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 are responsible for USH1J/DFNB48 deafness in 57 Pakistani families (27). The four human 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 mechanosensitivity (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 Ca$^{2+}$ through the second and third domains (8). Fluorescence energy transfer measurements indicate that binding of Ca$^{2+}$ alters the three dimensional conformation of CIB2, a feature characteristic of proteins involved in Ca$^{2+}$ signaling (8). Coimmunoprecipitation experiments revealed that CIB2 homomerizes 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 Ca$^{2+}$ 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 Ca^{2+}-buffering properties of CIB2, we hypothesize that this protein acts as a calcium buffer to maintain optimal Ca^{2+} concentrations in the stereocilia. CIB2 may sequester Ca^{2+} that enters a stereocilium through MET channels.

Upon activation, Ca^{2+} 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 resting tension of the link. Fast adaptation may occur when Ca^{2+} 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 Ca^{2+}-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 Ca^{2+}-dependent linkages between the cytoskeleton and plasma membrane of a stereocilium.

Intracellular Ca^{2+} 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 Ca^{2+} may regulate the formation of the tip links and transduction apparatus (42). It is therefore critical that stereocilia maintain very low Ca^{2+} concentrations at rest (25). Because stereocilia do not have specialized intracellular compartments to store Ca^{2+}, they must rely on Ca^{2+} buffers, the mitochondria belt beneath the cuticular plate, and the plasma membrane Ca^{2+-}ATPase (PMCA) to regulate Ca^{2+} levels (7, 25, 28, 36, 39). The majority of the Ca^{2+} 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 Ca^{2+} 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 Ca^{2+} concentration, not only by generating ATP, an energy source for PMCA pumps, but also by sequestering a large amount of Ca^{2+} (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 Ca^{2+} 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 Ca^{2+}-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 Ca^{2+} concentrations, the presence of a defective CIB2 that hinders Ca^{2+} 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.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

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