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Research Report

Mixing model systems: Using zebrafish and mouse inner ear mutants and other organ systems to unravel the mystery of otoconial development

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ABSTRACT

Human vestibular dysfunction is an increasing clinical problem. Degeneration or displacement of otoconia is a significant etiology of age-related balance disorders and Benign Positional Vertigo (BPV). In addition, commonly used antibiotics, such as aminoglycoside antibiotics, can lead to disruption of otoconial structure and function. Despite such clinical significance, relatively little information has been compiled about the development and maintenance of otoconia in humans. Recent studies in model organisms and other mammalian organ systems have revealed some of the proteins and processes required for the normal biomineralization of otoconia and otoliths in the inner ear of vertebrates. Orchestration of extracellular biomineralization requires bringing together ionic and proteinaceous components in time and space. Coordination of these events requires the normal formation of the otocyst and sensory maculae, specific secretion and localization of extracellular matrix proteins, as well as tight regulation of the endolymph ionic environment. Disruption of any of these processes can lead to the formation of abnormally shaped, or ectopic, otoconia, or otoconial agenesis. We propose that normal generation of otoconia requires a complex temporal and spatial control of developmental and biochemical events. In this review, we suggest a new hypothetical model for normal otoconial and otolith formation based on matrix vesicle mineralization in bone which we believe to be supported by information from existing mutants, morphants, and biochemical studies.

1. Introduction

Otoconia are complex calcium carbonate ($CaCO_3$) biominerals in the utricle and saccule of the vertebrate inner ear. Otoconia are embedded in a fibrous extracellular matrix (gelatinous membrane) which couples the force of gravity to cilia of the sensory cells. Bending of the cilia in response to linear accelerations initiates the neuronal response. Otoconia are required for normal balance and the sensation of linear acceleration (gravity). In mammals, the majority of otoconia are generated only in the embryonic ear and must be maintained throughout life. Human otoconia are subject to demineralization and to alterations in structure and composition because of aging, disease, and exposure to common pharmaceutical agents. Ross et al. (1976) reported that temporal bones examined from all patients over 50 years of

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age exhibited saccular otoconial degeneration, which increased in severity with advancing age (Ross et al., 1976). Typically, the affected otoconia appear pitted or hollowed out, and subsequently break into fragments, which can result in displacement of utricular otoconia into the semicircular canals. In the semicircular canals, ectopic otoconia may cause abnormal sensations of dizziness and loss of balance, a condition referred to as Benign Positional Vertigo (BPV) (House and Honrubia, 2003; Lim, 1984; Tusa, 2001; Welling et al., 1997). Vestibular problems, like BPV, are reported in about 9% of people 65 years of age or older (Oghalai et al., 2000). An NIH report (1991 Annual Report, National Deafness and Other Communication Disorders Advisory Board) states that balance-related falls account for more than half of the accidental deaths in the elderly. Despite such significant morbidity, and even mortality, relatively little is known about the development and maintenance of otoconia, and the genes and processes required to assemble these complex extracellular structures.

Studies of human otoconial development are rare and most commonly associated with syndromic developmental anomalies or other pathologies (Sanchez-Fernandez and Rivera-Pomar, 1984; Sanchez-Fernandez et al., 1989; Wright and Hubbard, 1982; Wright et al., 1979, 1982). The majority of studies on otoconial development to date have focused on the mouse, rat, and chick which all have similar inner ear and otoconial structures as humans. Recent studies suggest that the genes and processes required for inner ear and otoconial development are evolutionarily conserved in the formation of the inner ear and otoliths of the teleost fish, yielding another, easily manipulated, model system to those that can be used for examining vestibular formation and dysfunction.

2. Structure and development of the otoconial and otolithic membranes

The structure and development of the otoconial organs have been best described in the mouse and the rat. The otoconial complex can be divided into 3 layers (Fig. 1A). Beginning in the endolymphatic space, (1) the otoconial layer contains thousands of otoconia, 0.1 to 25 μm biominerals consisting of a glycoprotein/proteoglycan core surrounded by minute crystallites (Figs. 1B, C), and the fibrous proteins that attach them to the underlying matrix, (2) the gelatinous layer is glutinous and amorphous, and (3) the subcupular meshwork (also called the veils) is a dense reticular network of fibrillar proteins that surrounds the processes of sensory hair cells. This complex extracellular structure is established in mid-embryonic development within the aqueous environment of the endolymph.

Otoconial formation begins over the sensory epithelia of the utricle and saccule when the core proteins (Otoconin 90 (Oc90) and other "minor" otoconial proteins) coalesce into distinct structures, with faint rhombohedral shapes (Ballarino and Howland, 1982) at approximately embryonic day 14 (E14) in the mouse. Calcification of this proteinaceous otoconial precursor is rapid, with maximal mineralization occurring between E15–E16.5 (Anniko, 1980; Anniko et al., 1987). Otoconial growth is most likely mediated through accretion of new $CaCO_3$ crystals at the pointed tips of the calcifying otoconia; this is supported by the localization of the calcium binding protein calbindin d28K to these sites in developing avian otoconia (Balsamo et al., 2000). Otoconia achieve essentially full size by postnatal day 7 (Erway et al., 1986; James et al., 1969; Lim, 1973) and, in mammals, it is presumed that no new otoconia will be added after this point, although turnover of otoconial Ca^{2+} has been observed at a low rate in the adult rodent (Erway et al., 1986; Preston et al., 1975).

Teleost otoliths have a very different structure as well as some differences in the developmental processes required to generate them. In contrast to the thousands of small otoconial particles in mammals and birds, only three large otoliths form in fish. These "ear rocks" are initially dome-like in structure (Fig. 1D), but will, with age, take on complex shapes consistent with the shape of the underlying sensory maculae, and with the biophysical requirements of each maculae for the sensing of motion and sound (Das, 1994; Lychakov and Rebane, 2000). Otolith formation begins with the aggregation of free-floating protein core particles, identifiable at 18–20 h post-fertilization (hpf). Core particles are directed to either of the two developing sensory maculae through the action of ciliated cells that line the otocyst cavity (Riley et al., 1997) where they adhere to the modified stereocilia on the first hair cells (tether cells) at the anterior and posterior poles of the otocyst. These preotoliths are rapidly mineralized with the aragonitic polymorph of $CaCO_3$ by 24 hpf. The otoliths remain attached to the fibrillar otolithic membrane linked to the hair cells of the sensory macula (Fig. 1E) throughout the morphogenetic movements required to generate the utricular and saccular maculae. A third otolith will begin to form about 11–12 days post-fertilization (dpf) in the lagena (Riley and Moorman, 2000) and is believed to utilize the same matrix components and developmental pathways used during the formation of the utricular and saccular otoliths. Otoliths continue to grow throughout the life of the fish, with daily accretion of layers of extracellular matrix proteins and deposited $CaCO_3$ (Borelli et al., 2003a) (Fig. 1D, inset).

3. Formation of otoconia and otoliths requires a stepwise process

Normal generation of otoconia requires the orchestration of complex temporal and spatial developmental and biochemical events. In the mouse, the first seeding of $CaCO_3$ crystals takes place at E14 and otoconia with defined forms can first be observed at E15–16, but genetic evidence suggests that the processes necessary for normal otoconial development begin much earlier. Normal otoconial formation requires (1) the correct induction and formation of the otocyst; (2) specification and differentiation of the sensory maculae and sensory and supporting cells; (3) establishing the correct ionic environment that allows for normal export and processing of matrix proteins and ions; (4) production and export of the otoconial matrix proteins and gelatinous membrane; (5) assembly of a protein core from free-floating matrix proteins; and (6) locally increasing Ca^{2+} and carbonate (CO_3^-) concentrations to initiate crystal formation on the proteinaceous core. The above elements must occur in a specific order and within specific time points to develop correctly formed