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Electrophysiology
and Bioelectricity of Tissues

Cell-cell Communication

Part 1-2

Gap Junctions & Electrical coupling

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Cell to Cell Communication

- You have learned how a cell is capable of generating and maintaining an electric signal.
  - It is amazing how it is generated in our brain and sent to a finger to control its movement
- This part of the course will be focused on how this signal is propagated across cells, tissues and organs.
  - Threfore mostly we will talk about excitable tissues
- Special consideration will be taken on how this communication can be modulated.
  - More intercellular communication biophysical bases

Communication in biological systems

- 1) Cell to cell
  - Paracrine (signals like hormoes or growth factors)
  - Direct GJ
  - Direct Desmosomes and tight junctions
  - Direct Glycocalix on membrane proteins

- 2) Tissue/Organ to tissue/organ
  - Neuromuscular Junctions
  - Oxigen receptors to brain
  - Blood glucose to pancreas

- 4) Individual to individual
  - Senses
  - Sweat and hormones
- 5) Communities to communities
  - Duck and fish synchrony
  - Fire Flies

  - ALL OF THEM GO BACK TO CELL-CELL communicaiton and most of the times the Membrane is highly involved.

Bioelectricity

- As a resource for generating a communicating signals
  - Mostly all sensing organs
  - Defense signals (electric eel)
  - Plants Fly trap and mimosa pudica
- Which tissues use electrical signals in BIOLOGICAL systems
  - Reviewed mostly excitable tissues
- Synapses
  - In general chemical synapses have higher control and directionality.
- As a tool to quantify physiological activity
  - (to be filled by the students 2Ex). Examples on impedance, EKG's and phototransduction like in voltage dependent dyes and Optogenetics

Synapses

- Chemical
  - Not direct and need of a transmitter.
  - Mechanisms of release, cleft and receptors.
- Electrical
  - Mostly gap junctions
- Mixed
  - Fish neurons as an example

Mauthner Cells

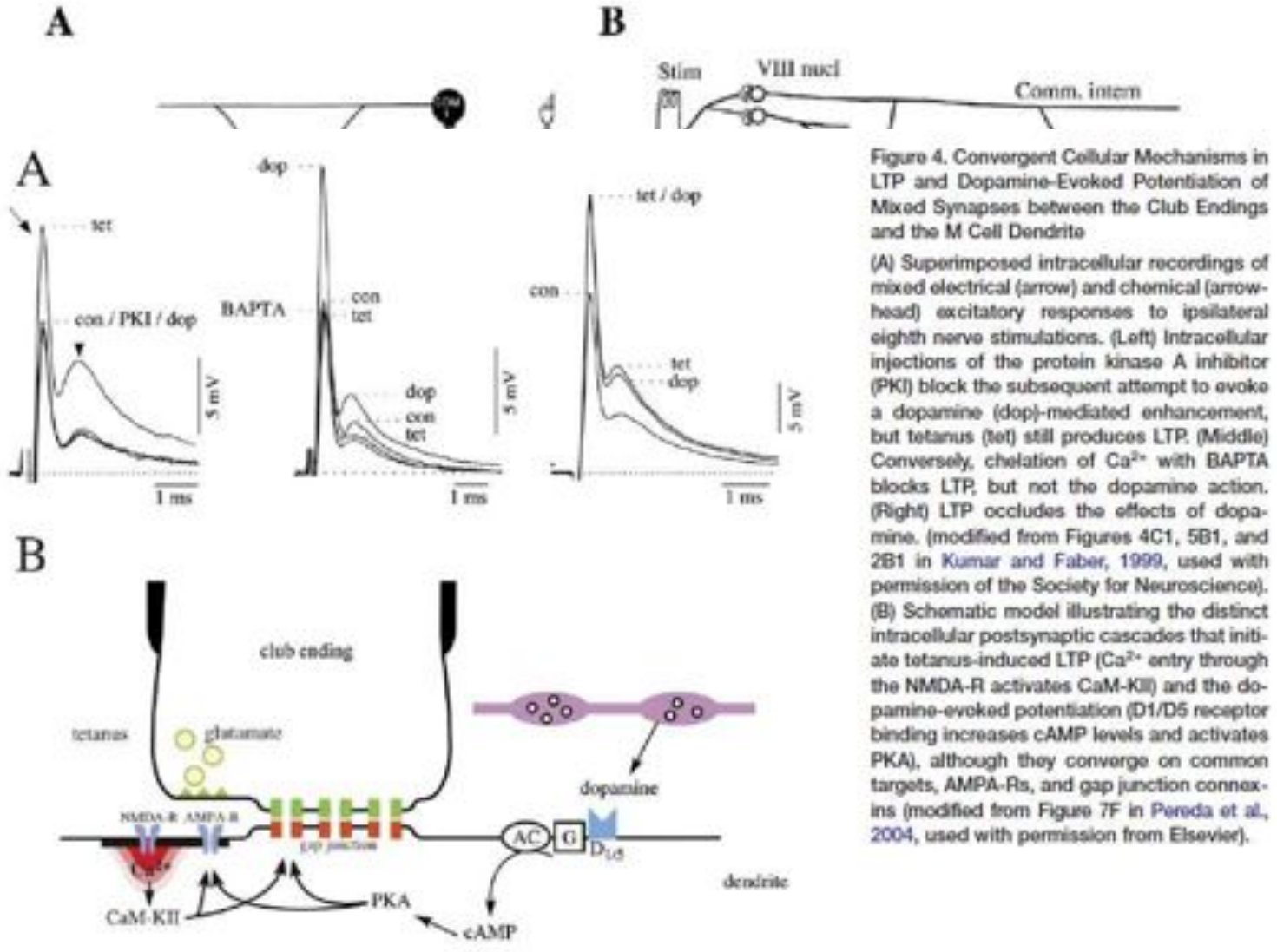


Figure 4. Convergent Cellular Mechanisms in LTP and Dopamine-Evoked Potentiation of Mixed Synapses between the Club Endings and the M Cell Dendrite

(A) Superimposed intracellular recordings of mixed electrical (arrow) and chemical (arrowhead) excitatory responses to ipsilateral eighth nerve stimulations. (Left) Intracellular injections of the protein kinase A inhibitor (PKI) block the subsequent attempt to evoke a dopamine (dop)-mediated enhancement, but tetanus (tet) still produces LTP. (Middle) Conversely, chelation of Ca^{2+} with BAPTA blocks LTP, but not the dopamine action. (Right) LTP occludes the effects of dopamine. (modified from Figures 4C1, 5B1, and 2B1 in Kumar and Faber, 1999, used with permission of the Society for Neuroscience). (B) Schematic model illustrating the distinct intracellular postsynaptic cascades that initiate tetanus-induced LTP (Ca^{2+} entry through the NMDA-R activates CaM-KII) and the dopamine-evoked potentiation (D1/D5 receptor binding increases cAMP levels and activates PKA), although they converge on common targets, AMPA-Rs, and gap junction connexins (modified from Figure 7F in Pereda et al., 2004, used with permission from Elsevier).

Korn and Faber, Neuron: 47 (2005)

Physiological Relevance and Diseases.

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

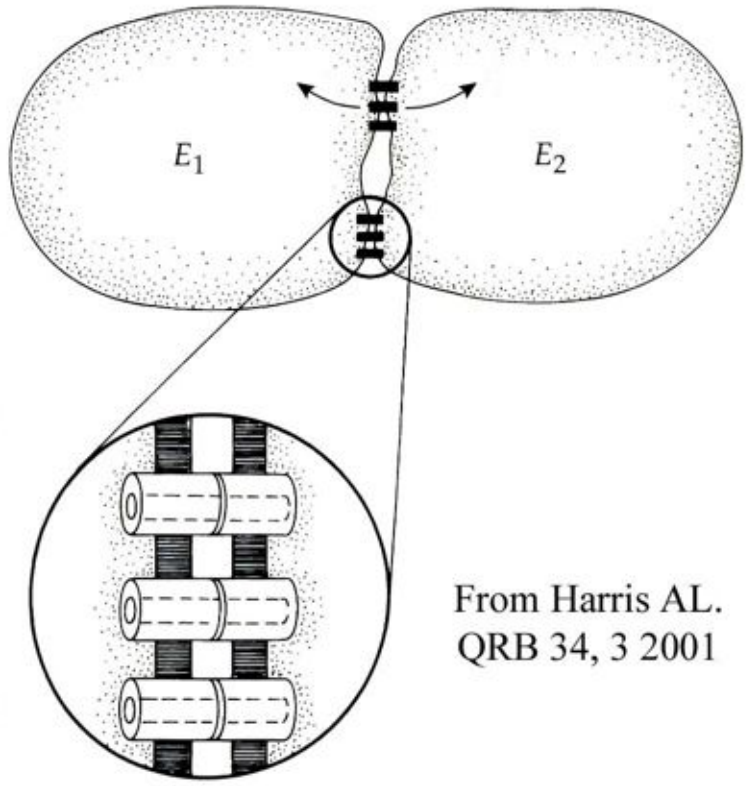
- In physiological conditions, permits the musculature from different regions of the heart to respond in a synchronous manner.

- In nervous tissue, coordination of electrical signals through gap junctions is necessary to generate brain circuits and rhythms.

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 cytoplasms of adjacent cells



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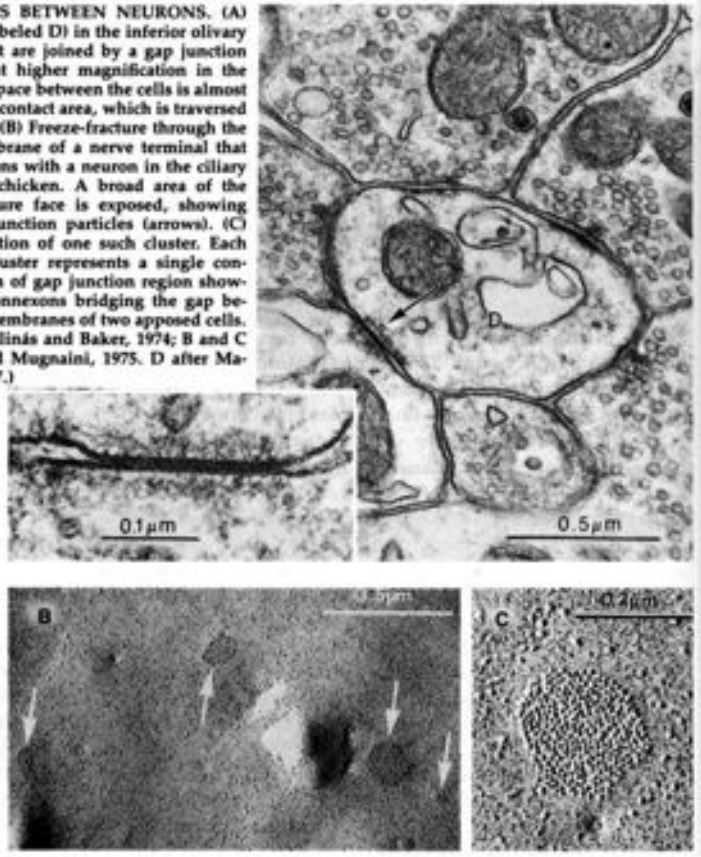
QRB 34, 3 2001



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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 membeane 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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Connexin Laboratory

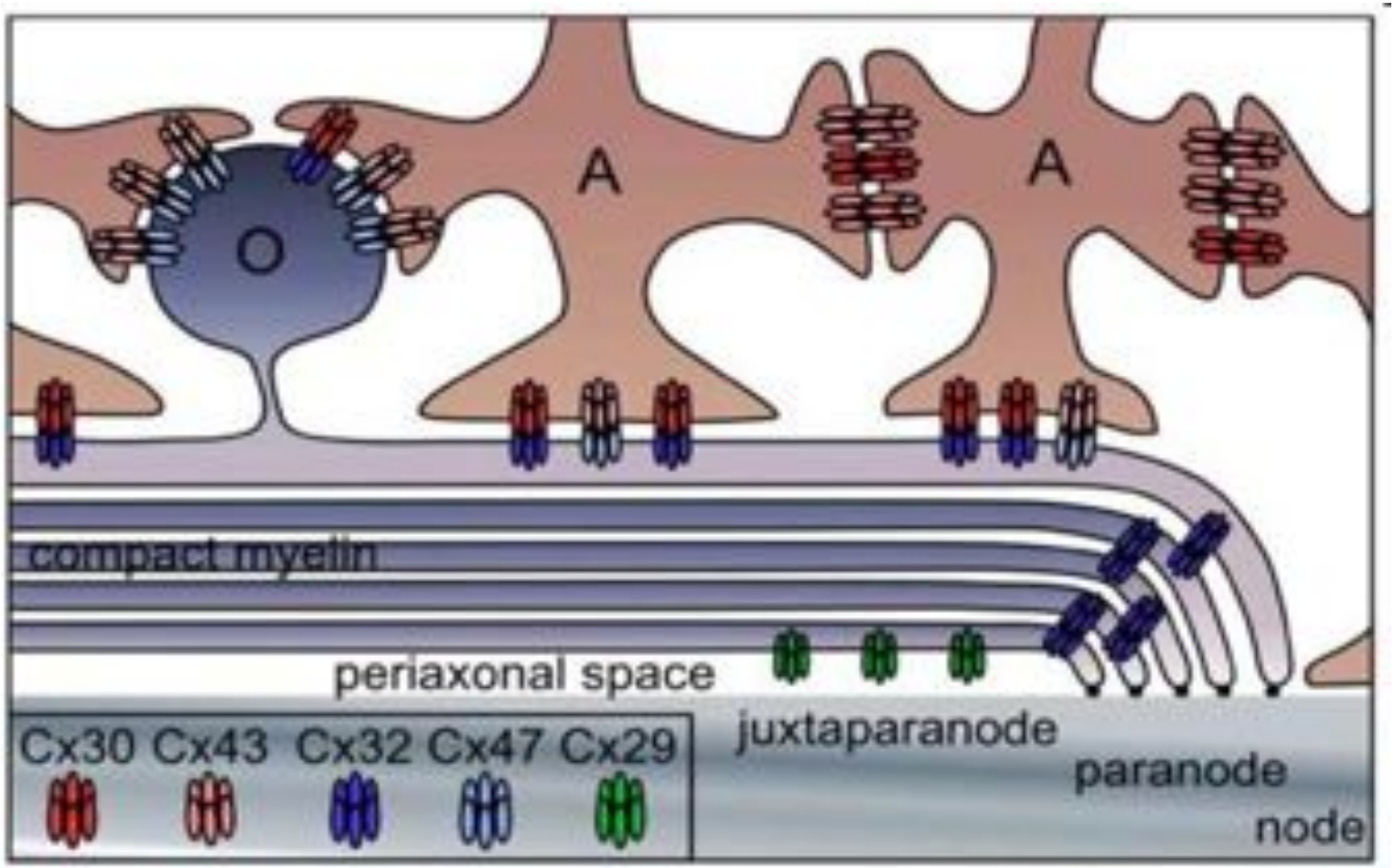
Distribution

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
Liver		Cx32, Cx26
Skin		Cx26
Smooth muscle		Cx43, Cx37
Eye lens		Cx46, Cx50

Homotypic, heterotypic and multi-heteromeric channels in the brain.



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

Connexin	Pathology	System	Possible mechanism
Cx40	Cardiac conduction defects and impaired regulation of vasodilation (Kirchhoff *et al.* 1998; Simon *et al.* 1998)	Mouse KO	Impaired cardiac electrical coupling
Cx43	Visceroatrial heterotaxia (defect in left-right asymmetry leading to cardiac malformations and multiple organ defects) (Britz-Cunningham *et al.* 1995) and hypoblastic left heart syndrome (Dasgupta *et al.* 2001)	Human	
	Perinatal lethal: defects of conotruncus and right ventricle leading to obstruction of cardiac outflow (Reaume *et al.* 1995; Sullivan *et al.* 1998)	Mouse KO	Disruption of neural crest cell migration
	Craniofacial abnormalities and delayed skeletal ossification (Lecanda *et al.* 2000)	Mouse KO	Osteoblast defect
	Small gonads, paucity of germ cells and immature follicles (Juneja *et al.* 1999)	Mouse KO	
	Structural defect in lens (Gao & Spray, 1998)	Mouse KO	Altered osmotic balance in the lens
	Diverse congenital abnormalities (spina bifida, anencephaly, myeloschisis, limb malformation, cleft palate, failure of hematopoiesis, cardiovascular deformity) (Becker *et al.* 1999)	Mouse, embryonic-knockdown	
	Defects in hematopoesis (Montecino-Rodriguez *et al.* 2000)	Mouse KO	
	Sudden cardiac death due to ventricular arrhythmia (Gutstein *et al.* 2001)	Mouse cardiac KO	Slowed ventricular conduction velocity and increased anisotropy
	Hypotension and bradycardia (Liao *et al.* 2001)	Mouse endothelial KO	Elevation of plasma NO
Cx45	Embryonic lethal: defective cardiogenesis and vasculogenesis (Kruger *et al.* 2000; Kumai *et al.* 2000)	Mouse KO	

Table 11. *Connexinopathies*

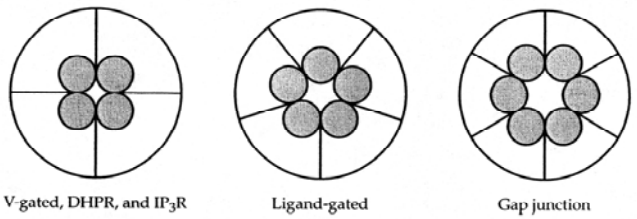
Connexin	Pathology	System	Possible mechanism
Cx26	Recessive non-syndromic deafness (DFNB1) (Kelsell *et al.* 1997)	Human	Impaired circulation of K^+ to endolymph via sensory hair cells, supporting cells and fibrocytes in cochlea
	Dominant non-syndromic deafness (DFNA3) (Kelsell *et al.* 1997)	Human	
	Palmoplantar keratoderma (PPK) (mutational overlap with DFNA3; abnormal callusing of palms and soles) (Kesell *et al.* 2000)	Human	
	Vohwinkel syndeome (VS) (mutational overlap with DFNA3; deafness and callusing of digits leading to autoamputation) (Maestrini *et al.* 1999)	Human	
	Embryonic lethal (Gabriel *et al.* 1998)	Mouse KO	Impaired transfer of glucose across the trophoblast layers of the placenta
Cx30	Dominant nonsyndromic deafness (DFNA3) (Grifa *et al.* 1999)	Human	
	Clouston's hidrotic ectodermal dysplasia (HED) (palmoplantar hyperkeratosis, hair and nail defects) (Lamartine *et al.* 2000)	Human	
Cx30.3	Ethrythrokeratoderma variabilis (EKV) (hyperkeratosis and red patches in skin) (Macari *et al.* 2000)	Human	
Cx31	Progressive high-tone deafness (Xia *et al.* 1998)	Human	
	Ethrythrokeratoderma variabilis (EKV) (hyperkeratosis and red patches in skin) (Richard *et al.* 1998)	Human	
	Dominant and recessive nonsyndromic deafness (Coucke *et al.* 1999; Liu *et al.* 2000)	Human	
	Peripheral neuropathy (Lopez-Bigas *et al.* 2001)	Human	
	60% embryonic lethal due to placental dysmorphogenesis (Plum *et al.* 2001)	Mouse KO	Reduced labyrinth and spongiotrophoblast size
Cx32	X-linked form of Charcot–Marie–Tooth disease (CMTX), peripheral demyelinating neuropathy) (Bergoffen *et al.* 1993)	Human	Impaired function of reflexive junctions between myelin layers (Scherer *et al.* 1995)
	Late-onset disorganization of peripheral myelin (Anzini *et al.* 1997; Scherer *et al.* 1998)	Mouse KO	Impaired function of reflexive junctions between myelin layers (Scherer *et al.* 1995)
	Enhanced susceptibility to hepatic tumors (Temme *et al.* 1997)	Mouse KO	
	Compromised hepatic glucose mobilization (Chanson *et al.* 1998)	Mouse KO	
	Enhanced susceptibility to chemical hepatocarcinogenesis; delayed liver regeneration (Omori *et al.* 2001)	Mouse liver KO	
Cx36	Cortical asynchrony; defect in retinal processing (Guldenagel *et al.* 2001)	Mouse KO	Disrupted electrical coupling

II. Molecular organization of a gap junction channel

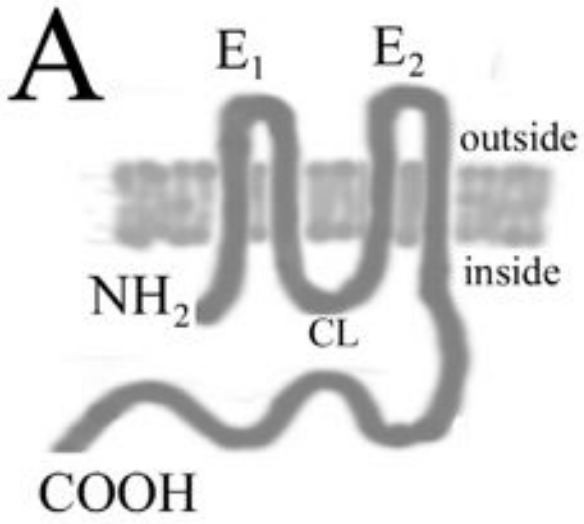
•Connexins are a family of homologous proteins that conform the intracellular channels.

•Currently >30 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.



Connexin26

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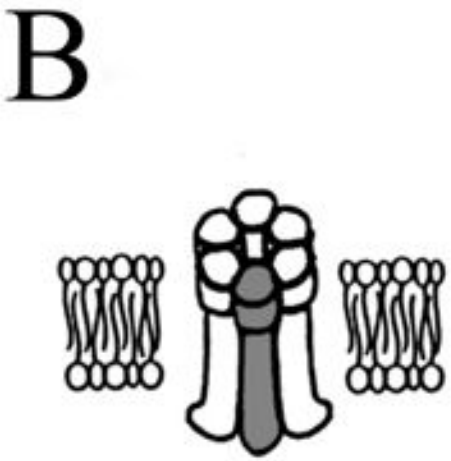
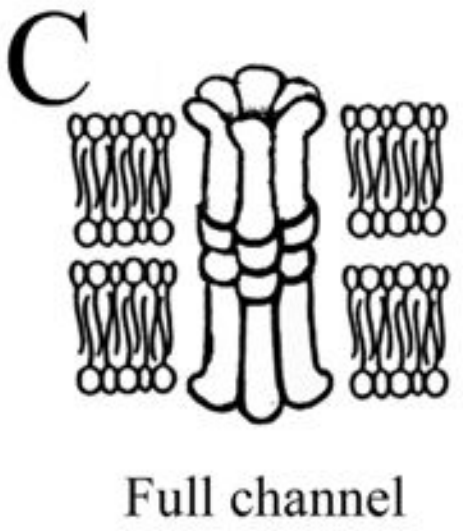
Connexin56





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Article ID jsbi.1999.4184, available online at http://www.idealibrary.com on IDEAL®

Gap junction channel ultra-structure

Expression, Two-Dimensional Crystallization, and Electron Cryo-crystallography of Recombinant Gap Junction Membrane Channels

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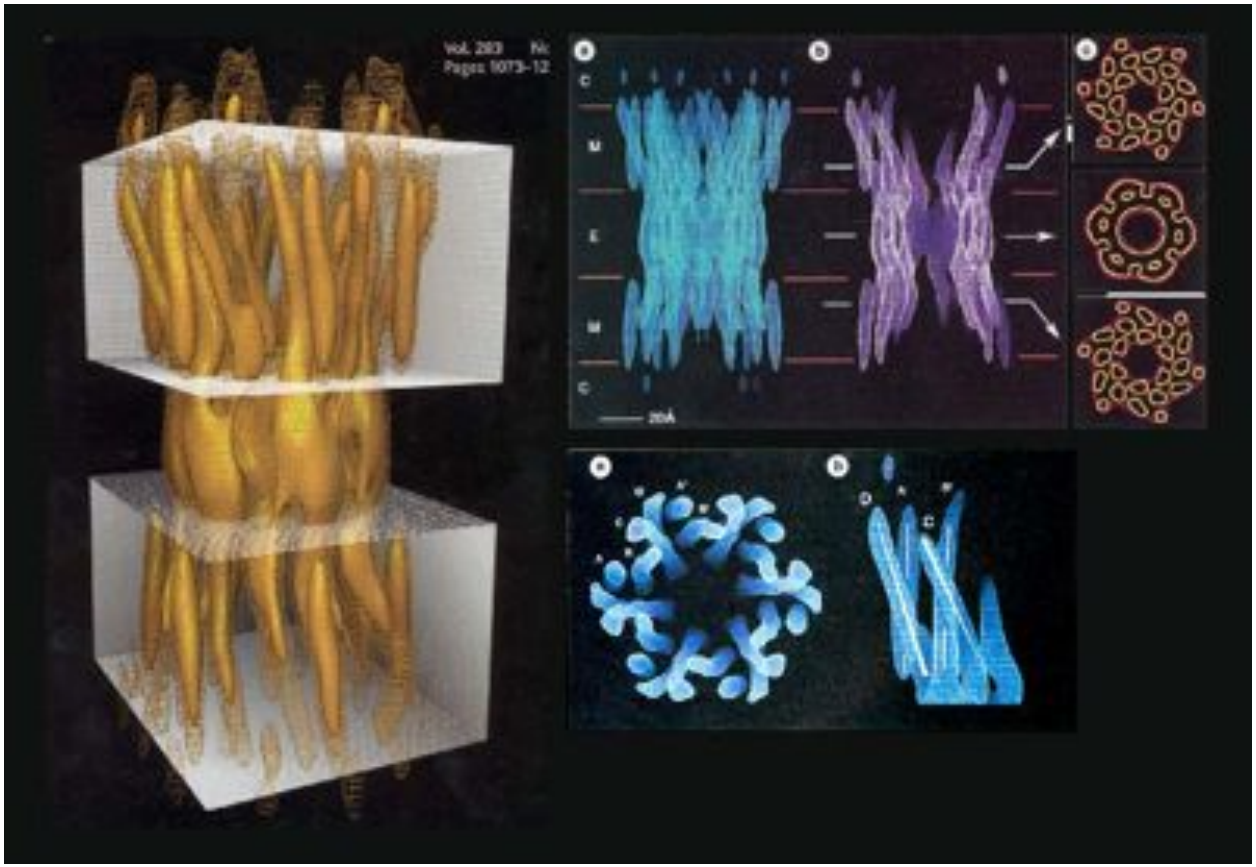

Yeager et al, Science 283, 1999

Connexin Laboratory

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ARTICLES

Structure of the connexin 26 gap junction channel at 3.5 Å resolution

Shoji Maeda[1], So Nakagawa[1], Michihiro Suga[1], Eiki Yamashita[1], Atsunori Oshima[2], Yoshinori Fujiyoshi[2] & Tomitake Tsukihara[1,3]

Gap junctions consist of arrays of intercellular channels between adjacent cells that permit the exchange of ions and small molecules. Here we report the crystal structure of the gap junction channel formed by human connexin 26 (Cx26, also known as GJB2) at 3.5 Å resolution, and discuss structural determinants of solute transport through the channel. The density map showed the two membrane-spanning hemichannels and the arrangement of the four transmembrane helices of the six protomers forming each hemichannel. The hemichannels feature a positively charged cytoplasmic entrance, a funnel, a negatively charged transmembrane pathway, and an extracellular cavity. The pore is narrowed at the funnel, which is formed by the six amino-terminal helices lining the wall of the channel, which thus determines the molecular size restriction at the channel entrance. The structure of the Cx26 gap junction channel also has implications for the gating of the channel by the transjunctional voltage.

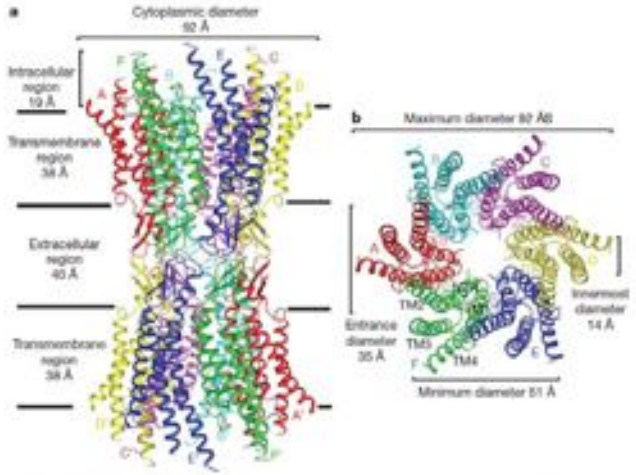

Figure 1 | Overall structure of the Cx26 gap junction channel in ribbon representation. The corresponding protomers in the two hemichannels, which are related by a two-fold axis, are shown in the same colour. **a**, Side view of the Cx26 gap junction channel. **b**, Top view of the Cx26 gap junction channel showing the arrangement of the transmembrane helices TM1 to TM4. The pore has an inner diameter of 35 Å at the cytoplasmic entrance, and the smallest diameter of the pore is 14 Å.

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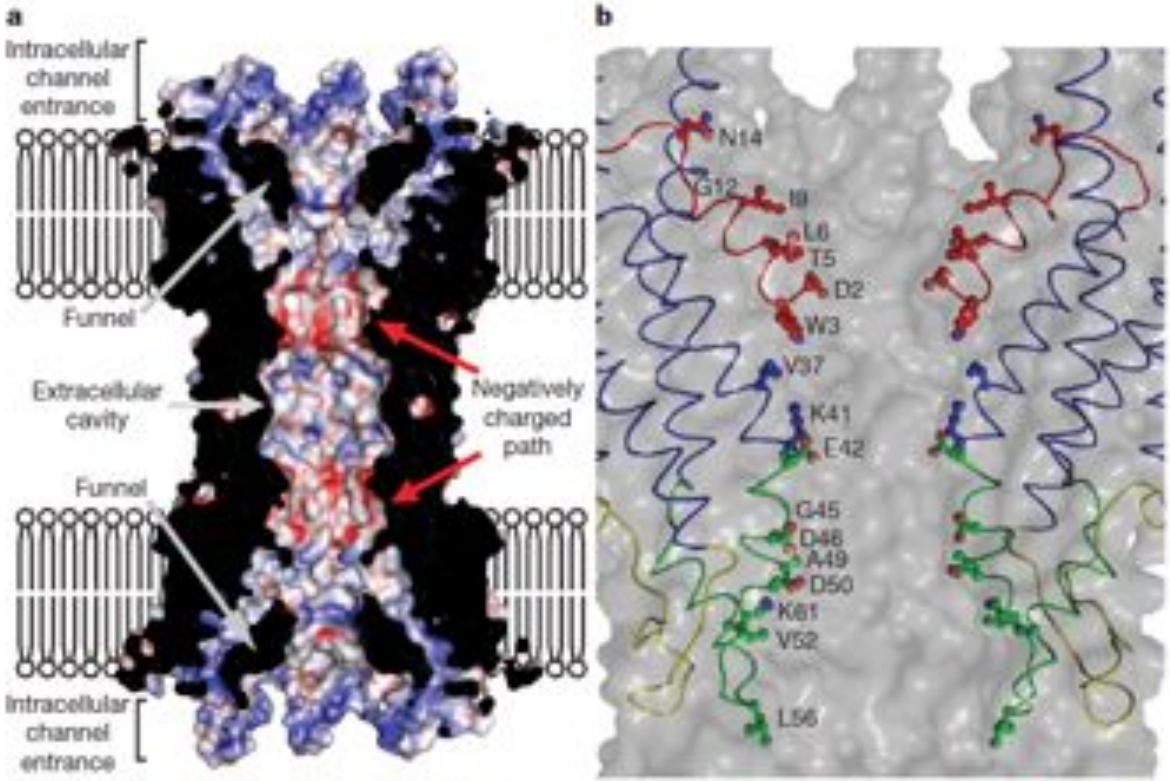
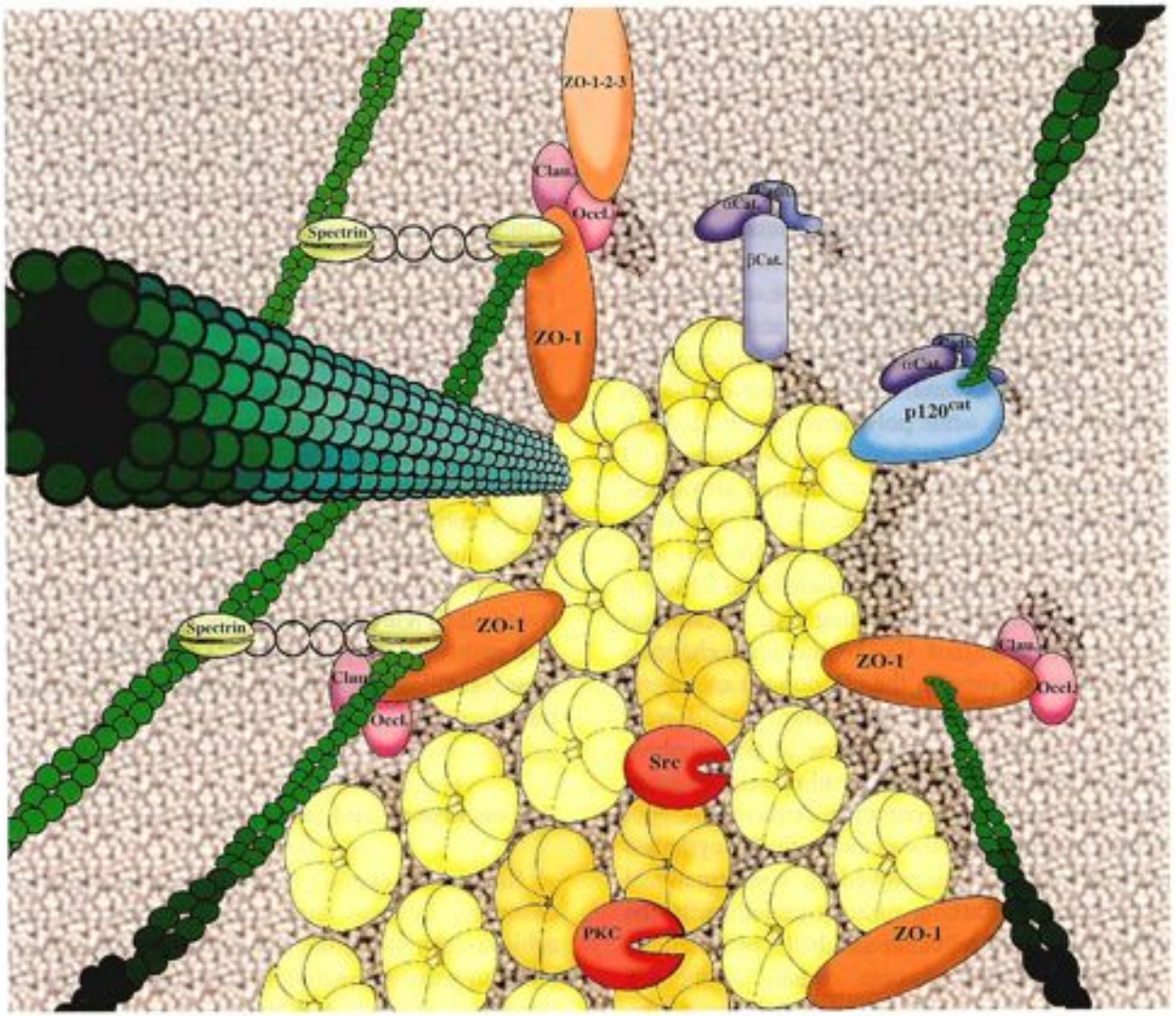


Figure 4 | Pore structure of the Cx26 gap junction channel. **a**, Vertical cross-section through the gap junction channel, showing the surface potential inside the channel. The channel features a wide cytoplasmic opening, which is restricted by the funnel structure, a negatively charged path and an extracellular cavity at the middle. Electrostatic surface potential of the Cx26 gap junction channel was calculated by the program APBS[43] as implemented in PyMOL under dielectric constants of 2.0 and 80.0 for protein and solvent regions, respectively. The displayed potentials range from −40 (red) to 40 (blue) kTe^{-1}. **b**, Pore-lining residues in a Cx26 gap junction channel. Side view of Cx26 gap junction channel pore; the main chain is depicted as a thin ribbon and side chains facing the pore as balls and sticks. For fine viewing, two subunits in the foreground are omitted in the surface representation and two further subunits in the background are omitted in the model depiction. The colouring is the same as in Fig. 3b.

Connexin channels are not alone

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



Connexin Laboratory

Regulation of intercellular communication

- It is simple

  Electrically we evaluate gj or junction conductance

$$g_j = n * \gamma_j * P_o$$

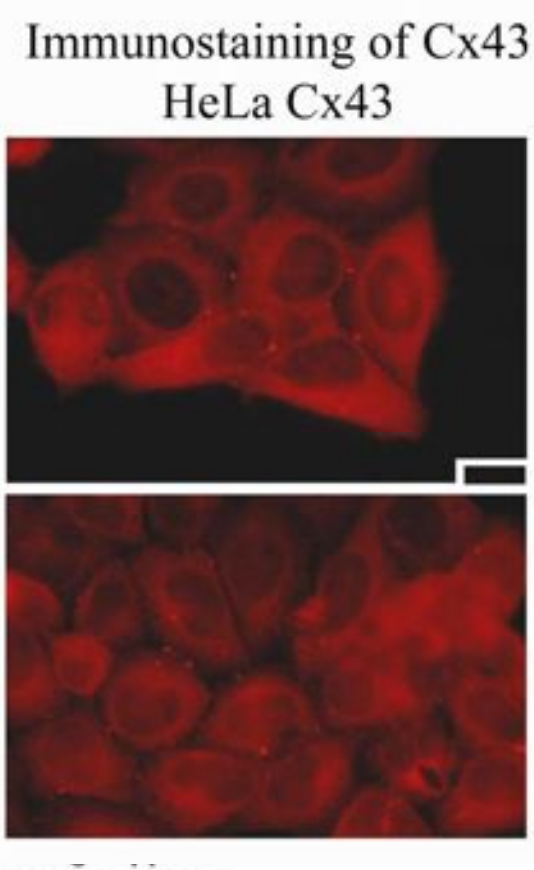
n = number of channels

γ_j = unitary conductance

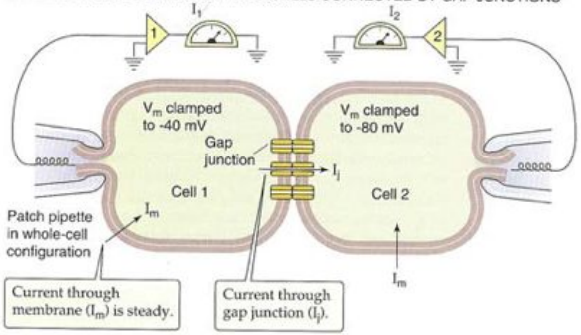
P_o = open probability

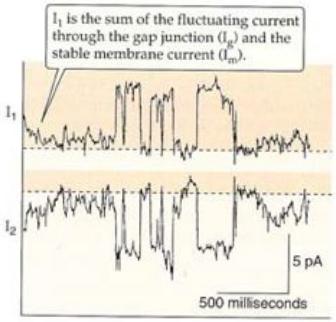
A GAP-JUNCTION CHANNELS IN APPOSING MEMBRANES

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



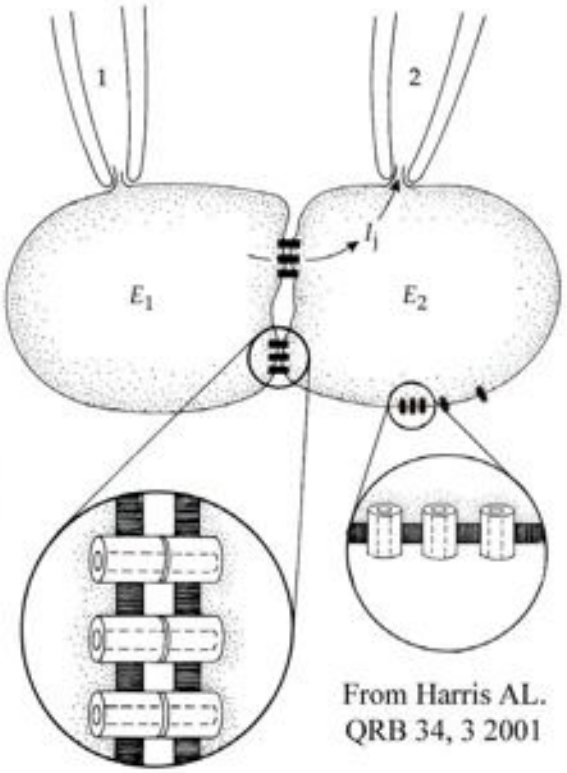
C ELECTRICAL COUPLING OF TWO CELLS CONNECTED BY GAP JUNCTIONS





Double whole cell voltage clamp and gating of gap junction channels

Unitary conductances of connexins



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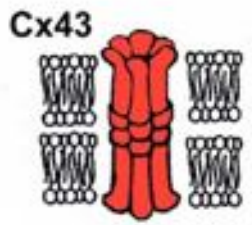
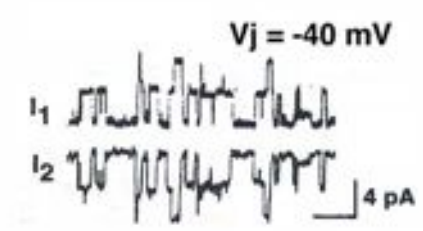
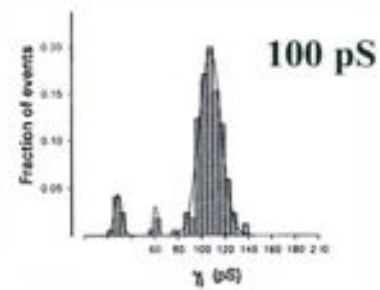
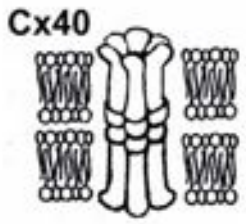
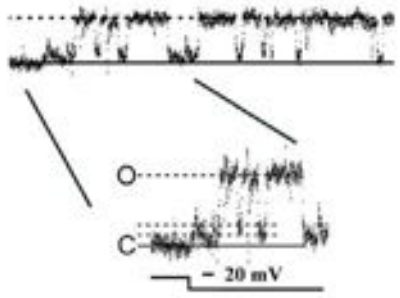
QRB 34, 3 2001

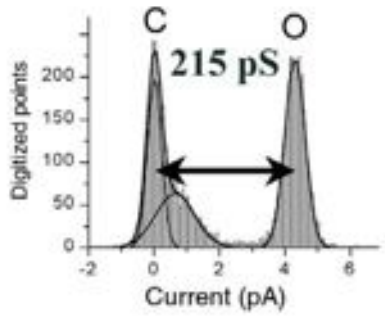


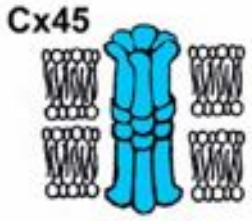


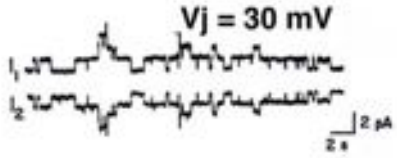


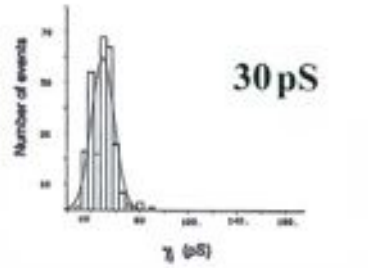












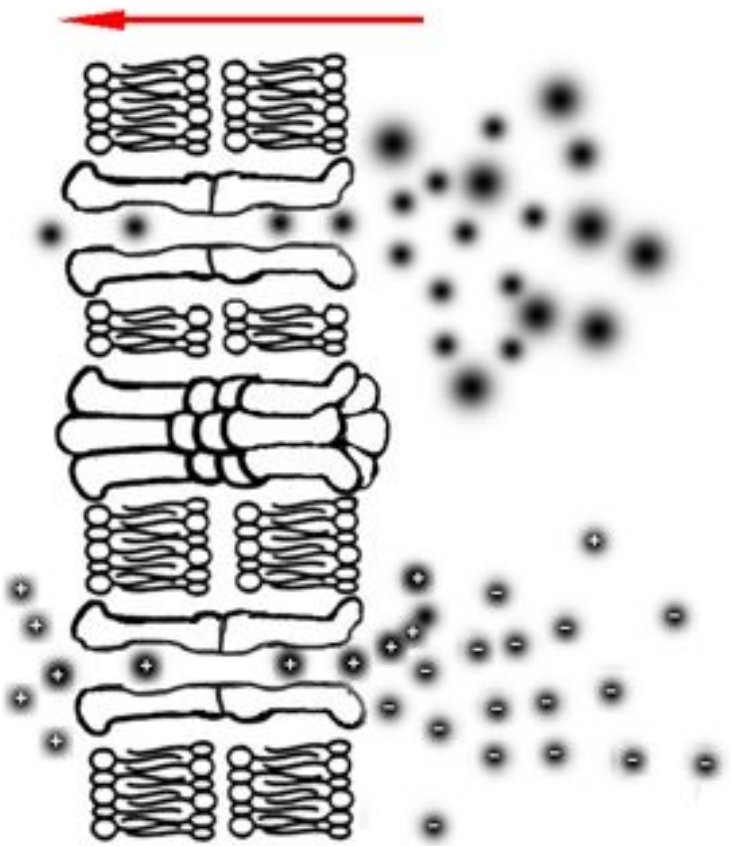
Connexin Laboratory

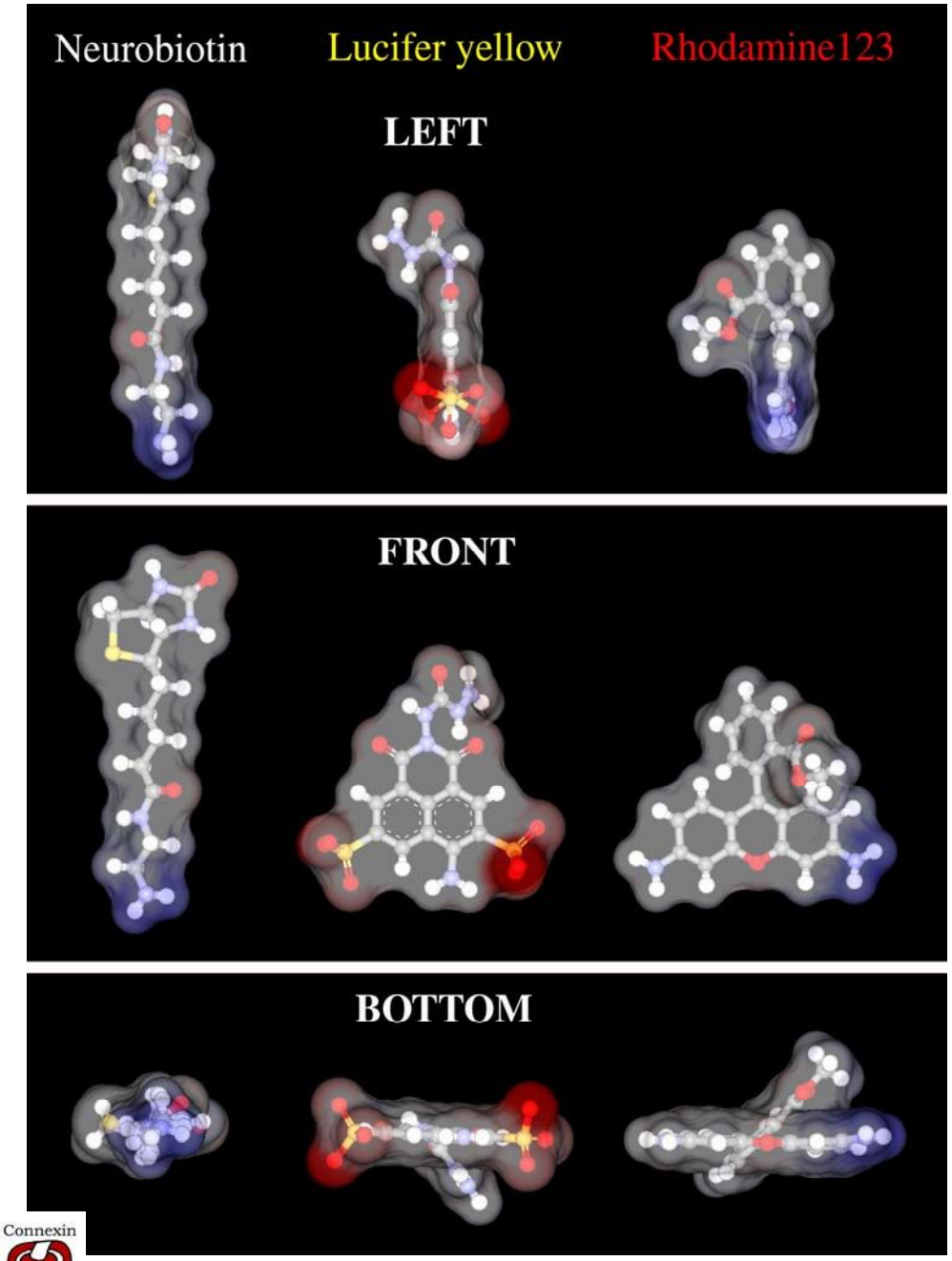
Permeance and selectivity

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 the channel
4) Particle-channel interaction and binding





Fluorescent molecular probes that help to test permeance

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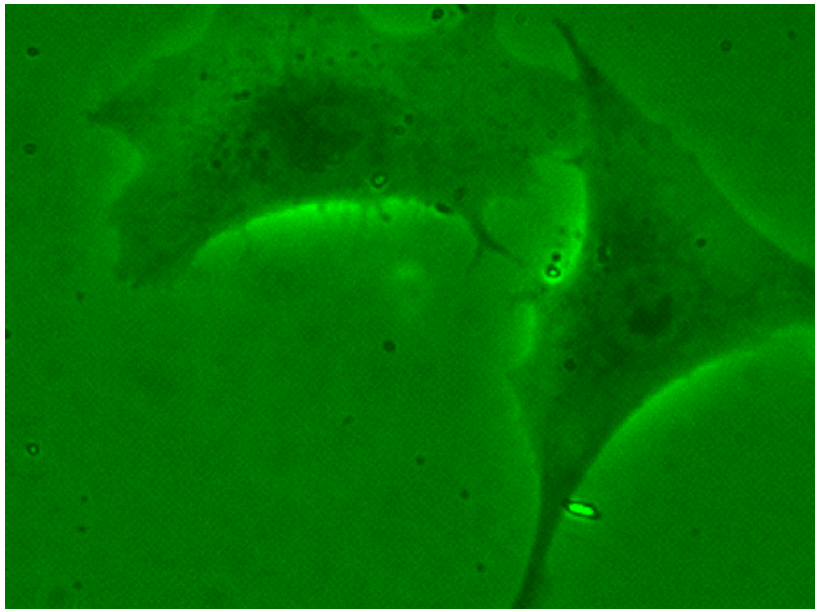
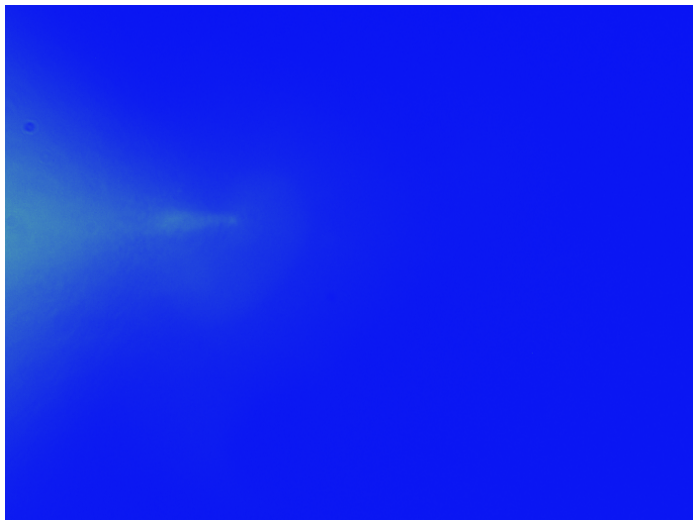
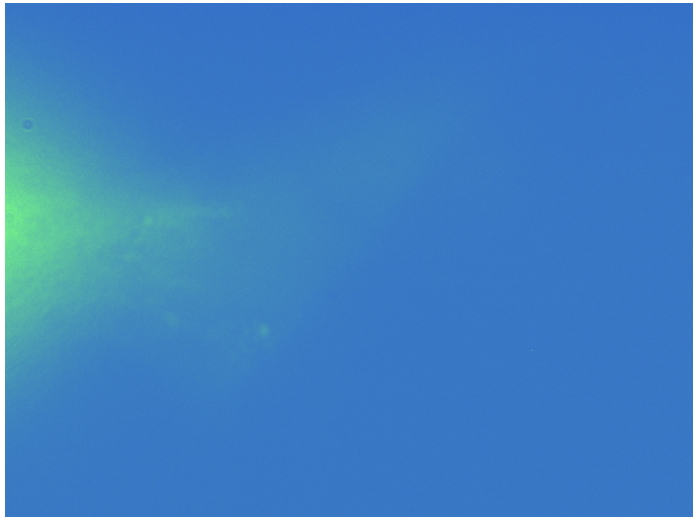
Molecular flux



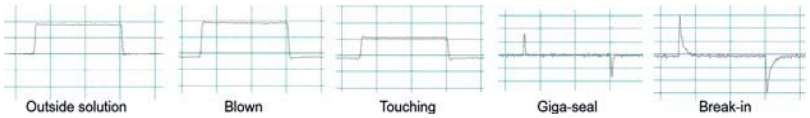


Homotypic Cx43-Cx43



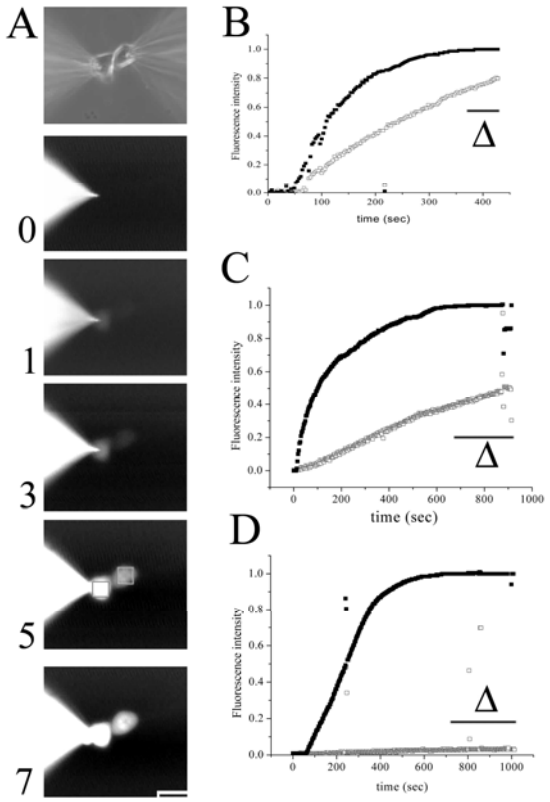
Homotypic Cx45-Cx45

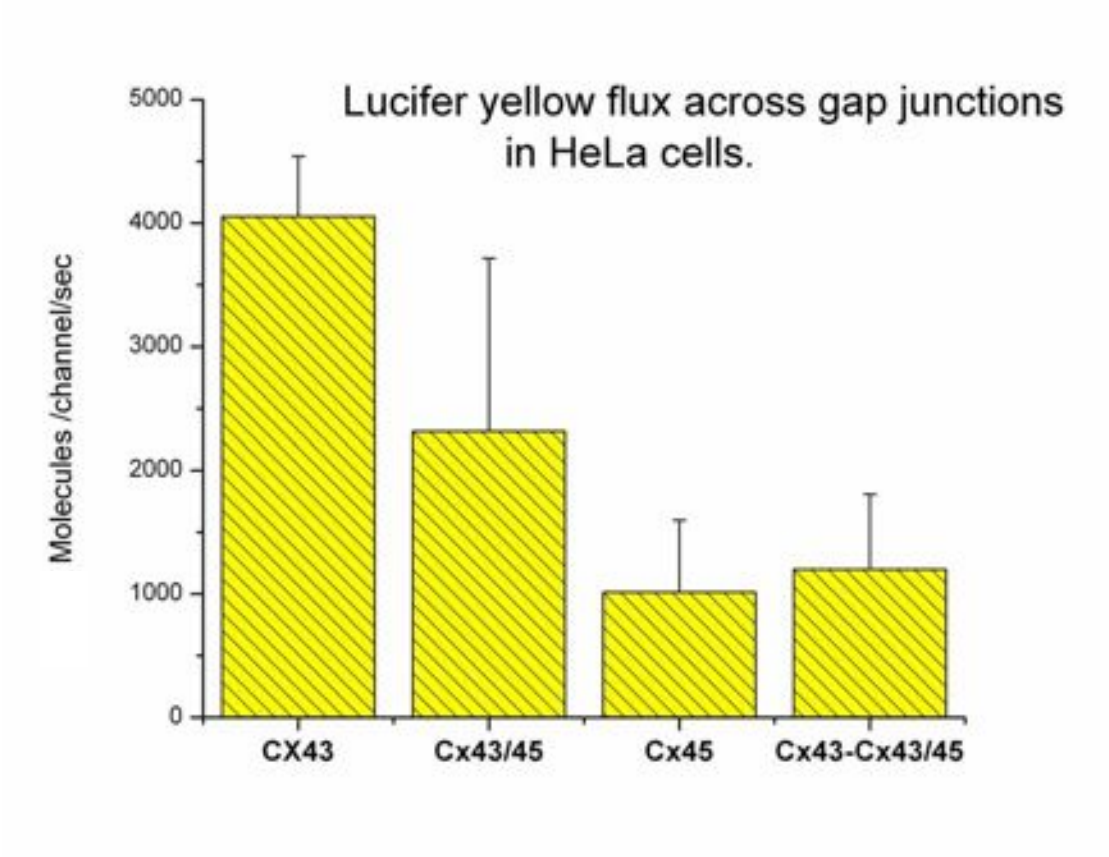
Current traces observed during the formation of a whole cell patch



Connexin Laboratory

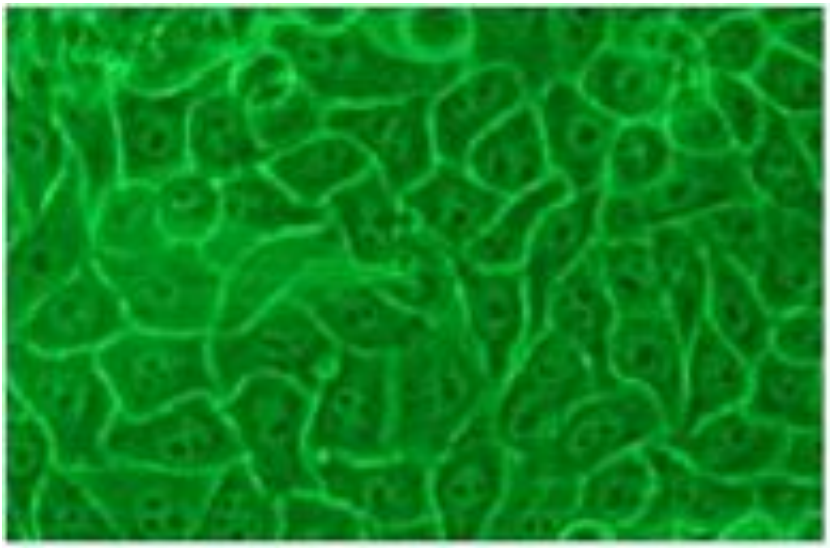
Molecular flux quantification

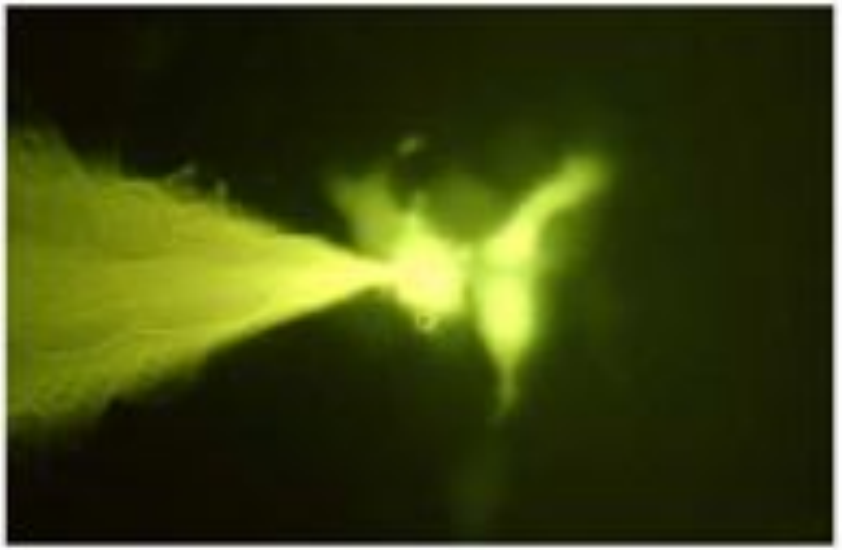




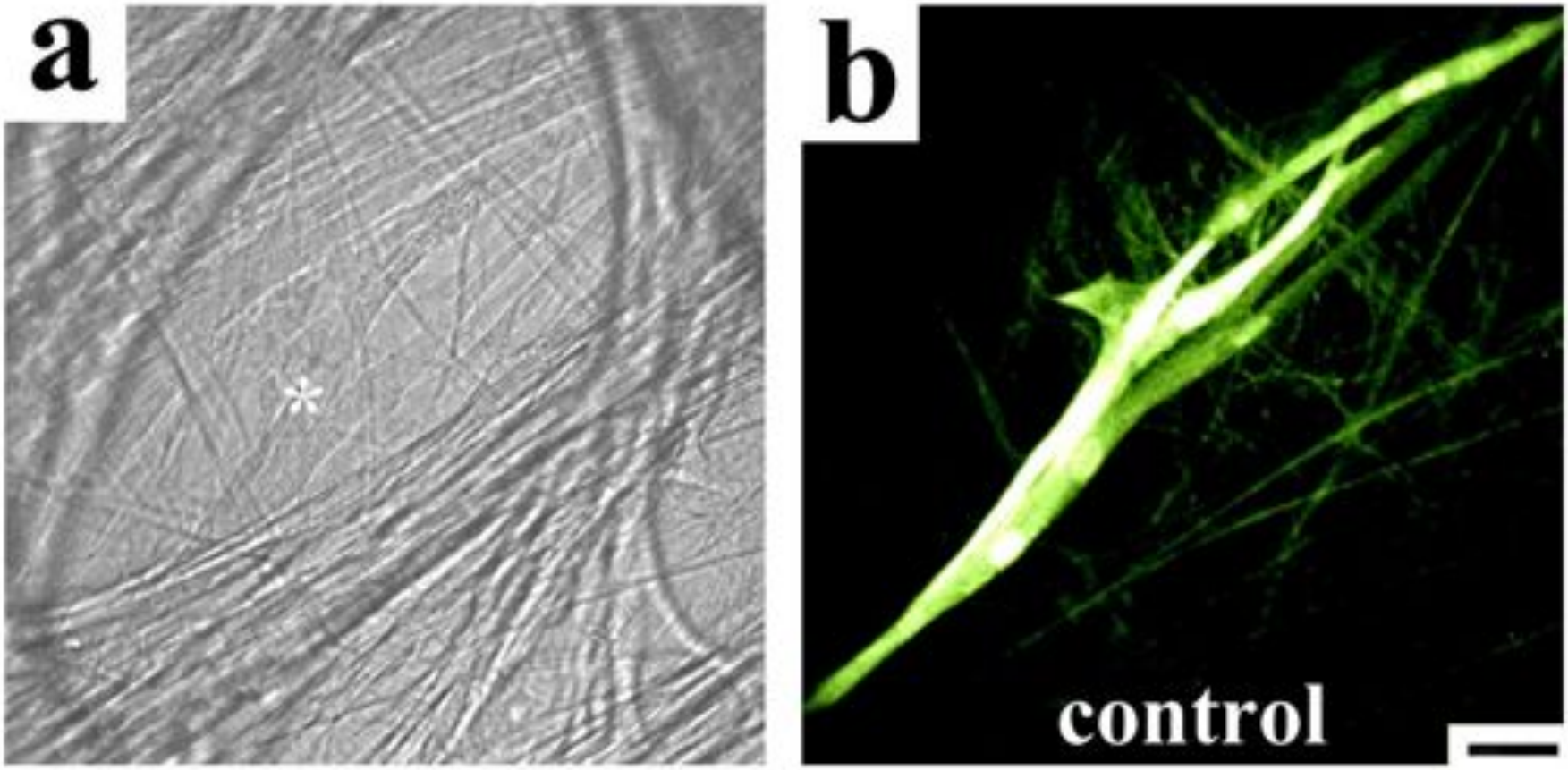
Connexin Laboratory

Intercellular communication is detected using fluorescent dyes



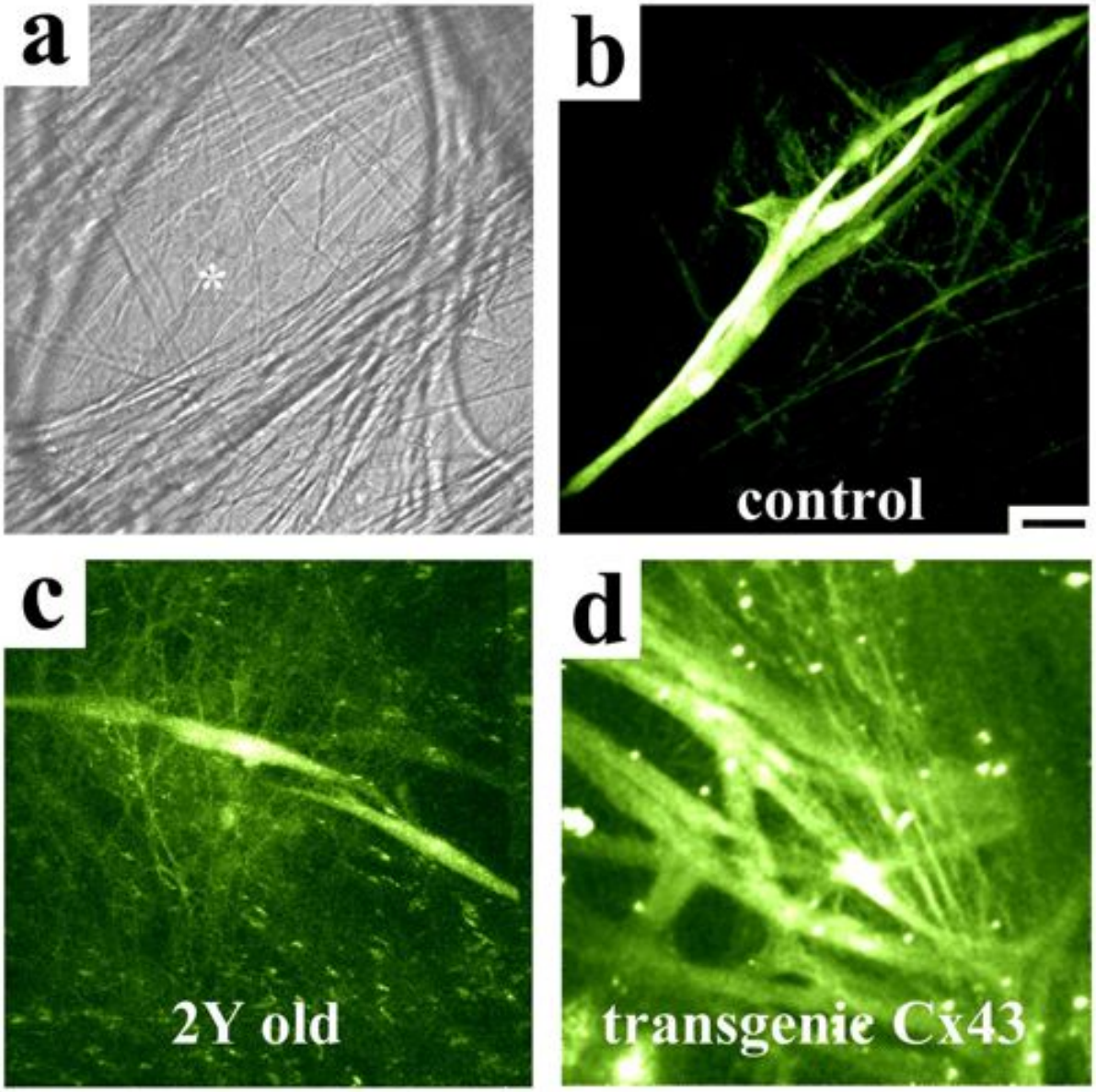


Lucifer yellow permeance in control murine atria



Connexin Laboratory

Lucifer yellow permeance in murine atria.

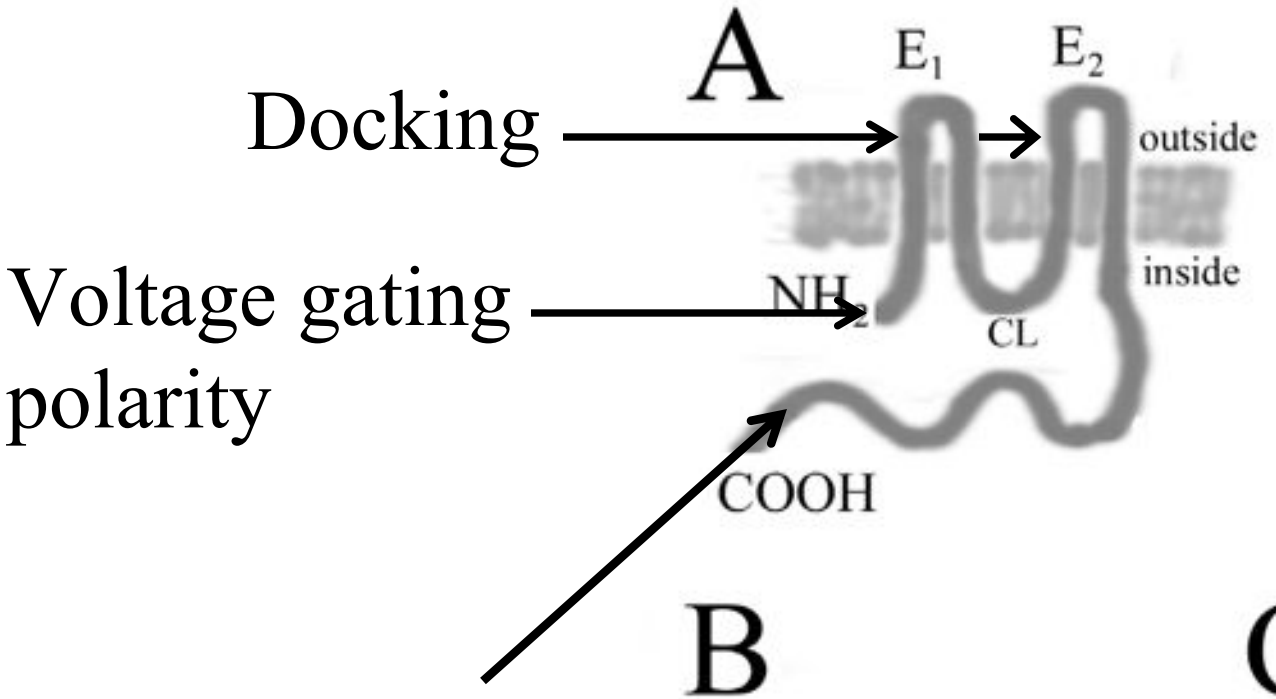


Connexin Laboratory

Gating of gap junction channels

- Gating by voltage
  - Trans-junctional and Trans-membrane
- Gating by intracellular pH
- Gating by protein phosphorylation

IV. Structure function relationship

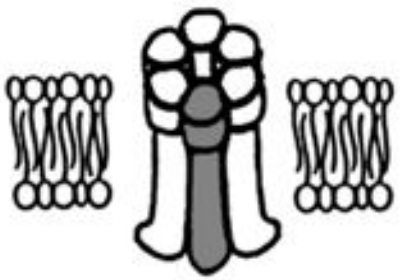


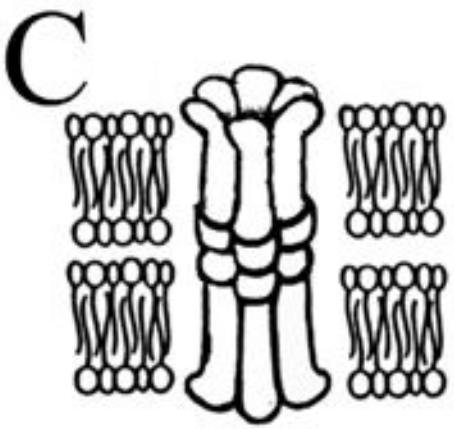
Connexin26

↓

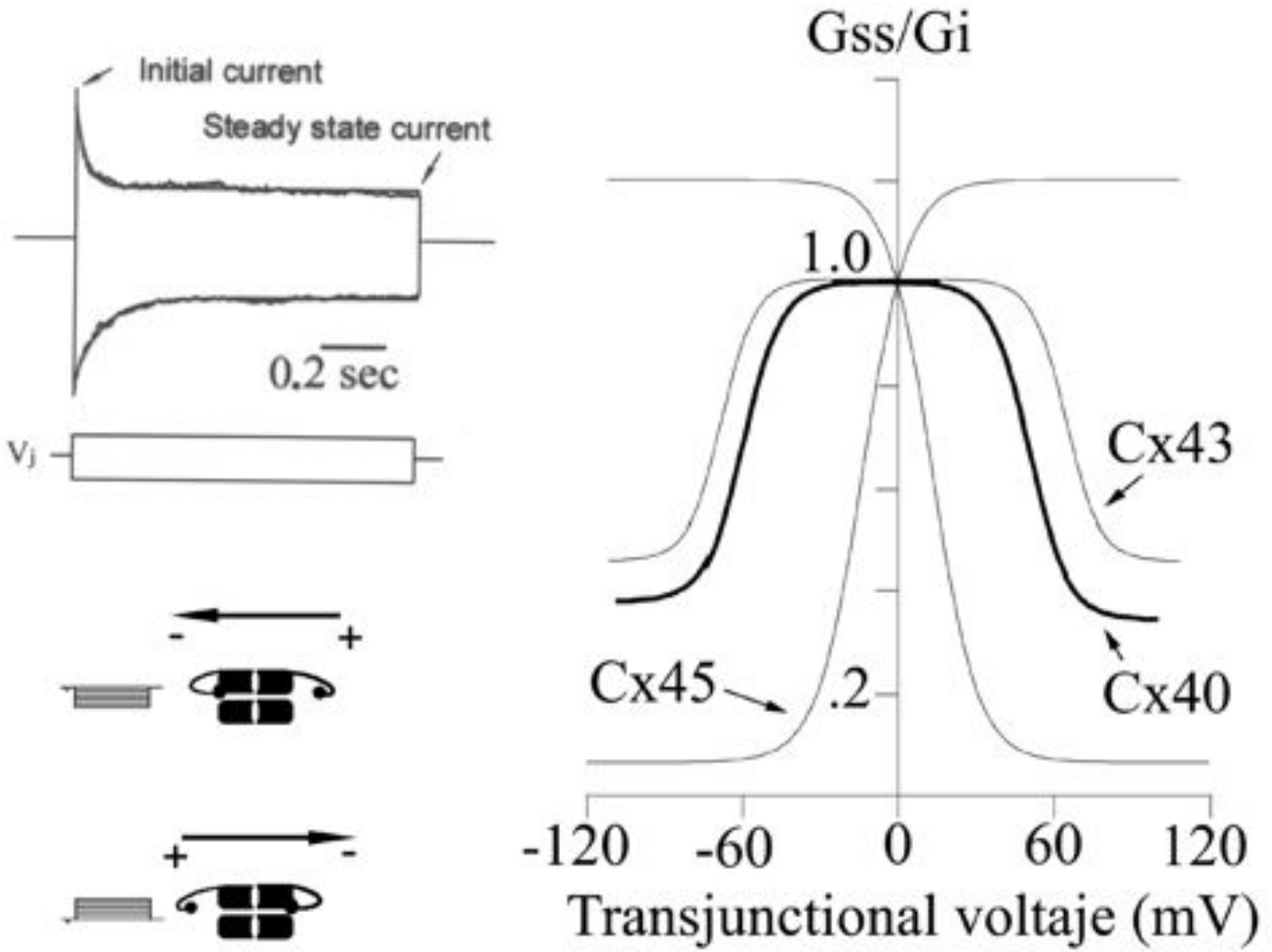
Connexin56

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





Transjunctional voltage dependence



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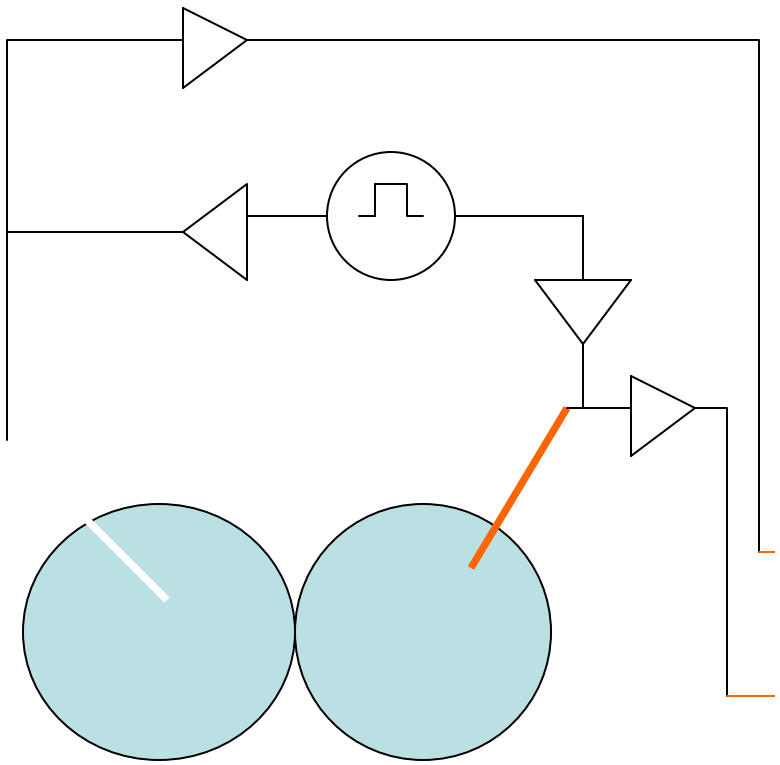
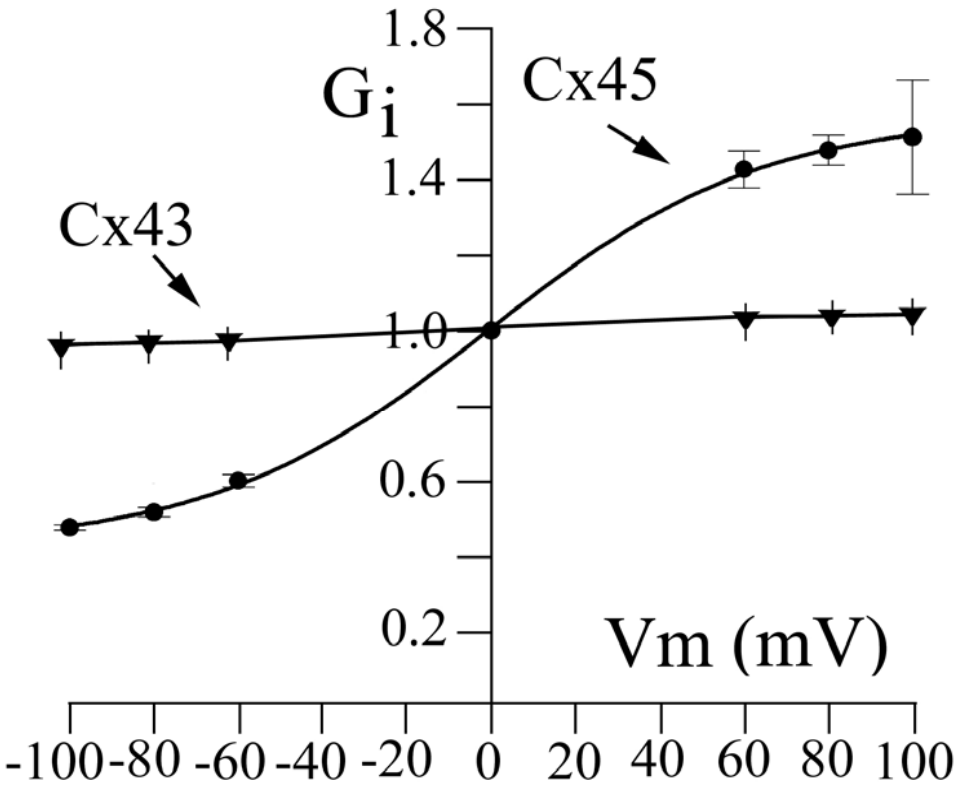
Gating by transmembrane voltage





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

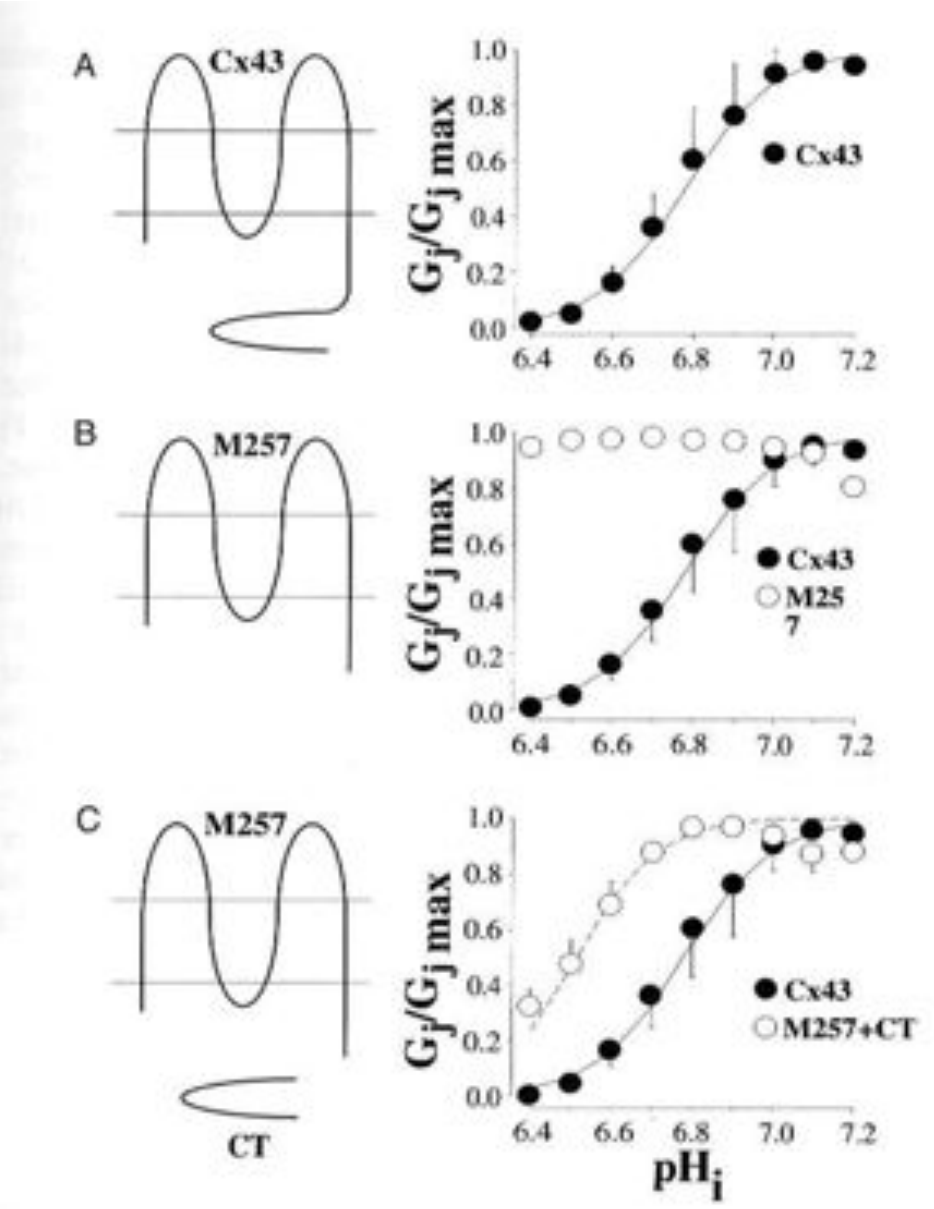
Connexin Laboratory

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.

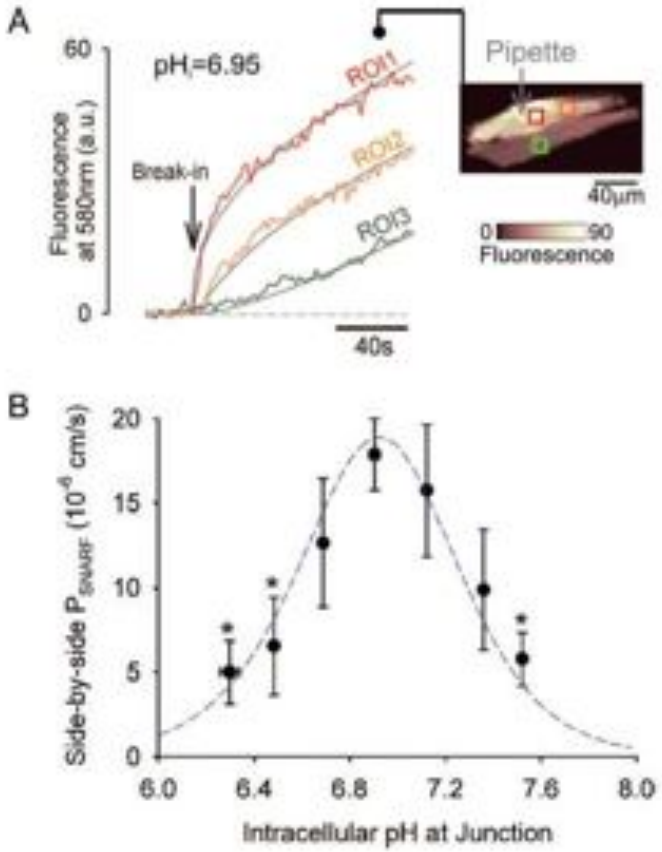
If the COOH tail is co-expressed, the gating by pH is re-established.

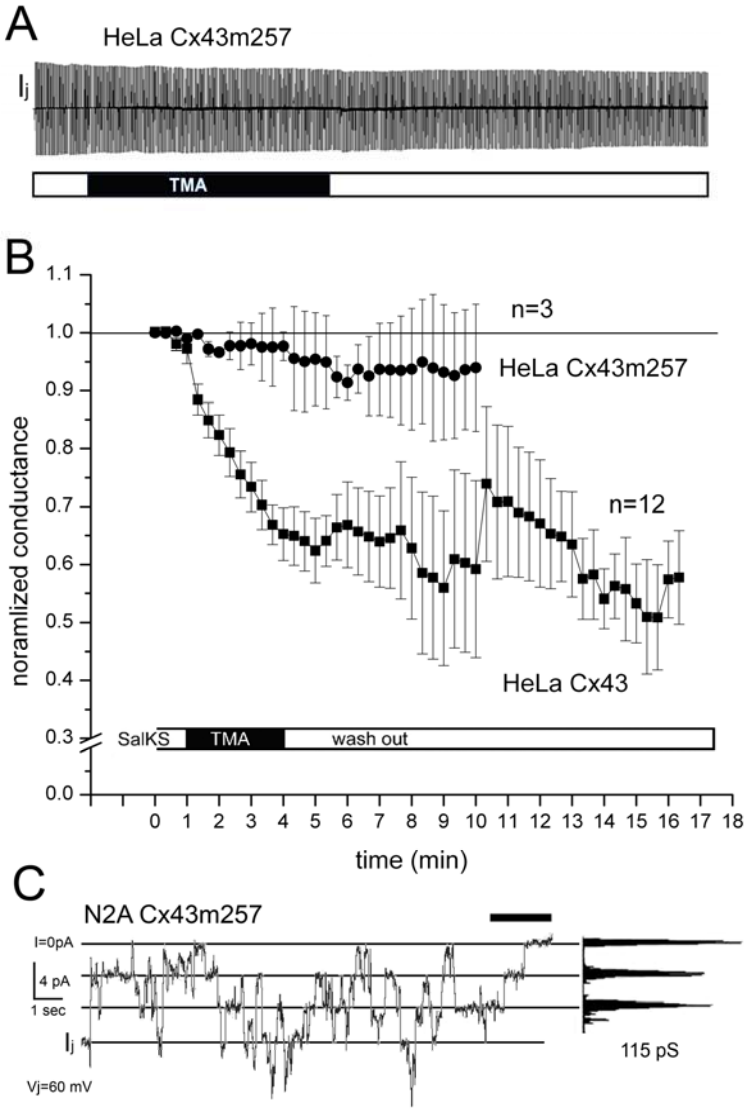


M. Delmar et al. Current Topics in Membranes (2000)

Alonso P. Moreno
Connexin Lab.
Krannert IUPUI

Connexins also gate for intracellular alkalosis





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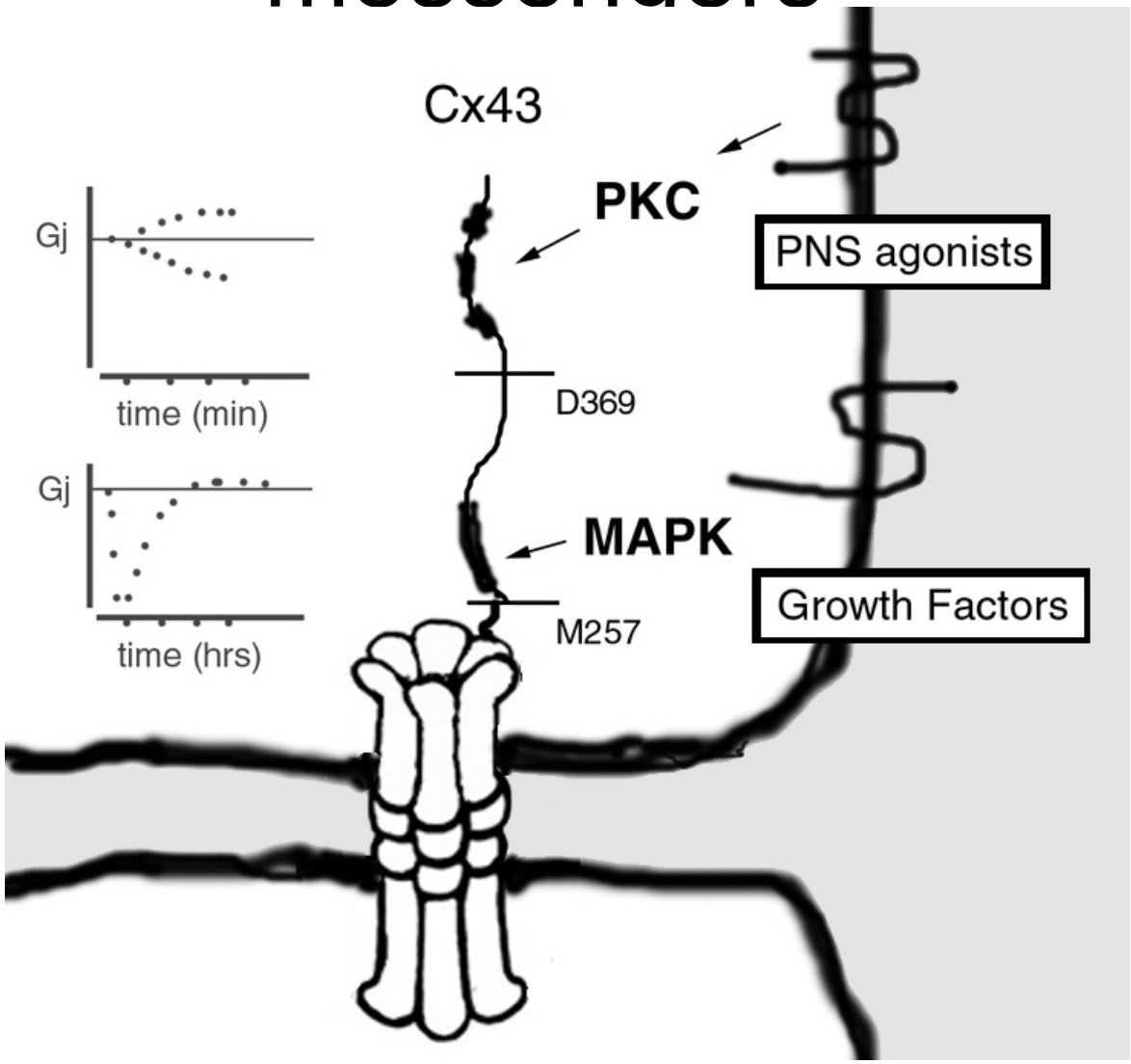
Gating through second messengers



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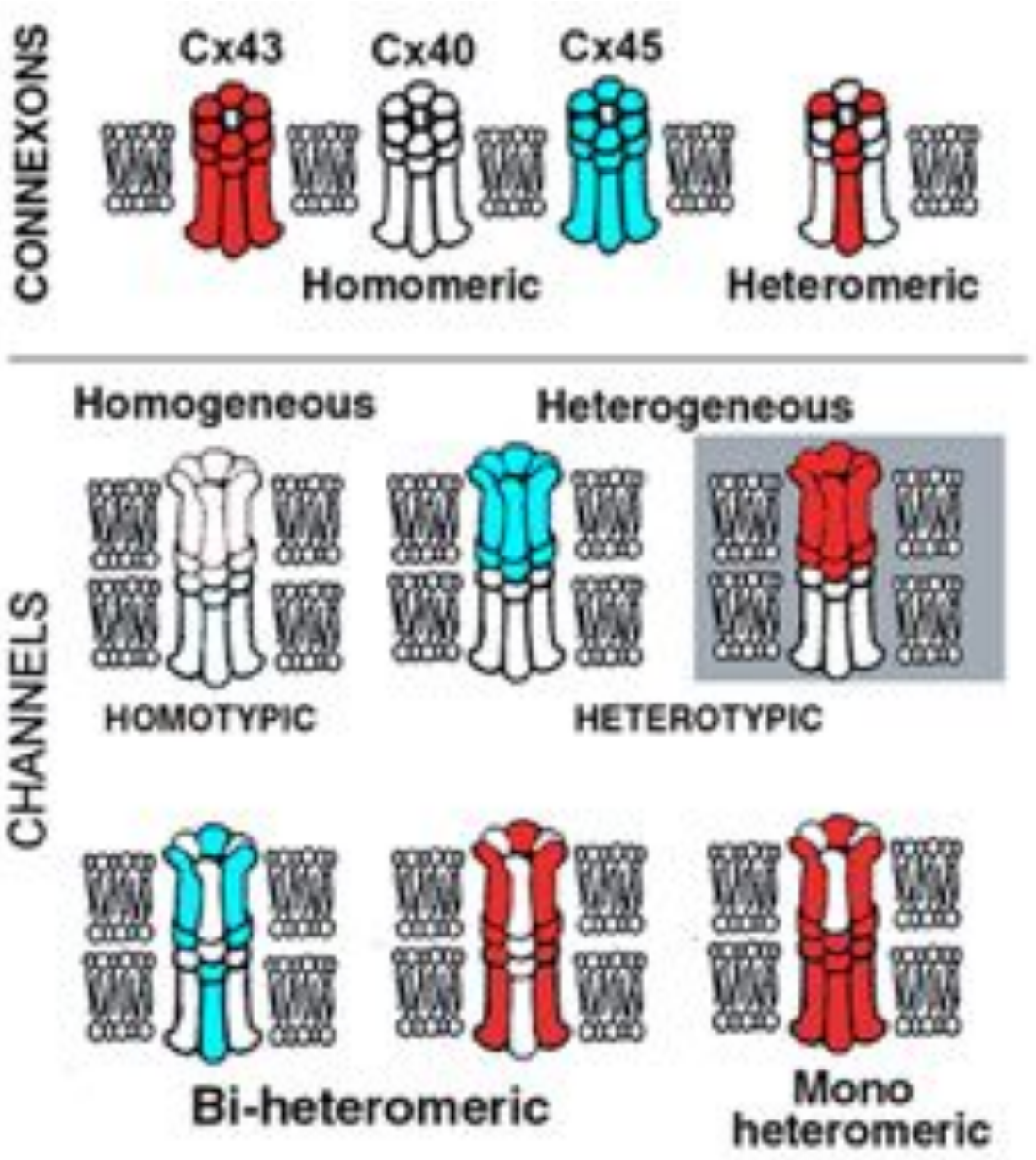
Gap Junction Channels Multiple Configurations

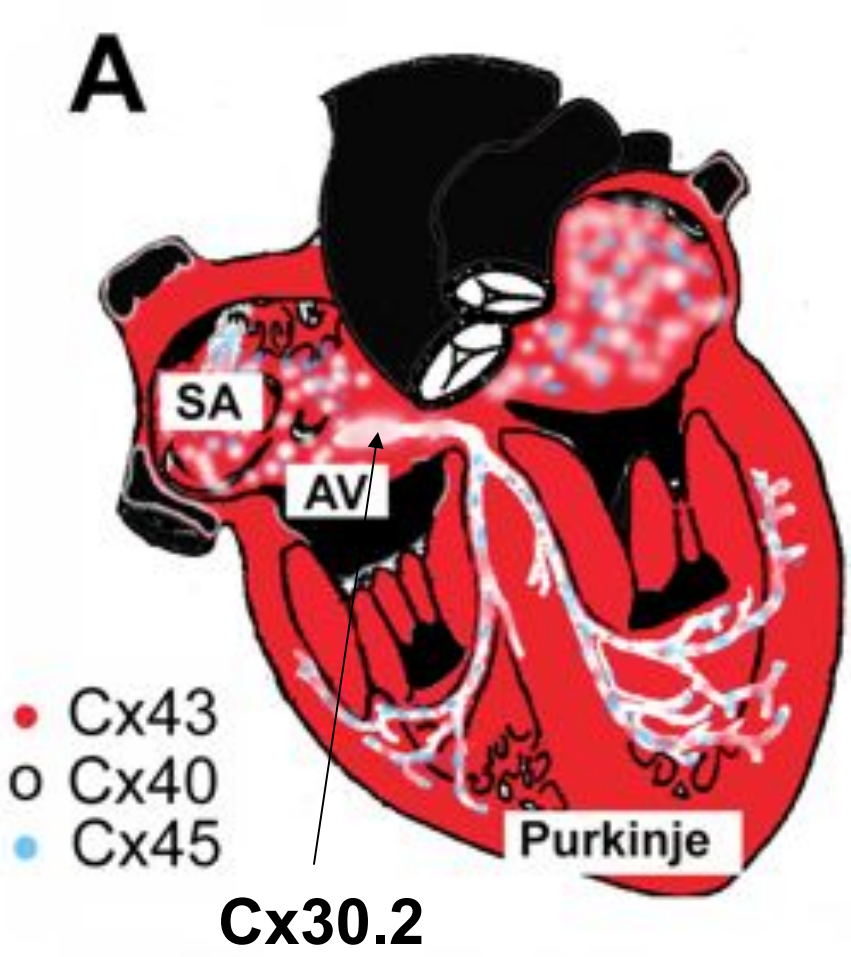


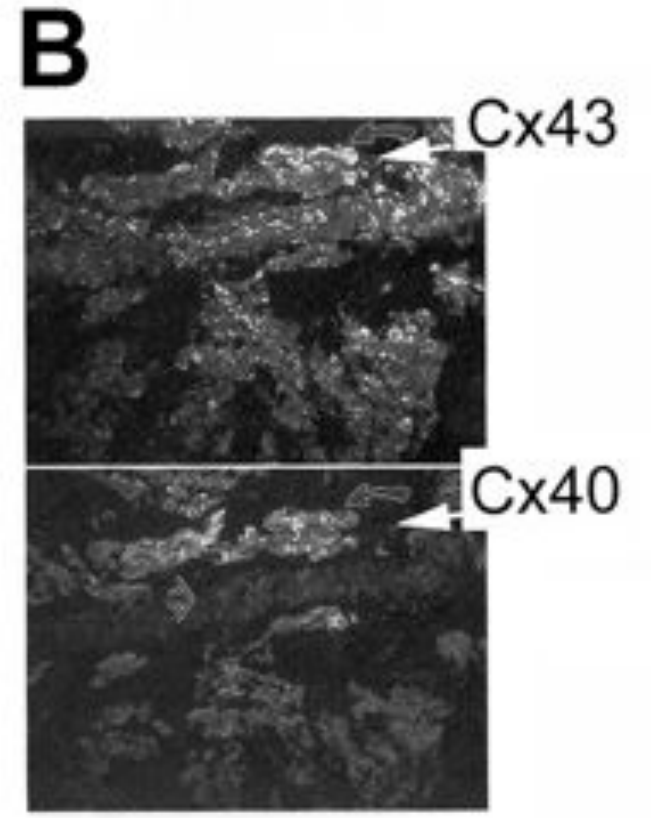
Connexin Laboratory

Multiple expression of connexins in a tissue

Connexins in the heart

Example of the co-expression of connexins

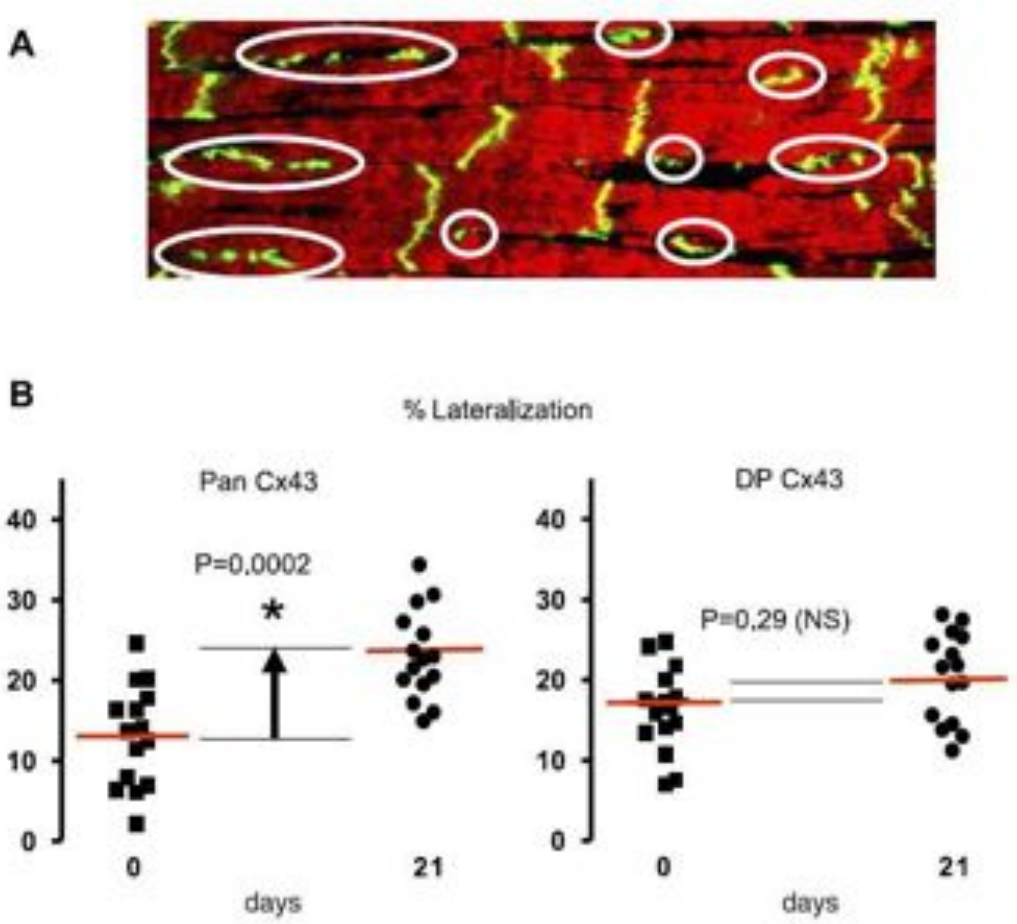


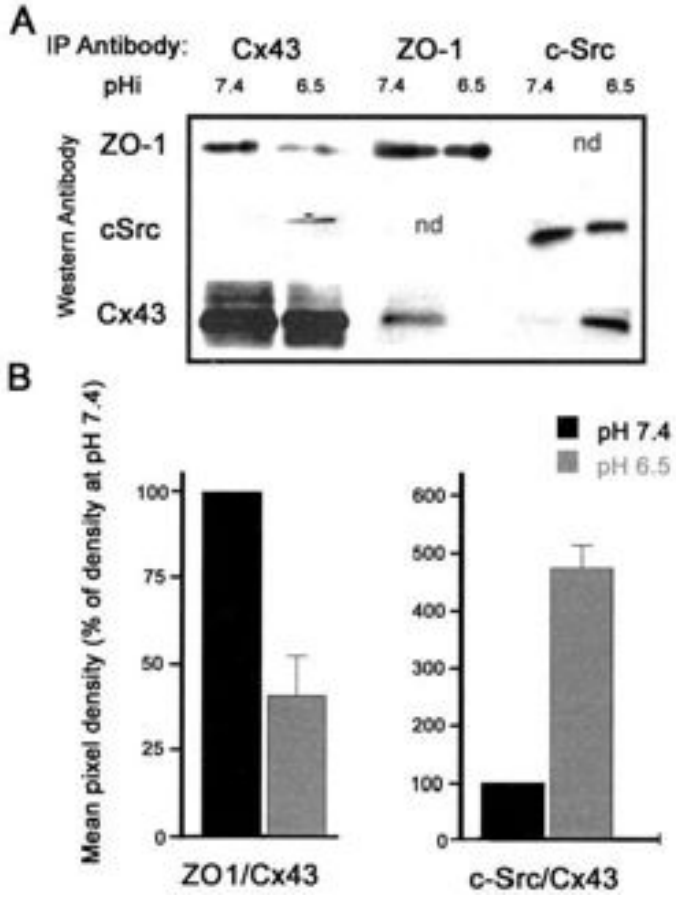


Canine sinus node
(Kwong et al, Circ Res. 1998)

Connexin Laboratory

Remodeling (long term ischemia or heart failure)





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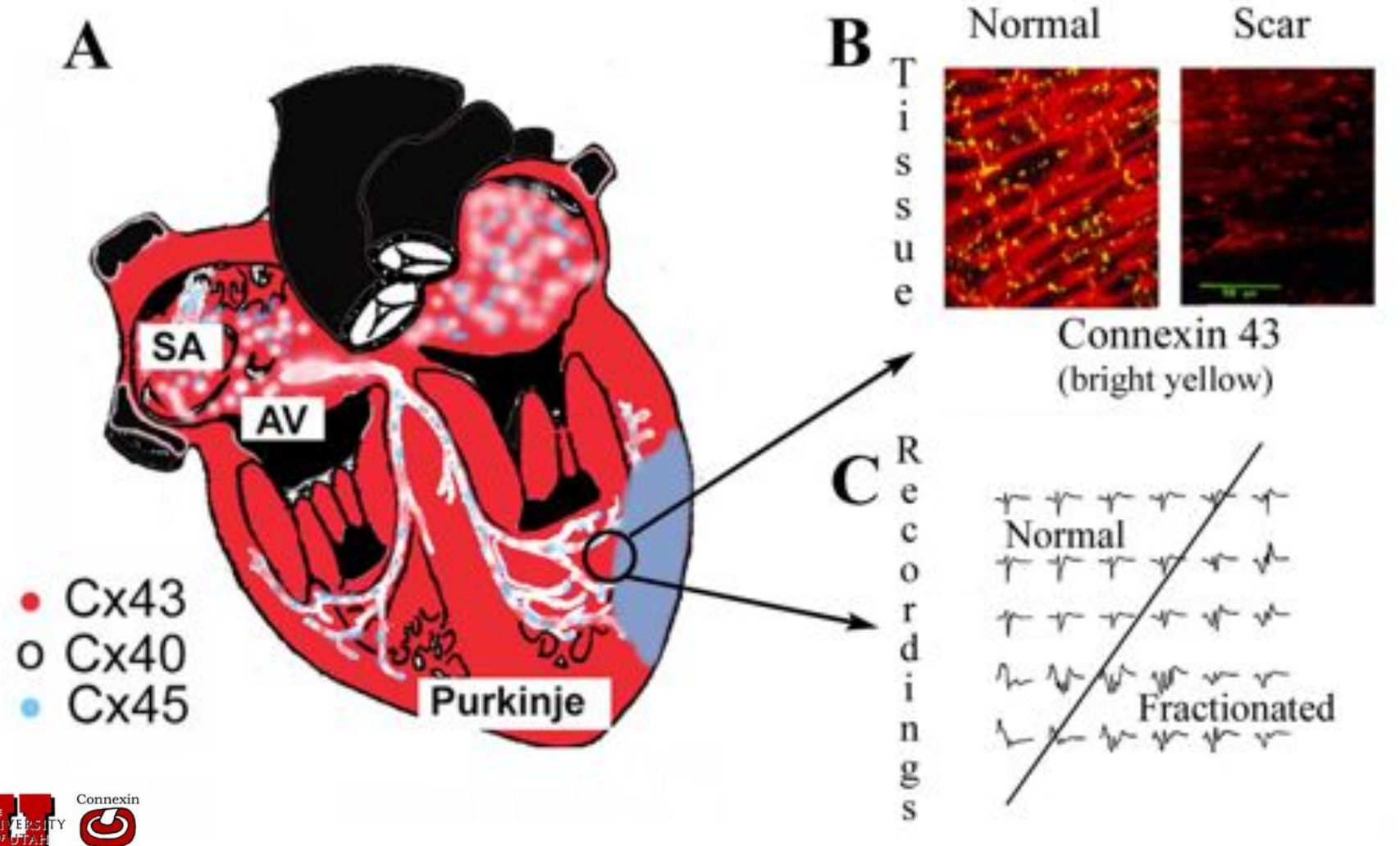
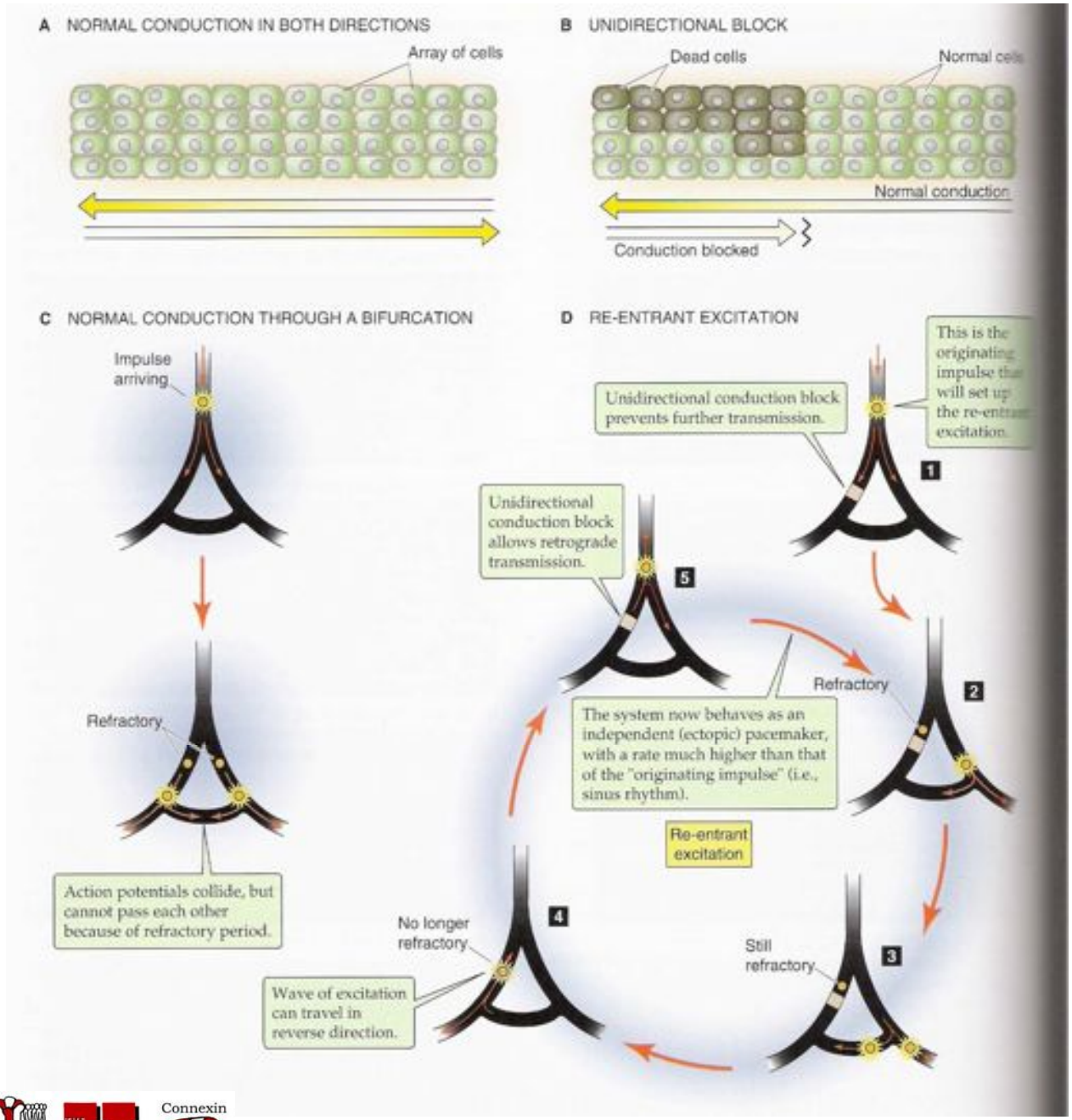
Connexins in the normal and infarcted heart



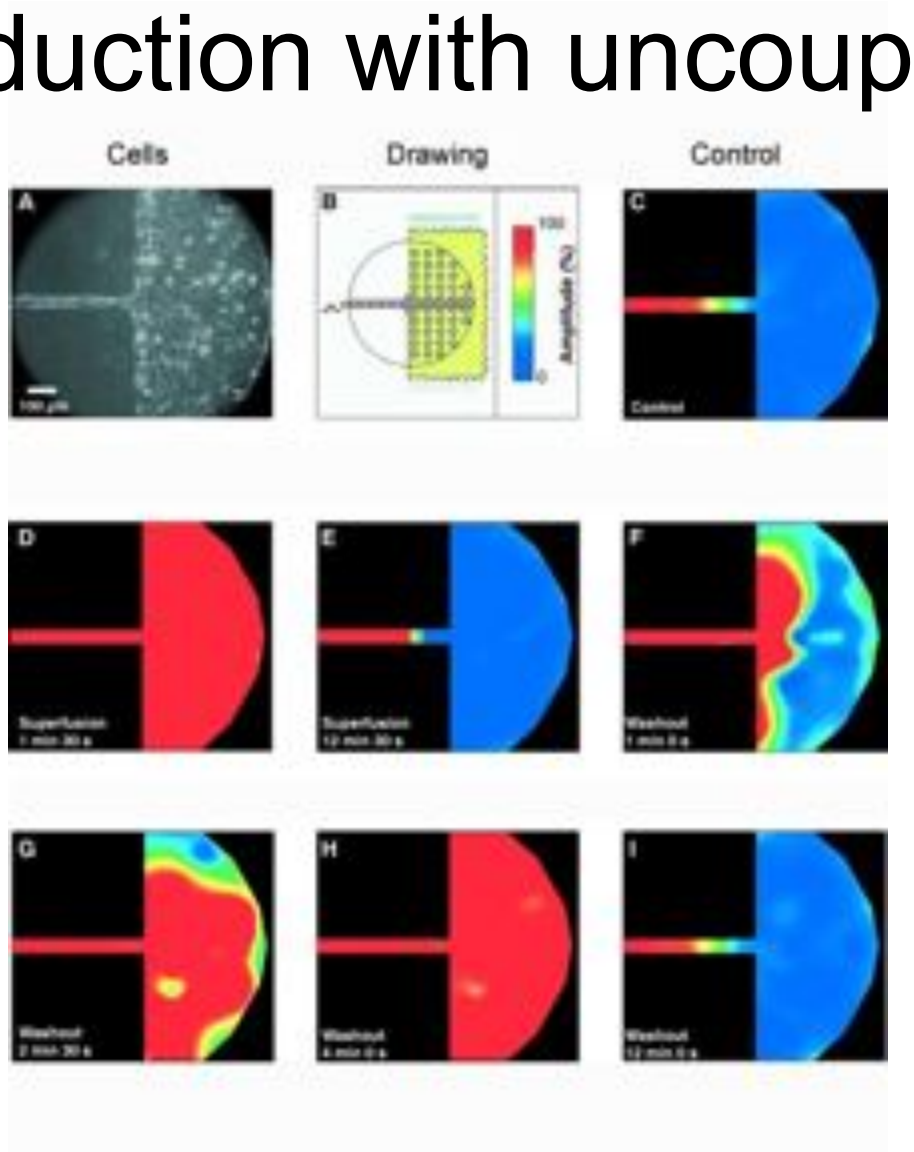
Connexin Laboratory



Arrhythmias

- Action potential
- Refractory period
- Membrane channel regulation

How to increase heart tissue conduction with uncoupling



Rohr et al, Science:275, 1997