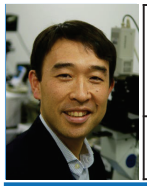
【Grant-in-Aid for Scientific Research (S)】

Principles of Molecular Action Mechanisms in Two-Step Ovular Guidance for the Pollen Tube



Principal Investigator	The University of Tokyo, Graduate School of Science, Professor HIGASHIYAMA Tetsuya　Researcher Number:00313205
Project Information	Project Number : 22H04980　Project Period (FY) : 2022-2026 Keywords : flowers, pollen tubes, two-step ovular guidance, live-cell analysis

Purpose and Background of the Research

●Outline of the Research

In the pistil of flowers, the pollen tube, a tubular cell growing from the pollen, precisely reach the tiny egg cell located at a long distance. Moreover, when there are multiple ovules (pre-fertilized seeds) containing an egg cell, each pollen tube is guided to each ovule in one-to-one manner. The study of pollen tube guidance has long fascinated biologists. Emerging evidences suggest that multiple molecules are provided as directional signals to pollen tubes from the ovule. The question of how the ovule uses multiple attractant signals to guide pollen tubes is not well understood, and understanding this question will be a significant advance in the field of plant science and cell biology as the elucidation of chemotropism. By live-cell analysis, we aim to understand two-step ovular guidance at the level of molecular action mechanisms.

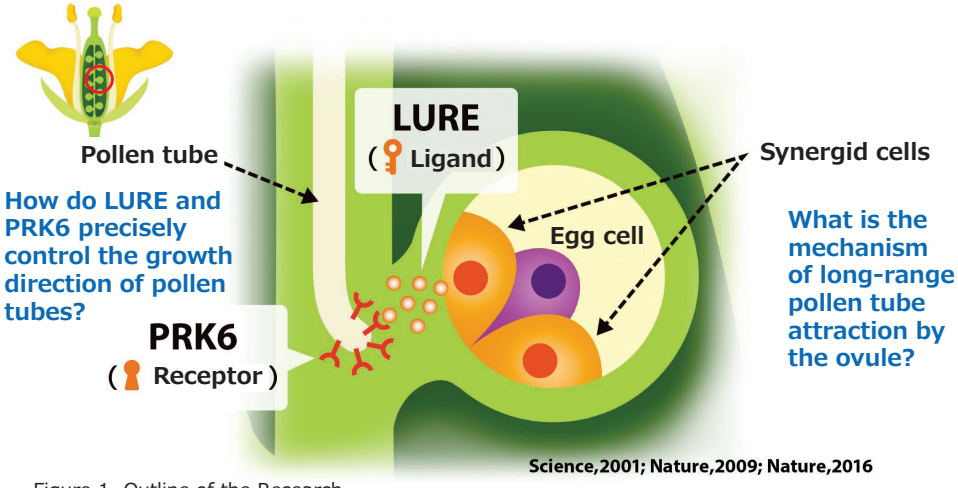


Figure 1. Outline of the Research

● Ovular short-range guidance

In the final step of pollen tube guidance by the ovule, two synergid cells on the side of the egg cell provide a short-range attractant signal for the pollen tube, the LURE peptide, which consists of about 70 amino acids. Synergid cells secrete multiple species of LURE and LURE-like peptides; LURE was found in a plant called *Torenia* (in which an egg cell and synergid cells protrude from the ovule). In *Arabidopsis*, we found a receptor for LURE1, PRK6 on the pollen tube tip (a single transmembrane receptor kinase). In *Torenia*, a bio-active sugar chain AMOR was found to be derived from the ovule to activate LURE pathway. However, how precisely LURE and PRK6 control the growth direction of pollen tubes in short-range and what is the mechanism of long-range pollen tube attraction by the ovule are big questions.

●Ovular long-range guidance

On the other hand, we have found CALL1 as a candidate for ovular long-distance attractant of *Torenia* (unpublished). CALL1 differs significantly from LURE in the following points: 1) it does not require pollen tube activation by AMOR, 2) the curvature of the attracted pollen tubes is very different, 3) the attraction distance by CALL1 and LURE are very different (several mm for CALL1 vs 0.2 mm for LURE), 4) Microfluidic devices that show attraction by CALL1 do not show attraction by LURE, and 5) no CALL1 attraction activity is observed in the bioassay for LURE. Therefore, the mechanism of attraction between LURE and CALL1 is likely to be very different. However, it is not at all clear how they differ.

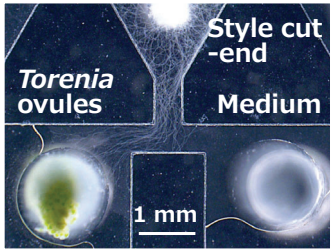


Figure 2. Long-range guidance in a microfluidic device (pollen tube exiting the cut end of the pistil are growing toward the left side where ovules are located)

Expected Research Achievements

In this study, multiple guidance derived from the ovule will be clarified at the level of molecular action mechanisms. Pollen tube guidance, as well as axon guidance in nerves, have been well studied as a model of chemotropism. Pollen tubes are well suited for studying real-time chemotropism with simple diffusion and concentration gradients of guidance molecules. Moreover, this study aims to elucidate multiple action mechanisms of ovule-derived guidance molecules. This work will provide significant insights into chemotropism.

At the tip of the pollen tube, various molecules are oscillatory regulated in tens of seconds to minutes.PRK6, a LURE receptor, is thought to signal to these cyclic regulatory pathways with the intracellular domain. PRK6 is a receptor-like kinase that, together with other members of the PRK family, acts on pollen tube growth itself. Perception of LURE by PRK6 has been suggested to induce accumulation of PRK6 toward the LURE side, thereby altering the direction of pollen tube elongation. Single molecule imaging will reveal how individual PRK6s change their behavior upon interaction with LUREs.

A sharp concentration gradient might be required for LURE to attract pollen tubes in short range. A microfluidic device for LURE is also being fabricated and will be compared with that for CALL1. On the other hand, to understand how long-range guidance molecules, such as CALL1, work to attract pollen tubes, it would be critical to identify the receptor that is the point of action of CALL1 (AMOR-independent). Simple calculations show that the concentration gradient of CALL1 in the microfluidic device is such that there is a difference of only a few molecules between the right and left pollen tubes. How it provides directional information to the pollen tube will be discussed based on receptor identification and downstream analysis. Long-range guidance is thought to be a signal that directs individual pollen tubes to the ovule prior to precise short-range guidance. It will be clarified how long-range signal is actually distributed in the pistil. Relation with repulsive process will be discussed as well.

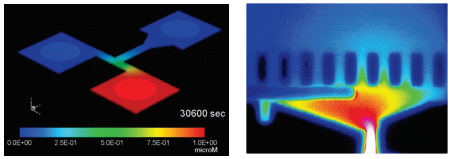


Figure 3. Concentration gradients in microfluidic devices showing long-range attraction (left) and short-range attraction (right)

Homepage Address, etc.	https://www.devcell-u-tokyo.bio/