Modeling Gap Junctions and Direct Cell-to-Cell Communication: A few Examples

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A story about:

- Cell-to-Cell Communication
- Gap Junctions (GJs) and GJ turnover
- (Molecular) Modeling!
Gap Junctions: Who has them and why?

Chlamydomonas Volvox
Hydra Planaria Crayfish Mouse Human

Human ovarian tube epithelium
Human airway epithelium.

(These cells have plasmodesmata, an analog structure used by plants to communicate)
Cell signaling occurs over long (A) and short (B, C, D) ranges: -- Four principle mechanisms to signal from one cell to another --

(A) ENDOCRINE
- endocrine cell
- receptor
- hormone
- bloodstream
- target cell

(B) PARACRINE
- signaling cell
- local mediator
- target cells
- target cells

(C) NEURONAL
- neuron
- synapse
- neurotransmitter
- cell body
- axon
- target cell

(D) CONTACT-DEPENDENT
- signaling cell
- membrane-bound signal molecule
- target cell

TV/Radio broadcast
Long-distance call; email
Flyer posted on selected message boards
Face-to-face communication
Cell-Cell Junctions Play a Key Role for Complex, Multi-Cellular Life

- Development and Morphogenesis
- Differentiation
- Cell and Tissue Function

- **Tight Junctions**
  Barrier Function and Cell Polarization

- **Adherens Junctions**
  Cell-Cell Adhesion and Cell Migration

- **Desmosomes**
  Tissue Strength

- **Gap Junctions**
  Direct Cell-to-Cell Communication

- Gap Junction Functions Include:
  - Coordination of heart beat
  - Onset of labor
  - Conduct neuronal signals through electrical synapses
  - Insulin secretion by pancreatic β-cells
  - Maintenance and modulation of cell-cell contacts
  - many more!

- Known diseases include:
  - Heart diseases
  - Neuropathies
  - Deafness
  - Lens Cataracts
  - Skin Disorders
  - Bone Malformations
Gap Junctions (GJs) mediate direct cell-to-cell communication

GJ channels are small and densely packed! Only 10 nm center-to-center spacing

- GJ Plaque
- GJ Channel
- GJ Hemi-Channel or Connexon made of 6 Connexin (Cx) proteins
Electron Microscopic (EM) Structure of Gap Junctions

Edge-on View:

Thin Section

Freeze Fracture

Negative Stain
Gap Junction Channel Function Demonstrated by Dye-Transfer Assays and Electrophysiological Measurements

Recombinant Cx26 connexons expressed and reconstituted from baculovirus-infected insect cells.

(From Falk, Buehler et al. EMBO J. 16:2703-16, 1997)
Connexins (Cxs) are polytopic trans-membrane proteins (21 different Cxs in Humans)

- Hydrophobicity Plots
- Anti-Peptide Antibody Mapping
- Protease Protection Assays
Gap Junction Plaques Assembled from Different GFP-tagged Connexins are Functional

(Transfected HeLa cells)

(From Falk, J. Cell Sci. 113:4109-20, 2000)
Gap Junctions: Many Interesting Questions for Modeling

(3 Examples)

(1) Why do GJ channels cluster and what holds the cluster together?

(2) How and where do connexons dock?

(3) What happens on the cytoplasmic surface of a gap junction that is endocytosed?
Gap Junctions are dynamic assemblies of channels
(Lateral Motion of Gap Junctions in the Plane of the Membrane; Surface Views)

60 frames, 30 sec apart, 30 min total time, looped

91 frames, 10 sec apart, 15 min total time, looped

75 frames, 30 sec apart, 37.5 min total time, looped

70 frames, 30 sec apart, 35 min total time, looped

(From Lopez, ..., & Falk, Cell Com. & Adhes., 8:237, 2001)
Weird, GJs have an unusually short half-life of only a few hours! (Newly synthesized Connexons are Recruited to the Periphery of GJ Plaques while simultaneously older channels are removed from plaque centers (Dendra2 – Photoconversion))

$T^{1/2} = \sim 2h$

(Lauf et al., PNAS 2002; Falk et al., MBoC 2009; Thevenin et al., MBoC 2018)
Gap Junctions internalize into one of the coupled cells to form cytoplasmic double-membrane vesicles termed Annular Gap Junctions (AGJs).

GJ turnover correlates with the short half-live of GJs and Cxs of 1-5 hours.
Biosynthesis and Degradation of GJs: A Continuous Dynamic Process

(From: Segretain & Falk, 2004, BBA, 1662:3-21, updated)
**Hypothesis:** ZO-1 release, de-/phosphorylation and ubiquitination opens up the Cx43-CT to allow AP-2/clathrin to bind to Cxs and internalize GJs

(In open, functional GJ channels the AP-2/clathrin binding sites are in-accessible/sterically blocked)

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**Qu.:** Structurally, what is different between new (functional) and old (non-functional) GJ channels; or why do old channels interact with the endocytic machinery and are endocytosed while new ones do not?

(from Falk et al., 2016, BMC Cell Bio 17:22)
Modeling Cx43 GJ Protein Structure

- Cx26 atomic resolution structure (Zukihara Lab)
- Cx43 7Å resolution structure (Yeager Lab)
- Cx26 structure (PDB: 2ZW5)
- Cx43 C-Terminal Domain (lowest energy NMR-solution structure) (PDB: 1R5S)
- Combined Cx26/Cx43 hybrid structure after some minimization (Sorgen Lab)
Membrane Lipid Composition:
- Outer Leaflet: 18 CHL1, 36 POPC, 36 PSM
- Inner Leaflet: 9 CHL1, 12 POPC, 30 POPS, 9 PSM
Top view

One-half of a GJ plaque

Side view
Molecular simulations using CHARMM-GUI

Animation of un-phosphorylated CT

Animation with serines 279, 282, and 368 phosphorylated

180 ns molecular dynamics simulation; 180 times repeated; ~ 28 sec total time

**Green spheres** depict the serine residues that can be phosphorylated
Molecular simulations

Animation of un-phosphorylated CT

Animation with serines 279, 282, and 368 phosphorylated

180 ns molecular dynamics simulation; 180 times repeated; ~ 28 sec total time

Green spheres depict the serine residues that can be phosphorylated
The Radius of Gyration indicates how compact a protein is; smaller is more compact!
Why does all this matter?

Preventing phosphorylation and other post-translational modifications on critical Cx43 C-terminal amino acid residues, interferes with balanced GJ turnover, and cause disease.
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