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Initial characterization of kinocilin, a protein of the hair cell kinocilium

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Abstract

A subtracted library prepared from vestibular sensory areas [Nat. Genet. 26 (2000) 51] was used to identify a 960 bp murine transcript preferentially expressed in the inner ear and testis. The cDNA predicts a basic 124 aa protein that does not share any significant sequence homology with known proteins. Immunofluorescence and immunoelectron microscopy revealed that the protein is located mainly in the kinocilium of sensory cells in the inner ear. The protein was thus named kinocilin. In the mouse, kinocilin is first detected in the kinocilia of vestibular and auditory hair cells at embryonic days 14.5, and 18.5, respectively. In the mature vestibular hair cells, kinocilin is still present in the kinocilium. As the auditory hair cells begin to lose the kinocilium during postnatal development, kinocilin becomes distributed in an annular pattern at the apex of these cells, where it co-localizes with the tubulin belt [Hear. Res. 42 (1989) 1]. In mature auditory hair cells, kinocilin is also present at the level of the cuticular plate, at the base of each stereocilium. In addition, as the kinocilium regresses from developing auditory hair cells, kinocilin begins to be expressed by the pillar cells and Deiters cells, that both contain prominent transcellular and apical bundles of microtubules. By contrast, kinocilin was not detected in the supporting cells in the vestibular end organs. The protein is also present in the manchette of the spermatids, a transient structure enriched in interconnected microtubules. We propose that kinocilin has a role in stabilizing dense microtubular networks or in vesicular trafficking.

Keywords: Kinocilin; Kinocilium; Stereocilia; Inner ear; Testis; Manchette; Microtubules

1. Introduction

The mammalian inner ear is a highly differentiated organ consisting of several sensory epithelia that are covered by acellular structures. The sensory cells are polarized in several different ways. First, they are epithelial cells with apical and basolateral membrane domains specialized for distinct functions; the apical pole is specialized for mechano-electrical-transduction and the basolateral membrane plays a number of different roles, that include neurotransmitter release and the shaping of receptor potentials. Second, the hair bundle, at the apex of the hair cell, is an asymmetric structure that is both morphologically and functionally polarized; the stereocilia in the hair bundle are arranged in rows of increasing height on the cell surface and the bundle responds optimally to deflections directed to and away from the row of tallest stereocilia. Third, the hair bundles exhibit planar polarity and are organized in a precise pattern with

Abbreviations: E, embryonic day; P, post-natal day; ES, ectoplasmic specialization

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respect to each other and to the organ as a whole. The kinocilium (or the basal body) is the first structure to polarize the apex of differentiating sensory cells. This structure, initially located at the center of the cell's apical surface, moves to the periphery whilst the asymmetric hair bundle is being established (Denman-Johnson and Forge, 1999), and then it and its associated hair bundle may rotate circumferentially as planar polarity becomes refined (Cotanche and Corwin, 1991; Dabdoub et al., 2003). The kinocilium is an enigmatic structure in several respects. First, this cilium has been reported as a primary cilium (e.g. a non-motile cilium with a 9 + 0 microtubule composition) by some authors (Kikuchi et al., 1988, 1989; Sobkowicz et al., 1995), while others depicted a 9 + 2 or an 8 + 1 microtubular organization (Sobkowicz et al., 1995; Kelley et al., 1992). Some authors have also reported a motile activity for this tubulin-rich structure (Flock et al., 1977). Second, the kinocilium is a permanent organelle of the vestibular hair cells, whereas it is transient in cochlear hair cells. In the mouse cochlear hair cells, the kinocilium regresses from P8 and eventually disappears at P12. Thus, the kinocilium has long been considered as a transient embryonic feature without any functional significance in the adult inner ear. However, links between stereocilia and kinocilia have been described in the vestibular hair cells of several species including mammals (Ernstson and Smith, 1986; Ross et al., 1987) and a recent report indicated that kinocilial links and inter-stereocilial tip links share morphological and biochemical properties (Goodyear and Richardson, 2003). These links could be directly involved in the development of hair bundles (Goodyear and Richardson, 2003). Additionally, mutations have been described in zebrafish that lead to the loss of the kinocilium in hair cells followed by the degeneration of the sensory cells (Tsujikawa and Malicki, 2004). Finally, whereas more than 15 proteins of the stereocilia have been identified, very few data are available concerning the molecular composition of the kinocilium. Two unconventional myosins are present in this structure; myosin VIIa has been described in the kinocilia of developing hair cells in the mouse cochlea (Wolfrum et al., 1998) and myosin Ic is present in frog vestibular hair cells (Hasson et al., 1997; Cyr et al., 2002). The function of these myosins within the kinocilium is still unknown.

The identification of genes specifically or preferentially expressed in the inner ear is a powerful approach for deciphering the molecular organization of the various cell types that make up the sensory epithelia. Here, we report the characterization of a cDNA identified in a subtracted cDNA library derived from vestibular sensory areas of the mouse inner ear (Verpy et al., 2000). The encoded protein is present not only in the vestibular and cochlear hair cells, but also in the pillar and Deiters cells of the cochlear sensory epthelium. The protein is also expressed in the germ cells of the testis.

2. Materials and methods

2.1. Animal handling

The care and use of animals followed the animal welfare guidelines of the "Institut National de la Santé et de la Recherche Médicale" (INSERM), under the approval of the French "Ministère de l'Agriculture, de l'alimentation, de la pêche et de la ruralité". All efforts were made to minimize the number of animals used and their suffering.

2.2. Subtracted cDNA library

The P0–P4 129sv mouse cDNA from saccule, utricle and ampullae was subtracted with the P0–P4 non-sensory cartilagenous and membranous parts of the semi-circular canals cDNA, with P2 liver and P15 dorsal root ganglion cDNAs using the RDA method (Hubank and Schatz, 1994) modified by Verpy et al. (2000).

2.3. Cloning of full-length Kino cDNA

The 514-bp L5 sequence was extended by rapid amplification of cDNA ends (RACE)-PCR. Oligo(dT)-primed double-stranded vestibular cDNA was prepared from postnatal day (P)0-P1 129sv mice, ligated to adaptors, and amplified as described (Cohen-Salmon et al., 1997). The 5′ and 3′ RACE-PCR products, obtained with primers 5′-374-AGCGGAAGTCTCTGGTGCTG-ATG and 5′-CCAGTCTCGGGGTCCTCC TTCAAG, respectively, were cloned into the pCR2.1-TOPO vector (Invitrogen) and sequenced.

2.4. Reverse transcription (RT)-PCR analysis

Total RNA from the brain, eye, inner ear, heart, lung, kidney, and small intestine of P2 swiss mice and from the testis and skeletal muscle of adult mice was prepared using the guanidium isothiocyanate procedure (Chomczynski and Sacchi, 1987). RT was carried out with 1 μg of total RNA in a final volume of 20 μl, as described (Zwaenepoel et al., 2002). 1/20 of the reaction product was PCR-amplified in a total volume of 25 μl using forward primer L5int2 (5′-29-GAGGCAGACCCACG-TCACTAGA) and reverse primer uL5 (5′-AAAGGG-GTCCTGTGGGTCTTTCC). PCR amplification of Hypoxanthine-guanine phosphoribosyltransferase (Hprt) mRNA was used as a positive control (primers 5′-576-GCTGGTGAAAAGGACCTCT and 5′-824-CACAG-GACTAGAACACCTGC). The reactions were carried out following a standard protocol (35 cycles), with the Expand High Fidelity PCR system (Roche Biochemicals).