Bioengineering 6003

Cellular Electrophysiology and Biophysics

Cardiac cell-cell Communication

Part 1

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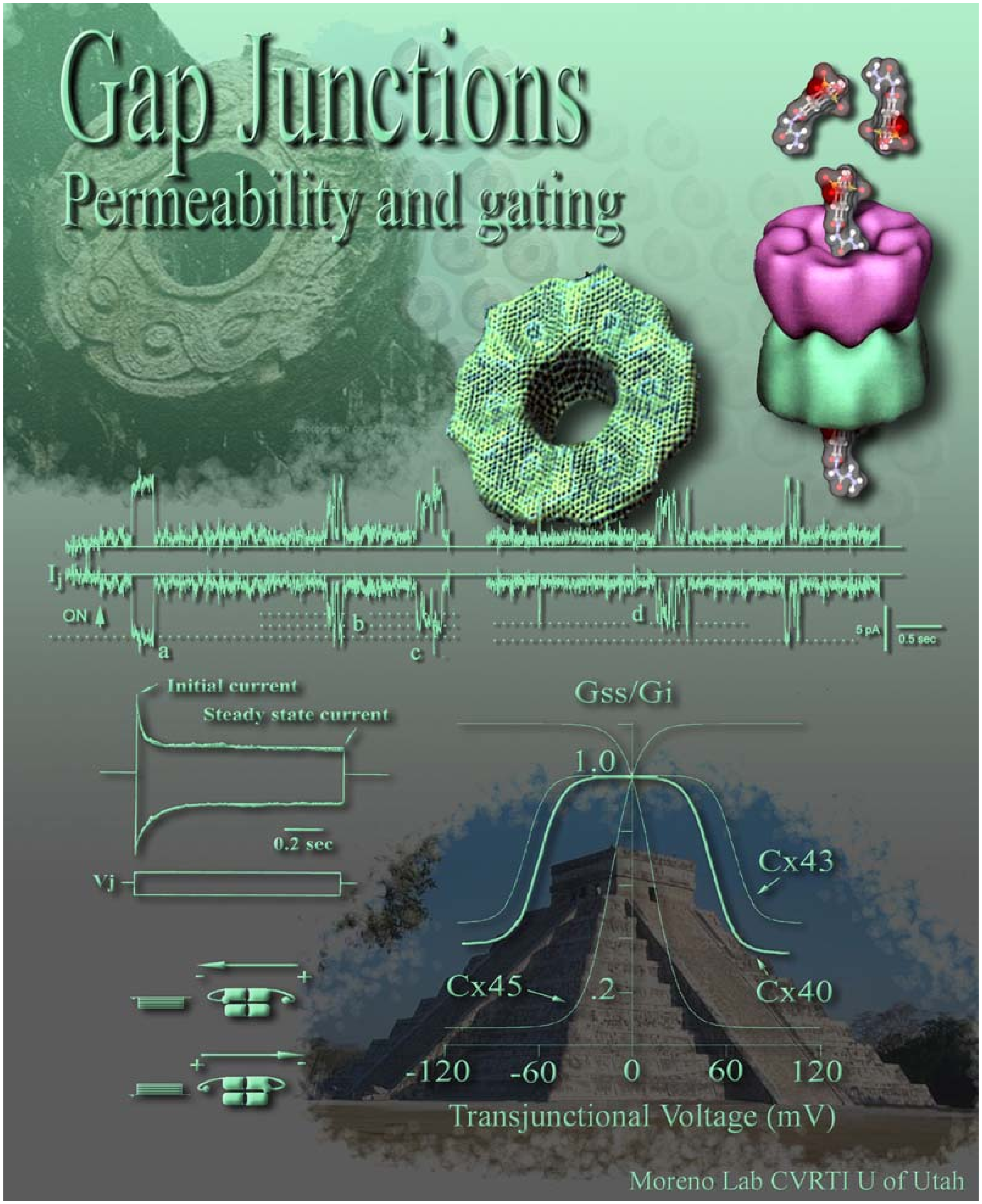


Gap Junctions
Permeability and gating
Ij
ON
a
b
c
d
5 pA
0.5 sec
Initial current
Steady state current
0.2 sec
Vj
Gss/Gi
1.0
.2
Cx43
Cx40
Cx45
-120
-60
0
60
120
Transjunctional Voltage (mV)
Moreno Lab CVRTI U of Utah

Physiological Relevance and Diseases associated with gap junctions.

Gap junctions allow the propagation of action potentials through the heart.

- In physiological conditions, the rapid propagation of action potentials through the heart permits the musculature from different regions of the heart to respond in a synchronous manner.
- Metabolites and other ions can cross between cell providing it with tissue homeostasis or cellular segregation during development

Cell-to-cell communication

- Functional junctions in invertebrates (Furshpan y Potter, 1959)
- Nexus in Heart (Dewey and Barr 1960)
- Plasmodesmata in Plants (Higginbotham 1970)

- Main protein of gap junctions (Saez-Beyer, 1986-87)
- Connexins (Beyer and Goodenough, 1989)
- Innexins in Invertebrates (Phelan, 1998)
- Multiple homologs of innexins in various taxonomic groups forced for a new name: Pannexins (Panchin, 2000)
- Viral homologs of pannexins have been found in PolyDNA viruses have been called Vinnexins (Turnbull and Webb, 2005)

Cell to cell communication through gap junctions (quick overview)

•Occurs when the cytoplasm of cells are in direct contact.

•The structures involved are intercellular channels.

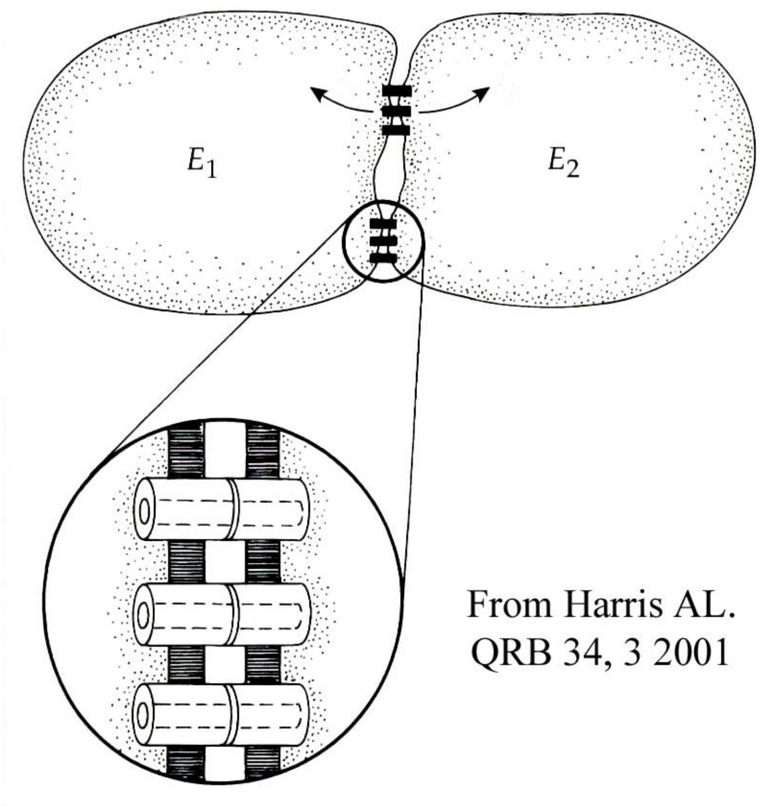
•Molecules and ions of different size and charge can cross.

•Max. molecular weight of particles that rapidly cross ~ 1200 Da

•Selectivity and gating depend on the constituent isoform.

•Signaling molecules can cross from one cell to another and can also regulate the communication between cells.

Gap junctions communicate directly the intracellular milieu of adjacent cells



From Harris AL.
QRB 34, 3 2001

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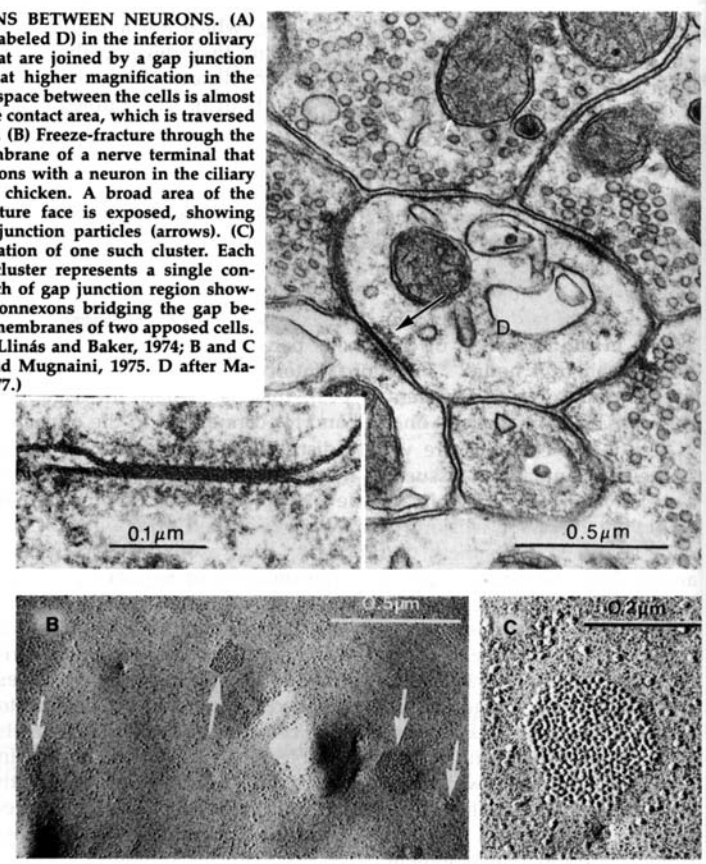
GAP JUNCTIONS BETWEEN NEURONS. (A) Two dendrites (labeled D) in the inferior olivary nucleus of the cat are joined by a gap junction (arrow), shown at higher magnification in the inset. The usual space between the cells is almost obliterated in the contact area, which is traversed by cross bridges. (B) Freeze-fracture through the presynaptic membrane of a nerve terminal that forms gap junctions with a neuron in the ciliary ganglion of the chicken. A broad area of the cytoplasmic fracture face is exposed, showing clusters of gap junction particles (arrows). (C) Higher magnification of one such cluster. Each particle in the cluster represents a single connexon. (D) Sketch of gap junction region showing individual connexons bridging the gap between the lipid membranes of two apposed cells. (A from Sotelo, Llinás and Baker, 1974; B and C from Cantino and Mugnaini, 1975. D after Makowski et al. 1977.)



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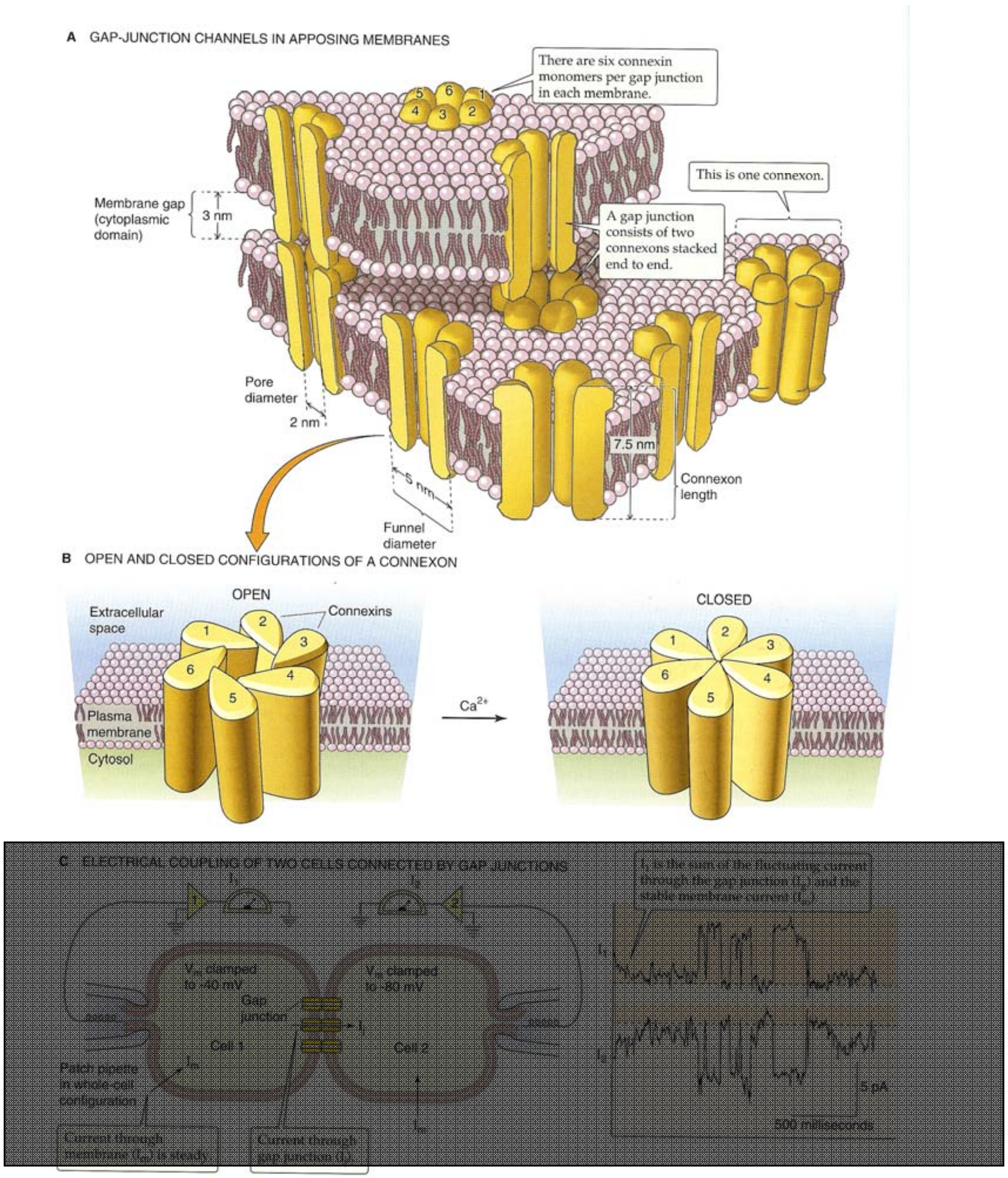
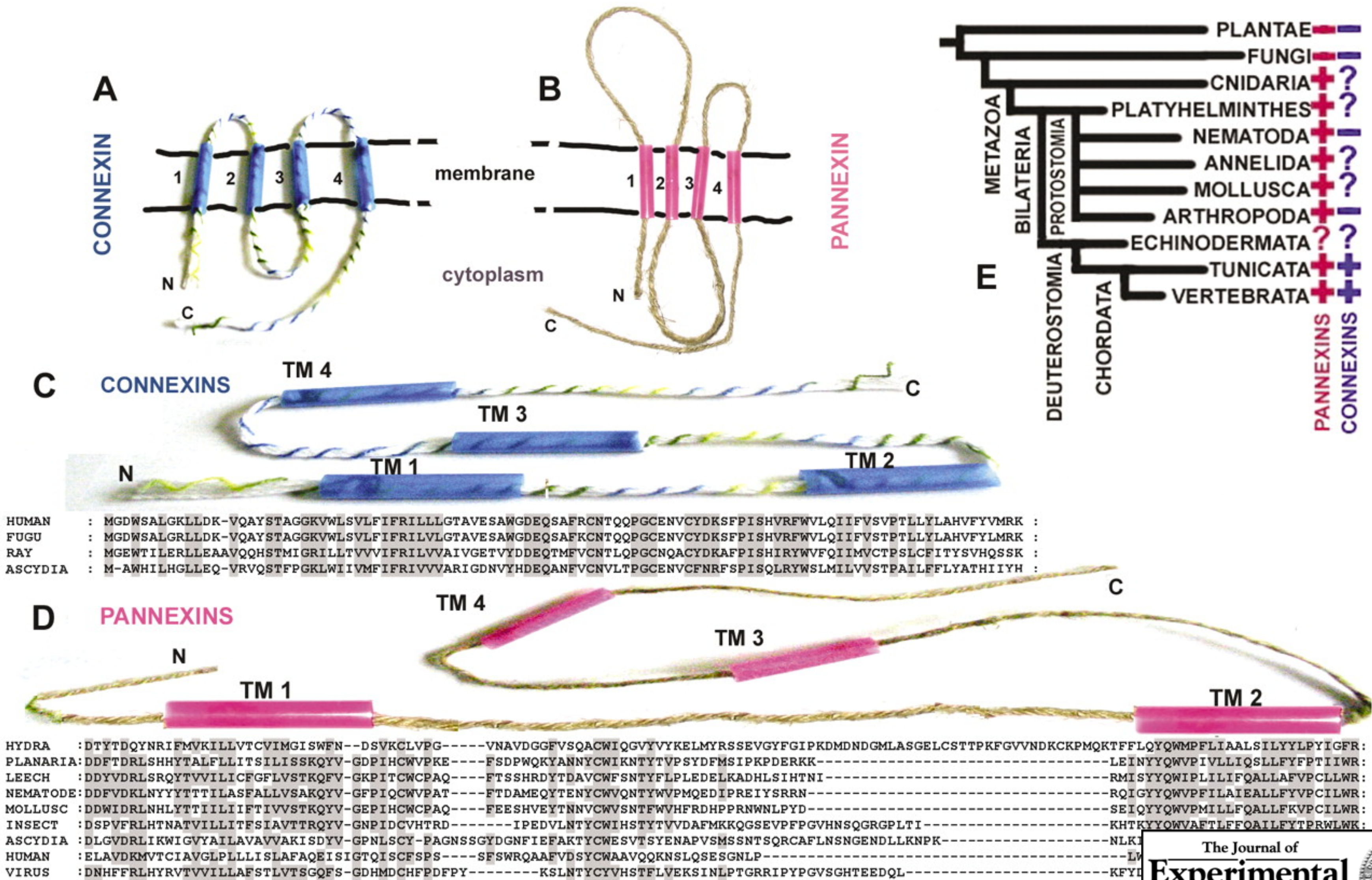


Structure of gap junction channels

Connexins and Pannexins Molecular Structure



Panchin, Y. V. J Exp Biol 2005;208:1415-1419

The Journal of Experimental Biology

Distribution

Gap junctions are present in almost all adult and embryonic tissues in vertebrates and invertebrates.
Important exceptions in mammals are the adult striated voluntary musculature and the blood free cells.

Some connexins are expressed preferentially in certain tissues

Brain	Neurons	Cx36
	Glia	Cx43, Cx32, Cx26
Heart		Cx40, Cx43, Cx45, Cx30.2
Liver		Cx32, Cx26
Skin		Cx26, Cx43
Smooth muscle		Cx43, Cx37
Eye lens		Cx46, Cx50, Cx43

Genetic diseases where connexins are involved

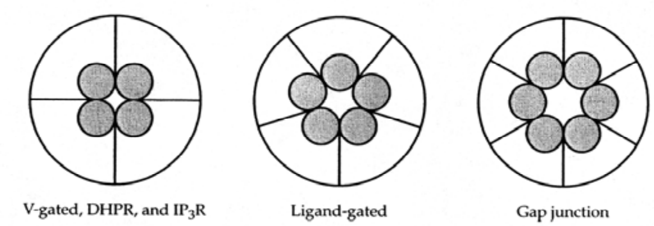
Cx26	Nonsyndromic deafness
Cx31	Aut. dominant Erythrokeratodermia
Cx32	Peripheral Neuropathy (CMTX)
Cx40	Aut. Heart conduction disorder
Cx43	Viceroatrial Heterotaxia
Cx46/50	Cataracts

Molecular organization of a gap junction channel

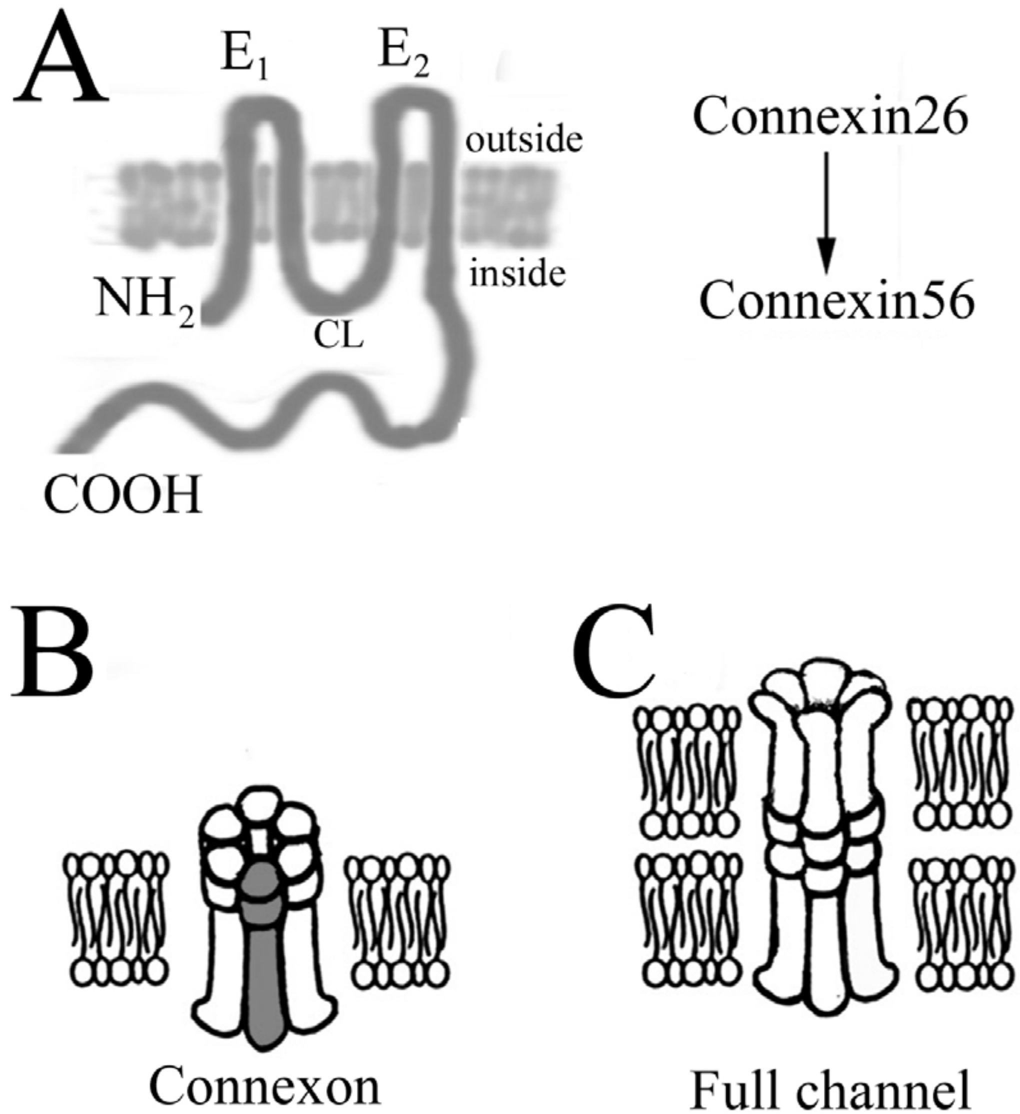
•Connexins are a family of homologous proteins that conform the intracellular channels.

•Currently 16 different connexins have been cloned from mammalian tissues. We know that there are only 22 in the human genome.

•Twelve subunits are necessary to form a complete

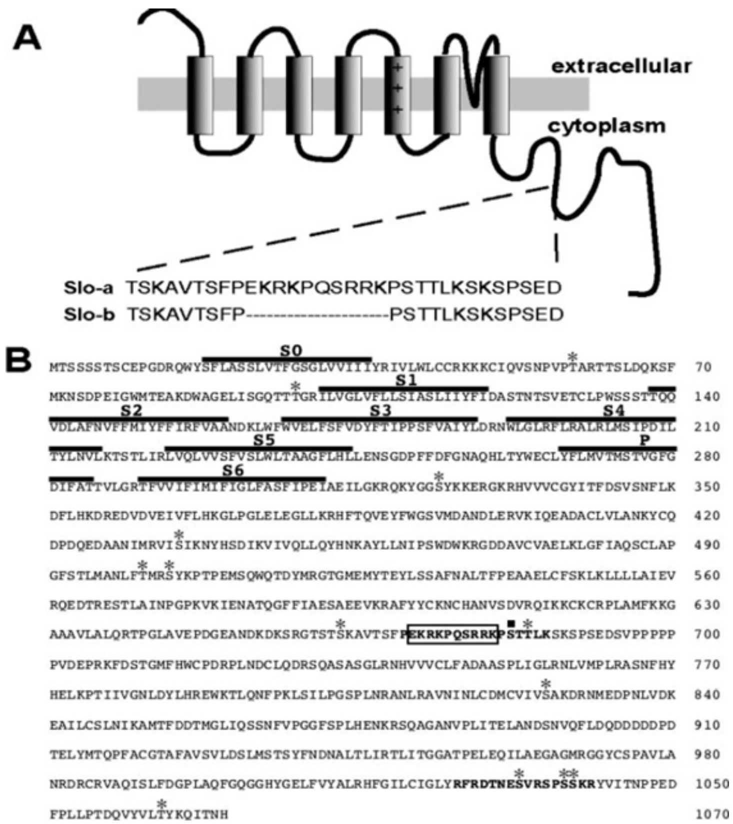


13.15 Symmetry of Different Channels Diagrammatic packing of four, five, or six subunits to make progressively larger pores. Abbreviations: DHPR, dihydropyridine receptor; IP_3R, IP_3 receptor.



K+ channels splicing

No splicing in gap junction channels



Six to seven transmembrane domains

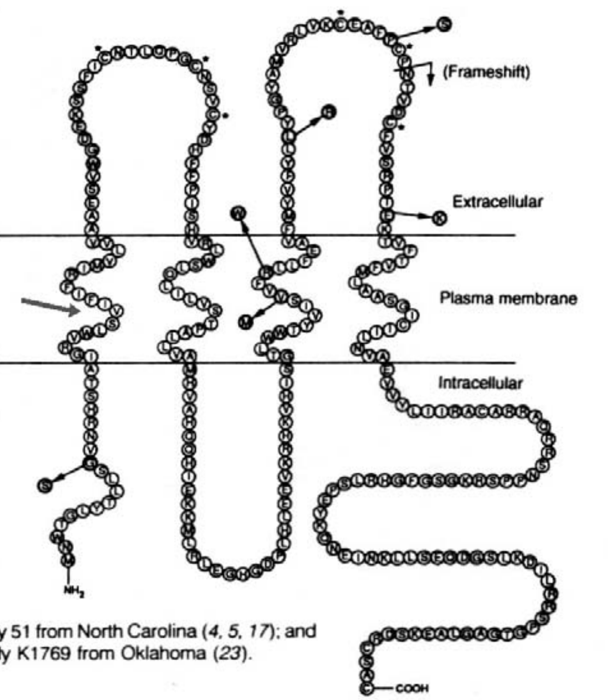
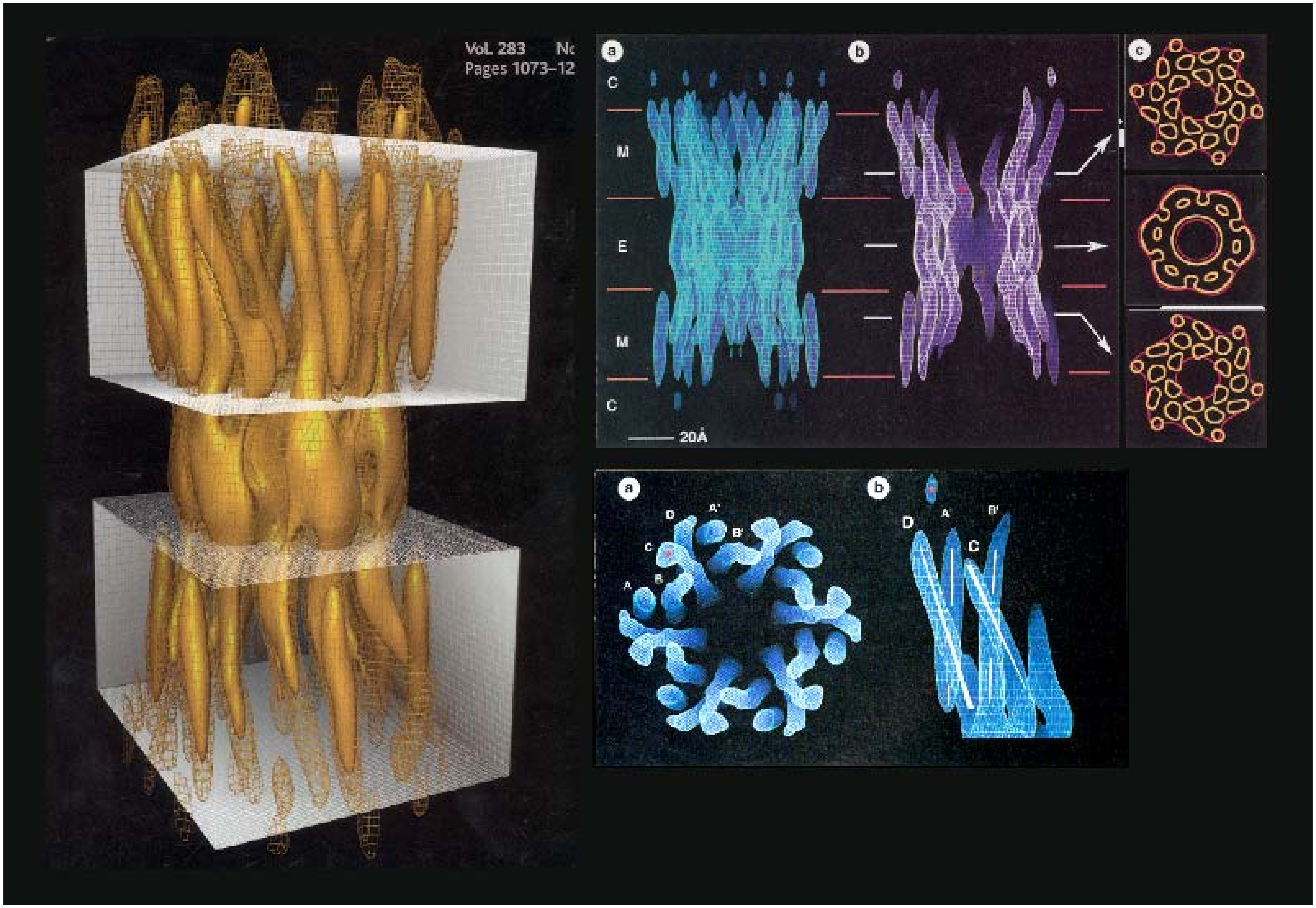


Fig. 3. Diagram of Cx32, showing transmembrane orientation, conserved cysteine residues (asterisks), and locations of the CMTX mutations (arrows) (*21*). The Cx32 structure is based on (*10*, *16*). The indicated mutations are as follows: G12S, GGC → AGC in family 58 from Belgium (*4*, *22*); V139M, GTG → ATG in families 221 and K1905 from South Dakota and Michigan, respectively [discussed in this paper and (*23*)]; R142W, CGG → TGG in family 243 from Pennsylvania (discussed in this paper); L156R, CTC → CGC in family 251 from Pennsylvania (discussed in this paper); P172S, CCC → TCC in family 133/K1852 from North Carolina (*5*, *23*); 175 frameshift, A insertion in family 51 from North Carolina (*4*, *5*, *17*); and E186K, GAG → AAG in family K1769 from Oklahoma (*23*).

SCIENCE • VOL. 262 • 24 DECEMBER 1993

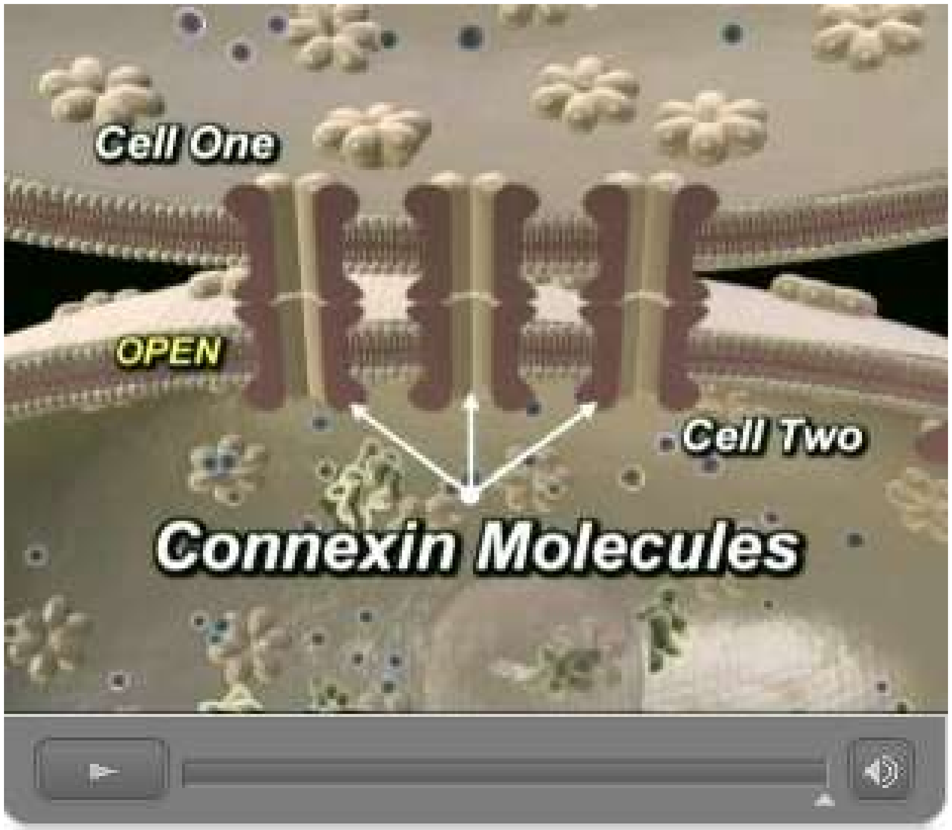
Four transmembrane domains

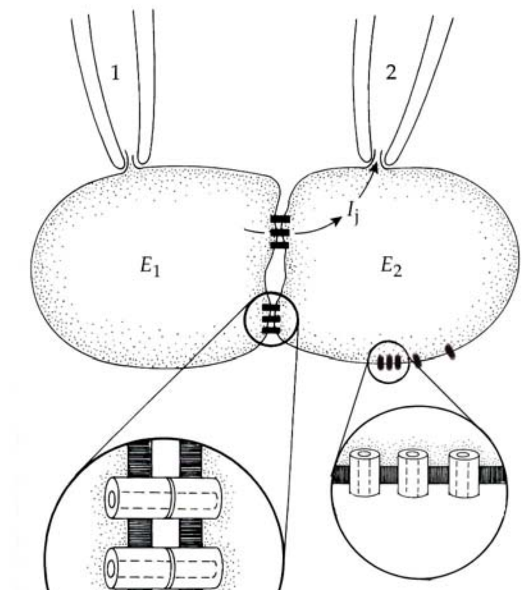
Gap junction channel ultra-structure



Yeager et al, Science 283,1999

Full channels and hemichannels





From Harris AL.
QRB 34, 3 2001

Pannexins: The unexpected cousins that provide membrane permeability?

They may be responsible for many published data indicating that Cx43 hemi-channels were the substrate for increases in membrane permeability during cellular stress.

- They form junction channels in oocytes and in between glia and other brain cells.
- They also form hemichannels, as connexins.
- They can be opened by cellular damage and free radicals.
- They are responsible for ATP release in neurons
- But their function in the heart has not been determined altough could be responsible for partial depolarization and hyperactivity during stress.

Regulation of intercellular communication

- It is simple

  Electrically we evaluate gj or junction conductance

$$\mathbf{g_j = n * \gamma_j * Po}$$

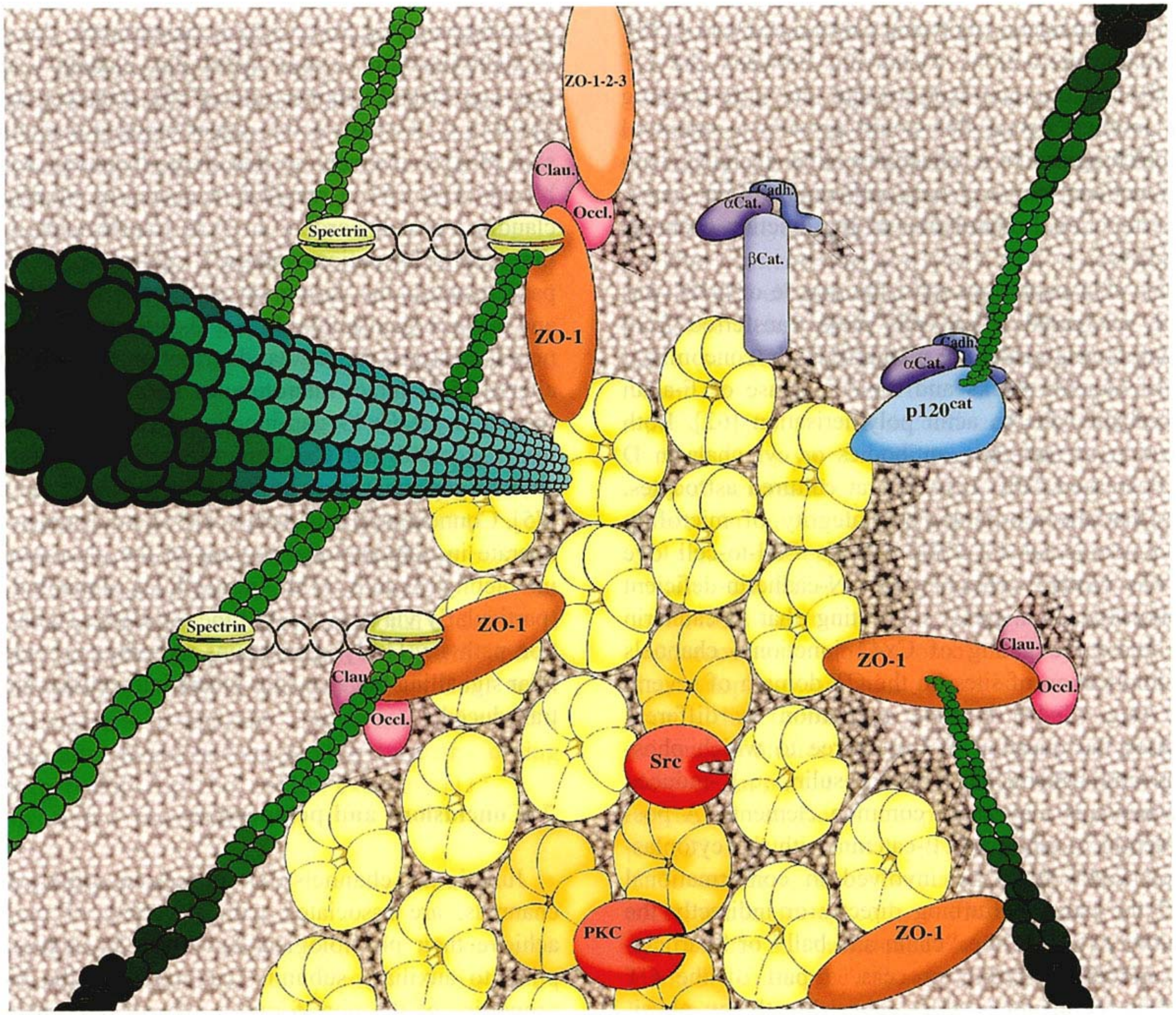
n = number of channels (Insertion-removal)

γ_j = unitary conductance (Phosphorylation)

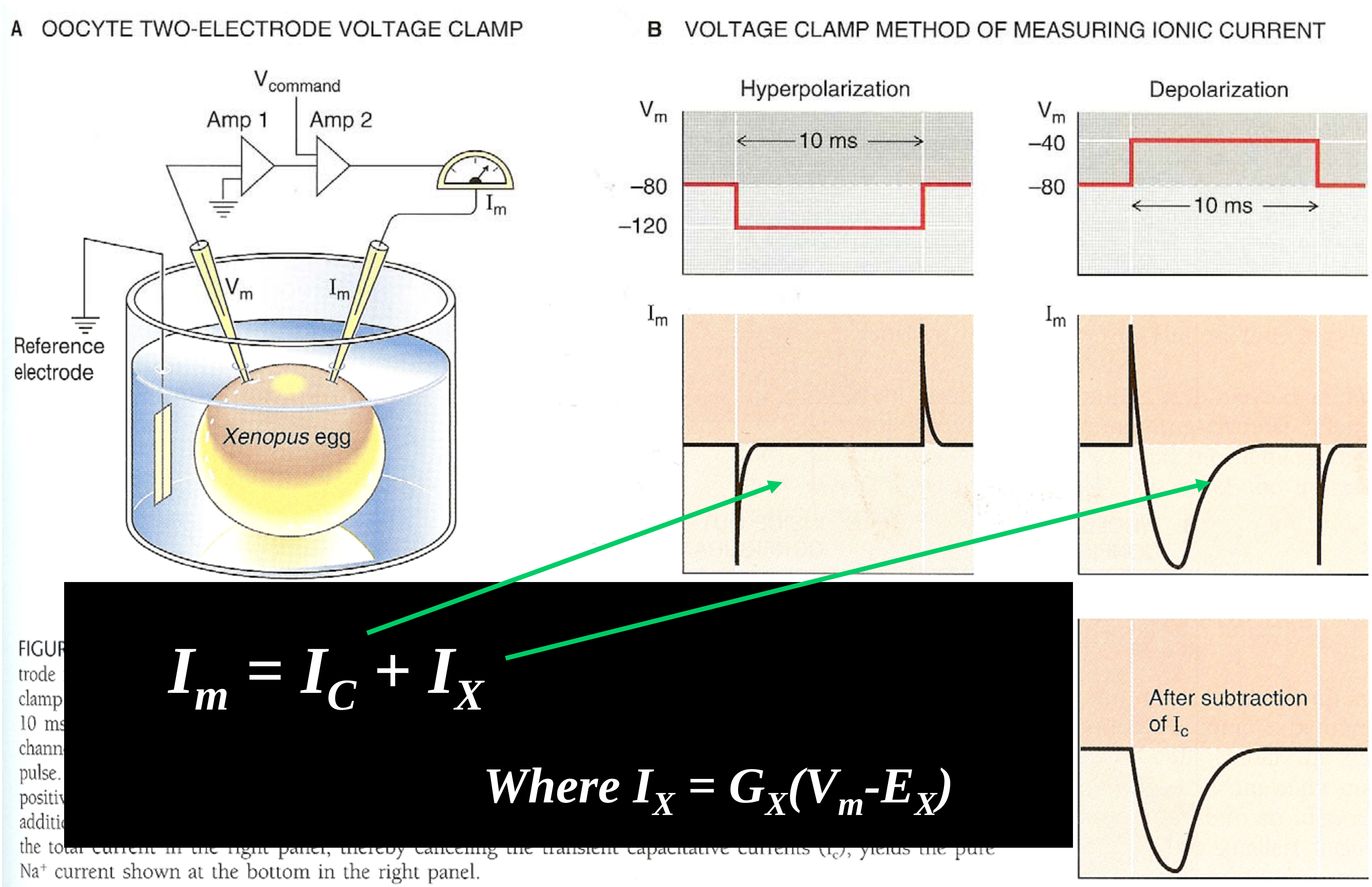
Po = open probability (gating e.g. pH, PO4)

Connexin channels are not alone

J.-C. Hervé et al. / Biochimica et Biophysica Acta 1662 (2004) 22–41



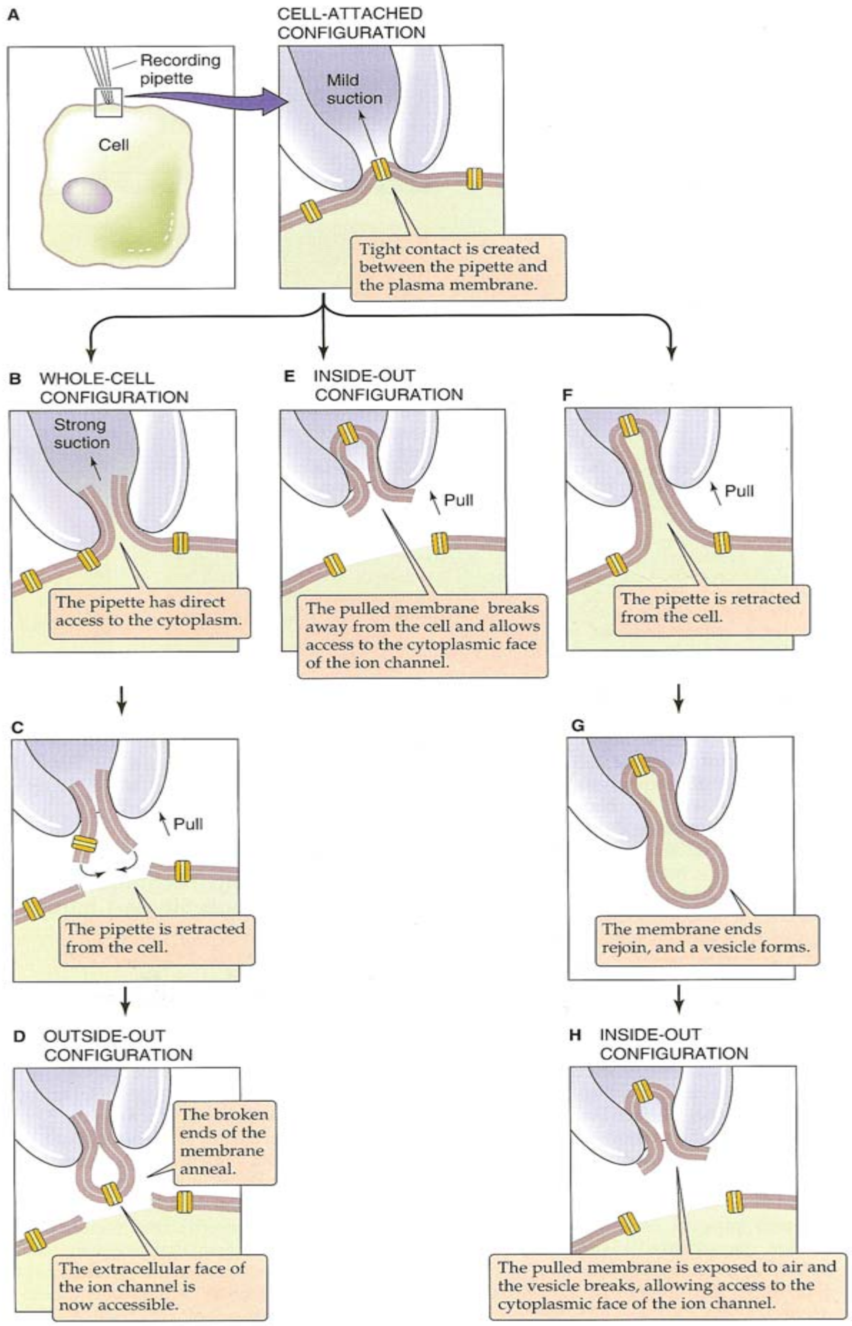
Whole Cell Voltage Clamp

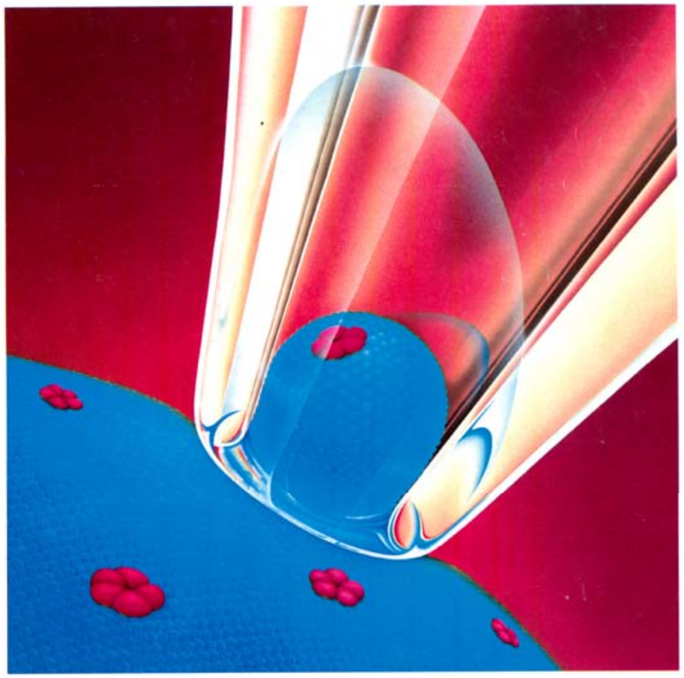


$$I_m = I_C + I_X$$

Where $I_X = G_X(V_m - E_X)$

FIGUR [redacted] trode [redacted] clamp [redacted] 10 ms [redacted] chann [redacted] pulse. [redacted] positi [redacted] additi [redacted] the to [redacted] Na^+ current shown at the bottom in the right panel.





Tiny pipette *isolates a pore-forming protein that allows signals to pass through cell membranes.*

The Patch-Clamp Technique to study for hemichannel function

Double whole cell voltage clamp and gating of gap junction channels

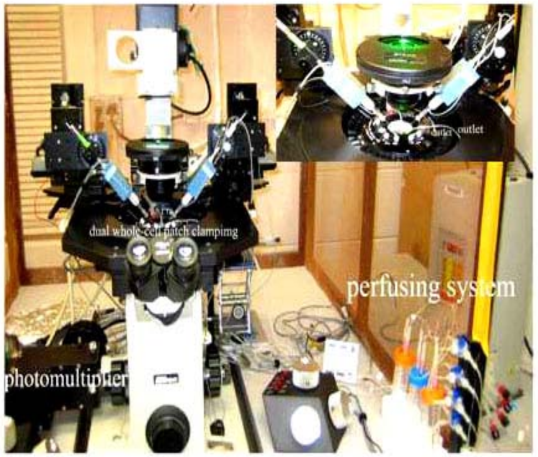
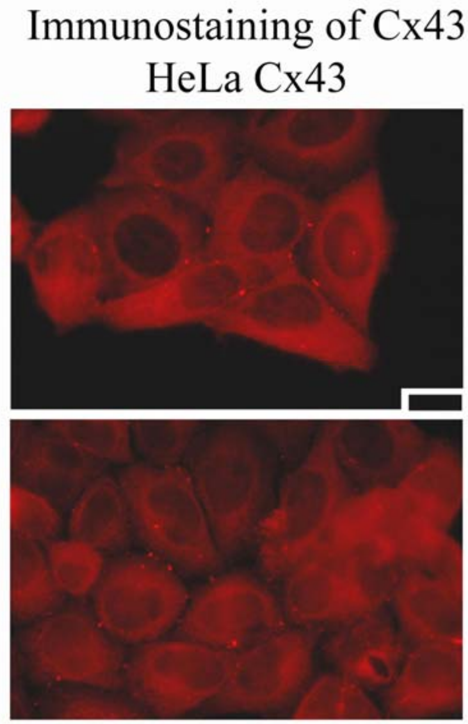
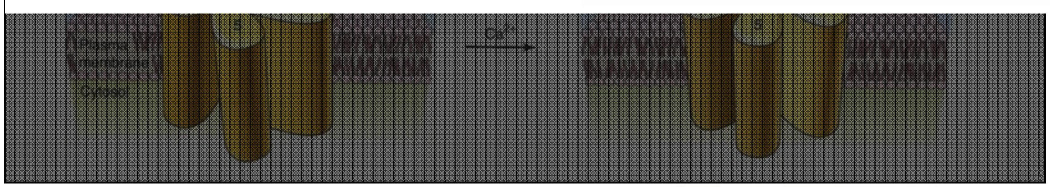
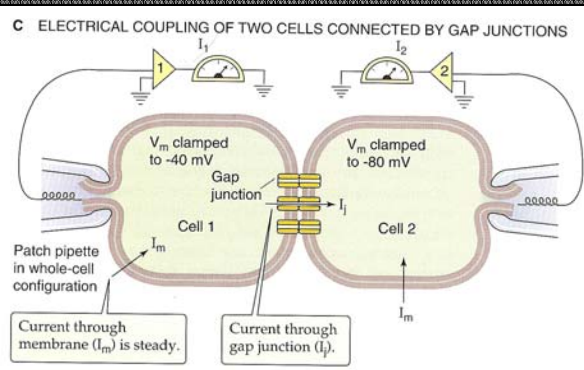
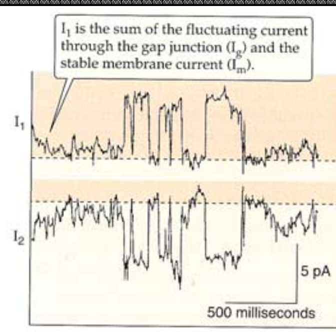


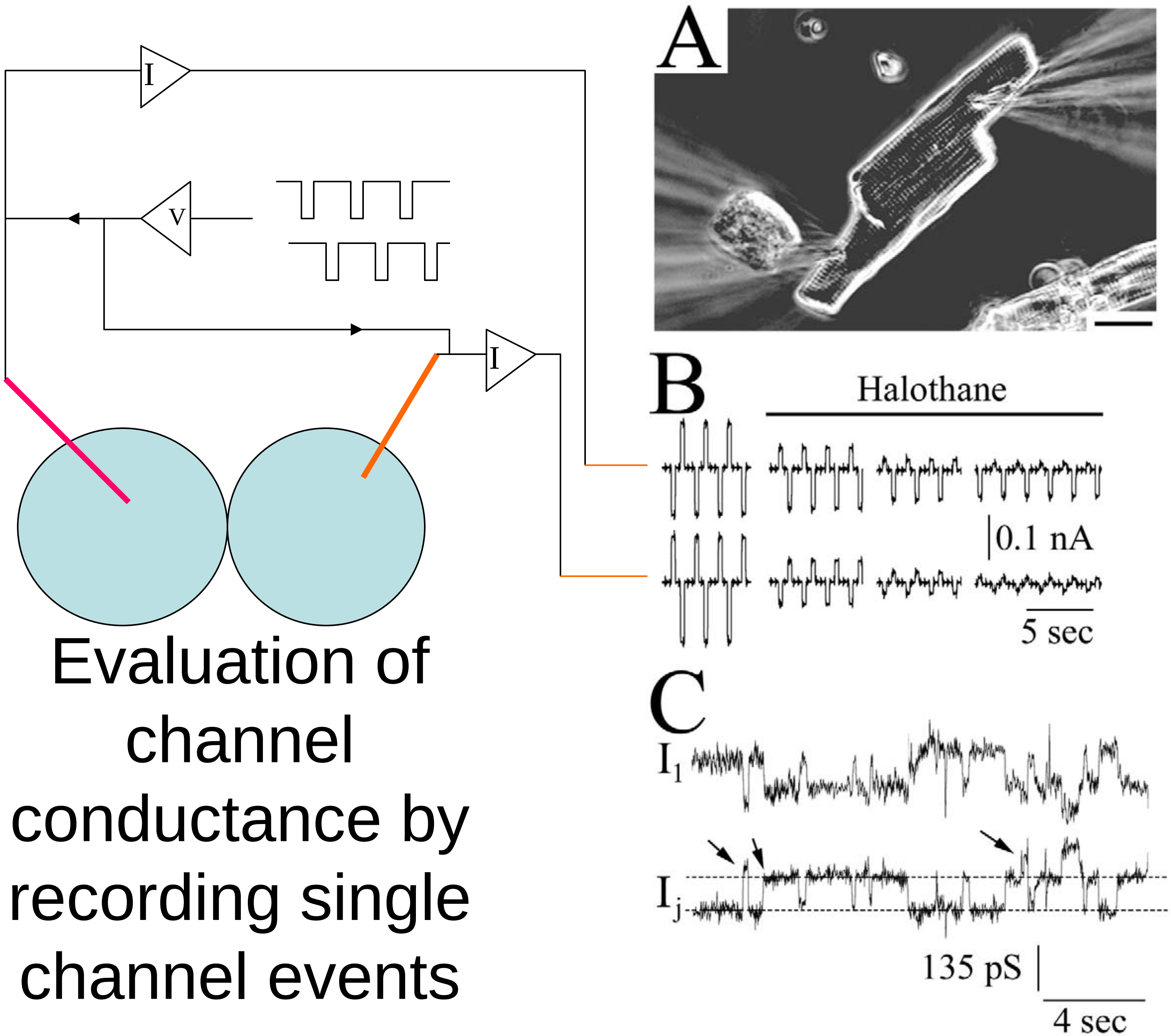
Figure 1. Double whole cell voltage clamp recording set up featuring the perfusion chamber and the photomultiplier required to detect pHi changes.





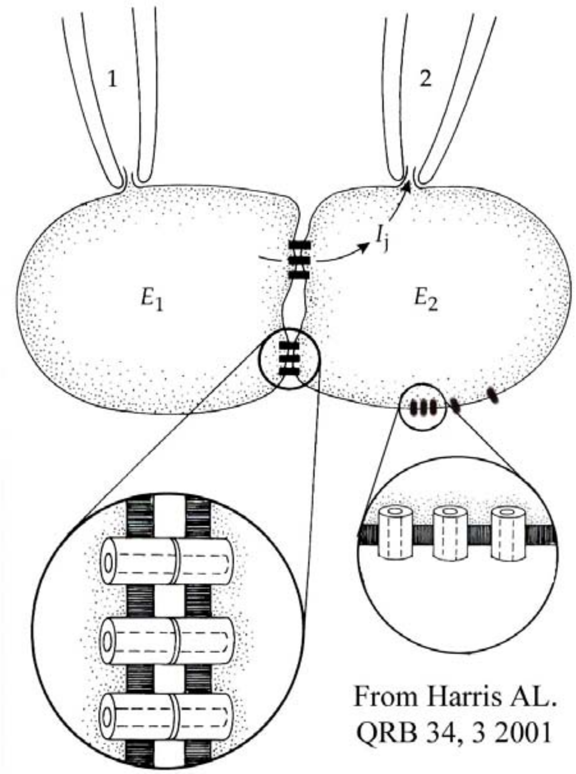




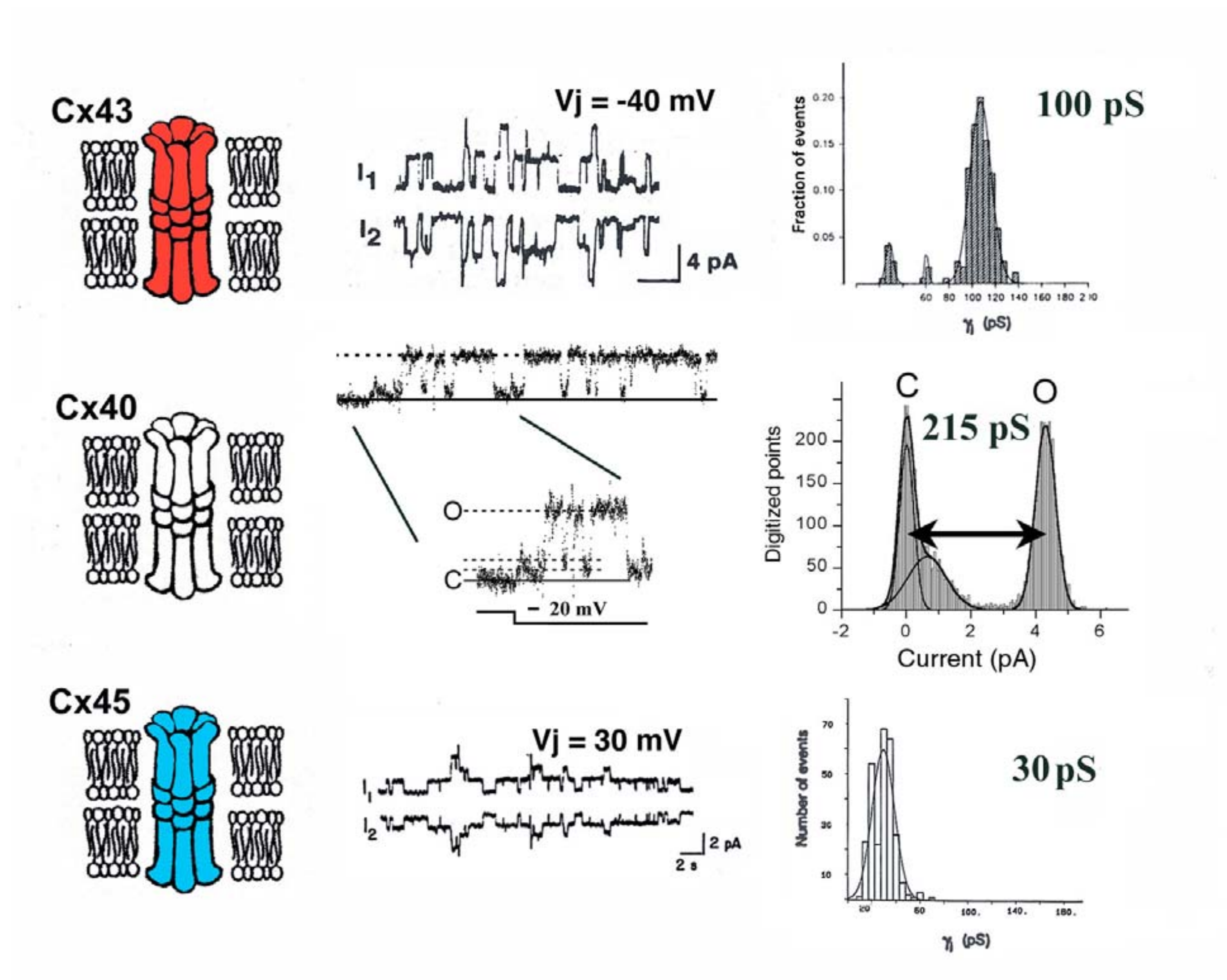


Evaluation of channel conductance by recording single channel events

Unitary conductances of connexins



From Harris AL.
QRB 34, 3 2001

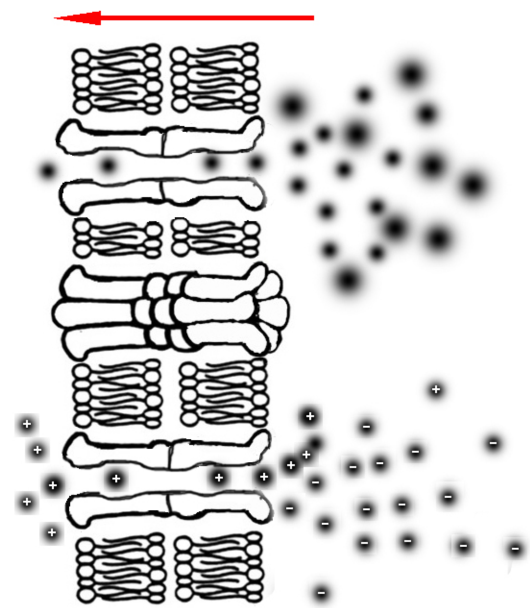


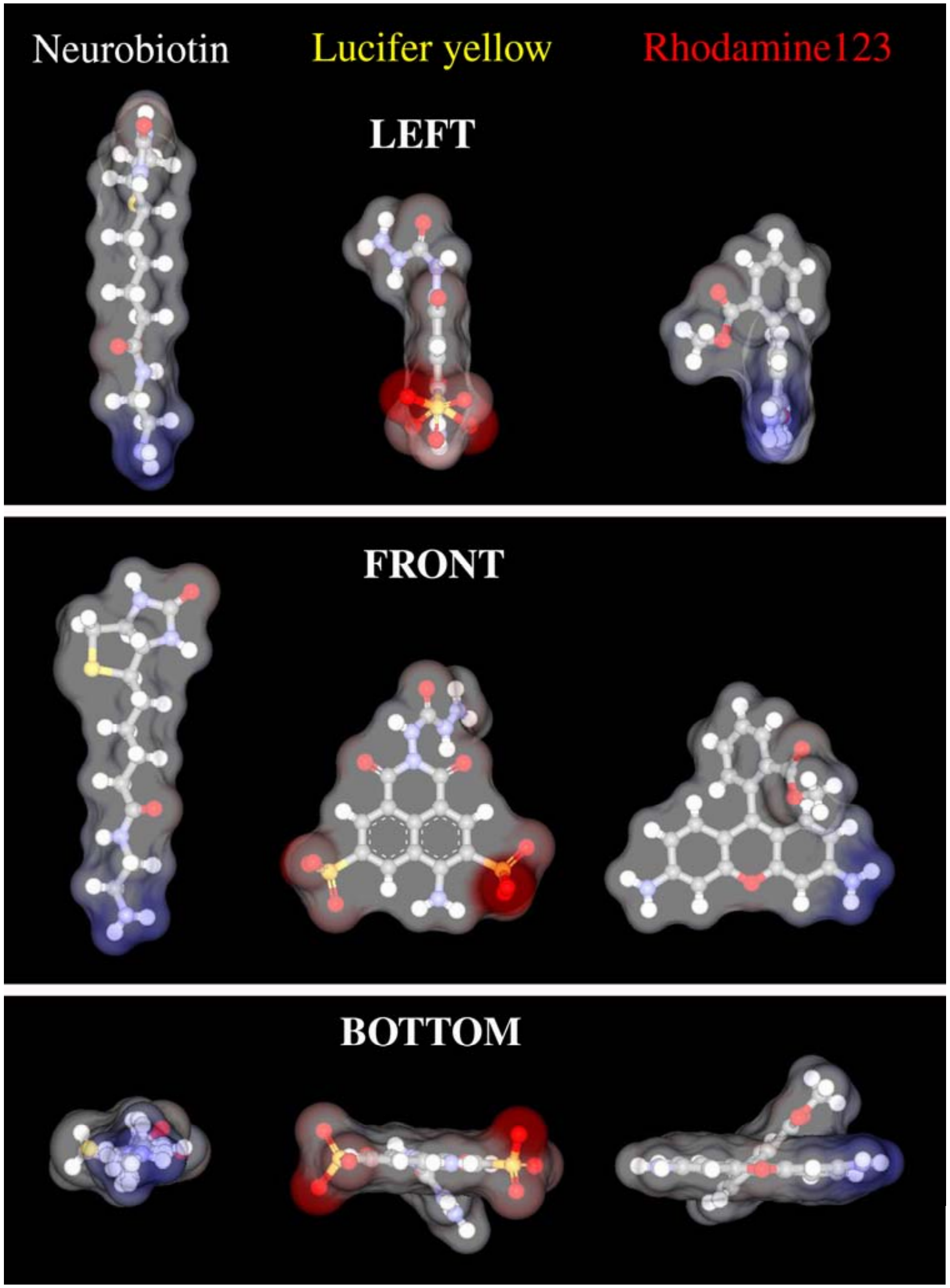
Permeance and selectivity

The perm-selectivity of molecules across gap junction channels is a complex phenomenon.

Various factors determine if a particle permeates across a gap junction channel

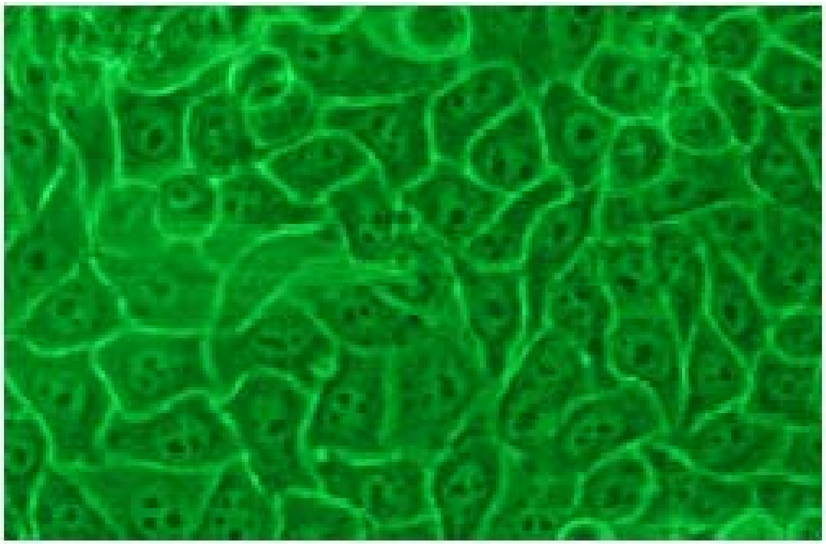
1) The size of the particle
2) The electric charge of the particle
3) Structure and isoform composition of th channel
4) Particle-channel interaction and binding

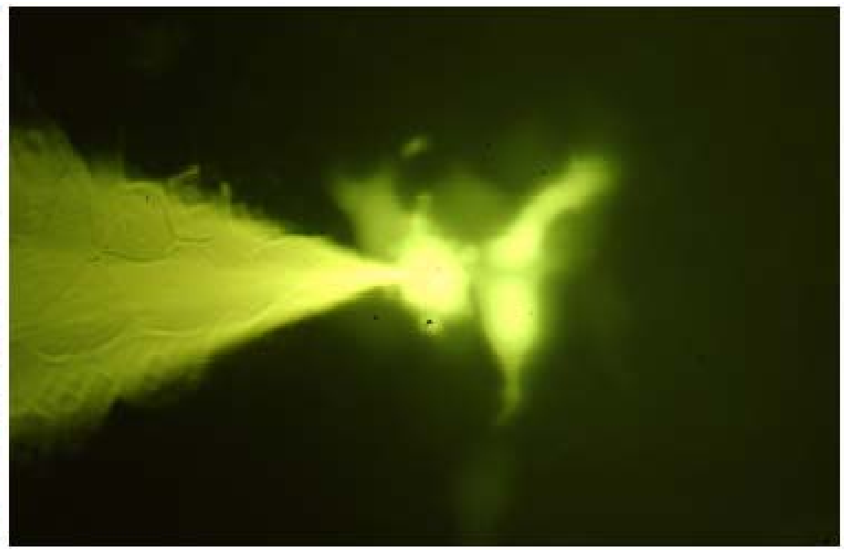




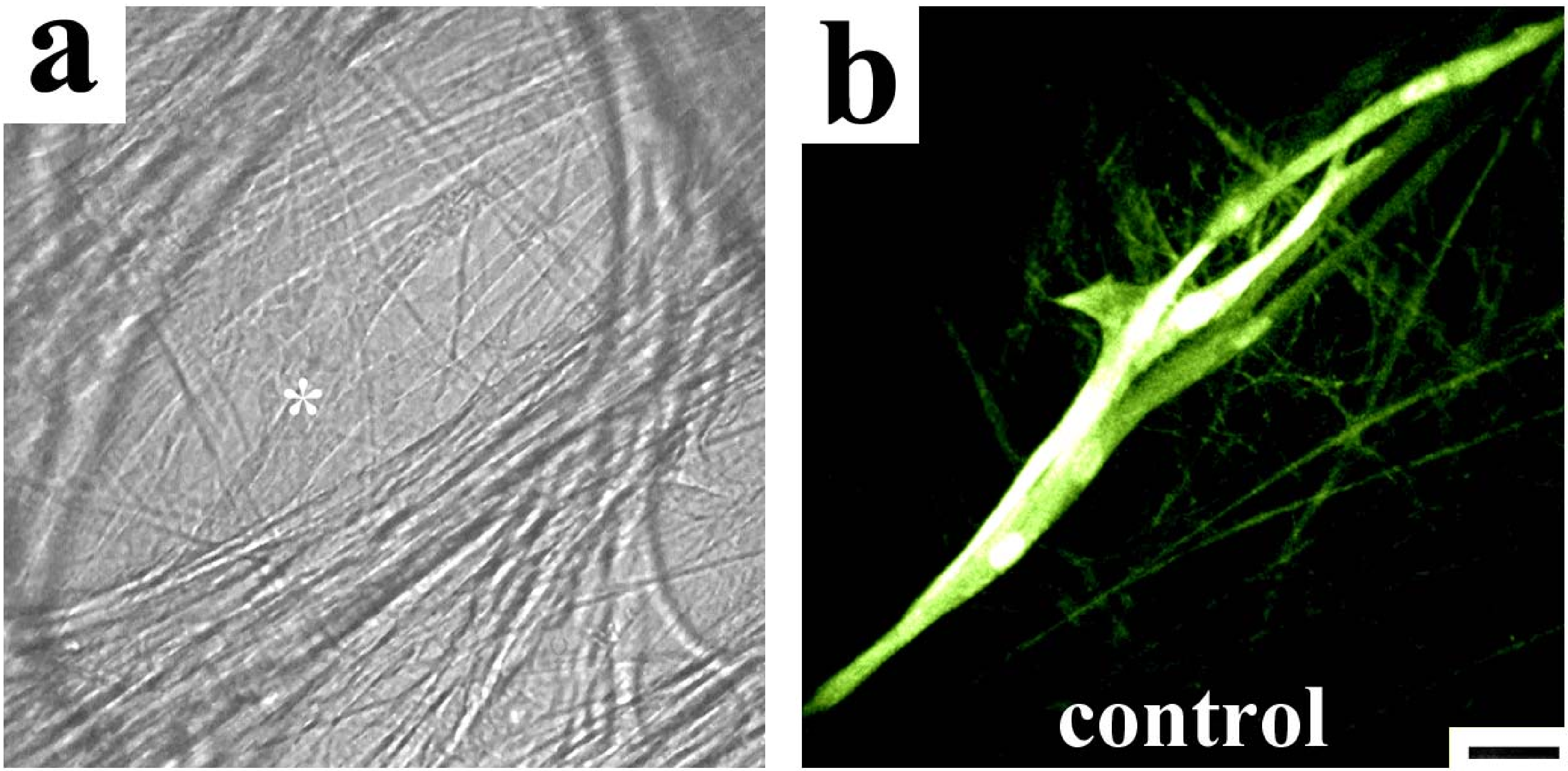
Fluorescent molecular probes that help to test permeance

Intercellular communication is detected using fluorescent dyes

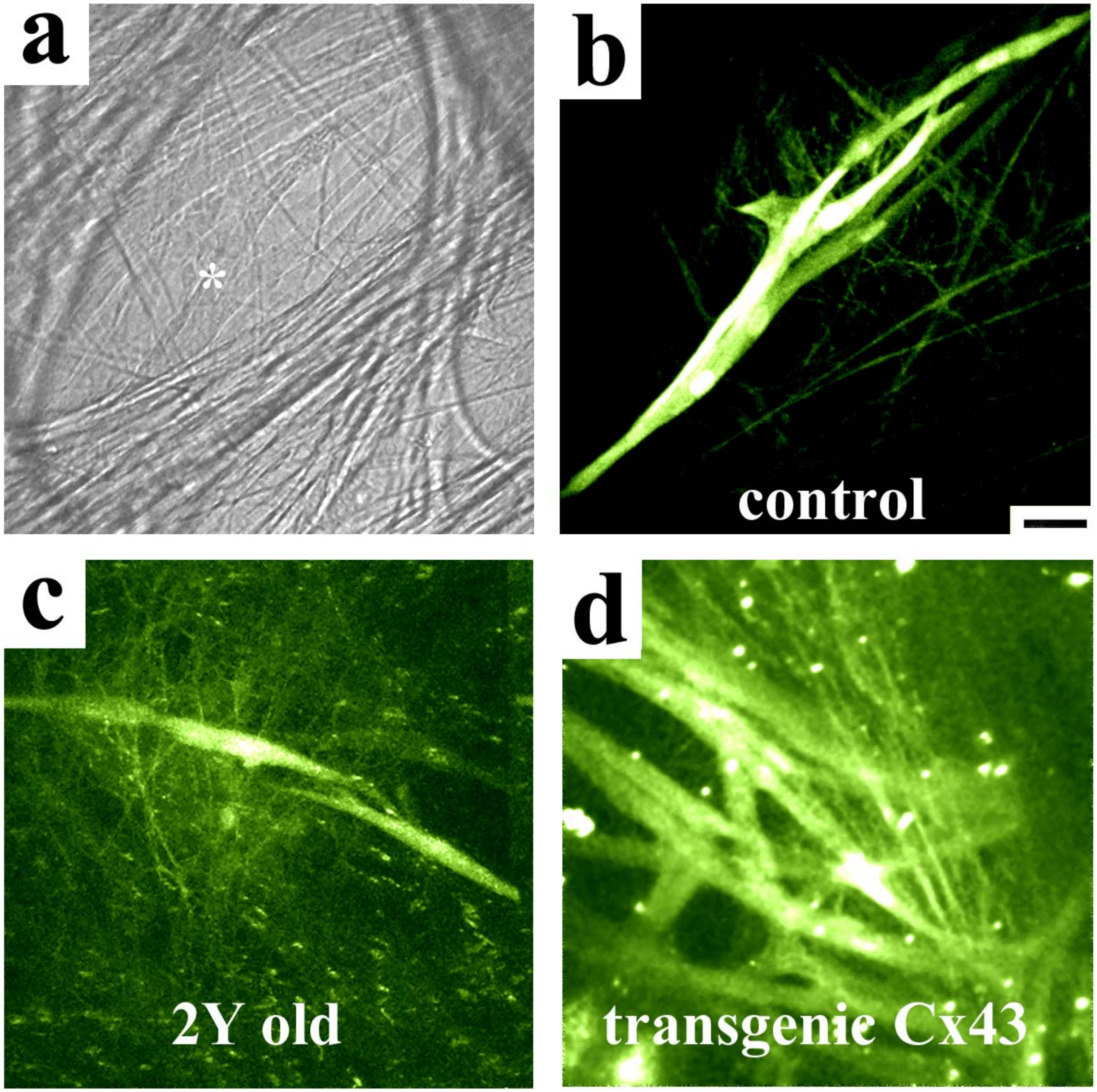




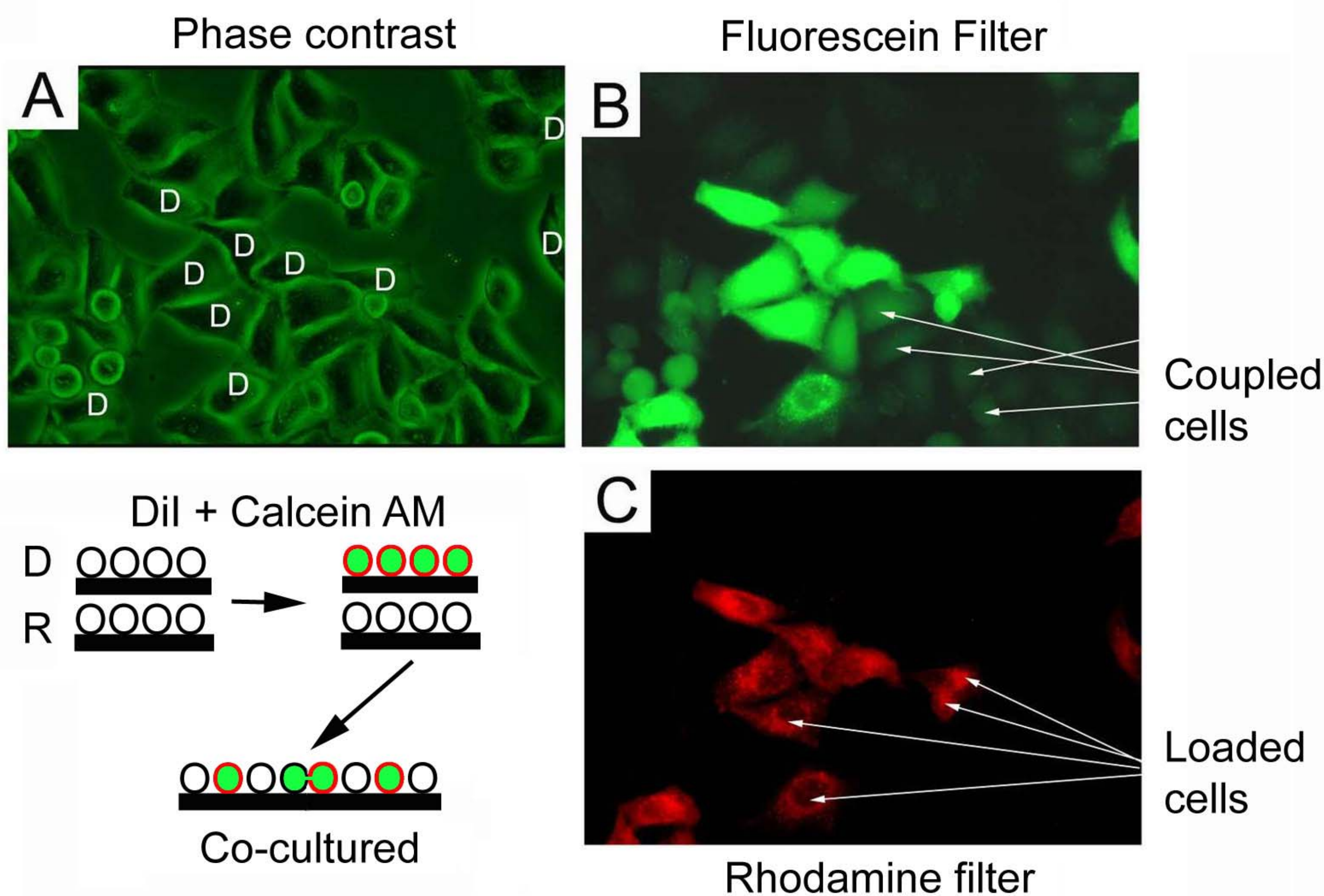
Lucifer yellow permeance in control murine atria



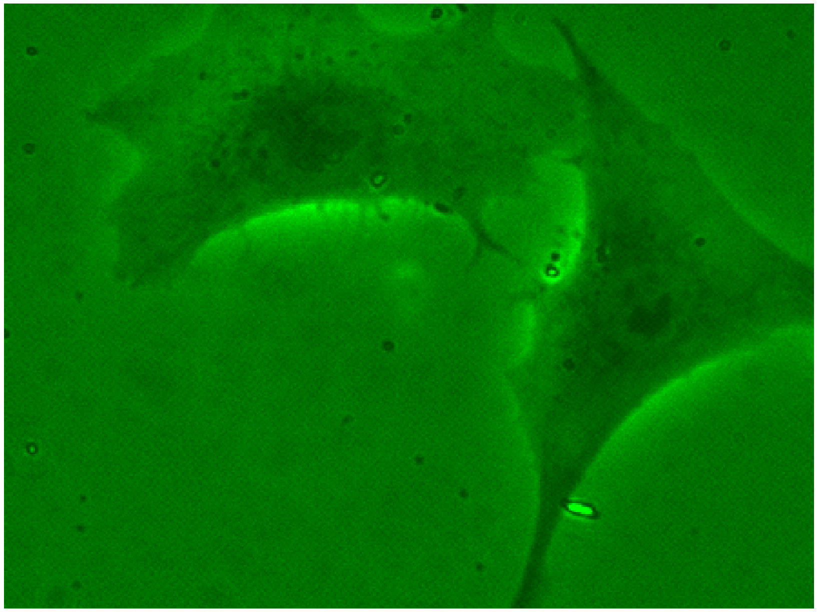
Lucifer yellow permeance in murine atria.

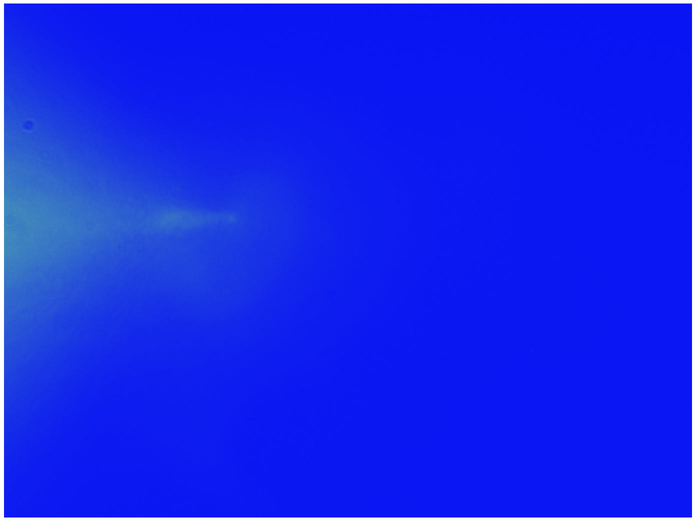


Permeance by cell drop

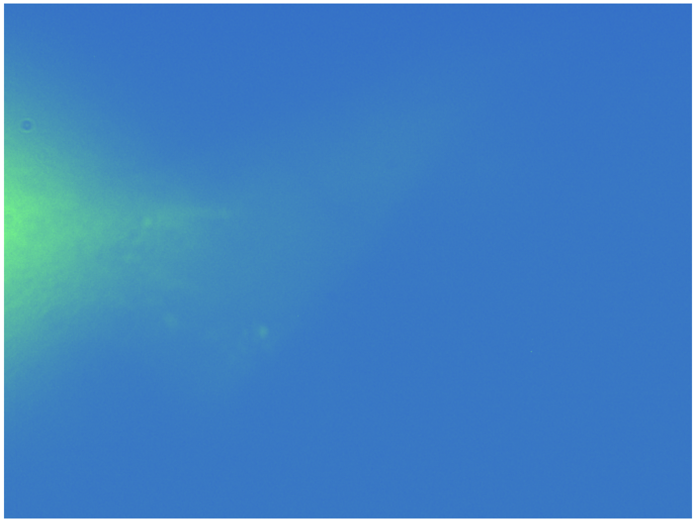


Molecular flux



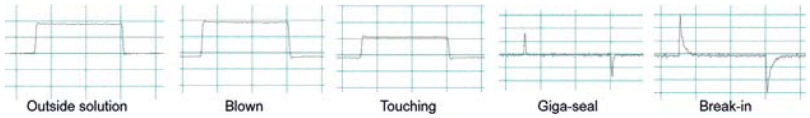


Homotypic Cx43-Cx43

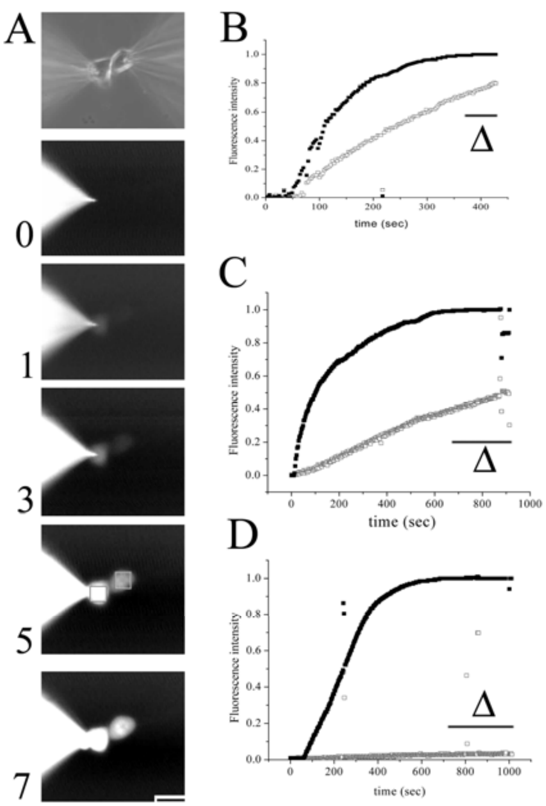


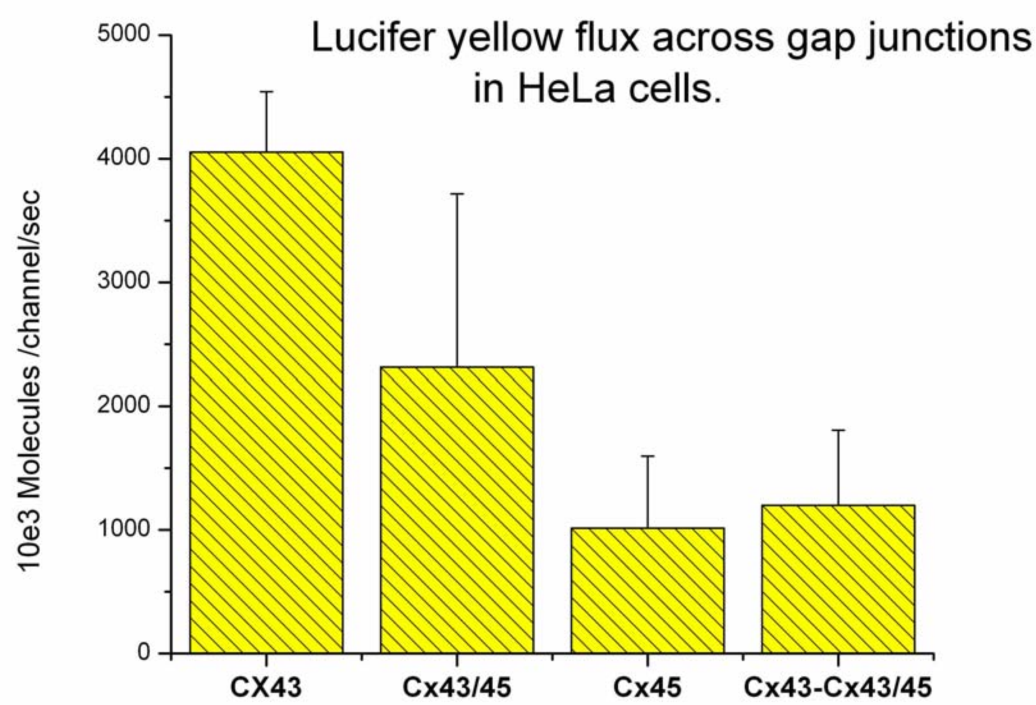
Homotypic Cx45-Cx45

Current traces observed during the formation of a whole cell patch



Molecular flux quantification





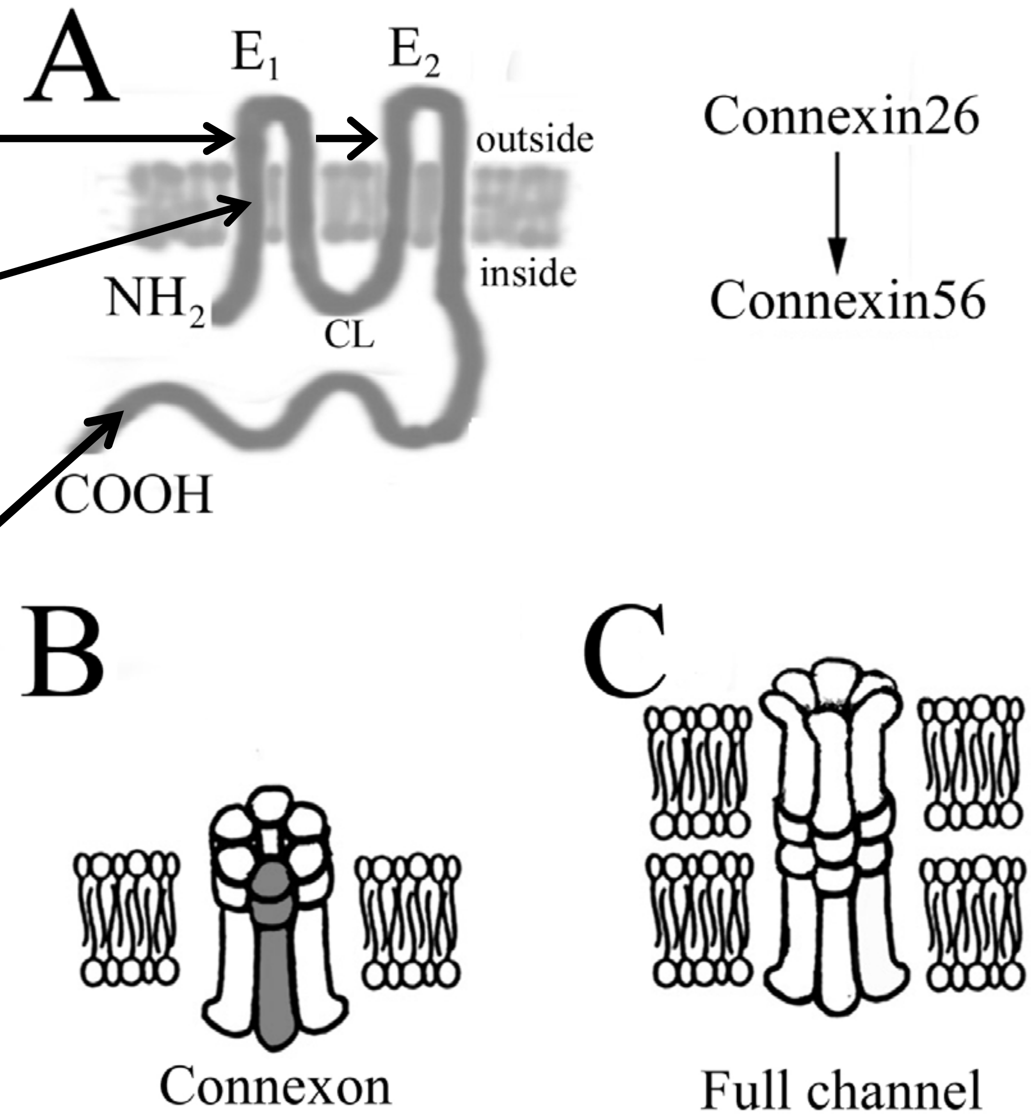
Gating of gap junction channels

- Gating by voltage
  - Transjuntional and transmembrane
- Gating by intracellular pH
- Gating by protein phosphorylation

Structure function relationship

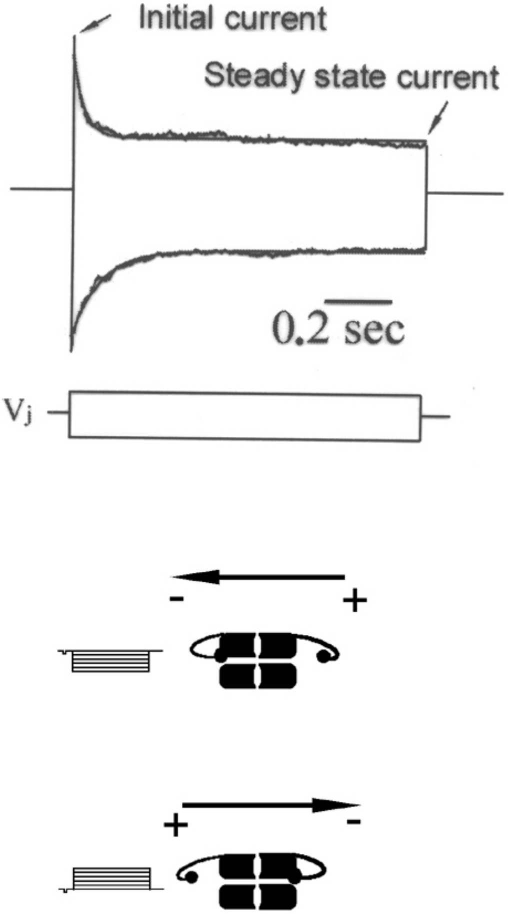
Docking

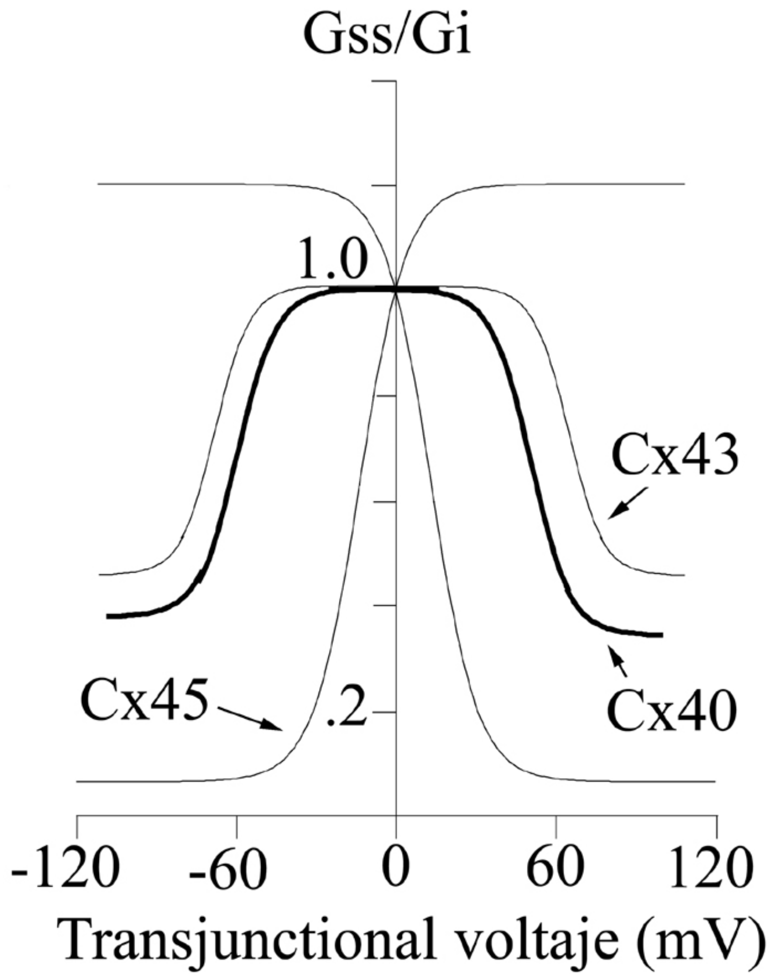
Voltage gating polarity



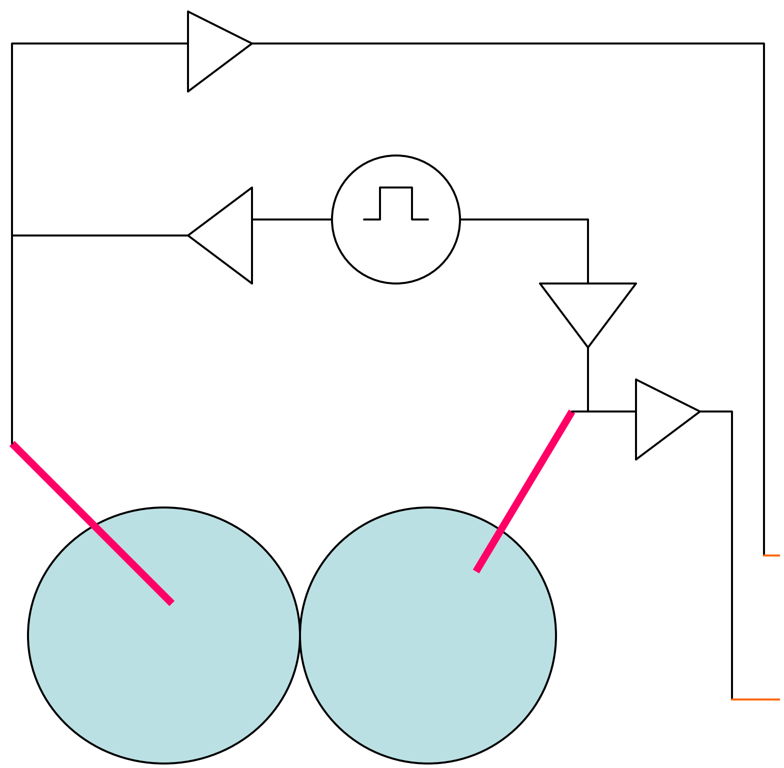
1. pH gating
2. Fast voltage gating
3. Phosphorylation

Transjunctional voltage dependence

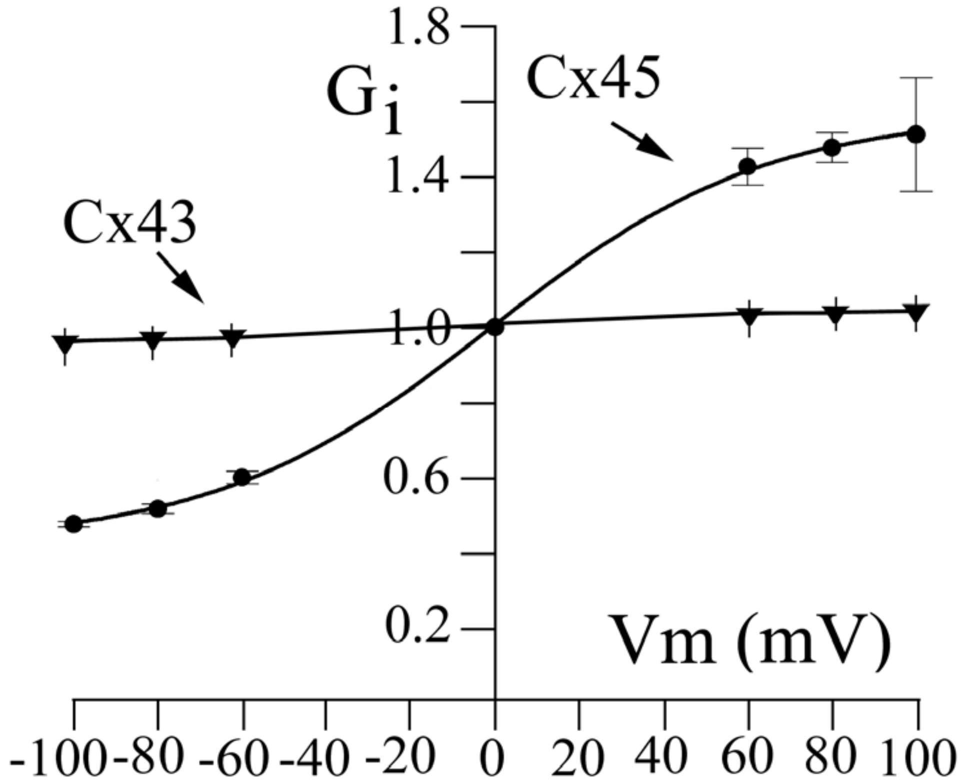




Gating by transmembrane voltage



Evaluation of changes in total conductance due to synchronous stimulation in both cells

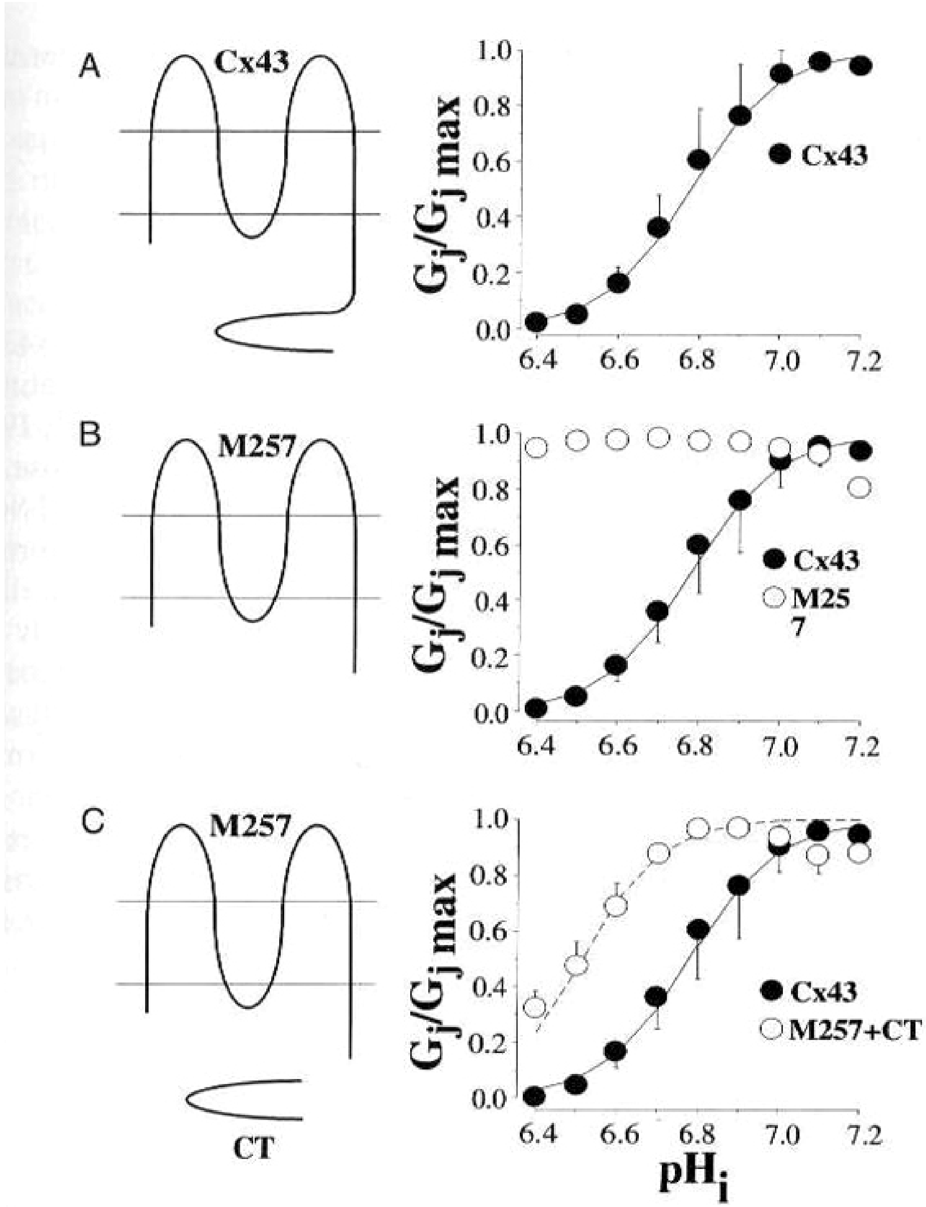


Gating by pH

The reduction of intracellular pH causes a reduction in the conductance of the junction (Gj/Gmax).

When the COOH tail is removed, there is no gating by pH.

If the COOH tail is co-expressed, the gating by pH is re-established.



M. Delmar et al. Current Topics in Membranes (2000)

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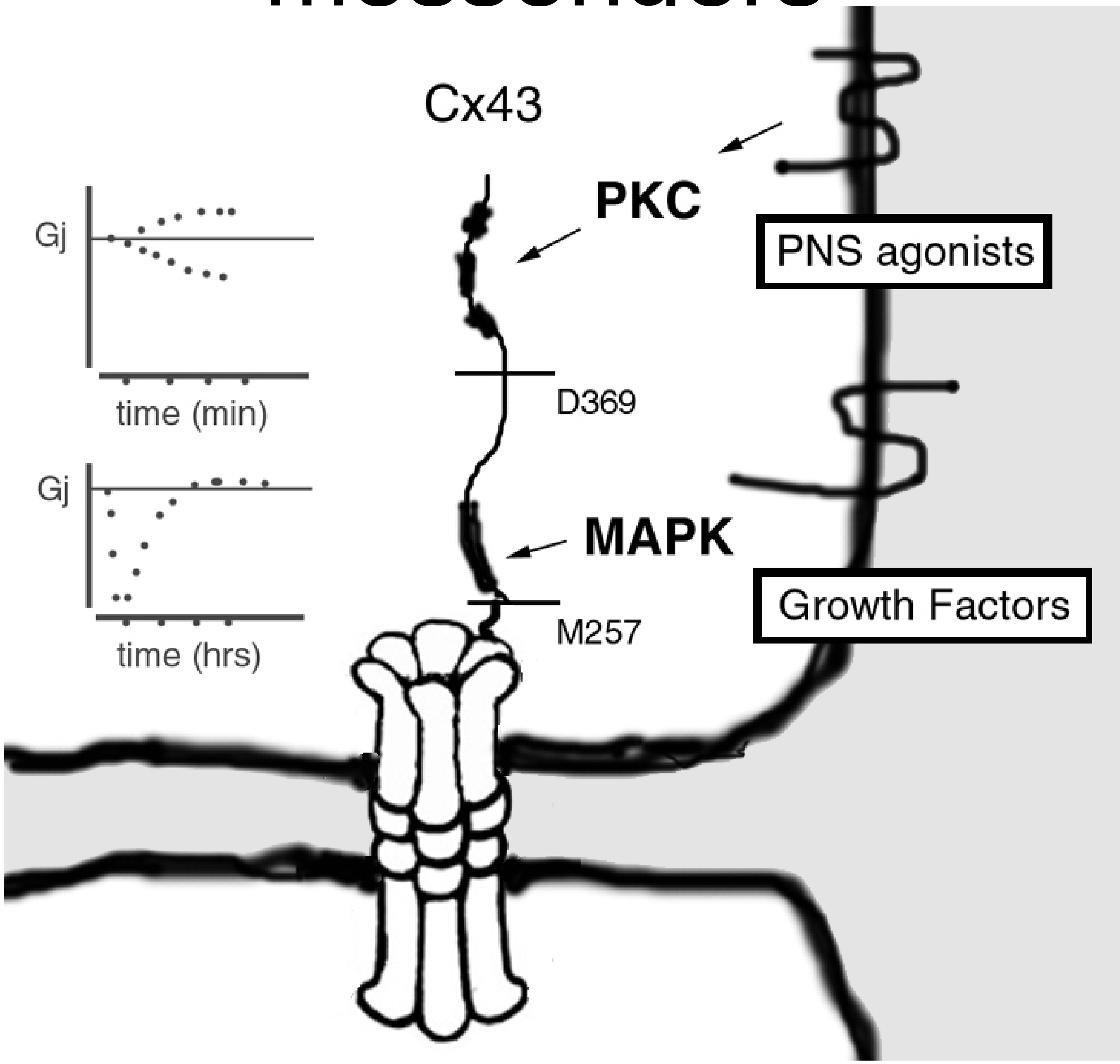
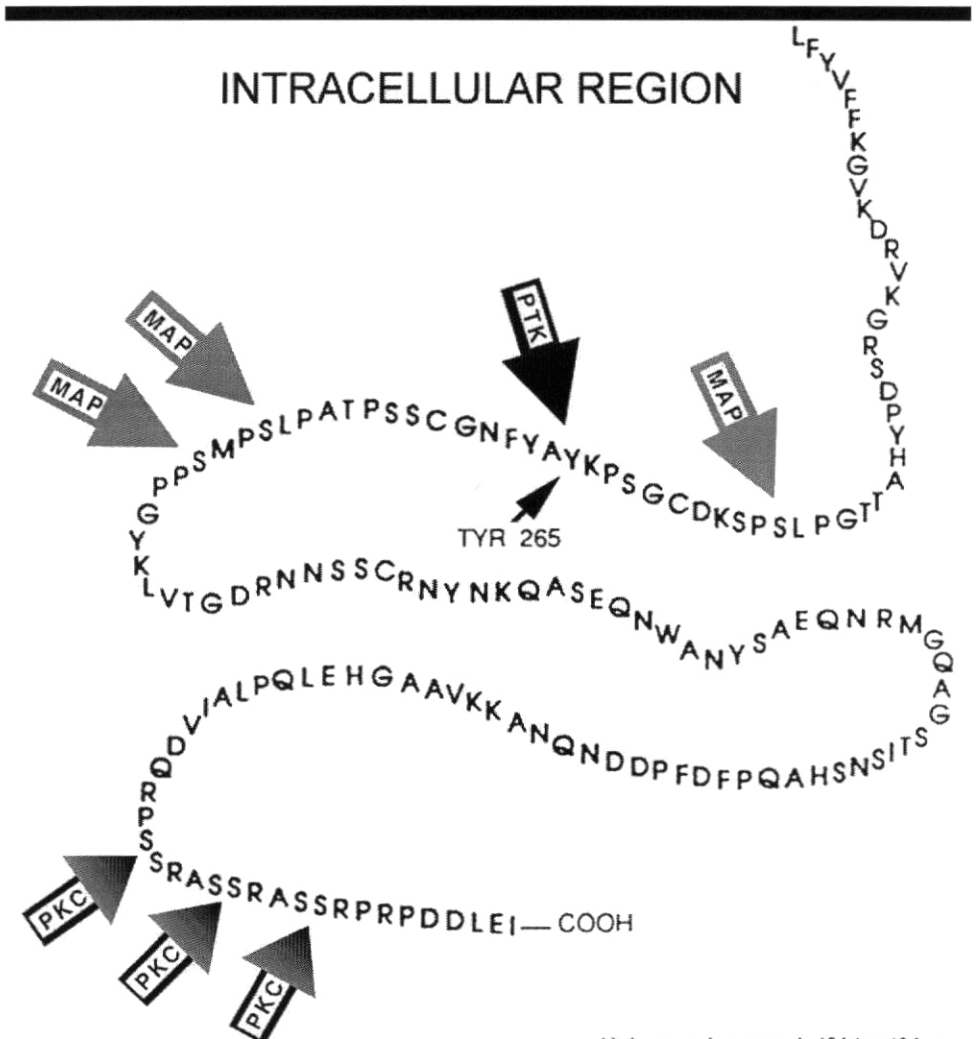
Gating through second messengers



Phosphorylation sites in Cx43 carboxyl tail



kic/moreno/...connexin43/struct6.jpg

Change in total coupling between neonatal myocytes or SKHep1 cells expressing Cx43.
Effects of different kinases

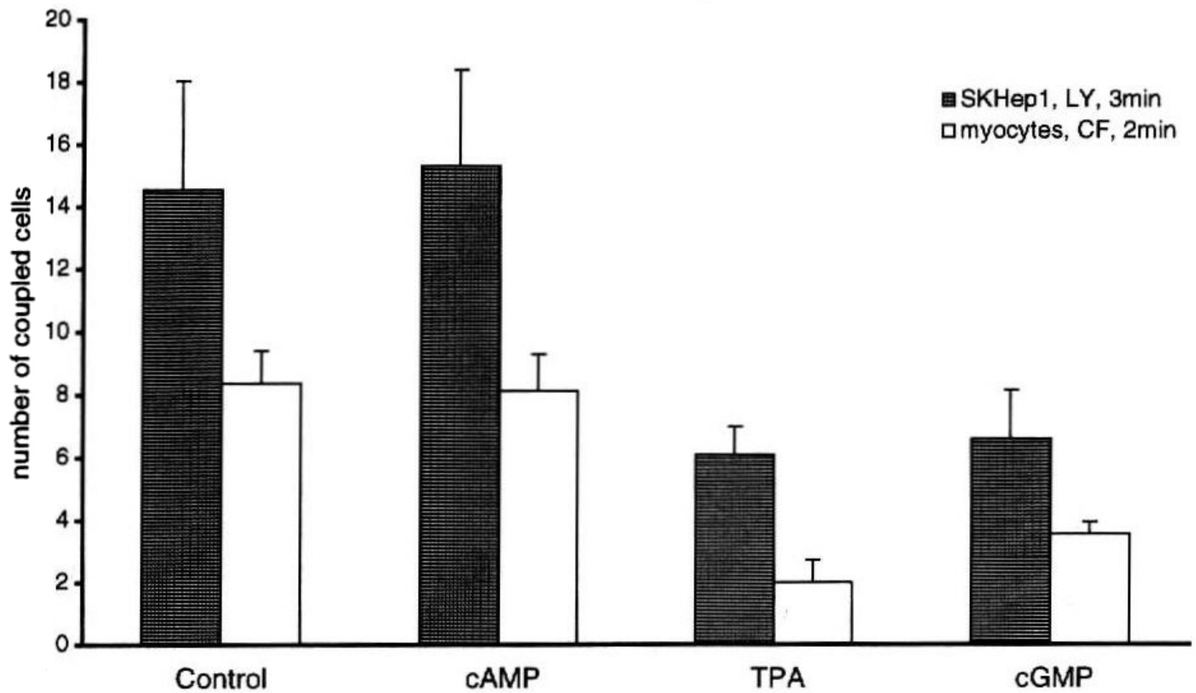


FIGURE 1 Dye permeability of connexin43 gap junction channels. One cell of a group was injected with a dye and the number of cells into which it had diffused after 2 min (6-carboxyfluorescein in rat neonatal cardiac myocytes; open bars) or 3 min (lucifer yellow in connexin43 transfected SKHep1 cells; hatched bars) was counted. Error bars depict SEM.

Shift in unitary conductance of Cx43 due to phosphorylation

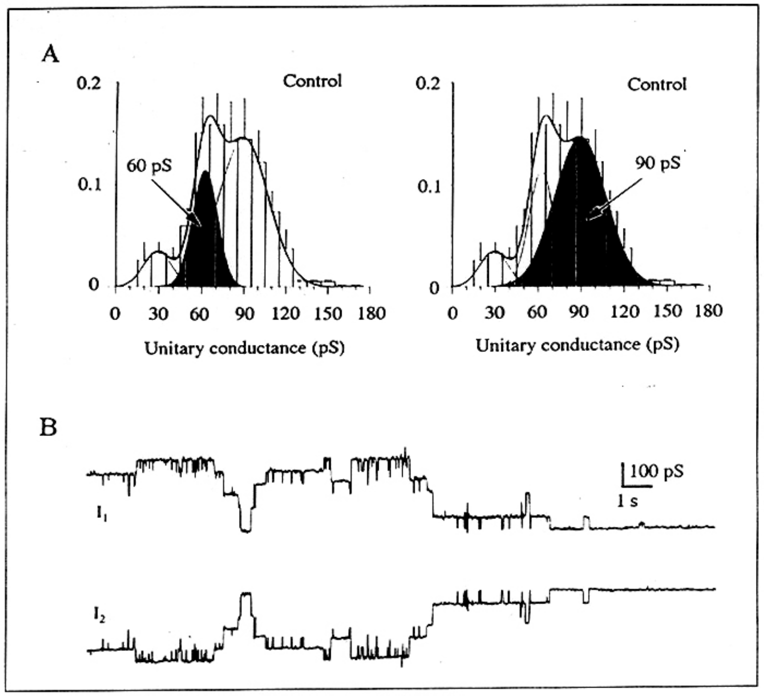


Figure 4 - *A*, Probability density function for single channel conductances recorded in pairs of SKHep1 cells transfected with the human cardiac gap junction cDNA, during halothane-induced uncoupling. The right and left histograms are identical and represent the average of 14 experiments in which relative frequency of events in 10-pS bins were normalized. Standard errors of the relative frequencies of events in all experiments are indicated on top of each bar. The solid curve on top of the histograms is the best fit for the sum of three Gaussian distributions centered at the following conductances: 29 ± 10 pS, 62 ± 9 pS and 89 ± 18 pS. The left histogram highlights the ~60-pS peak while that on the right the ~90-pS one. *B*, Single channel events recorded during halothane-induced uncoupling. Records like this were used to construct the histogram in *A*. The identical amplitude and opposite polarity of the current fluctuations recorded in both cells are used to ascertain the junctional nature of the single channel events.

eno et al **Connexin43 Gap Junction Channels** 1053

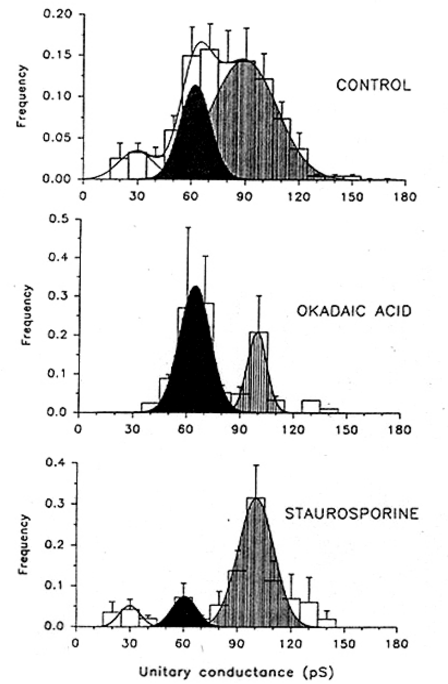
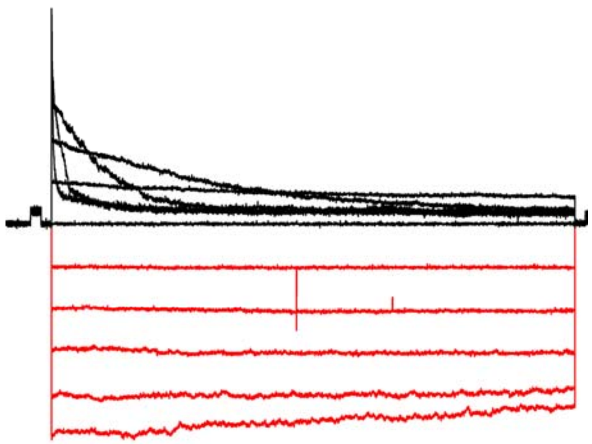
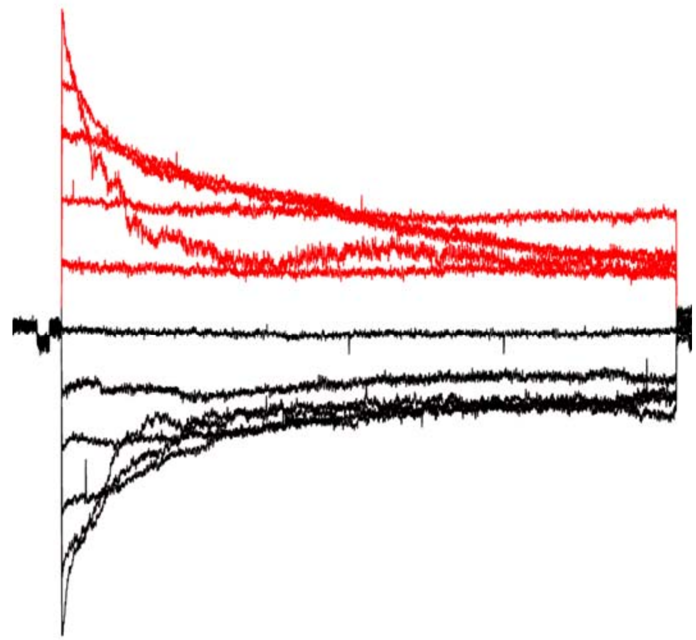


FIG 3. Frequency distributions of unitary junctional conductance (γ_j) events recorded under control conditions and after the application of the phosphatase inhibitor okadaic acid and the protein kinase inhibitor staurosporine. Top, Average of 14 experiments in which unitary conductances of junctional channels were measured after halothane application. The best fit to gaussian distributions was obtained with peaks at γ_j=29±10 (SD, 9% of total events), 62±9 (black area, 25% of total events), and 89±18 (shaded area, 66% of total events) pS. Middle, Average of four experiments in which 300 nmol/L okadaic acid was added to the bathing solution for 30 minutes to 1 hour before recordings were begun. Best fits correspond to γ_j=57±16 (71% of total events) and 103±7 (29% of total events) pS. Note shift in γ_j values to lower conductances. Bottom, Average of three experiments in which cells were treated with staurosporine (300 nmol/L) for 20 minutes; peaks occur at 30±6 (7% of total events), 61±7 (13% of total events), and 100±9 (60% of total events) pS. Note shift of the distribution of unitary conductances toward the highest γ_j values after treatment with staurosporine. All records are from cell pairs in which amplitudes of at least 100 unitary events were measured and are normalized with regard to the total number of events recorded in each experiment.

Problem: Identification of connexin isoform by voltage dependence



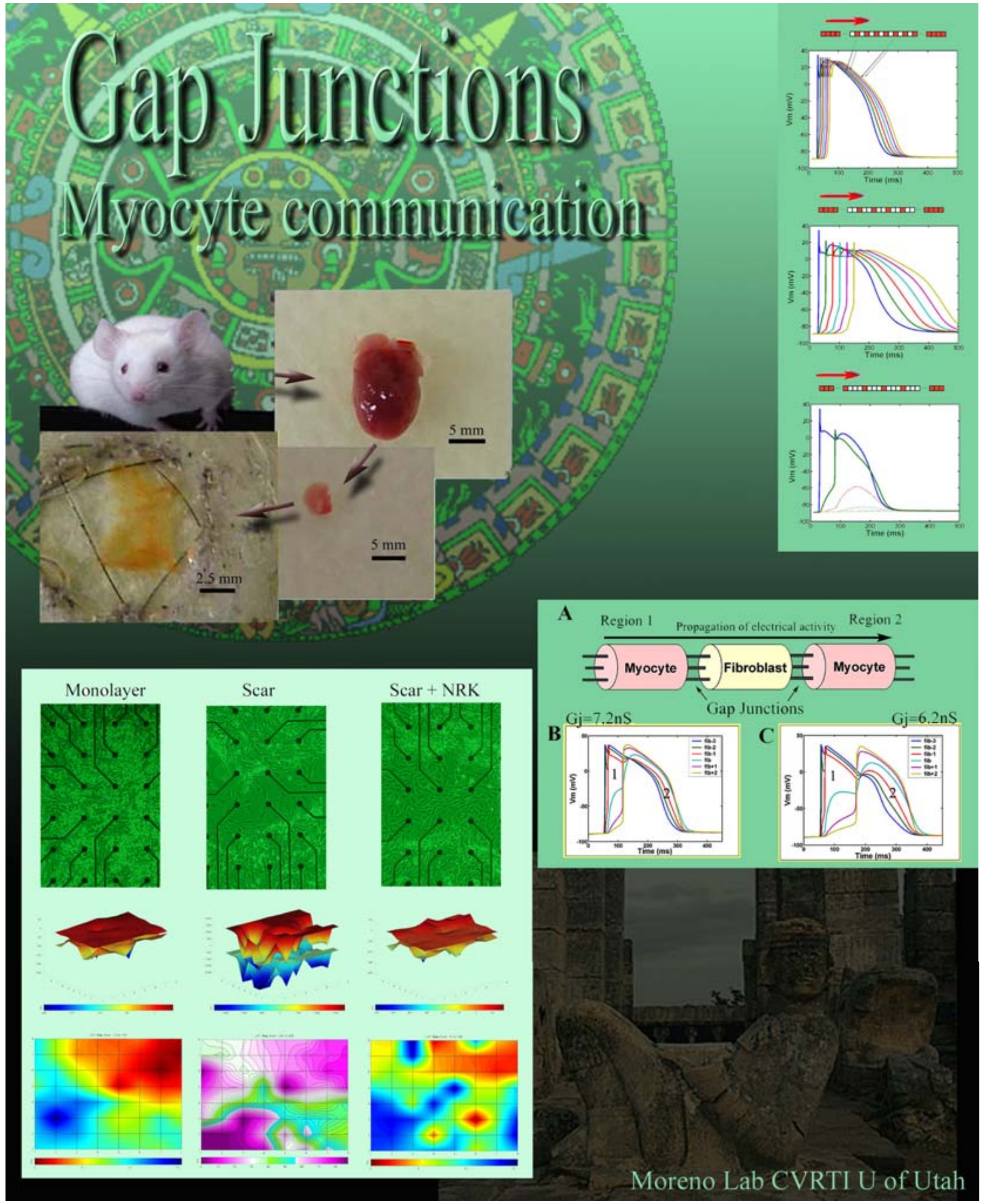


K+ vs Gj

K+	Gj
• S4 region is the recognized sensor for voltage	Depending on the connexin the sensor/effector could be the NH3 or COOH tails. The Polarity in some studied seems to be in M1-E2 region.
• Other subunits for modulation alfa, Beta	Many are coming up. Links to ZO1 and ZO2
• Gating by pH	Yes it does. Ball and chain?
• Gating is modulated by Phosphorylation	Phosphorylation gates or modulates
• Specific blockers	No specific bloquers. In general membrane lipophylic substances.
• Specific activators like Ca++ or ATP	pH

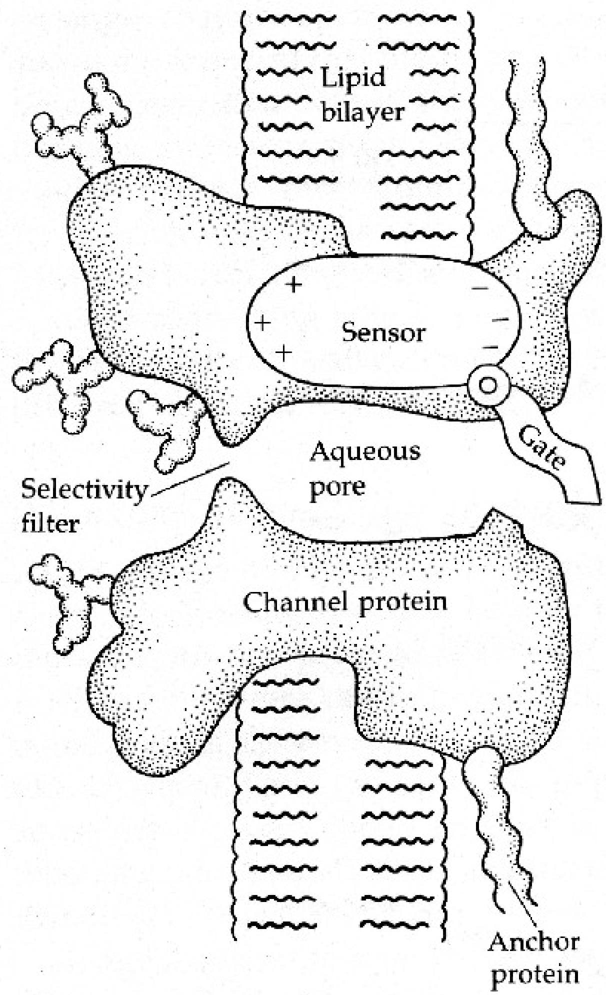
K+ vs Gj

K+	Gj
• Genetic origin genes-splicing	Each connexin a gene
• Six trans-membrane domains Some seven	Four trans-membrane domains Not known for any more
• Tetrameric	Hexameric
• Some families form heteromerics •	Some families form heteromerics Some form **heterotypic channels**
• S5-S6 forms the selectivity pore	So far we know that M2 and M3 are aligned along the pore
• Highly selective to K+ K 1000x :> Na •	Perm selective to large molecules ATP cGMP, cAMP even siRNA
• Unitary conductances from 4 to 15 pS	Unitary conductances from 5 to 400 pS.
• Activates and inactivates with Vm	Only inactivates and it is with Vm or Vj



Gap Junctions
Myocyte communication
5 mm
5 mm
2.5 mm
Vm (mV)
Time (ms)
A
Region 1
Propagation of electrical activity
Region 2
Myocyte
Fibroblast
Myocyte
Gap Junctions
Gj=7.2nS
Gj=6.2nS
B
C
Monolayer
Scar
Scar + NRK
Moreno Lab CVRTI U of Utah

Functional structure of a membrane channel



3 WORKING HYPOTHESIS FOR A CHANNEL

The channel is drawn as a transmembrane macromolecule with a hole through the center. The external surface of the molecule is glycosylated. The functional regions, selectivity filter, gate, and sensor are deduced from voltage-clamp experiments but have not yet been charted by structural studies. We have yet to learn how they actually look.