Bioengineering 6003
Cellular Electrophysiology and Biophysics

Cardiac cell-cell Communication
Part 1
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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 cells 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
Structure of gap junction channels
Connexins and Pannexins
Molecular Structure
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 channel.
K+ channels

splicing

No splicing in gap junction channels

Six to seven transmembrane domains

Four transmembrane domains

Fig. 3. Diagram of Co32, showing transmembrane orientation, conserved cysteine residues (asterisks), and locations of the CMTX mutations (arrows) (27). The Co32 structure is based on (10, 16). The indicated mutations are as follows: G12S, GGC → AGC in family 58 from Belgium (11, 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, CTG → GCC in family 251 from Pennsylvania (discussed in this paper); P172S, CCC → TCC in family 133/1852 from North Carolina (5, 20); 175 frameshift. A insertion in family 51 from North Carolina (4, 5, 17); and E186K, GAG → AAG in family K1769 from Oklahoma (23).
Gap junction channel ultra-structure

Yeager et al, Science 283, 1999
Full channels and hemichannels
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 although could be responsible for partial depolarization and hyperactivity during stress.
Regulation of intercellular communication

- It is simple
  
  Electrically we evaluate $g_j$ or junction conductance

  $$g_j = n \times \gamma_j \times Po$$

  - $n$ = number of channels (Insertion-removal)
  - $\gamma_j$ = 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 \( I_X = G_X(V_m - E_X) \)
The Patch-Clamp Technique to study for hemichannel function
Double whole cell voltage clamp and gating of gap junction channels.
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 the 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

Phase contrast

Fluorescein Filter

Coupled cells

Dil + Calcein AM

Co-cultured

Rhodamine filter

Loaded cells
Molecular flux

Current traces observed during the formation of a whole cell patch
Molecular flux quantification

Lucifer yellow flux across gap junctions in HeLa cells.
Gating of gap junction channels

• Gating by voltage
  – Transjuntional and transmembrane

• Gating by intracellular pH

• Gating by protein phosphorylation
Structure function relationship

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

![Graph showing voltage dependence with Cx43, Cx45, and Cx40]

- Initial current
- Steady state current
- Transjunctional voltage (mV)
- Gss/Gi

- Cx43
- Cx45
- Cx40
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 ($G_j/G_{max}$).

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.

Gating through second messengers

- Cx43
- PKC
- MAPK
- Gj (time in min)
- Gj (time in hrs)
- PNS agonists
- Growth Factors

Graphical representation showing changes in Gj over time with different time scales for minutes and hours.
Phosphorylation sites in Cx43 carboxyl tail
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: 20 ± 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 histograms 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.

Fig 3. Frequency distributions of unitary junctional conductance (γj) 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 γj = 29 ± 10 (SD, 9% of total events), 62 ± 9 (black area, 25% of total events), and 89 ± 16 (shaded area, 66% of total events) pS. Middle, Average of four experiments in which 300 nM okadaic acid was added to the bathing solution for 30 minutes to 1 hour before recordings were begun. Best fit correspond to γj = 57 ± 16 (71% of total events) and 103 ± 7 (29% of total events) pS. Note shift in γj values to lower conductances. Bottom, Average of three experiments in which cells were treated with staurosporine (300 nM/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 γj 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

- 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 blockuers. In general membrane lipophylic substances.

- Specific activators like Ca++ or ATP
  pH
<table>
<thead>
<tr>
<th></th>
<th>K+</th>
<th>vs</th>
<th>Gj</th>
</tr>
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<tbody>
<tr>
<td>Genetic origin</td>
<td>genes-splicing</td>
<td>Each connexin a gene</td>
<td></td>
</tr>
<tr>
<td>Six trans-membrane domains</td>
<td>Some seven</td>
<td>Four trans-membrane domains</td>
<td>Not known for any more</td>
</tr>
<tr>
<td>Tetrameric</td>
<td></td>
<td></td>
<td>Hexameric</td>
</tr>
<tr>
<td>Some families form heteromersics</td>
<td></td>
<td>Some families form heterermecics</td>
<td>Some form <strong>heterotypic channels</strong></td>
</tr>
<tr>
<td>S5-S6 forms the selectivity pore</td>
<td></td>
<td>So far we know that M2 and M3 are aligned along the pore</td>
<td></td>
</tr>
<tr>
<td>Highly selective to K+</td>
<td>K 1000x := Na</td>
<td>Perm selective to large molecules</td>
<td></td>
</tr>
<tr>
<td>ATP cGMP, cAMP even siRNA</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Unitary conductances from 4 to 15 pS</td>
<td></td>
<td>Unitary conductances from 5 to 400 pS.</td>
<td></td>
</tr>
<tr>
<td>Activates and inactivates with Vm</td>
<td></td>
<td>Only inactivates and it is with Vm or Vj</td>
<td></td>
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</tbody>
</table>
Gap Junctions
Myocyte communication

Monolayer
Scar
Scar + NRK

Region 1
Propagation of electrical activity
Region 2

Gj=7.3mS
Gap Junctions
Gj=6.2mS

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.