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Somatic motility and hair bundle mechanics, are both necessary for cochlear amplification?

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Abstract

Hearing organs have evolved to detect sounds across several orders of magnitude of both intensity and frequency. Detection limits are at the atomic level despite the energy associated with sound being limited thermodynamically. Several mechanisms have evolved to account for the remarkable frequency selectivity, dynamic range, and sensitivity of these various hearing organs, together termed the active process or cochlear amplifier. Similarities between hearing organs of disparate species provides insight into the factors driving the development of the cochlear amplifier. These properties include: a tonotopic map, the emergence of a two hair cell system, the separation of efferent and afferent innervations, the role of the tectorial membrane, and the shift from intrinsic tuning and amplification to a more end organ driven process. Two major contributors to the active process are hair bundle mechanics and outer hair cell electromotility, the former present in all hair cell organs tested, the latter only present in mammalian cochlear outer hair cells. Both of these processes have advantages and disadvantages, and how these processes interact to generate the active process in the mammalian system is highly disputed. A hypothesis is put forth suggesting that hair bundle mechanics provides amplification and filtering in most hair cells, while in mammalian cochlea, outer hair cell motility provides the amplification on a cycle by cycle basis driven by the hair bundle that provides frequency selectivity (in concert with the tectorial membrane) and compressive nonlinearity. Separating components of the active process may provide additional sites for regulation of this process.

Keywords

cochlea; active process; somatic motility; hair bundle; adaptation; auditory

Introduction

Comparative biology is an excellent tool for identifying selective pressures shaping the evolution of particular systems. The auditory system has conserved properties throughout evolution to overcome common physical obstacles (Figure 1), while modifying other properties for hearing sensitivity, frequency range, and frequency selectivity (Manley,

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Koppl, 2008). The evolution and modification of the system demonstrates how a comparative approach can identify driving forces behind particular traits. The primary function of the peripheral auditory system is to convert complex airborne vibration into its fundamental frequency components, transmitting information regarding frequency, intensity and timing to the central nervous system. Several independent mechanisms have evolved to accomplish this daunting task and the relative contribution of each to human hearing remains to be resolved. The purpose of the present work is to attempt to gain insight into mechanisms of cochlear amplification and how evolution may have driven the continued adaptations of the system. Given the abundance of excellent reviews on cochlear amplification, somatic motility, hair bundle mechanics, and comparative hearing (Ashmore, 2008; Dallos, 2008; Fettiplace, Fuchs, 1999; Fettiplace, Hackney, 2006; Hudspeth, 2008; Manley, 2000b; Manley, 2001), the present work will focus less on the mechanistic and historical details associated with each process and more on the strengths and weaknesses provided by the potential mechanisms.

Cochlear Amplification

The auditory system differs from other sensory systems in that it is thermodynamically limited; at least for free standing bundles like those of IHC, the energy associated with auditory detection is comparable to the energy associated with Brownian motion of the sensory hair bundle (the detector) (Bialek, 1987; Bialek, Schweitzer, 1985; de Vries, 1948a; de Vries, 1948b; Harris, 1968; Hudspeth, 1985). Given the similarity in threshold levels across species, this energy obstacle was surmounted, perhaps in different ways, by different auditory end organs. Having to detect such low energies at threshold may also be responsible for evolving active frequency selectivity mechanisms, as reducing the frequency range of any given detector will limit the thermal noise it must overcome. Post filtering amplification can then be used to further enhance the signal to noise ratio. The requirement of frequency selectivity prior to amplification is important and may be a driving feature that led to the conservation of the tonotopic organization across auditory end organs. Tonotopy is simply how a broad frequency range at the system level is produced by the concerted effort of individual sensory cells responding over narrow bands in an ordered manner. Tonotopy is present in species as diverse as crickets and katydids, attesting to its fundamental importance in auditory detection (Romer, 1983; Stumpner, Molina, 2006). Amplification and filtering alone are not enough to account for the dynamic range of auditory systems. The human auditory system not only detects extremely low level sounds, but also operates in a noisy environment and adapts to respond without saturation to sounds that encompass 12 orders of magnitude in intensity (Moore, 2004). To accomplish this feat, auditory systems use a nonlinear amplifier whose gain varies relative to the input intensity. When sounds are loud the gain is lowered, when sound is low, the gain is raised, thus providing modulation of input across a broad range of stimulus intensities (Johnstone et al., 1986; Rhode, 1978; Rhode, Robles, 1974). This feature, termed compressive nonlinearity, indicates an input-output function that follows a power law such that the output scales as one-third the power of input (Ruggero et al., 1997). Deprivation of metabolic energy to the auditory end organs results in a rapid loss of mechanical amplification with thresholds elevating as much as 60 dB, demonstrating that signal processing is not passive (Davis et al., 1989; Ruggero, Rich, 1991). Concomitant with this threshold elevation is a broadening of the frequency response (loss of tuning) and a loss of compressive nonlinearity, illustrating the interaction between these components and the highly metabolically active nature of the system (Hudspeth, 2008). Together these three properties, compressive nonlinearity, frequency selectivity and amplification, constitute the cochlear amplifier or active process. A fourth property, more a byproduct than a function of the active process, is the ability to provide mechanical energy that feeds back through the system to generate sound (Kemp, 1986; Probst et al., 1991). Termed spontaneous otoacoustic emissions (SOAEs), they are considered a hallmark of the

active process and demonstrate an active mechanical contribution by the cochlear amplifier (Brownell, 1990). Multiple mechanisms probably underlie the active process, and the relative contribution of each may vary between species. Thus mechanisms underlying the active process would be expected to produce each of the four associated characteristics (Hudspeth, 2008; Manley, Koppl, 2008).

Signal transduction

Several properties of signal transduction are common between auditory organs. Airborne vibration is converted, typically via the middle ear, into fluid vibrations that stimulate the inner ear sensory cells, called hair cells. Fluid vibrations can stimulate directly via fluid induced movement of the sensory hair bundles or indirectly by causing differential movement of the basilar membrane (BM) (upon which the hair cells sit) and the tectorial membrane (TM) (a membrane in which multiple sensory hair bundles are embedded). The method of stimulation that predominates in a given end organ largely depends on the specific end organ structure.

Hair cells are specialized epithelial cells whose apical pole has a protrusion of actin filled microvilli (termed stereocilia, though stereovilli would be a more appropriate term) of increasing height (Figure 2) (Tilney, Saunders, 1983). The size and shape of the hair bundle varies between and within endorgans, but typically there is a stair-cased array oriented such that deflection toward the tallest stereocilia opens mechanosensitive channels located near the tops of these stereocilia, and stimulation away from the tall end, closes channels (Figure 2) (Hudspeth, 1982; Hudspeth, Corey, 1977; Hudspeth, Jacobs, 1979; Jaramillo, Hudspeth, 1991; Shotwell et al., 1981; Tilney, Saunders, 1983). Mechanosensitivity is posited to be imparted by a thin filamentous link, the tip-link, composed of protocadherin 15 and cadherin 23 (Ahmed et al., 2006; Kazmierczak et al., 2007; Pickles et al., 1984; Siemens et al., 2004). This link connects the top of shorter stereocilia with the side of longer stereocilia, oriented along the staircase pattern providing directional sensitivity (Shotwell et al., 1981). Where investigated, mechanotransduction responses show an adaptive behavior, a reduction in response (current amplitude) during a continued stimulation (Figure 2) (Crawford et al., 1989; Eatock et al., 1987). Adaptation is implicated in a variety of functions that include: increasing the dynamic range of the hair cell, maintaining the hair bundle in its most sensitive position, providing mechanical filtering and amplification, and setting the hair cell resting potential (Eatock, 2000; Eatock et al., 1987; Farris et al., 2006; Fettiplace, 2006; Hudspeth, 2008; Ricci et al., 2005). Most of the proteins involved in mechanosensitivity and adaptation remain to be identified, so the specifics of the underlying molecular mechanisms have yet to be elucidated. As more molecules and mechanisms are identified, better experiments will be possible to discern the specific role of mechanotransduction and adaptation in establishing the cochlear amplifier. The functions associated with adaptation will in part dictate what mechanisms might be plausible; for example setting the operating point of the bundle is less kinetically demanding than providing tuning and amplification on a cycle by cycle basis.

Comparison of auditory properties

From insects to mammals, hearing evolved separately (Figure 1) resulting in differences in hearing organ structure, yet many commonalities exist between organs that may give insight into factors driving their development (Table 1). These evolutionary differences in hearing structure produced distinctive properties in frequency selectivity and hearing thresholds. The commonalities between these diverse species elucidate the underlying principles of cochlear amplification required for hearing sensitivity and frequency selectivity.

A comparison of performance across species reveals similarities between species. Hearing thresholds across species are comparable across a broad range of frequencies (Figure 3a). Tuning curves can be compared for different species using center frequencies near the lowest thresholds (Figure 3b). These curves have a Q_{10dB} associated with them, but because center frequencies vary considerably between species and Q_{10dB} values vary widely depending on frequency, the stated values are used for a rough comparison and cannot be strictly interpreted in terms of sensitivity or selectivity. The plot does demonstrate a similarity in tuning between species that is remarkable given the differences in end organ structures and mechanisms. The differences observed in performance appear to be more correlated to frequency range, for example the bird and mammal curves show sharper high frequency rolloffs; other differences such as thresholds may be associated with middle ear properties rather than end organ detection limits.

Insects have an auditory structure that is different from most other organisms studied. Insects have two types of hearing organs: an antennal organ and a tympanal organ. The antennal organ transduces vibrations of antennae, and the tympanal organ transduces vibrations of a tympanic-like membrane (Kossel et al., 2008; Yack, 2004). The organs do not possess the classical structure of a hair cell with an apical sensory hair bundle, but rather have a single cilium that acts as a mechanoreceptor. Despite the stark morphological differences, mechanoreceptors may have the same evolutionary origin dating to early metazoans (Manley, Ladher, 2008). Similar to many hearing organs, the tympanal organ possesses a tonotopic map with tuned receptors along the organ (Kossel et al., 2008; Oldfield, 1988; Oldfield et al., 1986). The tympanal organs are tuned to frequencies between 1 kHz and 100 kHz being limited only at lower frequencies (Kossel et al., 2008). Receptor cells of the *Ancistrura nigrovittata* exhibit a characteristic V-shaped tuning curve with a Q_{10dB} of 1.9 at the lowest threshold of 30 dB SPL at 20kHz (Figure 3; table 1) (Stumpner, Molina, 2006). The sensory organelle is also embedded in an apical extracellular structure (scolopale cap) (Slifer, Sekhon, 1975; Yack, 2004), similar to other hearing organs that have hair bundles embedded in a tectorial membrane. Despite insects evolving independently (Figure 1) they still possess a similar basis of hearing detection with mechano-receptor organelles embedded in an apical extracellular matrix and cells that are frequency tuned and organized in a tonotopic pattern. Whether this is convergence in problem solving or due to mechanoreceptors having similar origins is open to interpretation.

The amphibian auditory organ, like insects, is also distinct from the stem reptiles' auditory organs because it has two end organs: the amphibian papilla and the basilar papilla. Despite this alternative organization, common properties of tonotopy and frequency tuned receptor cells are conserved at least in the amphibian papilla (see van Dijk in this special edition). The change in organ structure correlates with a shift in the frequency range to 0.1 – 4.5 kHz with lowered thresholds of 10 dB SPL at 20 kHz and low Q_{10dB} (table 1, Figure 3) (Fay, 1988; Stiebler, Narins, 1990). With these changes, a hair cell having a distinct hair bundle composed of stereocilia that possess mechanically gated ion channels, graded stereocilia heights, and directional sensitivity emerges (Schoffelen et al., 2008). The similarity of this sensory cell to other chordate groups is discussed in more detail by Burighel et al., in this edition. The hair bundle for this cell type is also embedded in a tectorial membrane structure on its apical surface (Lewis, 1984).

In a primitive land animal, the turtle, often considered similar to the stem reptile papilla, but having evolved independently, additional features evolved at the system level. The basilar papilla, the turtle hearing organ, consists of hair cells sitting atop a flexible basilar membrane, a membrane found in all vertebrates derived from the stem reptiles. The basilar papilla organization is primitive, consisting of multiple rows of a single hair cell type, oriented similarly (Manley, 2000b; Miller, 1978b). The frequency range of the organ is

narrow (0.07 – 1 kHz) and thresholds are relatively high, 40 dB SPL at 200 Hz for the *Trachemys scripta*; however, the sharpness of tuning is increased to a $Q_{10\text{dB}}$ of 3 (table 1, Figure 3) (Crawford, Fettiplace, 1980; Fay, 1988). Higher thresholds in turtle have been ascribed to a thicker eardrum as opposed to a difference in detection at the sensory cell (see other papers in this edition). Auditory papilla hair bundles vary in size, with shorter stereocilia at high frequency regions and longer stereocilia at low frequency regions (Hackney et al., 1993), another characteristic found in all vertebrate auditory organs derived from stem reptiles.

Lizards become more complex with different hair bundle orientations and multiple hair cell types (Table 1). Some lizard basilar papilla hair bundles are oriented in opposing directions, particularly in the high frequency tuned cells (Manley, 2000a; Miller, 1973; Miller, 1978a). The frequency range of lizards stretches from 0.4 – 7 kHz. Thresholds lower to 5 dB SPL at 1.2 kHz for the *Tiliqua rugosa* and $Q_{10\text{dB}}$ is about the same as the turtle at 3.5 (Figure 3, Table 1) (Manley, 1990; Manley et al., 1988; Manley et al., 1990). The lizard has multiple types of hair cells. One hair cell type receives afferent and efferent innervations and is responsible for low frequency hearing (Manley, 1990; Manley, 2000b), and a second type of hair cell only has afferent innervations and is responsible for high frequency hearing. These hair cells are covered by apical sallets (much like strips of tectorial membrane) or by continuous tectorial membrane (Manley, 1990; Manley, 2000b). A third type of cell is not embedded in any apical membrane and is referred to as a free standing hair bundle cell (Mulroy, Williams, 1987). A fourth type of hair cell is found in the tokay gecko, may exist in other lizard species as well, is located on the neural high frequency region of the basilar papilla, is covered by a tectorial membrane, and has no innervation (Chiappe et al., 2007). The functional role of these cells remains unknown but it is not a large leap to hypothesize that these cells will be involved in a mechanical type of tuning and amplification that feeds back onto innervated cells.

Birds and crocodilia continue to increase the complexity of their auditory end organ structure. Frequency range is further extended (0.2 – 12 kHz) and thresholds continue to lower to –20 dB SPL at 4 kHz with high $Q_{10\text{dB}}$ reaching 5 for the *Tyto Alba* (Figure 3, Table 1) (Köpl, 1997). The basilar papilla contains hair bundles with varying angular orientations and the structure contains two distinct sets of hair cells based on cell morphology and neural innervation (Fischer et al., 1988; Gleich, Manley, 1988; Manley, 2000b; Tanaka, Smith, 1978; Tilney, Saunders, 1983; Tilney et al., 1987). Tall hair cells, located on the neural side of the basilar papilla, are innervated by both afferent and efferent nerves; whereas, short hair cells, located abneurally, are innervated solely by efferent nerve fibers (Fischer, 1994; Fischer, 1998). Additionally, a tuned basilar membrane motion is suggested (Gummer et al., 1987). Many properties in birds and crocodilia have parallels in the mammal, attesting to their significance as ascribed by the evolutionary convergence.

Mammals have what appear to be the most sophisticated auditory organization with the widest range of audible frequencies (0.02 – 100 kHz) and low thresholds (–10 dB SPL) with a $Q_{10\text{dB}}$ of 4.2 at 8 kHz in the *Cavia porcellus* (Table 1, Figure 3) (Fay, 1988; Pickles, 1984). The auditory organ consists of a coiled cochlea where all sensory hair bundles are oriented toward the center of the coil. The mammalian cochlea contains two types of hair cells, the inner hair cells (IHCs) that receive the majority of afferent innervations and the outer hair cells (Ehret, 1979; Morrison et al., 1975), (OHCs) that receive efferent innervation synapsing directly onto the hair cell (Warr, 1992). The basilar membrane motion is actively tuned and amplified by OHCs (Rhode, 1971; Rhode, Robles, 1974; Ruggero et al., 1997). Despite what at first appear to be major changes to the organization of the end organ, mammalian hearing is quite comparable to that of other species with sensitivities being comparable or in some cases less than that found in some birds and lizards.

As the mammalian system incorporates many structural properties found in other hearing organs, insight into function may arise from a better understanding of the commonalities of these various hearing organs. For example, the use of both free standing and embedded hair bundles, the divergence in innervation patterns between hair cells, and the apparent separation of functions, with one cell type being responsible for amplification and the other for signal transduction to the brain, are all common features found in species aside from the mammal. As mammals have the only hearing organ with a somatically motile cell type an important question becomes: are the preexisting mechanisms of cochlear amplification in nonmammalian systems lost or obsolete in the face of the development of electromotility, or are they incorporated and advanced by the emergence of a new mechanical component? What is the advantage of somatic motility over preexisting amplification mechanisms? The remaining portions of this article discuss the known mechanisms of amplification and tuning focusing on electrical resonance, hair bundle mechanics and outer hair cell electromotility, discussing the strengths, weaknesses and areas where more work is needed for each. Finally, a hypothesis is proposed that incorporates the strengths of each mechanism as a possible means of generating a mammalian amplifier.

Electrical resonance as an amplification mechanism

Electrical resonance is one of the first tuning and amplification mechanisms identified in auditory hair cells (Crawford, Fettiplace, 1978). The interaction of L-type calcium channels and BK-type potassium channels coupled with the membrane capacitance creates an inductive circuit oscillating about the cell's characteristic frequency (Crawford, Fettiplace, 1978; Crawford, Fettiplace, 1981; Fettiplace, Crawford, 1978; Fuchs, Evans, 1988; Fuchs et al., 1988; Hudspeth, Lewis, 1988; Pitchford, Ashmore, 1987). Tonotopic variations in the number of calcium and calcium-activated potassium channels as well as the activation and deactivation kinetics of potassium channels create a frequency map (Art, Fettiplace, 1987; Jones et al., 1999; Wu et al., 1995). Electrical resonance is a major tuning mechanism in turtles, frogs, and birds (Table 1, Table 2). A role in lizards remains to be established; electrical resonance has been observed in the lizard, but resonant frequencies do not correlate with characteristic frequencies of the cell (Chiappe et al., 2007; Eatock et al., 1993). Theoretical analysis of electrical resonance suggests that it can operate up to about 6 kHz (Wu et al., 1995), though even in higher frequency hearing organs like bird, resonant frequencies have not been directly measured from the hair cell above about 1 kHz; however preferred interval measurements in afferent fibers from bird suggest resonant frequencies up to 5 kHz (Köppl, 1997). No evidence for electrical resonance exists in mammalian hair cells. Electrical resonance is mentioned in the context of cochlear amplification because it is suggested to provide the tuning and amplification required to completely account for afferent fiber tuning curves; however, it has no mechanical correlate, and therefore, no spontaneous otoacoustic emissions are generated by this mechanism. Thus by definition, electrical resonance cannot be the only mechanism responsible for cochlear amplification in these species.

Paradoxically, the strongest data supporting a role for active hair bundle mechanics in cochlear amplification comes from end organs that exhibit electrical resonance (Fettiplace, 2006; Fettiplace et al., 2001; Hudspeth, 2005; Hudspeth, 2008). In fact, the first report of active, tuned hair bundle motions comes from the turtle auditory papilla and may reflect the first example of electromechanical feedback (Crawford, Fettiplace, 1985). Similarly, strong data supports active hair bundle oscillations in frog saccule hair bundles, another organ where electrical resonance is quite robust (Bozovic, Hudspeth, 2003; Hudspeth, Lewis, 1988; Martin et al., 2003). Yet no data exists investigating the interplay between hair bundle oscillations and electrical resonance. How hair bundle tuning operates with respect to

electrical resonance remains to be elucidated and may be important for defining the principles and limitations of electromechanical feedback.

Mammalian hair cells show no electrical tuning properties. In general, both outer and inner hair cells tend to behave as low pass filters, with low input resistances reducing the membrane time constant but no obvious frequency selectivity (Dallos, 1985; Russell et al., 1989). Thus electrical resonance represents an example of a totally cellular mechanism being responsible for tuning and amplification, yet it is completely absent in mammalian hair cells and replaced by what appears to be a more systems based tuning mechanism.

Hair bundles as the cochlear amplifier

That hearing thresholds and frequency selectivity are comparable across species attests to the argument that an active process exists in hearing organs other than mammals (Table 1, Figure 3). The ability to measure otoacoustic emissions from non-mammalian species further supports a mechanical mechanism of amplification with conserved properties (Koppl, Manley, 1993; Manley et al., 1987; Manley et al., 1996; Manley et al., 2001; Taschenberger, Manley, 1997; van Dijk et al., 1989). Pharmacological sensitivities of these same emissions between mammalian and non-mammalian species argue for some convergence in mechanism (Stewart, Hudspeth, 2000). To date, no somatically motile cells have been identified in non-mammalian hearing organs, leaving active hair bundle mechanics as the likely source of amplification (He et al., 2003). Alone, a negative data argument is weak; fortunately several elegant experiments performed in lizard clearly localize the site of the active process, the mechanism underlying otoacoustic emissions, to the stereociliary hair bundles and further demonstrate that the mechanism is calcium dependent (Manley, Koppl, 2008; Manley et al., 2001). By utilizing a lizard organ where hair bundles are oriented 180 degrees from each other, investigators were able to modulate electrically induced emissions with low frequency sound in a manner consistent with generation being localized to the sensory hair bundle. Additionally, modeling data suggests that the hair bundles play a larger role in cochlear amplification in birds than mammals due to differences in the morphology of the basilar papilla compared to the mammalian organ of Corti (Sul, Iwasa, 2009). These provocative data clearly point to the hair bundle as a potential site of cochlear amplification.

Data suggest the hair bundle has the attributes required for cochlear amplification. Perhaps the simplest evidence, albeit circumstantial, for the hair bundle involvement in cochlear amplification is data demonstrating tonotopic variations in mechanotransduction properties. In most species with tectorial membranes, hair bundles vary in height and number of stereocilia such that high frequency bundles are shorter and have more stereocilia than low frequency hair bundles, thus passively preparing them to respond best to their frequency range (Hackney et al., 1993; Koppl, Authier, 1995; Tilney, Saunders, 1983). Where investigated, the size of the mechanotransducer current varies tonotopically, being larger in high frequency cells (He et al., 2004; Kennedy et al., 2003; Ricci, Fettiplace, 1997; Ricci et al., 2005; Waguespack et al., 2007). The increase in current amplitude is in part due to a larger single channel conductance but also due to the increased number of channels associated with the larger number of stereocilia (Beurg et al., 2006; Ricci et al., 2003). Activation kinetics, where measurements are technically feasible, also show a tonotopic gradient as do adaptation kinetics, both correlating well with characteristic frequency of the afferent fiber (Ricci et al., 2005). Minimally, it may be argued that activation and adaptation can provide tuning to the hair bundle. Based on measured properties, the tuning provided by a single sensory hair bundle is relatively broad and inconsistent with the sharp tuning observed in afferent fibers (Ricci et al., 2005). One problem with direct interpretation of these measurements is that they are made under non-physiological conditions in terms of

temperature, endolymph composition, and endocochlear potential, so specific features related to tuning may not be directly interpretable. A second problem with direct interpretation of these measurements is that the bundles are unloaded, since the tectorial membrane is removed. From modeling data, the mass loaded onto the hair bundle can create sharper tuning of the hair bundle resonance (Dierkes et al., 2008; Nam, Fettiplace, 2008). Data from the lizard also support the argument that tectorial membrane attachments can enhance hair bundle tuning properties (Authier, Manley, 1995; Manley et al., 1988). Additional measurements of hair bundle interactions with the tectorial membrane are needed to separate hair bundle mechanics from tectorial membrane resonance properties in order to quantify the role of the hair bundle in frequency selectivity.

Hair bundles may provide considerably more to cochlear amplification than only frequency selectivity. Distortion product otoacoustic emissions (DPOAEs) are produced by the cochlea in response to two primary frequency inputs that generate additional frequency outputs often of the form $(2f_1-f_2)$ or (f_2-f_1) and are indicative of a functioning active process. DPOAEs were identified in individual hair bundles of frog sacculus and are thought to emerge from nonlinearities in the mechanotransduction process (Jaramillo, Hudspeth, 1993). SOAEs are present in a variety of nonmammalian species and argue for the existence of a mechanical amplifier (Koppl, Manley, 1993; Manley, Gallo, 1997; Manley et al., 1996; Taschenberger, Manley, 1997). Spontaneous hair bundle oscillations, a possible correlate to SOAEs albeit at much lower frequencies, have been identified in frog sacculus and turtle auditory hair bundles, and both the hair bundle oscillations and the SOAEs show similar sensitivities to pharmacological manipulations (Ricci et al., 1998; Stewart, Hudspeth, 2000). Hair bundles can amplify mechanical stimulations (Martin, Hudspeth, 1999) and can use mechanical noise to enhance sensitivity (Indresano et al., 2003; Jaramillo, Wiesenfeld, 1998). Hair bundles show compressive nonlinearity about the hair bundles optimal stimulation frequency (Martin, Hudspeth, 2001). Hair bundles can generate force that may feedback into the basilar membrane (Benser et al., 1996; Beurg et al., 2008; Howard, Hudspeth, 1988; Jaramillo, Hudspeth, 1993; Kennedy et al., 2006; Ricci et al., 2000; Ricci et al., 2002). However the amount of force a hair bundle can generate is typically small, considerably smaller than that generated by outer hair cell somatic motility. The force generation is limited, partly due to the sparse number of mechanotransducer channels present. More recent work in rat outer hair cells, however, indicates that these bundles can generate considerably more force than previously measured in the frog or turtle (Kennedy et al., 2005). Questions however have arisen about whether somatic motility played a role in the measured hair bundle movements (Beurg et al., 2008; Jia, He, 2005; Kennedy et al., 2006). Thus issues remain as to whether hair bundles can generate enough force to drive basilar membrane movements when mechanically loaded by the tectorial membrane. Proof of principle experiments in an *in vitro* gerbil preparation demonstrate calcium dependent movements, which are probably driven by the concerted efforts of active hair bundles (Chan, Hudspeth, 2005). An interesting experiment would be to probe hair bundle mechanics in the prestin mutant that no longer is motile as a means of quantifying the contribution of the hair bundle in the mammalian system (Dallos et al., 2008).

An additional issue regarding hair bundle mechanics underlying the active process is that the proposed mechanism for hair bundle force generation may not be fast enough. The general idea is that a region of mechanical instability is created (a Hopf bifurcation) that causes hair bundles to oscillate between energy minima (Hudspeth, 2008; Martin et al., 2000; Martin et al., 2003). Data supporting this hypothesis are strong. The factors suggested to be involved in creating the bifurcation are mechanotransduction channel gating compliance interacting with adaptation mechanisms (Hudspeth, 2005; Hudspeth et al., 2000). At present myosin 1c is implicated as underlying the mechanisms of slow and possibly fast adaptation (Gillespie, Cyr, 2004; Holt et al., 2002; Stauffer et al., 2005). Given the kinetic properties of myosin 1c,

it seems unlikely that high frequency responses could be generated. Similarly, a calcium-driven mechanism might not be expected to work up to 100 kHz, particularly if the calcium needs to diffuse away from the mechanotransduction channels to a distant binding site 10s to 100s of nm away (Beurg et al., 2009; Ricci et al., 1998). Recent arguments that myosin 1c underlies fast adaptation, a mechanism thought to involve myosin's interaction with calcium causing a tilting but not uncoupling from the actin seems in opposition to a role in amplification because the predicted movement and force generation are quite small (probably perpendicular to the basilar membrane motion) and unlikely to feedback to basilar membrane motion, so speed is gained at the cost of power and dynamic range (Stauffer et al., 2005). A better understanding of the molecular mechanisms of adaptation will give insight into the limitations and advantages of its application to cochlear amplification.

Despite the underlying issues with mechanism, direct measurements of hair bundle and mechanotransducer properties support the argument that hair bundles can operate at high frequencies. Activation kinetics of mechanotransduction are quite fast, currently faster than can be stimulated (rise times in the tens of microseconds), therefore, quite possibly capable of operating cycle by cycle at very high frequencies (Ricci et al., 2005). Faster technology is needed to clearly determine the kinetic limits of gating compliance. Adaptation rates in mammal are also quite fast, tens of microseconds for fast adaptation and milliseconds for the slow component, and these values are probably underestimates of the upper limits because adaptation rates are slowed by stimulus rise times (Kennedy et al., 2003; Ricci et al., 2005; Wu et al., 1999). Thus it appears that the kinetics of the measured processes are faster than the hypothesized molecular mechanism might support, but certainly in the appropriate range to serve as a component of the cochlear amplifier.

Earlier arguments demonstrated that limited thermal energy at threshold requires signal filtering and amplification. This argument holds regardless of the final function of the hair cell or the frequency range over which the hair cell responds. Hair bundle response properties are remarkably similar across hair cell types (see caveat regarding outer hair cells below) and end organs and can provide the amplification and filtering needed for signal detection. We hypothesize that this intrinsic mechanism is maintained throughout evolution and that further specializations have occurred in some species permitting higher frequency hearing and a separation of function into hair cells that transduce information centrally and those that amplify and filter signals at the periphery.

Two hair cell system

The simplest auditory end organs, like those in turtles and frogs have single hair cell types; hair cells receive both efferent and afferent innervation and hair cell properties vary in a tonotopically defined manner (Crawford, Fettiplace, 1980; Sneary, 1988a; Sneary, 1988b). In general, the hair cells are all doing the same thing, just at different frequencies. Birds, although still in a papilla, have two hair cells types based on both morphology and innervations. Short hair cells have broad apical surfaces and cuticular plate regions and receive efferent innervation whereas the tall hair cells receive both afferent and efferent innervations (Fischer, 1994). Recently a similar pattern was identified in a lizard (Chiappe et al., 2007). The functional significance of these two hair cell systems remains to be identified as no clear experimental paradigm has been able to separate functions between cell types. In the mammalian cochlea there is also a two hair cell system. A variety of differences have been identified between outer hair cells (OHCs) and inner hair cells (IHCs): (a) OHCs vary tonotopically in their mechanotransduction properties while IHCs do not (Beurg et al., 2006), (b) OHCs have hair bundles embedded in the tectorial membrane, IHC bundles are free standing (Lim, 1980), (c) OHCs show a tonotopic gradient in calcium buffering while IHCs do not (Hackney et al., 2005), (d) OHCs have high levels of Ca-ATPases in the

stereocilia while IHCs do not (Grati et al., 2006) (e) OHCs receive the majority of efferent innervations (Warr, 1992; Warr et al., 1997) while IHCs receive the majority of afferent innervations (Ehret, 1979; Morrison et al., 1975), and (f) OHCs show electromotility while IHCs do not (Ashmore, 2008). In addition, in mammalian systems, significant data supports the argument that OHCs are required for cochlear amplification. Selective damage to OHCs either by noise or toxin exposure elevates thresholds and reduces frequency selectivity (Ashmore, 2008). Although not conclusive, given that changes in basilar membrane mechanics are inevitable with OHC loss, OHCs certainly are strongly implicated as being involved in the generation of the cochlear amplifier. It is unclear at this point whether other two hair cell systems also assign cochlear amplification to one hair cell type though it seems plausible given the innervation patterns observed.

Somatic motility as the cochlear amplifier

Somatic motility was first described in the guinea pig (Brownell et al., 1985), and extended to all other mammalian species tested including human (Oghalai et al., 1998). In response to membrane voltage changes, OHCs change length (Brownell et al., 1985). Evidence suggest the movement is voltage, not current driven (Ashmore, 1987; Santos-Sacchi, Dilger, 1988). A novel mechanism of force generation was identified involving a membrane protein that changes conformation in response to voltage fluctuations (Zheng et al., 2000). The conformational change results in an altered membrane surface area leading to OHC length changes (Dallos et al., 1993; Iwasa, 1994). OHC length changes are in series with the basilar membrane and can augment or diminish sound evoked movements of this membrane depending on phase. A concomitant change in membrane capacitance was identified, similar to gating charge for ion channels and is a signature for the motility mechanism (Santos-Sacchi, 1991). The observation of somatic motility in OHCs served to focus the field on the potential of this process to underlie cochlear amplification. The identification of prestin, a member of the SLC26 family of anion-bicarbonate transporter proteins has allowed for a detailed analysis of the mechanisms of prestin action and also for a more direct investigation of the role of OHC motility in generating the active process. In this light, investigations of the functional significance of prestin are far ahead of those regarding hair bundle mechanics, where so little is known regarding molecular mechanisms.

Does somatic motility have the requisite properties of frequency selectivity, compressive nonlinearity and amplification that have been ascribed to cochlear amplification? To date, there is no data supporting the conclusion that OHC motility is tuned. There are no known tonotopic gradients in prestin levels or intrinsic properties of somatic motility that would make motility frequency selective. OHC motility can operate at very high frequencies under non-physiological conditions (Frank et al., 1999); however, given that it is voltage dependent and limited by the membrane time constant under physiological conditions, motility will not operate on a cycle by cycle basis much beyond 1 kHz. That it can physically work at much higher frequencies has led to several suggested means by which the membrane time constant can be circumvented (see Ashmore 2008, Hudspeth 2008, and Dallos, 2008). Possibilities such as chloride modulation via a stretch activated channel (Rybalchenko, Santos-Sacchi, 2003), extracellular current flow modulating prestin (Dallos, Evans, 1995) or gradients in intrinsic electrical properties of OHC all have merit, but none have been adequately tested in order to be accepted or refuted. Regardless of whether these clever possibilities hold water, they would simply allow the motility process to operate at high frequencies but would not provide frequency selectivity. Given that motility is a voltage-dependent phenomenon, one might posit that tuning would be provided by the electrical properties of the hair cell basolateral membrane, but as mentioned earlier no evidence exists for such a tuning mechanism. Compressive nonlinearity is a hallmark of the active process, yet here too, data does not support the argument that compressive

nonlinearity is a property of OHC motility. The voltage dependence of motility follows a Boltzmann function. It might be possible that variations in basolateral electrical or mechanotransducer channel properties of the OHCs might impose a compressive nonlinearity onto the OHC receptor potential and that this could feedback onto motility, but even here data is lacking. Finally, amplification is a critical component of the active process. Here OHCs excel, generating large forces and movements in series with basilar membrane motion, which in turn directly alters hair bundle motion. Forces are considerably larger than those measured thus far from hair bundle active mechanisms.

Unifying theory

The above arguments might imply that OHC motility is ill suited for providing cochlear amplification; however it seems unlikely that these mammalian specializations are for naught. Perhaps the difficulty is more with the assumption that a single cell or a single process need account for the entire system's abilities. This assumption stems from early work where tuning and amplification appear to be inherent to the sensory cell (see electrical resonance section). However as the complexity of the inner ear organ increased, processing appears to be less inherent to individual hair cells and more delegated to the organ. This seems to be the case particularly with the two hair cell system. Cells with no afferent innervations must be serving some type of feedback role, thereby modulating the output of the primary sensory cell. When considering the mammalian cochlea it seems more reasonable to assume that both hair bundle mechanics and somatic motility are important for the active process but perhaps in somewhat different ways. Consider that if amplification properties are not present in the freestanding mammalian IHC bundles, the work required of OHCs to lower threshold and provide frequency selectivity would be even greater. Similarly though, IHC hair bundle mechanics play little role in generating cochlear amplification as they are free standing bundles and the preponderance of data support the argument that the cochlear amplifier resides in the OHC (Ashmore, 2008). One possibility is that hair bundle mechanics can serve a specific intrinsic amplification process for a given bundle while OHC motility serves as a gain enhancement to the system. A simple scenario might be that hair bundle mechanics provide the tuning and compressive nonlinearity associated with the active process and that these features are directly mechanically amplified by OHC motility. In this sense OHC motility can provide a cycle by cycle (assuming the membrane time constant issue is circumvented) amplification to a hair bundle process that has the required tuning and compressive properties but is more limited in its force generating abilities, particularly at high frequencies (Figure 4). It also allows for all hair cells, not just OHCs, to possess an amplification mechanism. Indirect support of this possibility is the observation that OHC mechanotransduction has a limited slow component of adaptation. The slow component of adaptation is thought to provide the motor for hair bundle amplification, so perhaps the slower adaptation mechanism is replaced by somatic motility as the engine powering the process, alleviating limitations like calcium clearance while operating at high frequencies. This division of labor would provide an excellent means of limiting thermal noise amplification associated with Brownian motion of the hair bundle. If this possibility were true, it would be extremely difficult to separate between these two potential mechanisms because perturbation of one feeds back onto the other. Data from prestin knockout animals indirectly supports this hypothesis by demonstrating that distortion product otoacoustic emissions are present despite a loss of OHC motility (Liberman et al., 2004). The remaining DPOAEs were reduced as would be expected if the component providing gain were removed but also demonstrating the involvement of another mechanism, aside from OHC motility in DPOAE generation.

One problem, quite difficult to surmount, is identifying causal links between possible mechanisms of cochlear amplification. Given a system where many signal processing events

occur in series, alteration of any given step can lead to loss of function of the whole system, making it unclear whether the alteration left the other signal processing events intact. For example several clever prestin mutations, along with prestin knockouts have been used to argue that somatic motility can completely account for cochlear amplification. The knockout data are difficult to interpret based on changes in basilar membrane mechanics and on the cell death induced by the knockout (Cheatham et al., 2004; Liberman et al., 2002; Wu et al., 2004). However mutations that alter or remove the voltage dependent motility also lead to profound hearing loss implicating a critical role for somatic motility (Dallos et al., 2008). These provocative data certainly further implicate somatic motility as being an important component of the active process, but they do not causally demonstrate that motility alone is responsible for amplification. The above hypothesis, incorporating both hair bundle and somatic motility would result in similar findings to those reported because the hair bundle alone would not generate enough force to drive basilar membrane motion or the hair bundle position may be moved away from its most sensitive location reducing force generation by the bundle. It is important to recognize that the ability of the hair bundle to amplify is critically dependent on the resting position of the hair bundle; it must be poised at the Hopf bifurcation to generate requisite tuning and gain. Any perturbation of this position either directly or indirectly will reduce the ability of the hair bundle to provide filtering and amplification.

Tecta mice where the OHC bundles are no longer tethered to the tectorial membrane are deaf (Legan et al., 2000). It has been argued that the existence of electrically evoked emissions in these animals demonstrate that somatic motility is completely responsible for the active process because hair bundle movements would no longer be capable of moving the basilar membrane (Mellado Lagarde et al., 2008). Are electrically evoked emissions a complete representation of the active process or perhaps simply a means of probing an electrical component of mechanical amplification? The results obtained in these experiments could be accounted for by the above hypothesis that incorporates both mechanisms of amplification, losing the hair bundle contribution by uncoupling the tectorial membrane results in loss of mechanically induced cochlear amplification by bypassing the hair bundle while electrical stimulation activates somatic motility directly, bypassing the need for mechanosensitivity. So again the likely interaction between the modes of amplification make interpretation of data meant to impact only one mechanism more elusive.

It would seem that both hair bundle mechanics and somatic motility are important in establishing the cochlear amplifier in the mammalian cochlea. Each has advantages and disadvantages (Table 2). The significance of either mechanism is not diminished by the contribution of the other, and many important questions remain to be addressed. For example how do these two processes interact and feedback on each other? Being able to manipulate hair bundle properties at the molecular level will provide critical tools needed to address this question. How is the output of the OHC translated to the IHC, and more generally, in a two hair cell system, how do the cells interact?

In nonmammalian vertebrates, like birds and some lizards, where the cochlear amplifier appears to reside in the hair bundle, the most likely source for interaction is the tectorial membrane. Tectorial membrane mechanical properties have been investigated across a variety of species, and in all cases including lizards, birds, and mammals, gradients in mechanical properties were identified and indicative of a role in frequency tuning (Koppl, Authier, 1995; Richter et al., 2007; Weaver, Schweitzer, 1994). Genetically modified tectorial membranes can increase frequency tuning (Russell et al., 2007). Tectorial membranes are also reported as having resonant properties (Gummer et al., 1996). Theoretical analysis support the contention that loading the hair bundle can sharpen frequency tuning and also that the properties of the tectorial membrane can serve to

coordinate hair bundle motion and force generation (Dierkes et al., 2008; Nam, Fettiplace, 2008). Further investigations into the tuning and coupling properties of the tectorial membrane are needed as these properties are probably critically important to our understanding of amplification mechanisms. The properties of the tectorial membrane are possibly critical for the establishment of the cochlear amplifier by sharpening hair bundle tuning. Together, hair bundle mechanics, tectorial membrane properties, and outer hair cell motility probably combine to generate the mature mammalian cochlear amplifier.

Advantages of a two hair cell system

It has been argued that OHCs serve to extend the frequency range of the cochlea, but many examples of high frequency hearing exist in systems without OHCs, for example, ultrasound can be detected in a variety of frogs and many insects have high frequency hearing (Arch et al., 2008; Arch et al., 2009; Farris, Hoy, 2000; Faure, Hoy, 2000; Feng et al., 2006; Shen et al., 2008). You might argue that the cochlea is over-engineered for human hearing since our most utilized frequency range (for speech and localization) is comparably sensed by many species that do not have OHCs. Despite the numerous differences in end organ structures, frequency selectivity and thresholds are remarkably similar between species. Perhaps the differences employed in the mammal are not simply to overcome physical limitations but to serve some higher order function. The increase in parameter numbers, for example, two hair cells, motility, hair bundle mechanics, and a separation of innervations patterns, may provide more sites for regulation. Perhaps finer central control allows better selectivity of incoming signals.

The two hair cell system may allow for the functional separation of amplification and information transfer, which may result from a conflict arising at high frequencies between maximizing both mechanical amplification and voltage response (Chiappe et al., 2007). One population of cells may maintain a low resting probability of opening, 0.1–0.2, in order to maximize the voltage change during high frequency sine wave stimulation (like IHCs); whereas a second population of hair cells responsible for amplification maintain a much higher resting open probability, closer to 0.5 in order to maximize sensitivity (like OHCs). This is an interesting idea that is perhaps supported by the high concentrations of Ca-ATPases in OHC bundles as compared to IHC hair bundles (Dumont et al., 2001; Grati et al., 2006). This hypothesis is intriguing in that it is testable across all two hair cell end organs. It would be interesting, for example, to see if short avian or the noninnervated lizard hair cells have higher levels of Ca-ATPases in their stereocilia when compared to tall hair cells or innervated lizard hair cells. A corresponding change with the advent of the two hair cell system is that one population receives efferent innervations, probably the amplifying cells as in mammals, but not yet confirmed in other vertebrates.

In non-mammalian two hair cell systems, the efferent system may serve to detune and attenuate selective frequency ranges without altering spontaneous activity driven by the sensory cell. In single hair cell systems, efferent stimulation will reduce spontaneous activity in the nerve fiber while also detuning and attenuating. In the mammal it is possible that an additional level of regulation is available, where not only can spontaneous activity be maintained, but also amplification may be separately regulated from tuning, as hair bundle mechanics and somatic motility may be regulated separately. Separate regulation may be possible for example if second messenger levels of cyclic AMP are altered via efferent stimulation. Cyclic AMP can modulate the set point of the hair bundle with no known effects on somatic motility (Ricci, Fettiplace, 1997). The fact that IHC bundles are not directly coupled to the tectorial membrane supports the hypothesis that the resting position of the IHC bundle is unaffected by efferent stimulation to OHCs.

Conclusions

The physical limitations associated with audition have been overcome throughout evolution in a variety of ways. Several mechanisms have arisen to establish the sensitivity and frequency ranges observed. The presence of frequency discrimination, compressive nonlinearity, amplification, and otoacoustic emissions across these disparate hearing organs support the requirement of a common cochlear amplification mechanism to establish low thresholds and sharp tuning. Two mechanisms are proposed to be involved in generating cochlear amplification. Hair bundle active mechanics driven by channel gating and adaptation properties appear to be common across species, while in mammalian hair cells, somatic motility is thought to play a critical role. A new hypothesis is presented that suggests somatic motility interacts with hair bundle motility to provide cycle by cycle cochlear amplification. Hair bundle mechanics can provide frequency selectivity and compressive nonlinearity while somatic motility can provide amplification. Interactions with the tectorial membrane are posited to shape frequency response. It is proposed that the separation of amplification and tuning within hair cells and between hair cells provides multiple sites of regulation and control of inputs. Given the similarity in response properties across diverse species it might be that the specializations in mammalian hearing serve some higher order functions.

Abbreviations

OHC	outer hair cell
IHC	inner hair cell
SOAE	spontaneous otoacoustic emissions
DPOAE	distortion product otoacoustic emissions
TM	tectorial membrane
BP	basilar papilla
AP	amphibian papilla
BM	basilar membrane
SPL	sound pressure level
MET	mechanoelectric transduction

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Figure 1.

Evolution of hearing organs. An evolutionary tree of the various organisms discussed in this review shows that insects and amphibians are furthest from mammals, and stem reptiles (red) are the closest. Adapted from (Manley, Koppl, 1998).

Figure 2.

Principles of mammalian hair cell mechanisms. (A) Stereocilia at the apex of the hair cell are responsible for mechanotransduction. Positive deflection of stereocilia (black) causes an opening of the mechanotransduction channels leading to an influx of cations into the cell. The calcium component of the current drives an adaptation process, a reduction in current during a constant stimulus that is thought to underlie force generation by the hair bundle. Negative deflection of the stereocilia (red) causes transduction channels to close, triggering reverse adaptation back to the resting level. Asterisks indicate potential sites of force generation. (B) Somatic motility occurs in the lateral membrane of the hair cell (cross-hatched area). A hyperpolarization of membrane voltage leads to an increase in the lateral membrane surface area, hence an expansion of the hair cell. A depolarization in membrane voltage leads to a decrease in lateral membrane surface area and a contraction of the hair cell. (C) Cochlear mechanisms at work in the mammalian cochlea include active hair bundle motions coupled through the tectorial membrane (black arrow) and somatic motility (blue arrows) which feedback onto basilar membrane motion (red arrow).

Figure 3.

Comparison of threshold and tuning curves across vertebrate species. (A) Audiograms were obtained from (Fay, 1988) for all species except the bobtail skink which came from (Manley, 2000a) and the bush cricket from (Stumpner, Molina, 2006). All curves are behavioral audiograms except for the bobtail skink and the bush cricket which are neural audiograms. (B) Tuning curves for each organism were chosen with center frequencies near their lowest threshold in order to compare the shapes of tuning curves across species, therefore the sharpness of tuning ($Q_{10\text{dB}}$) cannot be strictly compared here because these values vary depending on center frequencies in different species. Center frequencies for the tuning curves and data sources are as follows: chinchilla 8.1 kHz (Ruggero et al., 1990), guinea pig 7 kHz (Pickles, 1984), mouse 10 kHz (Taberner, Liberman, 2005), chicken 1.7 kHz (Salvi et al., 1992), barn owl 4.2 kHz (Köppl, 1997), bobtail skink 1.2 kHz (Manley et al., 1988), turtle 330 Hz (Crawford, Fettiplace, 1980), frog 751 Hz (Stiebler, Narins, 1990), bush cricket 20kHz (Stumpner, Molina, 2006).

Figure 4.

Schematic representation of the unifying theory of cochlear amplification. (A) In some vertebrates, like turtle basilar papilla shown here, intrinsic mechanisms within the hair cell are enough to produce sharp tuning and amplification without basilar membrane tuning. Iso-intensity responses of the hair cell (blue) and basilar membrane (red) are schematized for 3 different intensity levels. Red arrows indicate direction of sound stimulation. (B) Hair cells of the mammalian cochlea alone are not able to feedback onto the basilar membrane, however the hair bundle has some intrinsic tuning properties providing it with compressive non-linearly and some tuning as compared to corresponding basilar membrane motion. (C) The introduction of a tectorial membrane further sharpens tuning with some amplification provided by the concerted effort of multiple hair bundles that can feedback onto basilar membrane. (D) The addition of somatic motility provides further gain to the system, which is fed back onto the basilar membrane. The gain is necessary to bring the motion above the level of the noise (cross-hatched area) in the system to make the stimulus detectable.

Table 1

Comparison of hair cell properties across species

	Insect	Frog	Turtle	Lizard	Bird & Crocodilia	Mammal

	Tympanal	AP	BP		Tall	IHC
	1-100kHz	0.1 – 1.4kHz	1 – 4.5kHz	0.4 – 7kHz	0.2 – 12kHz	0.02 – 100kHz
Freq Range for organ				1 – 7kHz		
Lowest Threshold in Figure 3	30 dB SPL	10 dB SPL	40 dB SPL	5dB SPL	-20dB SPL	-10dB SPL
Q _{10dB} at best frequency in Figure 3	1.9	2	3	3.5	5	5.5
Afferent Innervation	✓	✓	✓	✓	✓	✓
Efferent Innervation	✗	✓	✓	✗ _{but see *}	✓	✗
Embedded in Apical Extracellular Matrix	Scolopale Cap	TM	TM	TM	TM	TM
Basilar Membrane	None	None	Not tuned	Not tuned	Tuned	Tuned
SOAE	?	✓	?	✓	✓	✓
Electrical Resonance	?	✓	✓	✓ not @ CF	✓	✗
Active Bundle Mechanics	✓	✓ saccule	✓ saccule	✓	?	✓
Somatic Motility	?	✗	✗	✗	✗	✗
References	(Gopfert, Robert, 2003; Kossel et al., 2008; Mason, Faure, 2004; Stumpner, Molina, 2006; Yack, 2004)	(Fay, 1988; Megela-Simmons et al., 1985; Pritchard, Ashmore, 1987; Schoffelen et al., 2008; Simmons et al., 2006)	(Crawford, Fettiplace, 1980; Crawford, Fettiplace, 1981; Crawford, Fettiplace, 1985; Fay, 1988; Miller, 1978b)	(Chiappe et al., 2007; Eatock et al., 1993; Köppl, Manley, 1993; Köppl et al., 2004; Manley, 1990; Manley, 2009a; Manley et al., 1988; Manley et al., 1996; Manley et al., 2001; Wever, 1967) (Wibowo et al., 2009)	(Fay, 1988; Fuchs et al., 1988; Gleich, Manley, 2000; Köppl, 1997; Köppl et al., 2004; Manley, 1990; Patuzzi, Bull, 1991; Salvi et al., 1992; Taschenberger, Manley, 1997)	(Beurg et al., 2008; Brownell et al., 1985; Fay, 1988; Kennedy et al., 2005; Pickles, 1984; Taberner, Liberman, 2005)

Table 2

Properties of cochlear amplification

	Amplification	Frequency Tuning	Compressive Non-linearity
Electrical Resonance	Pro: - Amplifies signals when stimulated at resonant frequency	Pro: - Hair cell electrical tuning characteristics able to completely account for afferent tuning properties	Pro: - Present due to saturation with greater stimulus levels
	Con: - Resonant frequencies only up to ~ 6kHz	Con: - Upper frequency limit of ~ 6kHz	Con: - No mechanical correlate
Hair Bundle Mechanics	Pro: - Force generation allows amplification of incoming signal	Pro: - Can theoretically tune up to 100 kHz - TM allows concerted effort of OHCs and results in sharper tuning.	Pro: - Direct observations have been made
	Con: - Limited force generation, force perpendicular to basilar membrane motion	Con: - High frequency oscillations have not been shown directly	Con: - Limited operating range
Somatic Motility	Pro: - Amplify signals up to at least 79 kHz as motion is seen at these high frequencies - Large forces can be generated - Force in series with basilar membrane motion	Pro: - Theoretically can operate at high frequency Con: - No known frequency tuning or tonotopic variations - Limited by membrane time constant	Pro: Con: - No mechanism for generation of compressive nonlinearity known
	Con: - Amplification follows membrane potential, therefore is limited to about 1 kHz by the membrane time constant		