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Life's Machinery for Hearing

Electron Microscope Tomography Reveals Secrets of Ear's Molecular Machines

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One out of a thousand children in the United States is born deaf; ten percent of all people living in industrialized nations suffer from severe hearing loss—30 million in the U.S. alone. These are pressing clinical reasons to learn just how hearing works and why it fails.

"Hearing in humans is a remarkable faculty," says Manfred Auer of Berkeley Lab's Life Sciences Division. "It works over six orders of magnitude, from a whisper to the roar of a jet engine."

Hearing is also remarkable for its ability to adapt to constant loud noise yet still manage to pick out barely distinguishable sounds. And humans can pinpoint the source of a sound to within less than a degree: one ear hears the sound slightly before the other, and the brain calculates the direction from the offset. Since the difference in arrival times is less than a millionth of a second—a thousand times faster than most biochemical processes—hearing must depend on direct mechanical detection of sounds instantly translated into nerve signals.

The inner ear's hair cells are the key. Embedded in the epithelial lining of the cochlea, hair cells respond mechanically to sound vibrations and instantly convert these mechanical responses into electrical signals that trigger neurons in the brain.

The hair cells of the inner ear (shown in lower panel) are what make hearing possible.

Electron Beams and Zebrafish

Electron microscope tomography is a uniquely powerful tool for exploring biological structures at the level of these molecular machines. Unlike conventional two-dimensional microscopy, electron tomography tilts its target under the microscope's electron beam, yielding a series of projection images of the sample at different angles. From these, a computer constructs a three-dimensional image.

"Electron tomography bridges the gap between x-ray crystallography and the light microscope," says Auer. "X-ray crystallography can reveal the structure of proteins on the nanometer scale, while light microscopy can resolve organelles inside the cell to a couple of tenths of a micron. Most molecular machines fall between those limits."

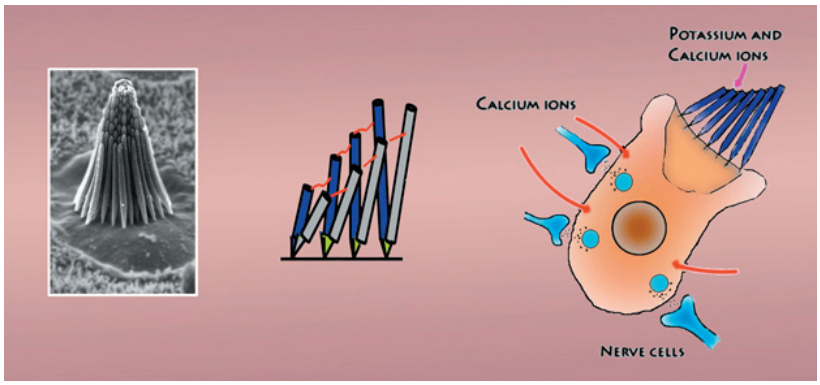
Much early microscopy of hair cells by Auer and others involved dissecting the inner ears of frogs, "but the frog system was limited by such problems as damage to tissues and sample preservation." Auer now uses a different animal system, wonderfully suited to electron micrography: zebrafish larvae.

"The fish are transparent, so it's possible to follow the development of their organs with a light microscope. The inner ear develops in the first day," he explains. "They are easy to preserve because the entire larva is tiny and can be frozen instantly under high pressure, so that no ice crystals form to damage the tissue."

Through genetic manipulation, short stretches of amino acids can be added to specific zebrafish proteins, which can be tagged with fluorescent, nontoxic labels. "In principle you can localize every tagged protein in any organ or cell," Auer says.

The part of the hair cell that mechanically responds to vibration is a bundle of fibers called stereocilia, sticking out of the top of the cell like a radical hairdo. In zebrafish the stereocilia are arranged in stair-step fashion. The tip of each lower fiber is attached diagonally to its neighbor by a fine filament called a tip link.

Vibration causes the fibers to lean over, stretching the tip-link filaments. This pulls open nearby channels in the fibers, allowing potassium ions to flow into the fiber and down to the body of the cell. The electrical balance between calcium and potassium ions in the cell is instantly changed, triggering a signal to adjacent neurons.



Bundles of stereocilia bend in response to vibrations in the inner ear. Tension on the tip links (red lines in center diagram) opens channels in the stereocilia to admit ions, instantly changing the electrical balance inside the cell and firing nerve cells.

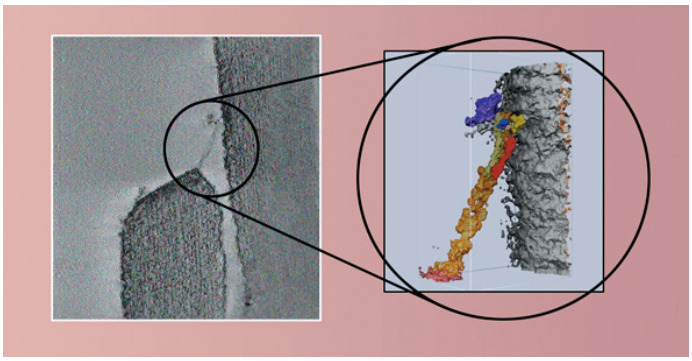
If the hair bundle remains bent by persistent noise, a higher level of calcium in the cell signals the structural protein myosin, also present in the stereocilia, to slide down along the actin fibers. By resetting the tension on the tip-link springs in this way, hair cells can adapt to sustained noise levels.

"Noise can stress the stereocilia bundle so much that the tip links break," Auer says. "They usually grow back in 24 hours—this is the rock-concert effect, where hearing loss is temporary. Loud noises can also shear off whole bundles of stereocilia. In mammals these can't regenerate, and the loss is permanent."

Finding a way to regenerate hair cells, says Auer, "is the Holy Grail of research. We're born with just 16,000 hair cells in the cochlea, and every passing subway train kills a few of them."

The micrographs that Auer and his colleagues use to construct electron tomographs resemble other microscopic studies of these structures, including blobs near the tips of the fibers that researchers customarily dismissed as "dirt." But, says Auer, "We think there is no such thing as dirt."

Because electron tomography allows "dissection in silico," Auer's group has been able to analyze these mysterious artifacts, finding hints of unsuspected structures—including whether there may be more than a single tip link between fibers, how tip links are structured, and what protein or proteins constitute the tip links.



Electron tomography reveals the three-dimensional structure of the tip link, providing valuable clues to its proteins.

"Until lately, the only protein firmly associated with stereocilia tip structures besides actin was myosin. Now we have 50 candidates—all because we could look at that 'dirt' in 3-D." Auer and his collaborators plan to publish their findings of just which proteins are involved in tip links and other structures soon.

And That's Just the Beginning

Because electron tomography can bridge the gap between ultrahigh-resolution protein structures and the large-scale organization of cells and tissues available to the light microscope, Auer says, "I would contend that electron tomography will play a major role in investigating all aspects of biology—in structural biology, cell biology, proteomics, biochemistry, physiology, pathology, evolution, everything. Once you have this new toy, you can apply it to all these questions."

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