2-22-2012

Visualizing actin regulatory proteins & the mechanoelectrical Ion channel in inner ear stereocilia

Chih-Tao Tu

ROCHESTER INSTITUTE OF TECHNOLOGY

A Thesis Submitted to the Faculty of
The College of Health Sciences and Technology

In Candidacy for the Degree of
MASTER OF FINE ARTS
In Medical Illustration

Visualizing Actin Regulatory Proteins & the Mechano-electrical Ion Channel in Inner Ear Stereocilia

By

Chih-Tao Tu
2/22/2012

Thesis Title: Visualizing Actin Regulatory Proteins & the Mechanoelectrical Ion Channel in Inner Ear Stereocilia

Thesis Author: Chih-Tao Tu

Chief Advisor: Professor Glen Hintz

Signature _____

Date: _____

Associate Advisor: Professor James Perkins

Signature _____

Date: _____

Associate Advisor: Dr. David Corey

Signature _____

Date: _____

Department Chairperson: Mr. Don Arday

Signature _____

Date: _____

ABSTRACT

My thesis was a continuation of the "3D Virtual Ear Animation" project for the Imagine RIT Festival held in spring 2010. I worked with Dr. David Corey, a Howard Hughes Medical Institute (HHMI) Investigator, whose research publications I had been reading since the spring of 2010. I learned about information that explains how hearing is transformed into neural signals ultimately perceived by the brain. Motivated by the presence of hearing-impaired students on the campus, I decided to continue this animation project. Another motivation for this topic was the lack of illustrations and animations that captured the latest discoveries, particularly the essential protein molecules that are involved. I had taken advantage of the discoveries made in Dr. Corey's laboratory. I expect it will be useful for teaching and educating the public on websites devoted to hearing loss. The goal was to illustrate the new molecular understanding of how hearing works. This will help the Deaf become more aware of the causes of hearing loss, and may encourage researchers to invent new solutions to hearing loss that are better than hearing aids.

INTRODUCTION

The inspiration for my thesis originated with my participation in a "3D Virtual Ear Animation" project for the "Imagine RIT" Festival in the spring of 2010. The goal of the project was to explain how hearing works. It involved a great deal of independent project planning and research, as well as group work, and personal interaction across different disciplines. They included Computer Graphics and Animation, Biomedical Science, Chemistry, and Medical Illustration. The key component of my project was the modeling and simulation of the hair cells transducing their movement into electrical signals that stimulate the auditory nerves. After a careful study of the publications by researchers of the Howard Hughes Medical Institute and the Lawrence Berkeley National Laboratory, I discovered that no one had solved the detailed three-dimensional molecular architecture of the mechano-electrical ion channel in the hair cells of the inner ear. To solve this problem, I adopted the model proposed by Dr. Roderick MacKinnon (Lehninger, 2004) because his model of the potassium ion channel resembles most closely the physiology of this mechano-electrical transduction.

When the animation project was completed, the science of the mechano-electrical transduction still intrigued me. During the summer, I was thinking of topics for my Master's thesis. This motivated me to study the movement of hair cells and the various proteins that are involved. Inspired by the presence of hearing-impaired students on the campus, including two of my peers, I decided to continue the animation for my thesis. Motivated by the lack of detailed illustrations and animations that captured the latest discoveries, particularly the essential protein molecule involved, I proposed to create more detailed and accurate models. I expect the animation may help the Deaf become more aware of the causes of hearing loss, and may encourage researchers to invent new solutions to hearing loss.

SCIENTIFIC BACKGROUND PART I

Unlike the widely studied visual system, a detailed mechanism for hearing has remained elusive to scientists for decades. Hair cells of the inner ear convert the mechanical stimulus of a sound wave into an electrical signal that is sent to the brain. These hair epithelial cells have a bundle of hair-like protrusions emanating from the top surface of the cell, named stereocilia. Stereocilia are connected by fine filaments, called tip links. These tips are stretched every time the hair bundle is deflected by a sound vibration. The tip link is, in turn, connected directly to mechano-electrical ion channel proteins that respond to the stretch by allowing an electrical current into the cell (Schwander et al., 2010).

The identity of the mechano-electrical transduction ion channel has been elusive. About seven years ago, a member of the transient receptor potential (TRP) family of ion channels, TRPA1, was proposed as a likely candidate for the transduction channel (Corey et al., 2004). However, a study in 2006 from the same group (Kwan et al., 2006) surprisingly showed that TRPA1 contributes to cold, mechanical, and chemical nociception but is *not* essential for hair-cell transduction. Other candidates for the transduction channel, transmembrane channel-like 1 and 2 (TMC1 and TMC2), have just been published (Kawashima et al., 2011) but confirmation is still needed. Despite the failure to find the ion channel, some proteins that regulate its transduction function have been identified. Among the many are cadherin 23 (CDH23) and protocadherin 15 (PCDH15). Around the same time, more proteins in hair-cell stereocilia were identified by the study of genes that are linked to deafness, and more were quantified by the technique of mass spectrometry.

The progress in understanding hair-cell transduction has been largely driven by the discoveries of genes that are linked to Usher Syndrome (USH). The discoveries have provided insights into the

mechanisms that are important for stereocilia development and their functions as mechanosensors (Schwander et al., 2010). Furthermore, scientists now have a better understanding of how the interactions between myosin motor proteins (MYO3A, MYO6A, MYO7A, MYO15A), actin binding proteins (twinfilin, espins), and adaptor proteins (harmonin, whirlin) regulate stereocilia development and function.

In summary, our understanding of the cellular and molecular mechanism of hearing has been enhanced both by the study of genes for Usher syndrome deafness, and by the identification with mass spectrometry of about 250 different proteins in stereocilia. Among the actin-binding proteins, twinfilin 2 is the major one in contributing to the staircase architecture of stereocilia by suppressing the length of the stereocilia filamentous actin (F-actin) core of the lower and middle row. TRPA1 was ruled out as the candidate for the mechano-electrical transduction channel; although new candidates have been proposed, the identity of the transduction channel remains elusive (David Corey, personal communication, December 21, 2011).

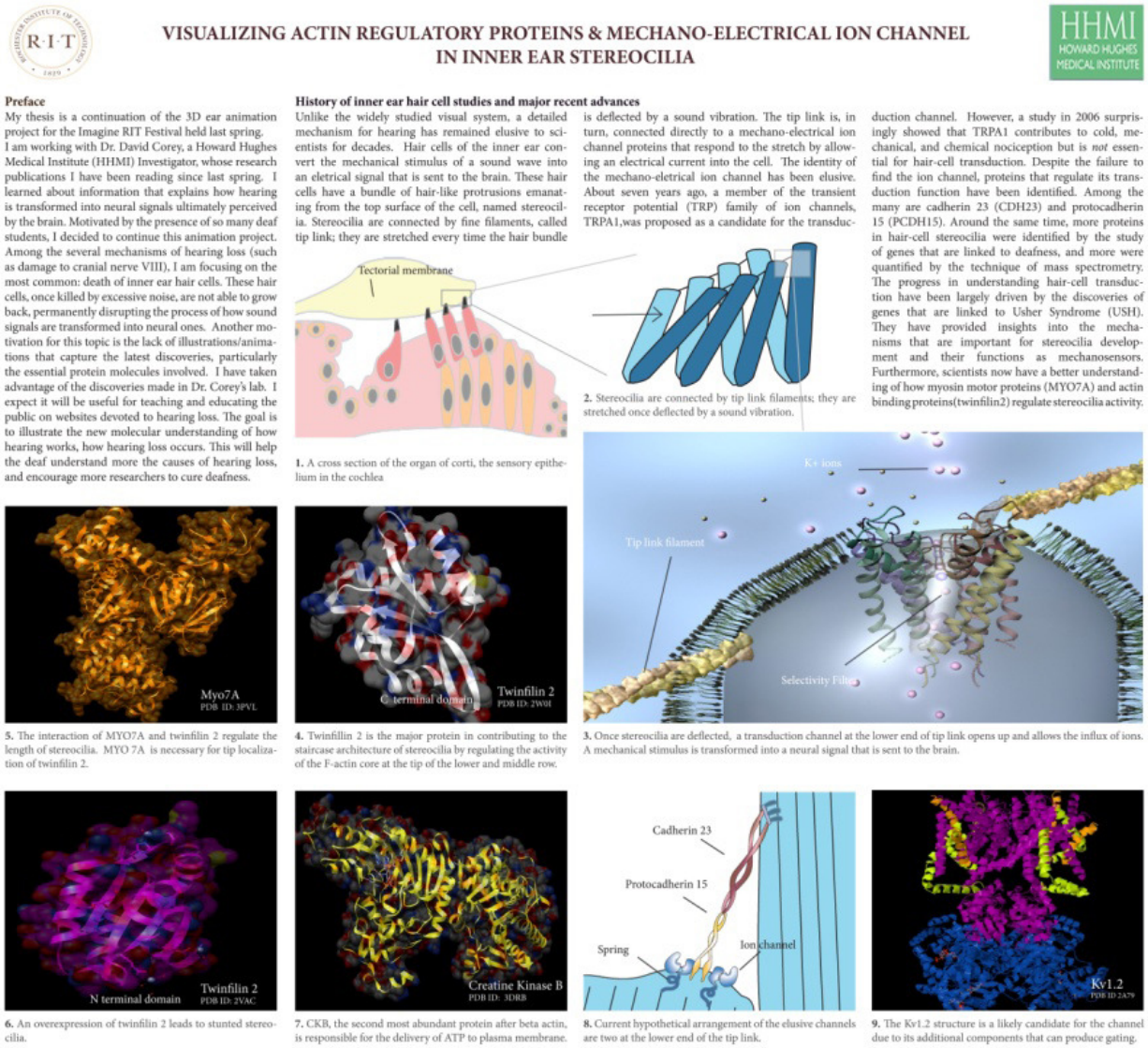


Figure 1. Final version of the poster for the thesis show in April 2011.

THE BODY OF WORK

The goal was to illustrate the new molecular understanding of how hearing works. For the sake of simplicity and conciseness, schematic versions were created first, followed by realistic and detailed versions for a more in-depth understanding. Both traditional and computer graphics mediums were used. I had displayed the traditional version as a poster at RIT's Bevier Gallery in the spring of 2010 and had put the 3D animation on my personal website at http://cias.rit.edu/~cxt6636/portfolio/moleculargraphics.html for a wider audience, particularly medical researchers and others interested in the subject. The 3D animation was made from Maya and Flash software. Maya expedited the modeling process and ultimately yielded a much better 3D representation. Flash enabled the animation on the website to process with greater ease and quickness. Also, besides the Protein Data Bank, I had used molecular visualization software, such as UCSF Chimera (Pettersen et al., 2004) and Visual Molecular Dynamics (VMD) (Humphrey et al., 1996).

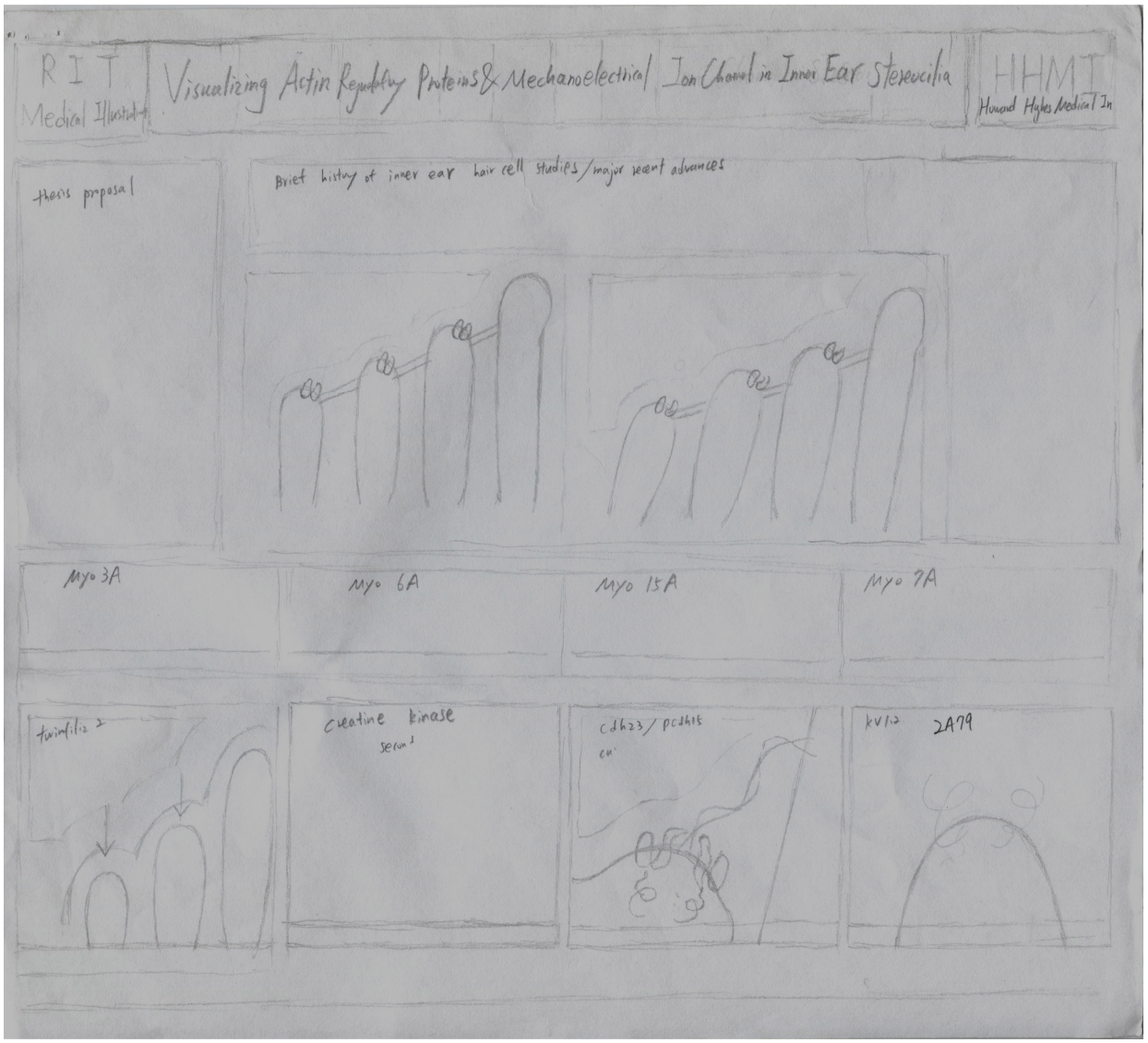


Figure 2. Early layout of the poster (scanned version).

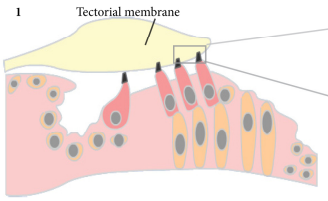
VISUALIZING ACTIN REGULATORY PROTEINS & MECHANO-ELECTRICAL ION CHANNEL IN INNER EAR STEREOCILIA

Preface

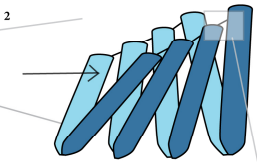
My thesis is a continuation of the 3D ear animation project for the Imagine RIT Festival held last spring. I am working with Dr. David Corey, a Howard Hughes Medical Institute (HHMI) Investigator, whose research publications I have been reading since last spring. I have conducted science background research, particularly finding information that explains how hearing is transformed into neural signals ultimately perceived by the brain. Motivated by the presence of so many deaf students at RIT, I decided to continue this animation project. Among the several mechanisms of hearing loss (such as damage to cranial nerve VIII), I am focusing on the most common: death of inner ear hair cells. These hair cells, once killed by excessive noise, are not able to grow back, permanently disrupting the process of how sound signals are transformed into neural ones. Another motivation for this topic is the lack of illustrations and animations that capture the latest discoveries, particularly the essential protein molecules involved. I have taken advantage of the discoveries made in his lab. I expect it will be useful for teaching and educating the public on websites devoted to hearing loss. Furthermore, the goal is to illustrate the new molecular understanding of how hearing works and how hearing loss occurs. This will help the deaf understand

History of inner ear hair cell studies and major recent advances

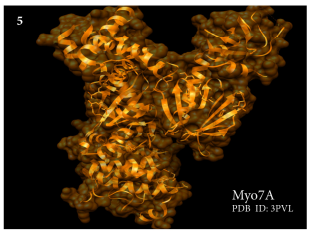
Unlike the widely studied visual system, a detailed mechanism of hearing remains elusive to scientists for decades. Hair cells of the inner ear convert the mechanical stimulus of a sound wave into an eletrical one that is sent to the brain. These hair cells have a bundle of hair-like protrusions emanating from the top surface of the cell, named stereocilia. Stereocilia are connected by fine filaments, called tip link; they are stretched every time the hair bundle is deflected by a sound vibration. And, tip link is, in turn, connected directly to a mechano-electrical ion channel proteins that respond to the stretch by producing an electrical current across the membrane. The identity of the mechano-eletrical ion channel has been elusive. About five years ago, a member of the transient receptor potential (TRP) family of ion channels, TRPA1, was proposed as a candidate for the transduction channel. In support of this result, a study showed that "knockdown of TRPA1 expression in embryonic hair cells using two different small interfering RNAs(siRNAs) largely abolished hair-currents"(Kwan,2006). However, a study in 2006 surprisingly showed that TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Despite the failure of finding the ion channel, proteins that regulate its transduction function have been identified. Among the many are cadherin 23 (CDH23) and protocadherin 15 (PCDH15). Around the same time, more proteins in hair-cell stereocilia were identified by the study of genes that are linked to deafness, and more were quantified by the technique of mass spectrometry.



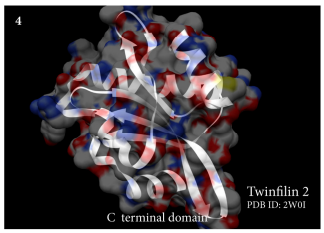
A cross section of the organ of corti, the sensory epithelium in the cochlea



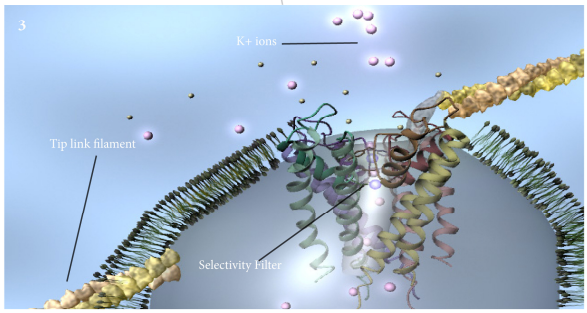
Stereocilia are connected by fine filaments, called tip link; they are stretched once the hair bundle is deflected by a sound vibration.



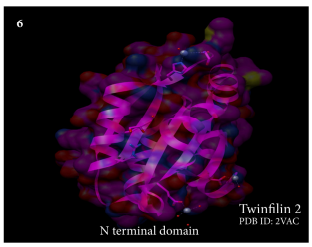
The interaction of MYO7A and twinfilin 2 regulate the length of stereocilia. MYO 7A is necessary for tip localization of twinfilin 2.



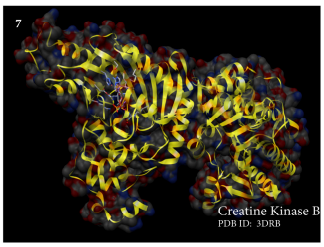
Twinfillin 2 is the major protein in contributing to the staircase architecture of stereocilia by regulating the activity of the F-actin core at the tip of the lower and middle row.



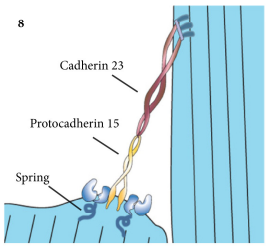
Once stereocilia are deflected, a transduction channel at the lower end of tip link opens up and allows the influx of ions. A mechanical stimulus is transformed into a neural one.



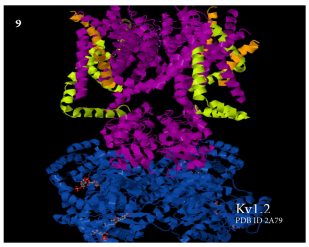
An overexpression of twinfilin 2 leads to stunted stereocilia.



CKB, the second most abundant protein after beta actin, is responsible for the delivery of ATP to plasma membrane PMCA2.



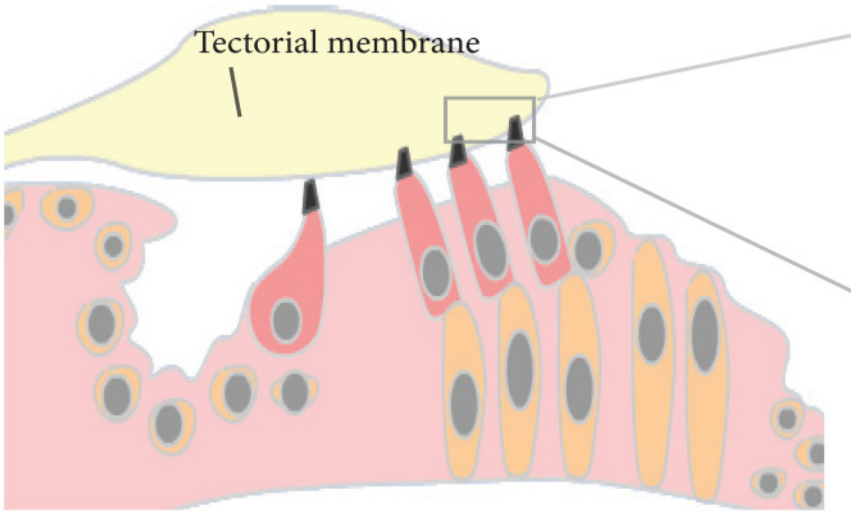
Current hypothetical arrangement of the elusive channels are two at the lower end of the tip link.



The Kv1.2 structure is a likely candidate for the channel due to its additional components that can produce gating.

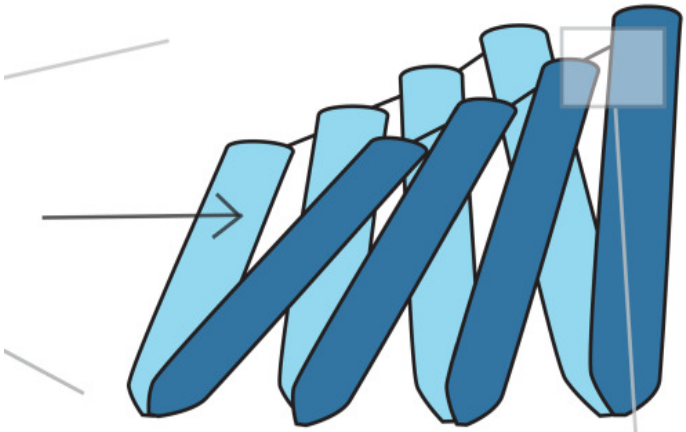
Acknowledgment: Glen Hintz, James Perkins, David Corey.

Figure 3. Late stage (see the differences in text and label format from the final version).



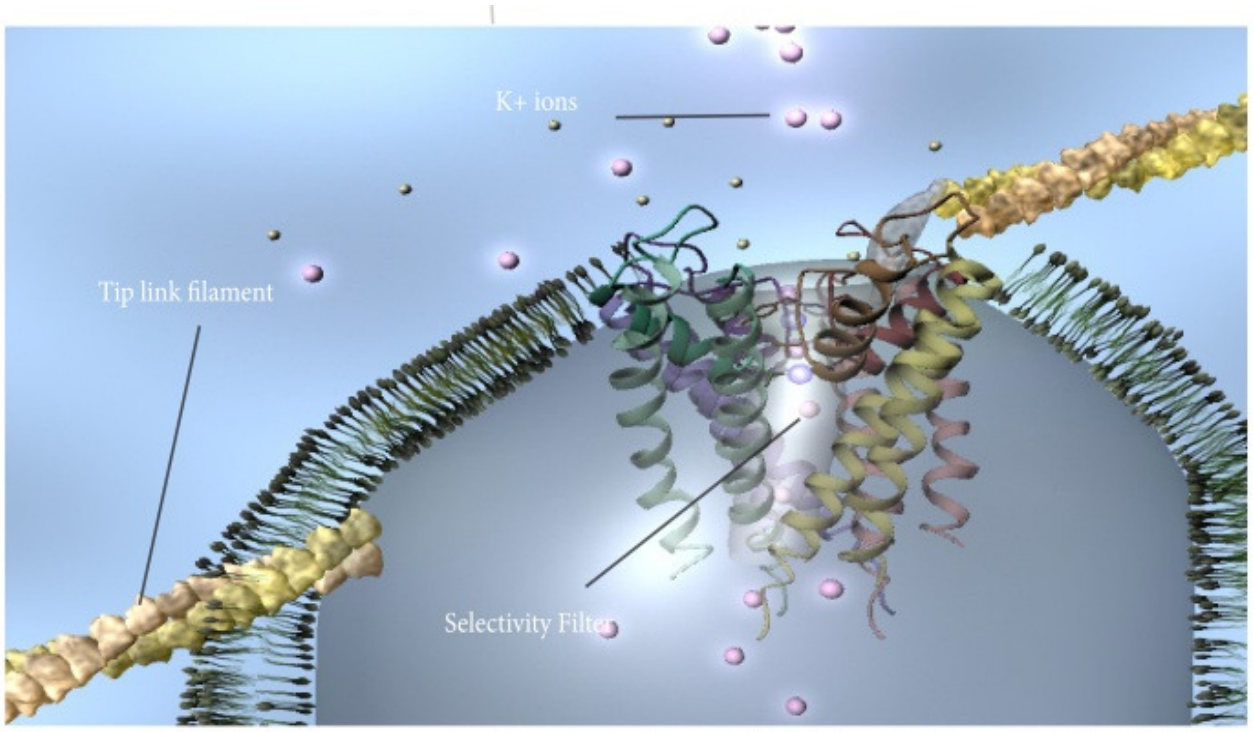
1. A cross section of the organ of corti, the sensory epithelium in the cochlea

Figure 4. For the cross section of the organ of Corti, Adobe Illustrator® was used to achieve a schematic and simplistic style (Schwander et al., 2010).



2. Stereocilia are connected by tip link filaments; they are stretched once deflected by a sound vibration.

Figure 5. To maintain consistent style, Adobe Illustrator® was used to create a schematic version of stereocilia (Corey, 2009).



3. Once stereocilia are deflected, a transduction channel at the lower end of tip link opens up and allows the influx of ions. A mechanical stimulus is transformed into a neural signal that is sent to the brain.

Figure 6. This is a render view of my ion channel animation in Maya.

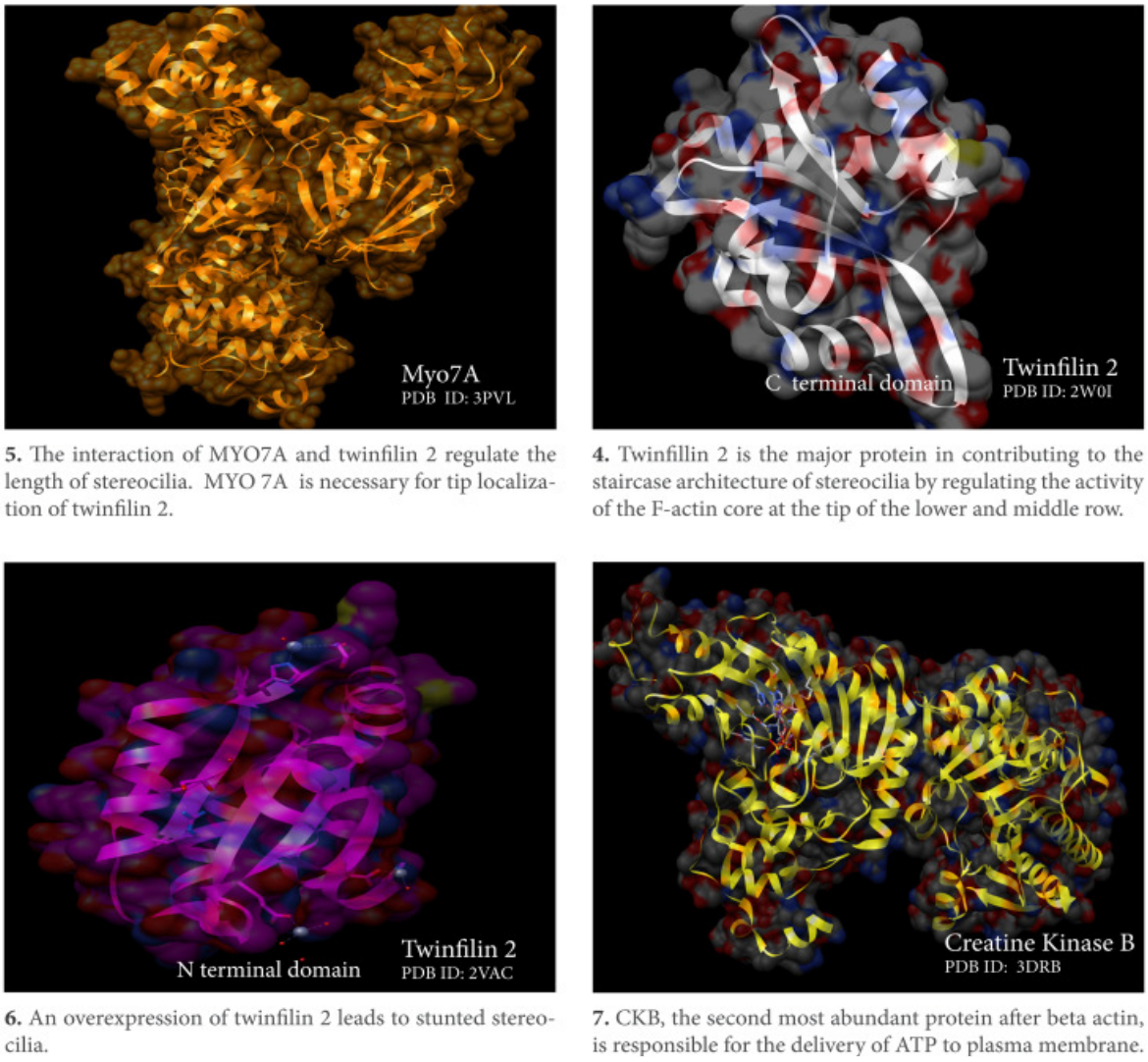
I made this animation based on the tutorials on the website of Molecular Movies. The molecular ribbon model was imported to the UCSF Chimera from the Protein Data Bank (www.rcsb.org). Each of the alpha helices was colored differently, distinguished from one another, then saved as a VRML file and opened in Cinema 4D, and finally in Maya for more detailed rendering and animation. As for the tip link filament that is made of cadherins, for its illustration I made it from individual actin monomers using "Duplicate Special" in Maya to make several copies and "Rotation Tool" to have them twisted. For the phospholipid bilayer I took advantage of the tutorial on the website of Molecular Movies (www.molecularmovies.com). For the individual ions, I used key frames in Maya to control the movement of each individual ion as it moves through the selectivity filter.

SCIENTIFIC BACKGROUND PART II

The progress in understanding hair-cell transduction has been largely driven by the discoveries of genes that are linked to Usher Syndrome (USH). The discoveries have provided insights into the mechanisms that are important for stereocilia development and their functions as mechanosensors (Schwander et al., 2010). Furthermore, scientists now have a better understanding of how the interactions between myosin motor proteins (MYO3A, MYO6A, MYO7A, MYO15A), actin binding proteins (twinfilin, espins), and adaptor proteins (harmonin, whirlin) regulate stereocilia development and function.

Myosin7a and twinfilin seem to interact, since a myosin7a deficiency results in a loss of twinfilin-2 at the tips of stereocilia. Actin treadmilling (see Notes) increases when myosin7a is absent. These not only suggest that myosin7a is involved in F-actin rearward flow (see Notes), but more important is that the interaction of myosin7a and twinfilin regulates stereocilia length. As a recent study shows, myosin7a is necessary for tip localization of twinfilin 2 (Peng et al., 2009). Moreover, the relative expression levels of twinfilin-2, whirlin, and espin-1 play an important role in organizing stereocilia in a staircase manner. Twinfilin-2 is expressed more strongly in the shortest stereocilia while espin-1 and whirlin are more abundant in the longest.

Peter Gillespie and his colleagues studied hearing loss in knockout mice of the cytosolic brain isoform of creatine kinase (creatine kinase B), which is the next most abundant bundle protein after beta actin (Shin et al., 2007). The result showed that “mice lacking brain creatine kinase have reduced hearing sensitivity" and that "the creatine kinase circuit is essential for high-sensitivity hearing"(Shin et al., 2007).



5. The interaction of MYO7A and twinfilin 2 regulate the length of stereocilia. MYO 7A is necessary for tip localization of twinfilin 2.

4. Twinfillin 2 is the major protein in contributing to the staircase architecture of stereocilia by regulating the activity of the F-actin core at the tip of the lower and middle row.

6. An overexpression of twinfilin 2 leads to stunted stereocilia.

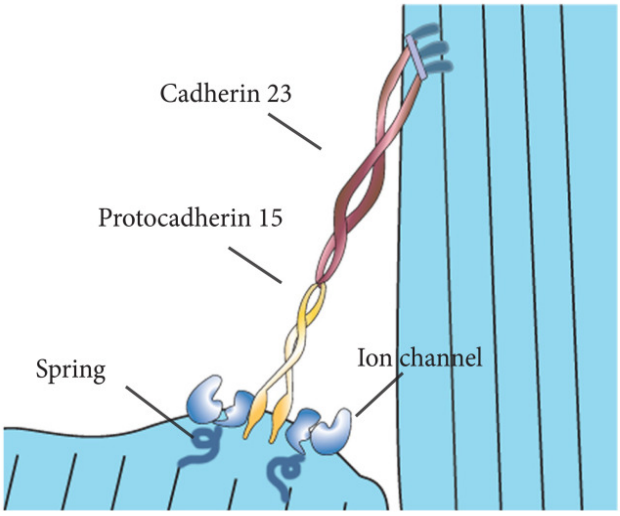
7. CKB, the second most abundant protein after beta actin, is responsible for the delivery of ATP to plasma membrane.

Figure 7. Myo7a, Twinfilin 2, and Creatine Kinase B.

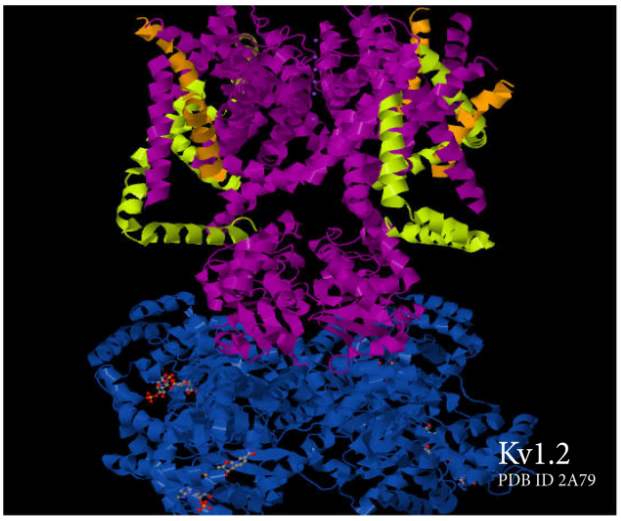
No one has solved the structures for Myosin 3A, 6A, and 15A (David Corey, personal communication, March 20, 2011), so they were not seen here. For these myosin motor proteins above, I used the UCSF Chimera to do the rendering, adding surface to show the general shape of the protein molecules. Each panel included both a ribbon diagram of the protein and a molecular surface. I used "Fetch by ID" command to retrieve the protein from the Protein Data Bank, selected the protein and viewed it as a ribbon model, and finally added a surface to the protein. I also adjusted the transparency of the surface to let the ribbon model show through, and assigned different colors to the ribbon model and its surface.

SCIENTIFIC BACKGROUND PART III

The identity of the mechano-electrical ion channel remains elusive because not only is it difficult to find, but also currently "there is not an antibody or a drug that can bind tightly to it, which could be used to purify it" (David Corey, personal communication, December 15, 2010). As far as the study of the transduction channel goes, the most up-to-date hypothetical arrangement of the transduction channel is two located at the base of each tip link (Corey, 2009). To model the transduction channel, I used the atomic structure of a simple bacterial potassium channel called Kcsa. However, this probably underestimates the size of the channel. "The Kcsa channel in 1BL8 is probably smaller than the hair-cell channel. You could use the Kv1.2 structure instead, which has the additional components that produce gating" (David Corey, personal communication, December 15, 2010).



8. Current hypothetical arrangement of the elusive channels are two at the lower end of the tip link.



9. The Kv1.2 structure is a likely candidate for the channel due to its additional components that can produce gating.

Figure 8. Current hypothetical arrangement (Corey, 2009) and an alternative model of the channel.

For current hypothetical arrangement of the channel, I used Adobe Illustrator® again to maintain a consistent style. I considered using Kv1.2 for the animation, but Dr. Corey commented that "the extra domains involved in its voltage-dependent gating would obscure the ion permeation" (David Corey, personal communication, December 20, 2011). I rendered it on the PDB website to distinguish it from other protein molecules because "it is only about the same size as candidate channels, but is not a candidate itself" (David Corey, personal communication, December 20, 2011).

SCIENTIFIC BACKGROUND PART IV

Many proteins had to be left out of the visualization, either because their position is not known or because there are just too many to show. "There are about 250 different proteins in a stereocilium. Actin is 40% of the total. There are no organelles in stereocilia with membrane--no vesicles, no endoplasmic reticulum, etc. There are no nerves inside a stereocilium. 97% of the electric current entering the tip makes it to the cell body within 6 microseconds" (David Corey, personal communication, January 27, 2011).

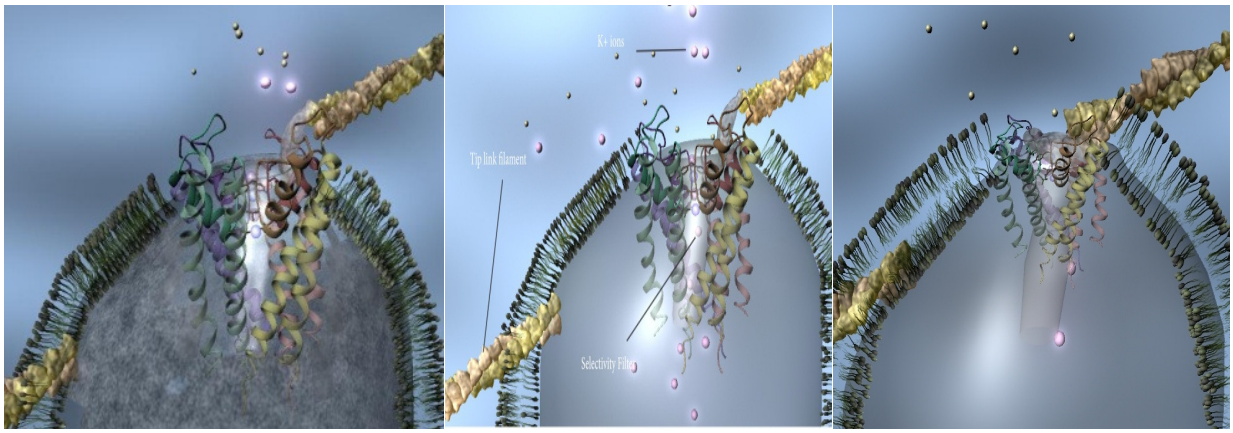
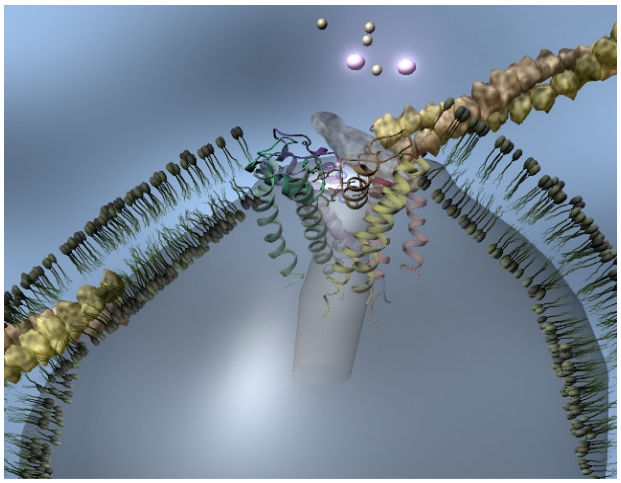


Figure 9. Different stages as of 05/10, 04/11, and 10/11(from left to right).

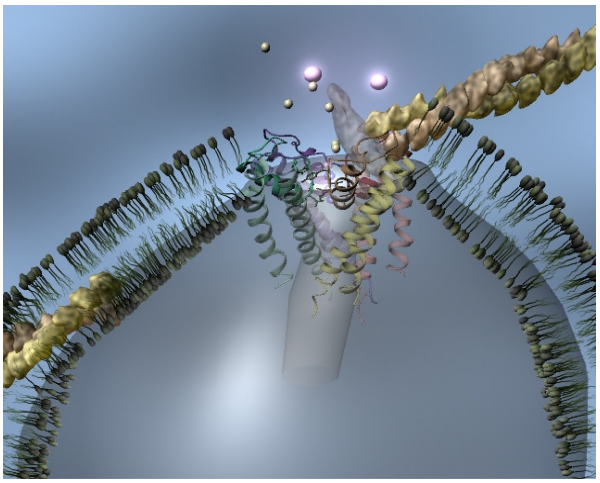
For the version of 05/10, I tried to include actin and other proteins inside the stereocilium, but after discussing with my advisors I decided to only focus on the transduction process here. Thus, for the version of 04/11, I cleaned up and added some light reflection to represent electric current passing down into the cell body. In addition, Dr. Corey commented that "[t]he channel is about five times bigger than it should be, relative to the lipid. You can use an alpha helix in the structure as a scale bar, the pitch is always 0.54 nanometer, and the thickness of a lipid membrane is 4-5 nm." For the version of 10/11, I made the adjustment by reducing the size of the ribbon model.

SCIENTIFIC BACKGROUND PART V

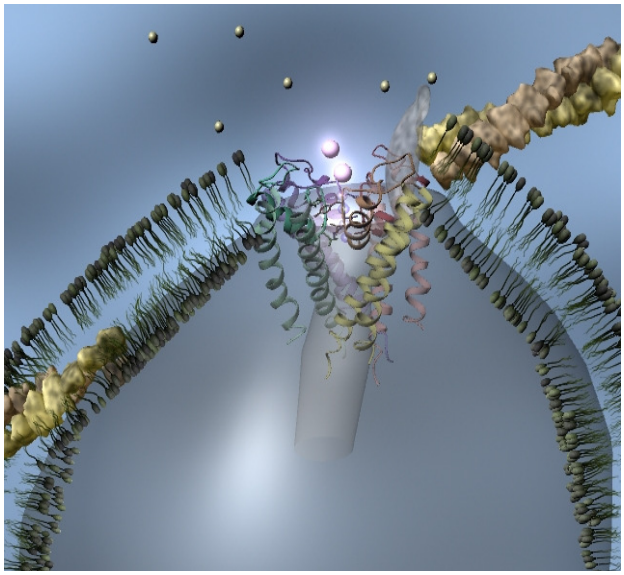
Cadherin 23 (CDH23) and protocadherin 15 (PCDH15) are two proteins that regulate the function of the transduction channel; they are the major components of the tip link filament (Pickles et al, 1984). In support of this model, "the hair bundle loses its mechanical sensitivity when tip links are broken"(Elledge et al., 2010). Sotomayor and his colleagues (2010) discovered with molecular dynamics simulations that CDH23 repeats appear stiff and that Ca^{2+} seems to determine the mechanical strength of these cadherins. A later study by another research lab mentioned that "these Ca^{2+} binding motifs are conserved in CDH23 and PCDH15, suggesting that tip links are rigid and not the elastic gating spring for the transducer channel" (Elledge et al., 2010). In agreement with this study, "Tip links appear in the electron microscope as stiff filaments that buckle under strain"(Elledge et al., 2010).



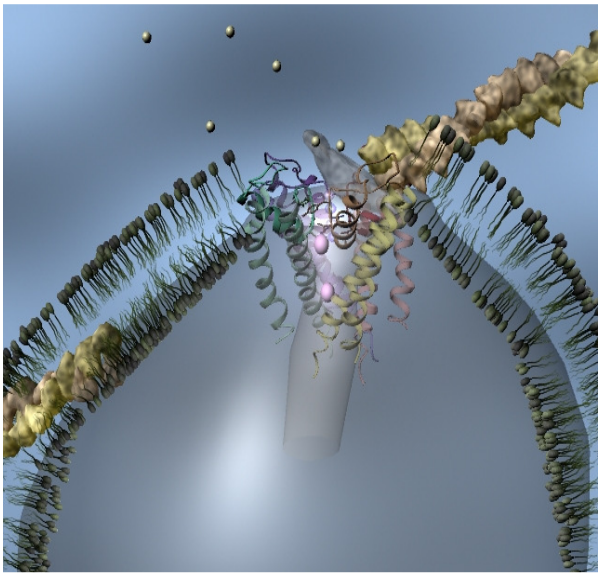
10a



10b



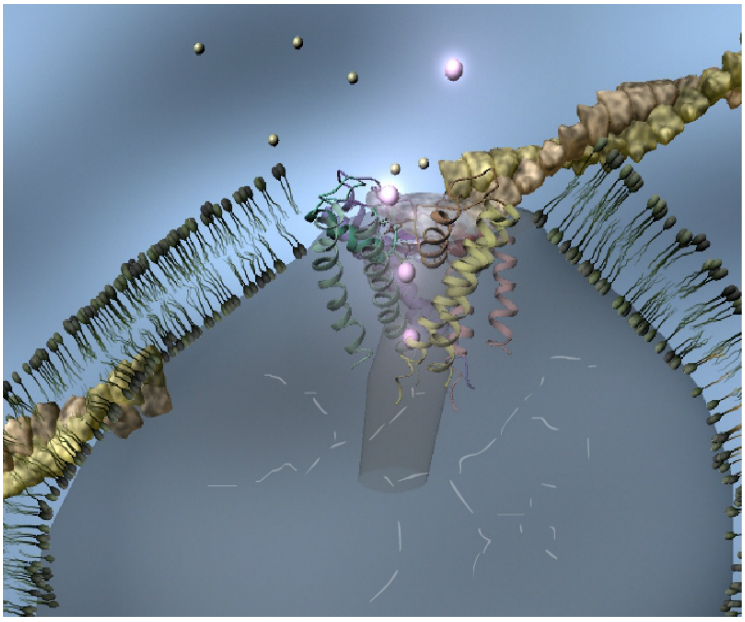
10c



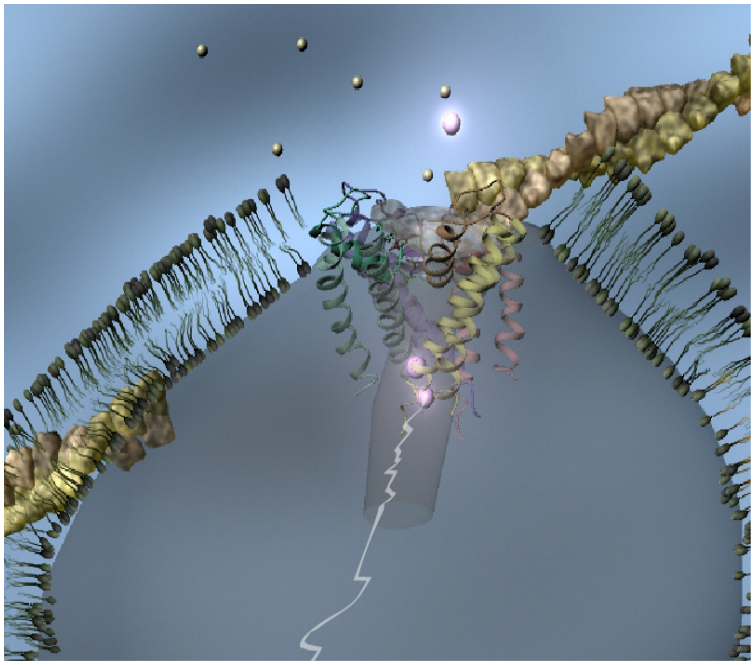
10d

Figure 10. The version (10/11): the animation of mechano-electrical transduction.

For the modified animation, I not only reduced the scale of the channel but also showed the randomness of the channel opening and closing. Since the tip links are rigid, I decided to use the "Rotation tool" in Maya to make them rotate as the animation proceeded.



11a



11b

Figure 11. Screen capture of the modified version in 02/12 (membrane re-positioned, light reflection eliminated, electric current added).

EARLY STAGE OF THESIS WORK

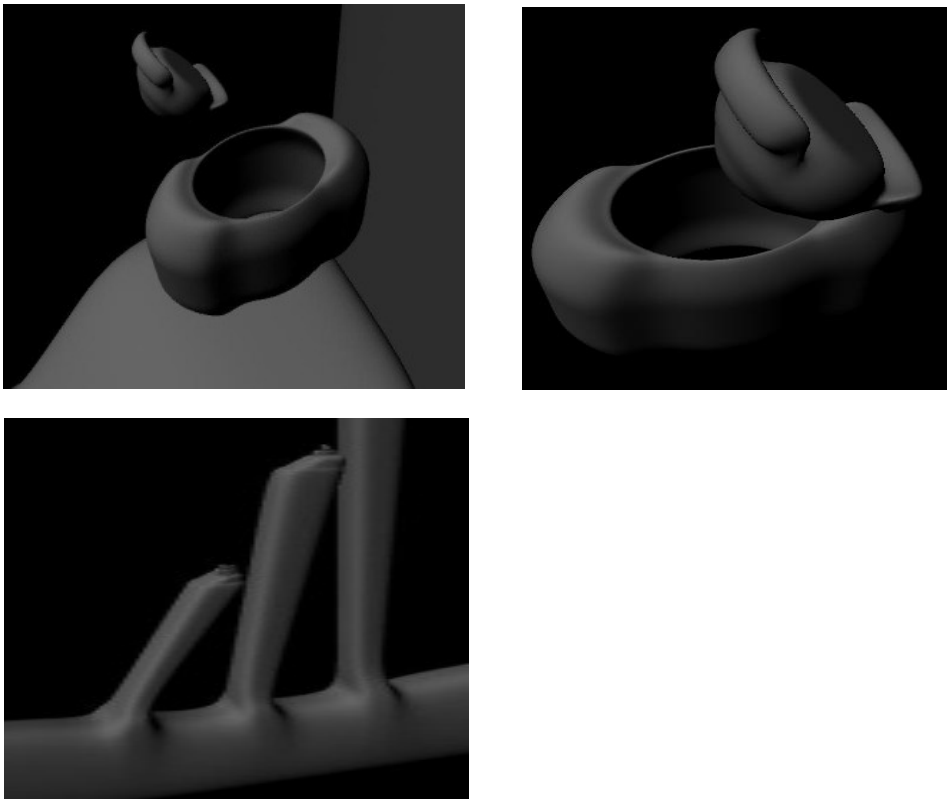


Figure 11. Hair cell and its cap in Maya as of 03/10

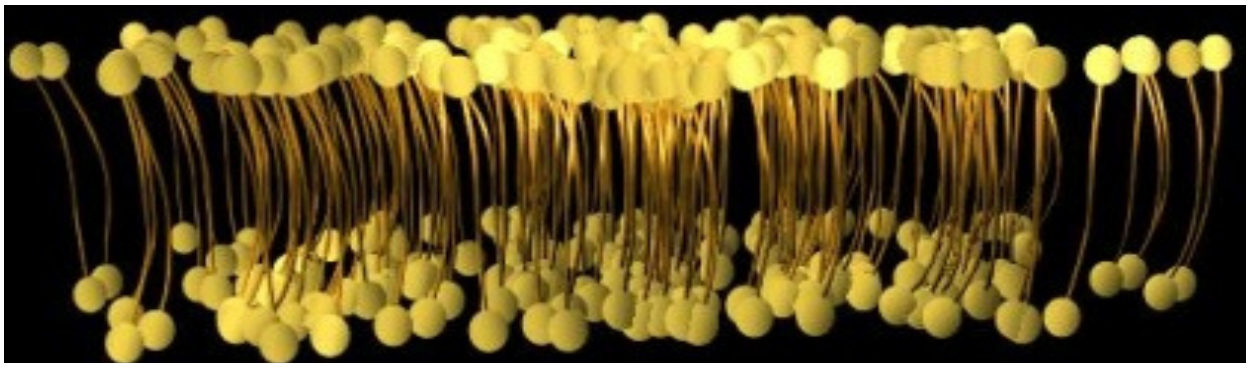


Figure 12. Lipid membrane in Maya from the tutorials on the Molecular Movies website in 4/10

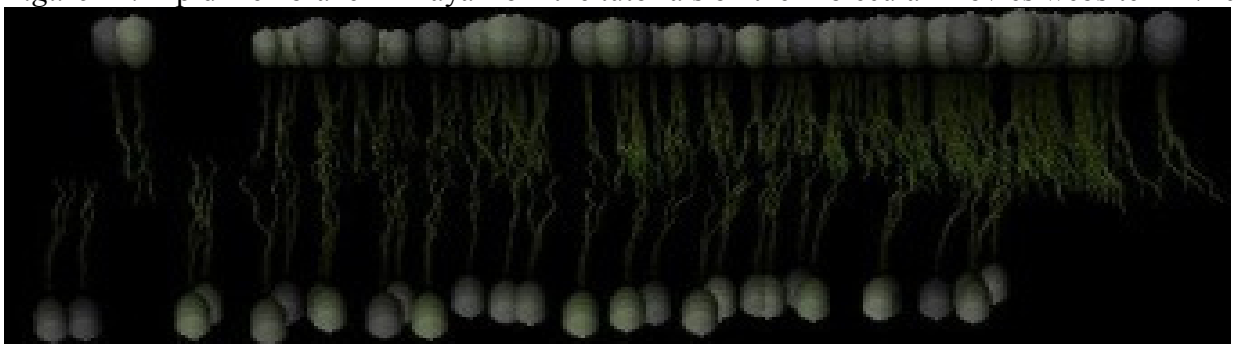


Figure 13. Lipid membrane made in Maya from the same tutorial and same website in 4/10

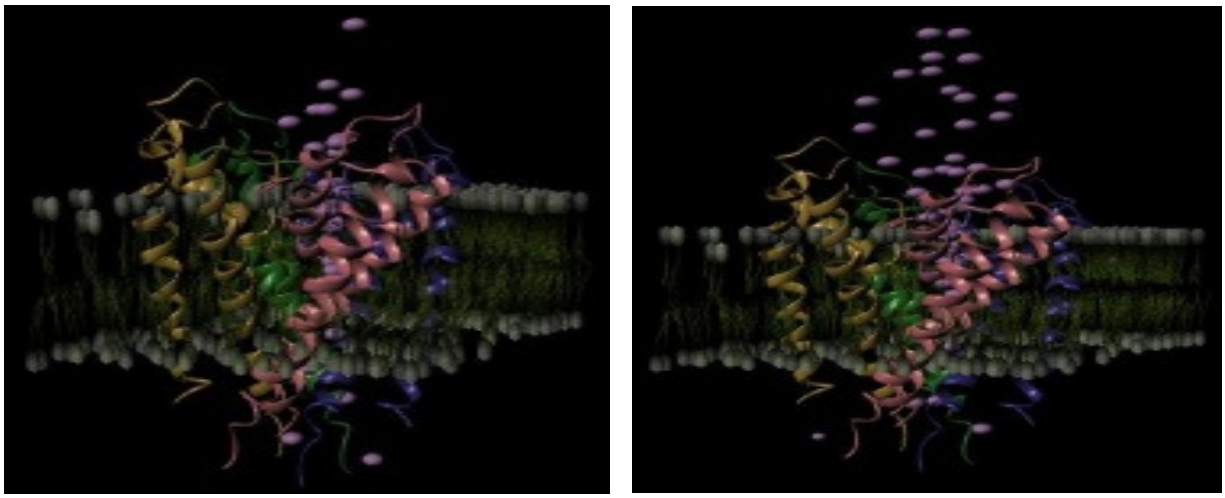


Figure 14. Early stage of the channel and lipid bilayer in 04/10

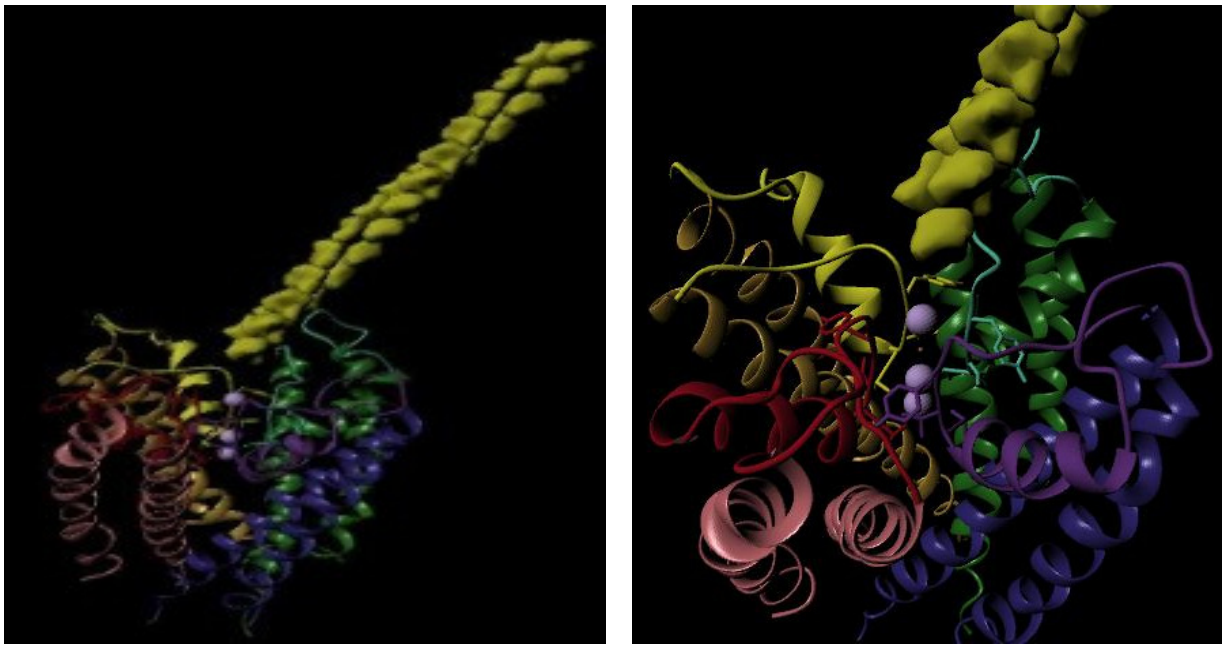


Figure 15. Early stage of the channel and the tip link

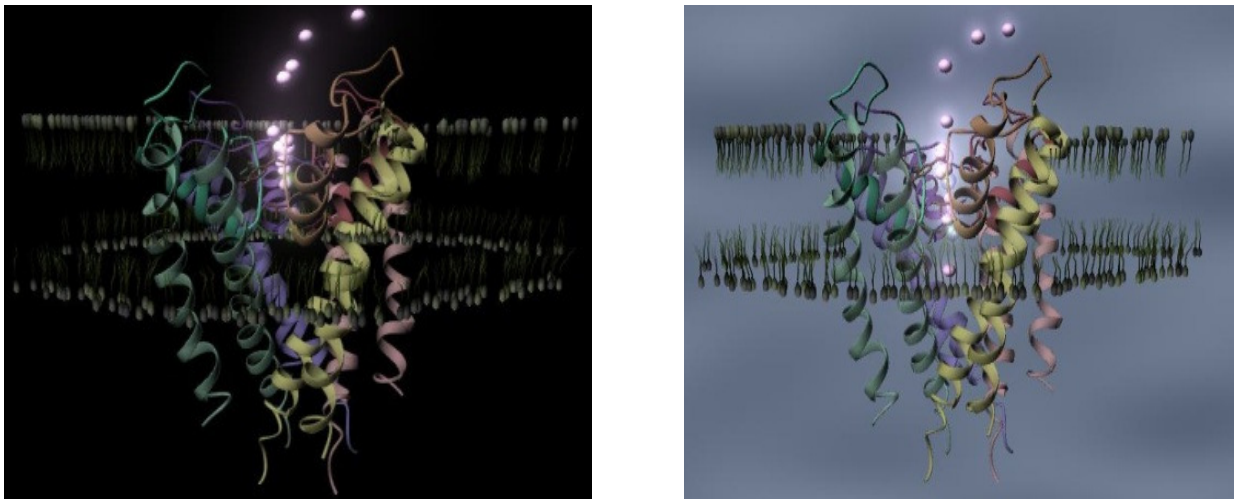


Figure 16. Use of "dynamic particles" in Maya for the movement of ions (change of background).

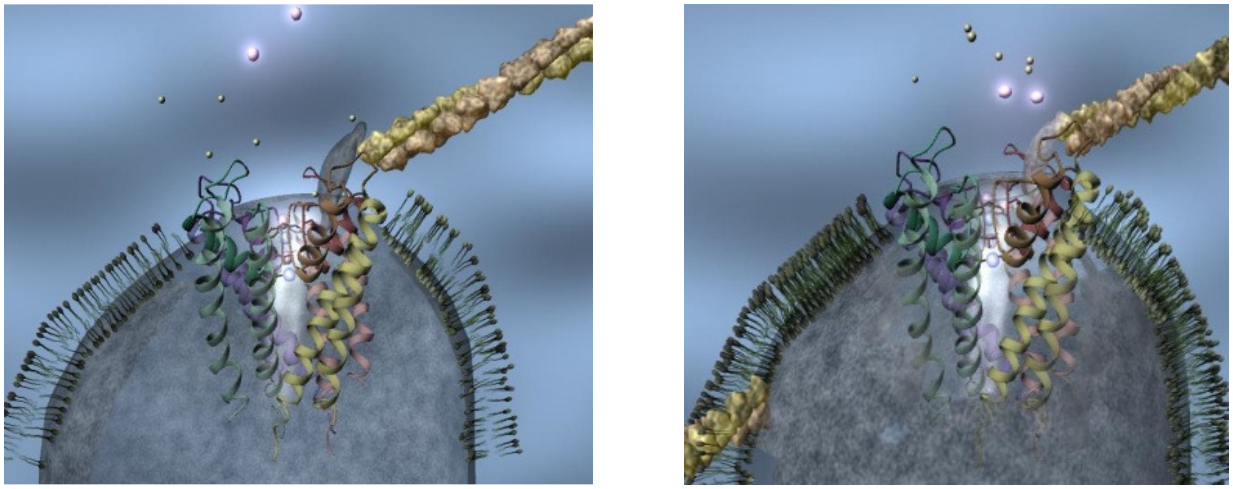


Figure 17. Late stage of the transduction channel with its cap, the tip link filaments, and lipids

CONCLUSION

I presented my thesis at RIT's Bevier Gallery in April of 2011. After the thesis presentation, I received a message from a student who had hearing difficulty commenting that "[my] work is cool!" After the presentation, I visited Dr. Corey in person for a more direct learning interaction and thanked him for his time and expertise. For the improvement of my thesis poster, he pointed out that I should be careful to represent different molecules at the same scale. I was impressed by how serious Postdoctoral fellows in his laboratory were about their research and how passionate two of them were to discuss and share ideas with me to make my animation better. Therefore, I had achieved both of my goals: helping the public become more aware of the causes of hearing loss and encouraging researchers to invent new solutions to cure deafness.

Reflecting back on the day of the "Imagine RIT" Festival last year, I remember that while a boy marveled at our animation his mother told me that the boy would like to become a scientist in the future. Now I have solidified my goal of becoming a biomedical and molecular illustrator/animator, using computer simulations to help the public and potential future scientists understand the intricacy of molecular and cellular dynamics.

FUTURE DIRECTIONS

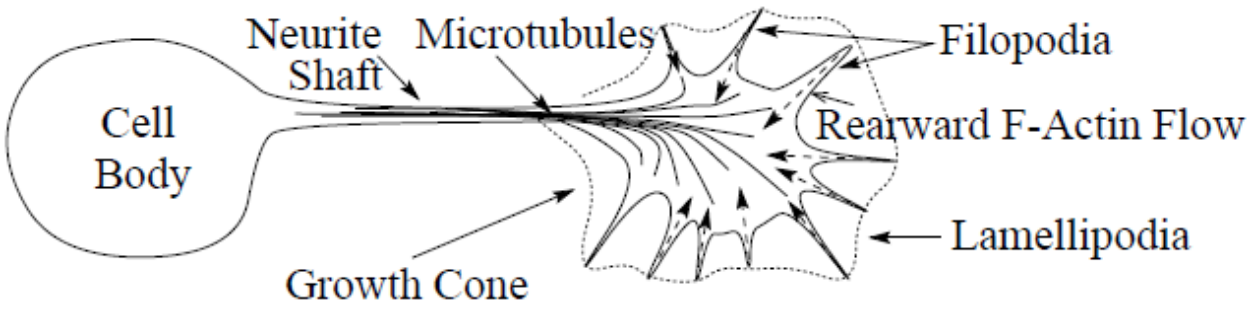
Because of my Master's thesis, I have become passionate about the study of the molecular and genetic basis of deafness. Research questions that I am interested in finding answers to include: the functions of hundreds of proteins in a stereocilium; the structures for the Myosin motor proteins, which with actin binding proteins and adaptor proteins regulate the staircase architecture of stereocilia; the gating of potassium channels, which have puzzled scientists for three decades or more; and the discovery of antibodies or drugs that could purify the channel.

I feel that I have become so immersed in creating the molecular models of stereocilia and ion channels that I want to pursue an advanced education to answer questions about proteins of stereocilia and to continue the quest for the identity of the elusive mechano-electrical ion channel. I am aspiring to work at an international publishing company and becoming a biomedical and molecular illustrator/animator. I hope to make the animation as close to reality as possible in the not-so-distant future, as well as become a researcher to explore the causes of hearing loss and deafness.

NOTES

Treadmilling is a phenomenon observed in many cellular cytoskeletal filaments, especially in actin filaments and microtubules. It occurs when one end of a filament grows in length while the other end shrinks resulting in a section of filament seemingly "moving" across a stratum or the cytosol. This is due to the constant removal of the protein subunits from these filaments at one end of the filament while protein subunits are constantly added at the other end (Alberts et al., 2002).

F-actin rearward flow: "Actin exists in two forms in the cell – as a monomer called globular actin (G-actin) and as a filamentous polymer called filamentous actin (F-actin). F-actin is a polar polymer of G-actin subunits held together. All of the subunits within an actin filament have the same polarity - they are all oriented in the same direction. Thus the two ends of a filament are different. One end of the filament is the pointed end (- end) and the other the barbed end (+ end). One important consequence of filament polarity is that the two ends of the filament have different functional properties" (Drubin, 2009). The figure below (Hely et al., 1998) shows an example of F-actin rearward flow in developing neurons with filopodia extending beyond the leading edge of lamellipodia. "The peripheral domain has few microtubules present and a high concentration of the F-actin polymer. Actin normally polymerizes at the leading edge of the growth cone, and then flows rearward towards the central zone at a rate of 3–6 m per minute"(Hely et al., 1998).



REFERENCES

1. Alberts, B., Bray, D., Lewis, J., *Molecular Biology of the Cell*, 4th Edition, Taylor & Francis, 2002

2. Corey, D.P. (2009). Cell biology of mechanotransduction in inner-ear hair cells. *Biology Reports*, F1000 Biol Reports 2009, 1:58 (DOI: 10.3410/B1-58)

3. Corey, D.P., García-Añoveros, J., Holt, J.R., Kwan, K.Y., Lin, S.Y., Vollrath, M.A., Amalfitano, A., Cheung, E.L., Derfler, B.H., Duggan, A., Géléoc, G.S., Gray, P.A., Hoffman, M.P., Rehm, H.L., Tamasauskas, D., Zhang, D.S. (2004) TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. *Nature* 432:723-30.

4. Drubin, David. Class Lecture. MCB130 Cell Biology. University of California, Berkeley, CA. March 2009.

5. Elledge, H.M., Kazmierczak P., Clark P., Joseph J.S., Kolatkar A., Kuhn P., & Müller U. (2010) Structure of the N terminus of cadherin 23 reveals a new adhesion mechanism for a subset of cadherin superfamily members. *Proceedings of the National Academy of Sciences of the United States of America.* 107:10708-12.

6. Hely, T.A. van Ooyen, A. Willshaw, D.J. (1998) A simulation of growth cone filopodia dynamics based on Turing morphogenesis patterns. *Proceeding IPCAT '97 Proceedings of the second international workshop on Information processing in cell and tissues* Plenum Press New York, NY, USA

7. Howard Hughes Medical Institute, Tip Links Pull Up the Gates of Ion Channels http://www.hhmi.org/senses/c130.html

8. Humphrey, W., Dalke, A. & Schulten, K., (1996). VMD - Visual Molecular Dynamics. *Journal of Molecular Graphics* 14:33-38.

9. Kawashima, Y., Géléoc, G.S., Kurima, K., Labay, V., Lelli, A., Asai, Y., Makishima, T., Wu, D.K., Della Santina, C.C., Holt, J.R., Griffith, A.J. (2011) Mechanotransduction in mouse inner ear hair cells requires transmembrane channel-like genes. *The Journal of Clinical Investigation*. 121:4796-809

10. Kwan, K. & Corey, D.P. (2006) TRPA1 Contributes to Cold, Mechanical, and Chemical Nociception but Is *Not* Essential for Hair-Cell Transduction. *Neuron,* 50, 277-289, April 20, 2006 DOI 10.1016/j.neuron.2006.03.042

11. *Lehninger Principles of Biochemistry* (4th edition.). W.H.Freeman Company. New York, New York (April 23, 2004)

12. Peng, A. & Heller, S. (2009). Twinfilin 2 Regulates Actin Filament Lengths in Cochlear Stereocilia. *The Journal of Neuroscience*, December 2, 2009, 29(48):15083-15088; doi:10.1523/JNEUROSCI.2782-09.2009

13. Pettersen E.F., Goddard T.D., Huang C.C., Couch G.S., Greenblatt D.M., Meng E.C., & Ferrin T.E. (2004). UCSF Chimera--a visualization system for exploratory research and analysis. *Journal of Computational Chemistry* 25(13):1605-12.

14. Pickles, J.O., Comis, S.D., & Osbome, M.P. 1984. Cross-links between stereocilia in the guinea pig organ of Corti, and their possible relation to sensory transduction. Hear. Res. 15:103-112. DOI:10.1016/0378-5955(84)90041-8

15. Protein Data Bank http://www.rcsb.org/pdb/home/home.do

16. Salles, F.T., Merritt, R.C. Jr., Manor, U., Dougherty, G.W., Sousa, A.D., Moore, J.E., Yengo, C.M., Dosé A.C., & Kachar, B. Myosin IIIa boosts elongation of stereocilia by transporting espin 1 to the plus ends of actin filaments. *Nat Cell Biol.* 2009 Apr; 11(4):443-50.

17. Schwander, M., Kachar, B., & Müller, U. (2010). The cell biology of hearing. *The Journal of Cell Biology*, *190* (1), 9-20.

18. Shin, J.B., Streijger F., Beynon A., Peters T., Gadzala L., McMillen D., Bystrom C., Van der Zee C.E., Wallimann T., Gillespie P.G. (2007) Hair bundles are specialized for ATP delivery via creatine kinase. *Neuron.* 53:371-86.

19. Sotomayor, M. & Corey, D.P. (2010) Structural Determinants of Cadherin-23 Function in Hearing and Deafness. *Neuron.* DOI 10.1016/j.neuron.2010.03.028

20. Understanding Hearing, Molecule by Molecule http://www.lbl.gov/publicinfo/newscenter/features/2008/LS-ear.html