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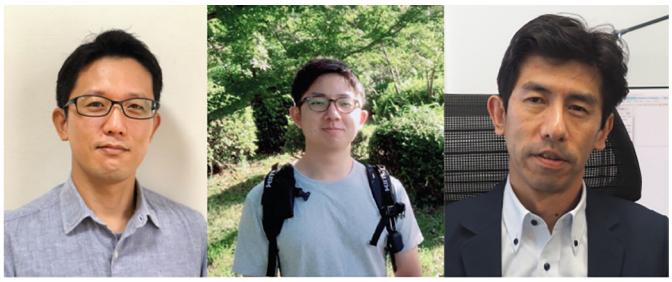
INTERVIEW

The people behind the papers – Tatsuaki Goh, Kaoru Sakamoto and Keiji Nakajima

During root development, the cells in the root cap transition from having a gravity-sensing function to becoming secretory cells and finally being shed. A new paper in Development describes the spatiotemporal dynamics of this process at both the cellular and subcellular levels, and identifies a key role for autophagy in organelle rearrangement and cell shedding. To find out more about the research, we caught up with first author Kaoru Sakamoto, first and corresponding author Tatsuaki Goh, Assistant Professor at Nara Institute of Science and Technology (NAIST), and corresponding author Keiji Nakajima, Professor at NAIST.



Tatsuaki Goh, Kaoru Sakamoto and Keiji Nakajima (L-R).

Tatsuaki and Keiji, can you give us your scientific biographies and the questions your labs are trying to answer?

TG: I did my PhD work on the endomembrane system in plants in Akihiko Nakano's lab at the University of Tokyo. During my PhD, I became interested in how dynamic behaviours of individual plant cells coordinate to form multicellular organs with specific functions. This led me to join Hidehiro Fukaki's lab at Kobe University as a postdoc, to study lateral root formation in *Arabidopsis* using long-term time-lapse imaging. I also had a chance to work in Malcolm Bennett's lab at the University of Nottingham, where I enjoyed working with people with diverse expertise. As an Assistant Professor at Nara Institute of Science and Technology (NAIST), I am currently working on the root growth dynamics by establishing a horizontal-axis motion-tracking confocal microscope system, in collaboration with Keiji and other lively lab members.

KN: I started my research career by studying molecular and structural biology of alkaloid biosynthetic enzymes in medicinal plants in Kyoto University. Later, I became interested in the spatiotemporal regulation of alkaloid biosynthesis in plant roots. As a further extension of my research interest, I joined Phil Benfey's lab in New York University, as a postdoc, to work on *Arabidopsis* root tissue patterning mediated by a mobile transcription factor. Currently, my lab at NAIST is working to elucidate how root cells integrate intrinsic genetic programs and external cues to acquire their specific functions. Technically, I am very interested in live imaging of cellular and subcellular dynamics in the living roots. This technique should make the *Arabidopsis* root an even better model system to gain new insights in plant development.

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Graduate School of Science and Technology, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan.
E-mail: goh@bs.naist.jp; k-nakaji@bs.naist.jp

Kaoru, how did you come to work on this project?

KS: As a master's student, I wanted to conduct cutting-edge biological research, and joined Keiji's laboratory at NAIST. Among the several projects on offer, I chose to analyse the developmental dynamics of plant root cap using time-lapse imaging. Luckily, our exciting data demonstrated a previously unknown role for autophagy in root cap development.

Can you give us the key results of the paper in a paragraph?

TG & KN: There are two key findings in our paper. One is the temporal dynamics of the subcellular rearrangement that enables the functional transition of the outermost root cap cells from the gravity-sensing cells to the secretory cells. Observations from our motion-tracking microscope clearly visualised the sequence of organelle rearrangement that accompany the functional transition of the outermost root cap cells. Although this change in subcellular structures had been previously described by electron microscopy studies, its spatiotemporal details have not been documented because of technical difficulty. The other main finding is the key role of spatiotemporally regulated autophagy in the organelle rearrangement and, somewhat unexpectedly, in the process of cell detachment. Our study clearly demonstrated the essential role of autophagy in plant development.

Pectin- and cellulose-degrading enzymes are implicated in cell separation in the root cap; do you think their secretion is regulated by autophagy?

TG: Yes, we certainly assume that possibility. Plant cells are tightly connected by cell walls and thus precise modification of cell wall structures is essential for cell detachment. Several cell wall-modifying enzymes are specifically expressed in the outermost root cap cells in *Arabidopsis*, including the pectinase RCPG and the cellulase CEL5. They should be secreted into the extracellular space via the membrane trafficking pathway. Since several key events for autophagosome formation require both the activation of ATG proteins and the membrane trafficking pathway, autophagy may affect the accumulation of the cell wall-modifying enzymes in the space surrounding the outermost root cap cells. We are currently testing this possibility.

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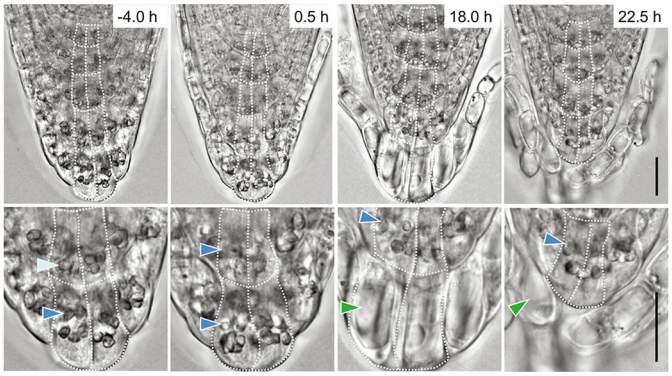

Organelle rearrangement in the outer root cap layers captured by time-lapse imaging.

Do you have a hypothesis on how autophagy is regulated in time and space in the root cap, and do you think that upregulating autophagy earlier would lead to premature cell shedding?

TG: Yes, we have a couple of hypotheses to be tested. Autophagy is induced by various abiotic and biotic stresses, such as nutrient starvation and pathogen attack, but they are unlikely to activate the root cap autophagy, because it can occur in plants growing on a sterile nutrient-rich medium. The root cap autophagy occurs with a high spatiotemporal precision, suggesting a more programmed nature of its control. The two BEARSKIN (BRN) transcription factors are known to promote the maturation of the outer root cap cells, but, again, are unlikely to regulate the root cap autophagy, because the mutant lacking two BRNs still exhibits organelle rearrangement in the root cap. A strong temporal association between the timing of autophagy activation and the cell detachment event instead suggests a regulatory mechanism mediated by mechanical and/or molecular signals derived from the cell detachment. As for the second question, early activation of autophagy may perturb the cell detachment behaviour, but I am not sure if it would result in premature shedding. Curiously, lack of autophagy rather enhances cell shedding.

When doing the research, did you have any particular result or eureka moment that has stuck with you?

TG: Yes. There were many such moments during this research because I observed something new almost every time I imaged. For example, during my first trial using the bright-field motion-tracking microscope, I could already observe the dramatic change in the subcellular organisation of the root cap cells. These changes were quite impressive and way faster than I thought. This exciting experience drove me to start this project, allowing me to have another exciting moment when I observed the cell detachment defects of the autophagy mutants. I had never expected this, because it was hard to link autophagy to the events occurring in the extracellular space. This finding could have been missed with conventional snapshot imaging.

And what about the flipside: any moments of frustration or despair?

TG: It was challenging to establish the motion-tracking microscopy system. Earlier, the system did not work as expected. For example, the tracking algorithm was sometimes cheated by the detached root cap cells and the system stopped following the root tip. Focus drifting and bleaching of fluorescent signals were other problems. We fixed many problems, one by one, to develop a better system over nearly 5 years in total. Now the system works nicely and has become an indispensable tool in our lab.

KS: The earlier version of our motion-tracking microscope was not equipped with a confocal unit, so I could not take fine images of autophagosomes and vacuoles. At that time, confocal time-lapse imaging was done manually – I had to take images every 6 hours for a few days using a conventional confocal microscope. Although it was tough to fight sleepiness, this allowed me to come up with the idea that the autophagy activation is spatiotemporally linked to the cell detachment process. Fortunately, we could later establish the high-magnification time-lapse technique to see the subcellular structures. The images I took manually are not used in this paper, but I have a good memory of the time I spent on capturing these images! Although I am now working on a totally different project at a sake brewery company [establishing a heterologous protein production system using koji (*Aspergillus oryzae*)], the experience I had during graduate study reminds me of the importance of time-consuming experiments and the attitude required to improve techniques.

Where will this story take your labs next?

TG & KN: This study proved the power of our motion-tracking microscopy system in visualising the cellular and subcellular dynamics in the tip of growing roots. We realised how finely the cellular and the subcellular structures are regulated in the root cap cells and how dynamically they can change. Seeing what's going inside the root cap cells is a key to understanding the mechanisms by which the root cap integrates diverse environmental cues to direct the root growth.

In a broader sense, we plan to apply this technology to visualise the dynamic and coordinated behaviours of root cells, and to further incorporate the techniques of computer vision for automated quantification. A similar approach has been taken by European researchers. Cell-level quantification of root growth dynamics combined with conventional genetic tools will open a door to understand plant growth dynamics, not only in our lab, but also in international collaborations.

Finally, let's move outside the lab – what do you like to do in your spare time?

TG: I enjoy playing tennis and skiing with my family. I practice to keep up with my sons!

KN: I like outdoor activities such as camping, hiking and cycling, but my daily fun is seeing pretty little plants growing in a small garden at my house.

KS: I often exercise at the gym. I go before work, and it clears my head and helps me get my work done.

Reference

Goh, T., Sakamoto, K., Wang, P., Kozono, S., Ueno, K., Miyashima, S., Toyokura, K., Fukaki, H., Kang, B.-H. and Nakajima, N. (2022). Autophagy promotes organelle clearance and organized cell separation of living root cap cells in *Arabidopsis thaliana*. *Development* **149**, dev200593. doi:10.1242/dev.200593