Usher proteins in inner ear structure and function

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Submitted 23 August 2013; accepted in final form 6 September 2013

Usher syndrome; CIB2; retinitis pigmentosa; inner ear; calcium buffer

Hearing impairment affects more than 28 million Americans. Usher syndrome (USH) is the most common cause of combined deafness and blindness, and a molecular diagnosis study suggested that one in 6,000 individuals in the United States is afflicted with USH (22). Clinically, USH is classified into three presenting subtypes, type I, II, and III based on the severity and age of onset (2). USH type I (USH1) is the most genetically heterogeneous: 14 loci have been mapped for USH, and genes for 11 have been identified (19, 27, 40). Although mutations in each of these genes cause USH, certain missense mutations cause only deafness or only retinitis pigmentosa (2, 27, 38, 40). Mutations in known genes and loci do not account for all of the known cases of USH.

Hearing and balance depend on hair cells, the polarized epithelial cells of the inner ear that have mechanosensitive hair bundles located at their apical poles. The hair bundle is composed of numerous stereocilia and, in all organs except the mature cochlea, a single kinocilium. Stereocilia are coupled to one another and with the kinocilium by a variety of links: tip links, horizontal top connectors, shaft connectors, ankle links, and kinocilial links (11). The tip links are the most crucial for mechano-electrical transduction (MET). Based on immunohistochemical studies cadherin 23 and protocadherin 15, two known USH proteins, are localized to the upper and lower parts of tip links, respectively (1, 21). Crystallographic structural evaluation revealed that the first and second ectodomains of cadherin 23 and protocadherin 15 interact with each other in a “handshake” mode to form the tip-link filaments (31). The current hypothesis is that the mature tip links are formed by protocadherin 15 and cadherin 23 (21), although during regeneration the transient but functional tip links are made of two protocadherin 15 isoforms (18).

USH proteins myosin VIIa, harmonin, and sans are also found at the upper attachment of the tip link (9, 12, 15). They are believed to form the complex that connects the upper end of the tip link to the actin cytoskeleton of the stereocilium. Dysfunction of these proteins causes defects of MET in the inner ear hair cells (9, 15, 24). Another USH protein, whirlin, is localized at the very tips of stereocilia (4). Whirlin interacts with myosin XVa, and both of these proteins are essential for the final stage of elongation of the stereocilia bundle (4). Although none of these proteins seem to be directly involved in MET (33), structural changes of the hair bundle associated with their dysfunction may significantly affect the MET in cochlear hair cells (34). In short, most of the USH proteins are associated with the broadly defined “tip link-MET complex” that is responsible for hair cell mechano-sensitivity (Fig. 1). In this minireview, we limit our focus to the roles of USH proteins in the hair cell stereocilia, even though some of these proteins are also important for signal transmission at the hair cell synapses (13, 14).

Recently, we reported that four different mutations in CIB2, a gene encoding a calcium and integrin binding protein, were responsible for USH1J/DFNB48 deafness in 57 Pakistani families and one Turkish family (27). The four human CIB genes encode small proteins CIB1, CIB2, CIB3, and CIB4; each contains three or four helix-loop-helix domains, also called EF hand domains. CIB2 contains only three EF hand domains and is able to bind Ca2+ through the second and third domains (8). Fluorescence energy transfer measurements indicate that binding of Ca2+ alters the three dimensional conformation of CIB2, a feature characteristic of proteins involved in Ca2+ signaling (8). Coimmunoprecipitation experiments revealed that CIB2 homodimerizes and interacts with whirlin and myosin VIIa and thus is a member of the USH interactome (27). In the rodent ear, CIB2 is localized in mechanosensory hair cell stereocilia. CIB2 inhibited ATP-induced Ca2+ responses in a heterologous expression system (27). Furthermore, experiments in zebrafish,
in which expression of CIB2 was inhibited, indicated that CIB2 is necessary for the function and/or proper development of neuromast sensory cells (27). Considering the localization and Ca2+-buffering properties of CIB2, we hypothesize that this protein acts as a calcium buffer to maintain optimal Ca2+ concentrations in the stereocilia. CIB2 may sequester Ca2+ that enters a stereocilium through MET channels.

Upon activation, Ca2+ influx through MET channels causes the subsequent decay of the MET response, a process called adaptation, that progresses on “slow” and “fast” time scales (3, 10). Slow adaptation is thought to result from “sliding down” the myosin motors that move the upper end of the tip link along the actin filaments of the core of stereocilium, generating restoring tension of the link. Fast adaptation may occur when Ca2+ enters a cell and binds either to the channel directly, thereby inactivating it (channel-reclosure model), or to another element that then undergoes conformational changes, thereby decreasing the tension applied to the MET channel (tension-release model) (10). Myosin-1c is thought to be involved in both slow and fast adaptation (17, 32). However, any Ca2+-binding protein that links the plasma membrane and the stereocilium actin core may, in theory, affect mechanical forces at the plasma membrane and thus influence the MET. For example, an unconventional myosin, myosin-7a, is abundant in the hair cell stereocilia and essential for MET adaptation (24). As CIB2 has an integrin-binding motif, it might also participate in Ca2+-dependent linkages between the cytoskeleton and plasma membrane of a stereocilium.

Intracellular Ca2+ is thought to regulate a number of processes in the hair cells, including MET adaptation, synaptic transmission, active force generation with hair bundle twitching (5), and outer hair cell electromotility. In addition, intracellular Ca2+ may regulate the formation of the tip links and transduction apparatus (42). It is therefore critical that stereocilia maintain very low Ca2+ concentrations at rest (25). Because stereocilia do not have specialized intracellular compartments to store Ca2+, they must rely on Ca2+ buffers, the mitochondria belt beneath the cuticular plate, and the plasma membrane Ca2+-ATPase (PMCA) to regulate Ca2+ levels (7, 25, 28, 36, 39). The majority of the Ca2+ that enters through MET channels is removed by PMCA (25, 39). Mutations of the PMCA pump modulate the hearing thresholds both in humans and in mice, implying that Ca2+ is a prime factor in hair cell pathology (23, 30, 35, 36). The mitochondria present beneath the cuticular plate of hair cells also play an essential role in balancing the Ca2+ concentration, not only by generating ATP, an energy source for PMCA pumps, but also by sequestering a large amount of Ca2+ (7). Lastly, hair cells of both cochlear and vestibular organs differentially express various calcium-binding proteins including calmodulin, calretinin, parvalbumin alpha and beta, and calbindin-D28K (16, 28, 29, 41). These mobile buffers are important in maintaining the optimal concentration of Ca2+ for proper functioning of calcium-signaling pathways.

Based on our recent studies that demonstrate that CIB2 localizes to stereocilia and interacts with the USH proteins myosin VIIa and whirlin, we hypothesize that CIB2 is a Ca2+-buffering protein essential for maintenance of calcium homeostasis in the mechanosensory stereocilia of inner ear hair cells. Because the MET current is regulated by stereociliary Ca2+ concentrations, the presence of a defective CIB2 that hinders Ca2+ sequestration is expected to affect the MET current. In contrast to all previously known mutations associated with USH, which affect the proteins involved in building the structure of the hair cell stereocilia bundles, CIB2 may affect calcium homeostasis in these sensory cells and thus represents a good target for potential therapeutic agents.

GRANTS

This work was supported by National Institute on Deafness and Other Communication Disorders Research Grants R01 DC-008861 and R01 DC-009434 to G. I. Frolenkov; R01 DC-012564 to Z. M. Ahmed; R01 DC-011803 and R01 DC-011748 to S. Riazuddin. Also, Z. M. Ahmed is a recipient of an RPB Career Development Award.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: Z.M.A. conception and design of research; Z.M.A. prepared figures; Z.M.A. and S.R. drafted manuscript; Z.M.A., G.I.F., and S.R.
REFERENCES


