Observation of actin dynamics in living cells

Actin filaments are involved in many aspects of cell morphogenesis and intracellular motility in plant cells. Actin dynamics are analyzed in a live cell using actin visualized transgenic plants expressing fluorescent protein-tagged actin-binding domains, such as GFP-talin and GFP-fimbrin. However, analyses are mainly conducted on the epidermal cells because the high resolution observation of inner cells, such as mesophyll cells, is difficult. Described below is our procedure for observing actin dynamics in the inner cells of *Arabidopsis* petioles.

Materials

Leaf petiole of GFP-talin-expressing transgenic line of *A. thalliana,* razor blade, silicon oil (KF-96-50cs, Shin-Etsu Chemical Co., Ltd), cover slip, glass slide

Specimen preparation and observation

1. Excise adult leaf petiole from 3 to 4 weeks-old seedling grown under standard culture conditions (23 C, 16L/8D).
2. Put the petiole into silicon oil. As the silicon oil has a low viscosity, it easily penetrates into the tissue and the airspace will be filled with the oil.
3. Using a razor blade, make a tissue section along the vascular bundle.
4. Prepare the specimen in the usual way, mounting with the silicon oil.
5. Incubate the preparation overnight under diffused light. During this period, damaged cells will die.
6. Select healthy, non-damaged cells and analyze actin dynamics under fluorescence microscope. We usually use x 63 objective (NA 1.4) for observation at high resolution. Dead or almost dead cells can be distinguished by the appearance of actin filaments (broken filaments, dots or diffused fluorescence). Damaged cells are distinguishable because chloroplasts in such cells show aggregation inside the cell. As fluorescence excitation light is usually too strong and toxic for live cell imaging, it is highly recommended to reduce the excitation light as much as possible in conjunction with the use of a low light camera. We are using a Roper HQ camera system.

Trouble shooting

If unsatisfactory results are obtained with the present procedure, attempt the procedure again using oil with a different viscosity.