

SUMMARY

Arabidopsis thaliana pollen tube growth serves as an excellent model elucidating the fundamental nature of unicellular polar growth. From a single pollen grain emerges a unipolar pollen tube fueled by the force of turgor pressure acting on the molecular machinery of cell wall dynamics. These promotional and inhibitory effectors are organized by a signalling network directed by ROP1. ROP1 is a regulator of many molecules, in particular RIC3, RIC4 and RIP1/ICR1 (Figure 1). RIP1/ICR1 targets SEC3, ultimately resulting in polar exocytosis. Exocytosis plays a key role in polar growth, but cell wall dynamics also limit this growth. One enzyme in particular, pectin methyl esterase rigidifies pectin, while its inhibitor promotes the plasticity of pectin, thus maintaining the conditions for polar growth.

ROP1 SIGNALLING NETWORK

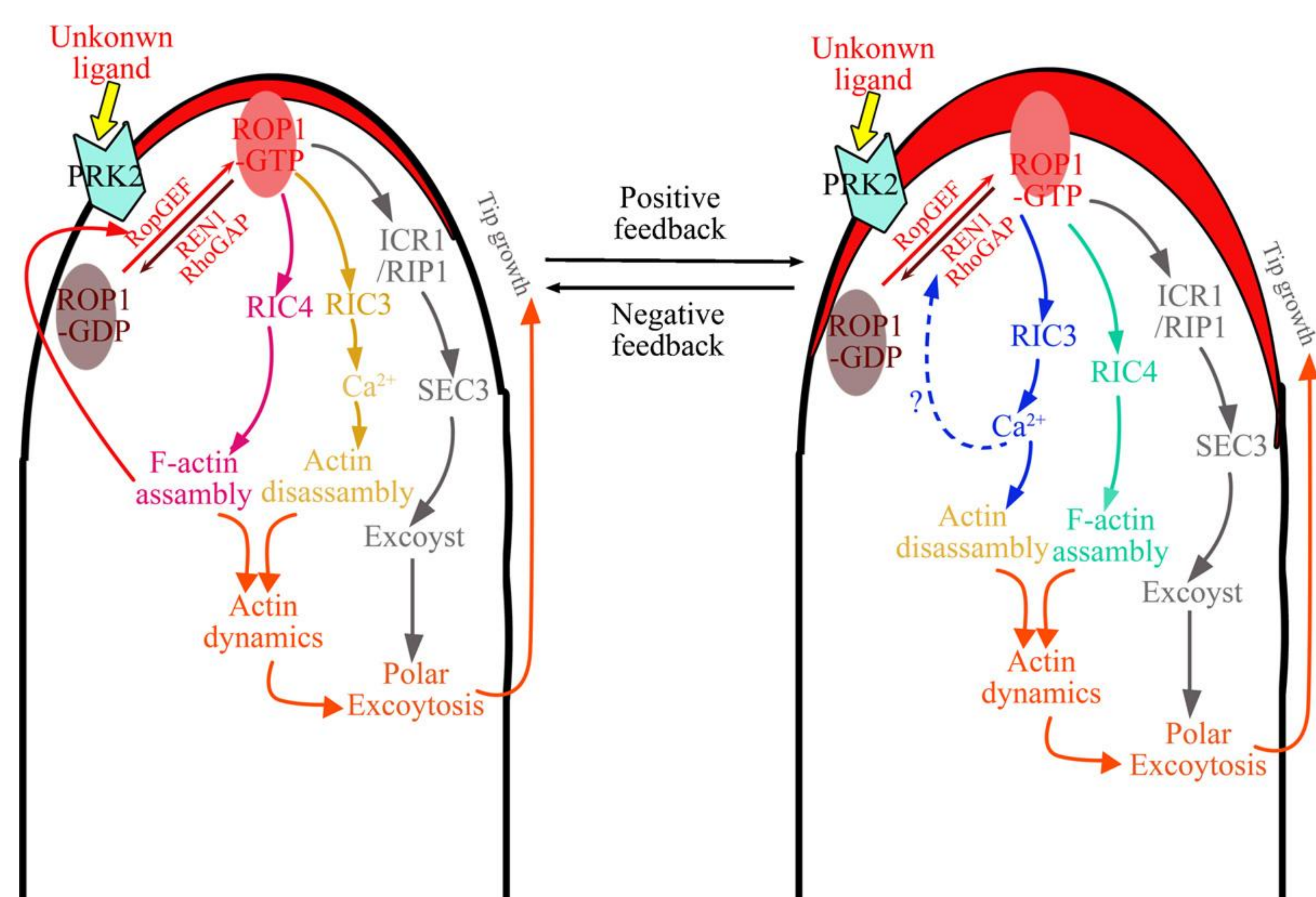


Figure 1: ROP1 Signalling Network, Courtesy of Dr. Zhenbiao Yang

PURPOSE

Spatiotemporally correlating the activity of pectin methyl esterase inhibitor (a key regulatory molecule in cell wall development) with timepoints of SEC3 activity will further define the oscillatory nature of pollen tube growth. This data will be incorporated into a simulation model. Unlocking the keys to polar growth will lead to breakthroughs in optimizing plant biomass and potentially tools to turn on and off polar growth at will.

SAMPLE IMAGE PROCESSING



Figure 2: Enhanced Video of Growing Pollen Tube

EXPERIMENTAL PLAN

A construct fusing SEC3-mCherry and PMEi-GFP will be inserted into pollen genome using tDNA. PMEi will be analyzed using a method called FRAP (Fluorescent Recovery after Photobleaching). A correlation will be made between the timepoints of activity and the spatial locations of the molecules. Analysis will be automated using CL Quant or superior tools as they are developed by our UCR IGERT Video Bioinformatics team.

COMPARISON RIP1-GFP

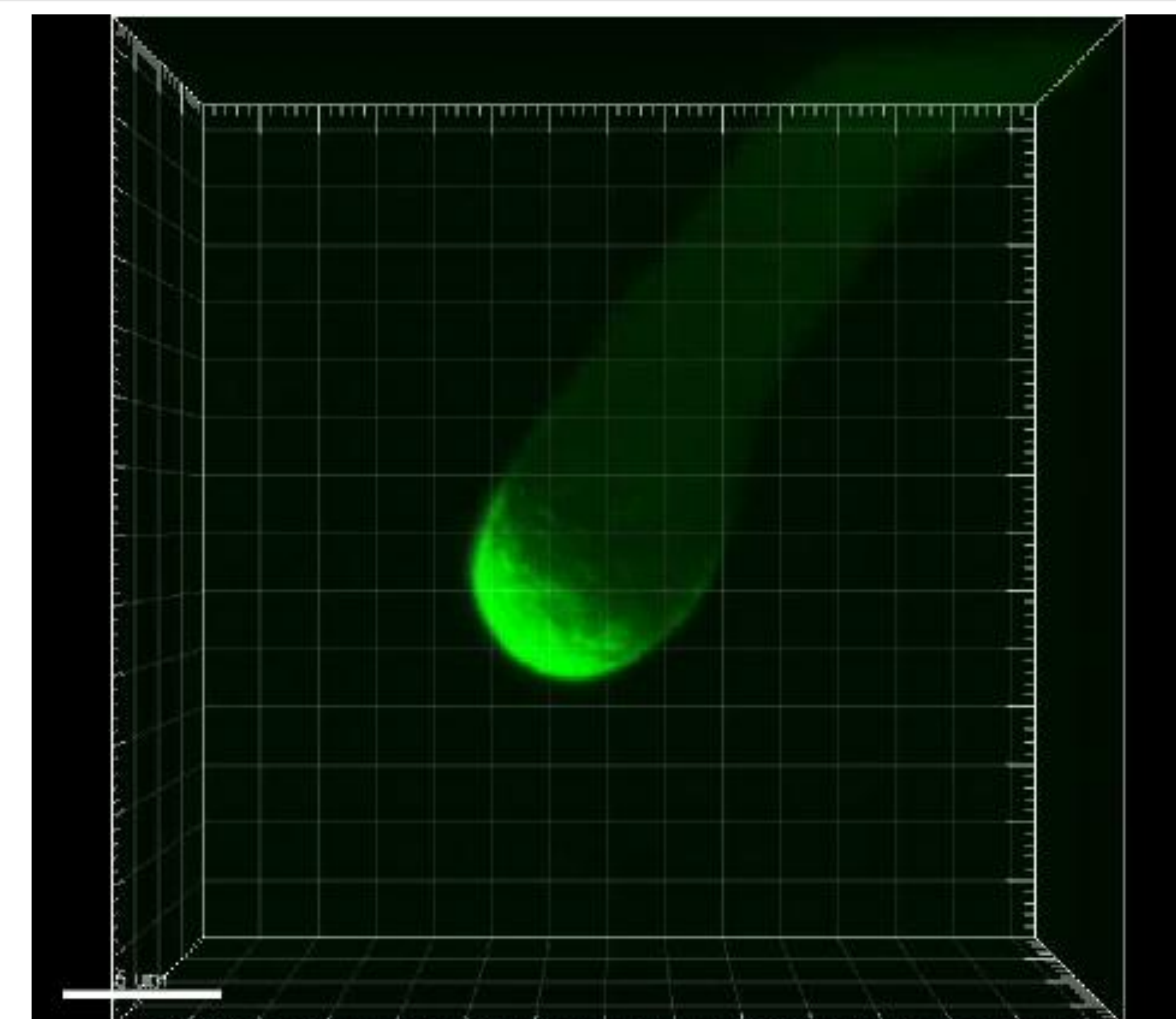


Figure 3: Bitplane RIP1-GFP Video Courtesy of Nan Luo

SEC3 & PMEi BACKGROUND

SEC3 is one of 8 subunits contributing to the exocyst, a protein complex that targets vesicles for exocytosis. PME de-esterifies pectin leading to increased cell wall rigidity. PMEi inhibits this activity, leading to increased softness of the cell wall, promoting growth dependent on cell turgor pressure. This inhibitor is concentrated at the apical tip of pollen tubes, making an excellent target for visualizing the dynamics of tip growth in relation to SEC3's location.

ACKNOWLEDGEMENTS

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