Calcium Selectivity in Channelrhodopsin Chimera is Governed by Electrostatic Interactions in Central Pore

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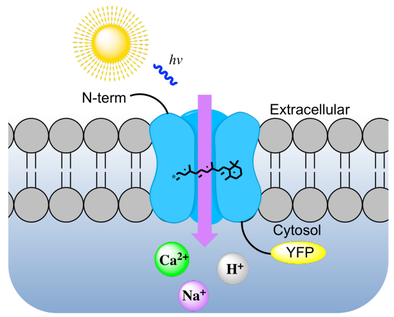
1. Introduction

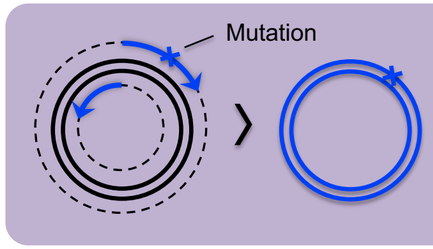
- Channelrhodopsins (ChRs) are directly light-gated cation channels
  - Channel pore opened by light-induced isomerization of bound all *trans*-retinal
- ChR1 & ChR2 found in eyespot of motile algae *Chlamydomonas reinhardtii* [1].
- C1C2 is a chimera of ChR1 + ChR2 used as a structural model for rational 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



2. Objectives

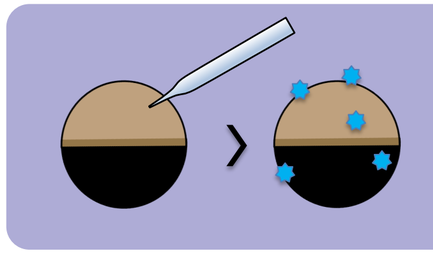
- Measure the functional effects of single-residue substitutions at conserved positions near central gate of C1C2
- How does electrostatic charge in the pore near the central gate impact calcium selectivity?

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



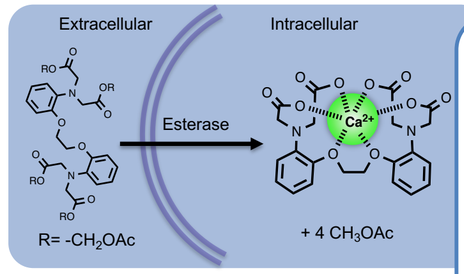
Site-Directed Mutagenesis & in *vitro* mRNA Synthesis

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



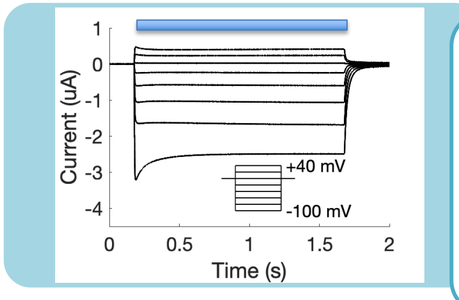
Functional Expression in *Xenopus laevis* oocytes

- 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



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



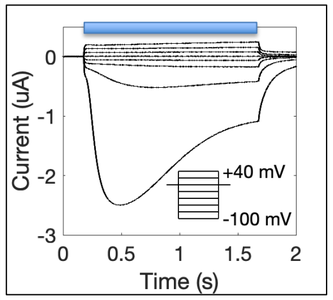
Two Electrode Voltage Clamp (TEVC)

- 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

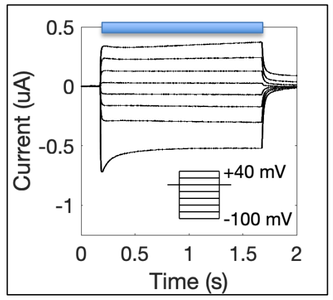
4. Results

C1C2 wild-type Ca^{2+} current

Before BAPTA-AM



After BAPTA-AM



P_X / P_{Na} ;	Permeability Ratios		
X =	C1C2 WT	N297D	N297V
H^+	1.42 x10^6	**0.81 x10^6**	**2.28 x10^6**
K^+	0.83	0.85	0.84
Ca^{2+}	0.29	**0.51**	0.28

5. 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

Acknowledgments

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References

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