, Mammano F, Carafoli E. A functional study of plasma membrane calcium pump isoform 2 mutants causing digenic deafness. *PNAS*. In press.

46. Frank G, Hemmert W, Gummer AW. Limiting dynamics of high-frequency electromechanical transduction of outer hair cells. *Proc Natl Acad Sci USA* 96: 4420–4425, 1999.

47. Frolenkov GI. Regulation of electromotility in the cochlear outer hair cell. *J Physiol* 576: 43–48, 2006.

48. Frolenkov GI, Mammano F, Belyantseva IA, Coling D, Kachar B. Two distinct Ca^{2+}-dependent signaling pathways regulate the motor output of cochlear outer hair cells. *J Neurosci* 20: 5940–5948, 2000.

49. Frolenkov GI, Mammano F, Kachar B. Regulation of outer hair cell cytoskeletal stiffness by intracellular Ca^{2+}: underlying mechanism and implications for cochlear mechanics. *Cell Calcium* 33: 185–195, 2003.

50. Fuchs P. The synaptic physiology of cochlear hair cells. *Audiol Neurootol* 7: 40–44, 2002.

51. Fuchs PA. Time and intensity coding at the hair cell's ribbon synapse. *J Physiol* 566: 7–12, 2005.

52. Fuchs PA, Glowatzki E, Moser T. The afferent synapse of cochlear hair cells. *Curr Opin Neurobiol* 13: 452–458, 2003.

53. Gale JE, Piazza V, Ciubotaru CD, Mammano F. A mechanism for sensing noise damage in the inner ear. *Curr Biol* 14: 526–529, 2004.

54. Geleoc GS, Corey DP. Modulation of mechanoeletrical transduction by protein kinase A in utricular hair cells of neonatal mice. *Assoc Res Otolaryngol Abst* 24: 242, 2001.

55. Gillespie PG, Cyr JL. Myosin-1c, the hair cell's adaptation motor. *Annu Rev Physiol* 66: 521–545, 2004.

56. Gillespie PG, Dumont RA, Kachar B. Have we found the tip link, transduction channel, and gating spring of the hair cell? *Curr Opin Neurobiol* 15: 389–396, 2005.

57. Glowatzki E, Fuchs PA. Transmitter release at the hair cell ribbon synapse. *Nat Neurosci* 5: 147–154, 2002.

58. Gold Hearing T2nd. The physical basis of the action of the cochlea. *Proc R Soc Lond B Biol Sci* 135: 492–498, 1948.

59. Grant L, Slapnick S, Kennedy H, Hackney C. Ryanodine receptor localisation in the mammalian cochlea: an ultrastructural study. *Hear Res* 219: 101–109, 2006.

60. Grati M, Aggarwal N, Strehler EE, Wenthold RJ. Molecular determinants for differential membrane trafficking of PMCA1 and PMCA2 in mammalian hair cells. *J Cell Sci* 119: 2995–3007, 2006.

61. Grati M, Schneider ME, Lipkow K, Strehler EE, Wenthold RJ, Kachar B. Rapid turnover of stereocilia membrane proteins: evidence from the trafficking and mobility of plasma membrane Ca^{2+}-ATPase 2. *J Neurosci* 26: 6386–6395, 2006.

62. Griesinger CB, Richards CD, Ashmore JF. Fast vesicle replenishment allows indefatigable signalling at the first auditory synapse. *Nature* 435: 212–215, 2005.

63. Hackney CM, Mahendrasingam S, Penn A, Fettiplace R. The concentrations of calcium buffering proteins in mammalian cochlear hair cells. *J Neurosci* 25: 7867–7875, 2005.

64. Hall A, Nobes CD. Rho GTPases: molecular switches that control the organization and dynamics of the actin cytoskeleton. *Philos Trans R Soc Lond B Biol Sci* 355: 965–970, 2000.

65. Hasson T, Gillespie PG, Garcia JA, MacDonald RB, Zhao Y, Yee AG, Mooseker MS, Corey DP. Unconventional myosins in inner-ear sensory epithelia. *J Cell Biol* 137: 1287–1307, 1997.

66. He DZ, Jia S, Dallos P. Prestin and the dynamic stiffness of cochlear outer hair cells. *J Neurosci* 23: 9089–9096, 2003.

67. Heidelberger R. Adenosine triphosphate and the late steps in calcium-dependent exocytosis at a ribbon synapse. *J Gen Physiol* 111: 225–241, 1998.

68. Hibino H, Kurachi Y. Molecular and physiological bases of the K+ circulation in the mammalian inner ear. *Physiology Bethesda* 21: 336–345, 2006.

69. Hill JK, Williams DE, LeMasurier M, Dumont RA, Strehler EE, Gillespie PG. Splice-site A choice targets plasma-membrane Ca^{2+}-ATPase isoform 2 to hair bundles. *J Neurosci* 26: 6172–6180, 2006.

70. Hirono M, Denis CS, Richardson GP, Gillespie PG. Hair cells require phosphatidylinositol 4,5-bisphosphate for mechanical transduction and adaptation. *Neuron* 44: 309–320, 2004.

71. Holt JR, Corey DP. Two mechanisms for transducer adaptation in vertebrate hair cells. *Proc Natl Acad Sci USA* 97: 11730–11735, 2000.

72. Holt JR, Corey DP, Eatock RA. Mechanoelectrical transduction and adaptation in hair cells of the mouse utricle, a low-frequency vestibular organ. *J Neurosci* 17: 8739–8748, 1997.

73. Holt JR, Gillespie SK, Provance DW, Shah K, Shokat KM, Corey DP, Mercer JA, Gillespie PG. A chemical-genetic strategy implicates myosin-1c in adaptation by hair cells. *Cell* 108: 371–381, 2002.

74. Housley GD. Physiological effects of extracellular nucleotides in the inner ear. *Clin Exp Pharmacol Physiol* 27: 575–580, 2000.

75. Housley GD, Greenwood D, Ashmore JF. Localization of cholinergic and purinergic receptors on outer hair cells isolated from the guinea-pig cochlea. *Proc Biol Sci* 249: 265–273, 1992.

76. Howard J, Hudspeth AJ. Compliance of the hair bundle associated with gating of mechanoelectrical transduction channels in the bullfrog's saccular hair cell. *Neuron* 1: 189–199, 1988.

77. Howard J, Hudspeth AJ. Mechanical relaxation of the hair bundle mediates adaptation in mechanoelectrical transduction by the bullfrog's saccular hair cell. *Proc Natl Acad Sci USA* 84: 3064–3068, 1987.

78. Hudspeth AJ, Gillespie PG. Pulling springs to tune transduction: adaptation by hair cells. *Neuron* 12: 1–9, 1994.

79. Hudspeth AJ, Lewis RS. A model for electrical resonance and frequency tuning in saccular hair cells of the bull-frog, Rana catesbeiana. *J Physiol* 400: 275–297, 1988.

80. Jagger DJ, Forge A. Compartmentalized and signal-selective gap junctional coupling in the hearing cochlea. *J Neurosci* 26: 1260–1268, 2006.

81. Jentsch TJ. Neuronal KCNQ potassium channels: physiology and role in disease. *Nat Rev Neurosci* 1: 21–30, 2000.

82. Jia S, He DZ. Motility-associated hair-bundle motion in mammalian outer hair cells. *Nat Neurosci* 8: 1028–1034, 2005.

83. Johnson SL, Marcotti W, Kros CJ. Increase in efficiency and reduction in Ca^{2+} dependence of exocytosis during development of mouse inner hair cells. *J Physiol* 563: 177–191, 2005.

84. Johnstone BM, Patuzzi R, Syka J, Sykova E. Stimulus-related potassium changes in the organ of Corti of guinea-pig. *J Physiol* 408: 77–92, 1989.

85. Kachar B, Parakkal M, Kurc M, Zhao Y, Gillespie PG. High-resolution structure of hair-cell tip links. *Proc Natl Acad Sci USA* 97: 13336–13341, 2000.

86. Keen EC, Hudspeth AJ. Transfer characteristics of the hair cell's afferent synapse. *Proc Natl Acad Sci USA* 103: 5537–5542, 2006.

87. Kennedy HJ, Crawford AC, Fettiplace R. Force generation by mammalian hair bundles supports a role in cochlear amplification. *Nature* 433: 880–883, 2005.

88. Kennedy HJ, Evans MG, Crawford AC, Fettiplace R. Depolarization of cochlear outer hair cells evokes active hair bundle motion by two mechanisms. *J Neurosci* 26: 2757–2766, 2006.

89. Kennedy HJ, Evans MG, Crawford AC, Fettiplace R. Fast adaptation of mechanoelectrical transducer channels in mammalian cochlear hair cells. *Nat Neurosci* 6: 832–836, 2003.

90. Kennedy HJ, Meech RW. Fast Ca2+ signals at mouse inner hair cell synapse: a role for Ca^{2+}-induced Ca^{2+} release. *J Physiol* 539: 15–23, 2002.

91. Kikuchi T, Adams JC, Miyabe Y, So E, Kobayashi T. Potassium ion recycling pathway via gap junction systems in the mammalian cochlea and its interruption in hereditary nonsyndromic deafness. *Med Electron Microsc* 33: 51–56, 2000.

92. Kikuchi T, Kimura RS, Paul DL, Adams JC. Gap junctions in the rat cochlea: immunohistochemical and ultrastructural analysis. *Anat Embryol (Berl)* 191: 101–118, 1995.

93. Kozel PJ, Friedman RA, Erway LC, Yamoah EN, Liu LH, Riddle T, Duffy JJ, Doetschman T, Miller ML, Cardell EL, Shull GE. Balance and hearing deficits in mice with a null mutation in the gene encoding plasma membrane Ca^{2+}-ATPase isoform 2. *J Biol Chem* 273: 18693–18696, 1998.

94. Kudo T, Kure S, Ikeda K, Xia AP, Katori Y, Suzuki M, Kojima K, Ichinohe A, Suzuki Y, Aoki Y, Kobayashi T, Matsubara Y. Transgenic expression of a dominant-negative connexin26 causes degeneration of the organ of Corti and non-syndromic deafness. *Hum Mol Genet* 12: 995–1004, 2003.

95. Lagostena L, Ashmore JF, Kachar B, Mammano F. Purinergic control of intercellular communication between Hensen's cells of the guinea-pig cochlea. *J Physiol* 531: 693–706, 2001.

96. Lagostena L, Mammano F. Intracellular calcium dynamics and membrane conductance changes evoked by Deiters' cell purinoceptor activation in the organ of Corti. *Cell Calcium* 29: 191–198, 2001.

97. Laird DW. Life cycle of connexins in health and disease. *Biochem J* 394: 527–543, 2006.

98. Lautermann J, ten Cate WJ, Altenhoff P, Grummer R, Traub O, Frank H, Jahnke K, Winterhager E. Expression of the gap-junction connexins 26 and 30 in the rat cochlea. *Cell Tissue Res* 294: 415–420, 1998.

99. Lelli A, Perin P, Martini M, Ciubotaru CD, Prigioni I, Valli P, Rossi ML, Mammano F. Presynaptic calcium stores modulate afferent release in vestibular hair cells. *J Neurosci* 23: 6894–6903, 2003.

100. Lenzi D, Runyeon JW, Crum J, Ellisman MH, Roberts WM. Synaptic vesicle populations in saccular hair cells reconstructed by electron tomography. *J Neurosci* 19: 119–132, 1999.

101. Liberman MC. Efferent synapses in the inner hair cell area of the cat cochlea: an electron microscopic study of serial sections. *Hear Res* 3: 189–204, 1980.

102. Liberman MC, Gao J, He DZ, Wu X, Jia S, Zuo J. Prestin is required for electromotility of the outer hair cell and for the cochlear amplifier. *Nature* 419: 300–304, 2002.

103. Lin HW, Schneider ME, Kachar B. When size matters: the dynamic regulation of stereocilia lengths. *Curr Opin Cell Biol* 17: 55–61, 2005.

104. Lioudyno M, Hiel H, Kong JH, Katz E, Waldman E, Parameshwaran-Iyer S, Glowatzki E, Fuchs PA. A "synaptoplasmic cistern" mediates rapid inhibition of cochlear hair cells. *J Neurosci* 24: 11160–11164, 2004.

105. Liu XZ, Ouyang XM, Xia XJ, Zheng J, Pandya A, Li F, Du LL, Welch KO, Petit C, Smith RJ, Webb BT, Yan D, Arnos KS, Corey D, Dallos P, Nance WE, Chen ZY. Prestin, a cochlear motor protein, is defective in non-syndromic hearing loss. *Hum Mol Genet* 12: 1155–1162, 2003.

106. Mammano F, Frolenkov GI, Lagostena L, Belyantseva IA, Kurc M, Dodane V, Colavita A, Kachar B. ATP-Induced Ca^{2+} release in cochlear outer hair cells: localization of an inositol triphosphate-gated Ca^{2+} store to the base of the sensory hair bundle. *J Neurosci* 19: 6918–6929, 1999.

107. Mammano F, Nobili R. Biophysics of the cochlea: linear approximation. *J Acoust Soc Am* 93: 3320–3332, 1993.

108. Marcus DC, Sunose H, Liu J, Bennett T, Shen Z, Scofield MA, Ryan AF. Protein kinase C mediates P2U purinergic receptor inhibition of K^+ channel in apical membrane of strial marginal cells. *Hear Res* 115: 82–92, 1998.

109. Marquis RE, Hudspeth AJ. Effects of extracellular Ca^{2+} concentration on hair-bundle stiffness and gating-spring integrity in hair cells. *Proc Natl Acad Sci USA* 94: 11923–11928, 1997.

110. Martin P, Bozovic D, Choe Y, Hudspeth AJ. Spontaneous oscillation by hair bundles of the bullfrog's sacculus. *J Neurosci* 23: 4533–4548, 2003.

111. Martin P, Hudspeth AJ. Active hair-bundle movements can amplify a hair cell's response to oscillatory mechanical stimuli. *Proc Natl Acad Sci USA* 96: 14306–14311, 1999.

112. Moller AR. Frequency selectivity of phase-locking of complex sounds in the auditory nerve of the rat. *Hear Res* 11: 267–284, 1983.

113. Moore DR. Anatomy and physiology of binaural hearing. *Audiology* 30: 125–134, 1991.

114. Morton-Jones RT, Cannell MB, Jeyakumar LH, Fleischer S, Housley GD. Differential expression of ryanodine receptors in the rat cochlea. *Neuroscience* 137: 275–286, 2006.

115. Moser T, Beutner D. Kinetics of exocytosis and endocytosis at the cochlear inner hair cell afferent synapse of the mouse. *Proc Natl Acad Sci USA* 97: 883–888, 2000.

116. Moser T, Neef A, Khimich D. Mechanisms underlying the temporal precision of sound coding at the inner hair cell ribbon synapse. *J Physiol* 576: 55–62, 2006.

117. Muresan V, Lyass A, Schnapp BJ. The kinesin motor KIF3A is a component of the presynaptic ribbon in vertebrate photoreceptors. *J Neurosci* 19: 1027–1037, 1999.

118. Nobili R, Mammano F. Biophysics of the cochlea. II: Stationary nonlinear phenomenology. *J Acoust Soc Am* 99: 2244–2255, 1996.

119. Nobili R, Vetesnik A, Turicchia L, Mammano F. Otoacoustic emissions from residual oscillations of the cochlear basilar membrane in a human ear model. *J Assoc Res Otolaryngol* 4: 478–494, 2003.

120. Nouvian R, Beutner D, Parsons TD, Moser T. Structure and function of the hair cell ribbon synapse. *J Membr Biol* 209: 153–165, 2006.

121. Oghalai JS, Patel AA, Nakagawa T, Brownell WE. Fluorescence-imaged microdeformation of the outer hair cell lateral wall. *J Neurosci* 18: 48–58, 1998.

122. Oliver D, He DZ, Klocker N, Ludwig J, Schulte U, Waldegger S, Ruppersberg JP, Dallos P, Fakler B. Intracellular anions as the voltage sensor of prestin, the outer hair cell motor protein. *Science* 292: 2340–2343, 2001.

123. Oliver D, Klocker N, Schuck J, Baukrowitz T, Ruppersberg JP, Fakler B. Gating of Ca^{2+}-activated K^+ channels controls fast inhibitory synaptic transmission at auditory outer hair cells. *Neuron* 26: 595–601, 2000.

124. Palmer AR, Russell IJ. Phase-locking in the cochlear nerve of the guinea-pig and its relation to the receptor potential of inner hair-cells. *Hear Res* 24: 1–15, 1986.

125. Parsons TD, Sterling Synaptic ribbon P. Conveyor belt or safety belt? *Neuron* 37: 379–382, 2003.

126. Pearson RA, Dale N, Llaudet E, Mobbs P. ATP released via gap junction hemichannels from the pigment epithelium regulates neural retinal progenitor proliferation. *Neuron* 46: 731–744, 2005.

127. Petit C. From deafness genes to hearing mechanisms: harmony and counterpoint. *Trends Mol Med* 12: 57–64, 2006.

128. Petit C, Levilliers J, Hardelin JP. Molecular genetics of hearing loss. *Annu Rev Genet* 35: 589–646, 2001.

129. Piazza V, Ciubotaru CD, Gale JE, Mammano F. Purinergic signalling and intercellular Ca^{2+} wave propagation in the organ of Corti. *Cell Calcium* 41: 77–86, 2006.

130. Puschner B, Schacht J. Calmodulin-dependent protein kinases mediate calcium-induced slow motility of mammalian outer hair cells. *Hear Res* 110: 251–258, 1997.

131. Raphael Y, Altschuler RA. Structure and innervation of the cochlea. *Brain Res Bull* 60: 397–422, 2003.

132. Raybould NP, Jagger DJ, Kanjhan R, Greenwood D, Laslo P, Hoya N, Soeller C, Cannell MB, Housley GD. TRPC-like conductance mediates restoration of intracellular Ca^{2+} in cochlear outer hair cells in the guinea pig and rat. *J Physiol* 579: 101–113, 2007.

133. Ricci AJ, Crawford AC, Fettiplace R. Tonotopic variation in the conductance of the hair cell mechanotransducer channel. *Neuron* 40: 983–990, 2003.

134. Ricci AJ, Fettiplace R. Calcium permeation of the turtle hair cell mechanotransducer channel and its relation to the composition of endolymph. *J Physiol* 506: 159–173, 1998.

135. Ricci AJ, Fettiplace R. The effects of calcium buffering and cyclic AMP on mechano-electrical transduction in turtle auditory hair cells. *J Physiol* 501: 111–124, 1997.

136. Ricci AJ, Kennedy HJ, Crawford AC, Fettiplace R. The transduction channel filter in auditory hair cells. *J Neurosci* 25: 7831–7839, 2005.

137. Ricci AJ, Wu YC, Fettiplace R. The endogenous calcium buffer and the time course of transducer adaptation in auditory hair cells. *J Neurosci* 18: 8261–8277, 1998.

138. Roberts WM, Jacobs RA, Hudspeth AJ. Colocalization of ion channels involved in frequency selectivity and synaptic transmission at presynaptic active zones of hair cells. *J Neurosci* 10: 3664–3684, 1990.

139. Robles L, Ruggero MA. Mechanics of the mammalian cochlea. *Physiol Rev* 81: 1305–1352, 2001.

140. Safieddine S, Wenthold RJ. SNARE complex at the ribbon synapses of cochlear hair cells: analysis of synaptic vesicle- and synaptic membrane-associated proteins. *Eur J Neurosci* 11: 803–812, 1999.

141. Saito K. Fine structure of the sensory epithelium of guinea-pig organ of Corti: subsurface cisternae and lamellar bodies in the outer hair cells. *Cell Tissue Res* 229: 467–481, 1983.

142. Saito K. Fine structure of the sensory epithelium of the guinea pig organ of Corti: afferent and efferent synapses of hair cells. *J Ultrastruct Res* 71: 222–232, 1980.

143. Santos-Sacchi J, Song L, Zheng J, Nuttall AL. Control of mammalian cochlear amplification by chloride anions. *J Neurosci* 26: 3992–3998, 2006.

144. Scemes E, Giaume C. Astrocyte calcium waves: what they are and what they do. *Glia* 54: 716–725, 2006.

145. Schnee ME, Lawton DM, Furness DN, Benke TA, Ricci AJ. Auditory hair cell-afferent fiber synapses are specialized to operate at their best frequencies. *Neuron* 47: 243–254, 2005.

146. Schultz JM, Yang Y, Caride AJ, Filoteo AG, Penheiter AR, Lagziel A, Morell RJ, Mohiddin SA, Fananapazir L, Madeo AC, Penniston JT, Griffith AJ. Modification of human hearing loss by plasma-membrane calcium pump PMCA2. *N Engl J Med* 352: 1557–1564, 2005.

147. Shah DM, Freeman DM, Weiss TF. The osmotic response of the isolated, unfixed mouse tectorial membrane to isosmotic solutions: effect of Na^+, K^+, and Ca^{2+} concentration. *Hear Res* 87: 187–207, 1995.

148. Shen J, Harada N, Nakazawa H, Yamashita T. Involvement of the nitric oxide-cyclic GMP pathway and neuronal nitric oxide synthase in ATP-induced Ca^{2+} signalling in cochlear inner hair cells. *Eur J Neurosci* 21: 2912–2922, 2005.

149. Spicer SS, Thomopoulos GN, Schulte BA. Novel membranous structures in apical and basal compartments of inner hair cells. *J Comp Neurol* 409: 424–437, 1999.

150. Spray DC, Ye ZC, Ransom BR. Functional connexin "hemichannels": a critical appraisal. *Glia* 54: 758–773, 2006.

151. Stauffer EA, Scarborough JD, Hirono M, Miller ED, Shah K, Mercer JA, Holt JR, Gillespie PG. Fast adaptation in vestibular hair cells requires myosin-1c activity. *Neuron* 47: 541-553, 2005.

152. Street VA, McKee-Johnson JW, Fonseca RC, Tempel BL,. and Noben-Trauth K. *Mutations in a plasma membrane Ca2+-ATPase gene cause deafness in deafwaddler mice Nat Genet* 19: 390-394, 1998.

153. Sziklai I, Szonyi M, Dallos P. Phosphorylation mediates the influence of acetylcholine upon outer hair cell electromotility. *Acta Otolaryngol (Stockh)* 121: 153-156, 2001.

154. Taschenberger H, Leao RM, Rowland KC, Spirou GA,. and von Gersdorff H. *Optimizing synaptic architecture and efficiency for high-frequency transmission Neuron* 36: 1127-1143, 2002.

155. Teubner B, Michel V, Pesch J, Lautermann J, Cohen-Salmon M, Sohl G, Jahnke K, Winterhager E, Herberhold C, Hardelin JP, Petit C, Willecke K. Connexin30 (Gjb6)-deficiency causes severe hearing impairment and lack of endocochlear potential. *Hum Mol Genet* 12: 13-21, 2003.

156. Thorne PR, Housley GD. Purinergic signalling in sensory systems. *Semin Neurosci* 8: 233-246, 1996.

157. Thorne PR, Munoz DJ, Housley GD. Purinergic modulation of cochlear partition resistance and its effect on the endocochlear potential in the Guinea pig. *J Assoc Res Otolaryngol* 5: 58-65, 2004.

158. Tolomeo JA, Steele CR, Holley MC. Mechanical properties of the lateral cortex of mammalian auditory outer hair cells. *Biophys J* 71: 421-429, 1996.

159. Vlajkovic SM, Thorne PR, Housley GD, Munoz DJ, Kendrick IS. Ecto-nucleotidases terminate purinergic signalling in the cochlear endolymphatic compartment. *Neuroreport* 9: 1559-1565, 1998.

160. Wangemann P. K(+) cycling and its regulation in the cochlea and the vestibular labyrinth. *Audiol Neurootol* 7: 199-205, 2002.

161. Wangemann P. K+ cycling and the endocochlear potential. *Hear Res* 165: 1-9, 2002.

162. Wood JD, Muchinsky SJ, Filoteo AG, Penniston JT, Tempel BL. Low endolymph calcium concentrations in deafwaddler2J mice suggest that PMCA2 contributes to endolymph calcium maintenance. *J Assoc Res Otolaryngol* 5: 99-110, 2004.

163. Wu YC, Ricci AJ, Fettiplace R. Two components of transducer adaptation in auditory hair cells. *J Neurophysiol* 82: 2171-2181, 1999.

164. Wu YC, Tucker T, Fettiplace R. A theoretical study of calcium microdomains in turtle hair cells. *Biophys J* 71: 2256-2275, 1996.

165. Yamoah EN, Lumpkin EA, Dumont RA, Smith PJ, Hudspeth AJ, Gillespie PG. Plasma membrane Ca2+-ATPase extrudes Ca2+ from hair cell stereocilia. *J Neurosci* 18: 610-624, 1998.

166. Zhang M, Kalinec GM, Urrutia R, Billadeau DD, Kalinec F. ROCK-dependent and ROCK-independent control of cochlear outer hair cell electromotility. *J Biol Chem* 278: 35644-35650, 2003.

167. Zhang Y, Tang W, Ahmad S, Sipp JA, Chen P, Lin X. Gap junction-mediated intercellular biochemical coupling in cochlear supporting cells is required for normal cochlear functions. *Proc Natl Acad Sci USA* 102: 15201-15206, 2005.

168. Zidanic M, Brownell WE. Fine structure of the intracochlear potential field. *I The silent current Biophys J* 57: 1253-1268, 1990.