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Differential distribution of β- and γ-actin in guinea-pig cochlear sensory and supporting cells

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Abstract

Sensory and supporting cells of the mammalian organ of Corti have cytoskeletons containing β- and γ-actin isoforms which have been described as having differing intracellular distributions in chick cochlear hair cells. Here, we have used post-embedding immunogold labelling for β- and γ-actin to investigate semiquantitatively how they are distributed in the guinea-pig cochlea and to compare different frequency locations. Amounts of β-actin decrease and γ-actin increase in the order, outer pillar cells, inner pillar cells, Deiters' cells and hair cells. There is also more β-actin and less γ-actin in outer pillar cells in higher than lower frequency regions. In hair cells, β-actin is present in the cuticular plate but is more concentrated in the stereocilia, especially in the rootlets and towards the periphery of their shafts; labelling densities for γ-actin differ less between these locations and it is the predominant isoform of the hair-cell lateral wall. Alignments of immunogold particles suggest β-actin and γ-actin form homomeric filaments. These data confirm differential distribution of these actin isoforms in the mammalian cochlea and reveal systematic differences between sensory and supporting cells. Increased expression of β-actin in outer pillar cells towards the cochlear base may contribute to the greater stiffness of this region.

Keywords: Isoactins; Actin filaments; Auditory system; Hair cells; Stereocilia

1. Introduction

Actin is a major component of hair cells and supporting cells in the inner ear. In the mammalian cochlea, various properties of these cells change systematically along the length of the cochlear partition (see e.g. Lim, 1986). These changes contribute to the processes by which complex sounds are decomposed into their component frequencies (Robles and Ruggero, 2001). Morphological changes in actin-containing structures along the cochlear length have been reported (Carlisle et al., 1988) but whether specific variations occur in the types and amount of actin present has yet to be investigated.

Three subtypes of actin, α, β and γ, are distinguishable by their differing isoelectric points on 2D gels (Vandekerckhove and Weber, 1978, 1981). Six functional actin genes have been described in humans, four of which encode the α- and γ-isoforms found in different muscle types (see e.g. Herman, 1993). The remaining two, *ACTB* and *ACTG1*, code for cytoplasmic β- and γ-actin which are found in a wide range of cell types including

Abbreviations: FITC, Fluorescein isothiocyanate; GS-PBS, goat serum in phosphate buffered saline; IHC, inner hair cell; MAP, microtubule associated protein; OHC, outer hair cell; PB, phosphate buffer; PBS, phosphate buffered saline; STBS, serum in TRIS buffered saline; TBS, TRIS buffered saline; TEM, transmission electron microscope; TRITC, tetramethyl rhodamine isothiocyanate

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hair cells and supporting cells (Slepecky and Savage, 1994; Nakazawa et al., 1995; Hofer et al., 1997). Mutations of the human γ-actin gene cause progressive non-syndromic sensorineural hearing loss, DFNA20/26 (Zhu et al., 2003; Wijk et al., 2003). Given the widespread distribution of γ-actin in the body, this is surprising and implies that it has a special role in hearing.

The cytoplasmic isoforms, β- and γ-actin, differ in proportion and distribution in various tissues (e.g. Otey et al., 1987) and interact with specific associated proteins (Sheterline et al., 1998). Changes in their expression result in cell phenotype changes, emphasizing their role in maintaining morphology (see review by Khaitlina, 2001). In the brain, β-actin is restricted to dynamic structures and is associated with cell processes e.g., dendritic spines and growth cone filopodia, whereas γ-actin is more ubiquitously distributed and occurs in relatively quiescent regions (Micheva et al., 1998).

In auditory hair cells, actin is found in the stereocilia, the cuticular plate and a circum-apical ring of filaments (Flock and Cheung, 1977; Flock et al., 1981; Tilney et al., 1980; Hirokawa and Tilney, 1982; Slepecky and Chamberlain, 1982, 1983, 1985, 1986). These networks contain different cross-linking proteins (Drenckhahn et al., 1991). Mammalian outer hair cells (OHCs) contain, in addition, an F-actin cortical lattice that lies between the plasma lemma and the layers of sub-surface cisternae (Flock et al., 1986; Bannister et al., 1988; Holley and Ashmore, 1988, 1990). In guinea-pig OHCs at least, there is also an actin-rich infracuticular network (Carlisle et al., 1988).

The β-actin isoform is known to be involved in dynamic maintenance of the hair bundle in rodents (Schneider et al., 2002; Rzadzinska et al., 2004). In chick, Hofer et al. (1997) reported that β-actin occurs in the stereocilia but not the cuticular plate, whereas γ-actin occurs in both, and suggested that the ratio of β:γ determines the final size and length of stereocilia. In mammals, decreasing stereociliary length (see e.g. Lim, 1986) and increasing stiffness of the hair bundles (Flock and Strelioff, 1984) towards the cochlear base might reflect changes in the ratio. The possibility that this ratio influences the structure and function of subcellular regions in other cell types makes it worth examining isoform distribution in mammalian hair cells and supporting cells in detail. We have therefore investigated the distribution of each isoform in different cell types and in two different frequency regions in the organ of Corti of guinea pig using post-embedding immunogold labelling.

2. Materials and methods

2.1. Animals and antibodies

Adult pigmented guinea pigs (500–850 g) exhibiting Preyer reflexes were killed with an overdose of sodium pentobarbitone (Vetalar®; 200 mg/kg IP), the bullae removed and opened. Cochleae were immediately perfused with, and immersed in, fixative for 2 h at room temperature and washed in phosphate buffered saline (PBS, pH 7.4). Subsequent processing varied according to the immunolabelling method. Animals were maintained and used in accordance with the "Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) and the UK Animals (Scientific Procedures) Act, 1986.

A mouse monoclonal IgG1 anti-β-actin antibody (clone AC-15, Product no. A-5441, Sigma-Aldrich, Dorset, UK) that recognises β-actin exclusively (Gimona et al., 1994) was used. A rabbit polyclonal antibody to γ-actin that does not cross react with β-actin (Otey et al., 1987) was the kind gift of Dr JC Bulinski. The latter antibody, which has been used in the chick (Hofer et al., 1997) and mammalian cochlea (Slepecky and Savage, 1994), was shown to be specific for cytoplasmic γ-actin in both.

2.2. Conventional transmission electron microscopy

Images from the cochleae of 10 normal animals were used for densitometric measurements and to illustrate the structures of interest. All cochleae were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 2 h, followed by 1% osmium tetroxide in the same buffer for 1 h, dehydrated through an ethanol series and embedded in Spurr resin as described previously (Furness and Hackney, 1985). Ultrathin sections 100–120 nm thick were cut, stained in 2% uranyl acetate in 70% ethanol and 2% aqueous lead citrate and examined in JEOL 100CX or 1230 transmission electron microscopes (TEMs) operated at 100 kV. Images were acquired on Ilford TEM film or with a Megaview III digital camera and analySIS® software. The electron density of the filamentous structures was analysed using Adobe Photoshop® v.7.0 in negatives digitised with a Canon 9900F scanner with transparency adapter. The Autolevel function was first applied to the scanned images in order to reduce variability between pictures from different material. This function selects the brightest and darkest pixels in an image and makes them white and black, respectively. The intermediate pixels are then assigned grey level values proportionately resulting in a similar spread of grey levels in pictures of similar structural content. We then determined the mean pixel grey level of these regions using the Histogram function.

2.3. Immunofluorescence labelling

Two cochleae from two animals were fixed using 4% (w/v) freshly dissolved paraformaldehyde in 0.1 M sodium phosphate buffer (PB, pH 7.4). Cochlear spirals (modiolus and organ of Corti) were excised in PBS,