Gap Junction Channels

Presented by: Ima Student
Overview

- Intracellular communication
- Human touch
  - Autosomal recessive deafness
  - X-linked Charcot-Marie-Tooth disease
  - Cx32 congenital demyelinated neuropathy
  - Cx50 congenital cataracts
- Transgenic mice lacking $\alpha_1$ Cx43
  - Principle heart gap junction
Three-Dimensional Structure of a Recombinant Gap Junction Membrane Channel

Vinzenz M. Unger, Nalin M. Kumar, Norton B. Gilula, Mark Yeager
Objective

- 3D analysis to explore transmembrane architecture.
- Previously 2D crystal analysis suggest 2 rings of $\alpha$ helices.
- Wild type and mutant connexins expressed in BHK cells
  - $\alpha_1$-Cx263T mutant
### Table 1

**Resolution**
- ~ 7.5 Å in membrane plane
- 21 Å vertical

**Range of tilt**
- 0° to 35.3°

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<th>Parameter</th>
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<td>Unit cell parameters</td>
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<td>Range of crystal tilts</td>
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<td>Range of underfocus</td>
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<td>Total number of fitted unique reflections†</td>
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<td>Overall weighted phase error‡</td>
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<td>Effective resolution cutoffs§</td>
<td>7.5 Å (in plane)</td>
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<td>21.0 Å (vertical)</td>
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Figure 1: Phase and Amplitude

Lattice Line (2,7) at 8.1 Å
Lattice Line (8,0) at 8.3 Å
Lattice Line (8,1) at 7.8 Å
Lattice Line (4,6) at 7.6 Å

Phase
Amplitude

z' [Å]
Figure 2a

- Tripartite arrangement
- ~150 Å thick
- “M” outer diameter ~70 Å
- “E” outer diameter ~50 Å
Figure 2b

- Vertical section
- Narrowing of channel occurs when crossing the lipid bilayer.
  - From 40 to 15 Å
- Center diameter ~25 Å
Figure 2c

- Red contours: $1\sigma$ above mean density; resolution 15 Å
- Yellow contours: $1.5\sigma$ above mean; resolution 17.5 Å
- 24 TM helices per connexin, 48 per channel.
Figure 3a: Helical packing arrangement.
Figure 3b

- C tilt and narrowing of the pore.
- C & B line the pore
- Cytoplasmic connections between helices?
Figure 4

Possible subunit boundaries
Identification of amino acid residues lining the pore of a gap channel.

Objective

- Identify pore-lining residues
  - SCAM
- Determine the pore lining helices
- Make helical assignments
  - Topology map \(\Rightarrow\) 3D model
SCAM

- Channel lumen facing protein domains
- Substitute cysteine for one a.a. at a time in domains.
- Add aqueous thiol reagent
- Measure conductance through channel
- Perform for open and closed states of the channel.
Figure 1: Paired oocyte perfusion system.
Mutants of Cx32

- 48 mutants total
- 3 Nonfunctional: W77C, W133C, T134C
- 36 Minimal changes in conductance (candidates)
- 7 “Reverse-gating” mutants
  - Cx32E146C
  - Cx32A88C
Figure 2

- Candidates
- Nonfunctional
- “Reverse gating”
- Altered channel properties
Figure 3: A-C

Wild type

Representative candidate mutants
Figure 3: A & D

Wild type

“Reverse-gating”
(heterotypic)
Figure 3F: Cx32E146C

- Nonfunctional channel homotypically or heterotypically with wtCx32
- Add DTT to mutant: wt junctional current restored.
Disulfide bond between E146C & C201
Cx32A88C

- Lethal to oocytes
- 10 fold increase in membrane conductance
- Current characteristic of open hemichannels
Minimal changes in transjunctional current when oocytes are cut and perfused. Partial loss of $V_j$ sensitivity.
Figure 5A: MBB

- Large thiol reagent, still too small to cause full channel blockage.
- Maleimide is an irreversible thiol reagent.
Figure 5B: Western Blot

- Lane 1: Cx32 from intact oocytes
- Lane 2: Cx32 perfused with MBB
- Lane 3: Noninjected oocytes exposed to MBB
- Lane 4: Perfused Cx32 not exposed to MBB
Figure 5: C & D

- Cx32 wt: conduction increases before & after treatment.
Figure 6

Standard Dual Oocyte Perfusion

M1
- S26C
- V27C
- 128
- F29C
- R32C
- L36C
- V37C
- S78C
- S85C
- T86C
- P87C
- A88C
- V91C
- A92C
- M93C
- wtCx32

M2
- V84C
- L89C
- L90C
- V91C
- A92C
- M93C
- wtCx32

% conductance change after MBB
-40-30-20-10 0 10 20 30 40

M3
- V136C
- H137C
- I138C
- V139C
- Y135C
- V140C
- V140C
- L143C
- A147C
- E146C
- F145C
- M150C
- Y151C

% conductance change after MBB
-40-30-20-10 0 10 20 30 40

M4
- A196C
- A197C
- S198C
- G199C
- L200C
- C201
- L202C
- wtCx32

% conductance change after MBB
-40-30-20-10 0 10 20 30 40
- Majority of reactive residues on M3
- M1: 4 “reverse-gating” reactive residues
- M2: reactive near cytoplasmic end or proline
- M4: reactive near extracellular end
Non-pore lining cystines

- From structure model only 2 helices thought to line the pore.
- Aqueous “crevices”?
Figure 7

Reactive sites eliminated as pore lining sites.
Figure 8: Summary
M2 & M3 (A & C) are the pore-lining domains in the open state.
- Not M1 (B)

Helical periodicity of reactive residues

Towards extracellular end reactivity of β-sheet pattern

F149C highest block reactivity
- Narrowing point of M3
Figure 9

M1 = B  M2 = A  M3 = C  M4 = D

A Model of a Gap Junction Pore

B Assignment of Helices to Unger et al. Model

Open state  Partial closed state
### Alignment of Reactive Sites in M3

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Discussion

- Why have a pore that is partially open all the time?
- Preference of cations over anions.
- Why preference of ATP over ADP?