Calcium Dynamics in the Stereociliary Bundle of Rat Cochlear Hair Cells

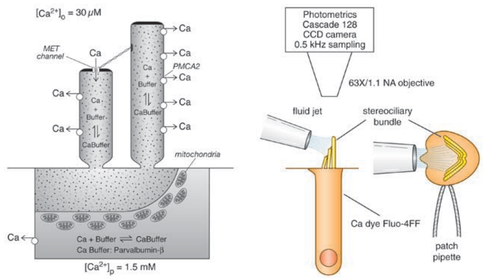
Jong-Hoon Nam[1], *Maryline Beurg*[2], *Carole Hackney*[3] *and Robert Fettiplace*[1]

[1]University of Wisconsin-Madison, [2]INSERM, Université Victor Segalen, Bordeaux, France and [3]Cambridge University, UK

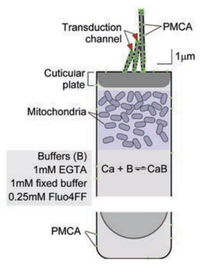
Introduction

Calcium plays a prominent role in OHCs, which possess 2 mM calcium-buffer, parvalbumin-β, and a high density of CaATPase pumps in the hair bundle. Mutation of the CaATPase causes deafness in mice (Street *et al.* 1998) implying calcium is a prime factor in hair cell pathology. The main calcium source is influx through the calcium-permeable mechanotransducer (MT) channels which are partly open at rest. Cytoplasmic calcium regulates MT channel adaptation and may influence OHC electromotility. A belt of mitochondria below the cuticular plate may act as a fixed calcium buffer, shielding the soma from large hair bundle calcium excursions. The aim was to quantify calcium transients originating from the MT channels and examine the mitochondrial contribution.

1 Apical inner hair cells (IHC) and outer hair cells (OHC) of P7 – P11 rats were patch clamped during bundle stimulation with stiff probe or water jet. Internal calcium measured with Fluo-4FF (K_D = 10 μM) imaged at 0.5 kHz with cooled CCD camera.



2 A computer model incorporates calcium influx through transduction channels, binding to mobile and fixed calcium buffers, uptake into mitochondria and extrusion by CaATPase pumps. Can the model explain the experimental results? Can the transient calcium flux affect the calcium landscape in the cell?



Calcium influx:

$$\left(\frac{dC}{dt}\right)_{influx} = -\frac{n_c i_{Ca}(t)}{2FV_C}$$

Calcium extrusion by PMCA and leakage:

$$\left(\frac{\partial C}{\partial t}\right)_{ext+leak} = \frac{v_{max,PMCA} A_C}{V_C}\left(\frac{C_0}{C_0 + K_{PMCA}} - \frac{C}{C + K_{PMCA}}\right)$$

Mitochondrial calcium transportation:

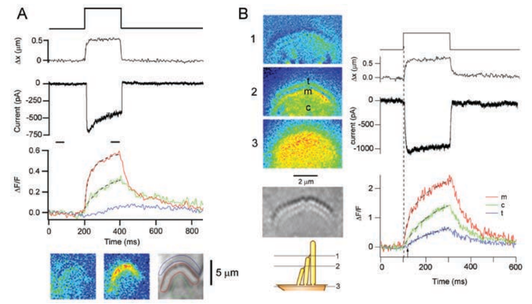
$$\left(\frac{dC}{dt}\right)_{mit} = -v_{max,uni}\frac{C^2}{K_{mit}^2 + C^2} + \frac{v_{max,NCX}}{\left(1 + K_{NCX}/C_m\right)}$$

$$\frac{dC_m}{dt} = \frac{\kappa_m}{\gamma_m}\left(\frac{dC}{dt}\right)_{mit}$$

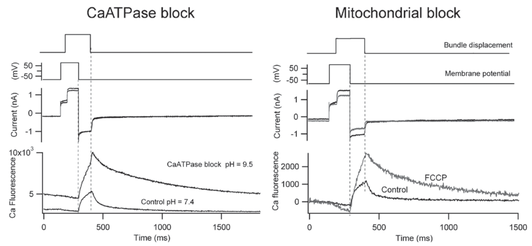
C = somatic free Ca concentration
C_m = free Ca concentration in mitochondria
n_C = number of channels in the compartment
i_{Ca} = single channel Ca current
F = Faraday constant
V_C = diffusible volume of the compartment
A_C = diffusible area of the compartment
κ_m = ratio of free to fixed Ca in mitochondria
γ_m = ratio of mitochondria to cytosol volume

C_0 = 0.1 μM
$v_{max,PMCA}$ = 0.67 μmolm^{2}s
K_{PMCA} = 0.5 μM
$v_{max,uni}$ =114mM/s
K_{NCX} = 40 μM
κ_m = 2.5x10^4
γ_m = 0.667

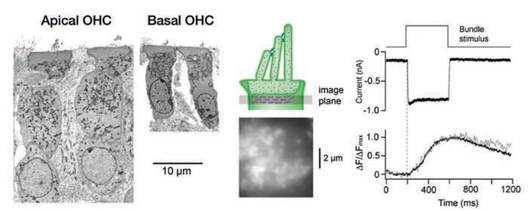
3 Calcium transients in an OHC (A) and an IHC (B). In IHC, calcium transients were larger and faster in row2 and row3 stereocilia than in row1 indicating absence of MT channels in row1 (Beurg *et al.* 2009).



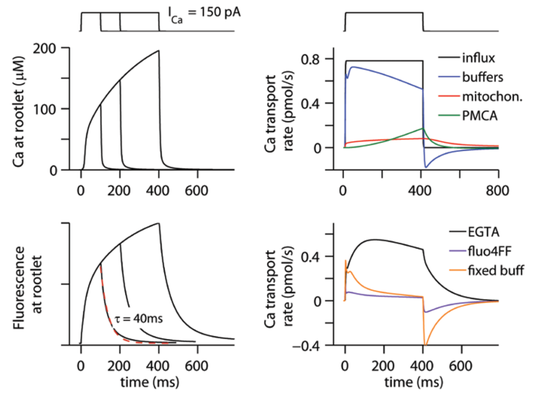
4 Recovery from a calcium load was slowed by increasing pH to block the CaATPase (Xu *et al.* 2000) or by adding 2 μM FCCP to eliminate mitochondrial membrane potential. Both treatments took 10 minutes to develop during which time resting calcium increased.



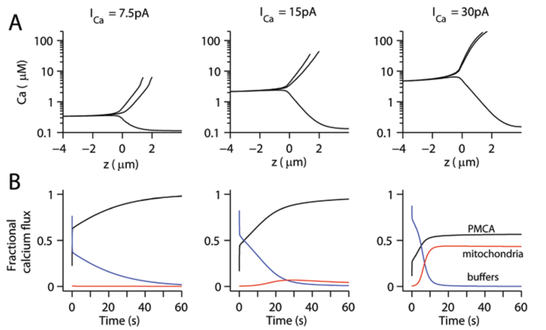
5 Imaging mitochondrial calcium using Rhod2AM showed the mitochondria see calcium influx due to MT channel stimulation. One experiment and the average of 5 others indicate fast calcium uptake and slower release with time constant of 1.1 sec.



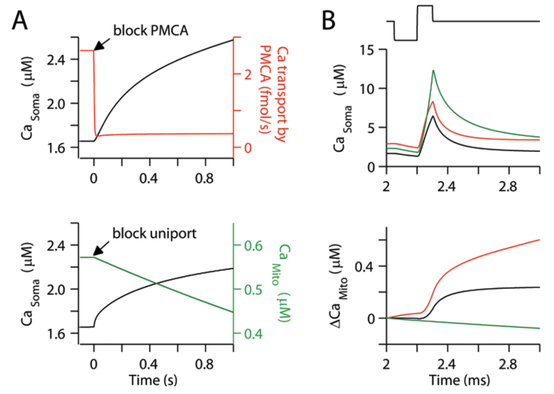
6 Simulation results matched the experiments when there was 1 mM fixed buffer with K_D = 10 μM. During transduction, calcium in stereocilia rose to hundreds μM. Within a fraction of a second of transient current, the buffers took up most calcium and determined the transient response.



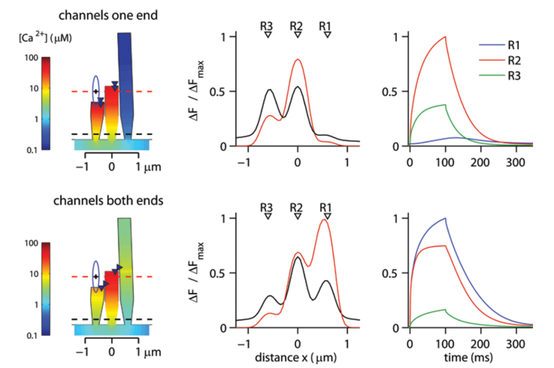
7 With 4000 Ca-pumps/μm^2, the simulated outer hair cell could sustain up to 10 % of maximal current *in vitro* (I_{Ca} = 15 pA). If influx exceeds Ca-pump capacity, calcium accumulates in the mitochondria, which may result in cell death.



8 Experimental results of Ca-pump or mitochondria inhibition were reproduced by simulations. Both treatments resulted in increased somatic calcium (C_{Soma}) and slowed the recovery from the stimulation. Ca-pump inhibition increased calcium loading of mitochondria.



9 Calcium distribution (left) and fluorescence intensity (right) are different according to the transduction channel location. Experimental results are consistent with the scenario of two channels at the lower end of tip link (top).



Work supported by NIDCD Grant RO1 DC01362 to RF.