Structure for a Physiologic Model: Electron Tomography of the Outer Hair Cell Lateral Wall

William J. Triffo (1,3), Kent L. McDonald (2), Manfred Auer (1), and Robert M. Raphael (3)

(1) Lawrence Berkeley National Laboratory and (2) University of California at Berkeley, Berkeley, CA, (3) Rice University, Houston, TX
Path to the Cochlea

Geisler (1998)
Cochlear Structure and Function

Geisler (1998)
Cochlear Structure and Function

Guyton & Hall (2000)
Organ of Corti

Gartner & Hyatt (2000)
SEM of Corti/OHCs
Cochlear Hair Cells

- Specialized, mechanosensitive epithelial cells
- Two populations: inner hair cells (IHCs) and outer hair cells (OHCs)
- General operation: deflection of hair bundle opens mechanically gated channels, modulating the intracellular potential
The Outer Hair Cell

Deflection towards tallest stereocilia: increase conductance, depolarize cell, cell shortens

Deflection away from tallest stereocilia: decrease conductance, hyperpolarize cell, cell lengthens
Jonathan Ashmore: http://www.physiol.ucl.ac.uk/ashmore/
Electrophysiologic model using Nernst-Planck model of charge transport

\[ \frac{dC_i}{dt} = -\nabla \cdot J_i, \text{ where} \]

\[ J_i = -D_i \nabla C_i - \frac{D_i z_i F}{RT} C_i \nabla V \]

<table>
<thead>
<tr>
<th>(g = S/m²)</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Na⁺</th>
<th>K⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>( g_{bundle _apical} )</td>
<td>6.875²,³</td>
<td>6.875²,³</td>
<td>20.088</td>
<td>22.320</td>
</tr>
<tr>
<td>( g_{bundle _lateral} )</td>
<td>0.0</td>
<td>0.0</td>
<td>0.5¹</td>
<td>0.5¹</td>
</tr>
<tr>
<td>( g_{lateral _wall} )</td>
<td>3.5⁴</td>
<td>3.5⁴</td>
<td>0.005¹</td>
<td>0.005¹</td>
</tr>
<tr>
<td>( g_{SSC} )</td>
<td>0.0</td>
<td>0.0</td>
<td>0.5¹</td>
<td>0.5¹</td>
</tr>
<tr>
<td>( g_{basal _pole} )</td>
<td>0.648</td>
<td>6.05</td>
<td>6.48</td>
<td>60.5</td>
</tr>
</tbody>
</table>

Conductances contribute to boundary conditions of the form:

\[ J_i \cdot n = g_i (V - V_{Nernst,i}) \]

1. Halter et al. (1997); 2. Geleoc et al. (1997); 3. He et al. (2004); 4. Santos-Sacchi et al. (1997).
Pipette stimulus at base: voltage clamp vs. time

Net current input under clamp:

Transmembrane potential vs. longitudinal position

Color corresponds to time (in seconds)
OHC Lateral Wall Structure

Model based on previous studies (Forge, Holley, Saito, Slepecky, Kachar, Furness, Hackney, Dieler, Ulfendahl, Kalinec):

1) plasma membrane, with intramembranous particles
2) cortical lattice – actin, spectrin, and pillar proteins
3) subsurface cisternae (SSC)

taken with permission from Brownell & Popel 1998
For Bearings

SEM image of OHCs in organ of Corti, illustrating the hair bundle.

Cartoon of OHC lateral wall, taken from Brownell et. al., 2001. Ax = axial space; ECS = extracisternal space.
Tomography Protocol

- Sample Preparation
- Data Acquisition
- Reconstruction
- Postprocessing
Conventional Fixation vs. HPF/FS

Glutaraldehyde/PFA fixation
- Pillar Protein
- Mitochondria
- Plasma Membrane

Cytoplasmic extraction, strong internal membranes.
(Typical preparation for TEM)

HPF/FS, prepared with Anping Xia, BCM
- Pillar Protein
- SSC

Dense cytoplasm, faint internal membranes.
Concept Behind Electron Tomography

JEOL 2010 series TEM

filament

e-beam

Sample chamber
Dual axis series, +/- 70 degrees
reconstruction in Z, 0.96 nm voxel
Data from HPF/FS mouse

Pillar Protein

SSC
Successive ~40Å planes parallel to membrane and actin lattice, UA only
Top down view of OHC lateral wall. At top, volume rendering of full volume from membrane to approx. 100 nm deep. Middle, isolated extracisternal space, approx. 30 nm thick. Bottom, extracted profiles of circumferential filaments, model radius = 8 nm. Scale bar = 100 nm.
Left column: Individual slices from reconstructed pillar proteins from HPF/FS mouse OHCs. At right, surface renderings of pillar/actin/membrane complex.
Pillars

• orientation of pillars
  – not all pillars are normal to the surface

• morphology of individual pillars
  – some thinner, some thicker than others
  – platform to test candidate proteins based on shape and dimension
    • requires classification and averaging
Setup for aspiration using dialysis tubing
HPF/FS of fresh g-pig OHCs

close-up of SSC stack, with apparent pattern in lumen.
Initial view of SSC substructure
En face view of subsurface cisternae, illustrating apparent periodicity within the cisternal lumen. Lattice spacing is approximately 16 nm.
Axial cross section of lateral wall; lumen of SSC has density running down the middle.
Tomogram of SSC, Axial cut, 4nm slice

- SSC memb
- cisterna
- cell membrane
- filament-SSC link
- circumenternal filament
Summary

circumferential filament

filament-SSC link

SSC membrane

PM

CL

SSC

ECiS

AC

CP

actin

spectrin

pillar

particles

SSC

cell membrane

SSC membrane
Ongoing Work

- Characterization of novel periodic density/structure spanning the lumen of the SSC
- Application of volume alignment and averaging approaches to lateral wall / hair cell structures to achieve higher resolution
- ‘unbending’ of curved surfaces within a tomogram
- Classification of pillar orientation and morphology

- Longterm goal: integrate structural results into models of OHC physiology
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