Do Plants Have a Rapid Block to Polyspermy?

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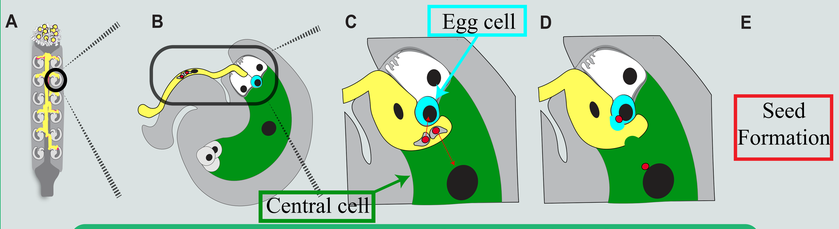
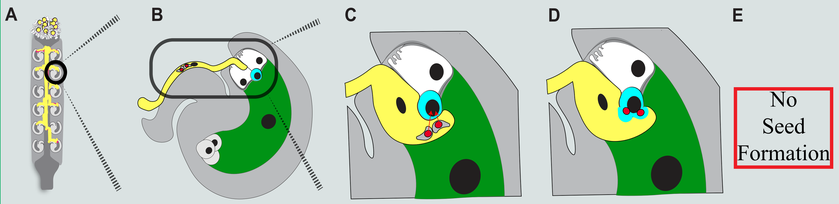
Introduction

Gamete pairing is essential for successful fertilization in *Arabidopsis thaliana*

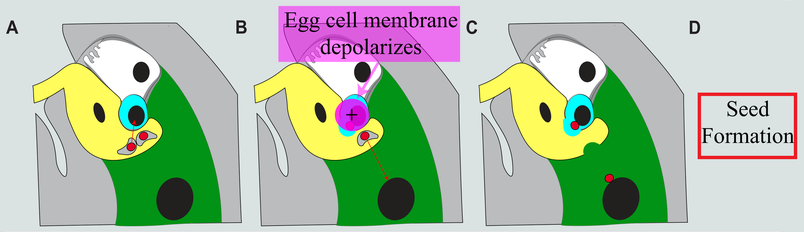


Polyspermy occurs when both sperm fuse with a single female gamete



Hypothesis

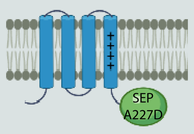
A single sperm fuses with either the egg or central cell causing a membrane depolarization event forcing the remaining sperm cell to fuse with the unfused female gamete.



The mechanism driving gamete pairing is essential for the success of a plant species as it ensures a viable amount of genetic information is passed to successive generations.

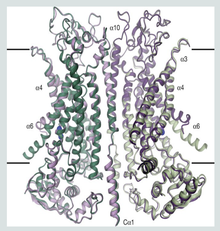
Aim

To address this hypothesis we are taking two approaches.The first approach involves utilizing genetically encoded fluorescent voltage sensors to test the plasma membrane of the egg and central cells for voltage potential changes at the moment of gamete fusion. The second approach is to analyze TMEM16A, a conserved chloride ion channel implicated in the block to polyspermy in animals, and to determine the role it plays during gamete pairing in *Arabidopsis thaliana*.



(Created in Biorender)

Voltage Potential Sensor

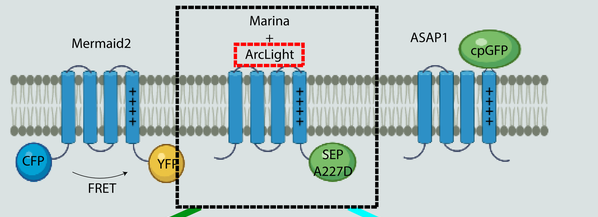


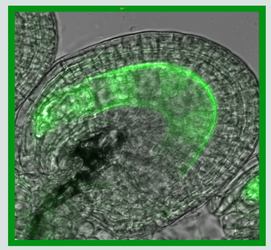
(Paulino et al. 2017)

Ribbon representation of TMEM16A

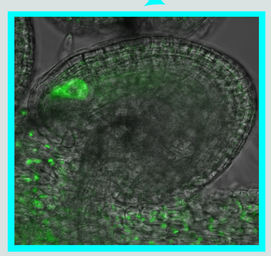
Methods/Results

Genetically encoded membrane potential sensors are used to quantify the change in membrane potential by measuring the change in fluorescence.



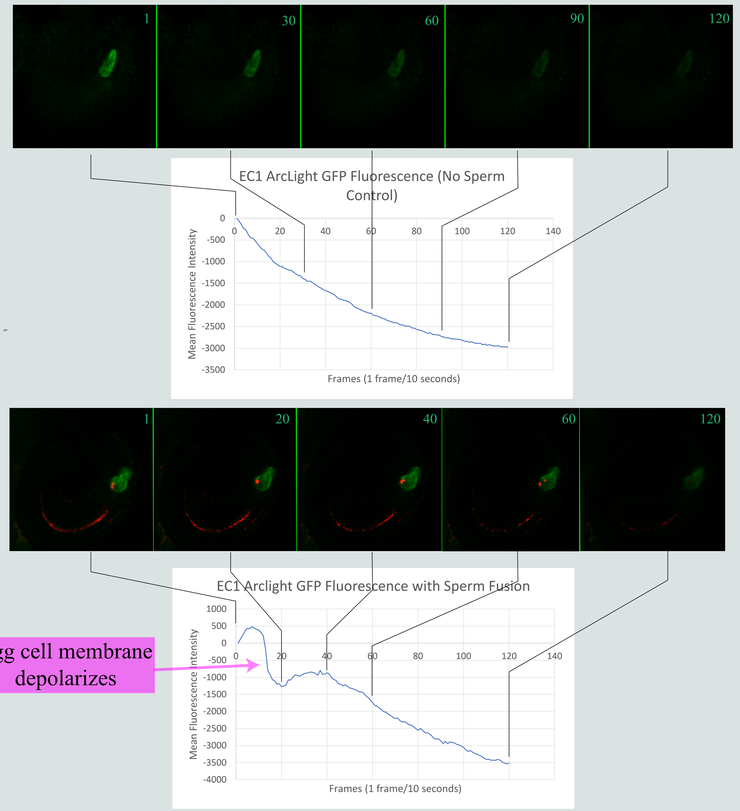


Central Cell Expressed



Egg Cell Expressed

Analysis of GFP fluorescence in an ovules containing ArcLight expressed in the egg cell with no sperm as a control and an RFP sperm fusion event.

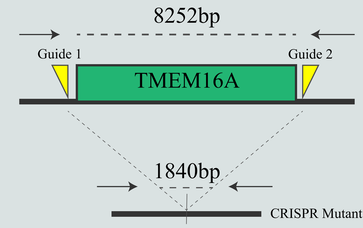


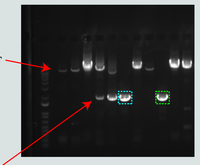
Methods/Results

Why TMEM16A? A table of expression values (RPM) for TMEM16A, EC1, and DD65 shows significant expression of TMEM16A localized in egg cell replicates.

Gene	Mean Central Cell Expression	Mean Synergid Cell Expression	Mean Egg Cell Expression
AT1G73020 (TMEM16A)	0.1	3.8	43.8
AT1G76750 (EC1)	0.9	0	48381.8
AT3G10890 (DD65)	4738.6	0	0

CRISPR mutagenesis of TMEM16A has produced mutants with a deletion of the entire TMEM16A gene. Early PCR genotype analysis suggests the presence of possible homozygous mutants.





Three possibly homozygous replicates obtained

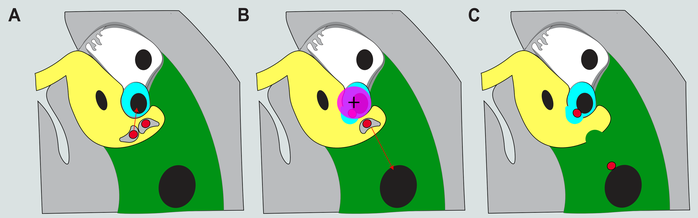
Sequencing results from the three mutants confirm the deletion of TMEM16A

Future Directions

1. Test for a plasma membrane potential change using the ArcLight sensor in TMEM16A CRISPR mutants
2. Test the protein localization of TMEM16A by tagging the C terminus with GFP
3. Test the central cell plasma membrane for depolarization upon sperm fusion

Conclusions

The results from monitoring GFP fluorescence on the egg cell using the ArcLight sensor suggest that there is a depolarization event that occurs upon sperm fusion with the egg cell. Depolarization across the plasma membrane of a female gamete is preliminary evidence for a rapid block to polyspermy in flowering plants. Analysis of membrane depolarization in TMEM16 CRISPR mutants should yield interesting results as TMEM16A is a conserved chloride ion channel implicated in the rapid block to polyspermy in animals.



The membrane depolarization seen in panel B can be seen as a sharp change in GFP fluorescence measured by the ArcLight sensor construct.

Acknowledgements

Johnson Laboratory: Nathaniel Ponvert, Mark Johnson, Sorel Ouonkap Yimga, Sherry Warner, Octavia Rowe, Plant Lab Members: Alison DeLong, Judith Bender, Microscope Core Director Geoff Williams, University of Connecticut: Laurina Jaffe, Funding: NSF Grants to MAJ