Press Release

Where males sense females in plants

Unraveling the unknown receptors and mechanism for fertilization in plants

March 10, 2016

Pollen tubes are attracted by LURE peptides, which are produced from ovules, to bring about fertilization. In their recent report published in *Nature*, a pair of plant biologists at Nagoya University has now revealed for the first time, the receptor in pollen tubes that is required to detect LURE. By uncovering this unknown mechanism in plant fertilization, this may lead to an improvement in the efficiency of pollen tube growth, which may thus result in an increased success rate of fertilization. In addition, this study may also generate new methods to enable cross-fertilization between different plant species to generate new crops.

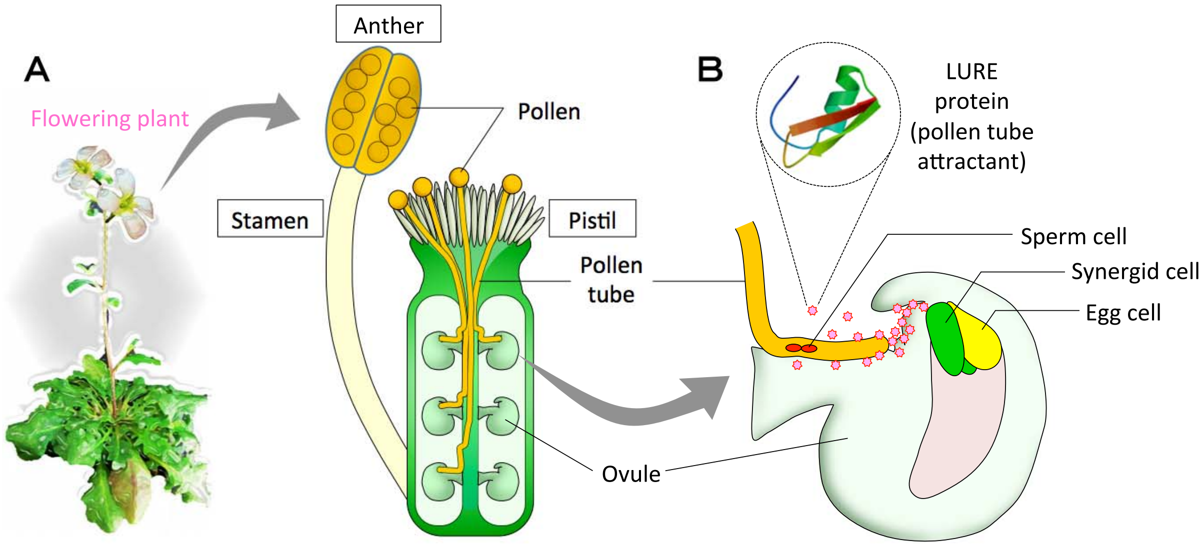


Figure 1. Pollen tube growth and guidance by the LURE peptide in *Arabidopsis*. (A) Image showing the stamen and pistil in *Arabidopsis*. When pollen formed at the tip of the stamen (anther) reaches the pistil, the pollen tube starts to grow towards the inside of the pistil. The pollen tube grows until it eventually reaches the ovule, where it fertilizes an egg cell and develops a seed. (B) The synergid cell, located next to the egg cell, produces the pollen tube attractant, LURE. Once the pollen tube reaches the synergid cell, it releases its sperm cells, which is accepted by the egg cell, thus leading to fertilization. The LURE peptide of *Arabidopsis thaliana* consists of about 70 amino acids and is effective for specifically attracting the pollen tube of *Arabidopsis thaliana*.

Nagoya, Japan – Dr. Hidenori Takeuchi and Professor Tetsuya Higashiyama of the JST-ERATO Higashiyama Live-Holonics Project and the Institute of Transformative Bio-Molecules (ITbM) of Nagoya University have succeeded in discovering a key kinase receptor in the pollen tubes (male) of flowering plants responsible for allowing the pollen tubes to precisely reach the egg cell (female) to enable successful fertilization, without losing its way.

Pollen tubes grow inside the pistil and deliver their sperm cell to egg cells, which are located deep inside the pistil, to bring about fertilization. Higashiyama's group has previously discovered a pollen tube attractant peptide, called LURE, which is produced by the ovule to guide the pollen tube towards the egg cell. Studies have shown that the structure of LURE differs for each plant species and is specific for each plant's pollen tube, i.e. each LURE peptide preferentially attracts the pollen tube of the same plant species. However, the exact mechanism on how pollen tubes detect LURE has been unknown up to now.

In this study, published online on March 10, 2016 in the journal *Nature*, Takeuchi and Higashiyama have discovered a receptor that is required for detection of LURE at the tip of the pollen tube for the model plant, *Arabidopsis thaliana* (thale cress). They also found that this receptor works with multiple receptors that have a similar structure, in order to precisely detect the signals transmitted from the pistil. By accepting the various signals sent from the pistil, the kinase receptors enable the pollen tubes to grow to a position inside the pistil where they can detect LURE. Subsequently, the pollen tubes are guided to reach the egg cell and pass on their sperm cells for fertilization.

"We believe that this study advances our understanding on the mechanism of fertilization between plant species," says Takeuchi, a postdoctoral researcher, currently at the Gregor Mendel Institute in Austria, who carried out this study. "Upon investigating the role of this receptor in further detail, we hope that this will lead to the development of techniques to alter the success rate in fertilization and improve the efficiency of seed production, as well as establish methods to enable fertilization between different species," says Higashiyama, project leader of the ERATO project and a Professor/Vice-Director at ITbM, Nagoya University.

Rice and soybeans that we eat on a daily basis are the seeds of plants and many vegetables develop from seeds. For plants to grow seeds, it is necessary for the male and female reproductive organs in plants to meet and fertilize. The male organ of flowering plants consists of pollen and the sperm cells within. Pollen develops into a pollen tube, which is a single cell with a tubular structure. The tip of the pollen tube (anther) extends and grows into the pistil. The pollen tube eventually reaches the egg cell deep inside the pistil, and passes the sperm cell to the egg cell to bring about fertilization.

The fact that pollen tubes are able to precisely find egg cells without losing its way may be the key element that supports our food supply. The meeting of male and female organs in plants is an extremely mystical and important event, but its exact mechanism is still full of mystery.

In 2009, Higashiyama and his colleagues discovered that a synergid cell, which is located next to the egg cell, produces molecules called LUREs that attract pollen tubes in *Torenia* plants. They also discovered similar LURE peptides in *Arabidopsis thaliana* in 2012.

"We found that the structure of LURE differs according to the plant species, and that LURE of a specific plant attracts pollen tubes of the same species, which preserves fertilization between the same species," describes Higashiyama. "Therefore, LURE has been identified as the key factor produced by the female organ to attract the male organ in plants."

Nevertheless, the mechanism on how pollen tubes can detect LURE, how the pollen tubes grow to a position inside the pistil where they can detect LURE, and the factors behind growth and responses of the pollen tubes have been unknown. Higashiyama's team decided to look into these questions by trying to unveil the key factors in pollen tubes that enable it to detect LURE.

"By using *Arabidopsis thaliana* as a model, we hypothesized that the 23 kinase receptors specifically localized on the membrane surface of pollen tubes could be candidates that are necessary to detect LURE," says Takeuchi. "I conducted bioassays of pollen tubes by deactivating the function of each kinase receptor and found that the PRK6 receptor was essential to detect LURE."

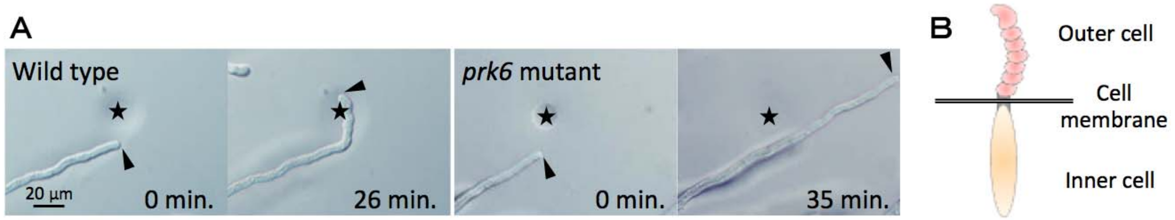


Figure 2. Comparison of wild type pollen tube attracted by LURE and *prk6* mutant pollen tube not attracted by LURE. (A) Wild type pollen tubes (tip shown by an arrow) are attracted by gelatin beads (star) containing LURE on a cultivating plate. On the other hand, a *prk6* mutant pollen tube, which was unable to make a PRK6 receptor did not respond to LURE. This shows that the PRK6 receptor in pollen tubes is crucial for detecting LURE. (B) Diagram of a PRK6 receptor protein. This transmembrane-type protein contains a leucine-rich repeat domain outside the cell and a kinase domain inside the cell.

For PRK6, there are actually multiple families of receptors that have a similar amino acid sequence. Upon deactivating the function of other PRK receptors, Takeuchi and Higashiyama found that the loss of various combinations of PRK receptors led to reductions in responses of the pollen tubes to LURE or hindered pollen tube growth. This coincides with previous reports that the growth of pollen tubes is induced by the PRK receptor responding to the signals sent from the pistil. Hence, the team found that PRK6 and its other PRK receptors work together to detect LURE as well as enable pollen tubes to grow to a position inside a pistil where it can detect LURE.

Takeuchi next studied how PRK6 sends signals within the cells of the pollen tube to understand how it responds to LURE. "When the pollen tube is growing in a straight direction, PRK6 is distributed equally across the cell membrane," explains Takeuchi. "I used fluorescently labeled PRK6 and upon addition of LURE to the pollen tube, I observed that PRK6 moves towards the area of cell membrane on the tip of the pollen tube that faces LURE. The pollen tube then changes its direction and starts to grow towards LURE." From these results, the team showed that PRK6 collects the factors necessary for pollen tube growth in the direction of LURE.

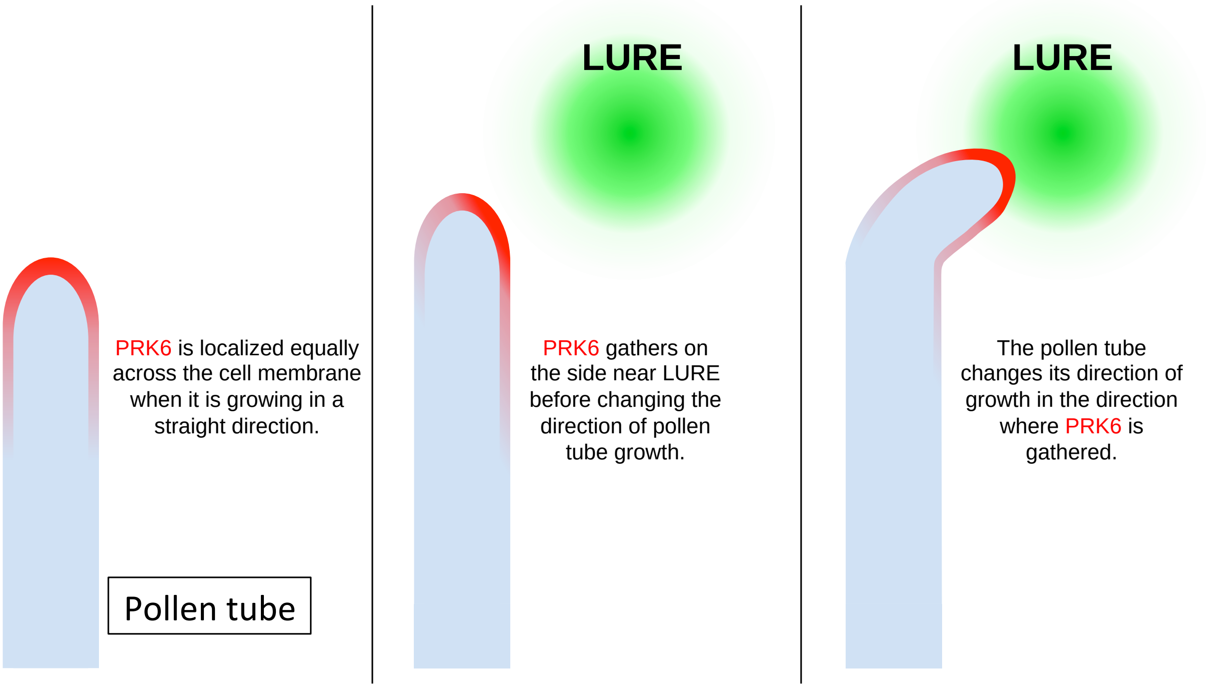


Figure 3. Change in PRK6 localization upon response to LURE. PRK6 is mainly localized in the cell membrane on the tip of the pollen tube. Before changing the direction of pollen tube growth in response to LURE, PRK6 was found to gather in the direction of growth. The direction of pollen tube growth may be determined by collecting the factors for pollen tube growth in the direction of LURE.

“Although the attraction of pollen tubes is considered to occur preferentially between the same species, we wanted to see whether if we can make it occur between different species,” says Higashiyama. Upon treatment of LURE from *Arabidopsis thaliana* to a pollen tube of a *Capsella rubella* (pink shepherd's-purse) plant, which is in the same Brassicaceae (Cruciferae) family as *Arabidopsis thaliana*, no response to LURE was observed.

“Interestingly, when we inserted the PRK6 gene of *Arabidopsis thaliana* into the pollen tube of *Capsella rubella*, it responded to the LURE of *Arabidopsis thaliana*,” says Takeuchi. “This data shows that the PRK6 receptor in the pollen tube is surely the key factor to detect LURE of the same species. We were also really excited to see pollen tube attraction occur between a pollen tube and a LURE of a different species,” say Takeuchi and Higashiyama.

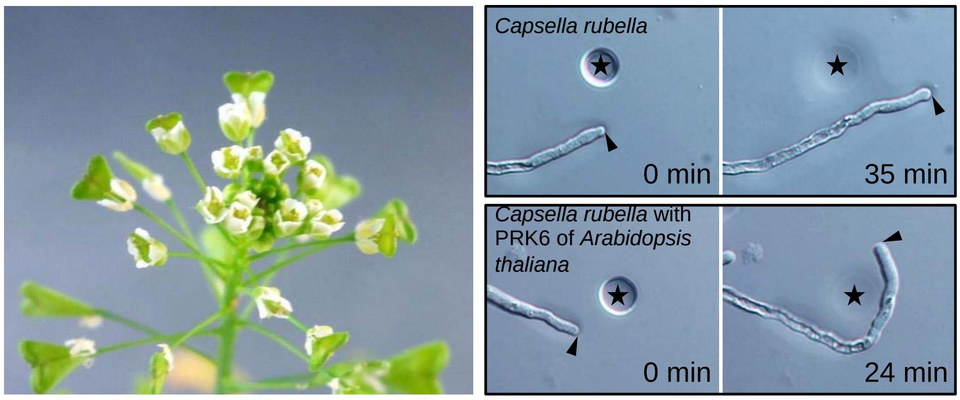


Figure 4. Insertion of PRK6 gene of *Arabidopsis thaliana* into *Capsella rubella*. *Capsella rubella* (photo) and attraction of its pollen tube. Although the pollen tube of *Capsella rubella* is not attracted to LURE beads (star) of *Arabidopsis thaliana* in its normal state, upon insertion of a PRK6 gene of *Arabidopsis thaliana* into the pollen tube of *Capsella rubella*, the pollen tube becomes attracted to LURE of *Arabidopsis thaliana*.

The generation of seeds through the fertilization of the pistil by the stamen has been known for over 2000 years ago and is an extremely important mechanism in agriculture. In addition, the fact that pollen tubes are attracted to the pistil organ has been discovered over 100 years ago. Since the discovery of the attractant molecule LURE, the disclosure of the mechanism of response to the protein has been sought. This study reveals that the PRK6 receptor in pollen tubes is the main factor for detection of and growth towards LURE.

“By further investigation on the family of PRK receptors, we hope to unveil the full mechanism of fertilization that occurs through the growth of pollen tubes and the detection of LURE,” say Takeuchi and Higashiyama.

“We also found in our studies that the insertion of a PRK6 receptor gene allows attraction of the pollen tube of a different species,” says Higashiyama. “This may have potential in developing new methods to enable fertilization between different species. By exploring molecules that target PRK receptors, this may lead to the production of agrochemicals that can improve seed production by increasing the fertilization rate. We also envisage that this study will trigger new research to enable fertilization between different species to create new and useful plant species that can contribute towards a sustainable food supply,” he continues.

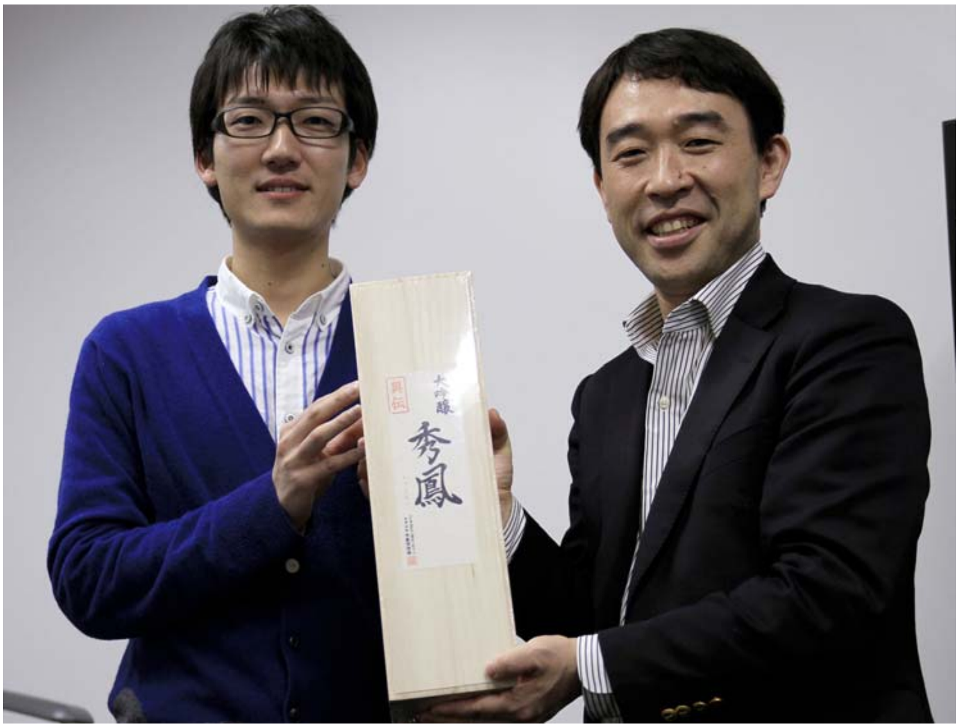
This article “Tip-localized receptors control pollen tube growth and LURE sensing in *Arabidopsis*” by Hidenori Takeuchi and Tetsuya Higashiyama, is published online on March 10, 2016 in *Nature*.
DOI: 10.1038/nature17413 (http://dx.doi.org/10.1038/nature17413)

About WPI-ITbM (http://www.itbm.nagoya-u.ac.jp/)

The Institute of Transformative Bio-Molecules (ITbM) at Nagoya University in Japan is committed to advance the integration of synthetic chemistry, plant/animal biology and theoretical science, all of which are traditionally strong fields in the university. ITbM is one of the research centers of the Japanese MEXT (Ministry of Education, Culture, Sports, Science and Technology) program, the World Premier International Research Center Initiative (WPI). The aim of ITbM is to develop transformative bio-molecules, innovative functional molecules capable of bringing about fundamental change to biological science and technology. Research at ITbM is carried out in a "Mix-Lab" style, where international young researchers from various fields work together side-by-side in the same lab, enabling interdisciplinary interaction. Through these endeavors, ITbM will create "transformative bio-molecules" that will dramatically change the way of research in chemistry, biology and other related fields to solve urgent problems, such as environmental issues, food production and medical technology that have a significant impact on the society.

JST-ERATO Higashiyama Live-Holonics Project (http://www.liveholonics.com/top.html)

Individual cells of multicellular organisms communicate with neighboring cells to maintain the organism. Each cell in a multicellular organism learns its role in the cell population through dynamic and intricate communication with surrounding and distant cells. We call this cell-to-cell communication as “holonic communication”. However, it is still unclear how cells actually communicate with each other in a living organism. The goal of this project is to understand holonic communication in a living, multicellular organism. For this purpose, our project sets up three research groups for optical technology, nano-engineering, and single-cell omics to make a new frontier in ‘live cell biology’ - the real-time analysis of intercellular signaling in multicellular organisms. For live-cell analyses with complete control under the microscope, various new technologies are expected to be developed such as live-cell and single-molecule imaging, manipulation techniques for cell and molecules, interdisciplinary studies of plant biology and engineering technologies, and nano- and micro-device engineering. These technologies will be applicable to other fields, not only scientific instruments but also diagnosis methods for medical care, reproductive medicine, and breeding techniques for agriculture.



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