

# Biophysics I (BPHS 4080)

Instructors: Prof. Christopher Bergevin (cberge@yorku.ca)

Website: <http://www.yorku.ca/cberge/4080W2018.html>

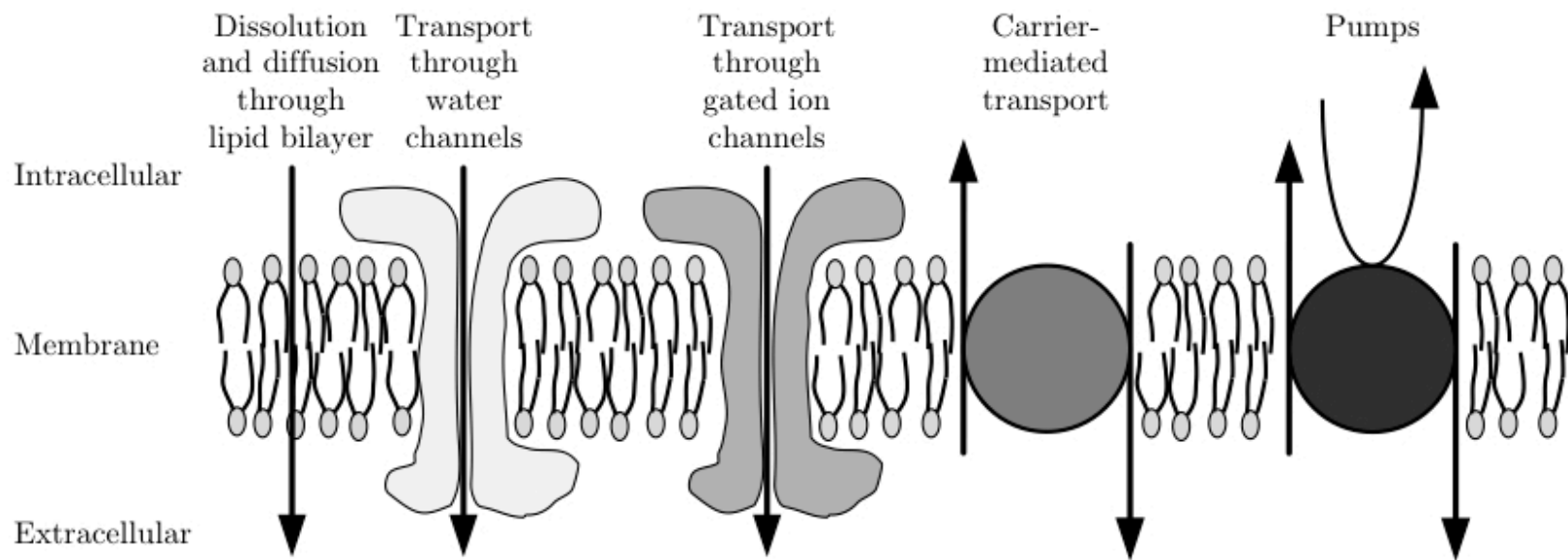


Figure 2.19

## Example → Auditory hair cells as RLC Systems

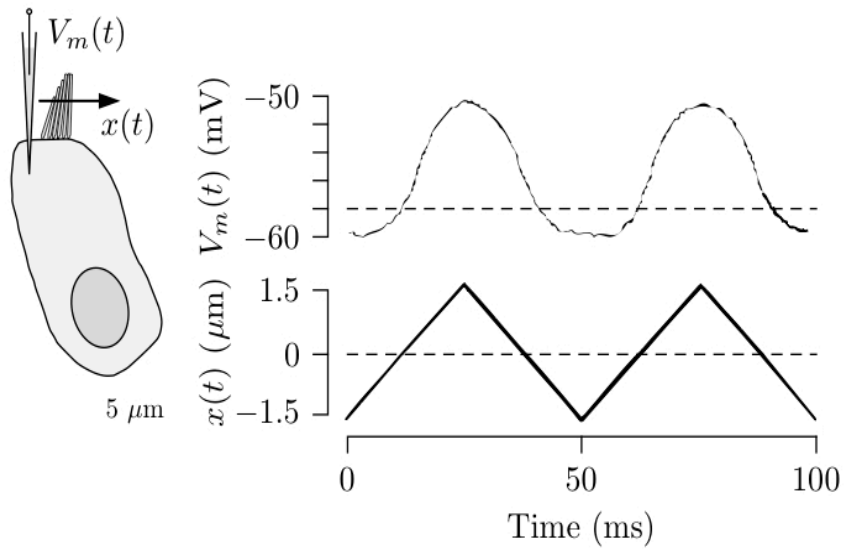
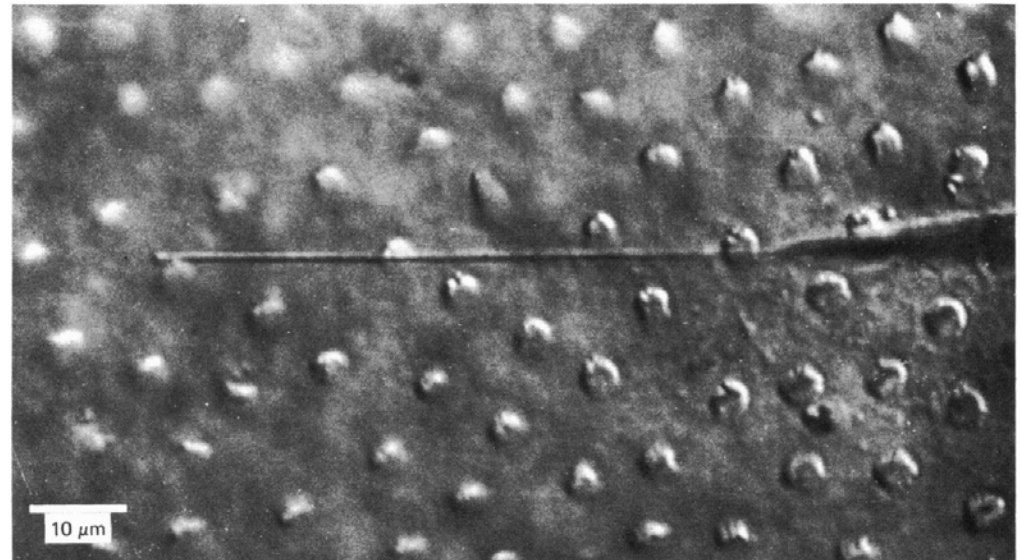
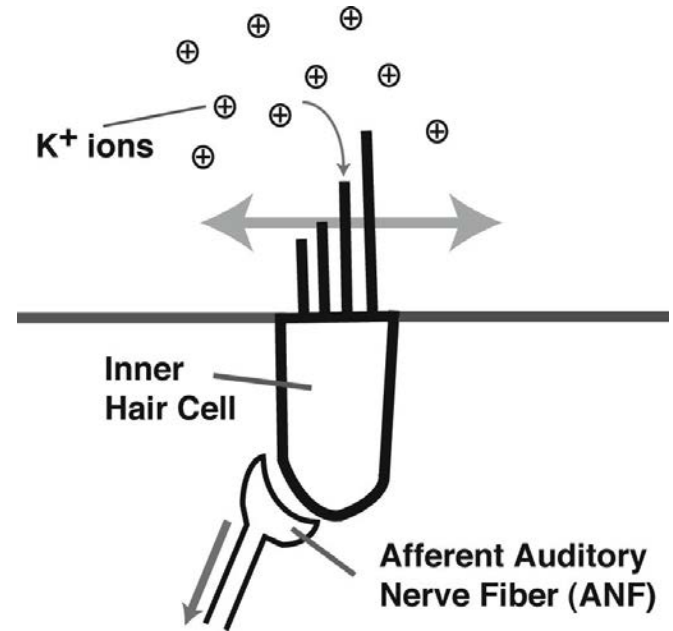
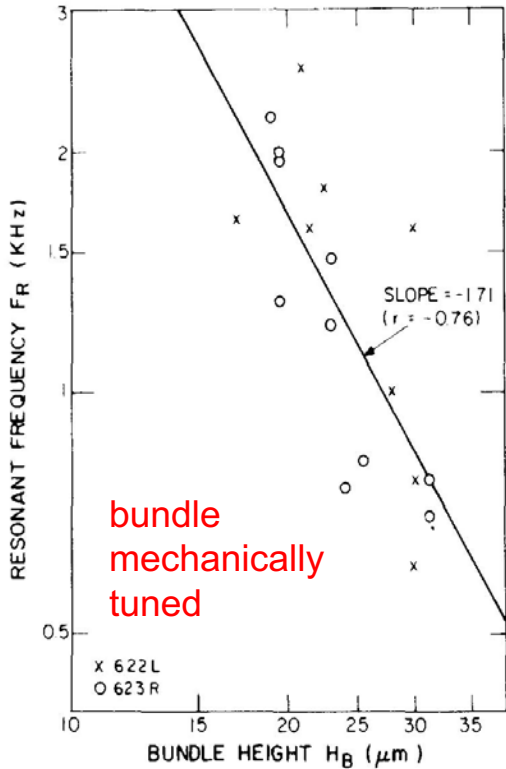


Figure 1.5

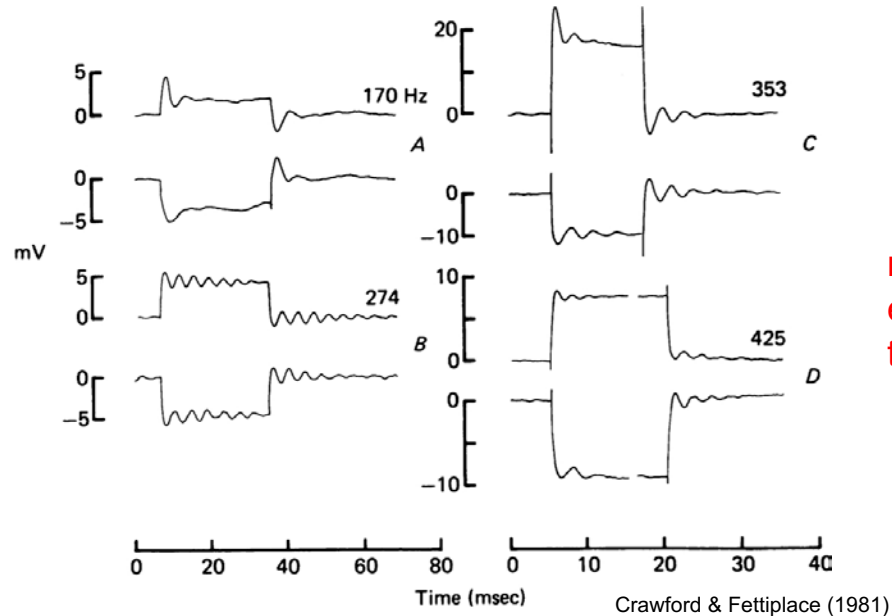


# Ex. Auditory hair cells

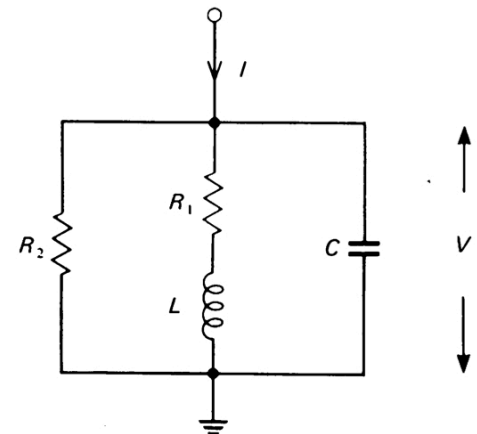
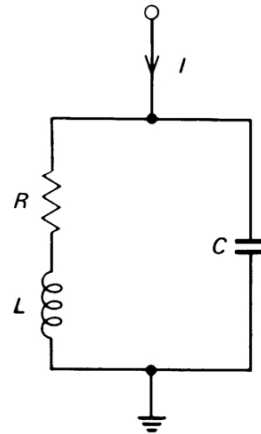


Frishkopf & DeRosier (1983)

bundle mechanically tuned



membrane electrically tuned



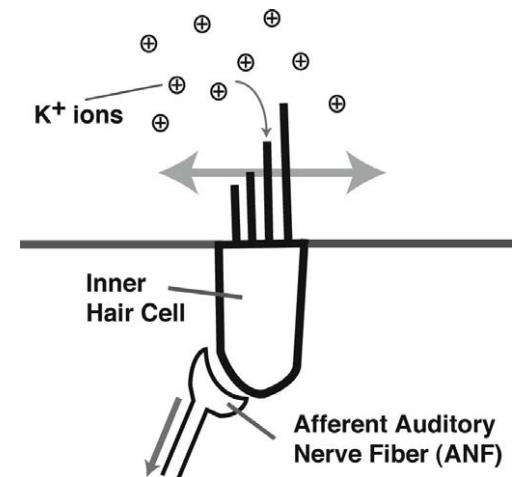
→ electrical measurements indicate both mechanical and electrical resonances

# A Molecular Mechanism for Electrical Tuning of Cochlear Hair Cells

Krishnan Ramanathan, Timothy H. Michael, Guo-Jian Jiang, Hakim Hiel, Paul A. Fuchs\*

Cochlear frequency selectivity in lower vertebrates arises in part from electrical tuning intrinsic to the sensory hair cells. The resonant frequency is determined largely by the gating kinetics of calcium-activated potassium (BK) channels encoded by the *slo* gene. Alternative splicing of *slo* from chick cochlea generated kinetically distinct BK channels. Combination with accessory  $\beta$  subunits slowed the gating kinetics of  $\alpha$  splice variants but preserved relative differences between them. In situ hybridization showed that the  $\beta$  subunit is preferentially expressed by low-frequency (apical) hair cells in the avian cochlea. Interaction of  $\beta$  with  $\alpha$  splice variants could provide the kinetic range needed for electrical tuning of cochlear hair cells.

SCIENCE VOL 283 8 JANUARY 1999



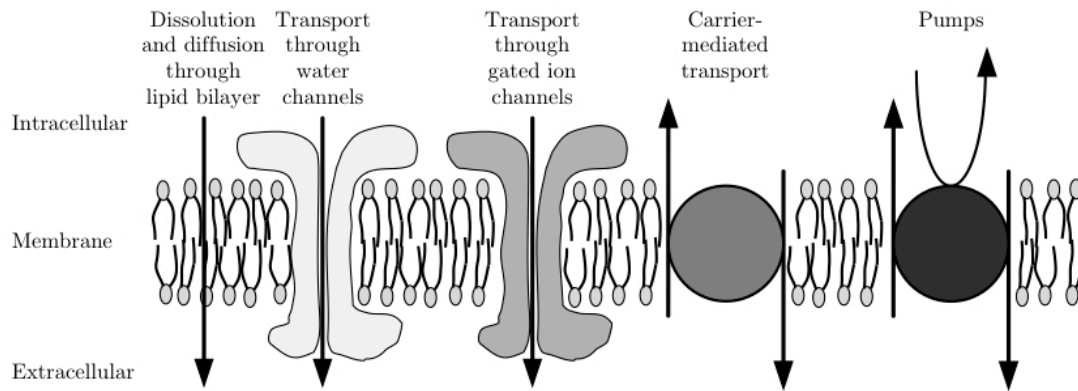
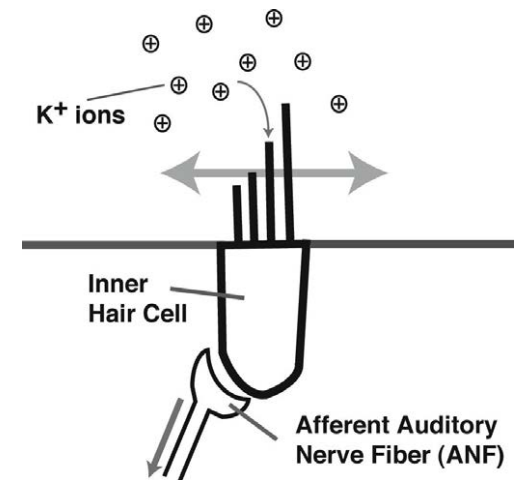


Figure 2.19



# Protein subunit

From Wikipedia, the free encyclopedia

In [structural biology](#), a **protein subunit** is a single protein molecule that assembles (or "*coassembles*") with other protein molecules to form a [protein complex](#). Some naturally occurring proteins have a relatively small number of subunits and therefore described as *oligomeric*, for example [hemoglobin](#) or [DNA polymerase](#). Others may consist from a very large number of subunits and therefore described as *multimeric*, for example [microtubules](#) and other [cytoskeleton](#) proteins. The subunits of a multimeric protein may be identical, [homologous](#) or totally dissimilar and dedicated to disparate tasks.



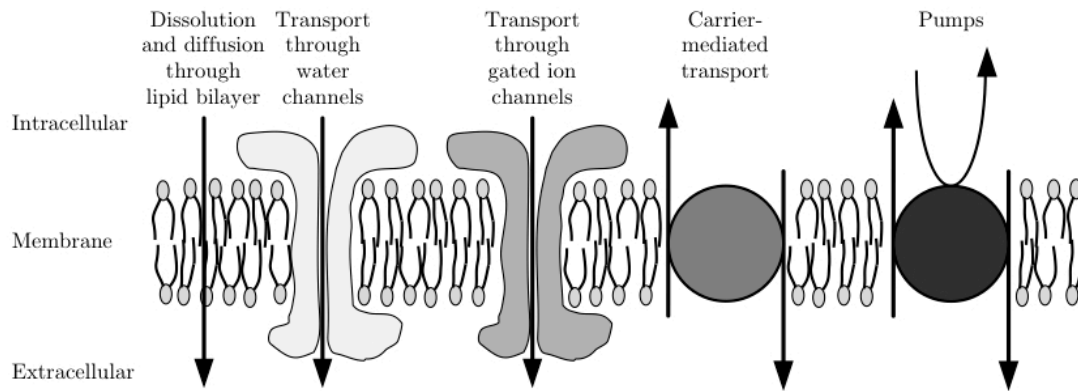
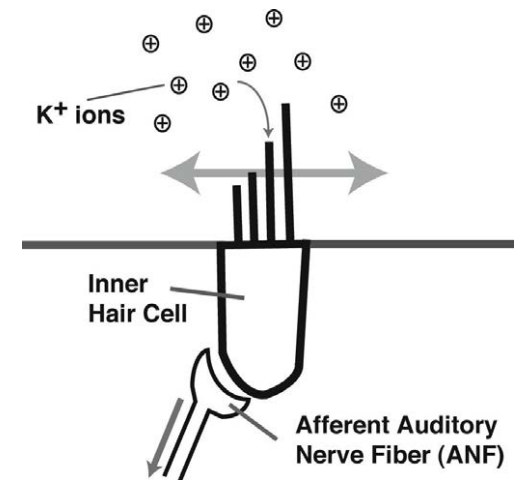


Figure 2.19

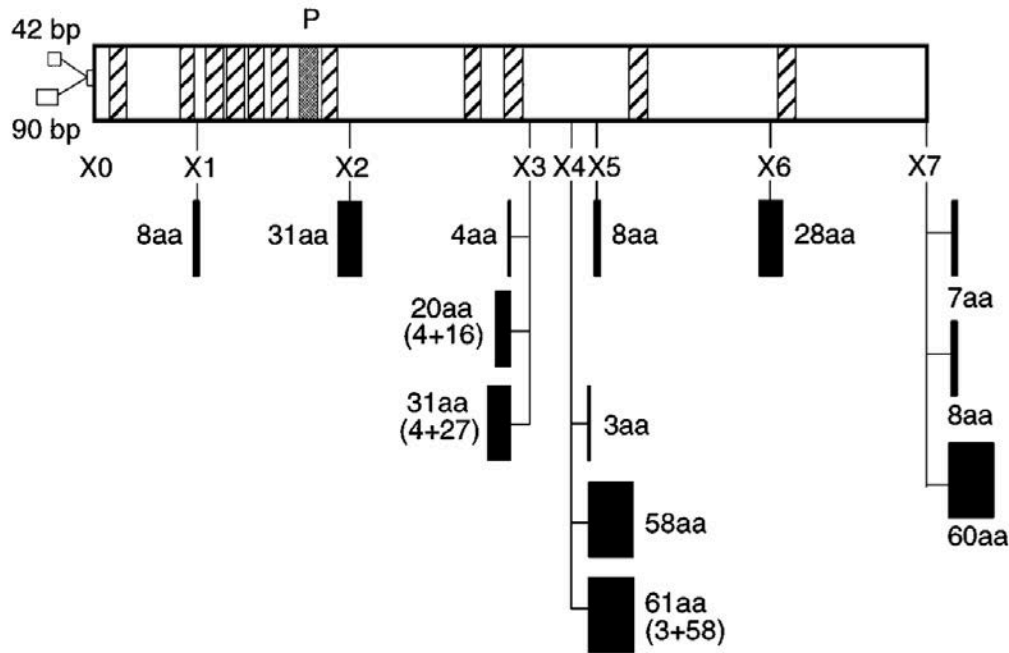


## Ion channel

From Wikipedia, the free encyclopedia

Ion channels are [integral membrane proteins](#), typically formed as assemblies of several individual proteins. Such "multi-subunit" assemblies usually involve a circular arrangement of identical or [homologous](#) proteins closely packed around a water-filled pore through the plane of the membrane or [lipid bilayer](#).<sup>[3][4]</sup> For most [voltage-gated ion channels](#), the pore-forming subunit(s) are called the  $\alpha$  subunit, while the auxiliary subunits are denoted  $\beta$ ,  $\gamma$ , and so on.

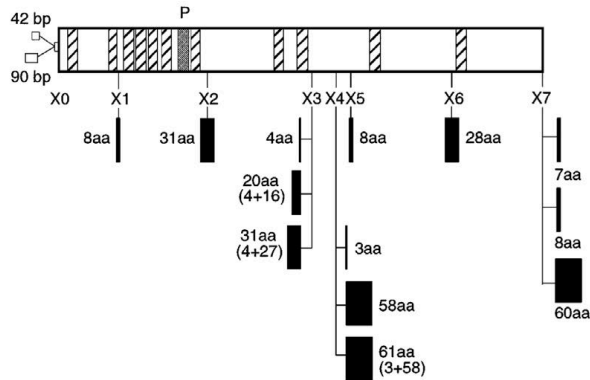
Cochlear frequency selectivity in lower vertebrates arises in part from electrical tuning intrinsic to the sensory hair cells. The resonant frequency is determined largely by the gating kinetics of calcium-activated potassium (BK) channels encoded by the *slo* gene. Alternative splicing of *slo* from chick cochlea generated kinetically distinct BK channels. Combination with accessory  $\beta$  subunits slowed the gating kinetics of  $\alpha$  splice variants but preserved relative differences between them. In situ hybridization showed that the  $\beta$  subunit is preferentially expressed by low-frequency (apical) hair cells in the avian cochlea. Interaction of  $\beta$  with  $\alpha$  splice variants could provide the kinetic range needed for electrical tuning of cochlear hair cells.



*Figure 2* Schematic diagram of the cochlear Slo channel  $\alpha$ -subunit open reading frame (~1150 amino acids in length), carboxy terminus to the right. *Hatched boxes* are putative amphipathic or transmembrane domains (S0–S10) based on data of Meera et al (42), and the *shaded area* is the pore (P). Eight splice sites, X0–X7, have been identified. Two 5' sequences have been identified (38,41). Both contain additional in-frame ATGs, but these are not necessary for producing a functional channel (36, 148) and may therefore be located in the untranslated region. At X7, three exons give rise to different carboxy termini of 7, 8, and 60 amino-acids (aa) (38–41; note a single residue difference in the 8 aa exon among these reports). X1–X6 may have no insert or contain a single exon or concatenated exons, the amino-acid sizes of which are noted below each site. X1, which is novel among all vertebrate and invertebrate BK transcripts, may contain an 8 aa insert (41); X2 may contain a 31 aa insert (40). X3 may contain a 4 aa (39–41), a 20 aa (41), or a 31 aa (39) insert. X4 may contain a 3 aa (39,40), a 58 aa (41), or a 61 aa (39) insert. The 58 aa insert is reported as 59 but includes a modified 5' flanking amino acid. X5 may contain an 8 aa insert (41) and X6 a 28 aa insert (40, 41; note the reported sequences differ by a single residue).

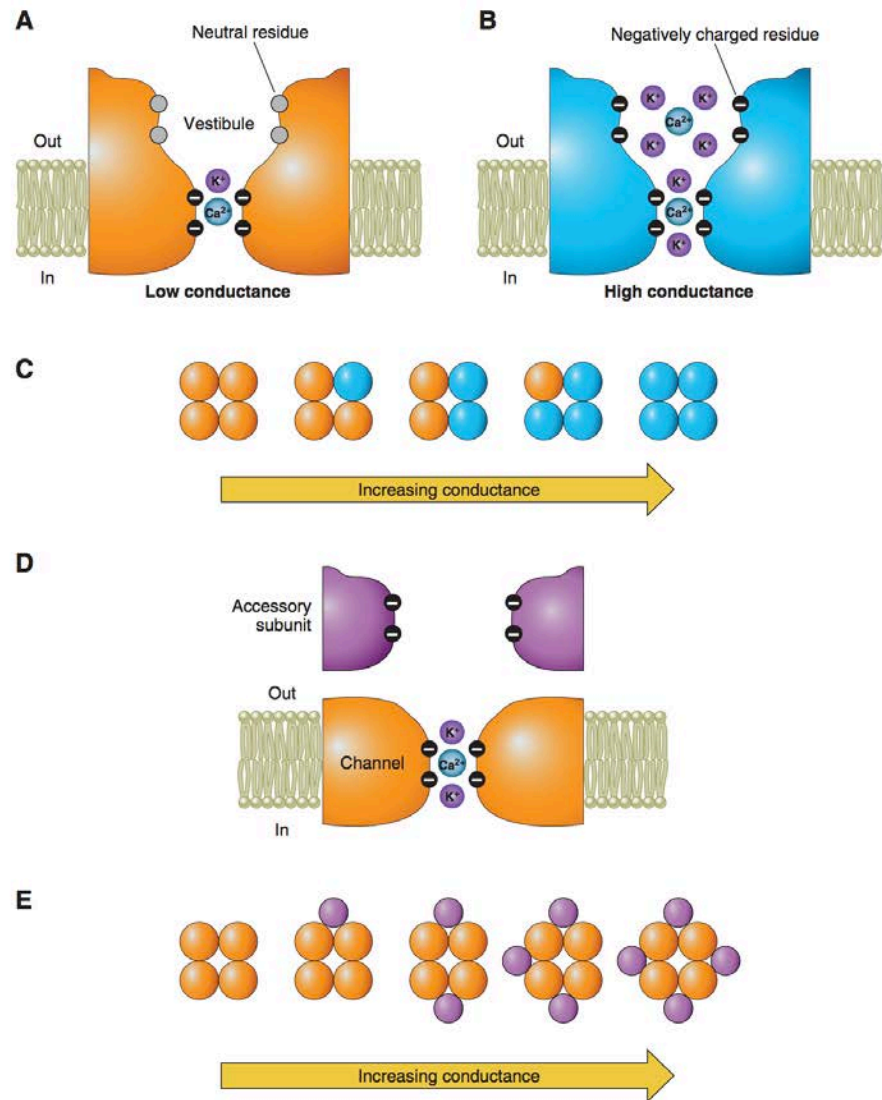
→ As knowledge expands, pictures like this become....





**Figure 2** Schematic diagram of the cochlear Slo channel  $\alpha$ -subunit open reading frame (~1150 amino acids in length), carboxy terminus to the right. *Hatched boxes* are putative amphipathic or transmembrane domains (S0–S10) based on data of Meera et al (42), and the *shaded area* is the pore (P). Eight splice sites, X0–X7, have been identified. Two 5' sequences have been identified (38, 41). Both contain additional in-frame ATGs, but these are not necessary for producing a functional channel (36, 148) and may therefore be located in the untranslated region. At X7, three exons give rise to different carboxy termini of 7, 8, and 60 amino-acids (aa) (38–41; note a single residue difference in the 8 aa exon among these reports). X1–X6 may have no insert or contain a single exon or concatenated exons, the amino-acid sizes of which are noted below each site. X1, which is novel among all vertebrate and invertebrate BK transcripts, may contain an 8 aa insert (41); X2 may contain a 31 aa insert (40). X3 may contain a 4 aa (39–41), a 20 aa (41), or a 31 aa (39) insert. X4 may contain a 3 aa (39, 40), a 58 aa (41), or a 61 aa (39) insert. The 58 aa insert is reported as 59 but includes a modified 5' flanking amino acid. X5 may contain an 8 aa insert (41) and X6 a 28 aa insert (40, 41; note the reported sequences differ by a single residue).

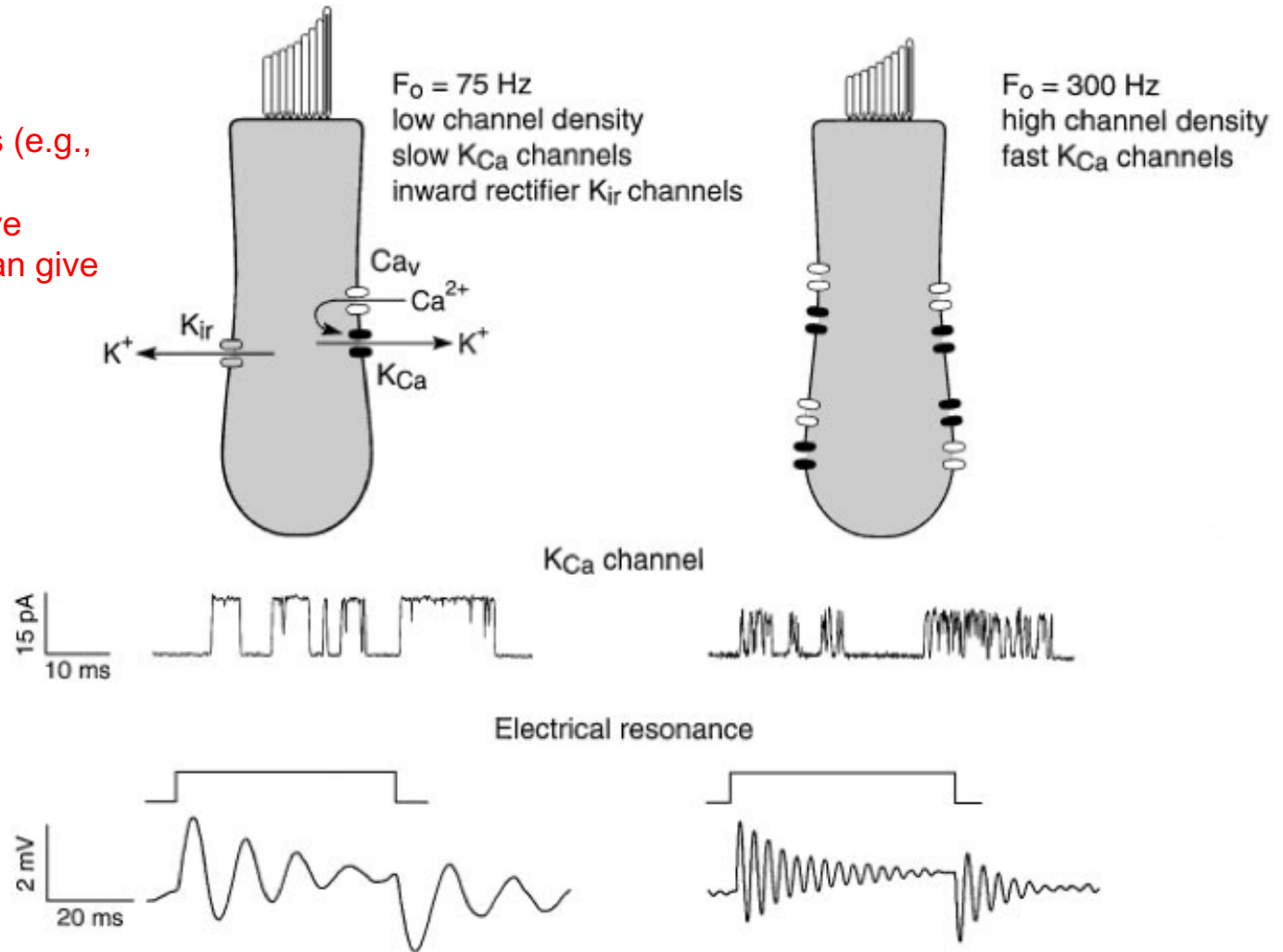
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**FIGURE 10.** Hypothetical structure of the MT channel illustrating the ion conduction pathway. **A:** cross-section of a channel showing a large vestibule on the external aspect of the channel and a tight selectivity filter lined with negatively charged residues at the cytoplasmic end of the channel. It is proposed that in the low-conductance isoform, there are critical neutral residues in the vestibule. **B:** in the high-conductance isoform of the channel, these neutral residues are replaced with negatively charged residues that cause accumulation of ions in the vestibule, hence increasing channel conductance [18]. **C:** one scheme for generating a range of single-channel conductances by mixing the low-conductance and high-conductance isoforms in **A** and **B** in a tetrameric channel, assuming properties are a linear function of subunit composition. **D:** in a second model, the MT channel comprises a pore-forming modulated by an accessory subunit with negatively charged residues to generate a high-conductance isoform as in **B**. **E:** a scheme using the second model for producing a range of unitary conductances, in which different numbers of accessory subunits, such as LHFPL5 or TMC1, associate with the channel to systematically vary its properties (see sect. VI B).

## Sensory cells of the inner ear can behave like RLC circuits

- Voltage-gated channels (e.g., calcium-activated “BK” potassium channels) have intrinsic dynamics that can give rise to electric tuning



*Figure 1* Schematic drawing of two hair cells from the turtle basilar papilla, with resonant frequencies ( $F_0$ ) of 75 and 300 Hz. The low-frequency cell has a longer hair bundle, and a low density of  $Ca^{2+}$  and  $Ca^{2+}$ -activated  $K^+$  ( $K_{Ca}$ ) channel complexes. The number of channel complexes increases with ( $F_0$ ). Beneath each cell are shown representative  $K_{Ca}$  (BK) single-channel records and ringing voltage responses to extrinsic current steps for cells tuned approximately to these two frequencies. The timing of the extrinsic current is shown above the voltage records. The single-channel records and the voltage ringing were from different sets of experiments (22, 23).

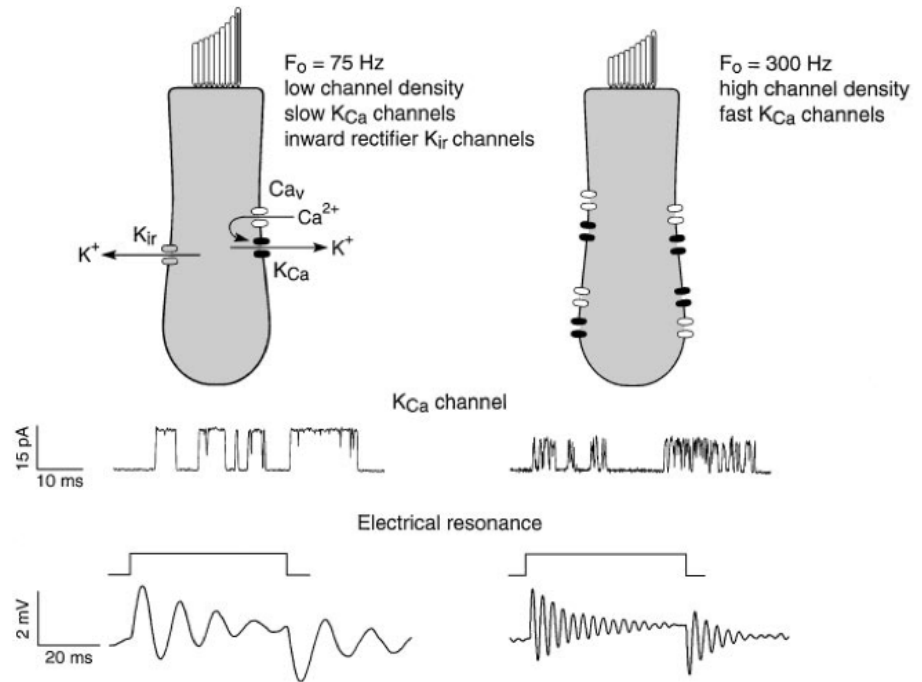
## Sensory cells of the inner ear can behave like RLC circuits

1. Mechanical motion deflects bundle, causing a transduction current to depolarize the cell

2. “Depolarization opens voltage-gated  $\text{Ca}^{2+}$  channels, promoting a rise in internal  $\text{Ca}^{2+}$  that activates BK channels”

3. “The large outward  $\text{K}^+$  current hyperpolarizes the membrane, closing the  $\text{Ca}^{2+}$  channels, which leads to the first cycle of the oscillation”

4. “As the cell hyperpolarizes and intracellular  $\text{Ca}^{2+}$  transients dissipate, the BK channels partially close, but due to the continued extrinsic current, the membrane swings positive to initiate another cycle of  $\text{Ca}^{2+}$  influx.”



“Since the BK channels are already partly activated, a smaller fraction of  $\text{K}^+$  current is recruited on the second cycle, which will have a smaller amplitude than the first. Because the  $\text{K}^+$  equilibrium potential ( $-80 \text{ mV}$ ) is negative to the resting potential ( $-50 \text{ mV}$ ), the BK channels behave as part of a negative feedback loop, but the time course of their activation delays the feedback and hence generates damped oscillatory responses. Such negative feedback also produces sharp tuning for sinusoidal stimuli, and the frequency at which the cell is maximally sensitive, the resonant frequency, should be influenced by the size and speed of the feedback.”

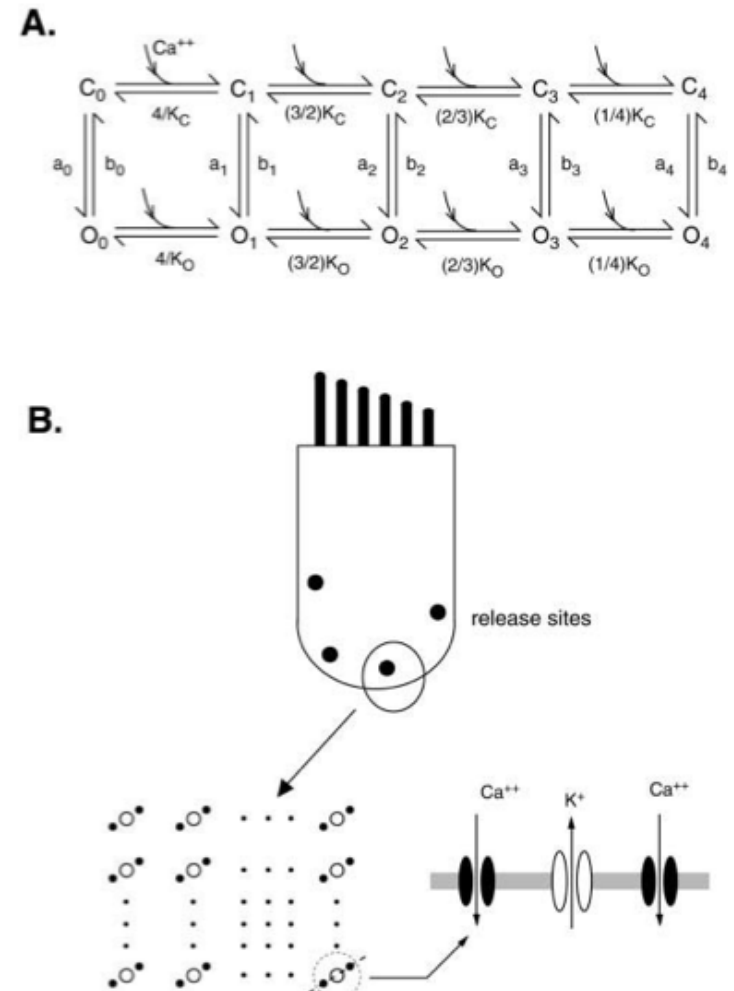
# Modeling Hair Cell Tuning by Expression Gradients of Potassium Channel $\beta$ Subunits

Krishnan Ramanathan and Paul A. Fuchs

The Center for Hearing and Balance, Department of Biomedical Engineering and Department of Otolaryngology, Head and Neck Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205-2195 USA

**ABSTRACT** The receptor potential of sensory hair cells arises from the gating of mechanosensitive cation channels, but its amplitude and time course also depend on the number and kinetics of voltage-gated ion channels in each cell. Prominent among these are “BK” potassium channels encoded by the *slo* gene that support electrical tuning in some hair cells. Hair cells tuned to low frequencies have slowly gating BK channels, whereas those of higher-frequency hair cells gate more rapidly. Alternative splicing of the *slo* gene mRNA that encodes the pore-forming  $\alpha$  subunit can alter BK channel kinetics, and gating is dramatically slowed by coexpression with modulatory  $\beta$  subunits. The effect of the  $\beta$  subunit is consistent with low-frequency tuning, and  $\beta$  mRNA is expressed at highest levels in the low frequency apex of the bird’s auditory epithelium. How might an expression gradient of  $\beta$  subunits contribute to hair cell tuning? The present work uses a computational model of hair cell-tuning based on the functional properties of BK channels expressed from hair cell  $\alpha$  and  $\beta$  *slo* cDNA. The model reveals that a limited tonotopic gradient could be achieved simply by altering the fraction of BK channels in each hair cell that are combined with  $\beta$  subunits. However, complete coverage of the tuning spectrum requires kinetic variants in addition to those modeled here.

**FIGURE 1** BK channel and hair cell models. (A) The allosteric (voltage-dependent MWC) scheme for BK channels has horizontal transitions that are calcium dependent and vertical transitions that are voltage dependent. The voltage-dependent MWC version assumes that each calcium-binding step has the same affinity. However, binding of calcium to the closed states may differ from the binding to the open states. The subscripts to the closed and open states indicate the number of calcium ions bound. Voltage-dependent gate movements between closed and open states are thought to occur allosterically with a single rate constant.  $a_x$  and  $b_x$  are the vertical transition rates that are dependent upon voltage.  $K_{Cx}$  and  $K_{Ox}$  are the dissociation constants for the binding of  $x^{\text{th}}$  calcium ion to the closed and open states respectively. (B) The hair cell model incorporates BK channels into functional units with two adjacent voltage-gated calcium channels. These are shown as clusters, as might occur at transmitter release active zones, but each BK channel is gated independently by its associated calcium channels. The number and kinetic properties of BK channels varies between model cells, but the ratio of two calcium channels to one BK channel remains constant, as do the gating properties of the voltage-gated calcium channels.





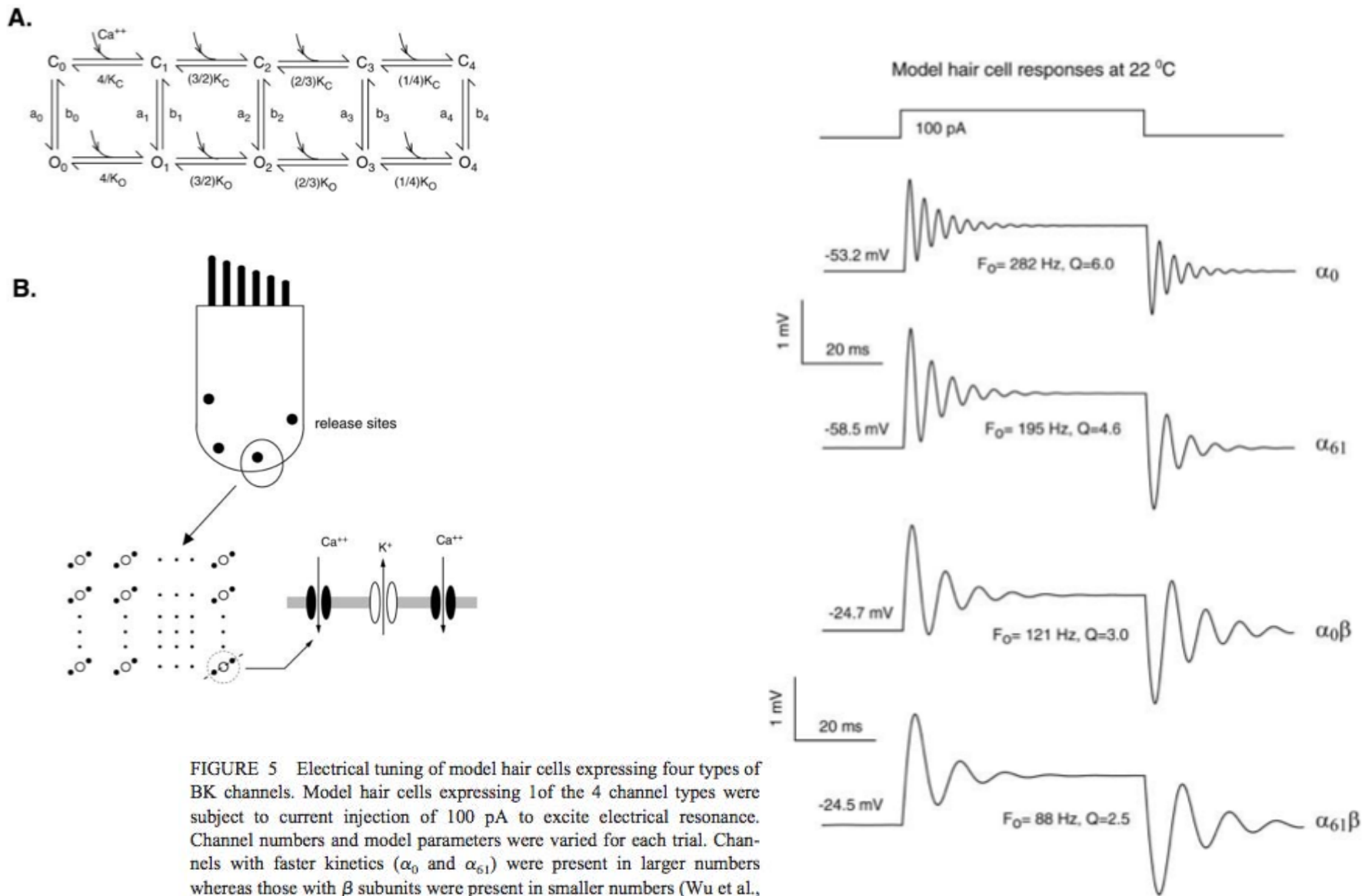
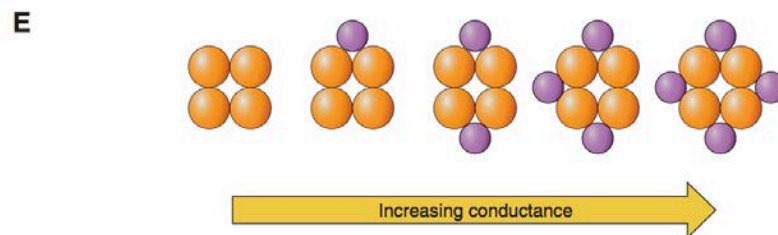
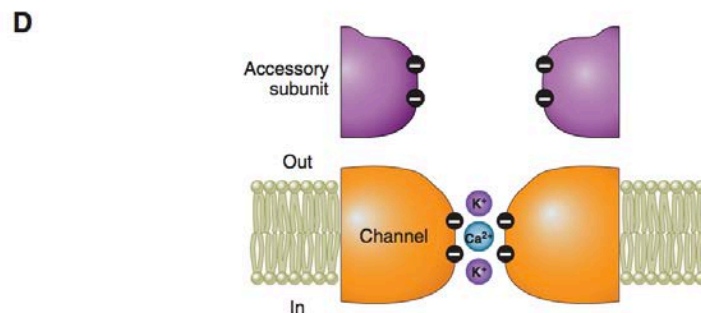
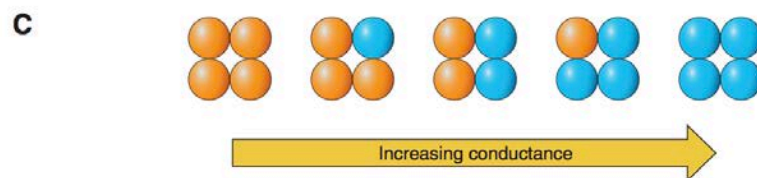
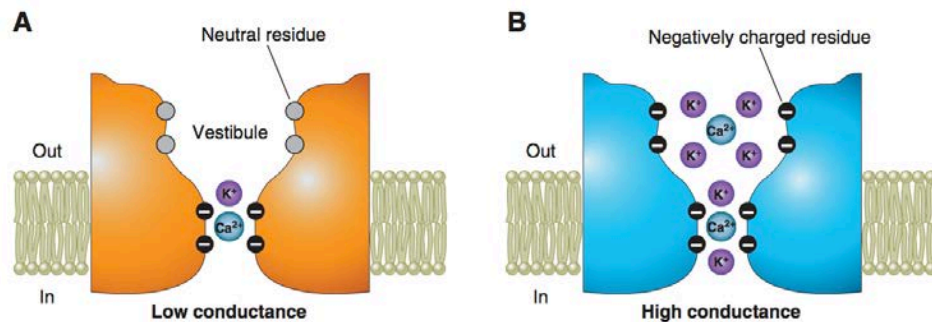
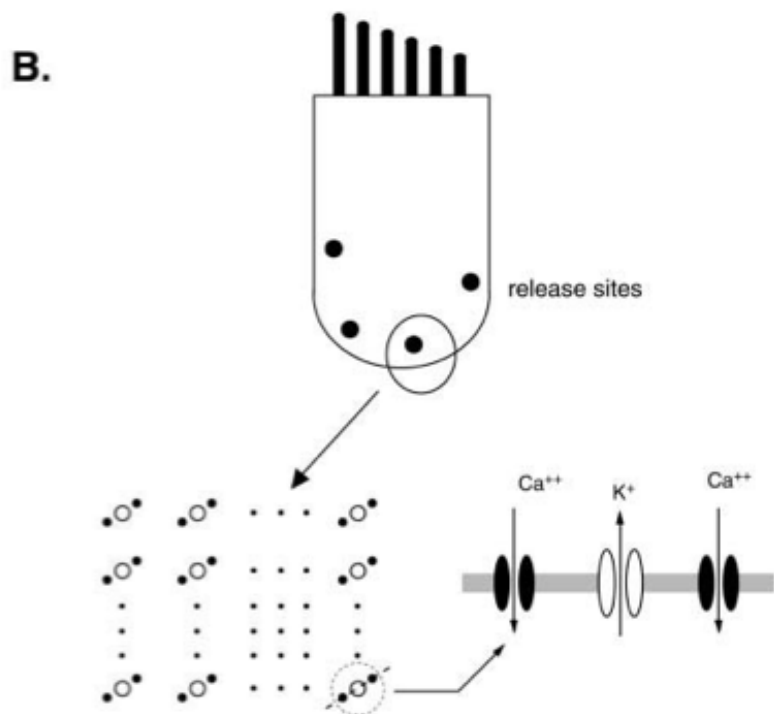
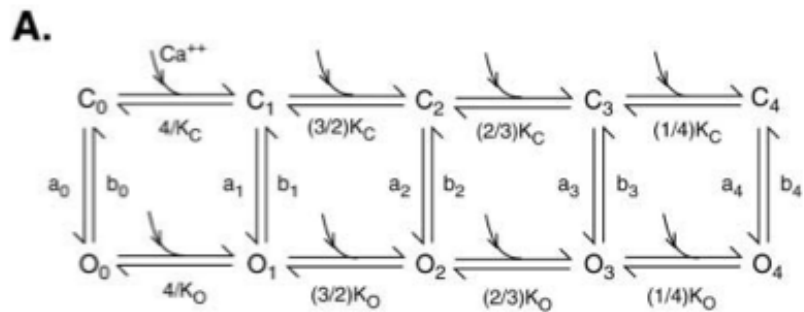
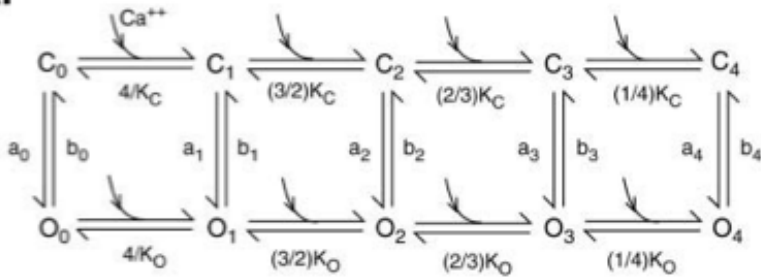


FIGURE 5 Electrical tuning of model hair cells expressing four types of BK channels. Model hair cells expressing 1 of the 4 channel types were subject to current injection of 100 pA to excite electrical resonance. Channel numbers and model parameters were varied for each trial. Channels with faster kinetics ( $\alpha_0$  and  $\alpha_{61}$ ) were present in larger numbers whereas those with  $\beta$  subunits were present in smaller numbers (Wu et al., 1995). The four channels span a electrical tuning frequency range from 88 Hz to 282 Hz. The resonant frequency and the quality of tuning for positive current injection are shown below each trace.  $\alpha_0$  and  $\alpha_{61}$  produced electrical resonance at resting hair cell voltages of  $\sim -55$  mV. Hair cells containing  $\alpha_0\beta$  or  $\alpha_{61}\beta$  resonated at resting potentials that were more depolarized ( $\sim -25$  mV).

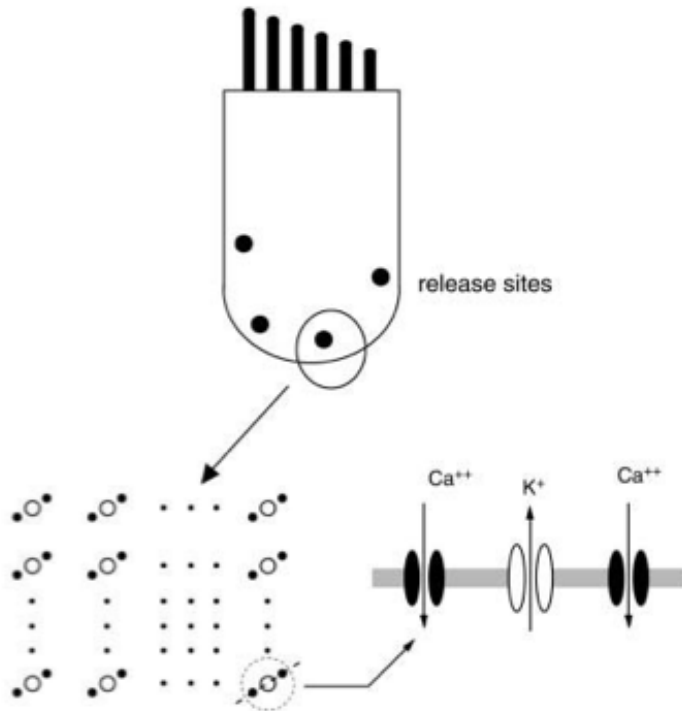




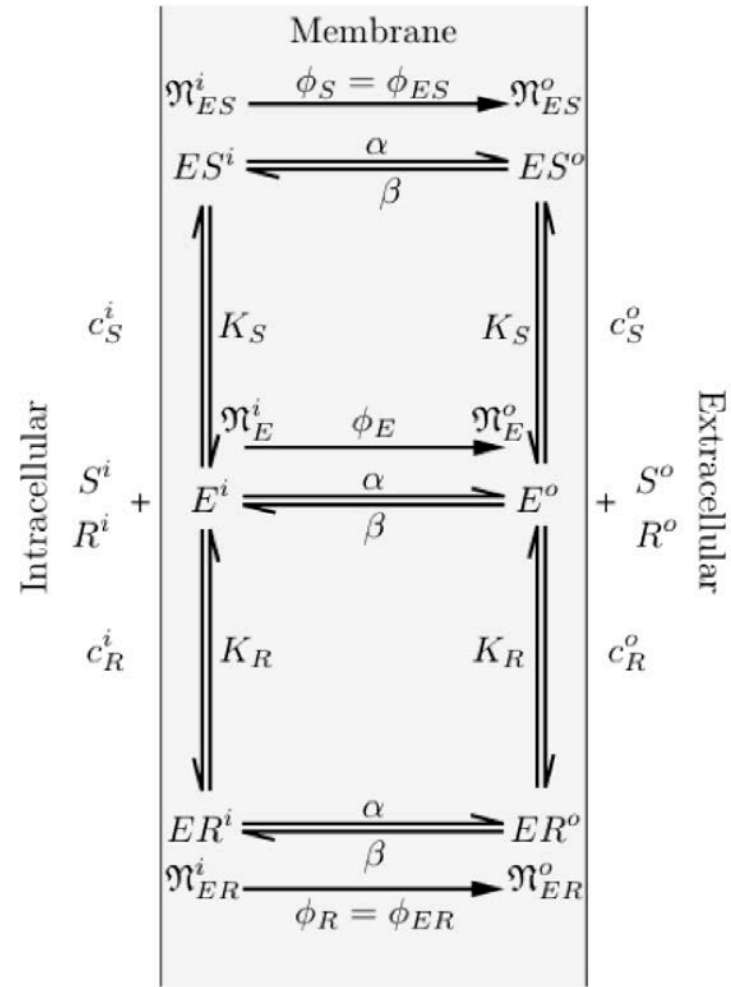
**A.**



**B.**

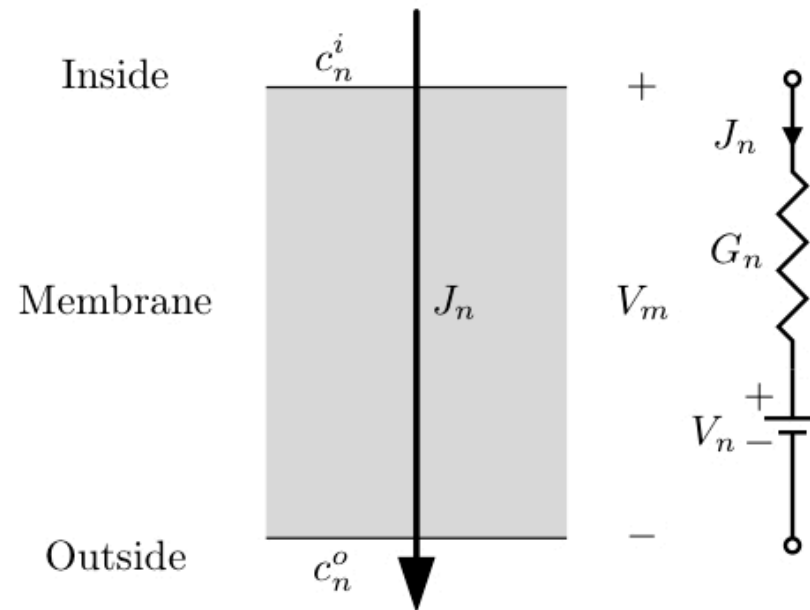


Kinda looks familiar already?



Looking ahead from this point.....

## Model of Steady-State Electrodifusion through Membranes



# Consider Different Charged Solutes in Parallel

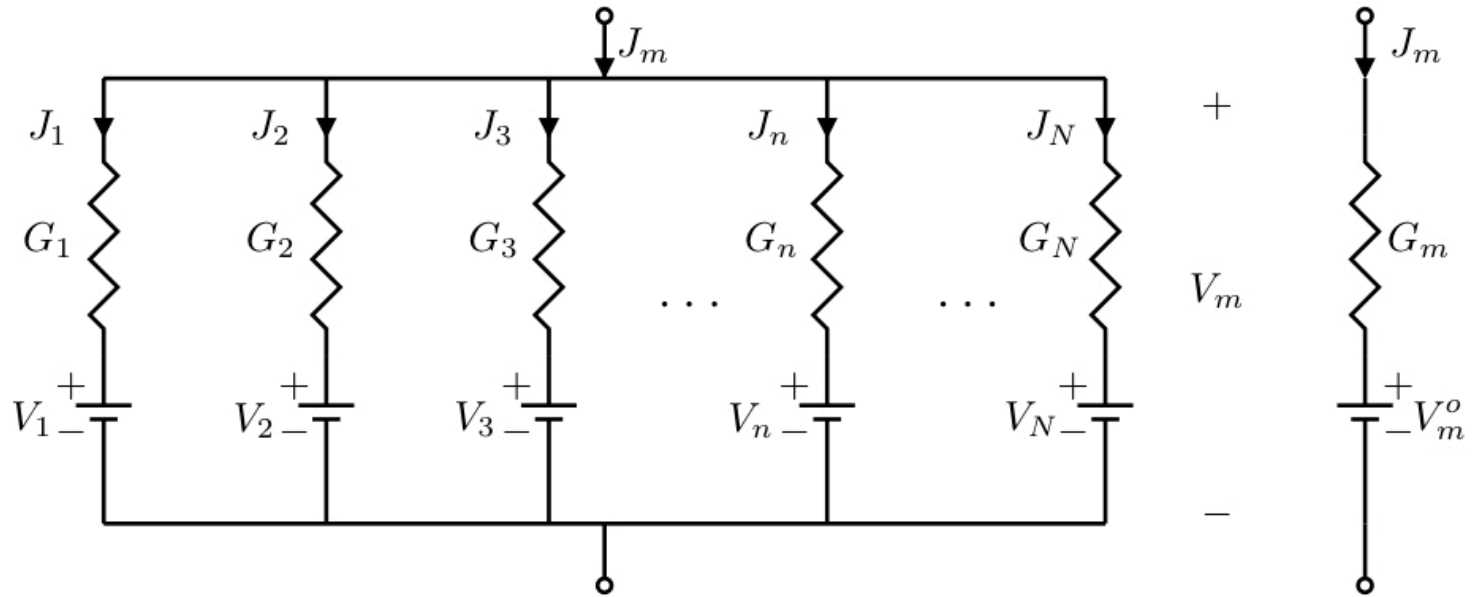
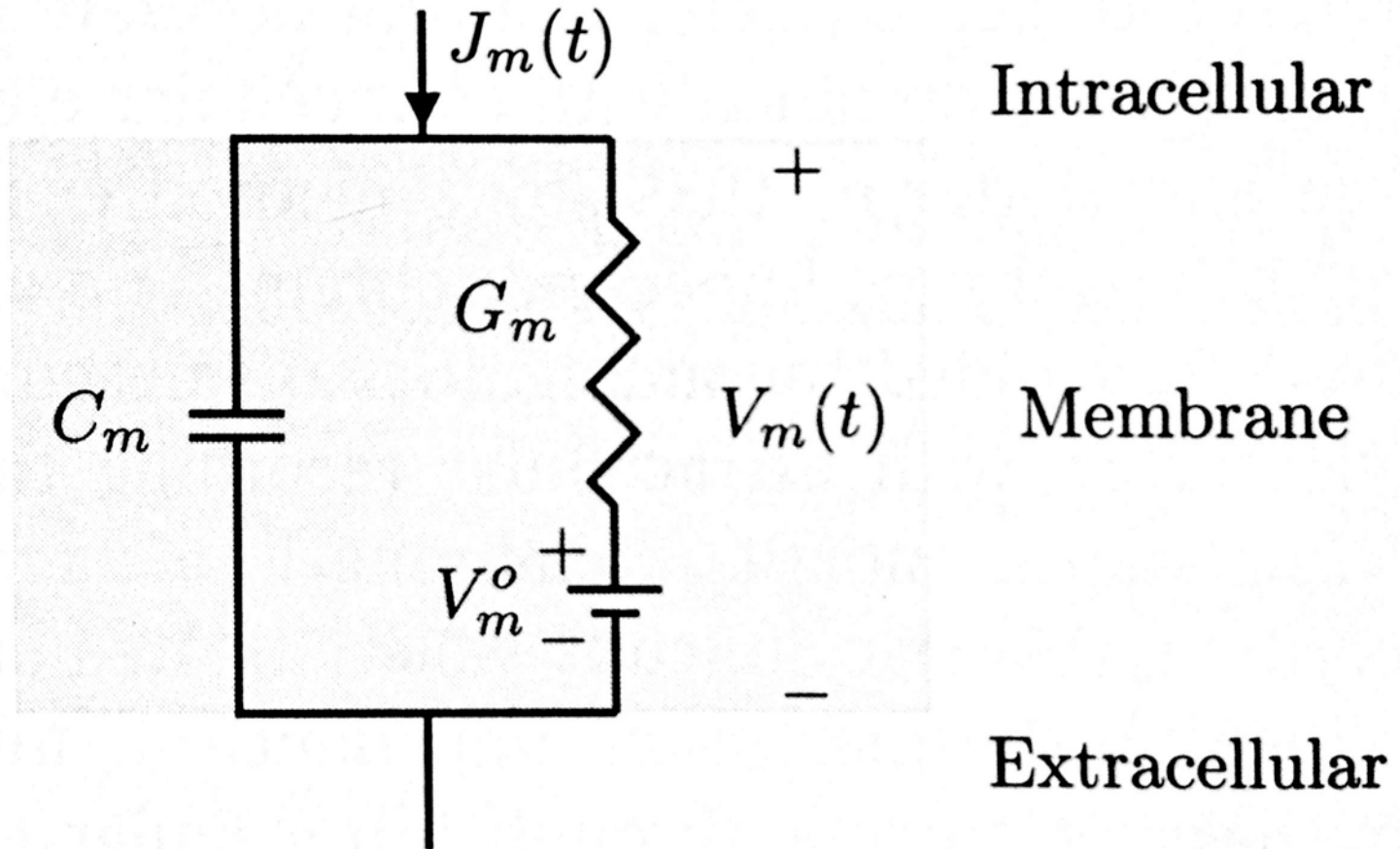


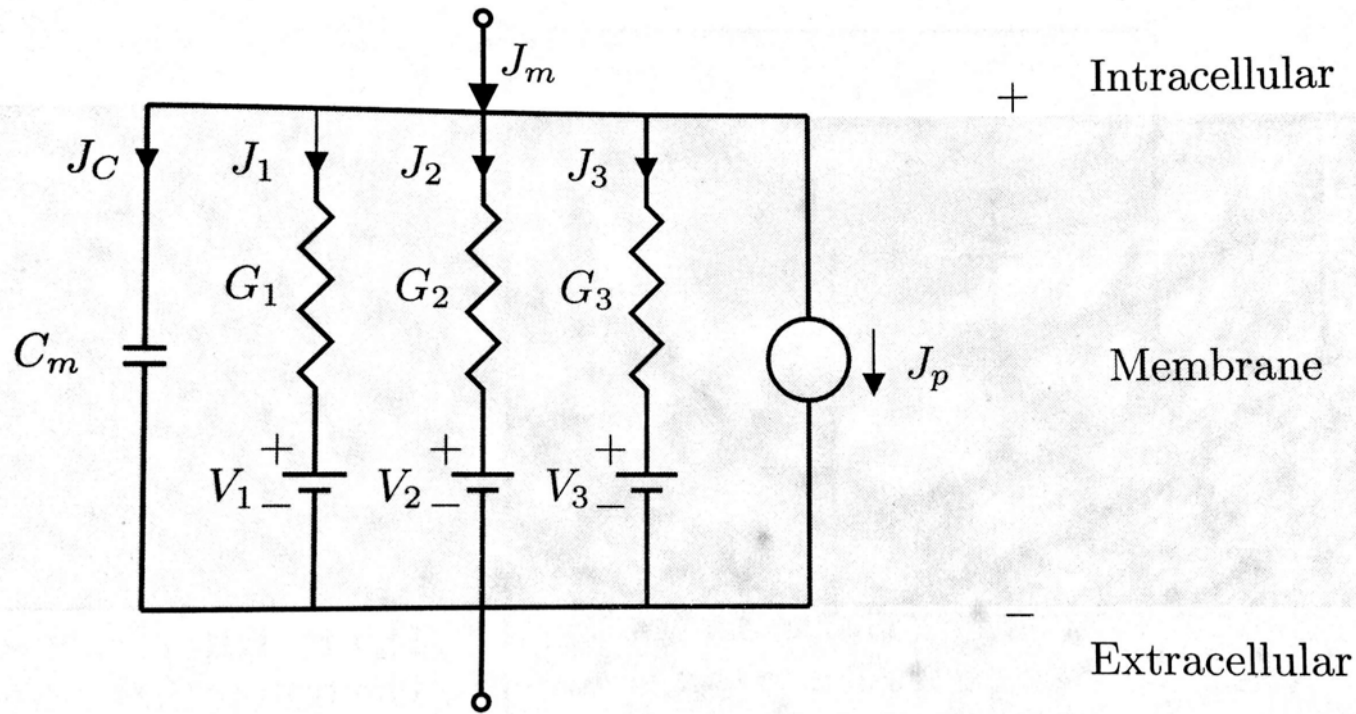
Figure 7.24

Equivalent

## Lipid Bilayer Acts as a Capacitor

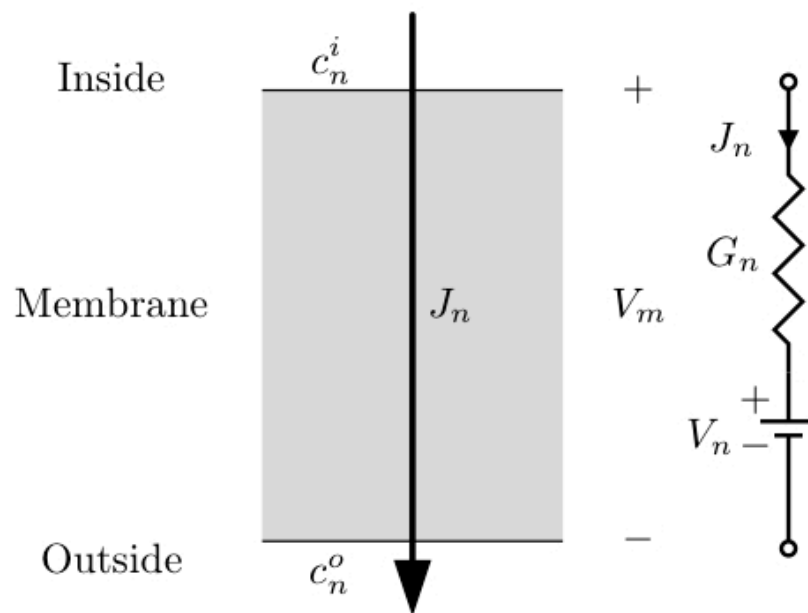


## Circuit Model for Membrane



**Figure 1.18** Macroscopic model of a unit area of membrane.  $C_m$  represents the capacitance of a unit area of membrane and represents the insulating properties of the lipid bilayer.  $G_n$  represents the conductance of a unit area of membrane for the  $n^{\text{th}}$  ion.  $V_n$  is the Nernst equilibrium potential of the  $n^{\text{th}}$  ion. The series combination of  $G_n$  and  $V_n$  represents conduction through a population of ion channels permeable to ion  $n$ .  $J_p$  represents the net current density due to other mechanisms, primarily the current carried by active transport mechanisms.

## Model of Steady-State Electrodifusion through Membranes





## Eqns. of Electrodifusion

### Nernst-Plank Equation

$$J_n(x, t) = -z_n F D_n \frac{\partial c_n(x, t)}{\partial x} - u_n z_n^2 F^2 c_n(x, t) \frac{\partial \psi(x, t)}{\partial x}$$

### Continuity

$$\frac{\partial J_n(x, t)}{\partial x} = -z_n F \frac{\partial c_n(x, t)}{\partial t}$$

### Poisson's Equation

$$\frac{\partial^2 \psi(x, t)}{\partial x^2} = -\frac{1}{\epsilon} \sum_n z_n F c_n(x, t)$$

## Nernst-Plank Equation → Electrodiffusion

current  
density

$$J_n(x, t) = \underbrace{-z_n F D_n \frac{\partial c_n(x, t)}{\partial x}}_{\text{diffusion}} - \underbrace{u_n z_n^2 F^2 c_n(x, t) \frac{\partial \psi(x, t)}{\partial x}}_{\text{electric drift}}$$

diffusion

electric  
drift

