

Hypothesis

Sequence similarity between stereocilin and otoancorin points to a unified mechanism for mechanotransduction in the mammalian inner ear

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Abstract

Background: Interaction between hair cells and acellular gels of the mammalian inner ear, the tectorial and otoconial membranes, is crucial for mechanoreception. Recently, otoancorin was suggested to be a mediator of gel attachment to nonsensory cells, but the molecular components of the interface between gels and sensory cells remain to be identified.

Hypothesis: We report that the inner ear protein stereocilin is related in sequence to otoancorin and, based on its localisation and predicted GPI-anchoring, may mediate attachment of the tectorial and otoconial membranes to sensory hair bundles.

Testing: It is expected that antibodies directed against stereocilin would specifically label sites of contact between sensory hair cells and tectorial/otoconial membranes of the inner ear.

Implications: Our findings support a unified molecular mechanism for mechanotransduction, with stereocilin and otoancorin defining a new protein family responsible for the attachment of acellular gels to both sensory and nonsensory cells of the inner ear.

Background

The cochlea and the vestibule, respectively, are responsible for hearing and balance in the mammalian inner ear. The tectorial membrane, an acellular gel, covers the surface of the organ of Corti within the cochlea. Similarly, otoconial and cupula membranes overlie sensory regions of the five organs constituting the vestibule. Sound-induced motion of the basilar membrane in the cochlea or head motion in the vestibule generates shear between the acellular gels and the apical surface of the sensory epithelia; the latter consist of both hair (sensory) and supporting (nonsensory) cells. Deflection of stereocilia bundles on

sensory hair cells causes membrane potential alterations that transduce mechanical information into electrical signals [1–3]. Mutations in genes encoding protein components of the acellular gels result in hearing and balance defects, highlighting the importance of these structures in mechanotransduction [2–10]. Therefore, there is considerable interest in identifying molecules that are responsible for attachment of the gels to the sensory epithelia.

Recently, two new genes specifically expressed in the human inner ear were described. *STRC*, a chromosome 15q15 gene mutated in families affected by non-syndro-

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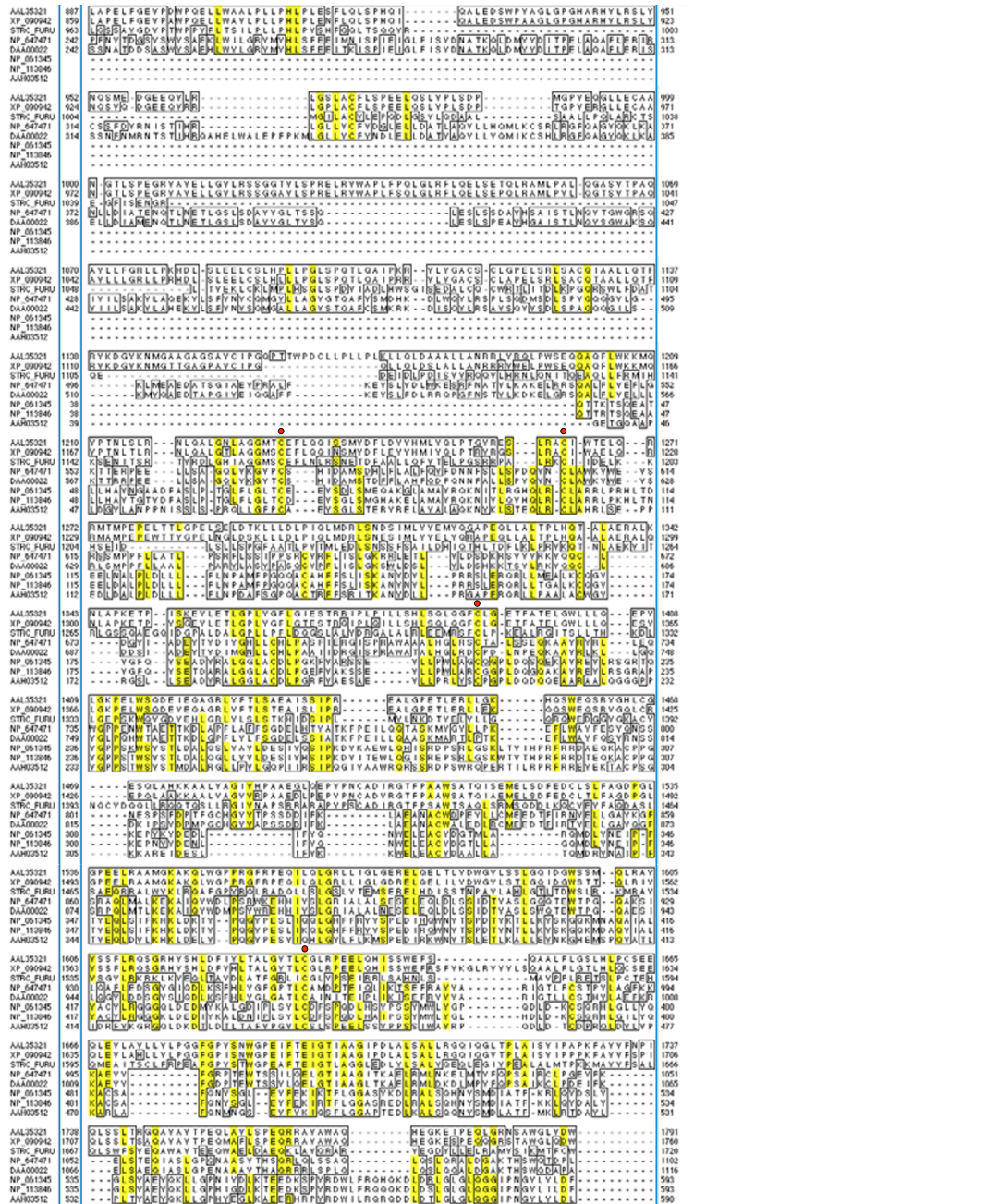


Figure 1

Multiple sequence alignment of stereocilin, otoancorin and mesothelin. Regions of homology are boxed, residues identical in more than 60% of the sequences are shaded in yellow and 4 cysteine residues conserved in all sequences are indicated by red dots. AAL35321, mouse stereocilin (1809 aa); XP_090942, human stereocilin (1778 aa); STRC_FURU, *Fugu rubripes* stereocilin (see below); NP_647471, mouse otoancorin (1137 aa); DAA00022, human otoancorin (1153 aa); NP_061345, mouse mesothelin (625 aa); NP_113846, rat mesothelin (625 aa); AAH03512, human mesothelin (621 aa). A gene encoding the putative *Fugu* fish homologue of stereocilin was identified by a BLAST [18] search of *Fugu* assembly release 2 (17.05.02) [19] with mouse stereocilin, matching sequences within scaffold 1525 (E-value 8.2×10^{-58}). GENSCAN [20] analysis of the genomic DNA was combined to local sequence alignments to known stereocilin sequences to yield a putative *Fugu* stereocilin homologue of 1994 amino acids, including an N-terminal signal peptide sequence and a predicted C-terminal GPI-anchor attachment site. STRC mutations resulting in truncation of stereocilin at amino acid positions preceding the start of the alignment with mesothelin were identified in human families affected by non-syndromal sensorineural deafness linked to locus *DFNB16* [1].

mic deafness at the *DFNB16* locus, was predicted to encode a polypeptide of 1778 amino acids of unknown function and with no homology to other proteins [11]. Mutations in the *OTOA* gene (chromosome 16p12.2) at locus *DFNB22* also cause autosomal recessive deafness. Immunofluorescence studies in mouse suggested that the *OTOA* gene product, otoancorin (1153 amino acids), mediates contact between the apical surface of nonsensory cells and acellular gels of the inner ear, the tectorial and otoconial membranes [12]. Otoancorin was found to share weak sequence similarity with megakaryocyte potentiating factor/mesothelin, a GPI-linked glycoprotein of unknown function [13,14]. Nevertheless, no similarity was reported between otoancorin and other known inner ear proteins.

Presentation of the hypothesis

We performed PSI-BLAST [15] searches of the NCBI non-redundant database (~1,035,000 sequences) with conservative parameters (low complexity filter, expectation value 1, word size 2, inclusion threshold 0.0005) and found highly significant similarity between ~900 C-terminal amino acids of stereocilin and otoancorin (E-values at iterations 1 and 4 $3e^{-14}$ and e^{-159} , respectively; Fig. 1). Furthermore, analysis with DGPI, a server for automatic detection of GPI-anchored proteins [16], suggested that stereocilin, like otoancorin, is a secreted GPI-anchored protein.

Acellular gels of the mammalian inner ear are attached not only to nonsensory cells, but also to hair bundles of sensory hair cells, composed of actin-rich stiff microvilli called stereocilia; the latter interaction is essential for mechanotransduction [17]. Since otoancorin is not localised on hair bundles, it was hypothesised that a different system, possibly integrins, is responsible for their interaction with acellular matrices [12]. In view of the fact that localisation of stereocilin in the inner ear is limited to sensory hair cells and, in particular, to the hair bundle [11], our findings strongly suggest that stereocilin may have a comparable function to otoancorin at the level of hair cells.

Testing the hypothesis

Our hypothesis could be tested by carrying out microscopic examination of mouse inner ear hair cell sections immunolabeled with antibodies directed against stereocilin; one would expect to observe labeling at sites of direct contact between the hair bundles of sensory hair cells and the acellular gels. In addition, GPI-anchoring of stereocilin could be confirmed by transfecting mammalian cells with the corresponding cDNA and comparing the localisation of stereocilin on the membrane of transfected cells in the absence and presence of PI-PLC.

Implications of the hypothesis

Our PSI-BLAST results suggest that, in contrast to what was previously thought, similar molecular interactions may be responsible for mediating attachment of acellular gels to the different cell types of the inner ear. Since these connections are important for mechanotransduction of sound, it is not surprising that both *STRC* and *OTOA* mutations lead to deafness in humans. In this context, it is of interest to note that reported *STRC* mutations [11] result in deletions affecting the conserved protein region shown in Fig. 1.

Abbreviations

GPI = glycosyl phosphatidylinositol

NCBI = National Center for Biotechnology Information

PI-PLC = phosphatidylinositol-specific phospholipase C

Authors' contributions

LJ recognised the sequence similarity between stereocilin and otoancorin. JP assisted the bioinformatic searches and methods. PMW assisted in coordinating this study, preparing the manuscript and maintaining a level of sanity.

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References

- Bryant J, Goodyear RJ, Richardson GP: **Sensory organ development in the inner ear: molecular and cellular mechanisms.** *Br Med Bull* 2002, **63**:39-57
- Petit C, Levilliers J, Hardelin JP: **Molecular genetics of hearing loss.** *Annu Rev Genet* 2001, **35**:589-646
- Steel KP, Kros CJ: **A genetic approach to understanding auditory function.** *Nat Genet* 2001, **27**:143-149
- Verhoeven K, Van Laer L, Kirschhofer K, Legan PK, Hughes DC, Schattman I, Verstreken M, Van Hauwe P, Coucke P, Chen A, et al: **Mutations in the human α -tectorin gene cause autosomal dominant non-syndromic hearing impairment.** *Nat Genet* 1998, **19**:60-62
- Mustapha M, Weil D, Chardenoux S, Elias S, El-Zir E, Beckmann JS, Loiselet J, Petit C: **An α -tectorin gene defect causes a newly identified autosomal recessive form of sensorineural pre-lingual non-syndromic deafness, *DFNB21*.** *Hum Mol Genet* 1999, **8**:409-412
- McGuirt WT, Prasad SD, Griffith AJ, Kunst HP, Green GE, Shpargel KB, Runge C, Huybrechts C, Mueller RF, Lynch E, et al: **Mutations in *COL11A2* cause non-syndromic hearing loss (*DFNA13*).** *Nat Genet* 1999, **23**:413-419
- Legan PK, Lukashkina VA, Goodyear RJ, Kossi M, Russell IJ, Richardson GP: **A targeted deletion in α -tectorin reveals that the tectorial membrane is required for the gain and timing of cochlear feedback.** *Neuron* 2000, **28**:273-285
- Simmler MC, Cohen-Salmon M, El-Amraoui A, Guillaud L, Benichou JC, Petit C, Panthier JJ: **Targeted disruption of *otog* results in deafness and severe imbalance.** *Nat Genet* 2000, **24**:139-143
- Moreno-Pelayo MA, del Castillo I, Villamar M, Romero L, Hernandez-Calvin FJ, Herraiz C, Barbera R, Navas C, Moreno F: **A cysteine substitution in the zona pellucida domain of α -tectorin results in autosomal dominant, postlingual, progressive, mid frequency hearing loss in a Spanish family.** *J Med Genet* 2001, **38**:E13-E16

10. Iwasaki S, Harada D, Usami S, Nagura M, Takeshita T, Hoshino T: **Association of clinical features with mutation of *TECTA* in a family with autosomal dominant hearing loss.** *Arch Otolaryngol Head Neck Surg* 2002, **128**:913-917
11. Verpy E, Masmoudi S, Zwaenepoel I, Leibovici M, Hutchin TP, Del Castillo I, Nouaille S, Blanchard S, Laine S, Popot JL, et al: **Mutations in a new gene encoding a protein of the hair bundle cause non-syndromic deafness at the *DFNB16* locus.** *Nat Genet* 2001, **29**:345-349
12. Zwaenepoel I, Mustapha M, Leibovici M, Verpy E, Goodyear R, Liu XZ, Nouaille S, Nance WE, Kanaan M, Avraham KB, et al: **Otoancorin, an inner ear protein restricted to the interface between the apical surface of sensory epithelia and their overlying acellular gels, is defective in autosomal recessive deafness *DFNB22*.** *Proc Natl Acad Sci USA* 2002, **99**:6240-6245
13. Bera TK, Pastan I: **Mesothelin is not required for normal mouse development or reproduction.** *Mol Cell Biol* 2000, **20**:2902-2906
14. Chang K, Pastan I: **Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers.** *Proc Natl Acad Sci USA* 1996, **93**:136-140
15. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ: **Gapped BLAST and PSI-BLAST: a new generation of protein database search programs.** *Nucleic Acids Res* 1997, **25**:3389-3402
16. **DGPI** [<http://129.194.186.123/GPI-anchor>]
17. Rueda J, Cantos R, Lim DJ: **Tectorial membrane-organ of Corti relationship during cochlear development.** *Anat Embryol* 1996, **194**:501-514
18. Altschul SF, Lipman DJ: **Protein database searches for multiple alignments.** *Proc Natl Acad Sci USA* 1990, **87**:5509-5513
19. Aparicio S, Chapman J, Stupka E, Putnam N, Chia JM, Dehal P, Christoffels A, Rash S, Hoon S, Smit A, et al: **Whole-genome shotgun assembly and analysis of the genome of *Fugu rubripes*.** *Science* 2002, **297**:1301-1310
20. Burge C, Karlin S: **Prediction of complete gene structures in human genomic DNA.** *J Mol Biol* 1997, **268**:78-94

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