Assembly of hair bundles, an amazing problem for cell biology

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ABSTRACT

The hair bundle—the sensory organelle of inner-ear hair cells of vertebrates—exemplifies the ability of a cell to assemble complex, elegant structures. Proper construction of the bundle is required for proper mechanotransduction in response to external forces and to transmit information about sound and movement. Bundles contain tightly controlled numbers of actin-filled stereocilia, which are arranged in defined rows of precise heights. Indeed, many deafness mutations that disable hair-cell cytoskeletal proteins also disrupt bundles. Bundle assembly is a tractable problem in molecular and cellular systems biology; the sequence of structural changes in stereocilia is known, and a modest number of proteins may be involved.

INTRODUCTION

The remarkable structure of the vertebrate hair bundle derives from an equally remarkable assembly process. Actin-rich stereocilia form, lengthen, and widen in a precisely determined order (Tilney et al., 1992), giving the bundle its asymmetric, staircase appearance (Figure 1). The importance of bundle assembly and structure is highlighted by the number of deafness-causing mutations, a subset of which are in genes that encode actin or actin-associated proteins (for examples, see Table 1). Assembly of the bundle involves multiple overlapping and interacting cellular processes, such as stereocilia lengthening and widening, each of which is responsible for one or more features of the final structure.

Tom Pollard has written eloquently on the reductionist-synthetic strategy to characterize complex biological mechanisms (Pollard, 2013). His first step is to frame a good problem—and I believe that hair-bundle assembly fits the bill. Like other subcellular processes, including cytokinesis or cell migration, bundle development is relatively circumscribed mechanistically and may require a modest number of proteins, perhaps 100 or fewer. As a reasonably simple systems-biology problem, it is both amenable to study with present technology and suitable as a model for more complex problems. I suggest that now is the time to use systems and cell biology to study how the bundle is built and that this problem is ideal for young cell biologists looking for a career-defining problem to solve.

DESCRIPTION OF HAIR-BUNDLE ASSEMBLY

First, I present what is known about molecular mechanisms of bundle assembly. Part of Pollard's first step is to define the problem of interest in enough detail that it can be interrogated using a molecular approach. Lew Tilney's landmark studies of hair-bundle development from the 1980s, using electron microscopy, remain the most comprehensive description of bundle assembly. Focusing on the chick cochlea, he and his colleagues identified four distinct stages of bundle growth (Figure 1A). In stage I, before embryonic day 8 (E8), a hair cell's progenitor undergoes its terminal division, and the hair cell begins to differentiate. In stage II (E8–E13), the kinocilium moves to one side of the bundle, stereocilia adjacent to the kinocilium elongate, tip links appear, and cross-linking of actin filaments increases. By the end of stage II, the bundle has a robust staircase separation of stereocilia length. In stage III (E13–E16), the kinocilium moves to one side of the bundle, stereocilia adjacent to the kinocilium elongate, tip links appear, and cross-linking of actin filaments increases. By the end of stage II, the bundle has a robust staircase separation of stereocilia length. In stage III (E13–E16), stereocilia lengthening stops, but the stereocilia begin to widen, from ~100 to as many as 900 filaments per stereocilium. During this time, the anchoring structure for the stereocilia—the cuticular plate—begins to form and the stereocilia bases taper, presumably essential for proper bundle flexibility. Finally, lengthening resumes in stage IV (E16–postnatal day 3, P3), stopping only when stereocilia reach their mature lengths. The cochlea encodes sounds of different frequencies using different hair cells; high frequencies are encoded at the proximal (basal) end, and low frequencies are encoded at the distal (apical end). Indeed, the number and length of stereocilia vary systematically from proximal to distal ends. Nevertheless,
the genetic strategy could easily miss many key proteins, including those whose activity can be compensated for by functional paralogues or those with pleiotropic effects, other approaches are needed to identify all functionally important proteins of the bundle.

Protein biochemistry offers a complementary approach. Development of methods for purification of hair bundles (Shepherd et al., 1989; Gillespie and Hudspeth, 1991) provided enough starting material to specifically examine which proteins are present in bundles. Although initial experiments used one-by-one examination of proteins by immunoblotting, development of modern mass spectrometry methods now allow parallel examination of thousands of proteins. Mass spectrometry analysis of purified bundles has identified hundreds of bundle-specific proteins from vestibular and auditory organs of the chick (Shin et al., 2013; Avenarius et al., 2014). Present challenges include determining which of these proteins are most important for bundle assembly and the features of bundle development in which they participate.

SPECIFICATION OF STEREOCILIA NUMBER AND POSITION

A close look at hair-bundle assembly with the parts list in mind allows us to associate some proteins with stages of morphogenesis. At early points during hair-cell differentiation, short stereocilia cover the apical surface of the cell; these cilia can nonetheless be distinguished from the shorter microvilli on adjacent supporting cells. The position of the kinocilium—an axonemal cilium (with 9 + 2 microtubule doublets)—may then specify bundle polarity. Stereocilia adjacent to the kinocilium first elongate, followed by succeeding rows (Tilney et al., 1992; Kindt et al., 2012). These results show the essential role of the kinocilium (or the basal body) in establishing subcellular planar polarity (Deans, 2013).

The number and placement of stereocilia can vary systematically along one or more tissue axes. How different cells specify stereocilia number is unknown, but an intriguing suggestion is that they arise as Turing patterns from simple reaction-diffusion mechanisms (Jacobo and Hudspeth, 2014). Stabilization of stereocilia by a Turing mechanism could complement developmental stimulation of stereocilia elongation; together these mechanisms would allow for growth of the rows followed by pruning of unstabilized stereocilia. Candidates for components for control of Turing patterns or stereocilia stabilization include RAC1 and its downstream effector p21-activated kinase (Grimsley-Myers et al., 2009, 2012), both of which are detected in stereocilia (Shin et al., 2013). Upstream control of those mechanisms may be exerted by Sonic hedgehog (SHH); eptopic SHH reduces the numbers of stereocilia in basal hair cells, which typically have many stereocilia (Son et al., 2015).

COMPONENTS OF THE HAIR BUNDLE

Pollard’s second step in characterization of molecular mechanisms is to develop a parts list. The auditory neuroscience field has been engaged in this problem for the last several decades, using both genetics and biochemistry to determine which proteins are present in hair bundles and which are functionally important. “Deafness genes,” which, when mutated, lead to deafness, are good candidates for key molecules, particularly if their protein products are present in bundles (Petit and Richardson, 2009; Dror and Avraham, 2009). Several dozen already identified proteins fit this category, and geneticists continue to slowly add more. Examples of key deafness genes that are expressed in hair bundles are listed in Table 1; locations for many of the proteins are shown in Figure 2. Because adjacent cells control bundle assembly very reproducibly (Figure 1B). The general principles of Tilney’s scheme for chick-bundle morphogenesis, especially the temporal and spatial segregation of stages of bundle development, are believed to apply to all hair-cell organs.
| Gene symbol | Gene name | Deafness mutant | Hair-bundle phenotype | Protein location |
|-------------|-----------|-----------------|-----------------------|-----------------|
| ACTB        | Actin beta | Actb knockout   | Progressive degeneration of hair bundles | Stereocilia shafts |
| ACTG1       | Actin gamma 1 | DFNA20/26, Actg1 knockout | Progressive degeneration of hair bundles | Stereocilia shafts |
| ANXA5       | Annexin V | Anxa5 knockout | None | Shafts |
| ATP2B2      | ATPase, Ca\textsuperscript{2+} transporting, plasma membrane 2 | Atp2b2 knockout | Progressive degeneration of hair bundles | Shafts (excluded from taper); concentration toward tips |
| CAPZ        | Capping protein (A1, A2, and B genes) | n/a | Unknown | Stereocilia tips |
| CDH23       | Cadherin 23 | USH1D, DFNB12 | Disorganized hair bundles | Tip links; transient lateral links; kinocilial links |
| DFNB31      | Whirlin | USH2D, DFNB31 | Short stereocilia | Tips; ankle links |
| EPS8        | Epidemial growth factor receptor pathway substrate 8 | Eps8 knockout | Short stereocilia | Stereocilia tips |
| EPS8L2      | EPS8-like 2 | Eps8L2 knockout | Progressive degeneration of hair bundles | Stereocilia tips |
| ESPN        | Espin | DFNB36 | Short and thin stereocilia; degeneration in the cochlea | Short ESPN splice forms throughout stereocilia shafts; long isoform (ESPN-1) at tips |
| FSCN2       | Fascin 2 | Fscn2\textsuperscript{R109H} | Progressive degeneration of hair bundles | Stereocilia shafts |
| MYO1C       | Myosin IC | Myo1c\textsuperscript{Y61G} | Slowed adaptation | Toward stereocilia tips |
| MYO1H       | Myosin IH | n/a | Unknown | Toward stereocilia tips |
| MYO3A       | Myosin IIIA | DFNB30 | Progressive hearing loss | Thimble-like pattern at tips |
| MYO3B       | Myosin IIIB | n/a | Unknown | Thimble-like pattern at tips |
| MYO6        | Myosin VI | DFNA22, DFNB37 | Fusion and elongation of stereocilia; apical membrane uplifting | Shafts and taper region |
| MYO7A       | Myosin VIIA | USH1B, DFNA11, DFNB2 | Disorganized hair bundles | Tip links; transient lateral links; kinocilial links |
| MYO15A      | Myosin XVA | DFNB3 | Short stereocilia | Stereocilia tips |
| PCDH15      | Protocadherin 15 | USH1F, DFNB23 | Disorganized hair bundles | Tip links; transient lateral links; kinocilial links |
| PLS1        | Plastin 1 | Pls1 knockout | Progressive degeneration of hair bundles | Stereocilia shafts |
| PTMRQ       | Protein tyrosine phosphatase receptor Q | DFNB84 | Fusion and elongation of stereocilia | Ankle region, stereociliary shafts |
| RDX         | Radixin | DFNB24, Rdx knockout | Progressive degeneration of hair bundles | Concentrated near base of stereocilia; activated RDX only found above the taper region |
| USH1C       | Harmonin | USH1C, DFNB18 | Disorganized hair bundles | Tip link upper insertion; tips in early development |
| USH1G       | Sans | USH1G | Disorganized hair bundles | Tip link upper insertion; tips in early development |

Most of these were initially flagged as being important because mutations in their genes caused deafness.

**TABLE 1**: Selected key proteins for hair bundles.
stereocilia of mice lacking MYO15A are only a few micrometers long (Belyantseva et al., 2005). In both auditory and vestibular hair bundles, adjacent stereocilia in a single rank do show increasing lengths, but the staircase spacing is only 100–200 nm (Stepanyan and Frolenkov, 2009). These observations suggest that MYO15A is required for Tilney’s first step of stereocilia elongation, that which occurs beyond the initial development of a short staircase.

The cross-linking protein espin (ESPN) is necessary for the second stage of stereocilia lengthening (Sekerkova et al., 2011); lengthening stops prematurely in mice homozygous for the jerker mutation, which disables Espn (Sekerkova et al., 2011). The longest splice form of ESPN, called ESPN-1, may exert its effects after transport to stereocilia tips by myosin IIIA or IIIB (MYO3A or MYO3B; Salles et al., 2009; Merritt et al., 2012). Short forms of ESPN elongate microvilli in cultured cells, however, suggesting that control of stereocilia length might involve multiple ESPN splice forms (Loomis et al., 2003).

**STEREOCILIA WIDENING**

In the chick cochlea, widening of stereocilia from ∼50 to >400 filaments occurs as a distinct step, sandwiched in time between the first and last lengthening phases (Figure 1, C and D; Tilney et al., 1980). By contrast, in the mammal, widening occurs concurrently with the second lengthening phase (Kaltenbach et al., 1994; Sekerkova et al., 2011). As with second-phase lengthening, widening during this period is dependent on ESPN; moreover, the tapered stereocilia
seen in jerker heterozygotes, with half the normal ESPN levels, sug-
ggest that widening occurs from the base of the stereocilium to the
tip (Sekerkova et al., 2011). This observation and the relationship
between the myosin III paralogues and the ESPN-1 splice form sug-
gests that widening may occur as myosin III motors transport ESPN-1
toward stereocilia tips, with ESPN-1 catalyzing barbed-end growth of
new filaments that are nucleated at stereocilia taper regions.

CONTROL OF STEREOCILIA LENGTH BY
MECHANOSENSATION

The remarkable precision of stereocilia length in adjacent rows begs
for local feedback control within the hair bundle; it would seem im-
possible for the cell to control lengths so reproducibly from the cy-
toplasm. Phenotypes of mutant hair bundles for which mechano-
transduction is known to be disrupted—for example, in Cdhr23 and
Ush1g mice—suggest that when mechanotransduction is disrupted,
length regulation is altered (Caberlotto et al., 2011).

Possible mechanisms for transduction control of stereocilia
length are few. Tip links—the extracellular filaments that gate the
transduction channels—are tensioned by myosin motors at the tip-
link upper insertion point (LeMesurier and Gillespie, 2005). The up-
ward force on tip links apparently puckers the membrane of a
shorter stereocilium enough to allow actin polymerization (Kachar
et al., 2000), producing a zone of actin turnover at tips (Zhang et al.,
2012; Drummond et al., 2015; Narayanan et al., 2015). Thus tip-link
force may elongate stereocilia, at least over a short distance.

There must also be a mechanism that opposes the upward
Tip link force, or stereocilia would elongate until the upper insertion
point reached the stereocilia tip. Myosin motors that control tip-link
tension—probably MYO1C or MYO7A—should be sensitive to
Ca2+; perhaps Ca2+ that enters at a stereocilium tip and diffuses
toward stereocilia tips, with ESPN-1 catalyzing barbed-end growth
of new filaments that are nucleated at stereocilia taper regions. Because
we expect that only a few hundred proteins are needed for bundle assembly, examining, for example, a 100 × 100
matrix of interactions would be straightforward with a variety of tech-
niques, including yeast two-hybrid assays (Bruckner et al., 2009)
and direct protein–protein binding assays (Syafriyanti et al., 2014), as well as with methods for testing the relevance of those interactions in vivo.

Finally, computational biologists need to develop mathematical
models that describe hair-bundle assembly. The models need to be
of sufficient complexity that meaningful hypothesis-testing experi-
ments can be inferred from them (Pollard, 2013). The advent of
CRISPR technology means that these testing experiments can be
conducted relatively quickly (Hsu et al., 2014; Incontro et al., 2014).

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A CALL TO ARMS

Although individual steps of hair-bundle assembly are beginning to
be understood, the problem begs for a systematic approach, given
the large number of overlapping and partially redundant molecular
mechanisms. Modern technology allows for experiments that could
only be dreamt of even a few years ago. As a systems-biology prob-
lem, assembly of the bundle seems highly tractable. What addi-
tional information is needed? Will this information require new
approaches? Pollard argues that three large areas of research—biochemical,
cellular, and structural—are needed for complete characterization of
a biological problem of interest. One need is for description of the
order of expression and appearance in stereocilia of key hair-bundle
proteins. Single-cell transcriptomics experiments now allow for a
temporal ordering of expression of genes in a cellular process (Durr-
uthy-Durruthy et al., 2014) and can be used to understand bundle
development. Complementary experiments showing development-
lar progression of protein expression are needed. The lack of coor-
dination of hair-cell development in vestibular organs (Goodyear
et al., 1999; Burns et al., 2012) suggests that these protein expres-
sion experiments will require single-cell mass spectrometry. Al-
though some efforts have been made in this direction (Wu and
Singh, 2012), detection of rare proteins in single hair cells will re-
quire increasing the sensitivity of mass spectrometry by at least 100-
fold. Given the spectacular advances in instrumentation in the last
two decades, this goal seems achievable.

We also need to know the activities, protein–protein interactions,
and activity-modifying posttranslational modifications of all proteins
important for bundle assembly (Pollard, 2013). Activities of many pro-
teins are known or can be inferred from better-characterized para-
logues. Because we expect that only a few hundred proteins are
needed for bundle assembly, examining, for example, a 100 × 100
matrix of interactions would be straightforward with a variety of tech-
niques, including yeast two-hybrid assays (Bruckner et al., 2009)
and direct protein–protein binding assays (Syafriyanti et al., 2014), as well as with methods for testing the relevance of those interactions in vivo.

Systematic characterization of all of these parameters is essential.

REFERENCES

Beurg M, Fettiplace R, Nam JH, Ricci AJ (2009). Localization of inner hair
cell mechanotransducer channels using high-speed calcium imaging.
Nat Neurosci 12, 553–558.

Belyantseva IA, Boger ET, Naz S, Frolenkov GI, Sellers JR, Ahmed ZM,
Giffith AJ, Friedman TB (2005). Myosin-XVa is required for tip localiza-
tion of whirlin and differential elongation of hair-cell stereocilia. Nat Cell
Biol 7, 148–156.

Bourgeois, C, Otto K, Gass P, Spindler KJ, Pagana JM, Andrade L, Kachar B, Choi D, et al. (2014). Correlation of actin crosslinker and capper expression levels with stereocilium growth phases. Mol Cell Proteomics 13, 606–620.

Belyantseva IA, Boger ET, Naz S, Frolenkov GI, Sellers JR, Ahmed ZM,
Giffith AJ, Friedman TB (2005). Myosin-XVa is required for tip localiza-
tion of whirlin and differential elongation of hair-cell stereocilia. Nat Cell
Biol 7, 148–156.

Bourgeois, C, Otto K, Gass P, Spindler KJ, Pagana JM, Andrade L, Kachar B, Choi D, et al. (2014). Correlation of actin crosslinker and capper expression levels with stereocilium growth phases. Mol Cell Proteomics 13, 606–620.

Belyantseva IA, Boger ET, Naz S, Frolenkov GI, Sellers JR, Ahmed ZM,
Giffith AJ, Friedman TB (2005). Myosin-XVa is required for tip localiza-
tion of whirlin and differential elongation of hair-cell stereocilia. Nat Cell
Biol 7, 148–156.

Bourgeois, C, Otto K, Gass P, Spindler KJ, Pagana JM, Andrade L, Kachar B, Choi D, et al. (2014). Correlation of actin crosslinker and capper expression levels with stereocilium growth phases. Mol Cell Proteomics 13, 606–620.
Campellone KG, Welch MD (2010). A nucleator arms race: cellular control of actin assembly. Nat Rev Mol Cell Biol 11, 237–251.

Deans MR (2013). A balance of form and function: planar polarity and development of the vestibular maculae. Semin Cell Dev Biol 24, 490–498.

Dor AA, Avraham KB (2009). Hearing loss: mechanisms revealed by genetics and cell biology. Annu Rev Genet 43, 411–437.

Drummond MC, Barzik M, Bird JE, Zhang DS, Lechene CP, Corey DP, Cunningham LL, Friedman TB (2015). Live-cell imaging of actin dynamics reveals mechanisms of stereocilia length regulation in the inner ear. Nat Commun 6, 6873.

Durruthy-Durruthy R, Gottlieb A, Hartman BH, Waldhaus J, Laske RD, Altman R, Heller S (2014). Reconstruction of the mouse otocyst and early neuroblast lineage at single-cell resolution. Cell 157, 964–978.

Gillespie PG, Hudspeth AJ (1991). High-purity isolation of bullfrog hair bundles and subcellular and topological localization of constituent proteins. J Cell Biol 112, 625–640.

Gillespie PG, Müller U (2009). Mechanotransduction by hair cells: models, molecules, and mechanisms. Cell 139, 33–44.

Goodyear RJ, Gates R, Lukashkin AN, Richardson GP (1999). Hair-cell numbers continue to increase in the utricular macula of the early posthatch chick. J Neurocytol 28, 851–861.

Grimsley-Myers CM, Sipe CW, Geleoc GS, Lu X (2009). The small GTPase Rac1 regulates auditory hair cell morphogenesis. J Neurosci 29, 15859–15869.

Grimsley-Myers CM, Sipe CW, Wu DK, Lu X (2012). Redundant functions of Rac GTPases in inner ear morphogenesis. Dev Biol 362, 172–186.

Hackney CM, Mahendrasingam S, Penn A, Fettsplace R (2005). The concentrations of calcium buffering proteins in mammalian cochlear hair cells. J Neurosci 25, 7867–7875.

Hsu PD, Lander ES, Zhang F (2014). Development and applications of CRISPR-Cas9 for genome engineering. Cell 157, 1262–1278.

Incontro S, Asensio CS, Edwards RH, Nicoll RA (2014). Efficient, complete deletion of synaptic proteins using CRISPR. Neuron 83, 1051–1057.

Jacobo A, Hudspeth AJ (2014). Reaction-diffusion model of hair-bundle morphogenesis. Proc Natl Acad Sci USA 111, 15444–15449.

Kachar B, Parakkal M, Kurc M, Zhao Y, Gillespie PG (2000). High-resolution structure of hair-cell tip links. Proc Natl Acad Sci USA 97, 13336–13341.

Kaltenbach JA, Falzarano PR, Simpson TH (1994). Postnatal development of the hamster cochlea. II. Growth and differentiation of stereocilia bundles. J Comp Neurol 330, 187–198.

Kindt KS, Finch G, Nicolson T (2012). Kinocilia mediate mechanosensitivity in developing zebrafish hair cells. Dev Cell 23, 329–341.

LeMasurier M, Gillespie PG (2005). Hair-cell mechanotransduction and cochlear amplification. Neuron 48, 403–415.

Loomis PA, Zheng L, Sekerkova G, Chandyaleket B, Mugnaini E, Bartles JR (2003). Espin cross-links cause the elongation of microvillus-type parallel actin bundles in vivo. J Cell Biol 163, 1045–1055.

Manor U, Disanza A, Grati M, Andrade L, Lin H, Di Fiore PP, Scita G, Kachar B (2011). Regulation of stereocilia length by myosin XVa and whirlin depends on the actin-regulatory protein Eps8. Curr Biol 21, 167–172.

Merritt RC, Manor U, Salles FT, Grati M, Dose AC, Unrath WC, Quintero OA, Yengo CM, Kachar B (2012). Myosin IIB uses an actin-binding motif in its espin-1 cargo to reach the tips of actin protrusions. Curr Biol 22, 320–325.

Narayanan P, Chatterton P, Ikeda A, Ikeda S, Corey DP, Ervasti JM, Perrin BJ (2015). Length regulation of mechanosensitive stereocilia depends on very slow actin dynamics and filament-severing proteins. Nat Commun 6, 6855.

Petit C, Richardson GP (2009). Linking genes underlying deafness to hair-bundle development and function. Nat Neurosci 12, 703–710.

Pollard TD (2013). No question about exciting questions in cell biology. PLoS Biol 11, e1001734.

Salles FT, Merritt RCJ, Manor U, Dougherty GW, Sousa AD, Moore JE, Yengo CM, Dose AC, Kachar B (2009). Myosin IIa boosts elongation of stereocilia by transporting espin 1 to the plus ends of actin filaments. Nat Cell Biol 11, 443–450.

Sekerkova G, Richter CP, Bartles JR (2011). Roles of the espin actin-bundling proteins in the morphogenesis and stabilization of hair cell stereocilia revealed in CBA/CaJ congenic jerker mice. PLoS Genet 7, e1002032.

Shepherd GMG, Barnes BA, Corey DP (1989). “Bundle-blot” purification and initial protein characterization of hair cell stereocilia. Proc Natl Acad Sci USA 86, 4973–4977.

Shin JB, Krey JF, Hassan A, Metlagel Z, Tauscher AN, Pagana JM, Sherman NE, Jeffery ED, Spinelli KJ, Zhao H, et al. (2013). Molecular architecture of the chick vestibular hair bundle. Nat Neurosci 16, 365–374.

Son EJ, Ma JH, Ankamreddy H, Shin JO, Choi JY, Wu DK, Bok J (2015). Conserved role of Sonic Hedgehog in tonotopic organization of the avian basilar papilla and mammalian cochlea. Proc Natl Acad Sci USA 112, 3746–3751.

Stepanyan R, Frolenkov GI (2009). Fast adaptation and Ca2+ sensitivity of the mechanotransducer require myosin-XVa in inner but not outer cochlear hair cells. J Neurosci 29, 4023–4034.

Syafizayanti, Betzen C, Hoheisel JD, Kastelic D (2014). Methods for analyzing and quantifying protein-protein interaction. Expert Rev Proteomics 11, 107–120.

Tilney LG, DeRosier DJ, Mulroy MJ (1980). The organization of actin filaments, stereocilia, and kinocilia in the organ of Corti. J Cell Biol 86, 244–259.

Tilney LG, Tilney MS, DeRosier DJ (1992). Actin filaments, stereocilia, and hair cells: how cells count and measure. Annu Rev Cell Biol 8, 257–274.

Wu M, Singh AK (2012). Single-cell protein analysis. Curr Opin Biotechnol 23, 83–88.

Zhang DS, Piazza V, Perrin BJ, Rzadzinska AK, Poczatek JC, Wang M, Prosser HM, Ervasti JM, Corey DP, Lechene CP (2012). Multi-isotope imaging mass spectrometry reveals slow protein turnover in hair-cell stereocilia. Nature 481, 520–524.