Precision patterning: How inner hair cells "hop" to it

Jemma L. Webber and Jaime García-Añoveros*

A combination of Notch-mediated lateral inhibition, mechanical forces, and differential adhesion generates a single row of alternating inner hair and supporting cells.

Our tissues are made up of communities of cells working together to perform a function beyond that which any individual cell could accomplish alone. These feats of nature require organizing the appropriate mix of cells, as well as coordinating their orientation and alignment relative to one another. Understanding how these cell communities self-organize remains a fundamental question in biology. In this issue of *Science Advances*, Cohen *et al.* (*1*) use a combination of live cell imaging of cultured tissue paired with mathematical modeling to address how precisely arrayed cellular patterns form in the mammalian cochlea, the spiral-shaped organ in the inner ear.

Arising from a disordered layer of uniform cells, the mature cochlear sensory epithelium assembles into parallel rows of sensory hair cells, with a single inner hair cell row and three to four outer hair cell rows respectively specialized for either sound transmission or amplification. The hair cells alternate with non-sensory supporting cells in a mosaic pattern, and a parallel row of structural inner pillar cells divides the inner and outer hair cell rows. This highly ordered arrangement extends along the cochlear axis and is required for proper auditory function. The predictability of this latticed arrangement, and the micrometric precision with which each cell is positioned and oriented, makes the cochlea an ideal organ in which to examine and model how tissues are organized during development.

The salt-and-pepper pattern of hair cells adjacent to supporting cells (and not other hair cells) is indicative of Notch-mediated lateral inhibition. Best studied in *Drosophila*, lateral inhibition models describe how near neighbors compete to obtain the default cell fate, in this instance, a hair cell, while the loser takes on a supporting cell fate. Indeed, genetic studies confirm the role of low/high levels of Notch signaling in hair/supporting cell development, respectively (*2*). However, although required for setting up the initial patterning, lateral inhibition alone cannot explain the precise patterning of parallel rows of hair cells found in the mature cochlea.

While cells are determining their fate, the sensory epithelium is also undergoing dramatic remodeling, decreasing in width and depth, and increasing in length, a process known as convergent extension. During this process, cells in the medial to lateral axis (from inner to outer hair cell rows) come under contractile pressure, which is resolved by cell movements and rearrangements. How these various events coordinate to generate parallel rows of cells was unknown prior to work from Cohen *et al.* (*1*, *3*).

The *Science Advances* paper describes the use of time lapse microscopy to examine inner hair cell rearrangements as the sensory epithelium transitions from a state of disorder to an organized array of cells. Tagging hair cells fluorescently (with Atoh1-mCherry as an emerging hair cell marker), Cohen *et al.* recorded initially disordered inner hair cells reorganizing themselves into a single row adjacent to the inner pillar cells. The authors observed that newly differentiated hair cells move toward the pillar cells, in a process that they suggest may be driven by physical pressure from cell division occurring in the surrounding tissue. Consequently, cellular rearrangements, including cell intercalations and delaminations, were revealed.

In addition to previously described cellular intercalations (*3*, *4*), in which cells squeeze between one another by shrinking and extending perpendicular apical junctions, the authors also noted a novel form of intercellular displacement that they term "hopping intercalation." Using a marker of tight junctions (Z01-GFP) normally found at the apical side of the cochlear epithelium, the authors find that hair cells first contact and form a junction with the inner pillar cells at the basolateral surface. This unusual contact appears to pull the cells together, while the tight junction moves apically, "zippering" up the inner pillar surface until a new apical footprint is formed adjacent to the inner pillar cell (Fig. 1, right).

As the new apical surface merges with the original, the cell can be described as having hopped under the intercepting cellular junction. It is speculated that the mechanically induced basolateral displacement affects the hair cells, and not the supporting cells, because the former are known to detach from the basement membrane (*5*). Finally, cells that fail to touch the inner pillar cell row were found to delaminate, disappearing from the epithelium, with the resulting gaps quickly filled in by neighboring cells.

To account for whether the described cellular events could explain inner hair cell alignment, the authors developed a series of mathematical models of the patterning process. Notch-mediated lateral inhibition simulated a disordered salt-and-pepper pattern of hair cells and supporting cells, but not an arrangement in a single file. Adding to the model the mechanically

Departments of Anesthesiology, Neurology, and Neuroscience, Hugh Knowles Center for Hearing Research, Northwestern University, Chicago, IL 60611, USA.
*Corresponding author. Email: anoveros@northwestern.edu

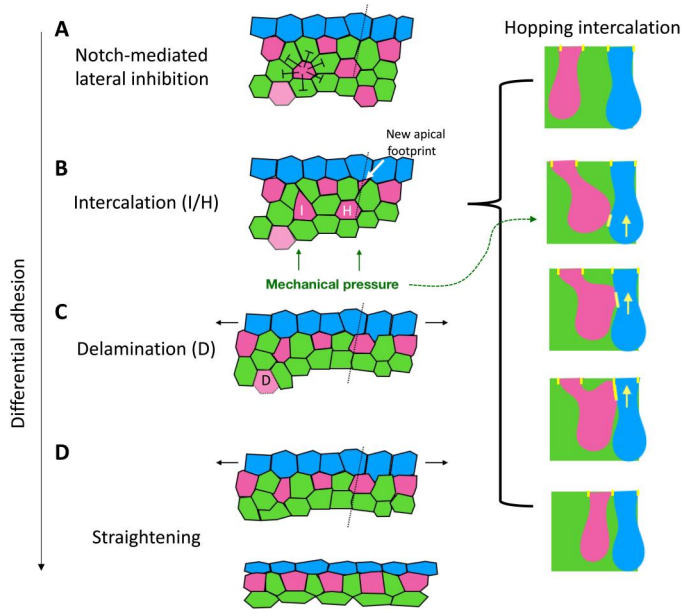

Fig. 1. Cell pattern formation in the human cochlea. Left: (**A**) Notch-mediated lateral inhibition drives formation of inner hair cells (magenta) and supporting cells (green), bordered by a single row of inner pillar cells (blue). Outer hair cells are not shown. (**B**) Pressure on the forming epithelium drives cellular intercalations (I) and hopping intercalations (H). (**C**) Hair cells not contacting pillar cells delaminate (D) and disappear. (**D**) Preferred adhesion between different cell types straightens out cell alignments. Right: A side view of hopping intercalation: Inner hair cells move toward and contact inner pillar cells basolaterally, forming a junction labeled by Z01-GFP (yellow). The junction moves up the surface of the inner pillar, until it forms a new apical footprint that merges with the original, juxtaposing the hair and inner pillar cells. Note that hair cells are detached from the basement membrane and hence malleable to basolateral displacement. Credit: Jemma Webber.

induced hopping and regular intercalations resulted in many hair cells aligning in a single file along the inner pillar cell row; however, several non-aligned hair cells persisted. Incorporating the delamination of out-of-row hair cells into the model resolved this, such that only aligned hair cells in the row adjacent to the inner pillar cells remained (see Fig. 1). Finally, modeling these events together with differential adhesion between pillar, hair, and other supporting cells predicted a straighter alignment of the row of hair plus supporting cells and the row of inner pillar cells. The modeling thus provided a virtual means of examining the contribution of the various phenomena observed by live imaging (and by prior molecular studies). The result is a holistic understanding of how a disorganized epithelium assembles a straight line of alternating (inner hair and supporting) cells.

The observations and model put forward by Cohen *et al.* (*1*) implicate a preassembled row of pillar cells as an "organizer" for the alignment of inner hair cells. First, hair cells appear to move toward the inner pillar cells, making contact before intercalation; second, in the absence of this contact, hair cells are eliminated from the epithelium. This is somewhat reminiscent of boundary capture that occurs in the notochord, whereby protrusive notochord cells that meet neighboring somitic cells are abruptly captured, ceasing movement to form a smooth interface in a process termed boundary-associated intercalation (*6*).

Cohen *et al.* suggest that physical pressure from surrounding tissues accounts for the drive toward the inner pillars. It is also possible that this process could involve a biochemical signal or a combination of the two. Given that presumptive hair cells are motile, with basal protrusions that could drive motility (*4*), it will be interesting to address whether movement toward the inner pillar cells depends on these processes. Of note, the authors find that the cell adhesion molecules, Nectin-1 and Nectin-3, are expressed differentially in hair and pillar cells, potentially promoting differential adhesion. Nectin-mediated capture of inner hair cells could not only ensure their alignment in a row but also prevent their escape by means of hopping intercalation into the outer compartment.

How the pillar cells are specified and aligned ahead of the inner hair cells remains to be elucidated. Avenues for future exploration include studying whether opposing signals or forces from the inner and outer compartments converge on the inner pillar cells to drive their specification and alignment, and whether preference for self-contact suffices for the self-assembly of an inner pillar row. Although these questions remain unanswered, the work by Cohen *et al.* reveals for the first time a framework to explain how inner hair cells assemble into a single file in the organ of Corti, the structure in the cochlea that triggers nerve impulses in response to sound vibrations. This is important, not just for the topic of hearing, whereby precise alignment of inner hair cells is needed for frequency determination of sound, but also more broadly for the field of tissue morphogenesis.

REFERENCES

1. R. Cohen, S. Taiber, O. Loza, S. Kasirer, S. Woland, D. Sprinzak, Precise alternating cellular pattern in the inner ear by coordinated hopping intercalations and delaminations. *Sci. Adv.* **9**, eadd2157 (2023).
2. R. Brown, A. K. Groves, Hear, Hear for notch: Control of cell fates in the inner ear by notch signaling. *Biomolecules* **10**, 370 (2020).
3. R. Cohen, L. Amir-Zilberstein, M. Hersch, S. Woland, O. Loza, S. Taiber, F. Matsuzaki, S. Bergmann, K. B. Avraham, D. Sprinzak, Mechanical forces drive ordered patterning of hair cells in the mammalian inner ear. *Nat. Commun.* **11**, 5137 (2020).
4. E. C. Driver, A. Northrop, M. W. Kelley, Cell migration, intercalation, and growth regulates mammalian cochlear extension. *Development* **144**, 3766–3776 (2017).
5. T. Tateya, S. Sakamoto, F. Ishidate, T. Hirashima, I. Imayoshi, R. Kageyama, Three-dimensional live imaging of Atoh1 reveals the dynamics of hair cell induction and organization in the developing cochlea. *Development* **146**, dev177881 (2019).
6. R. Keller, L. Davidson, A. Edlund, T. Elul, M. Ezin, D. Shook, P. Skoglund, Mechanisms of convergence and extension by cell intercalation. *Philos. Trans. R Soc. Lond. B* **355**, 897–922 (2000).

10.1126/sciadv.adg8662