Application Note
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The Samuel Roberts Nobel Foundation

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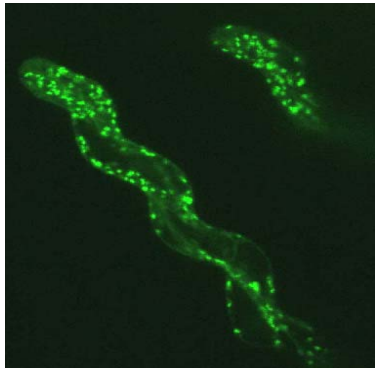
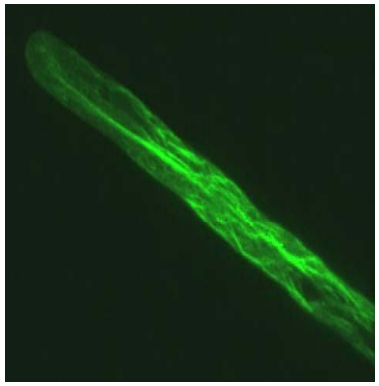
A class one ADP-ribosylation factor GTPase – activating protein is critical for maintaining directional root hair growth in *Arabidopsis thaliana*



Spinning disk confocal microscopy of an agd1-1 root hair expressing ST-YFP to label Golgi stacks.



Spinning disk confocal microscopy of a wild-type root hair expressing GFP-ABD2-GFP to label F-actin.

Dr Cheol-Min Yoo and colleagues at the Samuel Roberts Nobel Foundation are studying the fundamental mechanisms underlying the directionality of tip growth in plants. Membrane trafficking and cytoskeletal dynamics are important cellular processes that drive tip growth in root hairs. These processes interact with multitude signaling pathways that allow for the efficient transfer of information to specify the direction in which tip growth occurs.

In this study the team investigated the role of AGD1 (a class one ADP ribosylation factor-GTPase activating protein (ARF-GAP)) in maintaining straight growth in Arabidopsis root hairs. Initially, two mutants were identified using time lapse DIC microscopy and the team then investigated the differences in both the organelle motility and cytoskeletal dynamics between the wild-type and mutants. To evaluate whether the *AGD1* mutation affected organelle trafficking in root hairs, the team crossed *agd1-1* mutants with plants expressing a yellow fluorescent protein (YFP) used to fluorescently mark Golgi stacks in plants.

The PerkinElmer Ultra*VIEW* ERS spinning disk confocal microscope was used to acquire time lapse images of growing wild-type and *agd1-1* root hairs expressing ST-YFP. Images were acquired at 1 sec intervals for 60 sec. The images revealed that Golgi stacks were highly dynamic along the length of the root hair.

Using **Volocity Classification (Quantitation)** the team tracked individual Golgi stacks from the apical 20 µm and the basal region to obtain Golgi velocity measurements. In the basal region, the average velocity of Golgi stacks in *agd1-1* was slightly reduced compared to wild-type and this difference was statistically significant. More importantly, time-lapse spinning disk confocal microscopy revealed that Golgi stacks in root hairs of *agd1-1* occasionally extended into the root hair tip whereas Golgi stacks in wild-type root hairs maintained approximately a 10 µm distance from the extreme apex.

Through comparing Golgi and actin dynamics the investigation successfully shows that AGD1 is key for maintaining straight growth in Arabidopsis root hairs.

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