Introduction

- Channelrhodopsins (ChRs) are directly light-gated cation channels
  - Channel pore opened by light-induced isomerization of bound all-trans-retinal
- ChR1 & ChR2 found in eyespot of motile algae *Chlamydomonas reinhardtii* [1].
- C1C2 is a chimera of ChR1 + ChR2 used as a structural model for rational ChR design [2].
- Optogenetic research tool- enables precise control of excitable cells with light [3].
  - Vision restoration
  - Neuronal circuit mapping
  - Optogenetic pacemakers
- Wild-type channel is non-selectively permeable: $H^+ >> Na^+ > K^+ >> Ca^{2+}$
- Growing need for more selective ChRs for cell type-specific applications

Objectives

- Measure the functional effects of single-residue substitutions at conserved positions near central gate of C1C2
- How does electrostatic charge in the pore near the central gate impact calcium selectivity?

Measuring Ca$^{2+}$ selectivity of C1C2 wild-type and mutants expressed in *Xenopus laevis* oocytes via TEVC [4]

Site-Directed Mutagenesis & in vitro mRNA Synthesis

- Point mutations introduced into C1C2 gene via PCR: N297D, N297V
- mRNA synthesized in vitro using commercial kit

Pre-treatment with BAPTA-AM

- Influx of Ca$^{2+}$ through C1C2 could activate endogenous Ca$^{2+}$-activated Cl$^-$ channels (CaCCs)
- Oocytes briefly incubated with cell-permeant Ca$^{2+}$ chelator BAPTA-AM to buffer intracellular Ca$^{2+}$ and suppress contaminating Cl$^-$ currents

Functional Expression in *Xenopus laevis* oocytes

- mRNA of wild-type or mutant C1C2 injected into defolliculated oocytes
- Oocytes incubated for 48-72 hours to allow for expression of C1C2 on plasma membrane

Permeability Ratios

<table>
<thead>
<tr>
<th>$X$</th>
<th>$P_{X}$ / $P_{Na}$</th>
<th>Permeability Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1C2 WT</td>
<td>0.28</td>
<td>0.85 (\times 10^6)</td>
</tr>
<tr>
<td>N297D</td>
<td>0.29</td>
<td>0.83 (\times 10^6)</td>
</tr>
<tr>
<td>N297V</td>
<td>0.81 (\times 10^6)</td>
<td>0.51 (\times 10^6)</td>
</tr>
</tbody>
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2. Electrophysiological Measurements

- Membrane clamped at series of fixed potentials (subplot)
- C1C2 activated with blue light (blue bar) & whole cell current recorded
- Shifts in reversal potential reflect shifts in relative ion permeability through open channels

Conclusion

- BAPTA-AM treatment successfully suppressed activation of interfering CaCC's during Ca$^{2+}$ current recording for C1C2 wild-type and mutants
- Replacement of polar residue Asn297 with negatively charged Asp increased Ca$^{2+}$ permeability relative to Na$^+$ by 75% compared to wild-type
- A polar to non-polar substitution at the same position increased H$^+$ permeability relative to Na$^+$ by 61%, but relative Ca$^{2+}$ permeability was unaffected.
- Negative electrostatic charge near central gate is important for high Ca$^{2+}$ selectivity in C1C2

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References