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Structural Aspects of Slow Mechanical Adaptation in the Vertebrate Cochlea

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Anatomical and experimental data suggesting a slow adaptation of cochlear mechanics are summarized and discussed. All groups of terrestrial vertebrates, possessing advanced hearing—mammals, Archosauria (birds and crocodiles) and lizards—developed intrinsic cochlear specializations, which may adjust cochlear mechanics and therefore adapt hearing to different acoustic environments, or protect the cochlea from excessive mechanical stimuli. Mammalian outer hair cells, several types of supporting cells, hyaline and homogene in birds and crocodiles, and putative contractile cells of the cochlear lateral wall in mammals and in geckos may provide structural basis for the slow mechanical adaptation. Independent appearance of these specializations in animals that developed different cochlear designs may indicate that the maintainence of "mechanical homeostasis" is a common requirement for the highly organized hearing organ.

Mechano-electric transduction in the vertebrate hearing organs is mediated by mechano-sensitive channels located on the hair cell stereocilia, whose deflection produces an adequate physiological signal. The full dynamic range of the stereocilium transducer is narrow, since it covers approximately 2 angular degrees of hair displacement, or 100 nm of a total excursion at the tip of the hair bundle (Furness et al., 1997; Kros, Lennan, & Richardson, 1995; see Fettiplace & Ricci, 2003). Therefore, precise control of the mechanical input is required to keep the auditory hair cells within their operating range (Fettiplace & Ricci, 2003). Adaptation of the transducer channels has been suggested to be such a mechanism, providing control of the mechanical input in auditory hair cells (see Eatock, 2000; Fettiplace & Ricci, 2003). This process occurs *locally* in stereocilia, involves the stereocilium cytoskeleton and is mediated by Ca^{2+} ions entering through the opened transducer channels.

In addition to local adaptation, there are also active mechanical processes within the cochlea. These mechanisms may act at the level of the hair cells and surrounding sustentacular cells or even at the level of the entire cochlea and may participate in the regulation of the mechanical input to the hair cells by the adjustment of the cochlear micro- and macromechanics. In mammals, the fast cycle-by-cycle cochlear amplifier based on the somatic electromotility of the outer hair cells is known to affect basilar membrane motion (see Grosh et al., 2004; Nuttall & Ren, 1995). In nonmammalian vertebrates, the cochlear amplifier is thought to be based on fast active motions of the hair bundles (see Manley, 2001). A tonic modulation of the fast cochlear amplifier as well as the slow active mechanical processes within the cochlea may maintain cochlear "mechanical homeostasis" and adapt hearing to different acoustic situations or protect auditory sensory cells from ex-

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cessive mechanical stimuli. The slow mechanisms may operate over a time scale of tens of seconds or even longer. In this review we focus mainly on the putative structural basis of slow actin-mediated mechanical adaptations in the cochlea of mammals, birds, and reptiles. These mechanisms are still poorly understood, partly because it is not yet known how the changes in the mechanical properties of different cochlear structures may affect the mechano-electrical transduction in the hair cells (Dallos, 2003, also see Ulfendahl, 1997, and Robles and Ruggero, 2001).

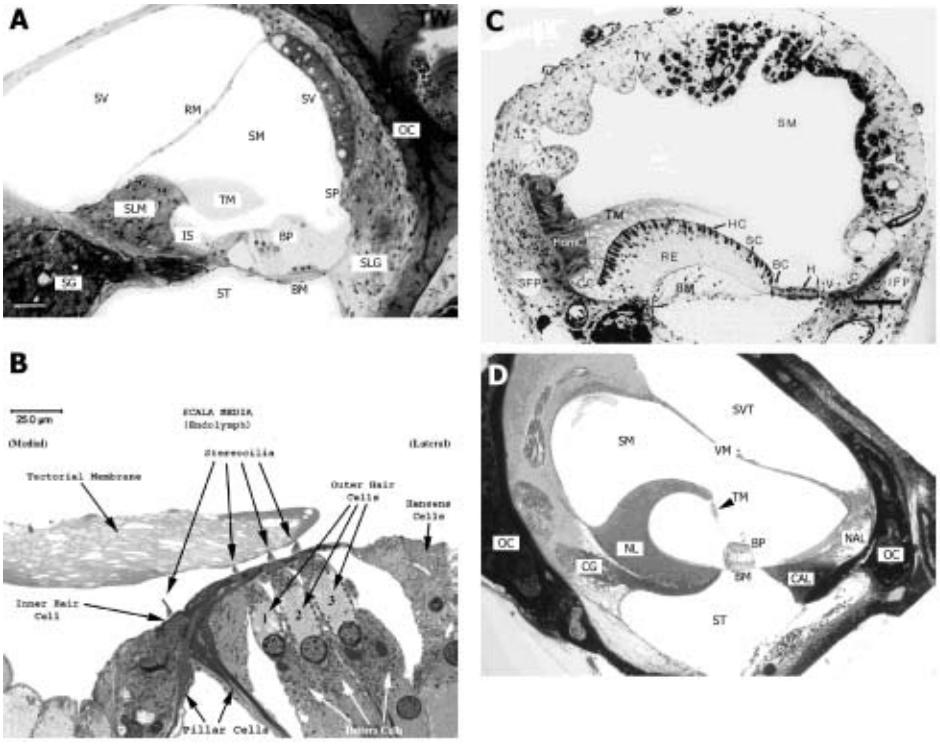

Figure 1. A structure of the cochlea in mammals, birds and lizards. (A) A cross section through the basal cochlear turn of mouse. Scale bar = 44 μm (modified from Ohlemiller et al., 2002; reproduced with permission from Elsevier). (B) A cross section of the mole rat organ of Corti (modified from Raphael & Altschuler, 2003; reproduced with permission from Elsevier). (C) A cross section through the cochlear duct of chick, scale bar = 100 μm (modified from Oesterle et al., 1992; reproduced with permission from Wiley & Sons); note a gradual decrease in the length of the hair cell from superior edge of the basilar papilla, where tall hair cells are located to inferior edge, where short hair cells are located. (D) A cross section through the cochlear duct of gecko *Teratoscincus scincus*, scale bar = 150 μm (from Ganeshina and Vorobyev, 2003; reproduced with permission from Wiley & Sons). BC – border cells, BM – basilar membrane, BP (RE) – basilar papilla (receptor epithelium), C - cuboidal cells, CAL – cartilaginous abneural limbus, CG – cochlear ganglion, HC – hair cells, TM –tectorial membrane, HP – habenula perforata, H – hyaline cells, HomC – homogene cells, IFP – inferior fibrocartilaginous plate, IS – inner sulcus, NAL – noncartilaginous abneural limbus (contractile region), NL – neural limbus, OC – otic capsule, RM – Reissner's membrane, SFP – superior fibrocartilaginous plate, SG – spiral ganglion, SP – spiral prominence, SLG – spiral ligament, SLM – spiral limbus, ST – scala tympani, TV – tegmentum vasculosum, SM – scala media, SV– stria vascularis, SVT – scala vestibuli, V - vacuole cells, VM – vestibular membrane.

Hearing organs of modern amniotes—reptiles, birds, and mammals—are finely tuned mechano-sensory organs, operating over a wide dynamic range (see Manley 2000, 2001). They provide auditory information to detect prey, predators

and mates, and to communicate by sound. The auditory sensory cells of higher vertebrates are located in a special compartment, the cochlea, which is separated from the vestibular part of the inner ear (the term "cochlea" is currently used for mammals as well as other amniotes, even though the latter do not have a spiral organ). The cochlea is supposed to be homologous in all amniotes (Manley, 2000) and is characterized by the presence of a movable basilar membrane housing mechanosensory hair cells (Figure 1). Arising from common ancestral form, the hearing organs of the main phylogenetic lineages of amniotes independently developed different sophisticated cochlear designs, enabling them to significantly improve hearing performance (Manley, 2000). If the maintenance of "mechanical homeostasis" is the common requirement for the highly-organized cochleae, we may expect to find this mechanism in all amniotes with well developed hearing. Indeed, the structures that exhibit properties compatible with slow mechanical adaptation are found in cochlea of mammals, birds, crocodiles and lizards. These structures include outer hair cells (present only in the mammalian cochlea), supporting cells, hyaline and homogene cells in birds and crocodiles, and putative contractile cells of the cochlear lateral wall. Active mechanical processes, which occur in the mammalian outer hair cells, have attracted scientists who study peripheral mechanisms of hearing over the last decades. By contrast, relatively little is known about the role of other cochlear components in active mechanical processes that may underlie mechanical adaptation in the vertebrate hearing organ. In this review, we briefly discuss the possible involvement of the sensory hair cells in slow adaptation of cochlear mechanics, and then review data on the role of nonsensory cochlear components in this process.

Fast Active Mechanical Processes in the Auditory Hair Cells

Vertebrate hair cells have a dual role in hearing—they mediate mechano-electrical transduction and provide mechanical amplification of low-level signals. The latter process is thought to be responsible for the broad dynamic range of the hearing organs. It is assumed that the cochlear amplifier in mammals is mediated by fast (i.e., operating in the microsecond time scale) somatic electromotility in outer hair cells (OHCs) (Brownell et al., 1985; see Dallos & Fakler, 2002; Geleoc & Holt, 2003; Santos-Sacchi, 2003). The electromotility is independent of Ca^{2+} and ATP, and is believed to be mediated by a molecular motor, prestin, located in the lateral membrane of the OHCs. The fast, cycle-by-cycle, electromotility supplements the energy of the sound stimuli and therefore enhances the sensitivity to weak signals (see Robles & Ruggero, 2001). In mammals, the OHC somatic electromotility is believed to generate a force capable of enhancing the basilar membrane motion, that is, to affect cochlear macromechanics (Grosh et al., 2004; Nuttall & Ren, 1995).

According to the current view, the somatic motility of mammalian OHCs is unique in that it does not occur in nonmammalian vertebrates (Brix & Manley, 1994; He et al., 2003a). However, the amplification of acoustic stimuli is a feature of hair cells that seems to have evolved early in vertebrate lineage. The phylogenetically ancient mechanism of amplification of mechanical stimuli is thought to be mediated by Ca^{2+}- dependent, myosin-based hair bundle motility, which is tightly linked to adaptation of the transducer channels (Bozovic & Hudspeth, 2003;

Crawford & Fettiplace 1985, Howard & Hudspeth 1987; Manley et al., 2001; Martin & Hudspeth, 1999; Ricci, Crawford, & Fettiplace, 2000, 2002; see Fettiplace & Ricci, 2003; Hudspeth, 1997; Manley, 2001; Ricci, 2003). The active hair bundle motion demonstrated in auditory hair cells in the turtle (Crawford & Fettiplace, 1985) has been suggested to represent a mechanical "cochlear amplifier" in auditory organs of nonmammalian vertebrates (Crawford & Fettiplace, 1985; Koppl & Yates, 1999; Manley, 2000; Martin & Hudspeth, 1999; Ricci et al., 2000, 2002; Yates, Manley, & Koppl, 2000; see Manley, 2001; Ricci, 2003). Although the active hair bundle motion in cochlea hair cells has been so far been demonstrated only in turtles, the phenomenon of otoacoustic emission (OAE) found in amphibians, birds, and lizards gives an indication of the existence of the cochlear amplifier in hearing organs of other nonmammalian vertebrates (Koppl & Manley, 1994; Manley et al., 1996; Manley & Gallo, 1997; Taschenberger & Manley, 1997; van Dijk et al., 1996). Moreover, the properties of the electrically evoked OAE in the lizard *Tiliqua rugosa* support the hypothesis of hair bundle motion as the origin of the cochlear amplifier (Manley et al., 2001).

It is thought that although the ancient mechanism of amplification based on the active hair bundle motility may persist in mammals, OHC somatic electromotility evolved in mammals to enhance high frequency hearing (Santos-Sacchi, 2003). It is important to note that the somatic motility of mammalian OHCs may also provide active negative feedback and hence attenuate signals (Zinn et al., 2000). Because the intensity of the low frequency sounds at the level of input to the cochlea usually significantly exceeds the intensity of high frequency sounds, the active amplification of low frequency sounds may not be needed (Zinn et al., 2000). Indeed, while the amplification of high frequency sounds in the basal part of the mammalian cochlea is prominent (see Robles & Ruggero, 2001), the amplification of low frequency sounds in the apex of the cochlea is either much less pronounced or absent (Hemmert, Zenner, & Gummer, 2000; Zinn et al., 2000). It has been suggested that in the cochlear apex the OHCs are required only for active attenuation (Zinn et al., 2000).

The ultimate result of OHC electromotility is a change of the angle of the stereovilli, which leads to either opening or closing of mechanosensitive channels. Depending on the relation of the stiffness of the basilar membrane and reticular lamina, the elongation of OHC may lead to cilia deflections of different signs (Dallos, 2003). Hence the electromotility of the OHC may provide either positive or negative feedback depending on the macromechanical properties of different components of the organ of Corti and basilar membrane. Because the mammalian cochlear amplifier operates within the limited dynamic range of the hair bundle displacement (Dallos, 2003), a precise adjustment of stiffness of all components of the organ of Corti, basilar and tectorial membranes is needed to maintain "mechanical homeostasis" within the cochlea. The control of gain and operating point of the cochlear amplifier may be provided by mechanisms located in hair cells as well as nonsensory cochlear components.

Modulation of the Hair Cell Mechanical Properties

In mammalian cochlea, phenomenon of the OHC "slow motility," that is, changes in the OHC length over the tens-of-seconds time scale, have been demon-

strated in different in vitro paradigms (Canlon et al, 1988; Dulon, Zajic, & Schacht, 1990; Flock, Flock, & Ulfendahl, 1986; Zenner, 1986). Unlike the fast electromotility, the slow OHC "motility" is calcium- and ATP-dependent and originally was thought to be mediated by an acto-myosin contractile system (Flock et al., 1986; Zenner, 1986). Increasing intracellular free calcium in isolated OHCs causes reversible elongation of the cells (Dulon et al., 1990, Frolenkov, Mammano, & Kachar, 2003). A specialized network of actin filaments, located beneath the OHC lateral membrane has been revealed in the guinea pig and is suggested as a structural basis for the OHC "slow motility" (Flock et al., 1886; Zenner, 1886). This cortical cytoskeleton, or "cortical lattice" consists of long circumferential actin filaments that are interconnected by short longitudinal spectrin filaments (Holley & Ashmore, 1988, 1990; see Holley, 1996). The slow calcium-dependent motile response of isolated OHCs is associated with changes in the OHC axial stiffness (Dallos et al., 1997; Dulon et al., 1990; Frolenkov et al., 2003). The OHC stiffness changes are thought to be mediated by spectrin links between adjacent circumferential actin filaments (see Holley, 1996). Although originally OHC "slow motility" has been considered as a result of active circumferential contraction-relaxation of the cortical cytoskeleton (Dulon, 1990; Flock et al, 1986; Zenner, 1986), further studies revealed that the OHC "slow motility" is operated by sophisticated mechanism(s), in which modulation of the OHC axial stiffness plays central role (e.g., Batta et al., 2003; He et al., 2003b; Holley & Ashmore, 1988, 1990; see Holley, 1996). It has been recently suggested that changes of the OHC length represent a passive mechanical reaction of the turgid OHC to Ca^{2+}-induced decrease in axial stiffness (Frolenkov et al., 2003).

Complex and multiple intracellular signaling pathways appear to mediate and regulate the OHC "slow motility." Evidence for the involvement of Ca^{2+}/calmodulin dependent protein phosphorylation has been provided by experiments demonstrating the blocking effect of the calmodulin and protein kinase inhibitors on Ca^{2+}-induced shape changes of the OHCs (Coling et al., 1998; Puschner & Schacht, 1997). Indeed, calmodulin has been shown to be present in higher amount in the OHCs compared to the IHCs, which do not exhibit the slow motility (Slepecky & Ulfendahl, 1993). Intracellular signaling pathways involving the small GTPases RhoA, Rac1 and Sdc42 have been identified as regulators of OHC slow motility elicited by acetylcholine (ACh), the major efferent neurotransmitter in the cochlea in vitro (see below; Kalines et al., 2000). Finally, nitric oxide/cGMP pathway involvement has also been recently demonstrated (Lin et al., 2003).

One of the first attempts to find a physiological correlate for the slow OHC motility was made by Zimmerman and Fermin (1996). In their experiments, exposure of the entire organ of Corti to artificial perilymph, which is known to induce shortening of isolated OHCs (Slepecky, Ulfendahl, & Flock, 1988), resulted in radial compression of the organ of Corti accompanied by the displacement of the reticular lamina and shortening of the OHCs. It has been proposed that regulated changes in the OHC shape in vivo may affect the passive cochlear mechanics (Zenner et al., 1990; Zimmerman & Fermin, 1996). Movements of the cochlear partition, or tonic force generation, may allow control of stiffness and/or geometry of the organ of Corti. Later, the radial compression of the organ of Corti in response to sound stimulation has been demonstrated by direct observation of organ

of Corti in the temporal bone preparations (Flock et al., 1999; Fridberger et al., 1998; Fridberger & de Monvel, 2003). The slow mechanical adjustments of the OHC length/stiffness may function as an automatic gain control and active protection against excessive mechanical stimuli (Zenner et al, 1990). It can be used for adaptation of the hearing organ to different acoustic environments or behavioral tasks. How may this putative mechanism function in vivo? OHCs receive extensive efferent innervation arising from the brainstem (see Smith, 1973; Spoendlin, 1985). Two major efferent subsystems have been identified in the olivocochlear bundle: the lateral olivocochlear system (LOC), targeting inner hair cells, and medial olivocochlear system, MOC, targeting OHCs. The major neurotransmitter of the olivocochlear efferent system is ACh (see Eybalin, 1993). Stimulation of the MOC elicits release of ACh by the efferent terminals at basal poles of the OHCs; the latter express acetyl choline receptors (AChR) with distinct pharmacological properties (Bobbin & Konishi, 1974; Elgoyhen et al., 1994; Housley & Ashmore, 1991). Binding of ACh to this receptor opens the ion channels and allows Ca^{2+} influx into the OHC cytoplasm (Elgoyhen et al., 1994; Housley & Ashmore, 1991). In vitro application of ACh to isolated OHCs made it possible to distinguish between several ACh-mediated Ca^{2+} effects: the fast effect (tens or hundreds of milliseconds) is manifested in Ca^{2+}-elicited K^+ efflux and the OHC hyperpolarization (Dallos et al., 1997; Housley & Ashmore, 1991). The slow effects (tens of seconds) include the OHC "slow motility" and changes in axial stiffness and require Ca^{2+} release from intracellular stores (Dallos, 1997; Frolenkov et al., 2003).

In vivo stimulation of the MOC system or direct application of ACh is known to produce inhibitory effects on inner hair cell receptor potentials (Brown & Nuttall, 1984), electrical responses of the cochlear afferents (Wiederhold & Kiang, 1970) and basilar membrane motion (Dolan et al, 1997; Murugasu & Russell, 1996a, 1996b). Fast and slow inhibitory effects of the MOC stimulation on the cochlear potentials have been distinguished (Sridar et al., 1995). The slow MOC inhibitory effect may be related to the slow mechanical responses of isolated OHCs (Dallos, 1997). Both fast and slow MOC inhibitory effects can be suppressed by ACh inhibitors and appear to be mediated by the same AChR (Sridar et al., 1995). Recently, a slow (10-100 s) effect of electrical stimulation of the MOC on sound evoked vibrations of the basilar membrane has been demonstrated and considered as a sequence of changes in the OHC mechanical properties (Cooper & Guinan, 2003).

It should be noted that in vitro application of ACh increases the magnitude of the OHC electro-motile response (Dallos et al., 1997; Sziklai et al, 1996; Sziklai & Dallos, 1993). Two explanations of the discrepancy between the in vivo and in vitro data have been proposed (Dallos et al., 1997). First, the local feedback system in the organ of Corti is extremely complex, and the mechanical changes in a large group of OHCs may produce an effect opposite to that intuited from the behaviour of a single element. Second, the somatic motility of OHC is not a principle variable in cochlear amplification. The hair bundle active motility may also contribute to the amplification of sound stimuli (Dallos et al., 1997). Finally, complex multiple actions of ACh, possibly mediated by different intracellular pathways and targeting different OHC intracellular components, may contribute to OHC axial stiffness (He et al, 2003b), and account for the differences between in vitro and in vivo data.

In nonmammalian vertebrates, the presence of efferent innervation of the auditory hair cells is well documented (birds: Fischer, 1992, 1998; Keppler et al., 1994; Takasaka & Smith, 1971; Tanaka & Smith, 1978; caiman: von During et al., 1974; lizards and snakes: Miller & Beck, 1988, 1990; turtle: Sneary, 1988). As in mammals, electrical stimulation of the efferent fibers suppresses auditory peripheral function (e.g., Art et al., 1982, 1985). The inhibitory effect of the efferent system is thought to be a consequence of the ACh-mediated, Ca^{2+}-elicited increase of K^+ conductance and hyperpolarization of the hair cells (turtle: Art et al, 1982, 1985; chick: Shigemoto & Ohmori, 1990; Fuchs & Murrow, 1992; Ohmori, 1993). Direct experimental evidence of efferent modulation of mechanical properties of hair cells of birds and reptiles is still lacking. However, some structural similarities between mammalian outer hair cells and a specific hair cell type characteristic for birds and crocodiles—short hair cells—make this hypothesis plausible (Takasaka & Smith, 1971). Moreover, in birds, a subpopulation of the short hair cells lacks afferent innervation (Fischer, 1992, 1998) and therefore may solely function as mechanical modulators (e.g., Manley 2000).

Putative Active Mechanical Processes in Cochlear Nonsensory Cells

In the vertebrate auditory (basilar) papilla, sensory hair cells are surrounded by supporting cells, which are traditionally believed to provide only passive mechanical support to the hair cells. However it has been shown relatively recently in mammals, birds and crocodiles that distinct supporting and other nonsensory cell types may also be involved in active (i.e., energy consuming) mechanical processes, which influence mechanical properties of hair cells and/or surrounding sound-conducting structures. These cells exhibit properties compatible with the hypothesis that they are involved in the slow adaptation of the cochlear mechanics.

Mammalian Deiters' cells provide direct mechanical support to the OHCs (Figure 1B). They mediate basal attachment of the OHCs to the basilar membrane. Their phalangeal processes, free of mechanical contact, extend to the endolymphatic surface of the sensory epithelium. The heads of the phalangeal processes establish tight junctions with the apical ends of the OHCs and represent an intrinsic component of the rigid reticular lamina (e.g., Kimura, 1975). Therefore Deiters' cells have an optimal geometrical position that would allow them to transmit displacements to the OHCs and thereby directly affect the set point of the cochlear amplifier. Dulon et al. (1994) have shown that Deiters' cells, isolated from the guinea pig cochlea, responded to the increase of intracellular calcium by a slow (minute time scale) extension of the phalanges and by increasing in their stiffness. The intracellular mechanism of this response is still unclear. Although it is well known that Deiter's cells, and especially their phalangeal processes, are enriched in actin (Flock et al., 1982; Slepecky & Chamberlain, 1983), there is currently no direct evidence that actin-mediated contraction accounts for the in vitro mechanical response. ATP applied extracellularly to isolated Deiters' cells induced a reversible motile response of the phalanges (Bobbin, 2001), probably mediated by P2X and P2Y ATP-receptors (Housely et al., 1999). It is still unclear whether ATP is a physiological stimulus for a mechanical response of Deiter's cells in vivo.

Another intriguing feature of Deiters' cells is the presence of direct innervation. Wright and Preston (1976) have described nerve fibers running in the vicinity of the third row of Deiters' cells in the apical turns of the guinea pig cochlea. These fibers were identified as efferent fibers, because they degenerated after cutting of the olivocochlear bundle. Furthermore, the vesiculated nerve endings synapsing on the supranuclear zone of Deiters' cells have been revealed with electron microscopy along the entire cochlear length in humans (Nadol & Burgess, 1994) and guinea pig (Burgess et al., 1997). Later, Fechner et al. (1998) did not confirm the complete degeneration of the fibers innervating Deiters' cells after chronic cochlear deefferentation. They suggested that only a minor fraction of the fibers innervating Deiters' cells belonged to the olivocochlear bundle. Further studies are needed to determine the origins, neurochemistry and the role of the Deiters' cell innervation in cochlear function. In the context of our discussion, it is tempting to speculate, that efferent innervation may provide a central control of the active adaptation of cochlear mechanics, mediated by Deiters' cells.

Another type of supporting cells in mammals—Hensen's cells (Figure 1B)—also contain an actin cytoskeleton (e.g., Slepecky, 1996). These cells receive innervation, and exhibit synaptic contacts (Burgess, 1997). However, so far there is no evidence of active mechanical responses in these cells.

Experiments using sound stimulation of the guinea pig temporal bone preparation (Flock et al., 1999) suggested that the putative mechanical adaptation may function locally, in the absence of central control. In these experiments, exposure of cochlear explants to high intensity tones elicited a reversible radial shift of the outer (dynamic) part of the organ of Corti, comprising the OHCs, Deiters' and Hensen's cell complex. The mechanical response coincided with elevation of thresholds of cochlear microphonic potentials without any signs of damage to the organ of Corti. The results have been considered as evidence for the presence of a protective mechanism against noise trauma, mediated by active contraction of the Deiters' cells (Flock et al., 1999).

Birds and crocodiles inherited a specific cochlear design, so called archosaur cochlear type, from their common ancestor (Figures 1 and 2; Manley, 2000) As it has been already mentioned, the archosaurian cochlea exhibits striking evolutionary parallels with the mammalian cochlea, which are reflected in the structural specialization of the auditory hair cells and their innervation pattern (Manley, 2000). A further structural parallel between mammalian and archosaurian cochlea types is the presence of non-sensory cells that receive direct efferent innervation (Takasaka & Smith, 1971) and may have contractile properties (Cotanche et al., 1992; Drenckhahn et al., 1991; von During et al., 1974; Odinokova & Prokof'eva, 1975). Hyaline cells occupy a portion of basilar membrane free from hair cells at the inferior (abneural) edge of the basilar papilla (Figures 1C and 2A; Held, 1926). Takasaka and Smith (1971) described acetylcholinesterase-reactive nerve fibers in close proximity to the hyaline cells of pigeon. It was later established that hyaline cells of birds are indeed extensively innervated by efferent nerve fibers and receive efferent synaptic inputs (Keppler et al., 1994; Odinokova & Prokof'eva, 1975; Oesterle et al., 1992). A number of examples, obtained with various staining techniques, provided consistent evidence that the hyaline cells are innervated by thick efferent fibers, which originate from the auditory brainstem (Code & Carr, 1994) and which innervate both short hair cells and hyaline cells

(e.g., Keppler et al., 1994; Takasaka & Smith, 1971; Zidanic, 2002; Zidanic & Fuchs, 1996). A similar pattern of efferent innervation and synaptic contacts has also been demonstrated in caiman (Drenckhahn et al., 1991; von During et al., 1974). Relatively little is known about the synaptic mechanisms and physiological effect of efferent signals on hyaline cells. Recently it has been shown that ACh acts through muscarinic receptors, whose activation causes mobilization of intracellular Ca^{2+} (Lippe et al., 2002). On the other hand, the α9 type of ACh receptor, specific for the mammalian OHCs and avian short hair cells, is highly expressed in the hyaline cell area (Hiel et al., 2000).

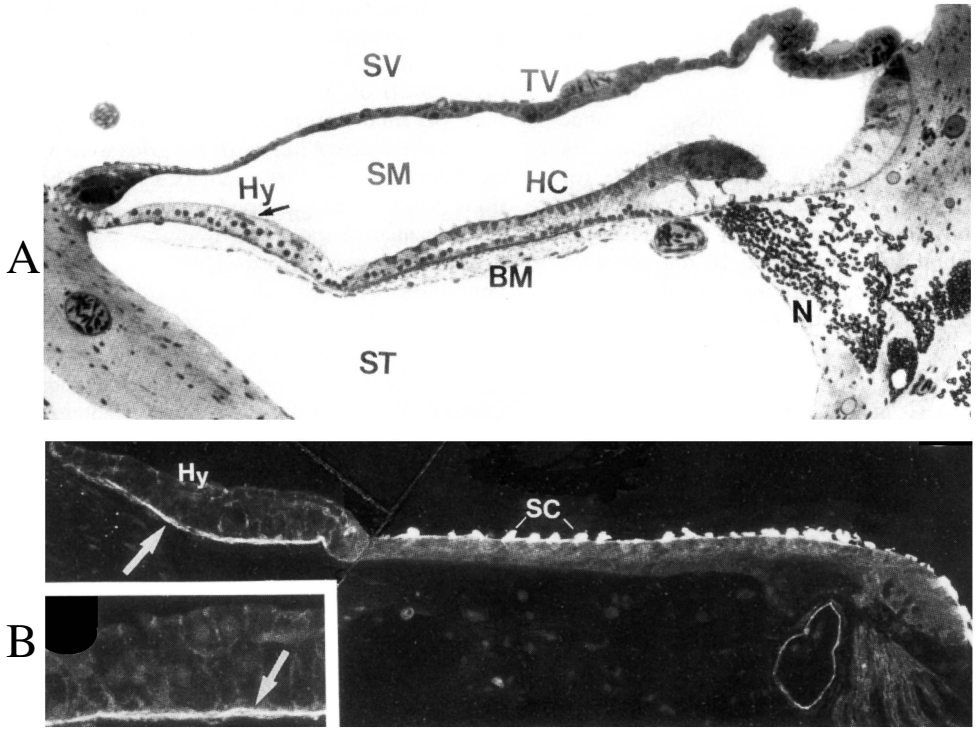

Figure 2. Expression of filamentous actin in the cochlea of *Caiman crocodiles.* (A) Toluidin-blue stained cross section through the mid-portion of the caiman cochlea. BM - basilar membrane, HC - basilar papilla with hair cells; HY - hyaline cells, N - nerve fibers, SM - scala media, ST - scala vestibuli, ST - scala tympani, TV - tegmentum vasculosum, X140. (B) Visualization of actin by phalloidin-rhodamine in cross section of the mid-portion of the auditory organ. Inset, is a higher magnification of hyaline cells (Hy). Arrows indicate strongly labeled basal portion of hyaline cells. Sc - stereovilli. Magnification X220, inset X440 (from: Drenckhahn et al., 1991; reproduced with permission from Elsevier).

Hyaline cells contain a prominent filament bundle network located in their basal portion and oriented transversely relative to the long axis of basilar membrane (caiman: Drenckhahn et al., 1991; chick: Cotanche et al., 1992). Filamentous actin, the smooth muscle form of myosin and α-actinin, has been demonstrated in the basal portion of the hyaline cells with phalloidin-rhodamine and immunostaining (Figure 2B; Cotanche et al., 1992; Drenckhahn et al., 1991). In chick, the bundles of actin filaments appeared to extend completely across the width of the hyaline cell region, forming large, multicellular cables. A tight attachment of the hyaline cells to the basilar membrane has been noted (Cotanche et al., 1992). More-

over, the structure of the basilar membrane under the hyaline cells differed from that under the sensory epithelium by a presence of wavy fibrils, oriented radially. All of these features of the hyaline cell-basilar membrane complex suggest that a radial contraction of the hyaline cells may be able to compress the basilar membrane in the radial direction (Cotanche et al., 1992; Drenckhahn et al., 1991). It has been hypothesized that hyaline cell contraction regulated by efferent fibers may, in turn, regulate the radial tension in the basilar membrane (Cotanche et al, 1992). This is likely to yield changes in the basilar membrane stiffness and as a consequence, a shift in the passive resonance properties of the cochlea. Hyaline cells also participate in the recovery of the basilar papilla after severe noise damage by fast migration into the hair cell damage areas (Cotanche et al., 1995). Hence, the hyaline cells may have both mechanical and regenerative functions in the cochlea. Because it is currently unknown whether the basilar membrane of birds is under tension, nor how the tension of basilar membrane affects its stiffness, further studies are needed to elucidate the role of hyaline cells in cochlear mechanics.

Besides purely mechanical processes, other cellular mechanisms operating in the cochlear supporting cells may contribute to the long-term adaptation of passive cochlear mechanics. Recently, for example, the molecular factors involved in the regulation of polymerization-depolymerization of actin filaments have been found in the cochlear supporting cells of mammals and birds (Heller et al., 1998; Oh et al., 2002; Schick et al., 2003). Vasodilator-stimulated phosphoprotein (VASP) and zyxin—the molecular factors that play an important role in the processes of focal cell adhesion and motility—have been demonstrated in the apical portion of mammalian pillar cells (Schick et al., 2003). Since the pillar cells have a central position in the organ of Corti and form a rigid bridge between inner and outer hair cells (Figure 1B), changes in their mechanical properties through a regulation of the actin cytoskeleton dynamics may alter the resonance properties of the entire organ of Corti. Another molecular factor—a novel protein called "homogenin" (Heller et al., 1998)—has been discovered in the chicken homogene cells, a specific avian cell type, anchoring the tectorial membrane (Figure 1C; Ganeshina, 1985; Jahnke, Lundquist, & Wersall, 1969; Retzius, 1884; Vinnikov et al., 1965). Homogenin belongs to the gelsolin family, which is known to regulate the extent of actin polymerization in response to Ca^{2+} and other cytoplasmic signals (Shafer & Cooper, 1995). Because homogenin was found to colocalize with filamentous actin in the apical portion of the chick homogene cells, it has been suggested that this molecular factor may participate in a system that adjusts the tension of the tectorial membrane (Heller et al., 1998). Indirect evidence suggesting that homogene cells undergo a mechanical stress arises from the fact that their intracellular cytoskeletal filaments are uniformly oriented along the cell axis, that is, at the right angle relative to the cochlear axis (Ganeshina, 1985). Moreover, the basal membrane of the homogene cells forms deep invaginations, and dense collagen bundles protrude into these invaginations from underlying connective tissue. These bundles are tightly attached to the homogene cell basal membrane and are oriented in the same (transverse) direction (Ganeshina, 1985). The entire structure appears to act as an anchor aimed at resisting uni-directional mechanical stress, indicating the most probable direction of the tectorial membrane vibration (Ganeshina, 1985).

It is important to note that the examples considered above and hypotheses suggesting a slow mechanical adaptation mediated by cochlear supporting cells are still highly speculative and based mainly on indirect evidence. Further studies are needed to confirm or dismiss the suggested mechanisms. However, the fact that similar structural specializations appeared in hearing organs of the diverged phylogenetic lineages of higher vertebrates may indicate a common functional significance of these specializations.

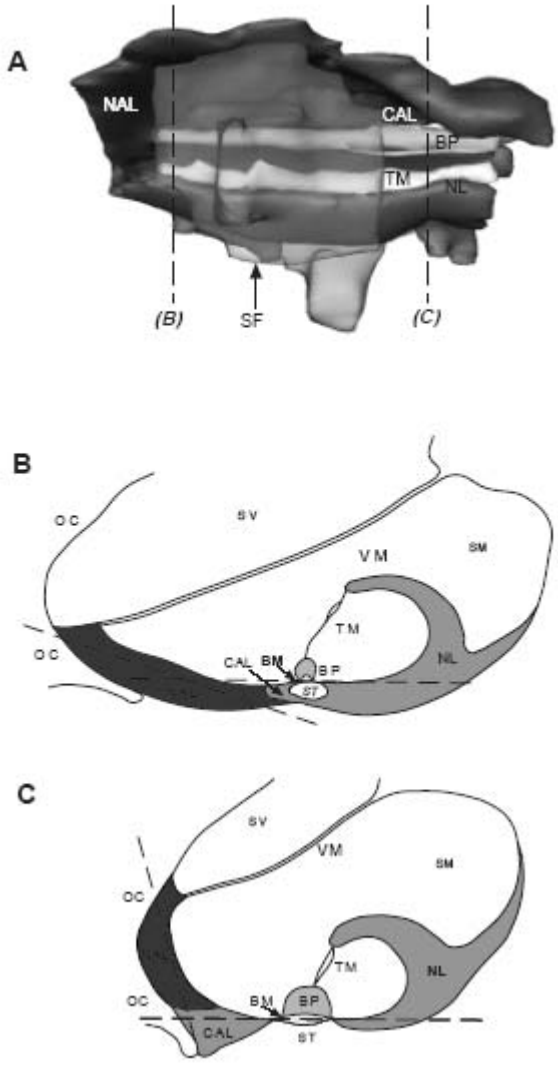

Figure 3. A contractile cochlear frame of the gecko *Teratocincus scincus*. (A) A three-dimensional reconstruction of the cochlea, lateral view (vestibular membrane is not shown), the middle ear stapedial footplate is shown transparent. (B,C) - outlines of the transverse sections through the cochlear duct in basal (B) and apical (C) parts of the basilar papilla. BP - basilar papilla, CAL – cartilaginous portion of abneural limbus, CG – cochlear ganglion, NAL – noncartilaginous portion of abneural limbus (contractile region), NL – neural limbus, OC – otic capsule, SM – scala media, ST – scala tympani, SV – scala vestibuli, TM - tectorial membrane, VM – vestibular membrane (from Ganeshina & Vorobyev, 2003; reproduced with permission from Wiley & Sons)

Putative Contractile Cells in the Cochlear Lateral Wall

In all groups of terrestrial vertebrates the cochlear part of the membranous labyrinth is enclosed in a bony otic capsule. Attachment of the membranous cochlea to the otic capsule occurs through a rigid cartilaginous-like tissue, which forms a cochlear frame (Ganeshina & Vorobyev, 2003). In mammals, the cochlear frame is represented by the spiral limbus and spiral ligament. In birds and crocodiles it is represented by the exterior and inferior fibro-cartilaginous plates, and in lizards by the neural and abneural (triangular) limbus respectively (Figures 1 and 3).

It is generally accepted that the cochlear frame has a purely passive supportive function. However, in various mammalian species and in geckos the lateral aspect of the cochlear frame (e.g., spiral ligament and abneural limbus respectively) has been suggested to possess contractile properties (Ganeshina & Vorobyev, 1991, 1992, 2003; Henson et al., 1984, 1985; Henson & Henson, 1988; Vorobyev & Ganeshina, 1992).

In the spiral ligament, a specific fibrocyte type has been described in the region where the ligament attaches to the otic capsule (Henson et al., 1984, 1985; Henson & Henson, 1988; Morera, Dal Sasso, & Jurato, 1980; Takahashi & Kimura, 1970). These anchoring cells, or "tension fibrocytes" (Henson & Henson, 1988; Henson et al., 1984) are characterized by a well-developed intracellular cytoskeleton, composed of a parallel array of filaments. The ends of these filaments come into close contact with extracellular fiber bundles that directly penetrate the bone of the otic capsule. The contact between intra- and extracellular filaments is mediated by specialized densities (plaques) in the plasma membrane of the anchoring cells (Henson et al., 1984). Because the system of the extracellular fiber bundles appears to be continuous through the basilar membrane-spiral ligament complex, it has been suggested that the marginal region of the spiral ligament may be related to elasticity of the complex (Voldrich & Ulehlova, 1982), and that the anchoring cells may create tension in the basilar membrane in the radial direction (Henson et al., 1984). Later, it has been demonstrated that the tension fibroblasts, in addition to having a high level of actin, specifically express myosin, tropomyosin and α-actinin, i.e., the proteins essential for contractility of the acto-myosin system (Henson et al., 1985). If the extent of the anchoring cell contraction is regulated, it may adjust the basilar membrane tension. Anchoring cells have been demonstrated in representatives of many mammalian orders (Henson & Henson, 1988). It is interesting to note diversity in the arrangement of anchoring cells, even among animals belonging to the same mammalian group. For example, in horseshoe bats, the actin-loaded anchoring cells are the only means by which the spiral ligament is attached to the cochlear wall in the basal turn, that is, the anchoring cells are arranged optimally to regulate tension of the spiral ligament-basilar membrane complex. However, in mustached bats the anchoring cells seem to take-up slack in the complex array of spiral ligament collagen fibers as they approach their bony attachment (Henson & Henson, 1988). It is tempting to speculate, that the observed diversity is associated with various requirements to mechanical adaptation within cochlea, which, in turn, may be determined by acoustic behaviour specific for these groups.

If the stiffness of the basilar membrane is, at least in part, determined by its tension in the radial direction, the spiral ligament may play an important role in

the regulation of mechanical homeostasis of the cochlea. However, some measurements of basilar membrane properties are inconsistent with this hypothesis. According to the original measurements of von Bekesy (1960) on cadaver preparations, the basilar membrane is not stretched. Later, Voldrich (1978) showed that, in a fresh preparation, the guinea pig basilar membrane is stretched in the radial direction. An implication of this finding is that the basilar membrane stiffness can be tuned by adjustment of its tension. However, Olson and Mountain (1994) showed that in gerbil the basilar membrane stiffness is likely to be insensitive to the basilar membrane deflection (the stiffness depends quadratically on the deflection), which means that even for the stretched basilar membrane the basilar membrane stiffness is likely to be independent of its tension. Finally, recent studies of the stiffness of gerbil basilar membrane indicate that *in vivo* stiffness measurements do not include the contribution from the active process (Emadi, Richter, & Dallos, 2004). This study does not completely rule out the possibility of regulation of the mammalian basilar membrane stiffness, because the possibility that some unavoidable damage occurred while obtaining the very first measurement cannot be excluded (Emadi et al., 2004)

The abneural (triangular) limbus of lizards corresponds by its position to the spiral ligament of mammalian cochlea. Typically, the abneural limbus is composed of a cartilage-like tissue (Miller, Kasahara, & Murloy, 1967; Wever, 1974, 1978). However, in the gecko *Teratoscincus scincus*, a part of the abneural limbus is replaced by a smooth muscle-like tissue composed of large tightly packed elongated cells, oriented in transverse direction relative to the long axis of basilar membrane (Figures 1D and 3; Ganeshina & Vorobyev, 1991, 1992, 2003). The myocyte-like cells are filled with filaments, whose thickness is characteristic of actin. Also, electron microscopic observation of the muscle-like tissue reveals small bundles of nerve fibers among the myocyte-like cells. ATP, delivered directly to the muscle-like cell cytoskeleton, specifically elicits reversible thinning of the tissue, suggesting contraction mediated by an acto-myosin system (Huxley, 1972). Moreover, application of noradrenaline to the contractile tissue, isolated in artificial perilymph, leads to slow relaxation of the myocyte-like cells (Ganeshina & Vorobyev, 2003; Vorobyev & Ganeshina, 1992). Three-dimensional reconstruction of the contractile region shows that it forms a curved ribbon extending along the basilar membrane axis between the otic capsule and cochlear duct epithelium (Figure 3). While in the basal portion of the cochlea it is oriented nearly in the plane of the basilar membrane, in the apical portion the ribbon is inclined relative to this plane (Figure 3). Therefore, it appears, that contraction of the tissue would lead to a complex “deformation” of the entire organ and affect different cochlear structures including basilar and vestibular membranes. Thus regulation of the contractile tissue tonus may adjust passive cochlear mechanics in the geckos. The contractile cochlear frame appears to be specific for gekkonoid lizards, since no muscle-like tissue was found in the cochlear frame of several agamid species (Ganeshina, unpublished data). How can the appearance of this specialized structure in a single lizard group be explained? Generally, the hearing organ of lizards exhibits a highly specialized cochlear structure (Manley, 1990, 2000). In the context of our discussion, two structural features characteristic of the lizard cochlea seem to be important. First, the central portion of the basilar membrane is thickened into a massive body, the fundus, or papillary bar. In contrast, its peripheral part attaching

to the limbus, is extremely thin: about 0.3-0.5 μm. Second, the tectorial membrane is oriented at an angle relative to the basilar papilla surface (Wever, 1978). This angle in geckos is about 90 degrees (Figure 3). Such a geometry requires that sound would elicit a pulling force instead of a shearing force developing between hair bundles and tectorial membrane as in cochleae of mammals or birds (compare Figures 1A, 1B, and 1C). This unusual cochlear design might make the cochlear structure very "fragile," that is, the cochlea could be easily damaged by sounds of high intensity. Indeed, sound overstimulation, which impairs hearing sensitivity in geckos, also results in detachment of the tectorial membrane from hair cell stereovilli over the entire basilar papilla (Wever, 1978). On the other hand, it is well known that among lizards, geckos possess the most advanced hearing, and that they are unique among other lizard groups by their ability to vocalize and use sounds for social communication (Manley, 1990; Marcellini, 1977; Wever, 1978). Some gecko species are able to emit very loud sounds that are detectable at a great distance (Marcellini, 1977). Therefore, it is reasonable to speculate that a specific mechanism evolved in geckos to protect their vulnerable hearing organ from the damage elicited by self-emitted sounds. A tonic damping effect of self-vocalization on cochlear microphonic potentials in echolocating bats has been demonstrated, and a cochlear mechanism of regulation and protection of the highly resonant cochlear partition, presumably mediated by the efferent MOC system, has been suggested (Xie & Henson, 1998).

Although the muscle–like tissue in the *Teratoscincus scincus* abneural limbus appears to be innervated, no synaptic contacts have been revealed between the nerve fibers and myocyte-like cells. Moreover, in a number of Australian gecko species, electron-microscopic observation of the muscle-like region did not reveal nerve fibres (Ganeshina, unpublished data). In mammals, tension fibroblasts of the spiral ligament also seem not to be innervated. Therefore, a regulation of the tonus of contractile cells in the cochlear lateral wall of mammals and geckos may operate in a different way, for example through stretch receptors in their plasma membrane.

It is interesting to note, that another striking evolutionary parallel between the mammalian spiral ligament and gecko abneural limbus is that both are likely to be also involved in ion transport, maintaining ionic gradients between endo- and perilymph (Ganeshina, 1991; Schulte & Adams, 1989; Spicer & Schulte, 1991).

Conclusions

We have reviewed data that suggest a structural basis for a putative mechanism of slow mechanical adaptation in the hearing organ of higher vertebrates. Mammalian outer hair (sensory) cells, nonsensory epithelial cells located on the basilar membrane, and specialized fibrocytes located in the connective tissue of the cochlear frame, exhibit structural features suggesting a regulated motility and/or development of tensile forces. Our discussion did not include data from a number of physiological studies, for example such phenomena as temporary threshold shift and sound conditioning (see Attanasio et al., 1998; Niu & Canlon, 2002; Quaranta et al., 1998). Analysis of these phenomena in the context of the putative mechanism of slow mechanical adaptation would help us to understand better how "mechanical homeostasis" is maintained in the cochlea under different

acoustic conditions. However, relevant information is not available for nonmammalian vertebrates.

According to the current view, the hearing organs of mammals, birds and reptiles independently developed structural specializations underlying high performance of their hearing organs (Ganeshina & Vorobyev, 1997; Manley, 1990, 2000). The comparative approach reveals striking evolutionary parallels in the structure of putative contractile components of the cochlea between different groups of higher vertebrates. The fact that different evolutionary lineages developed similar adaptation mechanisms despite the different cochlear designs may indicate that good hearing can be achieved only with a sensory apparatus whose mechanics are finely adjusted.

It should be noted that despite the long history of the idea of slow mechanical adaptation, there is still no direct evidence supporting this hypothesis. Many questions remain to be answered, and further studies are needed to elucidate the mechanisms, which allow some groups of vertebrates to perceive the complexity of the sounds offered by natural world.

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