My oh my(osin): Insights into how auditory hair cells count, measure, and shape

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The mechanisms underlying mechanosensory hair bundle formation in auditory sensory cells are largely mysterious. In this issue, Lelli et al. (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201509017) reveal that a pair of molecular motors, myosin IIIa and myosin IIIb, is involved in the hair bundle's morphology and hearing.

The mammalian cochleae are lined with hair cells, each with a precisely arranged, morphologically polar hair bundle that captures energy produced by sound stimuli. A fascinating problem in cell biology is how this elaborately structured mechanosensory hair bundle forms with precision to enable hearing. Each hair bundle is comprised of up to a few hundred actin-filled stereocilia, arrayed in a staircase-like pattern of increasing heights. Deflection of a hair bundle in the direction of the tallest stereocilium opens mechanically gated ion channels at the stereociliary tips, allowing an influx of cations from the endolymph bathing the stereocilia and thus depolarizing the cell. The staircase array of stereocilia forms by elongation of microvilli at the apical surface of the developing hair cell (Tilney et al., 1992). Over 20 years ago, Tilney et al. (1992) detailed the steps of stereociliogenesis as observed by electron microscopy. In this issue, Lelli et al. provide molecular insight into how hair cells count, measure, and shape stereocilia.

Loss of hair cells is a major cause of human hearing loss, which is often the result of genetic mutations affecting the development of stereocilia (Raphael, 2002). Mutations in genes encoding several different myosin motor proteins in stereocilia have been associated with human hearing loss, including myosin VIIA (Weil et al., 1995), myosin VI (Avraham et al., 1995; Melchionda et al., 2001), and myosin XVA (Wang et al., 1998). Additionally, loss-of-function mutations in *MYO3A*, encoding myosin IIIA, are responsible for hereditary progressive hearing loss DFNB30 (Walsh et al., 2002).

Myosins, the ATP-dependent motors that move along actin-based filaments, are typically composed of three functional domains: the head, the neck, and the tail domains (Krendel and Mooseker, 2005). Class III myosins are unconventional myosins that each have a kinase domain at their N terminus regulated by PKA phosphorylation and autophosphorylation (Kempler et al., 2007). The kinase activity of myosin III was shown to act on its own motor domain to reduce the motor activity. Myosin III proteins concentrate at actin-based cellular protrusions, such as stereocilia of inner ear hair cells (Dosé et al., 2003; Schneider et al., 2006). The two class III isoforms found in vertebrates, myosin IIIa and IIIb, differ toward their C termini: myosin IIIa is longer than myosin IIIb and has one additional actin-binding domain, myosin III tail homology domain II (3THDII). In cultured cells, whereas myosin IIIa localizes to the tips of filopodia on its own, previous work showed that myosin IIIb requires its interaction partner espin-1, an actin-binding protein, for localization to filopodia tips (Merritt et al., 2012). Mutagenesis studies performed on myosin IIIa revealed a relationship between the phosphorylation state of myosin IIIa and the length and density of filopodia (Quintero et al., 2013). How myosin IIIa and IIIb work individually or together to regulate stereociliogenesis remains to be investigated.

In this issue, Lelli et al. (2016) use *Myo3a* (*Myo3a*$^{-/-}$), *Myo3b* (*Myo3b*$^{-/-}$), and double (*Myo3a*$^{-/-}$*Myo3b*$^{-/-}$) knockout mice to dissect the complex roles of myosin IIIa and IIIb in hearing. Mice null for myosin IIIa, *Myo3a*$^{-/-}$, were initially normal but showed defects in hearing quality from 2 to 4 mo of age, as detected by auditory brain stem response measurements, which monitor the electrical response of the auditory pathway to short sound stimuli, but not by distortion product otoacoustic emissions, which test outer hair cell (OHC) function. These results indicated that inner hair cells (IHCs) are deleteriously impacted but that OHCs are not, and that these mice had progressive hearing loss. *Myo3b*$^{-/-}$ mutants had normal hearing, indicating that redundancy with myosin IIIa may obscure the role of this protein in single knockouts. Investigation of double knockouts, however, clarified the redundant functions of the two isoforms, as the mice were profoundly deaf at 1 mo of age.

Interestingly, Lelli et al. (2016) investigated the potential requirement for myosin IIIa in the maintenance of hearing. They generated conditional knockout (cKO) mice, *Myo3a-cKO*, in which myosin IIIa is inactivated postnatally in a *Myo3b*$^{-/-}$ background. These animals hear normally up to at least 6 mo of age. Therefore, myosin IIIa is required during a critical period in auditory system development but is not required to maintain hearing. The researchers further studied the relationship between myosin IIIa and myosin IIIb in the *Myo3a-cKO* mice and noted an increase in auditory brain stem response thresholds. This fascinating result likely indicates that inactivation of myosin IIIa elicits a pernicious effect of myosin IIIb at mature stages. Determining the mechanism

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by which myosin IIIb damages hearing in the absence of myosin IIIa and whether it is due to its lack of a single 3THDII domain may yield insight into the roles of class III myosins in hearing.

Given that myosin III proteins localize to the tips of stereocilia and are required for hearing, Lelli et al. (2016) explored the morphology of hair bundles lacking these proteins. Although double knockout *Myo3a*$^{-/-}$*Myo3b*$^{-/-}$ mice displayed normal positioning of the kinocilium, the cilium reflecting the initial position and polarity of the developing hair bundle, and of asymmetric cell division proteins Par-6 and $G\alpha i_3$, which constrain the shape and positioning of the hair bundle, many hair cells from these mice exhibited hair bundle abnormalities. These abnormalities were first described during embryonic auditory hair bundle morphogenesis, at which time 19% of IHCs and 81% of OHCs displayed misshapen bundles. Several of these misshapen IHC and OHC bundles also contained abnormally long, ungraded protrusions, which Lelli et al. (2016) referred to as "long amorphous" bundles. By birth, most IHCs displayed long amorphous bundles. Although most OHC hair bundles were abnormally shaped, they had developed normal staircase organization of their stereocilia heights, and the long amorphous protrusions were no longer present. Presumably, another molecular mechanism comes into play and "corrects" the protrusions to establish the staircase organization. Knockout of candidate proteins could be an interesting strategy to uncover this mechanism in the future. Furthermore, other stereociliary phenotypes of *Myo3a*$^{-/-}$*Myo3b*$^{-/-}$ mutant mice are also intriguing. Do myosin III proteins serve different mechanistic roles in IHCs and OHCs to give rise to different phenotypes? Or, are there other key factors that drive stereocilia into various degrees of hair bundle defects in the absence of myosin III proteins?

In *Myo3a*$^{-/-}$*Myo3b*$^{-/-}$ mice, many of the OHC bundles had abnormal side rows of extra stereocilia, which sometimes closed the bundle off into a circular shape. Interestingly, the stereocilia in these abnormal bundles were also significantly taller than in controls. Although the height of the tallest row of stereocilia in OHCs normally decreases after birth (Sekerková et al., 2011), the height of these stereocilia did not change in the *Myo3a*$^{-/-}$*Myo3b*$^{-/-}$ mice, leading to an increased height difference 9 d after birth. The increased height and number of stereocilia in *Myo3a*$^{-/-}$*Myo3b*$^{-/-}$ hair bundles is suggestive of unstable actin dynamics. The authors suggest that class III myosins stabilize the F-actin cores of the stereocilia, thus controlling their selective elongation by limiting their growth (Fig. 1). Surprisingly, class III myosins, which presumably climb the full lengths of the stereocilia to perch themselves near the tips, act to restrict their growth. This result seems to contrast with previous work in which myosin IIIa had been proposed to promote elongation of the stereocilia by transporting espin-1 to the stereocilia tips (Salles et al., 2009). Interestingly, Lelli et al. (2016) found that espin-1 was still properly targeted to the tips of stereocilia in *Myo3a*$^{-/-}$*Myo3b*$^{-/-}$ mutant mice. The researchers extended the study to another binding partner of myosin IIIa, retinophilin/MORN4. Surprisingly, MORN4 was normally targeted to stereocilia tips in *Myo3a*$^{-/-}$*Myo3b*$^{-/-}$ mutant mice. This suggests that there may be redundancy in the mechanisms of transport of stereocilia tip proteins. Whether or not distinct myosin isoforms interchange cargoes so that these cargoes are properly localized is a relevant avenue of future investigation. Furthermore, identification and knockout of class III myosin binding partners could help define the mechanism by which these proteins control elongation of stereocilia.

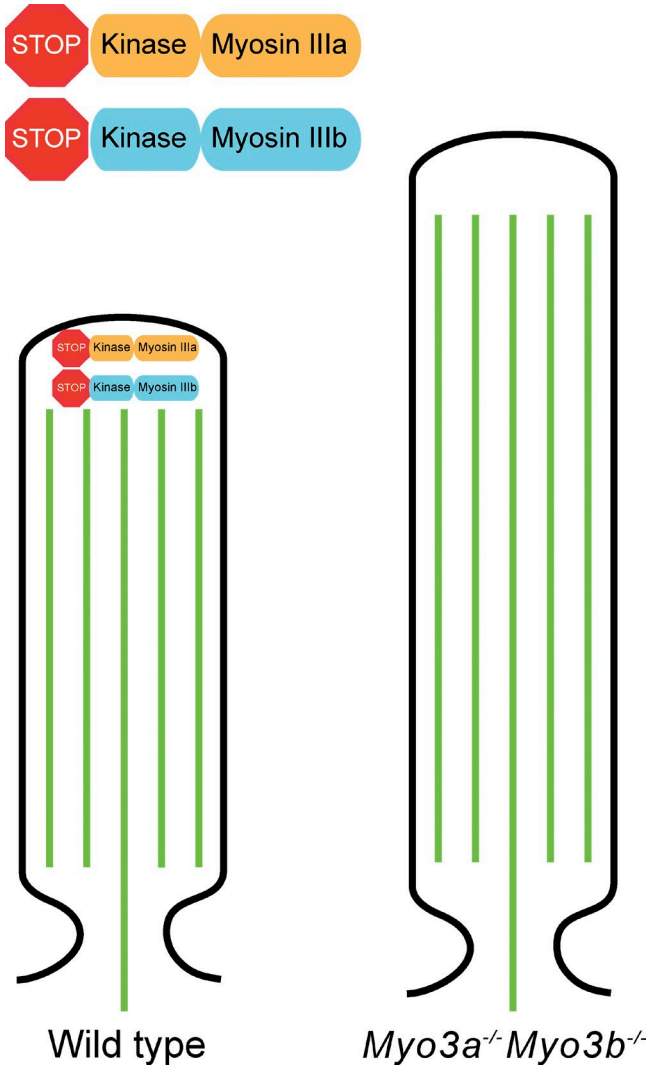

Figure 1. **Myosin IIIa and myosin IIIb regulate stereociliary length.** (top) Schematics of myosin IIIa and myosin IIIb. The stop signs indicate the role these proteins have in limiting the heights of stereocilia. (bottom) A single stereocilium with wild-type copies of myosin IIIa and myosin IIIb is shorter than a stereocilium from a *Myo3a*$^{-/-}$*Myo3b*$^{-/-}$ mutant when stereocilia from equivalent positions between hair bundles are compared.

In addition to characterizing the lengths of stereocilia, Lelli et al. (2016) noted transient defects in the numbers of stereocilia during development of hair cells within genetically altered mice. At birth, *Myo3a*$^{-/-}$*Myo3b*$^{-/-}$ mice had an increased number and density of stereocilia in both IHCs and OHCs as compared with controls. However, 9 d after birth, the number and density of the stereocilia had decreased to levels similar to those seen in control animals. Their results indicate that class III myosins help to control the initial selection of microvilli to become stereocilia, but a different, dominant molecular mechanism overrides this step and is likely involved in refining the number of stereocilia at later, postnatal stages. Identification of this mechanism by knockout of candidate molecules in the *Myo3a*$^{-/-}$*Myo3b*$^{-/-}$ mice would be of interest for future work. The defects, though transient, indicate that myosins are key molecular components that establish how cells count the number of protrusions to be produced.

In this work, Lelli et al. (2016) determined that myosins IIIa and IIIb are required for normal hair bundle development and hearing. These motor proteins influence the number and lengths of stereocilia to be produced and the overarching shape of the hair bundle. Evaluating the importance of the kinase domains of myosin IIIa and IIIb in hair bundle development is an important next step. The phosphorylation states of proteins in cellular protrusions, such as filopodia and stereocilia, can be regulatory. For example, phosphorylation of the actin cross-linking protein fascin 2b reduces this protein's capacity to lengthen filopodia (Chou et al., 2011). Moreover, phosphorylation of fascin 2b increases the exchange rate of this protein within zebrafish stereocilia (Hwang et al., 2015). Additionally, in other systems, PKA modulates a signaling cascade that activates the actin-severing protein cofilin to control actin-filament dynamics (Nadella et al., 2009). Future work will be needed to test whether signal transduction stemming from the kinase domains of class III myosins are consequential for hair bundle development.

Lelli et al. (2016) observed that the constitutive absence of both class III myosins leads to deafness as a result of hair bundle developmental defects. However, mice deficient for only one of the isoforms did not display such a striking hearing phenotype, indicating that myosins IIIa and IIIb compensate for the loss of one another in the developing cochlea. A role for myosin IIIa in the maintenance of stereocilia had been suggested by the discovery of loss-of-function mutations in *MYO3A* in patients with hereditary progressive hearing loss DFNB30 (Walsh et al., 2002). Remarkably, the analyses of *Myo3a*$^{-/-}$*Myo3b*$^{-/-}$ and *Myo3a-cKO Myo3b*$^{-/-}$ mice showed that the redundancy between myosins IIIa and IIIb is critical during hair bundle formation, but not during later, mature stages. Furthermore, the hearing impairment seen in *Myo3a-cKO* mice suggests that myosin IIIb exerts deleterious effects on hearing past the developmental stages of hair bundle morphogenesis. Although the mechanisms at work are unclear, these findings suggest that the down-regulation of *MYO3B* expression might be an effective way of preventing late-onset hearing loss in patients with *MYO3A* mutations.

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References

Avraham, K.B., T. Hasson, K.P. Steel, D.M. Kingsley, L.B. Russell, M.S. Mooseker, N.G. Copeland, and N.A. Jenkins. 1995. The mouse Snell's waltzer deafness gene encodes an unconventional myosin required for structural integrity of inner ear hair cells. *Nat. Genet.* 11:369–375. http://dx.doi.org/10.1038/ng1295-369

Chou, S.W., P. Hwang, G. Gomez, C.A. Fernando, M.C. West, L.M. Pollock, J. Lin-Jones, B. Burnside, and B.M. McDermott Jr. 2011. Fascin 2b is a component of stereocilia that lengthens actin-based protrusions. *PLoS One.* 6:e14807. http://dx.doi.org/10.1371/journal.pone.0014807

Dosé, A.C., D.W. Hillman, C. Wong, L. Sohlberg, J. Lin-Jones, and B. Burnside. 2003. Myo3A, one of two class III myosin genes expressed in vertebrate retina, is localized to the calycal processes of rod and cone photoreceptors and is expressed in the sacculus. *Mol. Biol. Cell.* 14:1058–1073. http://dx.doi.org/10.1091/mbc.E02-06-0317

Hwang, P., S.W. Chou, Z. Chen, and B.M. McDermott Jr. 2015. The stereociliary paracrystal is a dynamic cytoskeletal scaffold in vivo. *Cell Reports.* 13:1287–1294. http://dx.doi.org/10.1016/j.celrep.2015.10.003

Kempler, K., J. Tóth, R. Yamashita, G. Mapel, K. Robinson, H. Cardasis, S. Stevens, J.R. Sellers, and B.A. Battelle. 2007. Loop 2 of limulus myosin III is phosphorylated by protein kinase A and autophosphorylation. *Biochemistry.* 46:4280–4293. http://dx.doi.org/10.1021/bi062112u

Krendel, M., and M.S. Mooseker. 2005. Myosins: tails (and heads) of functional diversity. *Physiology (Bethesda).* 20:239–251. http://dx.doi.org/10.1152/physiol.00014.2005

Lelli, A., V. Michel, J. Boutet de Monvel, M. Cortese, M. Bosch-Grau, A. Aghaie, I. Perfettini, T. Dupont, P. Avan, A. El-Amraoui, and C. Petit. 2016. Class III myosins shape the auditory hair bundles by limiting microvilli and stereocilia growth. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201509017

Melchionda, S., N. Ahituv, L. Bisceglia, T. Sobe, F. Glaser, R. Rabionet, M.L. Arbones, A. Notarangelo, E. Di Iorio, M. Carella, et al. 2001. MYO6, the human homologue of the gene responsible for deafness in Snell's waltzer mice, is mutated in autosomal dominant nonsyndromic hearing loss. *Am. J. Hum. Genet.* 69:635–640. http://dx.doi.org/10.1086/323156

Merritt, R.C., U. Manor, F.T. Salles, M. Grati, A.C. Dose, W.C. Unrath, O.A. Quintero, C.M. Yengo, and B. Kachar. 2012. Myosin IIIB uses an actin-binding motif in its espin-1 cargo to reach the tips of actin protrusions. *Curr. Biol.* 22:320–325. http://dx.doi.org/10.1016/j.cub.2011.12.053

Nadella, K.S., M. Saji, N.K. Jacob, E. Pavel, M.D. Ringel, and L.S. Kirschner. 2009. Regulation of actin function by protein kinase A-mediated phosphorylation of Limk1. *EMBO Rep.* 10:599–605. http://dx.doi.org/10.1038/embor.2009.58

Quintero, O.A., W.C. Unrath, S.M. Stevens Jr., U. Manor, B. Kachar, and C.M. Yengo. 2013. Myosin 3A kinase activity is regulated by phosphorylation of the kinase domain activation loop. *J. Biol. Chem.* 288:37126–37137. http://dx.doi.org/10.1074/jbc.M113.511014

Raphael, Y. 2002. Cochlear pathology, sensory cell death and regeneration. *Br. Med. Bull.* 63:25–38. http://dx.doi.org/10.1093/bmb/63.1.25

Salles, F.T., R.C. Merritt Jr., U. Manor, G.W. Dougherty, A.D. Sousa, J.E. Moore, C.M. Yengo, A.C. Dosé, and B. Kachar. 2009. Myosin IIIa boosts elongation of stereocilia by transporting espin 1 to the plus ends of actin filaments. *Nat. Cell Biol.* 11:443–450. http://dx.doi.org/10.1038/ncb1851

Schneider, M.E., A.C. Dosé, F.T. Salles, W. Chang, F.L. Erickson, B. Burnside, and B. Kachar. 2006. A new compartment at stereocilia tips defined by spatial and temporal patterns of myosin IIIa expression. *J. Neurosci.* 26:10243–10252. http://dx.doi.org/10.1523/JNEUROSCI.2812-06.2006

Sekerková, G., C.P. Richter, and J.R. Bartles. 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. http://dx.doi.org/10.1371/journal.pgen.1002032

Tilney, L.G., M.S. Tilney, and D.J. DeRosier. 1992. Actin filaments, stereocilia, and hair cells: how cells count and measure. *Annu. Rev. Cell Biol.* 8:257–274. http://dx.doi.org/10.1146/annurev.cb.08.110192.001353

Walsh, T., V. Walsh, S. Vreugde, R. Hertzano, H. Shahin, S. Haika, M.K. Lee, M. Kanaan, M.C. King, and K.B. Avraham. 2002. From flies' eyes to our ears: mutations in a human class III myosin cause progressive nonsyndromic hearing loss DFNB30. *Proc. Natl. Acad. Sci. USA.* 99:7518–7523. http://dx.doi.org/10.1073/pnas.102091699

Wang, A., Y. Liang, R.A. Fridell, F.J. Probst, E.R. Wilcox, J.W. Touchman, C.C. Morton, R.J. Morell, K. Noben-Trauth, S.A. Camper, and T.B. Friedman. 1998. Association of unconventional myosin MYO15 mutations with human nonsyndromic deafness DFNB3. *Science.* 280:1447–1451. http://dx.doi.org/10.1126/science.280.5368.1447

Weil, D., S. Blanchard, J. Kaplan, P. Guilford, F. Gibson, J. Walsh, P. Mburu, A. Varela, J. Levilliers, M.D. Weston, et al. 1995. Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature.* 374:60–61. http://dx.doi.org/10.1038/374060a0