

OCTOBER 13, 2004

Channel Protein Converts Vibrations to Electrical Signal

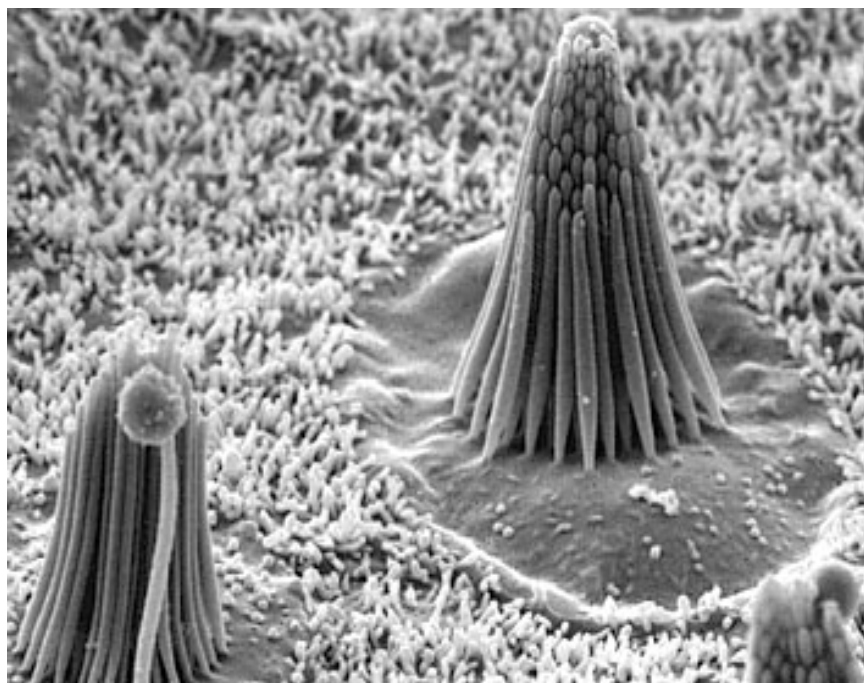


Image Title: Scanning electron micrograph of hair cells from the bullfrog inner ear, which contain the mechanically-gated ion channel TRPA1. - Laboratory of David P. Corey

Researchers have identified a molecule that can transform the mechanical stimulus of a sound wave into an electrical signal recognizable by the brain. The protein forms an ion channel that opens in response to sound, causing electrical impulses that communicate the pitch, volume, and duration of a sound to the brain.

Scientists have long suspected that such a molecule must exist in the tiny cilia extending from receptor cells in the inner ear. Now, researchers led by Howard Hughes Medical Institute investigator David P. Corey, who is at Harvard Medical School, have several lines of evidence that, in vertebrates, this mechanosensitive channel is formed by a protein known as TRPA1.

Certain features of the protein suggest that it may serve double, or even triple, duty in the inner ear, not only acting as an ion channel, but also forming a spring that allows the transduction machinery to stretch, and even amplifying incoming auditory signals. The work is published October 13, 2004, in an advance online publication of the journal *Nature*.

The cells that line the inner ear and convert mechanical sound vibrations into electrical impulses are known as hair cells □ named for the tuft of 30-300 cilia, or microscopic hairs, on each cell's surface. Thin filaments called tip links connect the channels in adjacent hairs, so that when a vibration stirs the bundle of cilia, the tip links are tightened and pull on the channels. Within 5 to 10 microseconds of this motion, channels in the hair cell open and allow ions to enter □ the first step in sending a sound signal to the brain.

"Part of the TRPA1 molecule is a long chain of ankryin repeats, and we think that the ankryin repeats actually form the elastic element that had been defined biophysically."

- David P. Corey

According to Corey, the rapidity of this response □ which is as much as 1,000 times faster than the opening of similar channels in the eye in response to light □ indicated to scientists that the channel must respond directly to the mechanical stimulus, rather than relying on a signal from another molecule. The speed of the response was determined more than 20 years ago in the laboratory of HHMI investigator A. James Hudspeth □ but since that time, no one had been able to identify the channel protein.

In the search for a molecule that might form the hair cell channel, the researchers turned to a family of ion channels known as TRPs, or transient receptor potential channels. "We thought the TRP family was a likely place to look for this channel, because many other TRP channels are involved in sensory transduction," Corey said. "In mammalian pheromone receptors, insect vision, hearing in flies, or touch in worms □ there were a lot of other TRP channels that seemed to be sensory." In addition, the selectivity and conductance of TRPs corresponded to what was already known about the unidentified hair cell channel.

TRPA1 was a particularly good candidate within this family, Corey said, because it has an architecture similar to a TRP channel that is mechanosensory in the fruit fly □ a molecule known as NOMPC, identified by HHMI investigator Charles S. Zuker. In other parts of the nervous system, however, the TRPA1 channel is activated by such stimuli as painfully cold temperatures and pungent chemicals like mustard, cinnamon oil, and wasabi, which seemed incompatible with mechanical activation in the ear.

The distribution of TRPA1 was one of the first clues that TRPA1 might indeed be the channel the researchers were searching for. They found that the *TRPA1* gene was expressed in the inner ear of the mouse, including in the hair cells. Not only was *TRPA1* in the right place to be the channel they were searching for, Corey said, it was also there at the right time. When the researchers tested for *TRPA1* gene expression in developing mouse embryos, they found that the gene became active when the embryo was about 16 days old □ just a day before hair cells become mechanically sensitive.

Next, the scientists looked for TRPA1 within the hair cell. In frogs and mice, a fluorescently-tagged TRPA1 antibody bound the tips of the cilia, where the mechanically activated channels were known to be located. This localization of TRPA1 changed, however, when the researchers treated the hair cells with a chemical that damages the mechanically-sensitive signaling pathway.

Other researchers had recently found that if a hair cell's tip links are chemically separated, the tip link protein, cadherin 23, is removed from the cilia within minutes. According to Corey, this suggests that once a hair cell senses that its signaling complex has been damaged, it rapidly recycles the components of that pathway so they can be reused or replaced. “So we said, look, if the tip-link protein goes away when you separate the tip links, maybe other parts of the transduction machinery will go away. And if TRPA1 is the right channel, it should go away.” In fact, TRPA1 did disappear when the tip links were removed □ further evidence for a role in that signaling pathway.

The next step was to determine how cells functioned without TRPA1, which the researchers tested by interfering with the production of the protein in both zebrafish embryos and hair cells from mice. To test function, the researchers bathed the embryos or the mouse cells in a solution containing a dye □ either yellow or red □ that can pass through the ion channel. The dye entered the normal cells, accumulating and causing them to glow. In cells where TRPA1 had been reduced, however, less dye was able to enter the cells - suggesting that without TRPA1, there was no channel for the molecule to pass through.

“A more direct way to measure the mechanical sensitivity of the cell is to measure the electrical response that it gives when you stimulate the cell,” Corey said. By putting a microelectrode on the mouse hair cells, the scientists could directly measure the amount of current flowing through the channels. With lower levels of TRPA1, current flow was diminished. In zebrafish embryos, they measured the voltage inside the developing ear, and found that this, too, was reduced when TRPA1 levels were low.

Given the evidence that TRPA1 plays an integral role in the conversion of a mechanical stimulus to an impulse that the brain can interpret, Corey is eager to determine whether defects in TRPA1 might play a role in inherited deafness. “At the moment there are no known deafnesses that map to the same chromosomal location as this channel,” he said, “but we are screening some unmapped deafness families.”

In addition to the evidence that TRPA1 forms a mechanically-gated channel in the hair cells, the structure of the protein suggests that it may play another role. Previous biophysical studies had indicated that a springy structure that stretches when a hair cell's cilia move pulled on the channels. Although it was once thought that the tip links might serve this function, the recent discovery of the tip-link protein, cadherin 23, suggested that their structure is too rigid for this role. "Part of the TRPA1 molecule is a long chain of ankryin repeats, and we think that the ankryin repeats actually form the elastic element that had been defined biophysically," Corey said. He plans further experiments to test this possibility.

Additionally, Corey suspects that TRPA1 might play a role in the amplification of sound signals, a process that increases sensitivity and improves the ability to distinguish between different frequencies. In mammals, hair cells not only respond to sound, but also amplify the vibrations for quiet sounds as much as 100-fold. One hypothesis for how they do this, first proposed by James Hudspeth, is that after the transduction channels open with each cycle of sound, they quickly snap shut, and the force of this snapping can push the cilia bundle. Like pushing a child on a swing, successive pushes can build up to a large oscillation, amplifying the sound. In addition, different hair cells amplify different frequencies, so this mechanism could create the sharp tuning in the ear that allows us to discriminate fine differences in pitch. If this model is true, TRPA1 may also be the amplifier protein.