Cambial Stem Cell System RIKEN ECL Research Unit（2024）
RIKEN ECL Unit Leader: Dongbo Shi (Ph.D.)

(0) Research fields

CPR Subcommittee**:** Biology
Keywords: Secondary growth, cambium, stem cells, environmental response, morphogenesis

(1) Long-term goal of laboratory and research background

Many plants can continue growing throughout their lifetimes. Stem cells play a crucial role in plants' continuous growth, with cambium stem cells in particular serving as the driving force behind secondary growth, contributing to the gigantism of terrestrial plants. Cambium stem cells perform remarkably diverse functions by maintaining themselves while differentiating into various cell types, coordinating with surrounding cells to modulate their activity in response to environmental factors such as the seasons. However, many underlying mechanisms remain unexplained. Our research unit aims to elucidate these mechanisms, thereby understanding the plant-specific nature of growth at the cellular level and exploring the differences in survival strategies between plants and animals.

(2) Current research activities (FY2024) and plan

(A) Elucidation of a novel molecular mechanism governing secondary growth in plants

This unit has previously studied the secondary growth process in plant hypocotyls (Figure 1) from a developmental biology perspective. In October 2024, we relocated from the University of Potsdam, Germany, to the RIKEN Centre for Sustainable Resource Science (Yokohama Office).
Previously, using single-nucleus RNA-seq analysis, we elucidated cell type-specific gene expression patterns during secondary growth in Arabidopsis thaliana hypocotyls and reported these findings in a preprint (Zhao et al., bioRxiv, DOI: 10.1101/2023.04.05.535530, 2023). This year, we also presented these findings at an international conference (Research achievements 3).

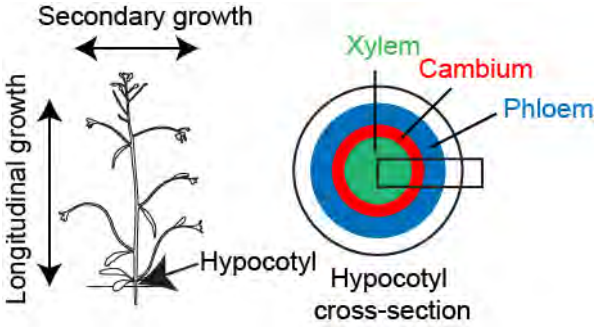


Figure 1 Schematic diagram of secondary growth of Arabidopsis thaliana hypocotyl

Future plan, 1) Single-cell RNA-seq analysis has identified a novel xylem-specific CLE gene. The CLE peptide family comprises over 30 genes identified in Arabidopsis thaliana, reported to perform various functions. While CLEs expressed in the phloem have been shown to regulate secondary growth, the function of xylem-expressed CLE genes remains unknown. Future work aims to elucidate the function of this CLE gene during secondary growth. By combining a localised gene manipulation system with immunostaining to visualise peptide localisation within tissues, we seek to quantitatively determine peptide dynamics in vivo and deepen our fundamental understanding of peptide hormone signalling.

(B) Further development of single-cell RNA-seq analysis technology in plants

Single-cell analysis is a high-resolution analytical technique that enables the acquisition of gene expression information, previously only studied at the tissue level, at the single-cell level. It is a powerful tool for tissues where various cell types are intermingled and cannot be isolated by dissection. Our unit reported the findings revealed using single-cell analysis in the aforementioned preprint. However, single-cell analysis in plants faces inherent technical obstacles, and its application remains limited at present. This year, we published a review summarising key considerations for performing single-cell analysis in plants, alongside technical introductions (Research achievements 1 and 2), thereby sharing the insights and techniques gained to date.

Future plan, One reason why single-cell analysis remains challenging across various plant species is the high cost involved. By advancing technological developments such as multiplexing, we aim to reduce the cost per analysis while simultaneously developing techniques that enable single-cell analysis across

diverse plant species and tissues.

(C) Control mechanisms of the cambium in Trees

In trees, cambial stem cells are the most abundant stem cells present throughout the plant body. However, much remains unclear about how these stem cells coordinate their self-renewal and differentiation throughout the seasons to generate functional tissues.

Future plan, Using poplar as a model tree species, research will be conducted to elucidate the control mechanisms of the cambium.

(3) Members

(RIKEN ECL Unit Leader)
Dongbo Shi

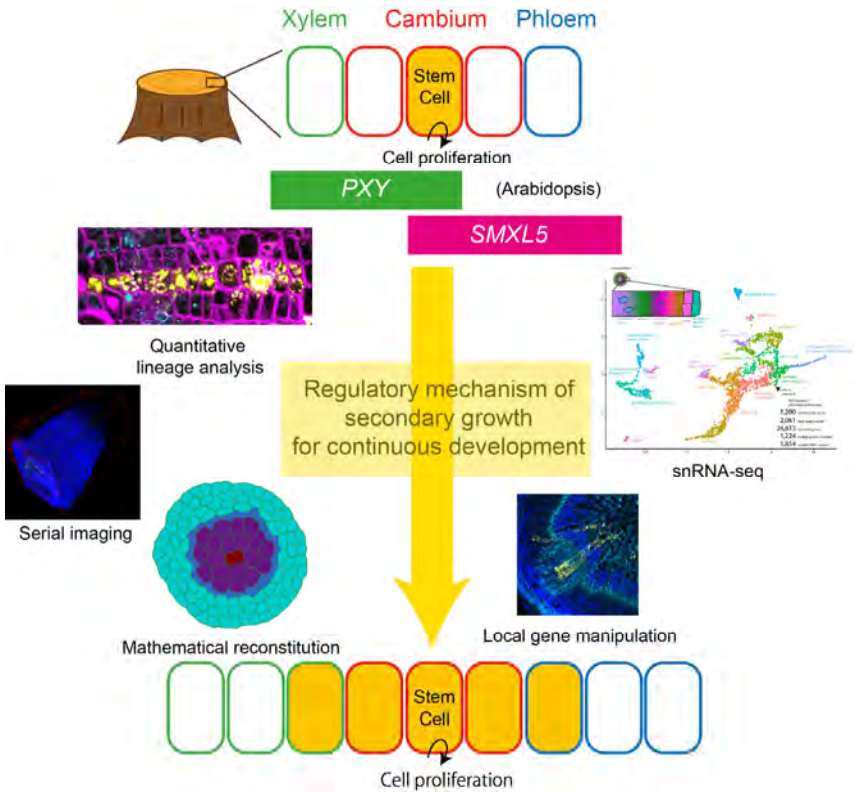
(Technical Staff)
Tomoko Kuriyama

(Student Trainee)
Hui Cao

(4) Representative research achievements

1. C. Grones, T. Eekhout, D. Shi, M. Neumann, LS. Berg, Y. Ke, R. Shahan, KL. Cox Jr, F. Gomez-Cano, H. Nelissen, JU. Lohmann, S. Giacomello, OC. Martin, B. Cole, JW. Wang, K. Kaufmann, MT. Raissig, G. Palfalvi, T. Greb, M. Libault, B. De Rybel, "Best practices for the execution, analysis, and data storage of plant single-cell/nucleus transcriptomics", **Plant Cell** 36(4), 812-828 (2024).
2. 石　東博,「植物核抽出　セルソーターによるシングル核解析に適した抽出」, **実験医学別冊　誰でも再現できる NGS「前」サンプル調製プロトコール　(羊土社)** 鹿島　誠, 伊藤　佑, 尾崎　遼／編(2024).
3. J. Zhao, K. Kaeufer, H. Cao, L. Lassen, T. Greb, D. Shi, "Revealing cambium stem cell behaviour during secondary growth in Arabidopsis thaliana with snRNA-seq", **Cold Spring Harbor Asia Conference FRONTIERS IN SINGLE CELL GENOMICS**, Awaji, Nov. 5-8th, 2024.

Supplementary



Overview of activities of Cambial Stem Cell System RIKEN ECL Research Unit in FY2024

Laboratory Homepage

https://www.riken.jp/en/research/labs/ecl/cambial-stem-cell-sys-riken-ecl/index.html
https://drshilabo.wordpress.com/