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Review

Stereocilia morphogenesis and maintenance through regulation of actin stability

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ABSTRACT

Stereocilia are actin-based protrusions on auditory and vestibular sensory cells that are required for hearing and balance. They convert physical force from sound, head movement or gravity into an electrical signal, a process that is called mechanoelectrical transduction. This function depends on the ability of sensory cells to grow stereocilia of defined lengths. These protrusions form a bundle with a highly precise geometry that is required to detect nanoscale movements encountered in the inner ear. Congenital or progressive stereocilia degeneration causes hearing loss. Thus, understanding stereocilia hair bundle structure, development, and maintenance is pivotal to understanding the pathogenesis of deafness. Stereocilia cores are made from a tightly packed array of parallel, crosslinked actin filaments, the length and stability of which are regulated in part by myosin motors, actin crosslinkers and capping proteins. This review aims to describe stereocilia actin regulation in the context of an emerging "tip turnover" model where actin assembles and disassembles at stereocilia tips while the remainder of the core is exceptionally stable.

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1. Introduction

The specialized auditory and vestibular sensory cells that detect sound, gravity and head position feature a bundle of hair-like projections called stereocilia that couple physical displacement to depolarization of resting membrane potential. This process, known as mechanoelectrical transduction (MET), is essential for hearing; consequently, stereocilia loss or degeneration causes deafness. In mouse models, defective stereocilia homeostasis is one primary cause of progressive and age-related deafness [1–7].

Mammalian auditory hair cells are not replaced if lost, possibly because of their intricate cell shape, position in a complex tissue or

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requirement to synapse with neurons. Thus, human hair cells must form precisely structured stereocilia bundles and also maintain those bundles for decades. Both events raise questions about the underlying molecular mechanisms, which are relevant to potential therapeutic approaches to treating hearing loss. Here, we first address how the bundle assumes its characteristic shape and cellular position, and next discuss how the stereocilia actin core is regulated to maintain mature stereocilia length.

2. Morphological and molecular structure of stereocilia bundles

2.1. General morphology and organization of bundles

The cochlea, which is the end organ of the auditory system, houses two types of sensory hair cells (Fig. 1). The inner hair cells (IHCs) communicate information to the auditory cortex, while outer hair cells (OHCs) amplify sound waves in the cochlea by rapidly oscillating in length [8,9]. Both cell types are stimulated by stereocilia deflections as small as a few nanometers. To detect these small movements stereocilia are organized into a bundle with three rows of stereocilia that increase in height in a stair-like fashion. From tallest to shortest, the rows are designated as row 1, 2 and 3. Within a row, stereocilia are very nearly the same height. The tips of stereocilia from the shorter rows (2 and 3) are connected to the sides of the adjacent taller stereocilia by tip links (Fig. 2). These thin filaments are formed from heterodimers of the transmembrane proteins protocadherin-15 (PCDH15) and cadherin-23 (CDH23) [10]. As the taller stereocilia are deflected backwards by force from sound waves, tension is transferred through the tip link, which then mechanically gates an ion channel housed in the tips of row 2 and 3 stereocilia [11]. Sodium, potassium and calcium ions enter through the open channel, depolarize the cell and trigger firing of associated afferent neurons for IHCs or cell contraction for OHCs [12].

The cores of stereocilia are composed of parallel actin filaments crosslinked at a frequency of about one crosslinker for every 10 actin subunits. They appear to run the entire length of the stereocilia, extending from the base to within a few nanometers of the membrane at the tip of the stereocilia [13–15]. The actin core is stiff and as a result the structure pivots, lever-like, at the base where the protrusion tapers as it joins the hair cell body. The actin filaments are regulated by several different types of binding proteins, with myosin motors and actin crosslinkers making particularly noteworthy contributions to stereocilia morphogenesis and maintenance.

2.2. The tip, shaft and base are distinct parts of a stereocilium

The tip of the stereocilium is a highly specialized domain (Fig. 2). Several proteins selectively localize to this region and are presumably part of the tip density that is evident in electron micrographs. Some of these, such as myosin XVa (*Myo15*), whirlin (*Whrn*), Epidermal growth factor receptor pathway substrate 8 (*Eps8*), myosin IIIa and IIIb (*Myo3a* and *Myo3b*) and espin-1 (splice isoform of *Espn*) are important for growth of the actin core [16–22]. Others, including CapZ, Eps8-like 2 (*Eps8L2*) and Twinfilin-2 (*Twf2*) have actin filament capping activity and likely regulate actin filament elongation [2,23–25]. Interestingly, the protein composition of stereocilia tips differs in the tallest row of the bundle compared to those in shorter rows. In particular, EPS8 is enriched in row 1 while EPS8L2 and TWF2 are largely restricted to rows 2 and 3 along with the long splice isoform of myosin XVa [2,24,26,27]. This localization pattern is likely important for generating or maintaining the staircase morphology of the bundle. In addition, the tips of the shorter row stereocilia are particularly notable as they also house functional mechanosensitive transduction channels [11].

The diameter of the stereocilia narrows near the cell body as only a subset of the actin filaments pass through this region. Within the taper, there is a rootlet structure that anchors stereocilia into the underlying cuticular plate in the cell body [14,28]. The rootlet depends on the actin binding protein TRIOBP to organize actin filaments into an array that is even more tightly packed than in the stereocilia shaft. Stereocilia lacking TRIOBP also lack rootlets and as a result are less rigid and more prone to damage [29]. In addition to having a specialized actin structure, the taper region also features a set of proteins that are required for stereocilia development and maintenance. Myosin VI, radixin (*Rdx*), chloride intracellular channel 5 (*Clic5*), and PTPRQ are critical for anchoring the membrane to the cytoskeleton because their loss results in stereocilia fusion, presumably because the membrane lifts from the base to cover multiple stereocilia cores [30–33]. Myosin VI also contributes to stereocilia development by reducing the number of CDH23 links found between stereocilia at early postnatal ages [34]. Additionally, Fam65b forms an oligomeric ring structure at the stereocilia base that is required to restrict taperin localization [35]. Taperin (*Tprn*), a protein of unknown function that is required for auditory maintenance [32,36], mislocalizes in overly elongated stereocilia that result from loss of Fam65b [35]. Together, these observations highlight that a structured base region regulates stereocilia development.

The stereocilium shaft is defined as the region between the base and the tip (Fig. 2). This region of the core is composed of highly crosslinked actin filaments notable for their uncommon stability. Important features in this region include the upper tip link density (UTLD) where the CDH23 portion of the tip link associates with an electron dense plaque. The UTLD depends on harmonin, a protein that localizes to the UTLD and can directly bind to F-actin [37]. Myosin Ic, myosin VI and myosin VIIa regulate MET adaptation, likely by applying force at the UTLD to adjust tip link tension [34,38–40].

3. Development of stereocilia

At an early developmental stage (~E14 in mouse) the apical surface of hair cells is decorated with two types of protrusions including numerous, small actin-based microvilli and a single microtubule-based primary cilium called the kinocilium. As development proceeds, the kinocilium migrates to the lateral side of the cell and adopts a position at the vertex of the developing stereocilia bundle [41]. Most of the microvilli are disassembled, leaving bare zones on the medial and lateral portions of the cell apex. Other microvilli presumably become stereocilia as the actin core is remodeled by a multitude of proteins including unconventional myosins, actin crosslinkers and actin capping proteins [23,42].

3.1. The kinocilium and bundle development

A central role for kinocilia in forming and orienting the chevron shape of stereocilia bundles is demonstrated by a number of mutations that perturb its structure and/or function [43,44]. For example, axoneme loss due to mutations in the intraflagellar transport (IFT) pathway result in bundles that are frequently misoriented such that the vertex of the bundle is not uniformly pointed along the medial-lateral axis. In addition, bundles are often split, circular or have a less defined V shape. The kinocilium responds to cues from the planar cell polarity pathway as well as cell intrinsic polarity pathways such that it is positioned between cortical domains marked by atypical protein kinase C (aPKC) and G-protein alpha i complex (Gαi) at the medial and lateral sides, respectively, of