Novel Function of Myosin XI-G in Gamete Nuclear Migration during Fertilization in Arabidopsis

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Background

- Fertilization in flowering plants comprises of fusion of two sperms with two female gametes. After fusion of gametes, sperm nuclei migrates to female gamete nuclei.
- Contrast to animal, sperm nuclear migration relies on filamentous actin (F-actin) meshwork movement in flowering plant.
- Apart from the involvement of a plant-specific small GTPase (ROP), ROP8 it is largely unknown how F-actin movement for sperm nuclear migration is regulated (Kawashima et al., 2014).
- The application of myosin inhibitor to Arabidopsis ovules arrests F-actin meshwork movement, indicative of myosin involvement (Kawashima et al., 2014) in F-actin movement.

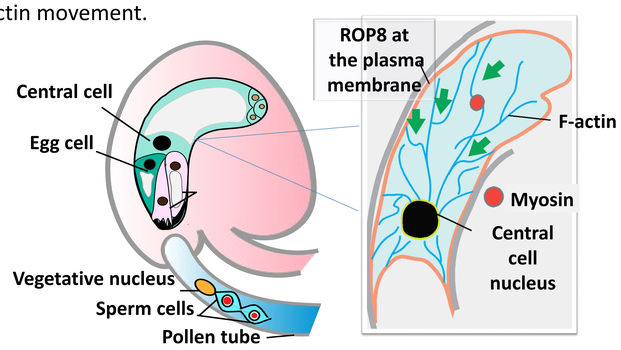


Figure 1. Fertilization mechanism in flowering plants with an emphasis on the F-actin meshwork movement and factors responsible for F-actin movement in the central cell.

Research questions

- Which particular myosin controls F-actin dynamics in the central cell?
- How is F-actin movement controlled by myosin?

Materials and methods

- All Arabidopsis plant lines used in this study are Columbia-0 (Col-0) ecotype.
- All constructs in this study is generated by multisite gateway cloning technology.

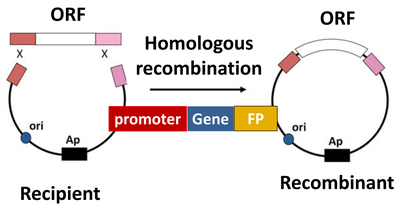


Figure 2. Schematic representation of the gateway cloning technology. This system use the homologous recombination technique for insertion of a particular open reading frame (ORF) into a vector body.

- All T-DNA mutants are obtained from ABRC seed stock.

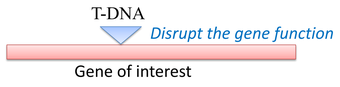


Figure 3. Schematic representation of T-DNA insertion in the genomic region of a gene of interest. The insertion site does not correspond to the actual inserted position.

- The interacting partners of myosin are found using Yeast two hybrid (Y2H) assay.

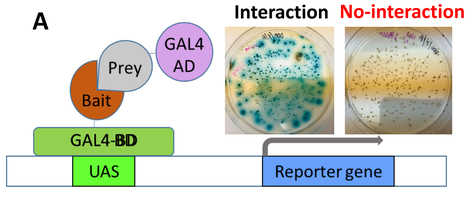


Figure 4. Schematic representation of Y2H assay. Y2H screening plates show interaction (blue colonies) & no interaction (white colonies).

Results and Future Direction

Which particular myosin control F-actin dynamics in the central cell?

The Class XI Myosin XI-G Plays a Major Role in the Active Movement of F-Actin Meshwork in the Central Cell

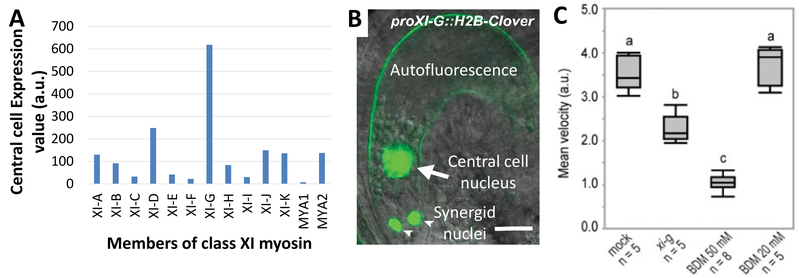


Figure 5. A. The bar diagram shows the RNA-seq expression value of class XI myosins in the central cell. **B.** The transcriptional activity of the Arabidopsis XI-G promoter is visualized by proXI-G::H2B:Clover (green). **C.** Mean velocity of F-actin dynamics in the central cell. Different letters indicate significant difference ($P < 0.01$, Tukey-Kramer HSD test).

How is F-actin dynamics controlled by XI-G?

1. Cytoplasmic streaming/Cellular dynamics?

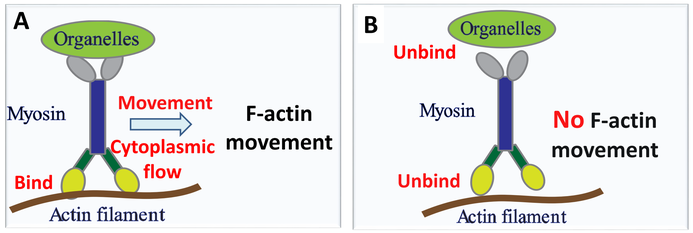


Figure 6. Possible models of F-actin movement by cytoplasmic streaming. **A.** Organelles bound to the myosin when move along actin filament, causes cytoplasmic streaming which in turn may also be responsible for the F-actin movement. **B.** Unbind myosin either with organelles or F-actin can not generate cytoplasmic streaming.

Sub-localization of organelles in the central cell

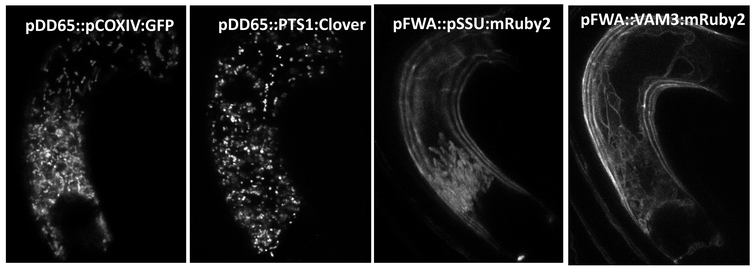


Figure 7. Sub-localization of organelles in the central cell. Organelles are visualized with their specific gene, pCOXIV; mitochondria, PTS1; peroxisomes, pSSU; plastids and VAM3; vacuoles under the control of central cell specific promoters pFWA/pDD65.

F-Actin dynamics is Controlled by a Non-canonical Function of the Myosin

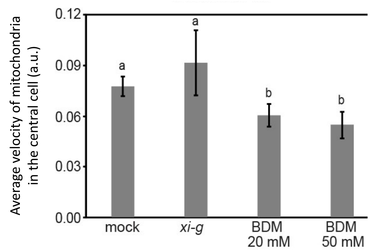


Figure 8. Average velocity of mitochondrial movement in the central cell. Error bars represent SEM. Different letters indicate significant difference ($P < 0.01$, Tukey-Kramer HSD test).

Work plan

- *Investigating co-localization of F-actin, and XI-G with peroxisomes and mitochondria in the central cell.*
- *Quantifying XI-G, peroxisomes and mitochondrial movement in the central cell.*

2. Movement of F-actin via XI-G using large organelles as a foundation?

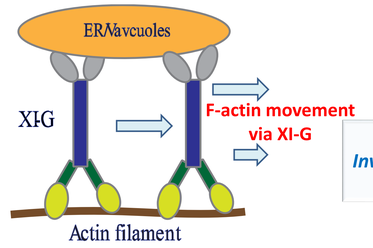
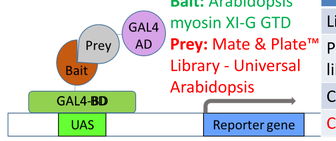


Figure 9. Hypothetical model of F-actin movement via XI-G using large organelles such as ER and vacuoles as a foundation.

Work plan

Investigating F-actin dynamics in the ER and vacuole mutant lines.

3. Novel mechanism?



Working Status	Colony number (cfu)
Library screening	45 million
Positive colonies from library screening	204
Confirmed interaction	54
Candidates working on	2

Selection criteria:
- Highest number of hits
- Similar expression pattern to XI-G

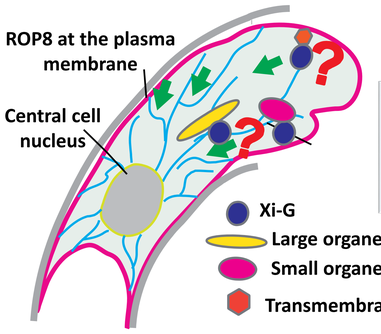
Table 1. Candidate interactors from Y2H assay with their AGI ID, known function and sub-localization

Common name	Known Function	Sub localization	Possible function
Gene A	Membrane transportation	Vacuole membrane	May connect vacuole and XI-G.
Gene B	Not known	Nucleus, cytosol	May act on the plasma-membrane to connect XI-G and actin

Work plan

- *Confirming their expressions in the central cell.*
- *Investigating F-actin dynamics and fertilization phenotype in their mutant lines.*

Conclusion



- *Myosin XI-G control F-actin dynamics in the central cell*
- *F-Actin dynamics Is Controlled by a Non-canonical Function of the Myosin either by cytoplasmic streaming/novel mechanism*

Figure 10. Factors controlling F-actin dynamics in the Arabidopsis central cell.

Acknowledgement & References

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Ali, M.F., Fatema, U., Peng, X., Hacker, S.W., Maruyama, D., Sun, M.-X., Kawashima, T., 2020. ARP2/3-independent WAVE/SCAR pathway and class XI myosin control sperm nuclear migration in flowering plants. Proc. Natl. Acad. Sci.

Kawashima, T., Maruyama, D., Shagirov, M., Li, J., Hamamura, Y., Yelagandula, R., Toyama, Y., Berger, F., 2014. Dynamic F-actin movement is essential for fertilization in Arabidopsis thaliana. eLife 3, e04501.